


Antioxidant Activity Test of Peel-off Mask of Ethanol Extract of Kitolod Leaves (*Isotoma Longiflora* L)

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Article Info	ABSTRACT
<p>Keywords: Kitolod leaves (<i>isotoma longiflora</i> L.), peel-off mask, antioxidant.</p>	<p>Free radicals are one of the main causes of skin cell damage, resulting in premature aging. Antioxidants play an important role in neutralizing free radicals, thereby maintaining skin health. Kitolod leaves (<i>Isotoma longiflora</i> L.) are known to contain flavonoids, alkaloids, saponins, and polyphenols that have antioxidant potential. This study aims to formulate 70% ethanol extract of kitolod leaves into a peel-off gel mask preparation and evaluate its physical stability and antioxidant activity. The research method is experimental with variations in extract concentration of 5%, 10%, and 15%. Physical evaluation of the formulation included organoleptic testing, homogeneity, pH, viscosity, spreadability, adhesion, and drying time, while antioxidant activity was tested using the DPPH method. The results showed that all formulations met the characteristics of a good peel-off mask preparation and remained stable for 14 days of storage. The antioxidant activity test using the method showed that the IC50 value of kitolod leaf extract was 52.84 ppm, which is classified as strong. Thus, the peel-off gel mask containing kitolod leaf extract has the potential to be developed as a natural cosmetic product with good antioxidant activity.</p>
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INTRODUCTION

The skin is the outer organ of the human body that acts as a protective barrier against various external factors. The skin is constantly exposed to the environment, such as ultraviolet (UV) radiation, air pollution, dust, humidity, and temperature fluctuations. The part of the skin that is most often exposed to these factors is the skin on the face. Facial skin has essential physiological and aesthetic functions, but its sensitive nature makes it prone to various problems, such as irritation, premature aging, and other skin health disorders (Widiati, 2014). Exposure to environmental factors, especially ultraviolet (UV) radiation, can cause damage to the skin. This damage is typically characterized by the appearance of scales, wrinkles, dryness, and brittleness. This condition makes the skin appear dull and wrinkled, and triggers premature aging, which is marked by the appearance of dark spots on the skin's surface (Nafiah et al., 2024).

Premature aging of the skin can be prevented through the use of ingredients with antioxidant activity in cosmetic formulations. Antioxidants play an important role by donating

electrons to oxidizing compounds or free radicals, thereby neutralizing and suppressing oxidative activity that damages cells. In skin care products, especially those categorized as anti-aging products, antioxidants are often used as the main ingredient due to their ability to protect the skin from the negative effects of free radicals. In general, antioxidants are divided into two groups, namely natural antioxidants and synthetic antioxidants. Natural antioxidants are found in plant sources, such as vegetables, fruits, and medicinal plants that are rich in polyphenols, flavonoids, and vitamins (Pisoschi & Pop, 2015). Meanwhile, synthetic antioxidants are chemically engineered compounds that are also often used in the food and cosmetics industries, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate, and ethoxyquin (Shahidi & Zhong, 2015).

Thus, this research can be said to be superior to other research, because this research uses plants that are rich in bioactive compounds such as flavonoids which can work as antioxidants that can protect the skin from air pollution and direct exposure to sunlight.

Kitolod leaves are known to contain various secondary metabolites such as flavonoids, alkaloids, saponins, polyphenols, and have strong antioxidant activity. Flavonoids are one of the most widely studied groups of secondary metabolites due to their ability to ward off free radicals. The content of these active compounds makes kitolod potentially useful not only as a medicinal plant but also as an active ingredient in natural cosmetics (Lestari et al., 2024). Peel-off gel masks were chosen as the dosage form because they have several advantages, including being easy to apply, providing a cleansing effect by removing dirt and dead skin cells, and being able to increase the penetration of active ingredients into the skin. The use of polyvinyl alcohol (PVA) as the main base allows the formation of an elastic film layer that is easy to remove after drying (Beringsh *et al.*, 2013). Thus, this research can be said to be superior to other research, because this research uses plants that are rich in bioactive compounds such as flavonoids which can work as antioxidants that can protect the skin from air pollution and direct exposure to sunlight.

Based on the above description, this study aims to formulate 70% ethanol extract of kitolod leaves into a peel-off gel mask preparation and evaluate its physical stability and antioxidant activity. This study is expected to contribute scientifically to the use of kitolod plants as natural cosmetic ingredients with antioxidant activity.

METHODS

This research is a laboratory experimental study aimed at testing the antioxidant activity of kitolod leaf extract (*Isotoma longiflora*). The study was conducted from May to July 2025 at the Natural Materials Laboratory of the Muhammadiyah University of Education (UNIMUDA) in Sorong.

Tools and Materials.

The tools used include Analytical scales (Wiggen Hauser), label paper, ruler, pencil, aluminum foil, plastic, measuring cup (Pyrex), stirring rod, chemical glass (Pyrex), hot plate, funnel, Erlenmeyer flask (Schott Duran), spatula, mortar, pestle, watch glass, maceration bottle, evaporating dish, vacuum rotary evaporator, pH meter (ATC) blender, flannel cloth, glass slide, and container.

The materials used Kitolod leaf extract, 70% ethanol, distilled water, polyvinyl alcohol (PVA), HPMC, glycerin, propyl paraben, methyl paraben, Diphenyl Picryl Hydrazine (DPPH), methanol p.a.

Working Procedure

Weigh 213 grams of dried kitolod leaves (*Isotoma longiflora* L.) and soak them in a glass jar by adding 70% ethanol solvent until all the leaves are submerged. Leave the mixture for 72 hours in a closed container lined with aluminum foil to protect it from sunlight, stirring occasionally. After that, it was filtered using filter paper. After the first extraction process was complete, the residue from the extraction was re-extracted for 2 days using the same solvent. The results of the first and second extractions were combined and then evaporated to obtain a thick extract. The filtrate was evaporated using a water bath at $\pm 50^{\circ}\text{C}$.

Peel-Off Gel Mask Preparation Formula

The peel-off mask preparation will be, made in a quantity of 50 g and will use the standard formulation developed in the study (Zebua, 2019) as described below :

Table 1. Calculation of the ingredients for the Kitolod leaf peel-off mask

Ingredient	formula		
	F1 %	F2 %	F3 %
Ekstrak daun kitolod	5 %	10%	15%
Polivinil alkohol	10%	10%	10%
HPMC	1%	1%	1%
Gliserin	12%	12%	12%
Propilparaben	0,05%	0,05%	0,05%
Metilparaben	0,2%	0,2%	0,2%
Aquadest	100 ml	100 ml	100 ml

Source: Primary Data, 2025

The masks will be made with three different formulations based on the concentration of kitolod leaf extract. Each gel mask will contain kitolod leaf extract with varying concentrations of 5%, 10%, and 15%, each weighing 50 g, using the same base composition.

Preparation evaluation

Homogeneity test

Homogeneity testing is carried out by applying a sample of the preparation onto a glass slide, then spreading it evenly for observation. A preparation is considered homogeneous if, after application, no coarse particles or granules are found to be unevenly distributed on its surface. The criteria for homogeneity are characterized by a uniform appearance of the preparation and no visible solid particles in the transparent medium (Gultom, 2019).

pH test

The peel-off mask preparation to be tested was prepared by dissolving 1 g in 10 ml of distilled water (10%). After that, the pH of the preparation was measured by adjusting the color change on the pH paper. To obtain good results, the peel-off mask must have a pH range of 4.5 to 6.5 (Hidayati, 2019)

Organoleptic test

Organoleptic testing was conducted using visual observation to assess the physical characteristics of the peel-off gel mask preparation, including its shape, color, and aroma (Pratiwi & Wahdaningsih, 2018).

Drying time test

A total of 1 gram of gel mask preparation from ethanol extract of kitolod leaves (*Isotoma longiflora* L.) was carefully placed on a 20 x 20 cm glass plate. Then, it was covered with another glass plate and weighted down until the total weight reached 125 grams. The diameter was measured after 1 minute. The ideal spreading rate is 5-7 cm (Slavtcheff, 2000)

Spreadability test

A total of 1 gram of gel mask preparation from ethanol extract of kitolod leaves (*Isotoma longiflora* L.) was carefully placed on a 20 x 20 cm glass plate. Then, it was covered with another glass plate and weighted down until the total weight reached 125 grams. The diameter was measured after 1 minute. The ideal spreading rate is 5-7 cm (Slavtcheff, 2000).

Adhesion test

Adhesion testing was conducted by weighing 5 grams of sample, placing it on glass, then covering it with another glass weighted with 500 grams and leaving it for 1 minute. The weight is then released downward until the samples separate, after which the time it takes for the two glass objects to separate is calculated. A good adhesion strength test value for the preparation is a separation time of more than 1 second (Syam 2021).

viscosity test

This test uses a Brookfield viscometer. A size 4 spindle is used with the spindle lock turned clockwise at a speed of 30 revolutions per minute (rpm), then the preparation is inserted and the spindle is dipped into the container until it reaches the limit mark and the value that appears is recorded as the viscosity. The viscosity is considered acceptable if it is between 3,000-50,000 cps (Sukmawati et al., 2013).

Antioxidant activity Test

Preparation of DPPH/Blanko Solution

A 0.4 mM DPPH solution is prepared by weighing 0.0157 grams of DPPH, then dissolving it in a 100 ml measuring container with ethanol p.a. to obtain a 0.4 mM DPPH solution (Muslihin et al., 2022).

Preparation of Kitolod Lest Extract Test Solution

The antioxidant activity of kitolod leaves (*Isotoma longiflora* L.) was measured by taking a 1000 ppm stock solution. Kitolod leaf extracts were taken in amounts of 0.1 ml, 0.2ml, 0.3ml, 0.4ml, and 0.5ml, then poured into 5ml measuring containers covered with aluminum foil. Next, 1.0 ml of 0.4 mM DPPH was added, then the volume was adjusted with p.a ethanol to the mark, thus obtaining concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. The mixture was left for 30 minutes, then the absorbance was measured using ultraviolet-visible spectrophotometry at a wavelength of 500 nm (Muslihin and Astuti 2022).

Preparation quercetin reference Test Solutio

A total of 1 mg of quercetin as a reference was weighed and dissolved with 96% ethanol solvent to a volume of 10 mL using a measuring flask to obtain a quercetin stock

solution with a concentration of 100 ppm. It was then diluted to 0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, and 0.5 ppm. Next, the absorbance was measured at a wavelength of 500 nm (Suyatmi et al., 2019). A 1000 ppm stock solution was prepared by weighing 10 mg of quercetin and dissolving it in p.a. ethanol while stirring and homogenizing, then making up the volume to 10 mL. Then, 0.1 ml was pipetted, homogenized, and the volume was adjusted to 10 ml with ethanol p.a. Next, each 10ppm stock solution was diluted by pipetting.

Absorption measurement

The blank solution, quercetin solution, and sample solution were recorded for their absorbance using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm.

Determination of IC50

The IC50 was calculated from the results of a linear regression curve between the % inhibition and the sample concentration series, namely the extract and quercetin.

Daya Analysis

The research data were analyzed by presenting them in tables and graphs, then described descriptively through narration. Data processing was carried out theoretically, namely by comparing the results of the peel-off gel mask evaluation test with relevant references or literature. The analysis of antioxidant activity test data was performed using a simple linear regression equation: $y = bx + a$, to determine the IC₅₀ value. The IC₅₀ value describes the concentration of extract capable of inhibiting 50% of free radical activity. Free radical scavenging activity against DPPH was calculated using the formula proposed by Rahmayani et al. (2013).

$$\% \text{ inhibition} = \frac{\text{blank absorbance}}{\text{sample absorbance}} \times 100\%$$

RESULTS AND DISCUSSION

Results

Tabel 2. Yield Results of Kitolod Leaf Extract (*Isotoma longiflora* L.)

Simplisia	Sample weight (Kg)	Weight of Powder (gram)	Extract weight (gram)	Yield (%)
Daun Kitolod	2,5 kg	213 gram	48 gram	17,6%???

Table 1 shows the weight of the crude drug used in this study, which was 2.5 kg, and the concentrated extract obtained was 48 grams, resulting in an ethanol extract yield of 22.54%.

Tabel 3. Results Of The Homogeneity Test Of The Peel-off Mask Preparation

Formula	Day		
	1	7	14
Formula 1	Homogeneous	Homogeneous	Homogeneous
Formula 2	Homogeneous	Homogeneous	Homogeneous
Formula 3	Homogeneous	Homogeneous	Homogeneous

Source: Primary Data, 2025

Homogeneity testing was conducted to ensure that the active ingredients and additives in the peel-off gel mask preparation were evenly mixed without any coarse particles or phase

separation. The results showed that all formulas (Formula 1, Formula 2, and Formula 3) had good homogeneity on days 1, 7, and 14.

Tabel 4. Results Of The pH Test Of The *Peel-Off* Mask Preparation

Formula	Day			Mean±SD	Standard pH
	1	7	14		
Formula 1	6	6	6	6±0	4,5-8
Formula 2	6	6	6	6±0	4,5-8
Formula 3	6	6	6	6±0	4,5-8

Source: Primary Data, 2025

Homogeneity testing was conducted to ensure that the active ingredients and additives in the peel-off gel mask preparation were evenly mixed without any coarse particles or phase separation. The results showed that all formulas (Formula 1, Formula 2, and Formula 3) had good homogeneity on days 1, 7, and 14.

Tabel 5. Results Of The Organoleptic Test Of The *Peel-Off* Mask Preparation

Formula Organoleptic		Day		
		1	7	14
F1	Form	Semi-solid	Semi-solid	Semi-solid
	Color	Dark brown	Dark brown	Dark brown
	Smell	Characteristic of the extract	Characteristic of the extract	Characteristic of the extract
F2	Form	Semi-solid	Semi-solid	Semi-solid
	Color	Dark brown	Dark brown	Dark brown
	Smell	Characteristic of the extract	Characteristic of the extract	Characteristic of the extract
F3	Form	Semi-solid	Semi-solid	Semi-solid
	Color	Dark brown	Dark brown	Dark brown
	Smell	Characteristic of the extract	Characteristic of the extract	Characteristic of the extract

Source: Primary Data, 2025

Organoleptic test results conducted on the three formulas showed that F1, F2, and F3 had relatively similar physical characteristics. All three formulas were greenish-brown in color, had a distinctive extract aroma, and were semi-solid in form. Observations from day one to day 14 showed no significant changes in the shape, color, or smell of the preparations. This indicates that the three formulas have good organoleptic stability during the storage period, so it can be concluded that the formula variations do not make a significant difference to the organoleptic parameters of the peel-off gel masks produced.

Tabel 6. Results of the Drying Time test of the *peel-off* mask preparation

Formula	Day			Mean±SD	Standard Time
	1	7	14		
Formula 1	17,07 Minutes	23,43 Minutes	24,46 Minutes	18,18±1,83 Minutes	15-30 Minutes

Formula	Day			Mean±SD	Standard Time
	1	7	14		
Formula 2	17,20 Minutes	25,12 Minutes	26,55 Minutes	24,99±1,50 Minutes	15-30 Minutes
Formula 3	20,27 Minutes	26,41 Minutes	27,11 Minutes	26,04±1,40 Minutes	15-30 Minutes

Source: Primary Data, 2025

The results of drying time testing on peel-off gel masks containing kitolod leaf extract showed an increase in drying time from day 1 to day 14. The average drying time on day 1 was 18.18 ± 1.83 minutes, increasing to 24.99 ± 1.50 minutes on day 7, and reaching 26.04 ± 1.40 minutes on day 14. This increase may be due to changes in the physical characteristics of the gel during storage, especially in the polymer matrix.

Tabel 3. Results of the Spreadability Test Of The *Peel-Off* Mask Preparation

Formula	Day			Mean±SD	Standard Spread Power
	1	7	14		
Formula 1	5,41 cm	6,22 cm	7,20 cm	5,16±0,11 cm	5-7 cm.
Formula 2	5,52 cm	6,57 cm	7,25 cm	5,16±0,20cm	5-7 cm.
Formula 3	5,77 cm	6,31 cm	7,81 cm	5,13±0,23cm	5-7 cm.

Source: Primary Data, 2025

Based on the results of the spreadability test of the peel-off gel mask containing kitolod leaf extract, the spreadability ranged from 5.41 to 7.81 cm during 14 days of storage. The spreading power values obtained in this study were mostly within this range, and although on day 14 there was a slight increase above 7 cm, this was still acceptable as it still supported ease of application (Garg et al., 2018).

Tabel 7. Results of the Adhesion Test Of The *Peel-Off* Mask Preparation

Formula	Day			Mean±SD	Standard Adhesion
	1	7	14		
Formula 1	01,36 Seconds	01,48 Seconds	02,62 Seconds	1,82±0,70 Seconds	1-3 Seconds.
Formula 2	01,93 Seconds	02,05 Seconds	01,74 Seconds	1,91±0,16 Seconds	1-3 Seconds.
Formula 3	01,64 seconds	01,29 Seconds	01,39 Seconds	1,44±0,18 Seconds	1-3 Seconds.

Source: Primary Data, 2025

Adhesion Test to determine the mask's ability to adhere when applied and perform its function of distributing the active ingredient onto the skin during the process until it dries. The greater the adhesion, the greater the diffusion of the active ingredient into the skin due to the longer bond between the preparation and the skin.

Tabel 8. Results of the Viscosity Test Of The *Peel-Off* Mask Preparation

Formula	Day			Mean±SD	Standar Viscosity
	1	7	14		
Formula 1	40,53	52,39	70,26	54,39±14,97	50-1000
Formula 2	42,40	55,33	76,30	58,01±17,11	50-1000
Formula 3	43,20	55,46	77,51	58,72±17,39	50-1000

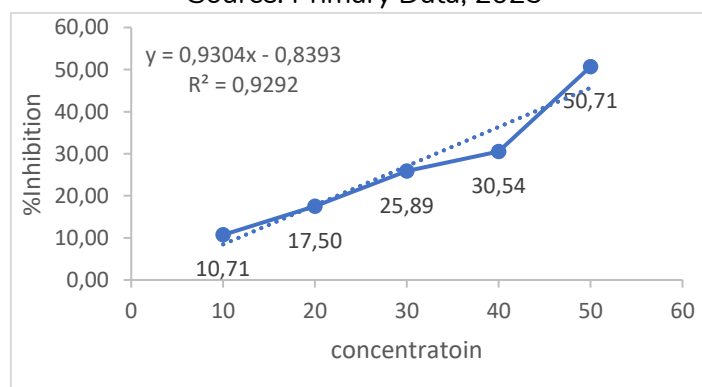
Source: Primary Data, 2025

The viscosity measurement results of the three peel-off mask formulas show that the viscosity values increased from day 7 to day 14. Formula 1 had an average viscosity value of 54.39±14.97 cP, formula 2 had 58.01±17.11 cP, and formula 3 had 58.72±17.39 cP.

Tabel 9. IC50 Values Of Kitolod Leaf Extract

No.	Concentration (ppm)	Absorbance	% inhibition	IC50 (ppm)
	DPPH	0.560		
1.	10	0.500	10.71	52.84
2.	20	0.462	17.50	
3.	30	0.415	25.89	
4.	40	0.389	30.54	
5.	50	0.276	50.71	

Source: Primary Data, 2025

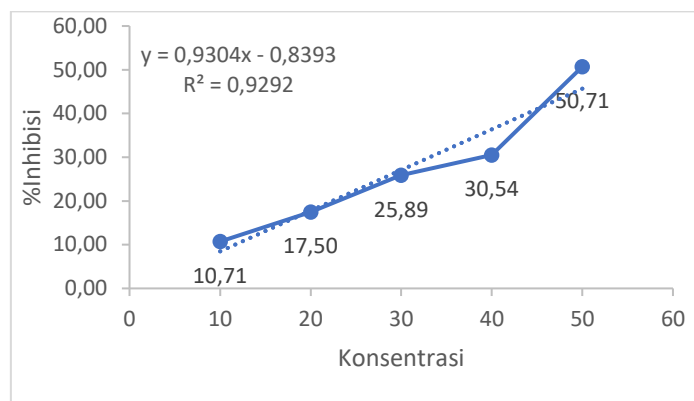


Gambar 1. Graph of Average Relationship of Sample Inhibition with Concentration

Tabel 10. Results of the homogeneity test of the *peel-off* mask preparation

No.	Concentration (ppm)	Absorbance	% inhibition	IC50 (ppm)
	DPPH	0.560		
1.	0,1	0.498	11.07	0.93
2.	0,2	0.483	13.75	
3.	0,3	0.450	19.64	
4.	0,4	0.13	26.25	
5.	0,5	0.400	28.57	

Source: Primary Data, 2025



Gambar 2. Graph of the Relationship Between % Inhibition of Quercetin and Concentration

Discussion

Antioxidant activity testing was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazil) method to determine the ability of ethanol extracts from kitolod leaves to capture free radicals. Based on Table 8, the antioxidant activity of the extract increased with increasing concentration. At a concentration of 10 ppm, an inhibition percentage of 10.71% was obtained, then gradually increased at concentrations of 20 ppm (17.50%), 30 ppm (25.89%), 40 ppm (30.54%), and reached the highest value at concentration of 50 ppm, namely 50.71%. This increase indicates a linear relationship between the extract concentration and the percentage of free radical inhibition. Meanwhile, Table 9 shows the antioxidant test results of the quercetin reference, which exhibits very strong antioxidant activity at < 50 ppm. Thus, these results confirm that quercetin is a flavonoid compound with very high free radical scavenging ability, in accordance with its basic properties as a polyphenol rich in hydroxyl groups.

Stability testing of peel-off mask preparations containing kitolod leaf extract was conducted with three replicates for each formula to ensure that the test results obtained were consistent, reliable, and accountable. Based on the results of the stability test of the peel-off gel mask containing kitolod leaf extract, it was found that the three formulas (F1, F2, and F3) had the same organoleptic characteristics, namely greenish-brown color, characteristic extract odor, and semi-solid form. The stable organoleptic consistency until the 14th day indicated no degradation of the extract or changes in the basic composition of the gel. Other physical parameters such as viscosity also showed stability, although there was an increase from day 7 to day 14 with an average value of 54.39±14.97 cP for Formula 1, 58.01±17.11 cP for Formula 2, and 58.72±17.39 cP for Formula 3. This increase is related to the ability of PVA polymers to bind water, making the gel matrix denser and thicker (Rowe et al., 2009). However, all formulas are still within the requirements of SNI 16-4399-1996, which is 50-1000 cP (Garg et al., 2002), so it can be ensured that the consistency of the mask is still suitable for application to the skin. The pH test results were also consistent at a value of 6 during 14 days of storage, which is still within the physiological range of the skin (4.5-8), safe to use, and does not cause irritation (Lambers et al., 2006). This pH stability indicates that there is no significant interaction between the kitolod extract and the gel base, consistent

with previous research confirming the stability of gel-based formulations at pH values close to neutral (Garg et al., 2018).

In addition, other physical tests also support the quality of the formulation. The drying time of the mask increased from 18.18 ± 1.83 minutes on day 1 to 26.04 ± 1.40 minutes on day 14, but was still within the ideal range of 15-30 minutes (Garg et al., 2018). The spreadability test showed a range of 5.41-7.81 cm, in accordance with the literature standard of 5-7 cm, with a slight increase above 7 cm on day 14, which is still acceptable as it continues to support ease of application. The adhesion test results showed variations between formulas, with Formula 2 having the highest value (1.91 ± 0.16 seconds) and Formula 3 the lowest (1.44 ± 0.18 seconds). Adhesion is quite important for prolonging the contact time of the preparation with the skin, thereby supporting the diffusion of the active substance (Lachman et al., 2008). The homogeneity test also showed an even distribution of ingredients without coarse particles, sediment, or phase separation up to day 14, confirming that the ingredient mixing process was carried out properly and that the gel base was able to maintain the stability of the mixture (Tranggono & Latifah, 2007). The results of this study are in line with research conducted by (Nurhayati, 2024), in which peel-off gel mask preparations with other herbal ingredients also showed good physical stability during storage. The study reported that the organoleptic characteristics remained stable without any changes in color, odor, or texture. Viscosity tests showed fluctuations in values, but they remained within the required range. Overall, all test results indicate that the peel-off gel mask made from kitolod leaf extract has good physical stability, is safe, and meets cosmetic standards, making it a potential candidate for development as a topical preparation with natural active ingredients.

The results of the antioxidant activity test using the DPPH method showed that ethanol extract of kitolod leaves was able to effectively scavenge free radicals. The inhibition percentage increased with increasing concentration, ranging from 10.71% at 10 ppm to 50.71% at 50 ppm. Linear regression analysis yielded an IC₅₀ value of 52.84 ppm, which falls within the category of strong antioxidant activity (50-100 ppm). This activity is thought to originate from phenolic compounds and flavonoids in kitolod leaves, such as gallic acid, catechin, and quercitrin, which act as hydrogen atom donors to neutralize free radicals. For comparison, quercetin was used as a standard with an IC₅₀ of 0.93 ppm, which means it has very strong antioxidant activity. These results confirm that although the antioxidant power of kitolod extract is lower than that of quercetin, this extract still has great potential as a natural antioxidant source. This potential can be utilized in the formulation of cosmetic products or health supplements, as it is able to suppress the activity of free radicals that play a role in the skin aging process and cell damage.

CONCLUSION

Overall, all stability test parameters indicate that the peel-off gel mask containing kitolod leaf extract has good physical quality and remains stable during 14 days of storage. This confirms that kitolod extract can be effectively formulated in peel-off mask preparations that meet cosmetic standards, and the antioxidant activity shows that the IC₅₀ value of kitolod leaf extract is 52.84 ppm, which is classified as strong. It is recommended that future researchers

formulate the extract into a more suitable dosage form with excellent antioxidant benefits, particularly in the form of cosmetic preparations. Further research is needed to test the antioxidant activity directly from the formulated preparations.

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