International Journal Of Health, Engineering And Technology (IJHET) *E-ISSN 2829 - 8683*
Volume 4, Number 6, March 2026, *Page. 2582 - 2595*
Email : editorijhess@gmail.com

Determination Of Sun Protective Factor (SPF) Values In N-Hexane, Ethyl Acetate, And Water Fractions Of Ethanol Extract Of Tamarind Eggplant (Solanum Lasiocarpum Dunal.) Peel With UV-VIS Spectrophotometry Method

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Abstract

Excessive UV exposure causes skin damage, so natural sunscreen alternatives are needed. This experimental descriptive study aims to identify and measure the Sun Protection Factor (SPF) value of ethanol extract and n-hexane, ethyl acetate, and water fractions from Solanum lasiocarpum Dunal fruit peel in vitro using UV-Vis spectrophotometry. The population of the entire skin simplicia from Tanjung Hulu, Pontianak; a sample of 500 g of powder (mesh 40) was extracted and fractionated. Instruments include a Thermo Scientific UV-Vis spectrophotometer (290-320 nm), rotary evaporator, TLC plate; SPF was calculated using the Mansur equation and Lambert-Beer validation linear regression. The results showed the highest SPF of ethyl acetate fraction (36.97 at 500 ppm, high category), while the ethanol extract (9.32), n-hexane (6.71), and water (6.76) were low. Phytochemical screening was positive for alkaloids, terpenoids, phenolics, and flavonoids. In conclusion, the ethyl acetate fraction has optimal potential for the development of sunscreen cosmetics.

Keywords: Ethyl Acetate Fraction, Phytochemicals, Solanum Lasiocarpum, Spectrophotometry, Sun Protection Factor.

INTRODUCTION

Acne is the most common skin problem in Indonesia, especially among adolescents, with a prevalence of up to 80-90% among those aged 15-18. As stated by Nurhadi Wartiningsih (2021), "One of the most common skin diseases suffered by people is acne." Propionibacterium acnes bacterial infection triggers this condition through the production of lipase, which breaks down triglycerides into free fatty acids, causing inflammation and blackheads.

The skin, as the largest organ, is susceptible to external stimuli, worsening acne due to P. acnes colonization. The prevalence of acne vulgaris rose from 8,563 per 100,000 people in 1990 to 9,790 in 2021. Antika (2020) explains, "Propionibacterium acnes is a normal bacterial flora on human skin that produces lipase that breaks down into triglycerides... causing inflammation and the formation of comedones."

Acne treatment relies on antibiotics such as doxycycline, but prolonged use can lead to resistance. Yu et al. (2024) reported, "53 (56.4%) and 52 (55.3%) isolates were susceptible to erythromycin and clarithromycin, respectively," indicating high resistance to macrolides. Erythromycin and clarithromycin resistance was high, while doxycycline remained effective despite the risk of disrupting the skin microbiota.

Topical anti-inflammatories such as nicotinamide or benzoyl peroxide are used, but natural alternatives with minimal side effects are needed. Kamala Permana (2020) stated, "Continuous use of antibiotics can lead to resistance." The dependence on synthetic antibiotics is pressing for a replacement with effective herbal remedies against P. acnes.

This study aims to formulate an anti-acne cream from neem leaf extract (Azadirachta indica A. Juss.), test its physical quality, and antibacterial activity against P. acnes at a concentration of 30-45%. The urgency of overcoming increasing antibiotic resistance, while its novelty in a stable cream formula with azadirachtin, nimbin, nimbidin, quercetin, and optimal concentration variations has not been widely explored. Andani (2021) found, "neem leaf extract was able to inhibit the growth of Propionibacterium acnes with an inhibition zone diameter of... 30% is 19.6 mm".

RESEARCH METHODS

This study uses a descriptive experimental research type that aims to identify and measure the Sun Protection Factor (SPF) value in ethanol extracts and n-hexane, ethyl acetate, and water fractions from the skin of sour eggplant (Solanum lasiocarpum Dunal.) in vitro using the UV-Vis spectrophotometry method (Azwanida, 2015). The experimental approach is applied through manipulation of independent variables in the form of sample concentrations (100–500 ppm) to observe their effect on the dependent variables, namely the SPF value and its protection category, according to the principles of quantitative methodology that emphasizes objective measurement and replication (Sugiyono, 2019). This method was chosen because it is suitable for testing the biological activity of natural ingredients such as fractionation and SPF evaluation, as recommended in pharmaceutical research (Harborne, 1998; Mirunalini & Arulmozhi, 2011).

The main instruments include a Thermo Scientific UV-Vis spectrophotometer for absorbance measurements at a wavelength of 290–320 nm (UV-B), a DLAB rotary evaporator, a Memmert water bath, a Kern analytical balance, cuvettes, and supporting tools such as a blender, an oven, and a silica gel 60 F254 TLC plate. Data analysis techniques include SPF calculations using the Mansur equation:

$$SPF = CF \times \int EE(\lambda) \times I(\lambda) \times Abs(\lambda)\, d\lambda$$

where CF is the correction factor, EE(λ) is the erythemal effect, I(λ) is the solar intensity spectrum, and Abs(λ) is the sample absorbance (Mansur et al., 1986). The obtained SPF values were then categorized into minimal, moderate, extra, maximal, or ultra protection levels (Damogalad et al., 2013). Qualitative data from phytochemical screening and TLC were analyzed descriptively, while quantitative data were processed using linear regression to validate the Lambert-Beer Law (Skoog et al., 2013).

The study population was all the acid eggplant skin simplicia (Solanum lasiocarpum Dunal.) collected from Tanjung Hulu, Pontianak, West Kalimantan, while the sample was 500 grams of dried simplicia powder (mesh no. 40) which was extracted and fractionated into ethanol extract and n-hexane, ethyl acetate, and water fractions. Sample selection was carried out using a purposive sampling technique based on the criteria of ripe fruit (dark orange), pest-free, and homogeneous to ensure population representation (Setiawan, 2020). The sample size was determined to allow three replications to reduce experimental variability (Kothari, 2004).

The research procedure began with plant determination at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, followed by sample collection (10 kg of ripe fruit), preparation of simplicia through sorting, washing, drying (40–50°C), grinding, and sieving, as well as testing for water content and drying loss (Ministry of Health of the Republic of Indonesia, 2008). Next, powder maceration was carried out (1:10 with 96% ethanol, 3×24 hours), concentration into a thick extract, standardization (water content, ethanol/metal free), multilevel fractionation (n-hexane 3×100 mL, ethyl acetate 3×100 mL, water), phytochemical screening (alkaloids, terpenoids, phenolics, flavonoids) (Harborne, 1998), TLC examination, and SPF testing at multilevel concentrations using UV-Vis spectrophotometry (Mansur et al., 1986). All steps are carried out in stages and documented to ensure the validity and reliability of the results (Sugiyono, 2019).

RESULTS AND DISCUSSION

Plant Determination

Plant determination was carried out at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University under the name UPF Solanum lasiocarpum Dunal, located in Tanjung Hulu, Pontianak, West Kalimantan.

Collection and Preparation of Raw Materials

Ten kg of ripe, orange-colored sour eggplant was obtained from Tanjung Hulu, Pontianak, West Kalimantan. The material was sorted, washed with running water, dried in an oven at 45°C for

approximately 8 hours, ground, and sieved using a 40-mesh mesh to obtain a homogeneous powder. Results of raw material collection and preparation.

Powder Characteristics Examination

Drying shrinkage test was performed on the powdered simplex of sour eggplant (Solanum lasiocarpum Dunal.) peel. Drying shrinkage test is a nonspecific parameter that is not directly related to pharmacological activity, but plays an important role in ensuring the quality, safety, and stability of the material to be used.

Table 1. Results of drying shrinkage test of sour eggplant skin powder

Parameters	Library	Test Results
Drying Loss	No more than 10% (Indonesian Ministry of Health, 2017)	7.62

Preparation of sour eggplant skin extract (Solanum lasiocarpum Dunal.)

The finely ground acid eggplant (Solanum lasiocarpum Dunal.) skin was then extracted using the maceration method. Based on various research literature and textbooks, the maceration method is widely used to extract bioactive compounds from natural materials such as terpenoids, alkaloids, and phenolic compounds because the procedure is relatively simple and effective in extracting active compounds with various types of solvents (Yunita, 2024). This method was chosen because the process and equipment used are easy and do not require special treatment.

500 grams of sour eggplant (Solanum lasiocarpum Dunal.) skin powder was placed in a maceration vessel, then 5000 mL of 96% ethanol was added at a ratio of 1:10. The soaking process was carried out for 3 days with occasional stirring to aid the diffusion of the active compounds into the solvent. Every 24 hours, the 96% ethanol solvent was replaced, then filtered using flannel and filter cloth to separate the filtrate from the dregs (Sari et al., 2021).

The resulting filtrate was then concentrated using a rotary evaporator at 40°C until a thick extract was obtained. The concentration process was continued using a water bath until a thick, concentrated extract was formed. The resulting extract was then weighed to calculate the extract yield (Sari et al., 2021).

Table 2. Results of making sour eggplant skin extract

Powder weight (g)	Extract Weight (g)	Extract Yield (%)
500	66.8	13.36

Extract Characteristics Examination

The finely ground acid eggplant (Solanum lasiocarpum Dunal.) skin was then extracted using the maceration method. Based on various research literature and textbooks, the maceration method is widely used to extract bioactive compounds from natural materials such as terpenoids, alkaloids, and phenolic compounds because the procedure is relatively simple and effective in extracting active compounds with various types of solvents (Yunita, 2024). This method was chosen because the process and equipment used are easy and do not require special treatment.

Table 3. Results of the test of the characteristics of sour eggplant skin extract

Parameter	Library	Reagent	Test Results
Water content	According to FHI, the water content for sour eggplant (Solanum lasiocarpum Dunal.) skin extract is no more than 8.9%.	-	2.67%
Ethanol Free	A positive test for ethanol-free extract is if there is no characteristic ester odor of ethanol (Rosidah et al., 2020).	H2SO4 + CH3COOH	No ester smell
Metal Free	Positive Pb if a yellow precipitate forms and positive Cd if a pink color forms (Rosidah et al., 2020).		
Lead (Pb)		K2CrO4	No yellow precipitate is formed
Cadmium (Cd)		NaOH	No white precipitate is formed

Water content

Determination of water content in the extract is carried out to determine the safe limit of water content allowed in the extract. Water content that is too high can increase the risk of the growth of microorganisms such as fungi, mold, and bacteria, thus potentially reducing the quality and biological activity of the extract during the storage process. In this study, the determination of water content of sour eggplant skin extract (Solanum lasiocarpum Dunal.) was carried out by weighing a 2 gram sample of the extract, then analyzed using a moisture balance tool at a temperature of 105°C.

The analysis results showed that the water content of the extract was 2.67%. This value is still below the maximum permissible limit, which is no more than 10%. If the water content exceeds this limit, changes in the chemical composition of the extract can occur, which will reduce the quality of the herbal medicine and increase its susceptibility to microbial growth (Shina et al., 2024). Calculation of the water content of the sour eggplant skin extract.

Ethanol Free

The ethanol-free test was conducted to determine whether or not there was any residual ethanol in the extract, considering that the extract used in the subsequent testing stages must be completely free of this solvent (Tivani et al., 2021). Ethanol is known to have antifungal and antibacterial activity, so an ethanol-free test is necessary to prevent false-positive results in the test treatment. Thus, the tamarind eggplant (Solanum lasiocarpum Dunal.) peel extract did not contain any residual solvent that could affect the results of the antibacterial activity test (Achmad et al., 2024).

Metal Free

Metal-free testing was conducted on the heavy metals cadmium (Cd) and lead (Pb). This examination aims to ensure that the extract does not contain certain heavy metals exceeding the established limits, considering their toxic and hazardous nature to health. The extract is declared positive for lead if a yellow precipitate forms, while the presence of cadmium is indicated by the formation of a white precipitate. Based on the results presented in Table 5, it shows that the extract of the skin of the sour eggplant (Solanum lasiocarpum Dunal.) was not detected to contain the heavy metals cadmium or lead.

Identification of Phytochemical Compounds of Extracts

Phytochemical screening tests using test tubes include alkaloid, terpenoid, phenolic and flavonoid tests.

Table 4. Results of phytochemical compound identification tests of sour eggplant skin extract

Secondary Metabolites	Reagent	Library (Color)	Results (Color)			
			Extract	n-hexane fraction	Ethyl acetate fraction	Water Fraction
Alkaloid	Mayer	White precipitate forms	(+) White precipitate forms	(+) White precipitate forms	(-) No sediment formed	(-) No sediment formed
Terpenoid	Liebermann-Buchard	Brownish red	(+) Red	(+) Red	(+) Red	(+) Red
Phenolic	FeCl3	Deep green, blue, red, blackish, or blackish blue	(+) Black	(+) Black	(+) Black	(+) Black
Flavonoid	NaOH	Red, yellow or orange	(+) Red	(+) Red	(+) Red	(+) Red
	HCl		(+) Red	(+) Red	(+) Red	(+) Red
	H2SO4		(+) Red	(+) Red	(+) Red	(+) Red

Fractionation of sour eggplant (Solanum lasiocarpum Dunal.) peel extract

A total of 10 grams of concentrated extract was dissolved in 100 mL of warm water, then placed in a separating funnel. The solution was then fractionated using 100 mL of n-hexane. The separating funnel was gently shaken while occasionally opened to release the formed gas, then left to stand until two layers were formed. The bottom layer was aquadest (polar) and the top layer was n-hexane (nonpolar). The two layers were separated and the n-hexane layer was taken. The process of adding n-hexane was repeated three times (3 replicates) to ensure maximum extraction of nonpolar compounds.

Next, 100 mL of ethyl acetate was added to the remaining water fraction, then shaken and left to stand until two layers formed: a layer of distilled water at the bottom and a layer of ethyl acetate at the top. The ethyl acetate layer was then separated. This process was also repeated three times (3 replicates) to obtain optimal semi-polar fraction results.

The results of each fraction were then evaporated using a rotary evaporator at a temperature of 40°C until a concentrated extract was obtained, and the concentration was continued using a water bath until a thicker extract was obtained (Shina et al., 2024).

Table 5. Fractionation results of thick extract of sour eggplant skin

Faction	Extract Weight (grams)	Fraction Weight (grams)	Yield (%)
***n*-hexane**	10 g	0.358 g	3.58
Ethyl Acetate	10 g	0.20 g	2.00
Water	10 g	5.66 g	56.6

TLC Examination of Extracts and Fractions

TLC analysis in this study was conducted to observe the separation patterns of compounds in extracts and fractions based on differences in their polarity levels, which are influenced by the interaction of the compounds with the mobile and stationary phases. This method is used as an initial step to identify the diversity of chemical components contained in the sample through the characteristics of the resulting chromatogram patterns (Peratiwi et al., 2023).

The spots formed on TLC generally do not directly show color, requiring observation using specific methods. Physical spot evaluation was performed using ultraviolet light at a wavelength of 254 nm to visualize the spots formed on the TLC plate (Rahmi et al., 2021). The TLC results are presented in Table 6.

Table 6. TLC Examination of Acidic Eggplant Skin Extract and Fractions

Secondary Metabolites	Motion Phase	Standard Comparative Value	Results (Color)			
			Extract	n-hexane fraction	Ethyl acetate fraction	Water Fraction
Alkaloid	*n*-hexane:ethyl acetate:toluene (6:2:2)	0.42	-	-	0.46	-
Terpenoid	chloroform-ethyl acetate (6:4)	0.62	0.78	0.76	0.8	0.78
Phenolic	*n*-hexane:ethyl acetate (9:1)	-	0.24	-	0.32	-
Flavonoid	*n*-hexane:ethyl acetate (3:7)	0.14	0.1	0.08	0.12	0.08

SPF Value Test Results with UV-Vis Spectrophotometer

The extract of sour eggplant skin (Solanum lasiocarpum Dunal.), n-Hexane fraction, ethyl acetate fraction and water that have been obtained are then made into a stock solution (1000 ppm)

after which it is diluted into 5 different concentrations, namely 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. The calculation of making the test solution can be seen in the appendix. The results of measuring the SPF value and the category of sunscreen effectiveness can be seen in Table 7.

Table 7. SPF Values and Sunscreen Effectiveness Categories

Test Sample	Concentration	SPF value	Category
Sour eggplant skin extract (Solanum lasiocarpum Dunal.)	100 ppm	1.89	Low
	200 ppm	3.78	Low
	300 ppm	5.67	Low
	400 ppm	7.43	Low
	500 ppm	9.32	Low
n-Hexane Fraction	100 ppm	1.58	Low
	200 ppm	2.8	Low
	300 ppm	4.14	Low
	400 ppm	5.56	Low
	500 ppm	6.71	Low
Ethyl acetate fraction	100 ppm	8.72	Low
	200 ppm	17.8	Currently
	300 ppm	26.7	Tall
	400 ppm	32.7	Tall
	500 ppm	36.97	Tall
Water Fraction	100 ppm	1.47	Low
	200 ppm	2.7	Low
	300 ppm	4.15	Low
	400 ppm	5.35	Low
	500 ppm	6.76	Low

Based on Table 7, the results of the SPF value measurements show that the tamarind eggplant skin extract (Solanum lasiocarpum Dunal.) as well as the n-hexane fraction, ethyl acetate fraction, and water fraction have activity as sunscreens as indicated by the SPF value at each concentration.

The SPF values of sour eggplant peel extract at concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm were 1.89; 3.78; 5.67; 7.43; and 9.32, respectively. All of these values are in the low category. However, an increase in the SPF value was observed with increasing concentration, indicating that the effectiveness of protection increased with increasing extract levels.

In the n-hexane fraction, the SPF values obtained at concentrations of 100 ppm to 500 ppm were 1.58; 2.8; 4.14; 5.56; and 6.71, respectively. Similar to the extracts, all of these values were still in the low category. However, a clear pattern of increasing SPF values was also observed with increasing concentration, indicating the contribution of non-polar compounds to sunscreen activity, although they did not yet provide high levels of protection.

Unlike the two previous samples, the ethyl acetate fraction showed the most significant sunscreen activity. The SPF value at a concentration of 100 ppm was 8.72 (low category), increasing to 17.8 (medium category) at 200 ppm, then 26.7; 32.7; and 36.97 at concentrations of 300 ppm, 400 ppm, and 500 ppm, which are included in the high category. These data indicate that the ethyl acetate fraction has the greatest potential as a sunscreen compared to the other fractions, especially at concentrations ≥300 ppm.

Meanwhile, the water fraction at concentrations of 100 ppm to 500 ppm produced SPF values of 1.47, 2.7, 4.15, 5.35, and 6.76, all of which are still in the low category. Although the SPF value increases with increasing concentration, its effectiveness remains lower than that of the ethyl acetate fraction.

The results of this study indicate that the higher the sample concentration, the higher the SPF value obtained. This indicates that increasing concentration is directly proportional to increasing effectiveness as a sunscreen. The ethyl acetate fraction had the highest SPF value, at 36.97 at a concentration of 500 ppm, making it the fraction with the most optimal sunscreen activity.

The high activity of the ethyl acetate fraction is thought to be related to the content of semi-polar compounds such as flavonoids and other phenolic compounds that dissolve in ethyl acetate.

Flavonoids are known to have a conjugated double bond system that allows electronic transitions to occur when absorbing ultraviolet (UV) radiation, both UV-A and UV-B. This ability to absorb UV radiation plays a role in reducing the intensity of UV exposure to the skin, thus providing a protective effect as a sunscreen.

DISCUSSION

This study aims to determine the sunscreen activity of the extract of the skin of sour eggplant (Solanum lasiocarpum Dunal.), as well as the sunscreen activity of the water fraction, ethyl acetate fraction, and n-hexane fraction of the skin of sour eggplant (Solanum lasiocarpum Dunal.). In addition, this study also aims to determine the Sun Protection Factor (SPF) value and the category of sunscreen protection produced from each of these fractions. To support the achievement of the research objectives, the initial stage carried out was the determination of the plant to ensure the accuracy of the identity of the species used as research material so as to minimize errors in sampling.(Hanifa et al., 2021)Accurate plant identification is crucial because species differences can influence secondary metabolite content and the resulting biological activity.

The next stage is the collection and preparation of raw materials, which begins with a sorting and washing process to remove dirt and foreign materials that could degrade the quality of the herbal medicine. Afterward, drying is carried out at a temperature not exceeding 50°C to maintain the stability of the active compounds and prevent damage from excessive heat.(Maslahah, 2024)The dried herbal ingredients are then ground and sieved to obtain uniform particle size. The grinding and sieving process aims to increase the surface area of the material, thereby increasing contact between the solvent and the material during the extraction process. This increased contact is expected to increase the efficiency of the extraction process and the resulting extract yield.

Powder characteristics were examined to determine the amount of weight loss due to the heating process, which is generally caused by the evaporation of water and other volatile compounds. Through the drying shrinkage test, the maximum acceptable weight loss limit can be determined so that the quality of the acidic eggplant skin (Solanum lasiocarpum Dunal.) simplex can be maintained. Based on the results obtained, the drying shrinkage value of 7.62% indicates that the water and volatile compound content in the powder is still within safe limits. This value has met the requirements of the Indonesian Herbal Pharmacopoeia, so the simplex is considered suitable for use in the extraction process without the risk of excessive microbial growth.

The extraction process was carried out using the maceration method using 96% ethanol as a solvent because this solvent is effective in extracting bioactive compounds such as alkaloids, terpenoids, and phenolic compounds. The extraction results showed a yield of 13.36%, which meets the requirements of the Indonesian Herbal Pharmacopoeia, which is ≥11.9%. The relatively high yield value indicates that the 96% ethanol solvent has good ability to optimally dissolve the active compounds from the acidic eggplant skin (Yohanes, 2022).

The extract obtained was then fractionated using solvents with different levels of polarity, namely n-hexane, ethyl acetate, and water, with the aim of separating active compounds based on their polarity. The resulting fractions were then tested for their sunscreen activity in vitro by determining the Sun Protection Factor (SPF) value. The SPF value obtained was used to determine the sunscreen protection category, so that the level of ability of each fraction to protect the skin from exposure to ultraviolet radiation can be determined. The difference in the resulting SPF value indicates that the distribution of active compounds in each fraction makes a different contribution to sunscreen activity.(Shina et al., 2024).

This test aims to determine the amount of weight loss due to the heating process, which is generally caused by the evaporation of water and other volatile compounds. Through the drying shrinkage test, the maximum limit of weight loss that is still acceptable so that the quality of the sour eggplant skin (Solanum lasiocarpum Dunal.) simple powder can be determined. In Table 3, the drying shrinkage value was obtained at 7.62%, indicating that the water and volatile compound content in the

powder is still within safe limits. This value meets the requirements of the Indonesian Herbal Pharmacopoeia, so the simple powder is considered suitable for use in the extraction process without the risk of excessive microbial growth.

The maceration method with 96% ethanol was chosen because it is effective in extracting bioactive compounds such as alkaloids, terpenoids, and phenolics. Maceration is a conventional extraction method carried out by soaking the medicinal plant in a solvent at room temperature, thus minimizing the risk of degradation of thermolabile active compounds due to heating. The soaking process allows the solvent to penetrate the plant cell walls, followed by the dissolution and diffusion of the active compounds into the solvent until equilibrium concentration is reached. In addition, the process of stirring or replacing the solvent during maceration can increase extraction efficiency by accelerating the contact between the solvent and the material. Compared with other extraction methods such as soxhletation and reflux that use continuous heating, maceration is considered safer for maintaining the stability of heat-sensitive phenolic and flavonoid compounds, although it requires a longer extraction time. The use of 96% ethanol as a solvent has the advantage of being semi-polar, allowing it to extract compounds with a wide range of polarities, from polar to semi-polar compounds. It is also relatively safe, easily evaporated, and suitable for the extraction of natural products. The combination of the maceration method and 96% ethanol solvent produced an extract yield of 13.36%, which meets the Indonesian Herbal Pharmacopoeia requirements of ≥11.9%. This relatively high yield indicates that the maceration method is capable of optimally extracting active compounds without damaging their chemical structure, and demonstrates the solvent's efficiency in extracting secondary metabolites from sour eggplant skin (Yohanes, 2022).

Determination of water content in the extract is carried out to determine the safe limit of water content allowed in the extract. Water content that is too high can increase the risk of the growth of microorganisms such as fungi, mold, and bacteria, thus potentially reducing the quality and biological activity of the extract during the storage process. In this study, the determination of water content of sour eggplant skin extract (Solanum lasiocarpum Dunal.) was carried out by weighing a 2 gram sample of the extract, then analyzed using a moisture balance tool at a temperature of 105°C.

The analysis results showed that the water content of the extract was 2.67%. This is in line with research conducted by Shina et al., 2024, where this value is still below the maximum permissible limit, which is no more than 10%. If the water content exceeds this limit, changes in the chemical composition of the extract can occur, which will impact the quality of the herbal medicine and increase susceptibility to microbial growth (Shina et al., 2024). Calculation of the water content of the sour eggplant skin extract.

Ethanol Free

The ethanol-free test was conducted to determine whether or not there was any residual ethanol in the extract, considering that the extract used in the subsequent testing stages must be completely free of this solvent (Tivani et al., 2021). Ethanol is known to have antifungal and antibacterial activity, so an ethanol-free test is necessary to prevent false-positive results in the test treatment. Thus, the tamarind eggplant (Solanum lasiocarpum Dunal.) peel extract did not contain any residual solvent that could affect the results of the antibacterial activity test (Achmad et al., 2024).

Metal Free

Metal-free testing was conducted on the heavy metals cadmium (Cd) and lead (Pb). This examination aims to ensure that the extract does not contain certain heavy metals exceeding the established limits, considering their toxic and hazardous nature to health. The extract is declared positive for lead if a yellow precipitate forms, while the presence of cadmium is indicated by the formation of a white precipitate. Based on the results presented in Table 5, it shows that the extract of the skin of the sour eggplant (Solanum lasiocarpum Dunal.) was not detected to contain the heavy metals cadmium or lead.

Based on the phytochemical screening results presented in Table 6, it shows that the extract and skin fraction of sour eggplant (Solanum lasiocarpum Dunal.) contain several groups of secondary metabolites, namely alkaloids, terpenoids, phenolics, and flavonoids. Positive results are indicated by the formation of color changes or deposits that correspond to the indicators of each reagent. The presence of these secondary metabolites indicates the potential biological activity of the extract and skin fraction of sour eggplant.(Ningsih et al., 2024).

In the terpenoid test using the Liebermann–Burchard reagent, the extract, n-hexane fraction, ethyl acetate fraction, and water fraction showed positive results, indicated by the formation of a red color. This color change indicates a reaction between the terpenoid compound and the reagent, resulting in a colored compound due to complex formation. These results indicate that terpenoid compounds are distributed throughout all fractions, including nonpolar, semipolar, and polar fractions (Putri, 2023).

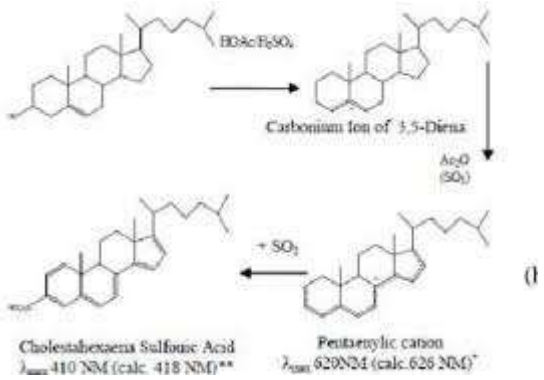

Figure 1. Reactions that occur whenTerpenoid Testing(Hanifa et al., 2021)

Testing for phenolic compounds using the $FeCl_3$ reagent showed positive results in the extract and all fractions, indicated by the formation of a black color. This color appears due to the formation of a complex between the phenol group and the Fe^{3+} ion, resulting in a characteristic color change. This indicates that the phenolic compounds in sour eggplant skin have broad solubility properties and can be distributed across various solvent polarities (Elsyana et al., 2019).

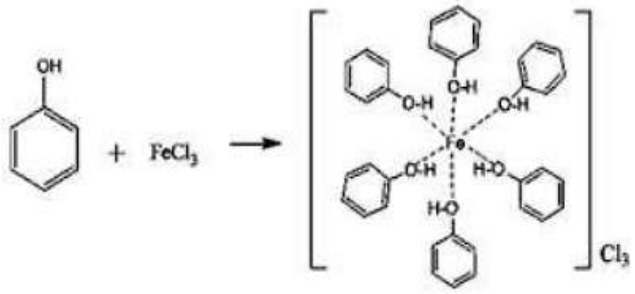

Figure 2. Reactions that occur during the testPhenol (Ramayani et al., 2021)

Flavonoid tests conducted using NaOH, HCl, and H_2SO_4 reagents showed positive results in the extract, n-hexane fraction, ethyl acetate fraction, and water fraction, indicated by the formation of a red color. The formation of this color indicates the presence of flavonoid compounds that undergo a reaction to form flavyllium salts under acidic or basic conditions, resulting in a characteristic color change. These results indicate that the flavonoid compounds in the skin of sour eggplant are distributed in various solvent fractions.(KA et al., 2022).

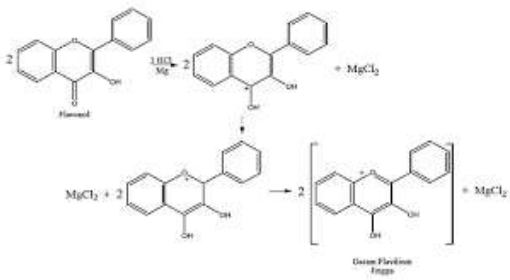

Figure 3. Reactions that occur during the flavonoid test with NaOH(KA et al., 2022)

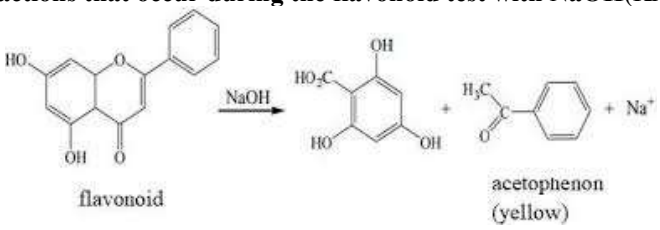

Figure 4. Reactions that occur during the Flavonoid Test with HCl
(KA et al., 2022)

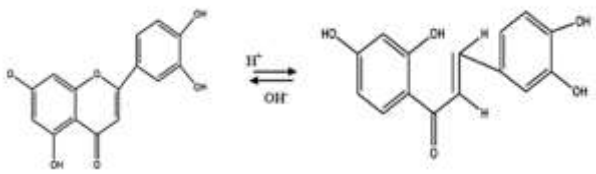

Figure 5. Reactions that occur during the flavonoid test with H2SO4(Desriyana and Iskandar, 2021).

Based on the results of the phytochemical screening, it can be concluded that the extract and fraction of sour eggplant skin (*Solanum lasiocarpum*Dunal.) contains various secondary metabolites with varying degrees of polarity. The distribution of compounds in each fraction is influenced by the solubility properties of the compounds in the solvent used during the fractionation process.(Desriyana and Iskandar, 2021). Documentation of phytochemical screening results is presented in Appendix 10.

Fractionation is a method used to separate and group chemical compounds in an extract based on their polarity. The liquid-liquid extraction process is based on the principle of "like dissolves like," meaning compounds tend to dissolve in solvents with similar polarity. The selection of fractionation solvents is carried out in stages, from nonpolar to polar solvents, with the aim of extracting nonpolar secondary metabolites using nonpolar solvents first. This is because polar solvents have a wider solubility range, allowing them to dissolve nonpolar, semipolar, and polar compounds (Shina et al., 2024). The concentrated extract of sour eggplant (Solanum lasiocarpum Dunal.) peel was then fractionated to separate compounds based on their differences in polarity.

The yield of the extract fraction is the ratio of the weight of the fraction obtained to the weight of the initial extract used. The yield value indicates the number of compounds successfully distributed into each solvent during the fractionation process. Based on the results obtained, the highest yield was found in the water fraction at 56.6%, while the lowest yield was found in the ethyl acetate fraction at 2.00%, followed by the n-hexane fraction at 3.58%.

The high yield in the water fraction indicates that the compounds contained in the extract of sour eggplant (Solanum lasiocarpum Dunal.) are dominated by polar compounds that are easily soluble in water solvents. Conversely, the low yield in the ethyl acetate and n-hexane fractions indicates that the content of semipolar and nonpolar compounds in the extract is relatively less. This is in line with research conducted by Maulana et al., 2025 which shows that polar compounds are more dominantly extracted by solvents with a high level of polarity such as water. The principle of fractionation is based on polarity, where compounds will tend to dissolve in solvents with an appropriate level of polarity (Maulana et al., 2025).

The appearance of fluorescence of a compound when observed under UV light at a wavelength of 254 nm indicates the presence of a compound with a conjugated double bond system. Under 254 nm UV light, the TLC plate will emit fluorescence, making it appear bright, while the compound spots will appear as dark spots due to ultraviolet light absorption (Peratiwi et al., 2023).

Based on the identification results using thin layer chromatography (TLC), it is known that the extract and fraction of the skin of sour eggplant (Solanum lasiocarpum Dunal.) show varying Rf values according to the type of secondary metabolite and the mobile phase system used. The differences in Rf values reflect variations in the polarity level of the compounds and their interactions with the stationary and mobile phases.(Shina et al., 2024)However, in some fractions no Rf values were detected, indicating that the target compounds were not separated or were not detected under the TLC conditions used. Calculation of Rf values of extracts and fractions.

Identification of alkaloids using the mobile phase n-hexane:ethyl acetate:toluene (6:2:2) with a standard Rf value of 0.42 showed that alkaloids were not detected in the extract, n-hexane fraction, and water fraction, while the ethyl acetate fraction showed an Rf value of 0.46. The undetectable Rf value in some fractions can be caused by the low concentration of alkaloid compounds in the sample or the incompatibility of the polarity level of the compound with the mobile phase system used, so that the compound is retained at the starting point or does not form an observed spot (Forestryana, 2020).

Identification of terpenoids using a chloroform:ethyl acetate (6:4) mobile phase showed that the extract and all fractions of the tamarind eggplant skin had Rf values ranging from 0.76–0.80. This is in line with research conducted by Wulandari et al. (2020), which stated that the use of a chloroform:ethyl acetate mobile phase was able to optimally separate terpenoid compounds in TLC analysis, indicated by relatively high and uniform Rf values in the extract and fractions. The similarity of these Rf values indicates that the terpenoid compounds have polarity characteristics that match the mobile phase system used, so they can be detected evenly in all fractions. Terpenoid compounds are known to have a fairly wide polarity range so they are easily distributed in various solvent fractions.(Rahmi et al., 2021).

In the identification of phenolic compounds using the mobile phase n-hexane:ethyl acetate (9:1), the Rf value was only detected in the extract and ethyl acetate fraction, while the n-hexane and water fractions did not show any spots. The absence of Rf values in certain fractions may be caused by a very low amount of phenolic compounds or because these compounds have a strong affinity for the stationary phase, so they do not move with the mobile phase. In addition, another possibility is that the phenolic compounds have been extracted more dominantly in the ethyl acetate fraction so that their content in other fractions is very small (Sari et al., 2023).

Flavonoid identification using n-hexane:ethyl acetate (3:7) mobile phase showed that flavonoids were detected in the extract and all fractions with Rf values close to the reference standard. However, small differences in Rf values between fractions may occur due to variations in flavonoid compound composition, differences in spot intensity, and the influence of the sample matrix which can affect compound migration on the TLC plate (Maulana, 2025).

In general, undetectable Rf values in some fractions indicate that the secondary metabolite content in those fractions is below the detection limit of the TLC method or is not compatible with the mobile phase system used. Other factors that can affect TLC results include sample concentration, silica layer thickness, mobile phase composition, and sample spotting technique. Therefore, TLC serves as a preliminary analysis method whose results are greatly influenced by testing conditions (Elisabeth, 2020).

Determination of the SPF value of the extract of sour eggplant (Solanum lasiocarpum Dunal.) n-hexane fraction, ethyl acetate fraction and water fraction was carried out in vitro using a UV-Vis spectrophotometer at a wavelength of 290-320 nm with the Mansur equation. This wavelength represents the wavelength of UV B rays (290-230 nm) which is in the erythmogenic region that can cause sunburn.(Peratiwi et al., 2023).

A sunscreen preparation is considered to have protective capabilities if it has an SPF value of at least 2. Preparations with an SPF value above 15 are categorized as ultra protection and are considered to have a good level of protection. An SPF value above 15 indicates a more optimal ability to protect the skin from the effects of ultraviolet light exposure, including the risk of long-term skin damage such as skin cancer. Furthermore, the higher the SPF value, the longer the skin can be protected from sun exposure.(Andini et al., 2023).

Based on Table 7, the results of the SPF value measurements show that the tamarind eggplant skin extract (Solanum lasiocarpum Dunal.) as well as the n-hexane fraction, ethyl acetate fraction, and water fraction have activity as sunscreens as indicated by the SPF value at each concentration.

The SPF values of sour eggplant peel extract at concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm were 1.89; 3.78; 5.67; 7.43; and 9.32, respectively. All of these values are in the low category. However, an increase in the SPF value was observed with increasing concentration, indicating that the effectiveness of protection increased with increasing extract levels.

In the n-hexane fraction, the SPF values obtained at concentrations of 100 ppm to 500 ppm were 1.58; 2.8; 4.14; 5.56; and 6.71, respectively. Similar to the extracts, all of these values were still in the low category. However, a clear pattern of increasing SPF values was also observed with increasing concentration, indicating the contribution of non-polar compounds to sunscreen activity, although they did not yet provide high levels of protection. In vitro determination of SPF values using a UV-Vis spectrophotometer (290-320 nm range and the Mansur formula) often produces varying SPF values between plant extracts due to differences in the presence of bioactive compounds that absorb UV light. This in vitro method is widely used because it is efficient and valid for the initial stages of exploring the potential of natural ingredients, although it generally produces lower SPF values than in vivo methods or clinical trials (Iskandar, 2022).The absorbance value is still acceptable because it is within the linear range of the UV-Vis spectrophotometer and shows a proportional relationship with increasing concentration, in accordance with the Lambert–Beer law, so it is valid for use in in vitro SPF calculations. The higher the absorbance value of a material to UV rays (especially UV-B in the range of 290-320 nm), the higher the Sun Protection Factor (SPF) value will be. High absorbance indicates that the material is effective in absorbing UV radiation, thereby increasing skin protection. (Fadila, 2023).

Unlike the two previous samples, the ethyl acetate fraction showed the most significant sunscreen activity. The SPF value at a concentration of 100 ppm was 8.72 (low category), increasing to 17.8 (medium category) at 200 ppm, then 26.7; 32.7; and 36.97 at concentrations of 300 ppm, 400 ppm, and 500 ppm, which are included in the high category. These data indicate that the ethyl acetate fraction has the greatest potential as a sunscreen compared to the other fractions, especially at concentrations ≥300 ppm.

Meanwhile, the water fraction at concentrations of 100 ppm to 500 ppm produced SPF values of 1.47, 2.7, 4.15, 5.35, and 6.76, all of which are still in the low category. Although the SPF value increases with increasing concentration, its effectiveness remains lower than that of the ethyl acetate fraction.

The results of this study indicate that the higher the sample concentration, the higher the SPF value obtained. This indicates that increasing concentration is directly proportional to increasing effectiveness as a sunscreen. The ethyl acetate fraction had the highest SPF value, at 36.97 at a concentration of 500 ppm, making it the fraction with the most optimal sunscreen activity.

The high activity of the ethyl acetate fraction is thought to be related to the content of semi-polar compounds such as flavonoids and other phenolic compounds that dissolve in ethyl acetate. Flavonoids are known to have a conjugated double bond system that allows electronic transitions to occur when absorbing ultraviolet (UV) radiation, both UV-A and UV-B. This ability to absorb UV radiation plays a role in reducing the intensity of UV exposure to the skin, thus providing a protective effect as a sunscreen.

CONCLUSION

This study successfully identified that the ethyl acetate fraction of the ethanol extract of sour eggplant (Solanum lasiocarpum Dunal.) peel has the most optimal sunscreen activity with the highest SPF value reaching 36.97 at a concentration of 500 ppm (high category), followed by a gradual increase from the low category at low concentrations to medium and high as the concentration increases. The ethanol extract and the n-hexane and water fractions showed lower SPF values (maximum 9.32, 6.71, and 6.76), remaining in the low to medium category, with a linear increase pattern that fits the Lambert-Beer Law. These findings are supported by positive phytochemical screening for alkaloids, terpenoids, phenolics, and flavonoids, especially in the ethyl acetate fraction, which contributes to UV-B absorption (290-320 nm). However, the main limitations lie in in vitro testing which produces relatively low SPF values compared to in vivo methods, as well as compound variability due to the single sample collection location.

Practical implications: The ethyl acetate fraction has the potential to be developed as a natural sunscreen active ingredient for environmentally friendly cosmetic products, especially for sensitive skin in tropical regions like Indonesia. Suggestions for further research include in vivo testing in animals or humans to validate efficacy and stability, isolation of specific compounds (main flavonoids) via HPLC, and cream formulation with skin irritation and storage stability testing for commercial applications. This approach will strengthen scientific evidence and support the local utilization of endemic plants in West Kalimantan.

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