



## ACTIVITY TEST OF NEEM LEAF (*Azadirachta indica* A. Juss) ANTI-DAMBRY SHAMPOO ON FUNGUS (*Malassezia furfur*)

Mandike Ginting<sup>1</sup>, Lilik Septiana<sup>2</sup>, Grace Anastasia Br. Ginting<sup>3</sup>

<sup>1,2</sup>Department of Pharmacy and Public Health, Institut Kesehatan Helvetia, Indonesia

<sup>3</sup>Department of Pharmacy and Health Sciences, Universitas Sari Mutiara Indonesia

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### ABSTRACT

**Background:** Dandruff is a disorder in the form of excessive flaking of dead skin on the scalp, sometimes accompanied by itching and inflammation. One of the causes of dandruff is the *Malassezia furfur* fungus. Neem leaves contain the main active ingredients of neem, namely azadirachtin, meliantriol, nimbin, nimbidin and salannin, substances that are known to be effective as insecticides. **Objective:** The aim of the study was to determine the formulation of anti-dandruff shampoo preparations of ethanol extract of neem leaves (*Azadirachta indica* A. Juss) and Activity Test against Fungus (*Malassezia furfur*). **Method:** The research method was experimental, which included taking plants, making simplicia, formula for neem leaf extract shampoo (2%, 4%, 6% and 8%), evaluating preparations and testing antifungal activity. **Results:** The results of the study were based on organoleptic tests, the preparations were white and brown in color, had a distinctive neem aroma and were viscous in shape; there is no coarse grain in the preparation; pH of preparations F0 (6.0), F1 (5.5), F2 (5.3), F3 (5.3) and F4 (5.2); foam height tests F0 (8 cm), F1 (8.5 cm), F2 (8.7 cm), F3 (9 cm) and F4 (9.5 cm); there is no itching, redness and swelling on the skin of the volunteers; stability test does not change color, aroma and shape of the preparation; antifungal test preparations F0 (0 cm), F1 (11.65 cm), F2 (12.73 cm), F3 (12.86 cm), F4 (13.61 cm) and K+ (22.8 cm). **Conclusion:** The conclusion of the research is that the ethanol extract of neem leaves (*Azadirachta indica* A. Juss) can be formulated as a shampoo that meets the requirements for physical evaluation and inhibits the growth of fungus (*Malassezia furfur*) at a concentration of 8% (13.61 mm) best.

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### Corresponding Author:

Mandike Ginting,  
Department of Pharmacy and Public Health  
Institut Kesehatan Helvetia, Indonesia  
Email: [mandike.ginting@gmail.com](mailto:mandike.ginting@gmail.com)

## 1. INTRODUCTION

Dandruff is still one of the causes of reduced self-confidence which can hinder the comfort of doing activities. Dandruff is a disorder in the form of excessive flaking of dead skin on the scalp, sometimes accompanied by itching and inflammation. The cause of dandruff can be excessive secretion of sweat glands or the role of microorganisms on the scalp which produce a metabolite that can induce the formation of dandruff on the scalp (1).

One of the fungi that cause dandruff is *Malassezia furfur* or *Pityrosporum ovale*. This fungus is actually a normal flora on the scalp, but in hair conditions with excessive oil glands, this fungus can thrive. In general, dandruff can be treated by using anti-dandruff shampoos that contain keratolytics, antimicrobials such as zinc pyrithione (ZPT), selenium sulfide, salicyl acid, imidazole derivatives, and sulfur. However, the use of chemical compounds for dandruff is very limited and can cause side effects, such as toxicity to eyes and hair become too dry (2).

*Malassezia furfur* is part of the normal flora, in the form of yeast and is found mainly in areas of the skin rich in sebum production. Predisposing factors include hot and humid environments, excessive sweating, tightly closed clothing, high plasma cortisol levels, immunosuppression, excess nutrition, and genetic factors (3).

Synthetic antifungal (antifungal) drugs are commercially known and relied upon in disease management. Among the synthetic antifungal drugs are amphotericin, griseofulvin, nystatin and ketoconazole. Synthetic medicines cause serious side effects and need a doctor's supervision, apart from being expensive, people have started using traditional medicines from herbal plants which are a source of domestic natural wealth and are worth extracting (4).

According to research by Cut Soraya et al (2019) entitled Antibacterial Effects of Neem Leaf Extract (*Azadirachta indica*) on the Growth of *Enterococcus faecalis* In-Vitro. The results of the phytochemical test showed that neem leaf extract (*Azadirachta Indica*) positively contained triterpenoids, phenolic compounds, tannins, steroids and saponins which act as antibacterials. The results of the antibacterial effect test of neem leaf extract (*Azadirachta indica*) showed the formation of an inhibition zone around the disc paper at each concentration. This proves that neem leaf extract has an antibacterial effect which has the ability to inhibit the growth of *Enterococcus faecalis* (5).

This neem plant is commonly found in several regions in Indonesia, including in Bali, NTB and its surroundings. Neem leaves have a very bitter taste. The main active ingredients of neem are azadirachtin, meliantriol, nimbin, nimbidin and salanin. This substance is known to be effective as an insecticide. Insects that eat plant leaves or that are directly exposed to neem extract sprays will experience disturbances in the body's metabolism, disrupting the metamorphosis process so that the metamorphosis of insects becomes imperfect until the severe ones die (6).

According to research by Adriani (2020) entitled test of antifungal activity from 96% ethanol extract of neem leaves (*Azadirachta indica* A. juss) against *Malassezia furfur* fungus, ethanol extract of neem leaves concentrations of 1%, 2%, 3%, 4% can inhibit growth the *Malassezia furfur* fungus with the largest inhibition zone was produced at a concentration of 4%, which was 24.5 mm (7).

## 2. RESEARCH METHODE

The method used in this study is an experimental method in the laboratory. Experimental is a study by carrying out experimental activities, which include making extracts, formulation of shampoo preparations, evaluating the quality of shampoo preparations and testing their activity which aims to determine whether there is an effect of anti-dandruff shampoo of ethanol extract

of neem leaves (*Azadirachta indica*) on the growth of *Malassezia* fungus Furfur using concentrations of 2%, 4%, 6%, 8% positive control and negative control.

The research was conducted at the Pharmacognostic Laboratory and the Cosmetology Laboratory of the Helvetia Health Institute and the USU Microbiology Laboratory from November 2021 to February 2022.

The sample used in this study was neem leaves (*Azadirachta indica*) obtained from Blang Paseh Village, Sigli City District, Pidie Regency.

The tools used in this study were aluminum foil, autoclave, mesh sieve, beaker glass, stir bar, bunsen, shampoo packaging bottles, petri dishes, vaporizer cups, erlenmeyer, measuring cups, incubators, calipers, loops, gauze, cotton, filter paper, parchment paper, label paper, laminar air flow, mask, mortar and stamper, water bath, pH meter, pipette, tweezers, rotary evaporator, gloves, horn spoon, spatula, tissue, digital scale (citizen), test tube, water bath.

The materials used were neem leaf extract (*Azadirachta indica*), sodium lauryl sulfate, CMC, propylene glycol, methyl paraben, cetyl alcohol, cocamid DEA, aquadest, SDA media (Sabouraud Dextrose agar), *Malassezia* Furfur mushroom, Mc Farland 0 standard solution, 5% and 0.9% NaCl solution, chlorhydrate, chloroform, HCl, dragendorf reaction, mayer, bouchardat, amyl alcohol, FeCl<sub>3</sub>, n-hexane.

### 3. RESULT AND ANALYSIS

#### Extraction

From neem leaves with a wet weight of 5000 g, 3500 g of simplicia powder was obtained, so that a 70% yield of simplicia was obtained. Extract from 500 g of neem leaf simplicia (*Azadirachta indica* A. Juss) which was carried out by maceration using 96% ethanol solvent obtained a condensed extract of neem leaves as much as 68 g, resulting in an extract yield of 13.6%

#### Characteristics of Simplicia

Table 2. Characteristics of Neem Leaf Simplicia Powder

No	Parameter	Hasil (%)
1.	Water content	8,74
2.	Water soluble essence content	21,33
3.	Ethanol soluble essence content	16,33
4.	Total ash content	5,66
5.	Acid insoluble ash content	0,83

#### Phytochemical Screening

Table 3. Results of Phytochemical Screening of Neem Leaf Ethanol Extract

Chemical Content	Reactor	Observation	Result	Conclusion
Alkoloid	Sample + Dragendorf	Brick red precipitate	+	Alkaloids +
	Sample + Mayer		+	
	Sample + Bouchardat	White precipitate	+	
Saponins	Sample + Hot water + HCL 2N	Blackish brown precipitate	+	Saponins +
		Formed foam	+	Flavonoids +
Flavonoids				
tannins	Sample + Mg powder + H <sub>2</sub> SO <sub>4</sub> (p) + amyl alcohol	Orange	+	Tannins +
Triterpenoids and steroids	Sample + FeCl <sub>3</sub>	Blackish green	-	Triterpenoids -

**Organoleptic Test**

Table 4. Organoleptic Observations of Neem Leaf Extract Anti-Dandruff Shampoo (Azadirachta indica A. Juss)

Formulas	Organoleptik		
	Color	Smell	Form
F0	Clear white	No smell	Thick
F1	Light brown	Typical neem aroma	Thick
F2	Light brown	Typical neem aroma	Thick
F3	Dark brown	Typical neem aroma	Thick
F4	Dark brown	Typical neem aroma	Thick

**Homogeneity Test**

Table 5. Observation of Homogeneity of Neem Leaf Extract Anti-Dandruff Shampoo (Azadirachta indica A. Juss)

Formulas	Homogeneity
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous
F4	Homogeneous

**pH test**

Table 6. pH Examination Results of Neem Leaf Extract Antidandruff Shampoo (Azadirachta indica A. Juss)

Formulas	pH	Information
F0	6,0	Sour
F1	5,5	Sour
F2	5,3	Sour
F3	5,3	Sour
F4	5,2	Sour

**Foam Height Test**

Table 7. High Foam Examination of Neem Leaf Extract Antidandruff Shampoo (Azadirachta indica A. Juss)

Formulas	Foam height(cm)
F0	8
F1	8,5
F2	8,7
F3	9
F4	9,5

**Irritation Test**

Table 8. Irritation Examination of Neem Leaf Extract Antidandruff Shampoo (Azadirachta indica A. Juss)

Parameter	Volunteer (+/-)														
	F0			F1			F2			F3			F4		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Itchy redness	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-



Homogeneity	F2	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem
	F3	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem
	F4	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem	Typical neem
	F0	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	F1	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	F2	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	F3	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	F0	6,0	6,0	5,9	5,9	6,0	5,9
	F1	5,5	5,5	5,4	5,4	5,4	5,3
	F2	5,4	5,4	5,4	5,3	5,3	5,2
	F3	5,4	5,3	5,3	5,4	5,3	5,2
	F4	5,3	5,2	5,2	5,3	5,2	5,2

### Antifungal Test

Table 12. Results of antifungal examination of anti-dandruff shampoo neem leaf extract (*Azadirachta indica* A. Juss) against *Malassezia furfur* fungus

No	Concentration	Testing			Average (mm)
		I (mm)	II (mm)	III (mm)	
1	F0	0	0	0	0
2	F1	10,8	11,9	12,25	11,65
3	F2	13,1	12,4	12,7	12,73
4	F3	12,85	12,85	12,9	12,86
5	F4	13,3	13,95	13,6	13,61
6	Control (+)	22,5	24,5	21,6	22,8

### Data Analysis

Table 13. Statistical Test Results Using the One Way ANOVA Test

ANOVA						
Diameter_Zona_Inhibition						
Between Groups	559,131	5	111,826	836,084	0,000	
Within Groups	1,605	12	0,134			
Total	560,736	17				
Between Groups	559,131	5	111,826	836,084	0,000	

## DISCUSSION

The ethanol extract of neem leaves uses the maceration method, because the extraction process does not use heat so it does not damage the thermolabile flavonoid compounds. This method is also considered economical (cheap) and easy to do (19). The solvent used is 96% ethanol, this solvent is used because the percentage of water is 4% and 96% ethanol can reduce contamination or the growth of microorganisms in the extract and can produce the optimal

amount of active ingredients where impurities are only on a small scale involved in the extracting fluid (20).

The essence content test was carried out using 2 solvents, namely ethanol and water which aims to determine the amount of substance dissolved in ethanol and water. From the research results, the ethanol-soluble extract content is 16.33%, which is less than the water-soluble extract content, which is 21.33%. Meanwhile, the total ash content test from neem leaf *simplicia* using a furnace obtained a result of 5.66%. Determination of the total ash content is carried out to determine the content of inorganic compounds in the *simplicia*, for example Mg, Ca, Na, and K. The ash content is influenced by several factors, including location of growth, soil, and pollution. While the test results for acid insoluble ash content using dilute hydrochloric acid is 0.83%. The acid-insoluble ash content test is used to determine the levels of inorganic compounds that are insoluble in acid (21).

Based on the results of the phytochemical screening, the ethanol extract of neem leaves contained alkaloids, saponins, flavonoids and tannins. Secondary metabolite compounds belonging to plants such as alkaloids, flavonoids and saponins have antifungal activity (11).

The results of the organoleptic examination of the shampoo preparations, the preparations made have a thick texture, have a variety of colors starting from F0 which is translucent white, F1 and F2 are light brown while F3 and F4 are dark brown, and have a distinctive smell of neem leaves. The higher the extract concentration contained in the shampoo preparation, the stronger the distinctive smell of neem leaves. The brown color of the resulting shampoo preparation is obtained from the dark brown color of neem leaf extract (22). The difference in color in the formula concentrations of 2%, 4%, 6%, and 8% was due to the addition of neem leaf extract. At concentrations of 2% and 4%, the colors are light brown due to the addition of neem leaf extract in small amounts, while at concentrations of 6% and 8%, the colors are dark brown, also supported by research related to shampoo conducted by Nielma Aulia et al., (2020) on skin extracts kaffir lime fruit with a concentration of 5%, 10%, and 15% which at a concentration of 15% has a stronger characteristic odor and a more concentrated color (12).

Based on the examination of the homogeneity test on shampoo preparations from neem leaf extract, it showed that the preparations made fulfilled the homogeneity test requirements in the absence of coarse grains in the preparations. In accordance with SNI No. 06-2692-1992 that a preparation is said to be homogeneous if during the homogeneity test no coarse grains are visible (13).

Shampoo preparations for topical use have a pH that corresponds to the pH of the scalp, namely 5.0-9.0. Based on the results of measuring the pH of neem leaf extract shampoo preparations, it was found that the pH resulting from the five shampoo formulations ranged from 5.2 to 6.0, meeting the scalp pH requirements set by SNI No. 06-2692-1992 which ranges from 5.0-9.0. Among the five anti-dandruff shampoo formulations of neem leaf extract, the highest pH was found in shampoos that did not contain neem leaf extract, namely 6.0, while the lowest pH was found in shampoos containing 8% neem leaf extract, namely 5.2. The pH measurement results showed that there was a decrease in the pH value between the shampoo without neem leaf extract and the shampoo with the addition of the extract, this was because the neem leaf extract was acidic (23).

Based on the results of measuring the foam height of the neem leaf extract shampoo, it was found that the height of the foam produced from the five shampoo formulations ranged from 8-9.5 cm, meeting the foam height requirements of 1.3-22 cm. Among the five anti-dandruff shampoo formulations of neem leaf extract, the highest foam was found in shampoos containing 8% neem leaf extract, namely 9.5 cm, while the lowest foam was found in shampoos that did not

contain neem leaf extract, namely 8 cm. From the results of foam height measurements, it was shown that there was an increase in foaming power between shampoo without neem leaf extract and shampoo with added extract, this is because neem leaf extract contains saponins.

Based on the irritation test of the preparation which was carried out on 15 volunteers, none of them showed signs of irritation such as redness, itching or swollen skin. These results indicate that the prepared neem leaf extract shampoo does not irritate the skin and is safe for the skin. This is because the pH of the shampoo preparation is good and is still in the pH range of the scalp (24).

Based on the results of the Brookfield Viscometer examination for neem leaf extract shampoo preparations, it was found that the viscosity produced from the five shampoo formulations ranged from 2013-2569 cP. meet the viscosity requirements of 400-4000 cP. Among the five anti-dandruff shampoo formulations of neem leaf extract, the highest viscosity was found in shampoos that did not contain neem leaf extract, namely 2569 cP, while the lowest viscosity was found in shampoos containing 8% neem leaf extract, namely 2013 cP.

From the results of viscosity measurements showed that there was a decrease in viscosity where the higher the concentration of the extract in the shampoo preparation, the lower the viscosity of the preparation. One of the factors that can affect the viscosity of the shampoo is the concentration of neem leaf extract used. The viscosity of the shampoo decreased with increasing concentration of neem leaf extract.

Room temperature stability was carried out for 4 weeks and evaluation was carried out every week including: organoleptic, homogeneity and pH tests. The organoleptic test results of the preparations were light brown to dark brown in color with a thick texture and a distinctive neem aroma that did not change in shape, color and aroma during the test. The homogeneity of the preparation can be seen from the presence or absence of coarse particles in the shampoo preparation. From the pH obtained, it can be concluded that shampoo can be used and does not cause irritation.

The cycling test was carried out for 6 cycles and each cycle was evaluated including: organoleptic, homogeneity and pH tests. The organoleptic test results of the preparations were light brown to dark brown in color with a thick texture and a distinctive neem aroma that did not change in shape, color and aroma during the test. The homogeneity of the preparation can be seen from the presence or absence of coarse particles in the shampoo preparation. From the pH obtained, it can be concluded that shampoo can be used and does not cause irritation.

Fungal testing was carried out at concentrations of 2%, 4%, 6%, 8% and as a comparison the negative control was shampoo base without neem leaf extract and the positive control was ketoconazole anti-dandruff shampoo. Among the five anti-dandruff shampoo formulations of neem leaf extract, the largest inhibition zone for fungi was found in shampoo containing 8% neem leaf extract, namely 13.61 mm, while the lowest inhibition zone was found in shampoos that did not contain neem leaf extract, namely 0 mm. In the positive control, an inhibition zone of 22.8 mm was formed.

From the test results it can be seen that the higher the concentration of neem leaf extract in the shampoo formulation, the greater the antifungal activity produced. This is due to the presence of chemical compounds contained in neem leaf extract, the effect of flavonoid compounds on mushrooms is by denaturing protein bonds in the cell membrane, so that the cell membrane becomes lysed and the possibility of flavonoids to penetrate into the cell nucleus. The entry of flavonoids into the cell nucleus can prevent fungi from developing, the flavonoid group can act as an antifungal because it has phenols which can denature proteins and can damage cell membranes which are irreversible (cannot be repaired anymore) (25).



Based on the results of the one way ANOVA test in table 11 above, it can be concluded that the average values of the five data groups (F0, F1, F2, F3, F4 and control (+) have a significant difference, this is evidenced by all sig. values  $0.000 < 0.05$ .

#### 4. CONCLUSION

The ethanol extract of neem leaves (*Azadirachta indica*) can be formulated into an anti-dandruff shampoo dosage form that meets the physical evaluation requirements; Neem leaf ethanol extract anti-dandruff shampoo can inhibit the growth of the *Malassezia furfur* fungus; The ethanol extract of neem leaves (*Azadirachta indica*) at a concentration of 8% (13.61 mm) was the most effective as an antifungal against *Malassezia furfur*.

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