

## Activity Test of Combination Binahong Leaf Extract (*Anredera cordifolia*) With Lemongrass Extract (*Cymbopogon citratus*) as Antifungal Against *Pityrosporum ovale*

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### ABSTRACT

*Pityrosporum ovale* is a microorganism thought to be the main cause of dandruff. Binahong and lemongrass are herbal plants that can cure various diseases. Based on usage empirical leaf binahong and lemongrass kitchen nutritious for remove dandruff, hair loss, diabetes mellitus, anti- cancer and treatment on infection skin caused by bacteria nor mushrooms. Binahong leaf extract and extract lemongrass contain metabolites secondary wrong only flavonoids. Flavonoids are effective as antimicrobials. Destination from study this The purpose of this study was to determine the antifungal activity of the combination of binahong leaf extract (*Anredera cordifolia*) and lemongrass extract (*Cymbopogon citratus*) against *Pityrosporum ovale* . This study used 5 test groups, which consisted of a combination group of binahong leaf extract and lemongrass F1 = concentration 50%:50%, F2 = concentration 37,5%:12,5%, F3 = concentration 25%:25% and F4 = concentration 12,5%:37,5%, and the control group was positive (ketoconazole 2%) and negative ( aquadest), with 3 repetitions. The materials used in this study were binahong leaf simplicia and kitchen lemongrass simplicia macerated using 70% ethanol solvent, resulting in a thick extract which was then tested for antifungal activity against *Pityrosporum ovale* by the method diffusion paper disc on Sabouraud Dextrose Agar medium . The parameter observed was the diameter of the growth inhibition zone of the fungus *Pityrosporum ovale* . Analysis using ANOVA (Analysis of Variance). The results of this study were binahong leaf extract and kitchen lemongrass extract positive containing flavonoid compounds. The combination of binahong leaf extract and citronella extract has the ability to inhibit the growth of the fungus *Pityrosporum ovale* with visible inhibition zones formed. The most effective concentration to inhibit the growth of the fungus *Pityrosporum ovale* was at a concentration of F1 = 50%:50% of 17.40 mm with a strong category.

**Keywords** : Binahong leaf, lemongrass, antifungal, *Pityrosporum ovale*.

## INTRODUCTION

The hair that adorns the human head is an aesthetic need, so many people are willing to spend time and even money to care for and improve hair health in order to maintain their appearance. One of the problems that can cause a person's confidence to decrease in their activities is dandruff (Diensi et al., 2021). Dandruff is one of the common hair problems affecting nearly 50% of the population at puberty of any gender and ethnicity. The severity of dandruff is influenced by age, especially during puberty and middle age (reaching a peak at the age of 20 years) and decreases during lasia (above 50 years) and is relatively rare and mild in children (Anwar et al., 2015). The characteristics of a person with dandruff, where the scalp condition occurs excessive exfoliation of dead skin cells, are generally in the form of white or yellowish flakes (Widowati et al., 2020).

*Pityrosporum ovale* is a microorganism that is thought to be the main cause of dandruff, this fungus is actually normal flora on the scalp, but in hair conditions with excess oil glands, this fungus can thrive. *Pityrosporum ovale* is a single-celled yeast or fungus that is a member of the genus member of the genus *Malassezia* sp, and belongs to the *Cryptococcaceae* family (Sakinah et al., 2015). Treatment of dandruff hair problems is one of them with herbal plants that contain antifungal compounds. one of which is the binahong and lemongrass plants.

Binahong (*Anredera cordifolia*) is a potential medicinal plant from the *Basellaceae* family. According to research (Ginting et al., 2021) phytochemical screening of binahong leaf extract contains alkaloid compounds, flavonoids, saponins, glycosides, teroids/terpenoids and tannins. The function of flavonoids is as antimicrobial work is an active compound that can kill *Candida albicans* fungi. Based on this study it can be concluded that anti-dandruff from binahong leaf extract with a concentration of 5%, 10% and 15% has given a very strong inhibition against the growth of *Candida albicans* fungus, which is one of the fungi that cause dandruff. So from this description, researchers are interested in using binahong leaves as a test material for antifungal activity against *Pityrosporum ovale*, because the *Candida albicans* fungus has similarities with *Pityrosporum ovale*, namely both fungi that cause dandruff (Yusuf et al., 2020).

According to (Arianto et al., 2018) another plant that is thought to potentially contain antifungal compounds is the kitchen lemongrass plant (*Cymbopogon citratus*). Kitchen

lemongrass is one of the plants that can produce the most important essential oil, because it contains high levels of citral (65% to 85%). So based on this description, the researcher is interested in using kitchen lemongrass stems which will be used as an extract as a test material for antifungal activity against *Pityrosporum ovale*. Testing the antifungal activity in this study is by using the disc diffusion method.

Based on this description, it encourages researchers to conduct research on "Test the Combination Activity of Binahong Leaf Extract (*Anradera cordifolia*) and Kitchen Lemongrass Extract (*Cymbopogon citratus*) as Antifungal Against *Pityrosporum ovale*".

## **TOOLS AND MATERIALS**

The materials used in this study were Binahong Leaf *Simplisia* 700 g, *Simplisia* Kitchen Lemongrass 500 g, 70% Ethanol, Aquadest, Ketoconazole 2% cream, Paper Discs, Sabouraud Dextrose Agar Media, *Pityrosporum Ovale* fungus culture, NaCl 0.9%, Magnesium 5 g, Concentrated.

The tools used in this study Rotary Evaporator, water Bath, Petri dish, beaker Glass, beaker Glass 350 ml, beaker Glass 50 ml, drop Pipette, stirring Rod, spatel, measuring Glass, distillation flask, 1000 ml porcelain cup, reaction tubes, reaction tube rack, plastic wrap 1 pcs, 1 cc syringe, 10 cc syringe, 5 cc syringe, Sterile cotton swab, Sterile Gauze, Filter Paper, Funnel, ose needle, L rod, analytical balance, hallway term, autoclave, incubator, bunsen.

## **METHOD**

### **1. *Simplisia* Procurement:**

Binahong (*Anredera cordifolia*) and kitchen lemongrass (*Cymbopogon citratus*) leaf preparations were obtained from PT Dipa Prasada Husada.

### **2. Preparation of sample**

Weighed 700 grams of binahong leaf *simplisia* and 500 grams of kitchen lemongrass *simplisia* then each was put into a black glass bottle. Then added 70% ethanol solvent for binahong leaves as much as 7000 ml and for kitchen lemongrass 5000 ml (1:10 ratio) and soaked for 3 times 24 hours while occasionally stirring. Filtered filtrate ethanol extracts of binahong leaves and kitchen lemongrass so as to obtain the filtrate and the dregs. Then, the filtrate of ethanol extract of binahong leaves and kitchen lemongrass extract was evaporated using a

rotatory evaporator with a temperature of  $<65^{\circ}\text{C}$  to obtain thick ethanol extract. The resulting thick extract is then weighed and the yield is calculated.

### **3. Phytochemical screening:**

Put the ethanol extract of binahong leaves (*Anradera cordifolia*), and ethanol extract of kitchen lemongrass (*Cymbopogon citratus*) sufficiently into a test tube. Magnesium powder and concentrated HCl were added to the test tube. The presence of flavonoids is characterized by the formation of yellow, orange to red color (Indah, 2006).

### **4. Tool Sterilization**

All equipment to be used was washed and dried, then wrapped in paper and sterilized using an autoclave at  $121^{\circ}\text{C}$  for 15 minutes. The microbiological test treatment process was carried out aseptically in an encase that had previously been sterilized with 70% alcohol and irradiated with UV light turned on for 15 minutes before use (Aziz, 2010).

### **5. Media Preparation**

Sabouraud Dextrose Agar (SDA) media as much as 30 grams was put into a glass beaker and then dissolved by adding 500 ml of distilled water, then heated to boiling on a water bath while being homogenized using a stirring rod. After boiling, the media was sterilized by autoclaving at  $121^{\circ}\text{C}$  for 15 minutes. Next, pour into petri dishes containing 15 ml of each petri and allowed to solidify (Irianto, 2006).

### **6. Fungal Culturing**

#### a) 0.5 Mc Farland standard

Put 9.95 mL of sulfuric acid ( $\text{H}_2\text{SO}_4$  1%) in a sterile tube. Added barium chloride ( $\text{BaCl}_2$  1%) as much as 0.05 mL. Homogenized, so as to get a turbidity of 0.5 Mc Farland. The turbidity of this solution is used as a turbidity standard for fungal suspensions.

#### b) Preparation of *Pityrosporum ovale* Fungal Suspension.

Fungal culture was taken using a sterile ose and then put into 10 ml of 0.9% NaCl solution, shaking and stirring until it reached a turbidity equivalent to 0.5 Mc Farland standard. The suspension of each fungus that has been shaken and stirred is immediately inoculated on a solid agar plate. The suspension was shaken and stirred just before inoculating each petri dish to prevent settling of the suspension.

### c) Treatment

*P. ovale* fungal suspension was taken as much as 0.1 ml and then spread aseptically using a sterile L rod on SDA media. Paper disk blank, then dipped in a combination of binahong leaf extract and kitchen lemongrass extract with variations of F1 = concentration 50%: 50%, F2 = concentration 37.5%: 12.5%, F3 = concentration 25%: 25% and F4 = concentration 12.5%: 37.5%, as well as making positive control (ketoconazole 2%) and negative control (aquadest). Paper disk blanks that have been dipped are taken and affixed to the top surface of the SDA medium, in a Petri dish. Then incubate at 37°C for 24 hours, observe the inhibition zone around the paper disk blank on Sabouraud Dextrose Agar media and measure the inhibition zone formed.

### **7. Preparation of Concentration Variations of Binahong Leaf Extract and Lemongrass Extract**

The concentration of the combination of binahong leaf extract (*Anradera cordifolia*) and kitchen lemongrass extract (*Cymbopogon citratus*), with variations of F1=50%:50% concentration, F2=37.5%:12.5% concentration, F3=25%:25% concentration and F4= 12.5%:37.5% concentration. Made by weighing the thick extract of binahong leaves and thick extract of kitchen lemongrass respectively 3 grams, 2.25 grams, 1.5 grams, and 0.75 grams then each concentration is diluted with distilled water solvent until the volume is 6 ml.

### **8. Antifungal Activity Test**

Antifungal activity test was carried out by diffusion method. This method is carried out using disc paper with a diameter of 6 mm. SDA media that has been heated is put into a Petri dish as much as 15 ml and then allowed to freeze. The test fungus was poured on the frozen SDA media. Paper disks were soaked in a combination solution of binahong leaf extract and kitchen lemongrass extract for 15 minutes, then placed on the surface of the media that had solidified. The media that has been filled with the test preparation is then incubated at 37°C, then observation and measurement of the inhibition formed at the 24th hour.

## **RESULT**

### **Antifungal Activity Test Results**

Antifungal activity test aims to determine the ability of the combination of binahong leaf extract (*Anredera cordifolia*) and kitchen lemongrass extract (*Cymbopogon citratus*) to inhibit the growth of *Pityrosporium ovale* fungi tested. The inhibitory ability is characterized by the formation of a clear zone

around the disc paper. This clear zone indicates the antifungal activity of the extract tested. Results can be seen in Table 1

**Table 1. Zone of Inhibition Results**

No	Treatment	Inhibition Zone Diameter (mm)			Average	Description
		Replication I	Replication II	Replication III		
1.	F1	18,36	20,03	13,81	17,40	Strong
2.	F2	13,22	13,46	9,44	12,04	Strong
3.	F3	10,39	11,27	9,39	10,35	Strong
4.	F4	9,53	10,91	7,71	9,38	Medium
5.	F5	31,31	30,30	31,04	30,88	Very Strong

Description: F1 (50%:50%); F2 (37.5%:12.5%); F3 (25%:25%); F4 (12.5%:37.5%); F5 (positive control) (Binahong Leaf Extract: Kitchen Lemongrass Extract)

**Table 2 Results of Statistical Analysis of Zone of Inhibition Values**

Treatment	Average ± S.D	P. value	Description
F1	17,40 ± 3,219		
F2	12.04 ± 2,254		
F3	10,35 ± 0,940	0,000	< 0,05
F4	9,38 ± 1,605		
F5	30,88 ± 0,522		

Description: F1 (50%:50%); F2 (37.5%:12.5%); F3 (25%:25%); F4 (12.5%:37.5%); F5 (positive control)  
 > 0.05 = no difference in inhibition zone results  
 <0.05 = there is a difference in the results of the inhibition zone

## DISCUSSION

Based on the results that can be seen in table 1, the combination of binahong leaf extract (*Anredera cordifolia*) and kitchen lemongrass extract (*Cymbopogon citratus*) at a concentration of (50%: 50%) obtained an average inhibition zone of 17.40 mm, a concentration of (37.5%: 12.5%) of 12.04 mm and a concentration of (25%: 25%) as much as 10.35 mm. This means that it has an average inhibition value of > 10 - 20 mm, so it can be said to be included in the category according to Greenwood (2005), namely strong inhibition. Meanwhile, the concentration (12.5%: 37.5%) has an average inhibition value of 9.38 mm, so it can be said to be included in the medium inhibition category. The higher the concentration (F1) is the wider the inhibition zone formed, although basically all concentrations of test substances have inhibitory response activity that does not exceed the positive control.

Statistical analysis of data in this study was carried out using the *Statistical Package for the Social Sciences (SPSS) version 22.0 for Windows application including One Way ANOVA test and Tukey-HSD univariant test.*

Based on the results of statistical analysis of the inhibition zone value of formula 1, formula 2, formula 3, formula 4 and formula 5, all data are normally distributed  $P > 0.05$  and the distribution of data shows homogeneous distribution  $P (0.074) > 0.05$ . The One Way ANOVA results show a significance value of  $0.000 < 0.05$ , meaning that  $H_a$  is accepted, then it shows that there is a difference in the effect of giving various concentrations of the combination of binahong leaf extract (*Anredera cordifolia*) and kitchen lemongrass (*Cymbopogon citratus*) on the value of the inhibition zone on the growth of *Pityrosporum ovale*.

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## **CONCLUSION**

Based on the results and discussion, the conclusions that can be drawn from this research are as follows:

The combination of 70% ethanol extract of binahong leaves (*Anredera cordifolia*) and 70% ethanol extract of kitchen lemongrass (*Cymbopogon citratus*) has antifungal activity against the growth of *Pityrosporum ovale*.

In the combination of 70% ethanol extract of binahong leaves (*Anredera cordifolia*) and 70% ethanol extract of kitchen lemongrass (*Cymbopogon citratus*) at a concentration of (50%: 50%) has the most effective antifungal activity against the growth of *Pityrosporum ovale* with an average inhibition zone diameter of 17.40 mm which is categorized as strong, in connection with that the higher the concentration given, the wider the inhibition zone or clear zone formed against the growth of *Pityrosporum ovale*.

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