

Activity Test of Anti-Dandruff Shampoo Preparation of Fragrant Pandan Leaf Extract (*Pandanus amaryllifolius* Roxb.) Against the fungus *Malassezia Furfur*

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Abstract

Dandruff is a scalp disorder caused by *Malassezia furfur* fungus, often treated with anti-dandruff shampoos. However, synthetic active ingredients in such shampoos may cause side effects like scalp irritation, dryness, or allergies. As an alternative, herbal formulations using fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.), known for their antifungal properties, can be developed. The objective of this study was to determine and analyze the best Fragrant pandan (*Pandanus amaryllifolius* Roxb.) leaf extract shampoo formulation and its antifungal activity against *Malassezia furfur*. The type of research conducted was a true experimental study with a Post-Test Only Control Group Design. In this study, there were treatment groups given a shampoo preparation of fragrant pandanus leaf extract at concentrations of 30%, 50%, and 70%, a negative control group of a shampoo base, and a positive control group of a shampoo containing 1% selenium sulfide under the brand name Selsun. The analysis used was the One-Way ANOVA test. The shampoo preparation of fragrant pandanus leaf extract demonstrated antifungal activity against *Malassezia furfur*, with inhibition zones obtained at a 30% concentration (15.83 mm), 50% concentration (18.68 mm), 70% concentration (21.89 mm), positive control (21.36 mm), and negative control (0 mm). The 70% Fragrant pandan (*Pandanus amaryllifolius* Roxb.) leaf extract shampoo preparation is the best formulation because it exhibits optimal physical characteristics and produces the greatest inhibition against *Malassezia furfur*.

Keywords: *Pandanus amaryllifolius* Roxb; *Malassezia furfur*; Shampoo; Antifungi.

INTRODUCTION

Human skin generally accommodates a variety of commensal microorganisms that can cause irritation or inflammation of the skin (Balato et al., 2019; Liu et al., 2023; Naik et al., 2025). One example of a chronic inflammatory condition of the scalp is seborrheic dermatitis, more commonly known as dandruff (Tao et al., 2025). Dandruff is a scalp condition that causes excessive exfoliation of dead skin cells in the form of white or yellowish flakes, which often manifests as itching and irritation on the scalp of the sufferer (Kumari et al., 2022; Mahajan & Handa, 2021; Trüeb & Gavazzoni Dias, 2023). Dandruff has become one of the most common scalp problems in society. Some previous studies have shown the global distribution of people with dandruff problems to be up to 50% of the total population (Widowati et al., 2020).

Dandruff occurs due to an infection caused by fungi; one of the fungi that often causes dandruff is *Malassezia furfur* (Angiolella et al., 2017; Meray et al., 2018; Narshana & Ravikumar, 2018; Sibi et al., 2023). *Malassezia furfur* is a lipophilic fungus that is part of the normal flora on the scalp, commonly found in adolescents and adults. The growth of this fungus increases beyond normal levels under hot and humid conditions, which causes the accumulation of unsaturated fatty acids so that inflammation occurs and leads to scalp desquamation, resulting in dandruff. In addition to causing dandruff, this fungus can be a causative agent of other diseases such as tinea versicolor, chronic skin diseases, and superficial skin infections.

Basically, dandruff can be managed using anti-dandruff shampoo. Anti-dandruff shampoos with active substances such as ZnPT (Zinc Pyrithione), piroctone olamine, and selenium sulfide are able to inhibit the growth of dandruff-causing fungi, with effectiveness that depends on the concentration of each of these active substances (Basarang et al., 2022). The use of the active ingredients mentioned above certainly has side effects that cannot be ignored, such as irritation to the scalp, dry hair, and hair damage, or can even trigger allergic reactions;

therefore, one alternative approach that can be used to treat dandruff is to use plants and herbal formulations that have anti-dandruff properties (Pingili et al., 2016).

One type of herbal plant that is quite abundant and is native to Indonesia is the fragrant pandan leaf (*Pandanus amaryllifolius* Roxb.) (Suryani et al., 2017). Fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) contain monoterpenes, alkaloids, and flavonoids that have been proven to have antifungal activity, meaning that fragrant pandan leaves have the potential to be used as an anti-dandruff agent (Fitri et al., 2016).

Based on a study previously conducted by Cut Ria, Sri, and Suwendar in 2016, entitled "Evaluation of the Potential Antifungal Activity of Ethanol Extract of Fragrant Pandan Leaf (*Pandanus amaryllifolius* Roxb.) against *Candida albicans* in vitro," it was shown that fragrant pandan leaf extract has antifungal activity against *Candida albicans*, with a minimum inhibitory concentration (MIC) of 3.75%, which produced an inhibition zone diameter of 1.25 cm (Fitri et al., 2016). This research was then supported by a study conducted the following year using different dependent variables; namely, in 2017, a study conducted by Lusi, Siti, and Nur, entitled "Development of Anti-Dandruff Hair Gel Preparation of Fragrant Pandan Leaf Extract (*Pandanus amaryllifolius* Roxb.) Using Viscolam as a Gelling Agent and Testing its Activity on *Pityrosporum ovale* Fungus," showed the antifungal activity of the anti-dandruff gel preparation at a pandan leaf extract concentration of 30%, which produced an inhibition zone diameter of 17.80 mm (Nurdianti et al., 2017).

Based on the description above, the author is interested in researching and developing an activity test of fragrant pandan leaf extract (*Pandanus amaryllifolius* Roxb.) formulated as a shampoo preparation based on Sodium Cocoyl Isethionate against the fungus *Malassezia furfur*. The purpose of this study is to determine and analyze the best formulation of fragrant pandan leaf extract (*Pandanus amaryllifolius* Roxb.) shampoo and its antifungal activity against *Malassezia furfur*. Specifically, this study aims to evaluate the physical characteristics of the shampoo preparation, including organoleptic properties, homogeneity, pH, and foam height, as well as to measure the inhibitory zone diameter of the extract against the growth of *Malassezia furfur* at various concentrations of 30%, 50%, and 70%.

The benefits of this research are expected to provide both theoretical and practical contributions (Narshana & Ravikumar, 2018; Ridder, 2017). Theoretically, this research contributes to the development of scientific knowledge regarding the potential of fragrant pandan leaf extract as an antifungal agent, particularly in the formulation of herbal anti-dandruff shampoo. Practically, this research is expected to provide an alternative solution for the community in overcoming dandruff problems using natural ingredients that are safer and have minimal side effects compared to synthetic chemical-based shampoos. Furthermore, the results of this study can serve as a reference for further research in the development of herbal pharmaceutical preparations and support the utilization of local Indonesian plants in the health sector.

METHOD

The type of research used is true experimental design research with a posttest only control group design research design. The bivariate data analysis used was the One Way Anova test, then a post hoc test was carried out using the Dunnett T3 test. The location of this research is in the Laboratory of the Faculty of Medicine and Veterinary Medicine, Nusa Cendana University, UPT Laboratory of the Faculty of Mathematics and Natural Sciences, Widya Mandira University, and the UPT Laboratory of the Faculty of Mathematics and Natural Sciences, Widya Mandira University. The research time was from August to October 2023. The fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) used in this study were obtained from Jl. Titus Nau RT 07/RW 02 Fatukoa Village, Maulafa District,

Malassezia furfur mushroom strain used in this study was obtained from Indilab Indonesia Jln. Soekarno Hatta KM 4 No. 12, Samarinda, East Kalimantan. Each part of the sample amounted to 5 groups, including 3 groups of fragrant pandan leaf extract shampoo formula with a concentration of 30%, 50%, 70%, positive control, namely Selsun shampoo with a selenium sulfide content of 1%, and negative control, namely shampoo base.

A total of 5 kg of fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) were cleaned by separating the impurities on the leaves through wet and dry sorting. The fragrant pandan leaves are then dried by drying without direct sunlight.

The dried pandan leaves are then mashed using a blender so that 600 grams of pandan fragrant leaf powder is obtained. Maceration of fragrant pandan leaves uses 70% ethanol solvent with a ratio of 1:5, namely 0.6 kg of fragrant pandan leaves and 3 liters of solvent. Fragrant pandan leaf powder and solvent are soaked for 3 days by stirring once a day. The maceration results were filtered using filter paper and 1.8 liters of fragrant pandan leaf extract was obtained. The liquid extract is evaporated using an Evaporatory Rotator Vacuum so that a thick 96.6 grams of fragrant pandan leaf extract is obtained. The ethanol-free test was carried out by reacting potassium dichromate ($K_2Cr_2O_7$) with ethanol in an acidic atmosphere, while the phytochemical test on pandan fragrant leaf extract included examination of alkaloids, flavonoids, saponins, and tannins.

The manufacture of the shampoo base is preceded by mixing surfactants first, namely, Sodium Cocoyl Isethionate and Cocamidopropyl Betaine. Menthol that has been mashed first is then added to the surfactant mixture and then followed by the addition of optiphen (preservative). Aquades and extracts are then added according to the predetermined formulation. All ingredients that have been mixed in one container are then homogenized in a waterbath while stirring using a stirring rod for 30 minutes.

The glass and media tools are wrapped in aluminum foil then sterilized in an autoclave at 121°C for 15-20 minutes. The ose needles and tweezers are sterilized by burning them over a direct fire using spiritus.

The fungal confirmation test was carried out using a 0.9% NaCl solution and the staining test was carried out using Lactophenol Cotton Blue (LPCB). Saboroud Dextrose Agar (SDA) media is made by cooking and sterilizing and then poured into sterilized petri dishes. The manufacture of mushroom suspensions uses 0.9% NaCl and 1-2 bacterial acids until it reaches the standard of 0.5 Mc Farland.

The antifungal test is carried out by placing 1 piece of disc paper on the mushroom culture in a petri dish with sterile tweezers. The disc paper had previously been dipped in a 30 µg/ml selenium sulfate shampoo for positive control, a shampoo base for negative control, and a 30-minute comparative control. Furthermore, all media are incubated into an incubator at 37°C for 24-48 hours. After incubation for 24-48 hours at a temperature of 37°C, observations were made on the petri dish, namely by looking at the growth of *Malassezia furfur* around the disc and calculating the diameter of the growth inhibition zone of each zone around the disc.

RESULTS AND DISCUSSION

Fragrant Pandan Leaf Extraction

A total of 5 kg of fragrant pandan leaves (*Pandanus amaryllifolius* Roxb.) were obtained with a thick extract of 96.6 grams.

Ethanol-Free Test

The colored reaction of the mixture of the extract, $K_2Cr_2O_7$, and H_2SO_4 indicates the extract does not contain ethanol.

Phytochemical Tests

The test results showed that fragrant pandan leaf extract (*Pandanus amaryllifolius* Roxb.) contained secondary metabolite compounds, namely alkaloids, flavonoids, saponins, and tannins that have an antifungal role.

Mold Confirmation Test

1. Tests with NaCl 0.9%, Tests with NaCl 0.9% showed positive results because they produced images in the form of hyphae and spores (spaghetti and meatballs appearance).
2. Lactophenol Cotton Blue (LPCB) staining, the results of Lactophenol Cotton Blue (LPCB) staining showed bluish test mushrooms with morphology showing positive results because they produced images in the form of hyphae and spores (spaghetti and meatballs appearance).

Evaluation of Fragrant Pandan Leaf Extract Shampoo Preparation

Organoleptic Test

The organoleptic test was carried out by observing and evaluating the shape, consistency, color, and smell of the fragrant pandan leaf extract shampoo preparation.

Table 1. Organoleptic Test Results of Fragrant Pandan Leaf Extract Shampoo			
Formula	Consistency	Color	Smell
Formula 1	Emulsion	Yellow	Typical base
		Greenery	Shampoo with
			slight aroma
			Pandan Leaves
			Squirt
Formula 2	Emulsion	Yellow	Typical leaves
			Fragrant pandan
Formula 3	Emulsion	Deep green	Typical leaves
			Fragrant pandan

Source: Primary Data from Research Results, 2025

Homogeneity Test

Observation of homogeneity test results on all preparations was considered stable in homogeneity parameters. This is based on the results obtained, namely that there are no solid particles or coarse granules in the shampoo preparation, and there are no clumping or uneven shampoo formers in the preparation. The results of the homogeneity evaluation of the preparation can be seen in Table 2.

Table 2. Homogeneity Test Results of Fragrant Pandan Leaf Extract Shampoo Preparation

Formula	Observation results	Interpretation
Formula 1	-	Homogeneous
Formula 2	-	Homogeneous
Formula 3	-	Homogeneous

Source: Primary Data from Research Results, 2025

pH Test

The pH determination of shampoo preparations is carried out using a pH meter. The number shown by the pH meter is the pH of the preparation. The pH value of the preparation that is safe for the skin is 4.5–6.5.

Table 3. pH Test Results of Fragrant Pandan Leaf Extract Shampoo

Formula	pH value	Interpretation
Formula 1	7,19	Not compliant with standard pH
Formula 2	6,33	As per standard pH
Formula 3	5,12	As per standard pH

Source: Primary Data from Research Results, 2025

Foam Height Test

The results of the foam height check in each preparation were carried out with the preparation shaken vigorously for 10 times by turning the measuring cup over regularly. The results of the foam height check can be seen in the following table 4.7:

Table 4. Foam High Test Results

Formula	Foam Height	Interpretasi
Formula 1	10.8 cm	Normal
Formula 2	9.6 cm	Normal
Formula 3	7.5 cm	Normal

Source: Primary Data from Research Results, 2025

Antifungal Test

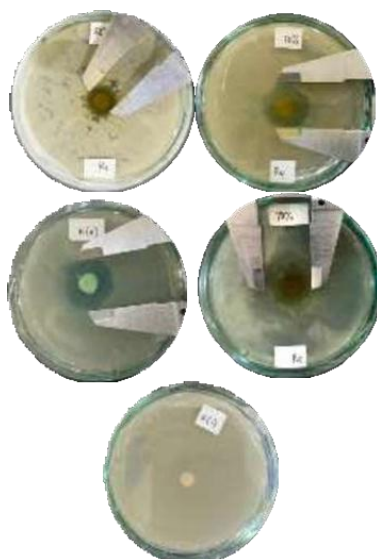


Figure 1. Antifungal Test Results of Fragrant Pandan Leaf Extract against *Malassezia furfur* Fungus

Source: Documentation of Research Results, 2025

Data Analysis

Table 5. Measurement Results of Buffer Zone Measurement of Fragrant Pandan Leaf Extract Shampoo Preparation against *Malassezia Furfur* Fungus

Groups Treatment	Diameter of the barrier zone (n)					Average
	1	2	3	4	5	
F1	12,13	12,36	17,15	14,56	14,88	15,83
F2	14,71	20,48	18,68	20,53	19,03	18,68
F3	19,76	24,76	22,01	23,16	19,76	21,89
K(-)	0	0	0	0	0	0
K(+)	20,5	21,5	22,2	21,2	21,4	21,36

Source: Primary Data from Research Results, 2025

Table 6. Test Results of One-Way ANOVA Diameter Barrier Zone Preparation of Fragrant Pandan Leaf Extract Shampoo Preparation against *Malassezia Furfur* Fungus

	Asym. sig	Remarks
Jamming Zone Diameter	0,000	There is a significant difference in the average

Description:

$p < 0.05$: there is a significant difference in average

Source: Processed Data Analysis Results, 2025

Table 7. Results of Post Hoc Analysis Using Dunnet T3 Diameter Inhibition Zone Preparation of Fragrant Pandan Leaf Extract Shampoo against *Malassezia Furfur* Fungus

Groups		Treatment Groups			
Treatment	30%	50%	70%	K(-)	K(+)
30%		0,099	0,004*	0,001*	0,006*
50%	0,099		0,352	0,000*	0,330
70%	0,004*	0,352		0,000*	0,099
K(-)	0,001*	0,000*	0,000*		0,000*
K(+)	0,006*	0,330	0,099	0,000*	

Description:

Stating that there is a significant difference ($p < 0.05$)

Source: Processed Data Analysis Results, 2025

This study was conducted to test the antifungal activity of fragrant pandan leaf extract (*Pandanus amaryllifolius* Roxb.) which is formulated into a shampoo preparation form. The antifungal potential test used was the Kirby Bauer method (disc diffusion) by measuring the barrier zone around the disc paper. The test mushroom used was *Malassezia furfur*.

The research began with the extraction process of pandan fragrant leaves using 70% ethanol solvent, then continued with the evaporation process to obtain a thick extract so that the content of metabolic compounds contained in fragrant pandan leaves could be analyzed. Based on the results of phytochemical tests, it was found that fragrant pandan leaf extract contains alkaloid compounds, flavonoids, tannins, and saponins. This is in accordance with the research of Suryani et al (2017).

Fragrant pandan leaf extract containing secondary metabolic compounds in this study was then formulated in the form of a shampoo preparation. The formulation consists of 3 types of shampoo formulations with different concentrations, namely 30%, 50%, and 70%.

The evaluation of the physical stability of the shampoo consisted of several tests, namely, organoleptic test, homogeneity test, pH test, and foam height test. The preparation of fragrant pandan leaf extract shampoo was tested for organoleptic parameters by evaluating its color, odor, and consistency. Based on table 7, all formulas have a stable emulsion consistency. Formulas F2, and F3 have a distinctive smell of fragrant pandan leaf extract, while F1 (concentration of 30%) still has a typical shampoo-based smell with a slight aroma typical of the extract. The smell and color difference are affected by the concentration of extracts contained in each formula. The results of homogeneity testing in this study show that all formulas do not show the presence of coarse grains, meaning that the three formulas have a homogeneous arrangement. The results of the pH test obtained a pH value of F1 of 7.19, a pH value of F2 of 6.33, and a pH value of F3 of 5.12. Since the normal skin pH value is 4.5-6.5, based on the test results, F2 and F3 formulas meet the requirements for skin pH, while F1 does not meet the skin pH requirements. Changes in pH can occur due to the temperature, type and level of surfactants contained in the shampoo formulation. If the pH of the scalp is below 4, it can cause inflammation, and can also cause acne on the scalp, but if the pH is too high, it can cause damage to the hair shaft, causing dryness of the scalp to trigger dandruff. The foam height test showed a stable increase in foam height, F1 (30%) with a foam height of 10.8 cm, F2 (50%) with a foam height of 9.6, F3 (70%) with a foam height of 7.5 cm. The higher the number of bases mixed, the higher the foam will be produced.

The last test carried out was an antifungal activity test of fragrant pandan leaf extract shampoo preparation with several concentrations, along with negative control in the form of shampoo base and positive control in the form of Selsun shampoo with 1% selenium sulfide content. The test was carried out on Sabouraud Dextrose Agar (SDA) media and obtained the results of measuring 30% concentration (15.83 mm), 50% concentration (18.68 mm), 70% concentration (21.89 mm), positive control (21.36 mm), and negative control (0 mm).

This study is compared to the research conducted by Lusi et al (2017) using the same test mushroom (*Pityrosporum ovale*) and is also made into a type of shampoo preparation. According to Lusi, the largest concentration (30%) resulted in an inhibition zone of 17.80 mm. This proves that the previous study is in line with this study which has the largest concentration (70%) formulated into shampoo, resulting in an inhibition zone of 21.89 mm.

Research by Sa'adah and Riana (2021) using fragrant pandan leaf extract tested on *Pityrosporum oval* mushrooms, using PDA (Potato Dextrose Agar) media showed concentrations of 10%, 20%, 30%, and 40% resulting in consecutive inhibition zones of 7.9 mm, 8.3 mm, 8.9 mm, 9.4 mm⁵⁵. However, in this study, the 30% extraction, which is the lowest extraction, has an inhibition force of 15.83 mm. One of the factors that affected the size of the inhibition zone in this study was the use of preservatives in the form of Phenoxyethanol/Caprylyl glycol (Optiphen) which contains active antimicrobials Phenoxyethanol equipped with boosters to increase broad protection against microorganisms, one of which is fungi.⁵⁶

The data analysis of the research results was carried out with the One Way Anova test. The results of the One Way Anova test were obtained with a value of $p = 0.000$ ($p < 0.05$) then H_0 was rejected and H_1 was accepted, it can be concluded that there is antifungal activity from the preparation of fragrant pandan leaf extract shampoo. Furthermore, a Post Hoc test was carried out using the Dunnett T3 test because the data were inhomogeneous ($p = 0.034 < 0.05$). In the results of the Dunnett T3 test, the treatment groups that had a significant average difference or $p < 0.05$, namely 30% formulation with 70% formulation, control (-) and control (+), 50% formulation with control (-), 70% formulation with 30% formulation and control (-), control (+) with 30% formulation and control (-), and control (-) with formulation 5 and control (+).

Based on the discussion above, the preparation of fragrant pandan leaf shampoo (*Pandanus amaryllifolius* Roxb.) has been tested to have antifungal activity so that it is proven to inhibit the growth of *Malassezia furfur* fungus, This result can then be concluded that the formulation of 30%, 50%, and 70% is fungistatic or has antifungal activity against the *Malassezia furfur* fungus with the most optimal and most powerful formulation as an antifungal to overcome dandruff is a 70% formulation.

CONCLUSION

The best concentration of fragrant pandan leaf extract shampoo preparation (*Pandanus amaryllifolius* Roxb.) is homogeneous, has a pH value of 5.12, foam height of 7.5 cm, and antifungal activity of 21.89 mm contained in the shampoo preparation formulation with a concentration of 70%. It is recommended that future researchers conduct long-term stability tests and clinical trials on humans to ensure safety and efficacy. Further development of other pharmaceutical dosage forms such as creams or gels from pandan leaf extract is also

suggested. The community can consider this herbal preparation as a safer alternative for treating dandruff with minimal side effects. For future research, it is recommended that long-term stability tests and clinical trials on human subjects be conducted to further validate the safety and efficacy of the preparation, alongside the exploration of other pharmaceutical dosage forms, such as creams or gels, derived from *Pandanus amaryllifolius* Roxb. extract.

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