Anti-Dandruff Shampoo Gel Formulation from Pandan Leaf Juice (*Pandanus amaryllifolius* Roxb.) As Antifungal *Malassezia furfur*

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Abstract. The tropical climate in Indonesia causes dandruff problems due to moisture in the scalp, so that fungi are easy to breed. The fungus that causes dandruff is Malassezia furfur. Compounds that can kill fungi are flavonoid compounds, where these compounds are contained in fragrant pandan leaves. So that it can be formulated into an anti-dandruff shampoo gel preparation. Pandan leaf juice is formulated as an active ingredient in shampoo gel preparations with varying concentrations of F0, F1, F2, and F3. Making fragrant pandan leaf juice using the filtration method, namely filtering twice, so that it can be filtered without leaving residue, so that no pandan leaves enter except the filtrate. As for the antifungal test using the disc diffusion method. Phytochemical screening test positive results containing flavonoids, alkaloids, saponins, tannins, essential oils, negative results on 10% NaOH flavonoids and Alkaloids with Dragendorf reagent. The organoleptic test of F0, F1, F2, and F3 had a gel texture, pandan aroma, and color from clear white to clear dark green. The pH test for F0 is 7, while F1, F2, and F3 are 5. The foam height test from F0, F1, F2, F3 is 4.2 cm, 4.5 cm, 5.5 cm 5.8 cm, respectively. Antifungal tests at concentrations of 12.5%, 25% and F1, F2, F3 were intermediate, while 50% were sensitive, while the control (+) was very sensitive. The fragrant pandan leaf juice can be formulated into an anti-dandruff shampoo gel preparation. The preparation of the anti-dandruff shampoo gel formula with pandan leaf juice fulfilled the physical properties test, namely the pH test, organoleptic test, and high foam test. Fragrant pandan leaf juice and shampoo gel formula were tested for antifungal activity and had antifungal inhibition at all concentrations.

Key words : [Fragrant pandan leaf juice, Pandanus amaryllifolius Roxb., anti-dandruff shampoo, physical properties test, antifungal test]

INTRODUCTION

Hair is a part of the human body located on the scalp, providing warmth, protection, and beauty (Nurhikma et al., 2018). Much time is spent on repairing and caring for hair to ensure it looks healthy and beautiful. Disorders of the scalp, such as sensitivity, hair loss, and dandruff, can inhibit normal hair growth (Limbani et al., 2009). Dandruff is a hair problem that often reduces an individual's self-confidence in carrying out daily activities (Mahataranti et al., 2012).

As much as 50% of the world's population experiences dandruff. Dandruff can affect all ethnicities and genders, but it is rare in children and more common in adolescents and adults (Putri et al., 2020). Dandruff is an unusual condition of the scalp, characterized by excessive flaking of the horny layer of the scalp, forming fine scales (Sukandar & Suwendar, 2006). Another name for dandruff is seborrheic dermatitis (Apriyani & Marwiyah, 2014).

Dandruff can occur due to air pollution, water pollution, lifestyle changes, poor hygiene and immune system, sweating, mental stress, and so on, which can cause fungal infections (Putri et al., 2020). In tropical countries, fungus is a skin problem that is difficult to treat, while Indonesia is a country with a tropical climate so where the humidity level is high and causes microorganisms to grow well (Suryani et al., 2020). According to Iskandar et al (2017), the fungus found in human hair and causes dandruff is Malassezia furfur.

Malassezia furfur is a fungus found on human skin under various conditions, and can cause systemic infections in individuals with immunocompromised conditions (Natalia et al., 2018). Malassezia furfur is a lipophilic fungus that acts as a normal flora on human skin. Disruption of the balance between the host and the fungus allows the fungus to thrive and develop from a yeast form into a pathogenic mycelial form (Sihombing et al., 2018). One way to treat and destroy Malassezia furfur on human skin is by using compounds from the fragrant pandan plant found in its leaves (Siregar & Topia, 2021).

The compounds contained in fragrant pandan leaves (Pandanus amaryllifolius Roxb.) are alkaloids, flavonoids, saponins, and tannins (Dasopang & Simutuah, 2016). According to Dewanti and Sofian (2017), the compound that plays the most important role in inhibiting fungi is flavonoids, while

other compounds contain chemical compounds that are considered to be able to be used as inhibitors of cancer growth, lower blood glucose levels, and as antibiotics (Bali et al., 2019). Fragrant pandan leaves have many benefits, including being used as a herbal medicine to minimize side effects, so that this plant can be used as a treatment for various infections caused by microbes, namely dandruff, so that pandan leaves can be formulated as an active ingredient in the manufacture of shampoo preparations (Siregar & Topia, 2021).

Anti-dandruff shampoo is a cosmetic preparation that can remove oil, dust, skin flakes, and other dirt from the hair. Shampoo has various forms or shapes, including liquid, gel, emulsion, aerosol, or those containing surfactants, so that it has detergency, humectant, and foam-producing properties (Faizatun, 2008). Shampoo preparations in gel form are more widely used because they have benefits, including a cool feeling on the skin, easy drying to form a film layer, which makes the washing process easier and easier to use (Sayuti, 2015). Gel preparations are semi-solid topical preparations that do not easily irritate the skin and are comfortable to use (Rosida et al., 2018).

According to research by Elifas et al. (2019) using experimental methods, it was proven that fragrant pandan leaf juice (Pandanus amaryllifolius Roxb.) was able to inhibit the growth of Candida albicans on SDA (Sabouraud Dextrose Agar) media with concentrations of 12.5%, 25%, 50%, and 100%. The most effective concentration was 100%. Pamudji et al. (2014) argued that the fungus that lives on human skin and causes dandruff is Malassezia furfur. An anti-dandruff shampoo developed with a formula consisting of 15% tea tree oil, 15% sodium lauryl sulfate, 2% cetostearyl alcohol, and 10% propylene glycol was proven to have activity against Malassezia furfur. Based on the background regarding the content of pandan leaves as an antimicrobial, pandan leaf juice which can inhibit the growth of microbes and fungi that cause dandruff, there is an idea to conduct further research on the ability of fragrant pandan leaf juice to overcome dandruff with the title "Formulation and activity test of anti-dandruff shampoo gel preparation of fragrant pandan leaf juice (Pandanus amaryllifolius Roxb.) against Malassezia furfur".

METHODS

Type of Research

This research is quantitative and employs experimental methods because it involves treatments. The treatments are applied to the independent variable, and the results are observed on the dependent variable. The study involved making an anti-dandruff shampoo gel from fragrant pandan leaf juice. The fragrant pandan leaf juice was the independent variable, and the physical properties of the anti-dandruff shampoo gel were then tested. This study used an experimental method, observing changes in one variable as a result of the treatment of another variable.

Research Design

The research design is the preparation for the research activities. In this experimental design, an anti-dandruff shampoo gel formulation will be created using pandan leaf juice as the active ingredient at concentrations of 12.5%, 25%, and 50%. The ingredients used in the anti-dandruff shampoo gel are pandan leaf juice, sodium lauryl sulfate, HPMC, methylparaben, propylparaben, propylene glycol, distilled water, and fragrance.

Population

The population used in this study was fragrant pandan leaves obtained from Kedungwinong Village, Sukolilo District, Pati Regency, Central Java Province.

Sample

The sample must be representative of the population, meaning it should be able to optimally describe the population's condition to ensure valid conclusions are drawn. There are two types of sample characteristics:

a. Inclusion criteria
   Fragrant pandan leaves must be dark green, fresh, without holes, not wilted, not yellow, and free from microorganisms.
b. Exclusion criteria
   Young pandan leaves, old, wilted or stale pandan leaves, yellow or perforated leaves, and leaves that do not meet the researcher's criteria.

Research Instruments

The tools used in this study were as follows: Petri dishes (Pyrex), beakers (Herma), measuring cylinders (Pyrex), spirit burners, parchment paper, autoclaves (Geared Gaude), ovens (Memmert), droppers, loop needles, test tubes (Pyrex), stirring rods, analytical balances (Ohaus), wooden clamps, Erlenmeyer flasks (Pyrex), tweezers, glass funnels (Pyrex), aluminum foil, pH paper, hotplates (Thermo), gauze, string, label paper, blenders, test tube racks, water baths, filter paper, UV-Vis spectrophotometer (Shimadzu UV Mini 1980), calipers, LAF, micropipettes, spatulas, and drygalski.

The materials used in this study were fragrant pandan leaf juice (Pandanus amaryllifolius Roxb.), SLS (Brataco), HPMC (Brataco), PPG (Brataco), Methyl paraben (Brataco), Propyl paraben (Brataco), Concentrated hydrochloric acid, magnesium powder, sulfuric acid, distilled water, Dragendorff reagent, Wagner reagent, FeCl3, NaOH, Sudan III, 2% ketoconazole anti-dandruff shampoo as a positive control, and the test microbes used were Malassezia furfur fungus, PDA media, potatoes, dextrose, whatman paper.

Data Collection Techniques

a. Plant Determination

The process of determining (collecting) fragrant pandan leaves (Pandanus amaryllifolius Roxb.) was carried out in Pati, Central Java. The determination process aims to establish the correct identity of the plant and avoid errors in collecting the research material. The process was carried out in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta.

b. Plant Sample Processing

The fragrant pandan leaves were washed thoroughly under running water and cut into 120g pieces. They were then ground using a blender, mixed with 5 ml of mineral water, placed in gauze, and filtered by squeezing the fragrant pandan leaves through the gauze, then filtered the juice again using a filtration method, and then transferred into a bottle. The resulting extract was fragrant pandan juice with a concentration of 100%. This concentration was prepared using the liquid dilution method (Elifas et al., 2019).

c. Phytochemical Screening Test of Sample

Phytochemical screening was conducted to determine the active compounds contained in fragrant pandan leaves (Pandanus amaryllifolius Roxb.). The following are some of the tests performed:

1. Flavonoid Test

The following are the various flavonoid tests:

a. Willstatter Test

1 ml of pandan leaf juice was measured, placed in a test tube, then 2 drops of concentrated hydrochloric acid (HCl) were added and shaken vigorously. Afterward, magnesium (Mg) powder was added and shaken vigorously. A sample is considered positive for flavonoids if there is intense foaming and the solution changes color to orange (Kazia et al., 2017).

b. Bate-smith Test

1 ml of pandan leaf juice was measured, placed in a test tube, then 2 drops of 2 N sulfuric acid (H2SO4) were added and shaken vigorously. A sample is considered positive for flavonoids if the solution experiences a marked color change to yellow, red, or brown (Kazia et al., 2017).

c. Flavonoid Test with 10% NaOH

1 ml of pandan leaf juice is measured, transferred to a test tube, and then 2 drops of 10% sodium hydroxide (NaOH) are added and shaken vigorously. A sample is considered positive for flavonoids if the solution experiences a marked color change to yellow, red, or brown (Kazia et al., 2017).

2. Saponin Test

1 ml of pandan leaf juice is measured, transferred to a test tube, then 5 mL of hot water and 2 drops of 2N HCl are added and shaken vigorously. After letting the mixture stand for 10 minutes, foam is observed. A sample is considered positive for saponins if foam is present, with a high intensity and consistent consistency for 10 minutes.

3. Alkaloid Test

   1 ml of pandan leaf juice was placed in each of two test tubes. 10 drops of 2 N H2SO4 were added to each tube and shaken vigorously. Dragendorff's reagent was added to the first tube, and Wagner's reagent to the second, and the samples were then observed. A positive result was obtained if the first tube (with Dragendorff's reagent) produced a red precipitate, and the second tube (with Wagner's reagent) produced a brownish precipitate (Kazia et al., 2017).

4. Tannin Test

   1 ml of the juice was boiled with 20 ml of water in a water bath, then filtered. A few drops (2-3 drops) of 1% FeCl2 were added to the resulting filtrate. The formation of a greenish-brown or bluish-black color indicates the presence of tannins (Dasopang & Simutuah, 2016).

5. Essential oils

   Essential oil testing is performed by measuring 1 ml of the juice, placing it in a test tube, and adding a drop of Sudan III reagent. A positive reaction is indicated if the solution turns red (Kurnianingsih et al., 2021).

d. Formulation of Anti-Dandruff Shampoo Gel with Pandan Leaf Juice

The formula for the anti-dandruff shampoo gel with pandan leaf juice is based on research by Mardiana & Safitri (2020). The formulation of the anti-dandruff gel can be seen in Table 1.

Table 1. Formulation of Anti-Dandruff Shampoo Gel with Pandan Leaf Juice

Bahan	FI (g)	FII (g)	FIII (g)	Basis (g)	Kegunaan
Pandan Leaf Juice (*Pandanus amaryllifolius* Roxb.)	3,75	7,5	15	(-)	Active Ingredients
SLS	3,99	3,99	3,99	3,99	Surfaktan
HPMC	0,399	0,399	0,399	0,399	*Gelling agent*
Metil paraben	1,8 mg	1,8 mg	1,8 mg	1,8 mg	Preservative
Propyl paraben	0,018	0,018	0,018	0,018	Preservative
Propilen glikol	1,930 ml	1,930 ml	1,930 ml	1,930 ml	Humectant
Aquadest ad	30 ml	30 ml	30 ml	30 ml	Solvent
Fragrance	qs	qs	qs	qs	Fragrance

e. Making Anti-Dandruff Gel Shampoo from Pandan Leaf Juice

A total of 0.399 grams of HPMC was developed using 8 ml of hot distilled water, stirred until a semi-solid mass was formed. Propylene glycol, along with methyl and propyl paraben dissolved in propylene glycol, was added little by little and stirred until a clear gel formed (mixture A). Sodium lauryl sulfate was first dissolved in distilled water, little by little and then stirred until a homogeneous mixture (mixture B). Mixture B was added little by little to pour into mixture A. Add pandan leaf juice (Pandanus amaryllifolius Roxb.) and make up the volume with distilled water to 30 ml (Mardiana & Safitri 2020).

f. Physical Properties Test Procedure for Anti-Dandruff Shampoo Gel

1) Organoleptic Test

   This organoleptic test is conducted by observing the appearance, color, aroma, and texture (Sambodo & Salimah, 2021).

2) pH Test

   The pH test aims to determine the safety of the product when used. A shampoo pH that is too acidic or too basic will irritate the scalp. The anti-dandruff shampoo gel is placed in a container, dipped in pH paper, and the color change on the paper is observed. The color indicated on the paper represents the pH value of the product. Anti-dandruff shampoo that meets the requirements stipulated in SNI No. 06-2692-1992 ranges from 5.0 to 9.0 (Sitompul et al., 2016).

3) Foam Height Test

   The foam height test aims to demonstrate the surfactant's ability to form foam. The foam height requirement is 1.3 to 22 cm. The foaming power test was carried out by making a 10% shampoo gel solution, shaking it 10 times, and recording the foam volume or height of the foam formed (Malonda et al., 2017).

g. Antifungal Activity Test Procedure for Anti-Dandruff Shampoo Gel

The steps for testing the antifungal activity of anti-dandruff shampoo gel are as follows:

1) Equipment Sterilization

   The glassware, petri dishes, and blue tips used in this antimicrobial activity study were first sterilized in an oven at 180ºC for 1 hour. The tweezers and loop needles were fired over direct flame. The media were sterilized in an autoclave at 121ºC for 15 minutes (Katili et al., 2020).

2) Preparation of PDA Media

   To prepare PDA media, 5 grams of PDA media were weighed, dissolved in 245 ml of distilled water, and the pH was checked (4.5-6.5). Afterward, 250 ml of distilled water was added and heated to a boil on a hotplate. After boiling, let it cool for a few minutes, then cover it with cotton wrapped in gauze. Tie the test tube tightly with several rubber bands and aluminum foil, then label the medium with the name of the medium. Sterilize using an autoclave at 121°C for 15 minutes. Open the lid and wait until the pressure reaches zero (Mardiana & Safitri, 2020).

3) Making Liquid PDL Media

   Weigh 20 grams of potatoes, finely slice them, and boil them in 80 ml of distilled water until they are soft. The potato extract is filtered, 2 grams of dextrose are added, and then more distilled water is added to make a 100 ml solution. The pH is checked (4.5-6.5). Then, 10 ml is poured into the prepared test tubes. Sterilize using an autoclave at 121°C and 1 atm for 15 minutes (Toy & Dhanang, 2019).

4) Preparation of fungal suspension in PDL media

   To prepare a suspension of Malassezia furfur, take one loop of Malassezia furfur and add it to a 10 ml PDL media suspension solution. Incubate at room temperature for 24-48 hours, then homogenize the mixture to remove any sediment. The sterilized PDL is placed in a cuvette as a blank and then inserted into a UV-Vis spectrophotometer to check its turbidity at a wavelength of 625 nm. The absorbance is then measured. The prepared fungal suspension is taken from one test tube, homogenized to remove any sediment, and then transferred to the cuvette. The absorbance is then measured, subtracting it from the sterile PDL. The absorbance should be in the range of 0.08-0.1, equivalent to the McFarland standard of 108 CFU/ml.

5) Preparation of positive controls and pandan leaf juice gel shampoo

   The positive control was prepared using anti-dandruff shampoo containing 2% ketoconazole, while for pandan leaf juice gel shampoo, including Formula 1, Formula 2, and Formula 3, the base was prepared by taking 2 ml of each anti-dandruff shampoo and adding 100 ml of distilled water (Mardiana et al., 2020).

6) Preparation of negative controls

   Negative controls were prepared using 1 ml of sterile distilled water. Negative controls were used as a comparison and solvent for the positive control and test solution (Sambodo & Salimah, 2021).

7) Antifungal test using the disc diffusion method

   A 100 µl fungal suspension with a cell density of 108 was taken and poured into a Petri dish containing solidified PDA media. The suspension was spread evenly with a Drygalski plate using the spread plate method, then allowed to absorb. Disc paper (whatman) no. 4 with a diameter of 6 mm was soaked in a positive control solution, negative control, pandan leaf juice at a concentration of 12.5%, 25%, 50%, shampoo base, formulation 1, formulation 2, formulation 3 until the solution was completely absorbed in the disc paper, then taken using tweezers and aired first after that it was attached to the media that had been inoculated by the fungus. One petri dish was filled with 1-3 disc papers, then the petri dish was labeled, for one petri dish filled with one concentration or formula replicated 3x, after which it was incubated in an incubator at room temperature for 24-48 hours, then the diameter of the clear zone around the disc paper was measured using a caliper (Katili et al., 2020). If the results show <5 mm then it falls into the resistant (weak) criteria, 5-10 mm falls into the intermediate (moderate) category, 11-20 mm falls into the sensitive (strong) category, >20 mm falls into the very sensitive (very strong) category (Novaryatiin et al., 2018).

Data Analysis

Data analysis in this study, using a linear regression equation using SPSS 20 series software, was conducted based on data obtained from the foam height and antifungal tests. Hypothesis testing included data normality and homogeneity testing. However, if the data were not normal or homogeneous, a one-way ANOVA with the Games-Howell post hoc test was used. If the hypotheses were not normal and

homogeneous, the Kruskal-Wallis test was used, followed by the Mann-Whitney post hoc test.

RESULTS AND DISCUSSION

1. Plant Determination

Plant determination is the initial step that must be completed before proceeding to the next stage of the research process. Plant determination is the process of determining the specific name or species of a plant using the Flora of Java book. This aims to ensure the correct identity of a plant or verify the authenticity of the material to be used in a study, thereby avoiding errors in collecting material for research.

Plant determination was conducted in the Pharmaceutical Biology Laboratory, Faculty of Pharmacy, Ahmad Dahlan University, Yogyakarta. The results of the plant determination were obtained from the Flora of Java book (Backer, 1965), confirming that the plant to be used for the study was indeed the fragrant pandan (Pandanus amaryllifolius Roxb.). The results of the fragrant pandan plant identification are as follows:

1b – 2b – 3b – 4b – 12b – 13b – 14b – 17b – 18b – 19b – 20b – 21b – 22b – 23b – 24b – 25b – 26b – 27b – 799b – 800b – 801b – 802a – 803b – 804a Pandanaceae (Family)

1b Pandanus (Genus)

1b – 25a – 26a – 27a Pandanus amaryllifolius Roxb. (Species)

2. Results of Making Pandan Juice

Pandanus juice is filtered using a double filtration method. This process is performed to ensure complete filtration without any pandan leaf residue entering the juice. To make fragrant pandan leaf juice, wash the leaves thoroughly under running water and cut them into 120g pieces. Then, blend them using a blender, add 5 ml of mineral water, transfer them to cheesecloth, and strain them by squeezing the leaves through the cheesecloth. The juice is then collected for testing and preparation. The results of making fragrant pandan leaf juice are shown in Table 2.

Table 2. Results of Juice Production

Pandan Leaves (gram)	Water Added (ml)	Juice Yield (gram)	Juice Color Result
120	5	20	Dark Green
120	5	20	Dark Green
120	5	20	Dark Green
120	5	20	Dark Green

Based on the results of making fragrant pandan leaf juice (Pandanus amaryllifolius Roxb.) at 100% concentration, the results obtained were 20 grams for every 120 grams of pandan leaves weighed and added with 5 ml of distilled water, in line with the research of Elifas et al (2019). Making the formulation and testing is less, it requires repetition up to 4 times in making pandan leaf juice.

3. Phytochemical Screening Test Results

Phytochemical screening is one method that can be used to identify the secondary metabolite compounds contained in a natural product. The obtained pandan leaf juice was then subjected to a phytochemical screening test to determine the several active compounds contained in the pandan leaf juice. The chemical compounds tested in this study were flavonoids, alkaloids, saponins, tannins, and essential oils. The results of the pandan leaf phytochemical screening are shown in Table 3.

Table 3. Results of the Phytochemical Screening Test of Pandan Leaf Juice

Compound	Test Result	Description	Color Theory
Flavonoid			
a. Uji willstatter	(+)	Yellow	Orange
b. Uji Bath-Smith	(+)	Brown	Yellow, red, or brown,
c. NaOH 10%	(-)	Green	Yellow, red, or brown,
Alkaloid			
a. Pereaksi Dragendroff	(-)	Blackish-green	Red precipitate

b. Pereaksi Wagner	(+)	Brown precipitate	Brown precipitate
Saponin	(+)	Terbentuk buih	Foaming persists for 10 minutes.
Tannin	(+)	Greenish-brown	Greenish-brown, blue-black
Minyak atsiri	(+)	Red	Red

Based on the results of phytochemical screening tests, fragrant pandan leaf juice (Pandanus amaryllifolius Roxb.) contains several compounds, including flavonoids. The Willstatter test using HCl and Mg reagents yielded a positive result, indicating a yellow color, consistent with research (Kazia et al., 2017). The Bath-Smith test using H2SO4 reagent yielded a positive result, consistent with research (Kazia et al., 2017). Meanwhile, the flavonoid test using 10% NaOH reagent yielded a negative result, consistent with research (Wahyuni et al., 2018).

Flavonoids are one of the largest groups of natural phenolic compounds and are found in all green plants. Flavonoids are compounds that can inhibit fungal growth (Dewanti & Sofian, 2017). The mechanism of action of flavonoids in inhibiting fungal growth can disrupt the permeability of fungal cell membranes. The hydroxyl groups present in flavonoid compounds cause changes in organic components and nutrient transport, ultimately resulting in toxic effects on fungi. Flavonoids act as antifungals by inhibiting mitochondrial electron transport, resulting in a reduction in the mitochondrial membrane potential. This inhibition can occur through proton inhibition in the respiratory chain, leading to decreased ATP production and fungal cell death (Balafif et al., 2017).

There are two types of alkaloid compounds: the first, alkaloids with Wagner's reagent and H_2SO_4, yielded positive results, consistent with (Kazia et al., 2017). Alkaloids with Dragendorff's reagent and H_2SO_4 yielded negative results, consistent with (Wahyuni et al., 2018). Alkaloids are a group of compounds widely distributed in almost all plant species. All alkaloids contain at least one nitrogen atom, which is usually basic and forms a heterocyclic ring. Most alkaloids are toxic, but some are useful in medicine. Alkaloids are colorless, often optically active, mostly crystalline, but only a few are liquid. Alkaloids function as drugs and powerful activators for immune cells that can destroy bacteria, viruses, fungi, and cancer cells (Wahyuni et al., 2018).

Saponin compounds reacted with hot water and HCl, yielding positive results, consistent with research (Kazia et al., 2017). A positive saponin test resulted in foam that persisted for 10 minutes. Saponins possess antimicrobial properties and are cytotoxic because they can alter the permeability of the microbial cytoplasm, causing cell lysis (Wahyuni et al., 2018).

Tannin compounds reacted with FeCl3, yielding positive results, resulting in a greenish-brown color, consistent with research (Dasopang & Simutuah, 2016). The mechanism of action of tannins as antifungals is by inhibiting the biosynthesis of ergosterol, the main sterol constituent of fungal cell membranes. Sterols are structural and regulatory components found in eukaryotic cell membranes (Arifin et al., 2018).

The essential oil compound reacted with Sudan III, yielding a positive result with the formation of a red color, consistent with research (Kurnianingsih et al., 2021). Essential oils are one of the chemical compounds in plants that have been shown to have antifungal potential. The antifungal mechanism of essential oils is that the phenol group in essential oils forms a complex with proteins in the cell membrane, resulting in coagulation (Astutiningsih et al., 2014).

4. Physical Characteristics Test Results for Anti-Dandruff Gel Shampoo with Pandanus Leaf Juice (Pandanus amaryllifolius Roxb.)

a. Organoleptic Observation Results

Organoleptic testing is conducted using the five human senses. Organoleptic testing aims to assess the physical appearance of a product, including shape, color, and odor. The organoleptic test for the gel shampoo includes color, aroma, and shape. The results of the organoleptic observation of the anti-dandruff gel shampoo with pandan leaf juice (Pandanus amaryllifolius Roxb.) are shown in Table 4.

Table 4. Organoleptic Observation Results

Formulation	shape	smell	color
F0	Gel	Pandan	Clear white
F1	Gel	Pandan	Clear green
F2	Gel	Pandan	Clear dark green
F3	Gel	Pandan	Clear dark green

Based on the results of organoleptic test observations conducted on anti-dandruff shampoo gel preparations, it can be seen that the base preparation without the addition of pandan leaf juice concentration produces a clear white gel preparation. This is because in F0, there is no pandan leaf juice. While in F1, F2, and F3, the preparation is clear green to clear dark green because the concentration of pandan leaf juice added is greater from F1 to F3.

b. pH Test Results

The pH measurement aims to determine whether the product is acceptable to the scalp's pH level. This can affect the safety and comfort of the shampoo gel preparation when used. If the scalp's pH is too acidic or too alkaline, scalp irritation can occur, causing discomfort when using the anti-dandruff shampoo gel preparation and exacerbating hair problems. The pH value of the gel preparation must fall within the scalp pH range stipulated by SNI 06-2692-1992, which is between 5.0 and 9.0. The pH test results for the anti-dandruff shampoo gel preparation using pandan leaf juice (Pandanus amaryllifolius Roxb.) are shown in Table 5.

Table 5. pH Test Results

Formulation	Result	Standart SNI	Description
F0	7	5,0-9,0	fulfill
F1	5	5,0-9,0	fulfill
F2	5	5,0-9,0	fulfill
F3	5	5,0-9,0	fulfill

The measurement results for each pH test on the anti-dandruff shampoo gel preparation decreased. This decrease or change in pH can occur due to environmental factors such as temperature during production, storage, which produces acids or bases, and pandan leaf juice can undergo oxidation (Putra et al., 2020). The shampoo base has a high pH value of 7. After adding pandan juice, the pH changes and decreases to 5, but still meets the requirements, so it is still safe to use on the scalp and is comfortable without causing irritation to the scalp in line with research (Malonda et al., 2017).

c. Foam Height Test Results

The foam height test aims to demonstrate the surfactant's ability to form foam. Foaming in shampoo is crucial. This is because foam keeps the shampoo in place on the hair, makes it easier to wash, and prevents hair strands from clumping together, causing tangles. According to Wilkinson (1982), a good foam height for topical preparations is between 1.3 and 22 cm. The foam height test results for the anti-dandruff shampoo gel made from pandan leaf (Pandanus amaryllifolius Roxb.) juice can be seen in Table 6.

Table 6. Foam Height Test Results for the Shampoo Gel Preparations

Formulation	**Mean ± SD**	**Foam Height Range**	**Description**
F0	4,2 ± 0,0577	1,3-22 cm	Meets Requirements
F1	4,5 ± 0,0577	1,3-22 cm	Meets Requirements
F2	5,5 ± 0,0577	1,3-22 cm	Meets Requirements
F3	5,8 ± 0,1528	1,3-22 cm	Meets Requirements

The foam strength of the three shampoo formulations increased. This increase was due to the increased concentration of pandan leaf juice in the shampoo formulation, as pandan leaf juice contains saponins. This is in line with research by Malonda et al. (2017), which found that saponins produce higher foam levels with higher concentrations of saponin-containing extracts.

Data from the foam strength study were analyzed using SPSS 20. The statistical results of the hypothesis test indicated that the data were not normally distributed and homogeneous. Therefore, the data were non-parametric, and a Kruskal-Wallis test was used to determine differences. The Kruskal-Wallis test showed a significant difference with a $p<0.05$ value. A Mann-Whitney post-hoc test was then used to determine differences between the three formulas.

The Mann-Whitney post-hoc test revealed that F1 and F2, F1 and F3, F1 and F4, F2 and F3, F2 and F4, and F3 and F4 showed a $p<0.05$ value, indicating significant differences

between the three formulas. This shows that there is a significant difference in the influence of all formulas, where the higher the concentration of pandan leaf juice as the active substance used, the higher the foam produced.

d. Antifungal Test Results

The antifungal effectiveness test aimed to determine the inhibitory effect of fragrant pandan leaf juice and an anti-dandruff shampoo gel formula on the growth of Malassezia furfur. Malassezia furfur is a fungus found on human hair and causes dandruff (Iskandar et al., 2017). The antifungal test used the disc diffusion method with PDA and PDL media. The disc diffusion method is the most frequently used method due to its simplicity and precision, identifying potential antifungal agents as indicated by the formation of a zone of inhibition (a clear area) around the paper disc.

PDA media was chosen because fungi can grow well on it and contains nutrients that meet the requirements for fungal growth media, including carbohydrate sources. Furthermore, PDA media was chosen because it supports the growth of fungi, which typically grow rapidly in acidic conditions compared to normal pH. The pH range for PDA is 4.5-6.5, resulting in a pH measurement of 5, which is within the PDA pH range (Basarang et al., 2020).

Potato dextrose liquid (PDL) is a liquid medium used to cultivate mushrooms. After that, a mushroom suspension is made and its turbidity is measured using a spectrophotometer with a wavelength of 625 nm and an absorbance limit ranging from 0.08 to 0.1, resulting in an absorbance result of 0.087. The antifungal effectiveness test of gel shampoo and pandan leaf juice was carried out by observing for a 24-48 hour incubation period, in line with the research of Toy & Dhanang (2019) that the mushroom incubation process is waited for 24-48 hours. The results of measuring the diameter of the inhibition zone in mushrooms can be seen in Table 7.

Table 7. Antifungal Test Results

Formulation and Concentration	Mean ± SD	Criteria	Description
12,5%	6,3 ± 0,27839	5-10 mm	Intermediate
25%	10,38 ± 0,11015	5-10 mm	Intermediate
50%	13,23 ± 0,20207	11-20 mm	Sensitive
F0	-	-	-
F1	5,26 ± 0,02887	5-10 mm	Intermediate
F2	7,41 ± 0,17559	5-10 mm	Intermediate
F3	9,26 ± 0,20817	5-10 mm	Intermediate
K+	23,31 ± 0,10408	>20 mm	Very sensitive
K-	-	-	-

Based on the results of the antifungal activity test, each of the three replicates showed that the 12.5% concentration of juice had an inhibitory power of 6.3, categorized as intermediate. The 25% concentration had an inhibitory power of 10.38, categorized as intermediate. The 50% concentration had an inhibitory power of 13.23, categorized as sensitive (strong). This is in line with research (Alfiah et al., 2015) that states that higher concentrations increase inhibitory power.

In formulas F0, F1, F2, and F3, the juice with varying concentrations and a shampoo gel base, Formula 1 had an inhibitory power of 5.26, categorized as intermediate. Formula 2 had an inhibitory power of 7.41, categorized as intermediate. Formula 3 had an inhibitory power of 9.26, categorized as intermediate. Formula 0, or the base, had no antifungal power because no juice was added to the formula.

The positive control used was 2% ketoconazole shampoo. Ketoconazole shampoo was chosen as a positive control because it is an imidazole antifungal with a broad spectrum. The results obtained from the 2% Ketoconazole positive control showed a large inhibition zone diameter with an average of 23.31, which is categorized as very sensitive (very strong). This means the positive control had the greatest inhibitory power compared to pandan leaf juice concentrations and other formulas, which were categorized as intermediate to sensitive. The negative control was distilled water, which was used to determine the effect of the solvent on the growth of the test fungus. The negative control test results showed no antifungal inhibitory power because distilled water does not contain any antifungal properties. Therefore, it can be concluded that the antifungal activity found in the juice and formula does not originate from the solvent used,

in line with research (Malonda et al., 2017).

Data from the antifungal effectiveness test were analyzed using SPSS 20. The statistical results of the hypothesis test showed that the data were normally distributed and not homogeneous. Therefore, the data were non-parametric, and a one-way ANOVA test was used to determine differences. A one-way ANOVA test showed a significant difference at $p<0.05$, indicating a significant difference. A Games-Howl test was used to determine the differences between each formula.

The non-parametric Games-Howl test revealed significant differences between all groups, except for the 12.5% juice mixture with Formula 2 (containing 12.5% juice mixture) and the 12.5% juice mixture with Formula 3 (containing 25%). This is because the 12.5% juice mixture, Formula 2, and Formula 3 have the same antifungal activity, meaning they have the same inhibitory power.

In the juice mixture groups with different concentrations, there were significant differences because the higher the juice concentration, the greater the inhibitory power. In the pandan leaf juice shampoo gel formula, there was a significant difference ($p<0.05$). This is because the concentration of juice added to the formula differs, resulting in different inhibitory powers. Therefore, the higher the concentration of juice added to the shampoo gel base formula, the greater the inhibitory power.

The juice, shampoo gel formula, and control (-) groups showed significant differences compared to the control (+). This was because the control (+) used a shampoo preparation containing 2% ketoconazole, where ketoconazole is an active chemical ingredient that has antifungal activity, resulting in greater inhibitory power compared to the other groups. The juice of pandan leaves, shampoo gel formula, and control (+) showed significant differences compared to the control (-) group ($p<0.05$), indicating a significant difference. This was because the control (-) used distilled water, which is neutral and does not have antifungal activity, in line with research by Malonda et al. (2017).

CONCLUSION

1. Pandan leaf juice can be formulated as an anti-dandruff shampoo gel.
2. Pandan leaf juice and the anti-dandruff shampoo gel can inhibit the growth of Malassezia furfur at all concentrations.
3. Pandan leaf juice meets the physical parameters of the shampoo, namely organoleptic tests, pH tests, and foaming height tests, making the shampoo safe and suitable for use.

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