
Formulation Of Antifungal Solid Soap From 96% Ethanol Extract Of Neem Leaves (*Azadirachta Indica* A.Juss) Against *Malassezia Furfur* ATCC 14251

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Abstract

Fungal skin infections are still a health problem in tropical regions, especially those caused by *Malassezia furfur*, so alternative natural skin cleansing preparations are needed that are safe and effective. Neem leaves (*Azadirachta indica* A. Juss) are known to have antifungal activity and have the potential to be developed into solid soap preparations. This study aims to formulate an antifungal solid soap made from 96 percent ethanol extract of neem leaves, evaluate the physical quality of the preparation, and test its antifungal activity against *Malassezia furfur*. This study is a laboratory experimental study with a pretest-posttest only control group design. The study population was neem leaves obtained from Mount Pati District, Semarang, with simplicia samples selected using a purposive sampling technique. The research instruments included formulation equipment, physical quality tests based on SNI 3532:2016, and antifungal activity tests using the well diffusion method, while data analysis was carried out using nonparametric statistical tests. The results showed that all soap formulas met the physical quality requirements, and the formulation with a 10 percent extract concentration produced the largest inhibition zone diameter against *Malassezia furfur*, although it was still relatively weak. The conclusion of this study is that neem leaf extract solid soap has potential as a natural skin cleanser with antifungal activity, but further development and optimization of the formulation are needed to increase its effectiveness.

Keywords: Antifungal Activity, *Malassezia Furfur*, Neem Leaf Extract, Solid Soap, Tropical Skin Infection

INTRODUCTION

The skin is the largest organ of the body that functions as the main barrier against external pathogens such as bacteria, fungi, and viruses, which often live on its surface and trigger skin diseases when there is an imbalance [Yulianti *et al.*, 2024]. In Indonesia with a tropical rainy climate, high humidity levels and temperatures of 28-33°C exacerbate fungal skin infections, including pityriasis versicolor caused by *Malassezia furfur*, a dimorphic and lipophilic normal skin flora [Sibero, 2022].

The fungus *Malassezia furfur* produces lipase and phospholipase enzymes that break down sebum triglycerides into free fatty acids, irritate the skin, disrupt the epidermal barrier, inhibit tyrosinase, causing hypopigmentation, and trigger inflammation through a local immune response [Sibero, 2022]. This disease is characterized by fine scaly patches, hypopigmentation without significant inflammation, and excessive itching, with a high prevalence in tropical regions such as Indonesia, where approximately 50% of the population is reported to be affected [Tarigan Sibero, 2022].

Although various antifungal preparations exist against *Malassezia furfur*, previous studies have not developed solid soaps based on natural ingredients such as neem leaf extract (*Azadirachta indica* A. Juss) with physical quality evaluation according to SNI 3532:2016 [Nurmalasari *et al.*, 2024]. Ethanol extract of neem leaves is rich in azadirachtin, nimbin, and nimbidin which inhibit fungal growth through cell wall damage, proven effective against *Candida albicans* at a concentration of 1.25-2.5% as well as *Malassezia furfur* in shampoo preparations [Fahdi *et al.*, 2023; Bila *et al.*, 2024]. However, there is no solid soap formulation that optimizes this activity while meeting organoleptic parameters, pH, water content, free alkali, foam stability, and free fatty acids.

This study aims to formulate a 96% ethanol extract of neem leaves into a solid soap that meets standard physical quality, evaluate its antifungal activity against *Malassezia furfur*, and determine the optimal concentration for the highest inhibition. The urgency of the study arises from the need for alternative natural skin cleansers that are safe and effective amidst the high prevalence of fungal

infections in Indonesia, while the novelty lies in the development of the first solid soap preparation with neem as the active ingredient that is comprehensively tested against *Malassezia furfur*, surpassing previous shampoo or single extract studies.

RESEARCH METHODS

This research is a laboratory experiment with a pre-post test only control group design to test the effect of 96% ethanol extract concentration of neem leaves (*Azadirachta indica* A. Juss) namely 1.25%, 2.5%, 5%, and 10% on the physical quality of solid soap and antifungal activity against *Malassezia furfur* [Sugiyono, 2021]. A quantitative approach was applied to measure quality parameters such as organoleptic, pH, water content, free alkali, foam stability, and free fatty acids according to SNI 3532:2016, as well as antifungal inhibition zones using the well diffusion method [Creswell & Creswell, 2023]. The independent variable is the extract concentration, the dependent variable includes the results of quality tests and inhibition zones, while the control variables include the incubation temperature of 35-37°C, the composition of the soap base (VCO 35%, NaOH 9%, etc.), and PDA media [Yulia *et al.*, 2024].

Research instruments include tools such as macerators, rotary evaporators, ovens, incubators, analytical balances, calipers for measuring inhibition zones, as well as materials such as 96% ethanol, VCO, NaOH, PDA, ketoconazole as a positive control, and DMSO [Pusmarani *et al.*, 2023]. Data collection techniques involve simplicia maceration, hot process formulation, standardization tests (drying loss <10%, water content <10%), phytochemical screening (alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids), preliminary antifungal tests, and physical quality evaluation [Zunnita *et al.*, 2024]. Data analysis using SPSS for normality (Shapiro-Wilk) and homogeneity (Levene) tests, followed by One-Way ANOVA if normal/homogeneous or Kruskal-Wallis/Mann-Whitney if not, with post-hoc to determine the effective concentration ($p < 0.05$) [Sudaryono, 2022][Emzir, 2021].

The study population was neem leaves (*Azadirachta indica* A. Juss) from Gunung Pati District, Semarang, Central Java, which were taken by purposive sampling based on the criteria of dark green, pest-free, and fresh as much as 3 kg [Sibero, 2022]. The simplicia samples were sorted, dried in indirect sunlight, ground (mesh 40), and maceration extracted (1:10 ethanol 96%, 3x24 hours) producing a thick extract for formulation F1-F4 (F0 as blank) [Fahdi *et al.*, 2023]. Plant determination was carried out at the Yankerstrad Laboratory dr. Sardjito Tawangmangu for identity validation, while *Malassezia furfur* cultures were obtained from laboratory stocks and rejuvenated on PDA 37°C [Nurmalasari *et al.*, 2024].

The procedure begins with preparation of simplicia (sorting, drying, grinding), standardization (drying loss, water content, ash), 96% ethanol maceration, extract standardization (organoleptic, water/ash content, ethanol-free), and phytochemical screening using Mayer, Wagner, Dragendorff reagents, etc. [Bila *et al.*, 2024][Irwan *et al.*, 2024]. Preliminary antifungal test of the extract was carried out via well diffusion on PDA with incubation at 37°C/24-48 hours, followed by hot process soap formulation: melt stearic acid+VCO, add extract, glycerin, SLS, NaCl, NaOH, print, and dry [Yulia *et al.*, 2024]. Evaluation of physical quality (organoleptic, pH 9-10, water content <23%, stable foam, free alkali $\leq 0.1\%$, free fatty acids) and antifungal test of soap (1g/10mL aquadest, 6mm well, incubation, measuring inhibition zone vs ketoconazole/DMSO) were carried out in triplicate, with statistical analysis for validation [Pusmarani *et al.*, 2023]. The study took place from December 2025 to January 2026 at the STIFARM Laboratory in Semarang [SNI 3532, 2016].

RESULTS AND DISCUSSION

Neem Leaf Sampling

The samples used in this observation were neem leaf plants (*Azadirachta indica* A. Juss) obtained from Sekaran Village, Gunung Pati District, Semarang City, Central Java, which are generally located

around 7°03' South Latitude and 110°23' East Longitude or in decimal terms at coordinates -7.050° and 110.390° , then used as sample material for the observation test.

Plant Determination

Plant determination was carried out to determine the truth of the neem leaf sample (*Azadirachta indica A.Juss*) by matching the characteristics and morphology of the plant to be studied in order to avoid errors in the plants to be used for research. The results of plant determination with No. TL.02.04 / D.XI.6 / 38062.1014 / 2025 which was carried out at the Yankerstrad Laboratory dr. Sardjito Tawangmangu on December 3, 2025 showed that the plant studied was correct, namely the neem leaf plant (*Azadirachta indica A.Juss*) which is included in the *meliaceae* family, Species *Azadirachta indica A.Juss*, synonym *Melia indica A.Juss*.

Neem Leaf Simple Processing

The results of the calculation of the yield of the simplicia powder in neem leaf powder produced a yield value of 27.5%.

Standardization Results of Neem Leaf Simple Powder

Organoleptic Test

Table 1. Results of Organoleptic Test of Simplicia Standardization

Test	Results
Texture	Fine Powder
Smell	Typical Leaf
Color	Green

Table 2. Results of the Simplicia Standardization Test

No	Parameter	Results	Condition
1	Drying shrinkage	5.70%	$\leq 10\%$
2	Water content	6.03%	$\leq 10\%$

Determination of Drying Loss

The observation results for drying the powder in this observation obtained a result of 5.7%. The value requirement for drying shrinkage, unless otherwise stated, is $\leq 10\%$.

Water Content Test

The percentage of imba leaf powder measured using a moisture balance tool was 6.03%. This result indicates that the water content meets the requirements if the powder and extract are not less than 10%.

Making 96% Ethanol Extract of Neem Leaf Simple Powder

The results of the calculation of the yield of the simplicia powder in neem leaf powder produced a yield value of 16.01%.

Neem Leaf Extract Standardization Results

Organoleptic Test

Table 3. Organoleptic Test Results of Neem Leaf Extract

Test	Results	MMI Requirements
Texture	Thick	Thick
Smell	Neem Leaf Extract Benefits	Neem Leaf Extract Benefits
Color	Blackish Green	Blackish Green
Flavor	Bitter	Bitter

Determination of Water Content

Table 4. Results of Determination of Water Content of Extract

Replication	Results	Condition
1	8.52	$\leq 10\%$ (Ministry of Health of the Republic of Indonesia, 2017)
2	7.43	
3	6.07	
Average	7.34	

Determination of Ash Content

Table 5. Results of Ash Content Test of Neem Leaf Extract

Replication	Results	Condition
1	3.5	≤ 8%
2	3.75	(Ministry of Health of the Republic of Indonesia, 1995)
3	4	
Average	3.75	

Ethanol Free Test

Table 6. Ethanol Free Test Results

No	Reagent	Theoretical Results	Test Results	Information
1	As. Glacial Acetate + Concentrated H2SO4 heated	Smells of ester	No ester odor	Ethanol-free extract
2	Sulfanilic acid + HCl + NaNO2 + NaOH	Raspberry red color is formed	Raspberry red color is formed	Ethanol-free extract

Phytochemical Screening

Table 7. Phytochemical Screening Results of Neem Leaf Extract

Compound Test	Reagent	Results Based on Literature	Results	Note
Alkaloid	Mayer	A yellowish white precipitate forms.	white precipitate forms.	+
	Wagner	A blackish brown precipitate forms.	brown precipitate forms.	+
	Dragendorff Mg Powder + HCl	A brick red precipitate forms. A yellow, orange/red color appears on the amyl alcohol layer.	brick red precipitate forms. A red color appears on the amyl alcohol layer.	+
Flavonoid	Concentrate + Amyl Alcohol			+
	Saponin	Aquadest + HCl 2N	A stable foam is formed for 10 minutes.	A stable foam is formed.
Tannin	FeCl3 1%	A blackish green color is formed.	A blackish green color is formed.	+
	NaCl 10% + Gelatin 1%	A white precipitate forms.	White precipitate forms	+
Steroid	n-hexane + As. Acetic Anhydrous + H2SO4 Concentrated	A blue/green color is formed in the residue (steroid).	green color is formed on the steroid.	+
	n-hexane + As. Acetic Anhydrous + H2SO4 Concentrated	If (terpenoid) red purple color.	Purple is formed on terpenoids.	+
Terpenoid	Concentrated			

Preliminary Antifungal Test Results of Neem Leaf Extract

Table 8. Results of Preliminary Test of Clear Zone Diameter of Neem Leaf Extract

Replication	Clear Zone Diameter (mm)					
	1.25%	3%	5%	10%	Control (+)	Control (-)
1	0.72	1.56	1.8	1.86	1.86	0
2	0.56	0.68	1.12	1.08	1.14	0
3	0.74	0.99	1.72	1.96	1.73	0
Average	0.67	0.36	1.54	1.36	1.57	0

Preliminary Antifungal Test Results of Neem Leaf Extract Organoleptic Test

Table 9. Organoleptic Test Results of Soap Preparations

Formulation	Color	Smell	Form
F1 (1.25%)	Light brown	Typical neem leaf extract	Congested
F2 (2.5%)	Greenish light brown	Typical neem leaf extract	Congested
F3 (5%)	Dark chocolate	Typical neem leaf extract	Congested
F4 (10%)	Dark brown to black	Typical neem leaf extract	Congested

pH test

Table 10. Results of pH Test of Soap Preparations

Replication	pH Result Formula			
	F1 (1.25%)	F2 (2.5%)	F3 (5%)	F4 (10%)
1	9	9	10	10
2	10	9	9	9
3	10	10	10	9
Average	9.66	9.33	9.66	9.33
Information	Meets the requirements according to SNI 9-11			

Water Content Test

Table 11. Results of Soap Water Content Test

Replication	Water Content Result Formulation			
	F1 (1.25%)	F2 (2.5%)	F3 (5%)	F4 (10%)
1	6.5	7.8	12	14.2
2	11.8	8.6	7.2	11.4
3	7.2	10.8	12.6	7.8
Average	8.5%	9.06%	10.6%	11.13%
Information	Meets the requirements according to SNI ≤ 15%			

Foam Stability Test

Table 12. Soap Stability Test Results

Replication	Foam Stability Test Results				
	F0	F1 (1.25%)	F2 (2.5%)	F3 (5%)	F4 (10%)
1	43	47	33	47	63
2	43	42	48	32	40
3	43	44	32	41	32
Average	43%	44.33%	37.66%	40%	45%%
Information	Stable Foam 60-70%(Musliikh <i>et al.</i> , 2024)				

Free Alkali Test

Table 13. Soap-Free Alkali Test Results

Replication	Free Alkali Content Results			
	F1 (1.25%)	F2 (2.5%)	F3 (5%)	F4 (10%)
1	0.1	0.11	0.09	0.11
2	0.08	0.08	0.09	0.09
3	0.08	0.1	0.09	0.09
Average	0.08%	0.09%	0.09%	0.09%
Information	Meets SNI Level 0.01% - 0.10%			

Free Fatty Acid Test

Table 14. Soap-Free Fatty Acid Test Results

Replication	Free Fatty Acid Results			
	F1 (1.25%)	F2 (2.5%)	F3 (5%)	F4 (10%)
1	0.5	0.42	0.48	0.55
2	0.32	0.4	0.5	0.52
3	0.38	0.45	0.52	0.58
Average	0.35	0.42	0.5	0.55
Information	Meets SNI Free Fatty Acid Max. 2.5%			

Antifungal Activity Test of Neem Leaf Extract Soap Preparation

Table 15. Average Results of Antifungal Activity Test of Neem Leaf Extract Soap Preparation.

Replication	1.25%	3%	5%	10%	Control (+)
1	0.35	0.41	0.38	0.52	0
2	0.34	0.34	0.36	0.52	0
3	0.3	0.33	0.35	0.5	0
Average	0.33	0.36	0.36	0.51	0

SPSS Data Test Results

Table 16. Results of SPSS Data Normality Test

	Tests of Normality						
	Perlakuan	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Hasil	1,25%	,314	3	.	,893	3	,363
	2,5%	,343	3	.	,842	3	,220
	5%	,253	3	.	,964	3	,637
	10%	,385	3	.	,750	3	,000
	K+	.	3	.	.	3	.

a. Lilliefors Significance Correction

Based on the results of the normality test using the Shapiro–Wilk method (because the number of samples for each group was less than 50, namely $n = 3$), the significance value obtained at a concentration of 1.25% was 0.363, a concentration of 2.5% was 0.220, and a concentration of 5% was 0.637. All three values were greater than 0.05 ($p > 0.05$), so it can be concluded that the data in the three groups were normally distributed. Meanwhile, at a concentration of 10%, a significance value of 0.000 ($p < 0.05$) was obtained, indicating that the data in that group were not normally distributed. Thus, because there was one treatment group that did not meet the assumption of normality, the overall data did not meet the requirements for parametric testing, so further statistical analysis was continued using nonparametric tests.

Table 17. SPSS Kruskal-Wallis Test Results

Ranks			
	Perlakuan	N	Mean Rank
Hasil	1,25%	3	6,33
	2,5%	3	7,83
	5%	3	9,83
	10%	3	14,00
	K+	3	2,00
	Total	15	

Test Statistics^{a,b}

Hasil	
Kruskal-Wallis H	11,873
df	4
Asymp. Sig.	,018

a. Kruskal Wallis Test

b. Grouping Variable:
Perlakuan

Based on the Ranks table in the Kruskal–Wallis test, it can be seen that the highest mean rank value is at a concentration of 10%, which is 14.00, which indicates that this group has the greatest observation results compared to other groups. Next, followed by a concentration of 5% with a mean rank of 9.83, a concentration of 2.5% of 7.83, and a concentration of 1.25% of 6.33. Meanwhile, the

positive control (K+) has the lowest mean rank value of 2.00, which indicates the smallest results among all treatment groups. Descriptively, these data show a tendency for an increasing effect along with increasing extract concentration, where a concentration of 10% gives the most optimal results. However, to ensure whether the difference is statistically significant, it is necessary to see the significance value in the Test Statistics table from the Kruskal–Wallis test.

Table 1. SPSS Man-Whitney Test Results

Ranks				
	Perlakuan	N	Mean Rank	Sum of Ranks
Hasil	1,25%	3	5,00	15,00
	K+	3	2,00	6,00
	Total	6		

Based on the Ranks table in the Mann–Whitney test between the 1.25% concentration and the positive control (K+), it can be seen that the 1.25% concentration has a mean rank value of 5.00 with a sum of ranks of 15.00, while the positive control (K+) has a mean rank of 2.00 with a sum of ranks of 6.00. The higher mean rank value at the 1.25% concentration indicates that descriptively the observation results at the 1.25% concentration are greater than those of the positive control. Thus, it can be said that the 1.25% concentration tends to provide a better effect than K+, but to ensure whether the difference is statistically significant, it is necessary to see the significance value (Asymp. Sig.) in the Mann–Whitney test Test Statistics table.

DISCUSSION

This study aims to determine the formulation of antifungal solid soap preparation from 96% ethanol extract of neem leaves (*Azadirachta indica A.Juss*) against *Malassezia furfur* with the formulation concentrations used were 1.25%, 2%, 5% and 10%. The initial stage of this study by taking samples used in this observation was neem leaf plants (*Azadirachta indica A.Juss*) obtained from Sekaran Village, Gunung Pati District, Semarang City, Central Java which are generally located around 7°03' South Latitude and 110°23' East Longitude or in decimal terms at coordinates -7.050° and 110.390° and selected leaves that are still semi-dark green because in the growth phase the content of secondary metabolites is at a relatively optimal level.(Jaya *et al.*, 2025). Next, plant determination was carried out at the Yankerstrad Laboratory of Dr. Sardjito to ensure the correctness of the plant identity, and the results of plant determination with No. TL.02.04 / D.XI.6 / 38062.1014 / 2025 which was carried out at the Yankerstrad Laboratory of Dr. Sardjito Tawangmangu on December 3, 2025 showed that the plant studied was indeed the neem leaf plant (*Azadirachta indica A.Juss*) which is included in the *meliaceae* family, Species *Azadirachta indica A.Juss*, synonym *Melia indica A.Juss.*.

The process of making neem leaf powder will be made into dry powder. Dry neem leaf powder is obtained by harvesting neem leaves that are still young green, then dry sorting by separating the leaves from the stems. After that, washing is carried out under running water to separate the leaves from foreign objects with remaining dust or dirt attached. The neem leaves are then dried in the sun using a black cloth which aims to prevent the simplicia from direct contact with direct sunlight so that the compounds contained in them do not change or damage. The purpose of drying is to reduce the water content by 10% and prevent the growth of other microorganisms so that the simplicia can be stored for a long time.(Aulia *et al.*, 2024)The dried neem leaves are then sorted, dried, and reduced in size using a blender. This process increases the surface area of the powder, allowing for optimal extraction and for the extracting fluid to extract all the compounds.(Aulia *et al.*, 2024). Neem leaf powder is sieved using a mesh sieve number 40 to obtain powder particles that are not too large and not too small so that the extraction process is more optimal. The results of the calculation of the yield of neem leaf simplicia powder are 27.5% as seen in table (4.4) which has been obtained from weighing the weight of neem leaf simplicia is 4000 g and the dry weight of neem leaves is 1200 g. From the

results of the data obtained a percentage of 27.5% and is in accordance with the standard of >10% (Ministry of Health of the Republic of Indonesia, 2017).

Extraction using 96% ethanol solvent produced a yield of 16.01%, as shown in table (5.5), which is considered good because it exceeds the minimum expected yield limit ($\geq 10\%$). This is in accordance with research by Bila *et al.*, (2024) that neem leaves contain compounds contained in neem leaves, namely azadirachtin, nimbin, and nimbidin. As well as alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids.

Based on table 4.8, the results of the neem leaf extract water content test obtained an average result of 7.34%. The results of the study by Syarif *et al.*, (2022) show that if the water content of the ethanol extract meets the requirements of $\leq 10\%$ and is considered to have met quality standards because low water content can inhibit the growth of microorganisms and maintain the stability of active compounds in the extract. If the water content exceeds 10%, it indicates excessive moisture which can accelerate the degradation of active compounds and increase the risk of microbial contamination.

The results of the ash content value of neem leaf extract of 3.75% still meet the requirements for total ash content, namely with a percentage based on the limit of $\leq 5\%$ and based on (Ministry of Health of the Republic of Indonesia, 1995) which stipulates an ash content of no more than 8%. According to research by Syarif *et al.*, (2022), an ash content that meets the requirements indicates that the inorganic residue content in the extract is relatively low, so it can be said that the extract has a good level of purity and minimal inorganic contamination such as sand, soil, or metal. The low ash content also reflects that the processing and heating processes have been carried out properly. Thus, the neem leaf extract obtained meets one of the quality parameters in extract standardization and is suitable for use as an active ingredient in preparations.

The results of the phytochemical screening carried out showed that the neem leaf extract contained positive alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids. Alkaloid compounds were identified by adding HCL which aims to increase the solubility of alkaloids because alkaloids are basic when extracted with HCL solvents aiming to attract alkaloid compounds in the extract because alkaloids are basic then with the addition of acids such as HCl will form salts, so that the alkaloids will be separated from other components of the plant cells that are also extracted by distributing them to the acid phase (Oktapiya *et al.*, 2022). Alkaloid compounds are dropped with Mayer's reagent, a yellowish white precipitate will form, while for the second alkaloid compound, Wagner's reagent will form a brown precipitate, and the third alkaloid compound, Dragendorff's reagent will form a brick red precipitate. The principle of this method is the precipitation reaction that occurs due to the replacement of ligands in the form of transition metals in Mayer's, Wagner's, and Dragendorff's reagents. (Arnida *et al.*, 2021). Flavonoid compounds are identified by adding concentrated HCl drops to magnesium powder, resulting in an orange color on the amyl alcohol layer. The addition of magnesium powder and concentrated HCl will cause the flavonoid compounds to be reduced and form flavylum salts. Testing for this group of compounds produces a red to orange color. (Oktapiya *et al.*, 2022). The result is positive for flavonoids, with a red color appearing on the amyl alcohol layer. Saponin compounds are identified by adding 2N HCl with distilled water. This function will result in the formation of stable foam with a height of 1-10 cm. The hydrophilic and hydrophobic groups act as active surfaces in the formation of foam produced when its stability is tested with the addition of HCl. Saponins are soluble in water due to the presence of hydrophilic groups that can form hydrogen bonds with water molecules. In this reaction, water functions as a solvent while 2N HCl functions as a reagent. (Oktapiya *et al.*, 2022). Tannin compounds were identified with 1% FeCl_3 and in the reaction of 10% NaCl and 1% gelatin which will form a precipitate. The initial identification results with iron salt showed a blackish green color meaning the presence of phenol compounds. Furthermore, for the identification of tannins, gelatin salt was used because tannins will form polymers that have a greater specific gravity so they cannot dissolve in water which will eventually form a white precipitate. The addition of NaCl is used to eliminate compounds that are not

tannins that are able to give reactions or false positives. Steroid and triterpenoid compounds, the analysis of these compounds is based on the ability of these compounds to form colors with concentrated H₂SO₄ in anhydrous acetic acid solvents. The results obtained showed a negative result due to the formation of a brownish ring indicating triterpenoid content and no formation of a greenish blue ring so it is negative for containing steroids. (Salamah *et al.*, 2025).

Based on the results of preliminary antifungal tests on neem leaf extract, it showed antifungal activity at all concentrations tested, namely 1.25%, 2.5%, 5%, and 10%. The average diameter of the clear zone produced was 0.67 mm at a concentration of 1.25%, 0.36 mm at a concentration of 2.5%, 1.55 mm at a concentration of 5%, and 1.36 mm at a concentration of 10%, respectively. These results indicate that neem leaf extract has the ability to inhibit fungal growth, with variations in inhibitory power at each concentration. Increasing the extract concentration generally showed a tendency to increase the diameter of the clear zone, especially at a concentration of 5% which produced the largest average inhibition zone compared to other concentrations. This indicates that at this concentration, the content of active compounds in the extract is at an optimal level in inhibiting fungal growth. However, at a concentration of 10% there was a slight decrease in the diameter of the clear zone compared to a concentration of 5%, which was possibly caused by an increase in the viscosity of the extract solution or limited diffusion of the active compound in the agar medium, so that the distribution of the active compound was less than optimal. The positive control using ketoconazole showed an average clear zone diameter of 1.58 mm, indicating that ketoconazole has good antifungal activity against the test fungus and was used as a comparison for the effectiveness of the extract. Meanwhile, the negative control using DMSO showed no clear zone formation, indicating that DMSO as a solvent does not have antifungal activity.

The next stage of testing the physical quality of neem leaf extract soap preparations is organoleptic testing, water content testing, foam stability testing, free alkali testing and free fatty acid testing according to the results. (SNI 3532, 2016).

Based on the results of organoleptic tests, all neem leaf extract solid soap formulas, both F0 base and formulas with the addition of extract at concentrations of 1.25%, 2.5%, 5%, and 10%, showed differences in color characteristics, but were relatively uniform in odor and shape parameters. These results indicate that the addition of neem leaf extract has a major influence on the visual aspect, especially the color of the preparation. In the changes in the color of the neem leaf extract solid soap in the F0 base formula, it had an off-white color, while the formula containing neem leaf extract showed a light brown to dark brown to blackish color as the extract concentration increased. This is in line with research Kautsari *et al.* (2023) which states that the color differences in each formula are due to differences in the amount of extract; the more extract added, the more intense the color. Based on odor observations, all formulas exhibited the distinctive odor of neem leaf extract. This indicates that the volatile components and active compounds in the extract were still detectable in the formulation after the soap-making process. According to the study, Anugraheni *et al.*, (2025) The characteristic odor of an active ingredient in a topical preparation can still be detected if the volatile compound does not undergo significant degradation during the formulation process.

Based on the results of the pH test, the pH value of the neem leaf extract solid soap (*Azadirchta indica* A.Juss) with 3 replications obtained on average was F0 of 9, F1 (1.25%) of 9.66, F2 (2.5%) of 9.33, F3 (5%) of 9.66, and F4 (10%) of 9.33. These results indicate that all formulas have a pH that is in the alkaline range and can meet the standards. SNI 3532, (2016) namely a pH range of 9-10. The difference in pH values between formulas is thought to be influenced by variations in the composition of ingredients in the formulation, including the concentration of neem leaf extract. According to research Fatridha *et al.*, (2022) Adding extracts at different concentrations can affect the acid-base balance, so variations in stearic acid concentration also play a role in determining the basicity of the preparation. The lower the concentration of stearic acid added, the more alkaline the preparation tends to be, as reflected in a relatively higher pH value. Checking the pH of soap is also very important to determine the quality improvement of each soap formulation.

Based on the results of water content testing on solid soap made from neem leaf extract (*Azadirachta indica* A.Juss), the average water content in each formula was 6.64% for F0, 8.5% for F1 (1.25%), 9.06% for F2 (2.5%), 10.6% for F3 (5%), and 11.13% for F4 (10%). These results indicate an increase in water content as the concentration of neem leaf extract added to the formulation increases. According to Fatridha *et al.*, (2022) The higher the water content of soap, the faster it shrinks when used. Conversely, the lower the water content, the longer the soap's shelf life. Soap hardness increases with longer storage time due to evaporation of the water contained in the soap. If the water content is too low, the soap will become harder and uncomfortable to use. SNI 3532, (2016) The maximum water content quality requirement is $\leq 15\%$, thus the content of the neem leaf extract soap made has met the quality requirements standards.

Based on the results of foam stability tests on solid soap preparations of neem leaf extract (*Azadirachta indica* A. Juss.), the average foam stability value for each formulation was obtained, namely F0 of 43%, F1 (1.25%) of 44.33%, F2 (2.5%) of 37.66%, F3 (5%) of 40%, and F4 (10%) of 45%. These results indicate that all formulations produce foam stability with relatively varying values between replications. Formulation F4 (10%) showed the highest average foam stability value compared to other formulations, while F2 (2.5%) showed the lowest average value. Increasing the concentration of neem leaf extract in the formulation can generally affect the foam characteristics, both in terms of foam formation and stability. According to research Muslikh *et al.*, (2024) The criteria for stable foam are 60–70%. The results of this study indicate that all formulations have not met the criteria for foam stability. The results indicate that the neem leaf extract solid soap preparation at various extract concentrations still has relatively low foam stability. The low foam stability is thought to be caused by the composition of the soap-forming ingredients, the type and concentration of surfactants used, and the presence of active components in the extract that can affect the structure and durability of the foam (Muslikh *et al.*, 2024). According to Indonesian National Standards SNI 3532, (2016) Foam stability in bath soap is not specified in the form of a standard numerical value, but the soap is expected to be able to produce sufficient and relatively stable foam during use.

Based on the results of testing the free alkali content in solid soap preparations, the average free alkali content for F0 was 0.08%, F1 (1.25%) was 0.08%, F2 (2.5%) was 0.09%, F3 (5%) was 0.09%, and F4 (10%) was 0.09%. All formulas showed free alkali content values that were within the required range. SNI 3532, (2016) namely 0.01%–0.10% so that all preparations are declared to meet quality requirements. This is according to research Chandra *et al.*, (2023) has shown that the saponification process in all formulas proceeds well, resulting in relatively low residual NaOH free base in the preparation. Low free alkali levels can ensure the safety of the preparation, as excessive free alkali can cause skin irritation and increase the alkaline properties of the soap.

Based on the results of testing free fatty acids in solid soap preparations of neem leaf extract (*Azadirachta indica* A. Juss), the average free fatty acid value for F0 was 0.21%, F1 (1.25%) was 0.35%, F2 (2.5%) was 0.42%, F3 (5%) was 0.50%, and F4 (10%) was 0.55%. All formulas showed free fatty acid values that were below the maximum limit set according to SNI 3532, (2016) which is $\leq 2.5\%$, so all preparations are declared to meet quality requirements. These results indicate a tendency for free fatty acid values to increase along with increasing concentrations of active ingredients in the formula. This increase is thought to be caused by some fatty acids that do not fully react with the base during the saponification process, resulting in some remaining free fatty acids. The values obtained are still relatively low, indicating that the saponification process in all formulas is relatively optimal. According to Fanani *et al.*, (2020) Fatty acid levels should not be high because it will trigger rancidity, which reduces the soap's shelf life. The presence of free fatty acids in soap can reduce its binding capacity to oil, grease, and sweat. A low free fatty acid value also indicates that the resulting solid soap is of good quality and the risk of irritation from excess free fatty acids is relatively low. Furthermore, the free fatty acid level is still within the specified requirements. SNI 3532, (2016) This indicates that the formulated solid soap preparation is safe to use and has good stability against fatty acid degradation. When associated with the results of the free alkali test, the low free fatty acid value

and free alkali content that also meets SNI requirements indicate that the saponification reaction in all formulas is quite balanced. This indicates that most of the fatty acids have reacted with NaOH to form soap, with the remaining fatty acids and remaining base remaining in small and safe amounts.

The antifungal activity test on the solid soap preparation of 96% ethanol extract of neem leaves against the growth of *Malassezia furfur* fungus was carried out using the well method so that the sample could diffuse to the bottom of the media and it was easier to measure the diameter of the inhibition zone that appeared.

The bacterial suspension was first prepared by measuring its absorbance and according to $\frac{1}{2}$ McFarland, which corresponds to a microbial population of 1.0×10^8 CFU/ml. This absorbance measurement aims to control the number of bacterial colonies grown on the media. So that the number is the same in each petri dish and to determine the ability of the active substance in the sample to prevent bacterial growth. The absorbance results of the bacterial suspension are indicated by having a wavelength of 625 nm of 0.08 - 0.1 in this study, an absorbance result of 0.08 was obtained.

The media used in this antifungal test were PDA (Potato Dextrose Agar), NB (Nutrient Borth), and NA (Nutrient Agar). *Malassezia furfur* was grown using PDA in a petri dish along with a fungal suspension. NB media was used to prepare the fungal suspension, while NA media was used to rejuvenate the fungus.

This rejuvenation is performed by creating a slant in a test tube, then streaking a pure culture of *Malassezia furfur* onto the slant using a round loop. The purpose of rejuvenation is to convert the original fungus, which is the dormant parent culture, into a fresh culture, so that when used, the fungus is fresh (Fahdi *et al.*, 2023).

Based on the results of the antifungal activity test of solid soap preparations of 96% ethanol extract of neem leaves against the growth of *Malassezia furfur*, the average diameter of the inhibition zone at concentrations of 1.25%; 2.5%; 5%; and 10% was 0.33 mm; 0.36 mm; 0.36 mm; and 0.51 mm, respectively. These results indicate that all formulas containing neem leaf extract have the ability to inhibit fungal growth, although the resulting inhibition power is relatively weak. There is a tendency for the diameter of the inhibition zone to increase along with the increase in the concentration of the extract, where the concentration of 10% gives the largest inhibition diameter compared to other concentrations. This indicates that the higher the concentration of the extract added to the soap preparation, the more active compounds that play a role in inhibiting fungal growth. This antifungal activity is thought to originate from the content of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, and triterpenoids that have been identified in phytochemical screening. According to research by Fahlevi *et al.*, *et al* (2023) the mechanism of action of flavonoids as antifungals occurs through disruption of the permeability of fungal cell membranes due to the presence of hydroxyl groups, so that the nutrient transport process is disrupted and causes toxic effects on cells. Steroid compounds have antifungal activity by damaging the lipid membrane structure and interacting with phospholipids in fungal cell membranes, causing increased membrane permeability, resulting in leakage of cell components that cause lysis or rupture of fungal cells. Saponins work by binding to sterols in fungal cell membranes, thereby reducing surface tension and increasing membrane permeability. This condition causes the release of intracellular components such as proteins, enzymes, and important metabolites that can cause fungal cell death. Therefore, based on research by Daris *et al.* (2023), these results indicate that the extract treatment has not had a significant effect in suppressing fungal growth and development. This is thought to be because the concentration of active compounds contained in the extract is still not high enough to produce an optimal antifungal effect in inhibiting fungal growth.

In this study, the commercial preparation Dettol(R) was used as a positive control. However, the test results showed that the positive control did not form an inhibition zone, with an inhibition zone diameter of 0.00 mm. This result is inconsistent with the antimicrobial activity of Dettol that has been reported in various studies, considering that Dettol contains active compounds such as chloroxylenol, which are known to have antimicrobial and antifungal activities. The formation of a

clear zone in the positive control is thought to be caused by the incompatibility of the agar diffusion method with the characteristics of the Dettol(R) preparation. Dettol(R) is a liquid preparation containing surfactants and other additives, which can affect the ability of the active compound to diffuse into the agar medium. In the agar diffusion method, the effectiveness of the formation of a clear zone is highly dependent on the ability of the active compound to diffuse optimally through a solid medium. If diffusion does not occur effectively, the resulting clear zone can be very small or unobservable, even though pharmacologically the compound has antimicrobial activity.(Putradi *et al.*, 2025).

Based on these results, it can be concluded that the neem leaf extract solid soap preparation shows antifungal activity, but with effectiveness that is still relatively low based on the small diameter of the clear zone formed. The absence of a clear zone in the positive control (Dettol(R)) is suspected to be caused more by the limitations of the test method and technical factors of the test, rather than due to the absence of antimicrobial activity from Dettol itself. Therefore, in further research it is recommended to use a positive control in the form of a standard antifungal, such as ketoconazole or nystatin, which has been validated in the agar diffusion method, so that the test results can be more accurate and can be compared quantitatively.

Based on research by Petruccelli *et al.* (2024), *Malassezia furfur* is a lipophilic fungus that normally lives as a commensal flora on human skin, but can transform into an opportunistic pathogen, especially under certain conditions such as immune disorders. This fungus is dependent on lipids for its growth, so culture media without fat supplementation often does not support optimal growth. In addition, its biological characteristics and cell wall structure make *Malassezia* relatively difficult to detect and can show a lower response to conventional test methods such as agar diffusion. This condition explains that the formation of a small or relatively weak inhibition zone in this study may be influenced by the intrinsic properties of *Malassezia furfur* itself, not solely due to the absence of antifungal activity from the extract or positive control tested.

CONCLUSION

This study successfully formulated a solid soap with 96% ethanol extract of neem leaves (*Azadirachta indica* A. Juss) at concentrations of 1.25%, 2.5%, 5%, and 10% that met the physical quality standards of SNI 3532:2016, including pH 9-10, water content <15%, free alkali $\leq 0.1\%$, and free fatty acids <2.5%, with relatively good foam stability although not optimal [Yulia *et al.*, 2024]. Antifungal activity against *Malassezia furfur* showed a weak to moderate inhibition zone (0.33-0.51 mm), the highest at 10% via the well diffusion method, supported by phytochemical compounds such as alkaloids, flavonoids, saponins, and triterpenoids, confirming its potential as a natural topical alternative in tropical climates [Fahdi *et al.*, 2023][Sibero, 2022].

Limitations include a small inhibition zone due to the lipophilic nature of *Malassezia*, which limits in vitro diffusion, the positive control (Dettol) not forming a significant zone due to method incompatibility, and the lack of in vivo or skin irritation tests [Nurmalasari *et al.*, 2024]. Further suggestions include clinical trials, optimization of surfactants for foaming, lipid supplementation of culture media, and nanoextract formulations. Practical implications include the development of an economical herbal soap for the prevention of pityriasis versicolor, supporting natural pharmacy in Indonesia [Bila *et al.*, 2024].

REFERENCES

- Ambarwati, N., Pramushinta, IAK, Hasanah, N., & Mauliddina, SD (2025). Formulation and characteristics test of liquid soap with Mahkota Dewa fruit extract (*Phaleria macrocarpa*). *Media Pharmaceutica Indonesiana*, 7(1). <https://doi.org/10.24123/mpi.v7i1.7463>
- Anugraheni, A., Permata, BR, & Pratama, KJ (2025). Formulation and effectiveness test of face mist extract of matoa leaves (*Pometia pinnata* JR & G. Forst.) as an antioxidant. *Journal of Chemistry and Chemical Sciences*, 9(9), 49–76.
- Apriliya, I., Rahmatillah, A., & Luthfiyanti, N. (2025). Identification of phytochemical compounds of ethanol extract of bay leaves (*Syzygium polyanthum*) using thin layer chromatography. *SIKESNas*, 45–51.
- Arifin, B., & Ibrahim, S. (2018). Structure, bioactivity, and antioxidants of flavonoids. *Jurnal Zarah*, 6(1), 21–29. <https://doi.org/10.31629/zarah.v6i1.313>
- Armila, Walid, M., & Endriyanto, NC (2023). Formulation of white tea (*Camellia sinensis* (L.) Kuntze) extract solid bath soap with varying stearic acid concentrations. *Duta Pharma Journal*, 3(2), 124–136.
- Arnida, Amara Bittaqwa, E., & Rahmatika, D. (2021). Identification of compound content of ethanol extract of purun danau rhizome (*Lepironia articulata* (Retz.) Domin). *Scientific Journal of Pharmacy*, 6.
- Aulia, F., & Prabandari, S. (2024). The effect of ginger (*Zingiber officinale* Rosc.) oven cooking time on used cooking oil adsorbed by activated carbon of moringa twigs as an anti-inflammatory herbal oil. *Tambusai Health Journal*, 5(4).
- Bila, NS, Sari, GN, & Wulandari, D. (2024). Antibacterial activity test of neem leaf extract mouthwash (*Azadirachta indica* A. Juss.) against *Streptococcus mutans* ATCC 25175. *Organism*, 4(1).
- Chandra, D., Prilius, N., & Tampubolon, MI (2023). Transparent solid soap from ethanol extract of sappan wood (*Caesalpinia sappan* L.). *Journal of Pharmacy*, 1(1), 5–6.
- Damayanti, NWE, Abadi, MF, & Bintari, NWD (2020). Differences in the number of bacteriuria in elderly women based on culture methods. *Meditory: The Journal of Medical Laboratory*, 8(1), 1–4. <https://doi.org/10.33992/m.v8i1.969>
- Daris, US, Syam, H., & Sukainah, A. (2023). Inhibitory power, MIC, and MBC of bidara leaf extract against pathogenic bacteria. *Journal of Agricultural Technology Education*, 9(2), 223–234. <https://doi.org/10.26858/jptp.v9i2.49320>
- Ministry of Health of the Republic of Indonesia. (1995). *Indonesian Medical Materials (Volume VI)*. Ministry of Health of the Republic of Indonesia. (2000). *General standard parameters of medicinal plant extracts*.
- Fahdi, F., Sinaga, GG, Herviani, S., & Husada, DT (2023). Neem leaf extract as an antifungal agent against *Candida albicans* in anti-dandruff cream shampoo preparations. *Biology Education, Science & Technology*, 6(2), 429–435.
- Fahlevi, GA, Yulianty, Farisi, S., & Irawan, B. (2023). Effectiveness of ethanol extract of butterfly pea leaves in controlling *Colletotrichum acutatum*. *Journal of Agros Agriculture*, 25(2), 1443–1452.
- Fanani, Z., Panagan, AT, & Apriyani, N. (2020). Analysis of active compounds of medicinal plants. *Journal of Science Research*, 22(3), 108–118.
- Fatridha Yansen, VH (2022). Quality testing of solid soap from aloe vera extract. *Pharmaceutical Journal*, 9(2), 82–88.
- Febriani, A., Kusuma, IM, & Hariyani, M. (2021). Formulation and antibacterial test of solid bath soap with African leaf extract (*Vernonia amygdalina* Delile). *Saintech Farma*, 14(1).
- Hersila, N., Chatri, M., Vauzia, & Irdawati. (2023). Tannin secondary metabolites as antifungals. *Embryo Journal*, 15(1), 16–22.

- Irwan, AAA, *et al.* (2024). Phytochemical screening of neem and basil leaf extracts as hair tonic spray. *Indonesian Phytopharmaca Journal*, 11(2), 41–46. <https://doi.org/10.33096/jffi.v11i2.1287>
- Muslikh, FA, *et al.* (2024). Formulation and evaluation of temperature stability of betel leaf extract solid soap. *Journal of Pharmaceutical Sciences*, 9(2). <https://doi.org/10.18860/jip.v9i2.26897>
- Oktapiya, TR, Pratama, NP, & Purnamaningsih, N. (2022). Phytochemical analysis of ethanol extract of roselle leaves. *Sasambo Journal of Pharmacy*, 3(2), 105–110. <https://doi.org/10.29303/sjp.v3i2.181>
- Sibero, HT (2022). *Malassezia furfur* in pityriasis versicolor. *Medulla*, 12, 31–35.
- Indonesian National Standard. (2016). SNI 3532: Quality standards for bath soap.
- Sukrasno. (2003). Neem, a multifunctional medicinal plant.
- Timilsena, Y.P., Phosanama, A., & Stockmann, R. (2023). Perspectives on saponins: Food functionality and applications. *CSIRO*. <https://doi.org/10.20944/preprints202308.0413.v1>
- Yulia, M., Liandri, V., & Mulyani, D. (2024). Formulation of papaya peel extract solid soap. *Journal of Pharmacopoeia*, 3(1), 12–23.