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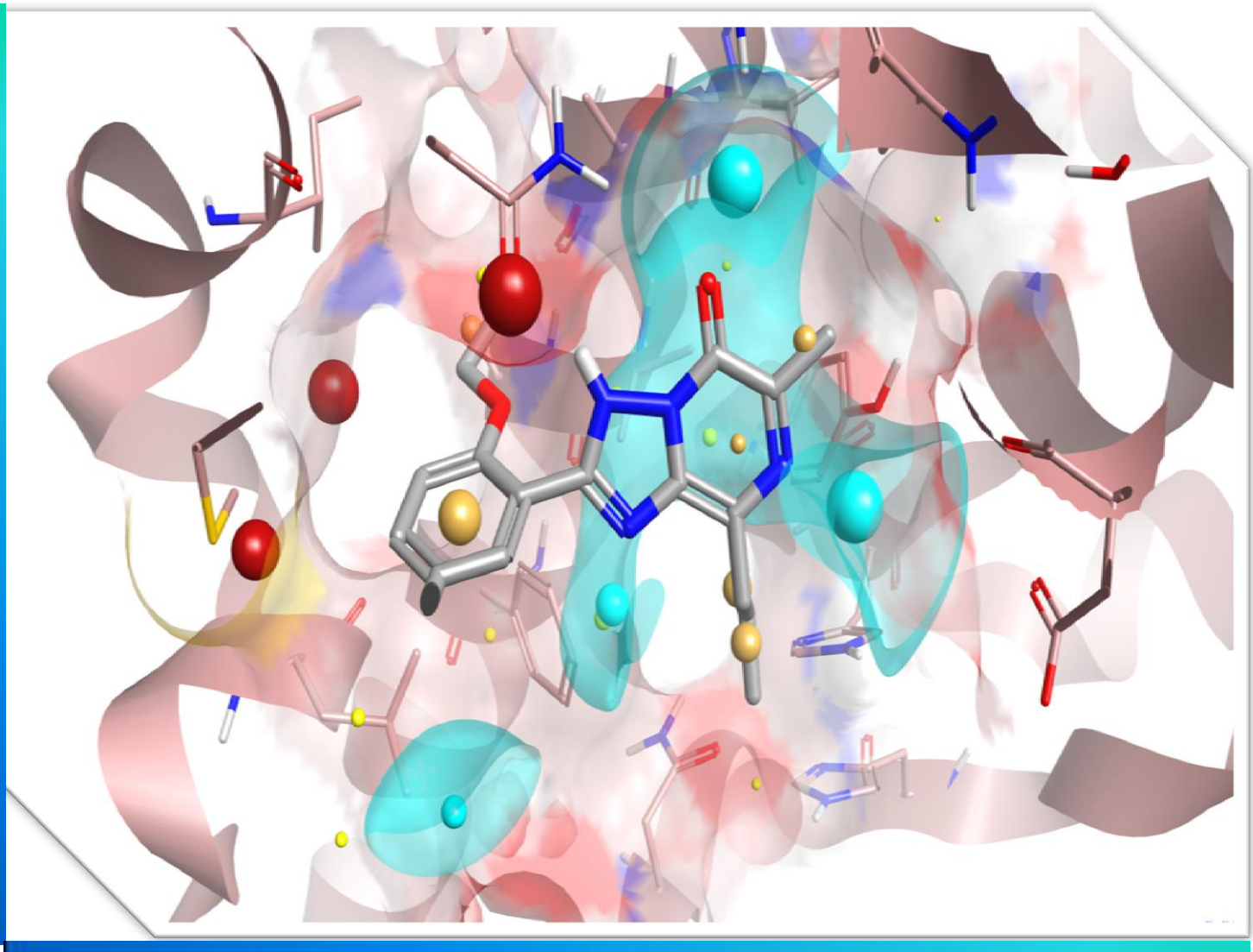
"Recent Advancement on Natural Product for Drug Design"



Volume 1, 2019

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The Effect of Ethanol Extract of Kasturi Citrus (*Citrus japonica* Thunb.) Peels on Cholesterol-LDL Levels in Male White Rats (*Rattus norvegicus*) Serum

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ABSTRACT

The effect of ethanol extract of citrus fruit (*Citrus japonica* Thunb.) peels on LDL cholesterol levels in serum of white male rat (*Rattus norvegicus*) has been studied. This research used 30 white male rat that divided into 6 groups, each group used 5 rats groups consisting of normal groups, negative group, positive group, and dose group that is dose 250, 500 and 1000 mg/kg BW. The normal group was given Na CMC suspension and standard feed. The negative group induced high-fat feed, standard feed and suspension of Na CMC. The positive group induced high-fat feed, standard feed, and atorvastatin suspension. The treatment group was given high fat feed-induced, standard feed, and suspension of ethanol extract of citrus fruit. The parameters are LDL cholesterol levels induced with high-fat feed containing a mixture of 2 mg cow brain and 2 ml duck egg yolk. The LDL cholesterol levels are calculating by using Assay Homogeneous method with a photometer (Mindray BA-88) at wavelength 600 nm. The data were analyzed by one way ANOVA (Analysis of Variance) and followed by the Tukey test. The result showed that ethanol extract of citrus Kasturi fruit can decrease LDL cholesterol levels by 19.18 % at doses of 500 and 25.48 % at a dose of 1000 mg/kg BW as indicated by significant difference with the negative control group ($p < 0.05$) and there was no significant difference with the positive group ($p > 0.05$).

Keyword : Ethanol Extract, Kasturi, LDL, Rats, Serum

1. INTRODUCTION

High cholesterol-LDL levels are a disorder of lipid metabolism called dyslipidemia [1]. Dyslipidemia promotes atherosclerosis and coronary heart disease (CHD) [2]. Based on Basic Health Research , coronary heart disease is the highest cause of death, namely 12.9% and is expected to increase to 23.3% in 2030 [3]. In Indonesia and several other countries, medicinal plants have been widely used in dealing with various diseases. One genus of plants that is often used as medicine is the genus Citrus. According to Okwu (2008) the genus Citrus has the potential for activity as an antioxidant, anticancer, anti-inflammatory, hypolipidemia, antihypertensive, antiulcer, antiaterosclerosis, anti-allergic and antimicrobial [4].

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Based on research on bioactive components from various types of citrus (citrus), it was found that kasturi orange (*Citrus japonica* Thunb.) rind had total flavonoids of 41.0±1.37 mg/g which consisted of flavonone (naringin, hesperidin and neohesperidin), flavones (diosmin, luteolin and sinensetin) and flavonol (routine, quercetin and kaemferol). In addition, it also has a carotenoid content of 0.737±0.029 mg/g and a pectin content of 62.1±2.36 mg/g [5]. Flavonoid compounds from the genus Citrus, such as hesperidin and naringin have hypocholesterolemic properties.

Ethanol extract of kasturi citrus rind is thought to have efficacy for lowering LDL-cholesterol levels, but no studies have looked at the effect of giving ethanol extract of kasturi citrus fruit on LDL-cholesterol reduction in experimental animals. The aim of this study was to determine the effect of giving ethanol extract of Citrus japonica Thunb. Citrus peel to decrease serum LDL cholesterol in male white rats (*Rattus norvegicus*).

2. METHODOLOGY

Materials

The materials used in this study are tools for chopping (knives and scissors), mortar and stanfer, drip plate, test tube (Pyrex®), drop pipette, a set of vacuum distillation devices (Clavenger Apparatus®), a set of rotary evaporator tools (Buchi®), centrifuges (Gemmy®), dark bottles, aluminum foil, analytical scales (Shimadzu Auw 220), animal scales, desiccators, glass beakers (Pyrex®), cotton, eppendorf tubes, measuring cups (Pyrex®), spatels, metabolic enclosures, oral syringes, filter paper, watch glass, micro pipettes, spikes, vials, and photometer devices (Mindray BA-88A). The ingredients used are citrus (*Citrus japonica*) fruit peel, 96% ethanol, feed, cow brain, duck egg yolk, aquadest, Na CMC, atorvastatin® 10 mg and LDL (Mindray) cholesterol-reagent.

Methods

Preparation of ethanol extract of kasturi citrus rind

As much as 10 kg of kasturi Citrus is first washed away from the dirt that is attached, then separate the peel of the citrus fruit from the flesh. The peel of the kasturi orange fruit is 2.55 kg. Then the peel of kasturi citrus fruit is chopped and dried air for 5 days to dry. The dried peel of kasturi citrus fruit is obtained as much as 650 grams. The dried samples were soaked with 96% ethanol until all samples were submerged in dark glass bottles and tightly closed with a capacity of 2.5 L. Soaking was carried out for 5 days (stirred every day) and repeated 3 times. The macerate is filtered with cotton and transferred into a tightly closed vessel. The macerate obtained was thickened by a rotary evaporator, so that the thick ethanol extract of the peel of the citrus fruit was obtained.

Preparation of Test Animal

Test animals are placed in individual cages, cage maintenance and cleaning are carried out every day. Acclimatization or stage of animal adaptation is carried out for 7 days by standard feeding and drinking ad libitum. Feeding animals as much as 10% of body weight [6]. The experimental animals used are animals that are healthy, have the same weight and age. Animals are declared healthy if during

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acclimatization do not show changes in body weight more than 10% and visually do not show symptoms that are not healthy. Test animals were grouped randomly so that the distribution of body weight was evenly distributed for all groups with variations in body weight not more than 20% of the average body weight [7].

Animal Test Grouping

Animals test were divided into 6 groups, consisting of one normal group, one positive (+) control group, one negative (-) control group and three treatment groups. Each group consisted of 5 rats. The treatment is as follows:

a. The normal group, each experimental animal was given standard feed for 28 days and was given Na CMC suspension on the 15^{th} to 28^{th} day.
b. Negative groups, each experimental animal was given standard feed and high-fat feed for 28 days and was given Na CMC suspension on the 15^{th} to 28^{th} day.
c. Positive group, each experimental animal was given standard feed, high-fat feed for 28 days and was given a comparative preparation atorvastatin on day 15^{th} to 28^{th} day.
d. Group dose 1, each experimental animal was given standard feed, high-fat feed for 28 days and was given ethanol extract of kasturi citrus peel at a dose of 250 mg/kg BW on the 15^{th} to 28^{th} day.
e. Group dose 2, each experimental animal was given standard feed, high-fat feed for 28 days and was given ethanol extract of kasturi citrus peel at a dose of 500 mg/kg BW on the 15^{th} to 28^{th} day.
f. Group dose 3, each experimental animal was given standard feed, high-fat feed for 28 days and was given ethanol extract of kasturi citrus peel at a dose of 1000 mg/kg BW on the 15^{th} to 28^{th} day.

Dosing Planning

The dosage of the test preparation (ethanol extract of kasturi orange fruit peel) used was 250, 500, and 1000 mg/kg BW given once a day. Whereas the comparative dosage dosage (atorvastatin) as a positive control used doses based on the usual dosage range in humans, 10 mg daily [8]. Converted to mice into: 10 mg x 0.018 = 0.18 mg / 200 g BW = 0.9 mg/kgBW/day.

Making High-Fat Feed

High-fat feed in the form of a combination of cow's brain and duck egg yolk with a ratio of 2 : 2 which is a mixture of 2 mg of cow's brain and 2 ml of duck egg yolk. High-fat feed is made by steaming the brains of the cow and mashed to form a pulp and then added duck egg yolk and homogeneous crushed. The induction of high-fat feed was carried out orally with a volume of 3 ml/head/day.

Preparation of ethanol extract suspension of kasturi citrus rind

Ethanol extract of kasturi citrus fruit was suspended with 1% Na CMC in distilled water. Weigh Na CMC then sprinkled on hot water in mortar, water used as much as 20 times the weight of Na CMC. Leave for about 15 minutes until Na CMC expands, then grind. Add the ethanol extract of the kasturi

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orange peel little by little into the mortar while being homogeneously crushed and enough with distilled water to the boundary mark.

Making Atorvastatin Suspension

The crushed Atorvastatin is suspended with 1% Na CMC in distilled water. Weigh Na CMC then sprinkled on hot water in mortar, water used as much as 20 times the weight of Na CMC. Leave for about 15 minutes until Na CMC expands, then grind. Add atorvastatin into the mortar little by little while grinding homogeneously and make enough with distilled water to the boundary mark.

Blood Sample Preparation

Mouse blood is taken as much as ± 2 mL through the orbital vein using a microhematocrit pipette, the blood obtained is left for 30 minutes and rotated on a centrifugation device at 3000 rpm for 10 minutes, then the serum will separate from the blood component and will be in the upper phase (supernatant). The separated serum was taken using a micro pipette, then inserted into the micro tube. Blood collection is done once, namely on the 29th day. Before taking blood, rats are fasted for 12 hours.

Measurement of LDL-cholesterol Levels

Measuring LDL-cholesterol levels using a homogeneous assay method with a photometer (Mindray®). Serum was taken as much as 12 µL with a micro pipette, blood was inserted into the micro tube and 900 µL reagent-1 was added. Mix and incubate for 5 minutes at 37 °C, then add another 300 µL of reagent-2. Mix and incubate for 5 minutes at 37 °C, read the absorbance at a wavelength of 600 nm with a photometer (Mindray BA-88A).

Data Analysis

Data obtained from observations of LDL-Cholesterol levels were analyzed statistically one-way Analysis of Variance (ANOVA) and followed by the Tukey Post Hoc test. This data is presented in the form of tables and bar charts.

3. RESULT AND DISCUSSION

The average LDL-cholesterol levels from the normal group, the negative group, the positive group, the dose group 250, 500 and 1000 mg/kg BW were 21.340; 27.745; 20.278; 23.397; 22.421 and 20.675 mg/dL. From the statistical calculation with one-way ANOVA, the results showed that there were significant differences between all groups ($p < 0.05$), so that it was followed by the Tukey Hoc Post test to see the differences between each group.

Based on the Tukey test, the results showed that there was a significant difference between the normal group and the negative group ($p < 0.05$). It can be interpreted that giving high-fat feed can increase serum LDL cholesterol levels in male white mice. Between the normal group with the positive group, the dose group 250, the 500 dose group and the 1000 mg/kg BW group did not have a significant difference ($p > 0.05$), which showed that the ethanol extract of the citrus peel can reduce LDL-cholesterol

levels in serum of male white rats induced by high-fat feed.

Table 1. The effect of ethanol extract of kasturi citrus (*Citrus japonica* Thunb.) peels on cholesterol-LDL levels in male white rats (*Rattus norvegicus*) serum

Number of Rats	LDL Cholesterol level (mg/dL)					
	Normal	**Negative**	**Positive**	**Dose 250 mg/kgBW**	**Dose 500 mg/kgBW**	**Dose 1000 mg/kgBW**
1	22.845	27.904	20.746	22.757	20.061	19.774
2	19.862	27.993	20.680	22.403	22.823	20.238
3	21.851	27.772	22.381	25.209	25.960	21.917
4	22.182	27.242	19.862	24.126	20.680	20.481
5	19.929	27.816	17.719	22.491	22.580	20.967
Average± SD	**21.334 ± 1.361**	**27.745 ± 0.294**	**20.278 ± 1.697**	**23.397 ± 1.228**	**22.421 ± 2.308**	**20.675 ± 0.817**

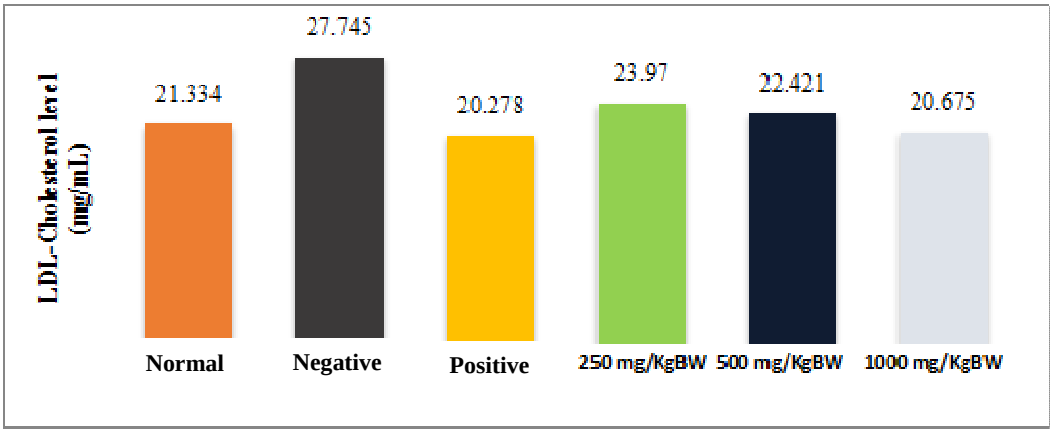

Figure 1. Average bar chart of LDL cholesterol in male white rat (*Rattus norvegicus*) serum

Based on the ANOVA test and the percentage reduction in LDL-cholesterol levels in groups of 250, 500 and 1000 mg/kg BW, which were also given a high-fat diet for 28 days showed a significant difference ($p < 0.05$) against the negative group, this means that administration the ethanol extract of kasturi orange peel in each group dose for 14 days, on the 15th day to the 28th day, can significantly reduce LDL-cholesterol levels. Ethanol extract of kasturi orange peel contains flavonoids, flavonoids will affect cholesterol concentration, especially LDL cholesterol levels, through barriers to cholesterol absorption and inhibition of HMG-CoA reductase enzyme activity. Thus giving ethanol extract can reduce the level of cholesterol-LDL. This study is in line with previous studies conducted by Kurowska et al. (2000) that the flavonoid compounds of citrus fruit can reduce LDL-cholesterol levels, triglycerides and can increase HDL-cholesterol levels [9].

Positive groups on 15th to 28th day were given atorvastatin suspension, atorvastatin can reduce LDL-cholesterol levels at a dose of 10 mg by inhibiting the enzyme HMG-CoA reductase which is an enzyme that plays a role in cholesterol synthesis in the liver. Based on research conducted by Abid et al.

(2016), atorvastatin can reduce LDL-cholesterol levels by 4.51% in hyperlipidemic mice [10].

Based on the ANOVA test and the percentage of LDL-cholesterol level reduction in the positive group with a dose of 500 and 1000 mg/kg BW there was no significant difference ($p > 0.05$), which means that the ethanol extract of citrus peel at doses of 500 and 1000 mg/kg BW can reduce LDL-cholesterol levels which are equivalent to the positive group given atorvastatin.

4. CONCLUSION

From the results of the research that has been done, it can be concluded that the administration of ethanol extract of the citrus fruit (*Citrus japonica* Thunb.) peel for 14 days can reduce LDL cholesterol levels in male white rats (*Rattus norvegicus*) at a dose of 250, 500 and a dose of 1000 mg/kg BW indicated by a significant difference with the negative group ($p < 0.05$). Based on statistical data on a dose of 500 and a dose of 1000 mg/kg BW, it has the same effect with atorvastatin 0.9 mg/kg BW in reducing LDL-cholesterol levels, so that the best dose in lowering LDL-cholesterol levels is a dose of 500 mg/kg BW because the dose is effective is a small dose that has a big effect.

5. ACKNOWLEDGEMENT

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The Best Extraction Methods of Wet and Dry Papaja (*Carica papaja* L.) Seed as Anthelmintics Effect on *Ascardia galli*

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ABSTRACT

Papaya seed in previous research is known has an effect of anthelmintic on *Ascardia galli*, but the effect of papaya seed just by traditional method i.e. boiling in water. To get the effective extraction methods, it still needs to be done many kinds of extraction methods of papaya seed, to know which one is the best. Methods of extraction in this research were maceration, soxhletation, and ultrasonic digestion. We used two kinds of papaya seed: wet and dry, using solvent: water, 70% ethanol, and n-hexane. Data of anthelmintic activity were percentage of worm death, LD_{50}, and relative potential to pyrantel pamoate as positive control. The result showed that the best extraction method of papaya seed which had highest in vitro effect on *Ascardia galli* worm was maceration method with 70% ethanol as solvent of wet papaja seed. It killed 60% of worm with LD_{50} 9.36 mg and its relative potential is 0.66 times pyrantel pamoate.

Keywords: Papaja seed, extract, anthelmintic

1. INTRODUCTION

Papaya seeds are one of the wastes that are not used by people, but in fact the results of preliminary studies show that papaya seeds are efficacious as anthelmintics (can kill worms in the intestine). Ardana et al. [1] showed the effectiveness of papaya seed powder in killing *Ascaris suum* worms in pigs, while Pattianakotta *et al*. [2] proved the effectiveness of ethanol extract of papaya seeds on *Ascardia galii* worms. Means papaya seeds can actually be used for the treatment of worms in children. In general, papaya seeds have been known to have antioxidant effects [3]. In order to be able to use it comfortably and easily, it is necessary to research and formulate this papaya seed extract in a stable and attractive modern drug dosage form for children, which has color and attractive aroma and good taste.

In this study we will look for extraction methods and solvents which will provide the most effective anthelmintic effect of papaya seeds. The design made was to try 2 types of simplicia papaya seeds, wet seeds and dried seeds. Each simplicia was then extracted using 3 kinds of extraction methods, maceration, ultrasonic digestion, and soxhletation. The solvents used are water, 70% ethanol and n-hexane. This design follows the research conducted by Himawan *et al*. [5] and Solhah [6].

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At this stage the research has not yet made an over-visible impact because basically it only gives an initial picture of the benefits of papaya seed extract, if it is successful in getting good preparations, efficacious and acceptable dosage forms for pediatric patients, then new dosage form can be applied to humans in standardized herbal forms [7]. The success of this research phase will be the basis for further research road maps, which are the basis for finding and obtaining active compounds from papaya seed extract. This active compound will be used as an extract marker. And the gain of this research is expected to get a dosage form. This preparation can be used for humans as well as for animals in veterinary dosage forms [8].

2. METHODOLOGY

Materials

Material used in this work is papaya seeds, aquadest, 70% ethanol, and n-hexane. Animal test used in this work is *Ascardia galli* worms. Tools used in this work is macerators, soxhlet, petri dish, rotary evaporator, and ultrasonic digest.

Methods

Preparation of extract

Simplicia of wet papaya seeds and dried papaya seeds was extracted with maceration, soxhletation, and ultrasonic digestion. In each method solvents were used aquadest, 70% ethanol and n-hexane solvents. The extraction results were concentrated using a rotary evaporator until it became a thick extract with certain parameters.

In vitro test of anthelmintics

Prepared petri dishes filled with each of 10 *Ascardia galli* worms. In the petri dish, normal saline (0.9% NaCl) was given which contained papaya seed extract with a level of 0.75; 1.5; 5.0; 7.5; 15; and 30%. Left for 15 minutes. Furthermore, it was observed the number of worm deaths in each petri dish, calculated as percentage of worm deaths.

3. RESULT AND DISCUSSION

Evaluation of papaya seed extract obtained included rendemen, organoleptic, flavonoid test, terpenoids, drying losses and ash residual. The highest extract yield was obtained by extracting with ultrasonic on wet papaya seeds with 70% ethanol solvent which was obtained 33% of rendemen. This extraction by ultrasonic is indeed the most effective way to get substances from simplicia because it occurs with a very effective dissolution process with ultrasonic vibrations. While the lowest yield was in the extract of wet seeds with n-hexane solvent soxhletation method. This happens because n-hexane is a nonpolar solvent, while wet seeds with polar conditions and soxhletation are heat methods with not too long immersion, even though solvent circulation occurs in this method.

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The organoleptic test results showed that all extracts were brownish in color, bitter taste, odor was not distinctive, because the smell of solvents was more pronounced, whereas for the solvent distilled water smelled of papaya. In phytochemical tests which include alkaloids, flavonoids, terpenoids, and steroids. All positive extracts of alkaloids and flavonoids. While the n-hexane extract was positive all tested, negative terpenoid and steroid water extracts.

For the loss on drying test because it was used as a standard, all extracts were thickened to water content (drying losses) not less than 6% not more than 8%. And all extracts were in the range of 6-8% LOD. For the highest levels of ash (residual spawning) was the ethanol extract with ultrasonic and the lowest level of n-hexane extract with the soxhletation method.

Table 1. Results of Qualitative Test of Extract

Materials	Methods	Solvents	Results				
			Alk	**Flav**	**Terp**	**Ster**	**Sap**
Wet Papaya Seeds	Macerations	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	-
		n-Hexane	-	+	+	+	+
	Soxhletation	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	+
		n-Hexane	-	-	+	+	+
	Ultrasonic Digest	Aquadest	+	+	-	-	+
		70% Ethanol	+	+	+	+	+
		n-Hexane	-	+	+	+	+
Dried Papaya Seeds	Macerations	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	-
		n-Hexane	-	+	+	+	-
	Soxhletation	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	-
		n-Hexane	-	-	+	+	-
	Ultrasonic Digest	Aquadest	+	+	-	-	-
		70% Ethanol	+	+	+	+	-
		n-Hexane	-	+	+	+	-

Note: Alk=Alkaloids; Flav=Flavonoids; Terp=Terpenoids; Ster=Steroids; Sap=Saponin

The results of anthelmintic test on *Ascardia galli* worms were obtained as shown in table 2. The results showed that the highest worm death was in wet seed extract with 70% ethanol maceration method. The average worm death in wet seed extract was relatively higher than the average worm death of dry seed extract. This shows that the anthelmintic active ingredient contained in papaya seeds will decrease if the seeds are dried. Furthermore, among the extraction methods it was seen that the killing power of the extract with the maceration method was on average higher than the worm death of the extract produced by another method, the ultrasonic method even though the yield was highest, but the worm death was

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low. This is presumably because there are too many other substances that do not have extracted anthelmintic properties that mask or reduce the effectiveness of the power to kill substances that have anthelmintic properties.

Then to compare the type of solvent to the killing power, it can be seen from the results that the worm death of the extract with 70% ethanol gives a higher average worm death compared to other solvents. In theory, semi-polar solvents make it possible to attract all efficacious substances from simplicia, in contrast to polar solvents which tend to attract only polar compounds, and nonpolar solvents only attract nonpolar compounds. Thus, it is assumed that the anthelmintic properties of papaya seeds are semi-polar compounds.

Table 2. Result of Test of Extract on *Ascardia galli*

Materials	Methods	Solvents	Anthelmintic effect		
			% Death	LD_{50} (mg)	Rel Pot
Wet Papaya Seeds	Macerations	Aquadest	27	65.65	0.09
		70% Ethanol	**60**	9.36	**0.66**
		n-Hexane	43	18.29	0.34
	Soxhletation	Aquadest	26	66.65	0.90
		70% Ethanol	53	12.10	0.52
		n-Hexane	37	28.98	0.22
	Ultrasonic Digest	Aquadest	23	70.56	0.08
		70% Ethanol	43	19.34	0.32
		n-Hexane	30	55.67	0.11
Dried Papaya Seeds	Macerations	Aquadest	23	78.65	0.08
		70% Ethanol	53	11.40	0.55
		n-Hexane	37	27.55	0.23
	Soxhletation	Aquadest	26	70.74	0.09
		70% Ethanol	53	12.22	0.51
		n-Hexane	37	30.32	0.21
	Ultrasonic Digest	Aquadest	17	129.45	0.04
		70% Ethanol	43	20.01	0.31
		n-Hexane	30	50.18	0.12

For the LD_{50}, the result showed that the lowest concentration was the highest for LD_{50}. Therefore, the highest of worm death gave the lowest of LD_{50}. It could be concluded that extract of wet papaya seed with 70% ethanol as solven give the best of LD_{50}.

Compare with pyrantel pamoate, the anthelmintic effect of extract could be said that it had adequate anthelmintic effect. So it is possible the papaja seed extract use as anthelmintic. For easier and practice on administration it can be made in the appropriate dosage form.

4. CONCLUSION

From the overall results of the test in this study obtained the best extract of its worm death on *Ascardia galli* worm was wet papaya seed extract with maceration extraction method using 70% ethanol.

The worm death of the extract was able to kill an average of 60% of worms with the extract content in 15% solution. The LD_{50} obtained 9.36 with relative potential 0.66.

5. ACKNOWLEDGEMENT

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Optimizing Streptozotocin Dose for Inducing Type 1 Diabetes Mellitus in Male Wistar Rats

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ABSTRACT

The preclinical antidiabetic study generally uses animal models with cytotoxic agent induction, e.g., streptozotocin (STZ). However, a different STZ dose (35-80 mg/kg) and routes of administration (intraperitoneally or intravenously) were mentioned before. This study has the aim to determine the optimal STZ intravenous dose for diabetes induction in rats. Sixty male Wistar rats (150-200 g) were undergone oral glucose tolerance test to examine their glucose homeostasis. A week later all rats were fasted for 8 hours and divided into six groups: STZ 40, 50, 60, 70, 80 mg/kg and control. Fasting blood glucose (FBG) level was measured on day 3, 7, 14 and 21 after STZ administration and was analyzed using a Kruskal-Wallis test. At day 21 all rats were sacrificed, and their pancreas was taken for histopathology examination. STZ given at 40,50,60, 70, 80 mg/kg resulted in death of 0%, 10%, 60%, 80%, 90% rats from each group. Three days after STZ administration there was a significant FBG increase in all STZ dose groups, except in 40 mg/kg group ($p < 0.05$). On the 7th day, the increasing FBG were persisted in 50, 60 and 70 mg/kg groups. On the 14th and 21st day, all rats FBG have decreased to <200 mg/dL. Histology examination revealed that a higher dose of STZ has resulted in lower Langerhans island number and diameter. The optimal dose of STZ to induce diabetic in male Wistar rats is 50 mg/kg. At this dose, hyperglycemia was persistent for seven days, and the mortality was minimal.

Key Words: optimal dose, streptozotocin, diabetes, Wistar rats.

1. INTRODUCTION

It was estimated that in 2014 more than 422 million adults are living with diabetes (global prevalence 8.5%). The increasing number of the diabetic patient was in line with increasing diabetic risk factors, such as overweight or obese. High glucose level also a risk factor for cardiovascular and other diseases leading to 3.7 million death before 70 years of age in 2012 [1]. The high level of diabetics incidence has encouraged the researcher to find antidiabetic remedies from various resources, one of them

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is from medicinal plants. It is common to use experimental animals with human-like diabetic physiological abnormalities to study the potential antidiabetic effect of the plant; The most common and frequent method was chemically-induced diabetes in rats or mice. A cytotoxic agent such as streptozotocin or alloxan was on the top list of the most widely used for diabetic induction.

Streptozotocin (STZ), a hydrophilic glucose analog, was more favorable than alloxan for animal diabetic induction because of it caused specifical damage to pancreatic beta cells. One hour after STZ administration, glucose concentration is increased, and plasma insulin was decreased. In this first hyperglycemic phase beta cells, morphology was changed and eventually result in inhibition of insulin secretion. This phase is persisted for 2-4 h. The second phase, the hypoglycemic phase, caused by hyperinsulinemia starts 4-8 h after induction and lasts for several hours. In this phase, glucose supplementation was needed to prevent animal death. Pancreatic cell already pyknotic and it was an irreversible condition. The last phase was a permanent hyperglycemic phase or diabetic phase that was seen 12-48 h after induction. In this phase pancreatic beta cell, the structure was utterly damaged [2].

In the implementation of diabetic induction using diabetes, there were several limitations, such as many variations in administration route and STZ dose from the literature. An extensive range STZ dose (35-80 mg/kg) was mention in various paper with two different routes of administration, intraperitoneally or intravenously [3]. STZ could be given in multiple small doses (35-40 mg/kg for 3-5 days) or single moderate or single large dose (> 45 mg/kg). Frequently used a single high dose of STZ given intravenously is 40-60 mg/kg. A single large dose STZ administration could destroy almost all pancreatic beta cells that mimicking diabetes type 1 condition in human [4–6].

Rats sensitivity to STZ was influenced by several factors such as strain, gender, diet, circadian rhythm. Moreover, different sensitivity to STZ in a subgroup of strain was also found. It is likely that it cannot be assumed that a strain from the same background will react to STZ in the same manner. Little genetic variability within the same age and gender group using the same STZ dose could result in different success induction rate. Furthermore, animals from different suppliers, outbred strain, or animals from a different generation in a colony can contribute to those difference sensitivity to STZ. Unidentified environmental conditions and stimuli also contribute to the different outcome of STZ-induced diabetes [4]. Therefore, preliminary experiments to determine optimal STZ dose for diabetes induction in our laboratory Wistar rats need to be carried out.

2. METHODOLOGY

Materials

Streptozotocin (Sigma-Aldrich, China), Glucose (Merck, Germany), glucometer with glucose dehydrogenase method (Accu-Chek® Performa, Roche, Germany), citric acid (Weifang Ensign Industry Co. LTD., China), monosodium citrate (Weifang Ensign Industry Co. LTD., China), sucrose (PT. Mawar Jaya, Indonesia), microscope (Olympus CX-21, Japan), Optilab® Advanced Plus software (Indonesia), syringe (OneMed, Indonesia), needle 30G 1/2 (BD Precisionglide, United States), rats feeding needle 18G (Indonesia), microcentrifuge tube 1,5 ml (OneMed, Indonesia), standard laboratory diet (19,5-21,5%

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protein, 5% fat, 5% fiber, 0,9% calcium, 0,6% phosphate, 3125-3225 kcal/kg, BR2 CP512, PT. Charoen Phokphand, Indonesia), insulin detemir (Levemir® FlexPen®, Novo Nordisk Production SAS, France).

Methods

Animals

Sixty male Wistar rats (*Rattus norvegicus*) with 150-200 g body weight (b.w) were obtained from a veterinarian breeder. Rats were transferred into the animal laboratory in Widya Mandala Catholic University, Surabaya, Indonesia and acclimatized for seven days. The animals were housed under 12 hours light/dark cycle, fed with standard laboratory diet and water ad libitum. These procedures have requested approval by Animal Ethics Committee at Integrated Research and Testing Laboratories, Gadjah Mada University, Yogyakarta, Indonesia.

Oral Glucose Tolerance Test

A week before the experiment, all animals undergo the oral glucose tolerance test (OGTT) to asses glucose homeostasis based on the postprandial blood glucose level. Oral glucose tolerance test method was modified from the previous study [7]. All rats were fasted for 18 hours, then weighed to determine glucose (2 mg/kg) doses. Blood was collected from the tail vein and tested with a glucometer as basal fasting blood glucose (FBG). Hereafter, 40% glucose solution was administered intragastrically with a feeding needle. Blood glucose level was measured again after 15, 30, 60 and 120 min. Rats with high glucose level (≥200 mg/dl) at 120 minutes after glucose administration were excluded from the study. The rest was divided into six different group, namely STZ 40, STZ 50, STZ 60, STZ 70, STZ 80, and a control group.

STZ Preparation

Streptozotocin (STZ) was stored in the freezer (-20^{o}C) before used. STZ is weighed according to the dose (40 to 80 mg) then dissolved in one mL freshly prepared cold citrate buffer (pH 4.5) in a microcentrifuge tube. During the STZ preparation, the container was maintained cold and protected from the light.

DM Type 1 Induction using STZ

All animals fasted for 8 hours (7.00 a.m - 3.00 p.m.). Afterward, rats were divided into six groups depending on body weight (the variation in each group ≤ 10%). Blood was collected from the tail vein and tested as basal FBG. The treatment group was given STZ 40, 50, 60, 70, 80 mg/kg. Meanwhile, the control group was given citrate buffer pH 4.5. All substances were administered intravenously through the tail vein. After STZ induction, all animals were given 10% sucrose in the first 24 hours to avoid death due to severe hypoglycemia. FBG level measurement was repeated on day 3, 7, 14, and 21. Pancreas were taken if the rat was not undergone rigor mortis. In this study, all rats with diabetic symptoms (fasting blood sugar ≥400 mg / dL) were given 2 U insulin detemir to prevent death from crisis hyperglycemia.

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Histopathological examination of the pancreas

Rats were euthanized by giving ketamine 200 mg/kg and xylazine 35 mg/kg intraperitoneally. Pancreas were collected for histopathological analysis. All portions of the pancreas were fixed in 10% buffered formalin solution for 24 h and then trimmed. The pancreatic tissue was gradually dehydrated in 70% alcohol, 80% alcohol, 90% alcohol, 96% alcohol, xylol, and liquid paraffin. The following step was tissue vacuuming and embedding. Pancreatic tissue was sectioned using microtome at a thickness of 2-5 µm and then processed with hematoxylin and eosin staining. The observation was carried out under a microscope with 400 times magnification. The Langerhans island diameter was measured using calibrated Image Raster 3® software.

Statistical Analysis

All data were statistically analyzed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., USA). All data were analyzed their normality of distribution using Shapiro-Wilk test before a specific test was accordingly performed. Difference analysis in blood glucose level and body weight were carried out using paired t-test or Wilcoxon signed rank test. Blood glucose level after STZ induction were analyzed using a Kruskal-Wallis test. A 0.05 level was adopted for any significant difference.

3. RESULT AND DISCUSSION

Oral Glucose Tolerance Test

Oral glucose tolerance test (OGTT) was the most common method to assess glucose homeostasis in rodents [7]. The ability of glucose tolerance can be maintained as long as the pancreatic beta cells can compensate by increasing insulin production. Hence the glucose tolerance test results can indirectly describe the function of pancreatic beta cells [8]. OGTT was performed one week before the series of this study was performed. All rats fasted for 18 hours before their basal fasting blood sugar levels were measured. After that glucose (2 g/kg) was administered and multiple blood glucose level measurements were performed at 15, 30, 60 and 120 minutes. There was a sharp increase in blood glucose level after 15 minutes. Sixty minutes after glucose administration, the blood glucose level was starting to decrease (Figure 1). There was a significant decrease in blood glucose level between 15 to 120 minutes ($p < 0,05$) and none of the rats have blood sugar level higher than 200 mg/dL. Consequently, all rats were included in this study.

STZ Dose and Mortality Rate

The administration of STZ in rats caused mortality in various number across the experimental group. Rats were mostly died (38,33%) within 0-5 days after STZ induction (Table 1). Two days after STZ administration, one rat from STZ 70 group was found dead. On day three, 14 rats from different experiment groups (five from STZ 60, four from STZ 70, and five from STZ 80 group) died at a different occasion. On day four, there were eight rats died (one from STZ 50 group, one from STZ 60, two from STZ 70 and four from STZ 80). The last recorded death was at day 20 when a rat from STZ 70 was found died. Overall, 24/60 (40%) rats were experienced death. From Table 2 it was known that the highest

mortality rate was found in the STZ 80 group (90%). A higher STZ dose may increase the risk of rats mortality [9]. It was already known that STZ given ≥75 mg/kg without insulin administration might cause natural ketosis and death within days after STZ administration [10].

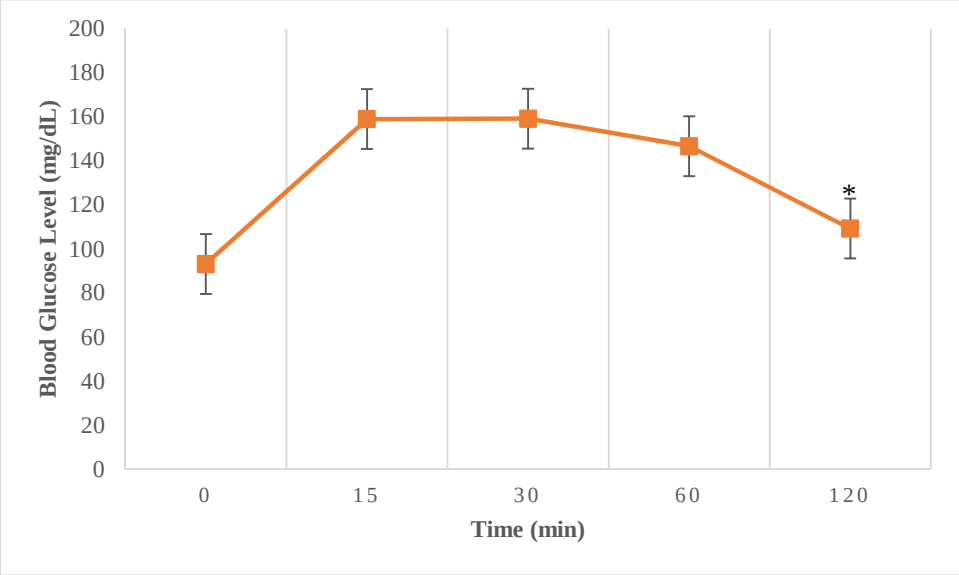

Figure 1. Blood glucose level during OGTT. Data express the mean ± S.E.M. for 60 rats. (*) show a significant different in FBG between 15 and 120 minutes (Wilcoxon signed rank test, $p < 0.05$).

Table 1. Rat mortality according to number of days after STZ administration

Days After STZ Induction	Number of Rats Found Dead	Number of Rats Left	% Mortality
0-5	23	37	38,3
6-10	0	37	0
>10	1	36	2,7

Table 2. Rat mortality according to STZ dose

Group	Number of Rats Found Dead	Number of Rats Left	% Mortality
Control	0	10	0
STZ 40	0	10	0
STZ 50	1	9	10%
STZ 60	6	4	60%
STZ 70	8	2	80%
STZ 80	9	1	90%

Body Weight after STZ Induction

Rats body weight was gradually decreasing after STZ administration (Figure 2). Significant decrease in body weight was found in STZ 50, 60, and 70 group at day 21 after STZ administration. Based on eye observation, physical activity and appetite in STZ-induced group were decreased.

Contrarily, their water consumption was increased. Those symptoms supposed to be a common phenomenon on day two and three after STZ administration. In that phase, rats may experience cachexia with decreased appetite, epistaxis, and hematuria it as well as hyperemia in the organs [11]. It was reported before that STZ administration can affect the body weight, particularly in Wistar and Sprague Dawley rats [12]. After STZ administration, male Wistar rats have more noticeable weight loss than female rats [4,12]. The body weight loss was proportional to the increase in STZ dose. Induction of T1DM with STZ causes glucose metabolism disorders with increased metabolism gluconeogenesis and glycogenolysis pathway [13]. Those metabolism change causes a decrease in bone and muscle mass after STZ induction, resulting in decreased body weight [5].

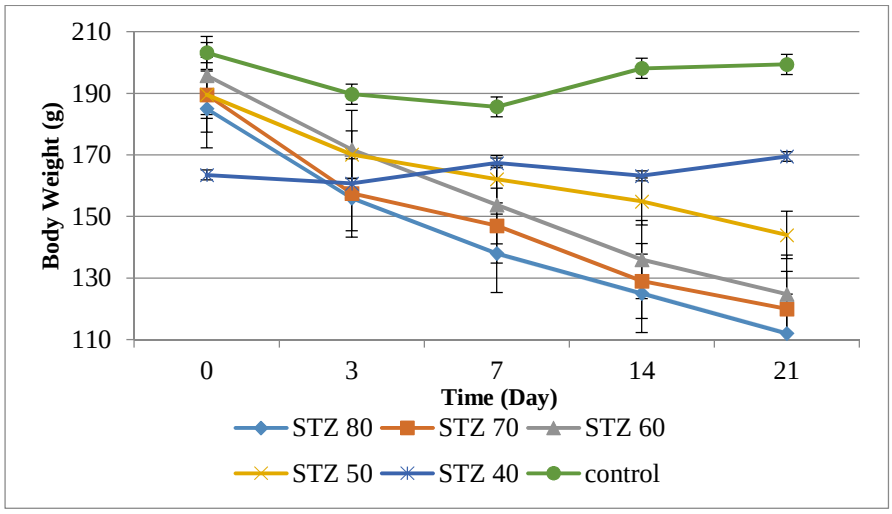

Figure 2. Body weight after STZ induction. Data express the mean ± S.E.M. Body weight was reducing significantly from day 0 to day 21 in STZ 50, 60, and 70 group (paired t-test or Wilcoxon test, $p < 0.05$). Only one rat in STZ 80 was survived on day 21 so the test result was not perfomed.

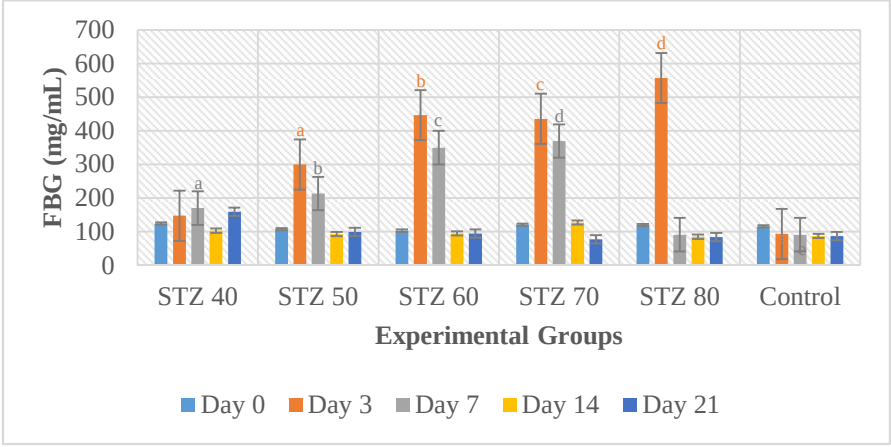

Figure 3. Fasting blood glucose level after STZ induction. Data express the mean ± S.E.M. Different letters indicate statistically significant different with control group on each day (Kruskal-Wallis, $p < 0.05$).

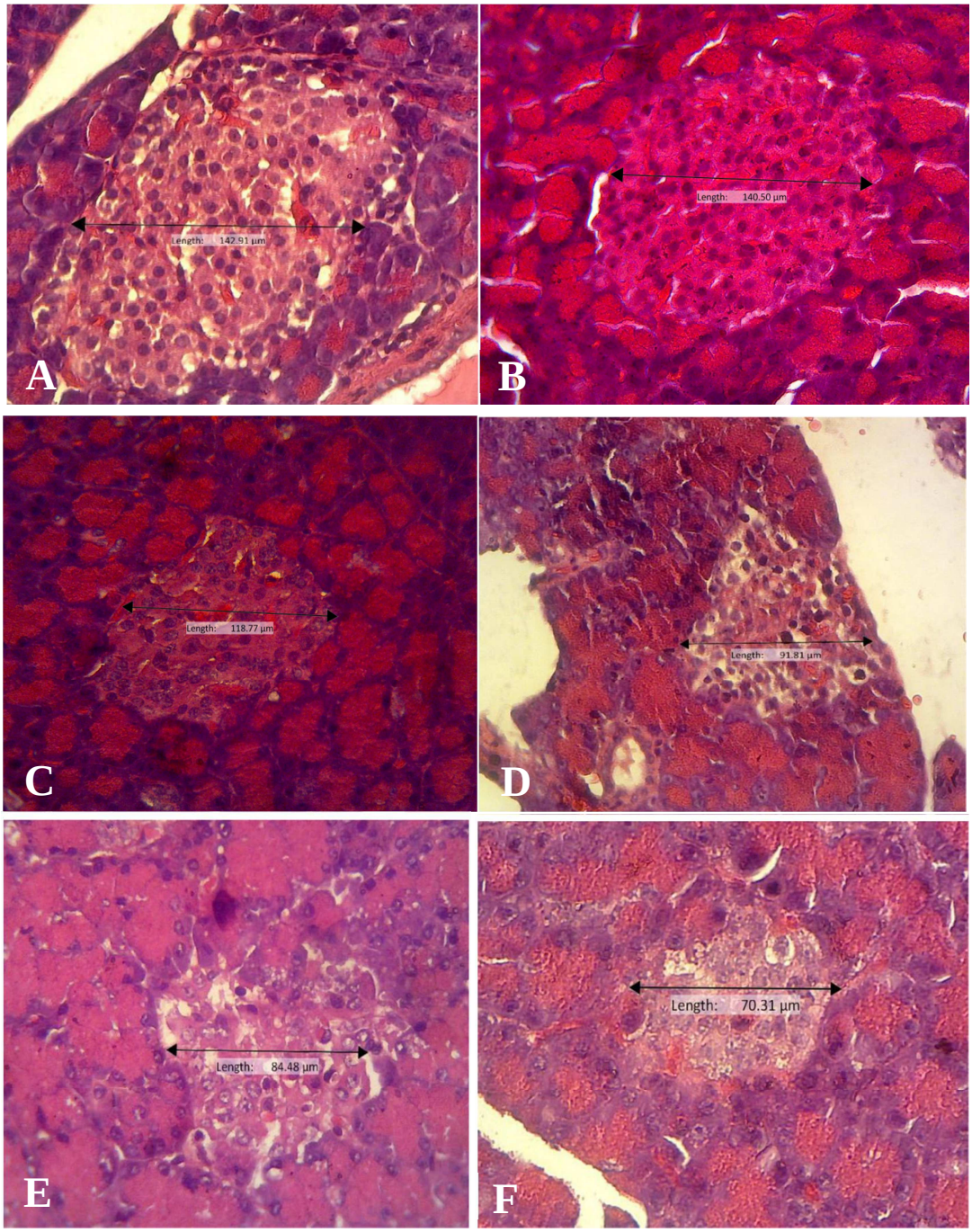

Figure 4. Histology of pancreatic rat cells in different experimental group: control (A), STZ 40 (B), STZ 50 (C), STZ 60 (D), STZ 70 (E), STZ 80 (F) (H&E staining, 400x magnification).

STZ Dose and Type 1 Diabetes Mellitus Induction Success Rate

Type 1 diabetes mellitus (T1DM) is a metabolic disorder related to glucose metabolism abnormalities. T1DM characterized by elevated blood sugar levels due to pancreatic beta cells damage. STZ may be used as a T1DM chemical inducing agent because of its specific action to pancreatic beta cells. STZ causes permanent damage to pancreatic beta cells resulting in permanent hyperglycemia as

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well. GLUT-2, an insulin receptor, helps STZ to enter the pancreatic beta cells. Also, pancreatic beta cells are more sensitive to glucose that facilitates STZ entry through GLUT-2. Therefore, STZ may cause toxicity in pancreatic beta cells. STZ is the material that causes DNA alkylation and increases oxidative stress. It also causes excessive glucose entry into beta pancreas cells that can cause cell damage [5].

FBG between groups at day 0 was found to be in normal range. Normal FBG usually lies between 95 to 100 mg/dL [14]. From day 2 -21, several rats of the various group were found dead (Table 1 and 2). Therefore, the number of rats examined for their FBG at each group was varied. Fig. Three showed that there was a sharp increase in blood glucose level in STZ 50, 60, 70, and 80 group on day three ($p < 0.05$). However, the increase was only survived until day seven. Their FBG returned to "normal" at say 14 and 21. Although the FBG level was decreased in all groups, our histopathology report showed a decrease in the number and diameter of the Langerhans island which proportionally to STZ dose (Figure 4). Those damage in Langerhans islands indicates a deterioration in insulin secretion by pancreatic beta cells [15]. However, pancreatic beta cells usually have a regeneration process to the cell damage, so that makes the hyperglycemia condition is less stable [13]. Second STZ induction may be needed to induce chronic diabetes mellitus in a longer antidiabetic experimental timeline.

At this present study, STZ 40 failed to induce diabetic state as their FBG always below 200 mg/dL (Figure 3). It was stated in a review that the administration of low dose STZ could not lead to persistent hyperglycemia [16]. The diameter of pancreatic islets of STZ 40 was also similar to the diameter of pancreatic islets of the control group (Figure 4). At low dose (20-40 mg/kg) STZ can be active at multiple administration regimentation [17].

4. CONCLUSION

STZ given at 40-80 mg/kg dose intravenously has a variable effect. STZ 40 has no diabetic effect while STZ 50 to 80 has actively induced T1DM. However, the mortality as a result from STZ administration was increased proportionally in according to STZ dose. Therefore, considering its effectivity and mortality rate, STZ 50 was the most effective and tolerable dose for inducing T1DM. At this dose, hyperglycemia was persistent for seven days and 10% mortality rate.

5. ACKNOWLEDGMENT

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Subchronic Toxicity Study of *Ipomoea batatas* L. Leaves Ethanol Extract on Albumin Level in Mice (*Mus musculus* L.) Serum

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ABSTRACT

Indonesian people have long known and used natural medicines, known as traditional medicines. *Ipomoea batatas* L. leaves is used traditionally for ailments in Indonesia and can be develope into standarized herbal medicine. Thus, toxicity studies are important to assess the toxic effects of plant. This study was conducted to see the toxic effects caused to the liver function by parameter measured levels of serum albumin, liver weight ratio and weight gain of experimental animals after repeated exposure for 60 days. This study used 20 white male mice were divided into 4 groups. Each group contained 5 animals consisting of the control group (NaCMC 1%) and extract with variations in doses of 150, 300, and 600 mg/kg body weight administered orally. On day 61 the animals were sacrificed and then measured levels of serum albumin with photometric test bromcresol green methods using Mindray® reagents and photometers instruments (Mindray® BA-88A) at a wavelength of 578 nm. The results showed that ethanol extract of leaves of purple sweet potato doses of 150, 300 and 600 mg/kg body weight on the serum albumin and a weight ratio of liver were analyzed statistically one-way ANOVA followed by Tukey's test did not differ significantly ($p > 0.05$) to control but differ significantly ($p < 0.05$) on weight gain were statistically analyzed by two-way ANOVA followed by Tukey's test.

Keywords: *Ipomoea batatas* L., serum albumin, mice

1. INTRODUCTION

Indonesian people have long known and used natural medicines, known as traditional medicines. One of the plants that grows in Indonesia is purple sweet potato (*Ipomoea batatas* L.). Traditionally purple sweet potato leaves are used as medicine for boils, fever and burns [1]. In vitro testing showed that the portion of sweet potato leaves significantly had higher phenolic levels and antioxidant activity compared to tuber parts [2]. Purple sweet potato leaves also contain vitamin A and vitamin C and contain several minerals such as magnesium, phosphorus, calcium, iron, sodium, potassium, and manganese [3]. In the case of developing natural ingredients medicine, it is necessary to know the effect of using these drugs on body safety. The drug safety assessment approach can be carried out by toxicity testing [4]. The toxic effects of drugs are more commonly seen in the liver, because liver plays a central role in metabolizing all drugs and foreign substances that enter the body [5]. clinical features of hepatotoxicity

include: hepatocyte integrity (SGOT and SGPT), bile excretion function (bilirubin, alkaline phosphatase, gamma glutamyl transpeptidase), and hepatocyte function (albumin, prothrombin time, ammonia) [6]. Examination of hepatocyte function illustrates the ability of the liver to synthesize proteins (albumin, globulin) and metabolize substances contained in the blood [7]. One of the most commonly performed categories of hepatocyte function checks is serum albumin measurement. Albumin is the main protein in human plasma and is produced by the liver around 12 g per day. Albumin serves to maintain plasma colloid osmotic pressure, binds various ligands, helps metabolize and transport drugs, anti-inflammatory, helps balance acid and base, antioxidants, maintains microvascular integrity, and anticoagulants. Reduced albumin synthesis signifies impaired liver function [8,9].

Based on the description above, a study was conducted on the effect of giving purple sweet potato (*Ipomoea batatas* L.) leaves ethanol extract using male white mice (*Mus musculus* L.) to see the toxicity it caused to liver function with measurement parameters serum albumin level, ratio of liver weight and increase in body weight of test animals. Giving this ethanol extract of purple sweet potato leaves will be given orally with a frequency of once a day for 60 days. Measurement of serum albumin was carried out using the Bromcresol Green Photometric Test method using a Photometer (Mindray® BA-88A) at a wavelength of 578 nm.

2. METHODOLOGY

Preparation of Ethanol Extract of Purple Sweet Potato Leaves

Purple sweet potato (*Ipomoea batatas* L.) leaves are sorted into separate pieces of dirt, then washed thoroughly. After that, it is dried by airing and not in direct sunlight. The dried purple sweet potato leaves are soaked in 96% ethanol for five days while stirring occasionally. The ethanol extract was filtered and the pulp was macerated again in the same manner for up to three repetitions. All ethanol extracts were collected, then concentrated using a vacuum rotary evaporator at 60ºC with a vacuum pressure of 175 mbar until the solvent evaporated until a thick extract was obtained.

Phytochemical Screening

Phytochemical screening of ethanol extract of purple sweet potato leaves includes examination of alkaloid, flavonoid, phenolic, saponin, terpenoid and steroid.

Preparation of Animal Experiments

The experimental animals used were healthy male white mice, as many as 20. Before the experiment the animals were acclimatized for 7 days. Animals are declared healthy if the spread of weight is evenly distributed for all groups with variations in body weight not more than 20% of the average body weight and visually do not show symptoms that are not healthy.

Preparation and Provision of Test Preparations

Male white mice were divided into 4 experimental groups where each group consisted of 5 mice. Groups 1, 2 and 3 were groups of mice given a dosage of 150, 300 and 600 mg/kg BW orally. Group 4

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was negative control given NaCMC with a concentration of 1% orally. The volume of test preparation given to mice is 1% of body weight. The test preparation is given every day with a frequency of once a day for 60 days.

Measurement of Test Animal Weight

Monitoring changes in the increase in body weight of test animals is carried out by weighing the body weight of the test animals 2 (two) times a week at 11 am for 60 days.

Sampling of Blood and Organ Hearts

After giving ethanol extract of purple sweet potato leaves for 60 consecutive days according to the dosage, on the 61st day all blood of mice was taken by cutting the blood vessels in the neck and then the blood was collected in a test tube and left for 30 minutes. Blood was centrifuged for 10 minutes at 3000 rpm. Serum fluid was separated using a micro pipette and inserted in the eppendorf tube, then serum albumin levels were examined by the Photometric Test Bromcresol Green method. In addition, the liver was taken and weighed to calculate the ratio of liver weight.

Measurement of Serum Albumin Levels

The amount of 5 µl of the test serum was reacted with 500 µl of Bromcresol Green Mindray® reagent and then homogenized at 37ºC and immediately measured its absorbance at a wavelength of 578 nm, read absorbance 5 minutes then using a Mindray® BA-88A photometer.

Calculation of Liver Organ Weight Ratio

The liver organs from mice that have been taken then weighed then calculated the ratio of liver weight to formula [10] :

$$\text{Liver Organ Weight Ratio} = \frac{\text{Mice Liver Weight}}{\text{Mice Body Weight}}$$

Data Analysis

Data from the results of serum albumin and the relative weight of the liver obtained were carried out by a one-way Analysis of Variance (ANOVA) test. Body weight was analyzed by two-way Analysis of Variance (ANOVA). If the results of further analysis of meaningful analysis are used Tukey Multiple Range Test (Tukey Multiple Range Test).

3. RESULT AND DISCUSSION

Based on the phytochemical screening, the ethanol extract of purple sweet potato (*Ipomoea batatas* L.) leaves showed that the extract contained chemical compounds in phenolic groups, flavonoids and terpenoids. The results of examination of serum albumin levels using the Bromcresol Green Photometric Test method according to the treatment dose are presented in Table 1.

Tabel 1. Mean (± SD) serum albumin levels of mice during the experiment

Group	Serum Albumin Level (g/dL)
Control (1% Na CMC)	3,269 ± 0,050[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 150 mg/kg BW	3,250 ± 0,075[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 300 mg/kg BW	3,268 ± 0,025[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 600 mg/kg BW	3,191 ± 0,021[a]

[a] The same superscript in the same column shows no significant difference ($p > 0.05$)

The serum albumin levels of mice obtained from the average control group, doses of 150, 300 and 600 mg/kg BW were 3,269; 3,250; 3,268 and 3,191 g/dL. Serum albumin levels obtained are included in the normal range of serum albumin for male mice which is 2.8-4.8 g/dL [11]. The results of one-way ANOVA statistical analysis of serum albumin levels showed that there was no significant difference from the administration of purple sweet potato leaves ethanol extract to controls, doses of 150, 300 and 600 mg/kg BW ($p > 0.05$). These results indicate that the ethanol extract of purple sweet potato leaves relatively did not cause toxic effects on hepatocyte function. Albumin is the most abundant protein in blood plasma and is responsible for regulating intravascular pressure [12]. Measurement of serum albumin levels is the most frequently performed examination of hepatocyte function [6]. Decreased serum albumin levels indicate the presence of chronic liver diseases such as cirrhosis and chronic liver failure [13]. Decreased serum albumin also occurs in cases of severe malnutrition, burns, acute and chronic inflammation, and malignancies [14,15,16,17].

After taking blood, the mice were dissected to make macroscopic observations of the organs of the mice, besides the liver was taken and weighed to calculate the value of the ratio of liver weight. From the results of macroscopic observations, there are some organs that have abnormalities compared to controls. In the group dose of 300 mg/kg BB, there is one mice with swollen intestine filled with air and in the 600 mg/kg BB group, there are two mice whose intestines are bulging filled with air. This shows that the test preparation of ethanol extract of purple sweet potato leaves affects or causes damage to intestinal organs from mice.

Tabel 2. Mean (± SD) liver organ weight ratio of mice during the experiment

Group	Liver Organ Weight Ratio
Control (1% Na CMC)	0,0537 ± 0,00247[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 150 mg/kg BW	0,0524 ± 0,00190[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 300 mg/kg BW	0,0521 ± 0,00331[a]
Purple Sweet Potato Leaves Ethanol Extract Dosage of 600 mg/kg BW	0,0505 ± 0,00384[a]

[a]The same superscript in the same column shows no significant difference ($p > 0.05$)

Table 2 shows the liver organ weight ratio of mice given suspension of purple sweet potato leaves ethanol extract dose 150, 300 and 600 mg/kg BW from the results of one-way ANOVA statistical analysis

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followed by the Tukey test which did not give a significant difference to the control ($p > 0.05$). Relative organ weight is a very sensitive and consistent indicator of toxicity. Organ weight ratio is usually a very sensitive indication of the effect on the liver [18]. From the study after 60 days of treatment of the liver did not show a significant difference, this showed that the ethanol extract of purple sweet potato leaves did not cause toxic effects that were evident in the liver tested.

Parameters of toxic effects can be seen from mortality, weight gain and relative weight of the liver and kidneys. Weight can provide a rough picture of general health conditions and is supporting data that describes health in experimental animals [18]. The results of measuring the increase in body weight of mice after being treated for 60 days are presented in Figure 1.

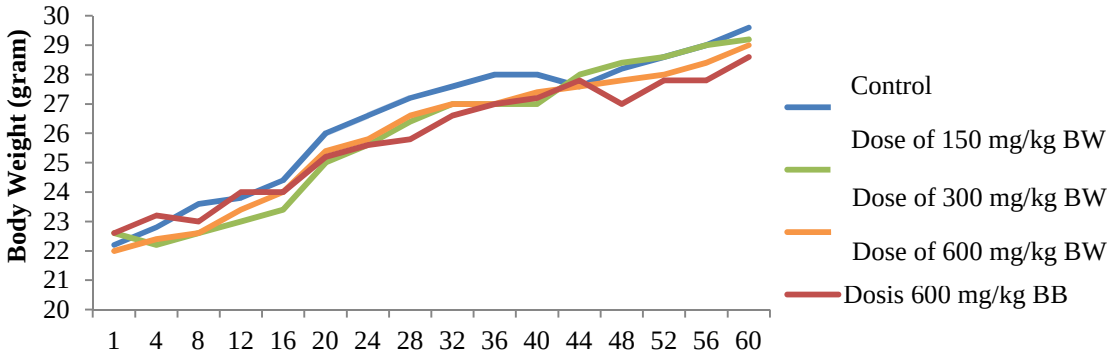

Figure 1. The average of increasing in body weight of male white mice for 60 days

In measuring changes in the weight gain of mice, a two-way ANOVA statistical analysis was used. The results of the two-way ANOVA statistical analysis showed that there were significant differences from the giving of purple sweet potato leaf ethanol extract to the control, dosages of 150, 300 and 600 mg/kg BW ($p < 0.05$). Based on the Tukey test it was found that the administration of ethanol extract of purple sweet potato leaves at a dose of 150, 300 and 600 mg/kg BW was significantly different from the control ($p < 0.05$) while giving ethanol extract of purple sweet potato leaves between doses of 150, 300 and 600 mg/kg BW did not differ significantly ($p > 0.05$). There was no significant difference in weight gain between doses of 150, 300 and 600 mg/kg BW indicating that the dose did not significantly affect the rate of weight gain. While the body weight of mice given treatment decreased the rate of weight gain significantly when compared to the control group.

The decrease in the rate of increase in body weight can be attributed to the content of flavonoids contained in the ethanol extract of purple sweet potato leaves. Flavonoids can inhibit cholesterol absorption, reduce LDL (Low Density Lipoprotein) levels, and increase HDL (High Density Lipoprotein) levels in the body [19]. In in vitro testing, flavonoid compounds namely astilbin can reduce cholesterol levels in the blood by inhibiting the action of the enzyme 3-hydroxy 3-methylglutaril coenzyme A reductase (HMG Co-A reductase), this enzyme catalyzes changes in HMG Co-A becomes mevalonic acid which is the first step in the synthesis of cholesterol. With the reduction of cholesterol available, the accumulation of cholesterol in the body's organs gets smaller and reduces the possibility of obesity or weight gain [20]. Flavonoid extracts isolated from gedi (Abelmoschus manihot) plants induced in wistar

strain male rats fed protein-rich feed had an anti-obesity effect by reducing rat body weight by 7.85% [21]. Decreasing the rate of increase in weight can also be caused by stress which can increase the speed of metabolism and excretion of nitrogen which results in endogenous proteins and fat reserves in the body being dismantled to become a source of energy, so that weight will decrease or tend to settle [22].

The administration of purple sweet potato (*Ipomoea batatas* L.) leaves ethanol extract at a dose of 150, 300 and 600 mg/kg BW caused a significant decrease in the rate of weight gain to control but the administration of ethanol extract of purple sweet potato leaves at a dose of 150, 300 and 600 mg/kg BW did not affect serum albumin levels and the liver organ weight ratio to control significantly.

4. CONCLUSION

Based on the results of this study, it can be concluded that the administration of purple sweet potato (*Ipomoea batatas* L.) leaves ethanol extract at doses of 150, 300 and 600 mg/kg BW on serum albumin levels and liver organ weight ratio in control male white mice did not differ significantly ($p > 0.05$) . This shows that the use of ethanol extract of purple sweet potato leaves is not toxic to hepatocyte and liver function significantly, but there was a decrease in the rate of weight gain at doses of 150, 300 and 600 mg/kg BW against controls, significantly ($p < 0.05$).

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The Eugenol Content from Essential Oils of Highlands and Lowlands *Ocimum sanctum* L. Leaves

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ABSTRACT

Ocimum sanctum L, known in Indonesia as kemangi, is one of essential oil producing plant with a fairly large eugenol content. The difference in where a plant grows can affect the content of chemical compounds from plants. The purpose of this study was to determine the eugenol content of essential oils of kemangi leaves that grow in the highlands and lowlands. The material was collected randomly from five regions of the highlands and five regions of the lowlands. Analysis of eugenol content was carried out by gas chromatography using the Stabilwax column, with the FID detector, N_2 and H_2 as mobile phases, 50 split ratio, 67.3 ml/sec flow path, temperature of detector and injector 230^oC, and 1 µl injection volume. The results showed that the eugenol content of essential oils of kemangi leaves from highland regions were greater with concentration of 43.87-61.81% than the lowland regions with concentration of 28.29-41.53%.

Key words: *Ocimum sanctum* L., kemangi, eugenol, lowland, highland

1. INTRODUCTION

Plants are a source of active compounds that are widely used as drugs, such as eugenol. Eugenol has pharmacological properties including anesthetic activity, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic, neuroprotective ability, hypolipidemic efficiency and antidiabetic effectiveness [1]. Eugenol is contained in many essential oils from various types of plants such as clove (*Eugenia caryophyllata* Thumb) and cinnamon (*Cinnamomum zeylanicum* Breyn), but the price are high. Another plant source that produces eugenol which is commercially cheaper is the *Ocimum* genus such as *Ocimum sanctum* [2]. *Ocimum sanctum* is a plant belonging to the Lamiaceae that grows in the tropics and subtropics [3]. In Indonesia, *Ocimum sanctum* known kemangi is commonly used as a vegetable. Kemangi is also used as a traditional medicine to cure stomach aches, fever, and eliminate bad breath [4,5]. Essential oil of *Ocimum sanctum* leaves contains eugenol, eugenal, carvacrol, metilchavicol, limatrol and caryophylline [3]. This plant can grow at lowland and highland areas. Differences in growing places can affect the compound content of a plant. This study was conducted to determine the eugenol content of *Ocimum sanctum* essential oil from several growing places in the highlands (above 700 m above sea level) and lowlands (up to 200 m above sea level). The essential oil of *Ocimum sanctum* leaves

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is extracted by steam distillation. Determination of eugenol essential oil content of *Ocimum sanctum* leaves was carried out by gas chromatography method.

2. METHODOLOGY

Materials

Fresh *Ocimum sanctum* leaves were collected from five highland areas (altitude> 700 m asl): Gunung Gede, Kuningan, Sukabumi, Lembang, Dago and from five lowland areas (altitude <200 m asl). All leaves were determined at the Biology Research Center, LIPI, Cibinong, Indonesia.

Methods

Essential oil preparation

Extraction of essential oils from leaves is carried out by steam distillation. Before distillation, leaves were wilted for 24 hours.

Gas chromatography analysis

Determination of eugenol content from essential oils was carried out by column chromatography using the Stabilwax® (Crossbond®) column, FID detector, N_2 and H_2 mobile phase, split 50 ratio, flow path 67.3 mL/sec, temperature detector and 230^{o}C injector, and volume injection 10 µL. Before determining the level of eugenol essential oil, analysis method validation was carried out which included system suitability test, linearity test, LOD and LOQ test, accuracy test and precision test.

3. RESULT AND DISCUSSION

Extraction of essential oils of leaves by steam distillation produced light yellow essential oil to brownish yellow in accordance with the *Ocimum spp* oil quality standard criteria [6]. The essential oil content of *Ocimum sanctum* leaves from the highlands and lowlands can be seen in **Table 1**.

Table 1. Essential oil content of *Ocimum sanctum* leaves from the highlands and lowlands

Altitude of land	Location	Essential oil content (%)
Highland	1. Dago	0.08
	2. Kuningan	0.13
	3. Gunung Gede	0.17
	4. Lembang	0.16
	5. Sukabumi	0.13
Lowland	1. Depok	0.10
	2. Jakarta Timur	0.36
	3. Tangerang	0.07
	4. Bekasi	0.15
	5. Bojong Gede	0.11

The validation of method resulted data that meet the requirements with RSD values <2%, linear (r = 0.9990), LOD = 71.54 µg/mL and LOQ = 21.46 µg/mL, accurate with percent recovery 99.17 - 101.51%

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and precise with relative standard deviation of 0.21%. The concentration of eugenol from essential oils of highland *Ocimum sanctum* ranged 43.87- 61.81% where the highest levels from the Lembang region (61.54%) and the lowest from the Dago region (43.87%). Statistical analysis was performed by Anova test. The result showed no significant difference of eugenol levels from the essential oils of *Ocimum sanctum* leaves in the highlands with $p > 0.05$ and significance > 0.05.

Concentration of eugenol from essential oils of lowland *Ocimum sanctum* ranged from 28.29 - 41.22% where the highest concentration from the Bekasi area (41.22%) and the lowest was from Bojong Gede (28.29%). Statistically analysis showed no significant differences in the concentration of eugenol from essential oils of lowland *Ocimum sanctum* with $p > 0.05$ and significance > 0.05, except eugenol concentration from Bojong Gede area with $p < 0.05$ and significance < 0.05. There was a significant difference between the concentration of eugenol essential oil *Ocimum sanctum* from the highlands and eugenol concentration from one lowland area: Bojong Gede. However, generally there is a difference between the levels of eugenol from the essential oils of the *Ocimum sanctum* leaves from the highlands and the lowlands. The height difference affects the air temperature and rainfall. The higher the place, the lower the air temperature and the higher the rainfall that will increase soil fertility [7]. The Differences in soil fertility in the highlands and lowlands cause the differences in the content of eugenol produced by *Ocimum sanctum* plants. The concentrations of eugenols in essential oil of *Ocimum sanctum* leaves are presented in **Table 2.**

Table 2. Eugenol content from *Ocimum sanctum* leaves from the highlands and lowlands

Altitude of land	Location	Eugenol content (%)
Highland	1. Dago	44.31
	2. Kuningan	53.45
	3. Gunung Gede	51.47
	4. Lembang	61.54
	5. Sukabumi	56.95
Lowland	1. Depok	40.05
	2. Jakarta Timur	36.86
	3. Tangerang	40.32
	4. Bekasi	41.22
	5. Bojong Gede	28.29

4. CONCLUSION

The eugenol content *of Ocimum sanctum* leaves from highland regions were greater with concentration of 43.87 - 61.81% than the low land regions with concentration of 28.29 - 41.53%.

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Quantification of Pyridoxine Level in Banana (*Musa paradisiaca*) Fruit with Processing Method Variation

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ABSTRACT

Traditionally, a banana (*Musa paradisiaca*) fruit is used to treat emesis on pregnant individual. Earlier, some research presented that the effect is probably caused by the pyridoxine content in the fruit, which is widely utilized to treat nausea and vomiting in pregnancy. It is important to acknowledge a better way consuming the fruit to achieve the best anti-emesis effect for a pregnant woman. This research aims to determine the level of pyridoxine in banana fruits with four different processing process. The study compares the pyridoxine composition in the mature fruit with and without any further heating treatment. The level was measured using HPLC method with the mixture of methanol and water for a mobile phase. The result shows that there is not a significant change of pyridoxine level in the sample before and after boiling. It was obtained that the content of pyridoxine in a before boiling mature banana fruit and in the fruit after boiling for 20, 30 and 40 minutes was 0.2530 and 0.2860; 0.3060; and 0.3646 mg/g, respectively. In conclusion, even though there is a slight increase, a differentiation of boiling time does not affect the level of pyridoxine in banana fruits significantly.

Keywords: Banana, heating, processing method, *Musa paradisiaca*, pyridoxine.

1. INTRODUCTION

Vitamins play an important role in human health, tough in a small amount [1]. Moreover, vitamins also can be used to treat some diseases or heal a disease's symptoms [2]. One of vitamins that is essential for human is pyridoxine (Sami et al, 2014). Besides its function in amino acid transamination [3], it also can delight nauseous and vomiting condition in a pregnant individual [4].

Nausea and vomiting happens in first trimester of pregnancy and occurs up to 80% of pregnancies. Combination of doxylamine and pyridoxine is currently the first line pharmacological therapy for nausea and vomiting of pregnancy. However, in some countries, the pyridoxine alone is recommended for the first line therapy the syndrome in pregnancies, such as in the United States and Australia [5]. Some studies report that the usage of pyridoxine alone has a significant result in minimizing nausea and vomiting symptoms compared to placebo in a double-blind randomized trial [6]. Furthermore, there is no evidence of adverse outcome in pyridoxine monotherapy in pregnancies [7].

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In Indonesia, traditionally, a pregnant individual is suggested to consume banana fruit to overcome nausea and vomiting condition. It is consumed with or without boiling process of the raw or edible fruit. The effect, possibly, is caused by pyridoxine content in it [8]. However, the level of pyridoxine content in banana could be affected by ways of the fruit consumption. It is obtained that pyridoxine is an unstable compound and can be degraded by light, heat, acids, alkali and oxidizing agents [9] [10]. Moreover, the substance is a water-soluble [11], so a boiling process probably can reduce its concentration in samples because of an extraction mechanism.

It is important to analyze the effect of boiling process on pyridoxine content in banana fruit to obtain the best way of banana fruit consumption in nausea and vomiting treatment in pregnancy. The level of pyridoxine in samples can be quantified using high-performance liquid chromatography (HPLC) [12]. About 40% higher vitamin B contents can be detected on an average than that by using classical microbiological methods [13].

2. METHODOLOGY

Materials

HPLC-grade solvents were used for analysis and obtained from manufacturer. Deionized water was used in all procedures and was carried out by means of a Millipore deionizer. The banana fruit was obtained from Pekanbaru, Indonesia.

Methods

Sample Preparation

Edible banana fruits, with its peel, were boiled in water (100°C) for 20; 30 and 40 minutes. The pyridoxine level was detected before and after boiling process.

Determination of Pyridoxine Level

The pyridoxine was extracted using buffer solution (a mixture of sodium salt of hexane sulfonic acid). The process was prepared at room temperature. Prior to sample injection the solution was filtered through a Milipore filter to remove any undissolved particle. Twenty microliters of the filtrate were injected into HPLC system. Quantification of pyridoxine level was accomplished by comparison to pyridoxine standards. Standard stock solution and calibration curve were prepared as reported by Anyakora et al (2008) [2]. Chromatographic separation was achieved on a reversed phase HPLC (Shimadzu) through the isocratic mobile phase (20-70 of methanol and the buffer) at a flow rate 0.75 ml/minute. Ultraviolet absorbance was recorded at 255 nm at room temperature.

3. RESULT AND DISCUSSION

Extraction

The pyridoxine from banana fruit sample was extracted in buffer solution from the mixture of sodium salt, hexane, sulphonic acid, glacial acetic acid and deionized water. Mixing sample in the buffer

was done by a vigorous shaking to make sure the homogeneity of the mixture. Hexane sulphonic acid in the buffer utilized for removing the bulky proteins in the food, hence fostering the proper dissolution of the vitamin [2]. The filtering process was done to to remove solid material before analyzing. The vitamin target is a compound that is soluble in the buffer.

Calibration curve of pyridoxine standard

The regression equation was achieved trough a calibration curve of standard with the concentration of 50; 100; 150; 200 and 250 ppm. It obtained a linear curve with regression coefficients value of 0.992. Figures 1 shows the calibration curve for pyridoxine standard. The regression equation was used to quantify pyridoxine concentration in sample.

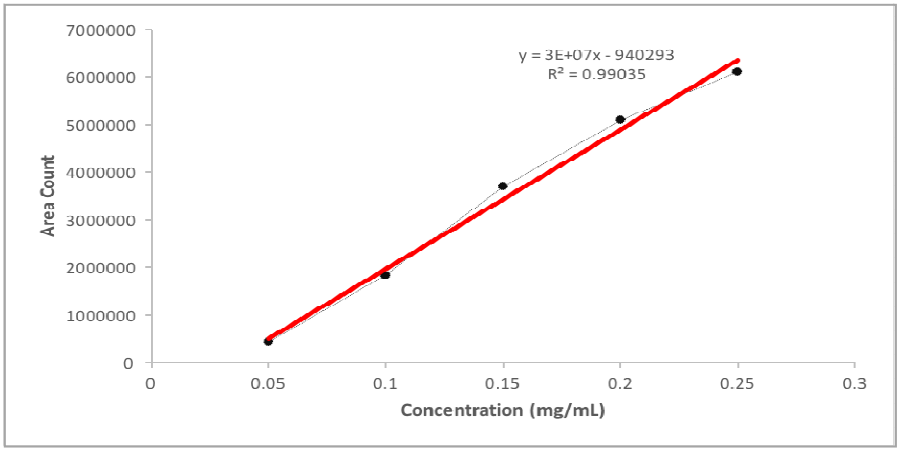

Figure 1. Calibration curve of pyridoxine.

Effect of boiling process on the level of pyridoxine

RP-HPLC can be used to analyzed the level of pyridoxine in banana fruit sample. Identification of the compound was done by comparing the retention time of sample and pyridoxine standard. In this research, the standard gave three peaks at 255 nm detection. The pyridoxine showed a peak at 9.926 minutes (250 ppm) (Figure 2).

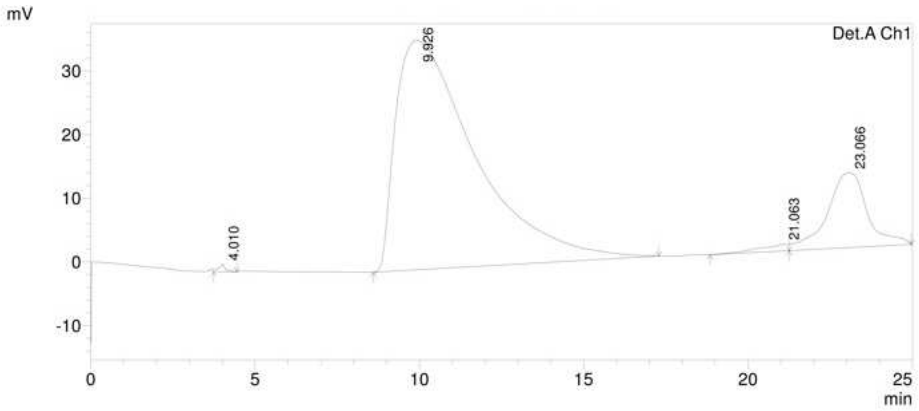

Figure 2. Chromatogram of pyridoxine standard concentration 250 ppm at 255 nm

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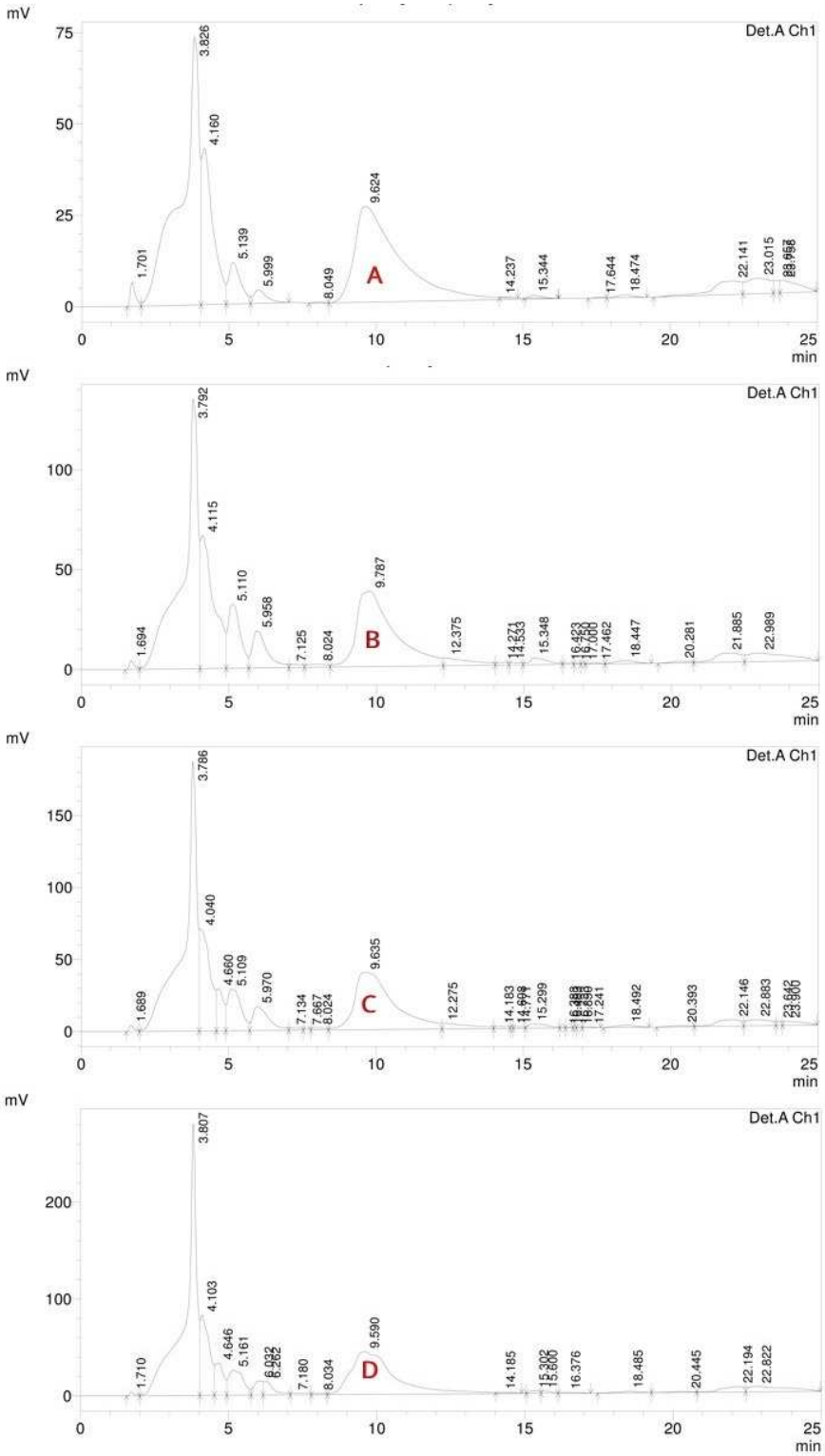

Figure 3. Identification of pyridoxine content in sample (A) before heating (B) after boiling 20 min (C) after boiling 30 min (D) after boiling 40 min.

All of sample contain pyridoxine with retention time of from 9.590 to 9.787 (Figure 3). It is obtained that there was no a significant change of pyridoxine level in banana fruit sample after boiling in water for 20 to 40 minute. The pyridoxine level in sample before boiling process and after boiling for 20,

30 and 40 minute was 0.2530; 0.2860; 0.3060; and 0.3646 mg/g, respectively. It can be seen that the level of pyridoxine after boiling increased slightly compared to the level before boiling. However, an increase of pyridoxine level did not significant statistically. Moreover, the concentration experienced a gradual increase after boiling process for 20, 30 and 40 minutes. Concentration of pyridoxine in the sample would increase with an increase of boiling time, but did not significant (Table 1). The heating process possibly could affect the level of pyridoxine in boiled banana fruit, but boiling the fruit without peeling the skin could help preventing the degradation process. Furthermore, the skin also prevented an extraction mechanism of pyridoxine in the sample into water.

Table 1. The level of pyridoxine in the sample.

Variable	AUC	Retention time	Concentration of pyridoxine (mg/g)
Without heating	2871602	9.624	0.2530
Boiling 20 min	3379615	9.787	0.2860
Boiling 30 min	3680188	9.635	0.3060
Boiling 40 min	4612640	9.590	0.3646

4. CONCLUSION

It is true that there is a slight increase of pyridoxine content by the boiling time prolongation, though the number is not significant. Moreover, boiling banana fruits without peeling its skin possibly could prevent the degradation process of pyridoxine content in it.

5. ACKNOWLEDGEMENT

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Antimicrobial Activity of Ethanol Extract of Pegagan Air (*Hydrrocotyle vulgaris* L.) Leaves against Pathogenic Bacteria and Fungi

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ABSTRACT

Plants of "*pegagan air*" (*Hydrocotyle vulgaris* L.) especially the leaves part is known to have medicinal properties. The active compounds that are contained in the leaves of "*pegagan air*" (*Hydrocotyle vulgaris* L.) are able to work as antimicrobial. The aim of this study is to explore the microbial activity of the ethanol extract of pegagan air (*Hydrocotyle vulgaris* L.) leaves against Gram negative bacterias (*Pseudomonas aeruginosa* and *Escherichia coli*), Gram positive bacterias (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and a fungi (*Candida albicans*) at concentrations of 5%, 10%, and 15%. Based on the antimicrobial test, ethanol extract of "*pegagan air*" leaves showed inhibition zone against all of tested bacteria and provided better inhibition to Gram negative bacteria. There is an effect of the concentration of ethanol extract of "*pegagan air*" leaves on the diameter of the inhibition zone ($p< 0.05$). But, it showed no inhibition zone against the tested fungi.

Keywords: *Hydrocotyle vulgaris* L., antibacterial, antifungal, inhibition zone

1. INTRODUCTION

Traditional medicine is medicine obtained naturally, generation-by-generation, based on ancestorial recipes, culture, beliefs, or local custom, whether it is be magic or traditional knowledge. Traditional medicine is beneficial for our health that the use of it keeps advancing because it is more accessible to the people, price or availability. Pegagan (*Centella asiatica* (L.) Urban) is one of the traditional medicine. It is a wild plant that has a worthy prospect to become a traditional medicine [1].

The "*pegagan air*" (*Hydrocotyle vulgaris* L.) can be explained in general that it contain some of the similar compounds in pegagan (*Centella asiatica* (L.) Urban) plant, includes secondary metabolites such as triterpenoids, flavonoids, tanin, and glycoside [2]. Some triterpenoids in "*pegagan air*" (*Hydrocotyle vulgaris* L.) are also found in pegagan (*Centella asiatica* (L.) Urban) among others are asiatate acid, madecasat acid, asiaticoside and madecasoside [3].

Research on the activity of pegagan (*Centella asiatica* (L.) Urban) plants against pathogenic bacteria that can cause disease has been widely carried out, while studies of antibacterial and antifungal activity of pegagan air (*Hydrocotyle vulgaris* L.) have never been reported. Due to the resemblance of the

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secondary metabolites in "*pegagan air*" (*Hydrocotyle vulgaris* L.) and pegagan (*Centella asiatica* (L.) Urban) and limited literature on the test of antibacterial and antifungal activity of the gotu kola plant (*Hydrocotyle vulgaris* L.). The aim of this study is to conduct antimicrobial activity of ethanol extract of pegagan air (*Hydrocotyle vulgaris* L.) leaves against some Gram positive bacterias (*Staphylococcus aureus* and *Staphylococcus epidermidis*), Gram negative bacterias (*Pseudomonas aeruginosa* and *Escherichia coli*), and a fungi (*Candida albicans*).

2. METHODOLOGY

Materials

The material used in this study is the gotu kola plant (*Hydrocotyle vulgaris* L.), Nutrient Agar (NA) media, Potato Dextrose Agar (PDA) media, disc ketoconazole 15 µg, disc chloramphenicole 30 µg, alcohol 70%, ethanol 96%, DMSO, NaCl physiologist solution, aquadest, sulfuric acid 2N, concentrated hydrochloric acid, iron (III) 1% chloride, chloroform, ammonia chloroform 0,005 N, magnesium metal, Liebermann-Burchard reagent, Dragendorf reagent, and Mayer reagent.

The tools used in this study are glassware, aluminum foil, bunsen, autoclave (Gea®), Petri dishes, erlenmeyer, dark bottles, funnels, hot plate, sonicator, incubator, blender, calipers, scissors, ose needles, gauze, corn yarn, cotton, parchment paper, Laminar Air Flow (JSCB-900SL®), UV-Vis spectrophotometer (Shimadzu®), oven (Memert®), tweezers, micro pipettes (Dragon Med®), drop pipettes, plates drops, spoons, test tube racks, rotary evaporator, vortex (Asone®), and analytical scales (Shimadzu®).

Methods

Preparation of test solution

The ethanol extract of pegaganair (Hydrocotyel vulgaris L.) leaves was dissolved in DMSO (Dimethyl Sulfoxide) to affordsolution with various concentrations of 5%, 10%, and 15% (b / v). The test concentration was made by weighing as much as 0.05 g; 0.1 g; and 0.15 g of extract with analytical scales, then each was dissolved in1 mL of DMSO with the help of vortex.

Determination of antibacterial and antifungal activity by the disc diffusion method

In solidified culture media, implanted discs which have been dripped with test solution based on each concentration of 10 µL. Then the Petri dish is closed using plastic wrap and incubated for 24 hours at 37^{o}C for bacteria and for 72 hours at 25^{o}C for the mushroom to be reversed. Microbial growth was observed and measured the inhibition diameter of growth formed using calipers. For comparison, 10 µL DMSO doped discs were used for negative control and positive control of 30 µg/disc chloramphenicol antibiotic discs for bacteria and 15 µg/disk ketoconazole for fungi.

3. RESULT AND DISCUSSION

The test microbes used were *Staphylococcus aureus* and *Staphylococcus epidermidis* representing Gram positive, *Eschericia coli* and *Pseudomonas aeruginosa* representing Gram negative and fungi

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Candida albicans. Aside from being representative of bacteria and test fungi, these microbes are used because they are a common cause of infections that occur and attack several parts of the human body [4-7].

Antimicrobial activity tests are carried out aseptically and use tools and media in sterile conditions. Before conducting the test, the test microbes to be used must first be rejuvenated, so that the test microbes to be used are in the exponential phase. In this phase microbes begin to grow optimally because in this phase available nutrients for microbes can grow well. Testing of antimicrobial activity of ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves was carried out by agar diffusion method.

This method was chosen because of the simple way of working and the results of testing antimicrobial activity can be known directly by measuring the surrounding clear zone [8]. The factors that can influence the results of antibacterial activity testing using the agar diffusion method include the type and condition of the test bacteria, the thickness of the moderate, incubating condition (temperature, time, pH), the speed of the substance diffuse in agar, the concentration of microorganisms, and the composition of the media [9].

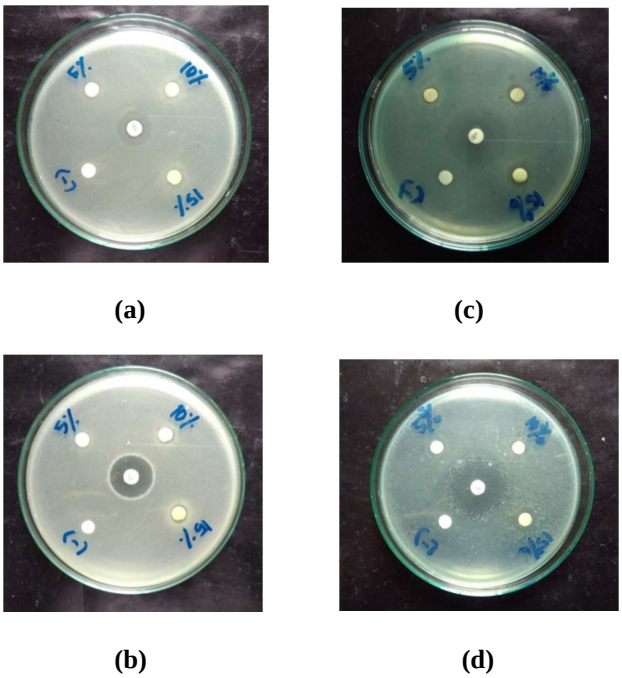
Ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves was made into three concentration variants namely 5%, 10%, and 15% which were dissolved using DMSO. DMSO is an organic solvent and is not bactericidal. In addition, DMSO can dissolve almost all polar and non-polar compounds, and can be used as solvent to obtain extract solution with a certain concentration. As a comparison of the antimicrobial activity of extracts, chloramphenicol 30 µg/disc and ketoconazole 15 µg/disc were used as positive control for antibacterial and antifungal test, respectively, and DMSO as a negative control.

Chloramphenicol is used as a positive control for bacteria because it is included in the broad spectrum antibiotic group that can inhibit the growth of Gram positive and Gram negative. While ketoconazole is used because it can inhibit the growth of various fungi *in vitro* [10]. Ketoconazole works by affecting the 14-alpha dimethylase enzyme which is a cytochrome P-450 enzyme needed in the process of changing lanosterol to ergosterol. With the disruption of the synthesis of lanosterol to ergosterol, the synthesis of triglycerides and fungal phospholipids is also inhibited. This makes the permeability of the cell membrane change and causes the death of the fungus[11].

Antibacterial activity can be seen by looking at the inhibitory power or the clear zone around the disc on the growth of bacteria in the agar medium that is already dense. The greater the inhibition diameter, the greater the antibacterial activity of the sample, and vice versa. The factors that can influence the test of antibacterial activity are the diffusion rate of different samples and the difference in bacterial response to the sample [12].

The diameter of the 15-20 mm inhibition zone is included in the strong category, 10-14 mm in the medium category, and 0-9 mm in the weak category [13]. The average diameter of the inhibition zone and the standard deviation of the ethanol extract of "pegagan air" leaves against *Staphylococcus aureus* for concentrations of 5%, 10%, and 15% are 7.43±0.33 mm (weak); 7.92±0.41 mm (weak); and 8.48±0.18 mm (weak), respectively. For *Staphylococcus epidermidis* bacteria, the activity produced is classified as weak with an average diameter of small to large concentrations of 7.45±0.04; 8.03±0.08; and 8.37± 0.13

mm. Against Gram negative bacteria, *Pseudomonas aeruginosa*, the activity is classified as weak with an average diameter of small to large concentrations of 7.43±0.15; 8.28±0.29; and 9.03±0.06 mm. Against *Escherichia coli*, the extract solution show the inhibition zone at concentrations of 5%, 10%, and 15% are7.77±0.26 mm (weak); 8.68±0.06 mm (weak); and 10.82 ± 0.45 mm (medium) (Figure 1).



(a) **(c)**

(b) **(d)**

Figure 1. The results of antibacterial activity test of ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves against (a) *Staphylococcus aureus*; (b) *Staphylococcus epidermidis*; (c) *Pseudomonas aeruginosa*; (d) *Escherichia coli*

In the fungal *Candida albicans* ethanol extract of "*pegagan air*" leaves (*Hydrocotyle vulgaris* L.) doesn't have the activity of as antifungal agent. The absence of activities antifungal agent characterized by the absence of the inhibition zone ethanol extract of "pegagan air" leaves on the concentration 5%, 10%, and 15%. In the test antifungal agent *Candida albicans* just control positive give the inhibition zone. Diameter inhibition zone formed is 34.77±1.06 (Figure 2).

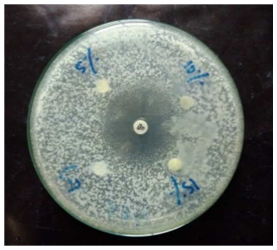


Figure 2. The resultofantifungal activitytest of ethanol extract of "pegagan air" (*Hydrocotyle vulgaris* L.) leaves against *Candida albicans*

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Based on the measurement of diameter of inhibition zone can be seen that the inhibition zones against Gram negative bacteria greater than Gram positive bacteria. These suggest that the ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves is more sensitive to Gram negative bacteria. The difference in the activity can be explained from the difference in the structure constituent of the cell wall of bacteria. The cell wall of Gram negative bacteria have a cell wall with a thin of peptidoglycan of the cell by 10% compared to Gram positive bacteria that have cell walls made of 50%-90% peptidoglycan, so that the antibacterial compound contained in the sample is able to penetrate the cell wall of Gram negative bacteria and to damage cell walls than Gram positive bacteria [8].

Activity antibacterial posed by the ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves can occur due to the content of secondary metabolites such as flavonoids, phenolics, and terpenoids. Flavonoids act as antibacterial agents with a mechanism of forming complex compound with proteins of the extracellular bacteria and dissolved so that it can damage the bacteria cell membrane followed by release of compound intracellular [12]. Flavonoids compound found in "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves is genestein. Phenolic compounds are also potentially as an antibacterial that cause the lysis of cellular components and damaging the mechanism of enzymatic bacterial cells [14]. In addition, terpenoids are also known to play a role as antibacterial, involving the breakdown of cell membranes by components lipophilic [15].

4. CONCLUSION

Based on the results, it can be concluded that the ethanol extract of "*pegagan air*" (*Hydrocotyle vulgaris* L.) leaves has the ability in hamper growth bacteria with the weak until moderate category. There is the influence in difference of concentration of ethanol extract of "*pegagan air*" leaves against diameter of inhibition zone in the test bacteria. The results show that ethanol extract of "*pegagan air*" leaves could not have the activity of antifungal against *Candida albicans*.

5. ACKNOWLEDGEMENT

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Formulation and *In Vitro* Evaluation of Sun Protection Factor of Dadap Serep (*Erythrina Subumbrans (Haks.) Merr*) Cream

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ABSTRACT

Dadap Serep *(Erythrina Subumbrans (Haks.) Merr*) contains bioactive flavonoid compounds. The chromophore group contained in flavonoids can absorb UVA and UVB rays. This study aimed to formulate a sunscreen cream from ethanol extract of dadap serep *(Erythrina subumbrans (Haks.) Merr*) leaves and to evaluate its sunscreen activity using spectrophotometry method. The concentrations of dadap serep extract were used in formulas I, II and III were 1.25%, 2.5%, and 5%, respectively. Evaluation of cream included the organoleptic test, homogeneity test, pH measurement, spreadability, viscosity, and sunscreen activity test. The results showed that FIII is the best formula with the physical characteristics are rather soft, homogeneous, pH of 5.860 ± 0.026, viscosity of 9255.32 ± 0.110 cps, spreadability of 5.117± 0.430 cm and the SPF value of 8.606 ± 0.350 with the ability of sunscreen in the maximum category.

Keywords: Dadap Serep, Cream, SPF

1. INTRODUCTION

Sunlight is an energy source that is beneficial to human life. However, continuous exposure to UV light can hurt health [1]. Sun exposure can cause erythema sunburn, edema, hyperplasia, immune suppression, DNA damage, photoaging, and melanogenesis. These changes can directly or indirectly cause cancer in the skin [2]. One way that can be done to reduce the negative impact of sunlight is by using sunscreen [3].

Sunscreen is a cosmetic ingredient that can physically or chemically inhibit the penetration of UV light into the skin. Sunscreen functions to absorb, reflect or spread sunlight that is in the area of UV radiation emission before being absorbed by the body. Sunscreen activity of a substance is determined by measuring the size of the sun protection factor, known as SPF (Sun Protective Factor). SPF defined as the amount of UV energy needed to give rise to MED (Minimal Erythemal Dose) on skin protected products or active sunscreen compared to the amount of energy needed to give rise to MED without the protection of products or active sunscreen [4].

The development of sunscreen leads to the use of natural materials. According to Hogade (2010) [5], flavonoid compounds can protect against UV light. The chromophore group contained in flavonoids can absorb light in the wavelength range of UV light in both UV A and UV B [6].

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Dadap serep (*Erythrina subumbrans (Haks.) Merr.)* is one of the plants that can develop as a sunscreen. This plant contains bioactive compounds, such as flavonoids, saponins, isoflavonoids, alkaloids, and lectins [7]. This study aimed to formulate and evaluate the sunscreen activity of sunscreen cream from leaves extract of dadap serep (*Erythrina subumbrans* (*Haks.) Merr.)* using spectrophotometric method.

2. METHODOLOGY

Materials

The materials used are dadap serep (*Erythrina subumbrans (Haks.) Merr.)* leaves, aquadest, cetyl alcohol, lanolin, stearic acid, Triethanolamine, glycerin, nipagin, nipasol, oleum rosae, ethanol 70% and ethanol 96% (pro analysis).

Methods

Preparation of simplicia

The leaves of dadap serep are collected and cleaned from the attached impurity. The leaves are washed thoroughly and drained, then the leaves are sliced up to ± 1 cm and dried in an oven at 45^{o}C-50^{o}C until the simplicia reaches a constant weight. The dry simplicia is then pollinated and stored in a tightly closed container, protected from light.

Extraction of dadap serep leaves

Extraction of leaves powder of dadap serep was performed by maceration method with 70% ethanol. The liquid extract obtained was concentrated with a rotary evaporator.

Formulation of dadap serep creams

The formula of sunscreen cream from leaves extract of dadap serep were presented in Table 1.

Table 1. Formula of sunscreen cream from leaves extract of dadap serep

Materials	F I	F II	F III
leaves extract of dadap serep	1.25	2.5	5
Cetyl alcohol	2	2	2
Lanolin	1	1	18
Stearic acid	5	5	5
Triethanolamine	1	1	1
Glycerin	10	10	10
Nipagin	0.2	0.2	0.2
Nipasol	0.2	0.2	0.2
Oleum rosae	0.001	0.001	0.001
Aquadest ad	100	100	100

The sunscreen creams from leaves extract of dadap serep were prepared following this procedure: The oil phase (cetyl alcohol, stearic acid, nipagin, and lanolin) was placed into a porcelain dish, then heated it on the water bath until it melts and stired until homogeneous. The water phase (TEA, glycerin, nipasol, water) was placed intoa porcelain dish, then mixture and preheated over the stirring until homogeneous. Then,

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the oil phase was added into the water phase with stirring until the mixture thickens and cools. The leaves extract was added into the cream base and stirred until homogeneous. Then, oleum rosae was added as a fragrance and stirred until homogeneous**.**

Evaluation of Dadap Serep cream

Organoleptic tests

Organoleptic tests are carried out to observe odor, color, texture.

Homogeneity

Homogeneity checks are carried out using the object's glass. A certain amount of the preparation is applied to a piece of glass or other transparent material that is suitable to produce a homogeneous and non-granular preparation.

pH measurement

pH determination using a pH meter. The pH meter is calibrated with a standard neutral buffer solution (pH 7.01) and a solution for acidic pH (pH 4.01) to show the pH value. Then the electrode is washed with distilled water and dried with tissue. The sample was made in a concentration of 1% which was weighed 1 g of the preparation and dissolved in 100 ml of distilled water. Then the electrode is dipped in the solution. Let the device show the pH value until constant. The number shown by the pH meter is pH of cream.

Spreadability

The spread test is carried out by placing 0.5 gram of preparation in the middle of the petri dish. Other Petri dishes are weighed and then placed on the preparation for 1 minute. Measure the diameter of the spread. Place Load 50, 100, 150, 200, 250, 300, 350, and 400 grams above the petri dish in a minute wait for 1 minute and measure the diameter of the spread of the preparation. Stop the addition of load when the preparation does not spread again.

Viscosity

The viscosity test was carried out using a rheosis viscometer.

SPF Determination

The SPF test was carried out by weighing 0.1 grams of dadap serep extract sunscreen and then diluted to 25.0 ml with etanol 96%. The samples were sonicated for 5 minutes, then centrifuged for 15 minutes. Measure The filtrate at wavelengths of 290-320 nm at intervals of 5 nm. The absorbance values were calculated by following equation:

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times abs((\lambda)$$

Where, CF = correction factor [4], EE (λ) = erythmogenic effect of radiation with wavelength λ, and Abs (λ) = spectrophotometric absorbance

3. RESULT AND DISCUSSION

The extraction of leaves of dadap serep was performed by maceration method. The extract yield was obtained from the extraction process was 17.53%. The results of this study have met the requirements, where a good extract yield value is ≥ 17.4% [8].

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Evaluation of sunscreen cream from dadap serep leaf included organoleptic test, homogeneity, pH, spreadability, viscosity, and SPF value. The parameters in organoleptic test included the smell, color and texture of the cream as presented in Table 2.

Table 2. Organoleptic test results of dadap serep creams

Organoleptic Test	Formula		
	I	II	III
Smell	Typical oleum rosae	Typical oleum rosae	Typical oleum rosae
Color	Young green moss	Young green moss	Moss green
Texture	Soft	Soft	rather soft

Based on the results of organoleptic tests, there are differences in the color and texture of formulated creams. The deferences are influenced by variations in the concentration of extract in each formula. The higher the concentration of the extract used, the more color it produces and the texture of the preparation denser. The typical odor of oleum rosae is due to the addition of perfume oleum rosae to each formula.

Homogeneity testing is done by applying cream preparations to a piece of glass. The formula I, II, and III showed that there were no clustered particles and the cream spreads evenly. It proves that the preparations produced are homogeneous as presented in Table 3. According to Syamsuni (2006) [9], a preparation is said to be homogeneous if it shows a similar arrangement and no coarse grain is seen.

Table 3. Homogeneity testing results of dadap serep cream

Formula	Result
F I	Homogeneous
F II	Homogeneous
F III	Homogeneous

The pH test is carried out to determine the acidity or alkalinity of the cream preparations. The pH values of the dosage are suitable according to the pH of the skin so as not to irritate the skin and be comfortable when used. According to Wasitaatmadja (2007) [10], the normal pH range of facial skin is 4.5-7. The result of pH measurement of the creams in Table 4 showed that the pH of all three formulas have met the requirements.

Table 4. pH test results of dadap serep creams

Formula	Result
F I	6.348 ± 0.072
F II	6.094 ± 0.082
F III	5.860 ± 0.026

The viscosity characterization of cream preparations aims to determine the viscosity value of preparation. The Determination of the viscosity of the cream preparation is using a rheosis viscometer Merlin VR II. The results of observations of viscosity characteristics presented in Figure 1. The concentration of extract dadap serep affects the viscosity produced — the higher the concentration of the

extract, the lower the viscosity. According to sunscreen dosage quality standards in SNI 16-4399-1996, viscosity (25˚C) for sunscreen preparations is 2,000 - 50,000 cps [11]. The results of the viscosity test for all formulas have met the requirements.

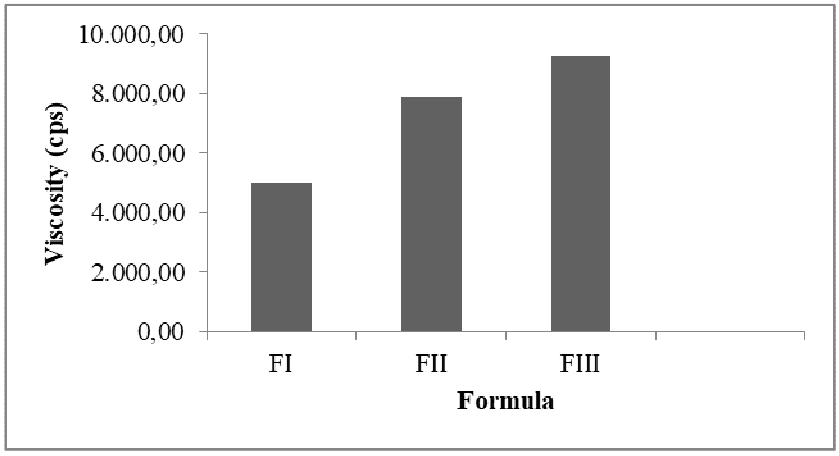

Figure 1. Viscosity of dadap serep creams

The flow types of preparations for FI, II, and III creams can also determine from the results of the preparation viscosity test. According to Martin et al. (2008), in general, semisolid preparations have Non-Newton flow properties, if analyzed with a viscometer and the results are plotted, various consistency curves are obtained which describe the existence of three classes of flow, namely plastic, pseudoplastic and dilated. The results of the viscosity test using a rheosis viscometer showed that the dadap serep leaf ethanol extract cream had plastic properties which were not through the origin (0,0). The curve explains the relationship between shear rate and shear stress, that is, a preparation that has the nature of plastic flow will flow with a small shear rate while the shear stress value given must be higher [12]. The results of the flow test are found in Figure 2.

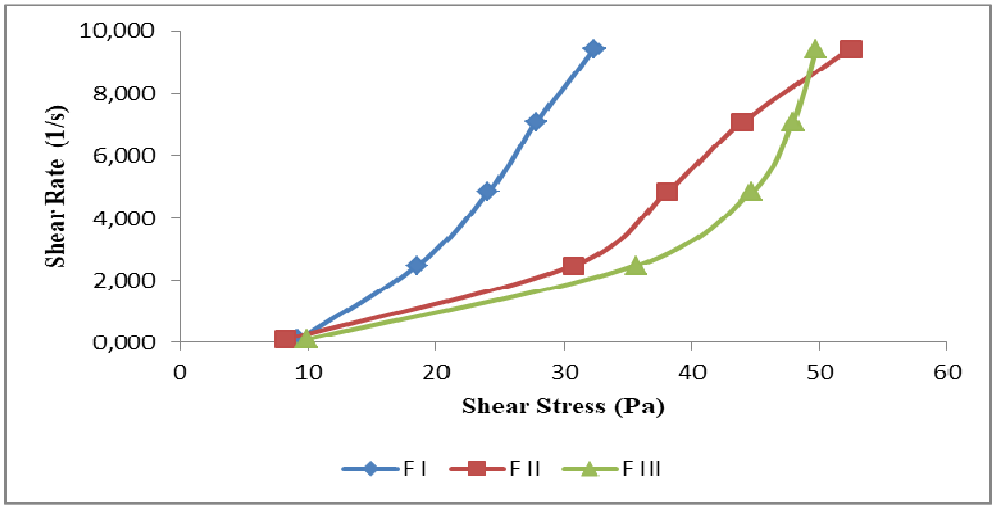

Figure 2. The result of flow test of dadap serep creams

The characterization of the spread of cream preparations aims to determine the ability of the

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distribution of preparations in the skin because it can affect the absorption and release of active substances in their use. The results of observations of the scattering characteristics of cream preparations for ethanol extract of dadap serep leaves are presented in Table 5.

Table 5. Test results of spreadability test of dadap serep creams

Formula	Diameter (cm)
F I	5.552 ± 0.114
F II	5.395 ± 0.143
F III	5. 117± 0.430

Based on table 5, it can be seen that the value of the spread of cream is influenced by the concentration of extract. The higher the concentration of the extract, the value of the dispersion power decreases. Thick preparations will be more difficult to spread when compared to preparations that have lower viscosity. According to Garg et al. (2002), the value of the diameter of the spread is good between 5-7 cm. Thus it can be concluded that FI and F II meet the requirements of good spread power [13].

One method to determine the activity of sunscreen for a substance is to measure the amount of sun protection factor, known as SPF (Sun Protective Factor). SPF is defined as the amount of UV energy needed to give rise to MED (Minimal Erythemal Dose) on the skin protected by products or active sunscreen compared to the amount of energy needed to generate MED without the protection of products or sunscreen [14].

Determination of SPF value of Dadap leaf ethanol extract was carried out using a UV-Vis spectrophotometer at UV B sunlight wavelengths (290-320 nm). According to the FDA (Food Drug Administration), the division of sunscreen capabilities is divided into five categories, namely Minimal, Medium, Extra, Maximum, and Ultra [15]. The results of determining the SPF value of Dadap leaf serep cream are found in Table 6.

Table 6. The SPF value of dadap serep creams

Formula	SPF value
I	3.312 ± 0.481
II	5.112 ± 0.004
III	8.606 ± 0.350

Based on table 6, it is known that the higher the concentration of Dadap leaf extract, the higher the SPF value produced is also higher. The dadap leaf extract cream at a concentration of 1.25% has the ability of sunscreen in the minimum category while in formula II the extract concentration of 2.5% has the ability as a sunscreen in the medium category. Formula III with a 5% extract concentration has the ability as a sunscreen in the maximum category. Flavonoids and phenolic compounds contained in Dadap Serep leaf extract have potential as sunscreens because they have a chromophore group. Chromophore absorbs high-energy ultraviolet light and releases energy in the form of low-energy rays thus preventing ultraviolet light from causing skin damage to the skin [16].

The SPF values of formulated creams did not reached the SPF value of 15. The FDA recommends the SPF value of sunscreens on cosmetic preparations of at least 15, because the SPF 15 is capable to filter UV B rays around 93.3% [17]. Therefore, the composition of the extract in sunscreen creams preparation needs to be reshuffled in order to have a maximum effect.

4. CONCLUSION

The results showed that F III is the best formula with the physical characteristics are rather soft, homogeneous, pH of 5.860 ± 0.026, viscosity of 9255.32 ± 0.110, spreadability of 5.117± 0.430 and the SPF value of 8.606 ± 0.350 with the ability of sunscreen in the maximum category.

5. ACKNOWLEDGEMENT

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Antioxidant Activity of Ethanol Extract of Durian (*Durio zibethinus* Linn.) Rind

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ABSTRACT

Durian (*Durio zibethinus* Linn.) is a commodity plant that has some essential benefits. Some studies, especially in parts of durian fruit have been reported to have antimicrobial, antioxidants and anti-inflammatory activities. Meanwhile, the durian wastes have not been fully explored. This research utilizes waste products from durian rind so it will give economic values. The potential of antioxidants contained in durian plants have been studied, but,the antioxidant activity of durian rind has never been reported. The aim of this study is to determine the antioxidant activity of ethanol extract of *Durio zibethinus* Linn. rind using the DPPH assay. The results showed that the ethanol extracts of durian rind showed a weak antioxidant activity with IC_{50} of 1009 ppm. Based on the results, it is known that the ethanol extract of *Durio zibethinus* Linn. rind has less potential antioxidant activity.

Keywords: Antioxidant, DPPH, *Durio zibethinus* Linn., IC_{50}

1. INTRODUCTION

Durian is known as “King of Fruit” and as a cultivar plants are consumed all over the world [1] also the differences variety of durian are practically have not been studied [2]. According to [3] The synergistic effect, which could exist between individual bioactive compounds, means that the antioxidant capacity may be higher than their sum, and not only individual bioactive compounds, but also the overall antioxidant capacity have to be determined. Some antioxidant assays give various and specifics antioxidant activity trends [4].

The presence of natural bioactive compounds is of interest to the cosmetic, pharmaceutical and food industries. Of course, it is a well-accepted fact that fruits based natural antioxidants can serve as natural protectants to prevent oxidative damage (from free radicals) and thus, slow down the occurrence of rancidity in foods [4, 5]. In Asian countries generally consume vegetables and tropical fruits that are relatively contain the source of antioxidant components with strong potential antioxidant activities. Durian can produces one of the active compounds. Some studies have reported the potency of biological activity of this plants. Therefore, in this study, we are interested to determine the antioxidant activity of the ethanol extract of durian rind using the 1,1-diphenyl-2-picrylhydrazil (DPPH) assay.

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2. METHODOLOGY

Materials

Durian (*D. zibethinus*.) rinds, Ethanol 96% (Bratacho), aquadest, *Rotary evaporator* (Stuart®), Oven (MMM Medcenter®), Pumpkin Measure, Beaker glass, Measuring cup, Erlenmeyer, Flacon, drops Pipette, volume pipette, drag ball, grinder, waterbath, dan glasses (Pyrex), analytical balance (Ohaus), micropipet (Rainin®), UV-Vis spectrophotometer (Dynamica Halo DB 30®), glass cuvette, DPPH reagents, and alumunium foil.

Methods

Preparation of simplicia and extract of durian rind

The making of simplicia and extraction are done in several stages as follows: the durian (*D. zibethinus* (Linn.)) rinds are washed and then dried using an oven at 60^{o}C. Simplicia of Durian (*D. zibethinus* (Linn)) rinds are made of powder by grinding. The durian (*D. zibethinus* (Linn.)) rinds which have dried as much as 1 kg are mashed to become powder. Extraction with 96% ethanol was performed by maceration method. Then, the macerate was concentrated with a rotary evaporator at a temperature of 50^{o}C with a speed of 50 rpm and then dried in an oven at 40^{o}C until a fixed weight was obtained [6]. The percentage of yield of solid extract obtained in this study was 4.08% (2,300 g of durian skin simplicia powder produced by solid extract of 94 g)

Phytochemical screening of durian rind extract

Phenolic Assay

A total of 40 mg of extract added with ten drops of $FeCl_3$ 1%. The formation of green, red, purple, blue or deep black color indicated the presence of phenolic compound [7].

Terpenoid Assay

Identification of terpenoid was carried out by dissolving the extract in 0.5 ml of chloroform, then adding 0.5 ml of anhydrous acetate and mixing with 2 ml concentrated H_2SO_4 through the tube wall. The formation of green to blue color indicated the presence of terpenoid [8].

Flavonoid Assay

Identification of flavonoids is made by dissolving the extract in hot ethanol and adding 0.1 gram of Mg powder and five drops of concentrated HCl. The formation of orange colour indicated the presence of flavonoids [8].

Tannin Assay

A test solution of 1 mL was reacted with a solution of 10% iron (III) chloride. The formation of dark blue or greenish black color indicated the presence of tannins [9].

Alkaloid Assay

Identification of alkaloids was carried out by Mayer and Dragendorff methods. A total of 0.5 grams of the extract was added with 1 ml of 2M HCl and 9 ml of aquadest, heated for 2 minutes, cooled and then filtered. The filtrate is divided into 3 parts, each added with Mayer and Dragendorff reagents. The positive results of alkaloid with Mayer reagent is characterized by the formation of white precipitate and positive

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results of alkaloid with Dragendorff reagent is characterized by the formation of light brown to yellow precipitate [8].

Saponin Assay

A total of 40 mg of extract was added 10 mL of water while shaking for a minute, then added two drops of HCl 1N. If stable foam is formed for ± 7 minutes, it indicated the presence of saponin [7].

Antioxidant Assay

The radical scavenging activity of extract in various concentrations we determined by using DPPH assay according to [10] with some modification. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Determination of Antoxidant Activity and Using the DPPH Method

As much as 12.5 mg of extract was dissolved in 10 ml PA ethanol in a 10 ml volumetric flask, as a mother liquor, so that a concentration of 1250 ppm was obtained, from a 1250 ppm mother liquor a diluent with a series of concentrations using multilevel dilution 1250, 625, 313, 156, 78, 39 and 10 ppm. Put 1.04 ml of sample solution added with 2.96 ml of DPPH solution. Incubated at room temperature for 20 minutes, after 20 minutes absorbance was measured using a Uv-Vis spectrophotometer the maximum wavelength obtained. The same treatment had done for the control solution (DPPH solution which does not contain sample solution), and as a comparative solution, ascorbic acid is used as reference [11]

Preparation of ascorbic acid and DPPH solution

Ascorbic acid (10 mg/ml DMSO) was used as reference. DPPH 0.1 mM solution was prepared by dissolving 4 mg of DPPH in 100 ml of ethanol.

Working procedure

Different volumes (2 – 20 µl) of plant extracts were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. Some 3 ml of DPPH was taken as control. Then, the percentage of radical scavenging activity of the plant extracts was calculated using the following formula:

$$\%\ RSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where, RSA is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + ethanol; *Abs sample* is the absorbance of DPPH radical + plant extract. Ethanol was used as a blank.

Furthermore, the IC_{50} value of the ethanol extracts of durian rinds was determined with 2 replications with 7 series of concentrations. Processing the data was performed by using simple linear regression analysis, according to [11]. Data was analyzed using Microsoft Excel.

$$y = ax + b$$

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Where,

x = The concentration of each durian leaves extract

y = Percentage of DPPH radical reduction (% inhibition).

3. RESULT AND DISCUSSION

Extraction

Some 11.7 kgs of wet sample of durian rinds were obtained from the Selat Village of Muaro Jambi Regency, Jambi. Then, the sample was dried using an oven at 50^{o}C to obtain 2.3 kgs of dried samples from the durian rinds. Furthermore, maceration and re-maceration of three times were carried out for three days using 96% ethanol and 94 grams of concentrated extract of durian rinds were obtained which was separated by macerated solvent using a rotary evaporator. The yield value obtained is 4.087%. The last, the ethanol extract of the durian rinds tested for its antioxidant activity [12].

Phytochemical screening

Based on the results of the phytochemical screening, it was shown that the extract of durian rinds contained a class of chemical compounds such as flavonoids, phenolics, alkaloids, saponins and tannins.

Table 1. Phytochemical screening result of durian rind extract

Phytochemical Tests	Result
Phenolic	+
Terpenoid	-
Flavonoid	+
Tannin	+
Alcaloid	+
Saponin	+

Antioxidant activity

Based on the antioxidant activity result, it is known that the Durian rinds possessed a weak antioxidant activity [13], with the IC_{50} of 1009 ppm. According to [14], antioxidant activity expressed as 50% inhibition concentration is a value that shows the ability of oxidation process inhibitors by 50%, on the other hands, the concentration needed to produce antioxidant activity is 50% [15]. The IC_{50} value is determined from the linear regression equation between the concentration of the test material with the average percentage of free radical deterrence from each concentration and its inversely proportional to antioxidant activity. The small number of the IC_{50} value indicates the higher antioxidant activity of a compound [14]. Based on the results, the% inhibition of durian rind extract (*Durio zibethinus* Linn) the greatest % inhibition was found at a concentration of 1250 ppm as shown in Figure 1.

In testing the antioxidant activity of ethanol and ascorbic acid as a positive control in addition to the determination of IC_{50} values also observed a color change that occurs when the sample solution (extract) and positive control (ascorbic acid) reacted with DPPH radicals, where the DPPH radical color that was originally deep purple will fade to yellow. This happens because there is a hydrogen atom donor from the sample containing an antioxidant compound and to the DPPH radical so that it is reduced to a

more stable form, DPPH-H (1,1-diphenyl-2-picrylhydrazine).

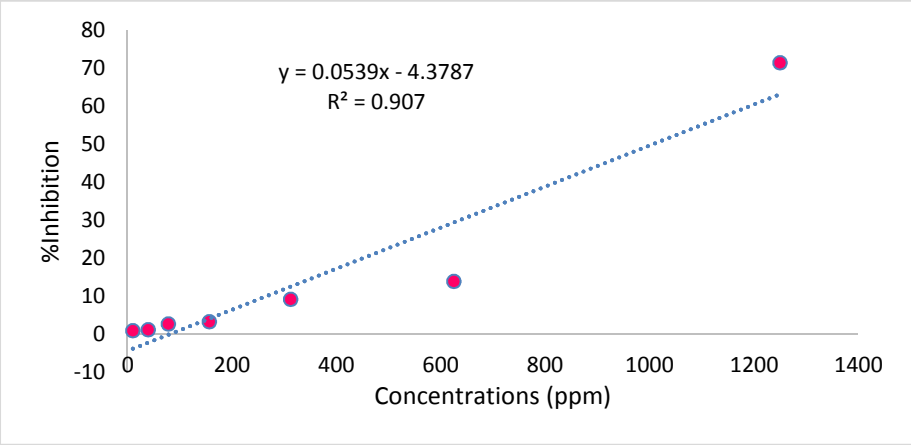

Figure 1. Linear regression between concentration and percentage of inhibition

There are some results of antioxidant activity in durian fruit wastes that has been also reported. Based on the research of [16], methanolic extracts of durian shell possessed IC_{50} values of 280.79, 154.67, 324.63, 770.52, 4.45, 102.37, 19.50, and 63.95 µg/mL for reducing power (Fe^{3+}), reducing power (Cu^{2+}), hydroxyl radical, superoxide anion radical, anti-lipid peroxidation, DPPH, $ABTS^{+}$, and ferrous ions (Fe^{2+}) chelating activity assays, respectively. Furthermore, on measuring the antioxidant capacities of durian fruit extracts reported an FRAP value of 741 µmol Fe(II)/100 g and 498 µmol TEAC/100 g from $ABTS^{+}$ assay [17]. Durian rinds consisted of much lipid in line with the research of [18, 19]. There is correlation between several compounds like flavonoids, phenolics (polyphenolic), it needs purification to separate between the secondary metabolites that contain in its. Therefore, it can give the best activities when it comes in purified products.

4. CONCLUSION

Based on this study, the ethanol extract of Durian (*Durio zibethinus* Linn.) rind appear to has less potential antioxidant. Further studies are needed on the separation the properties of secondary metabolites of Durian. Therefore, durian components (fruits, flesh, rind, seed, leaves and barks) contains high amount of bioactive compounds that need to be explored and there is still less literature that have been reported on the activity of fractions and isolates. Another works is also needed to develop this plant as a source of natural products.

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Antioxidant Activity of Red Dragon Fruit (*Hylocereus polyrhizus*) and White Dragon Fruit (*Hylocereus undatus*)

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ABSTRACT

Dragon fruit is one of the fruits that is efficacious as traditional medicine. Dragon fruit is a fruit that comes from Mexico, although it is not from Indonesia but dragon fruit is a newcomer fruit that is much loved by the public besides because its delicious fruit taste also has properties and benefits as well as high nutritional value. One of the benefits of dragon fruit is as an antioxidant. Antioxidant compounds are needed to protect the body from the danger of free radicals.In one-way ANOVA statistical analysis shows F_{count} = -1.204 while F_{table} is 0.05 = 161. In the calculation results it can be concluded that $F_{count}<F_{table}$. So, H_0 is accepted so it can be concluded that red dragon fruit extract has a higher antioxidant potential compared to white dragon fruit. Can be marked on percent inhibition, namely red dragon fruit extract has higher percentage of inhibition compared to white dragon fruit extract. Percentage inhibition is used to determine the antioxidant potential of red dragon fruit (*Hylocereus polyrhizus*) and white dragon fruit (*Hylocereus undatus*) using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Based on the DPPH assay, the red dragon and white dragon fruits showed percentages of inhibition of 78.07% and 68.89%, respectively, while the ascorbic acid showed percentage of inhibition of 94.60%.

Keywords: Antioxidant, DPPH, red dragon fruit, white dragon fruit.

1. INTRODUCTION

Modern medicines have been widely used, but even so traditional medicine still has a special position in society. One of them in Indonesian, the use of traditional medicine is still widely used in improving public health. Both vegetables and fruits plants have many medicinal properties [1].

Dragon fruit is one of the fruits that is efficacious as traditional medicine. Dragon fruit is a plant that comes from dry tropical climates. Dragon fruit growth is influenced by temperature, humidity, soil conditions and rainfall. The native habitat of dragon fruit comes from Mexico, North America and northern South America. However, dragon fruit has now been cultivated in Indonesian [2].

Dragon fruit is a fruit that comes from Mexico, although it is not from Indonesian but dragon fruit is a newcomer fruit that is much loved by the public besides because the delicious fruit taste also has

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properties and benefits as well as high nutritional value. One of the benefits of dragon fruit is as an antioxidant. Antioxidant compounds are needed to protect the body from the danger of free radicals [3].

Without us knowing it in our daily lives, we are often exposed to free radicals such as cigarette smoke, vehicle smoke, sun exposure, etc. Free radicals attack one molecule, the initially neutral molecule is converted to radical. This process causes chain reactions that can cause cell destruction. Various possibilities can occur as a result of the work of free radicals. For example, disturbances in cell function, damage to cell structures, modified molecules that cannot be recognized by the immune system, and even mutations. All of these disorders can trigger various diseases. Therefore, our body needs an important substance that is antioxidants that can help protect the body from free radical attacks and reduce its negative effects [3].

There have been a lot of studies on antioxidants in dragon fruit both in the study of the meat or on the skin, including antioxidant activity in red dragon fruit [4], antioxidant activity of red dragon fruit skin chloroform fraction [5], and testing of antioxidant activity in ethanol extract of super red dragon fruit peel [6].

Based on several studies that have been conducted on antioxidants in dragon fruit, it is necessary to do a study on the comparison of red dragon fruit (*Hylocereus polyrhizus*) with white dragon fruit (*Hylocereus undatus*) as an antioxidant. That way based on the above research can be known the antioxidant potential of red dragon fruit (*Hylocereus polyrhizus*) with white dragon fruit (*Hylocereus undatus*).

2. METHODOLOGY

Materials

The ingredients used in this study were red dragon fruit (*Hylocereus polyrhizus*), white dragon fruit (*Hylocereus undatus*), methanol p.a, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbic acid.

The instrument used in this study among others: UV-Vis Spectrophotometer (Shimadzu UV 1800), Rotary evaporator (Heidolph), analytical scales (Kenko), watch glass, beaker glass, filter paper, rod stirrer, chocolate bottle.

The plant samples used are red dragon fruit (*Hylocereus polyrhizus*) and white dragon fruit (*Hylocereus undatus*). These plants were taken at a dragon fruit plantation in Barelang, Batam City, Indonesia.

Methods

Preparation of Extracts

Red and white dragon fruit is cleaned from dirt, on the part of the dragon fruit meat is cut into small pieces then weighed as much as two kilograms extracted by maceration method by soaking the sample in methanol to cover all the surface of the sample. Soaking is done in a brown bottle and stored in a protected place from direct sunlight, for five days while occasionally stirring. Macerate was separated from the pulp by filtering and the pulp is macerated again. The maceration process was repeated three

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times. The macerate from the three processes was combined and then the solvent was evaporated *in vacuo* until a thick methanol extract was obtained.

Preparation of sample solution

The thick extract of the sample was weighed as much as 5 mg then dissolved in 5 ml of methanol in a 10 ml measuring flask to obtain a sample solution with concentration of 1 mg/ml [7].

Preparation of DPPH solution

As much as 1.97 mg of DPPH was dissolved in 100 ml of methanol p.a in a measuring flask to obtain a DPPH solution with a concentration of 0.05 mM [7].

Preparation of ascorbic acid solution

The thick extract of ascorbic acidwas weighed as much as 5 mg then dissolved in 5 ml of methanol in a 10 ml measuring flask toobtain a sample solution with concentration of 1 mg/ml [7].

Measurements of DPPH maximum absorbance

As much as 3.8 ml of DPPH solution 0.05 mM was pipetted and added with 0.2 ml of methanol p.a. Then the mixture was left for 30 minutes in a dark place. The absorptions of the tested solutions were measuredby a UV-Vis Spectrophotometer at a wavelength of 400-800 nm [7].

Antioxidant Activity Test

As much as 0.2 ml of sample solution was pipetted into a test tube. Then 3.8 ml of 0.05 mM DPPH solution was added. Themixture was homogenized with a vortex and for left for 30 minutes in a dark place. Absorption was measured by UV-Vis Spectrophotometer at a wavelength of 517 nm.The antioxidant activity of the sample was determined by the amount of DPPH radical uptake by calculating the percentage of DPPH absorption inhibition using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100\ \%$$

Caption : Abs. Control = Absorbance of DPPH

Abs. Sample = Absorbance of DPPH in tested sample solution

Statistical Analysis

The data obtained were analyzed statistically using one-way ANOVA test and narrated on the results and discussion and concluded.

3. RESULT AND DISCUSSIONS

One-way ANOVA statistical analysis showed F_{count} = -1.204 and F_{table} 0.05 = 161. Based on this calculation, it can be concluded that $F_{count} < F_{table}$ then H_0 is rejected so it can be concluded that red dragon fruit extract has higher antioxidant potential compared to white dragon fruit extract. Can be marked on percentage of inhibition, namely red dragon fruit has higher percent inhibition compared to white dragon fruit as shown in table 1.

Table 1. The result of DPPH assay

Sample	Absorbance		Average of absorbance	Inhibition (%)
	Repetition 1	Repetition 2		
White Dragon Fruit Extract	0.135	0.136	0.1355	68.89
Red Dragon Fruit Extract	0.096	0.095	0.0955	78.07
Ascorbic Acid	0.024	0.023	0.0235	94.60
DPPH	0.435	0.436	0.4355	

Antioxidant activity was carried out by weighing a sample of 5 mg then dissolved with methanol p.a, in a 10 ml volumetric flask. As much as 0.2 ml of sample solution was pipetted into the test tube and was added by 3.8 ml of DPPH solution. Before being measured it can be observed when the sample is added to DPPH solution if the sample containing DPPH antioxidant solution in purple will change color to yellow. Furthermore, the absorbance of control and sample were measured by UV-Vis spectrophotometry with the average results of red dragon fruit absorbance is 0.1355, white dragon fruit is 0.0955 and the average absorption of ascorbic acid is 0.0235. As for the inhibition percentage of red dragon fruit, white dragon fruit and ascorbic acid are 78.07%, 68.89% and 94.60%, respectively. Based on the percentage of inhibition, the antioxidant activity of red dragon fruit extract is higher than the white dragon fruit extract.

ANOVA is used in studies comparing two different sample groups. As was done in this study, comparing two different sample groups, namely red dragon fruit and white dragon fruit extracts. This one-way ANOVA calculation is used to determine the value of F_{table} and F_{count} where it can be determined if $F_{count} > F_{table}$ then H_1 is accepted and H_o is rejected otherwise if $F_{count} < F_{table.}$ Then, H_0 accepted and H_1 is rejected. Before determining the F_{count} and F_{table}, the hypothesis must be determined first. This hypothesis is a temporary answer to a problem. The answer still needs to be tested. The calculated F obtained is –1.204 while the F_{table} value is 161, then Ho is accepted. So it can be concluded that red dragon fruit with higher antioxidant content compared to white dragon fruit can be proven by the percentage of visible inhibition, the results showed 78.07% for red dragon fruit extract and 68.89% for white dragon fruit extract.

4. CONCLUSION

Based on this study, it can be concluded that the red dragon fruit extract has higher antioxidant potential compared to white dragon fruit extract with percentage of inhibition of 78.07% and 68.89%, respectively.

5. ACKNOWLEDGEMENT

With all humility, I want to say thank you profusely to Ms. Sri Hainil, M.Farm., Apt and Ms. Prof. Dr. Fatma Sri Wahyuni, Apt as the 1st supervisor and 2nd supervisors who have provided guidance, advice, solutions, and motivations that have been willing to take the time and guide me, so that I can finish thesis well.

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Preparation of Spray Nanoemulsions and Cream Containing Vitamin E as Anti-aging Product Tested *In Vitro* and *In Vivo* Method

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ABSTRACT

Skin is a layer covering body and protect body from negative effect and evironment factor. Some studies indicated that aging resulted by sun-light exposure and many other factors including free radical. Thus, it requires anti-aging product capable to resist and even to repair any disorder in skin caused by free radical. One of anti-oxidant required in one's body is vitamin E (*tocopherol*). The objective of this study was to formulate special preparation in spray nanoemulsion and cream containing vitamin E as *anti-aging* tested *in vitro* and *in vivo* method. This experiment used vitamin E *(alfa tocopherol)* as anti-aging component function and compare effectiveness of anti-aging made in preparation of spray nano-emulsion and cream containing vitamin E. Testing on preparation of spray nanoemulsion and cream vitamin E on skin and effectiveness anti-aging using skin analyzer applied on skin back of hand. Treatment took for 12 weeks using/applying each preparation twice daily. Parameters tested included moisture, avennes, pore and total spot. The result showed that vitamin E can be formulated in spray nanoemulsion and cream containing vitamin E with homogenous outcome, no irritation, pH ranged 6.7-7.16, stable during storage for 12 weeks, spray nanoemulsion has nanoparticle sized of 186,26 - 338,93 nm, spray nanoemulsion also had been shown penetrating into skin better than any preparation in cream and preparation spray nano-emulsion capable to show its effectiveness as a *anti-aging* product after treatment for 12 weeks than a preparation in cream. The results of this study suggest and indicated that be higher concentration of vitamin E and be smaller particle size it may produce effectiveness excellent as anti-aging.

Keyword : *aging, spray nanoemulsion, cream, vitamin E.*

1. INTRODUCTION

Skin is an organ to cover body and having the main function as a protection from variuos interferences and outside stimulation or environment [1,2]. The skin aging process can divided into intrinsic aging and extrinsic aging. Ultraviolet (UV) irradiation cause premature aging which is called as photoaging. It is caused by intrinsic process superimposed with degenerative charger to solar radiation. Premature aging may happen to everyone, especially in Indonesia with tropical climate so that the result of total exposure from the sun and it's possibility to expose with high potency for free radical. The Sun-

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light is one of factors for premature aging [3,4,5]. Anti-oxidant is a substance that is important for body to neutralize free radicals and to avoid disorder due to free radicals . The free radical scavenging activities such as hydroxyl, DPPH and anti-peroxidation [6,7,8,9].

Nanotechnology is a potentially the most important engineering revolution since the industrial age. Nanotechnology is science and technology conducted at the nanoscale. So far nanotechnology resulted in variants of formulations like nanoparticles, nanocapsules, nanospheres, nanosuspensions, nanocrystal, nano-erytrhosome and nanoemulsion. Nanotechnology is defined as creation and manipulation of materials at nanoscale level to create products [10,11,12]. Nanoemulsion drug delivery system are the alternative for the bioavailability enhancement of poorly soluble [13,14,15].

Anti-oxidant compound in food play an important role as a health protecting factor [15,16]. One of good anti-oxidants is vitamin E that is called *alfa tocopherol* [17]. This research used ester form of vitamin E as tocopherol acetate [18,19]. Based on the statement above, in this study it was evaluated that good penetration using Vitamin E as active substance provide it present as preparation in particle of smaller size of particle, in this preparation with *spray* nano-emulsion containing vitamin E [20]. Vitamin E has potential as a supplement as well as a source of antioxidants in pharmaceutical preparations such as emulsions, creams, ointments and gels. The aim of this study was to formulate a *spray* preparation nano-emulsion containing vitam in E compared with cream Vitamin E as anti-aging [21,22].

2. METHODOLOGY

Materials

The equipments used in this study included Particle size analyzer (PSA), Scanning Electron Microscope (SEM), High Performance Liquid Chromatography (HPLC) (Shimadzu LC 20AD), pH Meter (Hanna Instrument), spray bottle, skin analyzer (AramoHuvis), magnetic stirrer, magnetic bar, thermostat, glass wares, mortar and pestle, analytical balance and sonicator. All chemicals used were tocopherol acetate (Vitamin E), glycerin, Hydrogenated Castor Oil, Tween 20, Natrium EDTA, Sodium metabisulfit, parfume, propilen glicol, triethanol amine (TEA), vaseline, cetyl alcohol, stearic acid, gliyceryl monostearic, distilled water.

Methods

A spray formulated nano-emulsion Vitamin E and Cream Vitamin E was prepared with various concentrations (0%) as blank, 1%, 3% and 5% Vitamin E, each preparation was added additional substances such as surfactant with the same concentration in each tested material.

Preparation of emulsion and vitamin E nano-emulsion

Tween 20 was mixed with Tocopherol acetate (Vitamin E), then dissolved and added Hidrogenated castor oil as oil phase. The water phase was composed of aquabidest (distilled water) and glycerine. The oil phase and water phase was mixed slowly using magnetic stirrer and stirred for 60 minutes [23,24]. Oleum rosae was added to formula as perfume. The emulsion of vitamin E was sonificated using sonicator for 30 minutes [25,26].

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Preparation and formulation of cream containing vitamin E

All material was provided and weighed, and then separated into two layers that were oil phase and water phase. Oil phase consisted of vaseline, stearic acid, glyserine monostearate, and cetyl alcohol were smelted on heater at temperature of 70°-75°C, and then vitamin E was added into the smelted formula [27]. The water phase was compossed of aquabidest, propylene glycol, sodium acetate and TEA and dissolved with heater. The water phase and oil phase was milled in hot mortar with stirring slowly on a temperature of ±70°C until cream mass formed, then added oleum rosae as perfume sufficiently [28].

Stability Evaluation

The formula was stored in a transparent spray bottle and kept it at room temperature for 12 weeks. During storage, several examinations were done, and observed such as organoleptic, particle size in nano-emulsion and pH determination [29,30].

Anti-Aging Test using Skin Analyzer

The test of anti-aging activity was done with 12 volunteers that have been divided in each different vitamin E concentration. All volunteers's hand skin condition was pretested with various test parameters including moisture, evenness, pore, and spot number using skin analyzer and moisture checker. Nano-emulsion and cream vitamin E had been applied twice daily at night and mornings for 12 weeks. The change of skin condition was measured each week for 12 weeks using skin analyzer. The result was evaluated statistically using one way of anova .

3. RESULTS AND DISCUSSION

The pH values of preparation

The pH was measured to know the acidity of all prepared cosmetics. According to a regulation in SNI 16-4399-1996 that pH of skin moisturizer product should be ranged between 4.5-8.0 [5,29]. If the cosmetic product has pH very high or low it may cause an irritated skin [31,32,34].

Characteristics of nano-emulsion and cream containing Vitamin E

Based on data from the result of observation and stability test for 12 weeks indicated that the nano-emulsion prepared spray and cream vitamin E have a good stability for 12 weeks. Stability of a prepared pharmacy can be seen by any change of color, it's smelt and pH during storage [29,34]. The characteristics of vitamin E Spray and Cream can be seen in Table 1.

Table 1. Apperance of spray and cream vitamin E

Formula	Appearance			Formula	Appearance		
(spray)	Colour	Smell	Consistency	(cream)	Colour	Smell	Consistency
F1	White	Rose	Emulsion	F1	White	Rose	Semi solid
F2	White	Rose	Emulsion	F2	White	Rose	Semi solid
F3	White	Rose	Emulsion	F3	White	Rose	Semi solid
F4	White	Rose	Emulsion	F4	White	Rose	Semi solid

Note: F1 (Blank), F2 = 1%, F3 = 3%, F4 = 5% Vitamin E

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Homogeneity of prepared cream

The aim of homogeneity test was to show the materials distribution in the formula. Based on the homogeneity observation of cream formula had been shown not any coarse grains found on object glass[35]. so that should be concluded this formula was homogenous as presented in Figure 1.

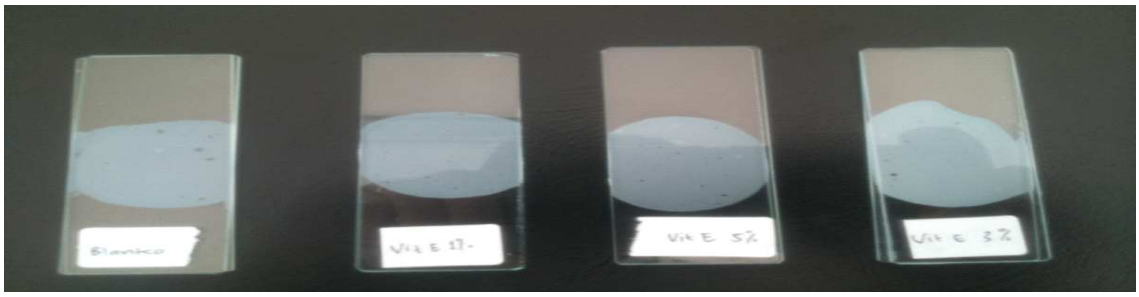

Figure 1. Homogeneity of prepared cream

Particle Size of nano-emulsion prepared spray (*by particle size analyzer)*

The result of particle size measured by Particle Size Analyzer (PSA) had been shown in Table 2.

Table 2. Spray Nanoemulsion Particle size

Formula	Before (initialy)		After 12 weeks	
	Size (nm)	Intensity (%)	Size (nm)	Intensity (%)
F2 (1%)	183.72	0.54	187.32	0.52
F3(3%)	186.26	0.53	308.17	0.91
F4(5%)	186.26	0.57	338.93	0.93

Based on the result that found it was that particle size of spray nano-emulsion had been changed after 12 weeks storage, but still persistent in nano size. That storage condition caused oxidation process and influence of surfactant components[34,35,36].

The result was estimated that nano-emulsion *spray* was stable after 12 weeks stored. The vitamin E concentration caused formula size particle had been changed but still within acceptable range of 2-500 nm size [38].

The Anti-Aging Activity

The anti-aging activity test using skin analyzer (Aramo) to measure parameter including moisture, evenness of skin, size of pore, and the spot total [34,35,36]. The anti-aging activity was started by measuring initial condition of back skin of hand each volunteers. The data obtained on each *anti-aging* parameter was analyzed statistically with ANOVA.

Moisture

The moisture in back hand of each volunteers was measured using moisture checker in Skin analyzer Aramo device. The result has been done and plotted to obtain a graphic indicated that early condition in back hand of each volunteers was close to approach dehydration (0 – 32). The result was measured by skin analyzer for 12 weeks had been shown to increase moisture caused by nano-emulsion spray and vitamin E cream. The result is presented in Figure 2 and 3.

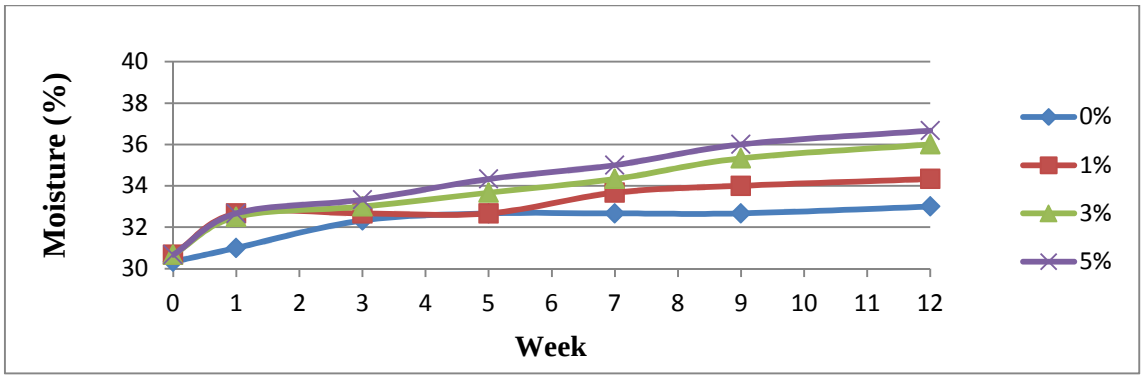

Figure 2. The effect of spray nanoemulsi containing vitamin E 0 % (blank), 1%, 3% and 5% on moisture for 12 weeks.

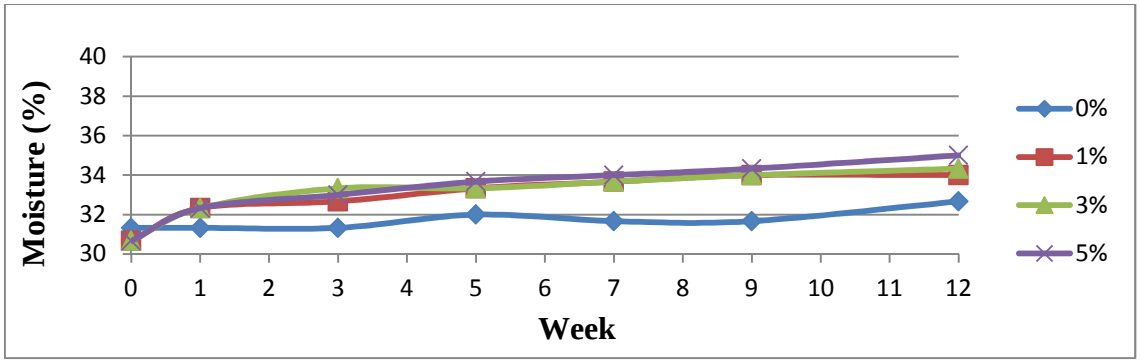

Figure 3. The effect of cream containing vitamin E 0 % (blank), 1%, 3% and 5% on moisture for 12 weeks.

Based on Figure 2 and 3 can be seen the result is a rising percentage of water content each formula every weeks measured. The result of statistical analysis by Anova showed that nano-emulsion spray 5% containing vitamin E is significantly different ($p \geq 0.05$) with all of nano-emulsion *spray* and cream vitamin E formula of every concentration.

The result of test suggested that the various concentration of vitamin E is influencing and profitable to skin healthy[36]. Vitamin E was usually used in purpose against dryness of the skin as anti-aging in sun-screen product[37]. The best sun-screen products should contain at least 1% Vitamin E. This study proved that Vitamin E resolved dryness and gave natural moisture on skin [37,38].

Evenness

Evenness skin of back hand of those volunteers was measured using device *skin* analyzer magnification lens 60% (normal lens) with a blue sensor. The result of statistical analysis also indicated that prepared nanospray group of 3% and 5% generated decreasing evenness percentage approaching almost the same as that of the condition a week backward, but if compared both groups was higher decreased percentage occurred on a prepared nano-emulsion spray group containing 5% Vitamin E compared to a prepared nano-emulsion spray group containing Vitamin E 3%. According to statistical

analysis was found to be significantly different ($p \geq 0.05$) from week-1 through week-12 on a prepared nano-emulsion spray containing Vitamin E 3% with 5%. The result can be seen in Figure 4 and 5.

The function of Vitamin E on skin is beneficial to restore structure of skin, to prevent aging, strengthen skin and got evenness on skin surface. In other research showed that Vitamin E is the best anti-oxidant component and evenness the skin, due to the nature of Vitamin E that dissolved in fatty material of skin and helpful to penetrate directly [4,5,31].

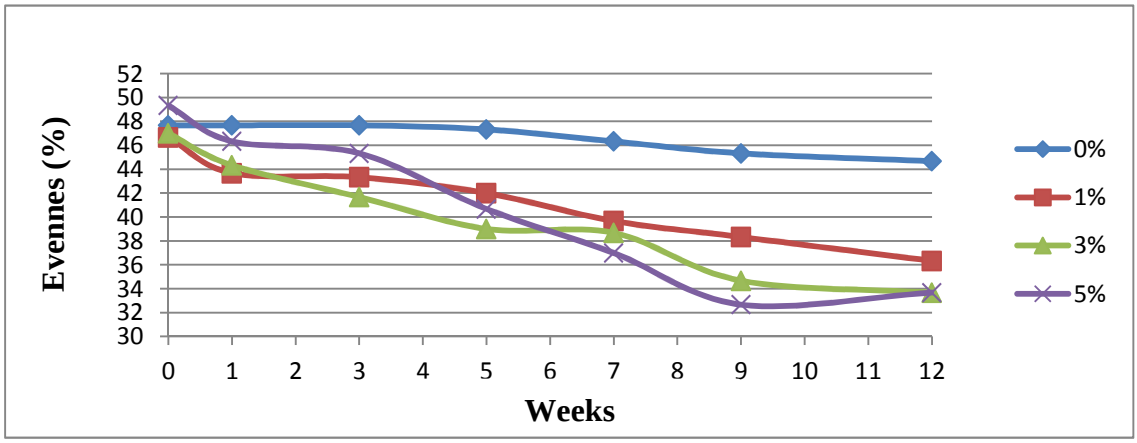

Figure 4. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% dan 5% on skin evennes for 12 weeks

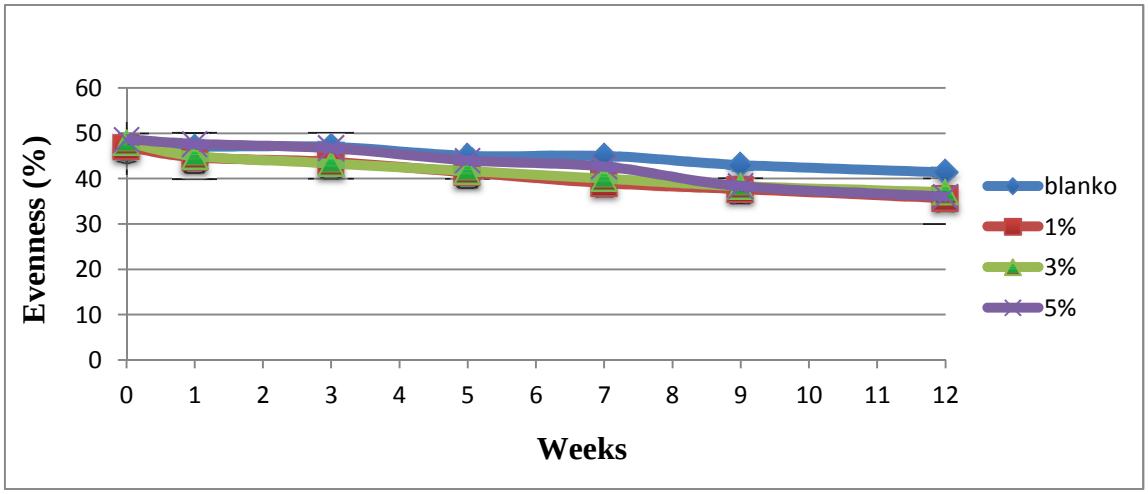

Figure 5. The effect of cream containing vitamin E 0 % (blank), 1%, 3% dan 5% on evenness for 12 weeks.

Pore

The existence of skin pore of back hand the volunteers was measured using the same skin analyzer device automatically its pore size in analysis included in reading [38,39].

The result presented is nano-emulsion spray group of Vitamin E 5% have decreasing compared with cream of Vitamin E 5% group since early week until week 12 presented in Figure 6 and 7. The graphic presented indicate that is a significantly difference ($p \leq 0.05$) between nano-emulsion spray group

and cream of Vitamin E. The result showed that in statistical analysis of Anova indicate significantly different ($p \leq 0.05$) between nano-emulsion spray group of vitamin E 3% and 5% with all of groups.

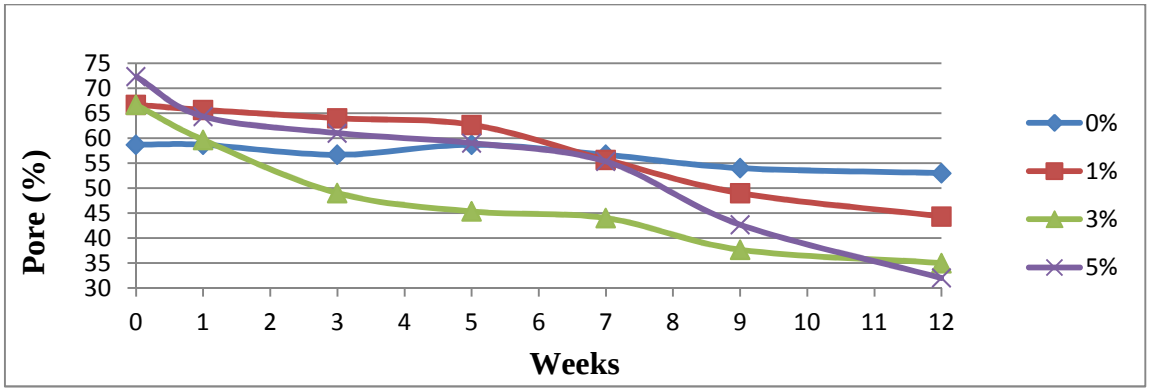

Figure 6. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% and 5% on pores for 12 weeks.

Pores may enlarge if exposure to sun-light, and acumulate died skin cell which may cause and triger emerging pimples and influence on pore size[37,38]. Vitamin E (*tocopherol*) can clean out died skin cell and stimulate formation new cell as well as neutralize or scavange free radical that may risky in skin damaged, so it may decrease pores of skin[23,24,25].

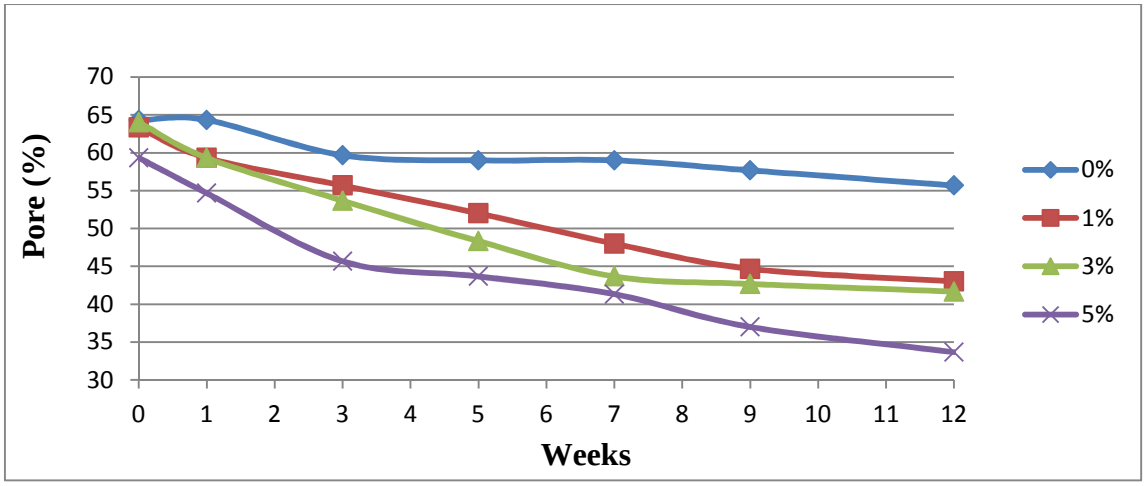

Figure 7. The effect of cream containing vitamin E 0 % (blank), 1%, 3% dan 5% for 12 weeks on pores.

Spot

Skin spot on back hand of volunteers was measured using skin analyzer device with magnifying lens at 60x (polarizing lens) in orange censor. The figure showed that initial skin condition of back-hand in all volunteers present many spots on skin (40 – 100).

The result of statistical analysis showed that there is significantly different ($p \geq 0.05$) between before and after treatment nano-emulsion spray group and cream of Vitamin E group. The result in statistical analysis found that occurrence is significantly different ($p \geq 0.05$) between a nano-emulsion prepared *spray* group 5% with all other group either nano-emulsion spray group and cream of vitamin E,

which means that nano-emulsion *spray* 5% become the best formula in reducing percentage total spots compared to other groups. The result can be seen in Figure 8 and 9.

Vitamin E could naturally keep skin health and also protect skin and hence as sun-screen, and this study proved that Vitamin E is also beneficial to generate of skin and natural moisturizer mainly on skin after exposure to sun-light, as well as to reduce any spots and also to keep skin healthy [23,26].

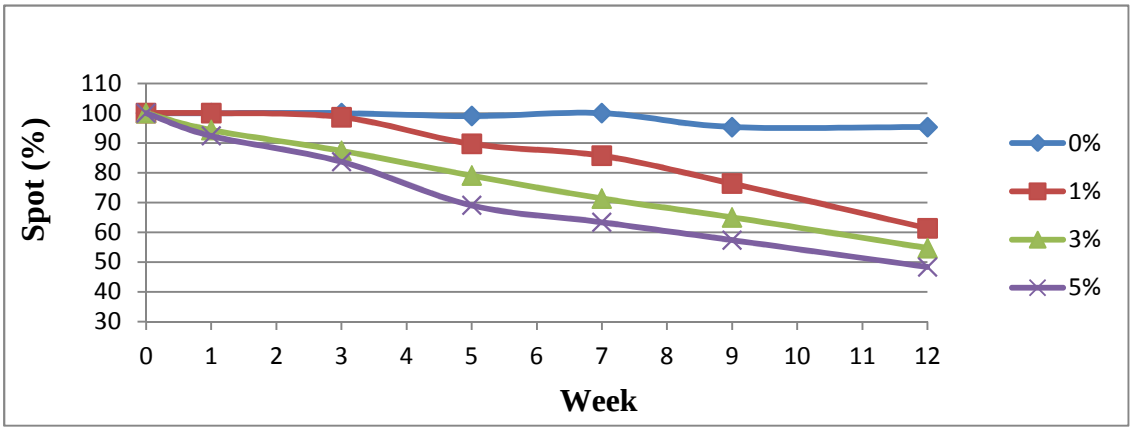

Figure 8. The effect of spray nanoemulsion containing vitamin E 0 % (blank), 1%, 3% dan 5% for 12 weeks on spot

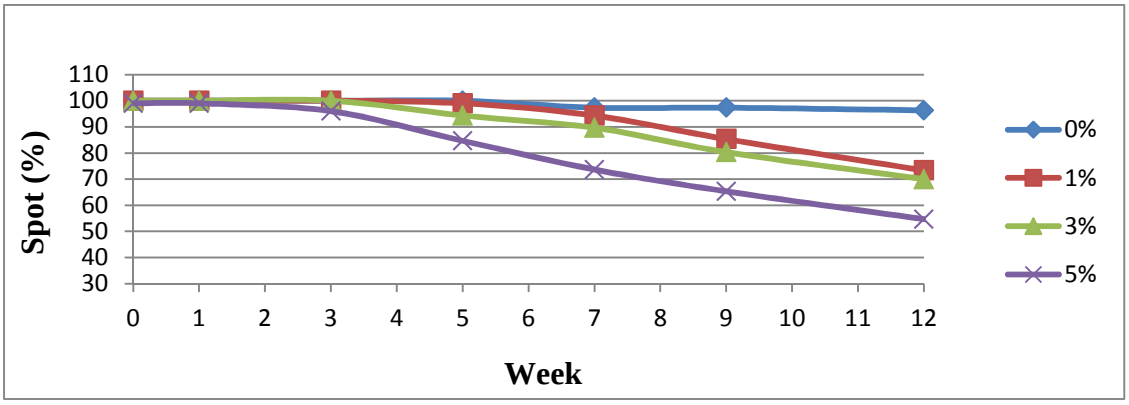

Figure 9. Graph the results of measurements of spot cream containing vitamin E 0 % (blank), 1%, 3% and 5% for 12 weeks.

4. CONCLUSSION

Vitamin E can be formulated into a nano-emulsion spray and cream and found to be in reliable stability. Vitamin E in any nano-emulsion spray proved to be helpful and function as anti-aging better than cream of Vitamin E. Activity of anti-aging of vitamin E in a nano-emulsion spray and cream are increasing on dependent concentration of vitamin E . The particle size is also significantly influence to the effectiveness of formula, that the smaller the size of particle the easier the formula to penetrate to the skin surface, so that may improve penetration of preparation into skin. The nano-emulsion spray vitamin E

5% proved to become the most beneficial formula in generating effectiveness as anti-aging product. for 12 weeks.

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