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Activity of Bangle Rhizome Extract (*zingiber cassumunar roxb.*) Inhibits the Growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes*

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ABSTRACT

Bangle rhizome extract (zingiber cassumunar roxb.) is an herbal plant widely consumed in Indonesia and also has broad medical properties. This research aimed to determine the yield of Bangle Extract (Zingiber cassumunar Roxb.) using the solvents Aquadest, Aceton, Ethanol 70%, Ethanol 96% and Methanol. Besides that, we find out the results of phytochemical screening tests and Minimum Inhibition Concentration (MIC). The research results on the yield of Bangle rhizome extract with the five solvents obtained the most significant yield, namely with distilled water, 7.2 percent. Based on the phytochemical screening test, Bangle rhizome extract was positive for containing secondary metabolites such as tannins, alkaloids, triterpenoids, steroids and saponins. The lowest MIC results for the Bangle extract were in a solvent called Aquadest at 6.25 mg/mL with a value of 0.049 mg/mL. They continued in 70% ethanol solvent with a titer at 3.125 mg/mL in the fungus Trichophyton rubrum and in the fungus Trichophyton mentagrophytes with a titer at 3.125 mg/mL. At the same time, the greatest MIC value was obtained in the solvents acetone, ethanol 96% and methanol with a value of 0.049 mg/mL for both fungi. The best solvents used in this research were high concentration organic solvents (acetone, alcohol 96% and methanol), but that toxicity needs further research.

Keywords: *Bangle rhizome extract (zingiber cassumunar roxb), MIC, Phytochemical Screening.*

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INTRODUCTION

Indonesia is a tropical country that has high humidity temperatures. These conditions are suitable for fungal growth. A disease caused by dermatophytosis fungus, which is a type of fungus that attacks keratinised tissue such as the callus layer of human skin, nails and hair. (1). Dermatophytosis is caused by dermatophyte fungi divided into three genera: Trichophyton, Microsporum and Epidermophyton. Various drugs can be used to treat dermatophytosis, but these drugs are often resistant to dermatophyte fungi (2). Microsporum and Epidermophyton. Transmission of this fungus can be direct or indirect. To cause disease, dermatophyte fungi must have the ability to attach to the host's skin, penetrate tissue survive and adapt to the temperature and biochemical environment of the host (3). Dermatophytosis is not a fatal disease but can become chronic and recurrent and is often resistant to anti-fungal drugs, so dermatophytosis can cause discomfort and reduce the quality of life for sufferers (4).

Systemic antifungals are an option for treating dermatophytoses, especially if the infection is widespread or topical treatment has failed. The discovery of new anti-fungal drugs has experienced rapid development, both topical and systemic, and is expected to reduce the prevalence of fungal infections (5). The high incidence of dermatophytes causes increased resistance to antifungals due to the use of antifungals that are not according to indications, and drug administration is not completed. Therefore, there is a need for anti-fungal alternatives from natural ingredients that have relatively few side effects and are safer (6).

Bangle rhizome is a natural ingredient that can become a systemic antifungal (Zingiber cassumunar Roxb.). Bangle rhizome (Zingiber cassumunar Roxb.) is is often used as a medicinal herb in local communities. The plant is easily found and cultivated, making it a potential traditional medicine. (7). Zingiber cassumunar Roxb Extract. It contains flavonoids, quinones, steroids, and triterpenoids. Flavonoid compounds are known to have valuable activities as antiseptics and antibacterials (8)

The antimicrobial activity of Zingiber cassumunar Roxb has been tested against Staphylococcus aureus ATCC 25925 and Microsporum canis using the disc diffusion method. Trying the action of bangle extract on the bacteria Staphylococcus aureus ATCC 25925 with a concentration of 4000-500 ppm had weak activity, and on the fungus Microsporum canis, the concentration had strong activity (9). Apart from that, Zingiber cassumunar Roxb. It can also inhibit the growth of Pseudomonas aerugenosa bacteria (10). Zingiber cassumunar Roxb 70% ethanol extract can also inhibit the growth of the fungus *Trichophyton rubrum* (11). The researchers wish to prove whether there is an effect of different solvents from Extract of Bangle Rhizome (Zingiber cassumunar Roxb.) at various concentrations for inhibiting the spread of dermatophyte fungi and determine the type of solvent and optimum concentration that can inhibit the growth of dermatophyte fungi.

MATERIAL & METHODS

Research Design

This research is a laboratory experiment to test the inhibition from Extract of Bangle Rhizome (Zingiber cassumunar Roxb.) against the spread of Trichophyton rubrum, and Trichophyton mentagrophytes by using five different types of solvents, namely Aceton, Aquadest, Ethanol 70%, Ethanol 96%, and Methanol. This research was conducted from March to October 2023 and was carried out in two different locations. The extraction process of bangle rhizomes with five types of solvents and phytochemical tests was conducted at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University. At the same time, the process of testing the inhibition of fungal growth of Trichophyton rubrum and Trichophyton mentagrophytes using the Minimum Inhibitory Concentration (MIC) method was carried out at the Microbiology Laboratory, Faculty of Health, MH University. Thamrin University. The sample used was a simplistic Bangle rhizome with characteristics obtained from Sukabumi, West Java. The Bangle rhizome has a brownish colour, uneven surface, and 2-5 mm thickness.

Research Materials, Tools, and Procedures

Extraction of Bangle rhizome (Zingiber cassumunar Roxb.)

The extract is made at the spice and medicinal plant research centre using the solvents Aquadest, Aceton, 70% Ethanol, 96% Ethanol and Methanol. Ground Bangle rhizomes are soaked in solvent in a ratio of 1:5 (1000 grams of Bangle rhizome powder: 5000 ml of solvent). Then, the Bangle rhizome and solvent are mixed with a mixer for 2-3 hours. This mixture is left for 24 hours. This mixture is then filtered with a filter to obtain a purified filtrate. Then, the filtrate is put into the rotatory evaporator. In a rotatory evaporator, the solvent is vacuumed and then distilled until it evaporates. Once all the solvent has evaporated, you will get a thick Bangle rhizome extract. The extraction result is called % rendement, which is calculated by calculating the ratio of the weight of the extract obtained to the weight of the simplisia in its raw form, times 100.

Phytochemical Screening Test

Phytochemical screening was done by mixing the extract with standard qualitative analysis reagents to test alkaloids, flavonoids, saponins, triterpenoids and tannins. These reagents show the secondary metabolite compounds contained in the extract by observing the colour test results.

a. Alkaloid Test

Alkaloid tests can be carried out with various reagents, such as Mayer's, Bouchardat's and Dragendorff's. Mayer's reagent is potassium tetraiodomercurate (II), and Dragendorff's reagent is a mixture of bismuth subnitrate in acidic conditions and potassium iodide to form Bismuth-Potassium Iodide. In contrast, Bouchardat's reagent is Iodine-Potassium Iodide. In this research, Mayer and Bouchardat reagents were used. The nitrogen in the alkaloid reacts with the potassium ion (K+) from potassium tetraiodomercurate(II) to form a precipitated potassium-alkaloid complex in the alkaloid test with Mayer's reagent. The presence of white or cloudy precipitates indicates positive results containing alkaloids. The alkaloid test uses Bouchardat's reagent; the reaction is relatively similar to the response in Mayer's reagent. Both produce complex salt deposits. In contrast to Mayer's reagent, Bouchardat's

reagent will form an orange-brown precipitate if it is positive for containing alkaloids. The Saponin Test is done by shaking the extract in a test tube containing 5 ml of hot H_2O solvent. A positive result will form persistent foam in the shaken extract solution (12).

b. Tannin Test

The tannin test was 2 drops of green to blackish blue ferric chloride (FeCl3) solution. Phenol Test can be done by mixing the alcohol extract with the NaOH reagent. Indications of the presence of phenol will be marked in red. Flavonoid Test The procedure in this research uses Mg and HCl reagents, or Zn and HCl can also be used. The appearance of a dark red colour indicates positive results. This colour is a reaction of Mg metal with flavonoids to form a complex. Glycoside Test: Add 1 ml of extract to glacial acetic acid, $FeCl_3$ and concentrated sulfuric acid and a purple ring forms, indicating the presence of glycosides (12).

c. Tyrpeneoid Test

In the terpenoid test, the presence of terpenoids is indicated by a colour change from purple or red to blue-green when 1 ml of extract is mixed with 2 drops of anhydrous acetic acid and 1 drop of concentrated sulphuric acid. The extract obtained is dissolved in water and then 3 to 4 drops of a solution of copper acetate are added. The presence of diterpenes is indicated by the formation of green colour. (13).

d. Steroid Test

The Steroid test was add chloroform and see the layer that forms, then the chloroform layer is dried. Then, add three drops of concentrated H_2SO_4. Then a blue colour will develop. The formation of blue dye can be observed at the edge of the drop plate (14).

e. Saponin Test

The saponin test was 1 gram of extract was added to warm water, shaken vertically for 10 seconds, and left for 10 seconds until foam was formed. Formation of 1-10 cm high foam that is stable for not less than 10 minutes, and when one drop of 2N HCl is added, the foam does not disappear. This indicates the presence of saponins (14).

Minimum Inhibitory Concentration (MIC) Test

Minimum inhibitory concentration (MIC) Microdilution method using Nutrient Broth (NB) media with Resazurin added to a microtitre plate to determine the MIC. First, NB (100 µL) was added to each well, and 100 µL of extract was added to the first well, then serial dilutions were made until the 12^{th} well. Then 10 µL of fungal suspension was added to each well and incubated for 24 hours at 37°C. The MIC value was observed by a colour change on the microtiter plate. The lowest MIC value of the extract was indicated as having the best antifungal activity.

RESULT & DISCUSSION

1. The Yield of Bangle Extract (Zingiber cassumunar Roxb.)

The maceration method has differences in temperature, type of solvent, and extraction time. The extract obtained from the extraction process is weighed to determine the yield. Based on Table 1, it can be seen that there are differences in the yield of Bangle rhizome extract (Zingiber cassumunar Roxb.)

Table 1. Yield of Bangle Extract (Zingiber cassumunar Roxb.)

No	Extract Bangle (*Zingiber cassumunar Roxb.*)	Rendement (%)
1.	Acetone Solvent	7,2
2.	Aquadest Solvent	6,4
3.	Ethanol 70% Solvent	9,4
4.	Ethanol 96% Solvent	6,3
5.	Methanol Solvent	5,9

The yield of extraction results in five different types of solvents produced different yields. The amount of yield depends on the solubility of the bioactive components. Table 1 shows that the highest yield was the yield using 70% ethanol solvent, so it is likely that the bioactive compounds contained in Bangle Extract (Zingiber cassumunar Roxb) (15).

2. Phytochemical Screening Test

Phytochemical compounds are secondary metabolite compounds in plants that have specific functions for humans. Identify these phytochemical compounds in this study; eight

types of phytochemical compounds were identified, which are thought to be found in Bangle Extract (Zingiber cassumunar Roxb.). These phytochemical compounds are alkaloids, saponins, tannins, triterpenoids and steroids.

Table 2. Phytochemical Screening Test Results

No	Solvent Type	Tanin	Alkaloid	Triterpenoid	Steroid	Saponin
1.	Acetone Solvent	+	+	+	+	+
2.	Aquadest Solvent	+	+	+	+	-
3.	Etanol 70% Solvent	+	+	+	+	-
4.	Etanol 96% Solvent	+	+	+	+	+
5.	Metanol Solvent	+	+	+	+	+

Information :
+: Positive for secondary metabolites
-: Negative contains secondary metabolites

Table 2 shows the results of the phytochemical screening test from the acetone solvent maceration method. 96% ethanol and methanol are the same, positive for containing alkaloid compounds, tannins, triterpenoids, saponins and steroids. In contrast, there are only four secondary metabolites in distilled water and 70% ethanol: tannins, triterpenoid alkaloids, and steroids (16).

Table 3. MIC Test Results of Bangle Extract Solvents Acetone, Aquadest, Ethanol 70%, ethanol 96% and Methanol Against *Trichophyton rubrum* and *Trichophyton mentagrophytes*

No	Solvent Type	Test Sample	
		Trichophyton rubrum	***Trichophyton mentagrophytes***
1	Acetone Solvent	0,049 mg/mL	0,049 mg/mL
2	Aquadest Solvent	6,25 mg/mL	6,25 mg/mL
3	Etanol 70% Solvent	3,125 mg/mL	3,125 mg/mL
4	Etanol 96% Solvent	0,049 mg/mL	0,049 mg/mL
5	Metanol Solvent	0,049 mg/mL	0,049 mg/mL

The lowest MIC results for Bangle extract were in Aquadest solvent at 6.25 mg/mL with a value of 0.049 mg/mL. They continued in 70% Ethanol solvent with a value of 3.125 mg/mL in the fungus *Trichophyton rubrum* and in the fungus *Trichophyton mentagrophytes* with an MIC value of 3.125 mg /mL. The highest MIC value was obtained in the solvent acetone, ethanol 96%, and methanol, with a value of 0.049 mg/ML for both fungi.

Alkaloid compounds are compounds that are basic because they contain nitrogen atoms. Testing for alkaloid compounds was carried out by adding hydrochloric acid and water. Hydrochloric acid and water are added to saturate the solution because alkaloids are essential, so they require a solution containing acid (1). The results of identifying alkaloid compounds with the addition of Mayer, Bouchardat, and Dragendorf reagents showed positive results with the formation of yellow, brown, and brick-red precipitates in each reagent. The precipitate is formed because the nitrogen compound binds to the K+ ion contained in each reagent (17). The difference in the colour of the precipitate with each addition of the reagent is due to the change in ligands in the form of metals contained in the Mayer, Bouchardat, and Dragendorf reagents (18). Alkaloids can have anti-diarrheal, anti-diabetic, anti-microbial and anti-malarial properties, but some alkaloid compounds can be toxic (19).

Saponins are compounds that have hydrophilic and hydrophobic groups (17) The results of the identification of saponin compounds can form foam because they have physical properties that are readily hydrolysed in water, causing foam when shaken (5). The principle of the test is based on the hydrolytic reaction of the saponins to form aglycone and glycone (18). Saponin can be efficacious in reducing surface tension to inhibit fungal growth (20). Flavonoids are phenol derivative compounds which are efficacious in lowering cholesterol and lipids because they are antibacterial (21)

Triterpenoids help the body to synthesise and repair cells, while steroids exhibit antibacterial, antifungal, antitumour, neurotoxic and anti-inflammatory properties. These two compounds are bioactive with antibacterial and antioxidant activity by isolating and identifying their effects. Triterpenoid compounds found in plants function as protection to resist insects and microbial attacks (22). Steroidal compounds can interact with the cellular phospholipid membrane, which is impervious to lipophilic compounds, leading to loss of membrane integrity and alteration of membrane morphology, eventually leading to membrane fragility and lysis. (23).

Differences in the MIC (Minimum Inhibitory Concentration) results of bangle extract in various solvents such as acetone, ethanol 96%, and methanol can be influenced by the following factors such as The first, Active Compound Content: Each solvent has a different ability to extract active compounds from the bangle. The content of these compounds in extracts from various solvents can vary, which may affect the extract's ability to suppress trichophytomous fungal growth (7). The second is the solubility of Active Compounds: Various active compounds in the bangle may be more soluble or more easily extracted in certain solvents. This means that extracts obtained from different solvents may have different amounts or types of active compounds, influencing their effectiveness in inhibiting fungal growth (24). The third is the chemical Properties of the Solvent: The chemical properties of the solvent (such as polarity, pH, etc.) can affect the extraction of specific compounds. It is possible that certain solvents, such as Trichophyton rubrum, are more efficient in extracting compounds that have antimicrobial activity against fungi (9). The last is about the interaction Between Extracted Compounds and Fungi: The interaction between compounds extracted from bangle and the Trichophyton rubrum fungus can vary depending on the type of compound present in the extract. This interaction can affect the extract's ability to inhibit fungal growth (5)

CONCLUSION

The lowest MIC results for Bangle extract were in Aquadest solvent at 6.25 mg/mL with a value of 0.049 mg/mL. They continued in 70% Ethanol solvent with a content of 3.125 mg/mL in the fungus *Trichophyton rubrum* and in the fungus *Trichophyton mentagrophytes* with an MIC value of 3.125 mg /mL. The highest value for the MIC was obtained in the solvent acetone, ethanol 96%, and methanol, with a value of 0.049 mg/ML for both fungi.

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