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Article

Antioxidant Effectiveness of Kapok Leaves Extract Moisturizer (*Ceiba pentandra* (L.) Gaertn.) with DPPH Method

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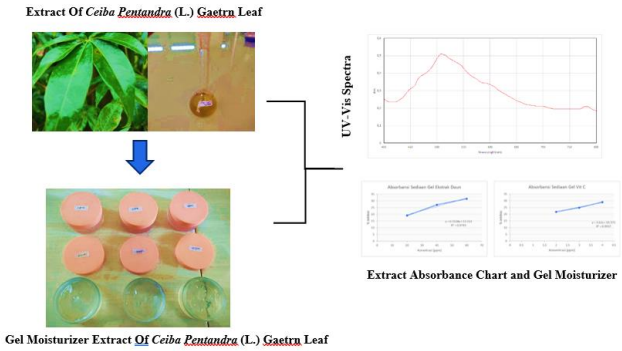
Abstract

Dry skin can often cause psychological discomfort. Moisturizer is a preparation that is used to improve dry skin. Kapok plant (*Ceiba pentandra* L. Gaertn) can be used as an active moisturizer ingredient. It can be used as a treatment, where the chemical content in kapok leaves are flavonoids, saponins, alkaloids, phenolic compounds, tannins, and terpenoids as antioxidants. This study aims to determine the antioxidant activity of kapok leaf extract and moisturizer preparations with the DPPH method using a UV-Vis spectrophotometer, the physical quality of preparations, and the antioxidant activity of moisturizer preparations using the DPPH method. Kapok leaves extract was prepared in various concentrations, namely 20 ppm, 40 ppm, and 60 ppm. The IC_{50} value of kapok leaves extract is 67.4007 ppm, which has vigorous antioxidant activity. Then, variations in the concentration of kapok leaves extract were put into the formulation of moisturizer preparations, and the results of moisturizer preparations of kapok leaf extract and vitamin C met the requirements of the physical quality test. The moisturizer preparation was continued and tested for antioxidant activity using the DPPH method with ascorbic acid as a comparison. The results of the IC50 value on the kapok leaves extract moisturizer preparation are 110.065 ppm, which is classified as having moderate antioxidant activity, while the vitamin C moisturizer preparation is 9.8417 ppm, which is classified as having extreme antioxidant activity.

Keywords: *Antioxidant, DPPH Method, Kapok Leaves, Moisturizer*

Graphical Abstract



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Introduction

Dry skin is a problem for millions and can often cause psychological discomfort and stress[1]. Indonesia has a tropical climate and the most exposure to sunlight[2]. One of the effects of sunlight can cause various damage to the skin layer, in which the skin becomes dry due to the evaporation of water on the skin's surface. Dry skin is characterized by a rough feel on the skin's surface, dull scaly, and stiff [3].

Moisturizer is a preparation used to improve dry skin[4]. This preparation can reduce Trans Epidermal Water Loss (TEWL) by forming a thin, fat layer on the skin's surface as a barrier, calming dermal nerve endings, and restoring skin softness[5]. Moisturizers that are in great demand are moisturizers with natural ingredients. The flavonoid content in moisturizers functions as an antioxidant, so it is suitable for use as a beauty product. A good moisturizer can moisten the skin and act as an anti-free radical, namely with flavonoid compounds that work as antioxidants in moisturizer formulations[6].

One plant with flavonoid compounds that have potential as antioxidants is the kapok plant (*Ceiba pentandra* L. Gaertn). The Kapok plant (*Ceiba pentandra* L. Gaertn) is one of the high-ranking plants identified as being used as a treatment, in which the chemical content in kapok leaves contains flavonoids, saponins, alkaloids, phenolic compounds, tannins, and terpenoids [7]. Natural plant antioxidant compounds are phenolic or polyphenolic compounds, which can be flavonoids, tocopherols, and polyfunctional acids. One plant with antioxidant potential is kapok (*Ceiba pentandra* L. Gaertn [8]. Somehow, at concentrations of 20 ppm, 40 ppm, and 60 ppm with an average IC_{50} value of 59.296 ppm so that at that concentration, the ethanol extract of kapok leaves can inhibit 50% of DPPH free radicals [9].

Antioxidants can be used to repair skin cells damaged by free radicals and to counteract free radicals. Antioxidants in cosmetic ingredients can provide moisturizing and brightening effects so that the skin does not only maintain its moisture but looks radiant[10]. The comparator used as a positive control in the DPPH method is vitamin C because it functions as a secondary antioxidant that captures free radicals and prevents chain reactions. Its antioxidant activity is also very high and easy to obtain. Moreover vitamin C is more polar than other vitamins[11]. Vitamin C has high polarity and effectively inhibits free radicals [12].

Currently, there is further scientific research regarding the antioxidant effectiveness of kapok leaves (*Ceiba pentandra* L. Gaertn) as a moisturizer. Kapok is known to have some bioactivity and has not been formulated in pharmaceutical preparations [13]. Based on this background, researchers are interested in conducting research on the antioxidant effectiveness of kapok leaves (Ceiba pentandra L. Gaertn) in moisturizing preparations using the DPPH method, with the hope that this research can be developed to see the potential of kapok leaves (*Ceiba pentandra* L. Gaertn) as an antioxidant in moisturizing preparations

Material and Methods

Materials and Instrumentation

Kapok leaves (*Ceiba pentandra* (L.) Gaertn.) were obtained from Mr. Sukimin's yard located in Dusun Krenggan RT.02/RW.04, Ngebong Village, Pakel sub-district, Tulungagung district, East Java and 70% ethanol solvent (ONEMED) for extract preparation. Mayer reagent, hot water, hydrochloric acid (HCl) (EMSURE®), Magnesium (Mg), 70% ethanol (ABSOLUTE), H2SO4 (EMSURE®), 10% $FeCl_3$, Acetone (EMSURE®), were used for phytochemical screening. For serum preparation, Natrosol, glycerin, DMDM Hydantion, DMSO were provided. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) powder was used for antioxidant test. In addition, we also prepared analytical balance (GOTO), blender (Philips), sieve number 80, simplisia container, maceration bottle, filter paper, hot plate (MASPION S-301), glassware (PYREX®), tube rack, dropper pipette, measuring pipette, analytical balance, parchment paper, mortar and stamper, mixer, waterbath (MEMMERT), stirring rod (PYREX®), pH universal (MACHEREY-NAGEL), viscotester (VT-04F Rion Co. , Ltd.), UV-Vis Spectrophotometer (Thermo-Scientific, Singapore), Rotary

Evaporator (Heidolph Laborata, China), glass object, adhesion tester, and tissue**.**

Preparation *Ceiba pentandra* Leaves Extract

The extraction sample used was kapok leaves (*Ceiba pentandra* (L.) Gaertn.). Samples were taken using a random sampling method by taking kapok leaves around Ngebong village, Pakel sub-district, Tulungagung district, East Java. Furthermore, the samples were sorted, washed, sharpened, dried with an oven of no more than 60°C, dry sorting, then sieved with sieve no. 80 until the sifted powder was exhausted to get the desired simplisia [14]. Simplia standardization test includes dyring shrinkage test and simplisia.

Extraction of Kapok Leaves and Phytochemical Screening

The extraction process of kapok leaves (*Ceiba pentandra* (L.)) is carried out using maceration, namely 500 grams of powder soaked with 3750 ml of 70% ethanol at room temperature for 5 days and occasionally cornered then the filtrate obtained is re-macerated and filtered using filter paper and replacement of new solvents[15]. All filtrates were then concentrated with a rotary evaporator until a thick extract of kapok leaves was obtained[5]. Calculate the obtained yield, the percentage of weight (b/b) between the yield and the weight of the simplisia powder used by weighing[16]. Phytochemical screening tests in this study include tannin, flavonoid, saponin and alkaloid tests.

Antioxidant Activities

Preparation of DPPH Solution: DPPH (standard) solution with different concentrations of 40 ppm DPPH up to 100 ppm. Determining the Optimum Wavelength of DPPH Solution: Determining the maximum wavelength of DPPH is conducted by pipetting as much as 2 mL of 40 ppm DPPH solution, put into a vial bottle that has been wrapped in aluminum foil, and added 2 mL of 70% ethanol, shaking and then allowed to stand for 30 minutes. Then, it was put into a cuvette and observed for absorbance at a wavelength of 400-800 nm. The highest wavelength was determined as the maximum wavelength[17].

Antioxidant Activity of Kapok Leaves Extract and Vitamin C. Antioxidant activity was determined from the IC_{50} value calculated using the DPPH method with a UV-Vis spectrophotometer. The viscous extract of kapok leaves (*Ceiba pentandra* (L). Gaertn) was made in 500 ppm stock solution, then made dilutions with 3 concentration series, namely 20 ppm, 40 ppm, and 60 ppm. Vitamin C 40,000 ppm (200mg/5ml) was made dilution of 3 concentration series namely 2 ppm, 3 ppm, and 4 ppm. Testing was done by pipetting 0.5 ml of sample solution from various concentrations. Then each was added 3.5 ml of 50 ppm DPPH in a closed test tube and allowed to stand for 30 minutes. The solution was homogenized and the activity absorbance was read using a UV-Vis spectrophotometer at the optimum wavelength. The absorbance results were used to calculate the percent of free radical silencing and then entered into the equation obtained from the linear regression curve to obtain the IC_{50} value [18].

Determining the Percentage of Antioxidant Activity and IC_{50} Value. The IC_{50} value can be calculated based on the percentage of silencing between the DPPH radical and the sample solution using the equation:

$$\% \text{ Inhibition} = \frac{(\text{A Blanko} - \text{A Sample}) \times 100\%}{\text{A Blanko}} \quad \ldots\ldots (1)$$

Description:

A Blanko = absorbance of DPPH radical absorption (blank) at wavelength.

A Sample = absorbance of sample uptake in DPPH radical at maximum wavelength.

The IC_{50} of each sample concentration was calculated using the linear regression equation formula. Sample concentration as x-axis and % inhibition as y-axis. From the equation:

$$Y = a + bx \quad \ldots\ldots (2)$$

To determine the IC_{50} value, it can be calculated using the formula:

$$IC_{50} = (50 - a)/ b \quad \ldots\ldots (3)$$

The parameter used for measuring antioxidant activity is the I_{C50} (Inhibition Concentration 50%) value, which is the concentration of the sample that can reduce the DPPH radical by 50%. The IC_{50} value is obtained from the results of inhibition

and concentration entered into the Microsoft Excel 2016 application [19].

Moisturizer Formulation

The standard formula used in this study was modified from formula[15]. The standard formulation can be seen in Table 1 and the modified formulation can be seen in Table 2.

Table 1. Standard formulation of moisturizer preparation[15]

Ingredients	Formulation (%)					
	F0	**F1**	**F2**	**F3**	**F4**	**F5**
Shallot Skin Extract	-	2	4	6	8	10
Carbopol	2	1	1	1	1	1
Glycerin	5	5	5	5	5	5
Propylen glycol	10	10	10	10	10	10
TEA	1	1	1	1	1	1
Methyl Paraben	0.1	0.1	0,1	0,1	0,1	0,1
Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Table 2. Modified formulation of moisturizer preparation

Ingredients	Formulation (%)					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Extract	0.02	0.04	0,06	-	-	-
Vitamin C	-	-	-	0,002	0,003	0,004
Carbopol	1	1	1	1	1	1
Glycerin	5	5	5	5	5	5
Propylene glycol	10	10	10	10	10	10
TEA	1	1	1	1	1	1
DMSO	7	7	7	7	7	7
Methyl Paraben	0.1	0.1	0.1	0.1	01	0.1
Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Description:
F1 : Formulation 1 with 0.02% extract concentration (20 ppm)
F2 : Formulation 2 with extract concentration 0.04% (40 ppm)
F3 : Formulation 3 with 0.06% extract concentration (60 ppm)
F4 : Formulation 4 with vitamin C concentration of 0.002% (2 ppm) as the positive control.
F5 : Formulation 5 with vitamin C concentration 0.003% (3 ppm) as positive control.
F6 : Formulation 6 with vitamin C concentration of 0.004% (4 ppm) as the positive control.
*The addition of the amount of kapok leaf extract solution and vitamin C was determined after the IC50 x 100 value was obtained.

Moisturizer Preparation

Based on the preparation of moisturizer gel[15]. The preparation of kapok leaf extract moisturizer gel (*Ceiba pentandra (L.)*) was carried out by weighing each ingredient first such as Carbopol, Glycerin, Propylene glycol, TEA, DMSO, Methyl Paraben and Aquadest. In (Mortar 1) carbopol is developed with distilled water in a mortar. After expanding, grind it first by adding TEA little by little to form a gel base. In (Mortar 2) DMSO and Methyl Paraben are dissolved in glycerin, stirred until dissolved. In (Mortar 3) Kapok leaf extract is crushed by adding some propylenglycol until the texture becomes soft and homogeneous [20].
A mixture of DMSO, Methyl paraben and glycerin (Mortar 2) was added to the gel base (Mortar 1).

The remaining propylene glycol was added to the base mixture (Mortar 1), grinded until homogeneous. Next, the kapok leaf extract (Mortar 3) was mixed into the gel base mixture (Mortar 1) and crushed until homogeneous. Add the remaining distilled water little by little. The gel that has been formed is evaluated for gel preparation[20].

Preparation of Moisturizer

Moisturizer from kapok leaf extract was weighed 2.5 grams and then put into a test tube. In the test tube 5 ml of ethanol was added then the tube was covered with black plastic. Shake the tube until the solution is homogeneous. Separate the solution by centrifuge for 10 minutes, filter until a clear filtrate is obtained [21].

Physical Quality of Moisturizer Preparations

The physical quality test of moisturizer preparation includes organoleptic test, homogeneity test, pH test, spreadability test and adhesion test[21].

Antioxidant Activity

The prepared sample was taken 3 ml and put into a 10 ml volumetric flask, 2 ml DPPH solution and 10 ml ethanol were added to the flask. Let it sit in a dark place for 30 minutes. Then the absorbance of the sample was read with a UV-Vis spectrophotometer at the optimum wavelength obtained[21]. The IC_{50} value can be calculated based on the damping percentage between DPPH radicals and the sample solution.

Results and Discussions

Drying Shinkage

Drying shrinkage testing in this study was carried out by gravimetric method. In the test, it was found that the percentage of drying shrinkage of kapok leaves (*Ceiba pentandra (L.) Gaertn*.) was 8.4%. It can be concluded that these results are in accordance with the drying shrinkage test reference for kapok leaf powder according to the Indonesian Herbal Pharmacopoeia, which is no more than 10%.

Simplisia Moisture Content

Good simplisia is in a dry condition where the water content does not exceed 10%. The water content test results obtained were 3.1%. The results obtained indicate that the simplisia used has met the predetermined requirements[14].

Extract Yield and Phytochemical Screening

The yield is the total weight of secondary metabolite compounds that have been extracted from a sample. The results showed that the yield of kapok leaf extract was 48.73%. This result meets the requirements of the Indonesian Herbal Pharmacopoeia, which is a yield of no less than 7.2%. The test results from the phytochemical screening of kapok leaves extract show that kapok leaves contain secondary metabolite compounds such as flavonoids, alkaloids, saponins and tannins.

Antioxidant Activity Test with DPPH

The results of the optimum wavelength of DPPH can be seen in Figure 1. The optimum wavelength of DPPH solution is 505 nm with an absorbance of 0.510. The results can be used for percentage inhibition and determination of IC_{50} using linear regression.

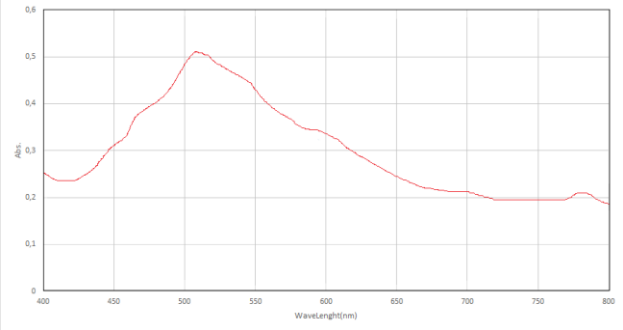
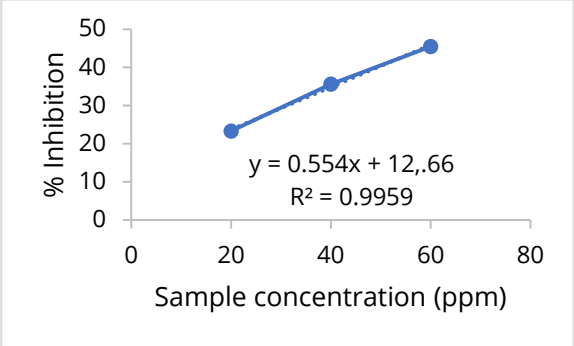


Figure 1. Absorbance spectrum of DPPH solution

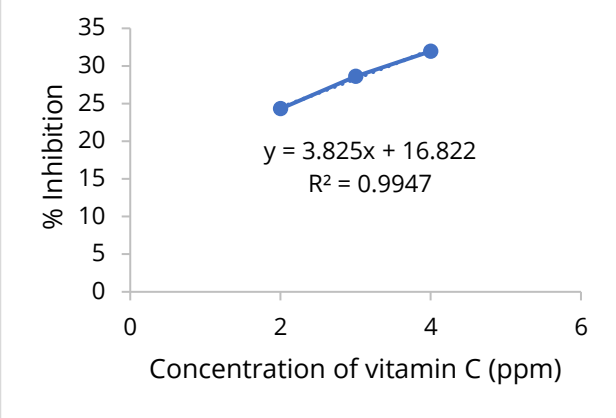
Antioxidant Activity

The results of the antioxidant activity test of kapok leaf extract and vitamin C can be seen in Table 3, which shows that vitamin C as a positive control antioxidant has an IC_{50} value of 8.67 ppm which is classified as very strong, while the IC_{50} value of kapok leaf extract is 67.40 ppm which is classified as strong. Somehow, these IC_{50} results,

kapok leaf extract has the potential as an antioxidant, which is then formulated in the form of gel preparations



(A)



(B)

Figure 2. Relationship curve between concentration of active ingredient and % inhibition. (A) Antioxidant activity of kapok leaf extract; (B) Antioxidant activity of vitamin C.

Table 3. Antioxidant test data of kapok leaves extract and vitamin C.

Sample	Sample Concentration (ppm)	Absorbance Average	% Inhibition	IC_{50} (ppm)
Kapok Leaves Extract	20	0.391	23.33 %	67.40007 ppm
	40	0.328	35.68 %	
	60	0.278	45.49 %	
Vitamin C	2	0.386	24.31 %	8.673 ppm
	3	0.364	28.62 %	
	4	0.347	31.96 %	

Quercetin Calibration Curve

In measuring the absorbance of total flavonoids for the determination of the calibration curve of quercetin at a wavelength of 450 nm, a linear regression equation is obtained in Figure 2, namely y = 0.001x + 0.1137. In the standard solution, a linear relationship was obtained between absorbance and concentration with a correlation coefficient value of 0.9904 where the value (R^2) which is close to 1 indicates that the regression equation is linear.

From the calibration curve equation in Figure 3, it can be used as a comparison to determine the flavonoid content in kapok leaf extract, by calculating the X value as the flavonoid content sought and the Y value of the absorbance of the kapok leaf extract of each concentration.

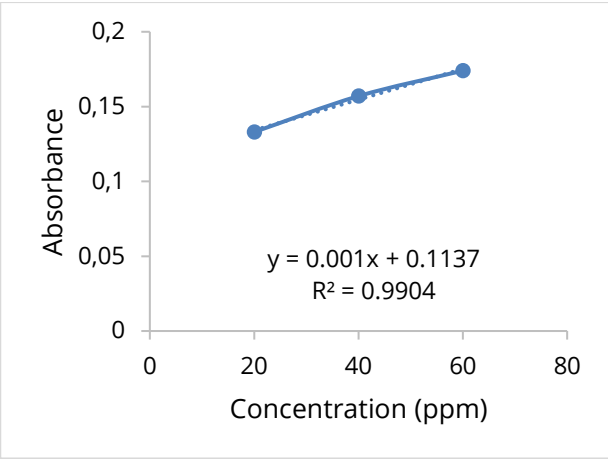
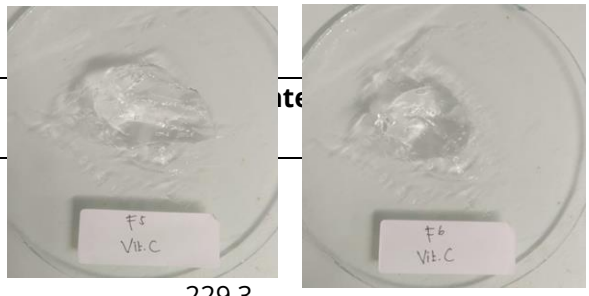


Figure 3. Linearity curve of quercetin concentration with its absorbance

It can be seen in Table 4 the results of each flavonoid content value of kapok leaf extract, where the optimum concentration is obtained at 60 ppm extract concentration with 229.3 µg/ml.

Table 4. Flavonoid content of kapok leaves extract

Sample Concentration	Absorbance Value
20 ppm	0.130
40 ppm	0.238
60 ppm	0.343

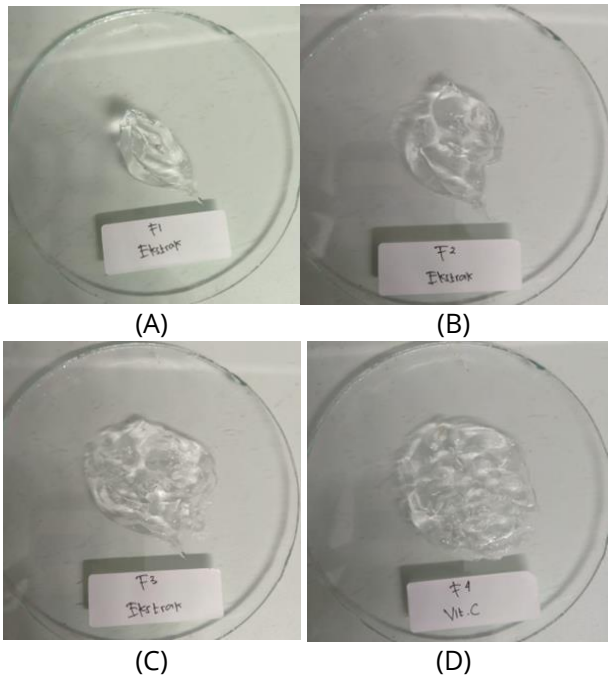


(E) 229,3 (F)

Figure 4. Physical appearance of moisturizer gel preparations. (A) F1 = Extract concentration 0.02%; (B) F2 = Extract concentration 0.04%; (C) F3 = Extract concentration 0.06%; (D) F4 = Vitamin C concentration 0.002%; (E) F5 = Vitamin C concentration 0.003%; (F) F6 = Vitamin C concentration 0.004%.

Physical Quality Test of Gel Preparations.

Organoleptic Test: The results obtained are that from the six preparations there are no significant changes; all formulations have a form consistency that is semi-solid and clear in color; the preparation does not have a significant aroma.



(A) (B)

(C) (D)

The pH test: The state of pH must be set in such a way that it does not interfere with the function of cell membranes and does not irritate the skin [22]. The pH value of the preparation that must be met is pH 4.5-6.5 (normal pH of the skin).

Homogeneity Test: The results of the homogeneity test on the gel preparation are homogeneous.

Spreadability Test: The requirements for a qualified spreadability test are 5-7 cm[22]. The results of the spreadability test on gel preparations can be seen in Table 6.

Adhesion Test: The requirement for adhesion is more than one second [23]. The results of the adhesion test of the gel preparation can be seen in Table 7.

Viscosity: The good viscosity value of the gel is 200 to 400 dPa-s[23]. The average results of the viscosity test on each gel preparation were 300 ± 0 dPas.

Table 5. Data from pH test of moisturizer gel preparation

Active Ingredient	Formulation	Concentration (%)	Average (pH) (Mean ± SD)
Kapok Leaves Extract	F1	0.02	5.7 ± 0.01
	F2	0.04	5.7 ± 0.01
	F3	0.06	5.7 ± 0.02
Vitamin C	F4	0.002	4.9 ± 0.02
	F5	0.003	4.9 ± 0.03
	F6	0.004	4.8 ± 0.01

Table 6. Data on the results of the spreadability test of moisturizer gel preparations

Active Ingredients	Formulation	Load (Mean ± SD)				
		0g	50g	100g	150g	200g
Kapok Leaves Extract	F1	5.1 ± 0.02	5.5 ± 0.01	6.2 ± 0.01	6.5 ± 0.05	6.6 ± 0.17
	F2	5.1 ± 0.02	5.6 ± 0.02	6.2 ± 0.04	6.4 ± 0.02	6.6 ± 0.02
	F3	5.2 ± 0.01	5.6 ± 0.02	6.2 ± 0.02	6.4 ± 0.17	6.7 ± 0.02
Vitamin C	F4	5.1 ± 0.17	5.5 ± 0.05	6.1 ± 0.02	6.3 ± 0.02	6.7 ± 0.17
	F5	5.0 ± 0.05	5.6 ± 0.01	6.1 ± 0.17	6.4 ± 0.01	6.6 ± 0.05
	F6	5,1 ± 0,05	5.6 ± 0.17	6.0 ± 0.07	6.4 ± 0.04	6.6 ± 0.05

Table 7. Adhesion test result data of moisturizer gel preparation

Active Ingredients	Formulation	Concentration (%)	Result (in Second) (Average ± SD)
Kapok Leaves Extract	F1	0.02	4.9 ± 0.01
	F2	0.04	4.8 ± 0.02
	F3	0.06	4.6 ± 0.04
Vitamin C	F4	0.002	4.8 ± 0.03
	F5	0.003	4.7 ± 0.04
	F6	0.004	4.7 ± 0.05

Antioxidant Effectiveness Test. The results of the antioxidant activity test of kapok leaves extract gel preparations can be seen in Table 8. Vitamin C preparations that serve as a comparison have very strong antioxidant activity levels because they have an IC_{50} value of <50 ppm, which is 9.18417 ppm. The antioxidant activity test of kapok leaf extract gel preparations in Table 8 is included in the moderate antioxidant category because it has an IC_{50} value of almost close to 150 ppm, which is 110.065 ppm.

Based on the results of antioxidant activity in Table 4 and Table 8, it is known that there is a decrease in the IC_{50} value of kapok leaf extract and vitamin C before and after gel preparation. The IC_{50} value of the preparation is higher than the IC_{50} value of the extract which means that the antioxidant effectiveness decreases after being made into a dosage form.

Table 8. Antioxidant activity test data of moisturizer gel

Sample	Sample Concentration (%)	Average Absorbance	% Inhibition	IC_{50} (ppm)
Kapok Leaf Extract	0.02	0.413	19.02 %	110.065 ppm
	0.04	0.372	27.06 %	
	0.04	0.349	31.57 %	
Vitamin C	0.002	0.402	21.78 %	9.8417 ppm
	0.003	0.383	24.9 %	
	0.004	0.362	29.02 %	

This study is in accordance with[20], the IC_{50} value of the preparation is higher than the IC_{50} value of the extract which means that the antioxidant effectiveness decreases after being made into a dosage form. An ingredient that may decrease antioxidant activity is Carbopol. Carbopol is a strong gel base and has high acidity[24]. Increased acidity can affect antioxidant activity by making phenolic compounds more stable and difficult to release protons that can bind to DPPH, antioxidant activity will decrease.

To overcome the decrease in antioxidant effects, there are several suggestions from researchers, one of which is to add additional substances such as BHT (Butylated Hydroxytoluene) which is a synthetic antioxidant that can maintain the overall quality of ingredients by protecting active substances, slowing down damage, rancidity, and discoloration caused by oxidation which can be used to maintain antioxidant effects if the antioxidant effect decreases after becoming a preparation[25]. Nevertheless, the kapok leaf extract moisturizer gel preparation produced still has antioxidant activity which is included in the moderate category.

Conclusions

Based on the results of antioxidant activity level test data, kapok leaf extract has strong enough activity with the acquisition of IC_{50} of 67.4007 ppm which is classified as strong. Physical quality tests of moisturizer preparations, namely organoleptics, pH, homogeneity, spreadability, adhesion, specific gravity, and viscosity of moisturizer preparations have met the requirements at concentrations of 20 ppm, 40 ppm and 60 ppm. The results of the antioxidant test of kapok leaves extract moisturizer preparation, the preparation has good effectiveness at the highest concentration of 60 ppm with an IC_{50} of 110.065 ppm which is classified as moderate.

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Author Contibutions

Conceptualization, A.M, D.P.T; Methodology, A.M; Validation, A.M, A.E.P; Formal Analysis, A.M, A.E.P, D.P.T; Investigation, M.S.S; Data Curation, M.S.S; Writing–Original Draft Preparation, A.M; Writing – Review & Editing, A.M, D.P.T; Visualization, M.S.S; Supervision, A.M, A.E.P

Conflict of Interest

There are no significant conflicts

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