

Research Article

Formula Variation of Hand Body Lotion Extraction of Lime Peel (*Citrus aurantifolia*) as an Antioxidant

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ABSTRACT

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Lime (*Citrus aurantifolia*) was one of the herbal plants were often used for cooking ingredients and also as a medicine. Lime had chemical contains such as flavonoids, and saponins could have function as antioxidants. The purpose of this study was to determine lime peel extract could be formulated in hand body lotion preparation and determine the antioxidant activity value of lime peel extract hand body lotion preparation. The method used in extraction was maceration using 96% ethanol solvent with a yield value of 11.6%. Test of flavonoid levels using UV-Vis spectrophotometry with quercetin standard obtained 1.13%. Evaluation of stability of hand body lotion preparation included organoleptic test, homogeneity test, pH test, spreadability test, adhesivity test and irritation test. Based on the results of the study, a good formula was obtained 0,1% and antioxidant activity was tested on the lime peel extract hand body lotion preparation 0.1% using the DPPH method and an IC₅₀ value of 159.91 ppm was obtained.

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INTRODUCTION

The skin is an organ that covers the entire human body and functions to protect the body from external influences, thus skin needs to be protected and maintained its health. The process of skin destruction is characterized by the appearance of wrinkles, scales, dryness, and cracks. One of them is caused by free radicals (Tika et al., 2014). The free radicals produced will cause DNA damage having impacts on continuous cell proliferation; therefore it becomes the beginning of the formation of cancer (Sari, 2015). Antioxidants are able to act as hydrogen radical donors or able to act as free radical receptors thus they can delay the initiation stage of free radical formation. One of the plants that has been known to prove its efficacy as an antioxidant is

lime (Permata et al., 2018). Lime peel contains flavonoids, alkaloids, and saponins. Lime peel contains abundant apigenin, rutin, quercetin, and kaempferol. The content of lime peel has efficacious as an antioxidant which includes in a group of flavonoids. Therefore, there is a need for skin care with a cosmetic product made from natural ingredients with antioxidant effects.

The use of natural antioxidants is easier to apply in topical dosage forms such as hand body lotions, masks, and creams. Hand body lotion is intended for external use as a skin moisturizing cosmetic preparation. Skin moisturizing cosmetics are generally in the form of liquid oil preparations or a mixture of oil in water which can be added or reduced by certain substances for special purposes (Wasitaatmadja, 1997). Based on this

description, the authors are interested in conducting research with the aim of knowing whether lime peel extract can be formulated in hand body lotion preparations and how much IC₅₀ is the formulation of lime peel extract hand body lotion as an antioxidant. The selection of this lotion preparation is based on its good spreading ability on the skin and easy to clean or wash, making it more practical. Based on the above background, therefore the author conducts on the research entitled the Formula Variation of Hand Body Lotion Extraction of Lime Peel (*Citrus aurantifolia*) as an Antioxidant.

MATERIAL AND METHODS

Materials

This study used some tools such as analytical balance, beaker glass, funnel, pH meter, object glass, rotary evaporator, filter paper, measuring flask, measuring cup, stirring rod, spatula, mortar and stamper, bunsen, watch glass, volume pipette, Erlenmeyer, spectrophotometer of UV-Visible. The materials used in this study included samples (lime peel extract), 96% ethanol, quercetin, 10% AlCl₃, potassium acetate, stearic acid, TEA, liquid paraffin, cetyl alcohol, glycerin, methyl paraben, propyl paraben, distilled water, DPPH, aluminum foil, Vitamin C, NaCl.

Methods

Preparation of Lime Peel Extract

The lime peel was weighed as much as 500 grams and then put into the macerator, and then 1.5 liters of 96% ethanol solvent was added into the macerator until the simplicia was completely submerged. The maceration process was carried out for 3x24 hours; the solvent was replaced with a new solvent until the resulting filtrate was clear. The extraction results obtained were concentrated using a vacuum rotary evaporator at a temperature of 38°C. The extraction process was continued using an oven at a temperature of not more

than 40°C, in order to obtain a thick extract in the form of a paste.

Determining Flavonoid Level

Preparation of Quercetin Solution-Weigh 100 mg of quercetin and dissolve it with ethanol into a volume of 100 mL.

Dilution of Quercetin-Dilution of quercetin was made with concentrations of 4, 6, 8, 10, and 12 ppm as a comparison solution.

Determination of Maximum Wave Length

Quercetin pipette 1 mL standard solution of quercetin in each concentration series. Add 10% AlCl₃ and add 0.2 mL, potassium acetate add 10 mL 96% ethanol. After that, it was incubated for 30 minutes at room temperature and measured the absorbance on spectrophotometry UV-Vis with a wave length of 430 nm.

Determination of Flavonoid Level

Samples of lime peel extract were weighed into 100 mg then dissolved in 10 mL of 96% ethanol. Take 1 mL of the test sample, add 0.2 AlCl₃ 10%, 0.2 mL potassium acetate, and add ethanol 96% add 10 mL. After incubation for 30 minutes, the absorbance was measured with a spectrophotometer UV-Vis at a wave length of 430 nm.

Formulation of Hand Body Lotion

Making handbody lotion is done by mixing the ingredients of the oil phase (stearic acid, cetyl alcohol, and liquid paraffin) and the water phase (triethanolamine, glycerin, and aquadest) then heated to a temperature of 70°C using a porcelain cup for the oil and water phases for the aqueous phase. Then the water phase and the oil phase were mixed slowly using a mortar and stamper that had been preheated. Stirring was done until a homogeneous lotion mass was formed, then methyl paraben, propyl paraben were added. Then the lime peel extract was added and stirred until homogeneous. Lotion put in a suitable container and tightly closed.

Table 1: Formulation of Lime Peel Extract Lotion

Material Composition	Function	Formula (%)				
		F1	F2	F3	F4	K(-)
Lime Peel Extract	Active	0.06	0.08	0,1	0.3	-
Stearic Acid	substance	2	2	2	2	2
Tea	Emulsifier	0.5	0.5	0.5	0.5	0.5
Liquid Paraffin	Emulsifier	5	5	5	5	5
Cetyl Alcohol	Moisturizer	3	3	3	3	3
Glycerin	stabilizer	5	5	5	5	5
Methyl Paraben	Humectants	0.1	0.1	0.1	0.1	0.1
Propyl Paraben	Preservative	0.02	0.02	0.02	0.02	0.02
Aquades add	Preservative	100	100	100	100	100
	Solvent					

Stability Evaluation

Organoleptic Test- Performed using the five senses to know the shape, color, smell, and taste.

Homogeneity Test- Adequate amount of lotion was taken from each formula and then smeared on a glass plate, palpated, and rubbed, the lotion mass had to show

a homogeneous composition such as no solid material which was felt on the glass.

pH test- The pH stick is dipped into the lotion extraction and then allowed to stand for a moment and the resulting color was adjusted to the color on the device. The pH of the extraction must follow a pH which ranges from 4.5 to 6.5 (Tranggono and Latifah, 2007).

Spread-ability Test- A total of 1 gram of the extraction was placed on a glass measuring 20x20 cm. Then it was covered with another glass and a weight was used on it until the weight reached 125 grams, the diameter was measured after 1 minute. The dispersion requirement was 5-7 cm (Gerg and AKS, 2002).

Adhesion Test- A total of 0.25 grams of the sample was placed on the object glass, then the object glass was mounted on it. The object glass was attached with a load of 1 kg for 5 minutes and then removed. After that, the load weighing 80 grams was released which had been installed on the test equipment (St. Rahmatullah et al., 2019).

Skin Irritation Test- The irritation test was carried out on female white rabbits with an experimental time of 72 hours. Each rabbit was shaved on the back and then smeared with 0.5 gram of sweet orange peel ethanol extract lotion, and covered with gauze. Erythema and edema were observed at the 24th hour and 72nd hour after exposure.

Antioxidant Activity Test

Making DPPH Solution- Weighing 20 mg of DPPH powder then dissolved it with ethanol p.a to the mark into a 100 ml volumetric flask.

Determining the maximum wave length- ml of DPPH stock solution was taken and put into a 5.0 ml volumetric flask then added ethanol at the mark and shake the mixture until it became homogeneous. (Molyneux 2004).

Determining the operating time- ml of DPPH stock solution was taken and put into a 5.0 ml volumetric flask and then ethanol pa was added to the mark.

Making extract solution of lime peel extraction- The thick extract was weighed as much as 50 mg and dissolved with ethanol p.a add the limit mark into a 50 mL volumetric flask. Then 5 series of dilutions were made into 100 ppm, 80 ppm, 40 ppm, 20 ppm, and 10 ppm.

Making the vitamin C extract solution- Vitamin C was weighed into 10 mg, put in a 100 mL volumetric flask and added ethanol p.a add a limit mark hereinafter referred to as mother liquor. The mother liquor was made into 4 concentration series, those were 8 ppm, 4 ppm, 2 ppm, 1 ppm..

Making the lotion extract solution- Weighed into 100 mg of the lotion extraction, then dissolved it with ethanol at the limit mark in a 100 mL volumetric flask. The lotion solution with a concentration of 1000 ppm

was made into serial dilutions of 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm.

Measuring IC₅₀

The IC₅₀ value was calculated based on the percentage of inhibition of the DPPH radical from each solution concentration and the linear regression line equation $y = a + bx$ was obtained.

DATA ANALYSIS

Based on the classification of Blois (1958) the level of antioxidant power with the DPPH method is categorized as follows:

Tabel 2: Tingkat Kekuatan Antioksidan (Blois, 1958)

Intensity	Mark of IC ₅₀ (ppm)
Very strong	<50
Strong	50-100
Moderate	101-150
Weak	>150

RESULTS AND DISCUSSION

Lime Peel Extraction (*Citrus aurantifolia*)

The result of maceration of lime peel (*Citrus Aurantifolia*) as much as 500 grams with ethanol solvent was obtained a thick blackish green extract.

Table 3: The Result of Marinade of Lime peel Extraction

Type of Extraction	Extract Weight (gms)	Marinade (%)
Ethanol	58	11.6

The extraction method used maceration process because it has several advantages, such as easy way of working, the simple tools used and suitable for materials that could not withstand heating (Depkes RI, 1968). The reason for using 96% ethanol solvent is because it has the same level of polarity as the compound taken.

Furthermore, the results of the maceration process was concentrated using a rotary evaporator. This process aims to concentrate a solution consisting of a non-volatile solute and a volatile solvent.

Determining the Flavonoid Level

The quantitative analysis used to determine the total flavonoid content in lime peel extract (*Citrus aurantifolia*) used quercetin as a standard solution. The first step that needs to be done is to determine the maximum wave length by measuring the standard solution of quercetin; the maximum wave length is 430 nm. In measuring the total flavonoid content, the absorbance value of the standard solution of quercetin was obtained, namely $y = 0.042 + 0.070x$ with a coefficient of determination (R²) obtained at 0.990.

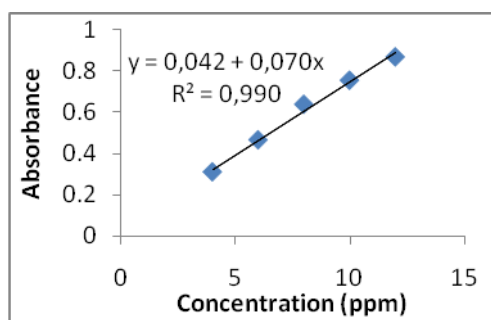


Fig.1: Quercetin Standard Curve

Table 4: Determination of Total Flavonoid Levels

Absorbance	Concentration (ppm)	Average (ppm)
0,860	11.68	11.36
0,812	11.00	
0,841	11.41	

Based on the results of the determination above, the average concentration was obtained into 11.36 ppm, then multiplied by 1000 times the dilution. Therefore the total levels of flavonoids in lime peel extract are 11,360 ppm or 1.13%.

The homogeneity examination aims to observe the presence or absence of coarse particles in the extraction. The homogeneity test was carried out on the five lotions, the results had good and homogeneous which is homogeneity. The ingredients used in making hand body lotion with lime peel extraction must be evenly dispersed in the extraction.

The measurement of pH value is carried out to determine the pH of the extraction. The pH values of the five lotion formulations ranged from 5.5 to 5.8. Due to the acidic nature of the lime peel, it affects the extraction. The pH value should not be too acidic because it can cause skin irritation and also should not

be too alkaline because it can cause scaly skin. The pH range of the extraction must follow the skin pH, which has range in 4.5 to 6.5 (Tranggono and Latifah, 2007).

The spread-ability of a good lotion is 5 to 7 cm. Good dispersion will make it easier to apply and evenly distribute lotion on the skin, and can increase comfort during use and can provide a maximum effect. From the five lotion formulations, the diameter of the distribution ranged from 6 to 6.8 cm.

The adhesion test was conducted to determine how much the lotion is able to adhere to the skin. There are no special requirements regarding the adhesion of semi-solid extractions, but preferably more than 4 seconds of adhesion is produced. From the results of the tests carried out on the five formulations, they meet the requirements of more than 4 seconds. The longer the lotion is attached to the skin surface, the longer the therapeutic effect given to the lotion preparation, because the preparation will be in contact with the skin surface longer, thus the absorption of the drug through the skin will be greater and provide an optimal effect.

Table 5: Results of Physical Stability Evaluation

Formula	Homogeneity	pH	Spread-ability (cm)	Adhesion (seconds)
K(-)	Homogeneous	5.8	6.5	5.0
F1	Homogeneous	5.6	6.0	4.5
F2	Homogeneous	5.7	6.4	4.5
F3	Homogeneous	5.5	6.8	5.1
F4	Homogeneous	5.5	6.6	4.9

The irritation test in this study refers to the guidelines from BPOM 2014 No. 875 on acute dermal irritation. The results of the irritation test for the preparation of lime peel extract lotion on rabbits showed no irritation with a score of 0 for erythema formation and a score of 0 for edema formation. It could be due to the pH of the extraction that meet the requirements of 4.5 to 6.5 and the absence of excipients required may trigger an irritant reaction.

Table 6: Results of Irritation Test

Treatment	Reaction		The Index of Primary Irritation	Notes
	Erythema	Edema		
K(-)	0	0		
F I	0	0		
Formula F II	0	0	0.0	There is irritation
F III	0	0		
F IV	0	0		

Antioxidant Test

In this study, the antioxidant activity test was carried out using the DPPH method. DPPH can be used because antioxidant compounds are able to reduce DPPH free radicals. Antioxidant activity testing was carried out using spectrophotometry UV-Vis at a wavelength of 516 nm (Molyneux 2004).

Antioxidant activity reading was carried out on 3 samples, those are lime peel extraction (*Citrus aurantifolia*), vitamin C and lotion formula 3. Each sample was made in several concentration series to obtain absorbance and % inhibition of DPPH. After obtaining absorbance data and % inhibition of the sample, IC₅₀ is calculated by entering the concentration as x and % inhibition as y thus the regression equation will be obtained.

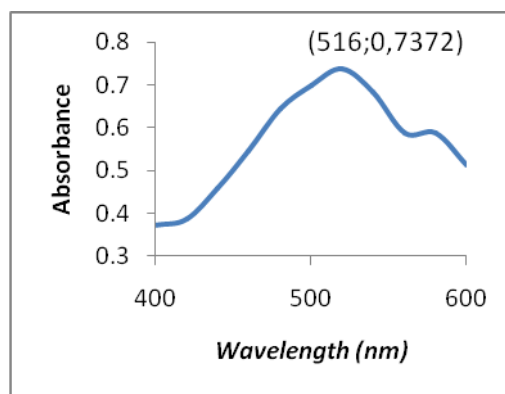


Fig.2: Maximum Wavelength of DPPH

Table 7: Results of Absorbance and % Inhibition

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (ppm)
Vitamin C	1	0.679	07.87	05.49
	2	0.589	20.08	
	4	0.415	43.69	
	8	0.231	68.66	
Lime Peel Extraction	10	0.668	09.36	61.47
	20	0.568	22.93	
	40	0.467	36.63	
	80	0.253	65.67	
	100	0.187	74.63	
The Lotion of Lime Peel Extraction	31,25	0.873	14.28	159.91
	62,5	0.748	23.12	
	125	0.549	43.58	
	250	0.262	73.07	

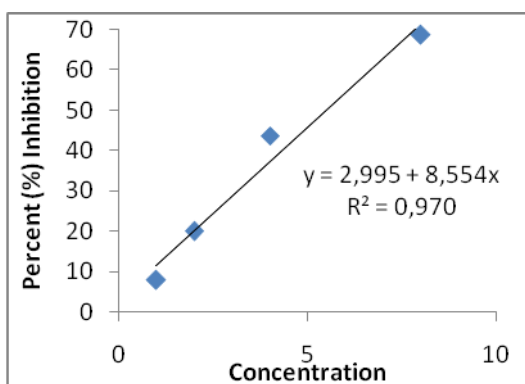


Fig.3: The curve percentage (%) DPPH inhibition for vitamin C

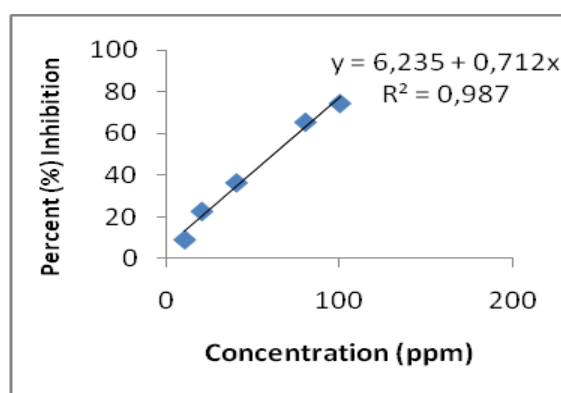


Fig.4: The curve percentage (%) of DPPH inhibition towards lime peel extraction

The testing of antioxidant activity of vitamin C was obtained IC₅₀ results of vitamin C with 5.49 ppm, which has meaning that it shows that vitamin C has very strong antioxidant activity because it has an IC₅₀ value of <50 ppm.

The testing of antioxidant activity of lime peel extraction (*Citrus aurantifolia*) was obtained the results of IC₅₀ from lime peel extraction (*Citrus aurantifolia*) of 61.47 ppm which has meaning that it shows that lime peel extraction has strong antioxidant activity because it has an IC₅₀ mark ranging from 50 to 100 ppm.

The test of antioxidant activity of the lotion carried out on 5 formulas was obtained IC₅₀ result of the lime peel extract lotion with 159.91 ppm, which has meaning that it showed the lime peel extract lotion had weak antioxidant activity because it had an IC₅₀ value of > 50 ppm.

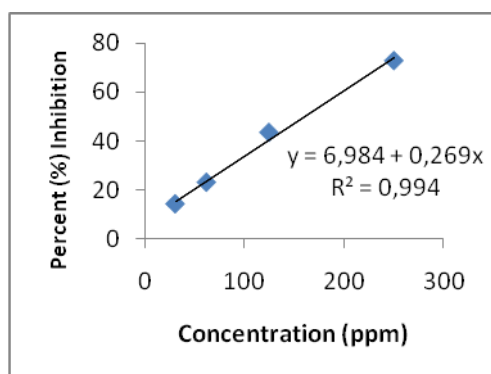


Fig.5: The curve of Percentage (%) DPPH inhibition for hand body lotion towards lime peel extraction

The test of antioxidant activity of the lotion carried out on 5 formulas was obtained IC₅₀ result of the lime peel extract lotion with 159.91 ppm, which has meaning that it showed the lime peel extract lotion had weak antioxidant activity because it had an IC₅₀ value of > 50 ppm.

By comparing the IC₅₀ value of lime peel extraction towards vitamin C, the antioxidant activity of the extract was lower than vitamin C, it could be the cause that vitamin C is a purer compound while lime peel extract contains various secondary metabolites (impure). The results of the IC₅₀ value of the hand body lotion extraction of lime peel in 0.1% formulation of 159.91 ppm which has meaning 0.159 ppm of the extract in the lotion can inhibit DPPH by 50%, while the lime peel extraction needs 61.47 ppm which able to inhibit DPPH as much as 50%. This can occur because the hand body lotion has other additives such as methyl paraben and propyl paraben which have antioxidant effects.

CONCLUSION

Lime peel extraction (*Citrus aurantifolia*) can be formulated into hand body lotion preparations in an extract concentration of 0.1%. The preparation of hand body lotion with lime peel extraction (*Citrus aurantifolia*) with a concentration of 0.1% has weak antioxidant activity with an IC₅₀ value of 159.91 ppm.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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