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Antioxidant Activity of *Theobroma cacao* L. Husk Ethyl Acetate Fraction in Peel-Off Mask Formulation Measured by the DPPH Assay

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Abstract

Cocoa fruit (*Theobroma cacao*) is one of Indonesia's most important commodities and is widely used as a raw material in various industries. Until now, cocoa fruit has primarily been used for its seeds, while its skin has not been widely explored. This study aims to formulate a peel-off mask from cacao husk and determine its antioxidant activity. The antioxidant test method used was the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Analysis results showed that the ethyl acetate fraction contained secondary metabolites, including flavonoids, tannins, and steroids. However, the peel-off mask formulation made with a combination of the ethyl acetate fraction of cocoa husk and polyvinyl alcohol was unstable due to changes in its characteristics during storage. Nevertheless, the resulting peel-off mask exhibited relatively strong antioxidant activity, with IC_{50} values of 6.819 ppm for the ethyl acetate fraction and 11.596 ppm for the mask. These results suggest that cocoa husk contains bioactive compounds that support strong antioxidant activity. Therefore, cocoa husk has great potential as an active ingredient in cosmetic formulations. However, further formula optimization is needed to improve the formulation's stability while maintaining optimal antioxidant activity.

1. Introduction

The skin is the largest organ in the human body, serving as the body's primary protector against various external factors, including ultraviolet (UV) exposure, pollution, microorganisms, and harmful chemicals. However, as age increases, the skin undergoes an aging process, both naturally (chronological aging) and due to environmental and lifestyle factors [1].

One of the primary mechanisms that can accelerate skin aging is oxidative stress, a condition in which an excessive number of free radicals or reactive oxygen species (ROS) are produced, surpassing the body's antioxidant defense capacity. ROS can damage the structure of DNA, proteins, and lipids, as well as disrupt collagen networks and skin elasticity. Such damage may lead to loss of elasticity, the appearance of wrinkles, hyperpigmentation, and signs of premature aging. To neutralize free radicals, the skin requires the support of antioxidants [1–3]. Naturally, the body has an endogenous defense system, comprising antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [4]. However,

when ROS production increases excessively, the body's antioxidant system alone is insufficient to neutralize them. Therefore, additional antioxidants are needed, either from nutritional intake or through topical application in skincare products. With growing public awareness and increasing research evidence regarding the dangers of synthetic substances, the use of natural antioxidants from plants has been expanding in the pharmaceutical industry. Various phytochemicals, including flavonoids, polyphenols, and natural vitamins, are widely studied for their potential to protect the skin from oxidative stress [5, 6].

One plant that has been proven to contain natural antioxidant activity is *Theobroma cacao*. Commonly known as the cocoa plant, *Theobroma cacao* produces fruit throughout the year. Indonesia is the world's third-largest cocoa producer, with an annual production of approximately 450,000 tons [7, 8]. This high production rate makes cocoa one of the most important plantation commodities. However, in its processing, the most commonly used part for industrial raw materials is the seed. Meanwhile, the fruit husk, which accounts for 70–75% of the total fruit weight, is often discarded or used only as animal feed and compost. The accumulation of cocoa husk waste can potentially cause environmental problems, including pollution, unpleasant odors, and the buildup of hard-to-manage organic waste [9]. Therefore, proper management of cocoa husk waste is necessary to minimize its negative environmental impact.

Several studies have demonstrated that cocoa husk contains various bioactive compounds with significant pharmacological potential, including antioxidants, antimicrobials, and anti-inflammatory properties [10–13]. Among these, the antioxidant properties of cocoa husk are particularly important, as they are directly related to preventing oxidative stress. Research by Indrianingsih et al. [11] reported that antioxidant activity tests using the DPPH method showed that methanolic extracts of cocoa husk produced IC_{50} values of around 41.3–44.5 µg/mL. Furthermore, Yahya et al. demonstrated that the ethyl acetate fraction of cocoa husk contained the highest levels of phenolics and flavonoids, showed the strongest antioxidant activity (EC_{50} = 9.61 µg/mL), and exhibited antimicrobial activity while remaining non-toxic. Overall, the evidence indicates that cocoa husk, especially its ethyl acetate fraction, is a sustainable and effective source of bioactive compounds for cosmetic formulations.

One suitable cosmetic dosage form for utilizing cocoa husk as a skin antioxidant is a peel-off mask. Peel-off masks are topical cosmetic preparations in gel form that, once applied, dry to form a thin elastic layer that can be easily peeled off. Peel-off mask formulations using natural ingredients are an innovation increasingly developed in the cosmetic industry due to their practicality, hygiene, and ability to remove dirt and dead skin cells from the skin surface [14, 15]. According to Nemati et al. [14], peel-off mask preparations that combine polyvinyl alcohol (PVA) with plant extracts rich in antioxidants have been shown to produce formulations with desirable physical characteristics, including a skin-compatible pH, stable viscosity, preserved homogeneity, and an ideal drying time. This provides an important foundation for developing peel-off masks from cocoa husk (*Theobroma cacao*) extracts.

However, the use of cocoa husk waste in peel-off mask formulations remains very limited and underdeveloped. Meanwhile, global trends in the cosmetic industry indicate increasing consumer interest in natural-based cosmetics that are safer and more environmentally friendly [5]. In addition, cocoa husk has been shown to contain various compounds with strong antioxidant properties, including flavonoids, tannins, and polyphenols. These compounds enable cocoa husk to act as a radical scavenger, neutralizing free radicals, preventing skin damage, and reducing signs of premature aging [12, 16, 17].

This study assessed antioxidant activity using the DPPH (*2,2-diphenyl-1-picrylhydrazyl*) assay. This assay is favoured for its simplicity, cost-effectiveness, high sensitivity, and minimal sample requirement [18]. Although it does not directly reflect the effects on human skin, the results provide a useful indication of the extract's ability to scavenge free radicals, suggesting its potential protective effect against oxidative damage in topical applications.

Thus, the development of peel-off mask formulations based on cocoa husk fraction represents an innovative alternative that not only provides tangible benefits in skincare but also serves as an environmentally friendly waste utilization strategy. This development also supports the concepts of green technology and circular economy by turning waste into high-value economic products. At the same time, it aligns with the principle of sustainability in the modern cosmetic industry, which increasingly emphasizes the use of natural ingredients that are safe, eco-friendly, and have minimal side effects. This research is expected to provide scientific contributions in the fields of pharmacy and cosmetics.

2. Materials and Methods

2.1. Preparation and Characterization of Simplisia

This study used a sample of cocoa fruit husk taken from Keumala District, Pidie Regency. The sample was

collected using purposive sampling with the criteria of ripe, yellow-colored cocoa fruits. Cut the cocoa fruit transversely and remove the seeds. Collect the cocoa husk, perform wet sorting, and wash with running water. Cut the husk into pieces approximately 1 cm in length, then air-dry at room temperature for 30 days. After 30 days, perform dry sorting on the cocoa husk samples, then grind them into powder using a blender.

The moisture content of cocoa husk simplisia was determined using the gravimetric method. A total of 2 grams of simplisia was placed into a pre-weighed porcelain dish, then oven-dried for 3 hours at 105°C. The dish was then transferred into a desiccator until it reached room temperature, weighed again, and the moisture content was calculated using Equation 1 [19]:

$$MC = \frac{W_i - W_d}{W_d} \times 100\% \quad (1)$$

where W_i is the initial weight and W_d is the after drying weight.

2.2. Ethyl Acetate Fraction Preparation and Characterization

Extraction of cocoa husk simplisia was carried out using the maceration method with sequential solvent fractionation. A total of 2.5 kg of cocoa husk simplisia was placed into a maceration container and soaked with 18.75 L of n-hexane for 5 days under dark conditions with occasional stirring. The filtrate was collected, and the residue was re-soaked with 6.25 L of n-hexane for 2 days. The residue was then soaked with 18.75 L of ethyl acetate for 5 days under dark conditions. After filtration, the residue was soaked again with 6.25 L of ethyl acetate for 2 days. The combined filtrates were concentrated using a rotary evaporator at 50°C.

2.2.1. Moisture Content Determination

The ethyl acetate fraction of cocoa husk was determined by placing 2-5 grams of the fraction in a previously weighed porcelain dish and then heating it in an oven for 3 hours at 105°C. After that, place the porcelain dish in a desiccator until it reaches room temperature, then weigh it. The water content was calculated using Equation 2 [19]:

$$WC = \frac{W_i - W_d}{W_d} \times 100\% \quad (2)$$

2.2.2. Total Ash Content Determination

Two grams of cocoa husk ethyl acetate fraction was placed into a pre-weighed porcelain dish, then ignited at 600°C until the carbon was completely burned off. The dish was cooled in a desiccator and weighed. Total ash content was calculated using Equation 3 [19]:

$$AC = \frac{W_a}{W_b} \times 100\% \quad (3)$$

where W_a is weight after incineration and W_b is the weight before incineration.

2.2.3. Water-Soluble Extract Content Determination

About 2–5 grams of cocoa husk ethyl acetate fraction were placed into a 100 mL volumetric flask and topped with chloroform-saturated water up to the mark. The solution was shaken for 6 hours and allowed to stand for 18 hours. The formed solution was filtered, and 20 mL of filtrate was evaporated at 105°C using a pre-weighed porcelain dish, then weighed. Water-soluble extract content was calculated using Equation 4 [19]:

$$EC = \frac{W_e \times 100}{W_s \times 20} \times 100\% \quad (4)$$

2.2.4. Ethanol-Soluble Extract Content Determination

The procedure for testing ethanol-soluble extracts was nearly identical to the procedure for testing water-soluble extracts. In this test, however, the fraction were placed in a 100 mL measuring flask, and 95% ethanol added up to the mark. The formulas used to calculate ethanol-soluble extract content shown in previous paragraph [19].

2.3. Phytochemical Screening

2.3.1. Alkaloid Test

Weighed 0.5 grams of cocoa husk ethyl acetate fraction, added 1 mL of 2 N HCl and 9 mL of water, then heated in a water bath for 2 minutes, cooled, and filtered. Three drops of filtrate were placed into three test tubes. Each tube was added with two drops of different reagents: Bouchardat's reagent, Dragendorff's reagent, and Mayer's reagent.

The formation of a brown-to-black color precipitate with Bouchardat reagent, orange precipitate with Dragendorff, and white-to-yellow precipitate with Mayer indicated a positive result for alkaloids. Alkaloid presence was confirmed if two positive reactions appeared [20].

2.3.2. Flavonoid Test

Cocoa husk ethyl acetate fraction was placed into a test tube and mixed with 5 drops of ethanol until homogeneous. Magnesium powder and 5 drops of concentrated hydrochloric acid (HCl) were then added. A positive result was indicated by yellow, orange, or red coloration [21].

Table 1. Formulation of the peel-off mask.

Ingredient	Concentration (%)
Cocoa husk ethyl acetate fraction	0.14
PVA (Polyvinyl Alcohol)	10
HPMC (Hydroxypropyl Methylcellulose)	1
Methyl paraben	0.2
Propyl paraben	0.1
Propylene glycol	15
Distilled water	Ad 100

2.3.3. Tannin Test

Approximately 500 mg of cocoa husk ethyl acetate fraction was soaked in distilled water for 15 minutes and then filtered. The filtrate was diluted until almost colorless, and 2 mL of it was placed in a test tube. Two drops of 10% FeCl3 were added, and color change was observed. A positive result was indicated by a green or blue coloration [22].

2.3.4. Saponin Test

Approximately 500 mg of cocoa husk ethyl acetate fraction was dissolved in 20 mL of hot distilled water in a test tube, and the mixture was shaken until a foam formed. Then, 1 drop of 2 N HCl was added. If the foam did not disappear, the sample was positive for saponins [20].

2.3.5. Steroid Test

Two mL of chloroform was added into the cocoa husk ethyl acetate fraction, followed by 0.5 mL of acetic anhydride and 2 mL of concentrated sulfuric acid through the test tube wall. A reddish-brown or purple color indicated triterpenoids, while green indicated steroids [23].

2.4. Peel-Off Mask Formulation

The formulation of peel-off mask was carried out by dissolving PVA (Polyvinyl Alcohol) in hot distilled water at 90°C (mixture I) and HPMC (Hydroxypropyl Methylcellulose) in distilled water (mixture II). Methyl paraben and propyl paraben were dissolved in part of the propylene glycol (mixture III), while the remaining portion was used to dissolve the cocoa husk ethyl acetate fraction. Mixtures II and III were then gradually added to mixture I while stirring until a homogeneous mixture was formed. The ethyl acetate fraction was added incrementally and stirred until a homogeneous mixture was formed.

Finally, distilled water was added up to the required volume, and the mixture was stirred again until a homogeneous peel-off mask preparation was obtained. The formulation of the peel-off mask using the ethyl acetate fraction is presented in Table 1.

2.5. Evaluation of Peel-Off Mask

Each peel-off mask formula was placed into a plastic container, tightly closed, and wrapped with aluminum foil. The container was stored in an oven at 45 ± 2°C for 24 hours and then transferred to a freezer at –5 ± 2°C for an additional 24 hours. This process was counted as one cycle, and the treatment was repeated for 6 cycles (12 days). The stability of the formulation was observed by monitoring changes in shape, color, odor, and phase separation [24].

2.5.1. Organoleptic Test

Organoleptic testing was conducted by observing the shape, color, and odor of the peel-off mask containing cocoa husk ethyl acetate fraction [14].

2.5.2. pH Measurement

pH testing was performed by dissolving 1 gram of the peel-off mask in 100 mL of distilled water, and then measuring the pH using a pH meter calibrated with a neutral buffer (pH 7) and an acidic buffer (pH 4). The electrode was rinsed with distilled water and dried before use. The pH value was recorded once stable readings were obtained [25].

2.5.3. Spreadability Test

The spreadability test was conducted by placing 1 gram of peel-off mask on a round glass plate, covering it with another round glass plate, and leaving it for 1 minute. The diameter of the spread was recorded. A 25-gram weight was then placed on top of the glass plate and left for 1 minute, and the diameter was recorded again. The process was repeated by gradually increasing the load up to a maximum of 125 grams, with the spread diameter recorded at each step [14].

2.5.4. Viscosity Measurement

Viscosity testing was conducted by placing 100 grams of formulation into a beaker, and the spindle and speed of the International Rheology Viscometer were adjusted according to test conditions [14].

2.5.5. Mechanical Properties Test

Mechanical testing was conducted by applying 4 grams of peel-off mask onto a glass plate and leaving it for 24 hours at room temperature until a film layer was formed. The texture analyzer was turned on for 15 minutes before ensure stability. The film was cut into strips approximately 2 × 5 cm in size and clamped onto a tensile

machine. The strip was pulled with a 100 kg load at a crosshead speed of 50 mm/min until it broke. The maximum tensile strength and elongation at break were recorded [26].

2.5.6. Drying Time Test

The drying time test was conducted by applying 1 gram of peel-off mask onto the inner forearm (7 cm in length and width). A stopwatch was used to record the time required for the preparation to dry and form a film layer [27, 28].

2.5.7. Irritation Test

The irritation test was conducted on 24 respondents by applying a peel-off mask to the inner forearm skin (approximately 7 cm in length and width). The preparation was observed for approximately 30 minutes, or until the mask had dried and formed a film layer. Afterward, the mask was peeled off, and the skin was examined for any signs of irritation [14].

The number of respondents was determined in accordance with standard guidelines for cosmetic safety testing. With a 95% confidence level, an expected incidence of mild irritation of 20%, and a margin of error of 15–16%, the calculated sample size was around 24–28 participants. Therefore, using 24 respondents in this study is considered adequate for evaluating the formulation's potential for irritation.

2.6. Antioxidant Activity Assay

Antioxidant activity was assessed using the DPPH method, as described by Thaipong et al. [29] with modifications.

2.6.1. Preparation of Sample Solution

To prepare 100 ppm stock solution, 0.0005 grams of cocoa husk ethyl acetate fraction and peel-off mask containing ethyl acetate fraction were each dissolved in ethanol in a 50 mL volumetric flask and stirred until homogeneous. From this stock solution, specific volumes (2.5, 2, 1.5, 1, and 0.5 mL) were pipetted into 10 mL volumetric flasks, then topped with ethanol to obtain serial concentrations of 25, 20, 15, 10, and 5 ppm, respectively.

2.6.2. Preparation of 0.1 mM DPPH Solution

Weighed 0.002 grams of DPPH powder and dissolved it in ethanol up to 50 mL.

2.6.3. Determination of Maximum Absorbance Wavelength of DPPH Solution

Two mL of DPPH solution were mixed with 2 mL of ethanol, shaken until homogeneous, and left in the dark for 30 minutes. The absorbance was measured using a UV-Vis Spectrophotometer at wavelengths of 400–800 nm with ethanol as blank.

2.6.4. Preparation of DPPH Standard Curve

To prepare a 40 ppm DPPH solution, 0.002 g of DPPH was dissolved in ethanol to a final volume of 50 mL and homogenized. Specific volumes (8, 6, 4, and 2 mL) of this stock solution were pipetted into 10 mL volumetric flasks and topped with ethanol to obtain serial concentrations of 32, 24, 16, and 8 ppm, respectively. Two mL of solution from each concentration series were mixed with 2 mL of ethanol, homogenized, and kept in the dark for 30 minutes. The absorbance was measured at the maximum wavelength using a UV-Vis Spectrophotometer with ethanol as blank. The test was repeated three times.

2.6.5. Antioxidant Activity Test

Two mL of each sample solution from the serial concentrations (25, 20, 15, 10, and 5 ppm) was mixed with 2 mL of 0.1 mM DPPH, homogenized, and kept in the dark for 30 minutes. The absorbance was measured using a UV-Vis Spectrophotometer at the maximum wavelength with ethanol as blank. Each test was performed in triplicate. The percentage of inhibition was calculated to determine antioxidant activity based on the inhibition of DPPH radical absorbance. The formula for % inhibition is shown in Equation 5:

$$I = \frac{A - A_1}{A} \times 100\% \quad (5)$$

where A is the absorbance of the control and A_1 is the absorbance of the sample.

Next, the 50% inhibitory concentration (IC_{50}) value was calculated using a linear regression equation, y = ax + b, obtained from the relationship between concentration and % inhibition, with y = 50.

3. Results and Discussion

3.1. Characterization of Simplisia

A total of 24.7 kg of cocoa husk yielded 4.32 kg of dried simplisia. The moisture content of the simplisia was determined using the gravimetric method to ensure its quality and stability, as high moisture content can facilitate the growth of microorganisms. Test results showed that the cocoa husk simplisia had a moisture

content of 6.75%, less than the Indonesian Ministry of Health's quality requirement of 10% [19].

3.2. Extraction and Characterization of Fraction

The ethyl acetate fraction of the cacao husk was obtained in an amount of 23.06 g, with a yield of 0.92%. Organoleptically, the extract exhibited a brownish-yellow colour, a distinctive odor, and a concentrated consistency. The fraction was characterized further by determining its moisture content, total ash content, water-soluble extract content, and ethanol-soluble extract content.

The water content was determined to establish the amount of water remaining in the fraction because high water content can affect its stability [30]. Test results showed that the water content of the ethyl acetate fraction of cocoa husk was 3.48%. The standard moisture content of an extract depends on its type; for dry extracts, an acceptable moisture content is less than 10% [31]. Total ash content was determined to provide an overview of the mineral content originating from the plant tissue and the initial extraction processes involved in producing the concentrated fraction. Analysis by heating at 600°C showed that the total ash content of the ethyl acetate fraction of cocoa husk was 8.57%.

Meanwhile, determining the levels of water- and ethanol-soluble fractions reveals the quantity of compounds that can be extracted using polar water and semipolar ethanol. Test results showed that the water-soluble fraction level of the ethyl acetate fraction of cocoa husk was 12.67%, while the ethanol-soluble fraction level was 44.67%. Based on these results, it is evident that the fraction is more soluble in ethanol than in water, with the compounds in the fraction being easily filtered using an ethanol solvent. This may be due to ethanol's universal nature, as it is capable of dissolving both polar and non-polar compounds [30].

3.3. Phytochemical Screening

Phytochemical screening of the ethyl acetate fraction of cocoa husk revealed the presence of flavonoids, tannins, and steroids. The flavonoid compounds in the ethyl acetate fraction are characterised by the formation of an orange-yellow solution when reacted with magnesium (Mg) and hydrochloric acid (HCl). This reaction occurs when Mg powder and concentrated HCl reduce the carbonyl group to an alcohol group, thereby forming colored hydroxy compounds. Test results for tannin compounds in the ethyl acetate fraction of cocoa husk are characterised by a change in colour of the solution to blackish green when reacting with 10% $FeCl_3$. This colour change occurs because one of the hydroxyl groups in the tannin compound reacts with the addition of 10% $FeCl_3$, resulting in a colour change. The presence of steroid compounds in the ethyl acetate fraction of cocoa husk is indicated by a change in colour to dark green when H_2SO_4 is added in an anhydrous acid solvent. This is because steroid or triterpenoid compounds can form colour complexes in strong acidic conditions [11, 16, 17, 32].

The results of this study align with those of Yahya et al., who reported that the ethyl acetate fraction of cocoa husk (TCEA) contained flavonoids, phenolic compounds (including tannins), and terpenoids. This fraction was found to have the highest bioactive content of all the fractions analyzed. Additionally, GC-MS analysis of the TCEA fraction revealed the presence of γ-sitosterol, stigmasterol, and campesterol, which are steroids. Therefore, the qualitative results and quantitative data from Yahya's research confirm that the ethyl acetate fraction of cocoa husk is a significant source of potential metabolites, particularly flavonoids, tannins, and steroids. These compounds contribute to antioxidant activity [11, 16, 17, 32].

3.4. Peel-off Mask Formulation

As shown in Table 1, the peel-off mask formulations containing the ethyl acetate fraction of cocoa husk utilized additional ingredients, including polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC), propylene glycol, methyl paraben, propyl paraben, and distilled water. The PVA and HPMC base ingredients played an important role in determining the quality of the preparation. PVA serves as the primary film-forming polymer, producing an elastic layer that adheres to the skin and provides a tightening and occlusive effect. When PVA is added to the ethyl acetate fraction mask from cocoa husk, it provides clean, firm skin and makes pores appear smaller after use. Meanwhile, HPMC acts as a thickening and gelling agent, affecting the consistency, viscosity, and comfort of the preparation when applied. In addition to these two main ingredients, propylene glycol is used as a humectant to maintain the formula's moisturizing properties and increase the film's flexibility, spreadability, and penetration of active ingredients into the skin. Methyl and propyl parabens are used as preservation agents to prevent microbial growth in the preparation and ensure the safety and stability of the product during storage [28, 33, 34].

3.5. Peel-off Mask Evaluation

Peel-off mask formulation containing ethyl acetate fractions of cocoa husk was evaluated using the cycling test method. The stability of the peel-off masks was tested by storing them at temperatures of 45°C and -5°C for 24 hours each over a period of 12 days. This method

Table 2. Result of the organoleptic test.

Observation	Cycle						
	0	1	2	3	4	5	6
Dosage form	-	-	-	-	-	-	+
Color	-	-	-	-	+	+	+
Odor	-	-	-	-	-	-	-

Table 3. Result of the pH measurement.

pH value	Cycle						
	0	1	2	3	4	5	6
	6.1	5.53	5.47	5.4	5.37	5.37	5.33

Table 4. Result of the viscosity measurement.

Testing	Viscosity (cP)
Before the cycling test	4480.10
After the cycling test	6319.92

assesses the physical resistance of the masks to extreme storage conditions accelerated over a short period. Parameters observed during the stability test included organoleptic characteristics, pH level, spreadability, viscosity, and mechanical properties. Additionally, the peel-off mask preparation went through drying time and irritation potential testing to ensure its safety.

3.5.1. Organoleptic Test

Organoleptic testing was conducted to observe the physical changes to the peel-off mask, including its shape, color, and odor. Based on the results shown in Table 2, the formulation was initially semi-solid, had a distinctive brown odor reminiscent of skin, and was transparent yellow, derived from the active ingredient, the brownish-yellow ethyl acetate fraction of cacao husk. During storage using the cycling test method, the ethyl acetate fraction peel-off mask showed physical changes. In the 4th cycle, the color changed to a paler shade and continued to fade, while in the 6th cycle, the consistency became thicker. These changes may have been caused by lipid oxidation and water evaporation during storage. These natural processes were accelerated by temperature, which promoted the formation of volatile aldehydes and ketones. Ultimately, this affected the product's stability and led to possible decomposition of the ingredients [35].

3.5.2. pH Measurement

pH testing was conducted to determine the pH of the formulation within the pH range of the skin. Peel-off mask formulation should have a pH comparable to that of the skin. If the pH of the preparation is too acidic, it can cause skin irritation. Conversely, if the pH is too alkaline, it can cause the skin to dry out [36]. According to Table 3, the initial pH value of the peel-off mask formulation was 6.1. However, it decreased to 5.53, 5.47, 5.4, 5.37, 5.7, and finally to 5.33 during the cycling test storage period. This decrease is assumed to be due to the degradation of the formulation ingredients [35]. Since human skin pH ranges from 4.5 to 6.5, the pH of the ethyl acetate fraction peel-off mask was still compatible with human skin pH.

3.5.3. Spreadability Test

Spreadability testing was conducted to determine how easily the gel mask could be spread on the skin. The spreadability value of the peel-off mask was 6.47-8.57 cm immediately after formulation, but decreased to 5.7-7.13 cm during storage. This change in spreadability is attributed to an increase in gel consistency. The gel thickened due to changes in the viscosity of the polymer matrix during storage [37]. Nevertheless, the spreading power obtained still meets quality requirements, varying between 5 and 7 cm. This indicates that the ethyl acetate fraction peel-off mask has remained stable and is comfortable to use, spreading evenly on the skin and allowing for optimal contact time with the active ingredients.

3.5.4. Viscosity Measurement

Viscosity testing was conducted to determine the viscosity of the formulated gel. The viscosity of the ethyl acetate fraction peel-off mask was tested using an International Rheology Viscometer with an R5 spindle at a speed of 150 rpm. The test was conducted in two stages: before and after the cycling test. Each stage had three repetitions. Based on the results shown in Table 4, the peel-off mask formulation had a viscosity of 4480.13 cP before the cycling test and 6319.92 cP after the test. These results show that polyvinyl alcohol and hydroxypropyl methylcellulose absorb the solvent during preparation, retaining the liquid and increasing its viscosity.

The ideal viscosity range for a gel is 2,000 to 4,000 cP; therefore, the results did not align with the viscosity requirements for the formulation. The increase in peel-off mask viscosity is influenced by the HPMC excipient used. HPMC can form new hydrogen bonds with water molecules and dispersed droplets, thereby densifying the gel structure and increasing viscosity during storage [25]. Additionally, other excipients, such as the hydrophilic PVA and the humectant propylene glycol, strengthen the interaction between polymers and attract water, thereby contributing to the increase in preparation viscosity.

3.5.5. Mechanical Properties Test

Testing the mechanical properties of peel-off masks made from ethyl acetate fractions of cocoa husk includes measuring tensile strength and elongation. Tensile

Table 5. Result of the mechanical property test

Testing	Tensile strength (Kg/cm²)	Elongation (%)
Before the cycling test	40.6	251.72
After the cycling test	41.0	263.66

Table 6. Result of antioxidant activity test

Sample	IC_{50} (ppm)			Mean of IC_{50} (ppm)
	P1	P2	P3	
FE	6.819	6.819	6.819	6.819
F0	410.925	413.924	410.825	411.891
F1	11.380	12.109	11.289	11.596

Descriptions: FE: ethyl acetate fraction of cocoa husk; F0: Negative control; F1: peel-off mask formulation

strength is measured to determine the maximum tension the film can withstand before breaking, and elongation is measured to determine the maximum length the film can stretch before breaking. As shown in Table 5, the average tensile strength of the mask before the cycling test was 40.6 kg/cm², and after the test, it increased to 41.0 kg/cm². Meanwhile, the elongation obtained before the cycling test was 251.72%; after the test, it increased to 263.66%. These results demonstrate that the formed film exhibits good cohesion, can withstand tension, and retains its elasticity after storage. Theoretically, matrix strength is influenced by hydrogen bonds between polymers. In this formulation, the interaction between the film-forming polymer, polyvinyl alcohol (PVA), and the thickening agent, hydroxypropyl methylcellulose (HPMC), strengthen the matrix structure, resulting in a strong, flexible layer [33, 37].

3.5.6. Drying Time Test

Drying time testing was conducted to determine how long it takes for the peel-off mask to dry and form a film layer. The drying time of the ethyl acetate fraction peel-off mask was 24.23 minutes. A good drying time for a formulation is between 15 and 30 minutes; therefore, the results show that the formulation still meets the requirements. The stability of this drying time is influenced by the presence of hydroxypropyl methylcellulose (HPMC) as a polymer matrix that binds water molecules, as well as propylene glycol as a hygroscopic humectant that maintains water availability in the system [28].

3.5.7. Irritation Test

Irritation testing was conducted to determine the safety of a peel-off mask containing the ethyl acetate fraction of cocoa husk for use as a topical preparation on human skin. Four out of 24 respondents (16.67%) experienced symptoms of irritation, including erythema, heat, and itching. Two respondents (8.33%) experienced erythema, one respondent (4.17%) experienced heat, and two respondents (8.33%) experienced itching. According to cosmetic safety references, mild irritation is not unusual. Therefore, the incidence of 16.67% observed in this study is still within an acceptable range for topical cosmetic formulations. These reactions are likely influenced by variability in skin sensitivity to active compounds or excipients among individuals [38].

This finding is also consistent with regulatory perspectives, which state that up to 20% of the general population may exhibit symptoms of sensitive skin or allergic contact dermatitis related to cosmetic use. Such reactions are among the most frequently reported, but are usually mild and reversible. Thus, the irritation response observed in this study can be considered tolerable and does not indicate a significant safety concern [38].

This study had two limitations: a relatively small sample size and a short-term observation period. Therefore, the results cannot fully predict the long-term safety profile of the formulation across a broader population. Future studies with larger sample sizes and longer observation periods are necessary to confirm the product's tolerability and safety.

3.6. Antioxidant Activity Assay

Antioxidant activity assay was conducted on the ethyl acetate fraction of cocoa husk and peel-off mask formulation containing the ethyl acetate fraction of cocoa husk using the DPPH method. This assay is based on the ability of antioxidant compounds to capture DPPH free radicals, which allows for the measurement of decreased DPPH absorption. The maximum wavelength obtained from the DPPH solution was 516.6 nm, which represented the maximum absorbance value. These results were consistent with the standard, which specifies a maximum wavelength of 515-520 nm for DPPH.

According to Table 6, the results of the antioxidant activity assay showed that the IC_{50} of the ethyl acetate fraction was 6.819 ppm, while the IC_{50} of the peel-off mask formulation was 11.596 ppm. These results indicated that both had very strong antioxidant activity. When compared to ascorbic acid, which has an IC_{50} value of 3.5 ppm [18], the activity of both fraction and the formulation was only slightly lower. This finding suggests that the antioxidant potential of the tested samples is remarkably high, approaching that of ascorbic acid, a well-known standard antioxidant.

This potential is closely related to the content of secondary metabolites, especially flavonoids, tannins,

and other phenolic compounds, in cocoa husk. These compounds work by donating electrons or hydrogen atoms to neutralize free radicals. Consequently, cocoa husk's ability to prevent oxidative stress, inhibit premature aging, and protect skin cells is directly affected. The difference in IC_{50} values is attributed to the interaction of bioactive compounds with excipients in the formulation, such as polyvinyl alcohol (PVA), hydroxypropyl methylcellulose (HPMC), and propylene glycol, which influence the release and availability of active compounds.

These results align with those of Yahya et al. [16], who reported that the ethyl acetate fraction of cocoa husk (TCEA) exhibited an EC50 of 9.61 µg/mL, the highest phenolic content (570.44 mg/g GAE), and the highest flavonoid content (4.34 mg/g QE) compared to other fractions. These characteristics directly contribute to TCEA's potent antioxidant activity. The results of Belwal et al. [12] study also prove that cocoa fruit peel is rich in polyphenols, flavonoids (e.g., catechin, epicatechin, and proanthocyanidin B2), methylxanthines (e.g., theobromine and caffeine), and phytosterols. These compounds synergistically provide high antioxidant activity by inhibiting lipid peroxidation and protecting against oxidative stress. Technical factors, such as extraction methods, have been shown to affect antioxidant capacity. For example, heat drying can reduce total phenolic content and antioxidant activity by 64–88%. In contrast, smaller particle size and microwave-assisted extraction can increase phenolic yield and antioxidant capacity [39].

Consistent with the various literature, the results of this study confirmed that the strong antioxidant activity of the ethyl acetate fraction of cocoa husk is mainly due to its high phenolic content. Although the formulation in the form of a peel-off mask showed a slight increase in IC_{50} values due to interaction with excipients, it did not change its antioxidant activity category.

This formulation enables the use of cocoa husk as a functional cosmetic that protects the skin from oxidative stress, prevents premature aging, and promotes natural skin health. However, stability issues were observed, including color changes, increased viscosity, and altered consistency. These issues highlight areas for improvement in the formulation of the peel-off mask. Future studies should focus on optimizing polymer ratios and incorporating stabilizing excipients to ensure consistent physical properties. Adjusting the pH level and preservative system may also enhance product stability. In preparation for potential large-scale production, a pilot study should evaluate the mixing efficiency, homogeneity, and reproducibility of batches to ensure consistent quality. Despite these challenges, the formulation shows promise due to its antioxidant-rich cocoa husk fraction [12, 16, 39, 40].

4. Conclusions

The ethyl acetate fraction of cocoa husk has been proven to exhibit very strong antioxidant activity, with an IC_{50} value of 6.819 ppm. Even when formulated into a peel-off mask, it retains very strong activity, with an IC_{50} value of 11.596 ppm. The flavonoids, tannins, and steroids content of cocoa fruit husk plays a major role in providing this antioxidant activity, making cocoa peel waste a potential source of valuable natural ingredients. The peel-off mask formulation generally meets quality parameters, as indicated by its pH and drying time, both of which are in accordance with standards. However, instability was found in its organoleptic properties, viscosity, and spreadability during storage. Irritation tests indicate that the formulation is relatively safe, with only a few subjects experiencing mild, tolerable reactions. These stability issues must first be addressed before commercial development can be considered. To enhance physical stability and maximize the recovery of bioactive compounds, further optimization of excipients, polymer ratios, and formulation strategies is recommended. This includes modifying PVA and HPMC concentrations to improve viscosity and spreadability, adding stabilizing agents such as humectants or antioxidants, and adjusting pH and preservative systems. Process improvements, including controlled mixing, optimized drying conditions, and the careful selection of extraction solvents and methods, are also recommended to better preserve bioactive compounds. While the in vitro antioxidant activity indicates potential benefits, additional in vivo studies, skin bioavailability assessments, and research on the mechanism of action are necessary to confirm any protective or anti-aging effects on human skin. Together, these formulation optimizations and additional studies provide a solid foundation for the future research and development of cocoa husk–based peel-off masks.

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