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ANTIOXIDANT ACTIVITY TEST OF BAOK FRUIT EXTRACT (*Limonia acidissima*) AS AN ADDITIONAL INGREDIENT IN MASK PREPARATIONS

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ABSTRACT

Facial masks are a popular skincare product, and natural ingredients are increasingly in demand due to their safety. Batok fruit (*Limonia acidissima*) is known to be rich in bioactive compounds such as flavonoids and polyphenols, which have the potential as antioxidants to fight free radicals that cause skin damage and premature aging. This study aims to test the antioxidant activity of batok fruit ethanol extract as a basis for developing additional ingredients in mask preparations. This study is an experimental study that begins with the preparation of simplex from batok fruit flesh. Extraction was carried out by maceration method using 96% ethanol solvent for 3x24 hours. The obtained thick extract was then tested for antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method with Vitamin C as a comparator. Determination of antioxidant activity was carried out by measuring the extract's inhibition value against DPPH free radicals using a UV-Vis spectrophotometer to obtain the IC_{50} value. The results of the characterization of simplex showed that batok fruit powder met the quality standards with a water content of 8.2%. The extraction process of 200 grams of simplicia produced a thick extract of 13.8 grams with a yield of 6.9%. The results of the antioxidant activity test showed that the ethanol extract of batok fruit has a very strong free radical scavenging ability, with an IC_{50} value of 13.2 ppm. Although this value is not as strong as the comparison of Vitamin C (IC_{50} 3.57 ppm), these results classify batok fruit extract as a very strong antioxidant (IC_{50} < 50 ppm). Thus, batok fruit extract has great potential to be developed as a natural active ingredient in facial mask formulations to protect the skin from oxidative damage.

KEYWORDS

Antioxidants, Batok Fruit, DPPH, Extract, Facial Mask

INTRODUCTION

Facial masks are a cosmetic product widely used for skin care. One natural ingredient with potential as an active ingredient in mask preparations is the batok fruit (*Limonia acidissima*). This fruit is known to contain bioactive compounds that can provide health benefits, including antioxidant activity (Fillah, 2023). Batok fruit is known to contain various bioactive compounds that are beneficial for health, including the ability to fight free radicals. Free radicals are molecules that can damage body cells and contribute to aging and various skin diseases (Ahsanuddin, Lam and Baron, 2016). Research by Bagul *et al*., (2019) showed that batok fruit extract contains flavonoids and polyphenols, both of which have significant antioxidant activity. Flavonoids, in particular, have been shown to protect skin cells from damage caused by UV rays and environmental pollution. This suggests that batok fruit can be an effective natural ingredient in skin care products.

Antioxidant activity is important for protecting the skin from free radical damage, which can lead to premature aging and various other skin problems (Wulantresna *et al.*, 2024). According to Packer *et al*., (1995), antioxidants play a crucial role in reducing oxidative damage caused by free radicals, thus helping maintain healthy skin.

A mask formulation using coconut fruit extract offers an innovative approach to skin care. Facial masks not only provide cosmetic benefits but can also improve overall skin health. According to research by Adhayanti *et al*., (2022), using a mask based on natural ingredients can increase skin hydration and reduce signs of aging. Research by Sari dan Indrayani (2020) indicates that coconut fruit extract contains

flavonoids and polyphenols, which have significant antioxidant activity. This activity is important in protecting skin cells from free radical damage.

Based on the above background, the researchers were interested in conducting a study entitled "Antioxidant Activity Test of Coconut Fruit Extract (*Limonia acidissima*) as an Additive in Mask Preparations".

MATERIALS AND METHODS

Research Design

This research falls into the experimental research category, where various parameters are evaluated to determine the effectiveness and safety of the resulting preparation (Rashid, 2022).

Research Location and Schedule

This research was conducted in the Chemistry Education Laboratory of the Faculty of Teacher Training and Education, USK, for extract preparation and in the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, USK, for antioxidant testing from June to July 2025.

Materials

The materials used in this study were coconut shell fruit, 96% ethanol, 10% NaOH solution, FeCl3, Wagner's reagent, Mayer's reagent, distilled water, DPPH powder, and vitamin C powder.

Equipment

The equipment used in this study included a UV-Vis spectrophotometer, rotary evaporator, analytical balance, blender, porcelain dish, Pyrex test tube, Pyrex volumetric flask, glass jar, test tube rack, volumetric pipette, dropper, aluminum foil, filter paper, large knife, horn spoon, spatula, and hand scoop.

Research Stages

1. Preparation of Simplisia
   Batok fruit obtained from Aceh Besar was gutted and dried in the sun. Once dry, it was ground using a blender and placed in a sealed container.
2. Simplisia Characteristics Test
   This test was conducted organoleptically, involving the use of the five senses to observe the form (powder, solid, liquid, thick), odor, color, and taste (Ministry of Health of the Republic of Indonesia,, 2000).
3. Material Preparation
   200 grams of batok fruit powder was taken and weighed.
4. Extraction
   Extraction was carried out using the maceration method using 96% ethanol. The batok fruit powder was soaked in 96% ethanol for 3 x 24 hours with occasional stirring to ensure homogeneity. The solution was then filtered three times, and the filtrates from the first through third filters were collected. The filtrate was then collected and evaporated using a rotary vacuum evaporator to obtain a thick extract.
5. Yield Calculation

Yield is calculated using the formula:

$$\% \text{ Yield} = \frac{\text{extract weight}}{\text{simplisia weight}} \text{ x } 100\%$$

Antioxidant Test

1. Reagent Preparation
   a. Preparation of 50 ppm DPPH stock solution
      5 mg of DPPH powder was taken and weighed, then dissolved in a 100 mL volumetric flask of standard methanol (Douw dan Wardani, 2023).
   b. Preparation of 1000 ppm vitamin C stock solution
      100 mg of vitamin C was weighed and dissolved in a 100 mL volumetric flask of standard methanol (Douw dan Wardani, 2023).
   c. Preparation of sample stock solution
      10 mg of sample was weighed, then dissolved in 10 mL of standard methanol, then stirred until homogeneous (Douw dan Wardani, 2023).

2. IC_{50} Determination
   a. Blank Absorbance Measurement
      A 3 mL DPPH stock solution was taken and its wavelength was measured using a UV-Vis spectrophotometer at a wavelength of 400-800 nm (Douw dan Wardani, 2023).
   b. Operating Time Determination
      A 3.0 mL 0.1 mM DPPH blank solution was added to 1.0 mL of vitamin C. The mixture was then homogenized for 1 minute using a vortex, followed by absorbance measurements every 5 minutes at a wavelength of 515.8 nm for 60 minutes (Rejeki *et al.,* 2024).
   c. DPPH Binding Activity Measurement with Vitamin C Powder

Each solution was prepared in five concentration series: 2, 4, 6, 8, and 10 ppm from the vitamin C stock solution. Pro-analyze methanol was then added to each solution to a total volume of 10 mL. 1 mL of the serial solution was taken and 2 mL of the DPPH stock solution was added. The solution was then incubated for 30 minutes, and the sample absorbance was measured with a UV-Vis spectrophotometer at a maximum wavelength of 515.8 nm (Douw dan Wardani, 2023).

d. Measurement of DPPH binding activity with samples and preparations

Each solution was prepared with five series of concentrations: 5, 10, 25, 50, and 100 ppm. To the sample stock solution, methanol was added p.a. to a volume of 10 mL. From this solution, 1 mL of the solution was taken and 2 mL of the DPPH stock solution was added. The solution was incubated for 17 minutes, and the sample absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 516 nm (Douw dan Wardani, 2023).

The percentage inhibition was calculated using the formula (Prasetyo, Kiromah and Rahayu, 2021):

$$\% \text{ Inhibition} = \frac{\text{Control Abs} - \text{Sample Abs}}{Control\ Abs} \text{ x } 100$$

The IC_{50} value was determined through linear regression between the percentage inhibition and sample concentration.

RESULTS

Results of Preparation and Characterization of Simple Drugs

Simple drugs were made from coconut pulp obtained from Aceh Besar, which was then dried and ground into powder. Characterization of the simple drugs was carried out to ensure the quality and standards of the raw materials used. The characterization results are presented in Table 1.

Table 1 Characterization Results of Coconut Fruit Simplex

Parameters Organoleptic	Standard Observation
Form	Fine powder
Color	Light brown
Odor	Distinctive aromatic, no musty odor
Taste	Slightly sour and astringent

Extraction was carried out using the maceration method using 96% ethanol solvent on 200 grams of coconut shell fruit powder. The resulting thick extract was then calculated for its yield.

Initial Weight of Simplex: 200 grams
Weight of Thick Extract: 13.8 grams
Extract Yield:

$$\% \text{ Yield} = \frac{\text{extract weight}}{\text{simplisia weight}} \text{ x } 100\%$$

$$\% \text{ yield} = \frac{13{,}8}{200} \text{ X } 100\%$$

% yield = 6,9 %

The resulting extract has a thick, blackish brown color and a distinctive aromatic odor.

Antioxidant Activity Test Results

Antioxidant activity testing was conducted using the DPPH method using a UV-Vis spectrophotometer. Vitamin C was used as a positive control.

1. Maximum Wavelength (λmax): The absorbance measurement of a 50 ppm DPPH solution yielded a maximum wavelength at 516 nm.
2. Operating Time: The stable reaction time between the antioxidant and DPPH was determined to be 17 minutes, after which there was no significant decrease in absorbance.
3. Absorbance and Percent Inhibition Measurement: Absorbance was measured for each concentration of the test solution (batok fruit extract) and the reference (Vitamin C). Percent inhibition was calculated based on the decrease in absorbance. The results are presented in Table 2.

Table 2 Results of Absorbance and Percent Inhibition Measurements

Sample	Concentration (ppm)	Rata-rata Absorbansi	% Inhibition
Batok Fruit Extract	50	0,164	78,66%
	40	0,228	70,39%
	30	0,307	60,09%
	20	0,340	55,89%
	10	0,399	48,18%

Calculation of IC_{50} Value: The IC_{50} value is determined from the linear regression equation between concentration (x-axis) and percent inhibition (y-axis). The calculation of the % inhibition can be seen as follows:

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

Description

Control Absorbance: The absorbance value of the DPPH solution alone (blank).

: 0.77

Sample Absorbance: The absorbance value of the mixture of DPPH and the test sample.

For example, for a concentration of 50: 0.164 and so on.

$$\% \text{ Inhibition} = \frac{0,77 - 0,164}{0,77} \times 100$$

$$= 78.658$$

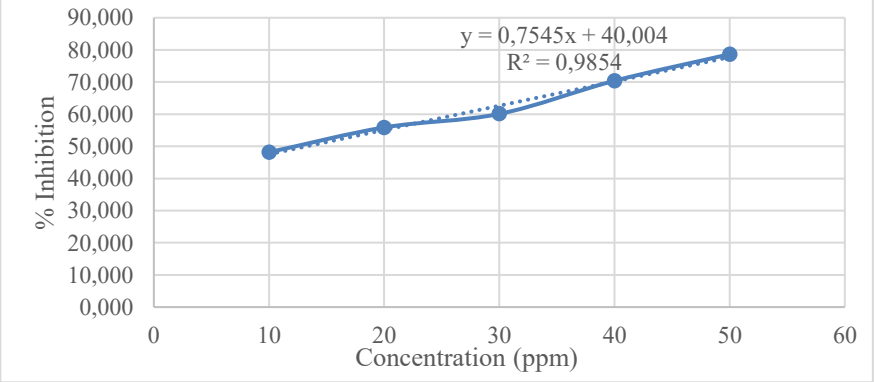

Figure 1. Graph of % inhibition in coconut fruit

Regression equation:
$y = 0.7545x + 40.004$
$R2 = 0.9854$
$IC_{50}(x) = \frac{50-c}{m}$
$IC_{50} = \frac{50-40,004}{0,7545}$
$IC_{50} = 13.25$ ppm

The research results showed that the IC_{50} value for the ethanol extract of coconut fruit was 13.25 ppm. The IC_{50} value indicates the concentration required to inhibit 50% of DPPH free radical activity. The lower the IC_{50} value, the stronger the antioxidant activity. For comparison, Vitamin C was used, which has an IC_{50} value of 3.57 ppm. Although the antioxidant activity of batok fruit extract is not as strong as pure Vitamin C, the IC_{50} value of 13.25 ppm indicates that this extract is included in the category of very strong antioxidants ($IC_{50} < 50$ ppm). This antioxidant power is supported by the content of bioactive compounds such as flavonoids and polyphenols in batok fruit, which are known to have the ability to fight free radical.

DISCUSSION

This study successfully tested the antioxidant activity of batok fruit extract (*Limonia acidissima*) as a first step in developing additives for face masks. Characterization of the medicinal plant (simple compound) indicated that the batok fruit powder met quality standards. Its water content of 8.2% is below the maximum threshold of 10% according to the Indonesian Herbal Pharmacopoeia. Distinctive organoleptic parameters also confirmed the authenticity and quality of the raw materials used. The extraction process, using a maceration method using 96% ethanol, resulted in a yield of 13.8%. Ethanol was chosen due to its universal properties and its ability to attract polar and semipolar compounds, including flavonoids and phenolics, which are suspected to act as antioxidants. This yield value indicates the amount of compounds successfully extracted from the medicinal plant and can vary depending on factors such as the source of the material, harvest time, and extraction method used.

Antioxidant activity testing using the DPPH method demonstrated that the ethanol extract of batok fruit has the ability to scavenge free radicals. The principle of this method is based on the color change of DPPH from purple to pale yellow after receiving a hydrogen atom from an antioxidant compound, which is measured as a decrease in absorbance. Antioxidant activity is expressed by the IC_{50} value, which is the concentration required to inhibit 50% of the activity of DPPH free radicals. The lower the IC_{50} value, the stronger the antioxidant activity.

The research results showed that the IC_{50} value of batok fruit extract was 13.25 ppm, while the reference vitamin C had an IC_{50} of 3.57 ppm. Based on the antioxidant strength category, an IC_{50} value below 50 ppm can be classified as a very strong antioxidant. Although its activity is not as strong as that of pure vitamin C, these results are very promising. An IC_{50} value in the strong category indicates that batok fruit extract has significant potential to fight free radicals. Free radicals are one of the main triggers of skin cell damage and premature aging. The following are antioxidant categories according to (Gane and Parkins, 1958).

Table 3 Antioxidant Categories

Category	IC_{50} Value (µg/mL or ppm)
Very Strong	< 50
Strong	50 - 100
Medium	100 - 150
Weak	150 - 200
Very Weak	> 200

Overall, the results of this study confirm that the ethanol extract of batok fruit (*Limonia acidissima*) has strong antioxidant activity. This activity is supported by the presence of secondary metabolites such as flavonoids and phenolics. Therefore, batok fruit extract has great potential for further development as a natural active ingredient in cosmetic formulations, particularly facial masks, aimed at protecting the skin from oxidative damage.

CONCLUSIONS

Ethanol extract of batok fruit (*Limonia acidissima*) has very strong antioxidant activity. This is evidenced by the IC50 value obtained of 13.25 ppm in testing using the DPPH method. This value indicates that batok fruit extract has significant potential to reduce free radicals. Thus, batok fruit extract has great potential for further development as a natural active ingredient in cosmetic formulations, particularly facial masks aimed at protecting the skin from oxidative damage.

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