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ANTIFUNGAL EFFECTIVENESS TEST FRAGRANT LEAF ETHANOL EXTRACT (*PANDANUS AMARYLLIFOLIUM ROBX*) AGAINST FUNGUS *PITYROSPORUM OVALE* IN VITRO

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ABSTRACT

Dandruff is one of the fungal infection problems that causes lack of confidence and discomfort due to complaints of itching that accompanies it. The fungus that causes dandruff is *Pityrosporum ovale*. *Pityrosporum ovale* has been reported to be resistant to the use of these azole drugs. One of the plants that has the potential as herbal medicine is pandan leaves (*Pandanus amaryllifolius Roxb*). The purpose of this study was to determine the antifungal activity of fragrant pandan leaf extract (*Pandanus amaryllifolius Roxb*) on the growth of *Pityrosporum ovale* in vitro. The research method used an experimental method with the extract concentrations used were 10%, 20%, 30%, and 40%. The antifungal testing method uses the agar diffusion method using the Kirby Bauer method. The results showed that pandan fragrant leaf extract with concentrations of 10%, 20%, 30% and 40% had moderate strength antifungal inhibition against *Pityrosporum ovale*. The largest inhibition zone was at a concentration of 40%, which was 9.43 mm. This indicates that the fragrant pandan leaf extract has antifungal effect on *Pityrosporum ovale* which causes dandruff. So that the results of this study can be used as a product to reduce the problem of dandruff.

I. INTRODUCTION

Indonesia is an archipelagic country located on the equator and has a tropical climate, making it possible for the development of infectious diseases caused by fungi. One of the most common fungal infections is dandruff. Dandruff is one of the problems on the scalp that occurs in almost half of the world's population regardless of gender and socio-culture. No resident in any geographical area is free without being affected by dandruff in daily life.

Dandruff is one of the fungal infection problems suffered by humans, although dandruff is not a life-threatening disease, but currently dandruff is a prominent problem among the general public. This is because for sufferers, dandruff can cause a lack of confidence due to cosmetic problems or aesthetic disturbances it causes and cause discomfort due to complaints of itching that accompanies it [1].

Dandruff belongs to seborrheic dermatitis is a common abnormal skin condition characterized by peeling and itching. Dandruff affects 50% of the population world. This disorder is caused by several factors, namely the activity of the sebaceous glands, the fungus genus *Malassezia* and individual sensitivity. The fungus that causes dandruff is Malassezia sp. One of the species is *Pityrosporum ovale* [2]. This fungus is actually a

normal flora in the hair, but there are several factors that affect the excessive growth of the fungus *Pityrosporum ovale* on the scalp, causing a scaly head [3]. Factors that influence the incidence of dandruff, among others increased sebum production in the sebaceous glands, individual susceptibility factors, environmental factors (temperature and humidity of the environment), and stress.

In *Malassezia sp*. (*Pityrosporum ovale*) has been reported to be resistant to the use of these azole drugs. Research in Japan reported that zinc pyrithione at sublethal doses was reported to be teratogenic and toxic to medaka fish (*Oryzias latipes*) [4]. Meanwhile, according to Stecher, Zinc pyrithione (ZPT) also has side effects such as dermatitis that occurs on the scalp and hair damage (hair loss, discolored, and broken).

The side effects of synthetic drugs and the 'back to nature' lifestyle that are starting to be widely developed by people in Indonesia are now encouraging researchers to look for alternatives. The alternative is in the form of active compounds from plants that can be used as antifungals and do not cause side effects. The plant was chosen because it was predicted to have an active compound as an antifungal, namely the fragrant pandan leaf (*Pandanus amaryllifolius Roxb*).

II. MATERIALS AND METHODS

II.1 STUDY SITE

This research was conducted at the Botany of Laboratory and Microbiology & Virology Laboratory of the Faculty of Pharmacy Institute of Health Medistra Lubukpakam. Samples were taken from Gg. Pantai Cermin Kiri Hamlet V, Pantai Cermin District, Bedagai Serdang, North Sumatra Province.

II.2 SAMPLING PROCEDURE

The sample used was 10 kg of Pandan Wangi Leaves (*Pandanus amaryllifolius Roxb*) obtained from Gg. Mirror Beach Kiri, Hamlet V, Pantai Cermin District, Serdang Bedagai Regency, North Sumatra Province. Sampling was done purposively, that is, without comparing with other areas.

Pandan leaves are washed with running water to clean them from dust and other foreign objects. Then chopped to facilitate the drying process. Drying was carried out in light indirect sunlight, using a black cloth as a cover over the sample. The dry product was then crushed using a blender machine, then sieved using a 40 mesh sieve so that the same size powder was obtained (Figure 1). Then, the dry powder was extracted using the maceration method [5].

Figure 1: Pandan Leaves (*Pandanus Amaryllifolius Roxb*).
Source: Authors, (2021).

II.3 MAKING FRAGRANT PANDAN LEAF ETHANOL EXTRACT

The simplicia powder of fragrant pandan leaf extract was carried out using 96% ethanol as a solvent. A total of 500 grams of simplicia powder of fragrant pandan leaves is put into a glass container, 3.75 liters of 96% ethanol is added, close the lid, leave for 5 days protected from light while stirring frequently, sprinkle, squeeze, wash the dregs with a sufficient amount of filter liquid. Transfer to a closed vessel, leave in a cool place, protected from light for 2 days. Precipitated pour or filtered. The results obtained were concentrated with a rotary evaporator at a temperature of 40°C until most of the solvent had evaporated and continued with the evaporation process on a water bath until a thick extract was obtained [6].

II.4 PHYTOCHEMICAL SCREENING

Phytochemical screening of simplicia powder includes examination of alkaloids, flavonoids, saponins, and tannins.

Alkaloids: A total of 0.5 grams of simplicia powder plus 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, the comparison on a water bath for 2 minutes, filtering and filtering. The filtrate used for the alkaloid test is as follows: a) 3 drops of filtrate plus 2 drops of Mayer's reagent solution will form a white or yellow lumpy precipitate; b) 3 drops of filtrate is added with 2 drops of Bouchardat reagent solution, a brown to black precipitate will be formed; c) 3 drops of filtrate is added with 2 drops of Dragendorff's reaction solution, a red or orange precipitate will be formed. Alkaloids are positive if they occur on the surface or turbidity in at least two of the three experiments above [7].

Flavonoid: A total of 10 grams of simplicia powder was added to 10 ml of hot water, boiled for 5 minutes and filtered in a hot state, into 5 ml of the filtrate added 0.1 grams of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Positive flavonoids occur if the color is red, yellow or orange on the amyl alcohol layer [8].

Saponins: As much as 0.5 grams of simplicia powder was put in a test tube, added 10 ml of hot water, brought to a boil and then shaken vigorously for 10 seconds, if 1-10 cm high foam is formed which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop. 2N hydrochloric acid indicates the presence of saponins [9].

Tanin: A total of 0.5 grams of simplicia powder was extracted with 10 ml of distilled water and then filtered, the filtrate was diluted until it was colorless. 2 ml of the solution is taken and 1-2 drops of 1% iron (III) chloride reagent are added, if a blackish blue or blackish green color occurs, it indicates the presence of tannins [10].

II.5 MAKING VARIATIONS OF ETHANOL EXTRACT CONCENTRATION OF FRAGRANT PANDAN LEAVES (*PANDANUS AMARYLLIFOLIUS ROXB*)

The ethanol extract of fragrant pandan leaves was made in concentrations by weighing the extracts of 100 mg, 200 mg, 300 mg, 400 mg, respectively. then each was dissolved with 10 ml of DMSO (dimethyl sulfoxide). The concentration of ethanolic extract of fragrant pandan leaves is 10%, 20%, 30%, 40%.

II.6 RESISTANCE TEST

Fragrant pandan leaf extract solutions as samples were made at concentrations of 10%, 20%, 30%, and 40%. As a positive control, ketoconazole 2% was used and a negative control was used DMSO 1%. Ethanol extract preparations from pandan fragrant leaves were tested for antifungal activity against *Pityrosporum ovale* using the disk diffusion method (Kirby-Bauer test) with positive and negative controls. SDA (Sabaroud Dextrose Agar) medium was made to be put into a petridish. Sterile SDA medium was put into a petri dish as much as 20-25 ml, then allowed to solidify. Make a mushroom inoculum/suspension solution by taking a few oses of *Pityrosporum ovale* isolate into 0.9% NaCl and compare the turbidity with 0.5 Mac Farland. Put 0.1 ml of *Pityrosporum ovale* suspension in a spread plate on its surface. Place the paper disc that has been soaked in a combination solution of Pandan Wangi leaf extract with various concentrations, 2% Ketoconazole solution and 1% DMSO solution on the surface of the SDA medium. Repeat in triples. Then incubated in an incubator for 3 x 24 hours at 37°C. The diameter of the inhibition zone formed (clear area around the paper disc without fungal growth) was measured with a caliper and expressed in millimeters [11][12].

III. RESULTS AND DISCUSSIONS

III.1 PHYTOCHEMICAL SCREENING

The class of chemical compounds on the simplicia powder of pandan leaves was carried out to obtain information on the class of secondary metabolites contained in it. The results of the examination of the Alkaloid, Flavonoid, Saponin, and Tannin compound groups (Table 1).

Table 1: Results of chemical compound group of pandan leaves simplicia powder.

No	Parameters	Information	Results
1	Alkaloid	Precipitates a white solid	+
2	Flavonoid	Orange solution	+
3	Saponin	formed foam	+
4	Tannin	Blue-black solution	+

Note: (+) = contain test's compound (-) = not contain test's compound.
Source: Authors, (2021).

Screening for alkaloids using chloroform and mayer reagents, the results are indicated by the presence of a white precipitate which means that the extract contains alkaloids. Alkaloids have been studied extensively for their biological activities and medicinal uses. Research that has been done shows that Alkaloids have significant pharmacological effects, such as anticonvulsant, analgesic, antifungal, anthelmintic, anti-inflammatory, antimalarial, anti-bacterial and cardiotonic [13].

Screening of flavonoids using Magnesium and HCl concentrated reagent, the results marked with the solution changes color orange, which means that there is a flavonoid extract. Research that has been conducted reports that fragrant pandan leaves contain flavonoids. Flavonoids are bioactive bases that are responsible for antimicrobial activity [14].

Testing of saponins on pandan pandan leaf extract showed positive results which were indicated by the formation of foam resulting from the reaction between saponin compounds and aquadest. Saponins have a high toxicity to fungi. The mechanism of antifungal activity is its interaction with membrane sterols [15].

Testing of Tannin compounds on fragrant pandan leaves using $FeCl_3$ reagent. Where a positive reaction is produced which is indicated by a change in the color of the solution to blue-black. This shows that the extract contains tannin compounds. Tannins are polyphenolic tannin compounds (commonly referred to as tannic acid). Tannins are recognized as outstanding antimicrobial compounds. Extensive analysis has revealed that tannins have antimicrobial properties by inhibiting the activity of microorganisms including fungi, yeasts and bacteria [16].

III.2 INHIBITORY TEST OF ETHANOL EXTRACT OF FRAGRANT PANDAN LEAVES (*PANDANUS AMARYLLIFOLIUS ROXB*) AS ANTIFUNGAL AGAINST PITYROSPORUM OVALE

Based on the research that has been done the results obtained in Table 2. The study was conducted in a positive control using ketoconazole 2%, negative controls using 1% DMSO, as well as variations Concentrations of ethanol extract fragrant pandan leaves that is 10%, 20%, 30% and 40%.

Table 2: The results of the inhibition test of fragrant pandan leaf extract (*Pandanus amaryllifolius Roxb*) as antifungal against *Pityrosporum ovale*.

Extract Concentration	Repetition I	Repetition II	Repetition III	Average	Information
DMSO 1% (Negative Control)	0	0	0	0	Weakness
Ketoconazole 2% (Positive Control)	9.5	9.8	10	9.76	Moderate
10%	7.7	7.9	8.0	7.86	Moderate
20%	8.3	8.5	8.8	8.53	Moderate
30%	8.5	8.8	9.0	8.76	Moderate
40%	9.2	9.5	9.6	9.43	Moderate

Source: Authors, (2021).

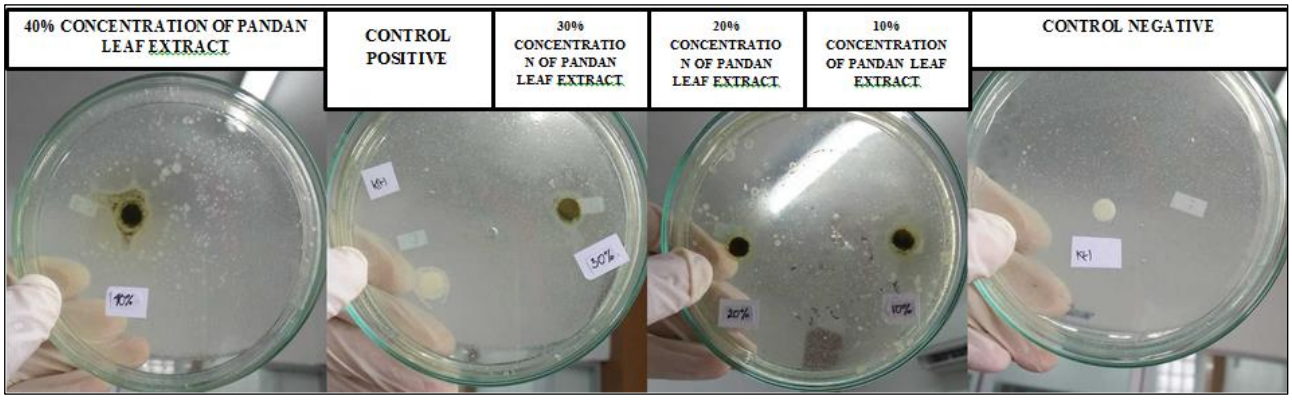

Figure 2: The results of the inhibition test were indicated by the formation of a clear zone around the colony.
Source: Authors, (2021).

In Figure 2 it can be seen that the negative control does not have a clear zone, this indicates that 1% DMSO does not have the ability to inhibit the growth of the fungus. This is also supported by previous research which stated that 1% DMSO at 24 hours growth could not significantly inhibit fungal growth. If DMSO is used >2% with a growth time of 48, 72, and 96 hours, it will inhibit the growth of the fungus [17]. Previous studies also reported that DMSO 2% significantly slowed growth and decreased growth in several *Candida* species tested, but DMSO 1% had no significant effect on growth kinetics. In addition, DMSO is a very polar and stable substance, so it is often used as a solvent and neutralizer [18].

In this study, the positive control used was 2% ketoconazole. The results showed that ketoconazole 2% had an antifungal inhibition of *Pityrosporum ovale* with an average of 9.76 mm (Table 2). Based on research that has been done, ketoconazole is an antifungal drug used to and with the help of oral fluconazole can kill fungus to 91% [19]. Ketoconazole is an antifungal that acts by inhibiting the cytochrome P450 14 α-demethylase enzyme which leads to the inhibition of lanosterol to ergosterol. This will change the permeability of the cell membrane. In addition, ketoconazole inhibits the biosynthesis of fungal triglycerides and phospholipids as well as peroxidative enzymes involved in fungal detoxification that cause cellular necrosis [20].

Based on the results of the study, it was shown that the ethanolic extract of fragrant pandan leaves at concentrations of 10%, 20%, 30%, and 40% had different inhibitory effects on *Pityrosporum ovale* (Figure 2). At a concentration of 10% it has an inhibitory power of 7.86 mm, a concentration of 20% has an inhibitory power of 8.53 mm, a concentration of 30% has an inhibitory power of 8.76%, and at a concentration of 40% has an inhibitory power of 9.43%. This shows that the greater the concentration of ethanolic extract of pandan leaves, the greater the inhibitory power produced.

According to Davis Stout [21], antifungal strength is classified into 4 groups, namely weak (<5 mm), moderate (6-10 mm), strong (11-20 mm), and very strong (> 20 mm). So, based on the research that has been done, it can be stated that of the 4 variations in the concentration of pandan fragrant leaf extract, the inhibition zone for *Pityrosporum ovale* is moderate, with the highest inhibition zone at a concentration of 40%. This is because the fragrant pandan leaf extract has bioactive compounds that act as antifungals such as alkaloids, flavonoids, saponins and tannins.

There is little information about the antimicrobial properties of the leaf extract of this plant. Previous research stated that pandan fragrant leaf extract was not effective in inhibiting the growth of the bacteria *Micrococcus (Staphylococcus) auereus* and *Escherichia coli*. This is possible due to the content of certain alkaloids in fragrant pandan leaves, such as Norpandamarilactonine-A,-B, Pandamarilactam3x,-3y, Pandamarilactone-1, PandamarilactonineA,-B,-C, Pandamarine, Pandanamine [22].

This is supported by previous studies that say that the fragrant pandan leaf extract at a concentration of 15% were able to reduce the Total Plate Count and Number of Mold on traditional foods. This is due to the chemical compounds present in the fragrant pandan leaves, namely Alkaloids, Flavonoids, Tannins and Saponins. Tannins are complex compounds that are soluble in water, polyhydric phenolic compounds that are toxic to humans fungi, bacteria, and yeast/yeast, as well as inhibit the growth of the virus. Alkaloids contain natural organic nitrogen base with a heterocyclic ring. This compound has antimicrobial properties due to its ability to damage DNA. Flavonoids have antimicrobial properties because of its ability to combine with bacterial cells extracellular membranes and proteins. Saponins is cytotoxic because it can change the permeability membrane so that lysis occurs from microbial cells [5].

Previous research on the antibacterial activity of pandan pandan leaf extract against bacteria (*S. sangnguinis, S. Mutans, S. Salivarius and P. gingivalis*) showed the presence of zones of inhibition resulting from various concentrations. The inhibitory power produced is due to the content of fragrant pandan leaf extract in which there are phenolic and flavonoid compounds [23].

IV. CONCLUSIONS

Based on the results of the study, it can be concluded that pandan fragrant leaf extract with concentrations of 10%, 20%, 30% and 40% had moderate strength antifungal inhibition against Pityrosporum ovale. The largest inhibition zone was at a concentration of 40%, which was 9.43 mm. These results were comparable to the zone of inhibition produced by ketoconazole 2% (positive control).

V. AUTHOR'S CONTRIBUTION

Conceptualization: Angelika Sinaga and Saadah Siregar.
Methodology: Saadah Siregar and Vincentia Ade Rizky.
Investigation: Angelika Sinaga and Riana Topia.
Discussion of results: Saadah Siregar, Vincentia Ade Rizky and Riana Topia.

Writing – Original Draft: Vincentia Ade Rizky and Riana Topia.
Writing – Review and Editing: Saadah Siregar and Angelika Sinaga.
Resources: Vincentia Ade Rizky and Riana Topia.
Supervision: Saadah Siregar and Angelika Sinaga.
Approval of the final text: Saadah Siregar and Vincentia.

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