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Antioxidant Effectiveness Test on The Formulation of Combination Facial Soap Ingredients of Ethanol Extract Lemon Peel *Citrus limon* and Forest Honey *Apis dorsata*

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Abstract

Citrus limon and Apis dorsata are claimed to have several active compounds namely: alkaloids, flavonoids, tannins and saponins. These compounds are known to have antioxidant activity in preventing skin damage due to free radicals and preventing premature aging. In this study, Citrus limon and Apis dorsata were formulated into facial soap ingredients. The purpose of this study was to find out whether the ethanol extract formulation in the lemon peel and the Apis dorsata forest honey has the effectiveness of antioxidants in preventing the free radicals. This research was an experimental study with physical and chemical evaluation of ingredients. The DPPH test was used to determine the antioxidant activity of the ingredients. This research used three types of formulation of Ethanol Ethanol Extract Formulation were lemon and honey forest, they were : FI (5%: 2%); FII (5%: 3%) and FIII (5%: 4%). The results of the DPPH test respectively were FI 6,16; FII 5,53 and FIII 4.39. The ingredients of Facial Soap Combination of Ethanol Extract Lemon Feel and Apis dorsata had the best antioxidant and antioxidant energy in Formula III with concentration of 5%: 4%

Keywords: *Formulation, Lemon Peel, Traditional forest honey, facial soap, antioxidant*

Abstrak

Buah lemon Citrus limon dan madu hutan Apis dorsata dilaporkan mengandung beberapa senyawa aktif yakni: alkaloid, flavonoid, tanin dan saponin yang diketahui memiliki aktivitas antioksidan yaitu mencegah kerusakan kulit akibat radikal bebas dan mencegah penuaan dini. Pada penelitian ini kedua bahan tersebut diformulasikan menjadi sediaan sabun wajah. Tujuan dari penelitian ini adalah untuk mengetahui apakah formulasi ekstrak etanol kulit buah lemon Citrus limon dan madu hutan Apis dorsata memiliki efektivitas antioksidan yang dapat mencegah radikal bebas. Penelitian ini merupakan penelitian eksperimental dengan evaluasi sediaan secara fisika dan kimia. Uji DPPH digunakan untuk mengetahui aktivitas antioksidan dari sediaan. Pada penelitian ini digunakan tiga jenis formulasi ekstrak etanol kulit buah lemon dan madu hutan yaitu: FI (5%:2%); FII (5%:3%) dan FIII (5%:4%). Hasil uji DPPH berturut-turut yaitu FI 6,16; FII 5,53 dan FIII 4,39. Sediaan sabun wajah kombinasi ekstrak etanol kulit buah lemon Citrus limon dan madu hutan Apis dorsata memiliki efek sebagai antioksidan dan daya antioksidan paling baik pada formula III dengan konsentrasi 5%: 4%

Kata kunci*: Formulasi, Kulit lemon, Madu hutan tradisional, Sabun wajah, Antioksidan.*

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INTRODUCTION

Free radical is one of example of reactive oxygen compounds that attack and bind to the surrounding molecular electrons. Excessive oxidation can give a damage to cells or tissues,

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autoimmune diseases, degenerative diseases until cancer[1]. Free radical is needed in normal amounts for the body health, while in excessive amounts will cause oxidative stress. Free radical is the main factor of causing the premature aging process (aging) in skin membrane[2]. Antioxidants are substances that can delay or prevent any various harmful effects of free radicals[3].

Antioxidants can be obtained both naturally and synthetically. Phenolic compounds like flavonoids, tocopherol, and functional acids are natural antioxidant types that are generally found in plants[1]. Lemon is one of the plants that can produce natural antioxidant because it contains vitamin C, citric acid, polyphenols, essential oils, flavonoids, bioflavonoids, kumarin and oils such as limonen[4]. Ethanol Extract of *Citrus limon* and citrus sinensis have antioxidant and antibacterial activity to gram -positive and negative bacteria[5]. The result of the Phytochemical test shows that lemon peel extract consists of saponin compounds, flavonoids, and vitamin C[7].

Honey is one of the natural food ingredients that contain sugar, organic acids, enzymes, amino acids, carotenoids, vitamins, minerals, and aromatic substances[8]. Honey can also be used to smooth the skin, prevent skin damage and premature aging because in honey there are various nutrients that have antioxidant activity. These compounds work together to protect normal cells and neutralize free radicals[9].

The skin is the outermost organ of the body. The skin has a function to protect the inside of the body from physical, mechanical, heat, cold, germs and bacterial disorders[10]. Facial soap has an important role for facial skin health. Cleaning the face is one of the methods to clean and to make healthy the face[11]. Choosing the facial soap must be adjusted to the type of skin for avoiding skin problems on the face such as the emergence of spots, dry skin, irritation, acne etc[12]. Based on these issues, the reseracher is interested in formulating and observing the antioxidant activity of lemon skin extract *Citrus limon* and *Apis dorsata* to facial soap ingredients.

METHODOLOGY

Material

The researcher used some equipments in this reserach such as strainers, blenders, maceration vessels, stirring rods, molds, porcelain cups, erlenmeyers (pyrex), measuring cups (pyrex), chemical beakers (pyrex), universal pH indicators, glass watch, glass preparations, glass 10x8 cm, stove, mortar and pestle, mixer

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(cosmos), micropipette, scale pipette, dropper pipette, water heater, tube shelf, spatula, UV-vis spectrophotometer, test tube, analytical scales, rion viscometer, and waterbath.

The materials used in this study were Aquades, black glutinous sticky rice (*Oryza sativa* glutinosa), lemon peel (*Citrus limon*), traditional forest honey (*Apis dorsata*), carbomer, propyl paraben, propylene glycol, sodium lauril sulfate, tea and oleum olivarum.

Method

1. Production Process of Ethanol Extracts Lemon (Citrus limon) and traditional forest honey (Apis dorsata)

Lemon skin extract is obtained by separating the skin from the fruit flesh, drying process of the fruit peel was implemented by aerated, reduced the size by the machine, the sifting, then, extraction processes was carried out by using the maceration method. Maseration extraction was carried out for 5 days with 96% ethanol solvent. After that, removing it 2 times. The results of the extraction were concentrated with a rotary evaporator[13]. 500 grams of traditional forest honey were added 96% ethanol, then a maceration was implemented about 5 days. The result of extraction was concentrated using a rotary evaporator[14].

2. Facial Soap Formulation Of Ethanol Extract Lemon Peel Citrus limon and Traditional Forest Honey Apis dorsata

Table 1. Facial soap formulation of ethanol extract lemon peel *Citrus limon* and traditional forest honey *Apis dorsata*

Composition	*Function*	*Control -*	*Formula*		
			Formula I (%b/b)	*Formula II (%b/b)*	*Formula III (%b/b)*
Lemon extract (*Citrus limon*)	active substance	-	5%	5%	5%
Traditional forest honey extract (*Apis dorsata*)	active substance	-	2%	3%	4%
black glutinous rice (Oryza sativa glutinosa)	Scrub	1.2%	1.2%	1.2%	1.2%
Carbomer	Basic	3%	3%	3%	3%
TEA	Alkalizing agent	0.5%	0.5%	0.5%	0.5%

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Oleum olivarum	Emolien	1.5%	1.5%	1.5%	1.5%
Sodium lauril sulfate	Surfactan	3%	3%	3%	3%
Propilen glycol	Humektan	15%	15%	15%	15%
Propil paraben	Preservative	0.1%	0.1%	0.1%	0.1%
distilled water	Solvent	100%	100%	100%	100%

Information :
F1 = 5% extract lemon peel and 2% traditional forest honey
F2 = 5% extract lemon peel and 3% traditional forest honey
F3 = 5% extract lemon peel and 4% traditional forest honey
K(-) = Negative control without extract lemon peel and traditional forest honey
K(+) = Positive control (Garnier).

The making process of facial soap formula, first the material used was weighed as needed. Sodium lauril sulfate (SLS), paraben propil and propylene glycol were mixed in mortar, then added oleum ovary (mixture 1). Carbomer was developed in hot water at 60-80 ° C and TEA was added and stirred quickly until homogeneous (mixture 2). Mixture 1 was inserted into the mixture 2, then stirring as good as homogeneous for 10 minutes. After that added glutinous rice and ethanol extract of lemon peel then stirred until homogeneous. Antioxidant Effectiveness Test of *Citrus limon* extract and honey forest *Apis dorsata*.

1. The evaluation of facial soap ingredients of Ethanol Extract Lemon peel (Citrus limon)

a. physical stability

Organoleptic test: Test the physical properties of facial soap by observing to the organoleptic which included appearance, smell, and color[15].

pH test: pH measurement was conducted by using a pH meter. Before using the pH meter, it was calibrate it with a buffer solution (pH 4.7 - 9.0) for every time a measurement was taken. The test was carried out 2 times for each formula. The formula should meet the pH range according to the pH skin between pH 8 - 11 so that, it was safe to apply to the skin. The formula was expected to avoid skin irritation[15].

Homogeneity test: It was used as much as 1 gram of pasta was smeared on the slide. The slide containing the sample was covered with another empty slide, then it was observed microscopically whether the base was homogeneous (the surface is evenly smooth) or not mixed, both before and after storage[16].

High foam test: Put 1 gram of emulgel into a test tube and added 10 mL of distilled water. Next, the mixture was shaken by inverting the test tube and then measuring the height of the foam produced was immediately

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carried out. Every 5 minutes of standing, the height of the foam produced was measured again According to Melian E, 2018 the standard for good foam ranges from 60% - 70% after letting it sat for 5 minutes.

$$\% \text{ Foam stability } = \frac{\text{final foam height}}{\text{Initial foam height}} \text{ x } 100$$

Spreadability test: As much as 1 gram of emulgel was placed in the middle of a round glass and a weight was placed on top, then left it for 1 minute. Then, the diameter of the preparation that spreaded in the round glass was calculated through observation (the average length or diameter of several sides). The weights used in this experiment were 25, 50, 100, 150, 200, 250 and 300 grams without weight. Each additional load was carried out for 1 minute and the diameter of the emulgel that spread was measured[16].

Viscosity test: The sample was added into the sample cup, and it was ensured that the sample was free of bubbles. It had spreaded evenly on the surface of the cup. The sample cup was put back on the viscometer, then the tool was turned on and left for a few moments until the viscosity value read on the tool display was stable and recorded[16].

Cycling test: The goal of the cycling test was to see whether phase separation occured in the preparation during the storage process. The stability check was conducted by using the freeze and thaw method by placing 2 mL of the preparation in 8 vials and closing tightly. Four vials were used as controls, stored at 25°C. The remaining 4 vials would be used for freeze and thaw by storing the vials at 40°C for 24 hours, then observing the organoleptic changes (1 cycle). Up to 6 cycles were carried out and the organoleptic changes were observed each cycle[17].

4. Antioxidant activity test of ethanol extract of lemon peel (Citrus lemon) and traditional forest honey (Apis dorsata)

a. The making process of DPPH solution

The DPPH powder was weighed and about 5 mg. Then, it dissolved using methanol solution p.a, after that put into a 50 mL volumetric flask and added with methanol to the boundary mark then homogenized and the solution was stored at low temperature and protected from light[18].

b. Making process of blank solutions and optimization of wavelengths

1 mL of 0.5 mM DPPH solution was taken and put it into a 50 mL volumetric flask. Then, it was added methanol solution up to the mark and then left it for 30 minutes at 37 degree celsius. Furthermore, the absorption

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was measured at a wavelength of 517.0 nm[18].

c. Making process of comparison solution (positive control) Vitamin C

5 mg of vitamin C (positive control) was weighed. Then it was dissolved with enough CO_2-free water, then put into a 50 mL volumetric flask and made up to the mark as the mother liquor. Then, serial dilution 2 was made; 4; 6; 8; and 10 and the absorption was measured at certain wavelengths[18].

d. Making process of emulgel test solution

Approximately 5 mg emulgel was weighed, then dissolved in 50 mL of methanol pro analysis (100 ppm concentration), this solution was the mother liquor. Then, made several series of concentrations (2; 4; 6; 8; and 10). From a series of concentrations, 2 mL was pipetted into a volumetric flask and DPPH solution (5 mg) was added in a 2:1 ratio, then made up to 10 mL. Then, it was incubated for 30 minutes at 37 degree celsius. Furthermore, it was measured using a UV-VIS spectrophotometer[18].

e. The Determination of IC_{50} value

The IC_{50} determination of antioxidant activity was carried out from the results of absorbance measurements from three concentration series resulting in % inhibition where these three % inhibitions were calculated based on the equation below:

$$\%\text{inhibition} = \frac{\text{Blank observation} - \text{sample observation}}{\text{Blank observation}} \text{x } 100\ \%$$

The inhibition of the three concentration series was followed by a linear calculation using the equation[19].

$$\mathbf{y = a + b\ x}$$

Information:

x = concentration (ppm)

y= inhibition percentage (%)

Antioxidant activity was expressed by inhibition concentration of 50% or IC IC_{50}, namely the sample concentration that could absorb 50% of the DPPH (1.1-Diphenyl-2-Picyrlhydrazyl) radical. The IC_{50} value was obtained from the x value after y with 50 [19].

5. Data Analysis

Antioxidant activity was indicated by the IC_{50} value and it was obtained from the linear regression equation of the concentration of the test solution with % antioxidant power. Vitamin C levels were taken obtained from the equation

Standard linear regression of vitamin C (Y = A+BX) where Y = percentage inhibition, B = slope, X = level, and A = intercept. The level of vitamin C in lemon peel (mg/g) was obtained by multiplying the x value by the dilution factor, namely the maximum wavelength measurement of

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517 nm was obtained so that absorbance readings could be taken.

RESULT AND DISCUSSION

RESULT

1. *The result of extraction of Citrus limon and Apis dorsata Traditional Forest Honey*

The weight of *Citrus limon* and *Apis dorsata* traditional forest honey was obtained from extraction.

Table 2. Yield result

Sample	solvent type	The weight of dry sample (g)	Condensed Extract Weight (g)
Citrus limon and *Apis dorsata* Traditional Forest Honey	ethanol 96%	500 gram	140,55 gram

2. *Organoleptic Test*

Table 3. Observation Results of Organoleptic Test

Facial soap formula	Observation					
	Before cycling			After cycling		
	Color	Smell	consistency	Color	Smell	Consistency
FI	dark cream	Unique	thick	dark beige	Unique	Thick
FII	dark cream	Unique	thick	dark beige	Unique	Thick
FIII	light beige	Unique	thick	light beige	Unique	Thick

3. *Homogeneity Test*

Table 4. The observation result of homogeneity

Facial soap formula	Observation	
	Before cycling	After cycling
FI	homogeneous	homogeneous
FII	homogeneous	homogeneous
FIII	homogeneous	homogeneous

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4. *Spreadability Test*

Table 5. The observation result of spreading

Facial soap formula	Observation	
	Before cycling	After cycling
FI	5.2 cm	5.6 cm
FII	5.3 cm	6.2 cm
FIII	5.3 cm	6.6 cm

The standard of spreadability: 5-7 cm

5. *pH Test*

Table 6. The observation result of pH

Facial soap formula	Observation	
	Before cycling	After cycling
FI	6.31	5.2
FII	6.85	5.3
FIII	6.95	5.3

pH concentration : 4.5 – 6.5

6. *Foam Height Test*

Table 7. The observation result of height test

Facial soap formula	Observation	
	Before cycling	After cycling
FI	65.71%	62.85%
FII	70%	66.66%
FIII	70.58%	67.64%

Good foam stability ranges from 60-70% after being left for 5 minutes.

7. *Viscosity Test*

Table 8. The observation result of viscosity

Facial soap formula	Observation	
	Before cycling	After cycling
FI	13100	5600
FII	8720	8150
FIII	5190	12500

Viscosity specification: 2.000-50.000 cPs

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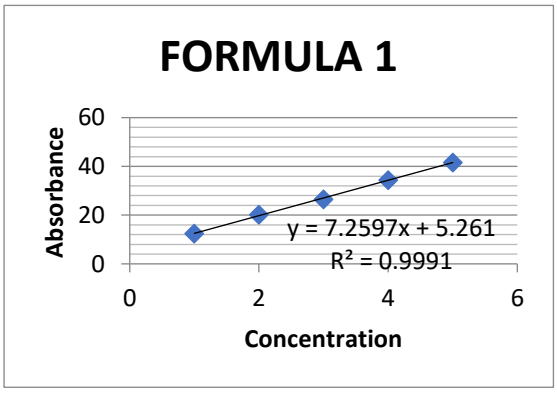
8. Antioxidant Activity Test

The antioxidant activity test applied the DPPH method as seen through the results of the percent inhibition which was the difference between the absorbance of the blanks. The following table showed the results of % inhibition.

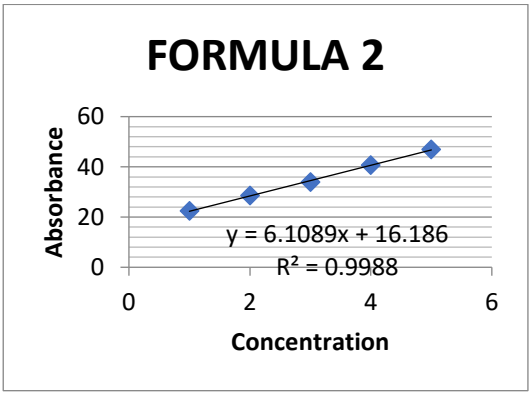
Table 9. The result of % inhibition Formula I, II dan III

No	Formula	Concentration	% Inhibition	IC_{50}
1	FI	10 ppm	41.94%	6.16265
		20 ppm	20.27%	
		30 ppm	26.53%	
		40 ppm	34.44%	
		50 ppm	41.67%	
2	FII	10 ppm	22.49%	5.5352
		20 ppm	28.60%	
		30 ppm	33.94%	
		40 ppm	40.67%	
		50 ppm	46.95%	
3	FIII	10 ppm	34.29%	4.39869
		20 ppm	38.91%	
		30 ppm	43.48%	
		40 ppm	48.19%	
		50 ppm	52.79%	

Information:
FI : The formulation of facial soap with the extraction of *Citrus limon* and *Apis dorsata* 5% : 2%.
FII : The formulation of facial soap with the extraction *Citrus limon* and *Apis dorsata* 5% : 3%.
FIII : The formulation of facial soap with the extraction *Citrus limon* and *Apis dorsata* 5% : 4%



(a)



(b)

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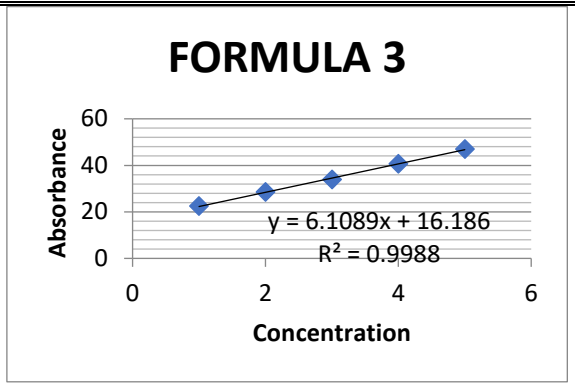

(c)

Figure 1. Curve % inhibition Formula I (a), Formula II (b) dan Formula III (c).

DISCUSSION

In this research, the samples used by the researcher were 140.55 grams of *Citrus limon* rind and *Apis dorsata* forest honey extracted using the maceration method. The filtrate result of *Citrus limon* and *Apis dorsata* forest honey produced a very thick ethanol extract.

In this study, a facial soap formulation was prepared with *Citrus limon* peel extract and *Apis dorsata* forest honey with concentrations of 5% : 2%, 5% : 3%, and 5% : 4% and without extract. In this experiment , the basic formula for facial soap was used which consisted of carbomer (base), TEA (alkalizing agent), oleum olivarum (emollient), black glutinous rice (scrub), sodium lauryl sulfate (surfactant), propylene glycol (humectant), propyl paraben. (preservative) and distilled water (solvent).

The DPPH (1,1-diphenyl-2 picrylhydrazyl) method was implemented to test the antioxidant activity of the preparations. The DPPH method was chosen because it was easy to be used, fast and quite thorough. The DPPH method could be used on solid and solution samples and it was not specific for certain antioxidant components. Free electrons in the DPPH radical provide maximum absorbance at a wavelength of 517 nm and showed a purple color[13].

In this study, 5 concentrations (10, 20, 30, 40 and 50 ppm) were made in each formulation with the aim of knowing the results of each existing concentration. The results obtained then averaged, so that from these average results, it could be seen how much antioxidant activity in the preparation was compared to the positive control of vitamin C. Vitamin C was used as a positive control because it was a secondary antioxidant that could capture free radicals, easy to obtain, could prevent chain reactions

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and vitamin C was more polar than the other vitamins[20].

A compound could be called as a very strong antioxidant if the IC_{50} value was less than 50, strong if the IC_{50} value was 50-100, moderate if the IC_{50} value was 100-150 and weak if the IC_{50} value was 151-200. The smaller the IC_{50} value, the higher the antioxidant activity[21]. Based on the results of the antioxidant effectiveness test for facial soap prepared with ethanol extract from *Citrus limon* and *Apis dorsata* forest honey against DPPH, it could be seen in table 9, the facial soap with *Citrus limon* peel extract and *Apis dorsata* forest honey has the potential as a very strong antioxidant because it has a high antioxidant value. IC_{50} with successive levels namely: FI 6.16; FII 5.53 and FIII 4.39.

CONCLUSION

The ethanol extract of *Citrus limon* peel and *Apis dorsata* forest honey can be formulated into facial soap ingredients. Both of them are chemically stable. The ethanol extract facial soap from *Citrus limon* peel and *Apis dorsata* forest honey is effective as an antioxidant in formula III with a concentration of 5% : 4%.

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