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RESEARCH ARTICLE

Analysis of pharmacists' knowledge and attitude in the pharmaceutical industry of halal certification and their readiness to produce halal medicine

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Abstract

Background: Since the issuance of Law 33 in 2014 concerning Guaranteed Halal Products, the government has an obligation to remind all parties that the law should be carried out properly in regards to the halal certification of medicine. **Aim**: This study aims to determine the attitude of pharmacists towards halal medicine and their readiness to produce it, as well as the relationship between their attitude and readiness. **Methods**: This study had a cross-sectional observational design, the research variable was the attitude and readiness of pharmacists to produce halal drugs. The sample used was 206 pharmacists who were carrying out professional practice in the pharmaceutical industry in East Java. The instrument used was a valid and reliable questionnaire. **Results**: The results showed that 51.54% of respondents agreed with halal certification on medicines and 58.74% of pharmacists were ready to produce halal medicine. **Conclusion**: The majority of pharmacists were in support of producing certified halal medicine. The attitude of these pharmacists was also related to their readiness to produce halal medicine.

Introduction

The world's need for halal products, including food, cosmetics, pharmaceuticals, services, and other products, will continue to increase along with the increase of the global Muslim population (Yusuf, Shukri & Yajid, 2016). Even today, the trend of halal products is not only developing in countries where the population is predominantly Muslim (Saha, Rifat, & Shimanto, 2019). Non-Muslim countries such as Japan and South Korea are becoming increasingly interested in halal products (Yusof & Shutto, 2014; Satriana & Faridah, 2018). In those countries, halal is considered to be the benchmark for guaranteeing food safety and quality (Saha *et al*., 2019). In the last five years, the general public awareness of halal products has begun to increase (Saha *et al*., 2019). There is also an increasing awareness among Muslims to avoid consuming all items containing non-halal ingredients, including medicinal products (Sadeeqa & Sarriff, 2014).

Pharmaceutical products rarely get a halal certificate, and there are only a few medicines with halal certificates (Aziz *et al.*, 2014). This makes the halal status of certain products unknown. The absence of detailed information about additional ingredients in the drug brochure is one of the factors that cause the halal drug status to be unknown (Aziz *et al.*, 2014). Medicinal products consist of a combination of active ingredients and excipients. The active ingredient is the

main ingredient used to treat disease, and there is usually only one of these in a product. Meanwhile, the excipient ingredients are additives, and there are usually more than one of these in a product. Excipients added to the active ingredient are used to provide good physical qualities, such as taking tablet form or acting as a diluent, flavouring agent, dye, binder, or preservative (Regenstein & Chaudry, 2003; Aziz *et al.*, 2014). The active ingredient and the excipients must both come from halal ingredients and be processed without any additional non-halal material sources (Khan *et al.*, 2013) because the halalness of medicinal products is determined by both of them.

Halal medicine can be defined as medicine that comes from halal materials, which can be sourced from plants, animals, or organic/non-organic substances starting from the process of preparation, production and extraction according to the Islam rules. In addition, it must be ensured that the drug is free from additives that are not halal (Khan & Shaharuddin, 2015). Halal certification for all medicinal products is needed in order to confirm the status of these products. The halal status will reduce the confusion and doubt of consumers to consume the product because it is in clear accordance with the teaching of their religion (Garg & Joshi, 2018).

The suspicion regarding the illegality of drugs that have not included a halal label is based on the fact that many of the additives come from pork. For example, pork gelatine is often used as a capsule shell. Gelatine is a component commonly used in food, cosmetics and pharmaceutical products because of its gelling properties (Rohman *et al.*, 2020). The consumption of food products from pork sources is strictly prohibited in Islam. Gelatine and collagen are important ingredients in products and become critical points in determining halalness. These ingredients come from cows, but a few also come from pigs (Yuswan *et al*., 2021). In addition, gelatine and collagen are widely used in various applications, such as in the food and pharmaceutical industries (Shabani *et al.*, 2015). Currently, Muslim scientists are still looking for alternative ingredients to use in halal medicine (Saha *et al.*, 2019). For instance, gelatine can be replaced with polysaccharides that are guaranteed to be halal (Faridah, 2019). Some polymers made from polysaccharides can be used as substitutes for gelatine materials; these include carrageenan, xanthan gum, maltodextrin, alginate, chitosan, gellan gum, and guar gum (Jana *et al.*, 2011). In addition, the halalness of pharmaceutical products is also determined by the process of production, packaging, storage, distribution and service. Besides having to be halal, everything that is consumed must also be safe, which means it cannot be harmful and must have good quality. This is commonly called "*Halalan Toyyiban*" in Islamic law (Aziz & Sulaiman, 2014).

The halalness of medicines is also determined by pharmacists being competent professionals in terms of pharmaceutical products. The role of pharmacists, both those who practice in the pharmaceutical industry and those who practice in the service, provide big hope to the community that they will ensure the availability of halal medicines. For pharmacists who practice in the pharmaceutical industry, select and prepare medicinal raw materials, carry out the production process, ensure that the products are of quality and ensure that the drugs produced meet the requirements according to applicable standards, including their halalness. Apart from halal medicines, it is important to initiate pharmacists from educational institutions in order to have sufficient human resources. However, until today the pharmacy curriculum regarding halal and haram medicines is still rarely discussed (Aziz *et al.*, 2014).

Pharmacists must have knowledge about halal and haram matters (Sarriff & Razzaq, 2013) so that those who practice in the service are able to explain to patients the availability of halal treatment options. These efforts can increase patient satisfaction and the trust that they have for their chosen medical services (Khan & Shaharuddin, 2015). Consulting services to find out halal information about the pharmaceutical preparations of products is part of a must-have skill for pharmacists (Syahrir, Rahem & Prayoga, 2019). Therefore, the aim of this study is to analyse the knowledge and attitude of pharmacists towards halal certification and their readiness to produce halal medicine.

Methods

Study design

This research was conducted in November 2018 in East Java using primary data and a questionnaire that had previously been tested for validity and reliability. The questionnaire given included several questions with three main topics: knowledge, attitudes and acceptance of halal drug certification. The research subjects were 206 respondents who were pharmacists working in the pharmaceutical industry in East Java. An ethics approval was not deemed necessary as the survey population did not include any-risk groups, and anonymity was assured to all participants. In addition, signed informed consent was obtained from all participants prior to data collection.

Population, sample, and sampling technique

The research population were pharmacists who attended a professional training event by the East Java Industrial Pharmacist Association in November 2018. The research sample was part of the population with the following inclusion criteria: pharmacists who were Indonesian citizens, willing to become respondents by filling out informed consent and who have worked in the industry with a minimum of five years of work experience. The sampling technique carried out was random sampling. The sample size was 206 pharmacists, and the sample size (n) was calculated using the simple random sampling formula with notation N (total population size), P (population proportion), and d (degree of error) as shown below (Ogston *et al.,* 1991):

$$\hat{Var}(\hat{P}) = \frac{P(1-P)}{n} \times \frac{N-n}{N-1}.$$

Z = 1.96; P = 0.5; d = 0.05; N = 440 pharmacists; thus, n = 205.34 = 206 pharmacists

Research variables

The research variables consisted of the independent and dependent variables. The independent variables were the knowledge and attitude of the pharmacists in the pharmaceutical industry in regards to halal certification. The dependent variable was the readiness of pharmacists in the pharmaceutical industry to produce halal drugs. The variable data were in the form of the respondents' answers to the questionnaire given to them.

Data analysis

The data from the answers to the questionnaire were tested for normality first using the one-sample Kolmogorov-Smirnov test. If it showed that the data were normally distributed, then the parametric test used for the correlation test was the Pearson test. If the data were not normally distributed, then the non-parametric test that was used for the correlation test was Spearman's rank test. After analysing the normality of the data, it turned out that the data were not normally distributed. Furthermore, to determine the relationship between the pharmacists' knowledge and attitude towards halal certification as well as their readiness to produce halal medicines, an analysis was carried out using the Spearman's rank correlation test. The Mann-Whitney test was also used to determine the differences in knowledge, attitudes, and readiness of respondents regarding halal certification and production of halal drugs while age, length of work, and ownership were analysed using the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

Results

Respondents characteristics

Characteristics of respondents were grouped based on the sex, age, length of practice, and ownership of the pharmaceutical industry in which they work. 69.9% of respondents in this study were women; this statistic is presented in Table I.

Table I: Respondents characteristics

Category	Frequency (n: 206)	Percentage (%)
Gender		
Female	144	69.9
Male	62	30.1
Age (years)		
≤ 35	135	65.5
36 - 45	47	22.8
46 - 55	20	9.7
≥ 56	4	2
Duration of practice (years)		
≤ 10	173	84.0
11-20	17	8.3
21-30	10	4.9
≥ 31	6	2.9
Pharmaceutical industry ownership		
Foreign investment (PMA)	24	11.7
Domestic investment (PMDN)	154	74.8
State-owned enterprises (BUMN)	7	3.4
Individuals	21	10.1

In this study, the majority of the respondents (65.5%) were 35 years old or less; this can be seen in Table I. There were 2% of respondents aged 56 years old or more. The majority of respondents (84%) had ten years or less as practice as a pharmacist in the pharmaceutical industry, and 2.9% had 31 years or more of work experience.

Respondents' knowledge of the halal drug certification

Respondents' knowledge about halal drug certification was divided into three categories: low, medium, and

high. Data on knowledge in Table II showed that the majority of respondents (48%) had low-category knowledge in regards to halal drug certification.

Table II: Respondent data

Category	Frequency (n: 206)	Percentage (%)
Respondents' knowledge		
Low	100	48.5
Medium	89	43.2
High	17	8.3
Attitude of respondents		
Disagree	19	9.22
Agree	107	51.94
Strongly agree	80	38.84
Respondents' readiness		
Not ready	10	4.85
Ready	121	58.74
Very prepared	75	36.41

The attitude of the respondents towards the halal drug certification

Based on the questionnaire given to the respondents, it was found that only 9.22% of respondents disagreed with the halal certification of drugs while the remaining 90.78% agreed. As much as 51.94% and 38.84% stated that they strongly agreed, as shown in Table II.

Readiness of respondents for halal drug certification

Only 4.85% of respondents stated that they were not ready to produce halal-certified drugs, as seen in Table II. The percentages that stated that they were ready and very ready were 58.74% and 36.41%, respectively. If combined, the ready and very ready respondents added up to 95.15%. This is a consequence of the prevailing laws and regulations, in which all industries have to prepare themselves to carry out certification and produce halal drugs. The author did not conduct an audit on each pharmaceutical industry but rather collected data through questionnaire answers given to respondents.

The data normality test can be seen in Table III.

Table III: Data normality test

Variable	Statistical test	*p*-value	Description
Age of respondents	Kolmogorov-Smirnov	0.0001	Data distribution is not normal
Duration of practice	Kolmogorov-Smirnov	0.0001	Data distribution is not normal
Knowledge	Kolmogorov-Smirnov	0.0001	Data distribution is not normal
Attitude	Kolmogorov-Smirnov	0.0001	Data distribution is not normal
Readiness	Kolmogorov-Smirnov	0.0001	Data distribution is not normal

Based on the statistical analysis shown in Table IV, there were no differences in knowledge, attitudes, and readiness related to certification and production of halal drugs between both genders. Likewise, there were no significant differences as a result of the variables of age and length of work, with each p-value being more than 0.05. This meant that gender, age, and length of work did not affect the knowledge, attitudes, and readiness of respondents towards the certification and production of halal drugs.

Discussion

The results of this study were in accordance with the characteristics of pharmacists in Indonesia, as seen in other research conducted by Satibi and the authors (2018) in different practice settings. For example, at the Health Center (Puskesmas) in 2018, the majority of pharmacists were women (72.2%) (Satibi *et al.*, 2018). Although jobs in the pharmaceutical industry are harder than in services, especially for female pharmacists, there are still more women who are interested in the industry because there are more female pharmacists in the East Java region. There were 76% of pharmacists in the East Java region were women based on data from the Indonesian Pharmacists Association of East Java (Syahrir *et al*., 2019). Syahrir's research that was carried out in various pharmaceutical practice settings also shows that 86% of respondents are female (Syahrir *et al.*, 2019).

Table IV: Data analysis

Characteristics	Categories	Knowledge M ± SD	Attitude M ± SD	Readiness M ± SD
Gender	Female	17.55 ± 5.49	25.02 ± 2.71	25.02 ± 2.63
	Male	18.74 ± 5.19	24.03 ± 3.30	24.32 ± 3.64
	Mann-Whitney (*p*)	0.289	0.068	0.052
Age	≤ 35	17.58 ± 5.47	24.95 ± 3,03	24.60 ± 3.11
	36 – 45	18.47 ± 5.73	24.83 ± 2.63	25.34 ± 2.61
	46 – 55	18.20 ± 4.19	24.70 ± 3.03	25.30 ± 3.03
	≥ 56	21.00 ± 7.00	23.33 ± 3.06	23.00 ± 1.00
	Kruskal-Wallis (*p*)	0.566	0.084	0.229
Duration of Practice	≤ 10	17.64 ± 5.42	24.87 ± 2.99	24.71 ± 3.00
	11 – 20	19.76 ± 6.18	25.47 ± 1.94	25.94 ± 2.49
	21 – 30	18.20 ± 3.61	25.10 ± 2.73	25.50 ± 3.81
	≥ 31	17.50 ± 4.04	23.00 ± 3.46	23.75 ± 0.50
	Kruskal-Wallis (*p*)	0.132	0.178	0.196
Knowledge	Spearman's rho (*p*)	-		0.566
Attitude	Spearman's rho (*p*)		-	0.0001

The practice conditions are related to the existing policies in Indonesia, namely the minimum requirement of three pharmacists in the pharmaceutical industry starting from the existence of Government Regulation number 51 of 2009 concerning pharmaceutical work (Syahrir *et al*., 2019). The regulation states that the person in charge of production, quality control, and quality assurance must be a pharmacist. This regulation came into effect on the 1st of September 2009, which was approximately ten years before the conduction of this research. Another thing is the recruitment of employees in the pharmaceutical industry in Indonesia usually has a maximum age limit of 35 years old, so it is natural that the majority of pharmacists working in the pharmaceutical industry are currently aged 35 or less. This research is the same as that conducted by Cambrey Nguyen in 2020, which stated that the majority of pharmacists (70%) who work in the industry are aged 35 or less old (Nguyen, 2020). This shows that most of the respondents were relatively young and at a very productive period. Based on the author's experience, most pharmacists that work in the industry are relatively young. After having experience in the industry and entering a more elderly age as well as having sufficient capital, people usually work in easier places such as in pharmacies or as lecturers at universities.

The results show that the respondents are mostly new pharmacists who started working in the pharmaceutical industry after the issuance of government regulation number 51 in 2009. Based on the ownership of the pharmaceutical industry, 74.8% of respondents' place of work was mainly in the domestic capital owner industry (PMDN). Meanwhile, the remaining 25.2% were spread across the pharmaceutical industry, foreign investment (PMA), state-owned enterprises (BUMN), and individuals.

The findings show that the respondents lacked knowledge regarding the concept of halal and halal drug certifications. This result is in accordance with what was conveyed by Eddy Yusuf and the authors (2016), where they stated that the biggest current challenge was the pharmacists' lack of understanding in the industry regarding the concept of halalness (Yusuf *et al.*, 2016). This is understandable because the existing pharmacy education curriculum has not included the concept of halalness as a part of the learning. In addition, training related to the halal

industry, especially in the pharmaceutical industry, has not been implemented much, unlike training related to International Organization for Standardization (ISO) management and good drug manufacturing methods that have been carried out by many training agencies and government institutions. Even professional organisations such as the Indonesian Pharmacists Association often hold training to improve pharmacists' competence in the industry, especially regarding how to make good medicines.

For respondents who had knowledge that was categorised high and very high in regards to halal, the possibility was that they were pharmacists who had already had a foundation of good religious understanding because of their religious family background or their activeness in Islamic activities. In this study, there were no data to reveal this. In regards to this, it is very necessary to adjust the pharmacist education curriculum to include material related to the halal concept and encourage training institutions or competent institutions such as Badan Penyelenggara Jaminan Produk Halal (BPJPH) to carry out training on an ongoing basis. It has to be implemented because training is needed in order to improve the competency of the resources needed by the industry, as stated by Norazmi & Lim (2015).

There were several reasons for the halal certification agreement, including the fact that the regulation had been established in 2014 and had become effective starting from the 17th of October 2019. Therefore, it has not been possible not to follow the applicable regulations in Indonesia while still producing drugs in the country. Another reason is the market has started to undergo an increase in demand for halal drugs from various hospitals, especially in areas with most populations were Muslim and in hospitals that have been declared as religious hospitals. With so much request for halal drugs, the pharmaceutical industry must adjust to these conditions by producing halal drugs; otherwise, it will die by itself because the products are not needed by customers. This research is in line with that conducted by Syahrir and the authors (2019), which states that the acceptance/attitude of pharmacists regarding halal drugs strongly agrees with the 89% index (Syahrir *et al.*, 2019).

Analysis of the relationship between knowledge and the readiness of respondents regarding certification and production of halal drugs obtained a value of p = 0.566 or p > 0.05, which means that there was no significant relationship between knowledge and readiness. The analysis of the relationship between attitude and readiness obtains values of p = 0.0001 and r = 0.602. This shows that there is a significant relationship between the respondents' attitude and readiness in regards to the certification and production of halal drugs. The relationship is a strong value of r = 0.602. From all these statistical analyses, it can be explained that the readiness to produce halal drugs is only influenced by the positive attitude of the respondents, while other factors in this study have no effect.

Conclusion

From the research conducted above, it can be concluded that pharmacists' knowledge regarding halal certification on drugs is still lacking. The attitude of the respondents towards the halal certification of drugs showed that 51.94% of respondents agreed with the halal certification of drugs and that 58.74% of respondents were ready to produce drugs with halal certification.

Conflict of interest

The authors state that there were no conflicts of interest in this study and the article.

Authors' declaration

The authors hereby state that the data and all contents presented in this article are original research results from the authors. Any liability for claims relating to the content of this article will be borne by the authors.

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RESEARCH ARTICLE

The impact of pharmacist shortage on the inventory management of medicines at primary healthcare centres in East Java, Indonesia

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Abstract

Background: Pharmacists are the only people authorised to manage the medicines inventory. However, in the case of pharmacist shortage, other personnel may take over this function. This is particularly the case in most primary healthcare centers (puskesmas) in Indonesia. **Aim**: To compare the outcome of medicine inventory management between pharmacists and non-pharmacists in primary healthcare centres (PHCs). **Methods**: A survey of 146 puskesmas in East Java was conducted involving 73 pharmacists and 73 non-pharmacist staff. This study was conducted from July to January 2020. Each respondent completed a questionnaire focusing on the inventory aspects of medicine management. **Results**: Purchasing accuracy is higher for pharmacists (90%) than for non-pharmacists (68%). Pharmacists manage the inventory more efficiently with only 2% of the drugs expired and wasted while non-pharmacist staff wasted 16% of the drugs and 18% of the drugs expired. **Conclusion**: The role of pharmacists in medicine inventories is vital as they carry out more efficient and accurate medicine management.

Introduction

The position of pharmacists in community health service facilities is very important because these are the places where people obtain safe, effective and high-quality drugs. Community pharmacists must ensure that medicines are efficiently regulated and available in sufficient quantities before focusing on providing clinical pharmacy services (van de Pol *et al.*, 2020). Over the years, several studies have shown that pharmacists spend a lot of time and money on pharmaceutical logistical setups (van de Pol *et al.*, 2019). Pharmaceutical logistic supply chains have unique characteristics so that it has different characteristics from other consumer commodity supply chains. The pharmaceutical supply chain faces uncertainty; for example, the demand for each drug is uncertain and can be affected by seasonal changes (Franco & Alfonso-Lizarazo, 2020). The supply chain of pharmaceutical products is characterised by high complexity, and the supply and delivery channels to customers are limited and highly regulated (Merkuryeva, Valberga & Smirnov, 2019). Since accuracy and skills are required in drug management, careful pharmaceutical management is directly linked to a country's ability to address public health problems (Uthayakumar & Priyan, 2013). In addition, health service facilities in the community are comfortable places for people to get information or advice from pharmacists regarding medication and drug use (Nunes, Anderson & Martins, 2015).

Management procurement, storage, and distribution in pharmaceutical preparation are very important for health care facilities and pharmaceutical companies (Uthayakumar & Priyan, 2013). Health service facilities can plan for drug procurement according to the needs

of the community around the health facility. The suitability of the management of pharmaceutical preparation with the needs and characteristics of health service facilities (Saha & Ray, 2019) will be achieved properly if managed by a pharmacist. The availability of drugs at the health centre is very important because the primary healthcare centre (*puskesmas*) is the leading health service facility. It is the closest to the community and is the main destination of the community for medical treatment (Athijah, Rahem & Setiawan, 2018), especially in the current era of National Health Insurance (JKN), where *puskesmas* are one of the first level health service providers (PPK I). In each sub-district, there is at least one *puskesmas*. Drug availability is an important factor to ensure rational drug use by patients. In addition, drugs also form a link between patients and health care facilities so they can encourage public trust in these health service facilities (Athijah, Rahem & Setiawan, 2018).

The initial planning step in a drug management series at health centres will affect the availability and suitability of the drugs and needs. Usually, stocking a number of drugs that is not close to the real needs is the main factor that causes empty or excess stock at the *puskesmas*. Therefore, it requires careful planning by all people in charge of the *puskesmas* pharmacies (Istiqomah & Satibi, 2012). Unsuitable drug stock at the *puskesmas* poses the risk of irrational treatment and will also increase cost, as well as potentially resulting in a large number of damaged or expired drugs. If the stock of drugs at the *puskesmas* is insufficient or below the required amount, patients do not receive the appropriate medicine, which results in irrational treatment. If the drugs are in excess or exceed the need, this will result in the drugs becoming damaged or expired because they were stored in the *puskesmas* for too much time (Rahem, 2017). In addition, if the management is not efficient, the number of expired drugs and the management cost will increase at the *puskesmas*.

Puskesmas are the Technical Implementing Units (UPT) of the district/city Health Service, so it is appropriate that drugs and consumable medical materials are obtained from the district/city Health Office or from regency/city pharmaceutical installations. Therefore, the adequacy of drugs at *puskesmas* are also determined by their availability at the institution. The planning and procurement of drugs in district/city pharmacy installations is based on data compiled from proposed planning for *puskesmas* and other health service facilities under the auspices of the Health Office. To accommodate for the recording and reporting that supports the evaluation of drugs and medical supplies, it is necessary to use an information system that is based on drug and transaction data for the process of purchasing and receiving drugs, drug distribution, and drug use and availability (Sanjaya *et al*., 2013). Information technology systems in the planning and procurement of drugs or what is often called e-purchasing and e-catalogue at health service facilities or the health office are very helpful to speed up the process which affects the availability of drugs (Ningsih, Fudholi & Sumarni, 2015).

The availability of drugs and consumable medical materials at the health centre depends on the competence of the drug administrator (Athijah & Rahem, 2018). In accordance with regulation, pharmacists are the ones who have the expertise and authority in relation to pharmaceutical work (Rahem, 2017). Moreover, there has currently been an expansion of the scope of pharmaceutical practice, which initially only focused on drug management; now, it is also obliged to carry out pharmaceutical care to ensure the success of therapy in patients. Drug management is not just how drugs are produced and distributed but also includes where patients get their drugs from, how to use them, from whom they get information about drug use and how they store them at home (Athiyah & Rahem, 2017). Therefore, pharmacists should run the pharmacy practice or be the person in charge of managing drugs at the *puskesmas*.

East Java Province had 960 *puskesmas* in 2016 (National Statistic Bureau, 2016). Unfortunately, not all *puskesmas* have a pharmacist as the person in charge of the pharmacy, and so there are still many other health workers or non-health workers who manage drugs in the East Java *puskesmas*. These conditions mean that it is very likely that there will be management differences that impact drug availability in the *puskesmas* managed by pharmacists and those managed by other personnel. Given that the competence and authority are not in accordance with the management of pharmaceutical preparation requirements, this study aims to analyse the differences in the suitability of the pharmacists and non-pharmacists that are planning the drug needs at *puskesmas*.

Methods

This study used a quantitative observational design, with a total of 146 respondents who were administering drugs at *puskesmas* in East Java. These were divided into two groups, namely 73 pharmacists and 73 non-pharmacist drug administrators. The selection of respondents was carried out by purposive sampling. Respondents managing non-pharmacist

drugs were selected from health centres that could be accessed via public roads and easily reached by car without having to walk. For the pharmacist respondents who were drug administrators, they were selected using the following inclusion criteria: they had to have been registered as members of the Community Pharmacist in *Puskesmas* Association (HISFARKESMAS) at the regional board of the Indonesian Pharmacist Association (PD IAI) of East Java and been participating in coaching related to responsible pharmacist practices carried out by the PD IAI of East Java. There were 73 pharmacists who fulfilled the criteria. Therefore, in order to have a balance, 73 non-pharmacist drug administrators from *puskesmas* were also taken. Ethics approval was not required as this was an evaluative study confirming the practice in inventory management. However, signed consent was obtained from all participants prior to conducting the survey.

Furthermore, the variable in this study was the management of drugs at the *puskesmas*. The management focuses studied were: the guidelines used as a reference in drug planning, planning time, the quantification method used as a consideration in planning, drug availability, the suitability of the drug requirement plan, expired drugs, damaged medicine, excess stock of drugs, and dead drug stocks.

Data collection was carried out from July 2019 to January 2020. The data collection technique was carried out using a validated questionnaire, which was given to the person in charge of drug management in each selected health centre. Respondents' answers to the questionnaire given were then recapitulated and analysed. Apart from descriptive analysis, inferential statistics were also applied to determine differences in drug availability and the suitability of RKO with drug availability between health centres managed by pharmacists and non-pharmacists. The statistical test used was the independent sample t-test.

Results

There were 83.6% female pharmacists and 69.9% female non-pharmacists included in this study (see Table I). The employment status of the majority of respondents in both the pharmacist and non-pharmacist groups was a civil servant. 67.1% of respondents in the pharmacist group were civil servants, and 69.9% of respondents in the non-pharmacist group were civil servants, as shown in Table I.

Table I: Characteristics of respondents

Characteristics	Pharmacists n (%); n:73	Non-pharmacists n (%); n:73
Gender		
Female	61 (83.6)	51 (69.9)
Male	12 (16.4)	22 (30.1)
Length of work as a drug administrator in years		
< 1	37 (50.7)	16 (21.9)
1 – 5	10 (13.7)	25 (34.3)
6 – 10	13 (17.8)	16 (21.9)
> 10	13 (17.8)	16 (21.9)
Status of Employment		
Public servant (PNS)	49 (67.1)	51 (69.9)
Non-Public servant (non-PNS)	24 (32.9)	22 (30.1)

The results of the study, as shown in Table II, demonstrated the similarities of several things that were done by pharmacists and non-pharmacists, namely: the guidelines used as a reference in planning along with the national formulary. Likewise, the planning time for both pharmacists and non-pharmacists was at the beginning of the year. Receiving drugs that are approaching their expiration date from the Health Office is an interesting experience. All respondents, both pharmacists and non-pharmacists, stated that drugs often reached their expiry date, but at the end of the year, there were more expired drugs in the inventories managed by non-pharmacists.

The results of the study shown in Table II demonstrate that the average availability of drugs at *puskesmas* was better when managed by pharmacists compared to those managed by non-pharmacists. It was found that there was up to 90% drug availability in *puskesmas* managed by pharmacists and up to 70% for those managed by non-pharmacists. Based on the statistical analysis shown in Table III, the value of $p = 0.000$ was obtained, meaning that there was a significant difference in the availability of drugs in the *puskesmas* that were managed by pharmacists and non-pharmacists.

Discussion

In this study, regarding drug administrators at the *puskesmas*, the pharmacist group was in accordance with the research conducted by Satibi and the authors (2018) that showed that the majority of pharmacists in the *puskesmas* were women (Satibi *et al*., 2018). On the other hand, the non-pharmacist drug administrators were in accordance with research conducted by Athijah and the authors (2018), which presented that the majority of drug administrators were women (60%) (Athijah, Rahem & Setiawan, 2018).

Table II: Research variables

Variables	Drug administrator at the *puskesmas*	
	Pharmacist	**Non-pharmacist**
Guidelines used as a reference in planning	National Formulary	National Formulary
Time to plan	Beginning of the year	Beginning of the year
Experience of receiving drugs that are almost expired from the Health Office	Often	Often
Quantification method used as a consideration in planning	Consumption, Epidemiologic ABC analysis, Non-essential Essential Vital (VEN)	Consumption
Average percentage of drug availability compared to those needed at the *puskesmas*	90 %	70 %
Average percentage of the suitability of the planned drug needs (RKO) with those needed at the *puskesmas*	90 %	68 %
Percentage of excess stock of drugs at the *puskesmas* (number of drug items with availability of more than 18 months of use divided by the number of all drug items at *puskesmas* x 100%)	10 - 20 %	10 – 40 %
Percentage of expired drugs at the *puskesmas* (number of expired drugs divided by the remaining stock of the drug item x 100%)	1 - 2 %	5 - 18%
Percentage of damaged drugs at the *puskesmas* (number of damaged drugs divided by the remaining stock of drug items x 100%)	1 - 2 %	2 - 16 %
Percentage of dead drug stocks in the *puskesmas* (number of drug items that never come out in one year divided by the number of all drug items in the *puskesmas* x 100%)	2 - 7 %	2 – 15%

Table III: Statistical analysis

Variables	Types of statistical test	Drug administrator		*p*-value
		Pharmacist **M ± SD**	**Non Pharmacist** **M ± SD**	
Average percentage of drug availability compared to those needed at the *puskesmas*	Independent Samples t-test	90 ± 2.224	70 ± 4.072	0.0001
Average percentage of the suitability of the planned drug needs (RKO) with those needed at the *puskesmas*	Independent Samples t-test	90 ± 2.291	68 ± 2.144	0.0001

Respondents' work experience differed between the pharmacist and non-pharmacist groups. For the pharmacist group, the majority (50.7%) had less than one year worth of experience. This is understandable because the recruitment of civil servants for pharmacists in a relatively large number of health centres has only been carried out after a circular from the Minister of Health of the Republic of Indonesia in early 2018 regarding the need for pharmacist placement at *puskesmas*. The experience in drug management in *puskesmas* for the non-pharmacist respondent groups were seen to be more evenly distributed in all groups, as seen in Table I. Although the majority (34.3%) had between 1 - 5 years of experience, there was not much difference with other groups. Meanwhile, the non-pharmacists with less than one year, 6 - 10 years, and more than ten years were all 21.9%.

Naturally, all humans will look for complete and qualified health service facilities, including facilities, infrastructures, and human resources, to carry out their health checks. Therefore, community health centres that have better facilities will be visited more by the community (Mustafa & Shekhar, 2020). *Puskesmas* are primary health care centres located in sub-districts and villages, so their existence plays an important role in providing the best health services to people in the *puskesmas* working area (Mustafa & Shekhar, 2020). Drug preparation is the most important part of the *puskesmas* facilities considering that drugs are part of the chain between patients and health services, and they can encourage public confidence in the health

service facility (Quick, 2012). Lack of drugs will cause customer dissatisfaction and increase workload (Heiskanen *et al.*, 2015), which will lead to the administrators implementing strategies that will optimise patient care (Donnelly *et al*., 2018). Otherwise, the health of the patients will be adversely affected, and conflicts could be caused between professionals (Abdelrahman *et al.*, 2016).

The reason why respondents still receive drugs that are approaching their expiration date is that at the local Health Office, there are no other drugs that aren't near their expiration date. Thus, patients accept the drug very compulsively because there are no other alternatives. This is a necessity considering that health workers who have the competence and authority related to the pharmaceutical practice are pharmacists. Their education has been almost completely devoted to understanding drugs in all of their aspects. As a result of their knowledge, skills, accessibility and ability to carry out good drug management, pharmacists are placed to ensure the availability of qualified, safe and efficacious drugs in all health facilities; this is in addition to the aspect of service as the pharmacists' expertise and authority (Canadian Pharmacists Association, 2004). Good drug management, such as careful selection of essential drugs, good quality assurance, sustainable procurement, supply management and rational drug use, all serve to optimise the use of limited government funds and optimise the provision of basic services for all people (Hogerzeil, 2006). Therefore, it is important to have a drug administrator at a health centre who has the competence and authority in accordance with the applicable law and regulation and who is a pharmacist. Pharmacists are important for public health as a whole and must be involved in the development and implementation of strategies to protect drug supply chains (Costantino, 2020). Pharmacists who are involved in purchasing drugs, especially at the *puskesmas*, are able to directly procure screen suppliers and ask about the availability of drugs at these suppliers. Companies with drug stock capabilities will be able to provide an uninterrupted supply of drugs even if production facilities experience problems (Costantino, 2020).

The availability of drugs in health facilities is something that absolutely should be controlled because drugs can save lives and improve public health. Most of the main causes of discomfort, disability and premature death can be prevented or treated with drugs, especially using vital and essential drugs (Quick, 2012). Thus, the shortage of drugs in health care facilities will affect the safety of patients who are undergoing outpatient and inpatient care. Drugs that are not available in health care facilities can cause drug-related problems, including patients not getting drugs and patients stopping taking drugs prematurely (Tuti, Athiyah & Utami, 2018).

The drug availability in relation to drug needs depends on the accuracy of the drugs needs plan (RKO) carried out by the implementer, especially considering their quantification (Athijah, Rahem & Setiawan, 2018). Quantification is the first step in the procurement process. Quantification is a process used to determine how many pharmaceutical products are required for procurement purposes. In addition, it also estimates how much money is needed to buy the item. Patient needs are estimated for specific conditions in the health facility, so the analysis should include contextual factors, such as available funds, human resource capacity, storage space capacity, and capacity to provide services. The purpose of quantification is to maintain the most cost-effective balance between service levels and costs (Quick, 2012).

In table II of the results of this study, the considerations applied by pharmacists as a determinant of quantification were: the combination of consumption and morbidity patterns in addition to the consideration of the VEN priority scale and ABC analysis. Meanwhile, the administrators only created the consumption patterns. If the quantification method carried out by the drug administrator was related to the suitability of RKO with drug availability, then it showed better results in the quantification of the combination of various methods when compared to only one method of consumption. Namely, 90% for the combination quantification method and 68% for the consumption method alone, as presented in Table II. Based on the statistical analysis shown in table III, the value of p = 0.0001 was obtained, meaning that there was a significant difference between the suitability of RKO and those required by pharmacists and non-pharmacists. This was in accordance with the research carried out by Rahem (2017), which showed that quantification using only one method of consumption has the potential for incompatibility between RKO and drug availability because disease patterns in an area can change depending on seasons and the replacement of medical personnel who practice in these health service facilities (Rahem, 2017).

The drug procurement process does not end after the drugs are ordered and received. Managing drug supplies at the *puskesmas* can be seen as a component of the procurement process that requires a management fund so that the quality of drugs stored at the *puskesmas* can be maintained. The objective of inventory management is to maintain the minimum amount of inventory required for services in order to control drug costs. Excess inventory can substantially increase management costs and potentially increase

the number of damaged and expired drugs, as well as drugs with out of control stock. A sufficient amount of drugs must be maintained so that there can be a continuity of drug service according to patient needs. A systematic process should be developed and maintained to check the expiration dates of drugs and remove expired drugs from the inventory. The type of expired drugs should be evaluated, as they may yield useful information that can be used as a basis for reducing availability, especially for drugs that are found to be consistently expired (Peterson, 2005).

The results of the study, as seen in Table II, showed that the percentage of excess drugs stocked in health centres managed by non-pharmacists was greater when compared to drug centres managed by pharmacists. Non-pharmacists stocked between 10 and 40% of excess drugs, and pharmacists stocked between 10 and 20% of excess drugs. In line with that, the number of expired drugs and damaged drugs was also larger when managed by non-pharmacists, ranging from 5-18% for expired drugs and 2-16% for damaged drugs. Meanwhile, for drugs managed by pharmacists, there was a maximum of 2% of damaged and expired drugs. A large number of excess drugs stocked resulted in a large number of dead drug stocks. In this study, the stock of dead drugs for health centres managed by pharmacists was 2 - 7%, while in the health centres managed by non-pharmacists, the stock of dead drugs ranged from 2 - 15%.

From all aspects studied, it has been shown that drug management at the *puskesmas* carried out by pharmacists is better than when managed by non-pharmacists. For this reason, it is hoped that the government will place pharmacists at the *puskesmas* considering that they have the ability to maintain an adequate supply of drugs in all conditions, including during a health crisis. Pharmacists have the capability to strategically address the risk of drug shortage and monitor supply from distributors, including directly dealing with drug manufacturers (Li *et al.*, 2020). Pharmacists can carry out assessments by talking to patients, preparing treatment plans tailored to the patients' needs, as well as explaining and educating about diseases, medications and lifestyle modifications. The pharmacists also prescribe therapy management plans for the patient. The pharmacists use their expertise to try and explain the reasons for therapy failure if that occurs. Pharmacists can communicate with doctors about medication to discuss problems with a patients' treatment (Chandrasekhar *et al.*, 2019). Pharmacists monitor adverse drug reactions (ADR), evaluate and analyse patients' symptoms and provide ADR information to physicians (Ying, Qian & Kun, 2020). Therefore, treatment becomes more rational, and the success of therapy can be increased. With the presence of pharmacists in health care facilities, including hospitals, there will be a significant decrease in the number of drugs used, resulting in a decrease in the cost of drug therapy (Aljbouri *et al*., 2013).

Conclusion

The role of pharmacists in managing drug inventories is very vital because they can manage them more efficiently and effectively so that the budget can be used very efficient as the planning for drug needs is more accurate, and loss due to damaged and expired drugs can be controlled. Based on the facts in this study that pharmacists are more competent in managing drugs compared to non-pharmacists, and referring to the prevailing law and regulation in Indonesia that those who have expertise and authority in carrying out pharmaceutical practices are pharmacists, it is recommended that all health centres in Indonesia recruit pharmacists.

Conflict of interest

The authors state that there were no conflicts of interest in this study, and the article.

Authors' declaration

The authors hereby state that the data and all contents presented in this article were original research results produced by the authors. Any liability for claims relating to the content of this article will be borne by the authors.

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IAI CONFERENCE

RESEARCH ARTICLE

Adverse drug reactions associated with successful treatment of multidrug-resistant tuberculosis patients in Cempaka Putih Islamic Hospital Central Jakarta

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Keywords
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Abstract

Introduction: Indonesia experiences Tuberculosis (TB) cases that are very complex and complicated, especially those dealing with Multi-Drug Resistance TB (MDR-TB). Its therapy using several types of drugs can cause several problems; one of them is an adverse drug reaction. **Aim**: To investigate the association between Adverse Drug Reactions (ADR) and the successful treatments of MDR-TB patients. **Method**: This was a retrospective study, the population in this study were all patients with medical and treatment records for MDR-TB. This sampling type is a purposive sampling technique. The statistical analysis used the chi-square test as the statistical test. **Results**: The significant correlation was between the incidence of ADRs and successful treatment with *p*=0.024; odd ratio=2.526; CI=1.193 - 17.892. **Conclusions**: This study may help in formulating strategies for the timely and aggressive management of adverse drug reactions. This can reduce the deferral of therapy and increase the clinical success rate.

Introduction

In Indonesia, the Multi-Drug Resistance Tuberculosis (MDR TB) cases from early 2009 to the first quarter of 2015 were 4,731. (Emil Ibrahim, 2018). Seventy-two per cent of these patients were currently continuing their treatment, while the remaining had recovered. MDR-TB is a condition in which Mycobacterium tuberculosis is resistant to Anti-TB Drugs (ATD), namely Isoniazid and Rifampin (Mulu *et al*., 2015). The World Health Organization (WHO) reported that there were 290,000 cases of MDR-TB in 2011 (WHO, 2016). In addition, there are 27 high burden countries for MDR-TB which represents 85% of the world burden of MDR-TB. Indonesia was in ninth place on this list. Indonesia's MDR-TB cases are increasing every year (Kamso *et al*., 2011).

Patient's non-compliance is the major contributor to drug resistance. A number of efforts were initiated, such as providing health education to TB patients and the general public about TB as well as rational prescribing and prescription review, which includes assessment of prescribing patterns, drug interactions, and adverse drug reactions (Lange *et al*., 2019).

The clinical characteristics of MDR-TB are more complex than sensitive TB due to using of category I and category II TB where Category 1 was for new smear-positive patients with pulmonary TB and Category 2 was for sputum smear-positive patients who have relapsed, who have treatment failure or who are receiving treatment after treatment interruption. In principle, the management of TB uses only one regimen of TB medicines for six months, while the management of MDR-TB uses a minimum of five medicines and lasts 18 to 24 months (Yang *et al*., 2017). Management of MDR-TB cases is often associated with the incidence of side effects ranging from mild to severe. The rationale

for anti-TB drug selection for accuracy is started from the most bactericidal with the lowest toxicity to highest toxicity (Jacobs, Ross, 2012; Yang *et al.,* 2017).

MDR-TB treatment with several types of drugs will cause problems in drug potentiation, as well as side effects. The response of each individual is unpredictable, but treatment should not stop just because of fear of the reaction that arises (El-Din *et al.*, 2015). The incidence rate of patients experiencing severe adverse events was 91.3% with line two anti-tuberculosis drugs, and the percentage of side effects in the mild category was 84.2% (Reviono *et al.,* 2014). It is a serious concern for patients, especially those who experience severe side effects if the monitoring of the side effects of the drug is not carried out properly (Mulu *et al.,* 2015).

This study was conducted in Cempaka Putih Islamic Hospital, which was assigned as one of the public hospitals for treating MDR-TB patients in Indonesia. According to the data from the medical records, the number of MDR-TB patients who are under treatment is 180 patients. For MDR-TB patients treated in the hospital, it seems that the number has increased from 2016-2018. In 2016, there were two patients, and in 2017, the number increased to 7 patients until 2018; there were 15 active MDR-TB patients who were receiving treatments. This is exacerbated by patients who are drug-resistant (WHO, 2016). So, one of the efforts of the hospital to be an effective and safe treatment is by placing the patients separately from other polyclinics to reduce exposure to other patients. Based on this background, the authors are interested in research to investigate the association between ADRs and treatment of MDR-TB in Cempaka Putih Islamic Hospital in Central Jakarta in 2020.

Methods

Design

A retrospective cross-sectional study evaluating 124 patients with MDR-TB who were treated in Cempaka Putih Islamic Hospital in Central Jakarta from January 2019 to December 2019 was conducted. The outcome of interest was defined as sputum culture conversion within three months, and we have analysed its association between adverse drug reaction and the outcome.

Population

This study is originally from medical and MDR-TB treatment records of patients who were treated in Jakarta Cempaka Putih Islamic Hospital in 2019. The type of sampling was carried out on a non-probability basis sampling with purposive sampling technique. That is, determining a certain consideration or criteria that must be attained (inclusion), such as all of the MDR-TB patients who have not consumed MDR ATD ever. Secondly, there were MDR-TB patients who consumed MDR ATD regularly at least three months from the earliest treatment, and the last one is patients who have a number of years more than 17 years old and less than 70 years old, have been willing to take part in the research and have signed the informed consent. Meanwhile, the exclusion criteria in this study are patients who had a history of other lung diseases; and MDR-TB patients who discontinued MDR ATD treatment three months after initial treatment. The number of samples carried out in this study was 136 people, but those who met the inclusion criteria of these patients were 124 patients who were observed during the six months that the study was carried out.

Data analysis

The statistical analysis used was a bivariate analysis to examine the relationship between drug side effects and the outcome of MDR TB patients using the Chi-square test (Hair *et al.,* 2010).

Ethics

The study design was approved by the ethics committee of Poltekkes Kemenkes Jakarta II and Cempaka Putih Islamic Hospital with the number LB.02.01/I/K.E./39/517/2020.

Results

Overall, 124 patients had received MDR-TB treatment with data on patient demographic characteristics consisting of 1) gender; 2) age; 3) occupation and history of comorbid diseases (see Table I).

The frequency distribution of MDR-TB patients based on gender shows that the number of male patients was greater than the number of female patients (56.45%). Based on the age category, there was a proportion of the productive age between 18 and 59 years old, with the highest proportion at 91.93%. The proportion of MDR-TB patients with a history of job status was the greatest; as much as 20.16% of the patients were with a history of employment as traders/labourers. And 32.6% of the patients were self-employed. Most of the patients' history status was in the comorbid category with other diseases percentage of 41.13%so that the most common types of comorbidity were diabetes mellitus (22.58%) and HIV infection (7.28%).

Table I: Sociodemographic characteristics

Sociodemographic characteristics	Number (n = 124)	Percentage (%)
Gender		
• Male	70	56.45
• Female	54	43.55
Age		
• Productive (18-59 years old)	114	91.93
• Less Productive (≥60 years old)	10	8.07
Occupational		
• Employed	99	79.84
• Unemployed	25	20.16
Job status		
• Traders/Laborers/Self-employed	40	32.26
	36	29.03
• Private employee	23	18.55
• Government employee	16	12.90
• Housewife	9	7.26
• Unemployment		
Disease status		
• Has no history of comorbidities	73	58.87
• Has a history of comorbidities	51	41.13
Types of comorbidity:		
• Diabetes mellitus	28	22.58
• HIV infection	9	7.26
• Hepatitis virus	5	4.03
• Hypertension	5	4.03
• Psychotic disorder	4	3.23

In Table II, it can be found that the frequency distribution of the results of the treatment and clinical characteristics of MDR-TB patients indicates the highest treatment. There are five treatment items (33.87%), with the type of cycloserine treatment being the most prescribed (74.19%). The most frequent ADR type in MDR-TB patients was gastrointestinal disorders in the stomach at 22.72%, and the most successful treatment of MDR TB patients was in the cured category (89.52%). In Table III, the relationship between the incidence of ADR and the outcome of success for MDR-TB patients has a significant value (p = 0.024; odds ratio = 2.526) and confidence interval (CI 95% = 1.193-17.892).

Table II: Descriptive summary for all variables of treatment pattern and adverse drug reaction type

Treatment and clinical characteristics	Total (n = 124)	Percentage (%)
Treatment regimen patterns		
• 2 items	1	0.81
• 3 items	18	14.52
• 4 items	41	33.06
• 5 items	42	33.87
• 6 items	10	8.06
• 7 items	12	9.68
MDR-TB drugs used	**Item = 573**	
• Levofloxacin	92	74.19
• Cycloserine	88	70.97
• Ethionamide	72	58.06
• Clofazimine	74	59.68
• Linezolid	60	48.39
• Bedaquiline	61	49.19
• Pyrazinamide	36	29.03
• Ethambutol	35	28.23
• Moxifloxacin	22	17.74
• Isoniazid	14	11.29
• Delamanid	11	8.87
• Kanamycin	8	6.45
Risk of ADR		
• Has an ADR	82	66.13
• Has not ADR	42	33.87
Level of ADR		
• Mild	47	57.32
• Moderate	23	28.05
• Severe	12	14.63
ADR Type		
• GI disturbance	20	16.93
• Peripheral neuropathy	19	16.13
• Psychiatric disorder	15	12.09
• Central nervous system	11	8.87
• Arthralgia, arthritis	9	7.25
• Dermatological effects	4	3.22
• Hyperthiroid	1	0.80
• Ototoxicity	1	0.80
Outcome of success MDR		
• Getting well	111	89.52
• Treatment Failure	13	10.48

Table III: Significant differences of adverse drug reactions associated with successful treatment

Adverse drug reaction type		Outcome of success for MDR-TB patients		*p* -value	Odd ratio (OR)	Confidence Interval (CI 95%)
		Getting well	Treatment failure			
ADR	Minor ADR	90 (72.58%)	5 (4.03%)	0.024	2.526	1.193-17.892
	Major ADR	21 (16.90%)	8 (6.45%)			

Note: Minor ADR type in comparison to not major type; model-adjusted estimates of odds ratio (OR) compared to reference groups, 95% Confidence Intervals (CI), and associated individual significance values (*p*) were reported.

Discussion

This study is in line with Erma's (2017) study which revealed that there was a case group of male patients diagnosed with TB that was more common in men than in women (Widiastuti *et al.,* 2017). The high number of men suffering from MDR-TB is because they have a high level of mobility which will affect their productivity. This is what allows for a wider risk of transmission. The high number of men with a history of dropping out of treatment is due to the tendency for men to have a lower regularity of treatment than in women (Gualano *et al.,* 2019). Characteristics of the age of MDR-TB patients are that they are mostly at reproductive age. This is in line with research conducted by Hyun-Oh Park (2016), in which the ages of patients are ranging from 14 to 42 years old. Even in the global report, the patients of MDR-TB cases are at the age of 15-55 years old (Tae Won *et al.,* 2017), so that in the occupational status category, patients are more likely to develop MDR-TB due to being more active in studying, working, or other activities and having difficulty taking medication according to schedule due to busy work, resulting in non-adherence to treatment (Mulu *et al.,* 2015).

Based on the results of research on comorbidities, there are comparable results between the proportion of patients who do not have a history of comorbidities and the proportion of patients who have a history of comorbidities. In Table I, there is Diabetes Mellitus as a comorbid disease in MDR-TB patients. Thus, TB patients with comorbid DM must be given clear and continuous information as well as persuasive communication about the importance of taking anti-tuberculosis drugs routinely. Always adhere to controlling blood sugar levels is also important to keep the blood sugar remain stable and well maintained. This is because patients who have abnormally high levels of sugar (glucose) in the blood can cause impaired phagocytosis, chemotaxis, reactive oxygen species (ROS), and cell function. This can reduce the immunity of TB sufferers and can increase cases of MDR-TB (Schmit *et al.,* 2017).

In Table II, the characteristics of the MDR-TB drug use regimen prescribed to MDR-TB patients have a pattern of two types of drugs up to seven types of drugs, The most widely used prescription is with five types of drugs, namely Levofloxacin (Lfx) + Cycloserine (Cs) + Bedaquiline (Bdq) + Linezolid (Lzd) + Clofazimine (Cfz) for as many as 15 patients. Based on the 2016 MDR-TB treatment guide, the doctor has done it well in providing this prescribing pattern. The guidelines state that the current MDR-TB ATD alloy choice is a standardised alloy, which at the beginning of treatment will be given the same to all MDR-TB patients (standardised treatment). MDR-TB management uses a minimum of five drugs and keeps going from 18 to 24 months. The management of MDR-TB cases is often associated with the incidence of side effects ranging from mild to severe so that many patients of MDR-TB start experiencing the side effects of disrupting the course of treatment (Kemenkes RI, 2014).

Levofloxacin, a third-generation fluoroquinolone with broad-spectrum activities, including gram-positive and atypical pathogens, inhibits topoisomerase II (DNA gyrase) and topoisomerase IV, which are required by bacteria for DNA replication. These drugs form complex bonds with each of these enzymes and bacterial DNA. This inhibition produces a cytotoxic effect on the target cells (Pranger *et al.,* 2019). Some fluoroquinolones are active against dormant and replicating bacteria. The mechanism of action of fluoroquinolones is different from other antimicrobials, such as beta-lactams, macrolides, tetracyclines, or aminoglycosides. Therefore, organisms' resistant to these antibiotics can still be sensitive to levofloxacin (D. Pranger *et al.,* 2012).

The MDR-TB treatment has a long treatment period (18-24 months). The number of drugs used for treatment is relatively higher than that of drug-sensitive TB patients. MDR-TB drugs can cause more side effects than ordinary TB drugs (Umi Fatmawati, 2019). In the study results, researchers evaluated the occurrence of ADR in 82 patients (66.13%). However, based on the observed level of adverse drug reaction, 14.63% of the patients required serious treatment (severe category). Based on these monitoring data, those side effects still have a lower frequency than that

of other types of side effects (mild or moderate category). So, discontinuation of therapy can be done with several other factors, for example, monitoring patients being treated for MDR-TB in the hospital, so that monitoring is needed for that, as well as stringent side effects and clinical pharmacy implementation in terms of rational drug selection (Merid *et al.,* 2019). The side effect that occurred in the results of this study was complaints of gastrointestinal disorders (GI) disorders after MDR-TB treatment (16.13%). This is in line with several MDR-TB treatment guidelines where gastrointestinal disturbances are most prominent after treatment with ethambutol, pyrazinamide, and para-amino salicylates.

Most GI side effects can be managed without stopping the drug, by increasing the dose, dividing the dose, or by taking antiemetics. If GI symptoms are not severe, H2 receptor blocking agents, such as lansoprazole, omeprazole, or antiemetic agents, may be added to the regimen, namely ondansetron. However, if symptoms, such as nausea, vomiting, or loss of appetite, are severe, then the administration of para-aminosalicylates can be stopped (Yates *et al.,* 2017).

Some of the drugs used for MDR-TB treatment can cause psychiatric disorders. Psychosis has been reported as a side effect of using Isoniazid (INH) ethambutol, fluoroquinolones, and cyclosporine (CS). Depressive psychosis has been reported as a side effect mainly associated with CS. It has been reported that the incidence of severe psychiatric manifestations includes hallucinations, anxiety, depression, euphoria, behavioural disorders, and suicidal thoughts or attempts to use cyclosporine drugs (Arif *et al.*, 2017). Cycloserine, a drug with suspected psychiatric ADR, was reported in 10% of cases where the drug was already discontinued in this study. Cycloserine-related neurotoxicity is likely due to reduced central nervous system (CNS) production of gamma-aminobutyric acid caused by inhibition of glutamate decarboxylase. In most of these cases, the drug is stopped, with rapid recovery of mental status and no recurring symptoms. Psychiatric symptoms appeared within the first two months of treatment in this study (Arif *et al.*, 2017). The increased risk of Central Nervous System (CNS) toxicity can be attributed to excessive use of the combination of the drug cyclosarin with ethambutol, isoniazid, and fluoroquinolones.

In addition to drug toxicity, psychosocial factors can influence psychological complications during MDR-TB therapy. Therefore, it is necessary to consider the patient's adherence to the treatment regimen. Various types of side effects of MDR-TB drugs can lead to the discontinuation or postponement in taking medication so that rational monitoring of drug assessment is needed.

In Table III, there is a significant correlation between the incidence of side effects of the drug with the successful outcome of patients (p<0.05; p=0.024), so the effect of ADR events have a tendency to be 2.5 times more likely to affect the success of treatment of MDR-TB patients when compared in the absence of ADR in treatment.

Another study found the successful treatment outcome profile showed that 59 cases (47.2%) were cured at the end of treatment; among them, ADRs were seen in 43 (72.88%). The death rate and progress to extensively drug-resistant TB (XDR TB) were 22.8%, 13.6% and 10.4%, respectively.

There was a significant (p<0.005) association between cure and ADRs (Dela *et al.,* 2017). Treatment outcomes were significantly better among those who experience ADRs. This is similar to a result found in a study done by Shin and the authors (2007) in Russia. A possible explanation is those patients who have side effects were followed more closely by TB providers and thereby adherent to treatment, thus increasing the likelihood of a favourable treatment outcome (Shin *et al.,* 2007). ADRs were significantly associated with non-treatment adherence (p = 0.001) and defaulter outcome (p = 0.002). A similar result is shown in a study done by Vishakha and Sanjay (2013).

The most common side effect of anti TB drugs was GIT manifestations, and the least complication was dizziness. Adverse reactions did not negatively impact treatment outcomes among individuals who were adherent to treatment. There were significant relationships between ADRs and co-morbid disease (p = 0.0001, PR = 1.871, 95% CI = 1.370-2.555). However, ADR treatment status based on the given guidelines does not have a statistically significant relationship with the rate of TB treatment cure (p = 0.172, PR = 2.028, 95% CI = 0.582-7.071). Although most adverse drug reaction in this study was managed successfully and not significantly affected outcome therapy, clinician and pharmacist should put more attention because there were some patients developed life-threatening adverse drug reaction such as hypokalemia and nephrotoxicity (Asril I & Soetikno V, 2019).

There are several limitations to this study. First, this kind of research is carried out retrospectively. Second, this study only evaluates patients from one MDR-TB referral hospital, where the total population reached is the respondent in this study by referring to random sampling techniques, and non-probability sampling can be carried out on a sample of 124 patients. Apart from the limitations of the study, we as researchers believe that our study provides important information about the side effects of second-line MDR-TB drugs, especially drugs that have a moderate to severe risk of side

effects. Some of the drugs used for MDR-TB treatment cause psychiatric disorders. Psychosis has been reported as a side effect of using INH, ethambutol, fluoroquinolones, and cyclosporine (CS). Depressive psychosis has been reported as a side effect mainly associated with CS. It has been reported that the incidence of severe psychiatric manifestations includes hallucinations, anxiety, depression, euphoria, behavioural disorders, and suicidal thoughts or attempts to use cyclosporine drugs (Arif *et al.*, 2017).

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IAI CONFERENCE

RESEARCH ARTICLE

The effect of ethanol extract from *Portulaca oleracea* on inhibiting total cholesterol on animal subjects

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Abstract

Introduction: Hypercholesterolemia occurs when cholesterol levels in the blood increases. Traditionally, *krokot* (*purslane*, *portulaca oleracea*) is used to treat cardiovascular disease. **Aim**: This research evaluated the effect of purslane extract to inhibit increasing of cholesterol levels. **Methods**: The ethanol extract dosage of purslane was 27.5, 55, and 110 mg/kg body weight (bw) and simvastatin 1.8 mg/kg bw were used as comparisons. The anti-hyper cholesterol effect test was done by feeding a high cholesterol diet and drinks containing 0.01% propylthiouracil. The test parameters were body weight and total cholesterol levels on days 0, 7, 14, and 21. **Results**: The results showed that the extract was able to prevent the increase in body weight compared to the control group (p<0.05) and that it could inhibit the increase of total cholesterol levels at day 14 and 21 compared to control group (p<0.05) and equivalent to simvastatin (p>0.05).

Introduction

Modernisation always changes lifestyles and habits, including excessive eating, doing too many activities, and too much smoking, but lack of resting. These changes can result in diseases including coronary heart disease and atherosclerosis, which occur when cholesterol levels in the blood increase. If blood cholesterol levels increase, then drugs which are called antihyperlipidemic (hypolipidemic), are needed to decrease the levels (Steyn & Damasceno, 1991). At present, available medicines are relatively expensive, making them unaffordable, especially for low-income people. Therefore, people began to turn to traditional medicine or known as going back to nature. Some plants have traditionally been used to treat hyper cholesterol disease, including *ceremai*, starfruit, purslane, and *binahong*. Purslane (*Portulaca oleraceae*), known as *krokot* in Indonesia, besides being used to feed animals, also has benefits for humans. Purslane can be used as an antioxidant, anti-diarrhoea, haemorrhoids medicine, anti-arthritic, anti-diabetic, hepatoprotective, nephroprotective, anti-inflammatory, anti-hypertension, and to help blood circulation (Jacob *et al*., 2017). The chemical content of purslane includes omega 3 fatty acid, alpha-linolenic acid, and eicosapentaenoic acid. Purslane herbs also contain vitamin A, vitamin C, vitamin B, and carotenoids. Other phytochemicals are alkaloids, terpenoids, organic acids, coumarins, flavonoids, essential oils, and polysaccharides (Uddin *et al*., 2014; Jacob *et al*., 2017; Petropoulos *et al*., 2019). The purslane-leaf extract showed a hypolipidemic effect in rats that were fed with high cholesterol diet (Shanker & Debnath, 2016). The effect of 70% ethanol extract from purslane leaves in rabbits fed with high cholesterol showed that the extract at doses of 200, 400, and 800 mg/kg bw per oral could decrease low density lipoprotein significantly compared to the control group (Movahedian *et al*., 2007). When 96% ethanol extract from purslane herbs at doses of 200, 400 and 800 mg/kg bw were given via intraperitoneal route, they also decreased the cholesterol and triglycerides levels even though the plasma concentration remained high and showed no

significant difference compared to the control group (Ashtiyani, 2016). Purslane stem extract also showed hypolipidemic effect on Wistar rats fed with hyperlipidemic diet that contained 20% fat, 1% cholesterol and 0.25% colic acid (El-Newary, 2016). Therefore, this research examined the effect of 50% ethanol extract from purslane herbs on hyper cholesterol rats.

Material and method

Collection of purslane herbs

The purslane herbs (Figure 1) used in this research were collected from Manoko plantations in Lembang, West Java, Indonesia. Determination of the purslane herbs was done at the Faculty of Biology, Padjadjaran University, with authentic identification No. 458/HB/01/2017.

Figure 1: ***Portulaca oleracea***

Extraction of purslane herbs

The extraction of the purslane herbs was done with 50% ethanol using continuous extraction (Soxhlet), and the thickening was done using a vacuum evaporator. The thickened extract was dried at ±60°C. The dried extract was pollinated with grinders, sifted, and stored in containers in drying cupboards at ± 60°C or in drying cupboards with a damp absorber (desiccant).

Examination of characteristics and phytochemical screening of Simplicia and purslane extract

Examination of the characteristics of Simplicia and the extract includes determining the water-soluble extract content, determining the ethanol-soluble content, determining the drying losses, determining the water content, and determining the total ash content. Phytochemical screening of Simplicia and the extract include examination of flavonoids, saponins, alkaloids, polyphenols, tannins, steroids and triterpenoids, quinones, monoterpenoids, and sesquiterpenoids (World Health Organization, 2011).

Antihipercholesterol effects assay

The experiment was conducted according to the procedure approved by the institutional ethic committee No. 6022/KEP-UNJANI/VII/2019. The experimental animals used were male Wistar rats obtained from the Animal Laboratory of Bioscience and Biotechnology Research Center of Institut Teknologi Bandung. The animals were divided into five groups, each consisting of four rats, namely the control group (CMC 0.5%), reference group (Simvastatin at a dose of 0.9 mg/kg bw), and purslane ethanol extract groups at doses of 27.5, 55, and 110 mg/kg bw. At the beginning of the study, the total cholesterol levels were determined. The test animals were induced exogenously and endogenously. Exogenous induction was done by giving high cholesterol foods. Endogenous induction was carried out by giving drinks containing propylthiouracil (PTU) 0.01% *ad libitum*. The composition of high cholesterol food was 1% pure cholesterol (Sigma-Aldrich, Cat. No. C8503), 20% goat fat, 5% duck egg yolk, 10% cooking oil, 10% beef liver, and standard feed ad 100%. The experiment was carried for 21 days, and the extract was given orally on a daily basis. Blood sampling and determining the cholesterol levels were carried out on days 7, 14, and 21. Blood sampling was done through the tail vein. Measurement of total blood cholesterol levels was carried out using an enzymatic reaction (reagent kit BioMaxima, Cat. No. 1-023-1000) using a Spectrophotometer (Clinicon 4010) at 546 nm. The collected data were analysed using *t-test* statistics.

Results

Examination of characteristics and phytochemical screening

The aim of examining the characteristics of purslane herbs was to standardise the materials and to find out the general criteria for the quality of materials to be used. The results of the characterisation of purslane Simplisia and ethanol extract are presented in Tables I and II. The results showed that both of the purslane herbs, Simplicia and ethanol extract, contained alkaloid, polyphenol, tannins, flavonoid, quinones, terpenoid, and steroids. Saponin was only showed in purslane Simplisia.

Table I: The results of the examination of the characteristics of purslane herbs

Parameter	Result (%)
Total Ash Level (% w/w)	25.00 ± 1.43
Water Soluble Ash Level (% w/w)	15.18 ± 1.14
Unsolved Acid Level (% w/w)	15.82 ± 0.08
Water Content (% v/w)	8.00 ± 0.01
Water Soluble Content (% w/w)	36.18 ± 0.76
Ethanol Soluble Content (% w/w)	12.99 ± 0.40

Antihipercholesterol effects assay

In the assay of the hypolipidemic effect of ethanol extract of purslane herbs, the body weight and total blood cholesterol levels of the subjects were observed. Observation results of body weight are presented in Table III, while the results of measurements of total cholesterol levels are depicted in Figure 2.

Table II. Phytochemical screening of Simplicia and ethanol extract of purslane herbs

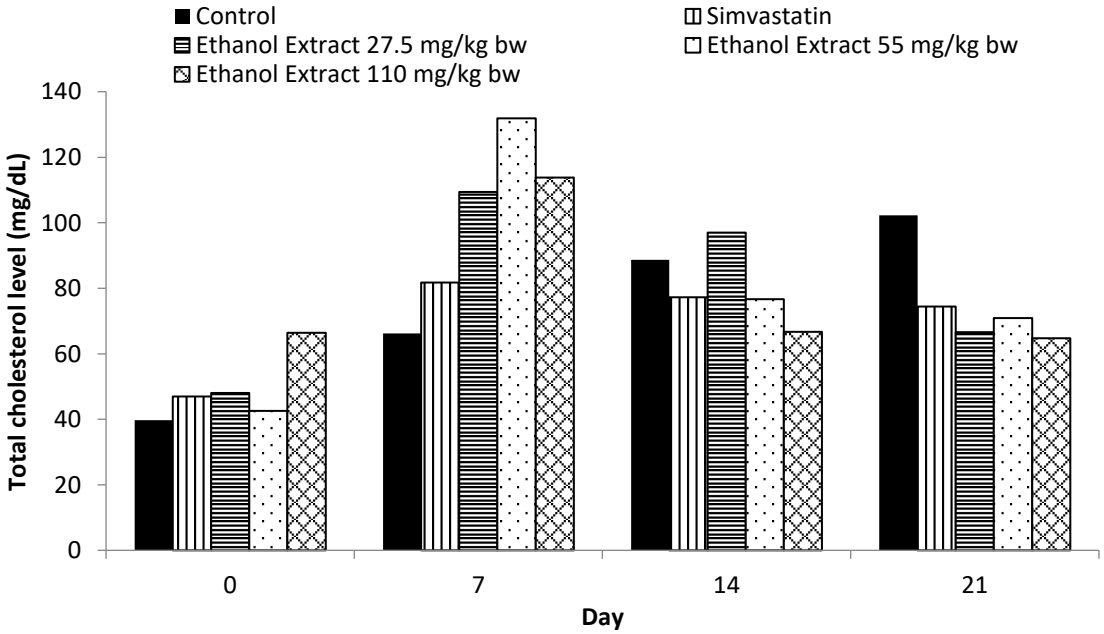
Compound	Result	
	Simplicia	Ethanol extract
Alkaloid	+	+
Polyphenol	+	+
Tannin	+	+
Flavonoid	+	+
Quinones	+	+
Saponin	+	-
Monoterpen and Sesquiterpen	+	+
Steroid and Triterpenoid	+	+

+: Positive contains test compounds
-: Negatives contain test compounds

Table III: Animal body weight on hypolipidemic assay

Group	Body weight (gram) at day			
	0	7	14	21
Control	255.3±47.8	262.5±53.2	290.0±50.3	298.0±50.6
Simvastatin	217.8±33.7	224.3±26.2*	241.5±25.7*	246.5±30.8
Ethanol extract 27.5 mg/kg bw	225.8±32.2	230.8±25.7*	244.0±20.2*	239.3±21.8*
Ethanol extract 55 mg/kg bw	215.3±21.3	221.0±26.0*	238.3±31.0*	210.8±36.4*
Ethanol extract 110 mg/kg bw	220.3±16.7	223.5±19.8*	240.8±18.6*	238.3±19.3*

n=4, *p<0.05 compared to control group using t-test



n=4, *p<0.05 compared to control group using t-test

Figure 2: Total cholesterol level on hypolipidemic assay

The observation of body weight showed that the ethanol extract was able to inhibit the increase in body weight from day 7 to day 21. Ethanol extract at a dose of 27.5 mg/kg bw was able to inhibit the increase in body weight by 10.77% on day 21. The administration of ethanol extract at a dose of 55 mg/kg bw was able to inhibit body weight gain by 18.84% on day 21. The administration of ethanol extract at a dose of 110 mg/kg bw was able to inhibit body weight gain by 8.58% on day 21. In contrast, the reference drug simvastatin was able to inhibit body weight increase by 3.55% on day 21. The optimal result of inhibition body weight gain was shown by ethanol extract at a dose of 55 mg/kg bw.

The measurement of total blood cholesterol level showed that the per cent relative level of total cholesterol in the serum of ethanol extract group dose 27.5 mg/kg bw on day 21 was 118.53% lower than the control group, while the total cholesterol level of the extract 55 mg/kg bw was 90.86% lower than the control group, and the total cholesterol level of the extract 110 mg/kg bw was 159.80% lower than the control group. This showed that the ethanol extract could apparently reduce the total cholesterol levels in the serum even though it was not different from the control group (p>0.05).

Discussion

In this research, the administration of purslane extract to inhibit the increase of total blood cholesterol level was carried out using exogenous and endogenous induction. The endogenous induction was carried out by giving cholesterol and PTU. The induction by giving pure cholesterol was done because it will contribute to the increase of cholesterol levels by as much as 70-80% on cholesterol levels in the liver, small intestines, and adrenal glands. The test animals were also given a high cholesterol diet with an increase of 10-30% (Dietschy & Siperstein, 1967). The induction was also done by giving PTU, which is a drug used to reduce thyroid levels. Clinical studies state that thyroid hormones will influence the formation of cholesterol, especially low-density cholesterol (LDL) (Abrams *et al.*, 1981). In testing using animals, this condition can increase body weight (Suzuki *et al*., 1979).

Purslane ethanol extract could prevent weight gain when given together with foods high in cholesterol. This study is in line with the research conducted by Hussein (2010), which stated that 95% ethanol extract of purslane obtained through extraction using Soxhlet also showed significant inhibition of body weight gain, blood glucose, triglyceride, total cholesterol, LDL-C, HDL-C, free fatty acids, and the atherogenic index levels in a dose-dependent manner on obesity-induced diabetic rats fed by a high-fat diet (Hussein, 2010). In contrast, research conducted by Shafi & Tabassum (2018) stated that 50% ethanol extract of purslane obtained through maceration could increase the bodyweight of animals in streptozotocin-induced diabetic (Shafi & Tabassum, 2018). This difference could be due to different extraction methods, in which this research used continuous heat extraction.

Different extraction methods will affect the compounds contained in the extract. Maceration is an extraction suitable for the isolation of thermolabile compounds, while the Soxhlet extraction at high temperatures and long extraction time can increase compound degradation due to temperature (Zhang *et al*., 2018).

The effects of the anti-cholesterol of purslane extract were likely to be the result of flavonoids. Flavonoids have antioxidant effects, and they can affect cholesterol concentration, especially LDL levels. Flavonoids will inhibit LDL oxidation, thereby reducing the likelihood of injury in the endothelial wall and reducing the risk of arteriosclerosis (Nijveldt *et al*., 2001).

One of the factors that influence the occurrence of hyperlipidemia is oxidative stress. Purslane contains high antioxidants, including alpha-tocopherol and ascorbic acid, so it is useful for reducing oxidative stress (Uddin *et al*., 2014). Vitamin A and carotenoids can prevent free radicals and LDL peroxidation. Beta carotene can lower blood cholesterol levels by preventing the HMGCoA reductase enzyme activity. Carotenoids also increase macrophage LDL receptor activity and reduce circulating LDL, inflammation, oxidative stress, and endothelial dysfunction (Malekmohammad *et al*., 2019). Purslane also contains omega 3 fatty acids, which are useful for increasing high-density lipoproteins and reducing blood viscosity.

It can be concluded that 50% ethanol extract of purslane herbs has the potential to inhibit the increase of total blood cholesterol levels in an animal model.

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RESEARCH ARTICLE

In vitro antimalarial activity assay of Ashitaba Leaf ethanolic extract (*Angelica keiskei*)

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Abstract

Introduction: The incidence of malaria is still very high in number in the world. Difficulty in treating malaria is caused by the resistance of malaria parasites to conventional drugs. An alternative treatment that can be used to treat malaria is to discover new drugs from natural ingredients. **Aim:** This study aimed to determine the activity of the Ashitaba leaf ethanolic extract as an antimalarial drug to *Plasmodium falciparum* strain 3D7. **Methods:** This study tested the activity of Ashitaba extract on the growth of *P.falciparum* in five concentrations, namely concentration of 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm, and 100 ppm. **Results:** The test results showed that the highest inhibitory effect was found on the concentration of 100 ppm with percent inhibition of 79.47 ± 26.91%. The 50% inhibition to parasites showed the half maximal inhibitory concentration (IC_{50}) value of 2.09 ppm, compared to the positive control of which the IC_{50} of chloroquine was 0.007 ppm. **Conclusion:** Ashitaba leaf extract can be considered to have very active anti-malarial activity, because it has an IC_{50} value of less than 5 ppm.

Introduction

Malaria is a dangerous disease caused by parasites that are transmitted to humans through the bite of a female Anopheles mosquito infected with the parasite (Sudoyo, 2009). In 2018, there were an estimated 228 million cases of malaria worldwide. The estimated number of deaths from malaria was 405,000 in 2018. Children under five years of age are the group most vulnerable to malaria, accounting for 67% of all malaria deaths worldwide. The African region bears the global malaria burden. In 2018, Africa was home to 93% of malaria cases and 94% of malaria deaths (Dinkes N.T.B., 2018).

Indonesia was reported as the third-highest number of malaria cases in the Southeast Asia region, amounting to 229,819 cases. Likewise, the number of deaths was 432 (WHO, 2020). Although the incidence of malaria has tended to decrease since 2000, there are still malaria outbreaks in seven provinces that have attacked 35 villages and caused the death of 211 residents. In Indonesia, the number of malaria sufferers tends to decline from year to year. However, several provinces in Indonesia still suffer from malaria, especially in the eastern part of Indonesia, namely Papua and West Papua, including West Nusa Tenggara (Depkes R.I., 2006).

The development and discovery of antimalarial drugs are expected to provide new drugs with potential and safe drug targets and mechanisms for humans. The emergence of drug-resistant *Plasmodium* species to antimalarials has urged researchers to look for new antimalarials to replace ineffective antimalarials. One of the efforts to find new antimalarials is through research on medicinal plants that are traditionally used by the community to treat malaria (Depkes R.I., 2008).

One of the plants used in an antimalarial treatment is the Ashitaba plant because this plant contains active substances that have medicinal functions. Research at the Osaka Pharmacy University in 1990 showed that the active ingredients contained in 100g of ashitaba were 0.25% xanthoangelol, 4-Hydroxyderricin 0.07% and 0.32% total chalcone. Ashitaba contains hexadecanoic acid 2.42%, palmitic acid 5.08%, xanthotoxin 3.12%, linoleic acid 9.17%, pyrimidine 2.70%, strychnidinone 3.18% and smenochromena 7.55% (Mustofa, 2009). Chalcone (1,3-diphenyl-2propen-1-on) is a compound that contains two aryl rings connected with ketones α, β unsaturated. Chalcone is an important intermediate in organic synthesis. The chalcone group is a structure common in plants that contain secondary metabolites of flavonoids (Handayani *et al.*, 2013). Chalcone has also been reported as a potential antimalarial agent (Hans *et al.*, 2010).

Material and method

Materials

The materials in this research were micropipette, Erlenmeyer flask, measuring cup, glass jar, analytical balance, laminar air flow (LAF), incubator, refrigerator, mix gas, pipette, petri-dish, well 96, sterile medium bottle, centrifuge, microscope, water bath, glass stirrer, oven, silica gel GF254, chamber, microtube, ethanol, and Ashitaba leaves (*Angelica keiskei* K), parasites used are *Plasmodium falciparum* strain 3D7 parasites, 50% dimethyl sulfoxide (DMSO), Roswell Park Memorial Institute (RPMI), 20% Giemsa colouring and 5% sorbitol, chloroquine.

Methods

Extraction of Ashitaba Leaves

Ashitaba leaf ethanolic extracts were made from 100 gram of Ashitaba simplicia leaves immersed in 400 ml of 70% ethanol mixture, stirred every three hours for 15 minutes for 24 hours. The immersion results were squeezed, then left for ten minutes before being evaporated at control temperature (50^{o}C) to obtain a thick extract.

Phytochemical screening

Wilstater test: Two ml of Ashitaba leaf extract solution was added with ten drops of concentrated hydrochloric acid (HCl) and a little magnesium powder. The orange-yellow colour indicates the presence of flavones, chalcone, and auron, based on the results of research by Sofa Fajriah & Megawati (2015).

Bate Smite-Metcalfe test: Two ml of Ashitaba leaves extract solution was added with ten drops of concentrated HCl then heated. The red colour indicates the presence of flavonoids.

Antimalarial activity assay

Sample preparation: One mg sample was dissolved in 100 µl DMSO (stock solution, concentration of 10,000 µg / ml). The serial dilution was made from a stock solution to obtain a final concentration of 1000 ppm, 100 ppm, 10 ppm, and 1 ppm.

Parasite preparation: The parasites used in this test were synchronized ring stage with parasitemia ± 1%.

Procedure: Two µl of the test solution with various concentrations was taken and put in each microwell, then 198 µl of the parasite was added until the test sample had a final concentration of the test sample of 100 ppm, 10 ppm, 1 ppm, 0.1 ppm, and 0.01 ppm. The microwell was then put in a chamber and given a mixed gas (5%, O_2, 5% CO_2 and 90% N_2). The chamber containing was incubated for 48 hours at a temperature of 37^{o}C. The culture was then harvested, and a thin blood smear was made with 20% Giemsa staining.

Statistical analysis

Blood smears that have been made were calculated by counting the number of infected erythrocytes every 1,000 normal erythrocytes under a microscope. The data were then used to determine the per cent growth and per cent inhibition. Per cent growth was obtained by the following formula:

$$\% \text{ growth} = \% \text{ Parasitemia} - D_0$$

Inhibition percentage was calculated as follows:

$$\% \text{ Inhibition} = 100\% - [(Xu/Xk) \times 100\%]$$

Where:

D_0 = Percentage parasitemia of infected red blood cell on day 0

Xu = Growth percentage of each sample

Xk = Growth percentage of negative control

Based on the per cent inhibition data, an analysis was made between the test concentration of the per cent inhibition using the SPSS program using probit log analysis to determine the IC_{50} value or the concentration of the test material that could inhibit the growth of parasites by 50%.

Results and discussion

The sample used in this study was Ashitaba leaves which were obtained from Sembalun Village, East Lombok, West Nusa Tenggara. The extraction method was maceration, i.e. to extract active compounds that can dissolve in a solvent-based on the degree of polarity of each solvent (Hans *et al.,* 2010). The distribution of organic solvents that occurs continuously into plant cells results in cell walls and membranes breakdown. This breakdown causes the active compounds in the cytoplasm to dissolve in organic solvents (Sofi & Megawati, 2015). Ashitaba leaf extraction resulted in about 18.6 grams thick brown extract. The qualitative test of the chalcone flavonoid compound was carried out using the Wilstater reagent (Figure 1). The colour change to orange in the sample that had been reacted showed that the Ashitaba leaf sample contained positive chalcone compounds. The colour change in the sample was due to the reduction process of Magnesium (Mg) with concentrated HCl so as to produce complex yellow-orange compounds in chalcones, flavonoids and auronols (Khoplar, 2008).

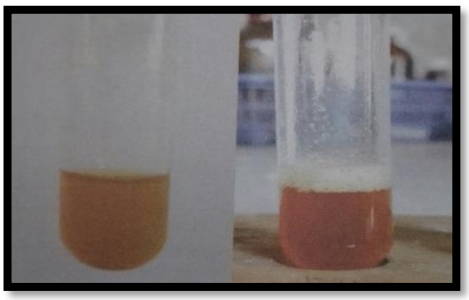

Figure 1: Test of flavonoid compounds with the Wilstater reagent

The test for flavonoid compounds using Bate Smite-Metcalfe reaction changed the colour of the sample to dark red after being reacted and heated, showing that the sample contained flavonoid compounds (Figure 2). This result is in line with research by Amalia (2017), showing that, in the phytochemical screening, Ashitaba plants contain flavonoids (Amalia, 2017).

Figure 2: Test of flavonoids with Bate Smite-Metcalfe reaction

The antimalarial activity test conducted in this study was an *in vitro* test. This *in vitro* test illustrated the antimalarial activity against *Plasmodium falciparum* parasites in the erythrocyte phase because the parasites were grown as if they were in the body's red blood cells. The parasite used in this study was *Plasmodium falciparum* Strain 3D7 which is sensitive to chloroquine (Amalia, 2017). The parasites were incubated for ± 48 hours, followed by the making of a thin blood smear slide. After drying, given 10% Giemsa staining was given. The number of erythrocytes with per cent inhibition was calculated by comparing the number of infected erythrocytes to 1,000 erythrocytes observed under a microscope. The results of the parasite culture observations showed that *Plasmodium falciparum* infecting red blood cells were at the trophozoite stage. Ring-form trophozoite has a ring-like shape with one or two small nuclei and is cytoplasmahalous (Ella, 2017). Antimalarial activity can be determined by calculating the percentage parasitemia obtained in the sample test, resulting in per cent growth, per cent inhibition, mean per cent inhibition, concentration log, and test dose, followed by probit analysis to obtain that the IC_{50} value. The results of the Ashitaba leaf antimalarial activity test can be seen in Table I.

Table I: Data on In Vitro Antimalarial Activity Test Results

Sample	Concentration (µg/ml)	Growth (%)	Inhibition (%)	IC_{50} (µg/ml)
Extract	100	3.23	-	2.09
	10	3.25	-	
	1	0.68	78.95	
	0.1	0.65	80.00	
	0.01	1.05	67.49	
Positive control (Chloroquin)	100	3.23	-	0.007
	10	3.25	-	
	1	0	100	
	0.1	0	100	
	0.01	0.3	90.71	
Negative control (DMSO)		3.24		

Table I shows the percentage parasitemia after the addition of Ashitaba leaf ethanolic extract from the highest concentration to the lowest one. The ethanolic extract of Ashitaba showed the highest per cent inhibition leaves at a concentration of 100 ppm with a per cent inhibition value of 79.47%. In comparison, the lowest one was found at a concentration of 0.01 ppm with a per cent inhibition value of 11.73%. In general, the higher the concentration of the extract given, the higher the per cent inhibition obtained to inhibit the growth of *Plasmodium falciparum*. These results are in line with research by Wardani (2019), showing that the highest

inhibitory effect to the growth of *Plasmodium falciparum* 66.36% resulted from the addition of the highest concentration, i.e. 100 ppm, while the lowest per cent inhibition was found at a concentration of 0.01 ppm with a per cent inhibition of 2.62% (Wardani *et al.*, 2019).

The parasitic resistance values obtained were then analysed using probit analysis. Probit analysis was used to determine the IC_{50} value. The IC_{50} value shows a concentration that can inhibit 50% of cell growth (Achmadi, 2010). The IC_{50} value of Ashitaba leaf extract was 2.09 ppm. The antimalarial activity of Ashitaba leaves fell into the very active category because the IC_{50} value was less than five ppm (Ilhami *et al.*, 2013). A research carried out by Wardani et al. in 2019 stated that there was an antimalarial activity Ashitaba plant, i.e. the stems and roots. Both stems and roots have antimalarial activity as indicated by IC_{50} values of 11.07 ppm and 16.09 ppm, respectively. In addition, the compounds contained in Ashitaba leaves that are believed to inhibit the growth of parasites are chalcone compounds, which belong to the flavonoid class (Wardani *et al.*, 2020).

Conclusion

Based on the results of the research that has been done, it can be concluded that the ethanolic extract of Ashitaba leaves has antimalarial activity because it has an IC_{50} of 2.09 ppm, which falls into the very active category in inhibiting 50% of the growth of the parasite *Plasmodium falciparum* strain 3D7.

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Conflict of Interest

The authors declare no conflict of interest.

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IAI CONFERENCE

RESEARCH ARTICLE

Identification of herbal products used by families in the campus of Darussalam Gontor University

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Keywords
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Abstract

Background: The development and the use of herbal medicinal products is increasing in Indonesia. **Aim:** To identify the safety of herbal medicine product used by families of Darussalam Gontor University (UNIDA) Gontor. **Method:** This research was conducted to identify herbal products that are used based on features, functions, and benefits. The method used was Rapid Assessment Procedure (RAP) with a qualitative approach. **Results:** The results showed there were 100 products used by 72 respondents. The level of product safety used based on raw materials was 96% and the efficacy claimed was 76%. The most type of product used was *jamu* (Indonesian indigenous traditional medicine) (94.7%). The range of understanding level of of respondents based on features and benefits was between good and very good. **Conclusion:** The use of herbal products by respondents can be viewed from the aspect of product safety levels based on raw materials (96%) and efficacy claims (76%). This was still classified as safe.

Introduction

Health development in Indonesia aims to increase awareness, willingness, and ability to live a healthy life for everyone to realise the highest degree of public health. The health effort can be carried out in the form of activities with promotive, preventive, curative, and rehabilitative approaches that are carried out continuously. As in Law No. 36, article number 48 of 2009 about health states that there are 17 health efforts, one of which is a traditional health service.

Herbal medicine can be used as a complementary therapy in health care facilities and is used by the community as a preventive, promotive, curative, rehabilitative, and palliative effort (Aditama, 2014). This is supported by the World Health Organisation (WHO), which recommends the use of herbal medicines to maintain health, prevent, and treat diseases, especially in chronic diseases and degenerative metabolic cancer (WHO, 2013).

The development of traditional health services using herbs today is increasing rapidly, in 2009 as much as 15.04%, in 2010 as much as 31.7%, in 2012 as much as 41.7%, and the latest data in 2018 as much as 44.3% (Kemenkes RI, 2018). Herbal medicines circulating in Indonesia are safe to consume with a record that has been registered in The Indonesian National Agency for Drug and Food Control, Republic of Indonesia (BPOM). However, herbal medicines that have been used for generations do not need clinical trials because they have been used for three generations or over 180 years (Parwata, 2016), such as *Tolak Angin* and *Diapet* (Utami, 2018).

Herbal medicine should not contain medicinal chemicals (*Bahan Kimia Obat*/BKO) because it can endanger health and could be fatal. Nowadays, many herbal medicines are clinically still not supported by strong and consistent evidence (Kamaluddin, 2016). The result shows that the use of herbal medicines among the families of University of Darussalam

(UNIDA) Gontor lecturers has been increasing rapidly in recent years.

So, the purpose of this study was to determine the level of safety of herbal products used by the family of UNIDA Gontor by identifying herbal related products based on features, functions, and benefits. This study also aimed to describe the use of herbal products in the categories of *Jamu* (Indonesian indigenous traditional medicine), standardised herbal medicine (*Obat Herbal Terstandard*/OHT), and phytopharmaca (*fitofarmaka*).

Theoretical review

Herbal medicines are raw materials or preparations derived from plants that have therapeutic effects or other effects that are beneficial to human health. BPOM classifies traditional medicine into three classes, namely *jamu*, standardised herbal medicine, and phytopharmaca, based on its scientific evidence.

Herbal products are unique in that there are features, functions, and benefits (Kotler & Keller, 2009). The feature is a characteristic of a product that is designed to enhance the function and consumer interest in the product (Arifin & Saidani, 2012). The function is a benefit obtained by consumers after using a product, that is, the suitability of the function listed in the product packaging (Juwandi, 2004). The benefit is the value obtained by consumers in using the product that has a high benefit ratio compared to the side effects caused (Rambat, 2001).

According to Government Regulation No. 69 of 1999 article 2 (1), every person who manufactures or imports packaged food in the territory of Indonesia must include a label inside and/or outside the food packaging. Also, based on Minister of Health Regulation No. 246 of 1990, article 1 (9) states that herbal products labels must include information in the form of 1) product name; 2) composition; 3) net weight; 4) name and address of the business actor; 5) expiry date; 6) rules of use; 7) date of manufacture; 8) side effects; 9) the symbol of *jamu*; 10) the dosage of use: 11) efficacy: 12) usefulness; 13) contraindication (if any); 14) registration number; 15) production code number; 16) specific ingredient information (if any), and alcohol content (if any).

Method

Research design

The design of this study used the Rapid Assessment Procedure (RAP) method with a qualitative descriptive approach. So, in this study, the authors identified herbal products used by respondents based on features, functions, and benefits.

Research place and time

This research was conducted at the campus of UNIDA Gontor, both for the families of lecturers who live in UNIDA Gontor Siman, as well as UNIDA Gontor Mantingan. The study started in September 2019 and ended in January 2020.

Research samples

The population in this study are the lecturers of UNIDA Gontor, with a total population of 250 people. The research sample that is used as a respondent uses a purposive sampling technique which is a method for selecting respondents by determining criteria included in the research category (Saryono & Anggraeni, 2010). The sample of respondents to be taken was done using the Slovin formula with an error rate of 10%. The degree of trust in this study is 90%.

$$n = \frac{N}{N\,(d^2) + 1}$$

n: Sample size; N: Large population; d: The degree of accuracy of the alleged magnitude of the sample = 0.1 (10%)

The results obtained based on the Slovin formula are 72 respondents from a total population of 250 people. The sample used in this study were 72 respondents and were homogeneous because the respondents lived in the same environment, so the respondents had the same level of communication and knowledge.

Ways of data collection

In this study, the authors conducted interviews with respondents by contacting respondents who used herbal products and filed a statement of willingness to be the respondent. Interviews were conducted face-to-face by visiting respondents in the office or at home. In this study, the authors brought tools such as pens, notebooks, and recorders to help facilitate data processing. At the end of the interview, respondents were asked to provide samples of the herbal products that were used as research documentation.

Data analysis and processing

The data obtained were analysed using a qualitative descriptive analysis which described the results that had been obtained by the authors during the interview.

The data processing was done using Microsoft Office Word and Microsoft Office Excel.

Stages of data processing are: 1) organising data obtained during interviews; 2) conducting data categorisation of the same type; 3) interpret data obtained to answer research problems and describe phenomena related to research; 4) evaluate interpretations to avoid misinterpretations.

Results and discussion

Overview of research subjects

Seventy-two respondents were included in this study. There were 73.6% of respondents male, and 26.4% of respondents were females. The respondents based on education level were dominated by lecturers who teach second-year undergraduate students (94.4%) than the third year (5.6%).

Licensing of herbal products

Table I shows licensing of 100 herbal products.

Table I: Types of licensing of herbal products

Licensing institution	Number of product
BPOM	25
BPOM & MUI	36
PIRT & MUI	16
PIRT	19

Note: BPOM = The Indonesian National Agency for Drug and Food Control; MUI = Indonesian Council of Religious Scholars; PIRT = Home Industry Food

Based on the features of herbal products, it can be viewed from four aspects. First, the safety of herbal products can be seen on the packaging label based on the raw materials used and claims of efficacy (see Table II).

Table II: Safety levels of herbal products

Licensing Institution	Raw materials		Efficacy claims	
	%	Category	%	Category
BPOM	25%	Secure	23%	Secure
			2%	Not secure
BPOM **&** MUI	36%	Secure	33%	Secure
			3%	Not secure
PIRT **&** MUI	16%	Secure	10%	Secure
			6%	Not secure
PIRT	19%	Secure	10%	Secure
	4%	Not secure	13%	Not secure

Secondly, the types of herbal products based on *jamu*, OHT, and phytopharmaca categories can be seen in Table III).

Table III: Types of herbal products

Category	%
Jamu	94.7%
OHT	5.3%
Phytopharmaca	0%

Third, the completeness of information listed on herbal products can be seen in Table IV. Eighty-three percent of products have completed information standards based on the type of license.

Table IV: Completeness of herbal product information

Licensing institution	%	Category
BPOM	25%	Complete
	0%	Not complete
BPOM **&** MUI	36%	Complete
	0%	Not complete
PIRT **&** MUI	16%	Complete
	0%	Not complete
PIRT	6%	Complete
	17%	Not complete

Fourth, the level of understanding of herbal medicine categories based on *jamu*, OHT, and Phytopharmaca can be seen in Table V.

Table V: Understanding level of herbal product

Respondents	Understanding level
A (1 Respondent)	*Jamu* and OHT
B (1 Respondent)	*Jamu,* OHT, and *fitofarmaka*
70 Respondents	Do not understand

The use of herbal products

The use of herbal products must have to match the benefits felt by consumers with the product claims listed on the packaging (see Table VI).

Table VI: The use of herbal products

Product	%
Supplement	71%
Preventive Form	19%
Help Treat	10%

Benefits

In the aspect of benefits, the use of herbal products is following the information contained in the packaging label. Although some products claim to cure, respondents only use it as a cure or prevent.

Discussion

Lecturers who teach at UNIDA Gontor are dominated by male lecturers. Many of them use herbal products. According to Nur (2004), male respondents tend to use the internet to search for information compared to social media, while female respondents tend to use the internet for social media. The university of UNIDA Gontor was only established in 2014, so that not many lecturers have doctoral degrees, and no one has graduated students at the doctoral level.

Distributing herbal products in Indonesia must have a marketing authorisation (Government Regulation, 2012) and include clear information on the packaging (Government Regulation, 1999). The licensing agency related to the circulation of a product in Indonesia can be through BPOM, MUI, and PIRT licensing is the highest licensing that takes care of drug and food control (BPOM, 2017). Indonesian Council of Religious Scholars (*Majelis Ulama Indonesia*/MUI) is a licensing institution based on the deliberations of Muslim scholars and scholars who determine the halal status of a product by Islamic law (Government Regulation, 2014). Home Industry Food (*Pangan Industri Rumah Tangga*/PIRT) is a licensing scope for district or city service for micro, small, medium enterprises and home industries (Nurwidiana, 2019).

In Table II, 96% of the products are classified as safe based on the raw materials listed on the packaging. This is very important, considering the number of cases related to herbal products that contain BKO and are fatal to health. There are 76% of products classified as safe, based on the claims of efficacy listed on the packaging. Herbal products with BPOM permission claim the product is only as a supplement and helps prevent or treat it, not as a medicine. Whereas the PIRT permit product can only claim as food, not as a supplement or medicine.

Based on the percentage of use of herbal products, respondents use more *jamu*, OHT and no one uses phytopharmaca. The use of herbal medicines by these respondents is in line with the results of Ahmad's study (2012) that most patients who seek treatment at the SJHM clinic prefer *jamu* rather than conventional medicines because of the perception of the efficacy of *jamu,* which was believed to be higher than capsule preparations. Phytopharmaca herbal products are herbal products that can be equalised as synthetic chemical drugs because they have been proven clinically (evidence-based medicine). Completeness of information in the label is important because it can guarantee the safety and authenticity of a product to consumers in using a product.

The level of understanding of respondents related to features and benefits fell in the range of understanding to very understanding. While the functions fell in the range of do not understand to understand. This is based on the ability of respondents to understand the active compounds contained in the product composition and the ability to analyse products that are categorised as insecure or contain BKO.

The use of herbal medicines by respondents based on function is also not in accordance with the complaints of the disease being suffered. Based on Table VI, most of the respondents used herbal medicines for the purpose of being a supplement. The reasons for using herbal medicines for these supplements are in accordance with the results of research by Panyod and the authors (2020), that food and herbal medicines can be used as complementary therapies to prevent infection, strengthen immunity and as antiviral agents.

Herbal products used by many respondents were classified as overclaimed, but the use of these products was limited to the table above. The respondent's answer related to the perceived benefits of making the body healthier, stamina, not easily hurt and tired when on the move. The reasons respondents use herbal medicines are the lack of side effects and even almost not found, minimize the use of chemicals, do not cause dependence effects, and can be used continuously to maintain health. Based on the perceived benefits, respondents will choose to use herbal medicines before using synthetic chemical drugs to help treat or prevent a complaint. The respondent's reason is in accordance with the results of Dewi's research (2019), the most widely used type of traditional medicine is herbal medicine (52.38%) on the grounds that people use traditional medicine because it is made from natural ingredients (37.50%).

For further research, it is necessary to test the levels of active substances in the products used by respondents to find out the level of truth by the packaging labels listed on the product and the need for making manuals or guidelines on how to choose herbal products that are safe, useful and guaranteed. As well as for the government to make a special policy regarding product truth standards with Home Industry Food permit status that can be accessed by the general public.

Conclusion

The use of herbal products by respondents from the aspect of product safety levels (96%) and efficacy claims (76%) are still classified as safe, the truth of information on product packaging labels is 83%, which is considered as truthful and the level of knowledge of respondents is based on categories jamu, OHT, and phytopharma are classified as low. Most of the use of herbal products is based on function as supplements (71%). Meanwhile, based on the benefits of using herbal products, it is safe. The use of herbal products based on the jamu category was 94.7%, OHT was 5.3%, and phytopharmaca was 0%.

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IAI CONFERENCE

RESEARCH ARTICLE

The remuneration of the community pharmacist in the developing world: the case in Indonesia

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Abstract

Introduction: The remuneration of pharmacist is critical to ensure sustainability of pharmacist services. There has been limited study about pharmacist remuneration in Indonesia. **Aim**: This study aims to investigate pharmacist remuneration system in Indonesia. **Methods**: A nationwide community pharmacy survey was conducted involving 7,000 pharmacies. Questions around remuneration models and amounts, types of incentives and other financial benefits structured the questionnaire. Descriptive analysis was used to evaluate the findings. **Results**: Of 2,087 pharmacists participated in the survey, only 1,952 respondents were recorded. More than half of respondents did not receive any particular fees designated to compensate provision of cognitive services. Fixed monthly salary predominantly formed the structure of remuneration system with less than half of the respondents received additional incentives to top up this monthly salary. **Conclusion**: The current remuneration system which mainly relies on monthly salary basis may not be sustainable to support provision of pharmacist-led cognitive services.

Introduction

Community pharmacist has been known as an important element of the healthcare system that provides both pharmaceuticals and services to the communities (Moullin *et al.*, 2013). Supporting health by enabling community pharmacists full potential in the delivery of cognitive services has become the main objective of pharmacy stakeholders in many Low and Middle-Income Countries (LMICs) (Miller & Goodman, 2016). The provision of pharmacist-provided patient care services, however, is challenging in many of these countries, given the pertinent barriers revolve around pharmacy and health system infrastructure (Scahill, 2014; Hermansyah *et al.*, 2016). One of the commonly perceived barriers is the lack of remuneration of the community pharmacist.

The remuneration of community pharmacists has been viewed as a facilitator for pharmacy practice change (Roberts *et al.*, 2006). The expanding role of pharmacists, which marks more progressive interventions of pharmacists in the fields of medication management, health promotion and disease prevention, implies a specific remuneration structure. The contemporary remuneration structure of pharmacists in LMICs, which highly relies on dispensing and medicines markup, may not be sustainable to support the implementation of cognitive pharmacist services (Hashemi-Meshkini *et al.*, 2013). This is also the case in Indonesia.

Multiple policy approaches have been established to change the practice of community pharmacy in Indonesia, including the provision of incentives in the form of minimum rates for pharmacist remuneration and payment for particular pharmacies working under the Universal Health Coverage programme (Hermansyah *et al.*, 2018b). The incentivisation policy may highlight a novel approach for developing community pharmacy in the context of LMICs as these incentives were not influenced by the volume and/or profit margin from selling the pharmaceuticals.

Nevertheless, the implementation has been far from satisfactory. The approach has been challenged by lack of coordination and lack of consensus leading to inconsistency and discrepancy of the actual payment for pharmacists. Apart from the troubled implementation, it is fair to say that the making of this policy did not take into account the characteristics and type of pharmacist remuneration in Indonesia (Hermansyah *et al.*, 2018b).

There is evidence that the successful wide-scale implementation of cognitive services has been limited by the lack of remuneration for providing cognitive services (Bernsten *et al.*, 2010; Houle *et al.*, 2014). Unless this barrier is addressed, it is likely that any policies or approaches concerning practice change will fail. Therefore, this study aims to investigate the pharmacist remuneration model in Indonesia.

Methods

Study design and settings

A cross-sectional study was conducted in the form of a survey of community pharmacists across all 34 provinces in Indonesia. Only the pharmacist in charge represented each pharmacy. This study obtained ethics approval from the Research Ethics Committee of the Faculty of Public Health at the authors' institution.

Study participants and recruitments

There were 25,339 community pharmacies in Indonesia at the time of the study conducted. Using a margin of error of 1% and a confidence level of 95% resulted in 7,000 pharmacists as a minimum sample size. A registered community pharmacist in charge (or so-called first pharmacist) was recruited to participate in this study. At first, the researchers used a cluster sampling method based on province distribution with pharmacist identity for randomisation was obtained from the local pharmacist association. The sampling technique was then expanded using accidental sampling to obtain more responses.

Study instruments

This study utilised a questionnaire as an instrument, which asked about the type and amount of pharmacist remuneration, other financial benefits obtained by pharmacists and pharmacist preferences with respect to the remuneration model. The questionnaire was developed based on the references, discussion among the researchers as well as considered the phenomenon and facts related to pharmacist remuneration in Indonesia. The questionnaire was then tested for validity to a panel of experts (eight persons representing pharmacist practitioner, biostatistician, pharmacist organisation, academic, policymaker and administrator). This is to ensure the face and content validity of the questionnaire. The questionnaire was subsequently pilot-tested for both face validity and reliability to 20 pharmacists with some minor changes for the final version, mostly related to wording and numbering of the questionnaire form.

Data collection

This study used both online and printed survey forms. The printed questionnaire was sent to some local pharmacist associations for a limited number. The main means for data collection was an online survey distributed through a number of social media applications, i.e. WhatsApp, Line, Facebook, Twitter, Instagram and Telegram. Survey Monkey was used as the platform for the online survey. Data collection was conducted from September 2018 to March 2019. Participants who completed the survey received two credits for the licensure requirement and were assigned for a lucky draw to win five android tablets. Participants only had one chance to fill out the survey. In case there were multiple answers from a similar pharmacy, only the latest response was recorded. The pharmacist in charge could participate in the survey or pass the questionnaire to another pharmacist who was representing the pharmacy. In addition, reminder notification was made each month and distributed via the pharmacist association and its network. Participants were required to provide consent prior to fill out the questionnaire.

Data analysis

All response was recorded by the Survey Monkey system which then exported to SPSS Version 22 for further data analysis. Descriptive statistics of the frequency (%) were used to describe the findings of the study.

Results

Of 2,087 pharmacists who participated in the study, only 1,952 pharmacists were deemed eligible. However, the number of complete responses in each question may vary. Table I summarises the characteristics of the respondents. The majority of respondents were female (78%), aged 21-30 years old (48%), completed pharmacist programme, i.e. four years of Bachelor of Pharmacy and one-year pre-pharmacist programme (90%), graduated after 2010 (62%) and got monthly paid roughly IDR 2-5 million (67%).

Table I: Characteristics of respondents

Characteristics	Frequency (%)
Gender (n = 1,767)	
• Male	391 (22%)
• Female	1,376 (78%)
Age in years (n = 1,211)	
• 21-30	586 (48%)
• 31-40	457 (38%)
• 41-50	123 (10%)
• 51-60	31 (3%)
• > 60	14 (1%)
Latest educational level (n = 1,210)	
• Pharmacist	1,097 (91%)
• Pharmacist and graduate programme	113 (9%)
Year of graduation (n = 1,209)	
• < 2000	100 (8%)
• 2000-2010	390 (32%)
• > 2010	719 (62%)
Take home pay received per month in IDR (n = 1,200)	
• < 1,000,000	14 (1%)
• 1,000,001 – 2,000,000	161 (13%)
• 2,000,001 – 3,000,000	379 (32%)
• 3,000,001 – 5,000,000	414 (35%)
• 5,000,001 – 10,000,000	156 (13%)
• 10,000,001 – 20,000,000	50 (4%)
• 20,000,001 – 30,000,000	16 (1%)
• > 30,000,000	10 (1%)

Table II shows the type of remuneration and/or benefits received by respondents. The respondents were commonly paid in the form of monthly salary (93%). Only less than half of respondents received fees for professional services (44%). In general, most of the respondents did not receive any other additional fees. However, they claimed that there is a remuneration increase periodically (62%).

Table III identifies pharmacists' preferences regarding the ideal remuneration model. When asked about whether a pharmacist is entitled to receive a fee for practice, the majority of respondents agreed (82%). Pharmacy owners, customers and the National health insurance agency (BPJS Health) are the top 3 payers preferred by the respondents to pay for pharmacist remuneration. With respect to the model of the remuneration, fee for service often sits in the most recommended model (5 out of 7) for paying pharmacists, followed by the capitation model (2 out of 7).

Table II: Type of remuneration of the respondent

Type of remuneration/benefits	Do you receive it?	
	Yes (%)	No (%)
Fee for professional services[1] (n = 1,168)	520 (44%)	648 (56%)
Monthly salary[2] (n = 1,179)	1,095 (93%)	84 (7%)
Fee per arrival[3] (n = 1,151)	516 (45%)	635 (55%)
Distribution of pharmacy profit[4] (n = 1,139)	443 (39%)	696 (61%)
Merit incentive[5] (n = 1,142)	198 (17%)	944 (83%)
Special holiday incentive[6] (n = 1,187)	1,057 (90%)	130 (10%)
Paid leave[7] (n = 1,178)	1,058 (90%)	120 (10%)
Periodical remuneration increase (n = 1,152)	712 (62%)	440 (38%)
Severance payment[8] (n = 1,108)	301 (27%)	807 (73%)

[1]Fees received for delivering professional services, including dispensing, counselling and medication review

[2]Monthly wages as employee pharmacist

[3]Fees paid every time employee pharmacist comes to work

[4]Fees received as part of profit made by the pharmacy

[5]Fees paid whenever pharmacist can achieve the specific target set by the pharmacy

[6]Annual mandatory incentive paid whenever employee pharmacist celebrating national religion festive such as Eid ul Fitr for Muslims or Christmas for Christians

[7]Fees given for approved leave, e.g. maternal leave

[8]Fees paid when the employee pharmacists resigned from their job

Discussion

There is an increasing need to deliver sustainable and high-quality health care to achieve the best possible outcomes in the most cost-effective fashion in Indonesia. This study argued that community pharmacy is in a unique position to offer the most cost-effective treatment to the general public. Not only cost-effective, but pharmacist also plays an important role to ensure that both pharmaceuticals and pharmacy services are delivered safely and effectively to the targeted population. However, results from this study show that despite the vital role of the pharmacist, compensation for their work has been minimal to support such a role.

Table III: Respondent's preferences regarding payment model

Question	Response	Frequency (%)
Is a pharmacist entitled to receive a fee for practice? (n = 762)	Yes	625 (82%)
	No	137 (18%)
Who should pay for pharmacist remuneration?		
Central government (n = 547)	Yes	255 (47%)
	No	292 (53%)
Local government (n = 519)	Yes	236 (46%)
	No	283 (54%)
National health insurance agency (n = 533)	Yes	307 (58%)
	No	226 (42%)
Pharmacy association / the Guild (n = 479)	Yes	149 (31%)
	No	330 (69%)
Customer / the patient (n = 586)	Yes	395 (67%)
	No	191 (33%)
Commercial insurance company (n = 472)	Yes	196 (41%)
	No	276 (59%)
Pharmacy owner (n = 624)	Yes	472 (76%)
	No	152 (24%)
What are the most suitable types of remuneration that should be paid by these parties?		
Central government (n =321)	Fee for service	58 (18%)
	Capitation	50 (15%)
	Subsidy	34 (11%)
	Others	
Local government (n = 296)	Capitation	46 (15%)
	Fee for service	45 (15%)
	Pay for performance	33 (11%)
	Others	
National health insurance company (n = 355)	Capitation	141 (40%)
	Fee for service	55 (16%)
	Pay for performance	18 (5%)
	Others	
Pharmacy association / the Guild (n = 232)	Fee for service	32 (14%)
	Pay for performance	27 (12%)
	Capitation	15 (6%)
	Others	
Customer / the patient (n = 414)	Fee for service	145 (35%)
	User charge	129 (31%)
	Pay for performance	29 (7%)
	Others	
Commercial insurance company (n = 268)	Fee for service	49 (18%)
	Capitation	43 (12%)
	Pay for performance	22 (8%)
	Others	
Pharmacy owner (n = 457)	Fee for service	124 (27%)
	Pay for performance	88 (19%)
	User charge	37 (8%)

Most pharmacists in this study only received a monthly salary and another minimum additional fee resulting in the range of IDR 2-5 million (USD 150-350) as the income that they bring home every month. Given their roles and responsibilities as mandated in the Presidential Decree 73 of 2016 (Hermansyah *et al.*, 2020), this seems inadequate to pay for pharmacist practice. Fairly speaking, such amount is equivalent to the minimum payment set by the government for the blue-collar worker, which absolutely highlights a contrasting spectrum of responsibilities with professionals such as pharmacists (Siregar, 2020). As the workload of pharmacists is increasing, particularly after the introduction of Universal Health Coverage which may imply an increased risk of their job, such amount of remuneration may not necessarily portray a proper compensation for the frontline healthcare workers like pharmacists.

The underlying objective of remuneration to pharmacists is to support them to deliver cognitive services. A practising community pharmacist is believed to have invaluable skills acquired from university-based training and experiential learning. Such predicate suggests that they deserve to be fairly compensated according to the standard of professional healthcare providers. This is why most respondents in this study believed that they should be paid for their professional contributions.

Pharmacists are also aware that they cannot rely on the government to pay for their work. Interestingly, pharmacy owners were commonly selected as the most recommended payer for pharmacy practice. This is perhaps related to the fact that the majority of pharmacists in Indonesia work as employee pharmacists (Hermansyah *et al.*, 2018a). Despite the policy that community pharmacy only operates under the full authority of pharmacists, ownership of pharmacy in Indonesia is not restricted to pharmacists only. Any individuals or companies can own a pharmacy leading to most pharmacies owned by non-pharmacist. Arguably, ownership may determine the vision and mission of a pharmacy in delivering pharmaceutical care, and to some extent, it may influence pharmacist remuneration structure (Athiyah *et al.*, 2019). With most pharmacists working as an employee, it might be challenging to negotiate remuneration, for instance, professional fee, unless the owners are aware the significance of delivering professional pharmacy services. This can be an insight for the existing employee pharmacists to convey a message that pharmacy services should be properly remunerated.

It is also not surprising that the customer sits in second place for the most recommended payer. In the short run, charging customers directly for service can be an

effective – and the easiest – alternative to collect remuneration for pharmacists. The pharmacy can determine the rate and the customer, or the patient is at the position of "less of freedom" given that they will require the services or the pharmaceuticals. However, pharmacies often offer services at a low charge or even most of the time, free of charge (Anderson & Thornley, 2014). This will be problematic for most pharmacies to initiate such payment. In the long run, charging customers can be feasible if the customers or patients recognise and experience the positive outcomes of the services. It may not be difficult to charge customers. What makes it difficult is to show the value and benefits behind the payment. Accordingly, fee for service is the perfect remuneration model for such case, which is also illustrated as the most selected remuneration model for community pharmacists in Indonesia.

Fee for service model is not new to community pharmacists in Indonesia. Prior to the implementation of Universal Health Coverage in 2014, the former insurance model in Indonesia used fees for services to pay pharmacy practice (Agustina *et al.*, 2019). This model took place between 1992 and 2013 in particular pharmacies affiliated with the national health insurance agency. Fee for service is the traditional payment model in many countries to remunerate pharmacists. The benefits are twofold; pharmacists can tailor particular services suit to patient's needs, and patients can opt for services and be flexible with the services provided by the pharmacists. However, the fee for service also exerts some disadvantages; particularly, it lacks accountability as there is uncertainty about the necessary service that should be provided to the customers and how it will cost the customers. There is no denying that fee for service is financially beneficial for providers, but in the long term, it is an unsustainable system that may lead to a lot of waste, unnecessary and perhaps inaccurate services.

The implementation of a national remuneration system is quite challenging in the context of Indonesia. The findings of this study showed that the level and type of remuneration varied among pharmacists. Implementing remuneration standards to community pharmacists is a multistage, collaborative process with a significant and complex interplay of stakeholders' interests. A pilot study focusing on learning the effective development and implementation of such standards might be required. The results from the pilot study can be an initial assessment to understand pharmacy service utilization, pharmacist acceptance and community pharmacy viability. In this process, remuneration planning and design are critical as it should encompass regulatory requirements, government responsibilities and pharmacy characteristics as most pharmacies in Indonesia are operated independently and owned by non-pharmacist. At the end of the day, the quality and competence of the pharmacist will determine the remuneration. An innovative pharmacist may have the potentials to gain more payment. However, it is not only about the payment; the positive outcome of the care is also substantial to help facilitating the ideal remuneration system for Indonesian pharmacists.

This study does have limitations that should be considered. First, this study used accidental sampling to recruit participants, which may not be accurate to portray the overall picture of the Indonesian community pharmacist. Second, the questionnaire was self-administered, highlighting that there is always a potential for recall and response biases from the respondents when answering the questions. Third and finally, it is also important to note that there is a variation of response between questions which may illustrate a lack of uniformity in drawing a conclusion for this study. Therefore, it is advised to interpret the findings of this study cautiously. Nevertheless, to the best of the authors' knowledge, this is the first nationwide survey collecting data about community pharmacist remuneration in Indonesia. This can be an important piece of information to support the existing incentivisation policy and to provide an overview of the remuneration model for community pharmacies in Indonesia. Further research is warranted to seek an effective model of remuneration in association with the outcome of the services.

Conclusion

Community pharmacists in Indonesia were commonly paid on the basis of monthly salary with a minimum additional fee provided to pay for the services. Such a remuneration model is indeed inadequate and not supportive to trigger professional and cognitive services in the community pharmacy. As pharmacists are uniquely positioned in the frontline of care, there is an imperative to properly compensate pharmacists considering their responsibilities, risks and qualifications.

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RESEARCH ARTICLE

Exploring pharmacist experience and acceptance for debunking health misinformation in the social media

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Abstract

Introduction: The increasing evidence of misinformation on pharmacy issues in Social Media (SM) may provide potential for pharmacist involvement. **Aim:** This study aims at exploring pharmacist experience and acceptance to debunk pharmacy misinformation in SM. **Methods:** Four Focus Group Discussions (FGDs) with 41 selected pharmacists were conducted. The FGDs collected participant's experience with misinformation, action taken and participant's acceptance for debunking misinformation. The FGDs were audio recorded, subsequently transcribed and thematically analysed. **Results:** The majority of respondents often clarified the misinformation. Pharmacist motivation, relationship with the sender, opportunities to response and ability to respond the misleading message are themes determining pharmacist acceptance for debunking misinformation in SM. **Conclusion:** Pharmacist has the potential to contain and prevent misinformation about health and pharmacy issues in SM.

Introduction

The use of Social Media (SM) for professional communication has gained popularity among pharmacists. A number of papers highlighted that pharmacists used SM for various purposes such as sharing information with the public and patients, disseminating research findings, communicating with other professionals and promoting debates in the fields of pharmacy (Benetoli *et al.*, 2015; Benetoli *et al.*, 2017; Hermansyah *et al.*, 2019). SM is without a doubt, offers the potential to improve patient care. However, the unfettered access to the internet and SM has also increased the spread of false claims or misinformation even faster and further than accurate information.

Information on SM can be perceived as a diverse mix of sound evidence, facts and phenomenon as well as a cacophony of opinion, pseudo-scientific research and falsehoods, which can propagate misinformation (Chou *et al.*, 2018). Misinformation as defined by Nyhan and Reifler (2010) is "cases in which people's beliefs about factual matters are not supported by clear evidence and expert opinion". Such definition has highlighted that misinformation contains incorrect information which may negatively affect the perceptions of the target/receiver. Health misinformation including hoax often appears in the discourse of SM.

The diffusion of misinformation has been driven by a number of factors including skepticism over the available treatment, distrust to institutions or involving stakeholders, lack of scientific evidence and poor access to information from the experts (Bode & Vraga, 2018). Information silos, echo chamber effects and controversies have often amplified misinformation with people rarely clarified the information leading to negative consequence to their health (Vraga & Bode, 2017).

The shifting role of pharmacist towards patient-centred care may push pharmacists to optimise their social media account in order to better communicate with the patients and communities. Moreover, the recent "infodemic" due to Covid-19 outbreak has suggested that pharmacist, who sits at the frontline of the healthcare services, can alter misperceptions that arise from incorrect claims of treatments for curing and preventing Covid-19 (Erku *et al.*, 2020).

The study of pharmacist role in debunking health misinformation is substantial to pharmacy practice, and it has been limited in the context of developing countries. In addition, responding to misinformation can be challenging to pharmacists implying the need to study the acceptance and strategy used by the pharmacist to counteract the circulated misinformation. Therefore, this study aims to explore pharmacist experience and acceptance to debunk pharmacy misinformation in SM.

Methods

Study design and setting

This study obtained permission from the Indonesian Pharmacist Association and was approved by the Board of Social and Political Affairs of East Java and Central Java Province. A qualitative study in the form of Focus Group Discussions (FGDs) was used to answer the objective of this study. The FGDs were conducted in four cities, namely Surabaya, Banyuwangi, Klaten and Semarang, from July to September 2019. Each city represents a unique characteristic and culture of the citizen. For instance, Surabaya and Semarang are the capital city of East Java and Central Java province, respectively. Both can be considered a metropolitan city which predominantly relies on trade and commercial sector. This is different to Klaten and Banyuwangi, which are more focused on tourism and agriculture as they are located relatively far from the capital city.

Participants

The researchers compiled a list of potential pharmacists as participants as recommended by the Indonesian Pharmacist Association and expanded with the names from researchers' networks and social media searches. The researchers purposively selected participants from a wide range of settings, including community pharmacy, hospital pharmacy, pharmaceutical industry and/or distributor, insurance company, health office and academician. A few of these participants were quite active in social media since they are appointed by the Ministry of Health as "Pharmacist Agent of Change", whose main task is to promote and influence good pharmacy practice. Shortlisted pharmacists were contacted about the FGDs. If the pharmacists agreed to the FGD, they were provided with an information sheet and consent form. Eligible participants were invited in 90-100 minutes FGDs. Each FGD was attended by 10-11 participants and was conducted in Bahasa Indonesia language. Participants received token money of IDR 200,000 (USD 15) in recognition of their involvement. Written consent was obtained prior to begin the FGDs.

Data collection

Participants were first asked to fill out a brief questionnaire asking about their past experience with misinformation circulated in SM and the action that they did to cope with the misleading message and the messenger. All researchers involved in the FGDs with one researcher acted as moderator. Subsequently, participants were invited to discuss their answers in the questionnaire. The discussion continued with another topic, including participants' activity in social media, their roles and strategies for debunking health misinformation in social media.

Data analysis

The FGDS were audio-recorded, de-identified and subsequently transcribed verbatim. Thematic analysis was used to uncover the findings. This began with each researcher independently reviewed the transcript, and audio recording iteratively coded the data and built emergent themes supported with illustrative quotes to reflect the themes. Once each researcher has completed this step, the findings were brought into a discussion within the research team. In this stage, all research teams would have to re-read transcripts to resolve any discrepancies with respect to the final themes. Final themes were agreed upon by all researchers.

Results

Overall, 41 pharmacists participated in the FGDs (10 males, 31 females). The majority of respondents (33 respondents) working in patient care settings. This includes community pharmacy, hospital pharmacy and public health centre. The remaining were working as academic (two persons), local health officer/administrator (four persons), in the pharmaceutical company (one person) and the insurance agency (one person). Respondents have mixed work experience. Fourteen had 1-5 years experience, eleven had 6-10 years experience, and sixteen had more than ten years experience. All participants had a WhatsApp account,

followed by Facebook (34 participants) and Instagram (31 participants) as the second and the third most used SM, respectively. Most respondents spent 1-3 hours in the SM daily (19 respondents).

There are four themes identified from the FGDs which affects the acceptance and respondents' experience for debunking health misinformation in the SM, namely 1) pharmacist motivation; 2) pharmacist relationship with the message sender; 3) opportunities to respond the misleading message; and 4) pharmacist ability to respond the misleading message. These themes are presented with illustrative quotations and a brief detail about the respondent characteristic.

Pharmacist motivation to respond to misinformation

Motivation is concerned with why people choose a particular course of actions over others and why they continue to do the actions, some of which is for a long time. With respect to health misinformation in pharmacy, it is the driving force by which pharmacists attempt either to counteract, to report, to share or even to ignore misleading information circulated in their SM.

One participant mentioned her calling as a pride pharmacist has encouraged her to counteract misleading information. She articulated clear reasons why pharmacists are needed for such roles as it is the pharmacist responsibility to debunk misinformation.

> *"I am a pharmacist, and it is my duty to educate community. (I) don't know who else will do (to debunk the misinformation)" (Female hospital pharmacist)*

Other participants, however, perceived not all misinformation must be clarified, particularly in a position where the individual pharmacist is prone to conflict, such as in a familial circle.

> *"I don't want to fall into a debate with my family. I knew that it was a hoax, but I chose to ignore it. Sometimes I did clarify, but it keeps coming. Even the hoaxes that I have clarified, they keep recirculating" (Female academic)*

The motivation of pharmacists to respond to misinformation is also determined by the experience of others, particularly from other healthcare professionals. A participant who works in a hospital pharmacy mentioned that she was not in agreement with the alternative therapies offered by her colleagues as it was not supported by scientific evidence. However, many of her friends, who are also healthcare providers, felt better after they took the therapy, which has made her share the information regardless of the truth behind the therapy.

> *"Some of my friends at the hospital, they are [name of healthcare profession], went to the alternative therapist. At first, I didn't see that it is right to do since it sounds mystical to me. But I saw that many of them were getting better, so I shared the information as well to those who desperately need the treatment" (Female hospital pharmacist)*

Pharmacist relationship with the message sender

A Pharmacist is also an individual who lives in an environment that may or may not recognise pharmacist status. The relationship between pharmacists and the member of the environment, i.e. families, communities, workmates or school mates may have determined pharmacist response to the health misinformation. This eventually will allow them to clarify misinformation directly with the message sender. The majority of participants viewed that their environment recognised their status as a pharmacist, which enabled them to counteract health misinformation.

> *"My family and my friends know that I am a pharmacist. They will contact or ask me when they found doubtful message about pharmacy" (Female local health officer)*

However, a conflict may arise with the sender, particularly if the sender is considered the elders in the family or those who are acknowledged as healthcare professionals. This is also problematic in the context of Indonesian culture

> *"I often clarified some hoaxes in my family WhatsApp group. But it is quite challenging if those who shared the hoax message is a health professional. I ever had a debate with my uncle and his son who is a [name of healthcare profession] about the benefits of vaccination…I showed them the journals…(until) my parents advised me not to do so" (Female hospital pharmacy)*

Opportunities to response the misleading information

Opportunities are defined as situation or condition that enables pharmacists to respond to the misleading information. The mounting pressure on pharmacists and pharmacy staff to dispense more medicines as well as to provide more services may have portrayed the challenges in the contemporary practice in Indonesia. This situation can be exacerbated by the spread of misinformation in the SM. One pharmacist mentioned that his workload had affected him to debunk the misleading information.

"I knew some (messages) are not correct. If I have the luxury of time, I shall response those (messages). But most of the time, I already tied up with the increased workload. No time to deal with (the messages), (I) just skip them" (Male pharmacist Agent of Change)

There are also pharmacists who really are committed to fighting against misinformation. One pharmacist claimed that she advocated a program to fight health hoaxes. However, the program was not sustainable as it was not included in the plan of the local government.

"There are many hoaxes related to health and these have concerned me. I made a program and promoted this program to the local government. For instance, I advocated the proper use of antibiotics. It was a success at the beginning. But at the end of the day, the program was discontinued…the program was not included in the government plan…it was exchanged by other priorities" (Female hospital pharmacy)

Opportunities to respond is also determined by the urgency. For example, a misleading message that may affect community pharmacy reputation is likely to be clarified as quickly as possible regardless of the time and the load of the pharmacist. This is to prevent a more devastating impact on the pharmacy operation. One participant mentioned her experience.

"There was a time when someone made a false claim in social media about the quality of product sold in my pharmacy…it was annoying me as it may ruin my pharmacy image. I am about to answer him but luckily the other pharmacists have responded" (Female community pharmacy)

Pharmacist's ability to respond the misleading message

Pharmacist's ability to effectively create a message that clarifies or counteracts the misleading information is also highlighted in the FGDs. Some pharmacists preferred to forward and share information from official media account of trusted sources, e.g. ministry of health and food and drug control agency, in order to debunk misinformation.

"There are public warnings from the official website or their social media accounts…I just shared them" (Male hospital pharmacy)

Creating a message that is effective to debunk misinformation is challenging to many of the participants. Some respondents viewed that making a short message with powerful words is a method to debunk misinformation. Other respondents used pictures on Instagram to draw attention from the public.

"I made a short message with powerful words. Some people were not aware with the danger (of the misinformation). Video can be an alternative, but people skipped them after three seconds when they found (it was) not interesting" (Female_community pharmacy)

"I made an Instastory about the use of Irbesartan (antihypertensive agent). I put a picture with some links provided (the medicine) to explain about the importance of taking it (Irbesartan)" (Female hospital pharmacy)

Reporting misleading information can be an option to respond to the misleading message. Whilst most respondents agreed that they had a role and are better positioned to report health hoaxes to the authorities, they did not choose this way as there was a lack of information about to whom and how the misleading information can be passed to the authorities.

"I never reported any hoaxes, never at all. (It is) simply because I didn't know where to report. All I did was just forwarded the (misleading) message to colleagues or friends who I knew she or he works for the government or the authorities. I don't bother myself to know whether it [the report] has been followed up or not" (Female community pharmacy).

Discussion

SM is increasingly becoming part of our lifestyle. This study demonstrates that pharmacists have used SM for supporting pharmacy practice which brings both risks and opportunities. The concern about misinformation, hoaxes and false information surrounding health and pharmacy issues may have put not only pharmacists at risk but also patients at greater risk. A pharmacist who is illustrated in many distinctive roles as a gatekeeper of care (Hermansyah *et al.*, 2018), the first point of call (Curley *et al.*, 2016), and the last healthcare worker to see the patients (in dispensing services) (Schindel *et al.*, 2017) can contain the health misinformation in the SM. This study, without a doubt, has added more discussions to such issues. However, what really matters is how pharmacists in the context of developing countries like Indonesia can consistently embrace this novel role amid the increasing pressure for practice change. Where would pharmacists go in contemporary practice?

With respect to the objective of this paper, it can be concluded that there is a potential for pharmacists to play an important role in clarifying hoaxes and

misinformation in SM. However, pharmacist acceptance to undertake such role is mixed, highlighting some limitations to implementation. Therefore, this discussion will focus on identifying limitations and devising strategies to overcome the limitations. There are three issues that need to be addressed by pharmacists based on the findings of this study, namely understanding how health misinformation is shared, evaluating the need to act and develop effective interventions, e.g. clarification, corrections or counter-message to debunk the falsehoods.

Typically, misinformation spreads as it is induced by scepticism, distrust, misperceptions and lack of access to reliable and trusted sources or information (Del Vicario *et al.*, 2016). These issues are closely related to the psychological and sociocultural factors of the recipients. The findings of this study revealed that misinformation could circulate in both exclusive environments such as family circles and in inclusive settings such as among workmates and even within the network of health providers. Pharmacists need to be aware of information exchange between the members of these communities. For instance, this study demonstrated that the elders are the ones who share the misleading information, which might be problematic given the cultural and familial hierarchy. This study argued that recognition as a pharmacist by the members of the communities is indeed critical to help determine the problems and communicate the remedies. Being a pharmacist suggests a strong status to refute false or misleading health information and supplied with evidence and appropriate sources to accompany the refutation. Apart from the status, pharmacists should also take into account the dynamics and the reception of the people. Avoid correcting people and focus on correcting the problem (Chou *et al.*, 2020). Pharmacists can employ straightforward efforts to respond to the misleading information with a risk of undermining the relationship with the sender. Alternatively, using "private" conversation can be an effective interpersonal approach. This is why evaluation of the action is the subsequent step to be conducted by the pharmacist.

In the situation where health misinformation has been widespread, accumulated and have the potential for devastating impact, as illustrated in the findings, pharmacist responses must be timely, strategic and evidence-based (Walter *et al.*, 2020). In addition, such a situation may also demand pharmacists to work with others, including the authorities, to contain the message. Pharmacist needs to identify who is the most vulnerable population and strategically intervening these groups if necessary. For instance, the case of misinformation related to vaccination or hypertension, as illustrated in this study, revealed the possibility for pharmacists to target people who are in need of vaccination and patients with hypertension, respectively. Pharmacists' focus is to lead these individuals to achieve their therapeutic goals and not to disengage with pharmaceutical care which may be detrimental to their health. Regardless of the state of the health misinformation – the urgency, the impact and the prevalence – pharmacists can always make an attempt to debunk health misinformation. However, it is also important to consider the backfire effect whereby the attempts proposed by the pharmacist can unintentionally discourage people (or the sender) and increase the acceptance of the misleading information (Peter & Koch, 2016). Therefore, developing an effective intervention is critical as the final process in debunking misinformation.

Pharmacist needs to carefully consider when and how to intervene. SM is a public space; therefore, communicating and clarifying misinformation in the SM is not only aimed to resolve the problem but also to sustain public trust in evidence-based health information (Kass-Hout & Alhinnawi, 2013). For instance, pharmacists might consider taking systematic improvements focusing on preventive action rather than correcting individuals in one situation. Nevertheless, a simple rebuttal can also be effective in a situation that requires a proactive response. It is also possible that pharmacist chooses not to respond at all, for example, when dealing with misinformation that most people do not believe it is real. Fairly speaking, there is no one size fits all. However, this study believes that "speak the truth" is, in fact, pharmacists' responsibility. Although this study agreed that not all pharmacists could embrace such commitments nor have the privilege (including time, workload, and communication manner) to speak based on evidence in the SM platforms, pharmacists cannot avoid the fact that their corrections or clarifications might have the meaning, particularly to the patient. This implies an imperative for pharmacists to overcome patients' confusions, concerns, and mistrusts as it is framed under the pharmacist-patient relationship. A proactive approach is more influential rather than expecting for the falsehoods to fade away.

Several limitations of this study must be noted. First, this study is not immune to selection bias in the recruitment of the participants. Maximum variation sampling is perhaps the alternative recruitment technique to obtain more comprehensive findings. However, there is also value for purposive selection as it may provide focused information about the case. Second, there is always an issue with the trust and credibility of participants' opinions in the qualitative study. This study cannot be highly confident how

participants deal with the misinformation in reality. Future research might be required to evaluate the actual implementation by the pharmacist.

Conclusions

Although there is still much to be learned, this study highlighted the important role of pharmacists in debunking health misinformation. Pharmacists can contain and prevent misinformation by strategically intervening with the public. However, some limitations have made the implementation challenging. Understanding how health misinformation is shared, evaluating the need for action and developing effective interventions are the keys to debunking the falsehoods.

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RESEARCH ARTICLE

The evident gap between actual and perceived facilities supporting value-added pharmacy services

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Abstract

Introduction: Whilst there is an emerging need to provide Value-Added Pharmacy Services (VAPS), the facilities in community pharmacy may not be supportive to implement this concept. **Aim**: This study aims to evaluate the actual and perceived facilities supporting VAPS. **Methods:** A cross-sectional survey was conducted to selected fifty pharmacies in Surabaya Indonesia. Respondents were asked about their agreement towards VAPS and facilities required for providing VAPS. The results were analysed using descriptive statistics. **Results**: All respondents agreed that pharmacy is in need to provide VAPS. In addition, they viewed the presence of facilities supporting VAPS are urgent. However, the availability of such facilities in the actual practice was relatively limited, highlighting underinvestment to provide VAPS. **Conclusion**: There is a gap between actual and perceived facilities supporting VAPS. This highlights more investments to upgrade pharmacy facilities.

Introduction

The role of pharmacists in the community has evolved over the years from compounding raw materials to medication management and public health-related pharmacy services, which encompasses a range of activities, including educating, screening, monitoring therapy and surveillance for both individual patients and communities (Hermansyah *et al.*, 2017). These services are not always related to the selling of medicines. Some published articles refer to these services as extended pharmacy services, which plays as an adjunct to the "traditional" pharmacist dispensing role (Edmunds & Calnan, 2001; Perraudin *et al.*, 2016). Some articles called these newly emerging services as Value-Added Pharmacy Services (VAPS) given their characteristics which add value to the medication use process and patient care outcomes (Malone *et al.*, 1993; Tan & Gan, 2016; Desselle *et al.*, 2019).

The definition of VAPS can be elusive as some researchers used VAPS to coin pharmacist or pharmacy services beyond the dispensing and professional consultations, whilst others might reflect VAPS as activities that add value to pharmacist work. The latter can exert a number of outcomes such as cost reduction effect and improved treatment, with some authors pointed at innovation and creativity to improve pharmaceutical care. Despite the elusive definition, experts-viewed delivery of VAPS can be substantial for health care and pharmacist role. In fact, some governments perceived VAPS as a "positive action to improve health care and to ensure a high-quality health system that is customer-oriented" (Tan & Gan, 2016).

The provision of VAPS, however, cannot be separated from the supports of facilities available at the pharmacy. For instance, pharmacies in Malaysia were reported to deliver VAPS in the form of drive-through services, pharmacy appointment systems and medicine by post (Tan *et al.*, 2015). These services were claimed to reduce waiting time, ease the refill of medications and increase patient convenience than conventional pharmacy services. Nevertheless, to smoothly apply such system, it

can be predicted that a community pharmacy needs to invest in some facilities which might not be commonly available in many typical pharmacies, i.e. software or application, spacious pharmacy setting and Short Message Service (SMS) push system as a reminder. In principle, VAPS can take in many different forms, but pharmacists and pharmacies ought to be ready for some changes, which is perhaps difficult and costly to do in the beginning.

Community pharmacy in Indonesia is also in the midst of change, particularly after the Universal Health Coverage was introduced in 2014 (Hermansyah *et al.*, 2018a). For example, Medication Therapy Management has increasingly become an acceptable norm to improve the outcomes of pharmacy services (Prasetio *et al.*, 2019). While innovations in health and pharmacy services have been driven towards cost containment, the core of pharmaceutical care, which focuses on patient's interests, remains intact. Some VAPS, such as courier services, telepharmacy and drug reminder systems, which might be costly, are still a new concept, and the general population might not fully utilize these services (Hermansyah *et al.*, 2020). However, adoption of these services in the future is perhaps inevitable, particularly after the Covid-19 pandemic has hardly hit the Indonesian health care system. Therefore, it is important to understand pharmacy preparedness to such changes, particularly related to the investment of facilities.

To the best of the authors' knowledge, there is a paucity of study that explores pharmacy facilities supporting VAPS in Indonesia. Therefore, this study aims to fill such gap by evaluating the actual and perceived facilities invested for the delivery of VAPS in the context of Indonesian community pharmacies.

Methods

Study design and setting

A cross-sectional study in the form of a survey was conducted between February and April 2019. This study included community pharmacies that operate in the public spaces, shopping centres, neighbourhood environments and community pharmacies affiliated with universities, i.e. teaching pharmacy in Surabaya, the second biggest city in Indonesia. One key respondent represented each pharmacy. They can be the pharmacist, the owner or the pharmacy staff. This study is low-risk research in which the only foreseeable risk is one of discomfort, particularly when completing the survey. Therefore, ethics approval was not deemed necessary.

Participants

Given the paucity of information regarding community pharmacies that fits the characteristics of this study, this study purposively selected 50 pharmacies. The researchers selected pharmacies that represents all five districts in Surabaya (northern, western, southern, eastern and central Surabaya), and these pharmacies were invited for the survey.

Data collection

A printed self-administered questionnaire was distributed by the researchers on hand. Initial contact was made prior to distribute the questionnaire. This includes a package of invitations to the survey, an information sheet and the questionnaire. When the pharmacy was able to participate, the package was provided, and informed consent was obtained in return. Pharmacy which withdrew from participation could not pass the questionnaire to another pharmacy, and no replacement survey was provided. In addition to the survey, reminder calls were made every month to non-respondents. No financial compensation was offered to respondents. Once respondents completed the questionnaire, they can send the questionnaire by post or asked the research team to pick it up.

The questionnaire was developed based on the references and discussions within the research team. The questionnaire used in this study was developed in two stages. The first stage was to conduct validity testing involving four experts, all of them had a background as academic pharmacists, to refine the face and content of the questionnaire. The questionnaire was then subsequently pre-tested to three community pharmacies (one independent pharmacy, one chain pharmacy and one pharmacy affiliated with the university). No major changes were made after the pre-test concluded. The final version of the questionnaire asked several questions, including respondent characteristics, perception towards VAPS and facilities supporting VAPS, the availability of the facilities and willingness to invest in facilities for delivering VAPS.

Data analysis

Descriptive statistics were used to report respondent characteristics and to highlight the findings. All data analysis was performed using Microsoft Excel.

Results

Of 50 pharmacies, 20 were deemed ineligible due to a number of reasons, such as two pharmacies were no longer in the business, one pharmacy withdrew its participation, and 17 pharmacies were not willing to participate in the study. Thirty respondents completed the

questionnaire. Table I shows the characteristics of the respondents. Respondents were dominated by females (83%), with the majority were aged between 19 and 39 years old (77%) and had a minimum qualification of bachelor's degree (93%). Pharmacists represented the predominant profession (94%). Most of the pharmacies have operated within ten years (60%), owned by individuals (60%) and operated as independent pharmacies (67%), with almost evenly split in terms of pharmacy location, in the sideways of the main road or in the residential (47%, 43%), respectively.

Table I: Characteristics of the respondents

Characteristics	Frequency (%)
Gender	
• Male	5 (17)
• Female	25 (83)
Age	
• 19-29	12 (40)
• 30-39	11 (37)
• 40-49	4 (13)
• 50-59	2 (7)
• > 60	1 (3)
Latest educational degree	
• Secondary degree	2 (6)
• Undergraduate	25 (83)
• Graduate	3 (10)
Workforce status	
• Pharmacist	28 (94)
• Non-pharmacist/technician	2 (6)
How long has the pharmacy been operated?	
• < 1 year	1 (3)
• 1-10 years	17 (57)
• 10-20 years	6 (20)
• > 20 years	6 (20)
Ownership	
• Owned by individual	18 (60)
• Owned by a company/institution	12 (40)
Type of operation	
• Independent pharmacy model	20 (67)
• Pharmacy franchise/banner group	10 (33)
Pharmacy location	
• Sideways of the main road	14 (47)
• In the neighbourhood/residentials	13 (43)
• In the shopping centre	3 (10)

Table II highlights respondents' perceptions of VAPS. All respondents agreed that a community pharmacy should provide VAPS, and therefore, investment for VAPS is substantial. Table III shows respondents' perception of facilities supporting VAPS and the availability of such facilities in the current pharmacy setting. In general, respondents viewed the importance of owning some facilities that may support VAPS. However, there are several facilities that were considered less important in relation to VAPS, such as pharmacy websites and vehicles for delivery. Accordingly, the availability of these facilities was not dominant in some pharmacies. In addition, there is a significant number of pharmacies (approximately 20%) that did not have software and cashless payment method to support VAPS.

Table II: Perception towards VAPS

Statement	Frequency (%)			
	Strongly agree	Agree	Disagree	Strongly disagree
Delivery of pharmacy services must be quick, practical, responsive and accessible	19 (63%)	11 (37%)	0	0
General public demands VAPS	17 (57%)	13 (43%)	0	0
Investment on facilities is important to support delivery of VAPS	11 (37%)	19 (63%)	0	0
The availability of facilities can boost innovation in services	10 (33%)	20 (67%)	0	0
Investment on facilities can improve management of pharmacy	15 (50%)	14 (47%)	1 (3%)	0
Investment on facilities can reduce waiting time	12 (40%)	18 (60%)	0	0
Investment on facilities can increase patient satisfaction	15 (50%)	15 (50%)	0	0
Investment on facilities can improve patient access to drug information and education	12 (40%)	18 (60%)	0	0

Table IV demonstrates the respondent's willingness to invest in facilities supporting VAPS. Despite the importance of the investment in the facilities, as illustrated in Table III, more than half of the respondents (60%) did not plan to install or upgrade facilities that were available in the current practice. The lack of space in the pharmacy (57%) and the shortage of workforce to provide VAPS (44%) were the top two reasons why pharmacies were reluctant to invest in delivering VAPS and facilities. On the contrary, pharmacies that were going to invest on facilities (40%) preferred to upgrade the software in order to keep up with the demand for providing VAPS.

Table III: Perception towards facilities supporting VAPS and the availability of the facilities at the pharmacy

Type of facility*	Is it important to support VAPS?				Is it currently available at the pharmacy?		
	Very important (%)	Important (%)	Slightly important (%)	Not at all important (%)	Yes (%)	No %	Why is it not available? ** (%)
Telephone	20 (67%)	10 (33%)	0	0	30 (100%)	0	N/A
Computer	20 (67%)	9 (30%)	1 (3%)	0	28 (93%)	2 (7%)	Computer is broken (50%) Waiting for new computer installation (50%)
Email address	13 (43%)	13 (43%)	4 (13%)	0	26 (87%)	4 (13%)	Not necessary to have (100%)
Pharmacy website	8 (26%)	11 (37%)	11 (37%)	0	12 (40%)	18 (60%)	Not necessary to have (85%) Pharmacy is currently developing website (15%)
Internet connection	19 (64%)	10 (33%)	1 (3%)	0	27 (90%)	3 (10%)	Not necessary to have (67%) Waiting for internet installation (33%)
Software for inventory management	23 (77%)	7 (23%)	0	0	26 (87%)	4 (13%)	Not necessary to have (50%) Waiting for software installation (50%)
Software for purchasing and selling record	23 (77%)	7 (23%)	0	0	26 (87%)	4 (13%)	Not necessary to have (50%) Waiting for software installation (50%)
Software for accounting and finance	17 (57%)	12 (40%)	1 (3%)	0	23 (77%)	7 (23%)	Not necessary to have (72%) Waiting for software installation (28%)
Software for supporting drug information	20 (67%)	10 (33%)	0	0	23 (77%)	7 (23%)	Not necessary to have (72%) Waiting for software installation (28%)
Patient medication record system / documentation	15 (50%)	14 (47%)	1 (3%)	0	19 (63%)	11 (17%)	Not necessary to have (72%) Lack of workforce (28%)
Cashless transaction payment / machine	13 (43%)	14 (47%)	3 (10%)	0	23 (77%)	7 (23%)	Not necessary to have (100%)
Air conditioning system	15 (50%)	12 (40%)	3 (10%)	0	24 (80%)	6 (20%)	Not necessary to have (100%)
Parking space	21 (70%)	9 (30%)	0	0	29 (97%)	1 (3%)	No additional space (100%)
Customer waiting room	19 (63%)	11 (37%)	0	0	29 (97%)	1 (3%)	Not necessary to have (100%)
Printed drug information materials	10 (33%)	20 (67%)	0	0	28 (93%)	2 (7%)	Not necessary to have (100%)
Vehicle for delivery	9 (30%)	13 (43%)	8 (27%)	0	17 (57%)	13 (43%)	Not necessary to have (100%)

*Facility that belongs to the pharmacy, not owned by individual
**Only for respondents answering No

Table IV: Willingness for investment on facilities supporting VAPS

Are you going to invest on more facilities to support VAPS?			
Yes = 12 (40%)		**No = 18 (60%)**	
Type of facilities invested	**Frequency (%)***	**Reasons for no investment**	**Frequency (%)***
• Upgrading software	5 (42%)	1. No space available in the pharmacy	10 (57%)
• Website	3 (25%)	2. Shortage of workforce	8 (44%)
• Laptop / computer	2 (17%)	3. Lack of funding to train current workforce (current workforce is not qualified to provide VAPS)	6 (33%)
• Television	2 (17%)		
• Vehicle for delivery	2 (17%)		
• Patient Medication Record	2 (17%)	4. Current facilities have met the minimum standard	6 (33%)
• Wi-Fi connection	1 (8%)		
• Email account	1 (8%)	5. Lack of funding for investment on facilities	5 (28%)
• Cashless transaction payment	1 (8%)		
• Children playground	1 (8%)		
• Barcode pricing system	1 (8%)		
• Membership card	1 (8%)		

*Respondents can answer more than one option. Frequency was calculated based on each Yes/No
Proportion e.g. three people answering for website scores 25% (3/12 x 100% = 25%)

Discussion

The provision of VAPS in a pharmacy setting is inevitable. The pharmacy must adapt to the contemporary situation, which is marked by shifting lifestyle and healthcare orientation from curative towards promotion and prevention. Such imperative, however, requires investment which sometimes is significantly affecting the operation and financial viability of the pharmacy. Therefore, pharmacists must seek balance and, to some extent is being forced to trade-off between more investments and maintain the current practice.

To the best of the authors' knowledge, this is the first study that acknowledges the importance of providing VAPS in Indonesia. The respondents involved in this study represented three main types of pharmacy affiliation in Indonesia, namely independent pharmacy, franchise pharmacy and pharmacy affiliated with university or teaching pharmacy. It is fair to say that these three types of pharmacy have their own orientation and mission, which will determine their intent to provide VAPS and investment on facilities supporting VAPS. For instance, the independent pharmacy model tends to maximize the pharmacy services and engagement with the customer rather than competing on price and variety of products as often found on the model of franchise pharmacy (Athiyah *et al.*, 2019). However, the similarity occurs as both pharmacy models are profit-oriented.

Conversely, teaching pharmacy much focuses in facilitating pharmacy students to acquire knowledge and skills prior to enter the practice field. This way teaching pharmacy cannot be fully profit oriented as they have mission for preserving the value of education in the practice.

The findings in this study reflected that there was a common agreement from the participating pharmacies regarding the need to change in the current practice. Respondents also agreed that investment for facilities supporting VAPS is significant for pharmacy operations. This may highlight that pharmacy, despite their business models and scale of economy, sees the future ahead will rely on the provision of VAPS (Singleton & Nissen, 2014). Such attitude implies the urgency for pharmacy to change. On the one hand, this is good news given that many pharmacies, particularly in the developing countries, including in Indonesia, were not aware or perhaps neglected the fact that pharmacies cannot do business as usual (Hermansyah *et al.*, 2012).

The shifting practice towards patient orientation in the form of VAPS and cognitive services should be part of the current paradigm of practice and streamlined into the pharmacy business model. On the other hand, change towards VAPS is not easy. The investment for providing VAPS may require an overhaul to pharmacy operation and such dramatic change is unlikely to happen in the majority of pharmacies (Doucette *et al.*, 2012). The existing facilities on the pharmacy may determine the state of investment required for providing VAPS. For instance, a pharmacy that already has a computer and internet connection may further invest in attracting larger customers by becoming an "online" pharmacy. By online in this particular meaning supports the broader recognition with which customers can have more access to contact pharmacy and to obtain pharmaceuticals and other products through a distant purchasing.

Despite the majority of pharmacies in this study already owned a number of facilities supporting VAPS, there is a concern that some facilities were not in place. This is particularly true for pharmacy websites, patient

medication record systems, and vehicles for delivery. Like the aforementioned, the website for a pharmacy can reflect a number of benefits. First, the availability of a website provides a venue for customers to stay connected with the pharmacy and be informed of the innovations as developed by the pharmacy. Second and more importantly, information on the website can go beyond the walls of the pharmacy. This will open up more possibilities not only to reach larger customers but also to provide care for a wider population (Bate & Hess, 2010). The latest report from the Indonesian Internet Provider Association found that 197 million people of Indonesian (approximately 77% of the population) used the internet every day, with the average time spent is eight hours per day (Indonesia Internet Service Provider Association, 2020). A good website, therefore, will give pharmacies the opportunity to grow. Likewise, the presence of patient medication records is essential to pharmacy practice. Good documentation of patient's medication serves the interest of the pharmacist, and it provides up-to-date information for continuity of care of the patients (Ojeleye *et al.*, 2013). The availability of vehicles comes with the innovation for telepharmacy and delivery services. The ongoing Covid-19 pandemic has witnessed dramatic change to pharmacy practice, with more pharmacies trailed to provide distant pharmacy care. Pharmaceutical deliveries have been seen as beneficial to cope with the situation, which limits face to face interaction between pharmacists and the customers (Koster *et al.*, 2020).

It is important to note that investing on facilities supporting VAPS is not on the agenda of most pharmacies participating in this study. On the one hand, this might reflect that the current facilities in the pharmacy can be assumed as sufficient to support the delivery of VAPS. Community pharmacy in Indonesia abides by the Presidential Decree 9 of 2017 regulating community pharmacy, including its facilities, operation and resources (Hermansyah *et al.*, 2020). This means pharmacies in this study not only has met the standard but also has the potential to provide VAPS. Therefore, further investment on facilities may not be necessary. However, on the other hand, the dynamics of change in the health and pharmacy sector in tandem with the ongoing Covid-19 infection in Indonesia may pose a challenge to community pharmacy viability in the future.

Delivering VAPS perhaps is not an option, but it is inevitable for a pharmacy. The lack of space, shortage of workforce and lack of skills which have limited pharmacy to deliver VAPS, as indicated in this study, might require reorganisation of the pharmacy structure and setting. Whilst spacious pharmacy outlet is always the "ultimate expectation", optimising the workplace condition and staffing support can be an alternative to most pharmacy settings in Indonesia (Hattingh *et al.*, 2016). This study argued that the option to optimise workplace conditions imposed advanced services and more attention towards safety and effectiveness. Likewise, staffing support particularly is directed to pursue continuing education which can reduce the errors and misses which may compromise patient safety (Hermansyah *et al.*, 2018b). Nevertheless, these approaches should be considered in light of pharmacy capacity.

Some limitations to this study should be noted. The number of participants and the response rate was low. As such, it is not possible to claim that this study represents the profile of community pharmacy in Indonesia. However, this study may provide background information about the pharmacy landscape in Indonesia with respect to the delivery of VAPS. There is also a possibility of social desirability bias where the respondents may perceive favourable responses in relation to the topic. This is possible, given the nature of the study. Other limitations are associated with the facilities included in the questionnaire, which differs from other studies focusing on VAPS. For example, drive-thru pharmacy is perhaps virtually non-existent in the Indonesian pharmacy sector. Only quite a few pharmacies offer such service. This means the applicability of this study may depend on the capacity, setting and organisation of the pharmacy. Further studies might be needed to address such issues.

Conclusions

There is a gap between actual and perceived facilities supporting VAPS. This highlights more investments to upgrade pharmacy facilities. However, investing on facilities cannot ignore pharmacy capacity and operation. As such, community pharmacies and pharmacists might need to consider optimising workplace conditions and better staffing supports.

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IAI CONFERENCE

RESEARCH ARTICLE

Description of medication adherence in hypertensive respondents at Mandalika Mataram elderly social centre

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Keywords
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Abstract

Introduction: Elderly people are at high risk of non-adherence to hypertensive therapy due to changes in body function and ageing processes. **Aim:** The purpose of this study is to explore medication adherence among hypertensive respondents at the Mandalika Mataram NTB Elderly Social Centre. **Methods:** This study is descriptive observational with a purposive sampling technic. The sample consisted of 30 respondents who met the inclusion criteria. Data were collected using the Modified Morisky Adherence Scale (MMAS-8). **Results:** The results showed that adherence among participants was high (23.3%), moderate (56.1%), and low (20.0%). Respondent adherence was associated with the role of health workers in monitoring drug therapy. Non-adherence was several factors, including side effects of the drug, complex drug regimens, and ageing.

Introduction

Hypertension is a non-communicable disease (NCD, not caused by microorganisms such as bacteria, fungi, viruses, and protozoa) that has a high prevalence in Indonesia. Weak control of risk factors affects the increase in cases per year. The results of Health Research (Riskesdas) in 2007, 2013, and 2018 reported an increasing prevalence of NCDs, particularly diabetes, hypertension, stroke, and joint diseases (Kemenkes RI, 2019). Hypertension is the second most prevalent NCD in West Nusa Tenggara after gastrointestinal infections. Visits for hypertensive complaints reached 214,080 in 2018 and 108,127 in 2019, illustrating the high incidence of hypertension in West Nusa Tenggara (Dinas Kesehatan NTB, 2018). In general, patients ≥ 60 years old can be given pharmacological therapy if systolic blood pressure ≥ 150 mmHg or diastolic blood pressure ≥ 90 mmHg. The blood pressure target to be achieved for systolic is < 150 mmHg or diastolic target < 90 mmHg (Strong Recommended-Grade A) (Muhadi, 2016).

Factors that affect hypertension in elderly respondents include aspects of life and changes in the cardiovascular system. These factors can increase the trigger of hypertension (Isnaini & Lestari, 2018). High-risk factors and poor control cause a higher incidence of hypertension. The medication adherence of elderly respondents is influenced by several aspects, including family support, health officer support, treatment factors, and respondent knowledge level (Pratama & Ariastuti, 2016). Adherence is a prerequisite for therapeutic effectiveness and the highest potential for improved hypertension control. Non-adherence to treatment is one of the leading causes of therapeutic failure (Hazwan, 2017).

Mandalika Elderly Social Center is a social service owned by the Province of West Nusa Tenggara. Of the 80 people who live there, 32 have hypertension. This

study was conducted at the Elderly Social Center to explore medication adherence among hypertensive patients at the Mandalika Mataram NTB Elderly Social Center.

Method

This descriptive cross-sectional study conducted in May 2019 recruited 30 elderly respondents with hypertension from the Mandalika Elderly Social Center, Mataram, NTB, using nonprobability sampling techniques. The inclusion was criteria were hypertension, non-dementia, age 60-90, and willingness to be interviewed and complete questionnaires. This survey used the MMAS-8 scale to measure medication adherence among participants. The data analysis was carried out to describe the characteristics of each research variable. The total MMAS-8 score was calculated by summing the answers, and adherence was categorised into 1) high adherence (score of 8); 2) moderate adherence (score of 6-7); and 3) Low adherence (score of 0-5) (Krousel-Wood *et al.*, 2009; Oliveira-Filho *et al.*, 2012).

Results

This research described the medication adherence among 30 hypertensive respondents who met the inclusion and exclusion criteria at Mandalika Elderly Social Center in Mataram, West Nusa Tenggara.

Respondents characteristics based on gender

Gender is one of the factors that influence blood pressure. Women tend to have hypertension more than men. The characteristics of the respondents based on gender can be seen in Table I.

Table I: Respondents characteristics based on gender

Gender	n	%
Men	4	13%
Women	26	87%
Total	**30**	**100%**

In this study, the incidence of hypertension was higher in women (87%), while it was 13% in men, suggesting that women are at higher risk of hypertension.

Respondents characteristics based on age

The characteristics of the respondents based on age can be seen in Table II.

Table II: Respondents characteristics based on age

Age	n	%
60-74 years	11	37%
75-90 years	19	63%
Total	30	100%

In this study, participants aged 75-90 years old were at higher risk of hypertension (63%) compared to those 60-74 years, indicating that older age increases the incidence of hypertension.

Respondents characteristics based on hypertension duration

The characteristics of the respondents based on the hypertension duration can be seen in Table III.

Table III: Respondents characteristics based on hypertension duration

Hypertension duration	n	%
> 5 years	13	43%
<5 years	17	56%
Total	30	100%

Of all respondents in this study, 56% were diagnosed with hypertension less than five years ago, and 43% were hypertensive since more than five years ago, indicating a higher proportion of respondents with hypertension since less than five years.

Medication adherence in respondents

Figure 1 shows medication adherence in respondents.

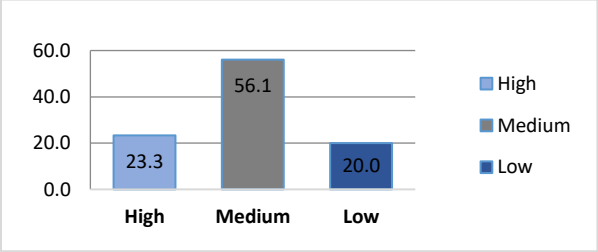

Figure 1: Medication adherence

The results showed three levels of medication adherence: high (23.3%), medium (56.1%), and low (20.0%), indicating that the highest percentage of respondents had medium medication adherence. This result is influenced by several aspects, such as health support, family support, attitudes, and age, in line with previous findings suggesting that knowledge, attitudes, and family support affect the hypertensive diet (Tarigan *et al.*, 2018).

Discussion

This study aimed to explore the characteristics of elderly hypertensive patients associated with medication adherence, namely, gender, age, and disease duration. Based on Table I, gender may lead to differences in the incidence of hypertension, higher among women, consistent with the findings of Anwar *et al.* (2019). After menopause, hormonal changes occur, such as a decrease in the ratio of estrogen and androgens, which causes an increase in renin, thus triggering an increase in blood pressure. Renin is an enzyme with a small protein released by the kidneys when arterial pressure drops very low. According to Klabunde (2005), the release of renin can be caused by the activation of the sympathetic nerves (activation via β1-adrenoceptor), decreased renal artery pressure (due to low systemic pressure or renal artery stenosis), and reduced salt intake to the distal tubule. Ageing is directly proportional to high blood pressure because, alongside ageing, structural and functional changes occur in the peripheral vascular system, responsible for controlling blood pressure. These changes include atherosclerosis, loss of elasticity of the connective tissue, and decreased ability of smooth muscle relaxation of blood vessels, which, in turn, reduces the ability of blood vessels to strain and stretch (Suzanne *et al.*, 2013).

Respondents have been experiencing hypertension for more than a year, as shown in Table II, so they understand the importance of taking the medication regularly and controlling blood pressure through an elderly *Posyandu* visit card once a month. Respondents may also be aware of risks and complications if they do not comply with their medication. The percentage of hypertensive respondents for less than five years is much higher than that of those over five years, in line with the findings of Anwar *et al.* (2019), showing that the distribution of hypertensive respondents for the period of 1-5 years is 57%.

Based on Figure 1, the adherence of most hypertensive respondents at Mandalika Elderly Social Center in NTB fell into the moderate category, i.e., 17 respondents (56.1%), followed by those with low (6 respondents, 20.0%) and high (7 respondents, 23.3%) adherence, indicating that elderly respondents need supervision or assistance from the healthcare professionals at the centre due to ageing and memory loss. This result is consistent with previous findings showing moderate adherence among elderly respondents in the working area of Puskesmas Air Putih Samarinda (41.0%) (Anwar *et al.*, 2019) and 40% among hypertensive respondents at the Wirobrajan Health Center Yogyakarta in August 2016 (Cahyani, 2018).

Medication adherence is essential to help respondents recover quickly from their illness (Sulistyarini & Hapsari, 2015). Side effects and type of drug are among the factors that affect adherence to medications. A complex therapeutic regimen will lower adherence rates (Lam *et al.*, 2007). Non-adherence leads to increased hospitalization length and risk of death. Some of the solutions include assessing the respondent's therapeutic needs, simplifying the therapeutic regimen, using long-acting medications, and supporting families and health workers (Burnier *et al.*, 2020). Medication adherence in elderly respondents is explained by the role of the family and health workers, in addition to excellent self-motivation. Improved compliance can be achieved by providing one-dose therapy and using a drug box for single-dose in elderly respondents.

Conclusion

The results showed that adherence among participants was high (23.3%), moderate (56.1%), and low (20.0%). Respondent adherence was associated with the role of health workers in monitoring drug therapy. Non-adherence was several factors, including side effects of the drug, complex drug regimens, and ageing.

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IAI CONFERENCE

RESEARCH ARTICLE

Hypoglycemic activity test on smooth pigweed (*Ammaranthus Hybridus L*) leaf water extract on male Wistar rats

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Keywords
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Abstract

Introduction: Type 2 diabetes mellitus consists of an array of dysfunction characterized by hyperglycemia. The activity of smooth pigweed (*Amaranthus hybridus L.*) leaves water extract on male Wistar rats. **Aims:** This research was started by supplying simplicia, making smooth pigweed leaves water extract, and testing the hypoglycemic activity of smooth pigweed leaves water extract on male Wistar rats. **Methods:** The glucose tolerance method was used to determine the hypoglycemic activity of smooth pigweed leaves water extract. Male white rats were divided into five groups of six rats each: a positive control group (0.5% of tragacanth suspension), a comparison group (Diabinese suspension at a dose of 22.5 mg/kg body weight (bw)), and three test groups at doses of 50 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw. **Results and conclusions:** The most significant hypoglycemic activity was seen with the dose of 150 mg/kg bw in comparison with the control group at 90 minutes.

Introduction

Diabetes mellitus is a non-contagious disease condition that arises from both relative and absolute insulin deficiency. Hyperglycemia is due to the absorption of glucose into inhibited cells and disrupted metabolism. Indonesian tropical forest medicinal plant species are used as raw materials in the herbal medicine industry, especially those against diabetes mellitus, such as smooth pigweed (*Amaranthus hybridus* L.), java plum (*Eugenia cumini* MERR), bilimbi (*Averrhoa Bilimbi* L.), and others. Knowledge of the medicinal and healing properties of a plant is generally based on natural clues or animal behaviour. For example, heart-shaped leaves cure cardiac disease, the yellow parts of a plant, such as turmeric and *temulawak*, are used in jaundice, sick animals that eat some types of plants indicate the plant is medicinal (Douglas *et al.*, 2000).

The traditional medicine industry and phytopharmacy have utilised various plant species as raw materials to treat several diseases, including fever, diarrhoea, malaria, hypertension, and thrush. (Anonim, 1989) Forest plants and gardens have many more medicinal potentials. Many people turn to alternative medicine for treating disease because they considered it safe with minimal side effects.

The study aims to determine the hypoglycemic activity of *Amaranthus hybridus* L water extract and the most effective concentration in lowering blood sugar levels in male Wistar rats.

Method

The study started with providing and characterising Simplicia, manufacturing smooth pigweed leaf extract,

and testing its hypoglycemic activity in male Wistar rats. Blood collection was carried out by cutting ± 0.5 cm of rat tail and measuring blood glucose levels in UV spectrophotometry (Evelyn, 1998).

The glucose tolerance method clinically used to diagnose diabetes was applied to determine the hypoglycemic activity of smooth pigweed leaf extract. (Gruben, 1981; Guyton & Hall, 1997) The male Wistar rats were divided into five groups of 6 mice, namely the positive control group (0.5% of tragacanth suspension), the comparison group (Diabinese suspension at a dose of 22.5 mg/kg body weight (bw)), and three test groups at doses of 50 mg/kg bw, 100 mg/kg bw, and 150 mg/kg bw, respectively (Hyne, K, 1987; Hoffbrand, 1996).

Materials

Ingredients

Test dosage (smooth pigweed leaf water extract), comparative substance (chlorpropamide 250 mg tablets), distilled water, standard glucose solution, suspended material (tragacanth), glucose reagent, NaCl 0.9%, alcohol 70%, oral glucose, gelatin, vanillin sulfate, dragendorf, NH4OH 10%, FeCl3, HCL 2N, alcohol, chloroform, Burchard Liebermann, ether, Mayer, Mg powder, filter paper (PT Eisai Indonesia, 1983).

Tools

Oral feeding tube, Eppendorf tube, centrifuge, scaled pipettes, UV-VIS spectrophotometry (Shimadzu UV type 1601), water stems, evaporation cups, crews, analytical balances (sartorius), 1 mL and 2.5 mL syringes, measuring glasses, stirring rods, ovens, rat scales, heaters, test tubes, micropipettes, desiccators, mortars, stampers, scissors, furnaces, glass funnels (Prof. Dr Arjatmo, 2016).

Experimental animals

Male, white, healthy Wistar rats, weighing around 200-300 g and aged about three months. The 30 rats were grouped randomly, using six rats per group: one positive control group, one comparison group, and three group tests (Roth, 1998).

Location and time of pick-up of smooth pigweed leaves (Amaranthus hybridus L)

Smooth pigweed leaves were collected from PT. Esai Sukabumi around January 2020, where the material was denominated in Herbarium Bogoriense, the botanical field of Biology Research Center-LIPI, Bogor. (Immaculata, 1991) The leaves were cleaned under running water, drained and dried by the wind away from direct sunlight. After they dried, the leaves were ground into powder (Immaculata, 1991).

Examination of the characteristics of smooth pigweed leaves

One gram of Simplicia was carefully weighed in a lidded scale bottle that had previously been heated at 105^{o}C for 30 minutes and maintained at this temperature (Ministry of Health and Social Welfare of Indonesian Drug Plant Invetaris, 2000). The simplicity in the bottle was flattened to form 5 -10 mm thick, then it was put in the drying room with the lid open and dried at a temperature of 105 ^{o}C until the weight remained. Every time before weighing, the bottle was let in a closed, cold state in the excavator at room temperature.

Determination of ash content

Approximately 2 g of Simplicia powder were inserted into the incandescent silicate crew at 500^{o}C, flattened slowly at 500^{o}C until the charcoal was depleted, then cooled and weighed. If the charcoal could not be removed, hot water was added then filtered through ash-free filter paper. Leftovers and filter paper were then heated in the silica crew until the weight was fixed, and then they were weighed. Ash levels were calculated against air-dried materials (Ministry of Health, 1995; Mutchler, 1991).

Making smooth pigweed leaves water extract

A total of 200 grams of smooth pigweed leaves were carefully weighed, wrapped in flannel cloth, added to 2 litres of distilled water, and boiled to half the volume. The procedure was repeated three times with 4 litres of filtrate. The extract was evaporated with the water handler until dry extract was obtained (Montgomery, 1993).

Comparative set-up

A total of 20 tablets were weighed. Their weight was 9.294 g; thus, the average weight of one tablet was 0.4647 g (Ministry of Health of the Republic of Indonesia, 1995). The tablet was finely ground in a mortar, weighed as much as 0.225 g, containing 121.04 mg of chlorpropamide, then dissolved in 50 mL of warm water. First, 0.25 grams of tragacanth was suspended in 50 mL of warm water while stirring, then 0.225 g of the comparative tablet was added to the suspension of the tragacanth little by little until they were perfectly mixed (PT Eisai Indonesia, 1983).

Testing hypoglycemia on smooth pigweed leaves water extract

Test animals were satisfied in advance for 18 hours then divided into five groups, namely the positive

control group, the comparison group, and three test groups: Dose 1 (50 mg/kg bw), Dose 2 (100 mg/kg bw), and Dose 3 (150 mg/kg bw). On the day of the experiment, all animals were weighed, identified, and placed into the restrictive box. Then, a blood test (t=0) was performed through the tail veins of the male Wistar rats. After it was ready, rats were given the test suspension orally. At the 30th minute, a snapshot of blood from the tail vein was taken, after which it was immediately given a glucose induction of 50% orally administered at a dose of 1 g/kg of body weight to all groups except the negative control group. After oral administration, blood tests were performed again at the 60th, 90th, 120th, 150th, and 180th minutes. Footage of blood accommodated in an Eppendorf tube was then centrifuged for 15 minutes at 3.500 rpm, and the clear supernatant was taken using a 20 micropipette. Blood serum was transferred into small tubes and added with 2 mL of glucose reagent to determine blood glucose levels. Measurement of blood glucose levels was carried out using a spectrophotometer at a wavelength of 546 nm.

Analysis

All data were evaluated statistically using ANOVA and t-tests using SPSS software. The decrease in blood glucose levels in the test group was measured by comparing the results obtained with the results from the positive control group.

Calculation

The formula for calculating blood glucose levels is as follows:

Blood glucose level = At/As x 100 mg/dl[13]

Description:

- A = Absorption at maximum wavelength
- At = absorption of test solution
- As = absorption of raw solution (standard glucose solution)

Application for ethical clearance

Ethical clearance was provided by the commission of research ethics for research involving living beings (humans, animals, and plants. Ethical clearance was submitted to the health research ethics committee of the Medical Faculty of Padjajaran University and Hasan Sadikin Bandung hospital. Ethical clearance was obtained by submitting the following documents: Form 1 (questions about complete and correct research), Form 2 (research information), Form 3 (letter of approval to participate in the study/informed consent), and Form 4 (the researcher's profiles and research proposal).

Results

Table I shows hypoglycemic activity test results of smooth pigweed by comparing one dose test group against the positive control group.

Table I: Hypoglycemic activity test results comparing one dose test group against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	84.92 ± 2.82	0.763
30	87.61 ± 1.39	87.35 ± 1.35	0.304
60	103.27 ± 1.36	97.53 ± 0.87	0.689
90	115.89 ± 0.03	107.12 ± 1.03	0.465
120	122.58 ± 5.60	99.49 ± 1.66	0.0001
150	115.71 ± 2.74	94.37 ± 1.90	0.0001
180	113.62 ± 7.17	95.43 ± 2.86	0.002

Table II shows hypoglycemic activity test results of smooth pigweed by comparing two dose test groups against the positive control group.

Table II: Hypoglycemic activity test results comparing two dose test groups against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	85.35 ± 2.70	0.982
30	87.61 ± 1.39	85.05 ± 3.28	0.136
60	103.27 ± 1.36	93.17 ± 1.52	0.0001
90	115.89 ± 0.03	104.06 ± 2.45	0.0001
120	122.58 ± 5.60	96.59 ± 1.37	0.0001
150	115.71 ± 2.74	94.52 ± 1.76	0.0001
180	113.62 ± 7.17	96.57 ± 1.14	0.003

Table III shows hypoglycemic activity test results of smooth pigweed by comparing three dose test groups against the positive control group.

Table III: Hypoglycemic activity test results comparing three dose test groups against the positive control group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Positive control	Dose 150 mg/kg bw	
0	85.38 ± 1.24	82.72 ± 0.85	0.004
30	87.61 ± 1.39	85.23 ± 1.94	0.025
60	103.27 ± 1.36	86.46 ± 1.32	0.0001
90	115.89 ± 0.03	96.14 ± 2.71	0.0001
120	122.58 ± 5.60	85.57 ± 2.00	0.0001
150	115.71 ± 2.74	84.79 ± 1.63	0.0001
180	113.62 ± 7.17	93.65 ± 2.24	0.002

Table IV shows the results of the hypoglycemic activity test of smooth pigweed by comparing the three-dose test groups against the comparison group.

Table IV: Hypoglycemic activity test results comparing three dose test groups against the comparison group

Time	Blood glucose levels (mg/dl)		Significance (Probability)
	Comparison group	Dose 150 mg/kg bw	
0	79.30 ± 14.93	82.72 ± 0.85	0.590
30	87.02 ± 2.01	85.23 ± 1.94	0.282
60	76.55 ± 1.44	86.46 ± 1.32	0.0001
90	73.41 ± 2.11	96.14 ± 2.71	0.0001
120	54.60 ± 1.76	84.57 ± 2.00	0.0001
150	72.50 ± 5.67	84.79 ± 1.63	0.001
180	72.01 ± 4.49	93.65 ± 2.24	0.0001

Discussion

In this study, a test on the characteristics of Simplicia and phytochemical filtering of smooth pigweed leaf (*Amaranthus hybridus* L.) was carried out. When examining simplicial characteristics, the total ash content was 17.7 %, and drying shrinkage was 2.5 %. The result of the phytochemical analysis showed that smooth pigweed leaves contain flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids (Montgomery *et al.*, 1993).

One component of smooth pigweed that can lower blood glucose levels is flavonoids, in accordance with the literature. The selection of the test group, Dose 1 (50 mg/Kg BW), Dose 2 (100 mg/Kg BW), Dose 3 (150 mg/Kg BW), was adjusted to use in the community (Sulistina G. Ganiswarna *et al.* 1995).

At minute 0 (t=0), the results showed that the initial blood glucose levels of the rat did not provide a noticeable difference between the control group and the test group.

At the 30th minute (t=30), no statistically noticeable difference was shown because the number of efficacious substances applied at doses of 50 mg/kg bw, 100 mg/ kg bw, and 150 mg/ kg bw did not affect blood glucose levels.

At the 60th minute (t=60), there was no noticeable difference of all three groups (50 mg/ kg bw, 100 mg/ kg bw, and 150 mg/ kg bw) compared to the control group.

At the 90th minute (t=90), the three groups showed a noticeable difference when compared to the control group because the number of efficacious substances that were applied had an effect on blood glucose levels.

At the 120th minute, the three groups showed a statistically noticeable difference when compared to the control group.

This study also used comparison tablets Diabenese 22.5 mg/ kg bw (containing chlorpropamide 121.04 mg) to see the equality of some variations of the spinach test given. In the third trial, the test group showed a lower hypoglycemic activity when compared to the comparison group (Diabenese), indicating there was no equivalence between the test dose and the comparison group (Toro Gelson, 1976).

Conclusion

The smooth pigweed plant contains flavonoids, saponins, tannins, alkaloids, steroids, and triterpenoids. At the characteristic examination of Simplicia, the ash content was 17.7 % and the drying shrinkage 2.5 %. All test doses given were 50 mg/ kg bw, 100 mg/ kg bw, and 150 mg/ kg bw, which produced a lowering of blood glucose levels. The highest drop in blood glucose levels was at the dose of 150 mg/ kg bw at the 90th minute (t = 90) and the 120th minute (t = 120). Furthermore, it was followed successively by the doses of 100 mg/ kg bw and 50 mg/ kg bw. Hypoglycemic activity for a dose of 150 mg/ kg bw is still lower when compared to that of the comparison group (Diabinese tablets containing 250 mg of chlorpropamide at a dose of 22.50 mg/ kg bw).

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IAI CONFERENCE

RESEARCH ARTICLE

Correlation between the level of knowledge of drug managers and drug management in several primary health centres in Malang regency

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Abstract

Background: Drug management is one of the primary health centre management activities that aims to ensure the continuity and affordability of pharmaceutical preparations. **Aim:** This study aims to determine the relationship between the level of knowledge of drug managers and drug management in several primary health centres of Malang regency. **Method:** The study was observational analytic using questionnaire instruments to analyse the level of knowledge of drug managers and three checklists to analyse drug management through three indicators of drug management: (1) conformity of stock to the national formulary, (2) conformity of stock to the disease patterns, and (3) the percentage of expired drugs. **Result:** There was no significant relationship between the level of knowledge and the first (p = 0.842), second (p = 0.236), and the third indicator (p = 0.361). **Conclusion:** Not all drug lists in the national formulary are required by each primary health centre. The inventory is adjusted to the consumption and epidemiology.

Introduction

Pharmaceutical service activities at primary health centres are divided into two aspects, i.e. drug management and pharmaceutical clinical services (Minister of Health, 2016). This study focused on the drug management aspect. According to the Ministry of Health (MoH) regulation number 74/2016, one of the objectives of drug management is to carry out service quality control, which affects the pharmaceutical clinical service process related to patient therapy. Efficient drug management relies entirely on robust healthcare systems. Sufficient staffs, viability fund, broad information systems, and synchronized healthcare teams and institutions are key components to secure the availability and accessibility of essential medicines (World Health Organization, 2015). Drug management consists of nine aspects, i.e. planning, procurement, receiving, storing, distributing, destruction and withdrawal, controlling, administration, and monitoring and evaluating processes. At primary health centres, it is carried out by pharmaceutical staff (Minister of Health, 2016). It can be measured through ten indicators based on the Indonesian MoH and Japan International Cooperation Agency (JICA), namely the suitability of drug availability with National List of Essential Medicines (NLEM) or national formulary, compatibility of drug availability with disease patterns, percentage and value of expired or damaged drugs, level of drug availability, the accuracy of drug demand, the average weight percentage of inventory variations, the average percentage of time vacancies of drugs, the percentage of drugs that are not prescribed, the accuracy of drug distribution, and the percentage of generic drug prescription writing (Minister of Health & Agency, 2010). In this study, researchers focused on three indicators of drug management: suitability of drug availability with the national formulary (indicator 1),

suitability of drug availability with disease patterns (indicator 2), and expired or damaged drugs (indicator 3). These three indicators are likely to represent and fulfill the nine aspects of drug management, and variations between Primary Health Centres (*Puskesmas*) tend to be minimal.

Pharmacy staff includes pharmacists and technicians who do pharmaceutical work. Pharmacy staff is the party directly related to the process of delivering drug information to patients. According to MoH regulation number 74/2016 Chapter 4 (Pharmaceutical Resources in Primary Health Center), at least one pharmacist acts as the person in charge and can be assisted by pharmacy technicians as needed (Minister of Health, 2016). However, research in 2011 reported that in all Indonesian primary health centres, only 17.5% had pharmacists, and 32.2% had no pharmaceutical staff at all (Herman *et al.*, 2011). Additionally, data from 2016 shows that out of 33 districts comprising 39 primary health centres in Malang Regency, 15 primary health centres have pharmaceutical staff (pharmacists and pharmacy technicians), and 24 primary health centres do not have pharmaceutical personnel (Ministry of Health, 2017). In this context, the urgency of the availability of pharmaceutical personnel, especially pharmacists, in implementing drug management at primary health centres is perceived.

One of the factors that can influence drug management is the level of knowledge of drug administrators. It is established that all pharmaceutical staff needs to improve knowledge, skills, and behaviour to increase their competence in pharmaceutical services at primary health centres (Minister of Health, 2016). Indeed, a study reported a positive relationship between the level of knowledge and drug management at Banyumas primary health centres (Aryani *et al.*, 2016). Based on the urgency of availability of pharmaceutical staff and their level of knowledge, both of which affect drug management in primary health centres, it was deemed necessary to explore the relationship between the level of knowledge and drug management in several primary health centres in Malang Regency.

Therefore, this study aimed to evaluate the knowledge level of drug administrators related to drug management theory, measure the level of drug management through three indicators, and determine the relationship between the knowledge level of drug administrators and drug management in several primary health centres in Malang regency.

Methods

This analytic observational study used a cross-sectional approach, where the relationship between variables will be evaluated through the data obtained by direct observation. It was conducted at 12 primary health centres in Malang Regency from March to June 2019. The instruments used were a questionnaire to assess the knowledge level of drug administrators and three checklists to measure drug management at the primary health centres. First, drug administrators completed the questionnaire then they were interviewed as supporting data. Finally, the researchers filled out a checklist adjusted to the data from the primary health centres. This research has also fulfilled the ethical clearance of the Health Research Ethics Commission of the Faculty of Medicine, Universitas Brawijaya, number 84/EC/KEPK-S1-FARM/03/2019.

Validity and reliability test

The validity and reliability of the questionnaire were tested before starting the study. The test was carried out on 15 samples outside the research sample using SPSS and MS Excel. Respondents for the validity test were all pharmaceutical personnel from five health centres representing five sub-districts in the city of Malang.

Sample

Respondents in this study were all drug administrators, both with pharmaceutical and non-pharmaceutical educational backgrounds. The total sampling technique was used. The determination of primary health centre samples was carried out using the clustered random sampling method, and 12 primary health centres in Malang Regency were obtained, representing each district, i.e. north, west, south, and east of Malang Regency.

Inclusion criteria

Included were drug administrators willing to participate, complete the questionnaire, and provide information related to data. The inclusion criteria for primary health centres was to provide research permits and documentations or archives of the data required.

Exclusion criteria

Exclusion criteria consisted of staff outside the pharmacy room. Primary health centres that were excluded were those outside Malang regency and those with poor administrative processes related to the availability of data.

Data analysis

This study involved two types of data, namely questionnaire data and data from the three checklists.

The questionnaire included 20 dichotomised (true or false) questions scored 1 or 0, put into percentages, and categorised into high, medium, and low (Arikunto, 2013). Moreover, three checklists for three indicators of drug management (suitability of drug availability with national formulary, suitability of drug availability with disease patterns, and percentage of expired drugs) were filled according to usage reports and drug request sheets (LPLPO) data, top 10 disease patterns in February 2019, expired drug list, and stock recording. The percentage of the three checklists calculated based on the predetermined formula (Minister of Health & Agency, 2010) were categorised into three categories: good, moderate, and poor (Azwar, 2012). After that, the normality test was carried out using the Shapiro Wilk method, followed by the correlation test using the Pearson Correlation method, each of which was analyzed using SPSS and MS Excel. From the correlation results obtained, a $p < 0.05$ indicated a significant relationship between the knowledge level of drug administrators and drug management at the health center, whereas a $p > 0.05$ indicated no significant relationship between the two variables (Arikunto, 2013).

Results

This study enrolled was 15 drug administrators from 12 health centres in Malang regency (Table I).

Table I: Characteristics of respondents

Parameter	Category	Number	Percentage (%)
Age	17 – 25	1	7.14
	26 – 35	4	28.57
	36 – 45	6	42.86
	46 – 55	2	14.29
	55 – 65	1	7.14
Educational background	Pharmacy high school	4	28.57
	Pharmacy diploma	7	50
	Bachelor of pharmacy	1	7.14
	Pharmacist	2	14.29
Working experience	< 12 months	1	7.14
	1–5 years	1	7.14
	5-10 years	6	42.86
	> 10 years	6	42.86

The analysis of the level of knowledge was carried out using a questionnaire with the percentages of correct answers categorised into low (≤ 55%), moderate (56-75%), and high (76-100%) (Arikunto, 2013). The average level of knowledge of drug administrators from 14 respondents in 12 primary health centres was 88.21%, indicating that most respondents were in the higher category. The 20-item questionnaire covered nine aspects of drug management, i.e., planning, procurement, receiving, storing, distributing, destruction and withdrawal, controlling, administration, and monitoring and evaluating processes. A summary of the results is presented in Table II.

Table II: Categorisation of the knowledge level

Category	Pharmacy room manager	
	Pharmacist (n=2) (%)	Non pharmacist (n=12) (%)
High	2 (100)	11 (91,67)
Moderate	0	1 (7,14)
Low	0	0

Drug management

Drug management was measured through three indicators, i.e. suitability of drug availability with national formulary, suitability of drug availability with disease patterns, and percentage of expired drugs.

Drug management based on suitability of drug availability with a national formulary

The results of the categorisation of Indicator 1 presented in Table III were based on the categorization calculation of Saifuddin Azwar (Azwar, 2012).

Table III: Drug management profile based on indicator 1

Category	Primary health centre (n = 12) (%)
Good	3 (25)
Fair	6 (50)
Poor	3 (25)

Drug management in this indicator was measured using checklist 1 in the form of a list of drugs in national formulary and the percentage of the number of drugs available at the primary health centres in February 2019. The percentage obtained was categorised into three categories: good if the score was ≥ (mean + 1.0), fair if (mean - 1.0) ≤ score < (mean + 1.0), and poor if the score was < (mean - 1.0) (Azwar, 2012).

Drug management based on suitability of drug availability with disease patterns

The results of the categorization of Indicator 2 presented in Table IV were based on the categorisation calculation of Saifuddin Azwar (Azwar, 2012).

Table IV: Drug management profile based on indicator 2

Category	Primary health centre (n = 12) (%)
Good	3 (25)
Fair	5 (41.67)
Poor	4 (33.33)

The disease patterns were limited to only the top 10 disease patterns in the primary health centres in February 2019 and analysed using checklist 2, and the percentage of the number of drugs available for treatment was calculated against the top 10 disease patterns to the number of drugs used. The availability for treatment of the top 10 disease patterns was according to the national formulary.

Drug management based on expired or damaged drugs

The results of the categorization of Indicator 3 presented in Table V were based on the categorisation calculation of Saifuddin Azwar (Azwar, 2012).

Table V: Drug management profile based on indicator 3

Category	Primary health centre (n = 12) (%)
Good	4 (33.33)
Fair	2 (16.67)
Poor	6 (50)

Drug management profile based on expired or damaged drugs was analysed using checklist 3, which was a list of expired or damaged drugs. The results were calculated through the percentage between the number of expired or damaged drugs and the number of drugs available at the primary health centres. Indicator 3 was carried out on expired or damaged drug data and the stock recorded in 2018. The percentage obtained was categorised into three categories: good if the score was < (mean - 1.0), fair if (mean - 1.0) ≤ score < (mean + 1.0), and poor if the score was ≥ (mean + 1.0) (Azwar, 2012).

Correlation analysis

Table VI shows the results of the correlation analysis between the levels of knowledge of each indicator in drug management at 12 primary health centres.

Table VI: Correlation analysis

Variable	Pearson correlation	Significance	Information
Indicator 1	0.065	0.842	Not significant
Indicator 2	0.370	0.236	Not significant
Indicator 3	0.289	0.361	Not significant

A correlation test was carried out on each indicator, and three correlations were obtained. The value of correlation 1 between the level of knowledge and suitability of drug availability with the national formulary was 0.065, with a significance of 0.842, corresponding to a very weak and not significant correlation (in the range of 0.001-0.199) (Jacob Benesty *et al.*, 2009; Arikunto, 2013).

The value of correlation 2 between the level of knowledge and suitability of drug availability with disease patterns was 0.370, with a significance of 0.236, indicating weak and not significant correlation (in the range of 0.20-0.399) (Jacob Benesty *et al.*, 2009; Arikunto, 2013).

The value of correlation 3 between the level of knowledge and percentage of expired or damaged drugs was 0.289, with a significance of 0.361, showing weak and not significant correlation (in the range of 0.20-0.399) (Jacob Benesty *et al.*, 2009; Arikunto, 2013).

The results of the 12 health centres show that knowledge levels do not have a significant effect on drug management, indicating that several other underlying factors could affect knowledge and drug management.

Discussion

The majority of respondents were 36-45 years old, were pharmacists, and worked for 5-10 years and more than ten years (Table I). According to Notoatmodjo (2007), age, education, and experience could affect the level of knowledge. Older age will further enhance one's experience in various domains and is directly proportional to the level of knowledge. However, age does not always describe a person's level of knowledge. Previous research reported no relationship between age and drug management among drug administrators

(Malahayati, 2016). Furthermore, educational background and working period are directly proportional to the level of knowledge. The higher the education and the longer the working period, the higher the level of knowledge (Katajavuori *et al.*, 2009).

Theoretically, the level of knowledge can be influenced by the characteristics of the respondents, such as age, educational background, working period, where the higher the age and working experience, the higher the level of knowledge. As for education, it is related to the acceptance of information, where higher education can increase knowledge (Triana *et al.*, 2014). In this study, age, educational background, and working period did not have a significant effect on the level of knowledge. The values obtained tended to be the same between one administrator and another, although they had different characteristics, indicating other factors can affect the level of knowledge of the administrators. The literature also states that the environment can also affect the level of knowledge related to a reciprocal interaction, which will then be responded to as knowledge by every individual (Notoatmodjo, 2007). Additionally, knowledge can also be influenced by the interests and activities of administrators. The higher a person's interest in a topic related to independent information retrieval, the more knowledgeable the person will be. The activity itself is known to have a significant relationship with the level of knowledge with a value of p = 0.015 (Harisman & Nuryani, 2012). However, in this study, no further analysis was carried out regarding environmental factors, interests, or activities of drug officers.

Based on the interview results, the high level of knowledge of drug officers was influenced by the socialization provided by the Malang Regency Health Office (DHO), notification of the latest information, and periodic evaluation of drug management in each primary health centre. The provision of this information can further increase the knowledge of officers manifested by the high percentage score of the level of knowledge obtained. The difference in the scores obtained could be due to ambiguities in the questionnaire related to the disparity between existing theories and actual field conditions.

Table III (Indicator 1) shows that three primary health centres (25%) were categorised as good (1 primary health centre with a pharmacist and 2 with pharmacy technicians), 6 (50%) were in the fair category (1 primary health centre with a pharmacist and 5 with pharmacy technicians), and 3 (25%) were in the poor category (pharmacy technicians only). It is noteworthy that the standard value of this indicator is 100% (Minister of Health & Agency, 2010), and the percentage range obtained from 12 primary health centres is 70.80% - 80.56%, with an average of 76.66%, indicating that the 12 primary health centres have not reached the set standard value. Several factors can influence, such as the basis for consideration of planning needs based on consumption patterns and disease patterns at the primary health centres, where consumption patterns will also be influenced by patterns of drug demand from prescribers in the implementation of primary health centre clinical services. Also, the demand from the primary health centre to the district pharmacy warehouse (GFK) sometimes includes drugs that are not in accordance with the national formulary but are needed by the primary health centres for services. There are also several conditions where the demand from primary health centres is not yet available at GFK, which can be due to various factors; thus, the percentage of suitability obtained will decrease. However, in this study, the researchers did not conduct further research on either the GFK or the Malang DHO.

Table IV (Indicator 2) shows that, out of the 12 primary health centres, 3 (25%) were categorised as good (1 primary health centre with a pharmacist and 2 with pharmacy technicians), 5 (41.67%) belonged to the fair category (1 primary health centre with a pharmacist and 4 with pharmacy technicians), and 4 (33.33%) were in the poor category (pharmacy technicians). The standard value for Indicator 2 is 100% (Minister of Health & Agency, 2010), and the percentage of the 12 primary health centres ranged between 68.75%-90.12%, with an average of 79.44%, revealing that all health centres have not reached the standards set. This result can be influenced by several aspects, including the pattern of drug consumption and drug availability at the GFK. However, regarding Indicator 2, the researchers only focused on the top 10 disease patterns, as it could not describe all cases of diseases in the primary health centres. The results of this study were in contrast with previous findings in southern Papua, showing the average suitability of drug availability with disease patterns in the district of 170.87%. This result indicates that all districts provide more types of drugs than the existing disease patterns in their regions, resulting in a waste of drug procurement budgets (Waluyo *et al.*, 2014).

Table V (Indicator 3) shows that out of the 12 primary health centres, 4 (33.33%) were categorized as good (2 primary health centres with pharmacists and 2 with pharmacy technicians), 2 (16.67%) were in the fair category (pharmacy technicians), and 6 (50%) belonged to the poor category (pharmacy technicians). The standard value for Indicator 3 is 0% (Minister of Health & Agency, 2010), and the percentage of 12 primary health centres ranged between 0%-14.44%, with an average of 7.22%. Only one primary health centre had reached the standard value. The results obtained could

be influenced by unpredictable changes in disease patterns, where demand was previously based on disease and consumption patterns. Some drugs that had been ordered were not used due to changes in disease patterns. Furthermore, in some conditions, changing prescribers can sometimes lead to the use of other drugs with the same indication. Hence, drugs often used by previous prescribers become unused by the replacing prescriber, thus being available and resulting in excessive supply from the GFK. These factors could lead to expired drugs. Poor compliance with the national guideline for drug disposition increases the risk of environmental contamination and the probability of consuming harmful pharmaceutical wastes by humans and animals, emphasising the need to develop drug management procedures for expired drugs to stop contaminations (Michael *et al.*, 2019).

The results of the analysis and discussion show that the relationship between the level of knowledge and each indicator tended to be weak and not significant, as knowledge did not significantly affect drug management in the 12 primary health centres. The level of knowledge of 14 respondents was in the high category, with an average value of 88.21%. Regarding drug management, the percentages of primary health centres categorized as good for Indicator 1, Indicator 2, and Indicator 3 were 25%, 25%, and 33.33%, respectively.

Conclusion

The correlation between the level of knowledge and drug management based on three indicators (conformity of stock to the national formulary, conformity of stock to the disease patterns, and the percentage of expired drugs) tends to be weak and insignificant. Most pharmacy staff at primary health centres have good knowledge of drug management theory, but only a small proportion could achieve the drug management target. Therefore, it is necessary to solve the following problems: 1) making available the drugs needed by the primary health centre but not listed in national formulary, 2) meeting the need for drugs according to the most prevalent disease patterns, and 3) adding drugs, such as anti-tuberculosis, ferrous sulfate tablets, and zinc tablets, which were dropped from the government as they were too excessive.

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IAI CONFERENCE

RESEARCH ARTICLE

The effect of stress level on the therapeutic outcomes of type 2 diabetes mellitus at the regional public hospital of West Nusa Tenggara province

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Abstract

Introduction: Diabetes Mellitus (DM) is a complex chronic disease that requires ongoing medical care with a multifactorial risk reduction strategy beyond glycemic control. Self-management, education, and support are essential to prevent acute complications and reduce the risk of long-term complications. Stress levels may affect fasting blood glucose (FBG) and 2-hours postprandial blood glucose (2HPPBG). **Aim:** This study aims to determine the effect of stress levels on the therapeutic outcomes of type 2 DM patients at the regional public hospital of West Nusa Tenggara province. **Methods:** This observational, cross-sectional research was carried out on a sample of 37 patients using the Perceived Stress Scale (PSS). Data analysis used a linear regression test. **Results:** The results showed that stress had a significant effect on FBG (p=0.038) and 2HPPBG (p=0.001) levels.

Introduction

Diabetes Mellitus (DM) is a disease in which blood glucose (simple sugar) levels are high because the body cannot release or use insulin sufficiently. Self-management, education, and support are essential to prevent acute complications and reduce the risk of long-term complications. A significant body of evidence supports various interventions to improve DM therapy outcomes (American Diabetes Association, 2020). According to Riset Kesehatan Data (Data Health Research), the prevalence of non-communicable diseases in 2018 has increased compared to previous years (Data Health Research, 2018). The prevalence consensus of the Indonesian Endocrinology Association reported that, in Indonesia, DM prevalence based on doctors' diagnoses in patients below 15 years has increased from 0.15% in 2013 to 0.2% in 2018 (Perkeni, 2015). In people above 15 years, and according to blood tests, it also increased between 2013 and 2018 (Data Health Research, 2018).

The psychological impact of DM, including treatment-related stress, is experienced by patients since the early stages of the disease and may last for years, given the chronic nature of the illness (Avci & Kelleci, 2016). Stress seems to highly influence diabetes because it affects the control and level of blood glucose levels (Glover *et al.*, 2016). During a stressful situation, the body response can be in the form of increased adrenaline, which eventually converts glycogen

reserves in the liver into glucose. Over time, high blood glucose levels may lead to complications of diabetes.

Stress and DM have a very close relationship, especially in urban residents. Life pressures and unhealthy lifestyles accompanied by rapid technological advances and various concomitant illnesses can cause a person's condition to deteriorate. DM patients who experience stress may have problems in controlling blood glucose (Golden *et al*., 2008; Knol *et al*., 2006; Richard *et al*., 2002). Stress levels in DM patients were measured using the Perceived Stress Scale (PSS), a 10-item questionnaire that identifies the respondent's stress description. This instrument was validated by Zaenal Arifin in 2011, with a validity and reliability value of 0.85 (Arifin, 2011). The measurement of diabetes stress plays an essential role in improving the quality of health and well-being of patients, especially at the regional public hospital of West Nusa Tenggara province, where the number of outpatients in 2018 reached 2.249 per year.

The objective of this study is to determine the effect of stress on fasting blood glucose levels (FBG) and 2 hours after meals (2hPPG) in outpatients with type 2 diabetes mellitus at the regional public hospital of West Nusa Tenggara province.

Methods

This study used an analytical observational method with a cross-sectional approach in determining the effect of stress on fasting blood glucose levels (FBG) and 2-hours after meals (2hPPG) in patients with type 2 diabetes mellitus (T2DM) at the Internal Medicine Department of the regional public hospital of West Nusa Tenggara province. It was performed from February 2020 to May 2020. The inclusion criteria were T2DM patients aged ≥46 years who had been taking oral antidiabetics for at least six months (with ICD code X E.11) before the stress measurements and were willing to sign the informed consent form. The exclusion criteria were deaf, illiterate, and pregnant patients. The final sample included 37 T2DM patients who met the inclusion criteria. This study had been approved by the ethics committee of the regional public hospital of West Nusa Tenggara province, Indonesia, number 070.2/13/KEP/2020.

The Perceived of Stress Scale (PSS) is a valid 10-item tool, covering both anxiety and depression, used to measure the response of individuals to stressful situations by direct observational interviews with patients (Arifin, 2011); validity and reliability test results were 0.85, similar to Arifin results (Arifin, 2011). It is an efficient scale to measure the relationship between stress appraisal and the risk for any disease (Vasanth *et al*., 2017; Al Kalaldeh & Abu Shosha; 2012). Data were collected through interviews and medical records or patients, which include name, age, gender, diagnosis, treatment, and laboratory data.

The data were analysed descriptively on SPSS 20.0 using patients' characteristics. Linear regression was performed to measure the effect of stress levels on FBG and 2hPPG.

Results

Subject characteristic

The characteristics of T2DM patients taken during the study included gender, patient age, and length of time the patient suffered from DM.

The effect of stress on blood glucose levels

Stress levels are associated with fasting blood glucose levels (FBG); the patients must be fasting for at least 10-12 hours, then blood glucose levels are measured 2 hours after eating (2hPPG) a meal. In this study, random blood glucose levels (measured at any time of the day without any conditions of fasting and eating) were not performed because the tests could not be completed simultaneously. This examination was administered four times a day: before eating and before bed to be performed independently. It did not describe long-term DM control (blood glucose control for approximately three months). Thus, it could not be used as a reference to see the relationship of stress with a patient's blood sugar levels. The normal range of random blood glucose levels is 80-144 mg/dl. This random blood glucose examination was administered only to overcome problems that arose due to sudden changes in glucose levels (Rachmawati, 2015).

Discussion

Our sample included more males (21 patients, 56.75%) than females (16 patients, 43.24%), different from the findings of Levine (2008), showing that women are more likely to experience endocrine-related diseases, such as diabetes mellitus and gestational diabetes mellitus (GDM) (Levine, 2008). Furthermore, 5-10% of women in productive age are prone to experience Polycystic Ovarian Syndrome (POS). This condition is associated with disrupted insulin secretion, insulin activity, and blood pressure regulation, an early sign of cardiovascular disorders.

T2DM generally occurs in middle-aged people and the elderly. Its prevalence and occurrence are associated

with older age, with about 50% of T2DM patients being over 60 years old (Yakaryılmaz & Öztürk, 2017). In our sample, 34 patients were more than 50 (91.89%), and 3 were less than 50 (8.10%), with an average patient age is 62 years old, consistent with the research conducted by Dunning (2009), explaining that the prevalence of DM increases with age, especially in developing and developed countries ranging from 10-20% at the age of 60-70 years (Dunning, 2009). Ageing may cause a decrease in pancreatic beta-cell function (Kalyani *et al.,* 2010). Pereira et al. (2008) emphasized that age is associated with insulin resistance and obesity in the elderly (Pereira *et al*., 2008).

Table I shows that 97.29% of patients had diabetes for more than six months. In a study conducted by Safitri (2016), 42.8% of patients had diabetes from less than five years (Safitri, 2016). The American Diabetes Association (2009) revealed that 32.6% of respondents had diabetes from 5-10 years (American Diabetes Association, 2009).

Table I: Initial data on the characteristics of the subject

Characteristics		n	Percentage (%)
Gender	Men	21	56.75
	Women	16	43.25
Age	<50 years	3	8.10
	>50 years	34	91.90
The long suffering of diabetes	6 months	1	2.70
	>6 months	36	97.30

The relationship between stressful experiences and controlling blood glucose levels is very different among individuals with T2DM. Stress can affect blood glucose levels directly (by acting on the neuroendocrine system) or indirectly (related to the duration of stress).

The effects of stress on the neuroendocrine system consist of stimulating the nervous system by activating the sympathetic-adrenal-medulla (SAM) followed by hypothalamic-pituitary-adrenal (HPA) activity. During stress, the sympathetic nervous system stimulates the adrenal glands of the medulla to secrete epinephrine and norepinephrine into the blood circulation. The activity of these hormones produces metabolic effects, i.e., increased metabolic rate and blood glucose levels (Lloyd *et al*., 2005; Champaneri *et al*., 2010).

Stress causes the hypothalamus to secrete Corticotrophins Releasing Factor, which releases adrenocorticotropin and stimulates the adrenal cortex to secrete glucocorticoid hormones, such as cortisol, thereby increasing the production of glucose by the liver and reducing its uptake by tissues. Cortisol affects the breakdown of carbohydrates, proteins, and fats through the gluconeogenesis process, which produces glucose as an energy source and plays a significant role in influencing body functions during the resting period (Hasan *et al*., 2014; Cosgorve *et al*., 2012).

The results of this study showed a significant relationship between stress levels and both FBG (p = 0.038 and r = 0.295) and 2hPPG (p = 0.001 and r = 0.508) in T2DM patients at the regional public hospital of West Nusa Tenggara province (Table II). This showed the higher the stress, the higher the FBG and with 2hPPG.

Table II: Linear regression analysis on the effect of stress levels on blood glucose levels

Domain	*r* and *p*-value	
	FBG	2hPPG
Stress level	r = 0.295	r = 0.508
	p = 0.038	p = 0.001

p < 0,05 means there is a significant effect.

The results are consistent with those of Lustman and the authors (2005), showing a relationship between stress, low self-care, and hyperglycemia (p=0.05) and between stress and increased haemoglobin glycosylate (HbA1c) after controlling for body weight (Lustman *et al*., 2005). Stress in T2DM patients may cause biochemical changes, such as hyperglycemia and the hypothalamus-pituitary-adrenal pathway activity (HPA-axis) (Llorente & Malphurs, 2007).

In 2008, Szoke reported a significant relationship between stress and diabetes, especially in women aged 20-39 years and men, showing more stress at a young age (Szoke *et al*., 2008). This difference could be due to differences in individual responses to stress and its description as measured by the PSS.

Furthermore, the correlation between stress and FBG and 2hPPG was positive, where the higher the stress, the higher the values. Also, the FBG correlation value was lower than that of 2hPPG.

When the study was conducted, measuring 2hPPG was a factor that had a considerable effect on stress. Indeed, waiting in a queue, tiredness from standing because of the limited number of chairs, and the unsatisfactory service at the hospital, made patients irritable and emotional and resulted in increased stress.

Conclusion

This study showed that higher stress significantly increases fasting blood glucose (FBG) and 2-hours postprandial glucose (2hPPG) levels.

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RESEARCH ARTICLE

A pharmacoeconomic study: cost-utility analysis of modern wound dressings *vs* conventional wound dressings in patients with diabetic foot ulcer

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Keywords
Cost-utility analysis
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Abstract

Introduction: Duration of treatment and outcome of therapy of diabetic foot ulcers are some of the factors that affect the quality of life and will require higher medical costs. **Aim:** This study aimed to choose an alternative wound dressing that provides the best utility at the most cost-efficient. **Methods:** The research method used was pharmacoeconomics with a patient's perspective. **Results:** The results showed the mean cost of modern wound dressings per visit was IDR 347,131, while that of conventional wound dressings was IDR 47,140. The quality of life with modern *vs* conventional wound dressing was significantly different ($p < 0.05$). The incremental cost utility ratio (ICUR) value was IDR 22,813 per quality of life (QoL). **Conclusions:** This study showed that modern wound dressings provide a higher quality of life at a higher cost. Indeed, it cost more than IDR 22,813 to change from conventional to modern wound dressings and increase 1 unit of quality of life, but patients obtained an additional 13.15 quality of life.

Introduction

According to the World Health Organization (WHO), diabetes mellitus (DM) combined with reduced blood flow and neuropathy (nerve damage) in the feet increases the chance of foot ulcer infections and the eventual need for limb amputation (WHO, 2020). In Indonesia, around 5.3 million people suffer from Diabetic Foot Ulcer (DFU), which is the most common cause of hospital admissions (80%) for DM (Hastuti, 2008). DFU is often overlooked, making its existing core concept imprecise; consequently, many patients develop osteomyelitis, even amputation (Misnadiarly, 2006). In 2010-2011, the incidence of amputation in Indonesia due to DFU increased sharply from 35% to 54.8% (Misnadiarly, 2006).

Diabetic ulcers are the most feared chronic complication for diabetes mellitus patients in terms of both the duration and cost of treatment. The latter costs three folds the treatment of diabetes mellitus without ulcers (Hastuti, 2008). In Indonesia, the cost of diabetic ulcer management is high, 1.3 million to 1.6 million IDR per month and 43.5 million IDR per year per patient (Hastuti, 2008).

Patients need more wound care from the onset of the wound, with 30 days required to prevent breakdowns, infections, and amputations because immediate intervention can save both the costs and the patient's leg (McGuire, 2014). According to the WHO, cost-saving and feasible interventions in developing countries include moderate blood glucose control, blood pressure control, and foot care (WHO, 2020).

The use of modern wound dressings, foam dressings, for example, has major advantages, including the ability to retain exudates, high absorption, effectiveness for wounds with excess fluid, reducing pain, ease to remove, and protecting the peri-wound area from

additional trauma (Jones *et al.*, 2006; and Hilton *et al.*, 2004). Furthermore, conventional wound dressings (wet-dry gauze with normal saline) cannot maintain a moist environment, required to provide optimal conditions for wound healing. Gauze can interfere with wound healing because it dries out and causes tissue damage when it is removed (Jones, Grey, & Harding, 2006). Additionally, conventional treatments take longer to heal (Allenet *et al.*., 2000). Several studies found that the healing efficacy of modern wound dressings is 100%, while that of conventional wound dressings is only 50% (Nurhaida, 2017). Other results showed that modern wound dressings, such as hydrogel, are three times more effective than 0.9% NaCl and that moist wound healing dressings are more effective than NaCl 0.9% + real honey (Purnomo *et al.*, 2014; and Riani *et al.*., 2017).

Unfortunately, modern wound dressings are more expensive than conventional wound dressings. Modern wound care provides better comfort and reduces the smell of the wound, but financially, conventional wound dressings are more cost-effective because they use health insurance from the government (Minarningtyas & Tami, 2018).

Therefore, it is necessary to conduct a pharmacoeconomic and cost-utility analysis on the use of modern wound dressings compared to conventional wound dressings using the quality of life of DFU patients measured by the DQOL (Diabetic Quality of Life) questionnaire and the average total cost of each wound dressing. This study aimed to perform a cost-utility analysis between modern versus conventional wound dressings in diabetic foot ulcer patients to determine an alternative cost-effective wound dressing that would provide the best utility or quality of life for diabetic foot ulcer patients.

Methods

This pharmacoeconomic research is analytical and observational and uses a cross-sectional approach. It has been reviewed and approved by the Health Research Ethics Commission University of Mataram No.:109/UN18.F7/ETIK/2020. This study compared the utility and cost of two treatments for diabetic foot ulcers, i.e. modern wound dressings and conventional wound dressings. The mean utility data were collected using the DQOL questionnaire, while the cost data were obtained from the average total cost from the patient's perspective. The cost components calculated consisted of direct medical and non-medical costs and indirect costs. Then, a cost-utility analysis was performed by calculating the value of the incremental cost-utility ratio (ICUR). The validity of the DQOL questionnaire had been tested with a validity value of r = 0.428-0.851 and Cronbach alpha 0.963 (Yusra, 2011). DQOL consists of 30 questions covering satisfaction, the impact of illness, concerns about physical function in addition to psychological and social problems. All answers are rated on a Likert scale, with DQOL scores categorized into low (less than 60), moderate (60-90), and high (more than 90) quality of life (Yusra, 2011).

The study population consisted of diabetic foot ulcer patients who needed wound dressings recruited from the AWCC Lombok wound care clinic and several public health centres (*Puskesmas*) in West Lombok Regency and Mataram City. The total sampling technique was used because the number of diabetic foot ulcer patients who needed wound dressings was small. The final sample included 16 patients; 11 used modern wound dressings, and 5 used conventional wound dressings. The patients' quality of life was monitored from their first visit to the clinic or public health centre until they recovered or no longer needed wound dressing. Hence, the mean utility and costs were calculated based on the total number of visits, i.e. 75 visits distributed as follows: 55 visits by patients with modern wound dressings and 20 visits by patients with conventional wound dressings. Informed consent was obtained from all the patients. The utility and cost comparisons were performed statistically using SPSS version 20 software.

Results

Overview of the utility of diabetic foot ulcer patients

In this study, demographic data collected were based on factors that affect the quality of life of diabetes mellitus patients, namely gender, age, education level, ethnicity, and marital status (Rubin, & Peyrot, 1999) in addition to the grade of diabetic foot ulcers and smoking status.

Diabetic foot ulcer patients who used modern wound dressings had various wound grades, ranging from 4, 3, 2, and 1, while those who used conventional wound dressings had grades 3 and 1.

Patients with modern wound dressings were only found at the AWCC Lombok wound care clinic, while patients with conventional wound dressings were only found at public health centres in West Lombok Regency and Mataram City. In other words, there were two different research locations. Currently, more patients prefer wound care clinics than public health centres, where they will receive a modern wound dressing even though they have to spend more money. Those who choose public health centres get a conventional wound dressing for free.

Table I shows that almost all patients with diabetic foot ulcers who used modern wound dressings had a high quality of life, except for those who were unmarried (they had a moderate QOL). Patients using conventional wound dressing who had a high quality of life consisted of those who had a high school education level or above and those who were employed.

Table I: Overview of the utility of diabetic foot ulcer patients

Demography of patients	Modern Wound Dressing			Conventional Wound Dressing		
	Total (n=11)	Percentage	Mean utility (n=55)	Total (n=5)	Percentage	Mean utility (n=20)
Gender						
Male	5	45.45%	104.38±9.49	3	60%	89.25±6.94
Female	6	54.55%	97.32±15.01	2	40%	84.25±7.59
Age						
< 46 years old	2	18.18%	106.00±5.46	2	40%	85.63±6.44
≥ 46 years old	9	81.82%	99.16±14.19	3	60%	88.33±8.14
Education						
<Senior High School	5	45.45%	94.42±14.67	3	60%	84.00±6.15
≥ Senior High School	6	54.55%	105.76±9.19	2	40%	92.13±6.77
Occupation						
Employed	10	90.91%	99.24±13.33	3	60%	90.00±7.81
Not Employed	1	9.09%	112.00±4.00	2	40%	83.75±5.60
Marital Status						
Married	10	90.91%	103.94±9.97	4	80%	87.38±7.21
Unmarried	1	9.09%	79.63±11.29	1	20%	86.75±4.57
Smoking Status						
Smoking	3	27.27%	102.33±9.96	2	40%	85.13±4.09
Not Smoking	8	72.73%	99.68±14.36	3	60%	88.67±8.93

Cost of modern wound dressing and conventional dressing

The cost calculation was carried out based on the patient's perspective. The calculated cost components were direct medical costs (wound dressing costs and wound care costs), direct non-medical costs (home care costs for modern wound dressing patients and transportation costs for conventional wound dressing patients), and indirect costs (loss of productivity cost). The loss of productivity cost was calculated based on the human capital approach, i.e., the number of days lost due to illness or treatment according to daily income (Setiawan, Endarti, & Suwantika, 2017).

Table II shows that the direct medical cost of conventional wound dressings was 0 IDR since patients underwent wound care at a public health centre free of charge. The direct medical costs were borne by the Social Security Administrator for Health (BPJS Kesehatan). Patients only incurred direct non-medical costs in the form of transportation costs from home to the public health centre. Even if the care is provided free of charge, the indirect costs (loss of productivity cost) create a financial burden. Meanwhile, the direct non-medical cost of modern wound dressings was high because patients received wound care at home. The average cost was IDR 347,131, or 7 times higher than the total cost of conventional wound dressings.

Table II: Cost of modern wound dressing and conventional dressing per visit

Cost components	Modern wound dressing (n=55) Total IDR	Conventional wound dressing (n=20) Total IDR	*p*-value
Direct medical cost	IDR 12,034,000	IDR 0	<0.0001
• Cost of wound dressing	(IDR 5,094,000)	(IDR 0)	
• Cost of wound care	(IDR 6,940,000)	(IDR 0)	
Direct non-medical cost	IDR 1,650,000	IDR 88,000	<0.0001
Indirect cost	IDR 5,408,223	IDR 854,795	0.009
Total cost	IDR 19,092,223	IDR 942,795	
Mean cost	IDR 347,131±129,309	IDR 47,140±39,183	<0.0001

Discussion

This study results show that modern wound dressings provided a high mean utility compared to conventional

wound dressings. Basic wound treatments rely heavily on antiseptics misuse and drying of the wound, resulting in lengthy, expensive, and painful care (Vuagnat & Comte, 2016). Complications experienced, such as diabetic ulcers, can result in a lower quality of life in diabetes mellitus patients, where these complications can result in physical, psychological, and even social limitations (Yusra, 2011). Patients with diabetic ulcers had a low quality of life as physical health is closely related to patient feelings about the pain and anxiety experienced, dependence on medical care, energy and fatigue, mobility, sleep and rest, daily activities, and work capacity (Utami, Karim, & Agrina, 2014). The quality of life of diabetes mellitus patients was significantly influenced (p<0.05) by gender, age, education, disease duration, including complications in the form of diabetic ulcers (Eristina, 2017).

The statistical results (Table II) showed significant differences in the direct medical costs, direct non-medical costs, indirect costs, and the average cost between modern and conventional wound dressings (p<0.05). A study conducted at Karanganyar General Hospital reported that complications significantly affected direct medical costs (p<0.05) and that the average cost of complications for diabetes ulcers was IDR 765,662.00±42,085.58 (Eristina, 2017). Another research conducted at Sanglah General Hospital Denpasar found that the average cost of modern wound dressings was IDR 335,500, not much different from the average cost of modern wound dressings in this study (IDR 347,131). Furthermore, in a study conducted in 2015 at Banyuasin Hospital, the unit cost of the service for hospitalised patients with diabetes mellitus complications was IDR 4,147,032.53. Previous research conducted between September and November 2019 concluded that the average treatment for type 2 diabetes mellitus with the complication of diabetic foot ulcers was IDR 29,139,247 (Tiara, 2012; Rahman, 2016; & Rondonuwu *et al.*., 2020). The differences in costs are influenced by the grade or severity of the wound (which requires more extensive therapy), cost of action, including accommodation costs in the hospital.

Cost-utility analysis

Table III shows a significant difference in the mean cost and the mean utility between modern wound dressings and conventional wound dressings (p=0.0001). Thus, the two methods yield different quality of life results, where modern wound dressings provide a higher quality of life than conventional wound dressings.

Table III. Cost-Utility Analysis between Modern vs Conventional Wound Dressing

Calculations	Modern wound dressing (n=55)	Conventional wound dressing (n=20)	*p*-value
Mean cost	IDR 347,131±129,309	IDR 47,140±39,183	<0.0001
Mean utility	100.4±13.27	87.25±7.45	<0.0001
Cost utility ratio (CUR)	IDR 3,457	IDR 540	
Incremental cost utility ratio (ICUR)	IDR 22,813		

The CUR and ICUR values were calculated after obtaining the results of the utility and cost calculations. The results of the CUR (Table III) show that modern wound dressings were in quadrant 1, while conventional wound dressings were in quadrant 3, so a cost-utility analysis was carried out by calculating the ICUR value. Modern wound dressings provided a higher quality of life at a higher cost than conventional wound dressings. The results of ICUR showed that it costs more than IDR 22,813 to change from conventional to modern wound dressings and increase 1 unit of quality of life, but patients obtained an additional 13.15 quality of life. Further studies comparing the GDP per capita, or the threshold value, or the willingness to pay are necessary to determine whether the addition is commensurate or not.

A study conducted in Germany reported that patients who used the new wound dressing (foam dressing) had a reduced mean frequency of dressing change by 1.3 times per week (from 4.6 to 3.3). The cost of dressings per change increased slightly, but the average cost of dressings per week was reduced by approximately 23% (Kronert, Roth & Searle, 2016). Another study conducted at Jss hospital, India, found that topical sucralfate was more cost-effective than conventional dressings, as it required a lower number of dressings and reduced hospital stay significantly (Preethi, & Dhanasekaran, 2019). Based on research conducted in the United States of America (USA), the incremental cost-effectiveness ratio of Dermagraft(R) (human dermal replacement) equals 38,784 FF, indicating the extra investment that the decision-maker has to accept for an additional ulcer healed with Dermagraft(R) compared with conventional treatment (Allenet *et al.*, 2000). However, it is different from the results of research in the UK reporting no difference in effectiveness and quality of life of N-A (a non-adherent, knitted, viscose filament gauze), Inadine (an iodine-impregnated dressing), both traditional dressings, and Aquacel, a newer product. The only statistically significant difference found in the health economic analysis was the cost associated with the provision of

dressings (mean cost per patient: N-A 14.85 pounds, Inadine 17.48 pounds, Aquacel 43.60 pounds) (Jeffcoate *et al.,* 2009).

Limitations of the study

The number of patients included in the evaluation was small, so the analysis in this study used the number of patients visits. Nevertheless, it would be beneficial to undertake further work in other wound care clinics and public health centres to increase confidence in the generalisability of the results.

Conclusion

This study showed that modern wound dressings provide a higher quality of life at a higher cost. Indeed, it cost more than IDR 22,813 to change from conventional to modern wound dressings and increase 1 unit of quality of life, but patients obtained an additional 13.15 quality of life. Further studies comparing the GDP per capita, or the threshold value, or the willingness to pay are necessary to determine whether the addition is commensurate or not.

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Conflict of Interest

The authors declare no conflict of interest.

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IAI CONFERENCE

RESEARCH ARTICLE

Dissolution profile of Curcumin from solid dispersion prepared at a high drug load of Curcumin using Poloxamer 407 as the carrier

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Keywords
BCS II
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Abstract

Introduction: Curcumin, a BCS II drug, suffers from poor bioavailability. Increasing curcumin dissolution is a way to increase its bioavailability. Solid dispersion formulation can be used to improve curcumin dissolution. However, the successful curcumin solid dispersion is limited to a relatively low drug load (< 20%). **Aim:** This study aimed to investigate the dissolution behaviour of curcumin at a higher drug load (27.9%, 42.3%, and 56.6%) using a surfactant carrier of poloxamer 407. **Methods:** The solvent evaporation method was employed to prepare high drug load solid dispersion of curcumin. A physical mixture of the corresponding solid dispersion formulation was prepared as a control. Drug load, dissolution behaviour in 180 minutes, and dissolution efficiency (DE180) were determined. **Results:** The results showed that incorporating curcumin into a poloxamer 407 solid dispersion significantly improves the dissolution rate of curcumin. In the solid dispersion formula, the dissolution behaviour of curcumin was found to be carrier-dependent.

Introduction

Curcuminoid, a mixture compound of curcumin, demethoxycurcumin, and bisdemethoxycurcumin, is widely known as a functional food and is obtained from the isolation of turmeric (*Curcuma longa*) rhizome and other species of Zingiberaceae. Extensive investigations revealed the beneficial effects of curcumin to cure several diseases with its main activities as antioxidant and anti-inflammation (Basnet & Skalko-Basnet, 2011; Abdollahi *et al.*, 2017). Although extensive *in vitro* and *in vivo* studies highlighted the potential activities of curcumin, a clear therapeutic benefit of curcumin in clinical studies remained questionable due to the poor bioavailability of curcumin after oral administration (Gupta, Patchva & Aggarwal, 2012). The poor bioavailability of curcumin has been attributed to the low aqueous solubility and dissolution; the solubility of curcumin in water was reported to be 11 ng/mL (Wang *et al.*, 1997).

An increased dissolution has been proposed to tackle the bioavailability problem of curcumin. Solid dispersion is one of the potential strategies to improve curcumin dissolution. Several factors enhance the dissolution rate of drugs, including increased surface area by reducing particle size, improvement of wetting, reduced agglomeration, and increased saturated concentration resulting from the amorphous form (Craig, 2002). Different types of carriers and methods are used to prepare solid dispersions (Leuner & Dressman, 2000). One of the carriers used in solid dispersion preparation is the poloxamer, a non-ionic triblock copolymer of polyoxyethylene-polyoxypropylene-polyoxyethylene, which has surfactant properties (Bodratti & Alexandridis, 2018). The dissolution enhancement rate of poloxamer

solid dispersion is achieved by improving the wettability and the ability of intermolecular interaction of poloxamer-drug to form a molecular dispersion (Ali, Williams & Rawlinson, 2010). This work aimed to investigate the dissolution behaviour of curcumin at a higher drug load (above 20% to less than 60%), using a surfactant carrier of poloxamer 407.

Material and Method

Material

Curcuma longa standardised extract (84.67% curcumin as detected by spectrophotometric method) was given by PT Phytochemindo Reksa, Bogor, Indonesia. Curcumin standard compound was purchased from Sigma. The poloxamer 407 was given by PT Konimex, Solo, Indonesia. Citric acid, methanol, ethanol, sodium dihydrogen phosphate dihydrate, and sodium lauryl sulfate (SLS) were purchased from Merck. Capsule shells of the size of 00 were purchased from Kapsulindo Nusantara, Indonesia. MiliQ water was supplied by our laboratory.

Method

1. Preparation of the solid dispersion formulation

The poloxamer 407 based solid dispersion (SD-PC) was prepared at 27.9%, 42.3%, and 56.6-wt % of the *C. longa* extract by a solvent evaporation method using a vacuum rotary evaporator. The formulas were coded as SD-PC 33, SD-PC 50, and SD-PC 67 assigned to each extract -wt. % of 27.9%, 42.3%, and 56.6-wt %, respectively in the solid dispersion formulations. Briefly, an accurate weight of *C. longa* extract and poloxamer 407 according to each code of formula was dissolved in ethanol at concentrations of 5 mg/mL and 45 mg/mL. Both solutions were stirred for 30 minutes to form a clear yellow solution. The ethanol was evaporated using a BUCHI vacuum rotary evaporator at 80^{o}C. The obtained viscous solution was then subsequently dried using a vacuum oven. The dried product was pulverized in a mortar and sieved using a 60-mesh size. Physical mixture formulations (PM-PC) were prepared at the drug load as that prepared in the SD-PC formulations. The extract and poloxamer 407 were gently mixed in a mortar using a spatula and sieved using a 60-mesh size. For the dissolution study, the SD powder was prepared into an approximately 500 mg capsule of size 00. The drug load of curcumin in the SD formulations was quantified using the calibration curve prepared from the serial concentrations of curcumin reference standard in methanolic solution. The linearity between the concentration and the absorption was confirmed with y=0.1349x + 0.0035 and a correlation coefficient of 0.9972.

2. Dissolution

The dissolution of the SD-PC or PM-PC prepared in capsules was evaluated using a SOTAX AT7 dissolution tester according to the USP type II dissolution method. For the dissolution study of lipophilic compounds like curcumin, 0.5% surfactant of SLS was added into 20 mM sodium phosphate buffer of pH 6.0. This pH value was chosen because the curcumin solution is highly stable at pH 6.0 (Wang *et al.*, 1997). The dissolution was carried out in 900 mL media with a paddle speed of 100 rpm and a temperature of 37±0.5°C. The samples of 2 mL were taken at predetermined time intervals of 0, 10, 15, 30, 45, 60, 90, 120, and 180 minutes. The withdrawn volume was replaced with the same volume of a fresh dissolution medium, maintained at 37° to keep the volume and sink condition constant. Curcumin in the dissolution sample was determined using the validated spectroscopic method at 430 nm against the blank sample of the dissolution medium. Curcumin concentrations were quantified based on the calibration curve at which the result shows linearity with a linear equation of y=0.1279x + 0.009 and a relation coefficient of 0.997.

3. Data analysis

The dissolution profile was determined by calculating the Dissolution Efficiency (DE) at 180 min, as in equations 1 and 2. The area under the curve of dissolved curcumin was calculated by the trapezoidal method, as shown in the following equations:

$$DE_t = \frac{\int_{t1}^{t2} y.\,dt}{y_{100}.t}) \times 100\% \qquad AUC = \sum_{i=1}^{i=n} \frac{(t_1 - t_{i-1})(y_{i-1} + y_i)}{2}$$

Equation 1 Equation 2

DE_t : Dissolution efficiency at time (t)

y : Area under the curve of dissolved drug at time t

y100.t : Rectangle area where 100% of drug dissolved at time t

Results

Table I shows the amount of curcumin content in the solid dispersion and physical mixture formulations. The % assay values, calculated based on the recovery at which the obtained curcumin contents were divided by the theoretical values and multiplied by 100%, were

between 94.33-99.48% for SD-PC and PM-PC formulations. The curcumin content for all SD and PM formulations was calculated for the relative standard deviation (RSD) values. The RSD values for all SD and PM formulations are between 0.12%-4.50%.

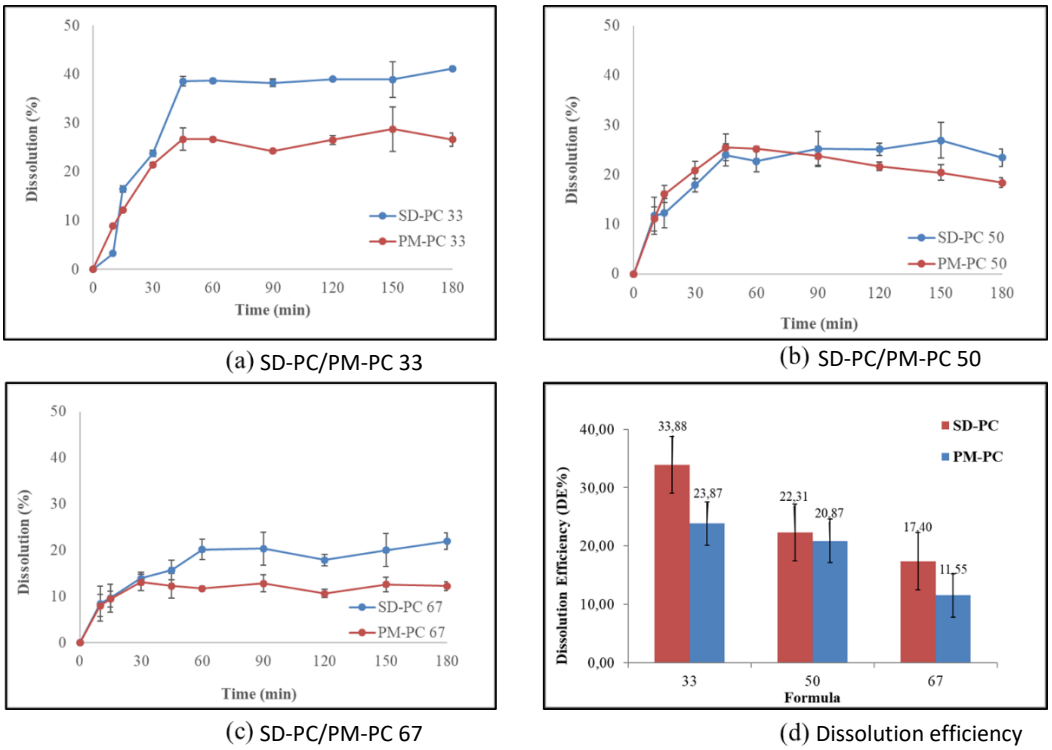
Table I: Curcumin content was observed in the solid dispersion (SD) and physical mixture

% weight of *C. longa* extract	Code	SD-PC			Code	PM-PC		
		% found	% assay	RSD		% found	% assay	RSD
27.9	SD-PC 33	27.59 ± 0.38	99.24	1.37	PM-PC 33	27.42 ± 0.32	99.48	1.16
42.3	SD-PC 50	40.87 ± 0.67	96.54	1.63	PM-PC 50	41.22 ± 0.71	96.38	1.72
56.6	SD-PC 67	56.29 ± 0.07	99.38	0.12	PM-PC 67	53.52 ± 2.41	94.33	4.50

Note: Data were presented as mean and SD of 3 assays. % assay was calculated by dividing the observed amount of curcumin by the theoretical amount of curcumin present in the formulation at the accordingly wt % of *C. longa* extract. The theoretical amount of curcumin was determined based on the curcumin content in the standardized *C. longa* extract.

The *in vitro* dissolution profile of the different solid dispersion formulations using poloxamer 407 and the respective physical mixture in 20 mM sodium phosphate buffer of pH 6.0 are shown in Figure 1 (a, b, and c). At the end of 180 minutes, 26.62%, 18.38%, 12.22%, 41.18%, 23.41%, and 21.92% were released from PM-PC 33, PM-PC 50, PM PC 67, SD-PC 33, SD-PC 50, and SD PC 67, respectively. The dissolution profile of SD and PM formulation show stagnant dissolution rate after 30 minutes (SD/PM-PC 33), 45 minutes (SD/PC-50), and 60 (SD/PC-PC 67).

DE_{180} was used to compare the dissolution profiles resulting from all SD and PC formulations and presented in Figure 1d, showing that all SD formulations increased the dissolution rate of curcumin compared to the physical mixture formulation as monitored in 180 minutes. It also revealed that the solid dispersion formulation increased the dissolution rate of curcumin as compared to the corresponding physical mixture formulation.



(a) SD-PC/PM-PC 33 (b) SD-PC/PM-PC 50

(c) SD-PC/PM-PC 67 (d) Dissolution efficiency

Figure 1: Dissolution behaviour of curcumin from SD and PM formulations

Discussion

As shown in Table I, the RSD values for SD and PM formulations are all below 5%. The data indicate that the solid dispersion and physical mixtures formulations are homogenous; independent assays of three samples taken from any dispersion showed an even drug distribution within each system. All formulas showed that % values between the founded curcumin content and the theoretical curcumin concentration were close, as reflected by a high % assay values of between 90 and 100%. Moreover, curcumin content in the SD-PC is quite close to the PM-PC, indicating that the curcumin remains preserved during the preparation processes of the solid dispersion formulations.

Surfactants can improve the hydrophobicity of poorly water-soluble drugs and increase the dissolution rate by increasing wettability. Solid dispersion formulation of *C. longa* extract using poloxamer 407 as carrier improves the dissolution profile of curcumin compared to the physical mixture formulation (Figure 1a, b, c) due to the improvement of wetting with the addition of poloxamer 407. A significant increase in dissolution rate was observed from the SD-PC 33 and SD-PC 67 compared to the corresponding physical mixture formulation (PM-PC 33 and PM-PC 67). The dissolution rate declined as higher extract concentration in the solid dispersion formulations was used (SD-PC 67). The release of curcumin during 180 minutes was about 40%; however, at higher extract concentration (SD-PC 67), the dissolution decreased to 20%. A lower dissolution rate at a higher drug load was also reported by other publications, suggesting that the dissolution mechanism is controlled by the carrier.

Dissolution efficiency (DE) is a dissolution parameter used to compare characteristics of the dissolution profile between formulas. The effect of increasing extract content in the solid dispersion formulation markedly reduced the dissolution rate of curcumin, as indicated by the DE values (Figure 1d). Lowering the dissolution rate by increasing the extracted content was observed in the solid dispersion formulation and demonstrated by the physical mixture formulation.

Conclusion

Poloxamer 407 is an effective carrier to increase the dissolution rate of curcumin during a 180-minute study in solid dispersion formulations containing C. longa standardized extract. An increased extracted content in the solid dispersion formulation results in reduced dissolution of curcumin dissolution. The highest dissolution rate of curcumin is found in the extracted content of 27.9 -wt% of curcumin of the SD-PC 33 formulation.

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IAI CONFERENCE

RESEARCH ARTICLE

In silico screening of mint leaves compound (*Mentha piperita L.*) as a potential inhibitor of SARS-CoV-2

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Keywords
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Abstract

Introduction: The novel coronavirus in Wuhan, China, was identified at the end of December 2019 and resulted in a global outbreak. Therefore, it is necessary to perform screening of compounds in herbal plants with antiviral potential against COVID-19. Mint leaves (*Mentha piperita L.*) were reported as one of the proposed samples, and this study was performed *in silico* to evaluate the antiviral activity of the content. **Methods:** The proposed mechanism of action includes the inhibition of SARS-CoV-2 proteins from binding with the receptor. Subsequently, several receptors associated with SARS-CoV-2 were validated, and the one with the code PDB 5R7Y and an RMSD value of 1.9974 Å was obtained using the YASARA application. This study was performed on 15 virtual mint leaves and five previously studied comparison compounds with inhibitory capacity. Therefore, docking started with the PLANTS application, and the results were visualised using PyMol to further identify the amino acids contained in the ligand, while the statistical t-test was used for comparison. **Results**: The study results showed the existence of active compounds in mint leaves, including rutin, hesperidin, and isorhoifolin.

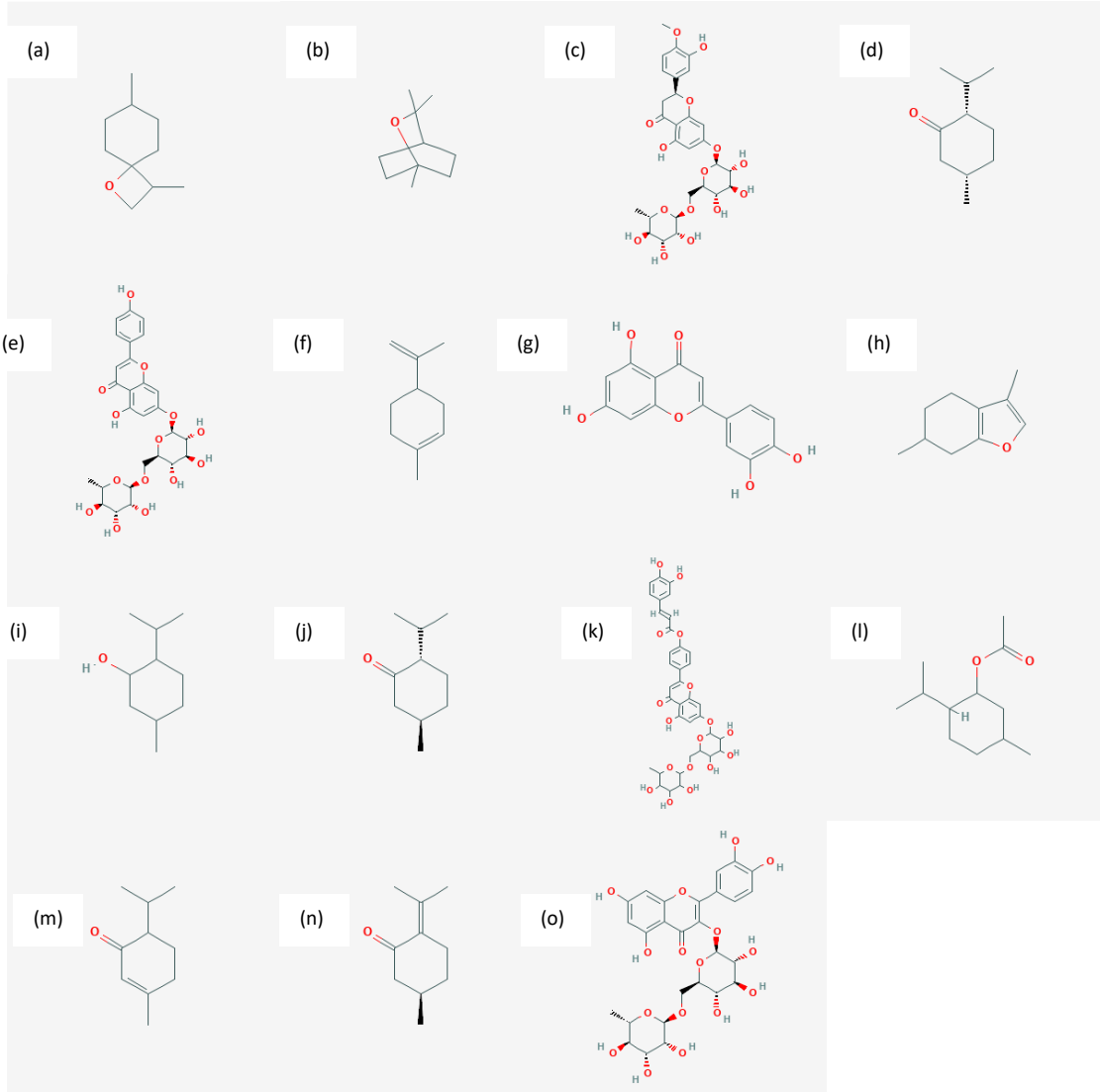
Introduction

Infectious disease is transmitted from one person to another, either directly or through an intermediary. This phenomenon is influenced by three principal factors, i.e., the host, agent (cause), and environment. The agent factors are possibly grouped into viral groups (influenza, trachoma, smallpox, and others), rickets (typhus), bacterial (dysentery), and protozoa (malaria, filaria, Schistosoma, and others) (Masriadi, 2016). The outbreak of mysterious pneumonia in Wuhan, China, in December 2019, spread to numerous surrounding cities and expanded globally. The virus responsible for this disease was later identified as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the World Health Organization (WHO) termed the disease Coronavirus Disease 2019 (COVID-19). A total of 28,276 confirmed cases with 565 deaths involving at least 25 countries were documented by the WHO as of 6 February 2020. The incidence of person-to-person transmission potentially occurs through droplet or contact transmission and is facilitated by the absence of any strict infection control measures or proper personal protective equipment for the first-line health workers at risk. There is currently no validated treatment, and the therapeutic strategy adopted is a symptomatic approach and supportive care by maintaining vital signs, oxygen saturation, blood pressure, and treating complications, including secondary or secondary infections or organ failure (Wu Y, 2019). Therefore, the performance of further research on the discovery of antiviral drugs is necessary (Tornery, 2020), including the use of medicinal plants expected to have antiviral activity. A virtual compound of the *Mentha piperita* L. plant, commonly known as peppermint/mint leaves, was used in this investigation. Furthermore, these samples have a high antioxidant content estimated to portray antimicrobial, anti-tumour, and anti-allergenic characteristics (Handayani, 2020). This research applied bioinformatics and molecular docking to

identify compounds of mint leaves with antiviral activity against COVID-19 using the *in silico* method. Also, samples with the best docking score data were obtained from the interaction between the active compounds and the receptor's 5R7Y protein. The results of the *in silico* screening provided insight into the activity of compounds on SARS-CoV-2 receptors, showing that some of them have antiviral effects against the novel coronavirus.

Material and method

The virtual structure of compounds in mint leaves *(Mentha piperita L.)* is shown in Figure 1 (3,7-dimethyl-1-oxaspiro (3,5) nonane, eucalyptol, hesperidin, isomenthone, isorhoifoline, limonene, luteolin, menthofuran, menthol, menthone, menthoside, menthyl acetate, piperitone, pulegone, rutin). The comparison compounds were arbidol, darunavir, chloroquine, lopinavir, and remdesivir. The virtual structure of the 5R7Y receptor was also obtained.



(a) 3,7-dimethyl-1-oxaspiro (3,5) nonane; (b) eucalyptol; (c) hesperidin; (d) isomenthone; (e) isorhoifoline; (f) limonene; (g) luteolin; (h) menthofuran; (i) menthol; (j) menthone; (k) menthoside; (l) menthyl acetate; (m) piperitone; (n) pulegone; (o) rutin

Figure 1: The structure of compounds in mint leaves

Receptor (protein) preparation

The protein complex demonstrated in the format (.pdb) was obtained from the Protein Data Bank (PDB) (RSCB, 2020) before subsequent preparation with the YASARA program. This procedure yielded three files, including

the protein. mol2, ref_ligand.mol2 and ligand.mol2 (Chowdhury, 2021).

Preparation of protein, comparison, and test ligands

The protein, comparative, test compound ligands were prepared using the MarvinSketch at pH 7.4 and saved as ligand_2D.mrv. Then, the conformational search was selected and the result saved with file type .mol2. This procedure was conducted for every single sample.

Optimise protein and set RMSD value

The prepared native ligands were then optimised with the protein crystal structure, using the PLANTS program to obtain a score. The best was then selected and stored in the form of a mole file 2. Subsequently, the optimisation result in terms of RMSD amount was calculated with reference to the experimental results or protein crystal structure using the YASARA program.

Docking comparison ligands

Docking was performed on the three files obtained from the protein preparation conducted later, using the PLANTS program. This approach aimed to obtain the best score for ligands of the comparison compound, which was consequently contrasted with test samples with topmost values.

Docking the test ligand against the receptor

The docking between each test compound ligand was performed using the PLANTS program. Then, the best score was contrasted with the top value for the comparison compound.

Visualisation of ligand and receptor interactions

The respective docking result files were created using the YASARA program (file type.pdb). These outcomes were subsequently visualised and interpreted to determine the initiated interactions using the VMD application.

Results and discussion

Analysis of the receptors used

COVID-19 is a recently discovered disease caused by SARS-CoV-2 (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). The infection in humans requires some receptors, which serve as an entry route. In addition, the virtual structure was obtained from the Protein Data Bank (RSCB, 2020), while the receptors for docking provided an RMSD value (Root Mean Square Deviation) less than 2.0 Å after validation.

Subsequently, a protein with the PDB code 5R7Y was used after authenticating several SARS-CoV-2 receptors, based on the results from PLANTS and YASARA applications, and an RMSD value of 1.9974 Å was obtained. These proteins were used because the validation results met the requirements, at less than 2 Å, preventing a far shift of the ligand position binding the protein (active side) because the conversion from 2 Å equals 0.2 mm, according to the diameter size range of an atom, measuring about 0.1 mm. In addition, smaller RMSD implies better ligand position prediction, which results from the relative closeness to the original conformation.

Analysis software used

The software used in this study was downloaded for free and separately to attain the desired docking simulation, comprising a combination of several different applications. Moreover, PLANTS is a docking software benchmarked internally in the Vrije Universiteit Amsterdam, medical chemistry research group with GOLD, a paid docking software routinely used at medicinal chemistry laboratories in Europe and the USA. The other supporting software used were YASARA for protein preparation and RMSD calculation, MarvinSketch for ligand preparation, and PyMol for bond visualization between amino acids and the active representative compounds.

Docking simulation results at the 5R7Y receptor

In this study, some already studied medicinal compounds were used for their antiviral activity against SARS-CoV-2, such as arbidol, darunavir, chloroquine, lopinavir, and remdesivir (Costanzo, 2020). The comparison compound was used as a measure for the ability of the test compound to act on the target receptor. Good activation ability was seen from a low or negative docking score. The docking score for each comparison compound was negative (arbidol: -89.2994, darunavir: -105,304, chloroquine: -81,3629, lopinavir: -98,3046, and remdesivir: -93.0524), showing that the five comparison compounds have a good affinity for the inhibition of the 5R7Y code enzyme used.

The tests were performed on 15 compounds in mint leaves (*Mentha piperita L.*) (Trevisan, 2017) and expected to demonstrate inhibition tendency against the protein coded for PDB 5R7Y. The affinity was also assessed using the molecular docking method performed based on the PLANTS application, with scores determined based on a ChemPLP value. This

factor is calculated with reference to the Gibbs free energy, where the smaller values for the test compound in contrast with the comparison indicates good receptor bond affinity. Moreover, the samples evaluated were obtained using the MarvinSketch application, and docking was performed using the PLANTS software to obtain the respective ChemPLP value.

The research identified three compounds from mint leaves (*Mentha piperita L.*,) with an affinity for inhibiting the SARS-CoV-2 code 5R7Y protein receptor (Gervasoni, 2020). These include Rutin, Hesperidin, and Isorhoifolin. Additionally, the respective ChemPLP values were lower than that of the chloroquine, darunavir, lopinavir, and remdesivir, while Rutin and Hesperidin specifically had lower values than arbidol. The findings indicate a more significant direct inhibition activity of rutin and hesperidin against SARS-CoV-2 protease enzyme receptors compared to others (Bellavite, 2020; Huynh, 2020). This also signifies a similarity in action mechanism between hesperidin, arbidol, and chloroquine. Therefore, both mint leaf (*Mentha piperita L.) in silico* constituents have the potential to be developed into antiviral drugs against COVID-19, although further in vivo research is also needed (Khan, 2020).

Statistic analysis

The ChemPLP docking score data showed higher activity in all three compounds compared to the five comparison samples at the protease receptor coded 5R7Y PDB. Furthermore, data analysis was performed using a statistical two-tailed paired T-test with paired samples from the same unit or group of units via Microsoft Excel. This approach was used to ascertain the suitability between the sample pair data tested. Based on the p-value, samples above 0.05 indicate the absence of any significant differences against the comparison. Conversely, the inverse is reported at $p < 0.05$.

Table I shows the different test and comparison compound pairs within the required $p < 0.05$, including Hesperidin-Chloroquine (0.0005), Hesperidin-Lopinavir (0.0153), Rutin-Lopinavir (0.0062), Rutin-Remdesivir (0.0159), and Isorhoifolin-Chloroquine (0.0240). This finding indicates significant differences between these five pairs, while others tend to not vary substantially.

Table I: Statistics results of active representative compounds

Test compound	Best score docking with the 5R7Y receptor	Comparison of compound	Best score docking with the 5R7Y receptor	*p*-value
Hesperidin	-91.2724	Arbidol	-89.2994	0.5607
		Darunavir	-105.304	1.1625
		Chloroquine	-81.3629	0.0005
		Lopinavir	-98.3046	0.0153
		Remdesivir	-93.0324	0.0808
Rutin	-90.0029	Arbidol	-89,2994	0,3473
		Darunavir	-105.304	2,1162
		Chloroquine	-81.3629	4.0763
		Lopinavir	-98.3046	0.0062
		Remdesivir	-93.0324	0.0159
Isorhoifolin	-84.5769	Arbidol	-89.2994	2.2113
		Darunavir	-105.304	4.3193
		Chloroquine	-81.3629	0.0240
		Lopinavir	-98.3046	4.1460
		Remdesivir	-93.0324	1.1469

Elucidation of the mode of binding of the representative compound active on the active site of the 5R7Y receptor

The amino acid bond visualisation in the binding pocket of the SARS-CoV-2 receptor protease enzyme was performed using PyMol software. This finding was described in three dimensions (3D), and the bonding distance between the active compound and the receptor was determined.

Table II and Table III show the analysis result using PyMol, where the amino acids obtained were thought to play an important role in the compound's affinity at the SARS-CoV-2 receptor, including GLN189 (glutamine), THR25 (threonine), ARG188 (arginine), CYS44, and CYS145 (cysteine). The residues produced demonstrated inhibitory characteristics, while the results of bond distance determination identified ligand-bound forms in hesperidin, Rutin, and isorhoifolin. The bonding generally occurs at a distance

of 1-5 Å, which is within the requirements for all samples. Furthermore, the developed bonds are similar to hydrogen bonds.

Table II: The interaction between test compounds and binding pocket protein with code 5R7Y

Compound name	Types of amino acids	Amino acid amount in the binding site
Rutin	HIS163, ASN142, GLN189, GLY143, THR26, CYS44, SER144, CYS145	8
Hesperidin	HIS163, THR25, GLN189, CYS44, ARG188	5
Isorhoifolin	GLU166, ASN142, GLN189, SER144, GLY143, CYS44, PRO39, CYS145, ARG188	9

Table III: The bond distance between the active representation compound and the bound amino acid

Compound name	Types of amino acids	Bond distance (Ã)
Rutin	GLN189	1.9
	CYS44	2.1; 1.8
	CYS145	2.4
`Hesperidin	THR25	2.3; 2.7
	GLN189	1.7; 2.1
	CYS44	1.9
	ARG188	2.3
Isorhoifolin	GLN189	1.9
	CYS44	2.1
	CYS145	2.1; 2.3
	ARG188	2.0

Visualisation of representative active compounds

Figure 2 shows a total of five amino acid residues at binding site of hesperidin. Meanwhile, the other four bind to the test ligand and are thought to play an important role in the compound's affinity towards SARS-CoV-2 receptor. The amino acids include THR25 with a distance of 2,3 Ã and 2,7 Ã, GLN189 at 1.7 Ã and 2,1 Ã, CYS44 at 1.9 Ã, and ARG188 at 2,3 Ã.

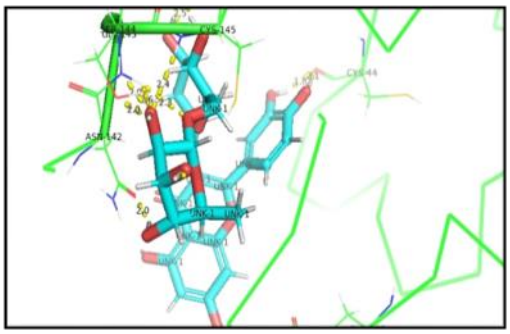


Figure 2: Hesperidin's 3D pose with bound amino acids

Figure 3 is an illustration of a total of eight amino acid residues at the binding site of Rutin compounds. Three were interacted with the test ligand and are thought to play an important role in the affinity towards SARS-CoV-2 receptor. In addition, the amino acids observed include GLN189 with a distance of 1.9 Ã, CYS44 at 1.8 Ã and 2.1 Ã, as well as CYS145 at 2.4 Ã.

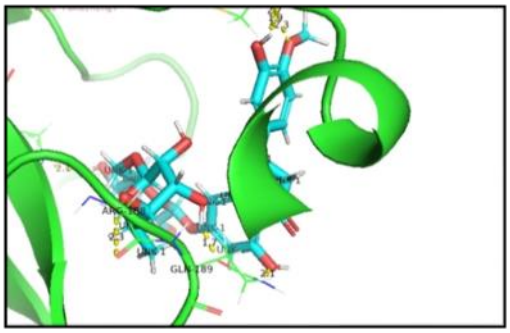


Figure 3: 3D pose of Rutin with bound amino acids

Figure 4 is an illustration of Isorhoifolin compound with a total of nine amino acid residues present at the binding site. Four were interacted with the test ligand, and are assumed to play an important role in the affinity aspect towards the SARS-CoV-2 receptor. In addition, the amino acids identified include GLN189 with a distance of 1.9 Ã, CYS44 at 2.1 Ã, CYS145 at 2.1 Ã and 2,3 Ã, as well as ARG188 at 2.0 Ã.

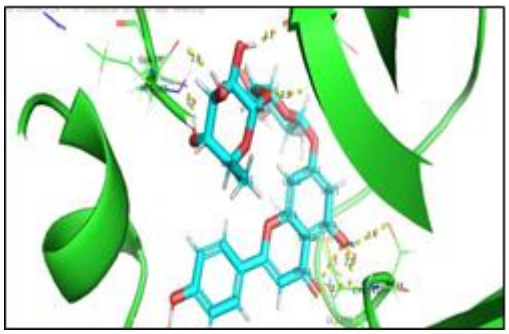


Figure 4: 3D pose of Isorhoifolin with bound amino acids

Conclusion

The research conducted *in silico* on virtual compounds of mint leaves (*Mentha piperita* L.) and molecular docking revealed the activity of these compounds and the potential for the development of antiviral agents to inhibit SARS-CoV-2. The three intrinsic representative active compounds in mint leave compounds, namely Hesperidin, Rutin, and Isorhoifolin, can individually inhibit proteases contained in the SARS-CoV-2 virus protease component. Further research experimental in vitro research on representative active compounds is recommended for the consequent development into SARS-CoV-2 antiviral compounds.

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IAI CONFERENCE

RESEARCH ARTICLE

Formulation and physical evaluation of facial cream preparations from Ceremai fruit juice (*Phyllanthus acidus (l.) Skeels*)

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Abstract

Introduction: Ceremai (*Phyllanthus acidus* (L.) Skeels) fruit contains AHA (Alpha Hydroxy Acids) compounds which are widely used as a moisturizer or as an exfoliating process in cosmetics production. It also contains vitamin C and flavonoids, both of which acts as whitening agents. **Aim**: This study aims to utilise a source of natural AHA active substances found in P.acidus in face cream preparations. **Methods**: The juice from P.acidus was dried by freeze-drying method and formulated into a cream with the preparation process evaluated for one month. **Results**: The observation results showed that after one month, there was no change in colour during storage, with the pH, viscosity and spreadability of the cream in the range 4.5-6.2, 2700-3996 cps, and 4.8-5.7 cm, respectively. **Conclusion**: P.acidus fruit juice containing AHA can be formulated into a face cream with good physical stability.

Introduction

Indonesia is one of the countries that lie along the equator; therefore, its climate is entirely tropical, which means that people are exposed to solar radiation every year compared to other countries not crossed by the equator. On the surface of the earth, sunlight consists of various spectra, including infrared light (> 760 nm), visible light (400-760 nm), ultraviolet light (UV) A rays (315-400 nm), UV B rays (290-315 nm) and UV C rays (100-290 nm). According to Agustin and the authors (2013), moderate amounts of radiation can provide a sense of comfort and health for the human body, while continuous exposure to high-intensity sunlight tends to interfere with skin health, thereby leading to changes in connective tissue, capable of causing premature ageing, skin cancer, hyperpigmentation, as well as black and scaly skin. In addition to continuous sun exposure, changes in facial skin colour can also be caused by lack of sleep, fatigue, dust and air pollution. To some people, the change in skin colour from fair to black is a very sensitive matter because it relates to appearance. Therefore, one of the techniques used to overcome dull skin and whitening facial skin is by using a face cream containing AHA (Alpha Hydroxy Acid), which is a carboxylic and hydrophilic acid group. AHAs are also known as fruit acids because they are commonly found in fruit, for example, citric, malic and tartaric acids in citrus fruits, apples, and grapes. AHAs which are not components of the fruit include glycolic acid from sugar cane and lactic acid from milk. It is also used as a moisturizer and exfoliates the skin from the top layer of the stratum corneum to the lowest through a keratolytic process (Tang & Yang, 2018).

Ceremai (*Phyllanthus acidus* (L.) Skeels) originated from India and belonged to the Euphorbiaceae family. This tree grows in light to heavy soils and is resistant to deficient or excess water. The roots contain saponins, tannins and toxic substances. The leaves, bark, stems, and wood comprises saponins, flavonoids, tannins and polyphenols, while the fruit contains vitamin C (Andrianto *et al*., 2017). Selpiana and the authors (2015) stated that the most commonly used parts of this tree are the roots, leaves and seeds, with the fruit widely used as sweets and food flavouring due to its sour taste. Therefore, it is necessary to carry out research on *P. acidus* fruit as medicine. Previous studies on *P. acidus* fruit led to several findings regarding its use as medicine, such as as an antipyretic, anti-diarrhoea, and analgesic (Afrin *et al*., 2015) hepatoprotective (Jain *et al*., 2010), and antioxidants (Andrianto *et al*., 2017).

P. acidus fruit contains vitamin C and flavonoids (Andrianto *et al*., 2017). According to Hanani (2017), flavonoids are the largest phenol group found in plants and comprise two or more hydroxyl groups with antioxidant activities that are good for the skin. Phenol is an alcohol compound, which reacts with strong acid oxidizing agents to form carboxylic acids. Meanwhile, vitamin C has the role of changing the paler melanin in the reduced form and prevents its formation by inhibiting dopa quinones, thereby brightening normal and pigmented skin (Kembun *et al*., 2012).

The water content contained in *P. acidus* fruit is 91.7 grams / 100 grams (Selpiana *et al*., 2015); therefore, this study used a sampling method from fruit juice. In addition, AHA compounds are soluble with high antioxidant content using a water solvent containing an LC_{50} value of 26.06 µg/mL (Andrianto *et al*., 2017).

This study aims to utilise a source of natural AHA active substances found in *P.acidus* fruit. The research included plant determination, making fruit juice samples using the freeze-drying method. The advantages of the freeze-drying method include maintaining sample stability, maintaining the stability of the material structure and increasing rehydration power (January & Martin, 2014). Phytochemical screening was carried out by testing flavonoids, alkaloids, steroids and terpenoids, saponins and tannins. The cream formulation uses excipient stearic acid, TEA (Triethanolamine), methylparaben, propylparaben, Oleum Cocos, glycerin, cetyl alcohol, and Oleum Rosae. Then performed a physical evaluation of the preparation, which includes organoleptic observation, pH measurement, spreadability test, viscosity test.

Methods

P. acidus fruit determination

Plant determination aims to examine the morphology of *P.acidus* plants, such as the colour, smell and taste through organoleptic tests and to use the Indonesian Institute of Sciences (LIPI), Bogor Herbarium.

Freeze-dried P. acidus fruit juice preparation

The manufacture of *P. acidus* fruit juice begins with wet sorting of *P. acidus* fruit; the goal is to separate impurities or other foreign materials from Simplicia. This step was followed by washing to remove impurities attached to the simplicia and the separation of the pulp from the seeds. The pulp that has been separated from the seeds is mashed, then squeezed using a filter to separate the filtrate from the residue. The juice that has been separated is then dehydrated using freeze-drying at a temperature of -5^0C for 24 hours (January & Martin, 2014).

Phytochemical screening (Sangi et al., 2018)

Phytochemical screening is carried out by determining the flavonoid, alkaloid, steroid and terpenoid, saponin, and tannin content.

Flavonoid test

Approximately 2 mL of *P.acidus* juice was pipette, put into a test tube and heated before ethanol was added. Magnesium and Hydrochloric acid (HCl 2N tape are added to the solution, and the formation of a red solution indicates the presence of flavonoids.

Alkaloid test

A total of 2 mL of *P.acidus* juice was put into a test tube, dissolved with 5 mL of chloroform and three drops of ammonia, with the addition of two drops of concentrated H_2SO_4 and divided into two test tubes. The first tube is added with three drops of Wagner's reagent, positive for alkaloids, assuming a brown precipitate is formed. The second tube is added with three drops of Mayer reagent and found to be positive for alkaloids assuming a white to yellowish precipitate is formed.

Steroid and terpenoid test

A total of 2 mL of *P.acidus* fruit juice was put into a test tube and dissolved in 0.5 mL of chloroform. Furthermore, 0.5 mL of anhydrous acetic acid was added with 2 mL of concentrated sulfuric acid dropped in the mixture through the test tube wall. The formation of a bluish-green colour indicates the

presence of steroids, while brown or violet rings indicate a terpenoid.

Saponin test

A total of 2 mL of *P.acidus* fruit juice was put into a test tube and added with 10 mL of hot water-cooled and shaken vigorously for 10 seconds. The inability of the foam to disappear after adding HCl 2N indicates the presence of saponin.

Tannin test

A total of 2 mL of *P.acidus* fruit juice was put into a test tube, and 1 mL of 10% ferric (III) chloride solution was added; the formation of a dark blue/greenish-black colour indicates the presence of tannins.

Cream formulation and manufacturing process

The formula for *P.acidus* fruit cream is shown in Table I.

Table I: *P.acidus* fruit cream formula

Ingredient	Amount	Function
• *P.acidus* L. fruit freeze drying extract	• 6,76 g	• Active ingredient
• Stearic acid	• 5,5 g	• Emulsifying agent
• TEA	• 1,5 mL	• Emulsifying agent
• Methylparaben	• 0,3 g	• Preservative
• Propylparaben	• 0,3 g	• Preservative
• Oleum Cocos	• 2,2 g	• Humectant
• Glycerin	• 1,8 mL	• Emulsifying agent
• Cetyl alcohol	• 2 g	• Thickening agent
• Oleum Rosae	• 2 drops	• Corrigen Odours
• Aquadest	ad 100 mL	• Solvent

The manufacturing process is done by mixing cetyl alcohol, stearic acid, propylparaben and Oleum Cocos, then heating it to melt and stirring until it is homogeneous (oil phase). Mixed with TEA, glycerin, methylparaben and add 30 mL of aqua dest until homogeneous (water phase). Mix the oil phase into the water phase until the mixture thickens and cools. Put the *P.acidus* fruit freeze drying extract that has been dissolved first with 40 mL aqua dest into the mixture. Add two drops of Oleum Rosae and the remaining distilled water, stir until homogeneous.

Evaluation of preparations

Organoleptic observation

Organoleptic observations were carried out by examining the colour, smell, shape and texture of the preparation.

pH measurement

pH measurement is carried out by dissolving the cream in water with a ratio of 1: 3, stirring until homogeneous and allowed to stand until it settles. The water is drawn and measured using a pH meter with the requirement for a good cream between 4.5 – 6.5.

Spreadability test

The formulated cream is carefully placed on the paper that is overlapped with transparent glass and left for a while. The area given by the preparation is calculated, and the cream is recovered with a glass that has a load equal to the amount of weight of each glass used and overwritten with a load of 50, 100 & 150 grams. Each load is left for 60 seconds because the wider the diameter, the better the spread.

Viscosity test

The cream was put into a cup glass container with its viscosity measured using an NDJ 5S viscometer before it is read and recorded. Furthermore, a cream is said to meet the viscosity requirements when it is in the range of 2000-50000 cps.

Results

P. acidus fruit was determined based on a letter from the Indonesian Institute of Sciences with a letter number B-1216/IPH.3/KS/X/2020, which explained that the sample used in the study was *Phyllanthus acidus* (L.) Skeels from the Phyllanthaceae family.

Phytochemical screening was used to determine the active substance content of *P.acidus* fruit is shown in Table II. The results of the phytochemical screening of *P. acidus* fruits showed that *P. acidus* fruit positively contained flavonoids, alkaloids, terpenoids, saponins, tannins and did not contain steroids.

Table II: Phytochemical screening results of *P.acidus* L. fruit

Secondary Metabolites	Result
Flavonoid	Positive
Alkaloid	Positive
Steroid	Negative
Terpenoid	Positive
Saponin	Positive
Tannin	Positive

Formulation and evaluation of preparations

The organoleptic test results showed that the cream base was white, while the *P.acidus* fruit cream sample

was brownish in colour. Base and sample rose-scented, semi-solid shape and texture; these characteristics were stable for five weeks. The results of organoleptic observations are shown in Table III.

Table III: Organoleptic test results of *P.acidus* fruit cream

Test Parameters	Time	Sample	Basis
Colour	Week 0	Brown	White
	Week 1	Brown	White
	Week 4	Brown	White
	Week 5	Brown	White
Smell	Week 0	Rose	Rose
	Week 1	Rose	Rose
	Week 4	Rose	Rose
	Week 5	Rose	Rose
Shape	Week 0	Semi-solid	Semi-solid
	Week 1	Semi-solid	Semi-solid
	Week 4	Semi-solid	Semi-solid
	Week 5	Semi-solid	Semi-solid
Texture of the preparation	Week 0	Semi-solid	Semi-solid
	Week 1	Semi-solid	Semi-solid
	Week 4	Semi-solid	Semi-solid
	Week 5	Semi-solid	Semi-solid

The results of testing the pH of the cream samples showed that the pH tends to increase, namely at the beginning of manufacture, it was 4.5, after a week of storage, it was 4.6, after four weeks of storage, it was 4.8, and after five weeks of storage was 6.2. The pH test carried out for five weeks are shown in Figure 1.

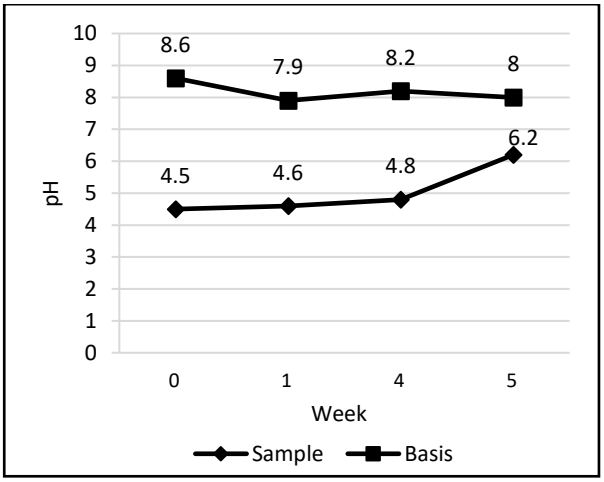

Figure 1: pH test chart

The results of the spreadability test of cream preparations showed that the spreadability of the cream samples was quite stable, namely, at the beginning of manufacture, it had a spreading power of 5.6 cm, after a week of storage, it was 5.4 cm, after four weeks of storage was 5.2 cm, and after five weeks of storage was 5.2 cm. The spreadability of the cream was carried out using two tests, namely the sample and the cream base, as shown in Figure 2.

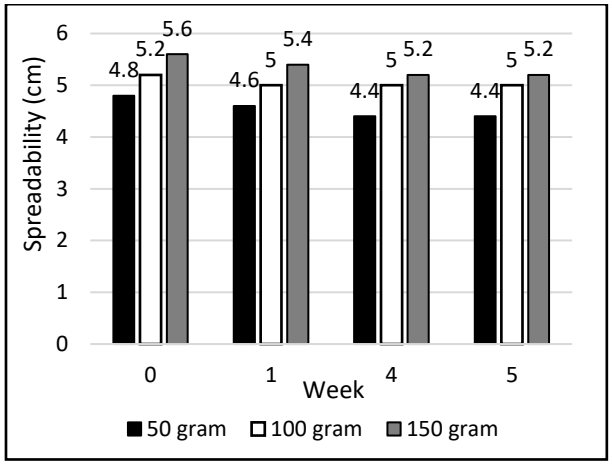

Figure 2: Spreadability test result chart

The viscosity test on the sample cream showed that the cream had a good viscosity, namely 2700 to 3996 cps for five weeks of storage. The viscosity test was carried out using a viscometer, as shown in Table IV.

Table IV: Viscosity data

Week	Test material	Viscosity
0	Sample	2700
	Basis	2304
1	Sample	3287
	Basis	3996
4	Sample	3996
	Basis	3996
5	Sample	3996
	Basis	3996

Discussion

Determination is a stage carried out in a study with the aim and purpose of knowing the truth regarding the identity of plants. The determination of *P.acidus* plants used as samples in this study was carried out at the Indonesian Institute of Sciences (Lembaga Ilmu Pengetahuan Indonesia (LIPI)). The results indicated that the sample used was a type of *Phyllanthus acidus* (L.) Skeels from the Phyllanthaceae family.

After the wet sorting and washing processes, the pulp and seeds were separated. The separated pulp is further blended to get a smaller particle size to obtain a larger sample surface area with the solvent. The greater contact with the solvent makes it easier for the solvent to attract the compounds contained in a sample.

The extraction method used to extract the active substance from *P.acidus* fruit is the squeeze method using a blender because the test material is not heat-resistant, and this method does not require a prolonged process (Rusdiaman, 2018). The fruit juice that has been separated is then dried using the freeze-drying process. The product advantages due to freeze-drying include maintaining stability, material structure, and increasing rehydration power (January & Martin, 2014).

The phytochemical test is used to analyse the content of secondary metabolites found in plants, with many benefits, such as protection from predators and parasites. Apart from being used by the plants themselves, secondary metabolites can also be used as medicine.

The data from the observation of the qualitative phytochemical test of *P. acidus* with parameters of the active phytochemical compounds, including flavonoids, steroids, terpenoids, alkaloids, saponins and tannins, are shown in Table II. The observations show that *P.acidus* fruit contains secondary metabolites that function as medicinal agents. Secondary metabolite compounds contained in *P.acidus* fruit include saponins, terpenoids, flavonoids and alkaloids. The reaction that occurs between the sample and the reagent leads to a change in colour and the formation of sediment (Habibi *et al*., 2018). The flavonoid test shows positive results due to the formation of a red colour after the addition of Mg and HCl, which reacts to form H_2 gas bubbles, while the concentrated Mg and HCl metals in this test function to reduce the benzopyrone core contained in the flavonoid structure, thereby forming a red or orange colour. According to Nuryanti and the authors (2016), the presence of flavonoid compounds in a plant extract forms red or orange salts with the addition of Mg and HCl.

Furthermore, the alkaloid test showed positive results with the formation of sediment after adding Mayer and Wagner reagents. A positive result of alkaloids in the Mayer test is indicated by the formation of a yellowish-white precipitate, which is a potassium alkaloid complex. Meanwhile, the addition of Wagner's reagent forms a brown precipitate caused by iodine reacting with ion I^- from potassium iodide to produce brown ion I_3^-. In the Wagner test, K^+ metal ions form coordinate covalent bonds with nitrogen in the alkaloids, thereby leading to the formation of a precipitated alkaloid potassium complex, which indicates a positive alkaloid result. The saponin test produces positive results with the formation of foam after adding hot water (Adhariani *et al*., 2018).

Steroid and terpenoid tests showed positive results with the formation of a violet colour change and negative for steroid tests after the addition of Lieberman Burchard reagent. The colour change after the addition of Lieberman Burchard's reagent was based on the formation of terpenoid compounds by H_2SO_4 in an anhydrous acetic acid solvent. The obtained colour difference is due to variation in groups on the C-4 atom (Marliana & Saleh, 2011).

The tannin test produced a positive result with the formation of a dark blue/black colour, which changed after the addition of $FeCl_3$ because tannins form complex compounds with Fe^{3+} ions (Ergina *et al*., 2014).

Evaluation of the properties of cream preparations aims to determine the stability of the preparation stored at room temperature, which includes evaluation of organoleptic, pH, spreadability and viscosity.

The organoleptic test aims to determine the colour, smell, shape and texture of the cream preparation, with the test results indicating that the base was white while the *P.acidus* fruit cream was brownish in colour due to the addition of *P.acidus* fruit extract. The observations made from week zero to four remained stable, with brown colour.

The pH test for cream preparations is used to determine the suitability of the degree of acidity of the cream formulas to ensure they can be applied to the skin. pH measurement using the Hanna HI 2211 pH meter was based on the National Agency of Drug and Food Control (BPOM) regulations regarding the classification of cosmetics containing AHA for group one with its content up to 10% using a pH range of 3.5 and above (Badan Pengawas Obat dan Makanan, 2006). In the pH test results, *P.acidus* fruit cream met the requirement because it was in the range of 4.6 to 6.2. In testing the pH of the cream, results that tend to increase were due to the possibility of an oxidation reaction of -OH molecules contained in citric and glycolic acid compounds found in *P.acidus* fruit cream. Meanwhile, based on the cream, the pH range between 8.6-8.0 tends to be alkaline due to the small amount of triethanolamine and stearic acid contained in cream preparations.

The viscosity test was carried out to determine the thickness of the cream preparation, which is the resistance of a preparation to flow; therefore, the greater the resistance, the higher the possibility of the viscosity to affect its distribution. The cream's viscosity was measured using the NDJ 55 viscometer, which obtained 2700 to 3996 cps in accordance with the requirements of a good cream (2000-50000 cps), while

the base was almost similar to the sample. In measuring the viscosity from week zero, there was an increase in viscosity from 2700 to 3996 cps, due to the rise in the proportion of the dispersed phase. Furthermore, an increase in the concentration of the emulsifier decreased the particle size. The greater the resistance, the greater the viscosity. Elcistia & Zulkarnaen (2018) stated that creams with high viscosity are difficult to pour into a container, while those that are too low are thin and tend to easily drip when applied to the skin.

The spreadability test of the cream aims to determine its ability to stick to the skin surface when used. The longer the stickiness of the cream to the skin, the more active the substances absorbed. Mukhlishah and authors (2016) stated that the dispersibility requirements for topical preparations are 5-7 cm. The results of the spreadability test for cream preparations showed that the results increased every week and met the requirements for good topical preparations.

Conclusion

P.acidus fruit juice containing AHA can be formulated into a face cream with good physical stability. The stability of the cream preparations from *P.acidus* fruit was good stability and in accordance with the standard requirements. The cream has a pH, viscosity and spreadability in the range of 4.6-6.2, 2700-3996 cps, and 5.2-5.6 cm, respectively.

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IAI CONFERENCE

RESEARCH ARTICLE

The evaluation of compounding prescription and its availability of a licensed product for children at a private hospital in Yogyakarta, Indonesia

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Abstract

Introduction: The availability of licensed drug formulas for paediatric patients is still limited, so compounded drugs still exist, especially in the form of divided medicinal powder. **Aim**: This study aimed to determine the profile of divided medicinal powder prescription and its availability in licensed drug formula for paediatric patients. **Methods**: This research was a cross-sectional study. Prescriptions containing order to compound divided medicinal powder at a ublic Hospital in Yogyakarta Special Region from January to March 2019, were collected and analysed descriptively. **Results**: The total collected prescriptions were 152. The total of active substances given to paediatric patients was 38. The most prescribed drug in the hospital was Triamcinolone. There are 34 active substances already available in licensed drug formulas for paediatric patients, so it is better not to be compounded. However, four active substances are not available as licensed product in the drug information handbook in Indonesia, so, it is reasonable to compound to provide a suitable medication (dose and dosage form) for paediatric patients.

Introduction

Providing medicine for paediatric patients is a huge challenge, including the lack of information on drug dosages and the availability of dosage to form the formulas. Medication errors and serious risks are also more common in children than adults (C. Wiedyaningsih *et al.*, 2012). Besides, the need for treatment in paediatric patients is certainly not the same as in adults because the physiology of the paediatric patients' bodies must be considered different in terms of pharmacokinetics, dosage, route of administration and adherence. That is the reason why a study for paediatric medication is required.

An extemporaneous preparation or compounded drug is commonly used as a medication for paediatrics (Widyaswari & Wiedyaningsih, 2012). Compounding is an activity of changing dosage form or mixing drugs into a new dosage form which is needed for the patient (Jackson & Lowey, 2010). It has a high risk and should be of concern because there are many undesirable events such as pharmaceutical problems, drug interactions, medication errors, quality of concoctions, and bacterial contamination problems. However, drug compounding is generally a solution to the limitation of drug formulas for paediatrics (C. Wiedyaningsih *et al*., 2012).

There are some alternatives to drug compounding for paediatric patients. Divided medicinal powder, syrup, dispersible powder/tablet, etc., are some dosage formulations that are commonly used. The study from Virginia (2014) showed that 73% of paediatric patients were more likely to get divided medicinal powder as a medication. Divided medicinal powder, also known as "puyer" in Indonesia, has several advantages, such as flexibility in dose adjusting, easy administration, and

simplicity to use (Virginia, 2014). However, it also has some disadvantages, including the possibility of adverse events, drug interactions, incompatibilities, and other risks (Rochjana *et al*., 2019).

A study of the profile and determinants of compounding services in the Yogyakarta Special Region showed that most community pharmacists (94%) dispensed prescriptions with compounding. Prescription-required compounding accounted for 11.55% of prescriptions dispensed within one month (Kristina *et al*., 2018). There is a high risk of medication error with compounding. This high compounding frequency rate and its risk make it important to do research on the profile of the compounding prescription in every health facility. The risk and negative effects of drug compounding should be minimised by using a licensed product for paediatric medication. Thus, it is necessary to carry out an analysis of the availability of licensed products for medicines formulated in a pharmaceutical installation. Studies have been conducted regarding the profile of the compounding prescription and also the evaluation of its availability as licensed products in a primary health facility in Yogyakarta (Widyaswari & Wiedyaningsih, 2012). In that study, it was found that there are still some drugs intended for children, not available in the form of a licensed formula.

This study aimed to determine and evaluate the compounding prescription profile, especially in divided medicinal powder form, and its availability as a licensed product in a public hospital in Yogyakarta Special Region. The results of this study are expected to provide long-term benefits in decreasing the frequency of compounding drugs for active substances available in licensed dosage forms for children.

Methods

This study was an analytical observation with a retrospective cross-sectional design. This study had an ethical clearance certificate from the Ethical Commission of the University of Respati Yogyakarta, no 130.3/FIKES/PL/V/2019. The population were all prescribed medicines in the hospital. The samples of prescriptions were collected by purposive sampling method in a pharmacy department at a public hospital in Yogyakarta Special Region. The inclusion criteria for the sample was that prescriptions contain an order to compound the divided medicinal powder for paediatric patients (0-18 years old) from January to March 2019. The exclusion criteria were unreadable prescriptions (illegible handwriting).

After the data were collected, they were analysed descriptively into two sections, including:

a. The profile of divided medicinal powder prescription:

   The profile analysis of prescription includes the characteristics of the subject, the age, the number of compounding, and the number of active substances contained in the preparations. The data were analysed descriptively and presented in the form of tabulation of frequency and percentage.

b. The analysis of the availability of a licensed product for paediatric patients:

The availability of the licensed product for paediatric patients (syrups, dry syrups, powder drops, suspensions, lozenges, and chewable tablets) was seen from the literature. The literature was MIMS Consultation Guidelines 2019/2020 and ISO Indonesia volume 52 (2019).

Results

In this study, the profile of divided medicinal powder prescriptions was analysed from all prescriptions that met the inclusion criteria. There are 152 prescriptions from the observation. In Figure 1, the profile of the subject was clearly shown that compounded drug was highly prescribed for paediatrics under five years old. Meanwhile, there are about 3% of the patients with the age range 12-18 years old also get a divided medicinal powder.

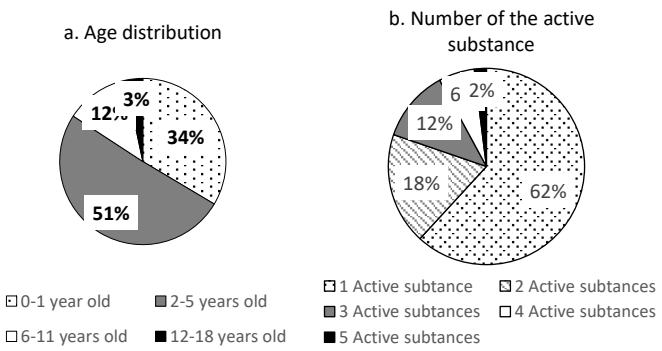

Figure 1: The profile of compounding prescription

The number of active substances that were compounded in one dosage form was also analysed. It was shown that more than half of all compounded drugs were containing one active substance. The highest number of active ingredients containing in a prescription were five substances; this kind of prescription-only appear in a low frequency (2%).

The availability of the licensed product for paediatric patients was analysed from all the active substances that compounded. The results showed that from 152

prescriptions obtained; there are 38 active substances prescribed for compounding (Table I). The top five frequently prescribed were Triamcinolone, Phenobarbital, Salbutamol Sulfate, Levotiroksin sodium, and Bromheksin. Most of them were drugs that indicated respiratory disease.

Table I: Active substances that undergo compounding along with the frequency of prescription

Active substances	Frequency	Percentage	Active substances	Frequency	Percentage
Triamcinolone	45	17.65 %	Ambroxol HCl	2	0.78 %
Phenobarbital	30	11.76 %	Methylprednisolone	2	0.78 %
Salbutamol Sulfate	25	9.80 %	Loratadine	2	0.78 %
Levotiroksin Sodium	17	6.66 %	Lisinopril	2	0.78 %
Bromheksin	15	5.88 %	Spironolactone	2	0.78 %
Terfenadine	12	4.70 %	Triheksiphenidil	2	0.78 %
Pseudoephedrine HCl	12	4.70 %	Ursodeoksikolat	2	0.78 %
Captopril	10	3.92 %	Risperidone	2	0.78 %
Piracetam	9	3.52 %	Vitamin B6 (Pyridoxine)	1	0.39 %
Furosemide	9	3.52 %	Ranitidine	1	0.39 %
Cefixime	9	3.52 %	Alprazolam	1	0.39 %
Phenytoine	7	2.74 %	Buspirone	1	0.39 %
Cetirizine	5	1.96 %	Haloperidol	1	0.39 %
Asam Folat	5	1.96 %	Amoxicillin	1	0.39 %
Diazepam	4	1.56 %	Mebendazole	1	0.39 %
Mebhydrolin	4	1.56 %	Clindamicin	1	0.39 %
Vitamin B1 (Thiamin)	4	1.56 %	Clobazam	1	0.39 %
Paracetamol	3	1.17 %	Levotiroksin	1	0.39 %
Niacin	3	1.17 %	Isoniazid	1	0.39 %

Discussion

The profile of divided medical powder prescriptions was analysed from the subject (patients) and the object (drug). In this study, 152 prescriptions suited the inclusion criteria to analyse its profile. The profile of the prescription was presented in Figure 1. It was shown that children from 0-5 years old were likely to get a divided medicinal powder as the medication. It is reasonable because, at those ages, they may have difficulty in taking a tablet or comply when given more than one drug (dosage form). Besides, the licensed product is also considered expensive (Setyani & Putri, 2019; C. Wiedyaningsih *et al*., 2012). This phenomenon was also shown in a study by Piliarta (2012), which stated that children under five years old tended to get compounded drugs than the older children (Piliarta *et al*., 2012). Meanwhile, there are about 3% of the patients with the age range 12-18 years old also get a divided medicinal powder. At this age, children should be able to get a pill or tablet as a medicine to reduce the number of compounded drugs.

The divided medicine powder can contain one or more active substances, which are mixed into one dosage form. The amount of active substances given to the patient depends on the severity and the doctor's diagnosis based on the symptoms and condition of the patient (C. Wiedyaningsih *et al.*, 2012). An analysis of the number of active substances in each prescription is useful to evaluate the prescription profile. There were 62% of prescriptions that were prepared with only one active substance. As seen in Figure 1b, the compounding order containing five active substances had the lowest frequency. This result shows that compounding practice in the hospital has compromised the potency of the compounded drug, thus increasing incompatibility and instability. The incompatibility and instability in the compounded drug will increase as more active substances are added (Setyani & Putri, 2019; C. Wiedyaningsih *et al*., 2012).

Compounding drugs using one type of active substance is usually carried out for several reasons, such as because of the limited licensed preparations for

children (syrup, drop) or because the available licensed preparations are not affordable (Setyani & Putri, 2019). In this study, the active substances that were compounded were recorded, and their frequency was calculated. From the 152 collected prescriptions, 38 active substances were compounded (Table I). These active substances were then examined for their availability in licensed products for paediatric patients.

Based on Table I, the five types of active substances mostly compounded into divided medicine powder were Triamcinolone acetonide as an anti-inflammatory drug, Phenobarbital as antiepileptic, Salbutamol as an anti-asthmatic drug, Levotiroxin Na as an antithyroid drug to treat hyperthyroidism, and Bromhexine as a mucolytic agent. In March 2020, it appeared that there are certain drug prescriptions that are higher than other months. It is because the weather and climatic condition in Yogyakarta Special Region from January to March 2020 was cold and rainy. It caused the children with low immunity to have a common cold, asthma, rhinitis allergic, etc. It was reasonable that these drugs have a high prescribing frequency. The results of this study are in line with the research of Wiedyaningsih and Oetari (2005), who also found that compounded drugs are prescribed primarily for the purpose of treating respiratory diseases and allergies.(Chairun & Wiedyaningsih. 2005).

The availability of licensed products for paediatric patients was analysed from all the active substances that were compounded. The results showed that from 152 prescriptions obtained, 38 active substances were prescribed for compounding (Table 1). The evaluation of their availability in licensed products for paediatrics based on the literature (MIMS Consultation Guidelines 2019/2020 and ISO Indonesia volume 52 (2019) showed that four drugs were not available for paediatrics. Specifically, these drugs were not available in a single composition with a suitable dosage form for children, and there was no available information on the paediatric dose. These four drugs were Thiamin, Niacin, Pyridoxine, and Buspirone. However, the other active substances that were available in licensed products were still compounded.

There are some limitations in this study that might be improved. Observation for the reason to compound the drug from the doctor should also be considered in this study. It will give more information why a lot of drug which already available in the licensed product was still compounded. Drug compounding has some issues about the stability of the compounded products, the accuracy in dose strength, and the lack of standard protocol (Gudeman *et al*., 2013; Kristina *et al.*, 2017). The pharmacy department should conduct a risk assessment of active substances that are routinely used or have a high frequency of compounding. It may prevent medication errors in the dispensing stage. If the results of the risk assessment and risk analysis show that there is a potential risk (either related to quality, efficacy, or safety) to the compounded drugs, it will be better if the drugs are delivered with the available licensed products to minimise or eliminate the risks (Jackson & Lowey, 2010).

Conclusion

The profile of divided medical powder prescriptions in this hospital from January to March 2020 were mostly written for paediatrics patients under five years old. The most compounded drug contained one active substance. There are 34 active substances available as licensed products for children. However, there are four active substances that are not available as licensed products for children, namely Thiamin, Niacin, Pyridoxine, and Buspirone. The results of this study are very useful for hospitals to identify what drugs are actually available in licensed products, to minimise the frequency of compounding and reduce the risk of medication errors in drug compounding. To minimise the risk of errors at the dispensing stage, it is recommended that these 34 active substances are delivered in available licensed products. Comprehensive research is needed to better describe the profile of compounding prescription, so it can assist pharmacists in hospitals in formulating strategic steps to reduce the risk of medication errors due to drug compounding.

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RESEARCH ARTICLE

The potential of banana fruit *Ranggap* (*Musa paradisiaca var. Troglodytarum*) as an excipient alternative to oral tablet dosage form

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Abstract

Introduction: Starch is one of the ingredients that has many benefits, including in the pharmaceutical field, especially as a pharmaceutical excipient in pharmaceutical formulations. **Aim:** This study aims to isolate, characterise, and formulate the starch of banana fruit (Musa paradisiaca var. Troglodytarum) into tablet dosage forms. **Methods:** The characteristics of the perceived banana starch can be said to be comparable to that of corn starch so that it is expected to be used as a source of starch which can be used as a pharmaceutical excipient. The starch of isolated banana fruit was used as a filler, binder, and crusher in the wet granulation method tablet formulations with concentrations of 2%, 3%, and 5%. **Results:** The physicochemical characteristics of starch isolated from banana fruit are considered to meet the requirements of pharmaceutical excipients required in the Handbook of Pharmaceutical Excipients 6th edition and the United States Pharmacopeia 32nd edition. **Conclusion:** Of the total formulas tested, tablets with binder content of banana starch 3%, 5% and 10% corn starch meet the tablet evaluation requirements.

Introduction

Most of the pharmaceutical companies in Indonesia have only carried out the stage of formulating the final product into pharmaceutical preparations, while 96% of the raw materials used are still imported from abroad. Formulated pharmaceutical preparations are a complex system, which consists of several components, including active pharmaceutical ingredients (API) and excipients. Some of the objectives of adding excipient include protecting the active substance, increasing the stability of the API, and increasing the safety and effectiveness of the preparation itself (Pawar, P.D., 2015).

According to the International Pharmaceutical Excipient Council, the excipient is a substance other than a drug that is included in the manufacturing process. In tablet dosage form, the US Pharmacopeia-National Formulary (USPNF) classifies excipients based on their function at the time of formulation, such as binders, disintegrants, and others (Chaudari, 2012). One of the excipients that are often used in formulations with function as a binder or disintegrants in tablet preparations is starch. Starch is one of the carbohydrates stored in plants and is found in many plant organs such as seeds, roots, fruits and tubers. It is widely used because it is easy to obtain, has inert properties, is cheap, and can be used as a filler, binder, crusher, and lubricant (Hu A. *et al*., 2015).

Starch is a compound that has a high molecular weight consisting of glucose polymers which are branched together with glycosidic bonds. Starch is one type of

important polysaccharide that is found in several plants that are spread in nature and can be extracted from its sources, such as cereals (rice, wheat, corn), tubers (cassava, sweet potato, potato), and palm stem pith. (sago, palm, new sago). Starch is composed of two different glucan chains, namely amylose (a linear polymer of D-glucose in a 1,4 glycosidic bond) and amylopectin (a branched polymer of d-glucose in 1,4 and 1,6 glycosidic bonds) (Hartesi, 2016).

Amylose is a straight-chain consisting of glucose molecules that bind to α-1,4-D-glycosidic. The number of glucose molecules on the amylose chain ranges from 250-350 units. The length of the polymer chain will affect the molecular weight of amylose, and the length of the polymer chain is strongly influenced by the starch source. The degree of amylose polymerisation ranges from 500-6000 glucose units depending on the starch source; the amylose structure can be seen in Figure 1.

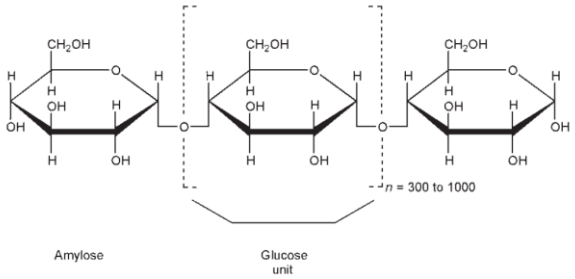


Figure 1: Amylose structure

The chemical structure of amylopectin is basically the same as amylose consisting of a short-chain α- (1,4) -D-glycosidic. The difference is that amylopectin has a high degree of branching and has a greater molecular weight in the presence of α-1,6-D-glycosidic bonds, where each branch contains 20-25 glucose units. The degree of polymerisation of amylopectin is also higher than amylose, which is between 105 to 3x106 glucose units (Hustiany, 2006). The structure of amylopectin can be seen in Figure 2.

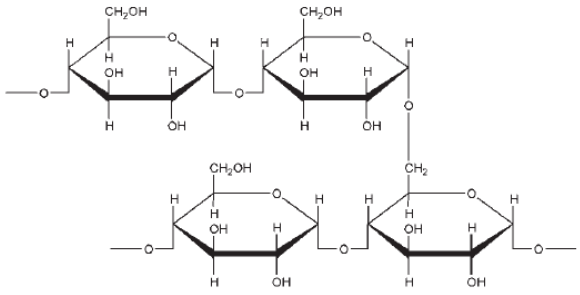


Figure 2: Amylopectin structure

Various attempts have been made to develop starch as an additional ingredient in tablet dosage formulations, for example, a starch made from cassava and durian seeds (Sapri, 2012), which function as binders and disintegrators.

Bananas are picked when the fruit is old but still green, with a total sugar content of 0.1% and starch up to 35%. In the fruit ripening process, there is an increase in total sugar because most of the starch is converted into sugar. The high starch content in bananas is expected to be used as a source of starch, which can then be used as a pharmaceutical excipient (Jime´nez-Herna´ndez, 2007).

Ranggap bananas (*Musa paradisiaca* var. Troglodytarum) is unique because it has a large fruit size and an upward bunches. Ranggap bananas are found in the foothills of Mount Galunggung, Tasikmalaya and other places, namely Lumajang, East Java. So far, it is known that the area of distribution is from Maluku to Papua, which is widely known by the surrounding community as Pisang Tongka Langit (E. Samson *et al.*, 2013).

The people around Mount Galunggung, ranggap bananas are considered commonly consumed not as bananas that are commonly eaten, but as a diabetes medicine and increase male vitality because they are not sweet.

In this research, the processing of the considered banana fruit into starch is carried out to increase the economic value for the community. Ranggap Bananas start producing at the age of 1–1.5 years. The flowering time happens throughout the season. Seven months after flowering can be harvested, the number of fruits is 5-8 per comb. The colour of the ripe fruit is brownish yellow; some are red according to the type. Fruit length reaches 17-23 cm, fruit weight 250-300 grams, and a diameter of 5-6.3 cm (Satuhu and Supriyadi, 2005). It can be an alternative for raw material needs, especially additives in the field of pharmaceuticals. The processing was also conducted to evaluate whether the starch from the banana fruit meets the Pharmaceutical Grade standards required by the USP and Handbook of Pharmaceutical Excipients. In this study, the starch from the isolated banana fruit was used as a binder in tablet preparation with metamizole as the drug model.

Methods

Plant determination

Ranggap Bananas (*Musa paradisiaca* var. Troglodytarum) were obtained from farmers in the foothills of Mount Galunggung, Tasikmalaya, with a fruiting age of seven months and estimated 10-15 days

before normal harvest. The banana was determined in Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Isolation procedure of banana starch

Isolation of the wild banana starch begins with peeling and soaking in water (1: 1) for an hour. Subsequently, chopping and soaking again with sodium metabisulfite solution with a concentration of 0.1 N for 16 hours were carried out by stirring several times.

After soaking, the ingredients were rinsed with distilled water and filtered using a filter cloth (Flannelette) to separate the starch and dregs. Then water was added to the dregs with a ratio of 1: 1 (w/v) to remove the remaining starch to be refiltered afterwards. The filtering process was repeated depending on the amount of starch until the filtrate was clear. The filtrate was allowed to stand for 12-16 hours so that it settles. After settling, the clear part was discarded. The precipitate was dried in an oven at 50^{o}C for 12 hours or until dry with a moisture content of less than 5%.

Characteristics of starch from banana fruit

Organoleptic test

The starch of isolated banana fruit was tested organoleptically, including smell, taste, colour, and visual shape with corn starch as a comparison.

Qualitative identification

An amount of 100 mg of starch from isolated banana fruit was analysed using several specific reagents to test for the presence of starch, including iodine to prove that the isolated sample was starch, and Fehling A and Fehling B reagents to prove that sugar has not been formed from the isolated starch (Prajapati *et al.*, 2013).

Fourier Transform Infrared (FTIR)

The starch of isolated banana fruit is pelleted by grinding with potassium bromide. Then, the Infrared spectrum of the sample was read with FTIR with corn starch used as a comparison. The measurements were made at a wavelength of 4000-400 cm^{-1}.

Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX)

The starch of isolated banana fruit was tested using Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX) to determine the surface morphology of the particles. The sample was placed on an aluminium stub with an adhesive conductive double-sided carbon pad and was then pressed so that the sample was evenly distributed and no air was trapped. The picture was taken with a voltage of 20.00 kV with various magnifications.

Particle Size Analyser (PSA)

The technique of measuring particle size and particle size distribution was by dispersing 10 mg of starch particles from isolated banana fruit each into 10 mL of distilled water and was then characterised using a PSA at a temperature of 25^{o}C.

Microscopic testing

The sample was prepared using liquid paraffin on a slide; then, the shape was seen using a light microscope.

pH testing

A banana starch sample of five grams was mixed with 40 mL of distilled water, shaken for 20 minutes, and centrifuged. The pH of the supernatant fluid was between 4.0 and 7.5 as measured by a pH meter (United States Pharmacopeia 32, 2009).

Test for heavy metal contamination and mineral content

Testing of the limits of heavy metal and mineral content was carried out using Atomic Absorption Spectrophotometer Spectroscopy (AAS). The heavy metals tested were Lead (Pb), Mercury (Hg), Arsenic (As), Tin (Sn), and Cadmium (Cd). Minerals that will be tested include potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and sodium (Na).

Microbial limit testing

A sample of one gram was dispersed in ten parts of a phosphate buffer solution pH 7.2 and was then homogenised using a vortex. Dilutions of 10^{-1} and 10^{-2} were made. The test for aerobic bacterial contamination was carried out using the Total Plate Number method. A total of 15-20 mL of Tryptone Soya Agar was poured into a petri dish, then 1 mL of the 10^{-1} and 10^{-2} dilution of the sample dispersion was added to the solid media. Petri dishes were incubated at 37^{o}C for 48 hours, counting the amount of aerobic bacterial contamination. The mould and yeast contamination test was carried out by using the Yeast Mould method using Sabouraud Dextrose Agar as much as 15-20 mL, which was poured into a petri dish. After solidifying, 1 mL of the 10^{-1} and 10^{-2} was a dilution of the sample dispersion added. Petri dishes were incubated at 20^{o}-25^{o}C for 72 hours. The amount of mould and yeast bacterial contamination was calculated (United States Pharmacopeia 32, 2009).

Physical properties of starch from Ranggap banana fruit

Bulk density determination

A sample of 10 g (W) was put into a measuring cup, the top surface of the powder was flattened and read the bulk volume (Vo) was read.

Determination of compressive density

A sample of 10 g (W) was put into a measuring cup, and the top surface of the powder was levelled. The tool was turned on and stomped 500 times, and the volume of the powder was read, then stamping a second time 750 times and reading the compressed volume (V_1). After knowing the bulk volume and compressed volume, the compressibility index was evaluated with the formula (Ohwoavworhua *et al.*, 2009). Compressibility(%)=(Bulk Volume-Compressed Volume) / (Compressed Volume) x 100%.

Determination of density

A dry, clean, and calibrated pycnometer was used by setting the pycnometer weight and paraffin weight. The test substance was entered into the pycnometer, and paraffin was added to the pycnometer to the maximum volume. The density was obtained by dividing the weight of the substance by the volume occupied by the substance in the pycnometer (Farmakope Indonesia V, 2014).

Moisture determination

Placed in an aluminium plate, the sample was weighed as much as 1 g. Samples were dried at 105^{o} C using a moisture balance (United States Pharmacopeia 32nd, 2009).

Determination of flow rate and angle of repose

The starch powder was put into a flow time test funnel. The flow time, height, and diameter of the powder coming out of the funnel were recorded to determine its angle of rest using millimetre graph paper. An angle of repose between 20°-40° and a flow time of more than 10 g/s indicates a good flow.

Tablet formulations

One application of the starch from the isolation of banana fruit can be used as additional material for tablet preparations. The tablet formula with the API of metamizole with the formula design is presented as in Table I.

Table I. Formulation of tablets

Ingredients	F1	F2	F3	F4
Metamizole	500 mg	500 mg	500 mg	500 mg
Banana starch	2%	3%	5%	---
Corn starch	---	---	---	5%
Amylum	5%	4%	3%	3%
Mg-stearate	2%	2%	2%	2%
Talc	1%	1%	1%	1%

Evaluation of tablets

The compressed mass that had been evaluated was compressed using a tablet machine with a tablet weight of 600 mg with a diameter of 12 mm and a hardness of 7-12 kg/cm^2. The resulting tablets were evaluated, repeated 20 times and tested statistically based on ANOVA, including uniformity of size, uniformity of weight, tablet hardness, disintegration time test, friability, friction, and dissolution. The results of the dissolution test were comparable with the PhEq-bootstrap software.

Results and discussion

Material collection and plant determination

The results of the determination showed that the plants examined were banana species (*Musa paradisiaca* var. Troglodytarum).

Isolation of banana starch

The starch obtained from the isolated banana fruit was 21.5%. This is consistent with the literature where bananas contain up to 35% starch (Jime´nez-Herna´ndez, 2007).

Characteristics of banana starch

Organoleptic examination

Organoleptic examination of the starch isolated from banana fruit was evaluated in terms of shape, taste, colour, and smell. The USP Standard and Handbook of Pharmaceutical Excipients for starch have characteristics such as odourless, white or pale white powder, consisting of very small round or ovoid granules, and tasteless. It shows the criteria that do not meet the standards organoleptically according to the sixth edition of the Handbook of Pharmaceutical Excipients, where the required colour is white, while the colour produced by

banana starch shows ivory white colour (See Figure 3a and 3b).

(a) (b)

Figure 3a: Corn starch; 3b: Banana starch

Qualitative identification

In simple identification using iodine solution, the sample of starch from isolated banana fruit gives a blue colour and the formation of a purple ring. This shows a positive result where the tested sample contains starch. In the swelling test, namely, by adding hot water to the starch sample, a thick starch solution was formed. Meanwhile, the addition of Fehling A and B solutions did not change colour. From these data, it can be concluded that the starch samples showed positive results according to the characteristics of starch, and no starch was formed into sugar.

Fourier Transform Infrared (FTIR)

In the infrared spectrum test, the starch samples from the isolation of banana fruit were tested with sodium metabisulfite and corn starch as a comparison for starch. Figure 4 shows almost the same spectrum. The results of observations using Fourier Transform Infrared (FT-IR) conclude that the infrared spectrum of the starch samples from isolated banana fruit compared to corn starch gives a spectrum with a similar pattern. This shows the similarity of the functional groups even though there are differences in wavenumber areas. (2600-2000 cm^{-1}). The infrared absorption spectrum shows the main absorption at wavenumbers 3363 cm^{-1}, 2931cm^{-1} and 1656cm^{-1}. The peak at a wavelength of 3363 cm^{-1} indicates an OH group, at a wavelength of 2931cm^{-1} indicates a C – H group, while at a wavelength of 1641.88 cm^{-1} indicates an OH bending, and at a wavelength of 1356.46 cm^{-1} indicates a C – H group bending. The test results shown in Figure 4, using Fourier Transform Infrared (FT-IR), shows that the starch samples are similar, but there are still differences in the fingerprint area. This difference may occur due to differences in starch raw materials.

Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDX)

SEM-EDX test was carried out to see the morphology of the starch from the banana fruit. Imaging results with magnifications of 500 and 1000times show that starch has an oval shape with relatively uniform size and shape. The imaging results with a magnification of 1000 and 5000 times, on the other hand, shows the surface shape of the starch that looks smooth and regular, as shown in Figure 5.

Particle Size Analyser (PSA)

Testing using the PSA aims to analyse the size distribution of the banana starch. From the analysis, it was found that the particle size was at 2-60µm. The starch sample from which the fruit of banana fruit was tested produced a normal distribution curve where the 28.70 µm starch particles dominated the sample; this result shows that the starch sample tested had a relatively uniform size and of a good particle size characteristic as shown in Figure 6.

pH testing

The results of pH testing using a pH meter against the 1% (w / v) dispersion in distilled water showed that the isolated banana starch had a pH of 6.82. The same result was also shown by corn starch, which had a pH value of 6.98. The 1% dispersion of the two samples shows a neutral pH value and meets the pH standards required by the Handbook of Pharmaceutical Excipients 6th edition, where the standard pH of starch is 4-7.

Test for heavy metal contamination and mineral content

The result shows that starch samples isolated from the banana fruit and the place where the banana plants grew were not contaminated by the presence of heavy metal waste. Metal contamination in a material isolated from plants depends on the environment in which it is grown, which can cause organ damage if consumed for a long time (Jaishankar e*t al.*, 2014).

Microbial limits

The test results of the Total Plate Number (ALT) for 24 hours and the Khamir Yeast Rate (AKK) for 27 hours showed that the microbial limit test of the starch samples from isolated banana fruit showed the total plate number (ALT) of 0.7 x 102 CFU g and Yeast Mould Rate (AKK) of 0.67 x 102 cfu/g. These results meet the requirements criteria according to the sixth edition of the Handbook of Pharmaceutic Excipients for ALT<103 cfu/g and AKK<102 cfu/g. The microbial contamination produced by starch products is influenced by several factors, including the storage process of the resulting product.

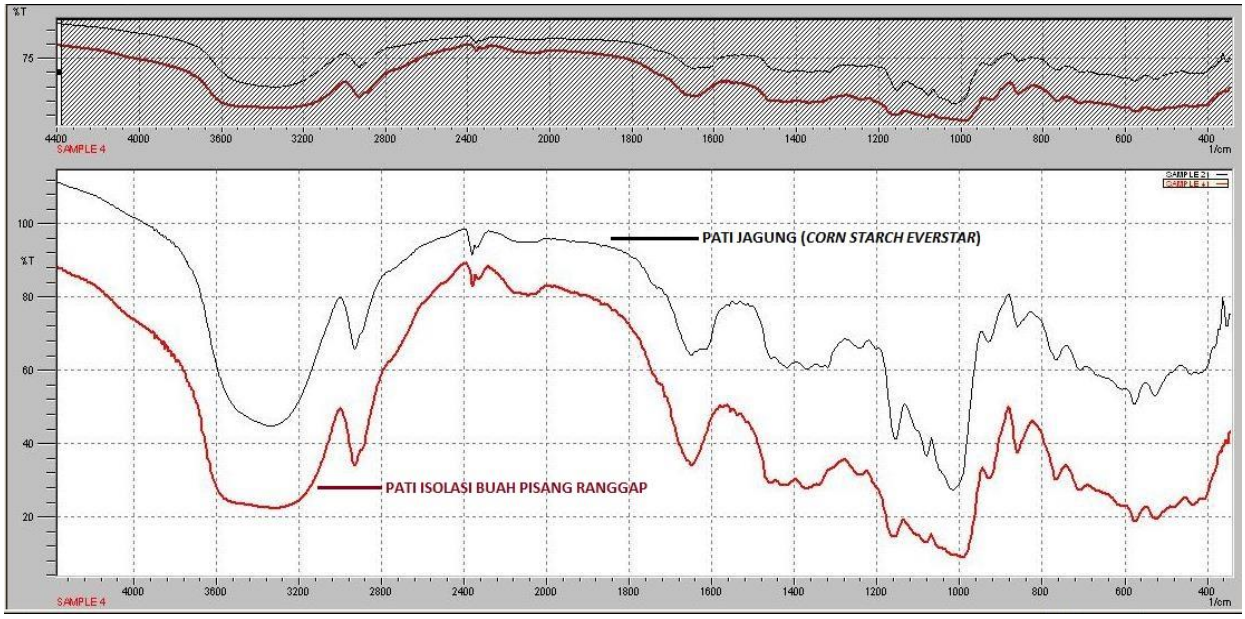
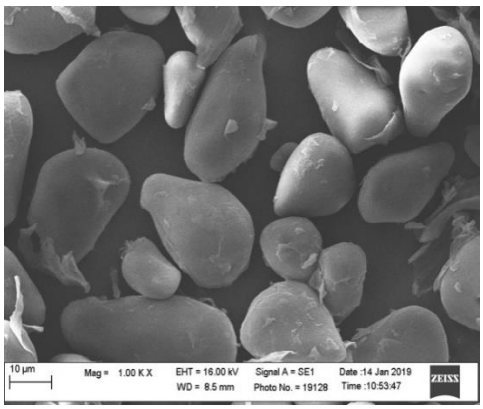
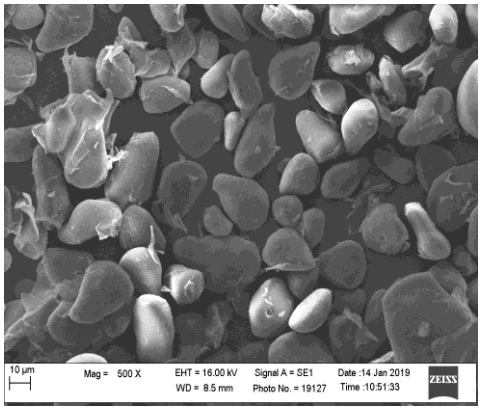


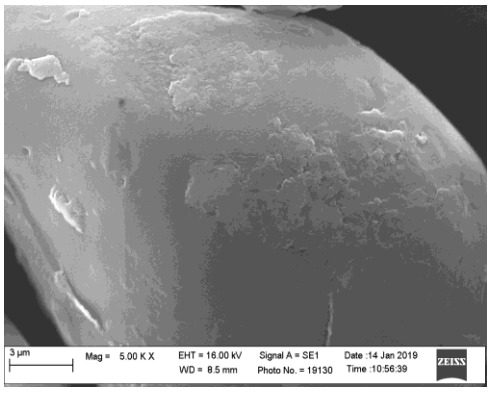
Figure 4: Results of Fourier Transform Infrared (FT-IR) Corn Starch (comparison) and Starch Banana Fruit



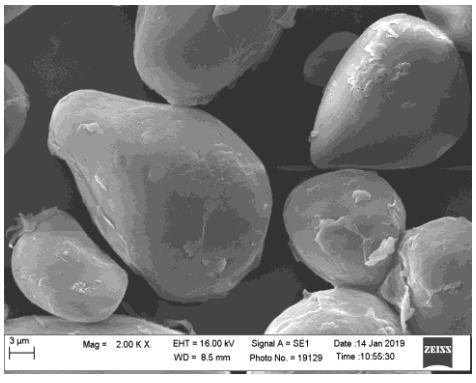
(a) 500x magnification



(b) 1000x magnification



(c) 2000x magnification



(d) Magnification of 5000x

Figure 5: Results of Scanning Electron Microscopy

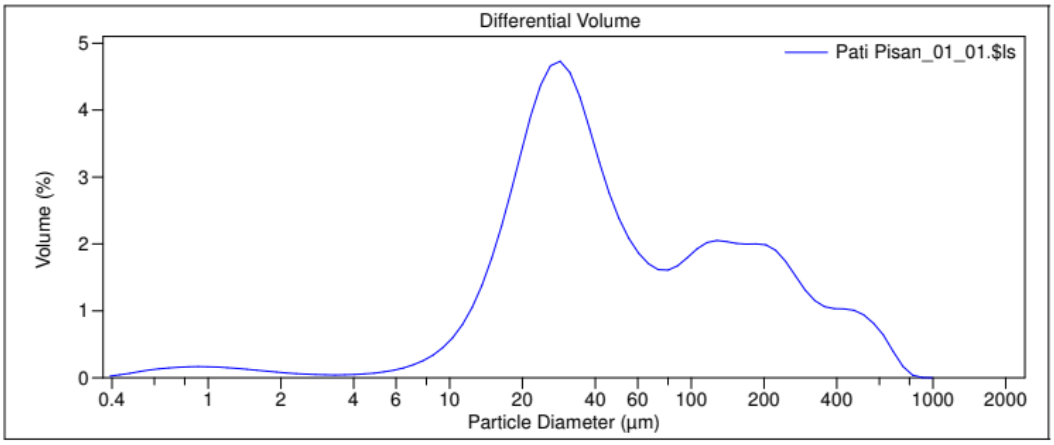


Figure 6: Results of the size distribution of banana starch

Sulfite residue testing

Sulfite residue testing was carried out to see the possibility of sulfite content remaining in the sample. This possibility can be caused by the use of sodium metabisulfite in the starch isolation process. The qualitative test was carried out by dissolving the sample with distilled water and adding barium chloride to the filtrate. The white liquid colour of the mist will be seen if the sample is positive for sulfite. The test results on the banana starch were considered to produce negative results.

Physical properties of granules

Testing of perceived banana starch includes examining according to the sixth edition of the Handbook of Pharmaceutical Excipients. In this test, the perceived banana starch was compared with corn starch. From the physical test data, it can be seen that the banana starch and corn starch tested showed quite good results when compared with the requirements, where banana starch and corn starch had a fairly good flow rate and angle of repose. This data supports the use of starch as an excipient for tablets by the direct pressing method. If it is necessary to increase the flow rate and the angle of repose that does not affect the physical properties of the active substance at the time of tablet making, it is better to use the wet granulation method (See Table II).

Tablet Formulations

The tablet formulation was carried out using starch from the isolated banana fruit as a binding agent in the form of starch paste. The process of making the mass ready for printing was done using the wet granulation method. Preparation of tablets with wet granulation has the advantage of increasing the particle size of the powder and the uniformity of the particle size so that it can improve the flow time of medicinal substances, especially those with poor flow times such as metamizole.

Table II: Physical properties of granules

Parameter	Banana starch	Corn starch	Standards of USP 32
Bulk density (g/cm^3)	0.56 ±0.0577	0.67±0.00223	0.45–0.58
Tapped density (g/cm^3)	0.70±0.1000	0.78±0.0215	0.69–0.77
True density (g/cm^3)	1.336±0.1155	1.447±0.0210	1.478
Compressibility (%)	16.26±1.4563	15.18±1.241	< 30
Haussner ratio	1.24±0.02165	1.18±0.0114	--
Flow rate (g/s)	4.59±0.04123	4.62±0.0123	--
Angle of repose	23.4^0 ±0.2130	28^0±0.0021	--
Humidity (%)	4.06±0.032	4.12±0.0210	< 12
pH	6.82±0.0012	6.98±0.0150	4.0–7.0

The binder made from starch from the isolation of banana fruit was made with a concentration of 2%, 3%, and 5%, while corn starch was used as a comparison binder with a commonly used concentration of 5%. Tablets were made using metamizole as the active ingredient, and starch in paste form was used as the inner phase binder. The active substance was added to the binder with each formula to obtain a mass that is easily clenched. The wet mass, which is easily clenched, was put into the granulator to granulate with 16 mesh. The granules were then dried at 40^0 C for 16 hours. The dried granules were then granulated again using 24 mesh. The results of granulation were tested for moisture content with a moisture content requirement of not more than 5%, and external phase substances such as starch, talc, and Mg Stearate were added.

All tablets in this test met the requirements for tablet evaluation, namely <0.8%. The results of statistical tests using SPSS 18 with the Anova method showed no significant difference, p=0.413 (p>0.05)

Granule flow properties

In testing the granule flow time, it gives good results, which is less than 10 seconds/100 grams. The test results obtained from the whole formula have a flow time of fewer than 10 seconds/100 grams. A good powder flow is needed for a uniform filling process into the hole of the tablet or dies so that it produces tablets with a uniform volume (Siregar, 2010). In testing the angle of rest, the whole formula has a value of angle of rest of <250, meaning that the flow is good. The angle of repose ranges from 25^0-$45^{0,}$ and the lower angle of rest indicates a better characteristic.

T*he Compressibility Index and the Haussner Ratio*

A good granule has a compressibility index of <21%. All the formulas were tested to produce a compressibility index of <21% so that it can be said to meet the requirements. The Haussner ratio was calculated by dividing the tapped density by the bulk density. The Haussner ratio value is said to be very good if the value is close to 1 and categorised as good if the value is <1.25. The higher the Haussner ratio value, the worse the flow of the powder/granule will be. From the test results, all formulas have a Haussner ratio of <1.25. This indicates that the Haussner ratio of the entire formula can be categorized as good. The results of the physical evaluation of the ready-to-compress mass (Table III).

Table III: Mass evaluation of the granule

Parameter	F1 (2%)	F2 (3%)	F3 (5%)	F4 (comparison)
Bulk Density	0.61±0.001	0.67±0,002	0.53±0,002	0.57±0,001
Tapped Density	0.71±0.002	0.80±0,001	0.65±0,002	0.69±0,001
Haussner Ratio	1.18±0,012	1.20±0,011	1.23±0,012	1.21±0,011
Compessibility	15.15±0,121	16.67±0,213	18.42±0,211	17.14±0,213
Angle of repose	34.61±1,230	30.06±1,211	32.08±1,113	29.47±1,212
Flowability (g/s)	13.66±1,111	15.54±1,211	15.07±1,112	14.50±1.211

The results of the evaluation of the friability of tablets F3 (banana starch 5%) and F4 (corn starch 5%) show that the required value is less than 1%, while the friability of F1 tablets (respondent banana starch 2%) and F2 tablets (banana starch 3%) shows friability results of more than 1%. Per cent friability is influenced by the amount of fine powder (fine powder) so that the compressed mass is not tightly bound at the time of pressing and will be released when there is friction between the tablet and the tablet or the tablet with the packaging.

Table IV. Evaluation of Metamizole tablet

Parameter	F1	F2	F3	F4
Weight (mg)	598.76 ± 5.42	603.11 ± 7.34	610.07 ± 4.98	597.91 ± 9.44
Hardness (kg/cm^2)	5 ± 1.20	8 ± 1.20	9 ± 1.20	8 ± 1.20
Thickness (mm)	0.58 ± 0.06	0.61 ± 0.08	0.63 ± 0.10	0.61 ± 0.08
Diameter (mm)	1.20 ± 0.01	1.20 ± 0.01	1.20 ± 0.01	1.20 ± 0.01
Disintegration time (minutes)	3.50 ± 0.012	9.15 ± 0.012	10.09 ± 0.012	5.44 ± 0.012
Friability (%)	1.18 ±0,011	1.74±0,012	0.22±0,021	0.60±0,0120

Dissolution

The tablet dissolution test was carried out on Formula 2, Formula 3 and Formula 4 tablets on the grounds that the tablets with these formulations had tablet characteristics that met the requirements for the dissolution testing process. The dissolution was carried out by method II; namely, a paddle using distilled water as much as 900 meters in a dissolution vessel and a rotation speed of 50 rpm with a temperature set at 37^0 C. Measurement of the metamizole wavelength absorption was based on literature at a wavelength of 260 nm (Table V and Figure 6).

Table V: Dissolution percentage of three Metamizole tablets with Banana starch and corn starch

Metamizole tablet with	Time (Minute)	1	5	10	15	20	30	40	50	60
3 % banana starch	% Dissolution	13.17± 0.0570	45.0 ± 0.100	69.11± 0.1155	83.40 ± 1.1234	88.84 ± 1.1134	96.73 ±1.1125	102.45± 1.1126	102.45± 1.1161	101.09± 1.1123
5 % banana starch	% Dissolution	14.53± 0.0653	37.53± 1.1116	73.73± 1.1115	80.67± 1.1123	86.80± 1.1132	96.73± 1.1114	97.01± 1.1132	98.50± 1.1121	99.59± 1.1116
5 % corn starch	% Dissolution	17.6± 0.0567	49.10± 0.1005	73.73± 1.1115	84.89± 1.1231	92.79± 1.1123	101.09± 1.1121	97.01± 1.1132	100.27± 1.1104	99.86± 1.1132

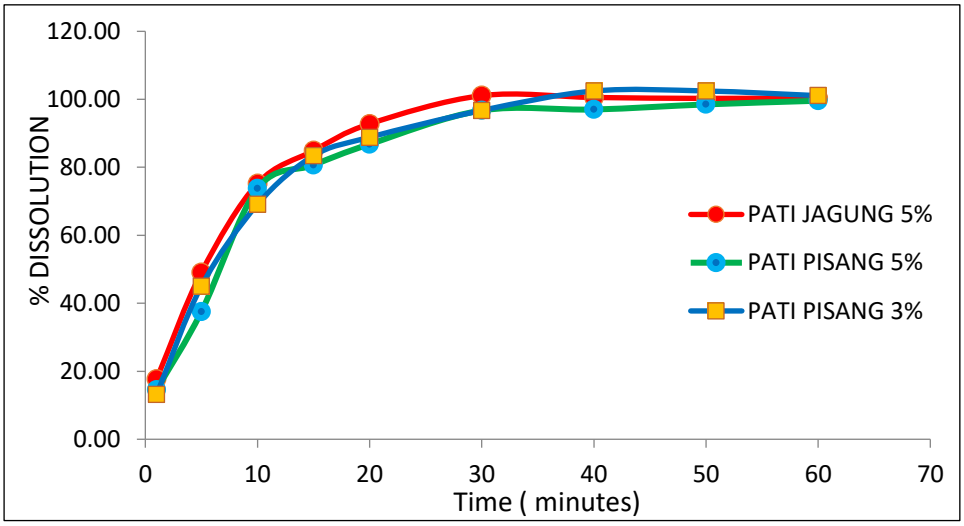


Figure 6: Dissolution profile of Metamizole tablets with 3%, 5% of banana starch binders and 5% corn starch

Indonesian Pharmacopoeia requires the release of metamizole tablets of more than 80% at 15 minutes. Based on the results of the dissolution test, the three sample formulas show a fairly good dissolution profile and meet dissolution requirements, but tablets formulated with banana starch are thought to show a slower dissolution process than corn starch. These results can be influenced by the disintegration power, causing the cohesiveness of the bonds between particles in the granules that are stronger than the formulation of corn starch tablets. The speed of disintegration affects the effectiveness of drug release for systemic absorption, but what is more influential is the speed of dissolution (J. Sinko, 2011). In this case, the binder concentration, viscosity, and swelling power of the binder affect the dissolution profile of the tablet preparation.

The similarity between tablet formulas can be seen from the calculation of the similarity value of the comparable dissolution test.

Conclusion

The starch of isolated banana fruit can be isolated by immersion technique with 0.1 N sodium metabisulfite solution for 16 hours and resulted in a yield of 21.5%. Starch isolated from banana fruit has functional characteristics that can be used as a pharmaceutical excipient especially in oral tablet formulation.

The results of the insulated banana fruit meet the additive standards Pharmaceutical Grade required by the USP and Handbook of Pharmaceutical Excipient.

Banana starch as a binder for metamizole tablets fulfills the requirements for a tablet dissolution test of more than 80% at 15 minutes. Based on the dissolution test results of the three sample formulas, the dissolution profile is quite good and meets dissolution requirements.

Reference

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IAI CONFERENCE

RESEARCH ARTICLE

Correlation between the antioxidant capacity of plasma and blood glucose level

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Abstract

Introduction: Oxidative stress on tissues can cause diseases such as diabetes mellitus (DM). **Aim:** This study aimed to pharmacologically evaluate the decrease in blood glucose levels and its relationship with the total antioxidant capacity of the blood compared to glibenclamide. **Method:** An experimental study with completely randomised designs was carried out. Rats were induced with streptozotosin followed by ethanolic extract for ten days. **Results:** The One-Way Anova test, showed that the increase of the total antioxidant capacity of plasma treated with ethanolic extract of *Tinospora cordifolia* and *Curcuma zanthorrhiza* was comparable in the same amount to glibenclamide (p=0.345), (p=0.289). There was a relationship between total blood antioxidant capacity and blood glucose levels, this linear association was expressed with the following mathematical equation: y = 20,253 - 2,946x. **Conclusion**: The antioxidant content of *Tinospora cordifolia*, *Curcuma zanthorrhiza*, and *Cinnamomum verum* has the potential to control blood glucose in diabetes mellitus.

Introduction

Diabetes mellitus can be caused by oxidative stress and oxidative damage in tissues. These can also cause other diseases such as atherosclerosis or rheumatoid arthritis. Patients with type 2 diabetes mellitus often have various tissues affected by oxidative stress, including pancreatic β cells (Tangvarasittichai, 2015).

Glucose can be oxidized before binding to proteins, as glycated proteins can be oxidized to produce reactive oxygen species (ROS) (Tiganis, 2011). Hyperglycemia exacerbates the formation of ROS by several mechanisms. ROS increase the expression and formation of tumour necrosis factor- α (TNF- α) and exacerbate oxidative stress. TNF-α can cause insulin resistance in many ways, such as by decreasing the autophosphorylation of insulin receptors, changing the substrate for insulin receptor 1 to inhibit insulin receptor tyrosine kinase activity, decreasing the sensitivity of glucose insulin transporter (GLUT-4), increasing the circulation of fatty acids, changing its function, ß cells and increasing triglyceride levels and by decreasing HDL levels. Previous studies have shown that TNF-α injection in healthy test animals will reduce insulin sensitivity due to hyperglycemia without decreasing plasma insulin levels (Dewanjee *et al.*, 2018).

Antioxidants can decrease free radical levels as proved by Luo and the authors (2019), and thereby reducing insulin resistance (Luo *et al.*, 2019). Antioxidants can decrease reactive oxygen species (ROS), which as a result reduces oxygen which will bind to free electrons released due to the electron chain leak. The reaction

between oxygen and free electrons produces ROS in mitochondria (Annisa *et al*., 2014).

Secondary metabolites found in plants can act as antioxidants; an example of these are flavonoids. Flavonoids derived from vegetables and medicinal plants have beneficial effects on diabetes by improving glycemic control, lipid profile, and antioxidant status. The antioxidants in flavonoids can donate their hydrogen atoms. Flavonoids will be oxidized and bind to free radicals so that the free radicals become more stable compounds (Ghorbani, 2017).

Several studies have been conducted on Indonesian herbs that are used for anti-diabetes in order to study their antioxidant activity. Most of these studies were conducted *in vitro*, such as a study conducted by Rui Wang, in which he examined the composition of volatile compounds in five species of cinnamon. In his research, it was known that the cinnamon antioxidant activity was 45.42% by using the DPPH method (Wang *et al.*, 2009). Cinnamon twig bark has the highest antioxidant activity compared to bark and branches assayed semiquantitatively by using the DPPH method (Ervina *et al*., 2016). The standardized extract of *Curcuma xanthorrhiza* and the active component *Xanthorrizol* significantly weakened the induction of a high-fat diet (HFD) against hyperglycemia and insulin resistance (Kim *et al*., 2014). Puranik conducted a study looking at the antidiabetic activity of *Tinospora cordifolia*. According to his study, *Tinospora cordifolia* had significant antidiabetic activity in diabetic rats by 40% to 80% compared to insulin (Puranik *et al*., 2010). Another plant that has antidiabetic potential is *Averrhoa bilimbi L* because its leaves contain flavonoids. Flavonoids function as antioxidants and antidiabetics (Alhassan & Ahmed, 2016). In previous studies that examined flavonoids in several Indonesian plants, researchers wanted to evaluate the pharmacological decrease in blood glucose levels by using herb extract. Its relationship with the total antioxidant capacity of the blood was studied and compared with glibenclamide which is widely used in diabetic treatment.

Materials and methods

Extract preparation

The extractions of *Cinnamomum zeylanicum*, *Tinuspora Cordifolia, Curcuma xanthorrhiza* and *Averrhoa blimbi L*. were carried out by soaking the samples in 70% ethanol in a ratio of 1:10 for 24 hours while stirring for the first two hours. Remaceration was also carried out once so that the active substance in the *Simplicia* could be optimally extracted.

Preparation of the test animals

As shown in Figure 1, 42 white male rats (*Rattus norvegicus*) aged seven to eight weeks were used. They weighed 179.29 grams on average and were divided into seven groups (six in rats in each group). The groups included K1 = normal rats, K2 = hyperglycemic rats (induced by streptozotocin (STZ) + nicotinamide), K3 = hyperglycemic rats (induced by STZ + nicotinamide) + glibenclamide, K4 = hyperglycemic rats (induced by STZ + nicotinamide) + ethanolic Extract of *Tinuspora Cordifolia*, K5 = hyperglycemic rats (induced by STZ + nicotinamide) + ethanolic extract of *Averrhoa blimbi L*, K6 = hyperglycemic rats (induced by STZ + nicotinamide) + ethanolic extract of *Cinnamomum zeylanicum*, K7 = hyperglycemic rats (induced by STZ + nicotinamide) + *ethanolic* extract of Curcuma *xanthorrhiza*.

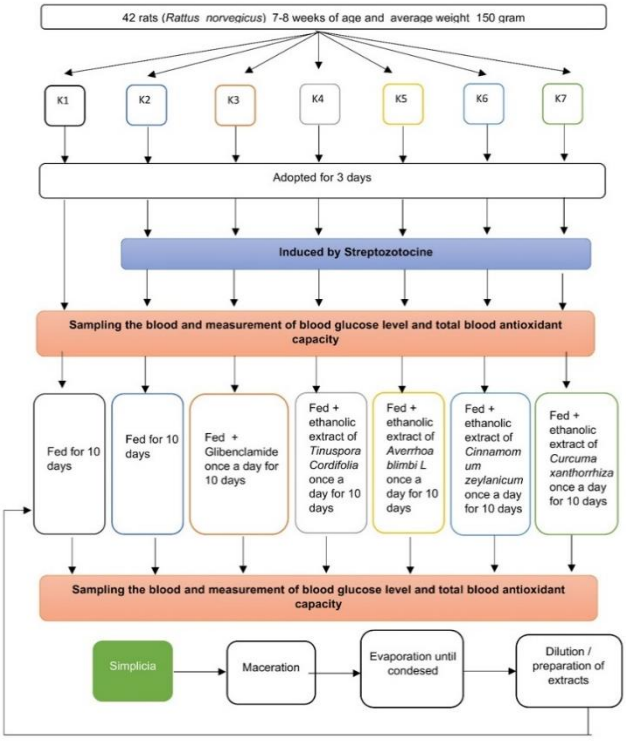


Figure 1: Experiment flow chart

Testing and experimental design

Firstly, all rats were conditioned to the laboratory conditions for three days. Then, the K2-K7 groups were given 45 mg of STZ per kg of body weight in order to make them hyperglycemic. Their blood glucose level was measured before and after STZ induction. Following this, the K3 group was given 0.09mg of glibenclamide per 200 g of body weight, K4 was given 90mg of ethanolic extract of *Tinuspora Cordifolia* per 200 g, K5 was given 15 mg of ethanolic extract of

Averrhoa blimbi L per 200 g of body weight, K6 was given 50 mg of ethanolic extract of *Cinnamomum zeylanicum* per 200 g of body weight, K7 was given 30 mg ethanolic extract of *Curcuma xanthorrhiza* orally per 200 g of body weight. At the end of the observation (day 11), the blood glucose level and total antioxidant capacity of plasma were measured.

Analysis

Reduction of the blood glucose level was calculated by subtracting the blood glucose level after ten days of the treatment from the blood glucose level before treatment. The same formula was also applied to measure the total antioxidant capacity of plasma. The mean differences of the blood glucose level and the total antioxidant capacity of plasma were analysed statistically using the One Way Anova test and LSD *post hoc* test with $\alpha = 0.05$ with SPSS statistic 25. The correlation between the total antioxidant capacity of plasma with the reduction of blood glucose level was analysed statistically by using regression in which reduction of blood glucose level acted as a dependent variable.

Result

Total antioxidant capacity of plasma before and after treatment

Figure 2 indicates that there was a significant difference between the mean antioxidant capacity of plasma between hyperglycemic rats that did not receive treatment and those that received ethanolic extract treatment. K1 and K2 were not treated with compounds that act as antioxidants. The antioxidant capacity improved in the group of rats that were treated with compounds for ten days, while the normal and hyperglycemic rats experienced a reduction (Figure 2). This meant that there was a decrease in free radical levels due to the ethanolic extract. The mean improvement of the total antioxidant capacity was different in the K3, K4, K5, and K6 groups, but the total antioxidant capacity value after treatment could be twice from the baseline (before treatment) or more, and it was observed to be statistically significant (p<0,05) using paired sample t-test pre and post-treatment (as shown in Table I). The comparative compound used was glibenclamide which is widely used to treat type 2 diabetes mellitus, and it was proved to successfully increase the antioxidant capacity of plasma. It indicated that the ethanolic extract of *Cinnamomum zeylanicum* was the strongest compound. It could increase the total antioxidant capacity of plasma better than *Tinuspora Cordifolia, Averrhoa blimbi L* or *Curcuma xanthorrhiza*.

The statistical analysis (Table I) revealed that the total antioxidant capacity of plasma between the K3 and K6 groups or between the K4 and K7 groups was not significantly different (p<0.05). This meant that the ethanolic extracts of *Cinnamomum zeylanicum* had the same antioxidant capacity as that of glibenclamide. Meanwhile, the ethanolic extracts of *Tinospora cordifolia* had the same antioxidant capacity as that of *Curcuma xanthorrhiza*.

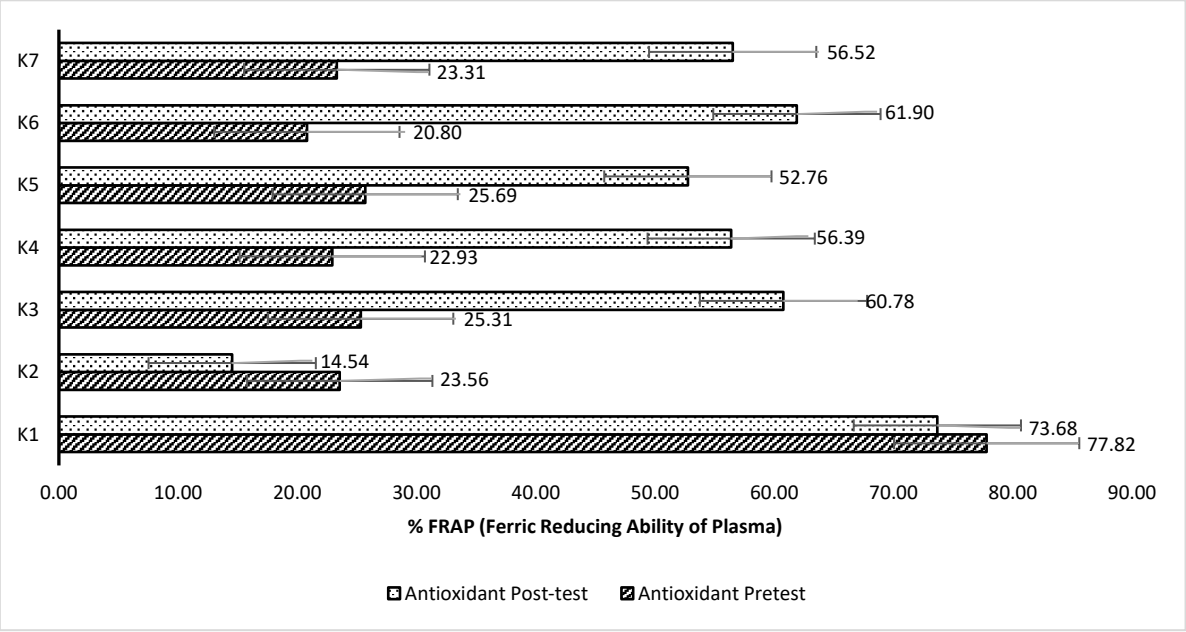


Figure 2: Total antioxidant capacity of plasma

Table I: Mean of the increase of the total antioxidant capacity of plasma and the reduction of blood glucose level

Group	Increase of the total antioxidant capacity of plasma (% FRAP)		Reduction of blood glucose level (mg/dL)	
	Mean	±SD	Mean	±SD
K1	-4.14	2.22	-2.91[a]	1.77
K2	-9.02	4.82	-4.56[a]	3.67
K3	40.10[a]	4.42	124.58[b]	7.05
K4	33.46[a]	2.46	117.68[c]	6.70
K5	27.07	3.52	108.68	7.69
K6	41.10	4.19	130.90[b]	3.43
K7	33.21[a]	2.91	123.01[c]	9.09

K1 = normal rats
K2 = Hyperglycemic rats (induced by STZ + nicotinamide)
K3 = Hyperglycemic rats (induced by STZ + nicotinamide) + Glibenclamide
K4 = Hyperglycemic rats (induced by STZ + nicotinamide) + Ethanolic Extract of *Tinuspora Cordifolia*
K5 = Hyperglycemic rats (induced by STZ + nicotinamide) + Ethanolic Extract of *Averrhoa blimbi L*
K6 = Hyperglycemic rats (induced by STZ + nicotinamide) + Ethanolic Extract of *Cinnamomum zeylanicum*
K7 = Hyperglycemic rats (induced by STZ + nicotinamide) + Ethanolic Extract of *Curcuma xanthorrhiza*
The same superscript letter showed that there were no differences between the groups (LSD *post hoc* ANOVA with *p-value* >0,05)

Blood glucose level before and after treatment

Figure 3 shows that the mean of the baseline blood glucose level (before STZ injection) in every group is placed on the same line. This means that the animals were healthy and homogenous, so they were able to be randomly separated into groups. After STZ injection, blood glucose levels in K2-K7 groups increased significantly and was placed at the same level. This meant that STZ successfully made the rats hyperglycemic and gave them type 2 Diabetes mellitus. Figure 3 also reveals that using ethanolic extract in treatment can decrease blood glucose levels significantly, although it has yet to be as effective as glibenclamide. It shows that ethanolic extract of *Cinnamomum zeylanicum* is the strongest compound at decreasing blood glucose levels compared to ethanolic extract of *Tinuspora Cordifolia, Averrhoa blimbi L* or *Curcuma xanthorrhiza*.

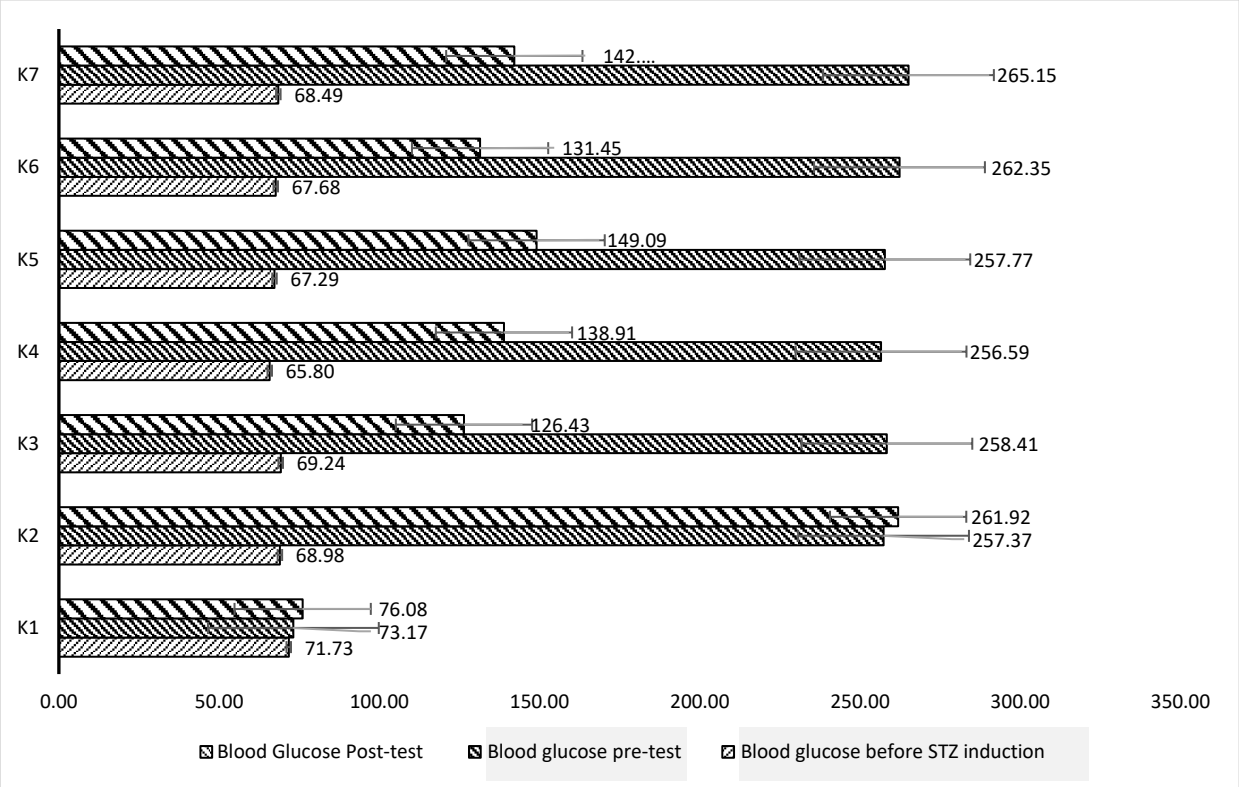
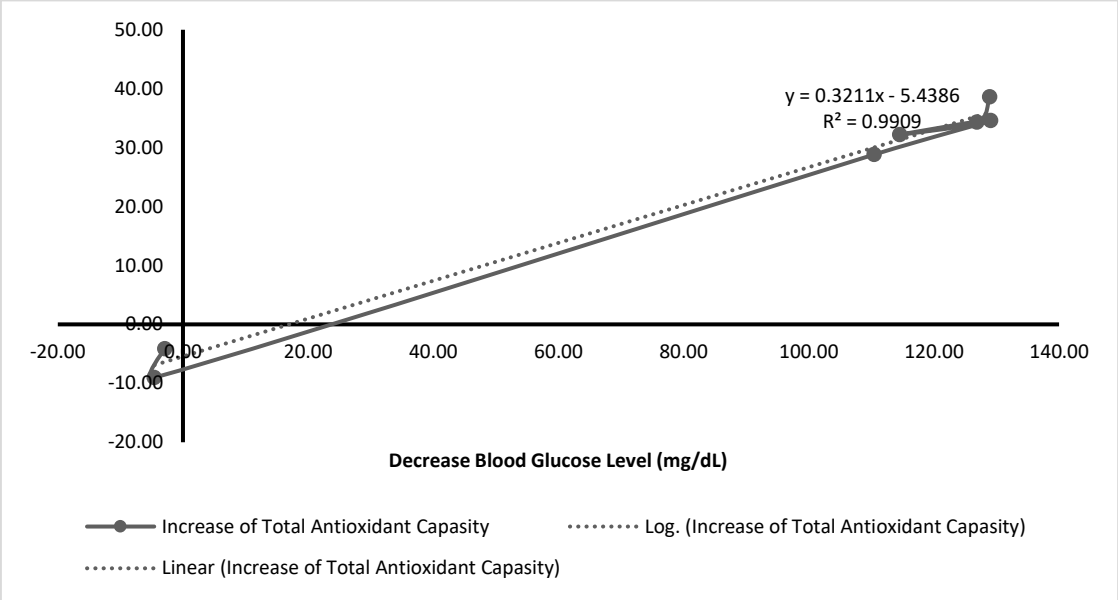


Figure 3: Blood glucose level before and after treatment

The correlation between the total antioxidant capacity of plasma and blood glucose level

The results of this study indicate that *Tinospora cordifolia* and *Curcuma zanthorrhiza* have equivalent potential to reduce blood glucose levels as glibenclamide, thereby increasing superoxide dismutase (SOD) activity and total antioxidant capacity in diabetic rats. This was approved by a linear relationship between the total antioxidant capacity of plasma and the glucose levels, which was inversely proportional to 96,67%. This states that there was a perfect negative linear relationship between the post-test mean of total antioxidant capacity variation and the mean of plasma glucose levels. The equation of this correlation, as shown in Figure 4, was y = 0,3211x – 5,4386. This means that for every 1mg/L of total antioxidant capacity of plasma (x) that is added, the glucose in the blood will decrease by 0.326 mg/dL.



Note: equation is y = 0.3211x – 5.4386 where y = blood glucose level, and x = antioxidant capacity of plasma

Figure 4: The correlation between total antioxidant capacity of plasma and blood glucose level

Discussion

Total antioxidant capacity of plasma was increased by treatment

Antioxidant potential was measured using the frap method. The principle of the frap method is based on the ability of the sample to transfer electrons to reduce the iron ion Fe^{3+} (ferro) to iron ion Fe^{2+} (ferri). Antioxidant capacity is one of the parameters that shows how much potential a substance has to act as an antioxidant (Pisoschi & Negulescu, 2012). The greater the total antioxidant capacity of plasma, the greater the ability of these compounds to act as antioxidants.

The total antioxidant capacity of the ethanolic extract from *Tinospora cordifolia*, *Cinnamomum zeylanicum*, and *Curcuma xanthorrhiza* is associated with the chemical compounds in these plants, which possess antioxidant activity. Polysaccharide compounds in the form of arabinogalactan, galacturonic acid, and neutral glucan found in *Cinnamomum zeylanicum* are known to act as antioxidants (Ghosh *et al*., 2015). *Cinnamomum zeylanicum* contains volatile oil with the main components being eugenol, cinnamaldehyde, and camphor which act as antioxidants, antimicrobials, and antidiabetic (Jayaprakasha & Rao, 2011). *Cinnamomum zeylanicum* bark and fruits contain proanthocyanidins which are flavonoids. In the ethanolic extract of *Tinospora cordifolia*, there are main components such as *tinocordioside*, *cordifolide* A, *palmatine*, *quercetin, β-sitosterol*, *heptacosanol*, and *syringin* (Kumar *et al*., 2018). One of the compounds that act as an antioxidant in *Tinospora cordifolia* is flavonoid quercetin. Quercetin is a 3-hydroxyl group flavonoid that neutralizes free radicals by one-step hydrogen atom or electron transfer followed by proton transfer during which they are oxidized (Lesjak *et al*., 2018). The essential oils contained in this plant are able to capture strong free radicals with DPPH with a total phenolic content of 28 ± 0.4 mg GAE / g (Naik *et al*., 2014).

The ability of free radical scavenging in *Curcuma xanthorhiza* is associated with chemical compounds contained in this plant, including curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which have strong antioxidant activity (Jantan *et al.*,

2012). Curcumin is the compound with the strongest antioxidant ability compared to demetoxycurcumin and bisdemetethoxycurcumin (Jayaprakasha *et al.*, 2006).

Blood glucose level decreased by the treatment

Rats that were given STZ-induced DM had similar pathophysiology as type 2 DM patients. STZ 2-Deoxy-2-[[(methylnitrosoamino)carbonyl]amino]-D-glucopyranose is a cytotoxic glucose analogue, and its cytotoxicity is derived from the ßcell selective action mechanisms (Islam *et al*., 2017). STZ is selectively accumulated in pancreatic β cells via the low-affinity GLUT2 glucose transporter in the plasma membrane (Gauthier, 2014; Jayasimha Goud & Swamy, 2015). The effects of STZ on glucose and insulin homeostasis reflect the toxin-induced abnormalities in β cell function. Initially, insulin biosynthesis, glucose-induced insulin secretion and glucose metabolism (both glucose oxidation and oxygen consumption) are all affected (Nagarchi *et al.*, 2015; Wu & Yan, 2015). At later stages of functional β cell impairment, gene expression and protein production deficiencies lead to the deterioration of both glucose transport and metabolism (Khaki *et al*., 2014; Kumar M, 2017).

The results revealed that all of the extracts made blood glucose levels decrease, and the best performance was obtained when *Cinnamomum zeylanicum* extract was used. The mean reduction of blood glucose level gained by using ethanolic extract in *Cinnamomum zeylanicum* is statistically significantly similar to the mean reduction obtained by using glibenclamide. Other experts reported that cinnamon extract plays a role in regulating blood glucose levels and lipids. It may also exert a blood glucose-suppressing effect by improving insulin sensitivity or slowing the absorption of carbohydrates in the small intestine (Abd *et al.*, 2010; Sartorius *et al.*, 2014).

Phytochemical screening on cinnamon bark Simplicia indicates that the Simplicia contains secondary metabolite compounds, namely tannins, phenolics, flavonoids, quinones, saponins, monoterpenes, and sesquiterpenes (Adisakwattana *et al*., 2011; Assefa *et al*., 2018). Flavonoids stimulate glucose uptake in peripheral tissues, regulate the activity and/or express the rate-limiting enzymes in the carbohydrate metabolism canal, and act as insulin secretagogues or insulin mimetics, possibly influencing the pleiotropic mechanisms of insulin signalling to ameliorate the diabetes condition (Cazarolli *et al*., 2008; Testa *et al*., 2016).

There is a correlation between the escalation of total antioxidant capacity of plasma and the decline of blood glucose level

Diabetes mellitus is characterised by hyperglycemia and average haemoglobin A1c levels (HbA1c) above 48mmol/mol (6.5%) for two to three months (Jean-Marie, 2018). This is caused by vascular dysfunction due to repeated exposure and pathologically high d-glucose concentrations (Domingueti *et al.*, 2016). The occurrence of vascular dysfunction is caused by disruption of the nitric oxide (NO) canal and an increase in oxidative stress, which will cause changes in glucose metabolism (Ghasemi & Jeddi, 2017). The results show that the proactive phytochemical exploration of *Tinospora cordifolia* and *Curcuma zanthorrhiza* in this study was obtained by antioxidant compounds from the measurement of the total antioxidant capacity, which is important for reducing glucose in the blood in Streptozotocin-induced DM rats. If hyperglycemia is not controlled in a Diabetes mellitus patient, it will cause further oxidative stress because hyperglycemia in diabetes mellitus leads to the excessive production of free radicals. This is characterised by an increase in malondialdehyde (MDA), peroxidation index, and a decrease in antioxidant protection in the body (Domingueti *et al*., 2016; Fouelifack *et al*., 2019).

The content of antioxidant compounds is determined by the presence of free –OH (hydroxyl) functional groups and carbon-carbon double bonds, such as flavones, flavanones, squalene, tocopherol β-carotene and vitamin C (Babu *et al.*, 2013). These bioactive compounds support the linear relationship between the decrease in blood glucose and the total antioxidant capacity so that they can prevent further vascular dysfunction in Diabetes mellitus (Hussain *et al.*, 2020). The total antioxidant activity from the results of this study illustrates the antioxidant status of STZ-induced rat blood samples, and it proves that the antioxidant response to free radicals is produced due to hyperglycemic conditions. Antioxidant activity describes the ability of an antioxidant compound to neutralise free radicals so that it can delay, slow down, and prevent the occurrence of free radical anti-oxidation reactions in lipid oxidation (Shahidi & Zhong, 2015). The mechanism of reducing blood glucose levels is carried out by stimulating the secretion of the insulin hormone, increasing glucose uptake from blood to tissues, oxidating glucose, and activating glycogen synthesis in the liver and adipose tissue (Lee & Jun, 2014; Bhatt *et al.*, 2016). Increased cumulative action of all the antioxidants present in plasma and body fluids *in vivo* will be able to balance oxidants and antioxidants. As a result, oxidative stress will decrease, and this will be marked by a decrease in glucose in the

blood (Birben *et al.,* 2012; Jamuna Rani & Mythili, 2014; Pruchniak *et al*., 2016).

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RESEARCH ARTICLE

Fluconazole-tartaric acid co-crystal formation and its mechanical properties

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Abstract

Introduction: The formation of co-crystal is widely studied to obtain more favourable physicochemical properties than the pure active pharmaceutical ingredient (API). The co-crystal formation between an anti-fungal drug, fluconazole (FLU), and tartaric acid (TAR) has been investigated and its impact on mechanical properties has also been studied. **Methods:** The co-crystal of FLU-TAR (1:1) molar ratio was prepared by ultrasound-assisted solution co-crystallization (USSC) method with ethanol as the solvent. Polarization microscopy was used to observe the crystal morphology. Meanwhile, powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) methods were used to characterise the co-crystal formation. The mechanical properties of the co-crystal, such as flowability and tabletability, were compared with pure FLU. **Results:** Photomicroscopes revealed the unique crystal morphology of the USSC product was different from the two starting components. The typical PXRD pattern was shown by the USSC product, which indicated the formation of FLU-TAR co-crystal. In addition, the DSC thermogram revealed 169.2°C as the melting point of the FLU-TAR co-crystal, which is between the melting points of FLU and TAR. It indicates that FLU-TAR co-crystal has better flowability and tabletability than pure FLU. **Conclusion:** FLU-TAR co-crystal is one of the alternative solid forms for a raw material in pharmaceutical tablet preparation because it has better mechanical properties than pure fluconazole.

Introduction

Currently, more than 70.0% of active pharmaceutical ingredients (APIs) are given in tablet dosage form since this form is stable and easy to use. Today, the direct compress method is widely used for the tablet manufacturing process as it is fast and it requires little equipment and personnel (Maghsoodi, 2012). However, tablet manufacturing that employs this method requires good mechanical properties of API, such as flowability and tabletability.

Modification of the crystal structure of an API due to different polymorphs can change the mechanical properties of an active pharmaceutical ingredient (API) (Upadhyay *et al*., 2013; Guadalupe Sánchez-González *et al.*, 2015; Yin *et al.,* 2016). To obtain polymorphic modifications from an API, however, is not easy. Sometimes, the polymorphic modification of an API is obtained accidentally from an experiment. Also, some of the polymorphic modifications obtained are less stable and could change to the more stable modifications during storage. One of the crystal engineering techniques that can be employed intentionally to obtain beneficial physicochemical properties is the formation of co-crystals.

Co-crystals are formed due to the presence of non-covalent bonds, including hydrogen bonds between an API and an excipient, which is also solid in the specific

stoichiometric ratio (Pan *et al.*, 2017). The co-crystal formation has a fascinating method. Apart from being able to increase solubility, dissolution rate, and bioavailability, it also can improve mechanical properties. Previous studies have succeeded in obtaining co-crystals that can improve the mechanical properties of APIs, including paracetamol (Kiarki *et al.*, 2009; Ahmed, Shimpi, & Velaga, 2016; Hiendrawan *et al.*, 2016), flufenamic acid (Joshi *et al.,* 2018), and telmisartan (Ratih *et al.*, 2018).

Fluconazole (FLU) is a bis-triazole derivative in the treatment of candidiasis and cryptococcal meningitis. The drug is given orally in capsule or tablet dosage form (Charoo *et al.*, 2014). However, FLU has poor flowability, so this drug has limitations in the manufacture of tablets by direct compression (Consiglieri *et al.*, 2010). Several fluconazole co-crystals have been made, but they are generally made to increase their solubility, including fluconazole co-crystals with malic acid, maleic acid, fumaric acid (Kastelic et al., 2010), dipicolinic acid, and adipic acid (Dayo Owoyemi *et al*., 2019). However, after a thorough search of the relevant literature, it was revealed that a study on the mechanical properties of fluconazole co-crystals is yet to be published. Therefore, the purpose of this study is to prepare fluconazole co-crystal with tartaric acid (TAR) as the co-former and investigates its impact on mechanical properties. Based on the chemical structure of the two components, both have a great potential to form a co-crystal. The chances of hydrogen bonding occur between the triazole group of fluconazole and the carboxylic group of tartaric acid.

Material and method

Material

Fluconazole was obtained from Viruphaksa, Hyderabad, India, while dl-tartaric acid and ethanol were purchased from Merck, Indonesia.

Preparation of Fluconazole-TAR (FLU-TAR) co-crystal

Fluconazole-tartaric acid co-crystal (FLU-TAR) was produced by the ultrasound-assisted solution co-crystallisation (USSC) method. A mixture of 3.06 g of fluconazole (10 mmol) and 1.5 g of dl-tartaric acid (10 mmol) was put into an Erlenmeyer flask and dispersed in 40 mL ethanol. The Erlenmeyer flask was placed on Branson ultrasonic 3510-DTH and was operated at 40-45^{o}C and a frequency of 42 kHz. After 20 minutes, the Erlenmeyer flask was removed from the ultrasonic and left for five minutes at room temperature. The habit crystal of the solid from the Erlenmeyer flask was observed to ensure that FLU-TAR co-crystal had been produced. Then, the solid was separated by filtration and dried at room temperature. Afterwards, the dried solid was stored in a desiccator.

Characterisation of FLU-TAR Co-crystal formation by polarization microscopy

The dried solid from the USSC product was placed on a closed slide, and the crystal morphology was observed under an Olympus BX-53 polarizing microscope. The photomicroscopes were taken using a digital camera (Optilab Advanced Plus) integrated into a polarizing microscope. The crystal morphology of the USSC product was compared with the crystal morphology of the recrystallised pure FLU and TAR in ethanol solvent.

Characterisation of FLU-TAR Co-crystal formation by Powder X-ray Diffraction (PXRD)

The powder X-ray diffraction pattern was determined for USSC products, pure FLU, and TAR. The data collection of powder X-ray diffraction pattern was carried out using the Panalytical Empyrean XRD system operated at 40 kV of generator voltage and 30 mA of generator current. The sample scanning speed was set at 2°/min with a measurement range of five to 45° of 20 angles.

Characterisation of FLU-TAR Co-crystal formation by Differential Scanning Calorimetry (DSC)

A total of three to five mg of dried solid from the USSC product was placed on an aluminium pan, and the DSC thermogram was recorded on the DSC-60 plus (Shimadzu, Japan). The heating rate was set at 10°/min and range from 30 to 200°C under nitrogen flow with a flow rate of 20 mL/min. DSC measurements were also carried out on pure FLU and TAR.

Evaluation of angle of repose and powder flow rate

Each of 20 g of pure FLU and FLU-TAR co-crystal was put into a funnel and left until all the powder flows and falls on a flat surface. The height (h) and radius (r) of the conical pile of powder were measured, and the angle of repose (θ) was calculated using equation 1.

$$Tg\ \theta = h/r \quad \text{(Equation 1)}$$

Flowability study

Carr's compressibility index and Hausner's ratio were calculated based on the values obtained from the determination of bulk density (ρ_b) and tapped density (ρ_t). The determination of bulk and tapped density was conducted using an automatic tapped density tester (ZS-2E Tapped Density Tester, China). Each 20 g of pure FLU and FLU-TAR co-crystal was placed in the cylinder

of the tapped density tester, and its volume (v_b) was recorded. Afterwards, a total of 500 beats were performed on each powder until the volume did not change and the volume was recorded (v_t). The bulk density, tapped density, Carr's compressibility index, and Hausner's ratio were calculated based on equations 2, 3, 4, and 5, respectively (Kaialy *et al.*, 2012b).

$$\rho_b = m/v_b \quad \text{(Equation 2)}$$

$$\rho_t = m/v_t \quad \text{(Equation 3)}$$

$$\text{Carr's compressibility index} = \rho_b/\rho_t \quad \text{(Equation 4)}$$

$$\text{Hausner's ratio} = (\rho_t-\rho_b)/\rho_t \quad \text{(Equation 5)}$$

Tabletability study

Tabletability studies were carried out by determining the tensile strength and elastic recovery percentage of and pure FLU and FLU-TAR co-crystal. Powder compaction was performed using the Athena manual hydraulic press (Athena Technology, India) and 11 mm-flat round tools (punch and die). The die was filled with 300 mg of powder and compressed into a tablet at ten to 60 kg/cm^2 of the pressure range. Every time the compaction process was performed, the punch and die were coated with 2% (w/w) magnesium stearate in ethanol. The diameter, thickness, and hardness of tablets were measured as soon as the tablet was ejected from the die hole. The tablets were allowed for 24 hours, and the diameter was measured again. A digital calliper was used to measure the diameter and thickness of tablets. The tablet's hardness was measured by the TBH-125 series hardness tester (Erweka, Germany). Tensile strength (σ) was calculated based on the values of diameter (D), thickness (T), and braking force (F) according to equation 6 (Kawashima *et al.*, 1994). The elastic recovery (ER) percentage was calculated based on the initial diameter (D_0) and the diameter after the tablet was stored for 24 hours (D) according to Equation 7. The appearance of the tablet was also observed after being stored for 24 hours.

$$\text{Tensile strength } (\sigma) = 2F/\pi DT \quad \text{(Equation 6)}$$

$$\text{Elastic recovery percentage } (\%ER) = (D- D_0)/D \quad \text{(Equation 7)}$$

Results

Polarisation microscopy

Photomicroscopes of USSC product and recrystallization results in ethanol of the two constituent components are shown in Figure 1. The crystal habit of dry solid from the USSC product was a plate-like crystal (tabular habit). The pure FLU and TAR crystal habits were needle-like crystals (acicular habit) and prism-like crystals (prismatic habit), respectively.

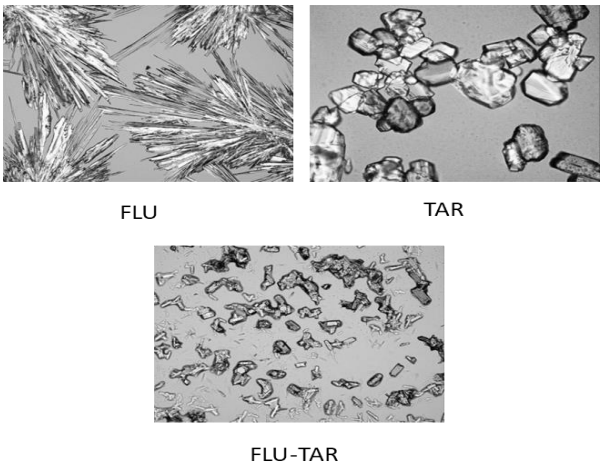

Figure 1: Crystal morphology of FLU, TAR, and FLU-TAR USSC product

Powder X-ray diffraction patterns

Figure 2 demonstrates the PXRD patterns of the FLU, TAR, and USSC products. PXRD pattern of the USSC product of FLU-TAR has typical peaks as indicated by the arrows at angles of 2θ = 7.7, 11.1, 16.5, 16.9, 17.6, 17.9, 19.1, 19.9, 22.1, 22.5, 23.2, and 24.0°.

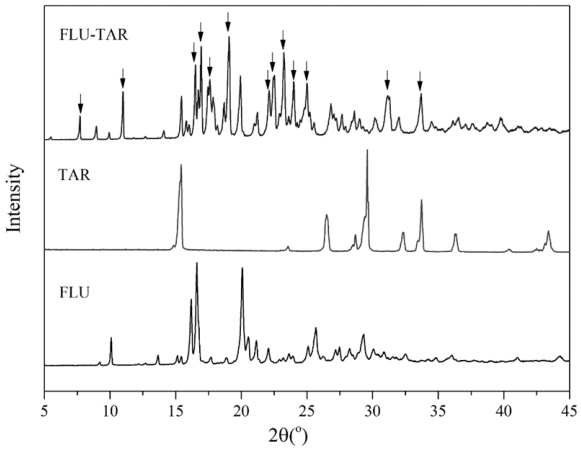

Figure 2: Powder X-ray diffraction patterns of FLU, TAR, and FLU-TAR co-crystal

Differential Scanning Calorimetry (DSC) thermograms

Figure 3 shows the DSC thermograms of FLU, TAR, and the FLU-TAR USSC products. The DSC thermogram of FLU showed two endothermic peaks (102.40 and 140.25°C), whilst the DSC thermogram of TAR revealed an endothermic peak at 173.17°C. The USSC product has an endothermic peak at 165.92°C.

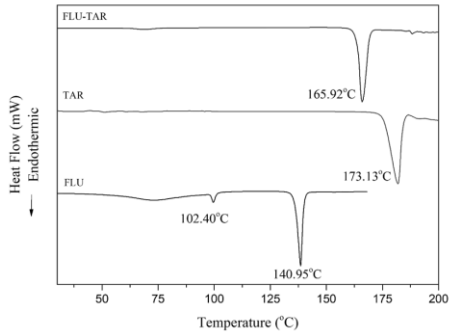

Figure 3: Differential scanning calorimetry thermograms of FLU, TAR, and FLU-TAR co-crystal

The angle of repose evaluation

The angle of repose of pure FLU and FLU-TAR co-crystal were 41.69±0.25 and 34.16±0.54, respectively.

Flowability study

The bulk density, tapped density, Carr's compressibility index, and Hausner's ratio of pure FLU and FLU-TAR co-crystal are shown in Table I.

Tabletability study

Figure 4 shows the tensile strength profile of pure FLU and FLU-TAR co-crystal at ten to 60 kg/cm^2 of compression pressure range, whilst both elastic recovery profile is shown in Figure 5. The appearance of pure FLU and FLU-TAR co-crystal tablets after being compressed at a compaction pressure of 50 kg/cm^2 is shown in Figure 6.

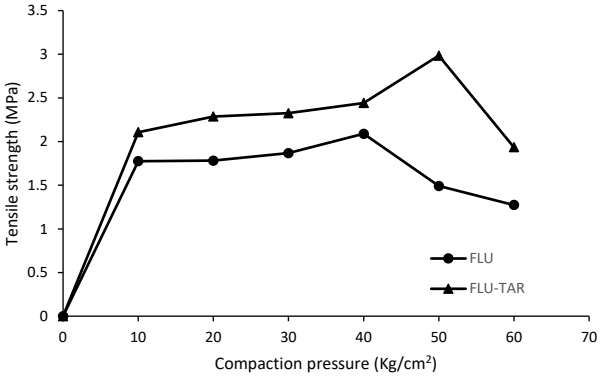

Figure 4: Tabletability profiles of FLU and FLU-TAR co-crystal

Table I: Flowability of FLU and FLU-TAR Co-crystal

Materials	Bulk density	Tapped density	Carr's compressibility index (%)	Hausner's ratio	Flow properties
FLU	0.505±0.005	0.726±0.010	30.2±0.20	1.437±0.007	Poor
FLU-TAR	0.469±0.013	0.545±0.017	14.1±0.32	1.163±0.004	Good

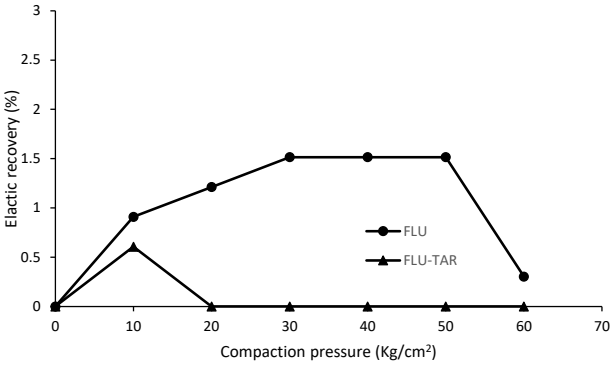

Figure 5: The elastic recovery of FLU and FLU-TAR co-crystal

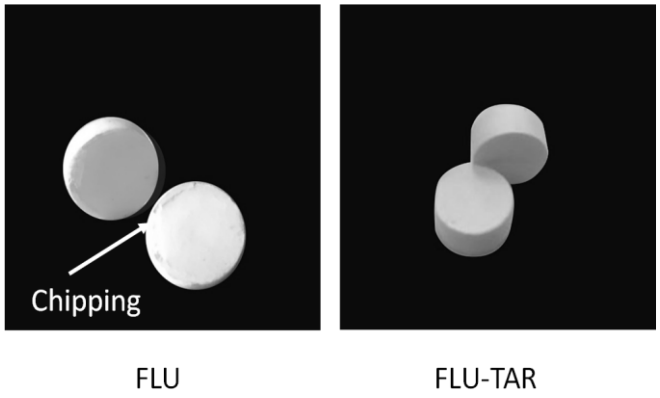

Figure 6: Appearance of FLU and FLU-TAR co-crystal tablets at 50 kg/cm^2 of compaction pressure

Discussion

The USSC method was employed to prepare FLU-TAR co-crystal since this method has a good effect on the scale-up process of co-crystal. Therefore, it can be applied to large-scale production processes. This method also has several advantages, namely the relatively short manufacturing time, the slow crystallization process that can produce a more regular crystal packing, less solvent is used, and compared to the grinding method, this method avoids crystal defects caused by the grinding process. In addition. the USSC method produces more uniform particles than any grinding method because the nucleation of co-crystal at low supersaturation in a solution occurs influenced by the presence of ultrasonic wave cavitation energy (Aher *et al.*, 2010; Zeng *et al.*, 2014). The solvent can only partially dissolve both the active substance and co-former to prevent each component from crystallizing individually as the solution is always in supersaturation (Thakuria *et al.*, 2013). Using the appropriate type and volume of solvent in the USSC method will produce a uniform and fine co-crystal form. In this study, ethanol was selected as a solvent in the co-crystal preparation as it fulfils all the criteria as a proper solvent in produce the FLU-TAR co-crystal where two components are soluble in this solvent.

Observation of crystal morphology using a polarizing microscope was conducted to identify the formation of FLU-TAR co-crystal using the USSC method. In the co-crystal formation process using the USSC method, the two constituent components (FLU and TAR) were converted into FLU-TAR co-crystal. This change can be known by observing the differences in the crystal habit of each of its constituent components with the habit crystal after the process was stopped. This difference in crystal morphology may indicate a change in solid form during the manufacturing process using the USSC method due to the intermolecular interaction between FLU and TAR.

PXRD is a reliable method to determine the interaction between two solid components that form a co-crystal by observing the difference in the PXRD pattern between the manufactured product and the respective solid components. The main peaks differ from the main peaks found in pure FLU and TAR, and this may reveal an interaction between the two solid components to establish a FLU-TAR co-crystal.

Differential Scanning Calorimetry (DSC) is a thermal analysis used to confirm the co-crystal formation since this method can show the thermal behaviour of a solid form, including changes in melting point. The DSC thermogram of FLU showed two endothermic peaks, one at 102.40°C due to dehydration of a water molecule from fluconazole monohydrate and another at 140.25°C in consequence of its melting point (Alkhamis, Obaidat, & Nuseirat, 2002). A typical endothermic at 173.17°C on the DSC thermogram of TAR is related to its melting point. Different from the thermal behaviour of the pure FLU and TAR, the DSC thermogram of the FLU-TAR USSC product showed no endothermic peaks at the melting temperatures of the two components. However, there was only one sharp endothermic peak located between the melting points of FLU and TAR at 165.92ºC. This thermal behaviour confirms that there was intermolecular interaction between FLU and TAR to produce FLU-TAR co-crystal. The sharp endothermic peak at 165.92ºC is related to the melting point of FLU-TAR co-crystal. The melting point below or between the melting points of the two initial components could indicate the formation of co-crystal (Batisai *et al.*, 2014).

The angles of repose of the pure FLU and the FLU-TAR co-crystal were 41.69±0.25° and 34.16±0.54°, respectively. It was found that FLU-TAR co-crystal had a better angle of repose than pure FLU. The angle of repose of FLU is in the poor flow category (46-55°), while the co-crystal FLU-TAR is in the passable or moderate flow category (36-40°) (Satpute & Tour, 2013).

It is crucial to know the mechanical properties of an API because it can be a consideration in determining the method used in the tablet manufacturing process. Flowability and tabletability are the mechanical properties, which are generally used as the basis for selecting methods in the tablet manufacturing process. The tablet manufacturing process by direct compress method requires good flowability and tabletability from an API. In this case, flowability is an important parameter in the manufacture of a pharmaceutical tablet. On the other hand, compressibility is the ability of a material to reduce the volume due to the pressure applied (Sun & Grant, 2001). Carr's compressibility index and Hausner's ratio could reveal the flowability of the powder. The flowability study shows the Carr's compressibility index of the FLU-TAR co-crystal is in the good flowability category (eight to 16%) while Carr's compressibility index of pure FLU is in a bad category (23-35%) (Satpute & Tour, 2013). The high Carr's compressibility index of pure FLU might be due to its needle-shaped crystal morphology that allows the aggregation of powders arising from the high mechanical forces interlocking. Conversely, a decrease in Carr's compressibility index of FLU-TAR co-crystal was caused by its tabular-shaped crystal habit that allows a decrease in the relative contact area between particles so that its cohesion properties decrease and result in less physical contact (Kaialy *et al.*, 2012a). Similar to Carr's compressibility index, based on the value of Hausner's ratio, the FLU-TAR co-crystal is also in the good flowability category (1.12-1.18), while pure FLU is in the poor flowability category (1.35-1.45).

The tabletability is defined as the ability of a powder or particle to be converted into a tablet with a certain strength under compaction pressure (Jain, Khomane, & Bansal, 2014; Tye, Sun, & Amidon, 2005). The tabletability profiles (tensile strength versus compaction pressure) of pure FLU and FLU-TAR co-crystal were measured in a compaction pressure range of ten to 60 kg/cm^2. The tabletability profile demonstrated pure FLU could be compressed to a compaction pressure of 60 kg/cm^2. However, the appearance of the tablet showed chipping at compaction pressures above 40 kg/cm^2. This condition is due to the needle-shaped crystal habit of FLU that has poor flowability with high electrostatic energy. This needle-shaped crystal habit has high internal friction, which causes a lot of space in the tablet mould that is not filled during the compaction process (Kaialy *et al.*, 2014). Conversely, FLU-TAR co-crystal can form a tablet without chipping under compaction pressures. FLU-TAR co-crystal has a higher tensile strength value compared to pure FLU, which indicates that FLU-TAR co-crystal tabletability is better than pure FLU. The tabletability profile showed that at a compaction pressure of 10 kg/cm^2, the FLU-TAR co-crystal has a tensile strength value of 2.108 MPa. The tensile strength increased steadily until a compaction pressure of 50 kg/cm^2 with a tensile strength value of 2.984 MPa, which is the maximum point of compaction or called the breaking point. After this point has passed, a material undergoes extensive fragmentation, which is called a brittle fracture (Jain *et al*., 2014). The tabletability profiles showed differences in the FLU and FLU-TAR co-crystal breakpoints. The tensile strength value of the FLU-TAR co-crystal was higher than that of FLU, and this indicates that the FLU-TAR co-crystal has better plasticity than pure FLU. To produce a good tablet, the tensile strength should be at least 2 MPa (Perumalla & Sun, 2014). With tensile strength values above 2 MPa, the FLU-TAR co-crystal should not encounter problems in the tablet manufacturing process by the direct compress method.

The percentage of elastic recovery can indicate the elasticity of various pharmaceutical materials to be compressed into tablets. The risk of capping or other defects during tablet development and manufacture can be overcome by testing the elastic recovery percentage of active pharmaceutical ingredients or excipients. The percentage of elastic recovery of the pure FLU is higher than that of the FLU-TAR co-crystal, and it indicates that the FLU-TAR co-crystal has better tabletability than a pure FLU. The more plastic powders can form, the greater the permanent bonds between particles after the compaction process, so they can show good tabletability (Chattoraj *et al.*, 2014). The properties of plastic and elastic deformation determine the ability of a material to be compressed. Particle rearrangement and deformation due to compaction pressure cause a bond area between particles during the powder compaction process (Sun, 2011). The amount of bond area between the particles remains constant after there is no compaction process and ejection of the tablet from a die that can produce a tablet intact.

Conclusion

The fluconazole-tartaric acid (FLU-TAR) co-crystal has been successfully prepared using the USSC method and ethanol as solvent. FLU-TAR co-crystal has a distinctive crystal morphology, powder X-ray diffraction pattern, and DSC thermogram. Moreover, the FLU-TAR co-crystal has better mechanical properties than pure fluconazole. Therefore, co-crystal can be used as a raw material in pharmaceutical tablet preparation with a direct compression method.

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IAI CONFERENCE

RESEARCH ARTICLE

Formulation and evaluation of Kirinyuh Leaf effervescent granules (*Chromolaena Odorata. L*) as an antioxidant

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Abstract

Introduction: Kirinyuh leaf is a widely grown plant in Indonesia, containing alkaloids, flavonoids, saponins, tannins, and steroids. Flavonoids are compounds that can capture free radicals or act as natural antioxidants. Effervescent granules can mask the bitter taste and simplify the dissolving process without involving manual stirring. **Aim:** The purpose of this study was to make and evaluate a formulation of effervescent granules of Kirinyuh leaf extract. **Methods:** The granule method was carried out by the wet granulation method. Granule evaluation included organoleptic test, water content test, dissolve time test, flow time test, pH test, and hedonic test. **Results:** Organoleptic test results showed similar granule size, slightly brownish colour, and characteristics of Kirinyuh leaf odour. When examining their quality, the granules produced met the requirements, with moisture content between 0.4% and 0.7%, dissolving time of 30-35 seconds, flow time test of 8-8.5 g/second, and pH of 5.6-5.8; the results of the hedonic test showed that the effervescent granule preparation was much preferred.

Introduction

In Indonesia, several plants, such as Kirinyuh, can be potentially used in traditional medicine. Despite being disturbing for other plants because of its ability to absorb water and nutrients, Kirinyuh has several benefits for human life. In addition to its use in agriculture as an organic fertiliser, biopesticide, and herbicide, it is traditionally used in medicine to treat wounds, diabetes, cough, and bleeding (Saputra *et al.*, 2017). Kirinyuh leaves are also known for their antioxidant and anticancer (against leukaemia) properties (Fitrah *et al.*, 2017).

Kirinyuh leaves contain alkaloids, flavonoids, saponins, tannins, and steroid compounds. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method used to test the antioxidant activity showed that the methanol fraction had the highest antioxidant activity, with an inhibitor concentration value (IC_{50}) of 9.57 ppm (Saputra *et al.*, 2017). Research findings revealed that the antioxidant capacity of the Kirinyuh leaf extract is 49.04% (Parnanto *et al.*, 2013). Flavonoids are compounds that can neutralise or capture free radicals (such as Reactive Oxygen Species - ROS) and act as natural antioxidants (Feronika, *et al.*,2019).

Effervescent granules are a mixture of acids and bases that, when added to water, produce foam and taste like soft drinks (Hassanbaglou *et al.*, 2012). The advantage of effervescence is that it can cover the unpleasant taste and give a fresh effect when consumed due to the carbonation process of acids and alkalis (Hayaza *et al.*, 2019).

Based on the background, this research was carried out to make and evaluate a formulation of effervescent granules of Kirinyuh leaves to be used as an antioxidant.

Material and method

The tools and materials used in this research were mortar-stampers, laboratory equipment, steam dishes, oven, analytical balance, mesh (14 and 16), pH meter, moisture analyzer, stopwatch, flow tester, Kirinyuh leaves, citric acid, sodium bicarbonate, lactose, polyvinylpyrrolidone (PVP K-30), and aerosol.

Determination

Determination was carried out at the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Material preparation

The plant material used was Kirinyuh leaves obtained from Cicarulang Village, Singaparna District, Tasikmalaya Regency, which had gone through wet sorting, washing, chopping, drying, and dry sorting processes.

Making effervescent granule

The acid component consisted of the dry extract of Kirinyuh leaves, lactose, and citric acid, stirred until they were homogeneous, then added a portion of PVP K-30 that had been dissolved with 70% alcohol until the mass could be clenched. The alkaline component, namely sodium bicarbonate, was moistened with the remaining PVP K-30 solution until the mass could be clenched into a fist. Each component was sieved with a sieve number 14, then dried in an oven at 40^{o}C for 8 hours. Each dry component was sieved again with a sieve number 16. Aerosil, acid components and alkaline components were mixed until they were homogeneous. Table I provides information on the granule formulation.

Table I: Kirinyuh Leaf extract effervescent granule formulation

Material	Formula 1 (g)	Formula 2 (g)	Formula 3 (g)
Kirinyuh Leaf	0.1	0.1	0.1
Sodium Bicarbonate	3	3	3
Citrate Acid	2.1	2.1	2.1
Aerosil	0.05	0.05	0.05
PVP K-30	0.2	0.3	0.4
Lactose	Add 10	Add 10	Add 10

Evaluation of effervescent granules

Organoleptic test

The organoleptic test process was carried out to see the physical appearance of the preparations by observing the colour, odour, and taste.

Water content test

A total of 10 g of granules were put into the moisture balance tool. First, granules were flattened, then the tool was turned on, then the data on the moisture contained in the granules were generated. Effervescent granules that met the moisture content requirements were granules with moisture content between 0.4% and 0.7%.

Flow rate test

A flow rate check was carried out by inserting some granules into the funnel, closed at the bottom, to produce good quality granules. The funnel was then opened slowly until all the granules came out and formed a heap on the graph paper. Granule flow was good if the time required to flow was 100 g/10 s (Anshory *et al*., 2007).

Point of quit test

The point of quit test was obtained by measuring the height and diameter of the granule pile.

Dissolve time test

The granule solubility test was done by putting the weighted sample in 200 ml of water. The time taken to dissolve the entire sample was calculated using a stopwatch. The good dissolving time of effervescent granules was less than five minutes.

pH test

The sample was dissolved in 50 mL of aqua dest in a beaker, then 200 mL of aqua dest were added, then stirred until evenly distributed. The pH of the solution was measured with a pH meter that had been calibrated.

Table II shows the quality inspection results of each formula.

Table II: Effervescent granule quality inspection results

Physical Properties	Formula 1 (g)	Formula 2 (g)	Formula 3 (g)
Water content (%)	0.45±0.02	0.47±0.01	0.48±0.03
Flow Rate Test (g/s)	8.27±0.01	8.31±0.01	8.32±0.01
Point of quit test α (°)	26.67±0.58	27±1.00	28±1.00
pH	5.8±0.10	5.6±0.17	5.7±0.10
Dissolve time test (second)	31.09±0.51	33.30±0.13	35.23±0.03

Hedonic test

Observations were made on colour, smell, homogeneity, and dispersibility by 35 respondents; they observed colour, smell, and taste changes in granule effervescent preparations.

Results

Organoleptic test

The organoleptic examination of all formulas was visible, and the colour of the preparation obtained was light green.

All formulations had a homogeneous appearance seen from the colour and size of the granules. The taste and smell of the preparation were typical of Kirinyuh leaves. Solubility test results met the requirements, dissolving in less than five minutes.

Hedonic test

After several evaluations of the preparation, a final evaluation consisted of the preference test. First, the organoleptic examination was done to find out which effervescent granule products the respondents preferred. Next, each respondent completed a questionnaire assessing the smell, colour, and taste of the preparation. The data obtained were analyzed using SPSS. The results of the Hedonic test showed that the granules produced were liked by many respondents. The formula respondents liked most was Formula 2.

Discussion

Inspection of the water content of the granules was carried out to determine the moisture or water content in the granules after drying, which would affect their flow properties.

The moisture content of the granules was measured with the Moisture Analyzer and met the requirement of 0.4%-0.7% determined by Anshory and authors (2007).

This test was carried out by flowing 100 g of granules through a funnel with three repetitions. A flow time of 100 g of granules ≤ of 10 seconds suggests the granules had a good flow rate, as shown by the flow velocity test in this study.

The flow rate was influenced by the point-of-quit; the smaller the point of quit, the better the flow rate (Lachman *et al.*, 2008). The results of the point-of-quit examination showed that all formulas met the requirements because they had an angle of rest between 25^{o} and 30° (Lachman, 1994).

The pH test results obtained from the effervescent granules ranged from 5.5 to 5.9. Therefore, based on the data obtained, the pH value of the three formulas had a good pH effervescence value.

Conclusion

The evaluation shows that the Kirinyuh leaf extract can be made into effervescent granules and has good results. Furthermore, the hedonic test findings showed that the effervescent granule extract was agreeable to respondents.

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RESEARCH ARTICLE

The potential effect of the green coffee extract on reducing atherogenic index in hyperlipidemic rats

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Abstract

Introductions: Dyslipidemia is a risk factor for atherosclerosis and cardiovascular disease. The high prevalence of dyslipidemia triggers the development of green coffee supplement products, which are claimed as cholesterol-lowering and slimming agents. Nonetheless, research data on the effect of taking green coffee supplement products, especially regarding cardiovascular function, is limited. **Aims:** To determine the potential effect of green coffee extract (GCE) on improving atherogenic index of plasma (AIP) and cardiac histopathology in hyperlipidemic rats. **Methods:** 24 rats were induced by high-fat feed for 21 days. Then, the rats were treated with a GCE, dose of 200, 400, and 800 mg/kg bodyweight for 14 days. The next day, blood was collected from the rats to take measurements of their serum lipid profile and calculating their AIP. The heart organ was created by using histopathological preparations. **Results:** Administration of GCE in all doses significantly reduced the AIP and improved cardiac histopathology in the hyperlipidemic rats. **Conclusions:** GCE can be developed as a cardio-protector.

Introduction

Dyslipidemia is a significant fat component disorder, which includes an increase in total cholesterol and Low-Density Lipoprotein (LDL) levels (called hypercholesterolemia), an increase in triglyceride levels (called hypertriglyceridemia), and a decrease in High-Density Lipoprotein (HDL) levels, or a combination thereof (DiPiro *et al*., 2014). This abnormal condition needs special attention because it is a risk factor for atherosclerosis which will lead to cardiovascular disease that comes from narrowing of blood vessels due to fat accumulation (Nelson, 2013). This disease caused by excess fat is a fairly common health problem.

In 2008, about 39% of 25-year-old had increased cholesterol levels globally. This increase in cholesterol was estimated to be the cause of 18% of cerebrovascular disease cases and 56% of ischemic heart disease in the world. The death rate caused by dyslipidemia disease affects 4.4 million people (WHO, 2002). Based on the 2013 Riskesdas Report, for people aged below 15 years old, there was 35.9% abnormal total cholesterol, 22.9% low HDL, 60.3% optimal-borderline high LDL and 15.9% high-very high LDL, abnormal triglycerides with high borderline categories of 13.0% and high-very high categories of 11.9%. East Java is in the top six for obesity cases and second place after DI Yogyakarta with a cardiovascular disease prevalence of 0.2% (Ministry of Health, 2013).

Certain factors can trigger the high prevalence of dyslipidemia to cause serious diseases. The interaction of genetic factors and environmental factors can cause hyperlipidemia (PERKI, 2013). Unhealthy lifestyles prevalent in today's society can also trigger fat accumulation in the blood circulation; these include the penchant for consuming food from fast-food restaurants and other fat-rich foods, lack of activity and exercise, alcohol consumption, obesity and a family history of hyperlipidemia (LIPI, 2009). Generally, this

disease does not cause symptoms in sufferers, but it was found that some patients had symptoms of chest pains, anxiety, sweating, and shortness of breath (DiPiro *et al*., 2014).

Dyslipidemia is one of the risk factors associated with coronary heart disease. Low HDL levels, as well as high levels of triglycerides and LDL, correlate with an increased incidence of coronary heart disease. Several lipid ratio parameters, such as the atherogenic index of plasma (AIP), Castelli's Risk Index, and the atherogenic coefficient, can be used to predict the risk of cardiovascular disease (Bhardwaj *et al*., 2013). Controlling these various lipid ratios, hopefully, can help the prevention of cardiovascular disease events and the management of dyslipidemia therapy.

In addition to the primary therapy with the statin class and lifestyle modification (LIPI, 2009; Walker, & Whittlesea, 2012), recently there are several products in the form of cholesterol-lowering supplements, slimming or weight loss on the market to reduce fat in the body. One of them is green coffee extract supplements. Green coffee is coffee that has not undergone a roasting process like black coffee and is known to have a higher chlorogenic acid content (Farah, 2012). It is also supported by the fact that Jember city is one of the five most significant contributors to Robusta coffee in East Java (Ministry of Agriculture, 2016).

Several studies have shown that the active compounds in coffee have beneficial effects, such as antioxidant (Sato *et al*., 2011), hepatoprotective (Ji *et al*., 2013), and antidiabetic effects (Ong *et al*., 2013). Besides, the active compounds in coffee can prevent the storage of carbohydrates and lipids (Shimoda *et al*., 2006). Chlorogenic acid in green coffee can increase the oxidation of fatty acids (Li *et al*., 2009). Research conducted by Shimoda and colleagues (2006) and Choi and colleagues (2016) proved that green coffee bean extract could cause weight loss in mice. Regarding lipid profile parameters, giving green coffee ethanol extract can significantly reduce LDL levels, increase HDL, and significantly reduce total cholesterol levels in white rats when induced with a high-fat diet (Setyono *et al*., 2014). Other research stated that green coffee extract could lower cholesterol and lower total triglyceride levels (Choi *et al*., 2016). Meanwhile, the latest study showed that administration of green coffee extract for 14 days in hyperlipidemic rats could significantly improve lipid profiles, except HDL levels which remained unchanged. It also enhanced the rat aorta histopathology even though it did not match normal conditions (Christianty *et al*., 2020).

So far, however, there has been no research data on the activity of green coffee extracts related to lipid ratio parameters to predict cardiovascular events. Therefore, this study aimed to determine the potential of green coffee extract in preventing cardiovascular disease based on atherogenic index parameters and histopathological cardiac features of the hyperlipidemic rats.

Materials and methods

Materials

Green bean coffee as a sample was obtained from KSU Buah Ketakasi, Sidomulyo, Silo District, Jember City. The materials used in the study included ethanol, aqua dest, HCl_2N, normal saline, NH_4OH, simvastatin tablet 10 mg (PT. Hexpharm Jaya), CMC Na, used cooking oil, quail egg yolk, BR II feed (PT Japfa Comfeed), formaldehyde, ether, triglyceride and HDL reagents, hematoxylin-eosin staining, enthelan, xylol. The experimental animals used were male Wistar rats, healthy, weight 150-250 g in weight, and around six to eight weeks old.

Methods

Preparation of green coffee extract

The green coffee extract was prepared by the maceration method. Dried green coffee beans were ground to a Simplicia powder, weighed in a certain amount and macerated using 96% ethanol 7.5 times the weight of powder. The resulting filtrate was concentrated using a rotary evaporator and then dried using an oven at a temperature of 50°C until a constant weight was obtained. Furthermore, the green coffee extract suspension was made in CMC Na 1% at a dose of 200 mg/kg, 400 mg/kg, and 800 mg/kg body weight.

In vivo activity assay

The whole procedure for the care and treatment of experimental animals had obtained the approval of the ethics committee of the Jember State Polytechnic with certificate number 615/PL17/LL/2018. A total of 25 rats were acclimatised first for seven days, then administered a high-fat diet, with a composition of quail egg yolk, using cooking oil and 0.01% propylthiouracil (PTU) orally for 21 days, except in the control group. Hyperlipidemic rats were then divided into several groups, namely the green coffee extract treatment group (dose 200 mg/kg, 400 mg/kg and 800 mg/kg body weight), and the positive control group was given simvastatin suspension 0.9 mg/kg BW and CMC Na 1% for negative control. All treatments were given orally for 14 days. On the 15^{th} day, the rats were killed, intracardiac blood was taken for measurement of lipid

profiles, and the heart organs were separated to make HE preparations.

Lipid profile measurements

The lipid profile measured was triglyceride and HDL levels. Triglyceride levels were measured using the enzymatic colourimetric method using glycerol-3-phosphate-oxidase (GPO-PAP). The test was started by mixing 10 µL of serum with 1000 µL of reagent and then incubated at 37°C for five minutes. The 1000 µL reagent mixture and 10 µL standard solution were treated the same, and then the absorbance was measured using a 546 nm wavelength Biolyzer-100TM photometer.

HDL levels were measured by the precipitation method and determined enzymatically. A sample of 200 µL was mixed with 500µL of HDL reagent, left to stand for 10 minutes at 15-25^{o} C, then centrifuged ten minutes at 4000g. The supernatant was removed from the residue within one hour. Next, pipette 100µL supernatant and mix with 1000 µL cholesterol reagent, incubated at 37^{o}C for five minutes. For the control, use 100 µL of aqua bidest with 1000 µL of cholesterol reagent, treated the same as the sample. The absorbance of the sample was measured within 60 minutes using a 546 nm wavelength Biolyzer-100TM photometer.

Atherogenic index determination

The atherogenic index was calculated based on the logarithm of the ratio between triglyceride levels and HDL levels in molar units (mmol/L), with the following formula (Frohlich & Dobiasova, 2003): Atherogenic index of plasma (AIP) = Log (TG/HDL)

Statistical analysis

The lipid profile data and AIP were expressed as means ± standard deviation ($\bar{x}$ ± SD). Between-group comparisons were performed using one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) procedure for multiple range tests. A value of $p < 0.05$ was considered significant.

Results

In this study, the induction of high-fat feed used a combination of quail egg yolk and used cooking oil (7:3) which was administered once a day, and 0.01% PTU suspension in distilled water given ad libitum. The induction was carried out for three weeks, and the results of the increase in lipid profile were obtained.

Based on the results shown in Table I, it is found that the triglyceride levels in the treatment group (both with green coffee extract and simvastatin) are lower than the negative control group. The results of statistical analysis using one-way ANOVA showed that there were significant differences ($p = 0.023$) between groups. The post hoc test with LSD showed that administration with green coffee extract had triglyceride levels that were significantly different from the CMC Na 1% group ($p < 0.05$) but not separate from the simvastatin group ($p > 0.05$). Between groups, the dose of the green coffee extract also did not show a significant difference. It means that treatment with green coffee extract (starting at a dose of 200mg/kg BW) can reduce triglyceride levels in hyperlipidemic rats, and it is equivalent to 0.9mg/kg BW of simvastatin. However, this was not the case for HDL levels, which between groups did not show a significant difference ($p = 0.379$).

The lipid profile (especially triglyceride and HDL level) data was then used to determine the AIP. Table I shows that the treatment with green coffee extracts for 14 days can reduce the AIP. According to one-way ANOVA analysis, as written in Table I, the AIP between groups has a significant difference ($p = 0.007$). The post hoc test with LSD showed that the green coffee extract group at various doses was significantly different from the CMC Na 1% group but not significantly different when compared to the simvastatin control group. Among the three doses of green coffee extract, the atherogenic index value was not significantly different. It means that green coffee extract (starting at a dose of 200 mg/kg BW) can reduce the AIP in hyperlipidemic rats, equivalent ability with 0.9 mg/kg BW simvastatin. However, based on the atherogenic index risk category classification, namely low (< 0.11), moderate (0.11 - 0.21), and high (> 0.21) (Dobiasova *et al.*, 2004), it was found that all groups had a low-risk category.

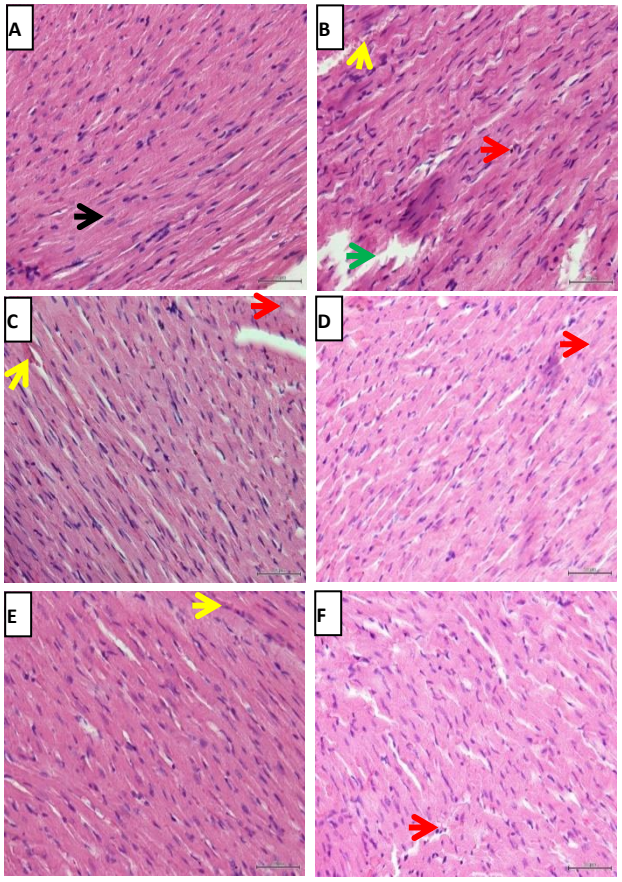
Table I: Profile of triglyceride and HDL level, and atherogenic index of plasma (AIP)

Groups	Mean level (mmol/L) ± SD		AIP	Interpretation (Based on risk category)
	Triglyceride	HDL		
Normal	0.61±0.32^{a}	0.91±0.20	-0.21±0.17^{a}	Low
CMC Na 1%	1.18±0.33^{b}	1.05±0.32	0.10±0.14^{b}	Low
Simvastatin	0.67±0.21^{a}	1.43±0.48	-0.33±0.19^{a}	Low
GCE 200 mg/kg bw	0.79±0.12^{a}	1.04±0.39	-0.15±0.19^{a}	Low
GCE 400 mg/kg bw	0.51±0.13^{a}	1.34±0.22	-0.42±0.06^{a}	Low
GCE 800 mg/kg bw	0.78±0.29^{a}	1.06±0.26	-0.18±0.21^{a}	Low

Different superscript letters indicate that there are significant differences between groups ($p < 0.05$); Anova test followed by LSD; GCE = Green coffee extract

The administration with green coffee extract and simvastatin could improve the cardiac histopathological feature of hyperlipidemic rats on

microscope observation. In normal rats, myocyte cells (longitudinal/elongated muscle fibres) were neatly arranged, parallel, and normal cell nucleus (→) (Fig. 1A). In the high-fat diet-induced rat, there were structural changes/degeneration in myocytes (→), vascular dilatation and congestion (→), and necrosis (→) (Figure 1B, 1C, 1D, 1E, 1F). The necrosis shown included pyknosis (cell nucleus shrinkage), karyorrhexis (destroyed cell nuclei), and karyolysis (cell nuclei disappeared). There was an improvement in the histopathological picture of cardiac myocytes in all treatment groups. Although some cells experienced necrosis and congestion, there was no degeneration of myocytes in the treatment group.



A. Normal rat heart muscle cells (myocytes), B. negative control (CMC Na 1%), C. positive control (simvastatin 0.9 mg/kg BW), D. GCE 200 mg/kg BW, E. GCE 400 mg/kg BW, F. GCE 800 mg/kg BW, → normal myocytes, → myocyte degeneration, → cell necrosis, → vascular dilatation and congestion.

Figure 1: Histopathology of rat heart, a cross-section with hematoxylin-eosin staining, magnification 400x

Discussion

The induction of high-fat feed used a combination of quail egg yolk and used cooking oil (7:3) which was administered once a day, and 0.01% PTU suspension in distilled water given ad libitum for three weeks successfully made hyperlipidemic model in rats. Quail egg yolk has the highest cholesterol content compared to other egg yolks, namely 2139.17 mg/100 g of eggs (Dwiloka, 2003; Aziz *et al.*, 2012). Used cooking oil contains saturated fatty acids and can increase the number of cells experiencing necrosis in rat hearts (Nurfadilah *et al.*, 2013). The addition of PTU is intended to damage the thyroid gland. Hypothyroidism can cause a decrease in the synthesis and expression of LDL receptors in the liver. That makes LDL circulate a lot in the plasma and drives hypercholesterolemia (Kapourchali *et al.*, 2014).

The green coffee extract could reduce the triglyceride level, but not with an HDL level. These results are in line with previous research, where green coffee extract could decrease all lipid profiles in high-fat feed induced rats, except HDL (Christianty *et al.*, 2020). Another study showed that the ethanol extract of green coffee beans at doses of 200 and 400 mg/kg BW could significantly reduce triglyceride levels (Shimoda *et al*., 2006). A slightly different result was found in the study conducted by Setyono and colleagues (2014), where giving green coffee ethanol extract at a dose of 400 mg/kg BW was more efficient in increasing HDL significantly in white rats induced by a high-fat diet. Still, there was no difference in lowering triglycerides (Setyono *et al*., 2014). This difference is influenced by its habitat and the processing of coffee. The difference in altitude where it grows will also affect the levels of active compounds in a plant, as well as the processing.

The increase in triglyceride levels is influenced by the rise in energy intake from the high-fat feed given. It can increase the activity of lipogenesis so that more free fatty acids are formed. The mobilisation of free fatty acids to the liver will also increase, and then it will be esterified with glycerol to form triglycerides. Meanwhile, HDL levels are more influenced by triglyceride metabolism due to lipoprotein lipase activity (Rodwell *et al*., 2018).

The green coffee extract could also lower the index atherogenic. Based on lipid profile, only the triglyceride level was affected due to differences in treatment, whereas HDL was not. Of course, it will affect the AIP, which is the result of the ratio logarithm of triglyceride and HDL levels. The higher the triglyceride level, the greater the atherogenic index value. Otherwise, the higher the HDL level, the smaller the AIP value. The AIP describes the distribution of lipids and lipoproteins in the body, which correlates significantly with the presence of risk factors for atherosclerosis, such as gender, age, dyslipidemia, and diabetes, as well as coronary angiography (Frohlich & Dobiasova, 2003). Also, the atherogenic index can be used as a parameter for routine daily monitoring, especially in patients with

other risk factors for cardiovascular disease (Niroumand *et al*., 2015). Compared to different parameters, the AIP has the most excellent sensitivity (84%) for predicting atherogenicity and the incidence of cardiovascular disease (Khazaál, 2013) and has the largest correlation coefficient compared to other lipid ratio parameters to the incidence of coronary artery disease (Bhardwaj, 2013).

From the results above, all of the groups are at low risk of developing a cardiovascular event. This was potentially due to the introduction of high-fat feed for only 21 days. The low atherogenic index in this study suggests that the risk of developing atherosclerosis and cardiovascular disease is still lacking.

The previous study showed that green coffee was known to improve inflammation and abnormalities in the heart, liver, and diastolic stiffness without increasing glucose sensitivity or lipid profiles in rats with metabolic syndrome (Bhandarkar *et al*., 2019a). Chlorogenic acid, as the main component of green coffee, can improve left ventricular diastolic stiffness by reducing collagen deposition and inflammatory cell infiltration in the left ventricle in high-fat feed rats (Bhandarkar *et al*., 2019b) and reducing interstitial collagen accumulation of the heart in an isoproterenol-induced myocardial infarction model in rats (Akila *et al*., 2017).

The AIP and cardiac histopathological feature improvement were presumed due to the main active compound in green coffee, namely chlorogenic acid. This compound is known to have a primary hypocholesterolemic effect and secondary effects such as atheroscleroprotective, cardioprotective, and hepatoprotective. The mechanism was presumed to increase the use of fatty acids in the liver through upregulation of peroxisome proliferation-activated receptor α (PPAR α) mRNA (Wan *et al*., 2012). Also, chlorogenic acid can activate AMPK (Ong *et al*., 2013), which causes metabolic responses, such as inhibiting fatty acid synthesis (through inhibition of fatty acid synthase) and increasing fatty acid oxidation (by increasing carnitine palmitoyltransferase (CPT) activity), inhibits lipolysis (through inhibition hormone-sensitive lipase (HSL)), and triglyceride synthesis (Meng *et al*., 2013).

Conclusion

This study concluded that green coffee extract has the potential to be developed as a protective agent against cardiovascular function through a series of advanced preclinical and clinical studies.

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IAI CONFERENCE

RESEARCH ARTICLE

Pandemic 2020: Economic pressure and evaluation of a primary health care innovation programme for type 2 diabetes mellitus treatment

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Abstract

Background: The COVID-19 pandemic has affected two vital sectors: the economy and health. Subsequently, people with type 2 diabetes mellitus (T2DM) face the dilemma of risking having a severe prognosis or non-compliance treatment. **Aim:** This study determines the relationship problems between the economic aspects and compliance behaviour in T2DM patients during the pandemic and how community health centres solve them. **Methods:** Data were collected from interviews with 20 T2DM patients and nine health workers in the Central Bogor region. The data were transcribed verbatim and analysed thematically. **Results:** Most patients tended to prioritise their economic condition. Besides, the community health centre has innovated an internet-based health service with particular policies to solve the problems. **Conclusion:** Economic pressure due to the COVID-19 pandemic has changed patients' mindsets. Community health centres respond with particular policies to sustain patient treatment adherence.

Introduction

Coronavirus disease 2019 (COVID-19) is a major problem affecting various countries, including Indonesia. In March 2020, the World Health Organization (WHO) declared the COVID-19 a pandemic (World Health Organization, 2020). Almost every sector in society has been affected, including the social, economic, political, cultural, defence, and security aspects. Governments have taken various measures to survive this pandemic situation (Kementrian Luar Negeri Republik Indonesia, 2020). This pandemic situation is also very closely related to patients with chronic diseases requiring regular medication. A specific type of patient prone to SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection is type 2 diabetes mellitus patients (Guo *et al.*, 2020). Type 2 diabetes mellitus (T2DM) is a risk factor that needs to be considered because it will accelerate the progression of COVID-19 and result in a worse prognosis (Hartmann-Boyce *et al.*, 2020; Singh *et al.*, 2020). Besides, T2DM caused the world's 1.5 million mortality rate in 2012 (Kementrian Kesehatan Republik Indonesia, 2016).

The pandemic has greatly disturbed Indonesia's economy, as reflected from the employee layoffs. Based on the Ministry of Manpower and BPJS Ketenagakerjaan data, 2.8 million workers were directly affected by COVID-19 (Badan Perencanaan Pengembangan Ketenagakerjaan, 2020). There were also 282 informal workers whose business was disrupted. Furthermore, according to the Indonesian Migrant Workers Protection

Agency, 100,094 Indonesian Migrant Workers (PMI) from 83 countries returned to Indonesia in the last three months. The International Monetary Fund (IMF) projects that Indonesia's unemployment rate in 2020 could be at 7.5%, an approximately 2.2% increase from 2019 (Agustiana, 2020; Badan Pusat Statistik, 2020).

Several studies suggest that the economic aspect's impact is related to various aspects of other people's lives, including the health aspect (Allen & Mirsaeidi, 2020; Pak *et al.*, 2020). On the health side, the number of visits to health checks in different regions with the non-covid condition experienced a decreasing pattern caused by the social restriction policy (Pradana et al., 2020). In Yogyakarta, there was a decrease in chronic disease health check visits by 43% from April to May 2020 (Aildasari *et al.*, 2020). Meanwhile, the number of visits to health checks in West Java were decreased by 50% (Azizah *et al.*, 2021).

Health checks are essential for monitoring patients with chronic diseases (Pradhan *et al.*, 2020). Limited access to health services presents risks for patient adherence to treatment. In T2DM, patients are expected to take medication and undergo regular checkups to prevent complications and worsening life quality, on the one hand (Aloudah *et al.*, 2018). On the other hand, it will be risky for a patient to visit a health facility for checkups during this pandemic (Apicella *et al.*, 2020; Orioli *et al.*, 2020). The potential risks that can occur during a pandemic for people with T2DM encourage the need for special monitoring of the therapy process, especially with the emergence of the Large-Scale Social Restriction policy, which has forced most patients to carry out their activities at home instead of outside (Koliaki *et al.*, 2020).

An in-depth review is required to probe the root problems and provide possible solutions in dealing with the therapy of T2DM patients, which needs monitoring and adherence. This study aims to identify the socio-economic situations that can affect patient treatment adherence and examine the functioning of innovative designs that health facilities need to implement to overcome a pandemic situation. The authors explored pre-implemented programs and policies by the Puskesmas (community health centre) in the Central Bogor area as a primary health facility to be used as a model for others. This study reviewed the socio-economic phenomena during the 2020 pandemic that have affected treatment compliance of T2DM patients due to government and community health centre policies.

Methods

This study used a qualitative phenomenological research design. The qualitative design was chosen to explore the in-depth perceptions and thoughts of the research objects, i.e. T2DM, regarding the situation faced during the 2020 pandemic. This research was conducted in the Central Bogor area, especially in the operational areas of Puskesmas Sempur, Puskesmas Belong, and Puskesmas Merdeka. It was carried out during March-August 2020. This area was chosen because it has diverse individual characteristics and is expected to provide different pandemic perceptions. The data collection area for this research is in a densely populated city centre.

Before conducting this research, ethics permission was obtained from the Ethics Committee of the Faculty of Medicine, University of Indonesia. Data were collected via in-depth interviews, observations, and focus group discussions with participants. In this study, the participants were T2DM patients who used to undergo routine checkups at the local health centre, active health workers, and the head of the local Puskesmas, all of whom had signed informed consent forms as proof of involvement in the study. The health workers involved in this study were doctors, nurses, and pharmacists who directly deal with the patient's treatment process. Nine health workers from the three community health centres and 20 patients participated in this study. The interviews were carried out with the patients, while focus group discussions were held with the health workers and heads of Puskesmas for data confirmation.

The research data were validated by triangulating information between the interviews' results and direct observation of patients with the focus group discussions. The interviews were carried out using the probing technique and double-check method, in which each participant's answer was reconfirmed during the interview, then all answers were summarised and concluded at the end of the interview. The double-check method is carried out by using two interview methods: telephone and face-to-face. Also, data were validated by comparing the patients' data recorded at the local health facilities. The data were analysed based on interview transcripts, and then thematic analysis was performed to identify the pattern in each participant's statements.

Results

The authors made direct observations at the residence of some patients spread over the Central Bogor area. In-depth-interview with the patients were conducted simultaneously with the observations. A total of 20 patients were willing to participate in the study. Geographically, according to the observations, each Puskesmas area provides an overview of the different

participant characteristics. The Puskesmas Belong area, near a marketplace, has patients whose occupations are traders or merchants, whose economic power relies heavily on sales. However, their dependency on work largely influences their treatment compliance. The Puskesmas Sempur and Puskesmas Merdeka areas have similar characteristics because they are near the main road, and the patients live in individual housing complexes on average. Most of them are company workers, while some open shops to support their economic activities.

Participant economic conditions during the pandemic

Generally, the patients live near each other in a large area. However, the roads are only one motorbike–wide, except for places such as the Ministry of Agriculture neighbourhood, which is in the proximity of the Puskesmas Merdeka or Sempur neighbourhood areas. Some patients who live in the Babakan area, close to Bogor Agricultural University, rent out rooms at boarding houses to earn money. Unfortunately, the universities have implemented distanced learning, leading to a decrease in their income since the students are absent and reduced sales in some patient-owned snack businesses, laundry services, and nearby food stalls. Some patients stated that they were reluctant to buy medicines due to financial difficulties. This economic problem pressures them and hinders them from seeking treatment, especially if they are uninsured.

> *'… I do not want to go to the clinic because I have not had a BPJS (National Health Insurance) yet. If there is no BPJS, I am afraid. I am not ready because if you go to the doctor and do not bring IDR 500,000, I am afraid the doctor will examine everything, and it will increase the cost….'*

Concerns about economic pressure (MW, complicated diabetes patient, adult)

> *'… In this situation, we have to think about ourselves, find food for ourselves, pay for the rent by ourselves; how do you sometimes feel confused when you pay rent because, in reality, you do not have any money…..'*

Concerns about economic pressure (S, complicated diabetes patient, adult)

Some patients who no longer have income chose to cut costs. As in the following statement, the patient tried to continue obtaining health care by downgrading his insurance membership class.

> *'… I do not make money already. I only get money from my children. So, in the end, I moved again to third grade (National Health Insurance), along with the three of us ….'*

Health coverage condition (ES, complicated diabetes patient, elderly)

Some patients admitted that they are reluctant to undergo necessary specific medical procedures as costly, which encouraged them to choose cheaper alternative therapies to improve their existing conditions.

> *'… It was a day when I want to be given a referral to Cipto Hospital, but I am lazy because going to Cipto required so much money for the doctor. In the past of 2004, you needed a minimum IDR 500,000.00. It was expensive. I refused it, so I did not go to Cipto. When I went to my home, at Anyar Market, there was a potion maker, I was given eight kinds of potions, boiled them into three glasses, I drank a little, sir, it destroyed my gallstone, sir, so what do you say like that water is flickering, urine that after eating it. Alhamdulillah, until now, sir, thank God….'*

Alternative medicine usage in patients (AA, complicated diabetes patient, elderly)

The patients always wanted to live a healthy life, especially those in productive periods. Productive-period patients who had dependents were pushed to work naturally to meet the needs of family life. The condition forced them to take medicine because they would not meet their family's needs if they cannot work because of diabetes.

> *'… If I die, what do my children do? Who takes care of him, so what should I do? I try to take medicine because I am afraid there is something with me. My child motivates me. I want to be healthy because of my kids ….'*

Concerns about adherence motivation (YA, complicated diabetes patient, adult)

Patients may have experienced confusion in choosing between money or being healthy because they believe that the two must go hand in hand. Without money, they believe that they cannot live and become cost-oriented in judging everything. Moreover, following inclusion in health programs, they would appreciate it if they were given money after joining the health program.

> *'… Patients sometimes do not take medication regularly because they do not get the knowledge or lack of funds to buy medicine. At the health centre or the Posbindu, the doctor explained that people with diabetes and high blood pressure regularly take their medication. After that, I got special money from the T2DM program because I diligently took medicine ….'*

Patient motivation in treatment adherence (NH, complicated diabetes patient, elderly)

The concept of the patients' mindset, which highlights everything based on economic factors, was confirmed by the health volunteers, who were representatives of the community health centres. The volunteers found it difficult to persuade the patients because no amount of money can be offered for joining the program. Patients were unaware of the benefits of the program. As far as the patients knew, they were willing to participate in these activities because transport money is given as compensation. Had they understood the program better, they could have seen this as an opportunity for them to have their health checked free of charge.

'…yes, when it comes to money, surely everyone will come, but for Posbindu, whose programs are not given money, I usually have to go around, so I have to pick up one by one. However, if there is transport money, it is given immediately to come….'

Concerns about adherence motivation (public health volunteer, Belong)

Patients also shared various views on the government; some trusted it and felt that it had done its job in bringing health access closer to the public. That condition is vital concerning the patient's view of the government-owned health facilities available in their environment. Different mindsets, criticisms, and suggestions are received by the government, depending on their experience.

'… The government should pay attention to the pattern of public health. So far, if there are patients who come, they are just checked, but those who cannot come. How is her fate? They should be visited, checked at his house because it is safe. This kind of situation will be difficult, especially if people have to queue, instead of the risk of infectious diseases?…'

Concerns about the health care system in the pandemic era (S, complicated diabetes patient, adult)

'… The government will not disappoint its people. They must think about health, prosperity, togetherness, community cohesiveness. So we do not have to blame the government. In any situation, especially the current situation, we should support the work of the government. The government cannot possibly torture its people. Maybe for others, there are cons, it does not matter. Of course, it is a form of input and support for the government….'

Opinion about the government in the health care system (UH, complicated diabetes patients, elderly)

'… At the moment, many government facilities have been repaired. BPJS, especially for treatment service, needs to be considered because many patients entering the hospital with BPJS must go home quickly before feeling healthy completely. However, in the future, hopefully, it will be better….'

Opinion about Universal Health Coverage in Indonesia (SM, complicated diabetes patient, adult)

Government policies

The patients also expected the government to fulfil their wishes.

'… I want to have a prosthetic leg. I used to get capital assistance from the government, but it is still lacking, and I cannot buy it until now. I still cannot buy it because the problem again is money…..'

Patient motivation in the treatment process (R, diabetes patient, adult)

'… I went to the Puskesmas and complained about the health workers' attitude. I said they were sick, but they did not have money. They do not want to be like that and only hope for government assistance. They want to be healthy, they want to have money like other people, but because it is God's destiny, I hope the Puskesmas does not differentiate between the services provided ….'

Concerns about the health care system (N, diabetes patient, adult)

The 2020 pandemic has significantly impacted how T2DM patients can obtain treatment. This condition is in line with the 'New Normal' policy by the government. Regarding T2DM, it has become more difficult for patients to monitor their blood sugar levels.

'… Right now, during the Corona pandemic, I feel scared, sir. I want to go to the Puskesmas, but I am afraid something will happen. So I did not go around, I just let it go….'

Concerns about a health condition in the pandemic era (SA, diabetes patient, elderly)

'… In the past, I often checked monthly, but now I do not. I usually wait for a doctor's referral to check the lab, but I cannot check it because of the current condition, even though I want to know my current health progress. However, because of the Corona, I was afraid of getting an infection….'

Concerns about a health condition in the pandemic era (M, diabetes patient, adult)

Some Puskesmas adopted the policy of eliminating blood sugar checking services in the Puskesmas network (supporting Puskesmas). Moreover, the said services are only provided at the central Puskesmas, eventually forcing them to halt diabetes testing. The long distance between the patients' homes and the

health centres has made it difficult for patients to attend checkups.

> *'… Because of Corona, I never checked my health. I want the pharmacy's control, but the pharmacy often closes quickly at 6:00 pm. I also do not take diabetes medicine now. Usually, at 6:00 am, I take my medication there and check everything, such as cholesterol, uric acid, and blood pressure. The last thing I remember, my sugar was high, 170. Before Corona, I used to control it every two weeks. However, until now, it has been a long time, about almost four months, I do not control it….'*

Concerns about a health condition in the pandemic era (S, complicated diabetes patient, adult)

> *'… In the Corona era, I think it is too risky to take medication, especially if there is no strict health protocol. I am afraid that if medical treatment can spread the coronavirus, which can infect the community. It is not enough if you only wear a mask. You need to wear gloves to cut the distribution of the virus. I think, therefore, health facilities such as Puskesmas must be repaired….'*

Concerns about the health care system in the pandemic era (ST, complicated diabetes patient, adult)

Relationship with the health aspect

The most visible problem was the access distance between the patient and the Puskesmas. The absence of blood sugar examination services at auxiliary Puskesmas during the pandemic led most patients to opt not to attend checkups there.

> *'… During this pandemic period, because the Puskesmas moved from Babakan to Sempur, I felt lazy. I have never been rechecked for health because it is very far from home ….'*

Medical problems in patients during the pandemic (N, complicated diabetes patient, elderly)

> *'… It is far away because usually in the house's Puskesmas, there is a laboratory section every day. Now I have to go to Merdeka Puskesmas. The Puskesmas near the house is closed now. It has been since the beginning of the pandemic ….'*

Medical problems in patients during the pandemic (D, complicated diabetes patient, adult)

> *'… The Puskesmas, which is far away, is also uphill. So it is pretty tiring too. There is a Puskesmas here too, but they cannot check sugar ….'*

Medical problems in patients during the pandemic (D, diabetes patient, adult)

Furthermore, the patients tried to change their diet. Some patients had opinions about what foods to avoid during this pandemic.

> *'… I do not eat chicken or any meat because I see it on television. I am worried that during the current Corona season, the food will catch the virus. So I have to be careful, sir, and the coronavirus is rapidly infecting diabetes patients. Alhamdulillah, I also take care of myself wearing a mask, sir ….'*

Concerns about a health condition in the pandemic era (AA, complicated diabetes patient, elderly)

Aside from diet, the pandemic has affected patients' patterns. Correspondingly, many patients felt that the pandemic has made them unable to exercise again. The physical distancing policy has led to the closure of many public places for exercise to prevent crowds, including several parks where patients usually do aerobic exercise or other sports. In the end, many patients felt feeling unwell because they were not used to not exercising during the pandemic.

> *'… I can run, also sometimes participate in healthy gymnastics in the field before there is the Corona. All of these activities are my usual routine ….'*

Concerns about a health condition in the pandemic era (S, diabetes patient, elderly)

> *'… I exercise regularly. Since there was this Corona, I could not get out, so I could not exercise, so I do not feel well because I usually exercise, and now I do not. ….'*

Concerns about a health condition in the pandemic era (S, complicated diabetes patient, adult)

Innovation for pandemic period adaptation

Health facilities were also affected during the pandemic period. In preparation, every health facility will implement a unique strategy to anticipate pandemic conditions. Puskesmas is a primary health facility that is close to society. In one year, the Puskesmas has presented health programs oriented towards public health. Some Puskesmas have innovated by using an online system or establishing a policy of taking medication for one month; this was, of course, adjusted to the conditions at hand.

> *'… In the Non-Communicable Diseases program, we use an online system to register and have a quota because of this pandemic period. For example, Monday, we have five people, then we will prepare their medical records; all kinds of equipment will then be handed over to the doctor. The doctor will give a prescription and send it to the pharmacy. When patients arrive, they take medicine directly. This system is a solution during a pandemic to*

minimise the risk of transmission to patients, especially with diabetes mellitus cases…..'

Health system innovation during a pandemic (Non-communicable diseases programmer, Merdeka)

As long as it was supported by the Internet and knowledge of basic computing system operation, the online system ran smoothly and precisely. Social media groups, such as WhatsApp, were used. Besides distributing medicine properly, healthcare providers also provided health information through social media groups:

'… To keep the patient doing physical activity at home, doctors share videos containing the exercise method on the WhatsApp [WA] group. We do this so that they can still exercise themselves at home. So there are several groups in the WA group, elderly and adults. Several exercise videos have been shared, elderly exercise, Tobelo exercise, Zumba exercise, and creative exercise. Here are also various health information. The problem is that we cannot provide direct counselling, especially if we have to do in-depth counselling....'

Health system innovation during a pandemic (Non-communicable diseases programmer, Merdeka)

Puskesmas need to innovate to ensure patients continue taking their medicines to control their blood sugar levels. Ease of access to medications must be secured, especially for patients who are BPJS participants. Puskesmas must guarantee that patients' rights are upheld, namely determining the latest diabetes mellitus status and obtaining medications.

'… Regarding treatment adherence problems, initially, we only gave the drug for two weeks. However, after an assessment was carried out during the pandemic period, it was found that many of our diabetes patients were elderly, which they were vulnerable to COVID infection. So we issued a policy of taking drugs to be once a month for their safety. However, we still inform patients if they feel that something is worsening; for example, they are not feeling well, they are welcome to take control at the Puskesmas….'

Health system innovation during a pandemic (Doctor, Belong)

Besides the online system innovation, policy adjustments were also made, like what Puskesmas Belong did, where it changed its policy for medication collection from 1-2 weeks to 1 month.

Discussion

The research transcript data indicated a strong relationship between economic factors and patient compliance behaviour. The COVID-19 pandemic situation has caused financial pressure that has impacted various sectors, including the health sector. Under economic pressure, patients have been forced to survive through their incomes. However, these incomes are prone to decline, pushing patients to be cost-effective, including in the health sector. Patients prefer to reduce drug purchases to reduce their spending. The data confirm that a person's economic condition would pressure the person (Cobden *et al.*, 2010; Cutler *et al.*, 2018; Lee *et al.*, 2006). This pressure can influence a person's thought process and attitudes to adapt by making efforts to save themselves (Block *et al.*, 2020; Yan *et al.*, 2020).

Economic pressures and changes in mindset

This research shows that the economy and health are strongly correlated (Aptel & Toren, 2020). This relationship ultimately impacts patient therapy. Economic limitations can force patients to choose alternative treatments instead of the anticipated medical actions. In financial stress situations, the patient struggles to stay afloat (Al-Hasan *et al.*, 2020). Healthcare costs make a large portion of the patient's expenditure, especially for patients with special conditions, such as diabetes with very high sugar levels, diabetic foot infection, and kidney deterioration due to diabetes (Andersson *et al.*, 2020; Visaria *et al.*, 2020). Patients do everything they can to survive with the existing limitations (Ding *et al.*, 2020; Hooft *et al.*, 2020). They have to support their families while living in fear of being unable to fulfil their basic daily needs due to the economic downturn. This fear ultimately affects the patient's treatment compliance behaviour (Hall, 2020; Markovski, 2020). The close relationship between the economy and health positively impacts the patient's life (Aptel & Toren, 2020). The patients believe that they cannot live without money. The thought processes presented in the present study are oriented towards money as an indicator of their economy. Economic pressure can change the patient's mindset (Nguyen *et al.*, 2020; Sajjad & Shahbaz, 2020).

Various things cannot be separated from the government's role in striving for public access to health services (Fafard & SJ, 2020; Hunger *et al.*, 2020). Patients and the government will have different opinions, depending on their experience (Frizelle, 2020). In the present study, the patients also expect the government to fulfil their wishes. The government serves the public and is obliged to meet the community's various needs (Greer *et al.*, 2020;

Ukhalkar, 2019). Patients who feel that their needs have been met will have a positive outlook (Putri & Wirawati, 2020). However, if they experience less pleasant services, they will criticise them until their expectations are met (Salokhiddinovich & Farrukh, 2020). The government can indeed be described as a place for patients to receive assistance for their survival, especially those who are not financially independent (Warner *et al.*, 2020).

The 2020 pandemic has significantly impacted how diabetes mellitus patients can obtain treatment. Many patient behaviours will change alongside the adaptation process in overcoming this pandemic (Acuña-Zegarra *et al.*, 2020; Bavel *et al.*, 2020). In the end, patients will also have to adapt to continue their normal activities (Weill *et al.*, 2020). This condition is in line with the government's New Normal policy. Changes in health facility policies due to the pandemic will directly impact the patients (Rosenbaum, 2020; Shayak *et al.*, 2020). Patients tend to follow the existing policies hoping that they can solve their problem. Many patients want to comply with treatment, but this conflicts with daily activities, work, and economic pressures.

Impact on health aspect dan innovations

One of the main problems is the patients' lack of access to the Puskesmas. During this pandemic, difficult access impedes patient communication with medical workers. An innovation regarding this issue needs to be made to allow patients to obtain adequate information and health services (Crespo-Gonzalez *et al.*, 2020). Health facilities must anticipate this condition by providing health services to patients and thinking about new strategies to sustain treatment adherence. In the case of T2DM, the patient's sugar level must be continuously monitored. Health policies will be taken in line with occurring developments (Raboisson & Lhermie, 2020). Every health facility will carry out unique strategies to anticipate pandemic conditions. During this time, the concern is handling things quickly and accurately, especially for COVID-19–infected patients in the area (Legido-Quigley *et al.*, 2020). Of course, the referral system and the like are prepared following Central Government policies.

T2DM is a specific comorbid disease that is highly risky during this pandemic. The risk of mortality will increase more in patients with diabetes mellitus (Zhou *et al.*, 2020); thus, it has to be controlled (Riddle *et al.*, 2020). Patients must do regular checkups to monitor the disease and take medicines. As a government-owned health facility, Puskesmas has a system that must align with existing policies and develop innovations to continue on its programs (Buffart *et al.*, 2020). Puskesmas must also carry out their functions optimally under government regulations and policies.

Puskesmas is a primary health facility that is close to society. In one year, the Puskesmas has health programs that are oriented towards public health. The programs run by Puskesmas must have the critical aim of improving health status, even during this pandemic. All forms of technical policies made must be able to support public health. In handling non-communicable diseases, in this case, T2DM, Puskesmas still has to ensure the patient's access to health services. The present study shows that an online system provides an easy coordination pattern with minimal risk. This system can be the safest choice because of its fast-paced nature, minimal physical contact, and easy operation (Allam *et al.*, 2020; Chen *et al.*, 2020). As long as it is supported by the Internet and knowledge of basic computing system operation, the online system will run smoothly and precisely (Smith *et al.*, 2020). Figure 1 shows how the community health centre built the innovation system.

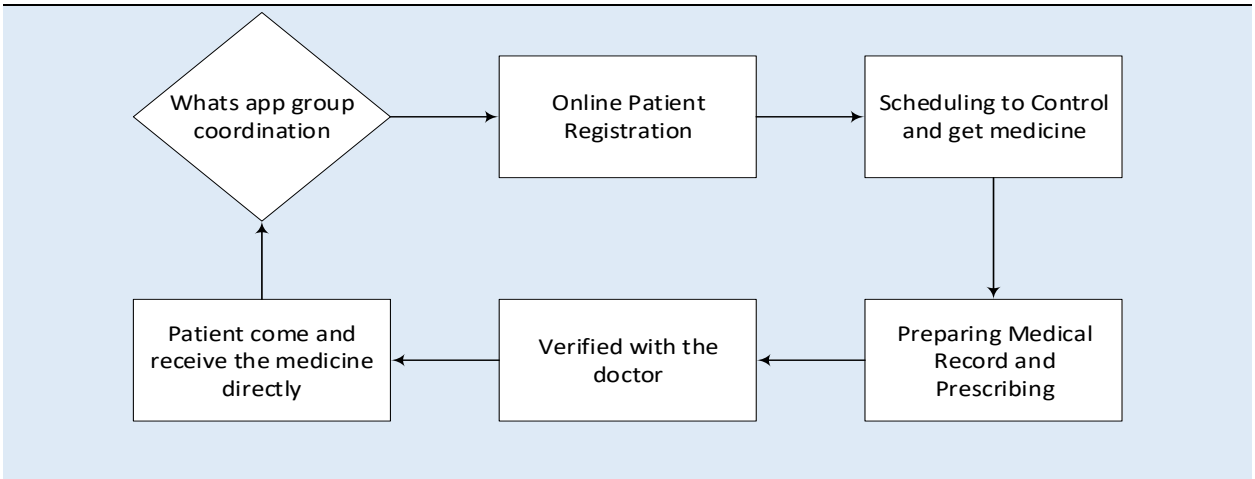

Figure 1: Health system innovation during pandemic COVID-19 at community health centres

The innovation system was built to ensure that patients can still carry out routine diabetes mellitus checks to ease monitoring. It can be said that the system's description is in line with the standard protocol for a routine examination at a health centre; the difference is that it is coordinated through the social media group WhatsApp. In pandemic conditions, contact with areas of infection, such as health facilities, must be minimised, including contact when patients register manually. The system is built to accelerate the administration process for patients to receive their medicines immediately without physical contact with the prescribing doctor. However, this creates a gap in the monitoring process; hence, the patient's diabetes condition cannot be observed directly since the consultation is performed online.

Telemedicine, a combination of technology and medical action that can be executed through social media applications such as WhatsApp, is a mechanism for which implementation is necessary during the pandemic to prevent the viral infection spread that is risky for T2DM patients (Budd *et al.*, 2020). WhatsApp is widely used because of its cheap and user-friendly features for many social classes. However, its capacity is limited to fewer than 300 members in a group. Another possible alternative is Telegram, where one group can accommodate up to 5000 members. Given sufficient numbers of members, the Puskesmas can consider Telegram as an alternative (Kamarudin *et al.*, 2020; Masoni & Guelfi, 2020).

Disseminating information via online coordination between health workers and patients will minimise the spread of false news (Budd *et al.*, 2020). The online system's weakness is that not all patients can fully utilise this facility, especially elderly patients who are not tech-savvy (Gerke *et al.*, 2020). However, this information can be relayed through the patient's family members, as they would help maintain its veracity. After all, the environment also plays a role in the patient's life (Fafard & SJ, 2020). Puskesmas needs to accommodate the accuracy of information so that it can be conveyed to the public. At the very least, this coordination should extend to the patient's family or closest relatives. Health cadres and community leaders can be persuaded to disseminate this information (Putri & Wirawati, 2020).

Efforts to achieve patient compliance are also the responsibility of the Puskesmas. Puskesmas need to innovate on making patients continue taking their medicines to control their blood sugar levels. As a public health facility, the Puskesmas must guarantee that the patient's rights are upheld, namely determining the latest diabetes mellitus status and obtaining medications. Another possible form of innovation is policy adjustments, as Puskesmas Belong did, which changed its policy of medication collection from 1–2 weeks to monthly. The Puskesmas stated that this was to prevent vulnerable patients from being exposed to COVID-19, as T2DM patients are high-risk patients. This change does have a drawback, namely, difficulties in controlling the patients' blood sugar levels. However, this problem can be anticipated by providing access for patients to be served whenever they need it. Decision-making and policy implementation is certainly not without risks (Greer *et al.*, 2020; Salokhiddinovich & Farrukh, 2020). Therefore, it is more appropriate to make decisions and policies based on minimal risk for patients while ensuring flexibility to change over time.

Study limitations

This innovation can be a form of vertical policy implementation to maintain health services' availability to patients. With this system, it is hoped that patients will continue to comply with treatment, even in a pandemic situation. This cutting-edge model is implementable, as many patients also have the technology to apply it. Nevertheless, this research is limited to the innovation model developed by the Puskesmas in the Central Bogor area; further analyses of other parts of the region or types of health facilities are required. The impact of the innovation on patient adherence problems should be examined, so further research is needed to evaluate the model's application. This implementation must be seen in various circles, where some patients are known to have neither the necessary tools nor the aptitude of technology for online treatments. The drug-taking policy also requires evaluation to assess the possible challenges of controlling patient adherence due to minimal contact with health workers.

Conclusion

This study shows that economic pressure can change the patient's mindset, significantly affecting medication adherence in type 2 diabetes mellitus treatment. The deteriorating financial condition discourages patients from prioritising routine health checks at health facilities because of reluctance to pay medical expenses. Concerning the problem of treatment compliance, three community health centres in Central Bogor Region implemented an innovation in the form of an Internet-based health service system with WhatsApp groups and a medication collection policy to minimise the risk of contact with areas of infection. This study still has limitations on the variety of innovations observed and the extent of their impact. Further

studies are needed to see other pilot projects during a pandemic and evaluate those carried out so far.

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RESEARCH ARTICLE

Acute toxicity study of the ethanolic extract of *Eleutherine bulbosa Urb* in Wistar rats

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Abstract

Introduction: *Eleutherine bulbosa Urb* is a plant species with medicinal properties, including anti-inflammatory, widely relied upon in traditional practices. For this reason, the present research was intended to assess and, thus, ensure the safety of this plant for conventional medicinal purposes using a toxicity test study. **Methods:** The acute toxicity test of the ethanolic extract of *E. bulbosa Urb* (EEEB) used the method adopted from the Organization Economic Cooperation and Development (OECD) guidelines 425 for testing Wistar rats. **Results:** During 14 days of the acute toxicity study, there were no significant changes in rat weight, no mortality, and no signs of toxicity after the oral EEEB administration at 2000 mg/kg body weight (bw). The limit test showed that the LD50 of EEEB was higher than 2000 mg/kg bw. **Conclusion:** EEEB has low toxicity because its LD_{50} is higher than the limit test results.

Introduction

More than 30,000 types of plants and 1,000 types of medicinal plants have been used in the traditional medicinal industry in Indonesia. Medicinal plants are, in general, forest plants that have been grown in yards and hereditarily used as traditional medicine since the era of ancestors. Recently, they have been widely developed as Indonesian traditional herbal medicine, namely "jamu", standardised herbal medicine, and phytopharmacy (Anam *et al*., 2013). "Bawang Dayak" (*Eleutherine bulbosa Urb.*) is an example of medicinal plants, nutritious for health but still scarcely used in community medicine. This plant is commonly found in South Kalimantan island, where the locals already use it as traditional medicine. Its bulbs are widely used for several therapeutic purposes. *E. bulbosa* Urb. effectively reduces cholesterol (Anjar, 2016) and has antihypertensive, immunomodulatory, and anti-inflammatory activities (Muthia & Astuti, 2018; Paramita & Nuryanto, 2018; Rauf *et al.*, 2018). The bulb extracts contain flavonoids, phenolics, saponins, and tannins (Andi *et al.*, 2013; Pratiwi *et al.*, 2013; Muthia *et al.,* 2021).

Acute toxicity testing is a preclinical test aiming to measure the toxic effects (degree of toxicity) of a compound or chemical that occurs immediately or shortly after it is delivered orally as a single dose or repeatedly within 24 hours (WHO, 2004). It is designed to quantitatively measure the Lethal Dose 50 (LD_{50}) of a substance. Its parameter includes the mortality of the test animals (Dipasqual, 2001). Medicinal plants must go through various testing processes for the safety of their consumption, one of which is the acute toxicity test (Syamsul *et al.*, 2015). As *E. bulbosa* Urb has many therapeutic and non-therapeutic properties, it is necessary to test its acute toxicity.

Methods

Plant collection and sample preparation

The *Eleutherine bulbosa Urb* plants were collected from Banjarbaru, South Kalimantan, and determined at the Herbarium Bogoriense, Biology Research Center, Indonesian Institute of Sciences (LIPI) Bogor, with the registration number 2244/IPH.1.01/If.07/XII/2019. The bulbs were separated, cleaned, washed, cut into small pieces, and dried by aeration. Afterwards, the dried bulb samples were ground to fine powders, which were later sieved using mesh number 16 and stored in closed containers.

Bulb extract preparation

The bulb powders obtained from the previous procedure were extracted by maceration for 24 hours, using 96% ethanol as the solvent (DepKes, 2014). The resulting filtrate was filtered, and the residual pulp was macerated twice using the same maceration procedure and solvent. The ethanolic extract was evaporated in a rotary evaporator at 45^{o}C and a water bath until a fixed weight was reached.

Approval from the animal ethics committee

The acute toxicity test was performed on seven healthy non-pregnant female Wistar rats aged 2-3 months old and weighing about 100-200 grams. The procedures involved were conducted after receiving approval from the institutional ethical committee University of Surabaya No: 141/KE/X/2020.

Acute toxicity test

As per the OECD Guidelines 425 (Up-and-Down Procedure) (OECD, 2001; OECD 2008), the test rats were kept in a standard condition for 15 days. The limit test for single peroral administration was conducted at 2000 mg/kg body weight (bw). The test rats were given no food three to four hours before the administration but had ad libitum access to water. After the prepared extract was given to one female rat, it was closely observed for any toxic effects in the first 30 minutes, 4 hours, and then regularly (at an interval of 24 hours) for 14 days. Food was provided one to two hours after the administration. If this test rat survived the procedure, the extract was given to four additional rats at the same dose and under the same conditions. These five test rats were labelled as the treated group. However, if it died, the main test to calculate the LD_{50} of responses was initiated. If three animals died, the limit test was terminated, then the main test was performed. The LD_{50} was greater than 2000 mg/kg bw if three or more test animals survived the procedure. Apart from the treated group, the experiment also used two other test rats as the control (vehicle-treated group). This group was given 1% carboxymethyl cellulose (CMC) gel orally, then, like the treated group, monitored for any toxic effects in the first 30 minutes, four hours, and at a regular interval of 24 hours for 14 days. The test rats that survived were examined for the onset of toxic reactions; their weights were also monitored and documented until the end of the study (OECD 2001; OECD 2008). The LD_{50} was computed in the Acute Oral Toxicity (AOT) 425 StatPgm software. After the experiment, the test rats that survived were anesthetised and sacrificed for histopathology.

Statistical analysis

The body weights were expressed as mean±SD, and the statistical significance between the treated and control groups was analysed using an independent-samples t-test on SPSS version 16. $p \leq 0.05$ reflected statistically significant differences.

Results

Behavioural pattern and body weight

Table I shows the test rats' weights in the control and treated groups. Table II presents the behavioural pattern of these rats observed after administering the ethanolic extract of *E. bulbosa Urb.*

Table I: Mean weight of the test rats in control and treated groups in the 14-day acute toxicity test

Treatment	Body weights (g)			
	Day 0	Day 1	Day 7	Day 14
Control	154.7±27.08	155.5±23.3	173.5±6.3	189.5±2.1
EEEB	153.8±17.7	147.8±15.6	169.8±12.6	173.6±17.8

Value provided as mean±SD (n=2) for control; (n=5) for treated group

Table II: Toxicity symptoms in control and treated groups in the acute toxicity test

Parameters	Symptoms of Toxicity							
	30 minutes		4 hours		24 hours		14 days	
	CG	TG	CG	TG	CG	TG	CG	TG
Fur & Skin	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N
Respiration	N	N	N	N	N	N	N	N
Convulsions	NF	NF	NF	NF	NF	NF	NF	NF
Tremors	NF	NF	NF	NF	NF	NF	NF	NF
Diarrhea	NF	NF	NF	NF	NF	NF	NF	NF
Mortality	NF	NF	NF	NF	NF	NF	NF	NF

CG = Control Group; TG = Treated Group; N = Normal; NF = Not Found

Acute toxicity test results

All rats used in the toxicity test of the ethanolic extract of *Eleutherine bulbosa* Urb., which were administered at 2000 mg/kg bw, showed no symptoms of toxicity and survived until Day 14 of the observation, meaning that the LD_{50} of this extract is higher than 2000 mg/kg bw. At this state, the LD_{50} fell into category 5: no symptoms of toxicity at a dose of 2000 mg/kg bw. Screenshots of the acute toxicity test results in the AOT 425 StatPgm software are presented in Figure 1.

Histopathological results

Treated rats were given the ethanolic extract of *Eleutherine bulbosa* Urb at a limit dose of 2000mg/kg bw. Liver and heart histopathological sections can be seen in Figure 2.

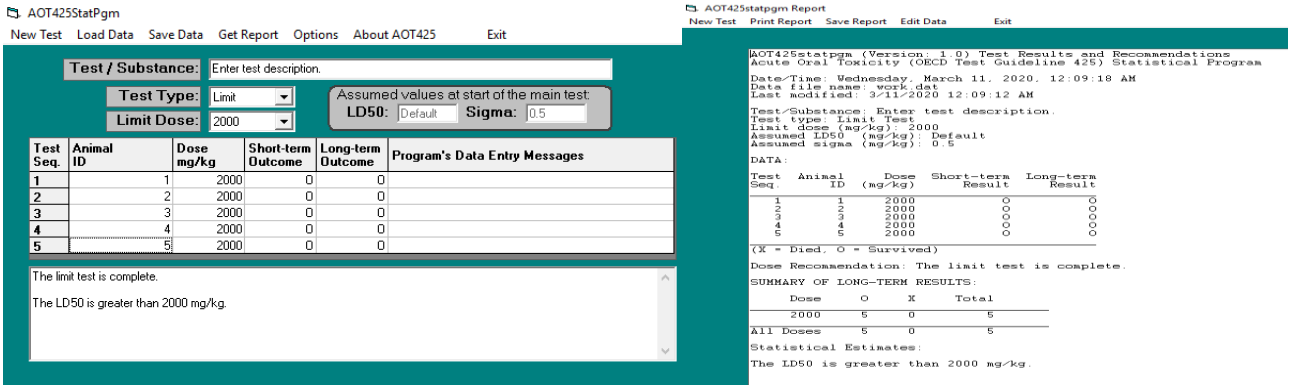
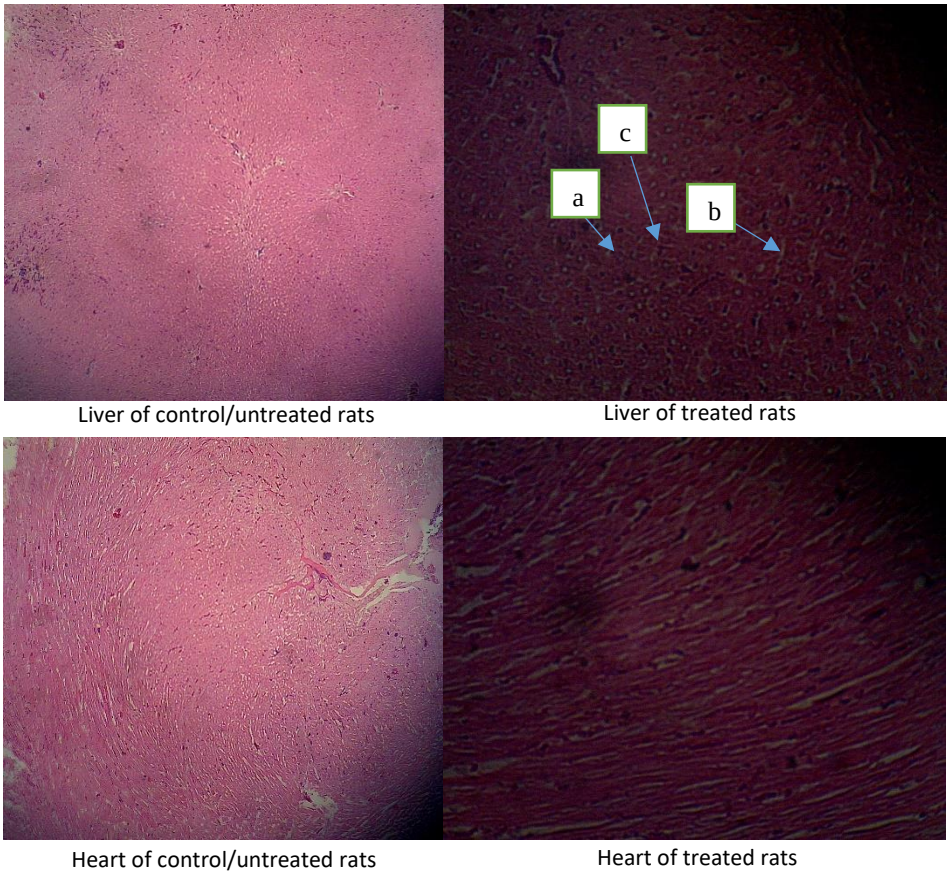


Figure 1: The AOT 425 StatPgm window for the acute toxicity test results



Liver of control/untreated rats

Liver of treated rats

Notes:

a) Normal hepatocyte
b) Hydropic degeneration
c) Hepatocyte necrosis

Heart of control/untreated rats

Heart of treated rats

Figure 2: Liver and heart histopathological sections of the control and treated rats (Hematoxylin and Eosin staining, 40x10 magnification)

Discussion

All test rats survived the toxicity study of the ethanolic extract of *Eleutherine bulbosa* Urb at a dose of 2000 mg/kg bw. The observations were made in the first 30 minutes up to 4 hours after the extract administration and then periodically for 14 days, meaning that the resulting LD_{50} of the ethanolic extract was from a dose higher than 2000 mg/kg bw.

As seen in Tables I and II, the control group gained weight throughout the 14 days of the toxicity study, while the treated group experienced fluctuating weights until the end of the observation. Weight changes are considered the manifestation of the toxic effects of a substance (Jothy *et al.*, 2011; BPOM 2014). Generally, the decrease in the weight of the body and internal organs are simple and sensitive indices of toxicity after exposure to toxic substances. Changes in body weight are indicators of drug and chemical adverse effects, considered significant if the loss is 10% from initial body weight (Vaghasiya *et al.,* 2011). The average body weight was analyzed using the statistical independent-samples t-test on SPSS, and no significant differences in body weight were found between the control group and the treatment group. The independent t-test of these weights resulted in a sig (2-tailed) value of 0.533, with $p > 0.05$. Wati *et al.* (2018) confirm no body weight change in the acute toxicity test, suggesting normal body metabolic processes (Klaassen, 2018). The acute toxicity of the ethanolic extract of *Eleutherine bulbosa* Urb was assessed in mice after orally administered at 1000, 2000, 3000, 4000, and 5000 mg/kg BW. These doses neither caused mortality nor show signs of toxicity (Hanh *et al.*, 2018).

In the OECD 425 guidelines (2008), toxicity can be reflected by changes in skin, hair, and eyes. Other signs include lethargy, convulsions (seizures), tremors, diarrhoea, and death of the test animals. In this study, the test rats were examined for any of these toxicity symptoms. During 14 days of observation, no such manifestations were found in the test rats. Also, the administration of the ethanolic extract of *E. bulbosa* Urb bulbs at 2000 mg/kg bw did not cause mortality. The LD_{50} of this extract was found to be higher than 2000 mg/kg bw, which, according to the criteria for preparations set by BPOM RI (2014), is classified as "mildly toxic". For this reason, it is safe to suggest that relevant national or state agencies for food and drug controls, especially the ones in Indonesia, can authorise the mass production of preparations made of this ethanolic extract.

The histopathological sections presented in Figure 2 show that the treated rat's liver exhibited hydropic degeneration and no hepatocyte necrosis. Liver fatty degeneration is damage to hepatocytes marked by morphological changes and decreased organ function due to fat accumulation in the cytoplasm of liver cells, as apparent from the clear microscopic patches of fat. Similarly, the heart of the rats treated with the ethanolic extract at a limit dose of 2000mg/kg bw showed normal myocytes (Aiyalu & Ramasamy, 2016).

Conclusion

The LD_{50} of the ethanolic extract of *Eleutherine bulbosa* Urb is higher than 2000mg/kg bw, and no toxicity symptoms have been found.

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RESEARCH ARTICLE

Evaluation of antidiarrheal effect of combination of Salam Leaves (*Syzygiumpolyanthum*) and Jackfruit Leaves (*Artocarpus heterophyllus Lam.*) infusum in rats induced by castor oil

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Keywords
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Abstract

Backround: Diarrhoea is a condition characterised by watery, loose stools that occurs more than three times daily. In Indonesian traditional medicine, salam leaves and jackfruit leaves have been used as herbal treatments for many conditions, including as antidiarrheal medicines. **Aims:** The aim of this experimental study was to evaluate the antidiarrheal effect of a combination of Salam leaves and jackfruit leaves infusum induced by castor oil in rats. **Method:** The rats were divided into nine groups, where the negative control group was given CMC 1 %, the positive control group was given tannins, and five test groups were given the infusum with five comparisons of each dose administered orally. Castor oil was used as a stimulant of diarrhoea. **Results:** The results show that with all combinations of salam leaves: jackfruit leaves have antidiarrheal effects with decreased frequency of defecation, faeces consistency, and faeces weight at ratios of 1:1, 1:2, 1:3, 2:1, and 3:1 compared to the negative control ($p < 0.05$). Phytochemical test of the infusum indicated positive tannins. The antidiarrheal effect of both infusums might be due to the presence of tannins, which have anti-secretory effect in the intestinal lumen. **Conclusions:** The treatment with combination of salam leaves and jackfruit leaves infusum in rats induced by Castor oil has an antidiarrheal effect. The best result is a mixture of salam infusum: jackfruit infusum with 3:1 ratio.

Introduction

Diarrhoea is a major health problem, especially in low-income countries, which includes Indonesia. In several provinces in Indonesia, the prevalence of diarrhoea is more than 7.0% (Riskesdas, 2018). Diarrhoea is a bowel movement of fluid that occurs more than three times a day or in frequency more often than that of normal people. One of the processes in the body that causes diarrhoea is a change in the motility of the gastrointestinal tract (Pandango *et al.*, 2018).

Chemical drugs, such as loperamide, can cause side effects, such as abdominal pain, nausea, vomiting, dry mouth, drowsiness, and dizziness. The existence of these side effects causes people to prefer efficacious medicinal plants as alternative medicine (Nurhalimah *et al.*, 2015).

Ethanol extract of Salam leaves (*S.polyanthum*) has an anti-diarrhoea effect in white mice (Mus Musculus) that have been induced by castor oil (Ambari, 2019). The chemical content contained in salam leaves (*S. polyanthum*) is tannin, which is known to be effective as an astringent, which can relieve diarrhoea by shrinking the intestinal mucous membrane (Sundari, 2010).

Jackfruit leaves (*A. heterophyllus* Lam.) is a traditional medicine that has been used empirically by the

community to treat diarrhoea. Traditionally, these leaves are used by boiling for traditional medicine (Anas *et al*., 2012). From the antidiarrheal activity of the ethanol extract of jackfruit leaves (Artocarpus heterophyllus Lam.) in mice, it was found that the antidiarrheal activity of both extracts was better than the standard antidiarrheal drug of loperamide (Anas *et al.*, 2012).

The aim of this experimental study was to evaluate the antidiarrheal effect of a combination of Salam leaves, and jackfruit leaves infusum induced by castor oil in rats. The parameters that were measured were the time of diarrhoea/onset of diarrhoea, frequency of diarrhoea, and consistency and number/weight of faeces.

Method

This experimental laboratory research was designed using a randomised post-test controlled design method. The research was conducted in June 2019 at Pharmacology Laboratory of Muhammadiyah Health Institute, Indonesia, using adult male rats (*Rattus norvegicus)* with ages between two to four months, with weights between 150-200 g, and good physical health conditions. The rats used were divided into seven treatment groups by random sampling. The amount of replication was determined by using Freeder's formula, and it found 27 research subjects with three rats as a negative control group, three rats as a positive control group, and the other seven rats as a treatment group. Diarrhoea activity data retrieval was done by placing the rats in individual containers for observation.

Prior to implementation, this research proposal had been approved by the Health Ethics Committee of the Faculty of Pharmacy, Ahmad Dahlan University, Jogjakarta, Indonesia, due to the use of rats as experimental animals. This research was conducted in three stages, namely the pre-treatment stage, treatment stage, and data retrieval stage. At the pre-treatment stage, a salam and jackfruit leaf infusum was made by mixing the 87.5 grams of fresh leaves powder with 300 mL of distillate water on a measuring cup then heated and held at a temperature of 90°C over a pot containing water for 15 minutes or until the water volume reached 100 mL, after which it was filtered using flannel.

Before treatment, the rats were acclimatised for two weeks with laboratory conditions for self-habituation during the study. At the treatment stage, the animals were divided into nine groups at random, Group I, II, III, IV, V, VI, VII, VIII, and IX. Group I was a Group with Castor oil alone; Group II (positive control) was a Group of rats given 1 ml/kg bw of tannin; then after one hour, they were given 1 mL of castor oil orally; Group III and IV were groups of rats treated with 87.5% salam and jackfruits leaves infusum, and after one hour, they were given 1 mL of Castor oil orally; Group V-IX were groups of rats treated with mixtures of salam leaf infusum and jackfruit leaf infusum (87.5%, 1:1, 1:2, 1:3, 2:1 and 3:1), then after one hour, they were given 1 mL of Castor oil orally. The data retrieval stage about diarrheal activities was done by observation, which included time of diarrhoea, frequency of diarrhoea, consistency and number/weight of faeces, and duration of diarrhoea. The response of each rat was observed at the 30^{th}, 60^{th}, 90^{th}, 120^{th}, 150^{th}, 180^{th}, 210^{th}, 240^{th}, 300^{th}, 360^{th} minutes after it was given the Castor oil. One-way ANOVA was used to analyse the results; $p < 0.05$ was considered statistically significant at the 95 per cent confidence interval.

Results

Chemical group test

Results of different qualitative chemical tests on Salam leaves (*S.polyanthum*) and Jackfruit leaves (Artocarpus heterophyllus Lam.) with $FeCl_3$ 5% and H_2SO_4 5% reagents showed the presence of tannin.

Antidiarrheal effect of salam and jack fruit leaves infusum was tested using nine groups of rats, in which each group consisted of three rats. Group II, the negative control group were given 1% CMC. Group I, as the positive control group, used tannin. The treatment Groups III, IV, V, VI, VII were given mixtures of salam and jackfruit infusum (1:1, 1:2, 1:3, 2:1 and 3:1), and the treatment Groups VII and IX were given salam infusum only and jackfruit infusum only, respectively. An hour later, each rat was given the Castor oil as much as 1 mL/rat.

The diarrheal activities of the rats in each group can be seen in Table I, in which consistency is converted into the following scores: 0) normal faeces; 1) soft faeces; 2) slimy/watery mass form of faeces; 3) not slimy/watery mass form of faeces.

All data parameters were normal and homogenous ($p > 0.05$). Based on the ANOVA test, the significance values between groups in diarrhoea parameters were $p < 0.05$ (Table II). This means that there were significant differences between groups.

To know which groups had significant differences, the next test done was the Post Hoc LSD test. From the results of the test analysis, significant differences were found in almost all of the parameters, except at Groups III and IX in weight parameters. The best result based on the *p*-value was Group VII (see Table III).

Table I: Rats diarrheal activities

Group	Rat	Total Frequency	Total consistency	Consistency average	Total weight (gram)
I (+)	1	4	10	1	2.25
	2	8	6	0.6	2.00
	3	7	7	0.7	1.19
II (-)	1	31	19	1.9	6.62
	2	26	21	2.1	7.57
	3	18	11	1.1	6.66
III (1:1)	1	16	16	1.6	4.83
	2	12	12	1.2	6.14
	3	15	15	1.5	7.12
IV (1:2)	1	13	8	0.8	4.40
	2	10	12	1.2	4.94
	3	5	0	0	5.79
V (1:3)	1	11	12	1.2	5.22
	2	10	9	0.9	4.43
	3	8	7	0.7	4.10
VI (2:1)	1	13	7	0.7	4.10
	2	9	8	0.8	4.32
	3	10	13	1.3	3.20
VII (3:1)	1	10	9	0.9	2.88
	2	9	10	1	2.25
	3	7	7	0.7	2.00
VIII (salam)	1	9	14	1.4	1.19
	2	10	9	0.9	6.62
	3	7	8	0.8	7.57
IX (j.fruit)	1	15	11	1.1	6.66
	2	9	15	1.5	4.83
	3	11	18	1.8	6.14

(I) Group of rats given Tannins as comparison and then 1 mL of Castor oil orally; (II) Group of rats given CMC 1% orally as a control and then 1 mL of Castor oil orally; (III) Group of rats given infusum of salam and jackfruit mixture (1:1) and then given 1 mL of Castor oil orally; (IV) Group of rats given infusum of salam and jackfruit mixture (1:2) and then given 1 mL of Castor oil orally; (V) Group of rats given infusum of salam and jackfruit mixture (1:3) and then given 1 mL of Castor oil orally; (VI) Group of rats given infusum of salam and jackfruit mixture (2:1) and then given 1 mL of Castor oil orally; (VII) Group of rats given infusum of salam and jackfruit mixture (3:1) and then given 1 mL of Castor oil orally; (VIII) Group of rats given infusum of salam only and then given 1 mL of Castor oil orally; (IX) Group of rats given infusum of jackfruit only and then given 1 mL of Castor oil orally.

Table II: One way ANOVA test result

Diarrhoea Parameter	*p*-value
Frequency of defecation	0.001
Faeces consistency	0.06
Faeces weight	0.001

Discussion

In this study, there were significant differences between Groups I and II, where the negative control group was given 1% CMC and Castor oil and the positive control group was given tannin before being given castor oil. The positive control group was significantly different from the negative control group in the weight of the stool defecation frequency and faeces consistency. This is consistent with the pharmacological theory, in which diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces (Horton, 1978).

In some diarrhoeas, the secretory component is predominant, while other diarrhoeas were characterised by hypermotility. It is logical because the autacoids and prostaglandins are involved in producing diarrhoea in humans. Other factors have been suggested to describe castor oil's diarrheal effect: Intestinal Na+ K+ ATPase inhibition reduces normal fluid absorption (Gaginella & Bass, 1978), adenylate cyclase activation or mucosal cAMP-mediated active secretion (Capasso *et al.*, 1994), prostaglandin production stimulation, and platelet-activating factor stimulation (Pintoy *et al.*, 1992).

Table III: Mean differences and *p* values based on ANOVA *post hoc LSD* test

Group	Frequency *p*-value	Mean differences frequency	Consistency *p*-value	mean differences frequency	Weight *p*-value	Mean difference weight
- vs +	0.0001	18.666	0.005	0.933	0.0001	5.136
- vs 1:1 (III)	0.0001	10.666	0.374	0.266	0.098	0.920
- vs 1:2 IV)	0.001	15.666	0.002	1.033	0.002	1.906
-vs 1:3 (V)	0.0001	15.333	0.017	0.766	0.0001	2.366
-vs 2:1 (VI)	0.0001	14.333	0.017	0.766	0.0001	3.076
-vs 3:1 (VII)	0.0001	16.333	0.011	0.833	0.0001	4.286
-vs salam (VIII)	0.0001	16.333	0.035	0.666	0.001	1.980
-vs j.fruit (IX)	0.0001	13.333	0.436	0.233	0.220	0.970

Importantly, it has been proposed that nitric oxide leads to castor oil's diarrheal effect (Capasso *et al.*, 1994). Castor oil, on the other hand, is well known to trigger diarrhoea due to its most active ingredient, ricinoleic acid, which triggers a hypersecretory reaction(Meite *et al*., 2009). The liberation of ricinolic acid from Castor oil results in irritation and inflammation of the intestinal mucosa, leading to the

release of prostaglandins, which stimulate motility and secretion (Labu *et al*., 2015).

Tannins have been proven to make the intestinal mucosa more resistant and decrease secretions; furthermore, they suppress the diarrhoea induced by Castor Oil (Hanwa *et al.*, 2007). Tannins present in the plant infusum (salam leaves and jackfruit leaves) are reported to inhibit the release of autacoids and prostaglandins, thereby inhibiting motility and secretion induced by Castor oil. All the treatment group results give significant differences, indicating that all of the groups (salam leaves, jackfruit leaves, or mixtures of salam leaves and jackfruit leaves) infusums had antidiarrheal effects in reducing faeces weight, defecation frequency, and faeces consistency. Hence, tannins as an active compound may be responsible for the antidiarrheal activity. The best result was in Group VII (salam: jackfruit with 3:1 ratio). This means that the optimum dose of inhibition of diarrheal activity from all treatment groups is a mixture of salam leaves and jackfruit leaves infusum with a 3:1 ratio.

Conclusion

The treatment with a combination of salam leaves and jackfruit leaves infusum in rats induced by Castor oil has an antidiarrheal effect. The best result is a mixture of salam infusum: jackfruit infusum with a 3:1 ratio.

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REVIEW

Self-medication and self-treatment with short-term antibiotics in Asian countries: A literature review

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Abstract

Introduction: The general public plays a role in the increase and spread of antimicrobial resistance by seeking self-medication with antibiotics. **Aim**: The purpose of this systematic review is to evaluate the prevalence of self-medication with short-term antibiotics in Asian countries. **Method**: A literature search was performed on Google Scholar, PubMed, and Science Direct databases in 2013-2020. **Results**: A total of 36 articles were included for full review and data extraction. The prevalence of self-medication with antibiotics in the articles reviewed was 50.8%. The main source of antibiotics without a prescription is from community pharmacies. The practice of self-medication with antibiotics is influenced by multifactor. **Conclusion**: Self-medication with antibiotics is a very common practice in Asian countries, contributing to the emergence of antimicrobial resistance which is no longer a threat but a terrible reality. This review provides an overview of the need for solutions to reduce self-medication with antibiotics behaviour in the community.

Introduction

The world is experiencing an epidemiological transition where the trend of non-communicable diseases tends to increase along with changes in people's life behaviour. However, the problem of communicable diseases cannot be completely resolved. One of the biggest threats to global health is the uncontrolled spread of epidemics due to highly pathogenic infectious diseases, especially those that easily cross borders and have the potential to endanger communities and their economies (WHO, 2015).

Antibiotics are the most commonly purchased drugs worldwide. The overuse, underuse, or misuse of antibiotics becomes a global issue that causes negative impacts. Antimicrobial resistance (AMR) results in the wastage of scarce resources and widespread health hazards. It is very critical health, social, and economic problem worldwide. Due to the clinical and socio-economic impacts of AMR, a strategy and action plan based on national efforts and international cooperation are needed to control and prevent AMR (Song, 2014).

Antibiotic resistance is perhaps the greatest threat facing the world in the field of infectious diseases, where it has the potential to cause greater death than cancer. The WHO report states that globally, the mortality rate due to antibiotic resistance in 2013 was 700,000/year, and it is predicted that in 2050 the mortality rate due to antibiotic resistance will be 10,000,000/year. The cumulative risk of economic expenditure is 100 billion USD/66 billion GBP. The direct and indirect impacts of antibiotic resistance will be felt in low-middle income countries in the Southeast Asia region. Every year, of the 14 million deaths that

occur in the Southeast Asia region, six million or about 40% are caused by infectious diseases, which also contribute to 42% of the loss of disability-adjusted life years. This burden requires urgent action to tackle antibiotic resistance, mitigating economic and health costs (WHO, 2016).

Half of all drugs are inappropriately prescribed, dispensed, or sold worldwide. Medicines are often used incorrectly, where 50% of all patients fail to take the drug and adhere to its therapy rationally. Irrational use of drugs is a major problem worldwide (Fresle, Hardon & Hodgkin, 2004). Self-medicated practice refers to the use of drugs to treat complaints of self-diagnosed disease without consulting a medical practitioner and without medical supervision. Weaknesses in health care systems, especially in developing countries, such as unfair distribution, high costs, limited access, lack of professional health care, unregulated distribution of drugs, and patient attitudes towards doctors, are some of the main drivers of self-medicated behaviour (Bhatta & Nepal, 2018).

In addition, it is widely shown that excessive use of antibiotics at the population level is an important risk factor for increased antibiotic resistance. Because patients do not know about antibiotics and are confused about their role, they must be told that most common infections do not require antibiotics and that these drugs can actually be dangerous. Antibiotic abuse that is prevalent in the community reinforces the importance of conducting research to develop effective strategies to stem the tide of antibiotic resistance (Singh, 2017). The objective of this literature search was to evaluate the prevalence and determinants of self-medication with antibiotics in Asian countries.

Methods

Search strategy

A literature search was performed on Google Scholar, PubMed, and Science Direct databases in 2013-2020. The search included a combination of "self-medication" OR "self-treatment" AND "antibiotics" AND "country where the study was conducted". The search was limited to peer-reviewed articles and reviews published in English. Search results from each database were exported to Microsoft Excel, merged, and sorted for removal of duplicate citations.

Study selection

Studies published in English are included in the review if they aim to assess the behaviour of self-medication with antibiotics in Asian countries. Studies on antiviral, antifungal, antiprotozoal, and topical antimicrobials are excluded. Original researches, research reports, case studies, and association reports were included for review. Abstract only articles, article reviews, editorials, systematic reviews, critical analysis, and narrative overviews were excluded. Articles were considered to be relevant based on the identification of self-medication with short-term antibiotics. Full-text articles were assessed for inclusion, and reasons were documented for all excluded papers.

Data extraction

Articles reporting community behaviour towards self-medication with short-term antibiotics were selected. Differences were resolved through discussion between authors. The authors excluded long-term antibiotics from this review, such as tuberculosis and antimalarial treatment.

A standard data extraction form was used to collect the following information: authors, year of publication, the country where the study was conducted, sample size, population sampled, prevalence, disease symptoms, main reasons of behaviour, where the information was obtained, source of drugs, and choice of antibiotics.

A database search returned 192 publications for review. 148 full-text publication meets the requirements, 114 were excluded for reasons of duplicate records, review articles, irrelevant topics, time of publication, systematic review, the article is not detailed, data is not systematically displayed which did not meet the authors' research objectives. Two additional articles were added following reference screening. The results were 36 articles that met the inclusion criteria (See Figure I).

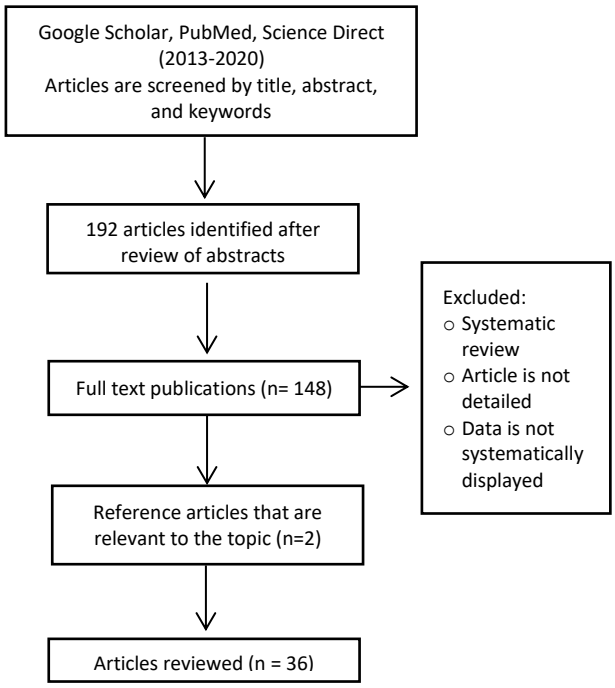

Figure 1: Flow chart of articles selection

Results

This study examined the prevalence of self-medication with short-term antibiotics from 29 countries out of a total of 48 Asian countries. Table I shows that there are 36 articles reporting on the main symptoms and the prevalence of self-medication with short-term antibiotics in Asian countries. The sample sizes ranged from 250 to 11,192 participants, and publication date ranged from 2013 to 2020; the sample population varies including the general public and other subgroup populations such as students (including medical and non-medical students); patients (attending pharmacies and other health facilities); age ≥ 18 years. The symptom categories reported to be various, and the symptoms most often treated with antibiotics were influenza or influenza-like syndromes. Prevalence (%) from the table means the percentage of the population that took part in self-medication with short-term antibiotics without consulting a medical doctor, i.e. using leftover antibiotics from previous treatments or getting antibiotics at the pharmacy without a prescription at a given time.

Table I: Articles reporting the prevalence of self-medication with short-term antibiotics usage in Asian countries

Authors, year	Countries	Sample Size	Population	Main Symptoms	Prevalence %
Barik, Islam, Kumar, &U. Haque, 2017	Bangladesh	4,100	Pharmacy patients	Fever, cold & cough, dysentery, diarrhoea and food poisoning, respiratory tract infection, UTI, toothache and viral disease	23.5
Biswas *et al*., 2014	Bangladesh	1,300	Pharmacy patients	Dysentery, diarrhoea, food poison, the common cold	26.69
Adhikari, Tshering, Tshokey, Wangmo, & Wangdi, 2017	Bhutan	692	General public	Common cold, any illness with fever	23.6
Prien, 2018	Cambodia	450	Outpatient of the referral hospital	Minor illness	49.4
Li, Lin, Wang, Xuan, & Zhou, 2018	China	11, 192	University student	Prophylaxis	63.1
Zhu *et al*., 2016	China	1,086	University students	Sore throat, fever, cough, runny nose	47.9
Manikandan, Muruganandhan, Priya, Shamsudeen, & Sujatha 2018	India		Dental clinic patients	Dental problems	71.2
Kapoor, Makhija, Nair, Nandigam, & Virmani 2017	India	531	Health science students	Sore throat, flu-like symptoms, skin infection	48
Kurniawan, Posangi, & Rampengan, 2017	Indonesia	400	Community health centre	Wound and skin infections	45
Widayati, 2013	Indonesia	559	Adults (household's survey)	Common-cold, cough, sore throat, headache, itching, toothache, fever	58
Ahmed, M, & Ali, 2019	Iraq	344	Pharmacy students	Headache, cough, and diarrhoea	62.79
Abedi, Dehghani, Emad, & Ghahramani, 2020	Iran	1,200	Outpatients of university dental clinics	Dental pain	42.6
Al Baz, Law, & Saadeh, 2018	Jordan	250	A refugee at health care	To cure or even prevent diseases	60
Yerubayev, 2019	Kazakhstan	472	General population	Cold, flu, sore throat, skin and wound infection,	63
Aboud & Awad, 2015	Kuwait	770	General population	Common cold, sore throat, cough, genitourinary infections, superficial wounds	27.5
Kitikannakorn & Phonlavong, 2018	Lao PDR	768	General population	Common cold, sore throat, and cough.	85

Authors, year	Countries	Sample Size	Population	Main Symptoms	Prevalence %
El-Kheir, Hanna, Mansour, Jamhour & Salameh, 2017	Lebanon	400	General population	Sore throat, common cold, fever	50
M. Haque *et al.*, 2019	Malaysia	649	University students	Runny nose, nasal congestion, cough, sore throat, fever, aches and pains, vomiting, diarrhoea, skin wounds	39.3
Kumar Sah, Kumar Jha, & Kumar Shah, 2016	Nepal	327	Nursing students	Common illness, save time and money	50
Shah *et al.*, 2014	Pakistan	431	Non-medical university students	Fever, pain relief, respiratory symptoms, gastrointestinal problems, urinary symptoms	47.6
Nazir & Azim, 2017	Pakistan	527	General population	Sore throat and flu	26
Gillani *et al.*, 2017	Pakistan	727	Non-medical university student	Gastrointestinal problems, pain relief, respiratory symptoms, fever, urinary problems	45
Abu Taha *et al*., 2016	Palestine	375	Adults	Cold, flu	38.7
Bulario, Cruz, Gutierrez & Pilapil, 2018	Philippines	390	Mothers of children < 18	Cough	42.05
Abdel-Rahman, Aljayyousi, El-Heneidy, Faisal & Kurdi, 2019	Qatar	596	University students	Previous illnesses that had similar symptoms	82 .
Alarifi, Alghadeer, Alhammad, Aljuaydi, & Babelghaith, 2018	Saudi Arabia	1,264	General population	Tonsillitis	34
Al Rasheed *et al*., 2016	Saudi Arabia	681	Adults	Cough, sore throat, common colds	78.7
Abdelrahman *et al.*, 2017	Saudi Arabia	1,028	Residents	Fever, common cold	37.9
Rathish *et al*., 2017	Sri Lanka	285	Medical students	Runny nose, flu	39
Al-kayali, & Haroun, 2017	Syria	436	Medical students	Headache, fever, and flu	60.5 40.9
Havanond, Hongsranagon, Pannoi, & Sirijoti, 2014	Thailand	396	Adults	Feeling unwell	33.84
Dönmez, Göv, & Güngör, 2018	Turkey	570	Nursing students	Colds and common cold, sore throat, toothache/swelling, fever, cough, stomach ache, weakness, hot urine, and skin infections	31.1
Abduelkarem *et al*., 2019	United Arab Emirates	315	General population	Sore throat, runny nose	31.7
Belkina *et al*., 2014	Saudi Arabia, Yemen, Uzbekistan	1,200	General education teachers	Cough and influenza	48.4 78.2 78.3
Ha, & Nguyen, 2019	Vietnam	1,000	Household survey (door to door survey in community)	Illness symptoms	83.3
Albawani, Abd-aziz, & Hassan, 2017	Yemen	363	Consumers attending community pharmacies	Cold, cough, diarrhoea, and fever	87.1

The authors also investigated the factors that influence self-medication with short-term antibiotic behaviour. Table II shows that 29 countries reported the main reasons for short-term antibiotic self-medication behaviour, source of information, source of where the drugs are purchased, and choice of antibiotics. When

exploring the data, not all of the studies the authors reviewed presented the percentage of choice of antibiotics for self-medication, and some studies did not present this data (N / A or data not available).

Table II: Main reasons, information from, sources, and choices of antibiotics

Studies	Main reasons	Information from	Sources	Choices of antibiotics
Bangladesh	Pre-experience, reduction of doctor's fees	Advice from traditional healers, own knowledge	Pharmacy	Azithromycin (24.3%) Metronidazole (12.3%) Ciprofloxacin (12.2%) Amoxicillin (9.2%) Cefixime (7.9%)
Bangladesh	Previous medication, other people's suggestions	Personal knowledge, past experience	Pharmacy	Metronidazole (50.43%) Azithromycin (20.75%) Ciprofloxacin (11.53%) Amoxicillin (10.37%) Tetracycline (7.49%)
Bhutan	Saving time and inconvenience of waiting in lines	Personal knowledge	Shared antibiotics; stock from previously used drugs,	Amoxicillin
Cambodia	Saving time, spending less money, living far from health facilities	Television, physician, radio, social network/ website, health care staffs, pharmacist, and newspaper	Pharmacy/ drug store	N/A
China	Success of previous treatment	Personal experience, health professional	Leftover, pharmacy, given by others	N/A
China	Convenience, cost saving, success of previous treatment	Personal experience, family member, pharmacist	Pharmacy, stock of previously used drugs	N/A
India	Success of previous prescription, availability of medicine, long waiting line in clinics, cost saving	Previous prescription, friends, family, internet, advertisements	Leftover, pharmacy	N/A
India	Success of previous prescription	Pharmacist	Pharmacy	B-lactams Fluoroquinolones Macrolides Tetracyclines
Indonesia	More practical way than seeking a doctor for a treatment, too busy to see a doctor, success of previous treatment, having not enough money to pay for the doctor visit	Old prescription	Pharmacy	Amoxycillin 68.3% Ampicillin 26.1%
Indonesia	Success of previous medication (54%)	Health professionals, friends/relatives, and drug leaflets.	Pharmacy, kiosks, drug stores	Amoxicillin Ampicillin Ciprofloxacin Tetracycline
Iraq	Quick relief desired, convenience, and avoiding waiting at clinics	Personal knowledge	Pharmacy	N/A
Iran	Severe pain, previous self-medications, and high costs of dental visits	Previous prescription	Pharmacy, leftover, family/ friends	Amoxicillin Metronidazole Cefixime Azithromycin Penicillin
Jordan	Long waiting hours of seeking medical advice	Medical advice	Pharmacy, leftover, share drugs	N/A
Kazakhstan	Success of previous medication	Medical advice, internet, friend/family	Pharmacy, leftover	N/A
Kuwait	Speedy recovery	Previous prescription, family/friends	Pharmacy, leftover, sharing with family	N/A
Lao PDR	Curing illness	Health care providers, relatives, television, radio and internet	Pharmacy	N/A

Studies	Main reasons	Information from	Sources	Choices of antibiotics
Lebanon, Beirut Tripoli	Curing illness	Health professional	Pharmacy	N/A
Malaysia	Cost saving and convenience	previous prescription	Pharmacy	Penicillin Doxycycline Clarithromycin
Nepal	Success of previous medication	Success of previous medication	Pharmacy	
Pakistan	Saving time and money, avoiding hassle of going to doctor, success of previous medication	Personal knowledge, old prescription	Pharmacy	Amoxicillin (41,4%) Metronidazole (30.5%) Ciprofloxacin (12.7%)
Pakistan	Success of previous medication	Previous experience, pharmacists, relatives/friends, doctors, leaflets	Pharmacy, leftover	Amoxiclav (40%) Ciprofloxacin (14%) Metronidazole (11%) Doxycycline (10%) Azithromycin (8%)
Pakistan	Saving time and money, avoiding hassle, success of previous treatment	Personal knowledge, friends, parents, pharmacist	Leftover, pharmacy	Metronidazole Ciprofloxacin Amoxicillin Co-Trimoxazole
Palestine	Preventing symptoms from getting worse	Doctors, pharmacists, friends/ relatives, websites, television.	Pharmacy, leftover, friends/ relatives	N/A
Philippines	Success of previous medication	Health centres and other sources	Pharmacy, health centres	Amoxicillin (50.25%)
Qatar	Success of previous medication	Pharmacist, old prescription	Pharmacy	N/A
Saudi Arabia	Success of previous medication	Previous doctor's prescription, advertisements from websites, social media, TV, or reading	Pharmacy	Amoxiclav (45.1%) Amoxicillin (39.9%)
Riyadh, Saudi	Curing symptoms	Friends, nearby pharmacy	Pharmacy	Amoxicillin Ciprofloxacin Penicillin
Saudi Arabia	Getting better more quickly	Physicians, pharmacists, friends/relatives	Pharmacy	N/A
Sri Lanka	Previous experience, no access to physician care	Previous knowledge, Physician, pharmacist, leaflet, relative/friend, internet	Pharmacy, relatives/ friends, households	Amoxicillin (56%)
Syria	Mildness of illness and time-saving	Pharmacist, previous doctor prescription, personal experience, friends' advice	Pharmacy, leftover of previous prescription	N/A
Thailand	Success of previous medication	Old packaging of antibiotics	Pharmacy	Amoxicillin
Turkey	Having no time to visit the doctor, success of previous medication, test fees, drug store and surrounding advice	Close friends and relatives	Maintained antibiotics at home, pharmacy	N/A
UAE	Previous experience with the disease	Previous personal experience, friend/relative, community pharmacist	Pharmacy, household.	Penicillins Macrolides Quinolones Cephalosporins Tetracyclines

Studies	Main reasons	Information from	Sources	Choices of antibiotics
Yemen, Saudi Arabia, Uzbekistan	Poor regulation, lack of access to health care, cultural beliefs	Pharmacist, friend, old prescription	Pharmacy	N/A
Vietnam	Believing in professional competences in buying antibiotics at drug store near home	health professional, family/friend	Pharmacy	N/A
Yemen	High cost of doctor consultation	Community drug dispenser, family member, personnel choice, old prescription, friends, media	Pharmacy	N/A

Discussion

This study shows that self-medication with short-term antibiotics behaviour is diverse in Asian countries (see Table I). Socio-demographic and economic factors have also been examined in some of these studies, providing information that the use of non-prescription antibiotics exists at all levels of socioeconomic status. However, the reasons for this behaviour, particularly those related to socio-cognitive or psychological factors, have not been explored. Therefore, more research is needed, especially the one focusing on these factors. This reinforces the importance of conducting research to develop effective strategies to stem the tide of antibiotic resistance.

Prevalence

The prevalence of self-medication with antibiotics (SMA) in this study was reported to be high, the lowest being 23.5% in Bangladesh (U. Haque *et al*., 2017) and the highest being 87.1% in Yemen (Albawani *et al*., 2017), with an average of 50.8%. Hence, this appears to be a health challenge in the region. This reinforces the results of health behaviour studies that are practised in most parts of the world, where more than 50% of antibiotics are purchased and used without a prescription (Morgan *et al*., 2011; Högberg, Muller, Monnet & Cars 2014; Auta *et al*., 2019).

The main reason for the large range of SMA prevalence may be due to differences in social, cultural, and economic status determinants. Differences are also caused by research methodology, research data collection, sample population, and time that might contribute to variations in the prevalence of SMA.

Socio-demographic and socio-economic factors

Most studies reported that the success of previous treatment influenced SMA, while other studies reported that low knowledge (Yerubayev, 2019); male gender (Emad *et al*., 2020); female gender (Zhu *et al*., 2016); age (Zhu *et al*., 2016); income (Abu Taha *et al*., 2016) were the ones influenced SMA. Other studies also reported that the level of education (Jamhour *et al*., 2017) and disease severity (Al-kayali & Haroun, 2017) were factors that were significantly related to SMA behaviour. In most studies, socio-cultural, economic, and demographic factors were reported to have an influence on the practice of self-medication with antibiotics. Older age groups were reportedly more likely to do SMA in China and Riyadh (Al Rasheed *et al*., 2016; Zhu *et al*., 2016), whereas in Tripoli (Lebanon), age did not have a significant effect (Jamhour *et al*., 2017). The effect of these factors is reported to be low or high depending on the research context. For example, studies in Palestine were reported to consider income with SMA practice (Abu Taha *et al*., 2016), while research in Yemen reported having no significant relationship with material status and monthly income (Albawani *et al*., 2017). Studies among people living in rural areas reported that factors, such as education level and socioeconomic status, were all related to self-medication (Phonlavong & Kitikannakorn, 2018). The study found how self-medicated participants had low levels of education, with almost half of them uneducated and most of them included in low socio-economic classes.

Information source, drug sources, and benefits of SMA

Information on antimicrobial agents on self-medication is obtained from a variety of sources. The majority of studies reported health workers, pharmacists, and family/friends/relatives as the main sources of information (see Table I). Other sources of information were the success of previous treatments (most studies), leaflets (Rathish *et al*., 2017), advertisements from websites, social media (Alghadeer *et al*., 2018), radio and television (Phonlavong & Kitikannakorn, 2018). Antibiotics were obtained from various sources, including pharmacies (all studies), leftovers (most studies), relatives/friends (most studies), and other health services. The advantages of self-medication with

antibiotics are that it is easy to get drugs at the pharmacies, saves money (Gillani *et al*., 2017) and time for doctor visits (Al-kayali & Haroun, 2017) and can cure symptoms (Al Rasheed *et al*., 2016). It turns out that the success of previous treatment, poor regulation, lack of access to health services, saving time or money are among the causes; similar findings are observed and reported worldwide (Lescure *et al.*, 2018).

Information and advice about antibiotics can come from common people (uninformed people). Several studies have shown that ordinary people, especially family members, relatives, or popular friends, can become the sources of information. Informal drug providers, such as traditional medicine traders and traditional healers, who are mostly not health workers, are also popular as drug advisers, especially in developing countries. The advice given by common people is mainly based on their experience in using antibiotics that are prescribed or not. This raises questions about the suitability and appropriateness of the advice given because their previous medical conditions may differ.

Health workers are expected to be able to provide education about medicine in a professional manner (Greiner & Knebel, 2003). The WHO states that pharmacists are health workers who have the responsibility to help people in their own treatment, including the use of antibiotics without a prescription, based on their knowledge of pharmaceutical care. Appropriate and consistent pharmaceutical services will increase the role and reputation of pharmacists in the wider community and can improve the health status of the community. In terms of time spent, pharmacists ideally prioritise providing pharmaceutical services, but there are often job conflicts as entrepreneurs who manage pharmacies with clinical pharmacy services neither reject nor question the reason for antibiotic use when requested (Hermansyah *et al.*, 2012). They tend to delay the client's idea of the need for antibiotics. Most pharmacy employees do not have professional qualifications and know very little about pharmacies. Pharmacists trained in integrated healthcare systems have had a significant impact on minimising irrational use of antibiotics in developing countries. The increasing role of pharmacists in developing countries has the potential to have a positive impact on global AMR issues (Gajdács, Paulik, & Szabó, 2020). Further research should be conducted to evaluate the attitudes and behaviours of healthcare practitioners towards antibiotic use.

Doctors also have a professional responsibility to provide information about medications, including the antibiotics prescribed for their patients. However, the excessive workload is often claimed as the main reason for not providing adequate information about the drugs prescribed to patients. Advice on the use of antibiotics given by common people or health workers can affect the behaviour of using antibiotics that are not prescribed in the community (Saqib *et al*., 2019). Therefore, this issue needs to be explored further.

Misuse of self-treatment antibiotics

The most common misuse of self-medication with antibiotics includes the inappropriate use of antibiotics for children, self-medication with antibiotics (Biswas *et al*., 2014), inappropriate duration of therapy of fewer than five days (Yerubayev, 2019), an incorrect indication that is used for viral infection (most studies), exchange/share of drugs (most studies), and wound healing (Kurniawan *et al*., 2017). The use of antimicrobials for viral infections, such as influenza and influenza-like symptoms, sore throat, and fever, was reported in nearly all studies. Other complaints reported were dysentery, diarrhoea, and food poisoning (Biswas *et al*., 2014; U. Haque *et al*., 2017). Antimicrobial agents commonly used for symptoms of viral infections included amoxicillin, ampicillin, metronidazole, azithromycin, ciprofloxacin, and tetracycline.

Several studies have shown a significant positive relationship between knowledge of antibiotics and antibiotic resistance and behaviour. Therefore, intervention studies are needed to educate the population. Because advice from medical personnel is positively related to knowledge and attitudes toward antibiotics, it is important to encourage medical staff to instruct patients. The study identified groups at high risk for poor antibiotic behaviour to increase the effectiveness of targeted interventions for reducing the abuse.

Key finding

Pharmacies are generally used as the main source of information for antibiotics that are obtained and used freely. However, community pharmacies often do not have adequate bio-medical knowledge about antimicrobial agents and disease processes. Therefore, a promotion at all levels of society is an important target for minimising SMA. The success of previous treatments tends to increase self-confidence in the ability to manage subsequent diseases without the need to consult a doctor. This is a potential risk factor for improper drug use because most patients do not have knowledge of the disease process and drugs used in self-medication. In the articles reviewed, side effects from SMA were rarely reported.

Recommendation

There is a need to develop more research (qualitative, quantitative, observational studies, prospective longitudinal studies, or retrospective studies) on factors affecting self-medication with antibiotics to optimally address public health problems. Some of the studies conducted in the majority of self-medication with antibiotics are cross-sectional, where several studies focus on knowledge, attitudes, and practices. Comprehensive qualitative research will have the benefit of increasing a deeper understanding of the phenomenon of self-medication with antibiotics. Therefore, randomised control trials (RCT) are recommended to explore the impact of self-medication with antibiotics on population groups. RCTs are essential for assessing the impact of self-medication with antibiotics on AMR to produce evidence that can guide the development of more effective health promotion strategies. Standard counselling protocols and policies regarding the prescribing and limiting of antibiotic release are important steps for controlling AMR.

Limitations

The limitations of this study are limited to the published literature, excluding potential results found in the "grey" literature.

Conclusions

Self-medication with antibiotics (SMA) is a very common practice in Asian countries. Self-medication with antibiotics is influenced by socio-cultural health factors and is often associated with poor spending and prescription practices. Self-medication with antibiotics practice is one of the most important factors contributing to the emergence of AMR, which is no longer a threat but a terrible reality.

This literature review provides an overview of the need for solutions to reduce SMA behaviour in the community, among others, by conducting pharmaceutical counselling, seminars, campaigns, pamphlets, and education through social media. Educational interventions targeting individuals and the community, in addition to increasing access to high-quality public health services and enforcement of regulations on the use of drugs without prescription, can also reduce the burden of infectious diseases and help reduce the challenges of SMA.

Conflict of interest

The authors have no conflict of interest associated with the material presented in this paper.

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Author contributions

Conceptualisation: IW, SB. Data curation: IW, SB. Formal analysis: IW, SB. Funding acquisition: None. Methodology: IW, SB. Project administration: IW. Writing original draft: IW, SB. Writing, review & editing: IW, SB, PJ, DP.

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IAI CONFERENCE

RESEARCH ARTICLE

Antibiotic use on paediatric inpatients in a public hospital in Bangil, Indonesia

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Abstract

Introduction: The importance of antibiotic use in a clinical setting was evaluated in order to support the global action plan to decelerate the spreading speed of antimicrobial resistance. **Aim:** This study aimed to evaluate antibiotic use among pediatric inpatients in Bangil public hospital, East Java, Indonesia. **Methods:** This study used a cross-sectional design. The data were obtained from medical records of pediatric patients admitted to a pediatric ward in 2017. Data were analysed using the anatomical therapeutic chemical classification system (ATC)/defined daily dose (DDD) method in conjunction with data sources from a locally developed bacterial map. **Results:** The results showed the paediatric patients were dominantly male (n=218; 54.2%) and mostly diagnosed with diarrhoea (n=87; 15.3%). Ampicillin-sulbactam was the most commonly used antibiotic (16.3%). The total DDD value was 66.1 DDD/100 bed-days, and ceftriaxone demonstrated the highest DDD value (10.3 DDD/100 bed-days). **Conclusion:** In conclusion, the use of antibiotics in the pediatric ward in Bangil public hospital was comparable to other studies conducted in Indonesia.

Introduction

Infectious diseases are a considerable contributor to morbidity and mortality in developing countries such as Indonesia. Some infectious diseases that are caused by bacteria remain public health concerns; these include pneumonia, urinary tract infections, diarrhoea, and tuberculosis. Antibiotics are used to treat bacterial infections. Antibiotic use in developing countries increased by 36% from 2000 to 2010 (Van Boeckel TP *et al.*, 2014). However, evidence shows that this increase has not been followed by appropriate antibiotic use. The inappropriate use of antibiotics can cause global health threats, especially with the emergence of antibiotic resistance. The notion of inappropriate use of antibiotics may include, but not be limited to, non-optimal prescribing, free use of antibiotics without a prescription, failure to take antibiotics, overuse of antibiotics, and excessive misuse of antibiotics (Ministry of Health of the Republic of Indonesia, 2011).

Among other subpopulations, the use of antibiotics in children is important. Two factors can explain the need for antibiotics in this particular population. Firstly, the immune system in children is not yet fully functioning. Secondly, children tend to be more exposed to pathogens due to their daily patterns of behaviour. However, many antibiotics that have been approved for adult use maybe not suitable for children due to the differences between these two populations, so the extrapolation of clinical data requires careful attention (Shea, Florini & Barlam, 2002).

Some efforts have been initiated to control the inappropriate use of antibiotics. The global campaign of Antibiotic Awareness Week initiated by the World Health Organization (WHO) can act as an example of

this (World Health Organization, 2018a). It is now mandatory that all Indonesian hospitals have an Antimicrobial Resistance Control Committee (PPRA) in clinical settings. The presence and activities of this committee are important factors for the hospital accreditation programme. Evaluating the implementation of antibiotics in the hospital can be carried out to control their usage. This evaluation can be done using a method developed by the WHO's collaborating centre in Norway, which employs the anatomical therapeutic chemical classification system combined with the defined daily dose (commonly known as the ATC/DDD method) (World Health Organization, 2018b). The objective of this study was to evaluate the use of antibiotics in the children population in a Bangil Public Hospital using the ATC/DDD method.

Materials and method

Study design and setting

This study was conducted using a cross-sectional research design in Bangil public hospital, Pasuruan Regency, East Java, Indonesia. Data collection and analysis was conducted from December 2018 to February 2019.

Sample and sampling method

All paediatric inpatients admitted to the Asoka Ward of the hospital from the 1st of January to the 31st of December in 2017 became the target sample. The inclusion criteria used to define the population for this study was hospitalised pediatric patients aged from one month to 14 years with complete patient digital medical record data. The medical record must have contained at least one antibiotic with its ATC code, the patient's identity (name, age, gender, financing status, admission date, discharge date, and diagnosis), and the profile of antibiotics (chemical sub-group, chemical name, dosage regimen, route of administration, and frequency of use). Patients with incomplete data, discharge from the hospital by family's request, and death were excluded from the analysis. This study applied a total sampling method to the patients that met the inclusion criteria.

Data collection and analysis

This study collected primary and secondary data. The primary data was gathered from an interview with a pharmacist involved in the hospital's antimicrobial resistance control programme (PPRA). The secondary data was retrieved from the hospital's Department of Electronic Data Management (PDE), Department of Medical Records, and Department of Infection Prevention and Control (PPI). The secondary data comprised the patient's digital medical record and bacterial sensitivity and resistance pattern. The antibiotics from the patient's data were further calculated based on the ATC/DDD method. The unit for the final calculation that represented the antibiotic use was in DDD/100 bed-days.

Ethical consideration

Ethical approval to conduct the study was granted by the Ethics Committee of the State Polytechnic of Jember number 2536/PL17/LL/2019. Permission to conduct the study was granted by Bangil Public Hospital (Number 445.1/3222/424.202/2018).

Results

This research collected data from 402 pediatric patients that met the inclusion criteria (Table I). Based on the characteristics of the admitted patients, there were more males than females, accounting for 54.2% (n=218) and 45.8% (n=184), respectively. Furthermore, the age group with the largest number of individuals was children aged from one month to two years, consisting of 52.2% (n=210). The top three diagnoses in this study were diarrhoea and gastroenteritis of presumed infectious origin (15.3%; n=87); bronchopneumonia, unspecified (13.5%; n=77); and tuberculosis of lung, without mention of bacteriological or histological confirmation (10%; n=57). Based on the financing status, the most widely used financing method for pediatric patients was the *Badan Penyelenggara Jaminan Sosial* (BPJS) health insurance, accounting for 40.3% (n=162).

A total of 3,730 antibiotic prescriptions were prescribed for 402 pediatric patients and covered nine chemical subgroups and 24 types of antibiotics (Table II). The most widely used antibiotic were penicillins, predominated by an ampicillin-sulbactam combination (16.3%; n=608). The second most used group of antibiotics were cephalosporins, especially cefixime (11.3%; n=422). Meanwhile, across all antibiotics used for pediatric patients, amoxicillin-clavulanic acid (0.1%; n=4) and spiramycin (0.1%; n=4) were rarely prescribed. The evaluation of antibiotic use using the ATC/DDD method in this research showed that the total DDD value of 24 antibiotics was 66.1 DDD/100 bed-days, ranging from 0.04 DDD/100 bed-days (spiramycin, J01FA02) to 10.3 DDD/100 bed-days (ceftriaxone, J01DD04), and the total length of stay (LOS) value was 1,829 days (Table III).

Table I: General characteristics of admitted patients

Characteristics	n	%
Gender		
Male	218	54.2
Female	184	45.8
Age category		
1 month-2 years	210	52.2
2-6 years	113	28.1
6-12 years	65	16.2
12-14 years	14	3.5
Diagnosis (ICD-10)		
Diarrhoea and gastroenteritis of presumed infectious origin	87	15.3
Bronchopneumonia, unspecified	77	13.5
Tuberculosis of lung, without mention of bacteriological or histological confirmation	57	10
Febrile convulsions	48	8.8
Fever, unspecified	33	5.8
Typhoid fever	27	4.7
Septicemia, unspecified	26	4.6
Urinary tract infection, site not specified	20	3.5
Acute upper respiratory infection, unspecified	13	2.3
Nausea and vomiting	11	1.9
Iron deficiency anaemia, unspecified	11	1.9
Septicemia, unspecified	9	1.6
Other diagnoses	149	26.1
Financing status		
BPJS health insurance	162	40.3
The Poor Statement Letter (SPM)	157	39.1
Non-insurance/pocket money	82	20.4
Other insurance	1	0.2

BPJS: *Badan Penyelenggara Jaminan Sosial* or Indonesian Universal Health Coverage, ICD-10: the 10th version of International Classification of Diseases

Table II: Profile of antibiotics used for paediatric inpatients

Chemical sub-group	Chemical name	n	%
Penicillins	Ampicillin-sulbactam	608	16.3
	Ampicillin	479	12.8
	Amoxicillin	204	5.5
	Amoxicillin-Clavulanic acid	4	0.1
Cephalosporins	Cefixime	422	11.3
	Ceftriaxone	378	10.1
	Cefotaxime	127	3.4
	Ceftazidime	9	0.2
	Cefadroxil	5	0.1
Antituberculosis agents	Isoniazide	276	7.4
	Pyrazinamide	224	6.0
	Rifampicin	186	5.0
	Ethambutol	7	0.2
Amphenicols	Chlorampenicol	371	10.0
	Thiamfenicol	88	2.4
Aminoglycosides	Gentamicin	96	2.6
	Streptomycin	60	1.6
	Amikacin	8	0.2
Macrolides	Erytromycin	95	2.5
	Azithromycin	11	0.3
	Spiramycin	4	0.1
Nitromidazole	Metronidazole	46	1.2
Penems	Meropenem	17	0.5
Lincosamide	Clindamycin	5	0.1
Total		**3,730**	**100**

Table III: Profile antibiotic use with ATC/DDD method

ATC code	Name	Standard DDD	Total DDD	DDD/100 bed-days
J01DD04	Ceftriaxone	2	189	10.3
J04AC01	Isoniazide	0.3	158	8.6
J01CR01	Ampicillin-Sulbactam	6	152	8.3
J04AB02	Rifampicin	0.6	110.3	6.0
J01DD08	Cefixime	0.4	120.5	5.6
J01BA01	Chlorampenicol	3	80.4	4.4
J01CA01	Ampicillin	6	79.8	4.4
J04AK01	Pyrazinamide	1.5	74.7	4,1
J01CA04	Amoxicillin	1.5 (PO) 3 (IV)	71.3	3.9
J01FA10	Azithromycin	0.3 (PO) 0.5 (IV)	43	2.3
J01FA01	Erythromycin	1	36.3	1.9
J01DD01	Cefotaxime	4	31.8	1.7
J01BA02	Thiamfenicol	1.5	29.3	1.6
J01GB03	Gentamicin	0.24	16	0.9
J01XD01	Metronidazole	1.5	15.3	0.8
J01GA01	Streptomycin	1	6	0.3
J01DH01	Meropenem	3	5.7	0.3
J04AK02	Ethambutol	1.2	2.9	0.2
J01DD02	Ceftazidime	4	74.7	0.1
J01GB06	Amikacin	1	2	0.1
J01CR02	Amoxicillin + Clavulanic acid	1.5	1.7	0.1
J01DB05	Cefadroxil	2	1.3	0.1
J01FF01	Clindamycin	1.2	1.3	0.1
J01FA02	Spiramycin	3	0.7	0.04
Total				**66.14**

Discussion

In general, the immune responses of T helper 1 (Th1) in the male paediatric population were generally thought to be weaker than those within the female pediatric population, thus making the males more susceptible to some pathogens (Muenchhoff & Goulder, 2014). In addition, behavioural differences between males and females also act as risk factors for infectious diseases. Other studies have also suggested that males in all age groups are more susceptible to airway infections than females. In males, it is likely that airway infections are severe and cause death, especially with pneumonia (Ostapchuk, Roberts & Haddy, 2004). Several reasons can explain why children under the age of six have a higher incidence of infection. Their immune systems are limited and do not yet produce antibodies in a fully functioning manner. This causes children to become prone to bacterial infections, thus requiring antibiotic treatment. When a child begins to grow up, their ability to fight infection will continue to improve, and they become less susceptible to bacterial infections. In

addition to their body's weaker immune system, children under the age of six tend to play in public areas, which increases their risk of being exposed to pathogens, and as a result, this will put them at risk of infectious diseases (Shea, Florini & Barlam, 2002; Simon, Hollander & McMichael, 2015).

High transmission rates of diarrhea disease in children can commonly be caused by poor environmental sanitation, contamination of food or beverages, an impaired immune system, inadequate intestinal flora, and a lack of gastric acidity.

The second most common diagnosis of the disease is unspecified bronchopneumonia, which is pneumonia that attacks the lower respiratory tract. Bronchopneumonia attacks the bronchi within the pulmonary alveolus and causes local inflammation in the pulmonary parenchyma. In Indonesia, basic health research carried out a regular nationwide survey in 2013 that reported that the highest pneumonia cases of pneumonia occurred in children aged one to four years (Ministry of Health of the Republic of Indonesia, 2013a). Pneumonia in children can be associated with several factors, including a disrupted immune system that puts them at risk of exposure to pneumonia-causing bacteria, an imbalanced nutritional intake, and exclusively breastfed as infants.

Tuberculosis (TB) of the lung, without mention of bacteriological or histological confirmation, was the third-largest diagnosis found in this study. Indonesia ranks third in the world for the highest number of TB cases, after China and India. Of the 264 million people in Indonesia (in 2017), the total TB incidence was 842,000, and the total HIV-TB co-infection incidence was 36,000. Because TB is contagious, children are at risk of infection when they live in the same household with a person with active TB, such as parents, or if they study in a school where a person with active TB has phlegm that comes out while speaking, sneezing, and coughing.

Based on the financing status, BPJS is a universal health coverage program run by the Indonesian government with an increasing number of users each year, reaching 171.9 million in 2016. This is due to the cooperation agreement between the hospital and BPJS in the National Health Insurance (JKN) program stated in Presidential Regulation Number 12 of 2013 and Regulation of the Minister of Health Number 71 of 2013 (Government of Indonesia, 2013; Ministry of Health of the Republic of Indonesia, 2013b).

The antibiotic profile results showed that ampicillin-sulbactam was the most commonly used antibiotic. Ampicillin-sulbactam is a broad spectrum β-lactam antibiotic combined with β-lactamase inhibitors that are intended for parenteral administration to overcome ampicillin resistance. Ampicillin-sulbactam works actively against Gram-positive bacteria that generally cause respiratory tract infections, skin infections, intra-abdominal infections, and soft tissue infections such as *Staphylococcus aureus*, *Acinetobacter*, *Enterobacter*, *Haemophilus influenza*, *Escherichia coli*, *Klebsiella*, *Streptococcus pneumonia*, *Streptococcus pyogenes*, and *Streptococcus viridans* (Lacy *et al*., 2009; Adnan *et al*., 2013). This is the first-line therapy used for severe pneumonia in children (≤ 5 years old) (World Health Organization, 2014).

Ampicillin, without sulbactam combination, actively fights bacterial infections caused by *Streptococcus, Pneumococcus*, non-penicillinase producing *Staphylococcus*, Listeria, Meningococcus, *Haemophilus influenza*, Salmonella, Shigella, *Escherichia coli*, Enterobacter, and Klebsiella (Lacy *et al*., 2009). Ampicillin is an alternative treatment for the shigella-induced diarrhoeal disease. It is the first-line therapy for paediatric inpatients aged ≤ 5 years old with severe pneumonia (Ostapchuk, Roberts & Haddy, 2004; Guarino *et al*., 2014; World Health Organization, 2014). This is in accordance with the antibiotic use guidelines formulated by the Bangil public hospital, where this study took place. Ampicillin is also used to treat meningitis and brain abscesses. The third most widely used antibiotic was cefixime. Cefixime is used to treat urinary tract infections, acute media otitis, and respiratory tract infections caused by the bacteria *Staphylococcus pneumonia*, *Staphylococcus pyrogens*, *Haemophilus influenza*, and Enterobacteriaceae (Lacy *et al*., 2009; Bradley *et al*., 2011). Furthermore, cefixime is used as an alternative treatment for diarrhoea caused by *Escherichia coli* and Shigella (Adnan *et al*., 2013; Bruzzese, Giannattasio & Guarino, 2018).

There are several studies similar to those conducted at Bangil public hospital. Research at Kariadi public hospital in Central Java carried out from August to December in 2012 showed a total DDD value of 39.4 DDD/100 bed-days, with ceftriaxone (10.6 DDD/100 bed-days) as the most antibiotic used (Febiana, 2012). Similarly, research at Soebandi Jember hospital during 2016 found a total DDD of 36.93 DDD/100 bed-days, with ceftriaxone (16.9 DDD/100 bed-days) as the most antibiotic used (Fathimatuzzahrah, 2016). In this present research, ceftriaxone also had the highest DDD, and this is thought to be related to the diagnosis of most diseases, namely diarrhoea and bronchopneumonia. Ceftriaxone is effective against Gram-negative bacteria, so it can be used as a treatment for diarrhoea caused by non-typhoidal Salmonella, and Shigella (Shea, Florini & Barlam, 2002; World Health Organization, 2018b). For severe pneumonia, ceftriaxone is a second-line

treatment for children who have failed first-line treatment (World Health Organization, 2014).

Conclusion

This study concluded that the pattern of antibiotic use in the children's patient wards of the Bangil Public Hospital in 2017 was similar to research conducted in several other hospitals in Indonesia, such as the Soebandi Jember hospital and the Kariadi public hospital. In addition, ceftriaxone was found to be the most commonly used antibiotic with the highest DDD value.

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IAI CONFERENCE

RESEARCH ARTICLE

The influence of extracts and fractions from matoa leaves (*Pometia pinnata*) on angiotensin I levels

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Keywords
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Abstract

Introduction: The matoa plant (Pometia pinnata) leaves can be used to treat hypertension. Matoa leaves are thought to have antihypertensive activity because they contain flavonoids. These flavonoids can reduce blood pressure that is modulated by the renin-angiotensin-aldosterone system (RAAS). It is suspected that matoa leaves have antihypertensive activity as they contain quercetin which is a compound that is presumed to be an angiotensin-converting enzyme (ACE) inhibitor. **Aims:** This study aims to determine which extracts and fractions from matoa leaves are able to decrease angiotensin I levels. **Methods:** The extraction was done by maceration with 96% ethanol solvent and fractionated by a liquid method using an n-hexane fraction solvent, an ethyl acetate fraction, and a water fraction. In this study, 21 male Wistar rats were used as test animals and divided into seven groups: Group I was the normal control, group II was the negative control (CMC-Na 1%), group III was the positive control (Irbesartan), group IV was given matoa leaf extract with 60 mg/200g body weight ratio, Group V was given 2.34 mg/g fraction of n-hexane, Group VI was given ethyl acetate fraction 9.54 mg/200g ethyl acetate fraction, and Group VII was given water fraction 7.98 mg/200g water fraction. The data obtained was analysed using the Shapiro-Wilk test, the Levene test, and analysis of variance (ANOVA). **Results:** The results showed that the angiotensin I levels induced by angiotensin II were more significant (p < 0,05) than those in the normal and negative groups. The ethyl acetate fraction showed a 23.6% decrease in angiotensin I level, which was close to the 24.8% decrease in the positive group. The extract from the matoa leaves showed a 17.2% decrease in angiotensin I levels which were close to the 20% decrease in the positive group.

Introduction

Hypertension is a cardiovascular disease that affects 34.1% of the population, based on data from the Indonesian Ministry of Health in 2018. The prevalence of hypertension in Indonesia in 2004 was around 13.4-14.6%, and in 2008 it increased to 16-18% (Riskesda, 2018). In people aged 18 and over, its prevalence was around 31.7% in 2007, 32.5% in 2012 and then decreased by 5.9% in 2013 (World Health Organization, 2012). Hypertension is a condition where the systolic pressure is equal to or higher than 140 mmHg, and the diastolic pressure or diastolic blood pressure is more than 90 mmHg (Kumar *et al*., 2014).

Matoa leaves contain *quercetin-3-O-rhamnoside* and *kaemferol-3-O-rhamnoside* (Suedee, Tewtrakul & Panichayupakaranant, 2013). These compounds are thought to be responsible for ACE inhibition in matoa leaves. The mechanisms that occur in ACE inhibitors can be used as a reference for the inhibitory mechanisms that occur in plant compounds. It is possible that the matoa leaves may be able to act as a hypertension drug (Suedee, Tewtrakul & Panichayupakaranant, 2013).

Extracts and fractions taken from matoa leaves have antihypertensive activity with an effective dose of 150mg/kg body weight (bw) (Purwidyaningrum, Sukandar & Fidrianny, 2017). In another study, an extract dosage of 300 mg was able to reduce blood pressure, and the best fraction of 30 mg ethyl acetate fraction induced by angiotensin II was able to reduce systolic, diastolic and mean blood pressure, as well as decrease the heart rate of male Wistar rats (Elisa, 2019). The classification of hypertension drugs is divided based on the mechanism by which they work,

including diuretics, sympatholytic drugs, and vasodilators that inhibit angiotensin action and production (Dipiro *et al*., 2017). Angiotensin receptor blockers (ARBs) work by preventing angiotensin II from binding to its receptors (AT1), which causes aldosterone elimination and vasoconstriction. This is then responsible for natriuresis and diuresis, which leads to a decrease in blood pressure (Kumar *et al*., 2014).

The aim of this research was to determine which matoa leaf extract and fraction (*Pometia pinnata*) can reduce angiotensin I level in angiotensin II-induced rats.

Materials and method

To begin with, 500g of matoa leaf powder was weighed and then put into a maceration vessel. Following this, 96% of ethanol was added using a 1:10 ratio (500 g of powder: five litres of ethanol). The matoa leaf powder was soaked in 3.75 parts (375 L) of 96% ethanol solvent before being covered and stored at room temperature for five days. It was protected from direct sunlight and shaken three times a day. After five days, the solution was filtered using a flannel cloth, and the maceration vessel was rinsed using the remaining 1.25 parts (125 L) of 96% ethanol solvent. It was then filtered again with the added solvent, using a flannel cloth and filter paper. The maceration results were then collected, and the waste was separated (Park *et al*., 2014). These results were concentrated using a rotary evaporator at a temperature of 40^{o}C to obtain a thick extract. The following formula was used in order to calculate the percentage yield.

Fractionation was carried out using the liquid-liquid extraction method. 15g of thick ethanol matoa leaf extract was dissolved in 96% ethanol solvent until mixed. The water and n-hexane solvent (1:1) were then added through separated funnels, and the solution was shaken and left standing. The n-hexane fraction was the filtrate at the top, and the water fraction was the filtrate at the bottom. The n-hexane fraction was separated, and the water fraction was collected. The resulting solution was concentrated using a rotary evaporator at a temperature of 50^{o}C. This treatment was repeated three times.

The animal subjects used in this study were white male Wistar rats. The rats were left to acclimatise to their surrounding environment for one week, and then they were weighed. In this study, 21 rats were used, and they were classified into seven test groups, with each test group consisting of three rats. The grouping of the test animals occurred as follows:

Group I was the normal control, group II was the negative control (CMC-Na 1%), group III was the positive control (Irbesartan), group IV was given matoa leaf extract with 60 mg/200g body weight ratio, Group V was given 2.34 mg/g fraction of n-hexane, Group VI was given ethyl acetate fraction 9.54 mg/200g ethyl acetate fraction, and Group VII was given water fraction 7.98 mg/200g water fraction.

Plasma was taken and added to ethylenediaminetetraacetic acid (EDTA), which acted as an anticoagulant and was then centrifuged for 15 minutes. After this, the angiotensin I levels were measured using an enzyme-linked immunosorbent assay (ELISA) cusabio kit using the indirect ELISA technique.

The differences in each group before and after treatment were analysed by means of a correction test. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's posthoc for parametric tests.

Where Ac is the absorbance of the control and As is the absorbance of the sample.

The IC_{50} calculation was obtained from the linear regression equation after calculating the percentage of inhibition of α-amylase enzyme activity of the test material with a concentration range of 2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, and 10 mg/mL. To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ was considered as statistically significant, alongside the Tukey Post-Hoc Test significance and 95% confidence interval. Linear regression measured the median inhibitory concentration (IC_{50}) to determine the inhibitory activities of α-amylase. IBM SPSS statistic version 22 was used for statistical analysis.

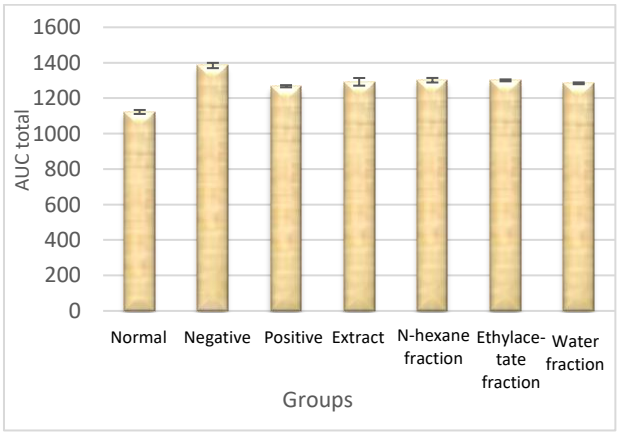

Figure 1: Total of AUC

Table I: The average angiotensin I level

Groups	Angiotensin I level (pg/ml)					
	T_0	T_1	T_2	T_3	AUC	% RAA1L
Normal	65.96±0.0	66.33±0.1	64.83±2.6	66.86±0.2	1122.35±11.2*	0
Negative	66.19±0.0	82.93±2.2	81.06±1.1	82.87±0.2	1384.46±14.8*	0
Positive	66.05±0.1	81.96±0.6	68.37±0.1	68.04±0.1	1267.57±6.2*	8.44
Extract	65.89±0.1	80.70±1.5	70.18±0.6	75.75±2.4	1292.39±21.7*	6.65
n-hexane fraction	66.19±0.1	80.70±1.7	74.04±0.0	73.31±0.0	1301.55±12.8*	5.99
ethyl acetate fraction	66.07±0.1	80.78±0.7	73.92±0.0	73.09±0.1	1300.75±5.5*	6.05
Water fraction	65.91 ±0.1	81.06±0.7	69.27±0.4	73.72±0.0	1284.12±5.1*	7.25

RAA1L = Reduce Activity Angiotensin 1 Level; *significant difference to negative control < 0,05

Results and discussion

The results obtained in Table I indicate an increase in angiotensin I level after angiotensin II (T1) induction and a decrease after T1 and T2 treatment. Measurements of angiotensin I levels were carried out before therapy (T0), 14 days after the angiotensin I induction period (T1), during the seven days of therapy (T2) and during the seven days following the therapy period (T3) for a total of 28 days of treatment. The area under curve (AUC) is the concentration of test preparation in blood plasma at different time intervals. The AUC in this study (Table I) was the total AUC of the average reduction in angiotensin I level over time intervals of T1 (day 14), T2 (day 21), and T3 (day 28).

The positive group, extract group, n-hexane fraction group, ethyl acetate fraction group, and water fraction group were significantly different from both the normal and negative groups. Giving matoa leaf fractions and extracts to male Wistar rats induced with angiotensin II could reduce their angiotensin I levels. The water fraction (7.98 mg/200g) and the positive control (Irbesartan) both similarly reduced angiotensin I levels.

The percentage reduction of angiotensin I levels in the treatment group was thought to be due to the chemical contents of the matoa leaves, namely flavonoids (quercetin) and triterpenoids/saponins. Quercetin can affect the renin-angiotensin-aldosterone system (RAAS), so it is thought to reduce both angiotensin I levels and hypertension symptoms in the rats that were induced with angiotensin II (Larson, Symons & Jalili, 2010). The use of 96% ethanol solvent is presumed to attract all the compounds from the matoa leaf extract. Ethanol is a very effective polar and non-polar solvent that can dissolve compounds, such as flavonoids, triterpenoids, and saponins.

The ethyl acetate fraction group showed a 23.6% decrease in angiotensin I level, which was close to the 24.8% decrease in the positive group. The matoa leaf extract group showed a decrease in 17.2% angiotensin I levels, which was close to the 20.2% decrease in the positive group.

Conclusion

Giving matoa leaves or leaf extracts to male Wistar rats induced with angiotensin II can reduce their angiotensin I levels. The water fraction (7.98 mg/200g) reduced angiotensin I levels which was the same result that was produced by the positive control (Irbesartan).

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IAI CONFERENCE

RESEARCH ARTICLE

The effect of biofilm formation on the outcome therapy of diabetic foot infections (DFIs) patients in the outpatient clinic and inpatient ward of Dr Sardjito General Hospital Yogyakarta

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Abstract

Introduction: Diabetes is a non-communicable disease with incidence rate of about 1.5 – 2.3% per annum with the most complication is Diabetic Foot Infections (DFIs). **Objective:** This research was conducted to describe the bacteria responsible for biofilm formation and its ability to cause DFIs in biofilm formation at Dr. Sardjito General Hospital as well as the therapy outcome. **Methods:** This research was conducted from September to November 2017. Specimens of samples were obtained from wound swabs of DFIs patients who met the inclusion and exclusion criteria (31 outpatients and 15 inpatients), and were then tested for culture and sensitivity and their ability to form biofilms. **Results:** The DFIs with the biofilm-producing bacteria (weak to moderate) have a different outcome compared to DFIs patients without biofilms.

Introduction

Diabetes is one of the chronic diseases caused by metabolic disorders of the body. This disease is one of the non-communicable diseases that continue to spread among individuals between 1.5% - 2.3% per annum (Al-Rubeaan *et al*., 2015). One of the complications that often occur in diabetes is Diabetes Foot Infections (DFIs). The number of DFIs patients is about 15% of the number of patients with diabetes (Aumiller & Dollahite, 2015). Nearly 85% of people with DFIs end up with amputation, whereby 40% of them can prevent the amputation through appropriate therapy and treatment. The management of DFIs includes reducing pressure on the foot (offloading), debridement and antibiotics administration. One of the challenges faced in managing DFIs is the formation of biofilms from bacteria that cause ulcers. Biofilms are thought to reduce the effectiveness of antibiotic use through several mechanisms, which ultimately leads to antibiotic resistance and delay in antibiotic penetration (Abbas *et al*., 2013; Banu *et al*., 2015). The management of diabetic ulcer antibiotic therapy with the biofilm-producing bacteria requires a specific strategy so that antibiotics are able to eradicate the infection-causing bacteria and accelerate wound healing (Abbas *et al*., 2013). This study was conducted to

describe the biofilm-producing bacteria and their ability to cause DFIs in Dr Sardjito General Hospital Yogyakarta (SGHY) and the outcome therapy to obtain the appropriate management therapy in overcoming bacterial infections in DFIs with biofilms formation.

Methods

This study was an observational study with a prospective cohort design which was conducted from September to November 2017 in the polyclinic and inpatient ward of Dr Sardjito General Hospital Yogyakarta (SGHY). The subject was the outpatients and inpatients who were diagnosed with DFIs. The inclusion criteria in this study were patients diagnosed with DFIs during the study period, aged ≥18 years old, who were examined for their DFIs age and had a complete medical record. Patients with malignancy and immune disorders were excluded from this study. The subjects involved in the study voluntarily agreed to take part, and informed consent was signed. A total of 31 patients in the outpatient clinics and 15 patients in the inpatient ward met the inclusion and exclusion criteria. The wound swab samples were taken when the wound was opened; after that, the culture and sensitivity tests were conducted to determine the profile of wound infecting bacteria and their sensitivity to antibiotics in accordance with the Clinical and Laboratory Standard Institute (CLSI) guidelines. In addition to culture and sensitivity tests, bacteria found in wound swab samples were tested for their ability to form biofilms in the laboratory. The culture and sensitivity tests and biofilm formation were carried out in the Microbiology laboratory of the Faculty of Medicine, Public Health and Nursing of Gadjah Mada University. The results of the culture and sensitivity tests and the biofilm formation ability were then given to the doctor who treated the subject as consideration for therapy. The subject was monitored for the development of the DFIs and their treatment until the wound improved, or the study was completed. Outcome assessment or wound repair was determined by the doctor who took care of the patient. The duration of wound repair was calculated from the time the patients were taken for basic ulcer swabs until the ulcer improved. The study was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia (KE/FK/0838/EC/2017 and KE/FK/1041/EC/2017).

Results

In this study, for three months, samples were collected from 31 patients in the outpatient clinics and 15 patients in the inpatient ward. The age range of subjects in this study was between 36 and 80 years, with an average age of 58.7 years (outpatient) and being 57.9 years old (inpatient), with a balanced number of male and female patients. The number of patients below 60 years of age and over 60 years was balanced in the outpatient care. In contrast, the inpatient care had more patients who were below 60 years old. The demographic data of the patients can be seen in Table I. In both outpatient and inpatient wards, most patients had a BMI of ≤ 25 Kg/m^2, suffering from diabetes with an average of more than ten years and the average occurrence of DFIs being less than six months. Almost all patients also experienced complications of Peripheral Arterial Disease (PAD).

Table I: Demographic profile of patients

Parameter	Outpatient	Inpatient
Average age (year)	58.8	57.9
Age ≤ 60 years old (%)	16 (51.6)	10 (66.7)
Age > 60 years old (%)	15 (48.4)	5 (33.3)
Male (%)	16 (51.6)	8 (53.3)
Female (%)	15 (48.4)	7 (46.7)
Average BMI (Kg/m^2)	23.9	23.5
BMI ≤ 25(%)	19 (61.3)	10 (66.7)
BMI > 25 (%)	12 (38.7)	5 (33.3)
Average DM duration (year)	12.2	10.1
≤ 12 years (%)	20 (64.5)	8 (53.3)
> 12 years (%)	11 (35.5)	7 (46.7)
Average wound duration (month)	5.8	3.2
≤ 6 months (%)	24 (77.4)	11 (73.3)
> 6 months (%)	7 (22.6)	4 (26.7)
Comorbidity*		
Total PAD (%)	28 (100)	15 (100)
Total hypertension (%)	20 (64.5)	7 (46.7)
Total Eye disease (%)	10 (32.3)	1 (6.7)
Total Kidney disease (%)	10 (32.3)	4 (26.7)
Total cardiovascular disorder (%)	6 (19.4)	0
Total stroke (%)	2 (6.5)	0
Total DVT (%)	2 (6.5)	1 (6.7)
Total other infection (%)	2 (6.5)	0

* Patients who suffered from more than one comorbidities

There were 27 bacterial isolates found in this study from outpatients and 14 bacterial isolates from inpatients, as presented in Table II. The ability of biofilm formation occurred in 8 of 27 bacteria in outpatients (29.6%) at a weak to moderate level. In inpatients,

there were 5 out of 14 bacteria with an ability to form biofilms at a weak level (35.7%). Almost all the biofilm-producing bacteria are Gram-negative bacteria (Table II).

Table II: The ability of bacteria to form biofilm and DFIs patient outcome therapy

Age of ulcer (month)	Bacteria isolated from ulcer	Biofilm strength				
1	*Pseudomonas aeruginosa*and*Klebsiella pneumonia* (MDR)	weak	Ceftazidime and metronidazole	Meropenem; Amikacin; Fosfomicyn	19	Bad
1.5	*Pseudomonas aeruginosa*(MDR)	weak	Ceftazidime and clindamycin	Amikacin	14	Bad
2.5	*Actinobacillussp*	weak	Ceftazidime and metronidazole	Meropenem; Fosfomicyn	17	Bad
5	*Klebsiellapneumoniae*	weak	Ceftazidime and metronidazole; Clindamycin	Amikacin and meropenem	17	Bad
12	*Morganellamorganii, Proteus mirabilis* (MDR)and*Klebsielaoxytoca*	weak	Ceftazidime and metronidazole;	Amikacin and meropenem	16	Good
					16.6±1.8	

The duration of the ulcer did not have any effect on the level of the biofilm, as shown in Table III. In outpatients, the longer the duration of DFIs, the broader the range of biofilm formed, from weak to moderate. Meanwhile, in inpatients who have suffered from DFIs for 5-48 months, the biofilms formed are all weak. There is no significant difference in the duration of healing between DFIs with and without biofilm-producing bacteria in outpatient. However, there is a significant difference in the duration of healing between DFIs with biofilm-producing bacteria and those with the non-biofilm-producing bacteria in hospitalized patients (10.1±3.5 days versus 16.6±1.8 days).

Table III: Statistical analysis

Outpatient	**Antibiotic duration (days)**	***p*-value**
DFIs with no-biofilm-producing bacteria	22.9±6.3	0.05
DFIs with biofilm-producing bacteria (weak-moderate)	22.0±3.9	
Inpatient	**Antibiotic duration (days)**	***p*-value**
DFIs with no-biofilm-producing bacteria	10.1±3.5	0.03
DFIs with biofilm-producing bacteria (weak-moderate)	16.6 ±1.8	

Discussion

Ageing can increase the risk of DFIs by two to four times. On the other hand, younger people have higher mobility than the older ones, who are at risk of getting new trauma or injuries. Age affects the duration of DFIs healing. Older patients have a longer healing time associated with a decrease in the inflammatory response, such as not immediately infiltrating T cells in the wound due to a disruption in chemokines production and decreased capacity of macrophage phagocytosis (Guo & Dipietro, 2010).

The risk of DFIs in women tends to be lower because they maintain and take care of their feet than men; besides, they have a lower risk of neuropathy than men (Al-Rubeaan *et al*., 2015). Wound healing also depends on hormones such as estrogen, testosterone and dehydroepiandrosterone (DHEA). Estrogen is related to matrix production, regeneration, inhibition of proteases, epidermal functions and is associated with genes related to inflammation so that their presence has an effect on wound healing (Horng *et al*., 2017).

Body mass index (BMI) can affect the speed of wound healing. In this study, the majority of patients with DFIs has a BMI of less than 25. The increase in BMI is directly proportional to the increase in the risk of DFIs in patients, as every 20 kg increase in weight can increase the risk of DFIs by 20%. This is because the fatter the patient, the greater the foothold and pressure on the feet compared to a slim patient (Sohn *et al*., 2010). In patients with obesity, adipose tissue secretes various molecules that can cause vascular disorders, including PAD. In addition, there is an increase in the working of the heart, which improve tissue perfusion; if the heart fails to perform perfusion, it can cause necrosis of the

tissue, which prolongs the healing process. In addition, patients with obesity are at risk of hyperventilation which can cause low oxygen level around the wound, leading to damage. In patients with PAD, perfusion can occur, which results in low antibiotic concentrations in the lower extremities, so that the healing process of DFIs is inhibited (Vella *et al*., 2016). The aggressive revascularisation therapy can increase reperfusion and accelerate wound healing in these patients.

A kidney disorder is a concomitant disease commonly found in patients with DFIs. Patients with renal impairment also discovered that PAD had a worse prognosis because PAD in patients with chronic kidney disorder was a poor predictor of wound healing (Prompers *et al*., 2008). In this study, there were 32.3% of patients with kidney disorder and PAD in outpatient care and 26.7% in inpatient care.

The average duration of the DFIs patients in this study suffered from diabetes mellitus was more than ten years, with an average duration of the wound being more than three months. In the prolonged duration of diabetes, the risk of complications, including diabetic ulcers, increased (Zoungas *et al*., 2014). The wound healing process in patients with diabetes was disrupted due to hypoxia, fibroblast and epidermal cells dysfunction, angiogenesis and neovascularization disorders, high metalloprotease level, neuropathy, and decreased immune resistance from the host (Guo & Dipietro, 2010). The duration of diabetes is also associated with the presence of complications in the form of neuropathy. The longer the duration of diabetes, the higher the patient's risk of developing neuropathy. In patients with neuropathy, neuropeptide level is lower; this decrease in neuropeptide level causes a long process of wound healing (Ackermann & Hart, 2013). The duration of the wound can also affect the speed of wound healing. Chronic (prolonged) wounds are associated with chronic inflammatory activity, ageing of fibroblasts, and growth of bacteria in wounds (Bosanquet & Harding, 2014).

Based on this study, in outpatient's clinics, the number of monomicrobial and polymicrobial bacteria tends to be similar. However, for the inpatients, the number of polymicrobial bacteria is higher. This may be due to the fact that the outpatients involved in the study were patients who have a more regular (weekly) check-up to the outpatient clinic, which prevents the accumulation of the growing bacteria, as there is more intensive debridement provided with a medical check-up at the clinic. One of such therapies to combat biofilm formation is using an agent that is capable of disrupting the multicellular structure of the biofilm (Deepigaa, 2017; Mendes *et al*., 2014). However, there is still a need for further research related to the effect of intensive debridement as a multicellular structure disrupter on the recovery of DFIs. The type of bacteria will also determine the level of the biofilm formed. *Pseudomonas aeruginosa, Citrobacter sp., E. coli, Proteus sp* and *Klebsiellaoxytoca* are known to be Gram-negative bacteria that are capable of forming biofilms in DFIs and Multi-Drug Resistance (MDR) organism (Abbas *et al*., 2013; Banu *et al*., 2015). The type of Gram-negative bacteria is in accordance with the results of the study.

Diabetes-associated foot ulcer infections are predominantly polymicrobial. Several bacterial can be part of the DFIs microbial, namely Staphylococcus, Pseudomonas, Streptococcus, Enterococcus, Corynebacterium, Acinetobacter, Prevotella, Porphyromonas, and members of the family Enterobacteriaceae. The predominant Gram-positive and Gram-negative species present in DFIs are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively (Abbas et al., 2013; Banu *et al*., 2015; Lipsky *et al*., 2008). In infected DFIs, because of deficient vascularization, antibiotics frequently reach the local ulcer microenvironment only at sub-therapeutic concentrations (Lipsky *et al*., 2008). Even when topically applied, antibiotics rarely reach bacteria that reside within mature biofilms at therapeutic concentrations (Lipsky *et al*., 2004). In addition, some antibiotics such as aminoglycoside contribute to the increase in the formation of biofilm by *P.aeruginosa* and *E.coli,* so that a strategy is needed to deal with this (Hoffman *et al*., 2005). The microbial cells growing within a biofilm are physiologically distinct from planktonic cells of the same strain. The overall resistance level in biofilms is distinct from the one observed at a cellular level (Stewart & Costerton, 2001). As a consequence, the antimicrobial concentration required to inhibit biofilms can be up to hundreds or even a thousand times higher than the corresponding concentration necessary to eliminate free-living bacterial cells (Ceri *et al*., 1999). The resistance of biofilms formed by Gram-positive strains was low against azithromycin and imipenem. Imipenem was the least affected by biofilms formed by Gram-negative bacteria. Vancomycin is unable to fight *S.aureus* and *Enterococcus faecalis* (LaPlante & Mermel, 2009). In addition, Ciprofloxacin was unable to eradicate the biofilm of *S.aureus*, *E.coli* and *P.aeruginosa* (measured by the ratio of MBEC/MIC expressed by ≥ 90% of the tested isolates) (Ceri *et al*., 1999). Several novel therapeutic strategies, namely bacteriophages, probiotics and antimicrobial peptides (AMP), are recently explored as potential alternatives to eradicate bacterial biofilms in DFIs. Antibiofilm agents in combination with antibiotics, for example, Ciprofloxacin, may be useful to overcome the high biofilm resistance to antibiotics. The synergistic effect of potential antibiofilm agents with Ciprofloxacin appears in several strains, namely *Acinetobacterbaumanii,* including

ambroxol, piroxicam, Manuka honey and grape vinegar (Abbas *et al*., 2013). An interesting observation from this study is the discovery of *Bulkholderia pseudomallei* bacteria, which can form biofilms at weak to moderate levels. *Burkholderia pseudomallei* are the causative agents of melioidosis, an infection common in Southeast Asia and other parts of the world. Clinical manifestations vary and may be entirely absent or may include acute septic shock and abscesses. Acute septic shock syndrome is common in patients with melioidosis and diabetes or chronic renal failure. The immune status of the host is an important factor in infection by B. pseudomallei. Susceptibility to melioidosis was found in hosts who were immunocompromised and/or had diabetes mellitus and other conditions *(Currie et al*., 2010). The stimulation of B. pseudomallei to produce biofilms resulted in upregulation of some genes to be more resistant to antimicrobial agents (Lee *et al*., 2010). B. pseudomallei in biofilm cells are highly resistant to ceftazidime, doxycycline, imipenem, and trimethoprim sulfamethoxazole. However, the drug resistant mechanism of biofilm is still unclear (Currie *et al*., 2010; Korbsrisate *et al*., 2005).

Improvement was observed in the outcomes of all the outpatients, while the inpatients had bad outcomes as the biofilms have been formed. The patient severity index has not been mapped in this study which is likely to complement the results of the study. The absence of differences in the duration of healing between the DFIs and biofilm-producing bacteria in outpatient is likely due to routine debridement of the ulcer. In addition to removing necrotic tissue, debridement also reduces bacterial colonization of the ulcer and damages the biofilm physically (Aumiller & Dollahite, 2015). In the future, DFIs can use combination of antibiofilm agents and antibiotics to improve the patients' therapeutic outcome.

Conclusion

Most of the bacteria that cause infection in DFIs at the Clinic of SGHY are Gram-negative bacteria. A total of 29.6% of bacteria in outpatients have an ability to form biofilms with a weak to moderate nature, while 35.7% of bacteria in inpatients have an ability to form biofilms with weak nature. In hospitalised patients, the presence of biofilms will prolong the healing of patients with DFIs.

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RESEARCH ARTICLE

The effect of loss-of-function allele (CYP2C19*3) with Clopidogrel efficacy in coronary heart disease patients

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Abstract

Introduction: Clopidogrel is the most widely prescribed antiplatelet for patients with coronary heart disease (CHD) who cannot take aspirin. Despite its effectiveness, Clopidogrel has several side effects caused by its metabolite. Clopidogrel resistance has been identified in some patients, and patient factors such as genetic polymorphisms in CYP2C19 may play a role in this resistance. The researchers wanted to look at CYP2C19*3 polymorphisms and platelet aggregation in CHD patients who were taking clopidogrel. **Methods:** This research used a cross-sectional design. The research enrolled CHD patients at a local hospital's cardiology unit with certain inclusion and exclusion requirements. In the clinical laboratory, CYP2C19*3 polymorphisms was investigated using polymerase chain reaction (PCR), and platelet aggregation will be measured using light transmission aggregometry (LTA). **Results:** This research enlisted the participation of 53 patients. The majority of the patients (68%) were men, with the highest age group being 60-69 years old. The most common comorbid disorder was hypertension. The result of CYP2C19*3 polymorphisms as follows: GA (75%), AA (21%), and GG (4%). Hypo-aggregation (89%) and normal-aggregation (89%) are seen in the majority of patients (11%). The authors were unable to locate the patient who had hyper-aggregation. **Conclusion:** According to descriptive research, CYP2C19*3 polymorphisms caused hypo-aggregation in more patients than normal aggregation in this study.

Introduction

The biggest health issues in the world nowadays are cardiovascular disease (CVD). Disorders in cardiovascular include conditions such as Coronary Heart Disease (CHD), Heart Failure, and Stroke. Cardiovascular disease is part of the Non-Communicable Diseases (NCD) and has surpassed cancer as the leading cause of death worldwide. Using data from the World Health Organization (WHO) in 2015, it was said that 31% of 56.5 million deaths in the world were caused by cardiovascular disease. Some of them are CHD and stroke with 42.3% and 38.3%, respectively. Deaths from heart disease occur in several countries, ranging from low-income to high-income countries. Indonesia is one of the developing countries with a high cardiovascular disease mortality rate (WHO, 2012).

In general, coronary heart disease can be divided into two, namely acute coronary syndrome and chronic coronary syndrome. The prevalence of CHD in Indonesia is still quite high, as evidenced by data submitted by the Basic Health Research by the Ministry of Health in 2014 showing the number of CHD patients in East Java based on a doctor's diagnosis by 0.5%, while based on symptoms and a doctor's diagnosis by 1.3% (Departemen Kesehatan, 2014).

The accumulation of atherosclerotic plaque in the endothelial walls of coronary arteries causes CHD. Plaque builds up on artery walls, reducing the blood flow that carries nutrients and oxygen. Ischemic symptoms are reported by CHD patients due to an imbalance in oxygen supply and demand. The goal of CHD therapy is to keep the plaque in the patient's coronary arteries stable by inhibiting the progression of atherosclerotic plaque and preventing plaque that is formed from rupture (Montalescot, 2013).

One of the treatments for CHD patients is antiplatelet therapy. In CHD patients, antiplatelet therapy helps to avoid or minimize platelet aggregation and/or plaque formation. Reduced platelet aggregation and/or plaque formation lowers the risk of thrombus in blood vessels, thus lowers the risk of ischemia, which can further lead to ischemic cardiovascular events. Clopidogrel is an antiplatelet agent that is given as a treatment for coronary artery disease in addition to Aspirin. The CAPRIE study (Clopidogrel *vs* Aspirin in Patients at Risk of Ischemic Events) shows slightly better effectiveness on Clopidogrel compared to Aspirin in preventing cardiovascular events in patients with previous myocardial infarction, stroke, or peripheral arterial disease.

Clopidogrel's pharmacodynamic effects are dependent on factors that affect its metabolism, which is one of its drawbacks (Aradi, 2014). Clopidogrel is a prodrug that must be oxidized by the cytochrome P450 system in the liver to produce active metabolites. Clopidogrel's antiplatelet efficacy is determined by pharmacodynamic factors linked to drug-metabolising enzymes, such as cytochrome P450 classes (for example, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5) and paraoxonase 1 (PON1). About 4-30% of patients treated with Clopidogrel show a low antiplatelet response or do not show an antiplatelet response. Clopidogrel's efficacy is influenced by a number of factors, including the patient's age, BMI (Body Mass Index), kidney disease, and genetic factors. From the genetic aspect, there are various CYP2C19 polymorphism profiles related to the metabolic process of the drug, where CYP2C19 is responsible for the metabolic activation of Clopidogrel, which is a prodrug to be transformed into active metabolites and loss-of-function alleles CYP2C19, which is directly related to the recurrence of cardiovascular disorders in patients receiving Clopidogrel. Clopidogrel resistance, or non-responsive Clopidogrel, is the term for this phenomenon. These patients, according to prior reports, have a higher risk of ischemic cardiovascular events.

CYP2C19*3 and CYP2C19*2 polymorphisms have the effect of reducing the effectiveness of Clopidogrel (loss-of-alleles) active metabolites in the systemic and anti-aggregation ability. Judging from the genetic polymorphism present in CYP2C19, patients treated with Clopidogrel can show a varied therapeutic response (Yin, 2011).

It is well known that Clopidogrel is an inactive prodrug that requires oxidation by the hepatic cytochrome P450 system to produce active metabolites. Due to mutations in the gene for the CYP enzyme, this can affect the effectiveness of Clopidogrel. Of these genes, the main focus is CYP2C19. Loss-of-function alleles, such as CYP2C19*2 and CYP2C19*3, are responsible for decreased activation of Clopidogrel and increased risk of recurrent heart disease in CHD patients.

The prevalence of CYP2C19 loss-of-function polymorphisms worldwide is 24% of the non-Hispanic white population, 18% of Mexicans, 33% of African Americans, and 50% of Asians. This population is a homozygous carrier that has a poorly metabolised CYP2C19, thereby reducing the antiplatelet effect of Clopidogrel (Kitzmiller, 2011). The prevalence of CYP2C19 loss-of-function polymorphisms in Asia is 24.2% of the Japanese population, 14.8% of the Korean population, 8.9% of the Han Chinese, 9% of the Malay population and 13% of the Indian population. Specifically, in Singapore, there are 10% of Chinese-Singaporean subjects and 9% of Malay-Singaporean subjects, and only 1% of Indian-Singaporean subjects (Chan, 2012).

The CYP2C19*3 is a loss-of-function allele, which is responsible for decreasing the activation of Clopidogrel. Clopidogrel concentrations in plasma are lower as a result of reduced clopidogrel activation, resulting in hyper platelet aggregation. The risk of persistent ischemia increases when hyper platelet aggregation occurs. Based on this background, and not many studies look at the genetic influence on the Clopidogrel metabolism process, the authors intend to conduct research by looking at the genetic influence on patients using Clopidogrel. The purpose of this study was to look at the effect of one of the alleles suspected of causing a decrease in the function of Clopidogrel (loss of function allele CYP2C19*3 polymorphisms). The effect of pharmacodynamic effects of Clopidogrel seen from platelet aggregation was measured using Light Aggregometry.

Methods

A cross-sectional design with descriptive analysis methods was used in this study. The aim of this study was to look at the relationship between CYP2C19*3 polymorphisms and platelet aggregation in CHD patients who were taking Clopidogrel.

Population of the study

The participants in this study were patients with coronary artery disease who received outpatient treatment at a local hospital. The sample used is drawn from a population that meets the following inclusion and exclusion criteria:

Inclusion criteria:

1. Patients who are willing to join the study
2. Patients using generic clopidogrel therapy
3. Patients who have high adherence to treatment
4. Patients taking clopidogrel therapy for one month

Exclusion Criteria:

1. Patients undergoing chemotherapy
2. Patients who have liver problems

Before beginning the study, all participants signed a written informed consent form. The local hospital ethics committees have given their approval to the research.

Genotyping

The CYP2C19*3 polymorphism profile was analysed by the Polymerase Chain Reaction (PCR) method. The sample used was a patient's blood sample that DNA isolation stages had previously been carried out. PCR is used for DNA amplification using specific carrier primers. The primers used for CYP2C19*3 DNA amplification are primary pairs 5'-TATTATTATCTGTTAACTAATATGA-3' and 5'-ACTTCAGGGCTTGGTCAATA-3'. PCR was carried out in the following stages: initial denaturation at a temperature of 94˚C for 2 minutes. After being denatured, it continues with 35 cycles consisting of:

a. Denaturation at 94˚C for 45 seconds
b. Annealing at 53 ° C for 40 seconds
c. Polymerisation at 72 ° C for 30 seconds
d. Final extension at 72 ° C for 5 minutes

After 35 cycles, the DNA amplicon is obtained. Followed by the digestion process, the CYP2C19*3 DNA amplicon was digested with the BamHI restriction enzyme at 37˚C and a Bovine Serum Albumin (BSA) buffer. Deactivation of the enzyme at 65˚C for 20 minutes. The next step was to obtain RFLP (Restriction Fragment Length Polymorphism) products. The obtained RFLP products are then separated in agarose gel solution (electrophoresis). The next step was the visualisation of electrophoresis results followed by staining in a solution of ethidium bromide (5 mg/mL) for ten minutes. Then the destaining step was carried out by immersing it in a solution of water for five-ten minutes. After observing the migration of DNA in the UV lamp transilluminator. Then agarose gel which has been electrophoresed, and DNA migration was observed in UV transilluminator and then photographed.

Platelet function test

In this study, the profile of platelet aggregation was seen through the Turbidimetry test conducted at the Prodia Laboratory. The sample used in this test is platelet-rich plasma (PRP) from CHD patients using clopidogrel therapy. Platelet aggregation was measured in PRP at 37°C by light transmittance aggregometry using an aggregometer (Model 700, Chrono-Log Corp., USA). The PRP was pre-warmed to 37°C for five minutes before the addition of the agonists, i.e. 5 µM adenosine diphosphate (ADP), 0.5 mM arachidonic acid (AA) and 2 µg / ml collagen (all from Chrono-Log Corp., USA). The aggregation response was monitored for at least five minutes, and the extent of aggregation was expressed as the percentage (%) aggregation calculated using Aggrolink software (Chrono-Log Corp., USA)

Results

This research looked at patients with Coronary Heart Disease (CHD) who were taking Clopidogrel as their only antiplatelet medication. The study was carried out at the District General Hospital's Cardiology Center. A total of 53 samples were collected. Table I shows the patient characteristics in this report.

Table I: Patient characteristics

Patient characteristics	n	%
Sex		
• Male	36	68%
• Female	17	32%
Age		
• 40-49	13	25%
• 50-59	12	23%
• 60-69	18	34%
• 70-74	5	9%
• More than 75	5	9%
Compelling indication		
• Diabetes mellitus	11	
• Hypertension	21	
• Dyslipidemia	5	
• Heart Failure	15	
• Asthma	1	
• Chronic Kidney Disease	2	
• Without compelling indication	8	

Blood samples for platelet aggregation analysis must be processed within three hours of taking a patient's blood sample. This is achieved to eliminate platelet

aggregation bias. Table II shows the outcomes of Light Transmission Aggregometry-assisted tests.

Table II: Profile of platelet aggregation with ADP as an inducer

Type of platelet aggregation	n	%
Hypo-aggregation	47	89 %
Normal aggregation	6	11 %
Hyper-aggregation	0	0

CYP2C19*3 Polymorphisms were obtained by PCR and electrophoresis, and the results are shown in Table III.

Table III: Profile of CYP2C19*3 polymorphisms

CYP2C19*3 polymorphisms	n	%
GA	40	75 %
GG	2	4 %
AA	11	21 %

The authors created a cross-tabulation between CYP2C19*3 polymorphisms and platelet aggregation. The result of cross-tabulation can be seen in Table IV.

Table IV: Cross-tabulation between CYP2C19*3 polymorphisms and platelet aggregation

CYP2C19*3 Polymorphisms	n (%)		
	Hypo-aggregation	Normal aggregation	Hyper-aggregation
GA	35	5	0
GG	2	0	0
AA	10	1	0

Discussion

The majority of the patients in the sample were male (68%), with the majority of them being between the ages of 60 and 69. There are a number of comorbid conditions, the most common of which is hypertension. Hypertension is a risk factor for coronary heart disease in principle. In the presence of hypertension, blood vessels may cause endothelial damage. Endothelial dysfunction, or damage to blood vessels, is the first stage of atherosclerotic plaque development. Patients with atherosclerotic plaque may experience complications such as coronary heart disease (CHD). According to the findings, almost all of the study participants had hypertension comorbidities, which supports the hypothesis that hypertension may be a cause of coronary heart disease.

Platelet aggregation measurements are measured using light transmission aggregometry (LTA). LTA is also known as optical turbidimetry or aggregometry. This LTA can be used to identify several disorders in platelet defects. Despite being used as a diagnostic tool, LTA can also be used to measure platelet function (Harrison, 2000; Michelson, 2004; Rand, 2003).

The decrease in optical density after stimulation of aggregation on platelet-rich plasma is used to measure platelet aggregation. This approach has many benefits, including the fact that it has been used in numerous studies for a long time, is predictable, and the instrument can be modified. There are some drawbacks to the benefits, such as the fact that it takes a long time, the sample preparation is very complex, and there is no simple standardisation (Favaloro, 2008; Lenk, 2013).

The agonists used in this test are ADP, collagen and epinephrine. Adding an agonist to this test aids to activate platelets. In this study, the only agonists observed were ADP. Clopidogrel drug inhibits platelet activation through inhibition of ADP binding to its receptors. ADP is removed from damage to blood vessels. ADP binding to P2Y12 receptors causes changes in platelet shape and induction of platelet aggregation through internal calcium movement. ADP binding to P2Y12 receptors plays a role in platelet aggregation response (Remijin, 2002; Koltai, 2017).

When the number of patients is divided by the degree of platelet aggregation, it can be shown that the number of patients with hypo-aggregation is much higher than the number of patients with normal platelet aggregation. The drug's pharmacokinetic and pharmacodynamic effects vary from patient to patient. Pharmacogenomic variation, which is genetic variation between people, is one explanation for the different responses. The enzymes involved in many stages of metabolism, such as the phase 1 reaction or the phase 2 reaction in drug metabolism, have been studied in pharmacogenomic studies. Cytochrome P450 (CYP450) is an enzyme that is involved in a variety of metabolic processes (Cacabelos, 2012).

Since the CYP2C group is responsible for about 20% of CYP450 substrate metabolism, genetic variation in one of the CYP2C9 classes induces different metabolism in certain CYP450 substrates (Speed, 2009; Liau, 2014; Wei, 2015; Brown, 2018).

According to the distribution of CYP2C19*3 polymorphisms in the study set, the findings of this study matches with Iddrisi and the authors (2018). The

CYP2C19*3 polymorphism discovered was: 76.67% GA allele and 18.33% AA allele, according to the report. Due to its high prevalence relative to other enzymes, the CYP2C19 polymorphism is one of the most studied in Asian populations (Adithan, 2003; Johnson, 2011; Lyon, 2012; Strom, 2012).

CYP2C19*3 is a loss-of-function allele that causes clopidogrel activation to be reduced. Clopidogrel concentrations in plasma are lower as a result of reduced clopidogrel activation, increasing the risk of platelet hyper-aggregation if plaque on the walls of blood vessels ruptures. As platelets hyper aggregate, the chances of a thrombus forming increase. The presence of a thrombus will result in a blockage of the coronary arteries, and a blockage of the coronary arteries is the result of ischemia. This ischemia is a manifestation of coronary artery disease.

In this study, chi-square analysis calculations cannot be performed to see the relationship between CHD patients who have CYP2C19*3 polymorphisms with platelet aggregation because there is a value of 0. According to theory, a loss-of-function CYP2C19*3 polymorphism could cause an increase in platelet aggregation. Clopidogrel metabolism into active metabolites is impaired due to CYP2C19*3 polymorphisms. Clopidogrel's active metabolites are essential for inhibitors of platelet activation to bind to P2Y12 receptors through ADP inhibition. In this study, CHD patients with CYP2C19*3 polymorphisms had mostly hypo-aggregation of platelets, which may be due to the presence of more than one CYP2C19 polymorphism in CHD patients.

It is also important to test clopidogrel drug concentration in CHD patients to determine the activity levels of Clopidogrel in the body. In different races, the CYP2C19*3 polymorphism is one of the strong determinants of decreased Clopidogrel active metabolites. Clopidogrel resistance is also influenced by the CYP2C19*3 polymorphism (Tresukusol, 2014). Based on Man and Chan's previous studies, the existence of CYP2C19* 3 polymorphisms has an effect on Clopidogrel metabolic processes in Asian patients undergoing PCI (Percutaneous Coronary Intervention). Clopidogrel metabolism effects result in a decline in active Clopidogrel metabolites, which may affect Clopidogrel's antiplatelet activity.

Polymorphisms in the CYP2C19*2 and CYP2C19*3 genes have been related to HPPR (High Post-Treatment Reactivity) in another study of patients with the acute coronary syndrome in Asian populations (Kim, 2009). Polymorphisms in the CYP2C19 gene are linked to high residual platelet reactivity, which raises the risk of major cardiovascular events (Yamamoto, 2011).

The development of thrombus in the coronary arteries is influenced by platelet reactivity. When a platelet is stimulated, the coagulation pathway opens, causing thrombin to be released. The presence of thrombin causes fibrin to activate, causing the thrombus to mature.

CYP2C19*3 polymorphism also has an effect on cardiovascular events. A study conducted by Jeong and the authors (2011) in patients with acute myocardial infarction in East Asia showed that the presence of CYP2C19*3 polymorphism was associated with an increase in cardiovascular events. Another study was conducted by Zhu (201)6 in patients undergoing Carotid Artery Stenting in Asia also showed a significant effect of CYP2C19*3 alleles on patient prognosis. Patients with CYP2C19*2 and *3 alleles have a higher ischemic event than those without.

Limitations of the study

Since we did not calculate plasma concentrations of Clopidogrel's active metabolite, we cannot provide clear proof of Clopidogrel's decreased antiplatelet efficacy in patients with at least one CYP2C19*3 variant allele.

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IAI CONFERENCE

RESEARCH ARTICLE

In vivo activity of *Phaseolus vulgaris* as an anti-hypercholesterolemic

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Abstract

Introduction: Hyperlipidemia occurs due to increased levels of lipids and cholesterol in the blood. Phytosterols, such as stigmasterol in *Phaseolus vulgaris*, can reduce blood cholesterol levels. **Aims**: The purpose of this study was to determine the activity of *P. vulgaris* in hypercholesterolemic rats. **Methods:** Nine groups underwent the anti-hypercholesterolemia test: control group, negative group, positive group, 150 mg/kg body weight (bw) and 300 mg/kg bw n-hexane extract groups, 150 mg/kg bw and 300 mg/kg bw ethyl acetate extract groups, and 150 mg/kg bw and 300 mg/kg bw ethanol extract groups. **Results:** All groups, except the control group, were given a high cholesterol diet to induce hypercholesterolemia (until the total cholesterol levels were higher than 200 mg/dL), followed by testing for ten days. The results showed that the 300 mg/kg bw ethyl acetate extract group had the best activity in reducing total cholesterol.

Introduction

Hyperlipidemia is a medical condition characterised by an increase in one or more of the plasma lipids (triglycerides, cholesterol, cholesterol esters, and phospholipids) and/or plasma lipoproteins (very-low-density lipoprotein and low-density lipoprotein), in addition to reduced high-density lipoprotein levels (Shattat, 2012). An increase in total cholesterol levels can lead to the risk of atherosclerosis (Owne *et al.*, 2015). The prevalence of dyslipidemia in adults aged ≥25 years in Indonesia was about 36% (33.1% for men and 38.2% for women) (Chao-Feng Lin *et al.*, 2018). Hyperlipidemia is one of the leading causes of cardiovascular diseases (Shattat, 2012). Green beans act as antioxidants, antidiabetics, diuretics, and antibacterials (Ratnayani & Puspawati, 2016; Raffaella *et al.*, 2018; Ramadhani *et al.*, 2020). Green beans contain glycoproteins, trypsin inhibitors, hemagglutinin, β-sitosterol, stigmasterol, allantoin, inositol, leukopelargonidin, quercetin, pelargonidin, cyanidin, kaempferol, petunidin, delphinidin, malvidin, and myricetin (Rahmawani, 2012). The chemical content of green bean extract includes alkaloids, carbohydrates, kumara, protein, amino acids, phenols, saponins, steroids, tannins, and terpenoids (Pascal *et al.*, 2017). In addition to their antioxidant properties that act as LDL reducers in the body, phytosterol components in green beans, such as stigmasterol, can reduce cholesterol levels by inhibiting HMG-CoA reductase, which will reduce LDL cholesterol synthesis. They can also increase HDL cholesterol levels. HDL cholesterol will carry excess LDL cholesterol in the bloodstream back to the liver so that HDL cholesterol prevents cholesterol deposition in the bloodstream, protecting blood vessels from the atherosclerosis process. Stigmasterol is the phytosterol in the ethanol extract of green beans as tested and analyzed by GC-MS (Sri Wahyuni & Ni Luk, 2016). *P. Vulgaris* showed to be an effective anti-hypercholesterolemic agent in ethanol extracts, but further research is needed from several extracts. Therefore, the researchers wanted to

explore the activity of *P. Vulgaris* extracts as anti-hypercholesterolemic agents.

Material and methods

Chemical material

The materials included are aqua dest, pulvis gummi arabicum as a suspending agent, n-hexane, ethyl acetate, 96% ethanol, high cholesterol feed (quail egg yolk, goat fat, cooking oil), propylthiouracil, simvastatin as a comparison drug, n-hexane, ethyl acetate, chloroform, hydrochloric acid, concentrated sulfuric acid, Dragendorf reaction, Mayer reagent, Bouchardat, magnesium powder, amyl alcohol, gelatin solution, iron (III) chloride, Liebermann Burchard reagent, sodium hydroxide, 10% vanillin in concentrated sulfuric acid, ether, 2N hydrochloric acid, and easy touch cholesterol test strips.

Plant material

Green beans were obtained from Cikurubuk Markat, Tasikmalaya, West Java. They were determined by the School of Life Sciences and Technology, Bandung Institute of Technology.

Extraction

The Simplicia of crushed green beans (*P. Vulgaris*) was weighed and then macerated in n-hexane, ethyl acetate, and 96% ethanol. In the first maceration, Simplicia was soaked in solvent until immersed for 24 hours and occasionally stirred (every six hours). The residue was separated from the filtrate and replaced with a new solvent. The process was repeated for 3x24 hours. The liquid extract was then collected in a beaker, and evaporation was carried out using a rotary evaporator to evaporate the solvent and obtain a thick, concentrated extract.

Animals experimental

A total of 36 Rattus male were divided into 9 test groups, namely: control group (not treated), negative group (1% PGA suspension), positive group (simvastatin suspension), group I (rats with induction + 150 mg/kg body weight (bw) n-hexane extract), group II (rats with induction + 300 mg/kg bw n-hexane extract), group III (rats with induction + 150 mg/kg bw ethyl acetate extract), group IV (rats with induction + 300 mg/kg bw ethyl acetate extract), group V (rats with induction + 150 mg/kg bw ethanol extract), group VI (rats with induction + 300 mg/kg bw ethanol extract). All groups except the control group were given high cholesterol diet for 30 days to induce hypercholesterolemia (until the total cholesterol levels were higher than 200 mg/dL), followed by testing for ten days. Before testing, the rats were fasted for eight hours and only given a drink. Then, the total cholesterol level was measured using the easy touch GCU and GCHb Test Strip method.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant intergroup differences of each parameter. A p-value <0.05 was considered statistically significant.

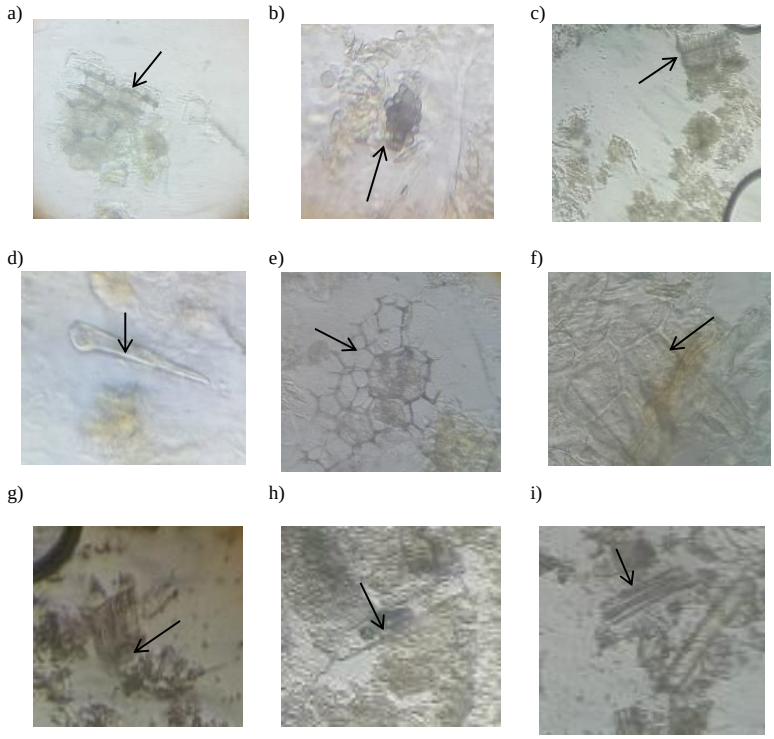
Results

The organoleptic macroscopic observations on the Simplicia of green beans showed a light brown powder with a distinctive odour and no taste. The microscopic observations of green beans Simplicia powder (*Phaseolus vulgaris L*) revealed endocarp, cell fragments containing aleurone grains and oil drops, dotted wooden vessels with ladder thickening, hair covering, endosperm cells, parenchyma cells containing starch grains, sclerenchyma fibres, xylem fibres with calcium oxalate crystal, and xylem fibres (Figure 1).

The results of standardisation of the water content parameters of the green beans Simplicia were carried out using the Azeotropic distillation method (Table I). In general, according to the Indonesian Herbal Pharmacopoeia, the water content of the Simplicia must not exceed 10%. A high water content (more than 10%) can cause the growth of microbes and fungi, thus reducing the quality of the powder and causing changes in enzyme work (Normalisa, 2018). Determination of total ash content is a way to describe the mineral content of Simplicia so that the parameters of the total ash content are related to the purity and contamination of a Simplicia (Tage, 2017). The results of this ash content meet the requirements, which was less than 11%. Determination of drying shrinkage is the percentage limit to the maximum range of compounds lost during the heating process (Tage, 2017). Our results showed an average of 9.33% drying shrinkage.

Table I: Standardisation of Simplicia

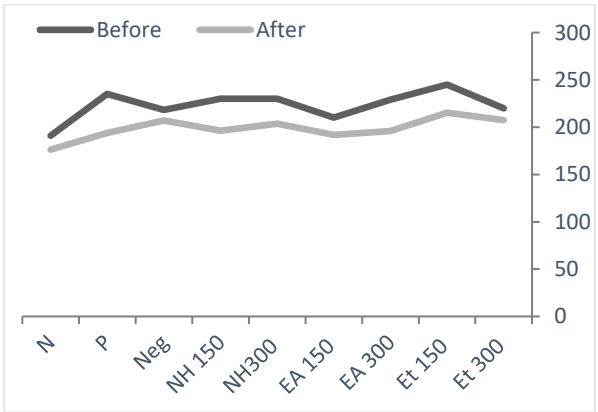
No	Parameter	Average level (%) ± SD
1	Water content	8 ± 0
2	Ash content	9,48 ± 0,112
3	Drying shrinkage	9,33 ± 0,41



a: Endocarp; b: Fragments with cells containing aleurone grains and oil drops; c: Wooden vessels with a thickening of the ladder; d: Conical cover hair; e: Endosperm cells; f: Parenchyma cells containing starch grains; g: Sclerenchyma fibers; h: Xylem fibers with crystalline ca oxalate crystals; i: Xylem fibers.

Figure 1: Microscopic observations of green beans

There were no significant differences in body weight ($p > 0.05$) between groups. However, there were differences in the mean between the groups after induction and after treatment using the test dose (Figure 2).



N: Normal (control); **P**: Positive; **Neg**: Negative group; **NH150**: N-Hexane 150 mg/kg bw; **NH300**: N-Hexane 300 mg/kg bw; **EA 150**: Ethyl acetate 150 mg/kg bw; **EA 300**: Ethyl acetate 300 mg/kg bw; **Et 150**: Ethanol 150 mg/kg bw; **Et 300**: Ethanol 300 mg/kg bw

Figure 2: Average body weight of rats before and after inductions

The 300 mg/kg bw ethyl acetate group rats did not show a significant difference ($p < 0.05$) with the 150 mg/kg bw ethyl acetate group rats, but there were differences in the average total cholesterol levels (Table II).

Tabel II: Effects of green beans on cholesterol levels in rats

Groups	Average of total cholesterol levels (mg/dL)
Normal	125
Positive (2 mg/kg bw simvastatin rats)	176
Negative	238*
150 mg/kg bw n-hexane rats	188.6
300 mg/kg bw n-hexane rats	160
150 mg/kg bw ethyl acetate rats	125.6*†‡
300 mg/kg bw ethyl acetate rats	114.3*†‡
150 mg/kg bw ethanol rats	157
300 mg/kg bw ethanol rats	136*†

* Significant difference with the negative control group ($p < 0.05$)
† significant difference with the positive control group ($p < 0.05$)
‡ Significant difference with the ethanol group 300 mg/kg bw ethanol rat group ($p < 0.05$)

The extract that was the most effective in lowering total cholesterol levels in rats was the 300 mg/kg bw ethyl acetate, showing it had better activity than simvastatin. Indeed, several active compounds of bean extract, such as alkaloids, polyphenols, flavonoids, and steroids, are known to have a cholesterol-lowering activity (Table III).

Tabel III: Phytochemical screening of green beans extract

No	Phytochemical	Reagent	Result			
			Simplicia	n- hexane	Ethyl acetate	Ethanol
1	Alkaloid	Dragendorff	+	+	+	+
		Mayer	+	+	+	+
2	Flavonoids	Mg, HCl, amyl alcohol	+	-	+	+
3	Quinone	NaOH	+	+	+	+
4	Monoterpenoid and sesquiterpenoid	Vanillin 10%	+	+	+	+
5	Steroids	Liebermann	+	+	+	-
	Tritepernoid	burchard	+	-	-	-
6	Saponin		+	-	-	-
7	Polyphenol	$FeCl_3$ 1%	+	-	+	+
8	Tannins	Gelatin 1%	-	-	-	-

Discussion

The active compounds in each solvent were different. The ethyl acetate and ethanol solvent contained flavonoids that were not detected in the n-hexane extract, indicating that the flavonoid components in the green bean extract contain polar flavonoids (Gazali & Nufus, 2019). The ethanol extract did not contain steroid metabolite compounds since steroids are composed of isoprene from long hydrocarbon chains and are non-polar. Ethyl acetate, as a semi-polar solvent, can attract polar and non-polar compounds (Putri *et al.*, 2013). Polyphenol metabolites were not present in the n-hexane extract, indicating that most phenolic compounds were polar compounds that dissolve in polar solvents (Fengel D & Wegener G, 1995). The multilevel maceration method was used for extraction because it could attract compounds based on the polarity of the solvent used. Thus, n-hexane would attract non-polar compounds, ethyl acetate would attract semi-polar compounds, and 96% ethanol would attract polar compounds without any interference of being extracted by other group compounds (Permadi, 2018).

Feeding rats with high-fat foods resulted in weight gain, accompanied by increased serum cholesterol levels (Muzdalifah, 2017). In addition to the high-fat diet that aimed to accelerate the increase in cholesterol levels, propylthiouracil (PTU) was added for the same purpose. The direct effect of PTU-induced hypothyroidism on lipoprotein metabolism is an increase in cholesterol levels, especially LDL-cholesterol caused by the metabolic suppression of LDL receptors, thus increasing LDL levels (Rahayuningsih, 2015). Flavonoid compounds act as hypolipidemic agents and antioxidants; they inhibit oxidative stress and reduce blood cholesterol levels (Wirawan, 2018). The mechanism by which flavonoid compounds can reduce total cholesterol levels and increase the number of LDL receptors in the hepatic cell membrane and extrahepatic tissue is the inhibition of 3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A (HMG-CoA) reductase, which can decrease cholesterol synthesis. They also reduce acyl-CoA cholesterol acyltransferase (ACAT) enzyme activity and cholesterol absorption in the digestive tract (Mutia, 2018). Alkaloid metabolite compounds can reduce cholesterol levels by inhibiting the activity of the pancreatic lipase enzyme, thus increasing fat elimination through faeces. As a result, fat absorption by the liver is inhibited, and fat cannot be converted into cholesterol (Arta *et al.,* 2017).

Other secondary metabolite compounds having a cholesterol-lowering activity are tannins; they inhibit the action of the HMG-CoA reductase enzyme and bind bile acids to the small intestine and phytosterols in steroids by inhibiting the binding of sterol regulatory element-binding protein (SREBP) with sterol regulatory element (SRE), a protein that plays a role in transcription of LDL receptor genes. This inhibition resulted in decreased activity of the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) and decreased chylomicron formation (Naim *et al.*, 2017).

Conclusion

Based on the research results, the activity of 300 mg/kg bw n-hexane extract, 300 mg/kg bw ethyl acetate extract, and 300 mg/kg bw ethanol extract can reduce total cholesterol levels in hypercholesterolemic rats.

The 300 mg/kg bw ethyl acetate was the most effective in lowering cholesterol levels.

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RESEARCH ARTICLE

Microencapsulation of *Jeringau Rhizome* essential oils (*Acorus calamus* L.) using β-Cyclodextrin

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Abstract

Background: The way to improve the stability of *jeringau rhizome* essential oils is microencapsulation using β-cyclodextrin. **Aim**: To determine the efficiency of coating the jeringau rhizome essential oil with β-cyclodextrin and examine its thermostability. **Method:** The microencapsulation method used was freeze-drying with a ratio of 1:20 and 1:30. **Results:** The microcapsule efficiency at the ratio of 1:20 and 1:30 was 81.67% and 60.70%, respectively. The thermostability test results showed that the degradation constant of 1:20 microcapsule at 50°C and ambient temperature was 0.0054 and 0.0029, respectively, with a half-life of 128.33 hours and 238.97 hours. Meanwhile, the degradation constant of 1:30 microcapsule was 0.0182 and 0.0080, with a half-life of 38.07 hours and 86.63 hours. **Conclusion:** The highest efficiency is in the ratio of 1:20 with a percentage of 81.67%. In the thermostability test, the 1:20 microcapsule was better protected and had a longer half-life than the 1:30 microcapsule.

Introduction

Indonesia is a tropical country with the highest biodiversity in the world (Murdopo, 2014), most of which are medicinal plants. There are 30,000 medicinal plants grown in Indonesia, but only 1,200 have been used as raw materials for herbal medicines (Salim & Munadi, 2017). The jeringau plant (*Acorus calamus* L.) is one of the medicinal plants that can be used in herbal medicine. It has many properties, and its rhizome is rich in essential oils.

The *Jeringau rhizome* essential oil contains active ingredients β-asarone (82%), colamenole (5%), colamen (4%), colameone (1%), methyl eugenol (1%), and eugenol (1%) (Kementan, 2012). The essential oil of *Jeringau rhizome* produces pharmacological effects and is used as a raw material for the cosmetic, food, and pharmaceutical industries. Huge benefits of *Jeringau rhizome* essential oil indicate that the *Jeringau rhizome* essential oil has the potential to be developed as a raw material with high selling power. However, this oil has not been entirely utilised because it has shortcomings, such as easy evaporation at room temperature, easy oxidisation, insolubility in water, and instability to environmental influences of oxygen, sunlight, and heat (Capelezzo *et al*., 2018).

The method used to improve the stability of essential oils is microencapsulation. Microencapsulation is a technique in confining a material using a particular coating material to protect the core material. The objective of microencapsulation is to protect the core material from environmental influences, improve the physicochemical properties of the core material, and maintain the stability of the core material in storage. The polymer used as a coating was β-cyclodextrin. Microencapsulation in β-cyclodextrins is an effective method for protecting active compounds against oxidation, heat degradation, and evaporation (Mahmudah, 2015).

According to Martin and authors in 2010, the best microencapsulation method of essential oil is freeze-drying. Freeze-drying is a method for volatile materials

due to the lower operating temperature, slow drying rate, and vacuum use (Martin *et al*., 2010). Microencapsulation prevents fungal and bacterial contamination of the core material, which is protected by the capsule wall; it also preserves flavour more and increases the added value of spices (Champagne & Fustier, 2007).

This study focused on the microencapsulation of jeringau rhizome essential oil using β-cyclodextrin coating. It is based on the previous research conducted by Cakrawati and the authors in 2018, where limonene was microencapsulated by the freeze-drying method using the β-cyclodextrin coating. Limonene microencapsulation helps mask the bitterness of the bioactive compound and protects against damage caused by oxygen, heat, or light. The bioavailability of bioactive compounds and organoleptic characteristics in microencapsulated products by freeze-drying is better due to the minimum use of heating. The results revealed that microencapsulation with a ratio of 1:20 had an efficiency of 80.52% (Cakrawati *et al*., 2018). A study by Ponce and the authors in 2010 indicated that the inclusion of complex thymol-β-cyclodextrin and cinnamaldehyde-β-cyclodextrin remained stable up to 75% during long storage time (Ponce Cevallos *et al*., 2010). Microencapsulation of the *Jeringau rhizome* essential oil with the β-cyclodextrin coating is expected to increase the development and use of Indonesian spices as a higher quality raw material.

Method

Essential oil distillation

The distillation was performed by steam and water method. A total of 2,000 g of the *Jeringau rhizome* was placed into a kettle/distillation pan filled with water. The distillation process was completed for four hours at a temperature of ± 100°C. The essential oil obtained was stored in a tightly closed bottle and protected from light.

Microencapsulation of the Jeringau rhizome essential oil

The microencapsulation method used was freeze-drying. The microencapsulation of *jeringau rhizome* essential oil using β-cyclodextrins was prepared in a ratio of 1:20 and 1:30. The microencapsulation was prepared by mixing ten grams β-cyclodextrin with 100 mL 70% ethanol using a magnetic stirrer at a speed of 500 rpm and a temperature of 40°C for 15 minutes. The mixture was taken as much as 20g and 30g. In each comparison, one gram of the *Jeringau rhizome* essential oil was added and continued stirring for four hours. Then, it was dried using a freeze dryer at a temperature of -80°C for 24 hours. The obtained microcapsules were weighed and stored in a brown vial bottle (Cakrawati *et al*., 2018). The results of microencapsulation and β-cyclodextrin were characterized using the Scanning Electron Microscope Hitachi TM-3000.

Determination of the efficiency of Jeringau Rhizome essential oil

The first step applied to determine the efficiency of the *Jeringau rhizome* essential oil microencapsulation was to make a standard curve using a UV-Vis spectrophotometer at a wavelength of 245 nm. The second step was to analyse the content of total oil in the microcapsule, i.e., the oil contained in the coating and on the surface of the microcapsule (Jayanudin *et al*., 2017). A total of 20 mg of 1:20 and 1:30 microcapsules were dissolved in 70% ethanol. Moreover, the absorbance was measured using a UV Vis spectrophotometer at a wavelength of 245 (Masrukan & Santoso, 2019). The third step was to analyse the surface oil in microcapsules. Surface oil is the oil on the surface of the microcapsule; its amount affects the efficiency value of the microcapsule. The more the surface oil, the less the microcapsule efficiency (Jayanudin *et al*., 2017). A total of 20 mg microencapsulated essential oil of Jeringau rhizome was dissolved in 5 mL of n-hexane. Then, it was shaken and filtered using filter paper. The absorbance of microcapsule filtrate was measured using a UV-Vis spectrophotometer at a wavelength of 245 nm (Masrukan & Santoso, 2019). The efficiency was calculated from the difference between the total essential oil content and the microencapsulated content surface oil. The efficiency of the microencapsulate was calculated by the formula (Handayani *et al*., 2018):

$$\% \text{ Efficiency} = \frac{\textit{Total Oil-Surface Oil}}{\textit{Total Oil}} \times 100\%$$

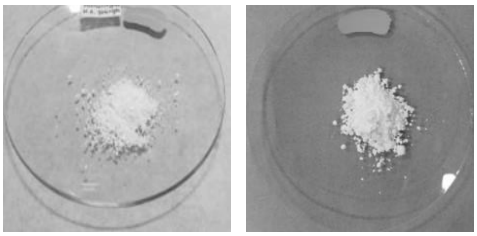
Microcapsule thermostability test

In each ratio, as much as 20 mg of microcapsule was put in 5 closed glass brown vial bottles. The thermostability test was administered at 50°C and ambient temperature. The absorbance measurements of essential oils were performed at 0, 24, 48, 72, and 96 hours with a concentration of 200 ppm, and the absorbance was measured at a wavelength of 254 nm (Wanda *et al*., 2017).

Result

Simplicia of the *Jeringau rhizome* was 2,000 g and yielded 12.5 mL of essential oil (6250%). The results of *Jeringau rhizome* essential oils microencapsulation using the freeze-drying method are presented in Figure 1. The

physical appearance of the 1:20 and 1:30 microcapsules is a white powder with a distinctive aromatic smell like the aroma of the essential oil. The 1:30 microcapsule powder has finer particles than the 1:20 microcapsule. The image of β-cyclodextrin SEM results (Figure 2) shows that the particles are irregular rectangular, with rough surface area and uneven size. It shows the morphological differences in shapes: β-cyclodextrin particles are much larger than the microcapsule particles.



(a) (b)

Figure 1: Microencapsulate 1:20 (a) and Microencapsulate 1:30 (b)

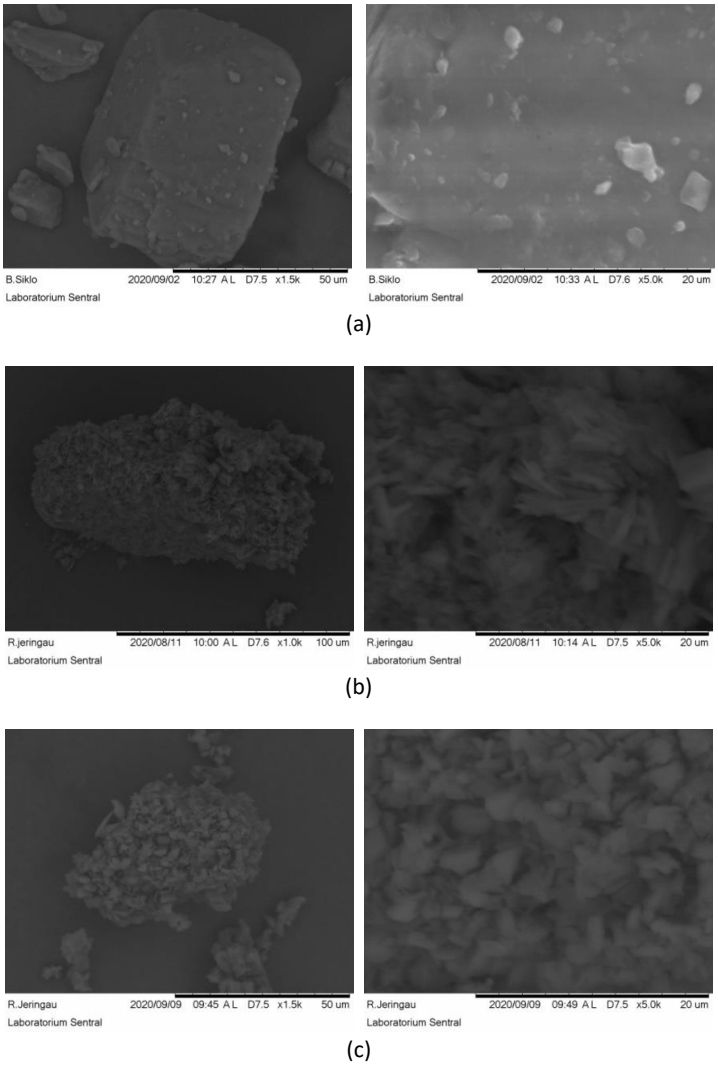


Figure 2: β-cyclodextrin (a), Microencapsulate 1:20 (b), Microencapsulate 1:30 (c)

The determination of *Jeringau rhizome* essential oil efficiency was performed to determine the percentage of essential oil in microcapsules. The result of the manufacture calibration curve showed a linear relationship between concentration and absorbance coefficient r^2=0.9993 and the linear regression

equation y = 0.0378x + 0.0204. Then, the absorbance value was checked at a wavelength of 245 nm to determine the concentration of total oil and surface oil for 1:20 and 1:30 microcapsules. The absorbance value obtained was plotted on the calibration curve until the concentration of total oil and surface oil in the microcapsule was obtained. The result of the microcapsule efficiency is shown in Table I.

Table I: Efficiency *Jeringau Rhizome* essential oils

Ratio	Total microencap-sulate (mg)	Total oil (mg)	Surface oil (mg)	Efficiency (%)
1:20	400.2	138.83	25.44	81.67
1:30	1371.6	99.53	39.12	60.70

The purpose of the thermostability test is to determine the degradation constant and half-life on the effect of 50°C and ambient temperature. This test was performed on microencapsulated and non-microencapsulated essential oils. The thermostability test results can be seen in Table II.

Table II: Results of degradation constants and half-life at 50°C and ambient temperature

Ratio	Degradation constants		$t_{½}$ (half-life)	
	50°C	Ambient	50°C	Ambient
Microencapsu-late 1:20	0.0054	0.0029	128.33 hours	238.97 hours
Microencapsu-late 1:30	0.0182	0.0080	38.08 hours	86.63 hours

Discussion

In this study, the essential oil yield of *Jeringau rhizome* was 0.63%, slightly different from previous findings, where Raina and the authors obtained 0.9% (Raina *et al*., 2003). The low yield of *Jeringau rhizome* essential oil in this study was due to several factors, including the place of growth, treatment or sample conditions, climate, light intensity, type of plant, and, most importantly, the distillation tool. If the device has a steam leak, the essential oil evaporates, thereby reducing its yield value. The *Jeringau rhizome* essential oil was brownish-yellow and had a distinctive odour according to the producing plants. In 2017, Rita and the authors stated that the physical appearance of the *Jeringau rhizome* essential oil in their research was also brownish-yellow and had a very sharp aroma (Rita *et al*., 2017).

The microencapsulation of *Jeringau rhizome* essential oils was performed using the freeze-drying method. This activity consists of two steps, i.e., the homogenisation process and freeze-drying. The homogenisation process was applied using two variations in the ratio of the coating material, i.e., β-cyclodextrin: 1:20 and 1:30. This coating could form inclusion complexes by introducing more hydrophobic compounds into the central cavity of the cyclodextrin molecule. In the process of forming the inclusion complex, there is an interaction between the functional groups of the essential oil compounds and the groups located in the cavities in cyclodextrin (Bestari, 2014).

During the homogenisation process, the stirring speed was 500 rpm, referring to the research of Pujiastuti and the authors in 2017, which explained that the stirring speed affects the particles' size. The greater the speed, the smaller the resulting particle size will be (Pujiastuti et al., 2017). However, Sirojuddin and the authors in 2015 argued that increasing the stirring speed increases the strength and frequency of collisions between particles, causing the breakdown of the coating material and the release of the core substance into the solvent (Sirojuddin et al., 2015). The second step was freeze-drying. It began with a freezing process and was continued with a drying process by sublimation. This mechanism is different from the usual drying process. Drying usually occurs through evaporation at high temperatures so that the dry part of the product forms a crust on the surface, which creates an obstacle for the diffusion of steam from the wet part to the environmental air. As a result of the normal drying process, the product has a dry crust outside and a wet centre. Freeze-drying is a sublimation mechanism at cold temperatures. Water vapour diffuses from the wet parts into the ambient air, forming a product that dries well and has finer particles (Hariyadi, 2013).

The results of the freeze-drying microencapsulation show that the 1:30 microcapsule powder has finer particles than the 1:20 microcapsules, consistent with the nature of β-cyclodextrins which can form hydrogen bonds with the surrounding –OH groups (Bestari, 2014). The more coating material was used, the more water molecules were bound, resulting in a powder with finer particles.

Li and the authors and Cakrawati and the authors stated that the particle morphology of the 1:20 and 1:30 microcapsules was irregular because of thermal expansion during drying and thermal stress (Cakrawati *et al*., 2018; Li *et al*., 2018). The morphological differences in the shape show that β-cyclodextrin particles are much larger than the microcapsule particles (Rakmai *et al*., 2018). The formation of

inclusion complexes between β-cyclodextrin and essential oils produces smaller and relatively finer microcapsule particles (Li *et al*., 2018). The change in particle morphology during the encapsulation process reveals the interaction between β-cyclodextrin and essential oils (Cakrawati *et al*., 2018).

The result of the microcapsule efficiency shows that the highest value was 81.67% at a ratio of 1:20, where the β-cyclodextrin coating in the *Jeringau rhizome* essential oil almost reached its optimum point. Asyhari explained that the greater the β-cyclodextrin concentration, the smaller the percentage of compounds absorbed, and the larger the microcapsule wall thickness, making water molecules easier to diffuse through the coating molecule and decreasing microcapsules efficiency (Asyhari, 2013). Table II shows that the higher the essential oil on the surface, the lower the efficiency obtained. The concentration of essential oils on the surface is helpful to see how much essential oil has been covered (Handayani *et al*., 2018). The purpose of microencapsulation is to protect the core material from evaporation and damage. It indicates that the uncovered essential oil will be more easily degraded, evaporated, and oxidised, which reduces its quality.

Thermostability test results demonstrate that the increase in temperature at 50°C resulted in a decrease in the absorbance of the *Jeringau rhizome* essential oil microencapsulate. The pattern of each temperature decreasing absorbance had a difference until the 96th hour, indicating that the increase in temperature causes a higher amount of degraded essential oil. While at ambient temperature, the absorbance value of encapsulated *Jeringau rhizome* essential oil could be read until the 72nd hour, it was not readable at a temperature of 50°C at 48 to 96 hours, indicating that terpenoid compounds in essential oils are not resistant to high-temperature heating. The storage of materials in the open air at high enough temperatures may cause physical and chemical changes to essential oils. One of the changes in the chemical properties of essential oil is through the oxidation process. The oxidation reaction in essential oils mainly occurs in the double bonds in terpenes. The thermolabile terpenoid compound will isomerize. The isomers can change the shape of the configuration or break the double bonds in terpenoid compounds, causing the absorption of light by the chromophore groups to decrease; hence, the absorbance value decreases further. The degradation constant is directly proportional to temperature. The increase in *k* value with increasing temperatures means a faster degradation rate of the essential oil (Sirojuddin *et al*., 2015). The storage temperature of 50°C is associated with the smallest degradation constant value compared to ambient temperature.

Based on Table II, the degradation constant of the essential oil 1:20 is smaller than the degradation constant of 1:30. It proves that the greater the percentage of essential oil absorption efficiency, the smaller the degradation constant value, and the longer the half-life of the essential oil. This result is in accordance with the findings of Mahmudah, showing that microencapsulation with β-cyclodextrins is an effective method to protect active compounds against heat degradation and evaporation (Mahmudah, 2015). The β-cyclodextrin coating used in the microencapsulation of essential oils forms a molecular inclusion complex between the essential oil and the β-cyclodextrin cavity. The inclusion complex can protect the essential oil during a stable storage period at low temperatures (Martin *et al*., 2010).

Further research is necessary for selecting other methods to refine the essential oil (to yield higher quantities), characterise it in microcapsules (mass spectrophotometry, differential scanning calorimetry, or x-ray diffraction), and test its stability (photostability test).

Conclusion

Some conclusions can be drawn from this study. The 1:20 and 1:30 microcapsule efficiency were 81.67% and 60.70%, respectively. The physical appearance of the 1:20 and 1:30 microcapsules is a white powder with a distinctive aromatic smell like the aroma of its essential oil. In the image of β-cyclodextrin SEM results, it is found that the particles are irregular rectangular, with rough surface area and uneven size. The thermostability test result of the *Jeringau rhizome* essential oil microcapsule for the 96th hours indicates that degradation constants of essential oil in the microcapsule 1:20 at a storage temperature of 50°C and ambient temperature were 0.0054 and 0.0029, and a half-life of 128.33 hours and 238.97 hours, respectively, while for the 1:30 microcapsule degradation constants were 0.0182 and 0.0080, and half-lives of 38.07 hours and 86.63 hours, respectively.

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IAI CONFERENCE

RESEARCH ARTICLE

IR spectroscopy coupled with chemometrics used as a simple and rapid method to determine the caffeine content of tea products

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Keywords
Caffeine content
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Abstract

Introduction: Tea is a popular beverage that comes from Camellia sinensis. Tea is generally categorised into four types: black tea, oolong tea, green tea, and white tea. These four types are distinguished based on the presence or absence of a fermentation process during their processing. One of the compounds that play a role in providing freshness to tea is caffeine. **Aims:** The purpose of this study was to determine the caffeine content in the tea samples that are on the market. **Methods:** This was done using the near-infrared (NIR)-chemometric method and using the TLC-Densitometry method as a comparison. Infrared (IR) spectroscopy combined with chemometrics has been developed as a simple method to analyse the caffeine content in a tea sample. IR spectra of tea samples were correlated with caffeine content using chemometrics. **Results:** In this study, the partial least squares (PLS) model of the NIR model that showed the best calibration with r-square was 0.958, and the root mean squared error of calibration (RMSEC) value was 0.070. The PLS calibration model of the NIR models was further used to predict the unknown caffeine content in commercial samples. The significance of the caffeine content that had been measured with NIR and TLC-Densitometry was evaluated using a two paired sample t-test. **Conclusion:** The caffeine content measured with both methods gave no significant difference.

Introduction

The leaves and shoots from tea plants are used to make drinks. There are four types of tea that are distinguished from each other based on their fermentation process. Black tea is fermented, while green tea is not. Oolong tea is made through a partial fermentation process (semi fermentation), and white tea is made by taking the youngest tea leaves, which are immediately evaporated and dried without using a fermentation process first (Hirthe *et al*., 2007).

Consumption of tea can provide general benefits to reduce fatigue, increase physical endurance and mental alertness, as well as by playing a role in the body's recovery process. People often consume tea for health and beauty purposes. Green tea is a type of tea that can be used for weight loss, and it also inhibits premature ageing (Sudaryat *et al*., 2015).

Caffeine is a methylated xanthine derivative alkaloid. The safe limit of caffeine consumption, according to BPOM (Indonesian Food and Drug Administration), is 150 mg/day, which is divided into at least three doses (BPOM, 2003). If caffeine is consumed in the right amount, the body will obtain the benefits. However, if caffeine is consumed excessively, this can trigger heart rate acceleration, feeling nervous or anxious and can even trigger insomnia. Thus, special attention to the levels of caffeine consumed must be taken by people whose bodies are lacking in caffeine tolerance, such as children, adolescents, and pregnant women (Belay *et al*., 2008).

Based on the side effects that arise if caffeine is consumed excessively, it is important to determine its content within tea products on the market. There are several methods that can be used to determine caffeine content, such as UV-Vis Spectrophotometry (Belay *et al*., 2008; Hasanah *et*

al., 2016; Navarra *et al*., 2017)), High-Performance Liquid Chromatography (HPLC) (Bae *et al*., 2015; Cunha *et al*., 2015; Jiang *et al*., 2015), and TLC-Densitometry (Ford *et al.*, 2005; Riswanto *et al.*, 2015; Torres *et al.*, 2015; Trianto *et al*., 2009). These methods have several disadvantages, namely requiring specific solvents and reagents. Researchers are currently being challenged to develop an alternative method, which is both fast and reliable. One method that has the potential to be used is near-infrared (NIR) spectroscopy as it has an easier sample preparation process, which does not involve any additional solvents or reagents (Roman *et al*., 2011; Shafirany *et al*., 2018).

The spectrum produced by NIR spectroscopy is quite complex, so it is difficult to interpret. To overcome this problem, a method known as chemometrics is needed. Chemometrics uses statistical and mathematical approaches to find the relationship between spectra data and chemical parameters of substances that are difficult to measure directly. This study aimed to create a calibration model using a NIR-chemometric technique in order to determine caffeine content from commercial tea products.

Methods

The materials used were commercial tea products (black tea, oolong tea, green tea, and white tea), caffeine standard, methanol, chloroform, and thin-layer chromatography (TLC) plates. The instruments used were a n^{o}60 sieve, a chamber, an ultrasonicator, micropipette capillaries, a CAMAG densitometer, a NIR device (Brimrose Luminar 3070), and The Unscrambler X 10.2 software.

Preparation of simulation sample

The tea sample simulation was divided into a training set and a test set collected from various shops, shopping centres, and traditional markets in Jember, East Java, Indonesia. Twenty tea product simulations were prepared as training sets consisting of three types of tea (black tea, green tea, and oolong tea). Four tea samples were prepared as test sets that consisted of black tea, oolong tea, and white tea, and three samples were used as the real samples. The samples used in the study can be seen in Table I.

Preparation of caffeine standard

50mg and 30mg of caffeine standard were weighed and then diluted in 25 ml of methanol. The standard caffeine mother solutions had concentrations of 2000 µg/ml and 1200 µg/ml, respectively. The 2000 µg/ml stock solution was diluted to concentrations of 500 µg/ml, 800 µg/ml, and 1000 µg/ml with methanol. Meanwhile, the stock solution (1200 µg/ml) was diluted to become 300 µg/ml and 600 µg/ml.

Table I: Code names used for the tea samples

No.	Code	Brand name of tea samples	No.	Code	Tea samples
(1)	PC	Poci	(15)	BTG	Cap Botol
(2)	SR	Sariwangi	(16)	SSG	Sosro
(3)	SS	Sosro	(17)	JWG	Jawa
(4)	DD	Dandang	(18)	2TG	2 Tang
(5)	KJ	Kepala Djenggot	(19)	XNO	Xiamen
(6)	GD	Gardoe	(20)	GLG	Galan 999
(7)	NG	Naga	(21)	TJ	Tong Tji
(8)	BD	Bandulan	(22)	ZTW	Zet White Tea
(9)	BT	Cap Botol	(23)	GG	Galan 999
(10)	PR	Prendjak	(24)	GP	Gopek
(11)	SM	Sarimurni	(25)	DD2	Dandang
(12)	SB	Cap Sepeda Balap	(26)	SS2	Sosro
(13)	CT	Tjatoet	(27)	XOO	Xiamen
(14)	IDG	Indomaret			

Determination of caffeine content by TLC-Densitometry method

The level of caffeine in the tea samples was determined using the comparison method (TLC Densitometry). The samples weighed 400 mg each, and this process was then replicated three times. The tea sample was diluted into 10 ml of methanol. The sample was inserted into an ultrasonicator and run for 10 minutes. The sample solution was then left to stand for 24 hours in the refrigerator in order to optimise caffeine for extraction and then filtered into the vial using filter paper.

All standard caffeine with sample solutions were put on the TLC plate using a capillary micropipette to measure out 2 µl. After the spot results were dried, the TLC plate was inserted into the chamber, which had been saturated by the chloroform: methanol (9.5:0.5). After the eluent had reached the limit, the plate was lifted and dried. The stain from eluation was scanned, and the purity of the spectra was produced using CAMAG densitometers. The caffeine content in the tea samples was calculated based on the scanning data.

Determination of calibration model

The NIR and Fourier-transform infrared (FTIR) spectra data of the training set samples were analysed quantitatively using partial least squares (PLS), principal component regression (PCR) and support vector regression (SVR) chemometrics through The Unscrambler X 10.2 software. Variable X (predictor) is an absorbance value of the infrared spectrum data that was correlated to variable Y (reference), which represented the caffeine value (% w/w) that was previously determined using the TLC-densitometry method as a comparison. The r-square value of 0.91 or greater and the smaller root-mean-square error (RMSE) value of the model indicated that the model chosen had the best predictive ability. The best calibration model was then tested by leave-one-out cross-validation (LOOCV) and 2-fold cross-validation (2-FCV) techniques. The model was validated by LOOCV by removing a set of sample data from the training set, and the rest of the data were used to create the new model. The 2-FCV was evaluated using the test set as an independent sample.

Application in a commercial sample

The spectra of commercial tea samples were determined by IR spectroscopy. The selected and validated model was then applied to the real sample. The caffeine content of the TLC-Densitometry method results was compared to the IR spectroscopy prediction of the caffeine sample. The results were then inputted into the SPSS Trial version 23.0 programme for further analysis.

Results and discussion

Samples were given identities using a code that was adjusted to each tea sample brand. The results of NIR and FTIR spectrum data are shown in Figure 1. The spectra data of all samples were used as predictors of the calibration model.

Determination of caffeine content by TLC-Densitometry

The wavelength used to scan the spots on the TLC plate using the densitometer was 277 nm. The caffeine content from each sample was expressed using % w/w. The level of % w/w of each tea sample was determined by converting the concentration value of the analyte that appeared in the form of a nanogram unit on the densitometer scan results to the initial weighing and dilution. The caffeine content obtained is displayed in Table II.

Table II: Results of R-square and RMSE values of the NIR model

Model	R-square Calibration	R-square Validation	RMSEC	RMSECV
PLS	0.9579	0.9579	0.0699	0.0663
PCR	0.6219	0.6078	0.2108	0.6054
SVR	0.5597	0.5243	0.2300	0.2175

Determination and validation of the calibration model

Three hundred twenty spectrum data values from NIR and 58 spectrum data values from FTIR were analysed quantitatively using chemometrics on the Unscrambler X 10.2 software. The spectra were analysed using the PLS, PCR and SVR techniques. The spectrum of the training set sample was correlated with the caffeine concentration for determining the calibration model. Spectra data as the variable y (response) and the value of caffeine content of the results of TLC-Densitometry expressed by % w/w as the variable x were used to predict the variable y (predictor). The best calibration model was the PLS model using NIR spectra because its R^2 and RMSEC values were 0.9579185 and 0.0698975, respectively (Table II). The calibration model showed a good R-value (above 0.91) (Lengkey *et al*., 2013) and a good RMSEC value. Thus, if the value gets smaller, it will also produce a better calibration model as it shows predictive results that are the same as or near the actual concentration (Trianto *et al.*, 2009). The slope parameter shows the average increase or decrease in the Y variable for an increase in one variable X (the slope size of a line). If the slope is positive, then the shape of the line will increase to the right, as shown in the results of this study. This value indicates that the PLS model of NIR has formed good regression linearity, which was the actual value, and the predictive value has a close correlation (Lengkey *et al.*, 2013).

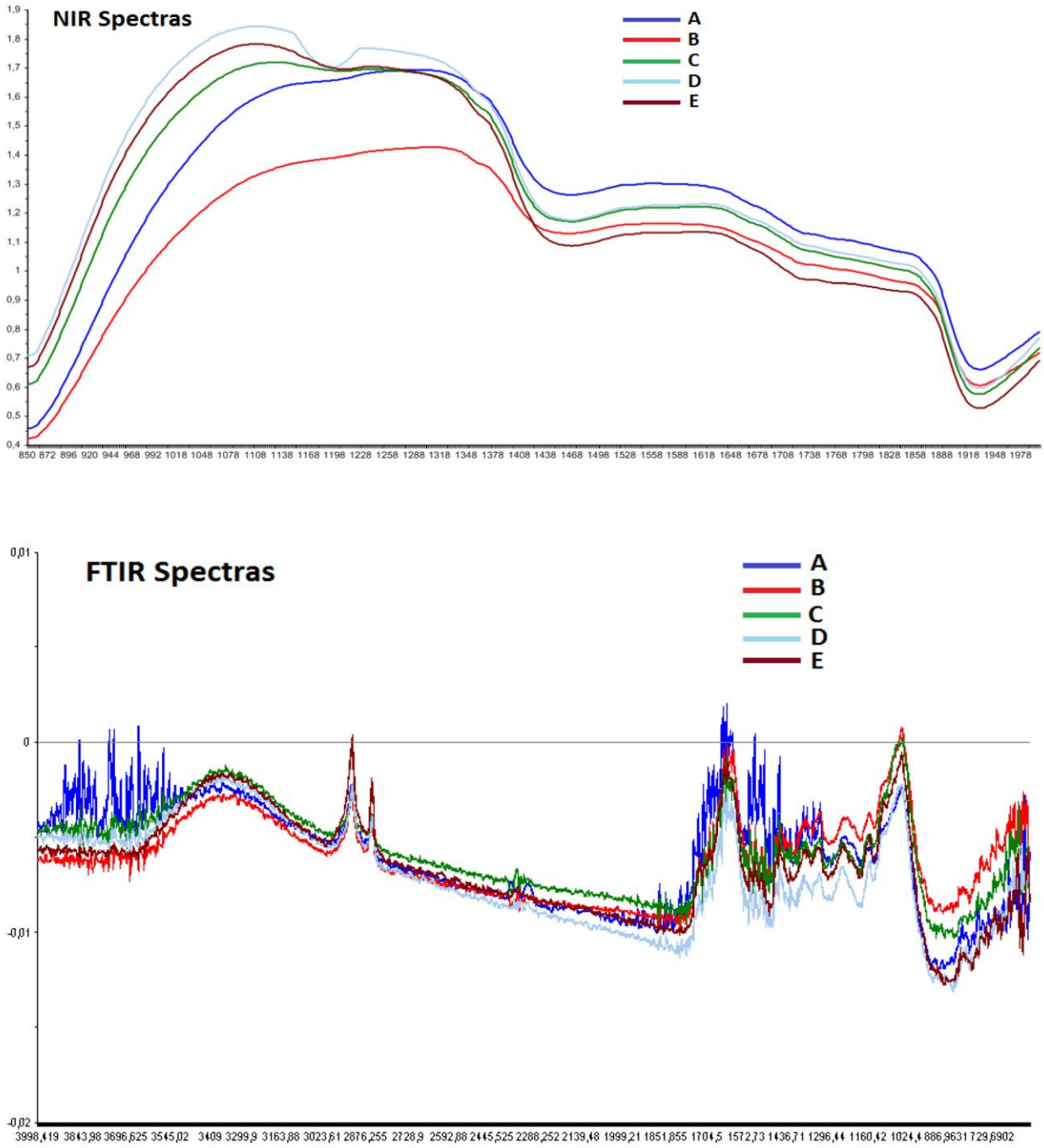

Figure 1: Spectra of NIR and FTIR, identity sample DD (A), KJ(B), PC (C), PR (D), XNO (E)

The calibration model using FTIR spectra had an R2 below 0.91, so it was the only calibration model using NIR spectra that was further validated. The results of the LOOCV PLS model of NIR showed that the R-square value was greater than 0.91, and the RMSE values were small (±0.06) (Table III). Meanwhile, the results of 2-FCV validation through the prediction of the test set sample showed that the R-square and RMSE values obtained were 0.9250847 and 0.0909219, respectively. Based on the results of LOOCV and 2-FCV validation, it can be concluded that the reliability or consistency of the prediction ability of the PLS calibration model NIR was well-formed, so it can be implemented in the actual sample.

Table III: Results of LOOCV PLS calibration model NIR

Number	Sample leaved	RMSE	R-Square
1	BD	0.0645	0.9486
2	JWG	0.0624	0.9478
3	XNO	0.0679	0.9432
4	SM	0.0660	0.9493

The PLS was validated as the best model calibration and was then applied to the real sample. The real samples that were obtained were scanned using a NIR spectrophotometer, and then the caffeine samples were determined using TLC-Densitometry. The caffeine content of TLC-Densitometry results was compared to the NIR scan caffeine levels by inputting both levels of data into the SPSS trial version 23.0 programme for further analysis. The mean value of % w/w caffeine resulting from the NIR spectroscopy method was compared to the results of the TLC-Densitometry method (Table IV).

Table IV: The comparison results from the NIR spectroscopy and the TLC-Densitometry

Sample code	Mean value %bw ± SD	
	NIR spectroscopy	TLC-Densitometry
DD2	1.28 ± 0.03	1.33 ± 0.01
SS2	1.52 ± 0.02	1.53 ± 0.03
XOO	1.33 ± 0.09	1.36 ± 0.03

Two paired t-test samples were used for the analysis in order to obtain information about whether there was a significant difference between the levels of real samples of NIR scan results with TLC-Densitometry. The results of the analysis of the two paired sample t-test showed that the significance value produced was 0.122 (> 0.05), so Ho was accepted. This meant there was no significant difference between the caffeine levels of real samples obtained from NIR and TLC-Densitometry.

Conclusion

The infrared spectroscopy method in the PLS calibration model of NIR spectroscopy-coupled with the chemometrics method can be used to determine caffeine content. The results of determining the caffeine content in both NIR Spectroscopy and TLC-Densitometry methods are the same or have no significant differences. The IR spectroscopy method is rapid, precise, accurate and eco-friendly.

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IAI CONFERENCE

RESEARCH ARTICLE

Acute toxicity test of 96% ethanol extract of *Syzygium myrtifolium* leaves in white mice (*Mus musculus*)

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Keywords
Acute toxicity test
Lethal dose 50% (LD_{50})
Syzygium myrtifolium leaves

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Abstract

Introduction: Acute toxicity effects appear within a short time following the oral administration of either a single dose or repeated doses of toxin within 24 hours. Acute toxicity testing involves the administration of a range of doses across several groups of experimental animals with one dose administered per group, followed by the observation of toxic effects and mortality. **Aims**: The purpose of this study was to determine the lethal dose 50 (LD_{50}) and acute toxicity of an ethanol extract of *Syzygium myrtifolium* leaves in white mice. **Methods:** Exposed groups consisted of a negative control group (carboxymethylcellulose sodium) and four treatment groups (500, 1000, 2000, and 4000 mg/kg body weight (bw)). Mortality was observed for 14 days following oral administration. **Results:** The results demonstrated an LD_{50} of 1995 mg/kg bw, categorised as moderately toxic. Observed toxic effects included white lesions in the lungs, blackened liver, organ swelling, and fluid accumulation in the abdominal cavity and thorax.

Introduction

Toxicity tests are classified as acute, subchronic, or chronic. They are designed to determine the safety of traditional medicines by detecting the toxic effects of a substance on a biological system and obtaining typical dose-response data. These data can provide insight into the potential human toxicity of the doses used in the test to determine a safe dosage in humans (Depkes RI, 2015).

Acute toxicity tests determine the lethal dose 50 (LD_{50}) of a compound. They are carried out by either single or repeated administration of the chemical compound tested, followed by observation of the test subjects for 24 hours.

One of the plants currently used as traditional medicine is the red shoot plant (*S. myrtifolium*). This plant contains flavonoids, phenols, and terpenoids, which have anti-tumour and anti-angiogenesis activities (Aisha *et al.*, 2013). According to Liniawati (2019), the *n*-hexane extract of red shoot leaves has triterpenoid compounds.

Toxicity testing of the 96% ethanol extract of red shoot leaves has been carried out *in vitro* with the Brine Shrimp Lethality Test (BSLT) method; the LC_{50} results were 171.59 ppm, indicating that the red shoot leaves were toxic (Haryati *et al.*, 2015). Research of *in vivo* toxicity is necessary to determine the safety of red shoot leaves. Therefore, an acute toxicity test was carried out *in vivo* by administering 96% ethanol extract of red shoots in experimental animals to determine the LD_{50} value and the maximum tolerated dose. These results may be an indication of potential toxicity in humans by extrapolation. This test generally uses two experimental animal models, with two administration routes and a single dose (Priyanto, 2010).

The LD_{50} value was determined using the Thompson and Weil formula. This method was chosen because it has a reasonably high level of confidence, is frequently used, and does not require a large number of

experimental animals. This method also uses a list of LD_{50} calculations to improve the accuracy of results. This study was conducted to determine the lethal dose 50 (LD_{50}) and the acute toxicity of a 96% ethanol extract of red leaves in white mice.

Methods

Materials

The tools used in this study included aluminium foil, glassware, funnels, filters, analytical scales, vacuum tray dryer, oral syringes, watch glasses, mouse cages, mortar, pestle, dropper, vial, spatula, ovens, plates, crucibles, animal scales, and stainless-steel surgical instruments. The materials used included *S. myrtifolium* leaves, distilled water, carboxymethylcellulose sodium (CMC-Na), 96% ethanol, white mice (*Mus musculus*), Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, gelatin, 10% sodium chloride, 1% and 3% ferric chloride, 80% methanol, 10% acetic acid, 2N hydrochloric acid, sulfuric acid, and anhydrous acetic acid.

Preparation of dried plants (Simplicia) and extract

A total of 3500 g of fresh *S. myrtifolium* leaves was cleaned of impurities, dried, and mashed. Then, 800 g of Simplicia powder was extracted using the maceration method. The Simplicia powder was placed into a dark bottle or macerator, then soaked with 96% ethanol in a 1:10 ratio of powder to solvent until the powder was completely immersed. The soaking process was carried out for 24 hours with occasional stirring. Macerate was then filtered, and the maceration process was repeated on the resulting pulp up to three times. The filtrate obtained was collected and evaporated using a vacuum tray dryer until a thick extract was obtained (Depkes RI, 2015). The viscous extract was stored in a tightly closed container and protected from light.

Preparation of experimental animals

This study utilized about 25 *Mus musculus* male mice, weighing between 25 and 40 g. First, the coefficient of variation (CV) of mouse body weight was calculated. Mice were then randomly divided into five treatment groups with five mice per group, then acclimatized for seven days in a cage. The mice were provided with standard food and drink (ad libitum) and individually weighed again after seven days. Furthermore, the final CV of mouse body weight was calculated to obtain relatively homogeneous mice (CV<15%).

Treatment of experimental animals

Mice were fasted for 24 hours prior to exposure. The experimental animals in each group were administered 0.4 mL of the following treatments:

1. Group I: 0.5% CMC-Na (the negative control)
2. Group II: 500 mg/kg body weight (bw) of *S. myrtifolium* leaves extract
3. Group III: 1000 mg/kg bw of *S. myrtifolium* leaves extract
4. Group IV: 2000 mg/kg bw of *S. myrtifolium* leaves extract
5. Group V: 4000 mg/kg bw of *S. myrtifolium* leaves extract

Mortality among experimental animals within 14 days post-exposure was determined. The deceased mice were then dissected to observe the effects of acute toxicity on their internal organs. The research protocol has been approved by the Ethical Committee for the Use of Experimental Animals, Faculty of Mathematics and Natural Sciences, Pakuan University (No.66/KEPHP-UNPAK/8-2019).

Calculation of LD_{50}

The LD50 value was calculated using the Thompson and Weil formula and the number of observed animal deaths in this study, as per the following equation:

$$\textbf{Log m = Log D + d (f + 1)}$$

Notes:

M: LD_{50} value
D: The lowest dose used
d: Log of the multiplier between dose concentrations
F: A value in the Weil table, determined by the specific mortality rate (r)

Results

Preparation of Simplicia and extract

The Simplicia has a characteristic greenish-red colour with a distinctive aroma and a slightly chewy taste. In the present study, 3500 g of fresh *S. myrtifolium* leaves provided a Simplicia powder yield of 27.14%. This result is consistent with previous research, which produced a 25% yield (Indriani *et al.*, 2020). Extraction of 800 g of *S. myrtifolium* leaves powder using 8 L ethanol 96% resulted in a thick extract of up to 339.58 g (42.45%).

Acclimatisation result

The CV of mouse weight was calculated, followed by acclimatization for seven days to allow for the adaptation of the mice to their new environment. Following acclimatisation, the CV of body weight was

recalculated. The CV value obtained is 10.176%, with average body weight as high as 30.56 g. CV value is considered homogeneous if it has a value <15% (Montgomery, 1991). The CV value provides a measure of the homogeneity of experimental animals based on body weight. It will also affect the quality of the data distribution: the smaller the CV values, the more homogeneous the data. After acclimatisation, mouse body weight increased, with no accompanying mortality, indicating that the mice had adapted to their environment.

Acute toxicity test results

This test uses the Thompson and Weil method. Qualitative data were obtained by observing whether mortality was present among the experimental animals in each treatment group, and quantitative data were obtained from the number of deaths in each group. Calculation of the LD_{50} value is based upon mortality among the experimental animals after 14 days of observation (Table I).

Table I: Number of deceased mice following 14 days of observation

Dose (mg/kg bw)	Number of mice	Number of deceased mice
500	5	2
1000	5	1
2000	5	2
4000	5	3

The results in Table I show two deaths in the 500 mg/kg bw group, one death in the 1000 mg/kg bw group, two deaths in the 2000 mg/kg bw group, and three deaths in the 4000 mg/kg bw group. The *r* values obtained from the number of deaths among mice given the ethanol extract of *S. myrtifolium* leaves were 2, 1, 2, and 3, respectively. Based on Thompson and Weil's LD_{50} calculation table, the *r* values of 2, 1, 2, and 3 have an *f* value of 1.0, which is then used to calculate the LD_{50}. The resulting LD_{50} value is 1,995 mg/kg bw, categorised as moderately toxic with a dosage ranging from 0.5 to 5 g/kg bw (Priyanto, 2010).

The administration of 96% ethanol extract of *S. myrtifolium* induced acute toxicity marked by mortality in experimental animals. Internal organ morphology was examined in the deceased mice (Figure 1), and toxic effects on the organs were demonstrated after administration of the thick extract. At doses of 500 mg/kg bw and 1000 mg/kg bw, white lesions on the lungs were observed along with a blackened liver, while doses of 2000 mg/kg bw and 4000 mg/kg bw caused almost all organs to swell and blacken alongside fluid accumulation in the abdomen and thorax.

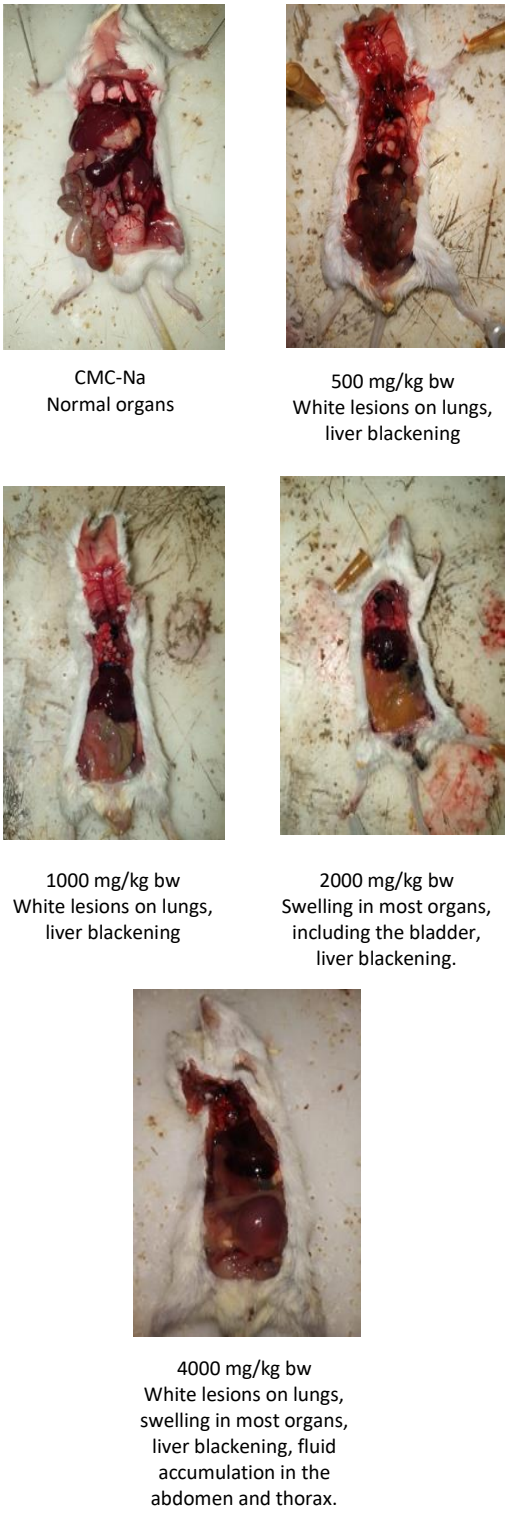

Figure 1: The effects of acute toxicity in internal organs of mice

Discussion

White lesions and swelling of the internal organs of mice are thought to be caused by necrotic damage to the tissue. Cells that experience necrosis can no longer revert to a healthy state and will proceed to die (Kumar *et al.*, 2007). The blackened liver observed in experimental mice is believed to be caused by disruption of the hepatocytes undergoing pycnosis. Damage to the lymph vessels causes fluid accumulation in the abdomen and thorax of mice. Lymph vessels play a role in the absorption of fluids and macromolecules from the tissues in the body and removing toxic substances after tissue damage occurs (Banks, 1993).

Toxic effects arise when absorbed toxins are transferred through the circulatory system to receptors. Oral administration of ethanol extract of red shoot leaves causes the active substances present in the red shoot leaves to be absorbed through the digestive tract, and these active substances then undergo distribution and metabolism (Katzung, 2002). Secondary metabolites of the red shoot leaves used in this study may be potentially responsible for the observed toxicity. These alkaloids and flavonoids act as stomach irritants and therefore cause digestive disturbance upon entering the body. According to Rita and the authors (2008), flavonoids are plant defence compounds that may be toxic. These compounds must first be isolated then tested to determine the toxicity of alkaloids. Saponin content in red shoot leaves is also believed to cause death and damage to the digestive organs of experimental animals. Saponins contain glycosides and can dissolve in water, thereby reducing activity within the digestive system. Decreased absorption will result in disruption of iron transport through mucosal cells. Red shoot leaves also contain tannin compounds known to cause toxic effects such as necrosis and bleeding. The higher the dose of extract administered, the greater the damage incurred to the organs of the experimental animal. In the present study, the 4000 mg/kg bw treatment group showed the most severe organ damage (Yunita, 2009).

Conclusion

The administration of ethanol extract of *S. myrtifolium* leaves showed a toxic effect and had an LD_{50} value of 1995 mg/kg bw and was categorised as moderately toxic. The toxic effect on the internal organs of mice is characterised by the presence of white lesions on the lungs, blackened liver, swelling of the organs, and fluid accumulation within the abdominal cavity and thorax.

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IAI CONFERENCE

REVIEW

The development of *Origanum vulgare L.* into nanoparticles in dosage forms

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Keywords
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Origanum vulgare L.

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Abstract

Introduction: *Origanum vulagre L.* is known for its abundant essential oil content with monoterpene and sesquiterpene derivatives. **Aims:** This research aims to gather comprehensive information about oregano and its potential to be developed into a nanotechnology drug delivery system. **Methods:** Literary studies were conducted using data obtained by searching through online literature sources. **Results:** Oregano is reported to contain active phytochemicals like esitronellol. In modern scientific literature, its extracts have been reported to have antidiabetic, analgesic, anti-inflammatory, anticancer and other potential properties. Further research needs to be done to ascertain the safety and therapeutic effect of this plant. The development of oregano's essential oil into nanoparticles in dosage forms can increase its solubility, stability, and pharmacological effects.

Introduction

Aromatic plants are considered to be very attractive plants because of their taste and pharmacological effects. Most of these aromatic species belong to the family *Lamiaceae*, whose distribution centre is located in the Mediterranean region. Within this family, oregano (*Origanum vulgare subsp. Vulgare*) is one of the most widely used species (De Falco *et al*., 2013). It is an important culinary herb in world trade and has been widely distributed in China and several Central Asian countries (Gong *et al*., 2014).

In the last few decades, oregano (*Origanum vulgare L.*) has become a valuable natural source that is used for maintaining human health. Oregano, in the form of dry herbs and essential oils, has been used in medicine for a long period of time and is one of the most widely used natural therapies (Gong *et al*., 2014).

Oregano is widely used as a traditional medicine to treat various diseases such as fever, jaundice, seizures, indigestion, and menstrual problems (Gong *et al*., 2014). Other benefits include preventing infection, treating stomach aches, as well as minor respiratory problems and itching of the skin caused by bacteria (Fardin & Sarina, 2017).

The essential oil compounds in *Origanum spp.* have been extensively investigated, and its differences between many other species have been reported. The chemical constituents that dominate *Origanum vulgare L.* are monoterpene and sesquiterpene derivatives (Gong *et al*., 2014). The essential oil from oregano has been shown to have antioxidant, antibacterial, antifungal, diaphoresis, carminative, antispasmodic, analgesic, and antimicrobial activity (De Falco *et al*., 2013).

Origanum species vary widely, both in their morphological properties and chemical composition. According to the latest taxonomy, there are six sub-species differentiated based on their morphological characteristics: *O. vulgare L. subsp. Glandulosum*

(Desfontaines) Ietswaart, O. vulgare L. subsp. hirtum (Link) Ietswaart, O. vulgare L. subsp. Gracile (Koch) Ietswaart, O. vulgare L. subsp. virens (Hoffmannsegg et Link) Ietswaart, O. vulgare L. subsp. vulgare L. and *O. vulgare L. subsp. viride (Boissier) Hayek* (Kosakowska & Czupa, 2018).

When oregano is in its natural form (Figure 1), it has woody stems with aromatic odours that reach 20-30 cm in height (Lukas *et al.*, 2010). The leaves have oval shapes with broader tips and have a length of 10-44 mm and a width of 5-25mm. These leaves are opposite each other on the stem (Force *et al*., 2000). The edges of the leaves are smooth, and the tips vary in shape ranging from pointed to rounded (Jerkovic *et al*., 2001). The flowers are clustered in whitish purple bunches with a length of 5-8 mm. Each flower has four stamens with four small seeds on the fruit (Kikuzaki & Nakatani, 1989). There have been many reports about the benefits of *Origanum vulgare L.* essential oil, but there are no studies that discuss it all. Therefore, the aim of this study was to collect complete and comprehensive information on the benefits of *Origanum vulgare L.* essential oil and its potential to be developed into nanoparticles in dosage forms.

Figure 1: *Origanum vulgare L.* (Wikipedia, 2020)

Method

In this paper, a literature study was conducted. The data obtained was in the form of qualitative data and quantitative data. The qualitative data obtained was described in narrative form, and conclusions were drawn from it.

The literature study in the review process of this article was carried out by utilizing online literature sources with the keywords: *Origanum vulgare L.*, oregano, chemical composition, pharmacological activity, and nanotechnology. The primary data sources used were PubMed, Research Gate, ScienceDirect, and Google Scholar. The inclusion criteria used were published national or international journals; written in Bahasa Indonesian or English. Meanwhile, the exclusion criteria were journals or references that were not valid and that had unclear sources.

Results and discussion

Chemical compounds of Origanum vulgare L.

The chemical compounds that dominate *Origanum vulgare L.* essential oil are monoterpene and sesquiterpene derivatives (Figure 2). According to research conducted by Gong and the authors, the main chemical compounds within oregano's essential oil are e-citronellol, citronellol acetate, thymol, trans-geraniol, eucalyptol, caryophyllene, eugenol methyl ether, caryophyllene oxide, carvacrol, and germacrene D (Gong *et al*., 2014).

In the research conducted by De falco and the authors (2013), the chemical compounds present in the oil were influenced by the planting system technique and growth. The oil from the *Origanum vulgare L.* plants that were grown in single rows was rich in sabinene, while those grown in double rows were richer in ocimenes. Meanwhile, oxygenated monoterpene derivatives were most abundant in terpinen-4-ol compounds (De Falco *et al*., 2013).

There was a higher content of total sesquiterpene hydrocarbons in fresh plants, with the most highly detected chemical components being β-caryophyllene, germacrene D and α-humulene. Among the oxygenated sesquiterpenes, spathulenol was the most abundant in all the oils and had a greater presence in the dry sample. As for phenolic compounds, carvacrol was the main constituent, and the concentration was higher in dry samples (De Falco *et al*., 2013).

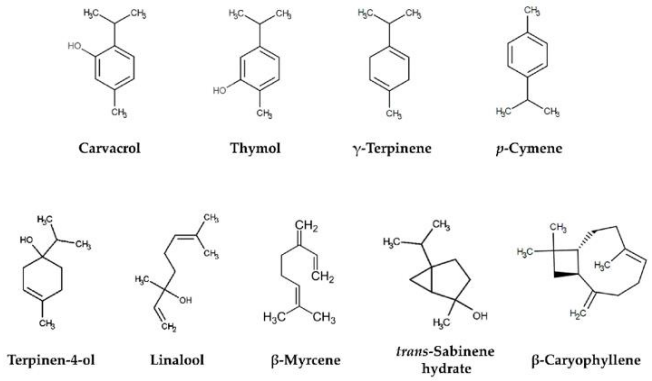

Figure 2: Chemical structure of the main compound from *Origanum vulgare L.* (Nayely Leyva-López *et al.*, 2017)

Pharmacological activity

Antidiabetic

In a study conducted by Lemhadri and the authors (2004), an *Origanum vulgare L.* anti-hyperglycemic activity test was carried out in diabetic rats, which were induced with STZ (65 mg/kg intravenous route). *Origanum vulgare L.* extract was administered at a dose of 20 mg/kg per oral, and its blood glucose-lowering activity was shown (Lemhadri *et al*., 2004). The antidiabetic effect of oregano extract works using a mechanism that inhibits the production of glucose in the liver or by inducing glucose utilization in peripheral tissues such as the muscle and adipose tissue (Eddouks *et al*., 2003).

Anxiolytic activity (antianxiety)

The anxiolytic effect of *Origanum vulgare L* was determined by carrying out the plus-maze test. In order to determine its sedative and muscle relaxant effect, an open field test and a horizontal wire test were performed. Mice were given oregano extract at doses of 50, 100, and 200 mg/kg intraperitoneally and diazepam as standard drugs. The test results proved that the oregano extract had an anxiolytic effect and the potential for minimal sedative effects (Mombeini *et al*., 2015).

Antioxidant activity

In a study conducted by Teixeira and the authors (2013), DPPH and FRAP (Ferric reducing antioxidant power) test methods were used to examine the essential oils and extracts from *Origanum vulgare L.* The results showed that both the essential oil and oregano extract had strong antioxidant activity. The strongest antioxidant effects can be affected by the phenolic components of the oregano essential oil and extract (Teixeira *et al*., 2013).

Antinociceptive activity (analgesic)

In a study conducted by Khaki and the authors (2013), the antinociceptive effect of *Origanum vulgare L.* extract was tested by providing a dosage range of 1, 3, and 6 µg/rat via intracerebroventricular injection. The test results showed that the oregano extract was able to produce antinociceptive and analgesic effects on the brain. Carvacrol is one of the ingredients of oregano which has an antinociceptive and analgesic effect (Khaki *et al*., 2013). This study is in accordance with previous studies where oregano extract was administered intraperitoneally and was able to produce analgesic effects when tested with a tail-flick test (Suleyman *et al*., 1996).

Antiurolytic activity

The antiurolytic activity of *Origanum vulgare L.* was proven by *in vitro* and *in vivo* tests. The *in vitro* test used rabbit urinary tracts, and the *in vivo* test used a mouse model. The *in vivo* test results showed that a dose of 10-30 mg/kg oregano extract was able to inhibit the occurrence of polyurea, crystalluria, oxaluria, increase in serum urea and creatinine, thus preventing the formation of crystals in the kidneys when compared to the control group. The inhibitory effect of oxalate crystal formation in the kidneys could also be influenced by the antioxidant activity of the oregano extract (Khan *et al*., 2011).

Anti-inflammatory activity

In a study conducted by Javadian and the authors (2016), the anti-inflammatory activity test of methanolic extract of *Origanum vulgare L.* was carried out using inflamed neural cells, namely glial and microglial cells. The test results proved that at a dose of 2.7 mg/mL of methanolic extract, oregano was able to provide the most optimal anti-inflammatory effect. In addition, at these doses, it also did have cytotoxic effects on normal cells. Thymol, as a component of oregano compounds, caused anti-inflammatory effects. The inflammatory barrier mechanism occurred by preventing the secretion of iNOS and TNF-α; these are the inflammatory mediators in the microglial cells (Javadian *et al*., 2016).

Antimelanogenesis

A study conducted by Liang and the authors (2010) found a new compound resulting from the isolation of the *Origanum vulgare L.* plant called origanoside (1), which is a new phenolic glucoside. Origanoside (1) was tested for its safety and for its antimelanogenesis effect *in vitro* and *in vivo*. The test results showed that at a concentration of 0-100 µg/mL, origanoside (1) was not toxic to normal cells. Meanwhile, the *in vitro* and *in vivo* test results showed that origanoside (1) was recorded to inhibit tyrosinase activity in cell culture at a dose of 10-20 µg/mL of 16.9–28.6% (melanoma cells B16). When applied to mouse skin, applying gel containing origanoside (1) for more than ten days showed whitening of skin cells (Liang *et al*., 2010).

Antifungal activity

In a study conducted by Adam and the authors (1998), the growth inhibition activity of human pathogens *Malassezia furfur*, *Trichophyton rubrum*, and *Trichosporon beigelii* were tested. Oregano extract with a dilution level of 1/50000 was able to produce better antifungal activity than extracts from *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa*. Within six hours of exposure, oregano extract was able to inhibit 95% of active metabolite fungal cells. The components that played the largest role in antifungal activity were carvacrol and thymol (Adam *et al*., 1998).

Memory boosting activity

Abbasnejad and the authors (2006) conducted a study on the effect of *Origanum vulgare L.* induced in male Wistar rats to reduce spatial learning errors. The male Wistar rats were trained in spatial learning tasks by regularly using the T-maze method. The mice that were given oregano extract injections at doses of 150, 300, and 450 mg/kg were able to show a lower error rate when compared to the normal group. This was observed based on the results of the electrophysiological recordings performed. This pharmacological effect can also be influenced by the effect of antioxidants and anti-acetylcholine esterase compounds such as ursolic acid (Abbasnejad *et al*., 2006).

Hepatoprotective activity

Origanum vulgare L. extract was tested on male Wistar rats induced by the CCL_4-induced hepatotoxicity to see its hepatoprotective ability. The doses of oregano leaf extract given ranged from 50, 100, 150 mg/kg p.o for 15 days. The hepatoprotective effect was best found at a dose of 150 mg/kg of oregano extract. This was indicated by lower aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels in liver blood serum compared to the normal and negative control groups. Researchers also found lower lipid peroxide levels in test mice (Sikander *et al*., 2013). In another study, it was shown that the flavonoids and triterpenoids contained by *Origanum vulgare L.* played an important role in protecting CCl4-induced liver oxidation (Röhrdanz *et al*., 2002).

Anti-cancer activity

In a study conducted by Kubatka and the authors (2017), the anti-tumour effect of *Origanum vulgare L.* was tested *in vitro* with MCF-7 cells and *in vivo* on female rats induced by n-nitroso-n-methyl urea. *Origanum vulgare L.* extract was administered orally at a dose of 3 and 30 g/kg. Based on the results from the *in vivo* tests, the oregano extract was able to reduce the tumour frequency by 55.5%, the tumour incidence by 44%, and the tumour volume by 44.5% when compared to the normal group. Meanwhile, the *in vitro* test results showed that the oregano extract was able to reduce the survival and proliferation rates of MCF-7 cells. In addition, the results of the oregano extract toxicity test showed that no severe side effects were caused, but special attention was needed as there was a slight increase in serum blood glucose levels (Kubatka *et al*., 2017).

Application of nanoparticle dosage form

Extracts from plants have natural properties, which means that they are more easily dissolved in oil bases. Meanwhile, active substances in the body will be more easily absorbed if they have a water-soluble form. Pharmaceutical dosage forms with nanotechnology applications such as self-nano emulsifying drug delivery systems (SNEDDS), gold nanoparticles and liposomes can overcome this problem. The small particle sizes with a range of 20-200 nm can increase the surface area and make it easier for active substances to penetrate the cell membrane towards the target cell in order to increase the effectiveness of the active substance (Chabib *et al*., 2020).

In a study conducted by Yi Zhao and the authors (2010), a SNEDDS was able to be formulated using the essential oil from *Curcuma zedoaria*. The resulting particle size had an average value of 68.3±1.6 nm and a zeta potential of -41.2±1.3 mV. Zedoary SNEDDS was stable in storage for up to 12 months, and the drug concentration in the blood was 2.5 times larger than conventional zedoary turmeric oil (Zhao *et al.*, 2010).

In another study conducted by Manconi M. and the authors (2018), liposome nanoparticles were able to be formed from thymic essential oil. The characterization results showed that the liposome had a particle size of 89 nm, a zeta potential of -60 mV, and had a small and unilamellar shape. Liposome carriers did not reduce the antioxidant and antimicrobial effects of *Thymus capitatus* essential oil (Manconi *et al*., 2018).

Based on both of these data, the essential oils from certain plants have been proven to able to be developed into nanoparticles in dosage forms, and these increase the solubility and stability, which results in pharmacological effects.

Conclusion

Origanum vulgare L. is an excellent medicinal plant that contains many bioactive phytochemicals. The main chemical compound contained in *Origanum vulgare L.* is an essential oil that is rich in monoterpene and sesquiterpene derivatives. In modern scientific literature, *Origanum vulgare L.* extract has been reported to have potential properties against various diseases as it has antidiabetic, anxiolytic (antianxiety), antioxidant, antinociceptive (analgesic), antiurolytic, anti-inflammatory, antimelanogenesis, antifungal, and anti-cancer activity, as well as the ability to improve memory. The essential oil from *Origanum vulgare L.* has the potential to be developed into nanoparticles in dosage forms.

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REVIEW

Therapeutic potential of *Cymbopogon schoenanthus* (L.) developed into nanoparticle technology

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Abstract

Introduction: One of plants that may have therapeutic potential is the Cymbopogon schoenanthus (L.) Spreng, also known as camel grass. **Aim:** This review aims to investigate and gather comprehensive information about camel grass plants and their potential to be developed into a nanotechnology drug delivery system. **Methods:** This review examined a variety of online literature. **Results:** It was found that camel grass contains essential oil such as piperitone. Piperitone is efficacious as an antioxidant, antimicrobial and anti-inflammatory, in addition to other properties . The development of camel grass essential oil into lipid-based nanotechnology preparations can improve its bioavailability, solubility, and stability, thereby improving its potential effectiveness.

Introduction

Cymbopogon schoenanthus (L.) Spreng is a natural herb that grows in tropical regions such as northern and western Africa, the Arabian desert, and Egypt. This plant is also known as *"Izkhir"* in Arabic, *"El bekhirai"* in Tunisian, and *"Tsabre"* in North African. *Cymbopogon schoenanthus (L.) Spreng* tends to grow in dry places such as the desert and is often used for camel feed, so it is also known as *"camel grass"* (Burkill & Dalziel, 1985; IUCN, 2005). In addition, this plant is also found at an altitude of 2000 meters or more in the province of Kiman, Iran (Amina et *al*., 2013).

Camel grass is shaped like a typical grass with a height of 60-90 cm (Figure 1). It grows in dry areas away from water sources. Camel grass has a distinctive taste and aroma due to its essential oils (Ben Othman et *al*., 2013). So, it is often used as a flavouring agent, fragrance, cosmetics, or perfume (Amina et *al*., 2013; Avoseh et *al*., 2015). The classification of camel grass plants is explained as follows (ITIS, 2020) :

Kingdom	: Plantae
Division	: Tracheophyta
Class	: Magnoliopsida
Order	: Poales
Family	: Poaceae
Genus	: Cymbopogon
Species	: *Cymbopogon schoenanthus (L.) Spreng.*

Figure 1: *Cymbopogon schoenanthus (L.) Spreng* (African Plants, 2020)

Cymbopogon schoenanthus (L.) Spreng contains essential oil, which is a typical source of monoterpenes such as piperitone and several other components known as intermedeol, δ-2-carene, and elemol (Pavlovic et *al*., 2017). Based on previous research, the chemical compounds of camel grass essential oil is efficacious as an antioxidant, antiacetylcholinesterase, antimicrobial, anti-inflammatory, spasmolytic, and contains other properties; Khadri et *al*., 2010; Pavlovic et *al*., 2017). However, currently, there are no articles that summarize specifically the content of chemical compounds, potential efficacy and benefits of camel grass. Therefore, this review article aims to collect comprehensive information about the efficacy of *Cymbopogon schoenanthus (L.) Spreng* and its potential development into pharmaceutical nanotechnology.

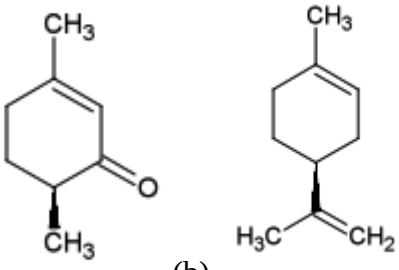
Methods

The authors utilised various online databases to carry out a literature review, such as PubMed, ScienceDirect, Research Gate, and Google Scholar. The search keywords used were *Cymbopogon schoenanthus (L.) Spreng*, camel grass, chemical compounds, therapeutic activity, and nanotechnology. The inclusion criteria were 1) published national and international articles; 2) English language; and 3) Indonesian language. The exclusion criteria were 1) unpublished articles; and 2) articles with invalid sources. The authors found and screened 35 references using the inclusion and exclusion criteria. The authors then used 27 references selected by the inclusion criteria to be reviewed in this article.

Results and discussion

Chemical compounds of camel grass

Camel grass has many chemical compounds. A study conducted by Pavlovic and the authors (2017) using camel grass from Sudan, showed in GC-MS testing that there were up to 45 different components in essential oils from camel grass. The flower and stem of the plant are used with camel grass flowers containing essential oils of 1.9-2.0% (v/w) compared to the stems of 0.2-0.6% (v/w). Piperitone makes up 47.7 to 71.5% of camel grass essential oil (Figure 2). Other compounds in camel grass include intermedeol (6.1-17.4%), δ-2-carene (4.5-10.0%) and elemol (5.2-9.0%) (Pavlovic et *al*., 2017). Studies on camel grass from Algeria also obtained similar results showing a compound composition of piperitone (63.3%), eudesmol (9.3%), δ-2-carene (4.9%), and elemol (6.9%) (Naima et *al*., 2016).



(a) (b)

Figure 2: Chemical structure of piperitone (a) and limonene (b) (Ganjewala, 2009)

Studies of camel grass harvested from Tunisia in June-July 2006 found that it contained 30 different components dominated by limonene (10.5-27.3%), β-phellandrene (8.2-16.3%), δ-terpinene (4.3-21.2%) and α-terpineol (6.8-11.7%) (Khadri et *al*., 2008). Hence, this shows that chemical compounds obtained from camel grass can be influenced by geographical conditions, seasons, and time of harvest (Khadri *et al*., 2008; Ganjewala, 2009; Naima et *al*., 2016; Pavlovic *et al*., 2017).

Pharmacological activity

Antioxidant

Antioxidant activity was tested by using the DPPH test method. This test illustrates the ability of the camel grass essential oil to capture free radicals from 2,2-diphenyl-1-picryl hydrazyl (DPPH) by releasing hydrogen atoms or electrons. The study conducted by Khadri and authors (2010) obtained IC_{50} values, ranging from 12.6±3.4 µg/mL to 26.4±6.8 µg/mL. Camel grass was harvested from 3 different types of places i.e. desert, mountain, and plant culture (Khadri et *al*., 2010). Due to its antioxidant ability, camel grass can be utilized in the food industry as a substitute for synthetic antioxidants (Khadri et *al*., 2008).

Anti-acetylcholinesterase

Acetylcholinesterase (AChE) is an enzyme that plays a role in inhibiting the transmission of nerve impulses in cholinergic synapses by hydrolyzing acetylcholine. When acetylcholine levels in the body are too low, it can result in neurological diseases such as Alzheimer, dementia, ataxia, and myasthenia gravis. The inhibitory activity of acetylcholinesterase can be found in many plants (Mukherjee *et al.,* 2007). *Cymbopogon schoenanthus (L.) Spreng* has been shown to have moderate anti-acetylcholinesterase activity with IC_{50} values between 0.23 mg/mL to 0.75 mg/mL (Khadri et *al*., 2010).

Antimicrobial

A study tested the antimicrobial activity of essential oils from camel grass against bacteria of the streptococci species, namely *S. mutans* and *S. sobrinus,* which commonly cause dental caries. The results obtained showed that its antimicrobial activity effectively inhibits the growth of *S. sobrinus* bacteria at concentrations of 4 mg/mL and 8 mg/mLwhilebacterial growth inhibition activity for *S. mutants* was seen at a concentration of 32 mg/mL (Khadri et *al*., 2010).

Cymbopogon schoenanthus (L.) Spreng also registered strong antimicrobial activity in studies on inhibitory zonesagainst *Enterococcus faecium* (21±1.4 mm), *Staphylococcus aureus* (19.5±0.7 mm), *Escherichia coli* (15±1.4 mm), *Salmonella typhimurium* (10.5±0.7 mm), *Streptococcus agalactiae* (12.75±0.3 mm), and *Candida albicans* (12±1.4 mm) (Naima *et al.*, 2016).

Anti-inflammatory

Anti-inflammatory activity can be found in camel grass essential oil, according to a study that conducted anti-inflammatory tests through carrageenan-tests on mice. Camel grass essential oils were administered intraperitoneally and found to significantly reduce paw oedema in doses of 50, 100, and 200 mg/kg, proving that camel grass essential oil has an effective anti-inflammatory effect in the acute inflammation phase (Talaei *et al.*, 2019). Other studies have shown that camel grass essential oil can inhibit the release of Nitric oxide (NO), which is an inflammatory mediator in RAW 264.7 cells, while IC_{50} test results show concentration values between 1.32±0.17 mg/mL to 1.38±0.04 mg/mL (Gomes *et al.*, 2017; Sukaboon *et al.*, 2019).

Spasmolytic activity

A study revealed that *Cymbopogon schoenanthus (L.) Spreng* essential oil has spasmolytic activity in the concentration range of 10-130 µg/mL. Testing was done by inducing acetylcholine in mice to observe effectiveness in inhibiting spontaneous contractions in rat ileum. The strongest spasmolytic activity was seen at a concentration of 130 µg/mL, which is equivalent to the maximum relaxant effect of atropine at a concentration of 6.4 µM (Pavlovic *et al.*, 2017). Another experiment method was also carried out to induce Potassium Chloride (KCl) (80 mM), which causes tonic contractions. Camel grass essential oil was found to inhibit the contraction effect of KCl by up to 19.67±20.26% at a concentration of 30 µg/mL (Pavlovic *et al.*, 2017).

Anti-stress activity

The anti-stress activity of camel grass essential oil was tested on SH-SY5Y human cells and heat-stressed HSP47-transformed cells. It was also tested in mice using the tail suspension test (TST) and forced swimming test (FST) methods. TST and FST tests are used as these are methods that most closely resemble the condition of depression in humans (Ben Othman *et al.*, 2013). Camel grass essential oil was found to protect these cells from stress-related disruption. In experiments using mice, camel grass essential oils at doses of 100 and 200 mg/kg administered orally were able to reduce the immobility time in the TST and FST tests indicating anti-stress activity.

Anthelmintic activity

Cymbopogon schoenanthus (L.) Spreng has a terpenoid compound that can inhibit the parasite growth phase. It has shown good anthelmintic activity as evidenced by four different tests: 1) Egg hatching assay (EHA) test; 2) larval development assay (LDA); 3) larval feeding inhibition assay (LFIA); and 4) larval escheatment assay (LEA) and in which the LC_{50} values obtained were 0.045 mg/mL, 0.063 mg/mL, 0.009 mg/mL, and 24.66 mg/mL, respectively. *Cymbopogon schoenanthus (L.) Spreng* is the best candidate for nematode control when compared to *Cymbopogon martinii* and *Mentha piperita* in the same study (Katiki *et al.*, 2011).

Insecticidal activity

The piperitone component in camel grass is known to inhibit the growth of neonatal eggs and larvae of *Callosobruchus maculatus,* whereas camel grass essential oil is also found to reduce the fertility of *C. maculatus* females by affecting the number of eggs produced (Ketoh *et al.*, 2006; Aous *et al.*, 2019).

Nanotechnology application

The use of nanotechnology in pharmaceutical preparations is able to cover the deficiencies of active substances, especially those derived from herbs or natural ingredients. Essential oils have an oil base, so they are more difficult to absorb in the body and have low bioavailability. Traditional processing of natural ingredients also usually requires a greater number of doses (Mukherjee *et al.*, 2015). Nanotechnology is divided into several dosage forms depending on the material used in the formulation and the method used for manufacturing. Generally, the nanotechnology form has a particle size between 20-200 nm, therefore it is often also referred to as nanoparticles. This preparation can improve bioavailability, solubility, and poor stability. This preparation also helps deliver active substances through cell membranes to reach action sites (Chabib *et al.*, 2020).

A study conducted by Ujilestari *et al.* was able to formulate essential oils from Cymbopogon citratus (lemongrass) in the form of spherical-shaped particles in self nano-emulsion drug delivery systems (SNEDDS). The characterization results showed the lemongrass SNEDDS preparation had a particle size of 20.7 nm and a polydispersity index (PI) of 0.378 (Tri Ujilestari *et al.*, 2018). Further studies also carried out an antibacterial test of *Cymbopogon citratus* in SNEDDS preparations and proved its effectiveness in inhibiting the growth of *Escherichia coli*, *Salmonella thyphimurium*, and *Lactobacillus acidophilus* bacteria (T Ujilestari *et al.*, 2019).

Another study conducted by Manju and the authors (2016), successfully formed a gold nanoparticle preparation from the essential oil of *Nigella sativa*. Its particle size range was 15.6-28.4 nm in a spherical, triangle, and hexagonal shapes. *Nigella sativa* SNEDDS was able to inhibit the growth of *Staphylococcus aureus* (16 mm) and *Vibrio harveyii* (5mm) at a concentration of 10 µg/mL. In the dosage range of 20-80 µg/mL, it was also shown to inhibit the biofilms of *S. aureus* and *V. harveyii* formed. In anti-cancer testing on A549 lung cancer cells, gold nanoparticles were able to inhibit the growth of cancer cells with IC_{50} values of 5-50 µg/mL (Manju *et al.*, 2016).

The results of the two studies above suggest that it is possible to develop camel grass essential oils into nanotechnology drug delivery systems. It is assumed that camel grass has the same essential oil profile as other plants, which can be developed into the nanoparticle dosage form.

Conclusion

Cymbopogon schoenanthus (L.) Spreng or camel grass is one of the medicinal plants mentioned in the Al-Quran and Hadith, which contains essential oils and substances like piperitone, intermedeol, δ-2-carene, and elemol. It is useful pharmacologically, containing antioxidant, anti-acetylcholinesterase, antimicrobial, anti-inflammatory, spasmolytic, anti-stress, anthelmintic, and insecticidal properties. Essential oils from *Cymbopogon schoenanthus (L.) Spreng* also has the potential to be developed into a nanotechnology drug delivery system.

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RESEARCH ARTICLE

Effectiveness of combination of Moringa Leaf Extract (*Moringa oleifera Lamk*.) and Papaya Seed Extract (*Carica papaya L.*) in reducing blood sugar levels of diabetic rats

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Abstract

Introduction: Moringa leaf extract and papaya seed extract contain flavonoids that can lower blood sugar levels. **Objectives:** The purpose of this study was to determine the antidiabetic effectiveness of moringa leaf extract and papaya seed extract and the effective dose of moringa leaf extract and papaya seed extract in reducing blood sugar levels. **Methods:** This study used 24 diabetic rats. Diabetes was induced by giving rats glucose 10% for four days. Measurement of blood sugar levels was carried out on the 0th, 5th, 8th, and 15th days. The pre-test (T1) and post-test (T2 and T3) blood sugar levels were measured, as well as the percentage of reduction at T2 and T3. **Results:** The result was that the treatment group with an extract combination of 700:500 showed decreased blood sugar levels on day 15 (57.24%), indicating antidiabetic effectiveness of the extract combination.

Introduction

Diabetes mellitus (DM) is a chronic progressive disease characterized by increased blood glucose levels (hyperglycemia) due to reduced insulin secretion and/or activity caused by insulin resistance. This disease increases the risk of death and decreases the quality of life due to various serious complications. The risk factors for diabetes mellitus are very diverse, and currently, the most common type of the disease is Diabetes Mellitus Type 2 (T2DM) (Yasin *et al.,* 2016). According to the International Diabetes Federation (IDF), the latest estimate of people living with diabetes in 2013 was 382 million, expected to increase to 592 million people in 2035.

The use of plants has been widely used in natural medicine to reduce blood glucose levels, particularly Moringa leaves (*Moringa oleifera Lamk.*) and Papaya seeds (*Carica papaya L.*). Flavonoids are the ingredients of Moringa leaves that have an antihyperglycemic effect. They stimulate pancreatic β cells and increase insulin secretion (Ambarwati *et al.*, 2014). Moreover, the papaya seed extract significantly affects the expression of Glucose Transporter 4 (GLUT4). GLUT4 is a protein that facilitates glucose transport, responsive to insulin in muscle and adipose tissue in both humans and rodents. A study showed that the most effective dose of Moringa leaf extract was 300 mg/Kg body weight (bw), and that of papaya seed extract was 500 mg/Kg; thus, in this study, doses of 300 and 500 mg/Kg bw were used (Wulansari *et al.*, 2017).

Based on the urgency of research, a single extract from each of Moringa leaf and Papaya seed has been used. So this research explored how effective the combination of Moringa leaf extract and papaya seed extract was in decreasing blood sugar levels.

Material and method

This research is experimental and compares blood glucose levels in male rats before and after the experiment. The method to extract the chemical contents in Moringa leaves and papaya seeds was maceration using 96% ethanol as a solvent. The antidiabetic effectiveness test was carried out by measuring blood glucose levels in white male rats that had previously been induced with glucose 10%. The tested animals were divided into six treatment groups: negative control, a positive control, glibenclamide 5 mg, and single extract or combined extract treatment groups.

Preparation of the Moringa Leaf Extract

Moringa leaf samples (*M. oleifera*) were sorted wet, washed, and then weighed (2 kg of wet weight). The simplicia of dried Moringa leaves was blended and sieved using a 40 Mesh sieve, then 300 grams were weighed and extracted by maceration, soaking the Simplicia of Moringa leaves with 96% ethanol solvent in a ratio of 1:5 for three days, while stirring. After three days, the Simplicia was filtered, and the residues were soaked again with a new filter liquid; this process was repeated three times. After the extraction was complete, the extract was filtered and concentrated with a rotary evaporator at 40° C. The overall thick extract obtained was put together and weighed to get the yield (Dewiyeti *et al.*, 2015; Jusnita *et al.*, 2019).

Preparation of the Papaya Seed Extract

Papaya seed samples (*C. Papaya* L) were sorted wet and washed under running water. Clean papaya seeds were wet weighed 2 kg. The weight of the Simplicia after drying was as much as 690 grams. The dried Papaya seeds were mashed using a blender to become powder, and then the sieving process was carried out using a 100 Mesh sieve. The sieved papaya seeds were then weighed as much as 425 grams and extracted by maceration, soaking the Simplicia of papaya seeds with 96% ethanol solvent in a ratio of 1:3 for three days while stirring, then the Simplicia was filtered, and the dregs were soaked again with a new filter liquid. After the extraction was complete, the extract was filtered and concentrated with a rotary evaporator at 50°C. The overall thick extract obtained was put together and weighed to get the yield (Ariani *et al.*, 2019).

Acclimatisation of test animals

Acclimatisation aimed to give the test animals time to try to adapt to their surroundings. It was carried out for 21 days, during which the rats were given a total of six grams of regular food per day in the form of pellets and drinking water *ad libitum*. Bottles of drinking water and the cage were cleaned every three days, and sawdust was changed every three days. The rats were weighed during adaptation or at day (-21) (Dewiyeti *et al.*, 2015; Theresia *et al.*, 2017).

Diabetic rats

The test animals used in this research were white male rats (*Rattus novergicus*). Before diabetes induction, the rats were satisfied for 12 hours, then weighed, and blood glucose levels were measured as initial blood glucose levels on day zero (T0). The rats were given glucose 10% glucose (w/v) solution for four days to trigger high blood sugar levels (diabetes mellitus). Before measuring blood sugar levels on the fifth day, rats fasted again for 12 hours, then weighed; their fasting blood sugar levels were measured after induction (T1). The rats were declared diabetic if the blood glucose levels after induction were 132 mg/dl (Ambarwati *et al.*, 2014).

Test animal treatment group

The 24 test rats used in this research were two months old with an average body weight of 150-250 grams. They were divided into six treatment groups with three repetitions each.

The treatment was carried out for ten days after diabetes from the fifth to the fifteenth day, during which weight and fasting blood glucose levels were measured twice, on the 9th day and the 16th day. Blood samples were taken from the test animal tails then dropped on the glucometer strip to get the final blood sugar level (T2).

Preparation of 10% glucose solution

A total of ten grams of anhydrous glucose were dissolved with 100 ml of distilled water in a 50 ml beaker. The solution was transferred into a 100 ml volumetric flask, the distilled water was added to the limit, then the solution was shaken until it became homogeneous.

Preparation of negative control

The negative control was prepared by preparing 1.5 grams of 1% Na-CMC in hot water then adding 150 ml of aqua dest.

Preparation of positive control

The positive control was prepared by dissolving 0.5 grams 1% Na-CMC in 5 ml hot water (10 x CMC Na)

while stirring, then adding 194.4 mg of glibenclamide powder and 50 ml of aqua dest.

Preparation of combination of Moringa Leaf Extract and Papaya Seed Extract

For the combination Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw, a stock solution of 350 mg/10 ml was prepared for the 350 mg/kg BW Moringa leaf extract and another one of 250 mg/10 ml for the 250 mg/kg bw Papaya seed extract.

For the combination Moringa Leaf Extract 700 mg/kg bw: Papaya Seed Extract 500 mg/kg bw, a stock solution of 700 mg/10 ml was prepared for the 700 mg/kg bw Moringa leaf extract and another one of 500 mg/10 ml for the 500 mg/kg bw Papaya seed extract.

Data analysis was carried out on SPSS version 24.0. One-way ANOVA was performed to calculate the average percentage of decreased blood sugar levels on the eighth (T2) and fifteenth (T3) days and compare these values to those of the 5th day (T1) in all groups of tested animals.

Results

A few drops of 10% NaOH solution were added to 0.5 grams of thick extracts until a colour change occurred to confirm the presence of flavonoid compounds in the Moringa Leaf Extract and Papaya Seed Extract (Rahayu *et al.*, 2015). The Moringa Leaf Extract turned yellowish, while the Papaya Seed Extract became yellow-orange. The ethanol-free test was carried out on both extracts to confirm they were completely free of ethanol by adding acetic acid solution and concentrated sulfuric acid solution; the mix was then heated until there was no smell of ester. Based on the results of the ethanol-free test, the Moringa Leaf Extract and Papaya Seed Extract did not contain ethanol.

The first measurement of blood sugar levels was carried out on day zero (T0). Then, diabetes was induced by giving white male rats glucose 10% for four days. After induction, blood sugar levels were measured on the fifth day (T1), the eighth day (T2), and the fifteenth day (T3) after treatment, using a glucometer test strip. The rat blood was taken from the tip of the tail after a 12-hour fasting period.

The average percentage of decrease in blood sugar levels at T2 (Day 8) was calculated by deducting blood sugar levels at T1 (pre-test) from T2 (post-test), dividing by blood sugar levels at T1, then multiplying by 100: ((T1-T2)/T1)*100. The average percentage of decrease in blood sugar levels at T3 (Day 15) was calculated using the same formula: ((T1-T3)/T1)*100.

This study showed that the best average percentage of decrease in blood sugar levels at T2 (Day 8) was 48.82%, seen with Group 3 (700 mg/kg bw of Moringa leaf extract), close to the percentage of the positive control treatment group (53.46%) (Table I). The dose of 700 mg/kg bw of Moringa leaf extract showed to be effective, with blood sugar levels close to normal values. The decrease in blood sugar levels was believed to be the result of the repair of pancreatic β cells by the flavonoids in Moringa leaves. These flavonoids can also function as an antioxidant that can reduce oxidative stress in cells, thus decreasing the pancreatic β cell damage process and accelerating the regeneration process of pancreatic β cells (Ambarwati *et al.*, 2014).

Table I: Percentage of average decrease in blood sugar levels on the eighth day (T2)

Treatment group*	Blood sugar level decreased (%)				Average (%) ± SD	*p*
	1	2	3	4		
Group1	4.44	9.42	6.66	4.62	6.29 ± 2.32	<0.0001
Group 2	52.94	54.44	54.28	52.17	53.46 ± 1.09	
Group 3	48.85	47.81	48.07	50.53	48.82 ± 1.23	
Group 4	31.51	34.86	37.54	37.9	35.45 ± 2.96	
Group 5	31.23	27.13	33.21	30.62	30.55 ± 2.53	
Group 6	48.22	46.56	48.85	45.74	47.34 ± 1.44	

*****Group 1: Negative control (Na-CMC 1%), Group 2: Positive control (Glibenclamide 5 mg), Group 3: Moringa Leaf Extract 700 mg/kg bw, Group 4: Papaya Seed Extract 500 mg/kg bw, Goup 5: Combination of Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw, Group 6: Combination of Moringa Leaf Extract 700 mg/kg bw: Papaya Seed Extract 500 mg/kg bw

The best average percentage of decrease in blood sugar levels at T3 (Day 15) found in this study was 57.24%, found in treatment group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract), close to the percentage of the positive control treatment group (61.49%) (Table II). The combination Moringa leaf extract and Papaya seed extract contain flavonoids that can be used as antioxidants; thus, papaya seeds can reduce blood sugar levels by decreasing the rate of glucose absorption in the periphery and by stimulating the ability of pancreatic β cells to produce insulation and repair pancreatic β cells (Wulansari *et al.*, 2017).

Table II: Percentage of average decrease in blood sugar levels on the fifteenth day (T3)

Treatment group*	Blood sugar level decreased (%)				Average (%) ± SD	*p*
	1	2	3	4		
Group1	10.37	14.13	16.14	14.59	13.81± 2.45	<0.0001
Group 2	63.66	60	60.71	61.59	61.49± 1.59	
Group 3	56.1	56.2	54.61	60.07	56.75± 2.33	
Group 4	47.47	46.74	47.59	51.98	48.45± 2.38	
Group 5	52.17	48.99	51.15	48.84	50.29± 1.64	
Group 6	56.42	56.47	58.24	57.81	57.24± 0.93	

*****Group 1: Negative control (Na-CMC 1%), Group 2: Positive control (Glibenclamide 5 mg), Group 3: Moringa Leaf Extract 700 mg/kg bw, Group 4: Papaya Seed Extract 500 mg/kg bw, Goup 5: Combination of Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw, Group 6: Combination of Moringa Leaf Extract 700 mg/kg bw: Papaya Seed Extract 500 mg/kg bw

Discussion

The identification test of flavonoid compound using NaOH 10% showed that the extract was positive for flavonoid, with the Moringa Leaf Extract turning yellowish and the Papaya Seed Extract yellow-orange. The ethanol-free test using the esterification method aimed to determine that the extract used did not contain ethanol. The results of the ethanol-free test showed that the Moringa Leaf Extract and Papaya Seed Extract did not contain ethanol, indicated by the absence of an ester odour of the Moringa Leaf Extract and Papaya Seed Extract.

Glibenclamide was used as a comparison because its short-term therapeutic effects were almost the same as the hypoglycemic effect of flavonoids present in Moringa Leaf Extract and Papaya Seed Extract, which increased insulin secretion from pancreatic β cells. Based on the decrease in blood sugar levels at T2 and T3, treatment Group 6 had the best outcomes of blood sugar levels, close to the positive control treatment group. For the results of the decreases in blood sugar levels at T2 and T3, one-way ANOVA and Tuckey test were performed to examine the difference in significance between groups.

The results of the one-way ANOVA statistical test on the percentage of average decrease in blood sugar levels showed significant differences between the treatment groups with a significance value of $p < 0.0001$ at T2 (Day 8) and T3 (Day 15). The Tuckey test at T2 showed no significant difference (0.907 > 0.05) between treatment Group 6 (700 mg/kg bw of Moringa leaf extract) and treatment Group 5 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract). Whereas in the Tuckey test at T3, there was no significant difference (0.063 > 0.05) between treatment Group 2 (positive control group) and treatment Group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract), no significant difference (0.999 > 0.05) between treatment Group 3 (Moringa Leaf Extract 700 mg/kg bw) and treatment group 6 (combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg BW of Papaya seed extract), and no significant difference (0.768 > 0.05) between treatment Group 4 (Papaya Seed Extract 500 mg/kg bw) and treatment Group 5 (combination Moringa Leaf Extract 350 mg/kg bw: Papaya Seed Extract 250 mg/kg bw).

Conclusion

This research found that the combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya

seed extract had the best antidiabetic effect, as indicated by the average value of decreased blood sugar levels on the fifteenth day, almost close to the mean value of the positive control group. Moreover, the single extract of Moringa leaves (700 mg/kg bw) showed better results than the combination of 700 mg/kg bw of Moringa leaf extract: 500 mg/kg bw of Papaya seed extract, as indicated by the values at eighth and fifteenth day.

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RESEARCH ARTICLE

Activity test of fruit and pomegranate seeds (*Punica granatum* l) as a hepatoprotector against white male Wistar rats

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Keywords
ALT
AST
Hepatoprotector
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Abstract

Introduction: White pomegranate (*P. granatum*) is a species of the Punicaceae which is thought as hepatoprotector based on its antioxidant secondary metabolite compoundst. **Aims:** The purpose of study was to determine the hepatoprotector activity of white pomegranate fruit and seeds ethanol extract on male white rats Wistar strain. **Methods:** This research is laboratory experimental which rats were grouped into 5 groups randomly. Normal, negative (given Carboxymethylcellulose sodium 1% orally), test dose 1,2,3 (given extract at a dose of 50 mg /200 g bodyweight of rat, 100 mg/200 g bodyweight of rat and 200 mg/200 g bodyweight of rat in Carboxymethylcellulose sodium 1% orally). Negative, test dose 1,2,3 induced with paracetamol 180 mg/200 g bodyweight of rat. **Results:** From the statistical analysis, it was found that a significant difference between the negative with test dose 1, 2, and 3. The best hepatoprotector activity was produced by the test dose 3 with the percentage reduction in AST levels 91.62% and ALT 90.20%.

Introduction

The liver is an organ that has the potential to experience damage due to various therapeutic chemicals and the environment because of its function in metabolic processes and the detoxification of chemicals that enter the body. The damage that occurs to the liver will disrupt metabolism in the body, causing homeostatic disorders (Akhlaghi & Bandy, 2009). Liver damage caused by acetaminophen, also known as paracetamol, occurs due to a metabolite of NAPQI (N-acetyl-p-benzoquinoneimine), which is highly reactive. Under normal circumstances, this reactive product quickly binds to the liver's glutathione levels, making it a non-toxic material. However, in a state of overdose, or continuous use that causes NAPQI production to continue to increase and is not proportional to glutathione levels, NAPQI binds to form macromolecules with liver cells resulting in liver cell necrosis, covalent binding levels that determine the level of binding to macromolecules in causing cell injury (Apriliani *et al.*, 2015). Hepatoprotectors are compounds or substances that can protect liver cells from toxic effects. Judging from the structure, compounds that are hepatoprotective include phenylpropanoid, coumarin, lignin, essential oils, terpenoids, saponins, flavonoids, organic acids, lipids, alkaloids and xanthines. Several natural antioxidant compounds such as flavonoids, terpenoids, and steroids have been pharmacologically studied to have hepatoprotective activity. The largest source of antioxidants in nature is the phenolic or polyphenol component, while the rest are nitrogen and carotenoid components (Atmani D *et al.*, 2015). Until now, there is no drug that specifically treats liver damage caused by drugs; therefore, it is necessary to conduct research to explore herbal medicines that can be used as hepatoprotectors. One of the herbal medicines being

explored is the fruit and seeds of the white pomegranate (*P. granatum*).

Pomegranate fruit and seeds are used to treat intestinal worms, and it is believed to have a hepatoprotective effect (Apriliani *et al.*, 2015). They also have antibacterial properties. Previous research has proven that the ethanol extract of red pomegranate at a dose of 500 mg/kg body weight (bw) of rats can inhibit liver damage because, at this concentration, the number of necrotic cells is low, there are no apoptotic cells, and the amount of fat degeneration is low (Isselbacher *et al.*, 2014). In this study, the researchers wanted to prove the activity of the white pomegranate fruit and seeds as hepatoprotectors.

Methods

Equipment

The equipment used included macerator, powder making tools, test tube, Erlenmeyer, evaporator, pipette, oral probe, micropipette, syringe 1 cc, scales, microcentrifugation and Effendrop tube.

Materials

The test materials used in this study included Simplicia of white pomegranate fruit and seeds, Diasys reagent kit consisting of AST and ALT reagents, 96% ethanol, 1% CMC, aqua dest, filter paper, paracetamol tablets, 2N HCl, acetone, powder boric acid, oxalic acid powder, ether, Mayer reagent, dragendroff reagent, NaOH, anisaldehyde-sulfuric acid or vanillin-sulfuric acid, Liebermann-Burchard, 1% $FeCl_3$, 1% gelatin.

Collection and determination of samples

The fruit and white pomegranate seeds were obtained from the Cilolohan area of the city of Tasikmalaya, and the determination was carried out in the Herbarium of the School of Life Sciences and Technology Bandung Institute of Technology.

Manufacture of Simplicia

The fruit and seeds of the white pomegranate (*P.granatum*) were collected first; then, wet sorting was carried out. The fruit and seeds of the white pomegranate (*P. granatum*) were cut into small pieces, then washed in running water until clean, then dried in an oven at 70°C. After drying, it was sorted again to separate it from other impurities. The fruit and pomegranate seeds then were dried in sunlight and covered with a black cloth with the dried fruit and seeds evenly pressed. The fruit and seeds of the dried white pomegranate (Punica granatum L) were then pulverised.

Extraction

Extraction was carried out by immersing 500 grams of white pomegranate fruit and seeds with 2L of 96% ethanol solvent. The solvent was changed three times.

Standardisaton of simplicia

Specific parameters used were organoleptic examination. Simplicia examination was conducted with four senses, including smell, taste, shape and colour of Simplicia.

Non-specific parameters: phytochemical screening

Phytochemical screening was conducted by examining the content of alkaloids, flavonoids, saponins, tannins and polyphenols, monoterpenes and sesquiterpenes, steroids and triterpenoids.

Preparation of test animals

A total of 25 male rats were acclimatized to the environment for seven days, and each cage was given husks and well maintained.

The classification of the test animals can be seen in Table I.

Table I: Classification of animals and treatments

Classification	Treatment
Negative	CMC 1% and Paracetamol 180 mg/200 g bw
Normal	Not treated
Test dose 1	White pomegranate fruit and seeds ethanol extract at a dose of 50 mg/200 g bw in CMC 1% and Paracetamol 180 mg/200 g bw
Test dose 2	White pomegranate fruit and seeds ethanol extract at a dose of 100 mg/200 g bw in CMC 1% and Paracetamol 180 mg/200 g bw
Test dose 2	White pomegranate fruit and seeds ethanol extract at a dose of 200 mg/200 g bw in CMC 1% and Paracetamol 180 mg/200 g bw

The rat blood serum was taken to measure the levels of aspartate transaminase (AST) and alanine aminotransferase (ALT). Blood was centrifuged at 2000 rpm for 15 minutes to obtain serum. A total of 100 µl of serum was mixed with 1000 µl of kit reagent. At room temperature, the mixture was measured on a photometer with a wavelength of 340 nm and a factor of 17,456 without incubation.

Statistical analysis

The data obtained were analysed for normality test, homogeneity test followed by one-way ANOVA test with a 95% confidence to identify significant differences between all test groups.

Results

Orgnoleptic examination

The organoleptic results showed a distinctive odour, sweet taste, and brown colour, which can be observed in both powders and extract forms (see Table II).

Table II: Results of the quality inspection of the Simplicia powder and crude extract

Examination	Simplicia powder	Simplicia extract
Color	Chocolate	Chocolate
Smell	Typical	Typical
Taste/shape	Sweet	Sweet

Phytochemical screening

The screening of phytochemical from the extract and powdered fruit and seeds of the white pomegranate (*P. granatum*) contained flavonoid, alkaloid, tannin, polyphenols, monoterpenes and sesquiterpenes (See Table III). This result coincides with earlier studies that state extract of fruit meat and seeds of white pomegranate contained flavonoids, alkaloids, and polyphenols (Apriliani, 2015).

The presence of flavonoids in the Simplicia of the fruit and seeds of the white pomegranate can be used as an antioxidant and hepatoprotector to protect the components of liver cells from free radicals produced by paracetamol. It will donate hydrogen atoms to free radicals so that they can inhibit and neutralise the occurrence of oxidation reactions. Flavonoids are active compounds included in antioxidant intermediates that act as hydrophilic and lipophilic antioxidants. The antioxidant mechanism of flavonoids is to capture ROS directly, prevent ROS regeneration and indirectly increase the antioxidant activity of cellular antioxidant enzymes (Akhlaghi & Bandy, 2019). Prevention of the formation of ROS by flavonoids is carried out in several ways, namely inhibiting the action of the enzymes xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, as well as chelating metals (Fe^{2+} and Cu^{2+}) so as to prevent redox reactions that can produce free radicals (Akhlaghi and Bandy 2019; Atmani *et al.* 2015).

Table III: Results of phytochemical screening of pomegranate seed powder and extract

Sample	Alkaloid	Flavonoids	Saponins	Tannins and Pholiphenol	Steroids and Triterpenoid	Quinone	Seskuiterpenoids and Monoterpenoid
Seed powder	-	+	-	+	-	+	+
Seed extract	-	+	-	+	-	+	+

Information : (+) = detected (-) = not detected

Measurement of AST and ALT levels

Transaminase is an amino acid catabolism process that involves the transfer of an amino group from one amino acid to another. In this transaminase reaction, the amino group of an amino acid is transferred to one of the three keto compounds, namely pyruvic acid, oxaloacetic acid and a-ketoglutarate, so that this keto compound is converted into amino acids while the original amino acids are converted into keto acids. Transaminase enzymes in serum do not have a function as an enzyme because, in the serum, there is no coenzyme and the right substrate. Transaminase enzymes present in serum are an indicator of tissue damage in certain diseases (Lotito SB, 2000).

The liver itself is able to secrete transaminase enzymes when the cells are damaged. High transaminase levels usually indicate liver abnormalities and necrosis. These enzymes enter the bloodstream. Transaminases are sensitive indicators of damage to liver cells.

Aspartate Transminase (AST)

Aspartate Transaminase (AST) is an enzyme found in heart muscle and liver, while moderate concentrations are found in skeletal muscle, kidney and pancreas. Low concentrations are also found in the blood; unless there is cellular injury, large amounts are released into the circulation. In cardiac infarction, AST will increase for a period of ten hours and reach its peak 24-48 hours after the infarction. AST will return to normal after 4-6 days if no additional infarction occurs. AST levels are usually compared with levels of other heart enzymes, such as CK (creatine kinase) and LDH (lactate dehydrogenase).

In liver disease, the levels will increase ten times more and remain so for a long time (Lotito SB, 2000).

AST serum is generally measured via photometry or spectrophotometry using a photometer or spectrophotometer or using a chemistry analyser. Reference values for AST in males are 0-50 U/L, while for females, it is 0-35 U/L (Lotio SB, 2000).

The AST level and the percentage decrease of AST level in the test groups can be seen in Table IV and Figure 1, respectively.

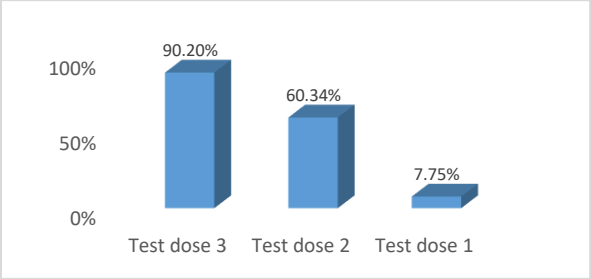

Figure 1: The percentage of decrease in AST level in test groups

Table IV: AST levels (mg/dL)

Rat number	Normal	Negative	Dose 1	Dose 2	Dose 3
1	22.22	86.21	66.24	28.51	6.27
2	27.51	73.11	72.22	24.22	8.13
3	32.11	74.13	54.21	36.18	7.29
4	31.17	82.18	63.31	29.04	6.32
5	23.42	83.42	70.11	31.36	5.43
Average± *SD*	**27.286±4.444**	**79.81±5.847**	**65.218±7.050**	**29.862±4.373**	**6.688±1.040**

Alanine Amino Transferase (ALT)

This enzyme catalyzes the transfer of amino groups like alanine and alpha-ketoglutarate acid. It is abundant in hepatocytes, and its concentration is relatively low in other tissues. Normal blood levels are 5-35 U/L, and ALT is more sensitive than AST (Lotito SB, 2000).

ALT and AST serum levels are elevated in almost all liver diseases. The highest levels are found in association with conditions causing extensive liver necrosis, such as severe viral liver hepatitis, toxin-induced liver injury, or prolonged circulatory collapse. A lower increase was found in mild acute hepatitis as well as in localized and diffuse chronic liver disease (Podolsky & Isselbacher, 2000). Levels drop suddenly in acute illness, indicating that the remaining source of the enzyme is depleted. If the damage by inflammation of the liver is only minor, ALT levels rise earlier and faster than AST levels.

In general, the ALT levels are higher than AST for acute liver parenchymal damage, while in the chronic process, it is the opposite (Lotito SB, 2000). Results of the ALT and AST measurements can be seen in tables 3 and 4.

The ALT level and the percentage decrease of ALT level in the test groups can be seen in Table V and Figure 2, respectively.

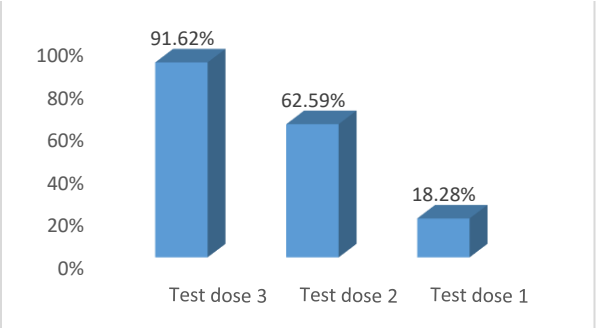

Figure 2: The percentage of decrease in ALT level in test groups

Table V: ALT levels (mg/dL)

Rat number	Normal	Negative	Dose 1	Dose 2	Dose 3
1	34.27	90.11	88.42	32.26	10.18
2	27.73	92.42	81.18	39.18	8.13
3	39.57	88.16	72.42	37.15	7.26
4	27.11	72.36	80.11	28.21	6.23
5	23.42	76.43	63.42	29.57	9.32
Average± *SD*	**30.42±6.436**	**83.896±8.92**	**77.4±9.524**	**33.274±4.751**	**8.224±1.272**

From Tables IV and V, it can be seen that paracetamol induction can increase AST and ALT levels, whereas, in the negative group, AST and ALT levels are greater when compared to the normal group.

Statistical analysis of AST and ALT levels

The test results were analyzed, including the normality test, homogeneity, ANOVA, and LSD test. Based on the Shapiro-Wilk test, it was found that the five treatment groups had AST and ALT data that were normally distributed ($p > 0.05$). In the homogeneity test using the Levene test, the results show that the data is homogeneous at AST ($p = 0.77$) and at ALT ($p = 0.11$). To see the difference in AST and ALT levels between treatment groups, a One Way Anova test was performed with the results of a significant difference ($p < 0.05$). Then the LSD test was carried out to see which group had a significant difference in Table VI.

From Table VI, it can be seen that there is a significant difference between the normal group and the negative group. The presence of a large dose of paracetamol in negative group adducts in liver proteins prior to hepatotoxicity is mainly dependent on P450-catalysed oxidative biotransformation to N-acetyl-p-benzoquinone imine (NAPQI) (Bessems, 2001). The negative group is significantly different from test doses 1, 2 and 3. This means that test doses 1, 2 and 3 are provided significant and effective hepatoprotection activity compared to the negative group. Test dose 3 was significantly different from normal, negative test dose 1 and test dose 2. This means that the dose of 3 (200mg / 200gram rat BW) is effective because the levels are close to normal levels. With a decreasing percentage of AST 91.62% and ALT 90.20%. In another study, AST and ALT levels were also lower than the negative control with pomegranate extract of 250 mg/kg BW and 500 mg/kg BW (Apriliani D, 2015).

Table VI: LSD test results levels of AST and ALT

Rat ID	Normal	Negative	Dose 1	Dose 2	Dose 3
Normal	-	SD	SD	NSD	SD
Negatie	NSD	-	SD	SD	SD
Test Dosage 1	NSD	NSD	-	SD	SD
Test Dosage 2	NSD	NSD	NSD	-	SD
Test Dosage 3	SD	SD	SD	SD	-

Description: SD : Significantly different ($p < 0.05$) ; NSD : Not significantly different ($p > 0.05$)

Conclusion

Based on the results of the study, the effect of giving ethanol extract of white pomegranate fruit and seeds (Punica granatum L) on Wistar male white rats induced by paracetamol can reduce AST and ALT levels. The best reduction was produced by the test dose group 3 (200mg / 200 g BW of rat), with a decreasing percentage of AST 91.62% and ALT 90.20%.In further testing, it is recommended to perform liver histopathology, and it is necessary to conduct research with another activity testing as a hepatoprotector.

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IAI CONFERENCE

RESEARCH ARTICLE

Comparing the quality of life of neuropathic patients treated with gabapentin and pregabalin at the neuropathic poly of the NTB provincial hospital in 2019

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Keywords
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Abstract

Introduction: Neuropathic pain is caused by the malfunctioning of the central nervous system or the peripheral nervous system. This pain is chronic and so it disrupts a patient's quality of life which can lead to them becoming frustrated. **Aim:** The purpose of this study was to compare the quality of life of neuropathic patients using either gabapentin or pregabalin at the neuropathic clinic of the Regional General Hospital of West Nusa Tenggara Province in 2019. **Methods:** This study used a cross-sectional study design. The sampling technique that was used was purposive sampling which was carried out by filling out the EQ-5D-3L and EQ-VAS questionnaires. **Results:** The results showed no significant difference between the quality of life of the patients using gabapentin and the patients using pregabalin as the EQ-5D-3L questionnaire had a value of $p = 0.683$. There was no significant difference between the quality of life between the gabapentin and pregabalin groups using the EQ-VAS questionnaire which had a value of $p = 1.000$.

Introduction

Pain is an inseparable part of human life. Apart from causing suffering, pain is a defence response of the body. According to the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience that is related to tissue damage. Neuropathic pain originates from the malfunctioning of the central nervous system or peripheral nerves, which can be caused by degenerative spinal diseases, diabetes, herpes zoster, AIDS, surgery, and strokes (Harden, 2005). The classification of neuropathic pain includes trigeminal neuralgia, neuropathic DM, post-stroke, and post herpes. Trigeminal neuralgia or nerve pain occurs in the trigeminal nerve area, and paroxysmal pain occurs in some parts of the face. Such pain is caused by eating, light touches such as washing your face, brushing your teeth, talking, starting and stopping suddenly, and activities that can be associated with anxiety (Reynolds, 2005). The European Federation of Neurological Societies (EFNS) recommends venlafaxine, duloxetine, amitriptyline, gabapentin, valproate, opioids (morphine sulfate, tramadol, oxycodone CR) and topicals as treatments for the disease (Argoff *et al.*, 2006). Neuropathic pain usually responds poorly to the analgesics standardly used by the World Health Organization (WHO), such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (Centre for Clinical Practice at NICE (UK), 2013).

Antidepressants and antiepileptic drugs are the first-line drugs used to treat neuropathic pain (Utami *et al*., 2013). Gabapentin has been shown to have analgesic effects when used as an antiepileptic drug. Gabapentin has been approved by the Food and Drug Administration (FDA) as an adjunct therapy for partial epilepsy and for the management of postherpetic neuralgia (Horizant Monograp*h*, 2012). Pregabalin (PGB) is a substance that is structurally analogous to gamma-aminobutyric acid (GABA), which is lipophilic and functionally unrelated to the neurotransmitter GABA. Based on clinical evidence, PGB is useful for treating epilepsy, psychiatric disorders, fibromyalgia and neuropathic pain (G *et al*., 2016). Gabapentin and pregabalin have antihyperalgesic and antinociceptive effects that reduce postoperative pain (Annisa, n.d.). The dose of pregabalin is 2-4 times smaller than that of gabapentin and is effectively used for neuropathic pain, usually at a dose of 150 mg (Annisa, n.d.). Neuropathic pain lasts a long time and makes patients frustrated, which decreases their quality of life. This condition highlights the need for drugs that help improve the quality of life of patients. This study aims to compare the quality of life of neuropathic patients that are treated with gabapentin and those that are treated with pregabalin at the Neuropathic Clinic of the NTB Provincial Hospital in 2019.

In the West Nusa Tenggara Province Hospital, pregabalin and gabapentin are included in the hospital drug formulary. A systematic review and meta-analysis of the use of pregabalin and gabapentin to manage neuropathic pain after a spinal cord injury resulted in proving that there was no significant difference in the effectiveness of either drug (Davari *et al*., 2020). However, no studies have been found that compare the quality of life of patients suffering from neuropathic pain that receive pregabalin or gabapentin therapy. Several previous studies that compared the quality of life of patients dealing with neuropathic pain due to strokes who were receiving gabapentin therapy with those receiving amitriptyline therapy also did not show a significant difference between both therapies. (Utami *et al*., 2013). This study aims to determine the quality of life of neuropathic patients using gabapentin compared to those using pregabalin. This study did not only deal with neuropathic stroke patients but patients suffering from neuropathic pain due to other causes such as diabetic neuropathy, post herpes, degenerative spinal disease, herpes zoster, and surgery at the Neuropathic Clinic of the West Nusa Tenggara Province Hospital in 2019.

Method

This research is an analytic observational study that used a cross-sectional study method. An observational analysis is used to determine the causal relationship between two observational variables. The forms of these relationships can be differences, relationships, or effects (Kukkar *et al*., 2013). This research aims to measure the quality of life of patients using the EQ-5D-3L and EQ-VAS questionnaires. The sample used in this study was composed of patients experiencing neuropathic pain at the Neurology Clinic of the Regional General Hospital of NTB Province from July to August 2019. The sample population was selected according to what researchers needed, based on inclusion and exclusion criteria.

The samples in this study were selected through a purposive sampling technique in order to sort out the subjects that met the inclusion and exclusion criteria. The subjects used between July and August were the total research sample. The independent variable in this study was which drug was used; this was either gabapentin or pregabalin. The dependent variable in this study was the patient's quality of life. The instruments used in this study were the EQ-5D-3L and EQ-VAS questionnaires that measured the quality of life of the patients. The Indonesian versions of these questionnaires were validated by the 2013 Indonesian EuroQol Group. The EQ-5D TM is a trademark of the EuroQol Group. This study has received approval from the Regional General Hospital of West Nusa Tenggara Province's ethics committee, and all research subjects have signed informed consent.

Results and discussion

A total of 20 patients met both the inclusion and exclusion criteria. Ten of these patients were treated with gabapentin therapy, and the other ten patients were treated with pregabalin therapy; both groups used the EQ-5D-3L and EQ-VAS questionnaires. The results were presented based on the characteristics of research subjects: sex, age and level of education. This can be seen in Table I.

Table I: Patient characteristics based on gender

Gender	N	%
Male	8	40
Female	12	60
Total	**20**	**100**

Table I indicates that there were 12 female subjects (60%) and eight male subjects (40%) dealing with neuropathic pain. Several studies have shown varying results depending on the sex distribution. Men and women have the same chance of suffering from neuropathic pain. The research data regarding the

patient's age was categorised into two levels: those over the age of 50 and those 50 or under. In this study, there were 16 people over the age of 50 and four people aged 50 or under. This distribution can be seen in Table II.

Table II: Patient characteristics based on age

Age	N	%
>50 years	16	80
≤50 years	4	20
Total	**20**	**100**

Based on Table II, 80% of the respondents were over the age of 50, and 20% of the respondents were 50 or under. The subjects were also classified into two groups based on their education level, with one group having been educated beyond senior high school and another group that had not. This distribution can be seen in Table III.

Table III: Patient characteristics based on education

Education	N	%
> Senior High School	4	20
≤ Senior High School	16	80

Table III shows that 20% of the respondents with diabetic neuropathic pain had been educated beyond senior high school, and 80% had not. This sample is therefore mainly predominated by patients with a level of education below high school. It is assumed that the level of education influences the way an individual responds to external forces. Someone who has received a higher level of education will respond more rationally than those with a middling or lower level of education (Asri, 2006). This fact is in accordance with the results of the study, which showed that when viewed from the latest education level, the number of respondents that had not been educated beyond a senior high school level was higher than the number of respondents who had been educated beyond a senior high school level.

This study used the EQ-5D questionnaire to determine the quality of life of the respondents. The EQ-5D questionnaire consisted of 2 types of questionnaires, one of which was the EQ-5D-3L questionnaire. The EQ-5D-3L had questions relating to five topics: the ability to walk/move, practice self-care, and participate in regular activities, as well as the level of pain/discomfort and anxiety/depression felt by the patient. It also classified the patients into three groups: those with no problems, those with several problems, and those with extreme problems.

Results from the EQ-5D-3L questionnaire showed that, on average, respondents who received gabapentin therapy perceived they had some problems, in which the most common problems were related to the ability to practice self-care and carry out regular activities, both with a score of 10%. For those who perceived having moderate problems, the highest level was in pain/discomfort with a result of 90%, the ability to walk/move as much as 50% and anxiety/depression as much as 50%, self-care 30% and 30% of the regular activities. For those who perceived no problems, the highest level was attained by the ability to walk/move, and regular activities with 60%, followed by the ability to walk/move and anxiety/depression, namely with 50%, and lastly was pain/discomfort with 10%. This shows that even though the patients feel pain, they can still take care of themselves.

On the other hand, respondents who received pregabalin had no correlation with the quality of life, which was indicated by their ability to walk/move, practice self-care, participate in regular activities, feel pain/discomfort, and anxiety/depression. For those who perceived having moderate problems, the most common problem was in pain/discomfort with 30%, followed by the ability to walk/move, do regular activities, feel anxiety/depression by 10%, and do self-care as much as 0%. Finally, the results showed that those without any problems had 100% ability to carry out self-care, followed by a score of 90% regarding the ability to walk/move and do regular activities, and the last level was those feeling 30% of pain/discomfort.

The resulting data was analysed using SPSS 16.0. The data generated a normal distribution in the normality test. Afterwards, it was analysed using an independent t-test in order to determine whether there was a difference or not in the mean (average) of the two independent data groups as a result of the use of gabapentin or pregabalin on neuropathic patients. The results of the research conducted using the EQ-5D-3L questionnaire can be seen in Table IV.

Table IV: Analysis of EQ-5D-3L questionnaire data

Group	n±mean rank	Normality	P
Gabapentin	15±16.20	0.046	0.683
Pregabalin	15±14.80		

Table IV shows that the answers from the EQ-5D-3L questionnaire after being analysed using the Mann-Whitney U test data were not normally distributed. There was no significant difference in the quality of life

of patients with neuropathic pain that were given gabapentin compare to those that were given pregabalin. This result was also reinforced by previous researches that indicated that gabapentin and pregabalin have antihyperalgesic and antinociceptive effects of reducing postoperative pain (Annisa, n.d.).

Neuropathic pain therapy generally aims to improve the quality of life of the patients by taking a holistic approach in the form of treatment of the pain triad. This therapy is carried out by a multidisciplinary team, and it involves the treatment of pain, sleep disorders and mood disorders. Common pharmacological therapies for neuropathic pain are analgesics, adjuvant analgesics, and pharmacological interventions (Snedecor *et al*., 2014). Several different therapies for neuropathic pain have been studied. Based on these clinical studies, the drugs that are recommended as first-line therapy for neuropathic pain include antidepressants (tricyclic antidepressant (TCA), serotonin-norepinephrine reuptake inhibitors (SSRI)), calcium channel α2-δ ligands (gabapentin and pregabalin), and topical lidocaine (Boyle *et al*., 2012).

Second-generation anticonvulsant drugs, such as gabapentin and pregabalin, are considered to have fairly good efficacy in dealing with neuropathic pain (Myr *et al*., 2015). Both of these drugs can be used as first-line therapy in patients with diabetic neuropathic pain who are contraindicated with the use of TCAs or who do not respond to the TCAs treatment (Backonja *et al*., 1998).

Gabapentin and pregabalin act by several mechanisms that can have pain-reducing effects in people with neuropathic pain. These two drugs are synthetic analogues of gamma-aminobutyric acid (GABA), which bind or act selectively on the α2δ subunit of the calcium channel (Myr *et al*., 2015). The effect that this has is the inhibition of the release of excitatory neurotransmitters, such as glutamate and noradrenaline. It also modulates the release of substance P (Imdad *et al*., 2013). Another mechanism that both of these drugs use is that they are antagonistic to the receptors N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5methyl-4-isoxazolepropionic acid (AMPA) (Myr *et al*., 2015). EQ-VAS is a 20cm vertical visual analogue scale used to assess the personal health of respondents, with the highest score being 100, which is labelled as 'the best health you can imagine' and with the lowest score being 0, which is labelled as 'the worst health you can imagine' (Hutapea *et al*., 2016). The resulting data is then inputted using SPSS 16.0. The resulting data were normally tested with the normality test and analysed after by the independent t-test in order to determine whether there was a difference in the mean (average) of the quality of life of the two groups of neuropathic patients that were independent or unrelated in terms of those treated with gabapentin and those with pregabalin. The results of the research conducted using the EQ-VAS questionnaire can be seen in Table V below:

Table V: Analysis of EQ-VAS questionnaire data

Group	Mean±SD	*p*
Gabapentin	60.00±13.33	1,000
Pregabalin	68.00±13.16	

Table V demonstrates that the average value of the quality of life of patients taking the drug gabapentin was found to be 60.00 ± 13.33 while the value of those who received pregabalin therapy was 68.00 ± 13.16. Systematically, there was no significant difference between the two groups, which was then strengthened by the result of a *p*-value of 1.000 ($p > 0.05$). This result proves that there was no significant difference in the quality of life between neuropathic patients treated with gabapentin and those treated with pregabalin based on imagined health.

The limitations of this study were the relatively minimal number of samples as well as a relatively short sampling duration, and the study subjects were patients with non-specific neuropathy pain. This study is the only one that has compared the quality of life of neuropathic pain patients that received gabapentin with those that have received pregabalin.

Conclusion

Based on the research conducted at the NTB Provincial Hospital, it is possible to conclude the following points:

There was no significant difference in the quality of life between the gabapentin group and the pregabalin group based on the EQ-5D-3L questionnaire (p =0.683, > 0.05).

There was no significant difference in the quality of life between the gabapentin group and the pregabalin group based on the EQ-VAS questionnaire (p = 1,000, > 0.05).

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IAI CONFERENCE

RESEARCH ARTICLE

Analysis of white pepper essential oil components using gas chromatography-mass spectroscopy

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Keywords
Essential oils
GC-MS
White pepper

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Abstract

Introduction: White pepper is an important commodity used to produce essential oils. Differences in the oil components are determined by which region the peppers used were sourced from. **Objectives:** The aim of this study was to analyse the components of the essential oils produced by white peppers from different regions and to examine the specificity of these peppers. **Methods:** This analysis was carried out using the chromatography gas – mass spectroscopy (GC-MS) method on essential oil samples made from peppers obtained from the Java, Sumatera, Kalimantan, and Sulawesi Islands. **Results:** The results showed that white pepper essential oil contains 22 components, and that *α-pinene, β–pinene, Δ-carene, sabinene, dl-limonene*, and *caryophyllene* were major compounds within the oils. Furthermore, it showed that *α-pinene, sabinene*, and *caryophyllene* compounds were most found in samples from the Kalimantan, Sumatra, and Sulawesi Islands respectively. Meanwhile, those from Java Island contained *β– pinene*, Δ-*carene*, and *caryophyllene* in similar quantities.

Introduction

Pepper crops form some of the most widely cultivated plantations in Indonesia. According to the 2017 statistics on the county's plantation commodities, this spice was also widely grown on the Java, Sumatra, Kalimantan, and Sulawesi Islands. Pepper plants produce black and white fruits, which can only be distinguished as a result of differences in post-harvest handling procedures (Zhao *et al*., 2005). White pepper can be used as a cooking spice, flavouring, meat preservative and traditional medicinal ingredient (Direktorat Jendral Perkebunan, 2019).

Essential oils are a mixture of low molecular weight molecules that are responsible for producing distinct aromas. The quality, freshness and uniqueness of the oils make them very valuable (Calo *et al*., 2015). Nonetheless, heat, humidity and oxygen greatly affect their stability, thus decreasing their quality. Essential oils are volatile and can be produced from the roots, leaves, stems, fruits, and seeds of various plants. They can also be obtained through distilling water vapour and from some animals, such as musk, sperm whales, and even microorganisms *(*Li *et al*., 2019).

The use of essential oils in the industry is quite extensive. In the food industry, they are widely used as flavouring ingredients or flavour enhancers (Calo *et al.*, 2015; Z. Liu *et al*., 2019). Meanwhile, in the pharmaceutical industry, they are widely used as antibacterial agents (Calo *et al*., 2015; Tamokou *et al*., 2017). These oils dissolve in fat underneath the skin because they are biodegradable and can be absorbed into blood vessels. In research carried out by Hu and the authors (2019) and Demirok Soncu and the authors (2018), it was shown that they act as very effective antifungal agents.

In the cosmetics industry, essential oils such as Patchouli oil, fragrant root and sandalwood are widely used as perfumes, while those made from pepper, ginger, cinnamon, cloves and coriander are widely used as cooking spices (Calo *et al*., 2015).

Essential oils are insoluble in water but are soluble in alcohol, ether, and fixed oils. Generally, they are

colourless and liquid at room temperature. The composition of these oils is very dependent on the species of plant that they were extracted from, their growth location, the time of harvest, and the extraction techniques used. Based on their chemical structure, the compounds within these oils can be classified as terpenes, straight-chain compounds without side chains, phenylpropanoids (benzene derivatives), and more (Moghaddam & Mehdizadeh, 2017).

Various factors can qualitatively and quantitatively affect the chemical composition of essential oils. One of which is the environment, as it can greatly affect the metabolic pathways of plants, which in turn leads to variations within their chemical components. In addition, plant age, rainfall, luminosity, soil composition, environmental pollution and microorganisms in the soil greatly affect the chemical components produced by plants (Fokom *et al*., 2019).

Essential oil analysis is generally carried out using gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) methods. GC is a chromatographic method that uses gas as a mobile phase (L. Liu *et al*., 2007).

Method

This study was carried out using experimental methods, starting with sample preparation, distilling the essential oils, determining the optimum conditions needed for GC-MS, and analysing the oils using GC-MS at optimum conditions.

Materials and tools

Materials: toluene, aqua, n-hexane and white pepper seeds samples from the Java, Kalimantan, Sumatera, and Sulawesi Islands. Tools: GC-MS Agilent Technologies 7890A, a steam distillation apparatus, and glassware.

Sample preparation

Raw white peppers were obtained from the Java, Kalimantan, Sumatera and Sulawesi Islands. These were purchased directly from the farmers on these islands. In order to further ensure the authenticity of the materials, a determination process was carried out in the Institute Technology of Bandung (ITB) Bioengineering Science School laboratory.

Essential oil distillation

The steam distillation method was used to distil these oils. This method resembled the boiling method; however, in this case, the water did not come into direct contact with the distilled materials.

Essential oils analysis with GC-MS

After determining the conditions needed for the GC-MS analysis, the essential oils were then analysed under the optimum conditions.

Result and discussion

Sample preparation

Based on the plant determination results, the sample proved to be white pepper.

The plant's taxonomy was as follows:

Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Piperales
Family: Piperaceae
Species: *Piper nigrum L*.
Synonym: *Muldera multinervis Miq*.

Distillation of the essential oils

The distillation process for the essential oils lasted 6 hours and was declared complete when the produced condensate no longer contained oil.

Analysis of essential oil components

The optimum conditions needed for the GC-MS oil analysis can be seen in Table I. Analysis of the essential oils using the GC-MS showed that the essential oils made from white peppers from the Java, Kalimantan, Sumatra and Sulawesi Islands contained almost the same components. Moreover, it showed that there were 22 component compounds within the oils. The essential oil components can be seen in Table II. White pepper chromatogram samples from the Java, Kalimantan, Sumatra and Sulawesi Islands can be seen in Figure 1.

Of the 22 compounds identified, the 6 with the largest compositions differentiated the essential oils based on which region they originated from. These six compounds were *alpha-pinene, beta-pinene, delta-3-carene, sabinene, dl-limonene*, and *caryophillene*. The white pepper from Java Island was more dominant in *beta-pinene, dl-limonene*, and *delta-3-carene* compared to those from the other islands. *Sabinene* was most commonly found in the essential oils from Sumatra Island, *alpha-pinene was most commonly found in the essentials oils from* Kalimantan Island, and *caryophyllene was most commonly found in the essentials oils from* on Sulawesi Island. This study showed that the differences in cultivation location affected the relative composition of the essential oils.

Table I: The optimum GC-MS conditions

Condition	Description
Gas chromatography	*Agilent Technologies* 7890A
The brand	HP-5MS, length 30m, diameter
Coulomb	0.25 mm
Carrier gas	Helium
Detector	Mass spectroscopy
Injection volume	1 mL
Injection technique	Split
Split ratio	25:1
Temperature programme:	
Injector temperature	250°C
Initial temperature	40°C
Temperature rate	10°C/minute
Final temperature	40°C
Intercept temperature	280°C
Detector temperature	250°C
Mass spectroscopy:	
Merck	*Agilent Technologies* 5975C
Mass range	40-550
Resolution	1188

Table II: Essential oil components present

Retention time (minutes)	Compound names
6.006	1-isopropyl-4-metthylbicyclo[3.1.0]hex-2-ene
6.130	Alfa pinene
6.401	Camphene
6.817	Sabinene
6.881	2-beta-pinen
7.079	Beta-Myrcene
7.338	I-Phellandrene
7.435	Delta.3 Carene
7.544	alfa-Humulene (CAS)
7.678	Benzene, 1-methyl-3-(1-methylethyl)-
7.744	dl-Limonene
8.242	y-Terpinene
8.731	Cyclohexene, 1-methyl-4-(methylethylidene)-
8.879	Linalool L
12.492	q-Elemen (CAS)
13.146	Copaene
13.388	Beta Elemenen
13.998	Caryophyllene
14.277	Guaia-1(5),11-diene
14.676	Alfa-Humulene
15.426	Beta-Seline (CAS)
15.630	Alfa-Selinene

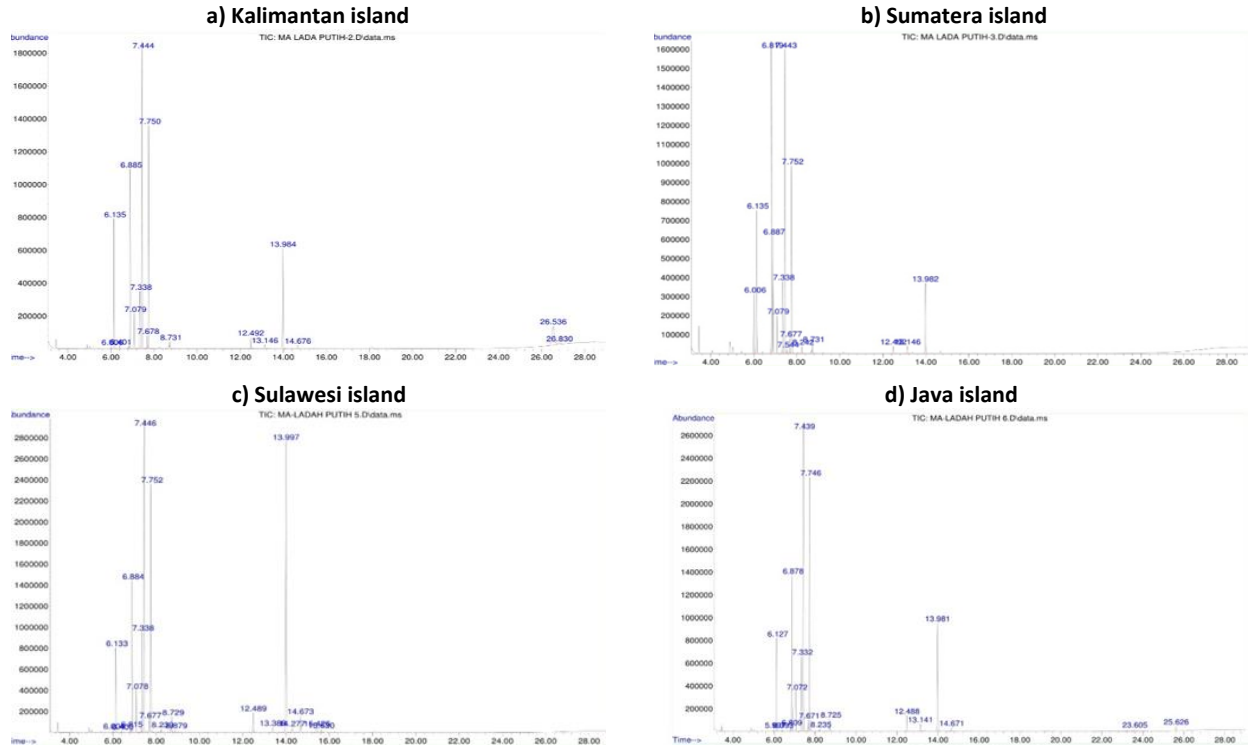

Figure 1: Chromatogram of white pepper's essential oils sample

The spectrum of the six main molecules can be seen in Figure 2. The identity of each molecule was determined based on the value of the spectrum match factor (MF) of the tested molecules against the data library. Match factor data can be seen in Table III. MF values that are close to 999 or greater than 700, indicate that the molecular identities are very compatible (Reichenbach *et al*., 2019). The data library used was the NIST11. The results were similar to those obtained by Liu in 2007. Liu, Song and Hu analysed the white pepper essential oil components using the GC-MS method without mentioning the location from which the pepper was obtained. They also showed that the main components of this pepper were *caryophillene, 3-carene, d-limonene, beta-pinene*, and *alpha-pinene*.

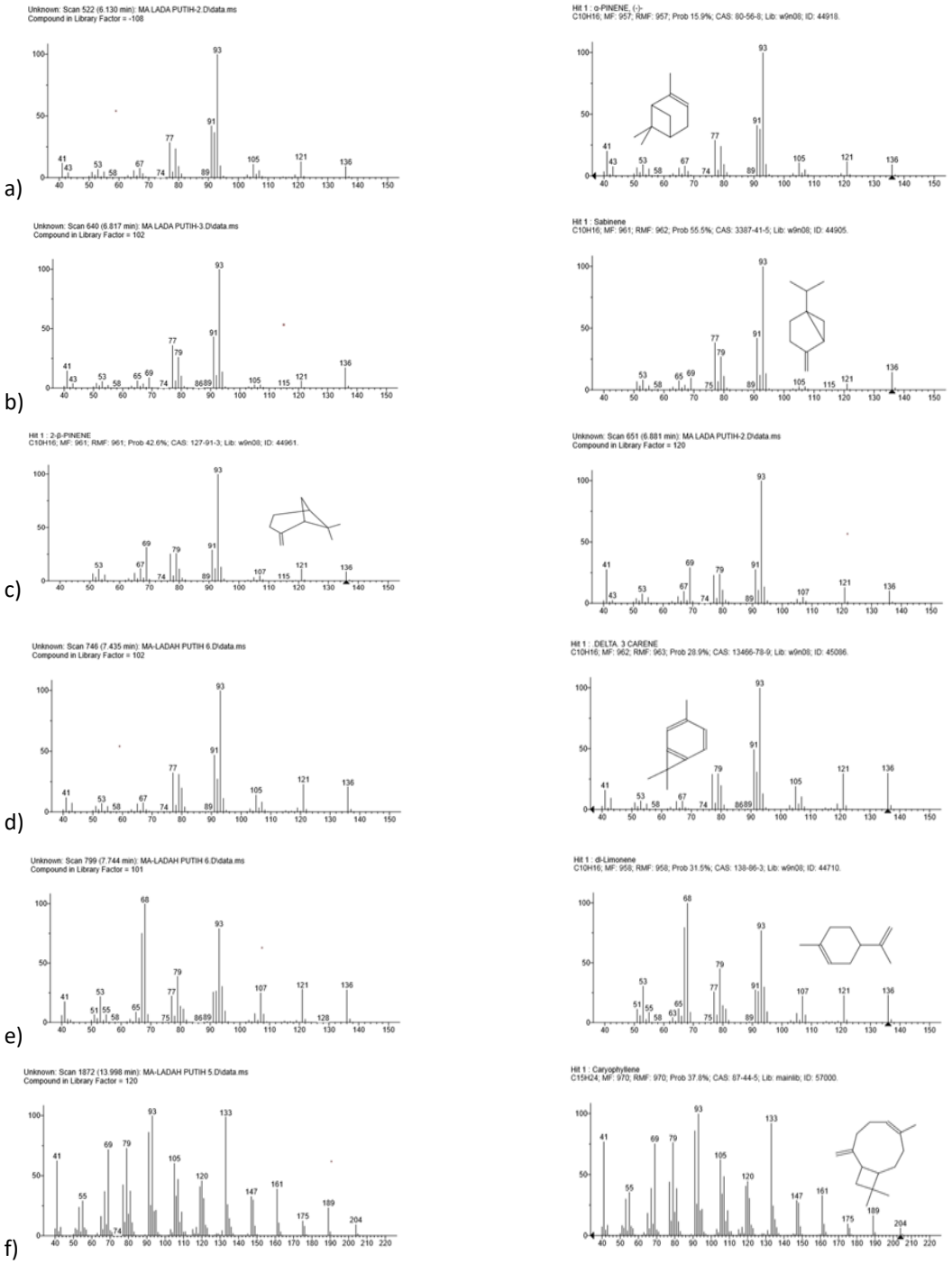

Figure 2: Spectrum of the six main molecules

Table III. Match Factor (MF) values of main molecules in the white pepper samples

No	Compound	Retention time	MF	%Area			
				Java	Sumatera	Kalimantan	Sulawesi
1	*α-pinene*	6,130	957	7,83	9,92	10,65	5,20
2	*Sabinene*	6,817	961	0,41	21,58	0,14	0,24
3	*Beta-Pinene*	6,881	961	13,03	8,18	14,82	9,48
4	*Δ-3-carene*	7,435	962	27,83	21,39	24,98	21,37
5	*dl-Limonene*	7,744	958	21,68	15,41	17,86	16,08
6	*Caryophyllene*	13,998	970	13,18	6,99	11,68	30,90

Conclusion

The white pepper essential oil from Java, Sumatra, and Kalimantan Islands contained 22 constituent compounds. In addition, those from different islands have specific compounds which relate to their differences in composition. *Alfa-pinene, Sabinene, and caryophillene* were dominantly found in white pepper from the Kalimantan, Sumatra, and Sulawesi Islands, respectively. Meanwhile, those from Java Island contained *beta-pinene*, *delta-carene* and *caryophillene* in similar quantities.

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RESEARCH ARTICLE

Formulation and physical properties of lotion Kalakai root ethanol extract (*Stenochlaena palustris Bedd*)

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Keywords
Ethanol extract
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Lotion
Physical properties test

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Abstract

Aim: To determine the physical (organoleptic, homogeneity, pH, dispersibility and adhesion) properties of the ethanol extract of Kalakai root lotion with a concentration of 1%, 2% and 3%. **Methods:** This study used the maceration method that was carried out by testing the physical properties of the lotion preparation with three concentrations, namely 1%, 2% and 3% using one type, namely the M/A type.
Results: It was found that at a concentration of 3% it was a formula for the ethanol extract of Kalakai root that met the quality parameters of the good physical properties of the lotion. **Conclusion:** Kalakai root extract concentration influenced the physical properties of the lotion preparation tested. The higher the kalakai root extract concentration, to a concentration of 3%, did not affect the pH value or homogeneity, but the kalakai root extract lotion preparation had higher results for the spreadability and adhesion.

Introduction

The skin controls body temperature, acts as a protective layer, allows a sense of touch and has melanocytes to filter out some of the potentially harmful ultraviolet (UV) radiation from sunlight (Fauzi & Nurmalina, 2012). The limited protection from UV light means the body needs antioxidants that are able to neutralise free radicals, which are otherwise very dangerous, to prevent damage to the exposed skin cells. This damage can lead to skin cancer; therefore, it is necessary to formulate a cosmetic preparation containing antioxidant compounds. Certain plants are known to have beneficial properties meaning they can be used as natural ingredients to protect the skin from the negative effects of sunlight. These natural substances can be extracted from the plants and serve as a potential source of sunscreen. The use of antioxidants can prevent various diseases caused by UV radiation; there are several active antioxidant compound groups, such as flavonoids, tannins, anthraquinones, cinnamates and other groups, that have been reported to have the ability to protect against UV rays (Indriani, 2018).

The search for these natural compounds is currently a major concern. A natural ingredient widely found in Borneo that grows in peat areas and has traditional medicinal properties is kalakai. Kalakai leaves and roots are widely used in traditional medicines, but it is known that other parts are also used as traditional medicines. Root K*alakai (Stenochlaena palutris)* has not been widely researched; therefore, there is minimal scientific data supporting the effectiveness of kalakai root as an antioxidant. Despite the minimal information for scientific publications, there are articles on the properties of the kalakai root (Adawiyah & Rizki, 2018). Research on antioxidant activity in kalakai roots, from work by Adawiyah and Rizki (2018), states that by using the DPPH and quercetin method as a comparison of the IC value of 50 kalakai roots growing on peat soil of 19.06 ppm and sandy soil of 24.40 ppm have very strong antioxidant activity. Adawiyah's research (2019) for the results of the SPF value of the ethanol extract of Kalakai root at a concentration of 350 ppm, the SPF antioxidant

values are 11 and 14. The accepted range to have an extreme level of ability as an antioxidant SPF is if the range is more than 11.

Antioxidants are important to maintain the quality product of food, health and beauty products. In the health and beauty sector, antioxidants function to prevent cancer, tumours, narrowing of blood vessels, premature ageing and others. In the body, antioxidants also inhibit the oxidation process, where the oxidation process that occurs continuously can cause various degenerative diseases and premature ageing (Sayuti & Yenrina, 2015).

The antioxidant activity and the SPF value from the existing research spurs the development of kalakai root in topical dosage forms that are acceptable to the community. The topical dosage form chosen in this study was lotion preparation. The lotion preparation was chosen because it was the most suitable pharmaceutical preparation for external use as protection. Its liquid consistency allows fast and even application on the skin surface, so it spreads easily and dries quickly after being applied and leaves a thin layer on the skin's surface (Lachman *et al*., 1994). This study aims to determine the physical properties (organoleptic, homogeneity, pH, dispersion and adhesion) of the ethanol extract of kalakai root with a concentration of 1%, 2% and 3%.

Method

The main ingredients used in this study were kalakai roots that grew on peat soil on Mahir Mahar Street, Palangka Raya City; other materials used were 70% ethanol, glyceryl monostearate, glycerine, Cera alba, liquid paraffin, nipagin, nipasol, twen 80, stearic acid, aqua rose and aqua dest.

The tools used in this study were analytic scales, pH universal, a water bath, a rotary evaporator for maceration tools, and a range of glassware.

Simplicia collection and processing

The process carried out in the manufacture of the first Simplicia was done by preparation by collecting Simpilicia, wet sorting, washing with running clean water, draining, chopping and drying. The drying process in this study was carried out by drying in the shade (dry-wind), then dry sorting was carried out, and the dry Simplicia was obtained in the form of a sieved powder using a sieve with a sieve number 14.

Preparation of the extract

Simplisia, containing Kalakai root powder weighing 900 g, was then extracted using the maceration method with 70% ethanol solvent with a ratio of 1: 8 for four days with a change of solvent every 24 hours. The liquid extract obtained was then separated from the residue using Whatman filter paper number 1 until the solution was no longer colourless. The liquid extract obtained was concentrated using a *rotary evaporator* with a temperature of 60°C for approximately eight hours, then evaporated on a water bath with a temperature of 60°C to form a thick extract.

Preparation of Kalakai Root ethanol extract lotion (Stenochlaena palutris Bedd)

Oil phase materials (glyceryl monostearate, stearic acid, liquid paraffin, Cera alba) were mixed by heating the materials at a water bath of 75°C until homogeneous. At the same time, the water phase (glycerin, TEA, twen 80, nipagin, nipasol and distilled water) were mixed. Next, the oil phase was put in a hot mortar then crushed while adding, a little by little, the water phase and crushed until the ingredients were mixed together. Finally, add aqua rose and kalakai root extract and crush it again until it was homogeneous and the lotion preparations are formed. The finished preparation was put in a closed container to avoid sun exposure, then evaluating the lotion preparation. For each variation of the kalakai root extract formula in this study, the concentration was 1% (F1), 2% (F2), 3% (F3).

Evaluation of physical properties of kalakai root extract lotion

The formula of the kalakai root ethanol extract lotion used is as described in Table I. in this study, the physical properties test carried out were an organoleptic test, homogeneity test, pH test, adhesion test and a spreadability test.

Organoleptic test

Organoleptic testing was carried out by observing the smell, color and texture of the lotion preparation (Anief, 1997).

Homogeneity test

The homogeneity test was carried out by weighing 0.1 g of the lotion preparation then placing it in the middle of a glass dish then flattening and covering it with another glass object (Pujiastuti, & Kristianti, 2019).

pH test

The pH test for lotion preparations uses universal pH. The lotion preparation was smeared on universal pH paper and replicated three times for each formula, and

the colour change on the pH paper observed and recorded. The colour that appears on the universal pH paper was then matched with the colour on the pH indicator found on the universal pH package (Pujiastuti, & Kristianti, 2019).

Adhesion test

The preparation was weighed at 0.1 g, placed in the middle of the glass dish and covered with another glass object, pressed using a heavy load (50 g weights) for five minutes. The tip of the cover glass object and the other edge of the glass object is attached to the clamp on the adhesive strength tester; then, the load support is removed. The length of time the two glass objects were separated from the test equipment was recorded as the attachment time for the preparation. This test was carried out for the three formulas (Please rephrase – meaning is unclear) (Pujiastuti & Kristianti, 2019).

Spreadability test

The spreadability test was carried out by weighing the lotion preparation at 0.5 g and then placing it in the middle of a round glass scale. It was then covered using a round glass which has been weighed, and the diameter of the spread was recorded. Weight loads of 50 g, 100 g and 150 g were added alternately for one minute, and the diameter of the spread was recorded. This process was repeated three times, and the same test stages were carried out for the three formulas. Then, another round glass was placed on top of the lotion and a weight of up to 150 g, left to stand for one minute for each additional weight, then the diameter of the spread was recorded. (Pujiastuti & Kristianti, 2019).

Data analysis

The result data of the tests of the physical properties of the lotion preparation formula were replicated three times to increase the accuracy of the experiment. The data obtained were compared with the physical properties of a good lotion preparation.

Result

The Kalakai root extract was made using 70% ethanol by maceration, and a thick extract was obtained weighing 8.00g, with a yield of 0.89% obtained from the kalakai root powder originally weighed at 900g. The lotion preparation formulas with variations in the concentration of kalakai root extract are presented in Table I. Organoleptic and homogeneity test results are presented in Tables II and III, while the results of the pH, adhesion and spreadability tests are presented in Figures 1, 2 and 3.

Table I: Lotion formulations for kalakai root extract with various concentrations of 1% (F1), 2% (F2) and 3% (F3)

Materials	F1 (1%)	F2 (2%)	F3 (3%)
Kalakai ethanol extract	0.50 g	1.00 g	1.50 g
Gliseril monostearate	2.75 g	2.75 g	2.75 g
Cera alba	1.35 g	1.35 g	1.35 g
Tween 80	1.75 g	1.75 g	1.75 g
Gliserin	5.00 g	5.00 g	5.00 g
Parafin liquidum	5.00 g	5.00 g	5.00 g
Nipagin	0.0075 g	0.0075 g	0.0075 g
Nipasol	0.0075 g	0.0075 g	0.0075 g
Acid stearate	2.00 g	2.00 g	2.00 g
TEA	2.00 g	2.00 g	2.00 g
Aqua rosae	3 drops	3 drops	3 drops
Aquadest	ad 50ml	ad 50ml	ad 50 ml

Organoleptic test results

The test was done by observing the smell, colour and texture of the preparation. The results of the three formulas obtained are presented in Table II. Formula 1 produced a brownish colour, had a distinctive smell of Kalakai extract and rose, a soft and smooth texture. Formula 2 produced a brown colour, had a distinctive smell of Kalakai extract and rose, and had a soft and smooth texture. Formula 3 produced a dark brown colour, a distinctive smell of Kalakai extract and rose and had a soft and smooth texture smooth.

Table II: Organoleptic test results

Organoleptic test	F1 (1%)	F2 (2%)	F3 (3%)
Colour	Light brown	Chocolate	Dark brown
Smell	The distinctive smell of Kalakai and Roseroot extracts	The distinctive smell of Kalakai and Roseroot extracts	The distinctive smell of Kalakai and Roseroot extracts
Texture	Soft and smooth	Soft and smooth	Soft and smooth

Homogeneity test

The homogeneity test was carried using 0.1 g of the lotion preparation by placing it on a glass object and observing it by moving it through uneven or coarse particles. Based on the three formulas, a homogeneous formula was obtained in the absence of harshness on the tested lotion, as presented in Table III.

Table III: Homogeneity test results

Replication	F1 (1%)	F2 (2%)	F3 (3%)
1	Homogeneous	Homogeneous	Homogeneous
2	Homogeneous	Homogeneous	Homogeneous
3	Homogeneous	Homogeneous	Homogeneous

pH test result

pH testing on lotion preparations using universal pH. Based on testing the pH value on the lotion preparation, the results obtained in formulas 1, 2 and 3 have a pH value of 7, as shown in Figure 1.

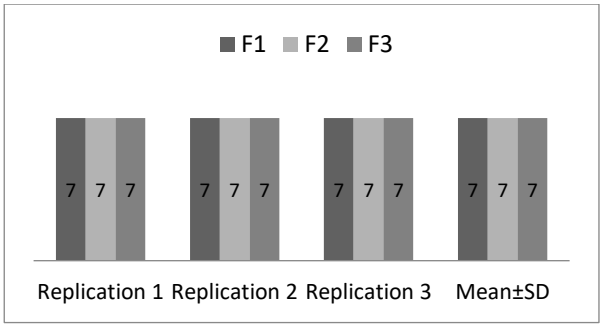

Figure 1: pH test results of Kalakai root extract

Adhesion test results

The adhesion test on lotion preparations obtained results in formula 1 with an average time of 2.4 seconds, formula 2 with an average time of 3.6 seconds and formula 3 with an average time of 4.2 seconds which is presented in Figure 2.

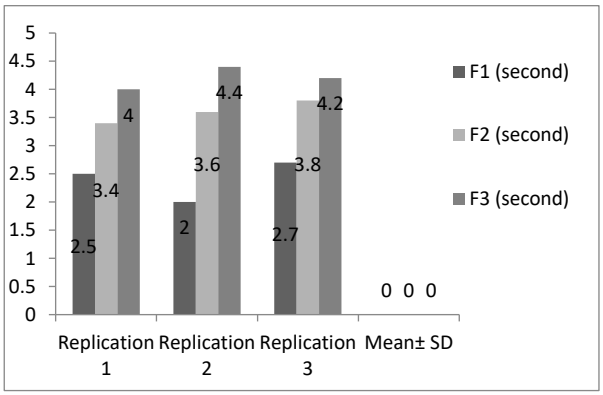

Figure 2: The result of Kalakai root extract adhesion test

Spreadability test result

The spreadability test on lotion preparations had averages of 9.73 cm for formula 1, an average of 9.03 cm for formula 2, and formula 3 with an average of 7.00 cm, which is shown in Figure 3.

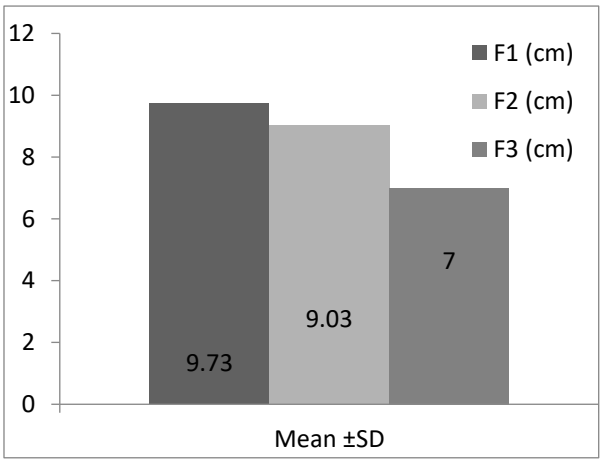

Figure 3: The spreadability test results of kalakai root extract

Discussion

The results of the ethanol extract of Kalakai root in this study used 70% ethanol due to its good penetration power for penetrating the cell wall. This allows the active compounds contained in the sample to be activated. It also has the ability to bind with compounds with a wide polarity range (polar to non-polar compounds); it is non-toxic compared to other organic solvents, and is not easily hydrolysed by microbes (Saifudin *et al*., 2010). The extract was made using the maceration method. Maceration is one of the simplest methods and is widely used, the principle is the diffusion process between the solvent and secondary metabolites due to the immersion of the sample in the solvent. After 24 hours, the solvent was removed and then process was then repeated. The solvent was replaced to speed up the extraction process as the solvents will become saturated. The solvent replacements are stopped when the solvent has shown a former colour or clear colour, which indicates that the compound has completely reacted (Adawiyah, 2018). Then evaporate the mixture until you get a thick extract. 8.00g of thick extract were obtained with a yield of 0.89%, obtained from the original 900g of kalakai root powder. The yield calculation determines the amount of kalakai root used to obtain the amount of extract and will help to improve the next experiment.

The yield of plant roots is generally not large, usually below 5%.

Organoleptic testing carried out on the three formulas of lotion preparations showed an increase in brownish colour with the increasing addition of Kalakai root extract, as well as all samples having a distinctive odour of Kalakai and roseroots. These observation aims to determine the physical characteristics of lotion preparations, so that, apart from being a factor parameter that affects physical and chemical changes, lotion preparations are also a comport parameter (Amatulla *et al.*, 2017).

The homogeneity test of preparation is influenced by the mixing process at the time of preparation (Pujiastuti, & Kristiani, 2019). Based on the research results, it is known that the increase in the concentration of Kalakai root extract in the lotion preparation does not affect the homogeneity of the Kalakai root ethanol extract lotion because all the ingredients are mixed homogeneously.

The pH test carried out on lotion preparations aims to determine the degree of acidity or alkalinity of a preparation, which can affect comport when used so as not to cause irritation to the skin. Based on the pH value testing of the Kalakai root extract lotion, the same pH value was 7, as shown in Figure 1. Based on the pH value, all formulas meet the pH requirements of skin moisturisers, namely 4.5-8.0 (SNI, 1996). The pH value is important to determine the acidity of the preparation, if it is too acidic, it will irritate the skin, and if it is too alkaline, it can cause scaly skin; the ideal pH for the skin is 4.5-7.0 (Wasitaatmadja,1997).

The adhesion test was carried out to determine the length of time the lotion adheres to the skin. If the lotion has low adhesion, then the desired effect is not achieved. In contrast, if the adhesion produced is strong, it will inhibit skin respiration (Megantara *et al*., 2017). Therefore it is important to conduct the adhesion test. The accepted requirement for adhesion time ranging is more than 4 seconds (Mulyani *et al*., 2018), which was only achieved by formula 3 (Figure 2). From this result, it can be concluded that the variation in extract concentration has an influence on the sticking time of the lotion. This is due to the ratio of the concentration of Kalakai root extract; the greater the concentration of Kalakai root extract, resulting in greater adhesion ability and longer adhesion time. This means that the ability of lotion preparations when more Kalakai root extract is added, the lotion adheres more to the skin so that the active substances contained in the lotion preparations will be better able to protect the skin from sun exposure (Ulandari, & Sugihartini, 2020).

The spreadability test was carried out to determine the dispersibility of the preparation when used on the skin. Good preparations are preparations that are easy to spread on the skin without applying great pressure. From the results of the tests carried out, only formula 3 meets the requirements because it has a topical spreadability ranging from five to seven centimetres (Dominica, & Handayani, 2019), as shown in Figure 3. The increase of Kalakai root extract concentration reduces its spreadability as the water content in the preparation decreases, so the lotion gets thicker (Ulandari & Sugihartini, 2020). In addition, the load resistance results in a larger spreading diameter. The wide distribution can show the ease of using the lotion on the skin (Pujiastuti, & Kristiani, 2019)

Conclusion

Kalakai root extract concentration influenced the physical properties of the lotion preparation tested. The higher the kalakai root extract concentration, up to a concentration of 3%, did not affect the pH value, homogeneity, but the increased kalakai root extract lotion preparation showed greater results for the spreadability and adhesion.

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IAI CONFERENCE

RESEARCH ARTICLE

Effectiveness of public service advertisements on the use of antibiotics in Pangkalpinang

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Keywords
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Abstract

Background: Previous studies have shown that the public needs information related to drugs from reliable sources of pharmaceutical personnel. One of the information media that has never been used is electronic public service advertisement. **Aim:** Study the effectiveness of public service advertisements on the use of antibiotics in Pangkalpinang City to find a medium that functions in reducing the number of antibiotic resistance. **Method:** This research was conducted in Pangkalpinang from December 2018 to September 2019 using a quasi-experimental quantitative approach with a time-series design. The sample of this research consisted of 400 people determined by accidental sampling techniques and analysed in univariate and bivariate using a dependent test. **Results:** There was a significant difference with the value of p = 0.0001 between the use of antibiotic respondents before (pre-test) and after airing of public service ads (post-test). **Conclusion:** Public service advertisements about antibiotics were effective in terms of antibiotics use.

Introduction

Antibiotics are the most widely used drugs for infections caused by bacteria. Various studies have found that about 40-62% of antibiotics are misused, such as for diseases that do not require antibiotics. A survey about the quality of antibiotic use in various parts of the hospital found that 30% to 80% were not based on indications (Hadi *et al.*, 2009). Antibiotic resistance harms many parties. Infectious diseases caused by bacterial resistance increase the time the patient suffers from the illness. Thus, if the patient is admitted to the hospital, the hospitalization costs will undoubtedly increase. According to the Centers for Disease Control and Prevention, every year in the United States, two million people get infected with bacteria that have become resistant to antibiotics, and at least 23,000 people die each year as a direct result of this resistance. The WHO data states that, in 2016, 480,000 new cases of multidrug-resistant tuberculosis (MDR-TB) have emerged in the world (WHO, 2016).

In Indonesia, public understanding of the benefits and impacts of using antibiotics is still weak, constituting a severe problem as the level of antibiotic use is quite alarming. People today freely buy and take medicines without a doctor's prescription. It is essential to impart knowledge about antibiotics to the community. Therefore, health workers, especially pharmaceutical workers, must work hard to increase public awareness regarding the proper and correct use of antibiotics. Research regarding community knowledge in the village of Penyamun, Bangka Regency, shows that the community still has in-depth knowledge about the use of antibiotics: 38 respondents (35.8%) had good knowledge, 25 (23.6%) had moderate knowledge, and 43 respondents (40.6%) had deep expertise (Septiana, 2016). These results are also in line with those of research regarding the use of amoxicillin in Penagan Village, Bangka Regency, showing that 54.27% of the community still lack knowledge (Zulaika, 2018).

Antimicrobial resistance (AMR) is now a global public health issue, projected to affect the longevity of people

and increase the health expenditure of countries. Its impact is expected to be higher in low- and middle-income countries, where healthcare systems are suboptimal and ill-equipped to deal with the issue. As antibiotic misuse is the primary driver for AMR, there is an acute need to create awareness among the general public, calling calls for a comprehensive communication strategy that considers the various drivers of AMR and associated solutions. In the short term, the focus of communication strategies should be to raise awareness in specific interest groups by channelling limited resources to achieve definite objectives, thereby improving the chances of behaviour change. The general public can be targeted at a later stage or as a second phase with specific strategies and messages (Mathew, Sivaraman & Chandy, 2019).

Public service announcements about the use of antibiotics have never been on television because antibiotics are hard drugs that have specific rules. Likewise, in the province of Bangka Belitung Islands, public service advertisements regarding the proper and correct use of drugs have never existed. Despite the expanding use of social media, little has been published about its appropriate role in health promotion and even less has been written about evaluation (Neiger *et al.*, 2012). The five-year strategy of the Department of Health outlined seven key areas to address antibiotic resistance; Public Health England (PHE) is responsible for improving surveillance, optimising prescribing practices, and educating doctors and the public about the risks of antibiotic misuse (Cully, 2014).

The findings of Ashe and the authors (2006) indicate that the educational poster had no effect on antibiotic use. Farmers, physicians, and patients need to recognize the value of antibiotics and protect this vulnerable resource. In the absence of enlightened self-interest, more effective policies are required because the current ones are insufficient. A global, multidisciplinary effort is needed to slow the development of antibiotic resistance; it will take more than one shepherd to prevent our commons from being overgrazed (Cully, 2014). Videotron is more effective and efficient than billboards and posters as it can reduce visual waste and does not take up much space (Aji, 2018). Therefore, it is necessary to study the effectiveness of public service advertisements on the use of antibiotics in Pangkalpinang City to find a medium that functions in reducing the number of antibiotic resistance.

Method

This research was conducted in Pangkalpinang City in December 2018-September 2019. It used a quasi-experimental quantitative approach with a time-series design, applying the pre-test and post-test methods. The population in this study consisted of the people of Pangkalpinang City. The sample size was calculated using the Slovin formula resulting in a sample of 400 respondents. The instrument used in this study was a questionnaire. Respondents read the explanation and then filled out written informed consent. Before taking primary data, the validity and reliability were tested at Sungailiat city. After that, four pre-test measurements for antibiotics use were carried out for each sub-districts of Pangkalpinang city at one-week intervals. During one month, public service advertisements were aired on Videotron in several strategic places in Pangkalpinang city. Furthermore, four post-test measurements were performed on the same respondents as well. The data analysis methods in this research are univariate and bivariate using the dependent t-test. This study has received ethical approval from the health research ethics committee of The Health Research Polytechnic of The Ministry of Health, Pangkalpinang.

Results

The demographic profiles of this study can be seen in Table I. The results of this study show that the respondents have used antibiotics before. Thus, it was manifest that the answers to the questionnaire corresponded to the reality of the actual use of antibiotics. The most widely used was amoxicillin, i.e. 398 respondents (99.5%). Other antibiotics used were clindamycin, chloramphenicol, metronidazole, ciprofloxacin, ampicillin/cloxacillin, and tetracyclines.

Table I: Demographics of respondents

Demographic profiles	Category	Total	
		n=400	%
Category of the highest education	Low (not attending school-Primary school-Junior high school)	134	33,5
	High (Senior high school-University)	266	66,5
Age	Youth (17-25 years old)	86	21,5
	Adult (26-45 years old)	180	45
	Elderly (>46 years old)	134	33,5
Job	Not working	194	48,5
	Working	206	51,5
Resources	1 media	387	96,8
	2 media	9	2,3
	3 media	4	1
Experience	Never	0	0
	Ever	400	100
Number of Usage	Once	192	48
	2 times	63	15,8
	3 times	145	36,3

The pre-test and post-test measurements show 365 people pre-test (91.3%) and 388 people -test (97%) (Table II). After conducting a t-test analysis on the use of antibiotics pre-test and post-test in all respondents, there was a significant difference, with a $p < 0.0001$ (Table III), indicating that public service advertisements are effective in reducing antibiotics misuse. The use of antibiotics categorised as "not good" at pre-test measurements was 8.8%, while at post-test measurements, this number decreased to 3%. Public service advertisements on the use of antibiotics effectively improved the use of antibiotics in the community of Pangkalpinang.

Table II: Description of the use of antibiotics in pre-test and post-test measurements

No	Sub-district	Pre-test				Post-test			
		Good	%	Not good	%	Good	%	Not good	%
1	Gabek	48	84.2	9	15.8	54	94.7	3	5.3
2	Gerunggang	55	96.5	2	3.5	55	96.5	2	3.5
3	Girimaya	49	86	8	14	53	93	4	7
4	Rangkui	56	98.2	1	1.8	56	98.2	1	1.8
5	Pangkalbalam	50	87.7	7	12.3	57	100	0	0
6	Taman Sari	55	94.8	3	5.2	58	100	0	0
7	Bukit Intan	52	91.2	5	8.8	55	96.5	2	3.5
	Total	**365**	**91.3**	**35**	**8.8**	**388**	**97**	**12**	**3**

Table III: Description of the results of paired samples test

		Paired differences					t	*df*	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the difference				
					Lower	Upper			
Pair 7	Average score of pre-test – average score of post-test	-0.813	1.333	0.067	-0.944	-0.681	-12.191	399	<0.0001

Discussion

The results of this study show that the respondents have used antibiotics before. Thus, it was manifest that the answers to the questionnaire corresponded to the reality of the actual use of antibiotics. The most widely used was amoxicillin, i.e. 398 respondents (99.5%). Our findings are in line with the results of another study showing that all patients (108 patients) have used antibiotics without a prescription and had a low level of awareness. The most purchased antibiotic without a prescription is amoxicillin (Fernandez, 2013). It is also the most widely used by community service participants, i.e. 11 people (45.83%) (Djuria & Sinulingga, 2019).

Analysis of data on the use of antibiotics in respondents shows that at the time of pre-test measurement, most people who misused antibiotics were in Gabek district, nine people (15.8%), while the least was in Rangkui district, one person (1.8%). In the post-test measurement, most people who misused antibiotics were in Gabek district, three people (5.3%), while the least was in Pangkalbalam and Taman Sari districts, non (0%), indicating that all of them used antibiotics appropriately.

This result indicates that most respondents are highly educated, namely 266 people (66.5%) (Table I). Education is needed to get information, for example, about things that support health to improve the quality of life. In general, the higher a person's education, the easier it is to retrieve information (Nursalam, 2010).

All respondents declared having received information about antibiotics use. The source of information was printed media (13 respondents, 3.25%), electronic media (15 respondents, 3.75%), but mainly non-media-based (health workers, neighbours, family, and friends), with 391 respondents (97.75%) (Table I). People with higher education will easily accept information, especially information about them (Nursalam, 2010).

Most respondents were adults 26-45 years old (180 participants, 45%) (Table I). Among older people, the memory factor considerably affects one's knowledge. The level of maturity and strength of a person depends on age. The elder would be more mature in thinking and working. Therefore, respondents will regularly increase their knowledge so that they can change their behaviour. Most respondents worked, namely 206 people (51.5%) (Table I). As work is a means to support one's and family life, working respondents earned

money and could buy antibiotics when they felt sick. Therefore, all respondents have used antibiotics.

A study showed a low awareness regarding prescription medicine, antibiotic use, and AMR among the general population in the highland provinces of Vietnam (Ha, Nguyen & Nguyen, 2019). These findings indicate the need for further systemic and didactic educational interventions targeting females, ethnic minorities, those with low education, low income, and those working in the agriculture/fishery/forestry sector in this setting to improve awareness about antibiotic use and resistance.

The t-test analysis showed a significant difference in antibiotics use in pre-test and post-test among all respondents (Table II), indicating that public service advertisements are effective in reducing antibiotics misuse. These results are consistent with previous findings on the influence of advertising messages, advertising sources/models, and ad execution on attitudes, showing that the three variables simultaneously have a positive and significant effect on the attitudes of faculty students (Mustika & Zakaria, 2012). Moreover, four public service advertisements received high enough attention from the public, namely: Traffic Orderly Ads, Amnesty Tax, Report Hendy, and Saber Pungli (Mukaromah, Yanuarsari & Pratiwi, 2017). Simultaneously, the attractiveness of advertisements, the quality of advertising messages, and the frequency of ad serving moderated by the effectiveness of advertisements have a significant effect on the attitudes of other people (Libradika, 2015).

It seems essential that future antibiotic awareness campaigns base their messages more rigorously on scientific evidence, context specificities, and behavioural change theory. A new generation of messages that encourage first-choice use of narrow-spectrum antibiotics is needed, reflecting international efforts to preserve broad-spectrum antibiotic classes. Evaluation of the influence of antibiotic awareness campaigns remains suboptimal (Huttner *et al.*, 2018).

In 2015, a study among the general population of the UK highlighted several issues to be considered when communicating the issue of AMR to the public (Trust, 2015). Another research shows that the framing of antibiotic resistance in the TV advertisement led to an increase in misunderstandings of what becomes resistant to antibiotics (Borgonha, 2019). The advertisement helped to highlight the vulnerability of antibiotics and create a new social norm about being a responsible antibiotic user. However, it was interpreted as childish by participants. It did not communicate the severity of antibiotic resistance or specific risk of antibiotic overuse to the audience or accurately reflect the audience's existing knowledge of antibiotic resistance and current behaviours. As the severity of antibiotic resistance was not conveyed, the advertisement did not motivate a change in antibiotic-seeking behaviours or attitudes among most participants. The findings highlighted knowledge gaps among study participants; they were unaware of the importance of completing the antibiotic course, and they thought that humans develop resistance, not bacteria (Borgonha, 2019).

Effective communication plays a remarkable role in improving community awareness about important healthcare issues (Mathew, Sivaraman & Chandy, 2019). But increasing awareness alone does not result in significant behaviour change unless the issues are addressed holistically. The messaging should be culturally relevant and adapted to the preferences of the target population. Even though a multi-stakeholder approach is preferred, specific leadership responsibilities should be assigned in the whole communication process. The role of champions and social influencers is essential in deciding the success of messaging, as their presence adds a layer of credibility to the whole exercise. In the case of AMR, it is pertinent that the messaging strategy should not be high-jacked by commercial entities who have conflicting interests in the sector. Even though professional and industry groups can be allies in a potential communication campaign on AMR, care should be taken to ensure that the process is free of any conflicts of interest. More importantly, it is pertinent to accept that awareness is just one part of the entire behaviour change process, and the targets for the communication campaign should not be restricted to raising awareness.

Finally, the application of findings in surveys and associated factors related to antibiotic use and AMR should primarily generate public health interventions and target specific groups to make progress in solving AMR problems and maximise the use of surveys (Kosiyaporn *et al.*, 2020).

Conclusion

Public service advertisements about antibiotics were effective in terms of antibiotics use.

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IAI CONFERENCE

RESEARCH ARTICLE

Satisfaction of drug information services implementation in Air Itam, Selindung, Girimaya, Gerunggang and Melintang health centre Pangkalpinang City

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Keywords
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Abstract

Background: Previous studies have shown that the service quality of the pharmaceutical drug information service has not been optimally implemented. This problem also occurred in Pangkalpinang city. Therefore, it is essential to conduct a study on the satisfaction of drug information service implementation to improve the quality of pharmaceutical services. **Aim:** Examining the satisfaction of drug information service implementation in Air Itam, Selindung, Girimaya, Gerunggang, and Melintang health centres Pangkalpinang city. **Method:** This observational research was conducted from March to December 2018 in Air Itam, Selindung, Girimaya, Gerunggang, and Melintang health centres, Pangkalpinang city. It involved 150 patients selected by accidental sampling techniques, and the bivariate analyses were performed using the dependent t-test. **Results:** The quality of drug information service implementation in all dimensions was good, and there were significant differences in tangibility ($p < 0.0001$), reliability ($p = 0.025$), and empathy ($p = 0.011$). All respondents were quite satisfied with the drug information service implementation in all dimensions, with a significant difference in the dimensions of tangibility ($p = 0.002$), reliability ($p = 0.045$), and empathy ($p = 0.045$). **Conclusion:** All respondents were quite satisfied with the drug information service implementation in all dimensions.

Introduction

The demands of patients and the community for the quality improvement of pharmaceutical service require an expansion from the old paradigm that is product-oriented (drug-oriented) to a new paradigm that is patient-oriented, with a philosophy of pharmaceutical care (Indonesian Ministry of Health, 2016a). This development can be an opportunity and a challenge for pharmacists to advance their competence to provide comprehensive pharmaceutical services, both managerial and clinical pharmacy (Indonesian Ministry of Health, 2016b). Based on Pharmaceutical Service Standards in Health Center, seven clinical pharmacy services must be carried out, i.e. assessment and prescription services, drug information services, counselling, patient visits (especially inpatient health centres), drug side effects monitoring, drug therapy monitoring, and drug use evaluation. Drug information service is a service activity carried out by pharmacists to provide accurate, explicit, and up-to-date information to doctors, pharmacists, nurses, other health professionals, and patients (Indonesian Ministry of Health, 2016a).

Research showed that pharmaceutical services implementation in the Province of Bangka Belitung

Islands has not been optimal, especially clinical pharmacy services in the form of drug information service (Djuria, 2017). Several other studies revealed that drug information services were not satisfactory in many cities, i.e. Health Centre in Pangkalpinang City (Djuria, 2013), Membalong Health Centre in Belitung Regency (Permadi, 2013), Gerunggang Health Centre in Pangkalpinang City (Defika, 2015), or not fully implemented, as in the Koba Health Centre in Bangka (Julimansyah, 2015). Also, the drug information service at Petaling Health Centre is not following the 2016 Standard for Pharmaceutical Services at Health Centre (Trisnawati, 2016).

However, these results oppose other findings showing that drug information services in some cities, such as Sungailiat (Septashary, 2014), Pasir Putih, Pangkalbalam, Taman Sari, and Kacang Pedang (Djuria, 2019), are satisfactory in all dimensions, particularly empathy. In 2015, Karolin reported a significant relationship between the quality of drug information service and patient satisfaction with pharmaceutical services, in particular, and health services, in general, at Pangkalbalam Health Centre, Pangkalpinang City (Karolin, 2015).

Therefore, researchers were interested in examining the satisfaction of patients with drug information services in other Pangkalpinang City Health Centres, namely, Air Itam, Selindung, Girimaya, Gerunggang, and Melintang.

Methods

This observational research was conducted between March and December 2018 at Air Itam, Selindung, Girimaya, Gerunggang, and Melintang Health Centres in Pangkalpinang City. The sample size for evaluating health services was 30 encounters between health workers and patients (WHO, 1993). Therefore, the sample of this study consisted of 150 respondents (30 respondents per health centre). The encounters sampling technique used the accidental sampling method. Patient encounters data were taken around the drug delivery/pharmacy area. Research data consisted of primary and secondary data. Primary data were collected by researchers directly from the field and processed by researchers, while secondary data were obtained from the Pangkalpinang City Health Office and Pangakalpinang City Health Centre. The questionnaire used for primary data collection consisted of five satisfaction variables, i.e. tangibility, reliability, responsiveness, assurance, and empathy. Before taking primary data, the validity and reliability were tested at the Pangkalan Baru Health Centre, and respondents read the explanation and then filled the informed consent form. This study consisted of the independent variable (implementation of drug information service) and the dependent variable (satisfaction with the implementation of drug information service). The data analysis methods were univariate and bivariate using the dependent t-test. This study has received ethical approval from the health research ethics committee of The Health Research Polytechnic of the Ministry of Health, Pangkalpinang city.

Results

The results showed that most respondents (54%) had low education, 49.3% were aged 18-40 years, old and 70% did not work (Table I). Also, the quality of drug information service implementation was satisfactory in all dimensions. Significant differences were seen in drug information service implementation in the dimensions of tangibility, reliability, and empathy (Table II). All respondents were quite satisfied with the implementation of drug information services in all aspects. There were significant differences in respondent satisfaction with drug information service implementation in the dimensions of tangibility, reliability, and empathy (Table III).

Table I: Demographics of research respondents

Variable	Category	n= 150	%
Highest education	Low (no school-elementary school-junior high school)	81	54
	High (senior high school-university)	69	46
Age	≤ 17 year	18	12
	18-40 year	74	49.3
	41-65 year	52	34.7
	> 65 year	6	4
Job	Didn't work	105	70
	Work	45	30

Table II: Description implementation of service

Dimension	Category	Pre-test		Post-test		*Sig* (2 tailed)
		n=150	%	n=150	%	
Tangible	Not Good	15	10	0	0	$p < 0.0001$
	Good	135	90	150	100	
Reliability	Not Good	5	3.3	0	0	$p = 0.025$
	Good	145	96.7	150	100	
Responsiveness	Not Good	11	7.3	5	3.3	$p = 0.134$
	Good	139	92.7	145	96.7	
Assurance	Not Good	2	1.3	2	1.3	$p = 1$
	Good	148	98.7	148	98.7	
Empathy	Not Good	15	10	4	2.7	$p = 0.011$
	Good	135	90	146	97.3	

Table III: Description of satisfaction

Group	Category	Pre-test n=150	%	Post-test n=150	%	*Sig* (2 tailed)
Tangible	Not satisfied	12	8	0	0	*p* = 0.002
	Enough satisfied	137	91.3	150	100	
	Satisfied	1	0.7	0	0	
Reliability	Not satisfied	4	2.7	0	0	*p* = 0.045
	Enough satisfied	146	97.3	150	100	
	Satisfied	0	0	0	0	
Responsiveness	Not satisfied	150	100	150	100	*p* = 0.083
	Enough satisfied	0	0	3	2	
	Satisfied	150	100	147	98	
Assurance	Not satisfied	0	0	0	0	*p* = 1
	Enough satisfied	150	100	150	100	
	Satisfied	1	0,7	1	0.7	
	Not satisfied	149	99.3	149	99.3	
	Enough satisfied	0	0	0	0	
	Satisfied	150	100	150	100	
Empathy	Not satisfied	12	8	3	2	*p* = 0.045
	Enough satisfied	137	91.3	147	98	
	Satisfied	1	0.7	0	0	
	Not satisfied	150	100	150	100	

Discussion

The research results show that the quality of drug information service implementation was satisfactory in all dimensions. Significant differences were seen in drug information service implementation in the dimensions of tangibility, reliability, and empathy. The results of this study are consistent with previous findings (George & Rao, 2005), showing an overall satisfaction of most respondents with drug information services; the queries answered by the centre were within acceptable quality limits.

This research reported the satisfaction of respondents with the tangibility dimension (physical evidence), reflected by the presence of magazines, leaflets, drug labels, and posters at the health centre. Respondents were also satisfied with the drug information answers of pharmaceutical personnel. Indeed, the results showed that pharmaceutical personnel is reliable in answering queries. The existence of significant deficiencies could be due to differences in the timeliness of service. A study in 2006 (Leksamana, 2006) reported that reliability includes the ability to provide promised services reliably, accurately, and timely. The empathy dimension includes attention, politeness, understanding consumer needs, and communication relationships (Wijayanti, 2008; James, 2006). Respondents reported that pharmaceutical officers have good communication skills; they pay attention and understand the needs related to their prescribed treatment.

All respondents were quite satisfied with all dimensions of drug information services implementation, with significant differences in satisfaction of tangibility, reliability, and empathy. This result contradicts previous findings (Rawn, 1999), showing that respondents reported a high level of overall satisfaction with Roche drug information services. In general, there was a good correlation between customer needs and customer satisfaction with the services offered. Areas of strength were courtesy of personnel, accuracy, and relevance to the inquiry, while response time required improvement. Suggested strategies for improvement consisted of expanding the range of topics available through the “Fax on Demand” system, raising customer awareness of this system, streamlining the process of handling information requests, and using a drug information website with an externally accessible database to reduce the workload of Roche Drug Information and Safety Department.

Previous research showed that drug information services provided by the pharmacy practice department of Kasturba Hospital, Manipal, catered to the need of health care professionals and eventually towards better patient care (George & Rao, 2005). Another study in Sudan (Fatherlrahman *et al.*, 2008) revealed that providing an acceptable drug information quality of service satisfied and retained users. Overall, the service received a positive evaluation. However, research in Latin America reported deficiencies, mainly a lack of objectivity among respondents (Fisher, Tavares & Pizzol, 2012).

The results of this study show that the drug information service given is still following the standards of pharmaceutical services at the Community Health Centre. According to these standards, services should include a bulletin, leaflet, drug label, poster, wall magazine, among others (Indonesian Ministry of Health, 2016a). A study in 1995 (Parasuraman, 1995) reported that the tangibility variable encompasses the availability of physical facilities, equipment, and room support facilities owned by service providers. Satisfaction with this variable can occur if the physical facilities are easily accessible and the appearance of employees is neat. In the sample of this study, respondents were somewhat satisfied because some aspects have not been fulfilled by the pharmaceutical officer, such as drug labels and posters from the Ministry of Health.

Of all respondents, 105 (70%) did not work as they were mainly housewives. While waiting, they usually direct their children towards objects of interest to them, such as wall magazines and leaflets. Therefore, respondents reported that such publications were of help.

Most respondents had low education levels (54%). Unlike people with high education, those with low education do not have high expectations. Therefore, it is easier for them to accept and be satisfied with the services provided. A previous study (Yuniarta & Suharto, 2011) revealed that the higher the education level, the higher the level of satisfaction with informed consent. Patients with low education might not understand the meaning of informed consent or the explanations given about the study objectives.

In this study, most participants were 18-40 years old (49.3%). Adults are usually more interested in immediately visible things. Therefore, the newsletters, wall magazines, posters, and leaflets are readily accepted by the respondents. Adults are motivated to learn according to their perceived needs and interests. This need for learning will be more oriented toward developmental tasks than social roles (Anonymous, 2018).

Measurement of the drug information service implementation in the reliability dimension, by statistically, there was a significant difference in satisfaction. The satisfaction of customers with service quality can be achieved by increasing the reliability to provide quality products and ease of access to goods/services (Juran, 1995). Providing services as promised using attractive product packaging are also predictors of customer satisfaction (Julianto, 2000). There was no significant difference in satisfaction because of the change in drug information services in the reliability dimension.

Satisfaction with the empathy dimension was significant in the implementation of drug information services as respondents could feel the increased attention of pharmacy officers. According to Yofa (2010), satisfaction with empathy is achieved when employees do not differentiate between consumers, provide solutions to problems, and understand the needs.

This result is supported by previous findings (Pascoe, 1983) showing that patient satisfaction can provide a dependent measure of service quality and serves as a predictor of health-related behaviours. Moreover, a previous study (Izzetin, 2019) showed that internet-based drug information services provided by clinical pharmacists contributed positively to users' satisfaction, indicating the importance of clinical pharmacists' involvement in this process.

Conclusion

This research reported that all respondents were quite satisfied with the Drug Information Service implementation in all dimensions.

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IAI CONFERENCE

RESEARCH ARTICLE

Medicine management in districts and primary health care centres (PHC) in the national health insurance (JKN) programme

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Keywords
e-procurement
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Abstract

Aim: This study aimed to identify medicine management in district health offices and primary health care centres (PHCs) after the national health insurance (JKN) programme implementation. **Methods:** A cross-sectional study was carried out by collecting documents related to medication management and in-depth interviews with the head of the PHC officials and JKN medicine management officers at the PHC in four provinces of Indonesia. **Results:** The results showed no regional policies related to medicine management; all policies were based on central policies. Medicine management in districts follows the procurement planning suggested by PHCs, which relies on disease patterns. Medicine procurement at PHCs is done by e-purchasing using an e-catalog. Medicines above IDR 200 million are purchased through catalogs provided by the procurement service unit (ULP), and those under IDR 200 million are obtained through a direct appointment. **Conclusion:** The storage of medicine requires more space and air humidity controlling. The reporting and monitoring of medications e-logistic system are based on 20 indicators and have not been carried out regularly. It is necessary to improve reporting and monitoring systems.

Introduction

The national health insurance (JKN) programme has been implemented in Indonesia since 2014, where the Social Security Administrator for Health (BPJS Kesehatan) is the appointed administering body, part of other national administering bodies. The national social security system is a procedure for administering social security programmes by several social security administering bodies (Law of the Republic of Indonesia Number 40 of 2004 Concerning the National Social Security System, 2004).

In this context, Primary Health Care centres (PHC), known as Puskesmas, are considered the main gatekeepers of the public primary health care system. Notwithstanding PHC, other private primary health care facilities should offer to cooperate with BPJS Kesehatan. PHC is a health service facility that makes public health and first-level individual health efforts, prioritizing promotion and prevention (MOH, 2019). It plays an optimal role as a gatekeeper in health services and is required to provide services for 144 diagnoses that should not be referred to the hospitals (Kedokteran, 2012).

Medicine is a substance or combination of active ingredients, including biological products, used to diagnose, prevent, cure, and improve human health (Law of the Republic of Indonesia, 2009).

The pharmaceutical and medical devices system is one of the sub-systems of the national health programme, aimed to ensure safety, efficacy, and quality aspects of pharmaceutical preparations, medical devices, and food, in addition to their availability and affordability (Indonesia, 2012). The implementation of the JKN

programme brings changes to the health system, including the pharmaceutical aspect. The ability to treat 144 diagnoses cannot be achieved unless necessary medicines are made available in PHCs.

Presidential Decree No.32 of the year 2014, concerning management and utilisation of JKN capitation funds, enacts the capitation fund to be directly transferred to the accounts of PHCs. However, if the status of the PHC does not allow it to have an account, then the capitation fund is transferred to the official district account before it is relayed to the PHC. As a result, PHCs should manage the fund according to the regulation stating that at least 60% of the amount should be allocated for health services costs and the remaining 40% for operational expenses, including medicines procurement. The changing in financing scheme has also changed the medicines procurement pattern, where PHCs own funds allow them to afford necessary medicines (Management of FKTP Capitation Funds, 2014).

Once the capitation funds are transferred, PHCs are authorised to manage it. However, appropriate competencies are needed to manage these funds appropriately. For example, the treasurers must have been trained to become familiar with the use of capitation funds. Additionally, there must be a certified procurement officer to procure drugs through e-purchasing (Ministry of Health, 2014).

Despite being authorised to procure medicines, PHCs still rely heavily on the district health office as the source of essential medicines. The situation is prolonged by the installation of a District Pharmacy at the District/City Health Office that procures medicines for all PHCs under their regional authority through large-scale e-purchasing.

E-Catalog is an electronic system that provides information about products, including lists, types, specifications technical, domestic component level (TKDN), domestic products, Indonesian National Standard (SNI), green industrial products, country of origin, price, provider, and other information related to goods/services (LKPP, 2018).

There is a need to find out how district health offices and PHCs procure medicines, considering the availability of capitation funds. The purpose of this study is to explore the management process of drugs in district health offices and primary health care systems from planning to provision, storage, monitoring, and evaluation. The availability and utilization of several indicators are also portrayed to get a comprehensive perspective of the pharmaceutical and medical devices aspects resulting from the JKN programme.

Methods

This cross-sectional study was carried out in four provinces of Indonesia (Yogyakarta, Bali, East Kalimantan, and East Nusa Tenggara (NTT) provinces), using a qualitative approach with in-depth interviews to determine the management of medicine in health centres. The regions chosen were considered to represent geographical patterns and population density associated with the capitation of PHCs.

The research samples to obtain secondary data (medicine management documents) consisted of 8 medicine managers of the District Pharmacy/City Health Offices and 16 medicine managers of Puskesmas from the four provinces selected, considering the status of BLUD and non-BLUD PHCs. The data collected consisted of documents related to medicine management at the PHCs, including planning, procurement, storage, monitoring, and evaluation.

Additionally, in-depth interviews were held with the Heads of the district pharmacy/city health office and the heads of PHCs, totalling 16 respondents. The whole conversation was recorded after getting permission from the participants.

Ethical approval

The Health Research Ethics Commission (KEPK), National Institute of Health Research and Development, Ministry of Health, Republic Indonesia, reviewed and granted this study ethics clearance (Number LB.02.01/5.2/KE.357/2016). Informed consent was obtained from all respondents prior to participation in the interview.

Results

Table I presents the respondents and their distribution per institution and province. The results show there is no regional policy related to medication management. The JKN programme policies related to drug management are dynamic, so regions need strong efforts to adjust to developments in drug management policies. Besides appropriate and dynamic policy objectives, laws were not enforced. For example, no sanctions were taken against suppliers regarding the late delivery of medicines to the region (Hermansyah *et al.*, 2018).

Table I: Characteristics of respondent

Respondents characteristics (n:10)	DIY	Bali	East Kalimantan	East NTT
Head of Pharmacy Institutions in the District / City Health	2	2	2	2
Managers in pharmacy installations in district / city health offices	2	2	2	2
Medicine managers at PHC	4	4	4	4
Head of PHC	2	2	2	2

In the four provinces, the policy was not implemented optimally, and the only existing regional regulation was related to capitation services. Medicine management in PHCs includes a plan for medication needs, prepared based on needs and disease patterns. JKN medicine procurement in PHC was done with capitation funds, but in the four provinces, it was also sourced from central funds (known as DAK funds) to cover JKN drug shortages (see Table II)

Table II: Medicine management in districts and primary health care (PHC)

Districts (Province)	Districts (Regency/ City)	Primary Health care (PHC)
Policy The Governor's policy regarding the JKN programme is Universal Health Insurance. Prov: Governor's Policy: JKBM / Balimandra. (integration into JKN). Prov: There is no specific regional regulation on medicine management in the implementation of JKN. Prov: There is no specific Regional Regulation on Medicine management for the Implementation of JKN, referring to the central policy "One gate Policy" with an integrated planning team	Medicine management refers to the Central Policy (one gate policy) by forming an Integrated Planning Team. There are no specific regulations on medicine management.	Referring to central and regional policies
Financing Costs from DAK APBD I (Buffer). Financing from DAK and central buffer assistance	Funds for drug purchases from DAK, APBD II, and APBD I.	PHC source of drug costs from APBD and JKN capitation (7% from 40% capitation)
Planning Based on the needs plan submitted by the district and the drug programme. Programme drug planning is carried out by the programme manager based on stock reports sent by the warehouse and programme requirements data.	District/city planning for medicine and health supplies based on the needs of the PHC based on the pattern of disease.	Drug planning based on disease patterns is proposed to the district from the APBD source.
Procurement Provinces, procurement for buffers, procurement of programme medicine based on recommendations from programme holders, are requested to the central government—procurement mechanism for e-catalogue medicine to PPTK (Technical Implementation Officer) and KPA, procurement.	Drug Procurement by the Health Office, Drug e-catalogue and non-e-catalogue submitted to PPK. Non-e-catalog <200 million through direct appointment (PL). Procurement of non-e-catalogue medicine> 200 million through auction to PPK (ULP)	Drug Procurement with capitation by PPK. Puskesmas where there is no procurement official yet, Puskesmas drug procurement is coordinated by the District / City Health Office
Storage Drug storage and distribution to districts/cities as a drug buffer	Medicines received by the recipient team are stored at the UPT Pharmacy. Drug Programme from the Central to the Province dropped to the district. Drug Storage with FIFO and FEFO systems	Drug Storage with FIFO and FEFO systems
Reporting Drug availability reporting: software (e-logistics). Reports on the availability of medicine and vaccines for 20 indicator medicine. There is still a manual reporting system.	Reporting the availability of medicine using software (e-logistics). Reports on the availability of medicine and vaccines for 20 indicator medicine are sent directly to the centre, namely to the Ministry of Health. For the drug programme, by each programme manager.	Drug reporting to District / City Health Office, Reporting

In the Yogyakarta Province, planning medicine in PHC was based on needs and disease patterns (ten major diseases). The PHC drug needs planning would then be reported to the district/city health office, to be summarised according to the health needs of the PHC in the city/regency area. Requests and procurement of JKN medicines were carried out by the city/district health office. JKN medicine procurement by

district/city health offices was done, considering the limited personnel who must meet the competence and certified procurement officials. The PHC was monitored by the district/city health office and other technical staff, not specifically for medicine management, at a frequency of three to four times a year. The monitoring showed the need to increase the frequency of money transfers from the Central Government and monitoring and evaluation from the provincial health office or the district/city health office. Some unavailable medicines are amlodipine, diazepam 5 mg, haloperidol, chloramphenicol, paracetamol 100 mg, amitriptyline 25 mg, acyclovir 5%, metoclopramide 10 mg, and propranolol 100 mg.

Bali province had never had a drug vacuum from planning, but with the e-catalogue, medicines were not always found although money was available; sometimes, their expiry date was too short. The medicines for JKN PHC must also be purchased through an e-catalogue. Medicines outside the procurement catalogue are procured through a direct nominal valuation mechanism when <200 million and those >200 million are bought through ULP. Drug storage and distribution in the PHC are based on First in First Out (FIFO) and First Expired First Out (FEFO) procedures, where storage conditions of medicines in the PHC storage do not meet space and humidity requirements. Reporting system and logistical monitoring and evaluation are only for 20 items of indicator medicine. Monitoring and evaluation of the PHC by the DHO has not been organised due to budget constraints. Drug shortages include glimepiride 1 mg, simvastatin, vitamin B complex, salbutamol, ambroxol, and paracetamol.

The distribution of medicines to the PHC is shown in Figure 1. In East Nusa Tenggara Province (NTT), PHC drug planning and drug programmes had special meetings, and the planning was based on the calculation of the remaining stock and usage from the year before. The meeting was held at the end of the year. The planning calculates 12-month needs. Drug procurement by the PHC refers to the national formulary, using the e-catalogue for health centres that have procurement officials. However, the obstacles encountered were that JKN medicines ordered in the first trimester often came late. Pharmacists or certified procurement personnel were still lacking, while for the expenditure process from capitation funds, there were rules that must be met, including certified procurement officials. Medicine management sources of funds from the BPJS had not fully funded medicines. In addition to getting JKN medicines with capitation funds, the PHC also received medicines from the district/city health office sourced from DAK funds. Accredited PHCs already had a medicine management SOP, contrary to those not accredited. Drug planning in the PHC was based on the needs and disease patterns in the regions. The distribution to sub-PHCs called *pustu* and *poskesdes* was carried out by the PHC drug manager. Obstacles or constraints in medicine management in JKN implementation include drug requests that sometimes could not be fulfilled. Unavailable medicines included dental products, amlodipine, and oxytetracycline.

In East Kalimantan province, programme drug requests from PHCs to programme holders are forwarded to the pharmacy section. For JKN drug procurement, although funds are available, medicines lack in the PHC and the district/city pharmacy warehouse due to an Extraordinary Event (KLB) or a distribution gap. The vehicle at the PHC is an ambulance. The storage method is the FEFO system with special conditions: when the drug is delivered, it must have a 24-month expiry date; those that expire before 24 months must have a certificate from the factory. Unavailable medicines include metformin, simvastatin, mefenamic acid, Glyceryl Guaiacolate (GG), CTM, ambroxol, and ethyl chloride for teeth.

Discussion

This study results indicate that the drug policy in the four provinces has not been implemented optimally, and the only existing regional regulation was related to capitation services. Medicine management refers to the Central Policy (one gate policy) by forming an Integrated Planning Team. Medicine management in PHCs includes a plan for medication needs, prepared based on needs and disease patterns. According to Raharni, government policies related to medicines management in the JKN era began with the national medicines policy, taking into account the benefits of medicines in JKN, both in terms of accessibility and affordability, with rational using of medicine (Raharni *et al.*, 2018). This finding seems to be consistent with the report, drug planning by method consumption and morbidity. The results indicated that the planning and procurement process were not appropriately applied. In the planning process, compliance with the national formulary was still lacking; changes in disease prevalence affected the accuracy of drug planning. (Aisah *et al.*, 2020). The planning and distribution of medicines had not been following the standards fully, with some indicators not meeting the standards, i.e., the accuracy of planning, planning irregularities, and drug availability (Boku *et al.*, 2019). The study reports that the drug requirement plan is calculated according to the average use of medicine, safety stock, lead time, and remaining stock. Lead time in Kediri Regency is based on the length of procurement process time up to drug reception; since it takes three months, the lead count time is three times the average usage (Sulistyorini, 2016).

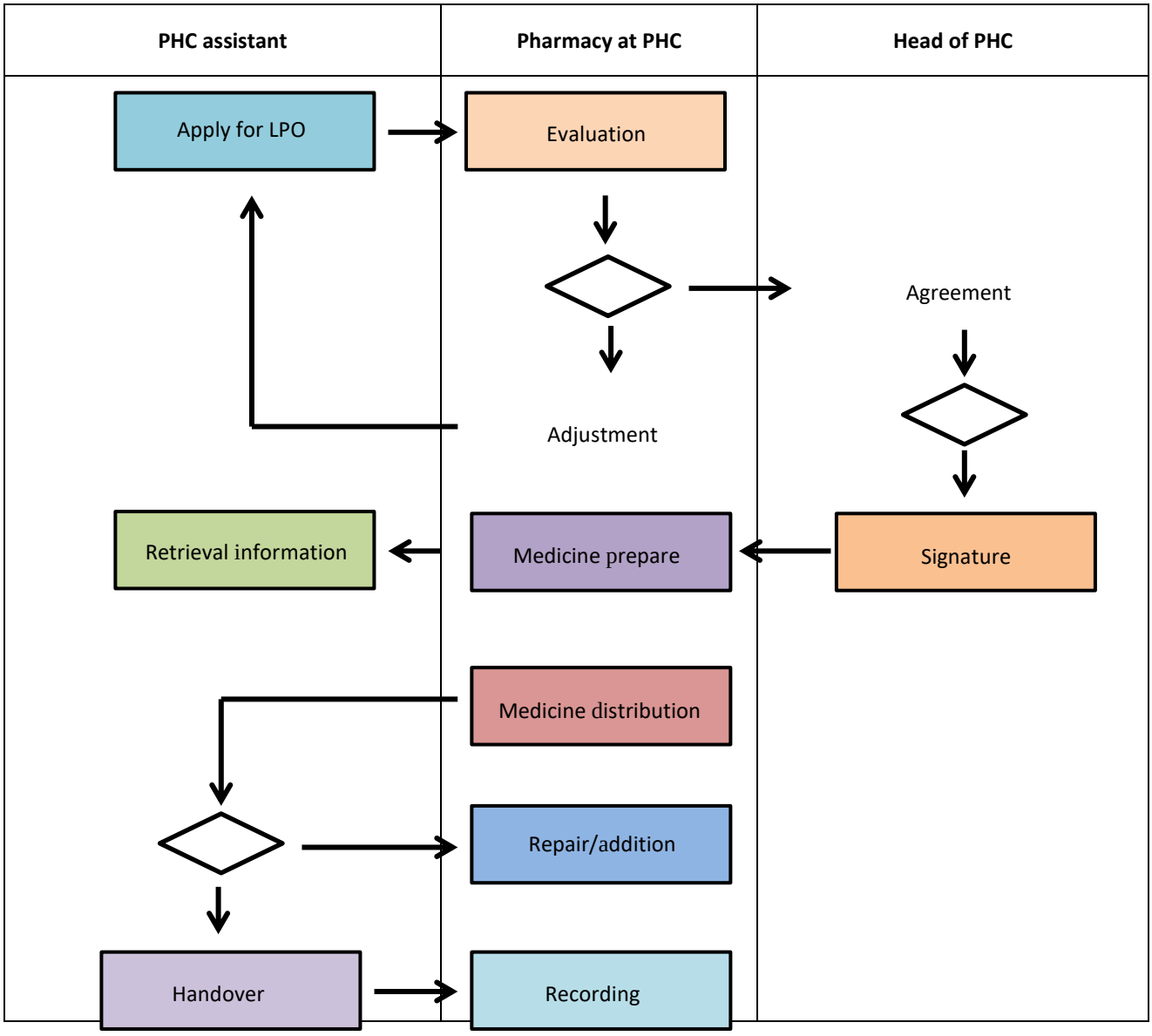

Figure 1: Drug distribution at PHC

In districts, drug procurement by the Health Office, drug e-catalogue, and non-e-catalogue are submitted to PPK. Non-e-catalogue medicines <200 million are bought through direct appointment (PL), while non-e-catalogue medicines >200 million are purchased by auction to PPK (ULP). This finding seems to be consistent with the report, drug procurement with e-purchasing, which is the most valuable IDR 200,000,000. Direct Procurement of Goods/Construction Work is the selection method to get Goods Providers/Construction Work/ Other Services, which is worth a maximum of IDR 200,000,000 (Presiden, 2018).

In provinces and districts, medicines received by the recipient team are stored at the installation pharmacy, using FIFO and FEFO storing systems. In PHCs, medicine storage also uses the FIFO and FEFO systems. This result seems to be consistent with previous findings showing that the storage process does not use the alphabetical system and the absence of a special cabinet for the medicine class of narcotics. In addition to this system, drug storage space is not sufficient to accommodate the stock of medicines, causing excessive accumulation of boxes due to inadequate facilities and infrastructure, such as shelves, trolleys, and human resources in the process of medicine management (Astriani, 2018). Drug procurement/demand at the PHC is in accordance with applicable regulations. Drug storage at PHC used the FEFO and FIFO methods (Indriawan *et al.*, 2014). The features that did not meet the standards were

selection, planning, procurement, percentage of available fund allocation (10,98%), and distribution (Oktaviani *et al.*, 2018). The medicine management system at the Pharmacy Warehouse of the Ngawi District Health Office was adequate, while shortages are often caused by empty stocks by PBF. (Hapsari, 2019)

The result showed that medicine management had not been fully in accordance with the standards. Of the seven measured indicators, one of them met the standard, namely, the percentage of the available fund compared with the cost planned. The other six indicators that did not comply with the standards were percentage of drug procurement with fund allocation, percentage of drug item planned, percentage of total quantities of an item, procurement frequency of each drug, frequency of uncompleted contract, frequency of delayed rate in payment by the hospital (Ulfah *et al.*, 2018).

Drugs are stored in pharmaceutical warehouses, some still not stored alphabetically. As for drug distribution, the frequency of once every two months is in accordance with the proposed request in the LPLPO; however, the drug distribution process is done by pick a ball from Medical Health Care to Health Department. (Lubis, 2017)

Some of the required activities cannot be implemented, and the lack of human resources results in the placement of health workers who do not comply with the required level of education. Based on this result, it can be concluded that the management of medicine in Danowudu Public Health Center needs to be implemented according to the regulation of pharmaceutical service standards in the PHC (Mailoor *et al.*, 2019).

The results showed that the drug management planning in the Health Office of West Muna Regency and Pharmacy Warehouse of West Muna Regency was based on epidemiological methods of drug supply, which are adapted to the disease pattern, as suggested, using request sheet and usage sheet. Storage of the medicine in local government clinics is still inadequate, but the storage has fulfilled the standards. The distribution of medicines has been appropriate with the procedure of management and eliminated the expired medicines (Rismalawati, 2015).

Reports on the availability of medicine and vaccines used 20 drug indicators, including medicines and vaccines that support maternal health, child health, disease prevention, and widely used basic health service medicines comprised in the National Formulary (Directorate of Public Medicine Development, 2015). In some districts, reporting is still manual. This finding seems to be consistent with previous reports, showing that there were 12 health centres (60.0%) that had good storage methods, and eight health centres (40.0%) were unfavourably rated (Poernomo *et al.*, 2019). Information System Medicine Distribution of Health Center Community in Pharmacy Stockroom of District Banjar consisted of a web-based, user-friendly platform. It covered reception and distribution, usage reports, online requests, data of available medicines and stock (Rismalawati, 2015).

The medicine management system at Kamonji Health Center was in accordance with the Ministry of Health RI No. 74 of 2016. However, there are still obstacles in the management process, such as lack of medications and inadequate drug storage rooms, still requiring supporting facilities for medicine management (Muthahara *et al.*, 2018).

Conclusion

The results show there is no regional policy related to medication management. The JKN programme policies related to drug management are dynamic, so regions need strong efforts to adjust to developments in drug management policies. In the four provinces, the policy was not implemented optimally, and the only existing regional regulation was related to capitation services. Medicine management in PHCs includes a plan for medication needs, prepared based on needs and disease patterns. JKN medicine procurement in PHCs was done with capitation funds, but in the four provinces, it was also sourced from central funds (known as DAK funds) to cover JKN drug shortages. Technical guidance and budgets in the four provinces are still limited. Finally, storage facilities are not yet in accordance with the humidity requirements, and monitoring and evaluation are not performed periodically.

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IAI CONFERENCE

RESEARCH ARTICLE

Antiseptic gel formulated from ethanol extract of Citronella grass (*Cymbopogon nardus)* using CMC-Na, arabic gums, and gelatin as gelling agents

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Keywords
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Abstract

Introduction: *The* ethanol extract *from* citronella grass (*Cymbopogon nardus*) has been found to contain saponins, phenolics (flavonoids), and polyphenols which have antibacterial activity. As a result, researchers developed an antiseptic gel that contains this ethanol extract. **Aims:** This study aims to find the optimum concentration of gelling agent required to make the gel and to use physical evaluations in order to understand whether combining gelling agents may increase the quality of the gel. **Methods:** The gel was made using a melting method, which mixed the base of gel with citronella ethanol extract at a temperature of 40°C to form a homogeneous phase. The gel evaluation was conducted using an organoleptic test, homogeneity test, dispersion test, pH test, and adhesion test. **Results:** The tests were carried out on 15 formulations respectively (R1, R2, and R3), and resulted in the production of clear, translucent yellow gel with a distinctive citronella grass odor. The homogeneity test showed that all formulations were homogeneous and contained no agglomerated particles. The gel dosage forms made with CMC-Na, gum arabic, and gelatin as gelling agents resulted in having a pH of 7 whilst gels made with a combination of gelling agents resulted in having a pH of 8. The combination of gum arabic and CMC-Na gelling agents showed an increase in spreadability of gel formulas at the same concentration of composition, of which the combinations were 1.25% gum arabic and 1.25% CMC-Na. The gel adhesion time was 0.2-2 minutes for all formula. **Conclusion**: Based on this test data, it can be concluded that the 15 gel formulations that resulted from this research are good and further testing can be performed to determine the most optimum and stable formula.

Introduction

The diversity of Indonesia's natural resources is marked by the abundance of herbs and natural medicinal plants. These plants are widely used for preservation, health care, and beauty. Citronella grass (*Cymbopogon nardus*) is an easily obtained plant in Indonesia; it is widely cultivated because it can have various pharmacological uses, such as producing antifungal and antibacterial effects. Citronella grass can easily be cultivated in gardens, and it is usually grown as a spice or medicinal plant. There are two types of lemongrass plants: lemongrass (*Cymbopogon citratus*), which is commonly used as a spice and citronella grass (*Cymbopogon nardus*).

Citronella grass extract has long been used as a traditional medicine for both oral and external use. Sore throats, colitis, gastritis, diarrhea, and stomach pains are treated by orally using the grass extract. It can also be used orally as a mouthwash (Wijayakusuma, 2001). On the other hand, rheumatic pain and skin diseases such as eczema are treated with the use of external drugs in the form of liniment (Oyen, 1999).

The active compounds of citronella grass are saponins, flavonoids, and polyphenols (Syamsuhidayat & Hutapea, 1991). The root from citronella grass can be used as a diuretic, diaphoretic and expectorant. Another one of its uses can be as an ingredient in mouthwashes and body warmers. Its leaves can be

used as a carminative, antipyretic, antispasmodic, stomachic as well as to treat postpartum (Sudarsono *et al*., 2002). The essential oil content of citronella grass is α-citral, β-citral, geraniol, myrcene, nerol, citronellal, terpinolene, geranyl acetate, linalool, terpinol, methyl-heptenone, borneol, linalyl acetate, limonene, and linalool isobutyrate. Citronella grass essential oil with active citral and geraniol components has antifungal activity (Tyagi & Malik, 2010; Khan & Ahmad, 2012). The contents of flavonoids, saponins, and citral compounds have antibacterial activity. Citronella grass could inhibit the growth of *C. Albicans* fungi because its chemical content included saponins, flavonoids, and tannins. Another study by Basuki (2011) has also found that the ethyl acetate extract of citronella grass has antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. These findings make citronella grass potentially effective as an antiseptic gel.

This research is expected to provide an optimal alternative gelling agent that will increase the effectiveness of the dosage forms. The type and concentration of gelling agent may affect the quality and stability of the prepared gel. In this research, there were three gelling agents (CMC-Na, gelatin and Arabic gum) that were used to make the citronella ethanol extract gel. The combination of gelling agents was also observed to understand the effects on the improvement of the gel's quality compared to the single gelling agent.

Materials and methods

The instruments used in this study were a rotary evaporator, an analytical balance, aluminium foil, a graduated cylinder, a beaker, a gel compartment, a hot plate, a stirring rod, a dropping pipette, test tubes, a cooling cabinet, a glass slab, and an adhesion tester. The materials used were citronella grass leaves and stems, CMC-Na, Arabic gum, gelatin, ethanol (70%), glycerin, sorbitol, triethylamine (TEA), methylparaben, and aqua dest. All materials used were laboratory grade and did not receive further treatment.

The citronella ethanol extract was obtained by maceration of citronella leaves and stems in 70% ethanol for two cycles, each of 24 hours. Afterwards, the extract was concentrated with the rotary evaporator at 60°C.

Preparation of Citronella Ethanol Extract Gel

Citronella ethanol extract gel was produced by preparing the gelling agent, adding preservatives and humectants, and finally adding the citronella ethanol extract, as shown completely in Figure 1.

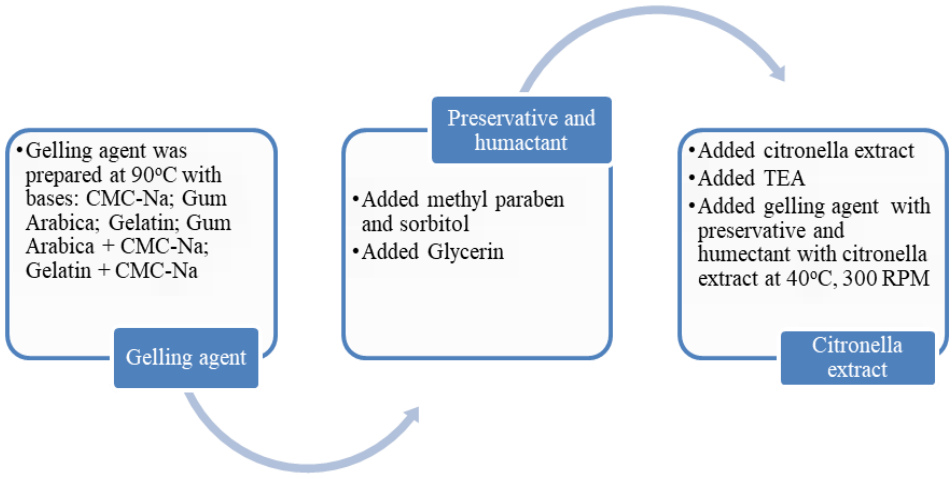

Figure 1: Schematic view of citronella ethanol extract gel preparation

Organoleptic test

An organoleptic evaluation was done by observing the texture, colour and smell of the gel in every formula. The high-quality gel should have been clear and transparent with a semi solid consistency (Ansel, 1989).

Homogeneity test

Physical evaluation of the gel has characterised the form of colour uniformity and the distribution of dispersed particles. These were conducted by applying samples to preparation glass. The high-quality gel should have shown homogenous dispersion.

Spreadability test

The diameter of the spreading gel was measured by putting weights above the gel. In the first test, 0.1 grams of each gel sample were spread on a glass slab and another glass slab was placed on top of the gel sample and the diameter of the spreading gel was measured. Afterward, a 20-gram additional load was placed on the gel sample for a minute before the diameter of the spreading gel was measured again. This process was continued by adding other loads of 50, 100, 150, and 200 grams gradually.

pH test

A universal pH indicator was used to measure the acidity of the gel. The indicator was dipped into each sample that had been previously separated and diluted. The pH of the gel was measured by the change of colour on the indicator. The pH must have been in a range that didn't cause any skin irritation.

Adhesion test

This evaluation was done by measuring the time each sample took to detach from a preparation glass slide on the adhesion tester. A 0.25-gram gel sample was spread on a preparation glass slide and covered with another slide. A 100-gram weight was added to the slide for 5 minutes. After that, the sample was mounted on the adhesion tester.

Results and discussion

The antibacterial effects of Citronella grass (*Cymbopogon nardus*) due to the citronellal and geraniol compounds has been studied. In this study, three different gel base formulas were used to develop citronella ethanol extract formulas. The materials used for the basis CMC-Na, gum arabic, and gelatin. This study was intended to prove that citronella ethanol extract gel could be made using 15 formulas of different gel bases (three times replicated). Each gel was evaluated by its organoleptic, pH, homogeneity, spreadability, and adhesion properties (See Table I).

Table I: Citronella gel formula (Note: each formula was replicated three times (R1, R2, R3))

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15
Citronella Extract	1%														
CMC- Na	0.5%	1.25%	2%	-	-	-	-	-	-	2%	1.25%	0.5%	2%	1.25%	0.5%
Gum Arabic	-	-	-	0.5%	1.25%	2%	-	-	-	0.5%	1.25%	2%	-	-	-
Gelatin	-	-	-	-	-	-	0.5%	1.25%	2%	-	-	-	0.5%	1.25%	2%
Glycerin	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%	20%
Sorbitol	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%	15%
TEA	5 drops														
Methyl Paraben	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Aquadest	Ad 50 ml														

Organoleptic test

Every formula resulted in producing a translucent yellow gel, except the formula that combined 2% CMC-Na and 0.5% gum Arabic, which resulted in a yellow gel. In addition, all obtained gels had a distinctive odour of citronella grass. The gel texture using gelatin and gum Arabic didn't show any difference with higher concentrations. However, the gel texture using CMC-Na as a gelling agent gets thicker and more viscous when used in higher concentrations. Each formula yielded clear and transparent gel even with a combination gel base (Figure 2 and Figure 3).

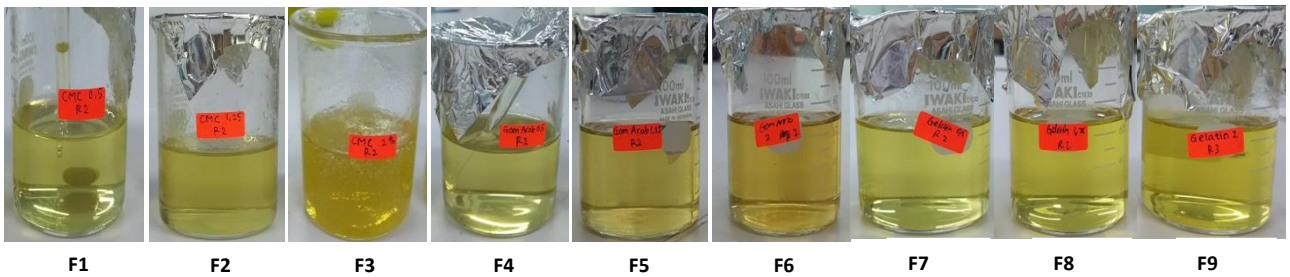

Figure 2: Citronella gel with single gelling agent

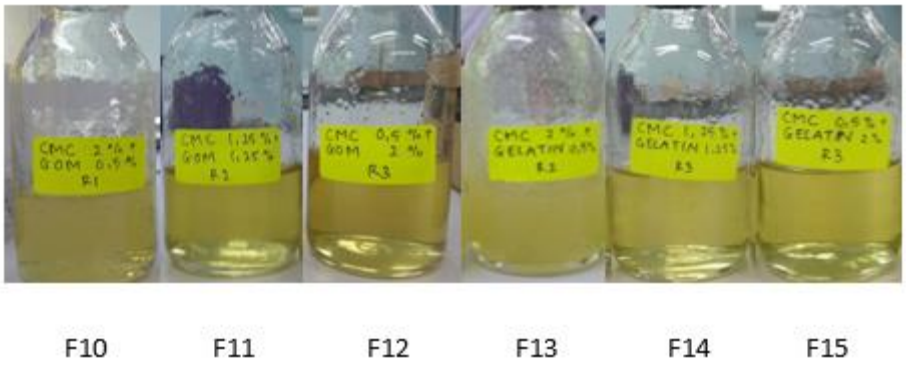

Figure 3: Citronella Gel with combination gelling agent

Homogeneity test

Every formula produced homogenous citronella ethanol extract gel, which was shown by no agglomerated particles in its dosage form. However, a different consistency was observed in higher concentration of CMC-Na (0.5%; 1.25%; 2%) (Figure 4).

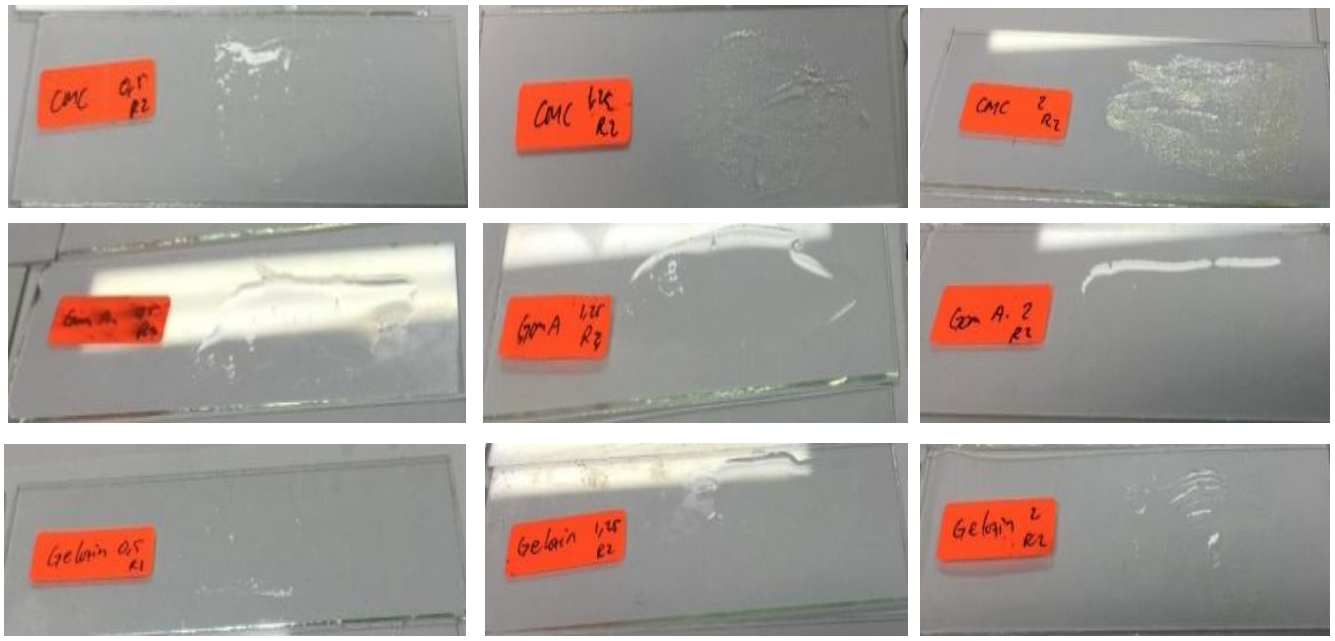

Figure 4: Homogeneity test of the formula

pH test

The pH value of each CMC-Na, gum arabic, and gelatin gel base was 7, while the base combination formulation resulted in increasing the pH value to 8. The dosage form which was safe to use on the skin without irritating was the one that had the same pH value as skin, which is between 4.5-6.5 (Draelos & Laurend, 2006). However, a pH value of 7 is still acceptable if the dosage form is proven not to cause skin irritation (Jamadar & Shaikh, 2017). Adjusting the amount of TEA affects the ph increase in the dosage form.

Spreadability test

The spreadability was influenced by the viscosity of the gel. If the gel had greater viscosity, then the spreadability of the gel was smaller (Martin, Swarbick & Cammarata, 1993). According to the evaluation results, the spreadability of F1, F2, and F3 varied inversely with the concentration of CMC-Na. The formulations with gum arabic and gelatin as gelling agents (F4-F9) showed relatively the same spreadability data despite their various concentrations. Moreover, the spreadability of gels made with gum Arabic and gelatin (F4-F9) was higher than the gels made with with CMC-Na. For the evaluation on the combination of gelling agents (F10-F15), the highest spreadability was shown by F11, which was the combination of 1.25% gum Arabic and 1.25% CMC-Na. According to the comparison of F2 and F11, the gum arabic and CMC-Na combination produced gel with higher spreadability than only using CMC-Na as a gelling agent. Based on the requirement, the spreadability of the good quality gel was between 5-7 cm; thus, it was assumed that F1, F4-F9, and F11 also had good spreadability.

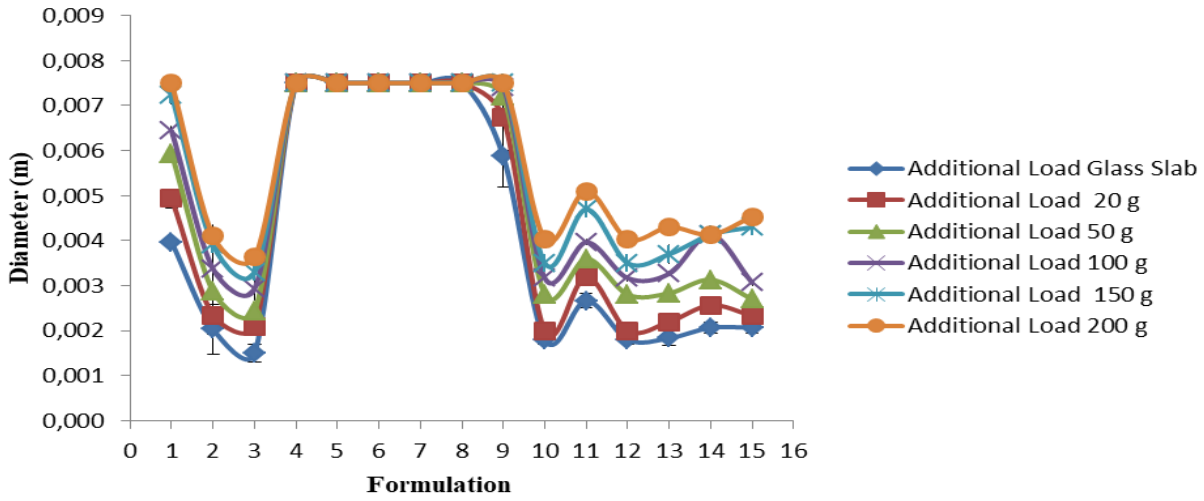

Figure 5: Spreadability test of the formula

Adhesion test

The ability of a gel dosage form to coat the skin did not interfere with the physiological function of the skin and did not completely clog the skin pores. These were the criteria for a good gel dosage form (Voigt R, 1984). The adhesion strength of a dosage form was determined by an indicator of time/duration for the active substance to exert an effect on the skin (Ansel, 1989). One of the factors that influenced adhesion was viscosity. If the gel had greater viscosity, then the adhesion of the gel dosage form was longer. The longer adhesion of the gel was expected to have a longer effect on the skin, and so the gel dosage form got better. The gel adhesion time ranged from 0.2-2 minutes for all formulations.

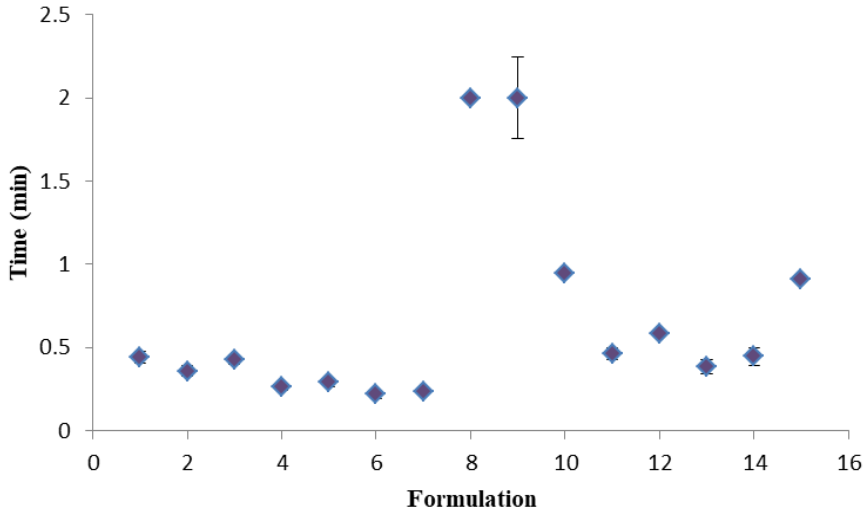

Figure 6: Adhesion test of the formula

Conclusion

Formulation development should be conducted to increase the effectiveness of a dosage form. Utilisation of different gelling agents affected the quality of the gel, and the optimum combination of gelling agents might be a better formula than the single gelling agent formula. The citronella ethanol extract gel formula that was developed in this study produced a good gel formula from the aspect of its physical appearance, pH (7 and 8), spreadability, and adhesion. However, the formula could be adjusted depending on its utilisation purpose. Moreover, a combination of gum arabic and CMC-Na as gel bases at the respective concentration of 1.25% showed an increase in spreadability. Further studies are required to determine the stability, viscosity, active compounds and antimicrobial activity of the citronella gel formula.

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IAI CONFERENCE

RESEARCH ARTICLE

Mother's knowledge and practices towards self-medication of fever among children under five years in Muncar Banyuwangi, Indonesia

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Abstract

Introduction: Self-medication is the use of medicines by individuals to treat mild symptoms or minor illnesses. It can overcome minor ailments and complaints, such as fever. Fever is a sign of disease often suffered by children under five years. Knowledge and self-medication practice of fever must be mastered well by the mother to handle this symptom correctly. **Aims:** This study aimed to explore mothers' knowledge and practices toward self-medication of fever among children under five years and the relationship between knowledge and practices. **Method:** This study was observational with a cross-sectional design. It involved 385 mothers from Muncar District Banyuwangi, Indonesia. It used a validated questionnaire to collect the data, including five categories, three for knowledge and two for practice. The relationship between knowledge and practice was analyzed by the Chi-square test. **Result:** The three categories of knowledge were good (83 respondents), sufficient (206 respondents), and insufficient (96 respondents), while the two categories of practice were good (213 respondents) and insufficient (172 respondents). The chi-square test yielded a p-value <0.001. **Conclusion:** This study revealed a significant relationship between knowledge and practice. Hence, the better the knowledge, the better the practice in fever self-medication.

Introduction

Self-medication is the use of medicines by individuals to overcome mild symptoms or minor illnesses, such as dizziness, flu, cough, pain, intestinal worms, diarrhoea, stomach ulcers, skin diseases, and others (Ministry of Health, 2007). Self-medication in Indonesia increased from 61.05% in 2014 to 71.46% in 2019 (Central Bureau of Statistics, 2020).

Fever, a symptom often treated with self-medication, is the condition when the body temperature is above >38°C. It is regulated by the hypothalamus in response to pyrogens. A high temperature leads to worse conditions. Fever above 41°C (hyperpyrexia) can cause various physiological and metabolic changes. Although it is a natural and general response of the body, fever itself is not the danger, but the underlying disease is (Adam, 2013). Fever often experienced by children makes parents uncomfortable with the situation and takes up their time and energy, especially when it is accompanied by chills, constant fussing, and crying. Several methods are used to reduce fever, from taking a fever-reducing medicine immediately to hurriedly taking the child to the doctor. Unfortunately, giving antibiotics without a precise diagnosis of the disease is common (Arifianto & Hariadi, 2017).

Handling fever with self-medication in children relies on the role of parents, especially mothers. Mother is an integral part of household management needed to take care of the children skillfully. Knowledge is a significant key, believed to have a strong relation to the practice. Raising the knowledge in fever self-medication will prevent inappropriate behavior towards the children and minimize inadequate responses to fever itself (Arica, 2011). Mothers should master knowledge about fever so that they handle it adequately.

Muncar District is part of Banyuwangi Regency with the largest population compared to other districts. It counts 133.705 people, including 9.875 children under five years old (Banyuwangi Public Health Office, 2017). A survey conducted in this region may represent other areas around. This study aimed to explore mothers' knowledge and practices toward self-medication of fever among children under five years and the relationship between knowledge and practices.

Material and method

Materials

This study used a validated questionnaire based on several studies, divided into three parts: sociodemographic, knowledge, and practice. The knowledge and practice sections were tested for validity and reliability and considered eligible (0.612 and 0.621).

Methods

This non-experimental (observational) study with a cross-sectional design involved 385 respondents from Muncar District, Banyuwangi Regency, and used convenience sampling. The data were collected at Integrated Health Post/*Posyandu* in four community health centres/*Puskesmas*. This research has received approval from the Research Ethics Committee of the Faculty of Dentistry, University of Jember (No.392/UN25.8/KEPK/DL/2019). All respondents had given their consent before participating in this study.

Data analysis

The knowledge data were divided into three categories:

1) Good if the respondent's value (x)> mean + 1 SD.
2) Sufficient if the respondent's value mean-1 SD <x <mean + 1 SD.
3) Insufficient if the respondent's value (x) <mean-1 SD.

The practice data were categorized into Good and Insufficient, using a T score. The formula for finding a T score is 50 + 10 (Z score). The Z score is obtained from the formula, Z = Mean / (Standard deviation (SD)):

1) Good if the respondent's T value> mean T.
2) Insufficient if the respondent's T value ≤ mean T.

The Chi-square test was used to determine the relationship between variables.

Results

Sociodemographics of respondents

This study involved 385 mothers who have children under five years old in Muncar District, Banyuwangi Regency. Most of them were 25-34 years old, a productive age range, and 80% were housewives with an income level below IDR 2 million. About half of them did not have an educational experience of fever self-medication in children (see Table I).

Table I. Sociodemography of respondents

Characteristic	Frequency, n=385	Percentage (%)
Age		
18-24 years old	70	18.2
25-34 years old	215	55.8
35-44 years old	100	26.0
Education		
Did not finished elementary school	26	6.8
Graduated from elementary school	64	16.6
Graduated from Junior High School	121	31.4
Graduated from Senior High School	130	33.8
Graduated from Vocational Program (Diploma)	9	2.3
Bachelor	35	9.1
Occupation		
Government employees	7	1.8
Private Employees	15	3.9
Entrepreneur	34	8.8
Retired/ Not working	9	2.3
Housewife	311	80.8
Others	9	2.3
Income		
Less than IDR 2,000,000.-	294	76.4
IDR 2,000,000.- or more	91	23.6
Educational/Counseling Experience		
Yes	183	47.5
No	202	52.5

Overview of fever self-medication

The majority of respondents (71.2%) did not have a thermometer, measured fever by putting their hands or palm against children's foreheads (70.9%), had given nonprescription fever medication (83.4%), principally paracetamol (89.5%), bought the medicine at a pharmacy (94.3%), preferred syrup as a dosage (87%), gave traditional medicine when their kid had a fever (73.5%), mainly turmeric (146; 45.1%) and shallots (129; 39.8%) (Table II).

Table II: The overview of fever self-medication

Characteristic	Frequency, n=385	Percentage (%)
Ownership of a thermometer		
Yes	111	28.8
No	274	71.2
Way of measuring fever		
Using thermometer	111	28.8
Placing a hand against forehead	274	71.2
Giving nonprescription fever medication		
Yes	321	83.4
No	64	16.6
Medicines (Respondents can choose >1 answer)		
Paracetamol	297	89.5
Ibuprofen	17	5.1
Aspirin	18	5.4
Form of Medicine (Respondents can choose >1 answer)		
Tablet	32	9.3
Powder (Puyer)	11	3.2
Syrup	300	87.0
Suppositories	1	0.3
Others	1	0.3
A place to buy medicine (Respondents can choose >1 answer)		
Pharmacy	312	94.3
Stall or Shop	5	1.5
Drug Store	14	4.2
Giving Traditional Medicine		
Yes	283	73.5
No	102	26.5
Traditional Medicines (Respondents can choose >1 answer)		
Turmeric	146	45.1
Curcuma	4	1.2
Shallot	129	39.8
Bitter Ginger (Lempuyang emprit)	2	0.6
Water of Green Coconut	37	11.4
Others	6	1.9

Respondents' knowledge and practice

Table III presents the level of knowledge and practice of mothers in fever self-medication. Most participants (80.5%) gave wrong answers about the definition of fever, 75.3% thought that antibiotics could be used as a self-medication for fever, 50.6% have never used a thermometer to measure fever, and 52.7% have never compressed a child's forehead using ice water and prefer to use warm water (Table IV).

Table III: Respondents' knowledge based on each item of the questions

Questions	Correct n (%)	Incorrect n (%)
Definition of fever, temperature >38^{o}C	75 (19.5)	310 (80.5)
Fever can cause dehydration	255 (66.2)	130 (33.8)
The duration of self-medication should not be more than three days	374 (97.1)	11 (2.9)
Antibiotics can't be used for the treatment of fever	95 (24.7)	290 (75.3)
Paracetamol can be used as a treatment for fever	350 (90.9)	35 (9.1)
Like an antibiotic, fever drug should be consumed until it runs out	253 (65.7)	132 (34.3)
Light clothing could help to reduce fever	291 (75.6)	94 (24.4)

Table IV. Respondents' practice based on each item of the questions

Question	Always n (%)	Often n (%)	Sometimes n (%)	Never n (%)
I use a thermometer to measure the fever in children*	67 (17.4)	45 (11.7)	78 (20.3)	195 (50.6)
Before giving medicines to my children, I read the dosage of the medicines and how to use the medicines on the packaging*	299 (77.7)	45 (11.7)	24 (6.2)	17 (4.4)
When my children have a fever, I prefer to compress my child's forehead using ice water rather than warm water†	69 (17.9)	52 (13.5)	61 (15.8)	203 (52.7)
I cover my children with a blanket when they have a fever†	38 (9.9)	36 (9.4)	87 (22.6)	224 (58.2)
If the fever is not cured after 3 days, I take the children to a health worker*	330 (85.7)	48 (12.5)	5 (1.3)	2 (0.5)

* positive statement, scoring: always=4, often=3, sometimes=2, never=1

†negative statement, scoring: never=4, sometimes=3, Often=2, Always=1

The relationship between knowledge and practice

The knowledge results were good (83 respondents), sufficient (206 respondents), and insufficient (96 respondents), while the practice results were good (213 respondents) and insufficient (172 respondents).

Table V. The relationship between knowledge and practice

Knowledge	Practice				*p*-value
	Good		Insufficient		
	n	%	n	%	
Good (n: 85)	62	74.7	21	25.3	<0.001*
Sufficient (n: 206)	125	60.7	81	39.3	
Insufficient (n: 94)	26	27.1	70	72.9	
Total (n: 385)	213	55.3	172	44.7	

*statistically significant relationship

Discussion

Most respondents were housewives from low-income households because the data were collected at the integrated health post (*Posyandu*), a community-based program to maintain maternal and child health in Indonesia, usually held monthly. Mothers of higher-income had low participation at *Posyandu*. Probably, they were working mothers, so they did not have time to attend *Posyandu* every month (Nazry *et al*., 2016).

About 70% of mothers placed their hands on their children's foreheads to measure temperature because they did not have a thermometer. It was not wise to rely on touch to measure fever. A thermometer is more preferred to give an objective measurement (CL Teng *et al*., 2008; Singh M *et al*., 2003).

The use of nonprescription drugs was high. It was consistent with a national survey showing that 71.76% of the population used self-medication (Central Bureau of Statistics, 2020). Paracetamol was the highest over-the-counter drug used to relieve fever, in line with a study in Pakistan that had a similar result (Aqeel et al., 2014). Aspirin is not recommended in children because of Reye Syndrome risks (Chapman & Arnold, 2020). However, it is still used by the community and was approved as an antipyretic and analgesic by the drug regulatory agency in Indonesia (Drug Regulatory Agency, 2014).

Since Indonesia is known for its rich herbal biodiversity, traditional medicines were used to cure fever. Turmeric (Curcuma domestica) contains several active compounds, such as curcumin, rich in flavonoids known to reduce fever (Chattopadhyay et al., 2004). Our results revealed that shallots were also widely used to treat fever. A previous study showed that shallots have an anti-inflammatory effect (Mohammadi-Motlagh *et al*., 2011).

Respondents had insufficient knowledge about fever definition. Fever is considered when body temperature is above 38ºC. However, it can differ based on the location of fever measurements: rectal >38 ℃, in-ear >38 ℃, mouth/oral > 37.5 C, and armpit >37.5 ℃. For clinical and research purposes, fever is often defined as a condition when the body's temperature reaches 38 ℃ or higher (Barbi *et al*., 2017). A wrong definition of fever could lead to fever phobia and unnecessary medicine use (Gunduz et al., 2016).

Self-medication for fever does not require antibiotics. Antibiotics are given to treat bacterial infections, while fever is generally caused by viral infections. The use of antibiotics can result in increased antibiotic resistance. In Jordan, a study reported that 14% of parents used antibiotics to reduce fever in their children (Athamneh et al., 2014).

Respondents with good knowledge tended to have good behavior, respondents with sufficient knowledge tended to have a good practice, and respondents with insufficient knowledge tended to have insufficient practice. These results indicated that the better the knowledge, the better the practice in fever self-medication. The Chi-square test yielded a p-value <0.001 showing a statistically significant relationship between knowledge and practice.

Conclusion

This study showed a significant relationship between knowledge and practice, indicating that the better the knowledge, the better the practice in fever self-medication. A follow-up intervention study is necessary to improve knowledge and practice of fever self-medication.

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RESEARCH ARTICLE

The comparison of the actual cost to case-mix of type 2 diabetes mellitus inpatient in Pandan Arang Boyolali hospital

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Abstract

Aim: The objective of this study was to determine the conformity of diabetes mellitus (DM) actual cost with the rate of Indonesia Case-Based Groups (INA-CBGs) among Universal Health Coverage (*Jaminan Kesehatan Nasional* - JKN) and non-JKN patients diagnosed with DM. **Methods:** This retrospective cross-sectional study was observational and analytic. It recruited DM inpatients with or without JKN. The data were analysed to identify the total actual cost of DM management among inpatients. **Results:** The results showed that the average cost for treating inpatient with diabetes mellitus at classes 1, 2, and 3 was IDR 2,677,003±906,406, IDR 2,881,023±851,483, and IDR 2,323,768±802,828, respectively. A positive difference of 378,509,126 was found between the actual cost and the INA-CBGs rate in 101 patients. There was a discrepancy between the real cost and the INA-CBGs rate for all treatment classes and the treatment levels. Factors influencing the real cost of treatment for DM were the length of stay and the severity level.

Introduction

Diabetes mellitus is a chronic disease that has become a global health problem. Several epidemiological studies showed an increased tendency of the incidence and prevalence of diabetes in various parts of the world. The World Health Organization (WHO) has categorized diabetes mellitus as a global disease. Statistical data estimated that 382 million adult people have diabetes; this number is expected to increase and reach 592 million by 2035. The growing diabetes problem presents a potential challenge to the development of health systems and economies in developing countries (Belma *et al.*, 2019).

Diabetes mellitus is known as the silent killer as it can attack all organs of the body and cause different complications, such as visual disturbances (including cataracts), heart disease, kidney disease, impotence, long-healing wounds (including gangrene), lung infections, blood vessel disorders, and stroke. Lifestyle modifications and medication intake reduce the incidence and severity of type 2 diabetes mellitus (T2DM) (American Diabetes Association, 2014).

A study conducted in 2014 estimated that in 2020 diabetes mellitus will increase the economic burden in Indonesia to exceed 1.27 billion. Health costs, the growing burden of diabetes mellitus, and the severity of rapidly evolving chronic complications have a significant long-term detrimental impact on health development and national economic growth (Finkelstein *et al.*, 2014).

Currently, the Indonesian government has implemented the National Health Insurance Programme known as JKN. The JKN programme aims to reform the health sector to overcome problems related to public health, limiting uncontrolled health costs and improving service quality (Eko Wahyu Basuki *et al.*,

2016). The Social Security Administering Body (BPJS) is a legal entity established to administer the JKN programme. The implementation of the BPJS programme in hospital services uses the INA-CBGs system (Indonesia Case-Based Groups). The INA-CBGs system is guided by the INA-CBGs rate, namely the number of claim payments by BPJS Kesehatan to health facilities for service packages based on the disease diagnosis and procedure groupings (Kemenkes, 2014).

The evaluation of the actual economic burden of the disease will provide a basis for the government for the long-term fiscal impact of chronic diseases for economic efficiency and the development of strategies, policies, or programmes on the health financing system (Zhuo *et al.*, 2013).

The economic burden of diabetes should be a concern of the National Health Insurance (JKN) implementation in the management of non-communicable diseases. Therefore, it is necessary to analyse the cost of diabetes mellitus. The objective of this study was to determine the conformity of diabetes mellitus (DM) actual cost with the rate of Indonesia Case-Based Groups (INA-CBGs) among Jaminan Kesehatan Nasional (JKN) and non-JKN patients diagnosed with DM at the Pandan Arang Boyolali hospital in 2017.

Methods

This retrospective cross-sectional study was observational and analytic with descriptive purposes. Data were collected from the medical records, the actual hospital fees, the case-mix system, and INA-CBG financing claims for inpatients with T2DM, with or without comorbidities, admitted at the Pandan Arang hospital Boyolali between January and December 2017. The data obtained were analysed using descriptive and quantitative methods. The study included JKN and non-JKN in patients with diabetes mellitus coded INA-CBGs E-4-10-I, E-4-10-II, and E-4-10-III.

Inclusion criteria

Medical record data of JKN patients diagnosed primarily with T2DM, claim files, and patient medical records with diagnostic codes INA-CBG's E-4-10-I, E-4-10-II, and E-4-10-III, complete details of claim filing fees, data for non-JKN patients diagnosed with T2DM based on details of treatment costs and classes.

Exclusion criteria

Medical record data of patients who died, patients discharged against medical advice, and incomplete patient data.

Results

Patient characteristics (Table I), according to the gender distribution, showed that females outnumbered males (60.4% in JKN patients and 66% in non-JKN patients).

Table I: Patients' characteristics

Characteristics	JKN patients		Non-JKN patients	
	n	%	n	%
Age				
12-25	4	3.96	-	-
26-45	6	5.94	5	11.36
46-65	67	66.33	23	52.28
>66	24	23.77	16	36.36
Gender				
Male	40	39.6	15	34
Female	61	60.4	29	66
Secondary diagnosis				
E11.0 (diabetes mellitus)	18	40.91	30	29.71
G73.0 (amyotrophic)	2	4.55	7	6.93
G63.2 (polyneuropathy)	1	2.27	3	2.97
H36.0 (retinopathy)				
H28.0 (cataract)				
N08.3 (diabetic nephropathy)	9	20.45	14	13.86
G99.0 (autonomic nephropathy)	1	2.27	6	5.94
M14.2 (diabetic arthropathy)	2	4.55	11	10.89
M14.6 (neuropathic diabetic arthropathy)	3	6.82	15	14.85
I79.2 (peripheral angiopathy)	3	6.82	5	4.95
N08.3 + I79.2	1	2.27	3	2.97
N08.3 + G99.0	2	4.55	2	1.98
N08.3 + M14.2	1	2.27	1	0.99
N08.3 + H28.0			1	0.99
N08.3 + G73.0			1	0.99
N08.3 + M14.6	1	2.27	2	1.98
G99.0 + I79.2	1	2.27		

These results were in line with those of Masni (2013), where the number of female patients with diabetes mellitus was greater than that of males (66.7% and 33.3%, respectively). According to Mauvais-Jarvis (2017), women are at higher risk of developing diabetes because, physically, they are more likely to increase their body mass index due to hormonal processes, i.e. monthly cycle syndrome (premenstrual syndrome) and post-menopause, both facilitating the accumulation of body fat and favouring the occurrence of T2DM in women. This finding is in line with that of Hein and the

authors (2018), reporting a higher incidence of diabetes among women. Regarding age, most participants with T2DM belonged to the 46-65 years age group (66.33% of JKN patients and 52.28% of non-JKN patients). Most diabetes patients were 40-59 years old, and 80% of patients in this age group were from developing countries, such as Indonesia. However, people less than 45 years old can have diabetes (Yosmar *et al.*, 2018). The results of this study were in line with those of the Association (2013), reporting that age over 45 years was a risk factor for diabetes mellitus due to a poor lifestyle, including unhealthy diet, lack of exercise, and lack of rest. With age, the risk of developing diabetes mellitus increases due to decreased glucose tolerance associated with reduced sensitivity of peripheral cells to the effects of insulin (Wahyuni *et al.*, 2012).

Patients with T2DM, particularly hospitalised patients, are mostly followed for comorbidities and complications in the secondary diagnosis due to long-time uncontrolled blood sugar levels during prediabetes. The most common complication is neuropathy, i.e. distal symmetric polyneuropathy, also known as neuropathic diabetes. Neuropathic diabetes is a sensory loss that starts distally and is characterized by the onset of pain (Feldman *et al.*, 2019). The findings from this study showed that most patients with T2DM had one or two secondary diagnoses. Most of the patients with T2DM have at least one complication (Zheng *et al.*, 2018).

Usually, hospitalised T2DM patients have various comorbidities and complications due to long-time uncontrolled blood sugar levels (Wahyuni *et al.*, 2012). Increased mortality and morbidity of these patients are caused by different macrovascular and microvascular complications that develop during the disease, especially when glucose control is poor. At the macrovascular level, T2DM patients experience hypertension and systemic heart disease more facilely. Tissue damage at the microvascular level is a primary factor in the progression of diabetic nephropathy and neuropathy (Association, 2013).

Table II shows that, among non-JKN patients, the highest average length of stay (LOS) was 1-4 days (26 patients, 59.09%), followed by 5-8 days (15 patients, 34.09%), and >8 days (3 patients, 6.82%). This finding indicates that T2DM patients without a secondary diagnosis had the lowest average LOS as they were treated at the Pandan Arang Boyolali Hospital, and all participants recruited from this site had no comorbidities. LOS is an essential indicator in determining the success of therapy for diabetes mellitus patients; it is related to the cost of care incurred by the patient. The shorter the time a patient is hospitalised, the more effective and efficient the service from the hospital is (Salim *et al.*, 2019). Predictor factors that influence LOS are patient characteristics, clinical conditions, medical action, patient management, and hospital administration problems (Lubis & Susilawati, 2018).

Table II: Length of stay distribution characteristics of JKN and non-JKN patients in 2017

Type	Characteristics	n	(%)
Non JKN	1-4 days	26	59.09
	5-8 days	15	34.09
	> 8 days	3	6.82
JKN	1-4 days	68	67.32
	5-8 days	32	31.69
	> 8 days	1	0.99

Real cost compatibility with INA-CBG rates

Based on the results obtained in Table III, there is a positive difference between the actual cost and INA-CBG rates at all severity levels because the INA-CBGs rates are higher than the actual cost.

Table III: The difference between total real cost and INA CBG rates for classes 1,2 and 3 at severity level I / II / III

Class	Category	n	Total actual cost (IDR)	Total INA-CBGs rates (IDR)	Difference (IDR)
1	E-4-10-I	9	16,481,701	46,267,200	29,785,499
	E-4-10-II	16	38,573,062	114,932,800	76,359,738
	E-4-10-III	14	49,348,351	124,822,600	75,474,249
2	E-4-10-II	12	27,034,770	73,885,200	46,850,430
3	E-4-10-I	14	16,770,430	51,408,000	34,637,570
	E-4-10-II	18	32,584,700	87,225,300	54,640,600
	E-4-10-III	18	41,134,960	101,896,000	60,761,040

The highest difference was at the second severity level IDR 76,359,738, because this level II included 16 episodes of treatment. Patients with severity II required lower medical costs and a shorter LOS than those from level III who had a more complex condition. The high difference found in class 1 severity levels II and III, reaching IDR 76,359,738 and IDR 75,474,249, was due to the use of the average cost of T2DM in these levels (IDR 2,410,816 and IDR 3,524,882, respectively) – see Table IV. Regarding patients at the Pandan Arang Boyolali hospital, the complications occurred during their hospitalisation, and they were treated accordingly. Thus, the INA-CBGs package automatically moved according to the rate of the most widely used treatment. Also, the smallest difference (IDR 29,785,499) was seen at the first level of severity because this level had nine episodes of treatment. This finding indicates that patients with severity III required more medical costs and a longer LOS, incurring more costs and less cost difference. Salim and the authors (2019) reported a significant difference in the average LOS between T2DM patients who experienced complications and those who did not. The difference in LOS affected the costs incurred by patients undergoing treatment in the hospital. The difference in the cost of therapy for T2DM patients with complications was influenced by the type of complication, the number of episodes of patient visits, and the different drugs used for each complication (Kusuma *et al.*, 2019).

Table IV: The comparison between average real cost and INA-CBG rates for classes 1, 2, and 3 at the I / II / III severity levels

Class	Level of care	Cost	Average (IDR)	Min (IDR)	Max (IDR)	*p*
1	E-4-10-I	Actual cost	1,831,300	1,010,315	2,949,902	0.0001
		INA CBGs Cost	5,140,800			
	E-4-10-II	Actual cost	2,410,816	1,594,440	3,359,498	0.0001
		INA CBGs Cost	7,183,300			
	E-4-10-III	Actual cost	3,524,882	2,529,622	4,791,366	0.0001
		INA CBGs Cost	8,915,900			
2	E-4-10-II	Cost Real	2,252,897	1,653,970	3,724,285	0.0001
		INA CBGs Cost	6,157,100			
3	E-4-10-I	Actual cost	1,197,887	705,760	1,543,379	0.0001
		INA CBGs Cost	3,672,000			
	E-4-10-II	Actual cost	1,810,261	1,200,681	2,834,460	0.0001
		INA CBGs Cost	5,130,,900			
	E-4-10-III	Actual cost	2,285,275	1,195,902	3,186,447	0.0001
		INA CBGs Cost	6,368,500			

This study showed a positive difference between the actual cost and INA-CBGs rates due to the conformity of medical procedures with the standards for an efficient and effective impact on patients and hospitals. The results of this study were consistent with those of Sari (2014), stating that the positive difference between actual costs and INA-CBGs rates demonstrates there was an effort to save on financing services for both hospitals and patients.

The INA-CBGs rates package provided was higher than the average actual cost of hospitalisation, serving the hospital because it had succeeded in providing therapy to patients effectively and efficiently. The remaining claims obtained by the hospital could be used: first, to cover or cross-subsidise patients whose total actual cost exceeded that of the INA-CBGs package and, second, as revenue for the hospital itself. The results of this study were in line with those of Sari (2014), who stated that the average actual cost of the I / II / III severity levels was lower than the INA-CBG package.

The treatment performed on JKN and non-JKN patients had differing costs, higher in non-JKN patients, lying in the medical action, including the charge of doctor's visits and the treatment. Besides, there were also differences in the costs of drugs and medical devices incurred by non-JKN patients, which were higher than those of JKN patients. In non-JKN patients, doctors could select more diverse drugs than JKN patients who were prescribed medications only from BPJS claims. The results of this study were in line with those of Islam & Rusdi (2014), reporting that the cost of inpatient treatment for T2DM patients was higher for non-JKN patients.

Factors affecting the actual cost

Table V shows a significant relationship between the severity level and LOS. It also reveals a significant relationship between severity levels and actual costs (p = 0.0001), with a moderate correlation (r = 0.600). The higher the severity of the patient, the longer the treatment needed. Therefore, patients received more treatments resulting in more costs, including supporting examinations, medical treatment, and hospitalisation. Overall, the services provided increased the total actual cost received by patients (Labovitz *et al.*, 2016).

Table V: Results of the bivariate correlation analysis of factors affecting the real cost of inpatient diabetes mellitus treatment

Factor	n	*r*	*p*
Age	101	0.152	0.129
Secondary diagnosis		-0.047	0.640
Severity		0.600	0.0001
LOS (Length of stay)		0.352	0.0001
Gender		0.018	0.855

Regarding LOS, the values of p = 0.0001 and r = 0.352 indicate a significant relationship between the secondary diagnose and the actual cost and a weak correlation. This result is in line with that of Juaella (2013), reporting that the longer the LOS, the more medical treatment performed, the more medicines needed to overcome the disease, and the more supporting examination costs, drug costs, and accommodation costs. Therefore, as a whole, it could increase the total actual cost.

Conclusion

Average actual costs of type 2 diabetes mellitus from the perspective of Pandan Arang Boyolali Regional Hospital for JKN patients for classes 1, 2, and 3 is 2,677,003 RP, 2,881,023 RP, and 2,323,768 RP, respectively. For non-JKN patients these costs are 3,301,001 RP, 2,597,435 RP, and 2,841,569 RP for classes 1, 2, and 3, respectively (Table VI).

For the actual cost with the INA-CBG rate, there is a difference in the total cost of JKN inpatients, which is significantly different among 101 patients. The actual cost at class 1 level I / II / III is 1,831,300 RP, 2,410,816 RP, and 3,524,882 RP, respectively, while, at class 1, the second level of severity was 2,252,897 RP, and, at class 3, the I / II / III severity levels were 1,197,887 RP, 1,810,261 RP, and 2,285,275 RP, respectively. This difference shows a positive indication, where the total actual cost is lower than the INA-CBG rate. Meanwhile, for the 44 non-JKN patients, the average actual costs at classes 1, 2, and 3 were 3,301,001 RP, 2,597,435 RP, and 2,841,569 RP, respectively.

Factors affecting the actual cost are LOS and the severity of type 2 diabetes mellitus coded INA-CBGs E-4-10.

Table VI: Comparison between the average actual cost of JKN and non-JKN patients

Type of payment	Average (IDR)	±SD	Min (IDR)	Max (IDR)	*p*
Class 1					
JKN	2,677,003	906,406	1,010,315	4,791,366	0.102
Non JKN	3,301,001	1,589,700	1,419,904	8,976,332	
Class 2					
JKN	2,881,023	851,483	1,653,970	3,724,285	0.810
Non JKN	2,597,435	736,575	1,310,853	3,993,891	
Class 3					
JKN	2,323,768	802,828	705,760	3,186,447	0.0001
Non JKN	2,841,569	2,889,817	990,030	10,593,021	

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IAI CONFERENCE

RESEARCH ARTICLE

Role of pharmacist in providing drug information and education for patients with chronic diseases during Transition of Care

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Keywords
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Abstract

Introduction: Community pharmacist can play an active role in improving care for recently discharged patient through provision of information and education and more importantly prevent readmission to hospital. **Aim:** This study aims to investigate the impact of pharmacist providing drug information and education for discharged patient with chronic disease. **Methods:** A survey was conducted between July to October 2019 involving 153 patients with hypertension and diabetes mellitus. Patients were purposively recruited from 11 pharmacies in East Java. A questionnaire was used to record patient's opinion regarding provision of drug information and education by pharmacist. The results were descriptively analysed. **Results:** Overall, patients mentioned that pharmacists mainly provided information about how to use the drugs (83%). The education provided by the pharmacist has improved patients' understanding about their disease state (70%) and influenced the compliance when using the medicine (68%). Patients acknowledged pharmacist's effort to ensure the positive outcome of the therapy. However, no data has been recorded whether such service may prevent patients from being readmitted to hospital. **Conclusion:** Pharmacist is at unique position in the transitions of care. Pharmacist can provide information and education that may contribute to improve patient's understanding and compliance.

Introduction

According to Law number 36 of 2009 on health, pharmaceutical practices that include manufacturing along with quality control of pharmaceutical preparation, security, procurement, storage and distribution of the drug, prescription-based drug service, drug information service and development of drug, medicinal ingredient and traditional medicine must be carried out by health workers who have expertise and authority in accordance with the provisions of law and regulation (Ministry of Health Indonesia, 2009). The health workers who have expertise and authority mentioned refers to the pharmacist. Management of pharmaceutical preparations in the health care system must be based on the needs and characteristics of the health service facility, which aims to minimise the cost and maximise the level of service to the community (Saha & Ray, 2019). Therefore, the pharmacist must always consider various characteristics of health facilities, including the culture of the people who use the health facility, both in normal condition and in the condition of the health crisis. In times of crisis, pharmacists are responsible for coordinating the supply of medicines and ensuring access to safe and quality drug supplies that are efficacious (Lemtiri *et al.*, 2020), with an amount that matches the needs of the health service facility.

Pharmacy, as one of the practice places for pharmacists, also becomes a strategic health facility as a partner for medical personnel or other health facilities to prepare drugs in that place. Therefore, it is necessary to have good cooperation between pharmacist in the pharmacy with other health workers in the hospital or other health facilities, especially in the time of health crisis. Hence, this strong relationship will be able to solve problems related

to optimal management of health products which is the main challenge during a health crisis such as a pandemic (Lemtiri *et al*., 2020). All pharmacies and supply chain leaders must have a strategy to address shortages and ensure an adequate supply of all drugs used, especially those used for a lifetime treatment by patients (Badreldin *et al*., 2020).

In addition to the management aspects as mentioned above, another responsibility of pharmacist in pharmacy is clinical pharmacy service as stated in the regulation of the Minister of Health of the Republic of Indonesia number 73 of 2014 on the standard of pharmaceutical service in pharmacy (Ministry of Health Indonesia, 2016). Through the clinical pharmacy service, a pharmacist in pharmacy must also ensure the success of patient therapy with roles such as disease prevention and infection control, adequate storage and supply of drugs, patient care, and support for health workers. The main responsibility of a pharmacist is to provide drug information to the health professional as well as to counsel patients (Visacri, Figueiredo, & Lima, 2020). The knowledge and understanding of the role of a clinical pharmacist by doctors need to be improved to build effective collaboration between pharmacists and other health workers. Therefore, it is necessary to advocate health service providers and stakeholders about the role of clinical pharmacy service in providing drug therapy management (Alhossan & Alazba, 2019).

Over the past few years, the pharmacist's role has evolved from a provider of the drug to a provider of service and information, and finally to a provider of patient care by actively participating in the treatment process. The pharmacist's next task is to ensure that the patient's drug therapy is indicated properly, is available most effectively, is the safest, and is comfortable for the patient (Merks *et al*., 2020). Thus, in fact, there are many roles of pharmacist in the aspect of clinical pharmacy service that can be done in order to increase the success of therapy, such as, a pharmacist can ensure that interaction between drugs occurs through prescription study, especially for a patient who gets a lot of drugs such as a psychiatric patient. In particular, prescription for a patient receiving multiple drugs needs to be thoroughly verified and examined, and pharmacists dispensing these drugs must ensure that drug interaction does not exist (AlRuthia *et al*., 2019).

Pharmacist in pharmacy is at the forefront of improving patient health, identifying treatment-related problems, and being involved in a decision about medication (Costantino, 2020). Several studies have shown that pharmacist intervention in a patient with chronic kidney disease leads to decreased Drug Related Problems (DRPs), polypharmacy, improved anaemia management, blood pressure, and quality of life (van Berlo-van de Laar *et al.*, 2020). Most of the drug-related problems are substantially reduced (Liu *et al*., 2020) with the involvement of community pharmacist in the treatment of the patient with chronic disease, in which this is also part of the pharmacist's role. The involvement of pharmacists in the community in diabetes mellitus service management programs is seen to be very beneficial by the program manager and the patient (Thakur *et al*., 2020). Therefore, to maintain health, this group of vulnerable patients is highly dependent on the expanding role of the community pharmacist in the management of chronic disease (Okoro, 2020).

Intervention by pharmacists in long-term care improves treatment outcomes by increasing medication suitability, reducing drug amounts, and leading to lower treatment costs (Sadowski *et al.*, 2019). A prospective study conducted by Chiarelli *et al.* (2020) related to the evaluation based on the integrated expertise of pharmacist and doctor confirms that drug-related problem often occurs in multi-comorbid elderly patients who are hospitalised so that integrated collaboration between pharmacist and doctor is needed in the process of optimising treatment (Chiarelli *et al*., 2020). The proportion of claims associated with administering the wrong drug and administering the wrong dose has decreased, indicating that pharmacists and medical personnel have made an improvement to reduce this type of medication error through various technological development efforts (Reiner, Pierce, & Flynn, 2020). Empirical data from several studies in community pharmacy show that medication management by a pharmacist leads to the identification of many Drug Therapy Problems (DTP) and positive responses from various parties to pharmacists for their success in solving DTP. Even when no DTP is identified, the patient still benefits from counselling and education during a visit to a community pharmacy (Salmasi *et al*., 2020).

Counselling, information, and education (CIE) by a pharmacist in community pharmacy is needed, considering that the success of therapy, especially in chronic disease patients, is largely determined by patient adherence to medication and drug use. Low adherence to treatment and lack of knowledge about the disease are the main factors for treatment failure (Chandrasekhar *et al.*, 2020). Providing information done by pharmacists can improve patient understanding of the disease and its treatment (Hutami & Rokhman, 2013), which is expected to affect the outcome of therapy for the patient.

So far, research data related to CIE by a pharmacist in community pharmacy are general. This means that the data are obtained based on all characteristics of patients who come to the pharmacy. For the benefits of CIE for a patient suffering from chronic disease for participants of the Social Security Agency (BPJS), specifically for contribution assistance participants (PBI) at a return-referral pharmacy, namely pharmacy that collaborates

with BPJS to serve return-referred patients, there has not been much research done. PBI patients are poor and incapable patients who previously do not adhere to the treatment due to constraints on cost. Since the enactment of the National Health Insurance, which is managed by BPJS, they have received contribution assistance from the government so that they can run free medical treatment at health service facilities in collaboration with BPJS and can also get free medicine from pharmacies that work with BPJS. Since they rarely do medication and rarely come to the pharmacy, this group of patients is unlikely to be exposed to such information by a pharmacist in the pharmacy. Thus, this research aims to "Investigate the impact of a pharmacist providing drug information and education for a discharged patient with chronic disease".

Methods

Study design

This study used a cross-sectional observational design, as the respondents were patients with chronic diseases, especially diabetes mellitus and hypertension, who obtained drugs at five independent pharmacies serving return-referral patients categorised as PBI participants in BPJS in East Java with the following inclusion criteria:

1. Patients with diabetes mellitus or hypertension;
2. Took medicine from July to October 2019;
3. Able to read and write;
4. Able to speak Bahasa Indonesia;
5. Willing to be research respondents;
6. Categorised as PBI participants in BPJS.

Ethics approval was not deemed necessary as the survey population did not include any-risk groups, and anonymity was assured to all participants. In addition, signed informed consent was obtained from all participants prior to data collection.

Sample

The sample size was 153 patients, the sample size (n) was calculated using the simple random sampling formula with notation N (total population size), P (population proportion), and d (degree of error) as shown below (Ogston *et al.*, 1991):

$$\hat{Var}(\hat{P}) = \frac{P(1-P)}{n} \times \frac{N-n}{N-1}.$$

Z = 1.96, P = 0.5, d = 0.05, N = 252 people with diabetes, so n = 1 52.42 = 153 respondents

The sampling technique was purposive sampling.

Research variables

The variables were the type of information provided by the pharmacist to the patient, the patient's understanding of the condition and the patient's adherence to consuming the drug. In addition, the independent variable was the patient's understanding, while the dependent variable was the patient's adherence to consuming the drug.

Research instrument

The instrument used in this study was a questionnaire. Before being used, the questionnaire was first tested for its validity and empirical reliability. Then the result of the questionnaire trial was analysed with statistics to determine the validity and reliability of the instrument. The instrument was declared valid if the correlation coefficient was > 0.3 and reliable if the Alpha Cronbach value was > 0.6 (Priyastama, 2017). The results of the reliability and validity tests showed that the instrument was valid with a correlation value greater than 0.3, namely from the nine statement items. The lowest correlation value was 0.421, and the highest was 0.844, with an Alpha Cronbach value of 0.822.

Data analysis

To determine the effect of respondent understanding on medication adherence, statistical analysis was performed using chi-square.

Results

In this study, the gender of the respondents showed that the majority was women, with 72.5%, as shown in Table I. The majority of respondents' ages ranged from 41 - 50 years old with 37.9%, 51 - 60 years old with 26.8%, and above 60 years old with 29.4%, as presented in Table I. As seen, the patients of chronic diseases range from a very productive age, even starting at the age of under 40 years.

Table I: Characteristics of respondents

Characteristics	Frequency (n: 153)	Percentage (%)
Gender		
Female	111	72.5
Male	42	27.5
Age (years)		
≤ 40	9	5.9
41 – 50	58	37.9
51 – 60	41	26.8
> 60	45	29.4
Smoking habit		
Not smoking	137	89.5
Smoking	16	10.5
Comorbidity		
Diabetes mellitus	110	71.9
Hypertension	43	28.1

In this study, there were several materials that were asked of respondents regarding the information provided by the pharmacist to the patient when taking drugs at the pharmacy. Table II showed that of the six information provided by the pharmacist to the patient, based on the respondent's answer to the questionnaire given, the most frequent information related to how to use the drug was 83% of respondents answered always. Next was information related to the importance of adherence in using the drug to control the illness, which was 71.9%.

Table II: Opinions of respondents related to information provided by the pharmacist at the pharmacy

Statements	Respondents' answers (%)		
	Never n (%)	Occasionally n (%)	Always n (%)
The pharmacist explains the right place to find safe, qualified, and efficacious medicine	99 (64.7)	52 (34)	2 (1.3)
The pharmacist explains how to use the drugs in each drug given to you	0 (0)	26 (17)	127 (83)
The pharmacist explains how and where to store drug in your home	13 (8.5)	68 (44.4)	72 (47.1)
The pharmacist explains how to destroy/dispose of the drug if there is a broken or expired drug at home	63 (41.2)	72 (47)	18 (11.8)
The pharmacist explains that your illness requires long-term treatment	7 (4.6)	51 (33.3)	95 (62.1)
The pharmacist explains the importance of adherence in using drugs to control the disease you are suffering from	2 (1.3)	41 (26.8)	110 (71.9)

Respondents' understanding of all information conveyed by pharmacists, and specifically related to understanding the need for long-term treatment for the diseases they suffer from, the majority of respondents said they understood, with the percentage of 70% respectively as shown in Table III. Respondents adherence to using drugs after getting information from pharmacists increased; this was indicated by the respondent recognition of the questionnaire answers in using drugs; 68% answered obediently.

Table III: Respondents' understanding and adherence in using drugs

Question	Respondents' answers	
	No, n (%)	Yes, n (%)
Do you understand everything the pharmacist explains?	46 (30)	107 (70)
Do you understand that your illness requires long-term treatment?	46 (30)	107 (70)
Are you obedient in using the medicine after getting information from the pharmacist?	49 (32)	104 (68)

Discussion

The result of this study should be a concern, especially for health workers, to always provide education and counselling to the community so that they can live a healthy lifestyle, given that as the main factor in the occurrence of chronic diseases such as diabetes mellitus and hypertension and cardiovascular is a lifestyle (Cokro *et al.,* 2018).

Several other similar studies also have similarities related to age range, as in research conducted by Alghamdi and the authors (2017). This is probably because women are usually more diligent in carrying out a health check at health care facilities both to the public health centre and to the hospital so that more women were chosen as respondents. This study is in accordance with several previous studies that stated that the majority of patients with chronic disease, especially diabetes mellitus (DM), are women, as in the study of Silarova and the authors (2018) and research by Moawad and the authors (2014).

The majority of hypertensive patients are also women, according to the research result of Athiyah and the authors (2019). Smoking is a risk factor for health problems in the community because it is associated with the incidence of several diseases, including hypertension (Sohn, 2018). In several studies, it has been shown that smoking will increase the incidence of stroke in hypertensive patients (Safiri & Ayubi, 2017). In addition, smoking is also a cause of failure to control blood sugar for DM patients and also contributes to the initial incidence of DM-related complications in addition to the direct toxic effects of smoking (Gerber *et al.*, 2013). The results of this study indicated that the majority of respondents did not smoke, with 89.5% as in Table I. This was because the majority of respondents studied were women. It is generally known that usually women do not like smoking (Athiyah *et al.*, 2019). If we look at the number of male respondents and respondents who smoked in Table I, it showed that not all male respondents smoked because the number of

smokers was only 16 respondents while the number of male respondents was 42 people.

Meanwhile, at least the information provided by the pharmacist was related to the right place to get safe, quality, and efficacious drugs. This condition occurs because pharmacists think that the success of therapy is greatly influenced by adherence and the correct use of drugs (Rahem *et al.*, 2019) so that the informational pressure point is directed at the correct use of the drug by the pharmacist. In addition, the failure of therapy and the emergence of side effects of treatment in a patient with chronic diseases such as diabetes mellitus and hypertension is because patients often forget to take medication (Stanton-Robinson *et al*., 2018), so that pharmacist considers this information as a part that must always be conveyed to the patient to improve therapy outcomes. While related to the information where the place to get the medicine is safe, qualified, and efficacious, perhaps the pharmacist thinks it is not too principle because this patient is a BPJS participant with the PBI category, which is believed by their pharmacist to take the drug at the pharmacy because in the pharmacy they get the medicine for free.

To determine the effect of understanding medication adherence, chi-square statistical analysis was performed. The results of statistical analysis showed that the value of p = 0.0001, meaning that the respondents' understanding of how to use the drug had a significant effect on medication compliance. This study is in accordance with the results of research by Rahem and the authors (2019) and Stanton-Robinson and the authors (2018), which present that information affects understanding and subsequently affects adherence to drug use. As previously explained that medication adherence has the potential to increase the success of therapy, so providing information by the pharmacist in this study has a great opportunity to control blood sugar in people with diabetes and control blood pressure in people with hypertension. This study is also in accordance with the results of research by Suradnyana and the authors (2018), which state that the provision of education has an effect on the knowledge of patients with diabetes and affects adherence to drug use.

Conclusions

Pharmacist is at a unique position in the transitions of care. Pharmacists can provide information and education that may contribute to improving patient's understanding and adherence.

The community pharmacist is the last health worker a patient sees because, after that, the patient goes straight home. For this reason, patients' understanding regarding treatment information becomes very critical. Therefore, it is advisable for all pharmacists in community pharmacies to always provide education and information to patients who come to the pharmacy.

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IAI CONFERENCE

RESEARCH ARTICLE

Proximate analysis on animal feed granules composed of raw material from fish innards wastes

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Abstract

Introduction: Fish innards contain 14.01% protein, 20% lipid, 4.75% ash, and 60.62% water. Fish innards are formulated into granules for practicality in their application as animal feed. **Aim:** This research on the proximate analysis of animal feed granules composed of raw material from fish innards wastes used a descriptive quantitative method. **Results:** The result indicated that the water content measured using the thermogravimetric method was 6.62%, the ash content observed using the dry ashing method was 10.25%, the protein content checked using the biuret method was 37.03%, fat content using the soxhlet method was 6.13%, and carbohydrate content measured using phenol sulfate method was 26.14%. **Conclusion:** These findings show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfill the regulation of animal feed content based on SNI-8509-2018.

Introduction

Fish is one of the food sources with many nutritional benefits. It is rich in essential amino acids, unsaturated fats, vitamins, and minerals and is easily digested (Wibowo & Darmanto, 2014). Besides producing useful products to fulfil food, industry, and daily needs, fish production also generates wastes, reaching up to 500,000 tons each year (Harianti, 2012). Wastes resulting from the fish industry consist of fish that are no longer good to consume or process, fish innards, and other non-commercial parts. These wastes pile up every day due to the lack of skills of the people handling them (Komariyanti & Surachman, 2018). These unutilized fish wastes can cause environmental pollution (Hildawianti, Vanny & Abram, 2017) as they become ideal media for microbes to grow, causing unpleasant odours (Jayanti, Herpandi & Lestari, 2018).

Fish innards are wastes resulting from the fish industry that, if not used, will cause harm to the environment, health, and economy (La Apu, 2017). In Indonesia, several types of research were conducted on the use of fish innards, one of which is to transform fish processing wastes into animal feed (Komariyanti & Surachman, 2018). Other research explored the utilization of fish waste as animal feed raw material (Sihite, 2013).

The formulation of artificial animal feed is based on the producer's considerations. Animal feed production should acknowledge animal nutritional needs, sources, raw material quality, and economic value (Niode & Nasriani & Irdja, 2017). Based on SNI-8509-2018, content requirements of quality animal feed are as follows: 12.00% water (max), 14.00% ash (max), 16.00% crude protein (min), and 14.00% carbohydrates (min) (Standar Nasional Indonesia, 2018). Based on several studies in Indonesia, which found protein and fat contents in fish innards and used them as animal feed, this research aims to do a proximate analysis on animal feed granules composed of raw material from fish innards wastes.

Method

The material used was fish innards, thick H_2SO_4, H_2SO_4 52%, aqua dest, alcohol 95%, petroleum ether, glucose, phenol, biuret reactor, and BSA (Bovine Serum Albumin). Formula modification of other researchers entitled: "Optimization of Tapioca Flour and Molasses Flour in Cat Food and Dog Food Pellets From Fish Innards Wastes Raw Material with the Factorial Design Method".

Ash content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed (B1). Five grams of sample were put into the formerly weighed cup and burned on a Bunsen burner, a smokeless stove. It was then put into an ashing furnace to be burned at 400°C until greyish ash was obtained or the sample had fixed weight. Then the furnace temperature was increased to 550°C for 12-24 hours. The sample was then cooled in a desiccator for 30 minutes and weighed (B2) (Hafiludin, 2011). Ash content can be calculated as follows:

$$\text{Ash content (\%)} = \frac{\text{B2}-\text{B1}}{\text{sample weigh}} \text{ x } 100\%$$

Water content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed. Five grams of sample (B1) were weighed on the cup and then dried in an oven at 100-105°C until the fixed weight was obtained (8-12 hours). The sample was then cooled in a desiccator for 30 minutes and then weighed (B2) (Hafiludin, 2011). Water content can be calculated as follows:

$$\text{Water content (\%)} = \frac{\text{B1}-\text{B2}}{\text{Sample weight}} \text{x } 100\%$$

Protein content determination

Standard solution preparation

One gram of Bovine Serum Albumin (BSA) was dissolved in distilled water in a volumetric flask 10 mL up to the designated mark to obtain a standard solution of 10% W/V.

1. Optimum wavelength determination
   Five per cent BSA Standard solution was put in a test tube by sampling 2.5mL of BSA d with 0,8 mL biuret reactor, and distilled water was added to make a total of a 5 mL solution. The solution was allowed to sit and react for ± 10 minutes; the absorption was then measured at a wavelength of 450-600 nm. The maximum absorption of the wavelength was recorded.
2. Standard curve making
   Six test tubes were prepared. The first one was filled with a blank solution (solvent), while the five others were filled with BSA standard solution at concentrations of 1, 2, 3, 4, and 5%, and completed with 0.8mL aqua dest to a total volume of 5 mL. The solution was allowed to sit for 10 minutes, and then each absorption was measured using a UV-VIS spectrophotometer at the maximum wavelength.
3. Measurement of the protein content of the sample
   Each sample weighing 25 grams was put into a beaker added with 250 mL of distilled water, smoothly ground, and filtered with a filter paper.

Protein content measurement was carried out as follows: 2.5 mL of protein sample was added to 0.8 mL of biuret reactor and completed with distilled water to a total of 5 mL solution. The solution was then vortexed let sit for 30 minutes to make a perfect purple. The maximum absorption of the wavelength was measured and recorded (Keppy & Allen, 2016).

Protein content calculation was obtained by:

$$\text{Protein content} : \frac{\textbf{Protein Weight}}{\textbf{Sample Weight}} \textbf{ x } \mathbf{100}\%$$

Protein weight: Sample volume x Protein concentration of a sample

Fat Content Determination

A round bottom flask was made fat-free using alcohol 95%, and 3 grams were wrapped with a filter paper and put into a Soxhlet tool. Then 200 mL of petroleum ether was put into the round bottom flask, and the Soxhlet toolset was connected to continue with sample filtering for 8 hours until the sample became clear. The solvent in the round bottom flask was evaporated until almost dry. Then it was put into an oven at 100°C for 30 minutes, then cooled in a desiccator for 30 minutes. The fat was then weighed (Suriani, 2015).

The fat content calculation was obtained by:

$$\text{Fat content (\%)} = \frac{\textbf{(B}-\textbf{A)}}{\textbf{Sample weight}} \textbf{x } \mathbf{100}$$

Carbohydrate content determination

Sample preparation

One gram of animal feed pellet sample was added to 10 mL of aqua dest while stirring, then 13 mL H_2SO_4 52% were added while stirring for 20 minutes using a magnetic stirrer and put into a test tube. Aluminium foil was used as a lid to cover the tube. One hundred mL of aqua dest were added and filtered into a 250 mL volumetric flask then aqua dest was added to the volumetric flask up to the designated mark.

Carbohydrate content measurement

Standard glucose solution was prepared at concentrations of 0, 100, 200, 300, 400, and 500 ppm. Each solution measuring 0.5 mL was put into separate flasks, then soaked in water, then 0.5 mL of phenol 5% and 2.5 ml of thick H_2SO_4 were added carefully and slowly along the wall of the flasks. Those solutions were allowed to sit for 10 minutes, then vortexed before being allowed to sit for 20 minutes. The absorption was then measured using a spectrophotometer at the wavelength of 490 nm. The linear equation was then made as a standard curve. Sample measuring was done by putting 0.5 mL of sample solution into a flask, then soaking it in the water, then adding 0.5 mL of phenol 5% and 2.5 mL H_2SO_4 slowly. The following process was the same as it was with a standard glucose solution, then the measurement value was plotted on the standard curve (Bintang, 2018). Carbohydrate content calculation was obtained by:

$$\text{Percent of glucose (\%)} = (G)/W \times 100\%$$

G = glucose concentration (g); W = sample weigh (g)

Results

In this research, the average contents were as follows: 10.25% ash, 6.62% water, 37.30% protein, 6.13% fat, and 26.14% carbohydrate (Table I). The samples of ash, water, protein, fat, and carbohydrate are shown in Figures 1-5.

Table 1: Proximate analysis result of animal feed granules composition

Content	Average (%)
Ash	10,25
Water	6,62
Protein	37,30
Fat	6,13
Carbohydrate	26,14

Figure 1: Sample of ash content

Figure 2: Sample of water content

Figure 3: Sample of protein

Figure 4: Sample of fat

Figure 5: Sample of carbohydrate

Based on the standard curve solution absorption, linear regression equation was y = 0.148 x + 0.088 (Figure 6).

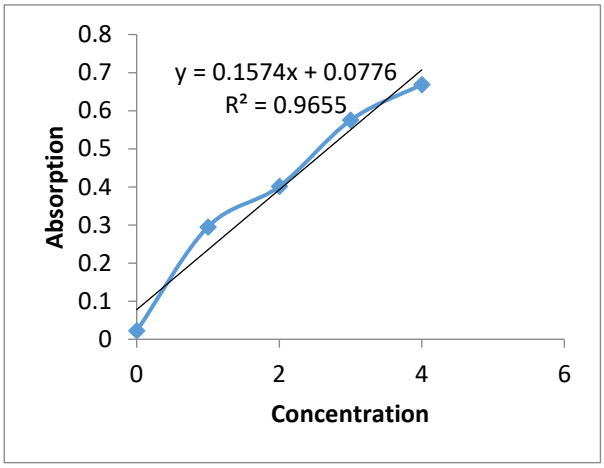

Figure 6: BSA Standard curve

Based on absorption measurement of glucose standard solution, the regression value obtained was y = 0.006x + (-0.066) (Figure 7).

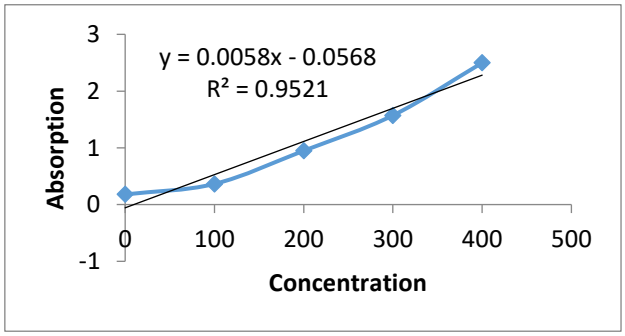

Figure 7: Glucose Standard curve

Discussion

Ash content determination of animal feed granules was done through the dry ashing method using a furnace to determine how good a process was in animal feed production (Widarta, 2015) and find the nutrient value of animal feed granules composition. In this research, the average amount of ash content with three replications was 10.25%. Referring to SNI-8509-2018, this amount should not exceed 14%. Hence, our results complied with SNI-8509-2018.

Water content determination of animal feed granules was done through the thermogravimetric method to find the nutrient content of the animal feed and check whether it meets the standards (Widarta, 2015). Water content determination is crucial because excess water can affect the appearance, texture, and taste of animal feed and support the growth of bacteria, mold, and yeast, resulting in the change of the animal feed. In this research, the average amount of water content with three replications was 6.62%. According to SNI-8509-2018, it should not be higher than 12%. Thus, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The protein content of animal feed granules was determined through the biuret method, based on radiation absorption, using a spectrophotometer to measure protein content quantitatively, find the nutrient content in the sample, and verify whether it meets the standards Niode & Nasriani & Irdja, 2017). The biuret reactor forms complexes, which help identify the substances. The $CuSO_4$ produced by heating urea had a similar structure to that of the peptide bond of protein. The principle of the biuret reactor is based on the existence of a reaction between copper sulfate and alkalis with other compounds resulting in a distinct purple-blue (Machin, 2012) or red-violet or blue-violet (Purnama & Retnaningsih & Aprianti, 2019) solution. In this research, the average amount of protein content was 37.30%. Referring to SNI-8509-2018, this amount should not be any less than 16%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The fat content of animal feed granules was determined using a Soxhlet, which extracted fat using petroleum ether solvent, to calculate calories in animal feed (Pargiyanti, 2019). In this research, the average amount of fat content with three replications was 6.13%. According to SNI-8509-2018, this amount should not be any less than 2%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

Carbohydrate content determination of animal feed granules used the phenol sulfate method. The principle of this method is that simple sugar and oligosaccharides

can react with phenol resulting in a stable yellowish-orange or orange colour. The standard carbohydrate used in this study was glucose. Based on absorption measurement, the regression value obtained was y = 0.006x + (-0.066). What follows is the glucose identification reaction by adding phenol and sulfate acid, which form the colour complex. In this research, the colour complex formed was orange. Based on Figure 8, the reaction shows that glucose was hydrated by thick sulfate acid to form hydroxymethyl-furfural. The colours obtained by hydroxymethyl furfural were various, ranging from orange-green to brown and purple, depending on the glucose concentration of the sample. In this research, the average amount of carbohydrate content with three replications was 26.14%. Referring to SNI-8509-2018, the amount should not be any less than 14%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

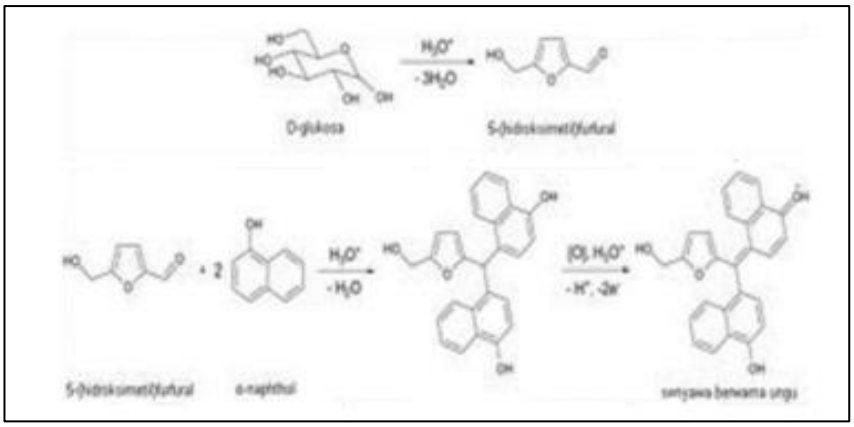

Figure 8: Glucose identification reaction with phenol-sulfuric acid (Wiyantoko, Rusitasari, Putri, dan Muhaimin, 2017)

Conclusion

The findings of this study show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfil the regulation of animal feed content based on SNI-8509-2018.

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IAI CONFERENCE

RESEARCH ARTICLE

The therapeutic outcomes and adverse drug reactions study of Clozapine on Schizophrenia inpatients in the Grhasia psychiatric hospital Yogyakarta, Indonesia

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Keywords
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Abstract

Introduction: Clozapine is an antipsychotic agent used in schizophrenia recurrence or when other antipsychotics are not effective. **Aims:** This study aims to determine the therapeutic outcome and adverse drug reactions of clozapine in schizophrenia disorder among hospitalised patients. **Methods:** A retrospective cross-sectional study was conducted between January 2018 and December 2019 using inpatients' medical records from the Grhasia Psychiatric Hospital, Yogyakarta. The therapeutic outcome was measured with the PANSS-EC scale, while adverse drug reactions of clozapine were analysed theoretically as per the literature. **Results:** The average decrease in the PANSS-EC score was 8.27, and the average duration to achieve this decrease was 2.5 days. The combination of typical-atypical antipsychotics could reduce the highest PANSS-EC score of 11-15 (41%). The adverse drug reactions of clozapine were tremor, weight gain, obesity, leucopenia, hyperglycemia, and hypercholesterolemia, among other effects.
Conclusion: Clozapine is effective in improving positive and negative symptoms, but its use needs close monitoring.

Introduction

Schizophrenia is a mental disorder characterized by positive, negative, and cognitive symptoms, causing psychological, social, economic, and other problems in patients (Patel *et al.*, 2014). Antipsychotic agents are the most used medications for relieving schizophrenia symptoms (Andreasen & Black, 2006). They are classified as typical (first generation) and atypical (second generation) antipsychotics. Generally, the therapeutic outcome of antipsychotic agents is their potential effect to improve schizophrenia symptoms based on patients' characteristics. However, they may cause adverse drug reactions related to their mechanism of action (Chrismon & Buckley, 2015).

Clozapine is an atypical antipsychotic agent used in schizophrenia recurrence or when other antipsychotics are not effective. It can be prescribed as monotherapy or combined with other antipsychotics and has many adverse drug reactions, ranging from mild to severe. Clozapine improves resistance to schizophrenia, but its use requires close and individual monitoring. Similar to other atypical antipsychotics, clozapine has adverse drug reactions, including weight gain, metabolic syndrome (major), and extrapyramidal syndrome (minor). It can also cause haematology disorders, such as neutropenia. In practice, the use of clozapine needs laboratory examination, particularly for long-term therapy. Previous studies reported that metabolic syndrome prevalence caused by clozapine was 50-60%, occurring in patients who have no prior risk factors for this condition (Meyler,

2016; Ventriglio *et al.*, 2019). Clozapine also causes agranulocytosis in 1% of patients; this low prevalence is, however, dangerous and may lead to death (AphA, 2019). Furthermore, the risk for developing agranulocytosis can be detected as early as within the first three months of treatment (Alldredge *et al.*, 2013). Several other life-threatening adverse drug reactions are caused by clozapine, whether in the short or the long term use (Meyler, 2016), but data from Indonesia are still limited.

Based on these explanations and the scarcity of studies in Indonesia, updated research is essential to explore the therapeutic outcomes and adverse drug reactions of clozapine, particularly among the Indonesian population that presents many sociodemographic differences. It is noteworthy that the government policy in schizophrenia medication also contributes to the use of the drug.

Therefore, this study aimed to explore the therapeutic outcome and adverse drug reactions of clozapine among schizophrenia patients in the hospital setting.

Methods

Study design

This retrospective cross-sectional study was conducted between January 2018 and December 2019, using medical records of patients from the Inpatient Unit of the Grhasia Psychiatric Hospital, Yogyakarta. The data included patient characteristics, drug use patterns, therapeutic outcomes, and adverse drug reactions of clozapine.

Therapeutic outcome was measured with the PANSS-EC scale, while adverse drug reactions of clozapine were analysed theoretically using the Drug Information Handbook (2019) and Meyler's Side Effect of Drugs (2016). Data were analysed descriptively. The Positive and Negative Syndrome Scale (PANSS) is a tool that assesses positive and negative symptoms of schizophrenia and general psychopathology (Kay *et al.*, 1987). The Positive and Negative Syndrome Scale-Excited Component (PANSS-EC), developed later by (Montoya *et al.*, 2011), is one of the most simple-to-use and intuitive scales to assess agitated patients. The tool consists of five items exploring excitement, tension, hostility, uncooperativeness, and poor impulse control, rated from 1 (not present) to 7 (extremely severe). The score ranges from 5 to 35; mean scores equal to or higher than 20 clinically correspond to severe agitation (Montoya *et al.*, 2011).

Material

Inclusion criteria were hospitalised patients who had been diagnosed with schizophrenia between January 2018 and December 2019 and taking clozapine as a monotherapy or combined therapy. Exclusion criteria were patients with incomplete or unclear data from their medical records. Data sampling used the purposive sampling method.

Data analysis

The data were analysed descriptively based on patients' characteristics, medication patterns, therapeutic outcomes, and adverse drug reactions.

Results

Table I describes patient characteristics, including age, gender, and occupation.

Table I: The characteristics of patients based on gender, age, and occupation

Variable	Number of patients	Percentage (%)
Gender		
Male	74	74
Female	26	26
Age (years old)		
Male		
0-20	3	4
21-50	52	72
51-65	17	24
Female		
0-20	0	0
21-50	19	68
51-65	9	32
Occupation		
Employed	27	27
Unemployed	73	73
Total	**100**	**100**

The total number of participants was 100 patients, meeting the minimum sample size required. The results revealed more male patients than females (72% *vs* 28%), to a previous study in The Psychiatric Hospital in Bali, Indonesia (Gemilang *et al.*, 2017). No differences were found between male and female patients, although the new cases of schizophrenia were male patients (Ochoa *et al.*, 2012).

Most patients were in the productive age between 21–50 years, with more males than females in this age bracket. This result is in line with previous findings showing a higher prevalence of adult patients. Theoretically, adults may experience schizophrenia symptoms due to their

responsibilities and problems (Aryani & Sari, 2016; Hariyanto *et al.*, 2016).

The majority of patients were unemployed (73%), similar to results from the Psychiatric Hospital in Central Sulawesi, Indonesia, reporting that 72% of patients were unemployed (Fahrul *et al.*, 2014). Schizophrenia affects the cognitive function of patients, which contributes to difficulty in being hired in work and doing work well besides a negative stigma in the society because of the disorder (Drake, 2018).

The medication pattern

The medication pattern includes two phases: acute and stabilization. The acute phase is when the patient is carried to the emergency unit, while the stabilization phase is when the patient is moved to the bed site. The main difference between them is the targeted symptoms. The acute phase focuses on acute symptoms, while the stabilization phase focuses on relieving positive and negative symptoms and maintaining remission across the bed site.

Antipsychotics, whether typical or atypical, can be used alone or combined. Table II shows that combined therapy was used more than monotherapy. Theoretically, typical antipsychotics are effective in relieving positive symptoms but are similar to atypical antipsychotics in treating positive symptoms (Durand & Barlow, 2007). The results also showed that atypical antipsychotics were used more than typical antipsychotics because they had other benefits, including better negative symptom relief and less risk of adverse drug reactions (Sadock *et al.*, 2014; Ikawati, 2014).

Table II: The pattern of medication patterns

Type of medication	Type of antipsychotics	Drug name	Number (cases)	%	Total % category
Monotherapy	Typical	Haloperidol (inj.)	2	1.7	5.9
	Atypical	Clozapine	2	1.7	
		Risperidone	3	2.5	
Combination of 2 antipsychotics	Typical- Typical	Haloperidol (tab.) + Chlorpromazine	2	1.7	50
		Haloperidol (tab.) + Chlorpromazine + Haloperidol (inj.)	1	0.8	
		Haloperidol (tab.) + Trifluoperazine	1	0.8	
	Atypical- Atypical	Risperidone + Clozapine	31	26.3	
	Atypical- Typical	Clozapine + Haloperidol (tab.)	10	8.5	
		Clozapine + Haloperidol (inj.)	2	1.7	
		Clozapine + Haloperidol (tab.) + Haloperidol (inj.)	4	3.4	
		Risperidone + Chlorpromazine	2	1.7	
		Risperidone + Haloperidol (inj.)	4	3.4	
		Clozapine + Trifluoperazine	1	0.8	
		Risperidone + Trifluoperazine	1	0.8	
Combination of 3 antipsychotics	Typical- Typical	-	0	0.0	43.2
	Atypical- Atypical	Olanzapine (tab.) + Aripiprazole + Clozapine + Olanzapine (inj.)	1	0.8	
		Olanzapine (inj.) + Aripiprazole + Clozapine	1	0.8	
	Typical- Atypical	Risperidone + Clozapine + Haloperidol (tab.)	5	4.2	
		Risperidone + Clozapine + Haloperidol (inj.)	29	24.6	
		Risperidone + Clozapine + Haloperidol (tab.) + Haloperidol (inj.)	9	7.6	
		Olanzapine (tab.) + Risperidone + Chlorproazine	1	0.8	
		Risperidone + Chlorpromazine + Haloperidol (inj.)	2	1.7	
		Clozapine + Risperidone + Trifluoperazine + Haloperidol (tab.)	1	0.8	
		Aripiprazole + Clozapine + Haloperidol (inj.)	1	0.8	
		Clozapine + Fluphenazine (inj.) + Haloperidol (inj.)	1	0.8	
Combination of 4 antipsychotics	Typical-Atypical	Sulpiride + Clozapine + Risperidone + Haloperidol (inj.)	1	0.8	0.8
		Sulpiride + Clozapine + Risperidone + Haloperidol (inj.)	1	0,5	
		Sulpiride + Clozapine + Risperidone + Trifluoperazine	1	0.5	
		Clozapine + Olanzapine (tab.) + Risperidone + Chlorpromazine	1	0.5	
Total			**198**		**100**

Therapeutic Outcomes

Therapeutic outcomes were evaluated by assessing schizophrenia symptoms relief using the PANSS-EC scale. The pattern of decrease in PANSS-EC scores is listed in Table III. The average decrease in PANSS-EC scores was 8.27 days, and the average duration to achieve this decrease was 2.5 days. Table IV shows the pattern of decrease in PANSS-EC scores based on the types of antipsychotics used.

Based on Table IV, monotherapy with typical antipsychotics could decrease PANSS-EC scores by 6-10 points; haloperidol was highly effective in decreasing PANSS-EC scores, reaching 10 points (one patient). Combined typical-atypical antipsychotics could achieve the highest decrease (11-15 points) in PANSS-EC scores (41%). Thus, the combination of different medications could relieve acute symptoms with an effective decrease in PANSS-EC scores.

Table III: The patterns of the decreases in PANSS-EC score

Decrease in PANSS-EC score	Duration (days)	Number of patients	%	% per scale
0	1	1	1	1
1-5	1	12	12	21
	2	4	4	
	3	2	2	
	4	3	3	
6-10	1	28	28	64
	2	20	20	
	3	4	4	
	4	2	2	
	5	3	3	
	9	1	1	
	12	1	1	
	16	2	2	
11-15	1	3	3	14
	2	8	8	
	4	1	1	
	5	3	3	
	8	1	1	
	9	1	1	
Total		**100**	**100**	**100**

The average of the decreases in PANSS-EC score is **8.27**

The average of the durations of PANSS-EC score decrease (days) is **2.5**

Table IV: The Patterns of the decreases in PANSS-EC score based on types of antipsychotics

The type of antipsychotics	Scale of the decrease in PANSS- EC score	Number of patients	%
Typical	6-10	1	1
Atypical	1-5	1	1
	6-10	2	2
	11-15	1	1
Typical-Typical	0	1	1
	6-10	2	2
Atypical-Atypical	1-5	7	7
	6-10	15	15
	11-15	5	5
Typical-Atypical	1-5	13	13
	6-10	41	41
	11-15	11	11
Total		**100**	**100**

Table V presents the patterns of clinical symptom relief. Clinical symptoms included positive and negative symptoms in schizophrenia.

Table V: The patterns of clinical symptoms relief in the stabilisation phase

Clinical symptoms		Number (cases)	% of the relieved state (%)	% of clinical symptoms (%)
Positive symptoms	Delusion	33		
	Relieved	31	94	16.2
	Not relieved	2	6	
	Hallucination	46		
	Relieved	36	78	22.5
	Not relieved	10	22	
	Poor impulse control	15		
	Relieved	14	93	7.4
	Not Relieved	1	7	
	Disorganised speech	11		
	Relieved	9	82	5.4
	Not relieved	2	18	
Negative symptoms	Alogia	65		
	Relieved	49	75	31.9
	Not relieved	16	25	
	Blunted affect	18		
	Relieved	18	100	8.8
	Not relieved	0	0	
	Avolition	6		
	Relieved	5	83	2.9
	Not Relieved	1	17	
	Anhedonia/Asociality	9		
	Relieved	8	89	4.4
	Not relieved	1	11	
	Attention impairment	1		
	Relieved	1	100	0.5
	Not relieved	0	0	
Total		**204**		**100**

Adverse drug reactions

Adverse drug reactions in this study were determined by looking at SOAP (Subjective, Objective, Assessment, and Planning) notes and laboratory examination results in the medical records of hospitalised patients. The interpretation was based on the list of adverse drug reactions of clozapine in literature (Drug Information Handbook 17th Edition and Meyler's Side Effects of Drugs 16th Edition). The summary of adverse drug reactions is presented in Table VI.

Table VI: The types of adverse drug reactions

Adverse drug reaction	Number of patients (n = 88)	Percentage (%)
Cardiovascular		
Hypotension	6	6.8
Hypertension	4	4.5
AV block	1	1.1
Central Nervous System		
Headache	2	2.3
Insomnia	1	1.1
Gastrointestinal		
Nausea and vomitting	6	6.8
Diarrhea	3	3.4
Constipation	2	2.3
Metabolism		
Weight gain	26	29.5
Hyperglycemia	25	28.4
Elevation in Blood glucose level	15	17.0
Hypercholesterolemia	5	5.7
Obesity	4	4.5
Hematology		
Decrease in Leukocyte	26	29.5
Decrease in ANC (Absolute Neutrophil Count)	23	26.1
Leukocytosis	19	21.6
Elevation in leukocyte	16	18.2
Leukopenia	2	2.3
Muskuloskeletal		
Tremor	28	3.8
Lethargy	4	4.5
Muscle rigidity	3	3.4
Dystonia	2	2.3
Dyskinesia	1	1.1
Myalgia	1	1.1
Others		
Hypersalivation	5	5.7
Diaphoresis	1	1.1

Discussion

Alongside a psychosocial rehabilitation program, antipsychotic agents are the drugs of choice to treat schizophrenia. Studies suggested that atypical antipsychotics resulted in better treatment retention and were more effective in preventing schizophrenia relapse than typical antipsychotics (Juleha *et al.*, 2019).

In this study, combined therapy was preferred over monotherapy. The most used treatment was a combination of two antipsychotics (50%), mainly risperidone + clozapine (26.3%). According to Juleha and the authors (2019), risperidone is the most prescribed atypical antipsychotic (55%). Among atypical antipsychotics, only risperidone and aripiprazole have evidence of efficacy and can be used as the first-line therapy in schizophrenia treatment (Dipiro *et al.*, 2017). This study showed that clozapine was the most used medication in combination with risperidone. A previous study reported that clozapine (38%) was the second most commonly prescribed atypical antipsychotic, in monotherapy or combined with other antipsychotics, to treat schizophrenia resistance (Juleha *et al.*, 2019). Clozapine showed to be superior in managing treatment-resistant schizophrenia or among patients with suicide risk (Dipiro *et al.*, 2017). Schizophrenia therapeutic guidelines recommend clozapine or combined antipsychotics but prefer clozapine as monotherapy in patients with refractory schizophrenia (Buchanan *et al.*, 2010).

In the study, the second most prescribed treatment was a combination of three antipsychotics (43.2%), i.e., risperidone + clozapine + haloperidol (injection) (24.6%). Haloperidol is used to treat positive symptoms mainly (Kay & Singh, 1989). Similarly, Indriani and the authors (2019) had found that this combination was the most used in schizophrenia patients (risperidone + clozapine + haloperidol).

Schizophrenia presents with one or more of the following signs: delusions, hallucinations, disturbed thinking and talking, behavioural disorders, and negative symptoms. Treatment effectiveness is measured with instruments, such as the PANSS-EC (Patel *et al.*, 2014). The results showed that the average decrease in the PANSS-EC score was 8.27, and the average duration to achieve this decrease was 2.5 days. Similarly, a study conducted by Ayuningtyas and the authors (2018) in Prof. Dr. Soerojo Psychiatric Hospital, Magelang, Indonesia, reported that clozapine significantly decreased PANSS-EC scores as monotherapy or in combination, using post hoc ANOVA (p= 0.05). This study also showed that combined typical-atypical therapy resulted in the highest PANSS-EC scores decrease (11-15, 41%). It also revealed that a combination of different medications improved acute symptoms and lowered PANSS-EC scores.

In this study, 78% of patients showed improvement in hallucination after taking their medication. Moreover, the most common negative symptom in the study was

alogia (31.9%), with 75% of patients showing relief. According to a previous study, the mean differences of the PANSS subgroups were more significant in the clozapine group than in the typical agents' group, which were in decreasing order: general psychopathology, anergia, positive, and negative symptoms. In the typical group, signs were (in decreasing order): general psychopathology, positive, and negative symptoms. This finding showed that both treatments (typical and atypical groups) improved positive symptoms more than negative symptoms (Sharafi, 2005). Some studies found that the efficacy of clozapine is clinically significant on negative symptoms but is delayed compared to its efficacy on other symptoms evaluated by the PANSS. Hence, both positive and negative symptoms appear to be improved with clozapine. Additionally, research suggests that the improvement of negative symptoms is directly related to positive symptoms after clozapine therapy (Sharafi, 2005).

Combined therapy involving clozapine can result in increased adverse drug reactions. The most commonly reported were tremor (28 patients), weight gain (26 patients), leukocytopenia (26 patients), and hyperglycemia (25 patients), as well as other adverse drug reactions.

Cardiovascular effects

Adverse drug events related to the cardiovascular system included hypotension (6 patients), hypertension (4 patients), and atrioventricular (AV) block (1 patient). The mechanism of clozapine-induced hypertension was assumed to be caused by alfa-2 adrenoceptors blockade (Meyler, 2016). Furthermore, hypotension is an adverse drug reaction commonly found in patients taking clozapine, as 9% of these patients might experience orthostatic hypotension (De Berardis *et al.*, 2018). It usually occurs as early as within the first 4-6 weeks of treatment; this effect can be tolerated by patients (Iqbal *et al.*, 2003). Paradoxically, cardiovascular effects such as tachycardia are not uncommon in patients using clozapine, often leading to palpitations (Yuen *et al.*, 2018).

Central nervous system effect

This effect involved insomnia (1 patient) and headache (2 patients). According to *Drug Information Handbook 17th Edition*, clozapine can cause headaches with a prevalence of 7% (AphA, 2019). The low number of patients with headaches in this study could be likely because physicians did not report it in the medical record.

Gastrointestinal effects

In this study, gastrointestinal effects included nausea-vomiting (6 patients), diarrhoea (3 patients), and constipation (2 patients). Constipation incidence of clozapine was 14-25% (AphA, 2019). The results of this study were similar to the theory, where the prevalence of anticholinergic symptoms (including constipation) was 20% as clozapine is a strong M1 muscarinic antagonist (Alldredge *et al.*, 2013; Meyler, 2016). Also, nausea prevalence was 11% in patients taking clozapine. This effect was due to the anticholinergic activity of clozapine, causing a delay of transit time in the gastrointestinal tract, a decrease in diet intake, and a direct effect on the hypothalamus (Iqbal *et al.*, 2003).

Metabolism effect

Metabolism effects included weight gain (26 patients), hyperglycemia (25 patients), elevation in blood glucose levels (15 patients), hypercholesterolemia (5 patients), and obesity (4 patients). Metabolic syndrome was determined by laboratory examination results. Most examinations were done one time only upon admission but could be carried out more than once in patients with some comorbidities. Obesity was found in all patients. Previous studies concluded that clozapine affected weight gain significantly in patients taking clozapine compared to controls (Rummel-Kluge *et al.*, 2010; Dayabandara *et al.*, 2017). The relation between dose and weight gain was not clear. The results of a study among 50 schizophrenia patients using 100, 300, or 600 mg/day of clozapine over four months showed that the increase in doses was linear with the weight gain of patients. Patients gained as much as 4.4 kg at 600 mg dose, 2.6 kg at 300 mg dose, and 1.3 kg at 100 mg dose (Meyler, 2016).

There was no standard related to weight gain due to clozapine, with patients showing varying results. This condition is assumed to be caused by the relation between weight gain and the serotonin polymorphism system (De Luca *et al.*, 2007; Sicard *et al.*, 2010).

Hyperglycemia

Of the total sample, four patients had abnormal fasting blood glucose levels, likely caused by medications and history limitations. Further studies are necessary to examine the effects of clozapine on blood glucose levels. Besides, these patients had been diagnosed with schizophrenia for 6-18 years, which might indirectly increase blood glucose levels. Other contributing factors included patients' behaviour, diet, and physical activities that could affect the metabolism.

The results of this study showed that clozapine use (whether acute or chronic) was related to insulin sensitivity, high blood glucose levels, and low insulin plasma levels, reflected by decreased insulin secretion (Liu *et al.*, 2017). A previous study reported increased

blood glucose levels with an average clozapine dose of 362 mg/day. The prevalence of metabolic syndrome caused by clozapine was 60% with a dosage of 615 mg/day, and there was no significant correlation between metabolic syndrome prevalence and clozapine dosage, which found that metabolic syndrome prevalence was 51.9% (Vancampfort *et al.*, 2013; Ventriglio *et al.*, 2019). Age, medication duration, and length of schizophrenia also contributed to metabolic syndrome.

Hypercholesterolemia effects

In this study, cholesterol laboratory examination was done in only 23 patients, and this examination was also only one time for each patient. Among them, five patients had abnormal cholesterol levels. All five patients had used clozapine for more than three years. The schizophrenia patient tended to have a high appetite and limited physical activity so that they increased weight gain potency causing metabolic syndrome like dyslipidemia. It needs lipid level monitoring mainly when atypical antipsychotics started, and it is carried out in the early medication, 12 weeks after treatment, and every five years during medication (Alldredge *et al.*, 2013).

Haematology effects

Adverse drug reactions related to haematology effects included a decrease in leukocyte (26 patients), absolute neutrophil count (23 patients), leukocytosis (19 patients), and increase in leukocyte (16 patients) as well as leukopenia (2 patients).

There is no explanation about the correlation between leucopenia or agranulocytosis risk and dosage of clozapine, but several case reports confirm agranulocytosis cases appearing in the patients taking clozapine at doses of 500 mg/day. This effect is reversible when clozapine is discontinued (Meyler, 2016). For that reason, leucocyte laboratory examination must be done before and during treatment, with clozapine monitoring every two weeks in the second 6 months of treatment, then every month during clozapine use. If leukocyte count is less than 2000 cells/mm^3, then clozapine treatment must stop until leukocyte is normal (Crismon *et al.*, 2015).

Musculoskeletal effects

The most common adverse drug reaction was tremor (28 patients), one of the clinical manifestations of extrapyramidal syndrome. Atypical antipsychotics have minimum extrapyramidal effects compared to typical antipsychotics. The reported tremor prevalence caused by clozapine was only 6% (AphA, 2019). Two patients using clozapine monotherapy had tremors. They had been taking clozapine for 2 and 5 years, respectively. Tremors occurred most frequently in patients using the combination clozapine + risperidone. This effect is related to the receptor affinity, and clozapine has a weaker binding with the D2 receptor than risperidone; thus, risperidone is more likely to cause extrapyramidal syndrome (Divac *et al.*, 2014).

Other effects

Other adverse drug reactions appeared in the study, i.e. hypersalivation (5 patients) and diaphoresis (1 patient). Hypersalivation is a common effect of clozapine, with an incidence rate of 10-23%. In this study, hypersalivation was most commonly detected among patients taking clozapine at a dose of 100 mg/day; this effect also occurred at doses lower than 25 mg/day. Theoretically, hypersalivation is dosage-dependent. The higher the dosage of clozapine, the higher the hypersalivation. The mechanism of hypersalivation is a decrease in larynx peristalsis due to muscarinic (M4) receptor agonists and alfa-2 receptor antagonists (Meyler, 2016).

These adverse drug reactions generally occur within the first months of treatment, prompting patients to withdraw from the medication. Often the main reason for discontinuing clozapine treatment is its intolerable adverse drug reactions. Hence, it is essential to manage adverse drug reactions adequately to maintain therapeutic outcomes. Dose adjustment can considerably help in minimizing the occurrence of adverse drug reactions. Usually, patients who have frequent but non-severe adverse drug reactions, such as increased appetite, sedation, enuresis, or hypersalivation, do not require adjustment (reduced doses) or drug intervention. Nevertheless, it is always recommended that routine assessment and blood monitoring be carried out before and after the initiation of clozapine to prevent severe adverse drug reactions (Pharmaceutical Services Programme, 2018).

This study has several limitations. First, most patients took combined therapy; thus, adverse drug reactions could be due to either antipsychotic. Second, the researchers interpreted the patient symptoms during inpatient care based on the literature. Third, this study could not determine a causal relationship and other factors related to adverse drug reactions in clozapine-treated patients. Furthermore, some assumptions and interpretations were made as the description of adverse drug reactions was not written clearly in the medical records.

Further prospective studies taking into account these limitations are necessary to confirm the results of this study.

Conclusion

The average decrease in the PANSS-EC score was 8.27, and the average duration needed to achieve this decrease was 2.5 days. The combination of typical-atypical antipsychotics could reduce the highest PANSS-EC score of 11-15 (41%). In this study, 78% of patients showed improvement in hallucination after taking their medication. Moreover, the most common negative symptom in the study was alogia (31.9%), with 75% of patients showing relief.

Adverse drug reactions of clozapine were tremor (28 patients; 31.8%), weight gain (26 patients; 29.5%), obesity (4 patients; 4.5%), leukopenia (26 patients; 29.5%), hyperglycemia (25 patients; 28.4%), elevation in blood glucose levels (15 patients; 17.0%), and hypercholesterolemia (5 patients; 5.7%), in addition hypersalivation, diaphoresis, and cardiovascular, central nervous system, hemotology, and musculoskeletal effects.

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IAI CONFERENCE

RESEARCH ARTICLE

Enhancing solubility and antibacterial activity using multi-component crystals of trimethoprim and malic acid

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Abstract

Aim: To improve the solubility and antibacterial activity of trimethoprim (TMP) by preparing its multicomponent crystals with malic acid (MA). **Methods**: Multicomponent crystals of TMP-MA were prepared by solvent co-evaporation. The solid-state properties were characterised by powder X-ray diffraction (PXRD), differential thermal analysis (DTA), Fourier transform infrared (FT-IR) spectroscopy, and scanning electron microscopy (SEM) analyses. The solubility was investigated in an aqueous medium, while the antibacterial activity against *Escherichia coli* was investigated using the agar disk diffusion method. **Results**: The PXRD pattern of the TMP-MA binary system differed from the starting materials, supporting the formation of a new crystalline phase (equimolar ratio). The DTA thermogram showed a single, sharp, endothermic peak at 212.5 °C attributable to the TMP-MA multicomponent crystal's melting point. FT-IR spectroscopy showed a solid-state interaction involving proton transfer between TMP and MA. The multicomponent crystal displayed a 2.5-fold higher solubility and had increased antibacterial activity compared to TMP alone. **Conclusions**: The TMP-MA binary system forms salt-type multicomponent crystals that significantly increase solubility and antibacterial activity. Multicomponent crystal formation is a viable technique for modifying the physicochemical properties of active pharmaceutical ingredients.

Introduction

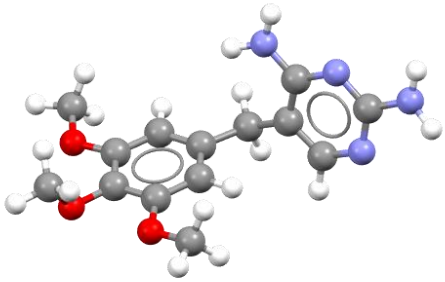
Trimethoprim (TMP) (Figure 1A), a 5-substituted-2,4-diaminopyrimidine, is a well-known antibiotic that functions via selective inhibition of bacterial dihydrofolate reductase. TMP is used to treat urinary tract infections caused by various gram-positive and gram-negative bacteria (Hawser *et al*., 2006). Due to poor aqueous solubility, the drug has been designated as class II and low solubility–high permeability by the Biopharmaceutical Classification System (Li *et al*., 2005). Poor water solubility results in incomplete drug absorption in the gastrointestinal fluid, reducing the drug's pharmacological effectiveness (Kawabata *et al*., 2011). Previous studies have sought to enhance the solubility of TMP through various methods, including through the formation of solid dispersions, inclusion complexes and spherical crystallisation (Guptat *et al*., 1991; Pawar *et al*., 1998; Li *et al*., 2005).

The solid-state properties of biologically active compounds significantly influence their physicochemical, mechanical, and pharmacological effectiveness (Sheth & Grant, 2005; Byrn & Henck, 2012). The formation of a multi-component crystal phase is a promising technique to improve solid-state properties and thereby enhance solubility and dissolution rate (Dwichandra Putra *et al*., 2016; Zaini *et al*., 2019). The multi-component crystal phase is a unique crystalline phase containing more than one component, such as active pharmaceutical ingredients and complementary molecules (e.g. solvents or pharmaceutical excipients), arranged stoichiometrically. The molecules in the crystal lattice are bonded by non-covalent intermolecular interactions, such as hydrogen bonding

and van der Waals forces. Multi-component crystals of pharmaceutical materials include cocrystals, salts, hydrates, and solvates (Cherukuvada *et al*., 2016; Berry & Steed, 2017).

Incorporating active pharmaceutical ingredients into multi-component crystals has been shown to enhance their physicochemical properties, including solubility and dissolution rate (Dwichandra Putra *et al*., 2018; Yuliandra *et al*., 2019), compressibility (Rahman *et al*., 2012; Paramanandana *et al*., 2020), physical and chemical stability (Vangala *et al.,* 2011; Sopyan *et al*., 2017), bioavailability, and pharmacological effectiveness (Shete *et al*., 2015; Yuliandra *et al*., 2018). Multi-component crystals of TMP have been extensively investigated, including a cocrystal with sulfamethoxazole and salts of trimethoprim with cinnamic acid, acetic acid, formic acid, maleic acid, and mefenamic acid (Bryan *et al*., 1987; Prabakaran *et al*., 2001; Umadevi *et al*., 2002; Muthiah *et al*., 2006; Zaini *et al*., 2017; Bhattacharya *et al*., 2020). However, to the best of our knowledge, there are no reports on the formation of multi-component crystals of TMP with malic acid (MA) nor an investigation into its physicochemical properties. MA (Figure 1B) is a dicarboxylate acid derivative used for flavouring food and as a pharmaceutical agent. MA is currently recognised as a safe excipient by the Food and Drug Administration (Ober *et al*., 2013).

(A)



(B)

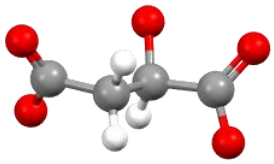


Figure 1: 3D structures of A) trimethoprim and B) malic acid

This study sought to prepare multi-component drug crystals of TMP and MA via solvent co-evaporation. The solid-state properties of the multi-component crystals were characterised by scanning electron microscopy (SEM), powder X-ray diffraction (PXRD), differential thermal analysis (DTA) for thermal behaviour, and Fourier transform infrared (FT-IR) spectroscopy. The solubility and antibacterial activities of the TMP-MA multi-component crystals were evaluated and compared to that of TMP alone.

Materials and methods

Materials

TMP was obtained from PT. Waris (Jakarta, Indonesia; Imported from Shouguang Fukang Pharmaceutical Co., Ltd., batch no. A-20111307093). MA, methanol p.a., sodium dihydrogen phosphate, nutrient agar (NA), and HPLC grade acetonitrile were purchased from Merck KGaA (Darmstadt, Germany). Sodium chloride (0.9 %) was obtained from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Ethyl alcohol (70 %) and distilled water were purchased from Brataco (Jakarta, Indonesia).

Preparation of multi-component crystals of TMP-MA

Equimolar TMP (0.01 mol, 2.9 g) and MA (0.01 mol, 1.37 g) were combined in a glass beaker, dissolved in methanol and stirred to form a clear solution. The solvent was evaporated at an ambient temperature for 48h. The TMP-MA multi-component crystals were stored in a desiccator for further characterisation.

Characterisation of solid-state properties

Powder X-ray diffraction analysis

PXRD analysis was performed using an X-ray diffractometer with monochromatic CuKα (PANalytical Inc., Almelo, The Netherlands) and a generator voltage and current of 40 kV and 30 mA, respectively. PXRD patterns were recorded from 10–40° on the 2 θ at a step size of 0.02°.

Differential thermal analysis

Thermograms of TMP, MA, and the multi-component crystal were obtained using an FP85 TA Cell differential thermal analyser (Mettler Toledo, Greifensee, Switzerland). Samples (~2–3 mg) were placed in aluminium pans, sealed, and heated from 50–250 °C at a rate of 10 °C/min.

Fourier transform infrared spectroscopy analysis

FT-IR spectra of TMP and the TMP-MA multi-component crystal were obtained using a 1600 Series FT-IR spectrometer (PerkinElmer, Rodgau, Germany). Samples (~2–3 mg) were mixed with dry potassium bromide and scanned from 4000–400 cm^{-1}.

Scanning electron microscopy analysis

The crystal habits of TMP, MA, and the multi-component crystal were investigated by SEM (JSM-6360LA, JEOL, Ltd., Tokyo, Japan). The sample was glued onto a metal stub with double-sided adhesive tape and sputter-coated with gold/palladium (Au/Pd 80/20 %) under vacuum before analysis.

Solubility test

Excess TMP and its multi-component crystal were added to an Erlenmeyer flask containing distilled water (100 mL). The samples were shaken at an ambient temperature for 24h using an orbital shaker. The samples were then filtered through a 0.45-micron membrane filter, diluted with the mobile phase, and injected into an HPLC column (Hitachi, Ltd., Tokyo, Japan) with a flow rate of 1 mL/min to measure the concentrations. Acetonitrile and sodium dihydrogen phosphate (pH = 3) with orthophosphate acid (70:30) were used as the mobile phase. TMP was detected by UV light at a wavelength of 287 nm. The retention time (t_R) of TMP was 1.4 min. All experiments were conducted in triplicate.

In vitro antibacterial activity study

The antibacterial activity of TMP and the TMP-MA multi-component crystal against *Escherichia coli* as a model bacterial species was investigated. NA medium was prepared (20 g in 1 L distilled water) and sterilised in an autoclave at 121 °C and 2 atm for 15 min. Bacteria were cultured at 37 °C and incubated for 24h. Bacterial colonies were swabbed, suspended in normal saline solution, vortexed, and incubated for two hours until the turbidity of a McFarland standard was achieved (3 x 10^8 CFU/mL). Antimicrobial activity assays were performed using the agar disk diffusion method with incubation at 37°C for 24h. Test compounds were prepared in multiple concentrations (1, 5, 10, and 20 %), and the diameters of the zones of inhibition were measured. All assays were performed in triplicate.

Data analysis

The data from the experiment are presented as mean ± SD. Statistical analysis for the diameter of inhibition before and after the formation of multi-component crystals was performed by using a paired t-test. The significance level was taken at a 95% confidence interval. The analysis was carried out using IBM SPSS Statistics version 26 (IBM, New York, USA).

Results

Solid-state characterisation

PXRD patterns were obtained for TMP, MA, and the multi-component crystal phase of TMP and MA (Figure 2). TMP displays specific diffraction peaks at 2 θ = 11.81, 15.14, 15.94, 17.40, 17.87, 18.18, 22.21, 22.47, 28.08, 30.74, and 33.44°. This indicated that the solid phase had a high degree of crystallinity. MA also demonstrates highly characteristic peaks at 2 θ = 16.70, 17.16, 28.08, and 32.14°. The TMP-MA multi-component crystal shows a unique PXRD pattern, lacking the diffraction peaks of isolated TMP and MA and displaying new peaks at 2 θ = 11.47, 12.30, 14.20, 18.72, 20.91, 23.07, 25.09, 27.07, and 29.02°.

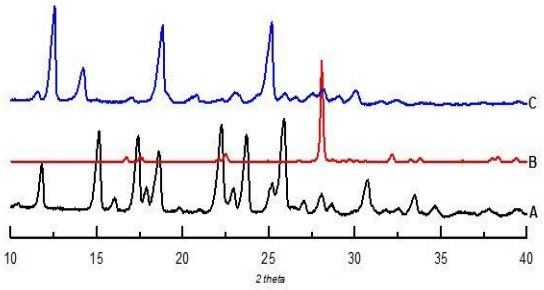


Figure 2: PXRD patterns of A) trimethoprim, B) malic acid, and C) the multi-component crystal phase of trimethoprim and malic acid

The thermodynamic behaviour of TMP, MA, and the multi-component crystal phase of TMP-MA is shown in Figure 3. The TMP thermogram exhibits a single, sharp endothermic peak at 203.4 °C, corresponding to the melting point of solid TMP. MA display a melting point of 143.5 °C, followed by thermal decomposition and evaporation above 180 °C. The DTA thermogram of the TMP-MA multi-component crystal is noticeably different from the starting materials (Figure 3C). A unique endothermic peak of the multi-component crystal can be seen at 212.5 °C. Meanwhile, no thermal events occurred before the melting point of the multi-component crystal.

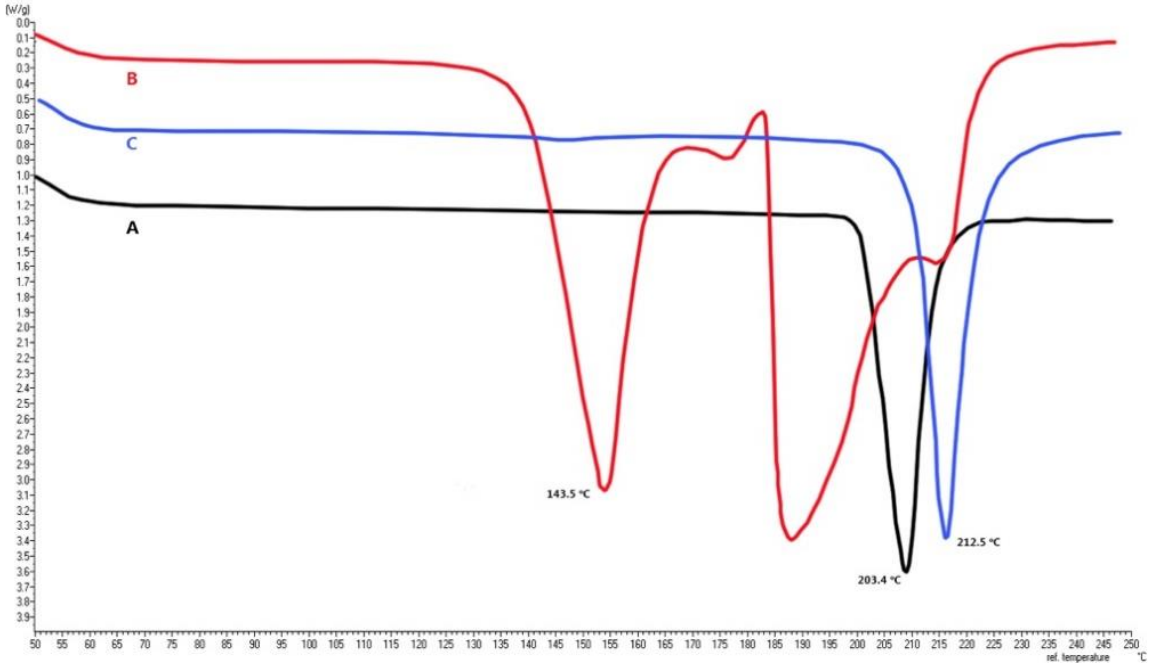


Figure 3: DTA thermograms of A) trimethoprim, B) malic acid, and C) the multi-component crystal phase of trimethoprim and malic acid

The FT-IR spectrum of TMP (Figure 4A) shows N – H stretching frequencies at 3470.15 and 3318.25 cm^{-1} and an N – H bending mode at 1632.86 cm^{-1}. Pivotal changes in the vibrational modes were observed in the FT-IR spectra of the multi-component crystal (Figure 4B). The N – H stretching modes shifted to 3400.56 and 3180.83 cm^{-1}, and N – H bending occurred at 1685.06 cm^{-1}. The MA carboxylate anion band appears at 1590.20 cm^{-1}, indicating proton transfer between the MA carboxylate anion and TMP.

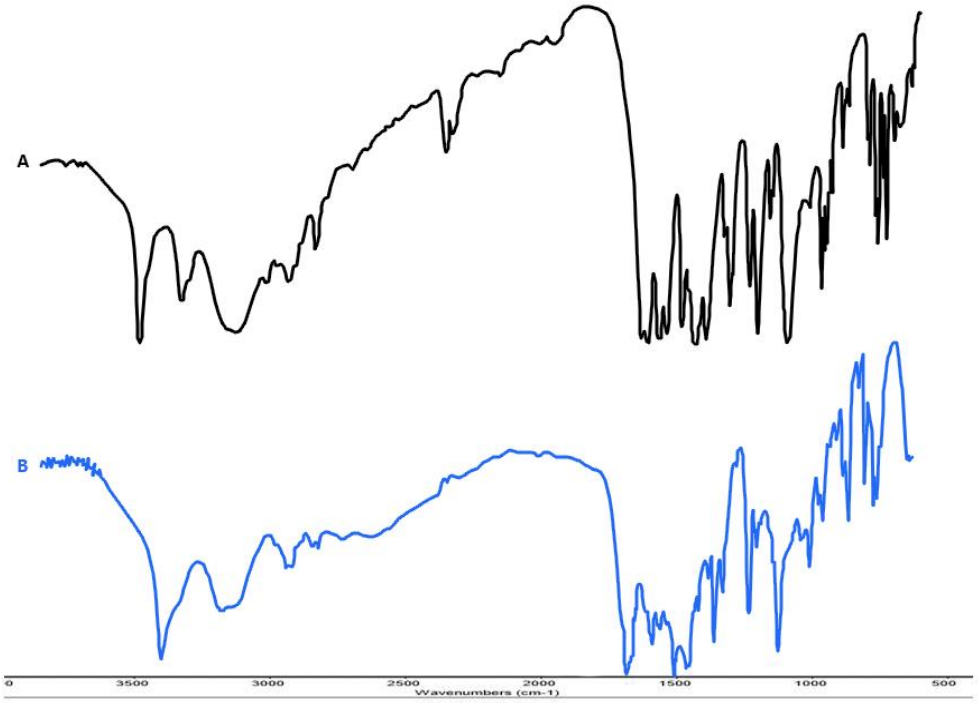


Figure 4: FT-IR spectra of A) trimethoprim and B) the multi-component crystal phase of trimethoprim and malic acid

The crystal habits of TMP, MA, and the TMP-MA multi-component crystal were imaged via SEM (Figure 5). TMP appears as fine, irregular-shaped particles, while MA appears as large, agglomerate-shaped particles. The multi-component crystals of TMP-MA (Figure 5C) are significantly different from the starting materials and occur as long rod-shaped particles.

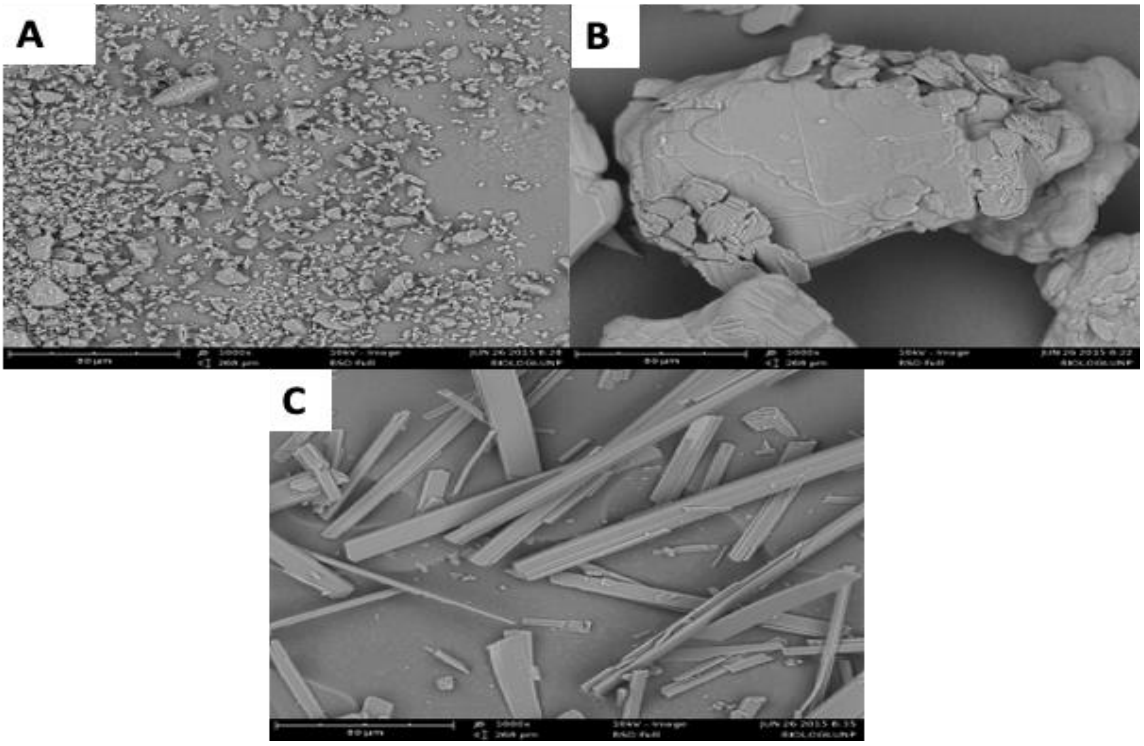


Figure 5: SEM micrographs of A) trimethoprim, B) malic acid, and C) the multi-component crystal of trimethoprim and malic acid (1000x magnification)

Solubility studies

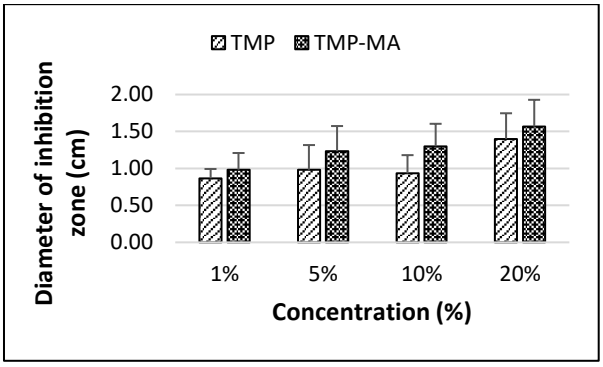
The results of the solubility studies are presented in Table 1. The data show the multi-component crystal of TMP-MA was 2.5-fold more soluble than TMP alone.

Table 1: Solubility of trimethoprim (TMP) and its multicomponent crystal with malic acid (TMP-MA) (n = 6).

Compound	Solubility ± SD (mcg/mL)
TMP	19.04 ± 0.05
TMP-MA	47.43 ± 0.07

In vitro antibacterial activity study

Treatment with TMP-MA multi-component crystals resulted in a notably larger (22%) zone of inhibition than TMP alone (Figure 6). Importantly, this increase was obtained for every concentration tested. The statistical analysis showed a significant increase in the inhibition zone after the formation of multi-component crystals of TMP-MA (p = 0.01).



Paired t-test showed a significant increase in the inhibition zone after the formation of multi-component crystal of TMP-MA ($p < 0.05$)

Figure 6: Antibacterial activity of trimethoprim (TMP) and trimethoprim-malic acid multi-component crystals (TMP-MA) against *E. coli* (n = 24)

Discussion

Poor aqueous solubility has been the major obstacle in the development of drugs, especially in the path of drug delivery. It makes it difficult for the sufficient concentration of drug molecules to be delivered at their sites of action. This issue has become more important as oral ingestion is currently the most common and convenient route of drug administration (Savjani *et al*., 2012). The formation of multi-component crystals is a promising approach to enhance the solubility of poorly water-soluble drugs such as trimethoprim (TMP). The present study aimed to prepare the multi-component crystals of TMP with malic acid (MA) and study their solubility and *in vitro* antibacterial activity.

PXRD is a reliable technique for characterising the solid-state properties of active pharmaceutical ingredients. PXRD patterns of a solid can be considered as fingerprints of the crystal phases; therefore, it can confirm the formation of new crystalline phases through the intermolecular interactions between two solid phases. A multi-component crystal phase is formed if the PXRD pattern of the binary mixture is distinct from its starting components (Karagianni *et al.,* 2018; Thakral *et al*., 2018). The PXRD pattern of the present study (Figure 2) showed specific diffraction peaks of TMP, which are similar to a previous report (Zaini *et al*., 2017). The unique pattern of TMP-MA, which established new peaks, supports the formation of a distinct crystalline phase composed of both TMP and MA. The formation of a multi-component crystal of an active pharmaceutical ingredient and coformer (salt-type or cocrystal) can be predicted using the ΔpKa rule. If the difference in pKa between the active pharmaceutical ingredients and coformers is ≥ 3 then the binary system tends to form a salt-type multi-component crystal, whereas a cocrystal form if ΔpKa < 3 (Cruz-Cabeza, 2012; Loya *et al*., 2019). TMP is a weak base (pKa = 7.3), while MA is a weak dicarboxylic acid (pKa = 3.4) (Molu & Yurdakoç, 2010; Uslu & Kırbaşlar, 2010). The ΔpKa for TMP and MA = 3.9, which suggests that the binary system will assume a salt-type multi-component crystal phase.

Thermal analysis is a simple and rapid method for determining the formation of a multi-component crystal phase (Lu *et al*., 2008). DTA was used to evaluate the thermodynamic behaviour of intact materials and their multi-component crystal phase. The melting point of MA and its thermal decomposition and evaporation above 180 °C are in agreement with previous work (Fernandes *et al*., 2019). On the other hand, the unique endothermic peak of the multi-component crystal (212.5 °C) indicates the formation of a new solid phase. The absence of thermal events before the melting point of the multi-component crystal suggests that it was either non-solvate or anhydrous. These results are also supported by the PXRD analysis.

FT-IR spectroscopy is an important method for confirming the formation of multi-component crystals. Changes in vibrational mode energies can suggest intramolecular interactions between the functional groups of the active pharmaceutical ingredients and excipients (da Silva *et al*., 2016). The FT-IR spectrum of TMP (Figure 4A) showed similar properties with a previous study (Bettinetti *et al*., 1983). MA is an organic acid (dicarboxylate acid) containing several carboxylate and hydroxyl functional groups, which act as proper coformers, interacting with target functional groups of the drug (a weak base) through non-covalent interactions, such as hydrogen and van der Waals bonds (Chadha *et al*., 2016; Cugovčan *et al*., 2017). TMP and MA interact via N – H ····· O hydrogen bonding between the TMP cation's protonated pyrimidine moiety and MA carboxylate anion. Previous studies have elucidated the structures of the multi-component crystal phase of TMP with various organic acids (Bettinetti *et al*., 1983; Bryan *et al.,* 1987; Muthiah *et al*., 2006; Prabakaran *et al*., 2001; Umadevi *et al*., 2002). The intermolecular interaction pattern is similar to our finding.

Co-crystallisation of multi-component crystals from organic solvent can produce a crystal habit differing from its starting materials. The crystal habit is affected by various factors, including solvent type, evaporation temperature, and degree of supersaturation. A change of crystal habit during multi-component crystal formation can influence the dissolution rate, flowability, and mechanical properties of active pharmaceutical ingredients (Serrano *et al*., 2016; Sathisaran & Dalvi, 2018). The crystal habits of TMP, MA, and the TMP-MA multi-component crystal were imaged via SEM (Figure 5). The new crystal habit seen at the multi-component crystals of TMP-MA (Figure 5C) is a strong indicator of the formation of a multi-component crystal phase (Basavoju *et al*., 2008).

Solubility plays a significant role in the gastrointestinal absorption of orally administered pharmaceutical agents. Drugs that are poorly soluble in water generally have limited bioavailability in the systemic circulation. Modifying the solid-state properties of active pharmaceutical ingredients through the formation of multi-component crystals with suitable coformers is a promising technique to improve solubility and pharmacological effectiveness (Kawabata *et al.,* 2011; Berry & Steed, 2017). The primary advantage of this approach is the ability to maintain the drug in a solid crystalline phase and sustain its thermodynamic stability. In addition, this technique does not change the pharmacophore structure of the active

pharmaceutical ingredients (Dwichandra Putra *et al*., 2018).

The present solubility studies showed that the multi-component crystal of TMP-MA was 2.5-fold more soluble than TMP alone. Several mechanisms can explain the improved solubility of TMP in the multi-component crystal. For example, the incorporation of soluble excipients as coformers in the multi-component crystal phase can increase the solubility of poorly soluble drugs. In addition, the formation of a salt-type multi-component crystal of TMP-MA improves solubility as a result of a higher affinity in water. Salt-type multi-component crystals immediately dissociate in an aqueous medium to cations and anions (Dwichandra Putra *et al*., 2016; Yuliandra *et al*., 2019; Zaini *et al*., 2019).

Agar disk diffusion was used to determine whether the TMP-MA multi-component crystals displayed increased antibacterial activity compared to TMP alone. This technique benefits from simplicity, affordability, ability to test against a wide number of bacterial species, and ease of analysis (Balouiri *et al*., 2016). The present study showed a 22% larger inhibition zone of multi-component crystal TMP-MA as compared with the intact TMP. Some recent studies also report the improvement of antibacterial activities through the formation of multi-component crystals of antibacterial agents. For example, the minimum inhibitory concentration of sulfaguanidine against *E. coli* is improved from 35 to 25 µg/ml after its cocrystal formation with thiobarbituric acid (Abidi *et al*., 2018). Sulfamethazine, another antibacterial agent from the sulfonamide class, shows a two-fold higher inhibition rate against the same bacterial species when prepared as a cocrystal with *p*-aminobenzoic acid (Pan *et al*., 2019). A previous study also finds similar results for this antibacterial agent when cocrystallised with aminosalicylic acid (Serrano *et al*., 2016). These results suggest that the formation of multi-component crystals is a propitious strategy to improve the efficacy of drugs, such as antibacterial agents, with poor aqueous solubility.

Conclusions

This study shows that the formation of a multi-component crystal phase between TMP and MA successfully improves the solubility and antibacterial activity of TMP. The solid-state properties, including the PXRD pattern, thermal behaviour, and crystal habits of the multi-component crystal of TMP-MA are remarkably different from that of TMP alone. Meanwhile, the formation of multi-component crystals of TMP-MA significantly improves the antibacterial activity *in vitro*.

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RESEARCH ARTICLE

Sambiloto (*Andrographis paniculata* Nees.) leaf extract activity as an α-Amylase enzyme inhibitor

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Keywords
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Abstract

Introduction: Sambiloto (*Andrographis paniculata*) is an antidiabetic medicinal plant that acts by inhibiting the α-amylase enzyme. Andrographolide, the active compound of sambiloto leaf, is insoluble in water but dissolves in ethanol. **Aim:** This study compared the *in vitro* activity of aqueous extract and ethanolic extract of sambiloto leaf with the α-amylase enzyme. **Methods:** The inhibitory activity test of the α-amylase enzyme was carried out using the ultraviolet-visible spectrophotometric method by measuring the absorbance of the remaining starch, which forms a blue complex with iodine-iodide. **Results:** The inhibitory activity of the α-amylase enzyme of the aqueous extract of sambiloto leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL). The results of the statistical tests showed significant differences (p <0.05) between the inhibitory activity of the α-amylase enzyme acarbose and the activity of both extracts.

Introduction

The prevalence of diabetes in Indonesia is relatively high and needs comprehensive prevention efforts. Using medicinal plants that have traditionally been employed by people in Indonesia is a means to overcome diabetes. Antidiabetic medicinal plants help maintain normal blood sugar levels. Inhibition of carbohydrate digestion and absorption is one of the strategies for managing blood sugar levels. The α-amylase enzyme plays a role in converting carbohydrates into sugar; thus, inhibiting its activity can suppress the formation of blood sugar (Saad *et al.*, 2017). Sambiloto (*A. paniculata*) has a high level of bitterness; its main constituents include diterpenoids, flavonoids, and polyphenols. Among the single compounds extracted from *A. paniculata*, andrographolide is the major one in terms of bioactive properties and abundance. It is slightly soluble in ethanol and almost insoluble in water. Andrographolide in leaves, stems, or whole plants can be extracted with ethanol (Chao & Lin, 2010). Andrographolide and ethanol extracts of *A. paniculata* leaf showed antidiabetic, hypoglycemic, and antioxidant activity in mice with type 2 diabetes mellitus (DM) (Subramanian *et al.*, 2008). Generally, people use the *A. paniculata* as a medicinal plant by boiling it in water or preparing it according to the Indonesian Traditional Medicinal Herb Formulary (Formularium Ramuan Obat Tradisional Indonesia) (Departemen Kesehatan Republik Indonesia, 2017).

Aqueous extract from some plants showed alpha-amylase inhibiting activity (Bhutkar and Bise, 2012). This study compared the *in vitro* activity of aqueous extract and ethanolic extract of *A. paniculata* leaf with the α-amylase enzyme. Various *in vivo* and *in vitro* methods can be used to examine new antidiabetic drugs. *In vitro* test methods were carried out by testing the inhibitory activity of α-amylase and alpha-glucosidase enzymes (Patil *et al.*, 2012).

Methods

The materials used are sambiloto (*A. paniculata*) leaf

obtained from PT HRL Internasional, East Java in the form of powder, α-amylase enzyme (SIGMA), amylum, andrographolide (SIGMA), 70% technical ethanol, ethanol pro analysis (E. Merck), toluene pro analysis. (E. Merck), chloroform pro analysis (E. Merck), methanol pro analysis (E. Merck), double-distilled water, dimethylsulfoxide pro analysis (E. Merck), acarbose tablets, amylum, sodium phosphate, and sodium chloride. The α-amylase enzyme inhibitory activity test was carried out according to Bhutkar and the authors (2018) with few modifications. The potato starch (1% w/v), 1ml of drug solution (extract, acarbose), 1 ml of α-amylase enzyme (1% w/v) and 2 ml of acetate buffer (0,1M, 7,2 pH) were mixed. The mixture was incubated for one hour, then a 0.1 ml iodine-iodide indicator was added to the mixture. The absorbance measurement used a Shimadzu UV-Vis spectrophotometer using a wavelength of 568.5 nm. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = (As-Ac/As) \times 100$$

Ac is the absorbance of the control; As is the absorbance of the sample.

The IC_{50} calculation was obtained from the linear regression equation after calculating the percentage of inhibition of α-amylase enzyme activity of the test material with a concentration range of 2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, and 10 mg/mL. To compare treatments, analysis of variance (ANOVA) was used, and $p < 0.05$ was considered as statistically significant, alongside the Tukey Post-Hoc Test significance and 95% confidence interval. Linear regression measured the median inhibitory concentration (IC50) to determine the inhibitory activities of α-amylase. IBM SPSS statistics version 22 was used for statistical analysis.

Results

At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α-amylase enzyme activity than the aqueous extract. The additional concentration of the test materials increased the percentage of inhibition of the α-amylase enzyme activity (Figure 1). Acarbose tablets showed a higher percentage of inhibition than both extracts. The aqueous extract and the ethanolic extract of *A. paniculata* leaf showed an *in vitro* inhibitory activity of the α-amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. The thin layer chromatogram of the *A. paniculata* extract showed a spot that is similar to the andrographolide spot (Figure 2).

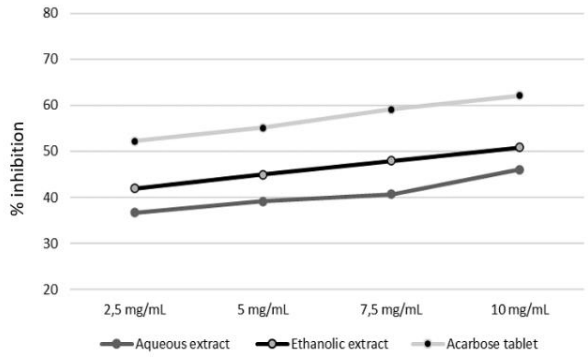
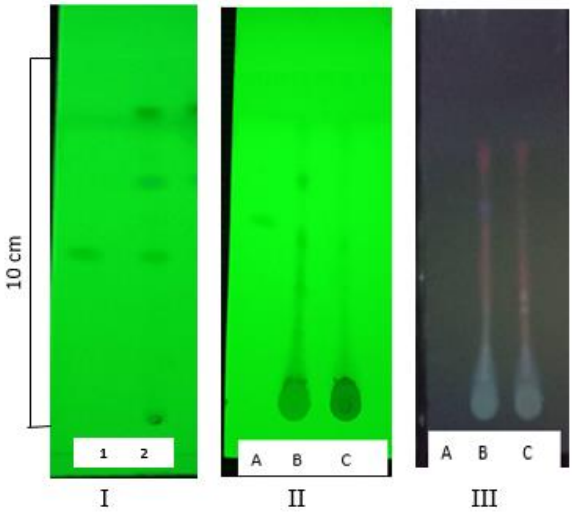


Figure 1: Per cent α-amylase inhibition activity of the aqueous extract, ethanolic extract, and acarbose tablet



(1)Andrographolide; (2)*A. paniculata* leaf powder; (A)Andrographolide; (B)The ethanolic extract of *A. paniculata;* (C)The aqueous extract of *A. paniculata*; (I) and (II) UV_{254} nm detection; (III) UV_{365} nm detection.

Figure 2: Thin layer chromatogram

Discussion

The inhibition of aqueous extract and ethanolic extract of *A. paniculata* leaf against α-amylase enzyme activity was tested *in vitro,* with acarbose being used as a positive control. Acarbose was chosen because of its inhibitory mechanism of action of the carbohydrate hydrolyzing enzyme. The chemical structure of acarbose is similar to that of amylum, which acts as a substrate, where both compounds have a benzene ring and a hydroxyl group that plays a role in binding to the active site of the enzyme; thus, a competitive inhibition mechanism of the enzyme activity can occur (Wright, 2003; Takahama and Hirota, 2017). The decreasing intensity of blue colour in the iodine-amylum complex

is due to the reduced amylum substrate hydrolyzed by the α-amylase enzyme. The additional concentration of the material tests increased the percentage inhibition of α-amylase enzyme activity (Figure 1). At the same concentration, the ethanolic extract of *A. paniculata* leaf showed a higher percentage of inhibition of the α-amylase enzyme activity than the aqueous extract. The ANOVA followed by the Tukey Post-Hoc test (p <0.05) showed a significant difference in the percentages of inhibition of the ethanolic extract of *A. paniculata* leaf, the aqueous extract of *A. paniculata* leaf, and acarbose tablets, on the activity of the α-amylase enzyme.

The level of inhibitory activity against the α-amylase enzyme is expressed as 50% inhibition concentration (IC_{50}), which is the concentration of the test material that can inhibit the enzyme activity by 50% (Subramanian *et al.*, 2008). The value of the percentage inhibition of the test materials is used to calculate IC_{50} by using the linear regression equation formula to determine the equation y = bx + a. The IC_{50} value obtained for the aqueous extract of *A. paniculata* leaf was 14.203 ± 0.112 mg/mL, the IC_{50} value for the ethanolic extract of the *A. paniculata* leaf was 9.253 ± 0.116 mg/mL, while the IC_{50} value of the acarbose tablet was 0.983 ± 0.036 mg/mL. The inhibitory activity of the α-amylase enzyme from the ethanolic extract was greater than that of the aqueous extract. It seems that the active compound that functions as an α-amylase enzyme inhibitor in the leaf of *A. paniculata* is in both extracts, but the amount of the compound in the aqueous extract is lower than that in the ethanolic extract. Qualitative testing used the Thin Layer Chromatography method using Chloroform: methanol (9:1) as mobile phase and silica gel GF 254 nm as stationary phase, and it detected the presence of andrographolide (Figure 2).

The *in vivo* antidiabetic activity of *A. paniculata* aqueous extract had also been reported; a significant reduction in blood glucose level was shown when hyperglycemic rats were treated with 50 mg/kg body weight aqueous extract of *A. paniculata* (Husen *et al.,* 2004). Considering that both extracts showed inhibitory activity of the α-amylase enzyme, andrographolide can be in both ethanolic and aqueous extract, thus suggesting that andrographolide is responsible for the inhibitory activity of the α-amylase enzyme. The overall activity of plant extracts can result from mixtures of compounds with synergistic, additive, or antagonistic activity. It seems that they are more effective than purified compounds due to beneficial "synergistic" interactions (Caesar and Cech, 2019). The inhibitory activity of the α-amylase enzyme from the aqueous extract of *A. paniculata* leaf can occur due to the presence of andrographolide compounds itself, or it can be due to compounds other than andrographolide, present in the extract, that have positive interactions. Further studies are necessary to examine the effect of the combination of compounds in aqueous extract and ethanolic extract of *A. paniculata* leaf as an effort to optimize the safety and benefits of using *A. paniculata* leaf extract. The ethanolic extract of *A. paniculata* leaf had higher α-amylase inhibitory activity than the aqueous extract but lower than acarbose tablets. The aqueous extract and the ethanolic extract of the *A. paniculata* leaf showed *in vitro* inhibitory activity of the α-amylase enzyme with IC_{50} values of 14.203 ± 0.112 mg/mL and 9.253 ± 0.116 mg/mL, respectively. Although having less activity than ethanolic extract, the aqueous extract showed an *in vitro* inhibitory activity of the α-amylase enzyme; it is thus recommended to prepare *A. paniculata* using water.

Conclusion

Both aqueous extract and ethanolic extract of sambiloto (*Andrographis paniculata* Nees.) showed inhibitory activity of the α-amylase enzyme. The inhibitory activity of the α-amylase enzyme of the aqueous extract of *A. paniculata* leaf (with the IC_{50} value of 14.203 ± 0.112 mg/mL) was lower than that of the ethanol extract (with the IC_{50} value of 9.253 ± 0.116 mg/mL).

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IAI CONFERENCE

RESEARCH ARTICLE

Drug interactions in patients with hypertension at Persahabatan hospital in 2015

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Abstract

Introduction: Hypertensive disease can cause various complications, such as cardiovascular disease, stroke, diabetes mellitus, and kidney failure. To overcome these complications, patients are given polypharmacy therapy, which can potentially lead to drug interactions. **Aim:** The purpose of this study was to determine the potential incidence of drug interactions in hypertensive inpatients at the Persahabatan Hospital in 2015. **Methods:** The research is a descriptive study with retrospective data by purposive sampling technique using secondary data, medical records of hypertensive inpatients. The study of drug interactions was conducted theoretically, based on a literature study using Drug Interaction Facts 2014. **Results:** The results showed out of 174 hypertensive patients, 141 (81.0%) had potential drug interactions, with a total of 1444 cases. The highest drug interactions were at three levels of significance in 383 cases (26.5%), with 554 cases (38.4%) of pharmacodynamic mechanisms.

Introduction

Drug interactions involve the effect of one drug being altered by the presence of other drugs, food, drink, or other chemicals. Possible drug interactions appear when a patient is simultaneously given more than one drug, with the likelihood increasing depending on the concentration of the drug administered. Nowadays, with the increasing complexity of therapy, polypharmacy is widely used and has a great potential of causing drug interactions. Antihypertensive drugs can increase the likelihood of the occurrence of clinically significant drug interactions (Nidhi, 2012).

Based on the data from basic health research, the prevalence of hypertension in Indonesia in 2013 diagnosed by health workers showed that there was an increase from 7.6% in 2007 to 9.5% in 2013. This diagnosis was based on interviews, in which patients were asked if they had been diagnosed by health personnel or whether the patients were taking any hypertension medication/s. The prevalence of hypertension based on scientific measurements, however, showed a decrease from 31.7% in 2007 to 25.8% in 2013. The assumption of a decrease in the prevalence based on measurements is estimated because the gauges used in 2007 were no longer produced in 2013, or because public awareness had improved in 2013, which can be seen from the increase in the prevalence of hypertension diagnosed by health personnel based on interviews (Badan Penelitian dan Pengembangan Kesehatan, 2013).

A study conducted on inpatients suffering from hypertension, diabetes mellitus and hyperlipidaemia at Haji Hospital in Jakarta from January to June 2013 showed that potential interactions occurred in 48 patients (48.4%), while there were no interactions found in 49 patients (51.6%). The most common type of drug interaction mechanism was a pharmacokinetic type, at 73.9%, and the level of pharmacodynamic interaction was 26.1% (Jauhari, 2014). Another research at Dr Soeradji Tirtonegoro Klaten hospital from January to June 2009 showed that 55 patients had drug interactions, with a total of 104 cases. The number

of events based on the pharmacokinetic interaction mechanism was 28 (26.92%); pharmacodynamic interactions numbered 43 events (41.4%), while there were 33 incidents (31.7%) of unknown interaction mechanisms (Andriyanto, 2011).

The hypertensive disease can lead to various complications, such as cardiovascular disease, stroke, diabetes mellitus, and kidney failure (Baxter, 2008). To overcome these complications, patients are usually given polypharmacy therapy, which can potentially lead to drug interactions, which may increase the risk of toxicity or reduce the therapeutic effect of hypertensive drugs. In addition to overcoming the complications of hypertension, patients receiving polypharmacy therapy are also the presence of accompanying patient illnesses, which could potentially lead to drug interactions. It is, therefore, necessary to study drug interactions in hypertensive patients.

Methods

A cross-sectional design was used by accessing patients' medical records retrospectively and purposively. The data used were secondary data obtained from the search results of the potential of a drug interaction based on medical record status and therapy received as inpatients at Persahabatan Hospital during 2015. Sampling using the Krejcie and Morgan formula (Tatro, 2014). One hundred seventy-four patients met the inclusion and exclusion criteria.

Sample population

The population comprised inpatients diagnosed with hypertension at Persahabatan Hospital. The sample consisted of the medical records of hypertensive patients at the hospital in 2015 who met the inclusion criteria.

Inclusion and exclusion criteria

The inclusion criteria included inpatients 1) male and female; 2) diagnosed with hypertension at Persahabatan Hospital in 2015; 3) aged over 18; 4) medical records were complete, clear and legible; and 5) had received at least two drug treatments and hypertension drugs. The exclusion criteria included inpatients 1) diagnosed with hypertension, who were suffering from malignant diseases such as cancer; 2) pregnant women, and 3) patients were dead.

Data analysis

After collecting the data, the researchers conducted a descriptive analysis by screening the drug interactions using Drug Interaction Facts 2014 (Tatro, 2009). The percentage of patients with potential drug interactions based on significance level and interaction mechanism was illustrated.

Results and discussion

Drug interactions

The following is the patients' drug interaction data based on the Drug Interaction Facts 2014. As shown in Table I, it was found that the total number of patients with potential drug interactions was 141 (81.0%). In comparison, those without potential drug interactions were 33 (19.0%) out of the total of 174.

Table I: Number of patients

Type of patients	Number of patients (n: 174)	Percentage
Patients with potential drug interactions	141	81.0%
Patients without potential drug interactions	33	19.0%

Percentage of drug interactions based on level of significance

As can be seen in Table II, the total number of potential drug interactions was 1,443, with the most cases at Level 3 of significance, at 383 (26.5%).

Table II: Drug Interactions Based on Level of Significance

Level of significance	Number of cases (n: 1,443)	Percentage
Level 1	323	22..4%
Level 2	213	14.8%
Level 3	383	26.5%
Level 4	212	14.7%
Level 5	312	21.6%

Level of significance grade 1

The highest percentage of drug interaction occurs within the significance Level 1 group was those between spironolactone and ACE inhibitors, with a total of 62 cases; spironolactone-captopril, with 59 cases (18.3%); spironolactone-lisinopril, with two cases (0.6%); and ramipril-spironolactone, with one case (0.3%). The level of significance of the interaction between spironolactone with the ACE inhibitor group is at the level of significance of 1, with a major degree of

interaction and documented *probable*, which means this effect is potentially life-threatening, capable of causing permanent damage, and is highly likely to occur, but is not clinically proven (Tatro, 2009).

The combination of spironolactone and ACE inhibitors results in increased serum potassium concentrations in high-risk patients (renal failure) and an unknown interaction mechanism (Tatro, 2009). The recommended procedure is to monitor kidney function and potassium serum concentrations in patients who were receiving this drug combination and reduce dosage if necessary (Tatro, 2009). Combinations of these drugs should be avoided for patients with a glomerulus filtration rate of less than 30 mL/min (Baxter, 2010).

Level of significance grade 2

Drug interaction with the highest level of significance at Level 2 was aspirin with the ACE inhibitor group, with a total of 85 cases, consisting of 81 cases of captopril-aspirin (38.0%), three cases of aspirin-lisinopril (1.4%), and one case of ramipril-aspirin (0.5%). ACE inhibitors with aspirin interact with mechanisms by which aspirin inhibits the prostaglandin-mediated synthesis of vasodilatation. The significance level interaction of these drugs is at Level 2, with a moderate degree of interaction and documented suspected, which means that the effect may lead to deterioration of the patient's clinical status, therefore requiring additional therapy, and a prolonged hospital stay is possible, some data support, but more studies are required for verification. Effects resulting from drug interactions include cases of the effects of ACE inhibitors being reduced. In this case, the recommended procedure is to monitor blood pressure and the patient's hemodynamic parameters, to decrease the aspirin dose to below 100 mg/day, to switch to non-aspirin antiplatelet, or to replace the ACE inhibitors with angiotensin receptor blockers (Tatro, 2009).

Level of significance grade 3

At this level, the drug interaction with the greatest significance was furosemide with ACE inhibitors, with 183 cases comprising 176 cases (46.0%) of furosemide-captopril, two cases (0.5%) of ramipril-furosemide, and five cases (1.3%) of lisinopril-furosemide. Furosemide with ACE inhibitors interacts with the interaction mechanism by which the ACE inhibitors inhibit angiotensin II production, so the effects of furosemide are reduced (Tatro, 2009). In addition, furosemide increases the nephrotoxicity of ACE inhibitors (Lacy *et al*., 2009). The significance level interaction of these drugs is at Level 3, with a minor degree of interaction and documented suspected, which means the effect may be undetectable, but should not significantly affect the outcome of therapy and may occur, some data exists to support this, but more studies are needed (Tatro, 2009). The recommended procedure is to monitor the electrolyte and weight levels of patients receiving this combination (Tatro, 2009). British National Formulary recommends that inpatients taking furosemide at doses greater than or equal to 80 mg per day should consider temporarily suspending diuretics or lowering diuretic doses before adding ACE inhibitor therapy or that ACE inhibitor doses should start at very levels to avoid orthostatic hypotension (Baxter, 2008).

Level of significance grade 4

Drug interaction with the highest significance at Level 4 was $CaCO_3$ with proton pump inhibitors, with 59 cases, including 39 cases (18.4%) of $CaCO_3$-omeprazole and 20 cases (9.4%) of $CaCO_3$-lansoprazole. The significance level interaction of $CaCO_3$ with proton pump inhibitors is Level 4, with a moderate and intermediate degree and documented *possible*, which means the effect may lead to worsening of the clinical status of patients, the need for additional therapy, and likely lengthening of the stay in the hospital, but the data on this is very limited. The mechanism of the interaction of $CaCO_3$ with proton pomp inhibitors means the absorption of $CaCO_3$ is decreased so that the effect of $CaCO_3$ therapy is also reduced. The recommended procedure is to increase the dose of $CaCO_3$, especially in elderly patients (Tatro, 2009).

Level of significance grade 5

The highest level of drug interaction at Level 5 was furosemide-aspirin, with 160 cases (51.3%). The significance level of this interaction is level 5, with a minor interaction degree and documented *possible*, which means the resulting effect undetectable, but should not significantly affect the outcome of therapy and may occur, but the data is very limited. The mechanism of furosemide-aspirin interaction is unknown in cases when the furosemide effect is weakened in cirrhotic, and no clinical intervention is needed in response to the interaction between these two drugs. However, patients with cirrhosis and ascites who require furosemide need to be paid attention to aspirin usage (Tatro, 2009).

Percentage of a drug interaction based on the drug interaction mechanism

The drug interaction mechanism consists of pharmacokinetic and pharmacodynamic processes. Pharmacokinetic interactions involve the absorption, distribution, metabolism, and excretion of drugs. In

contrast, pharmacodynamic interactions involve the interaction of one drug with another, affecting the workplace (receptor) and leading to physiological disorders (Baxter, 2008).

As shown in Table III, drug interactions based on drug interaction mechanism of total drug interactions as many as 1,443 cases of drug interaction is the most of the mechanism of pharmacodynamic interaction as much as 554 cases (38.4%).

Table III: Drug Interactions Based on Interaction Mechanism

Interaction mechanism	Number of cases (n: 1,443)	Percentage
Pharmacokinetic	425	29.5%
Pharmacodynamic	554	38.4%
Combined Pharmacokinetic and Pharmacodynamic	41	2.8%
Unknown	423	29.3%

Drug interactions with pharmacokinetic interaction mechanisms

The greatest number of drug interactions with the pharmacokinetic mechanism was related to spironolactone-aspirin, with 81 cases (19.1%). The significance level of this interaction is level 3, with a minor degree of interaction and documented suspected, which means the resulting effect undetectable, but should not significantly affect the outcome of therapy and may occur, some data support, but more studies are needed (Tatro, 2009).

Spironolactone-aspirin interacts with the mechanism in which salicylate (aspirin) inhibits tubular renal secreting canrenone, which is an active metabolite of, meaning the diuretic action of spironolactone is reduced. However, data suggest that low-dose aspirin decreases the effect of spironolactone is still not studied (Tatro, 2009). It is possible that low-dose aspirin has benefits greater than being cardioprotective and reducing the effects of spironolactone in hypertensive and coronary artery disease patients (Baxter, 2008). The recommended procedure is to monitor blood pressure and sodium levels in chronic patients receiving spironolactone and salicylate (aspirin). An increase in the dose of spironolactone may be needed to restore its therapeutic effect (Tatro, 2009).

Drug interactions with pharmacodynamic interaction mechanisms

The most significant drug interaction with the pharmacodynamic mechanism was furosemide with ACE inhibitors, with 183 cases (33.0%), consisting of 176 cases (31.8%) of furosemide-captopril, two cases (0.4%) of ramipril-furosemide, and five cases (0.9%) of lisinopril-furosemide. Furosemide with ACE inhibitors interacts with the mechanism by which the inhibitors inhibit angiotensin II production, so the effects of furosemide are reduced (Tatro, 2009). In addition, furosemide increases the nephrotoxicity of ACE inhibitors. The interaction significance level of these two drugs is level 3, with a minor degree of interaction and documented suspected. The recommended procedure is to monitor the electrolyte and weight levels of patients receiving this combination (Tatro, 2009).

Drug interactions with combined pharmacokinetic and pharmacodynamic interaction mechanisms

Drug interaction based on the combined pharmacokinetic and pharmacodynamic interaction mechanism only included 41 cases of aspirin-insulin. The significance level of this interaction was 2, with a moderate degree of interaction and is documented probable, which means that the effect could lead to deterioration of the patient's clinical status, the need for additional therapy, and a possible prolonged stay at the hospital; it is very likely to occur, but has not been clinically proven. The interaction mechanism of insulin-aspirin means the basal concentration of insulin is increased, and the acute response of insulin to glucose increases, so the insulin effect is also increased. The recommended procedure is to monitor blood glucose concentrations and decrease insulin doses if necessary (Tatro, 2009).

Drug interactions with unknown interaction mechanisms

The highest number of drug interactions with an unknown mechanism was that of furosemide-aspirin, with 160 cases (37.8%). The significance level of these two drug interactions is 5, with a minor degree of interaction and documented possible, which means the resulting effect undetectable, but should not significantly affect the outcome of therapy, but the data are very limited. The mechanism of furosemide-aspirin interaction is unknown in cases when the furosemide effect is weakened in cirrhosis and ascites patients. In general, no clinical intervention is needed in response to the interactions of these two drugs. However, in patients with cirrhosis and ascites who require furosemide, they need to be paid attention to aspirin usage (Tatro, 2009).

Conclusion

Hypertension inpatients at Persahabatan Hospital in 2015 consisted of 95 (54.6%) men and 79 (45.4%) women. Those with potential drug interactions numbered 141 (81.0%), with those with no potential drug interactions 33 (19.0%). The total number of drug interaction cases was 1,443. In terms of significance of interaction, 323 cases (22.4%) were at level 1; 213 cases (14.8%) at level 2; 383 cases (26.5%) at level 3; 212 cases (14.7%) at level 4; as and 312 cases (2162%) at level 5. Interaction based on the pharmacokinetic mechanism comprised 425 cases (29.5%), with 554 cases (38.4%) based on the pharmacodynamic mechanism; 41 cases (2.8%) based on the combined pharmacokinetic and pharmacodynamic mechanism; and 423 cases (29.3%) on unknown mechanisms. It is necessary to study drug interactions in other hospitals as a comparison in order to improve hospital pharmacy services in the field of clinical pharmacy, especially in relation to drug interactions. In addition, experimental studies on drug interactions are needed, for which documented doubts and further evidence is necessary.

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