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Activity of Bangle Leaf Extract (*zingiber purpureum roxb.*) Against the Growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes*

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ABSTRACT

Bangle rhizome extract (zingiber purpureum roxb.) is a herbal plant that is widely consumed in Indonesia. Bangle rhizome extract (zingiber purpureum roxb.) has broad medical properties such as diuretic, anti-hypertension, brochodilator, immunomodulator, analgesic, anti-inflammatory, anti-oxidant, anti-tumor, anti-diabetic, anti-microbial and anti-fungal. The aim of this research was to determine the yield results of extracts using the solvents Aquadest, Aceton, Ethanol 70%, Ethanol 96% and Methanol. And to find out the results of phytochemical screening tests and active ingredient content using GCMS. The stages of this research include the extraction process, phytochemical screening test and GCMS. The results of research on the yield of Bangle rhizome extract with the five solvents obtained the largest 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 Bangle extract were in Aquadest solvent at 6.25 mg/mL with a value of 0.049 mg/mL and 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. while the highest MIC value was obtained in the solvent acetone, Ethanol 96%, Methanol with a value of 0.049 ml/ML for both fungi.

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

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INTRODUCTION

Indonesia is a tropical country that has high humidity temperatures. These conditions are good for fungal growth. A disease caused by dermatophytosis fungus, which is a type of fungus that attacks keratinized tissue such as the stratum corneum of human skin, hair and nails. Dermatophytosis is caused by dermatophyte fungi which are divided into three genera, namely Trichophyton, Microsporum and Epidermophyton. There are various types of drugs that can be used to treat dermatophytosis, but often these drugs are resistant to dermatophyte fungi. The use of natural ingredients as antifungals is an alternative that can be used as a treatment. One natural ingredient that can be used is bangle rhizome (Zingiber cassumunar Roxb.). Bangle rhizome (Zingiber cassumunar Roxb.) is one of the plants that is often used as herbal medicine by the community. This plant is easy to find and cultivate, so it is a traditional medicine that has the potential to be explored for its benefits. Zingiber cassumunar Roxb Extract. contains flavonoids, quinones, steroids and triterpenoids. Flavonoid compounds are known to have useful activities as antiseptics and antibacterials.

Dermatophytosis is caused by dermatophyte fungi which are divided into three genera, namely Trichophyton, Microsporum and Epidermophyton. Transmission of this fungus can be direct or indirect. In order to cause disease, dermatophyte fungi must have the ability to attach to the host's skin, penetrate tissue and survive and adapt to the temperature and biochemical environment of the host (3). Dermatophytosis is often work-related (4). This disease is not fatal, but is chronic and recurrent, and is often resistant to anti-fungal drugs, so that dermatophytosis can cause discomfort and reduce the quality of life for sufferers (2). Systemic antifungals are one of the treatment options for dermatophytosis, especially in cases of widespread infection or failure of topical therapy. Currently, 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.

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Bangle rhizome (Zingiber cassumunar Roxb.) is one of the plants that is often used as herbal medicine by the community. This plant is easy to find and cultivate, so it is a traditional medicine that has the potential to be explored for its benefits. Zingiber cassumunar Roxb Extract. contains flavonoids, quinones, steroids and triterpenoids. Flavonoid compounds are known to have useful activities as antiseptics and antibacterials and this content is quite a lot in Zingiber cass umunar Roxb (6).

The antimicrobial activity of Zingiber cassumunar Roxb has been tested. against Staphylococcus aureus ATCC 25925 and Microsporum canis using the disc diffusion method. Testing the activity 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 (7). Apart from that, Zingiber cassumunar Roxb. It is also able to inhibit the growth of Pseudomonas aerugenosa bacteria (8). Zingiber cassumunar Roxb 70% ethanol extract. Also able to inhibit the growth of the fungus Trichophyton rubrum (9). Based on previous research that proves Zingiber cassumunar Roxb. Having anti-microbial power, the researchers wish to prove whether there is an effect of different solvents from Bangle rhizome extract (Zingiber cassumunar Roxb.) with various concentrations on inhibiting the growth of dermatophyte fungi and determine the type of solvent and optimum concentration that can inhibit the growth of dermatophyte fungi.

MATERIAL & METHODS

The tools used in this research were GCMS, analytical scales, funnels, Erlenmeyer, jars, measuring pipettes and measuring cups. The materials used for Rhizome Bangle were purchased from distributors and then determined at the Udayana Bali Pharmacy Faculty, which were then extracted at the Jakarta II Health Polytechnic Faculty of Pharmacy using the solvents Aquadest, Aceton, 70% Ethanol, 96% Ethanol and Methanol. Determination of 100 grams of Bangle Rhizomes obtained from the distributor, then determination was carried out at the Udayana Faculty of Pharmacy, Bali to ensure the correctness of the plants used. Extraction of Bangle rhizome extract is made at the spice and medicinal plant

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research center 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 filtered 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. % Results=(Extracted Weight (final))/(Simple Weight (initial)) x 100% Phytochemical analysis Phytochemical screening was carried out by mixing the extract with standard qualitative analysis reagents to test alkaloids, flavonoids, saponins, triterpenoids and tannins. These reagents play a role in showing the secondary metabolite compounds contained in the extract through observing the color test results. Alkaloid Test Alkaloid tests can be carried out with a variety of reagents, such as Mayer's, Bouchardat's and Dragendorff's reagents. Mayer's reagent is potassium tetraiodomercurate (II), Dragendorff's reagent is a mixture of bismuth subnitrate in acidic conditions and potassium iodide to form Bismuth-Potassium Iodide, while Bouchardat's reagent is Iodine-Potassium Iodide. In this research, Mayer and Bouchardat reagents were used. In the alkaloid test with Mayer's reagent, the nitrogen in the alkaloid will react with the potassium ion (K+) from potassium tetraiodomercurate(II) to form a potassium-alkaloid complex which is precipitated. Positive results containing alkaloids are indicated by the presence of white or cloudy precipitates. The alkaloid test uses Bouchardat's reagent, the reaction that occurs is relatively similar to the reaction 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. Saponin Test Saponin testing is carried out by shaking the extract in a test tube containing 5 ml of hot H2O solvent. A positive result will form persistent foam in the shaken extract solution. Tannin Test The tannin test is carried out as follows: The extract is added with 2 drops of green to blackish blue ferric chloride (FeCl3) solution (Hanani, 2017). Phenol Test Phenol testing can be done by mixing the alcohol extract with the NaOH reagent. Indications of the presence of phenol will be

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marked in red. Flavonoid Test The procedure in this research uses Mg and HCl reagents or Zn and HCl can also be used. Positive results are indicated by the appearance of a dark red color. This color is a reaction of Mg metal with flavonoids to form a complex. Glycoside Test Add 1 ml of extract to glacial acetic acid, FeCl3 and concentrated sulfuric acid and a purple ring forms, indicating the presence of glycosides. Tyrpeneoid Test 1 ml of extract is added with 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid, the color change from purple or red to blue green indicates the presence of terpenoids. The extract taken is dissolved in water and 3-4 drops of copper acetate solution are added. The formation of a green color indicates the presence of diterpenes (Tiwari et al., 2011). Steroid Test Results and Discussion Add chloroform and see the layer that forms, then the chloroform layer is dried. Then add 3 drops of concentrated H_2SO_4. Then a blue color will form. The formation of a blue color can be observed at the edge of the drop plate (Hanani, 2017). GCMS Analysis Analysis of Bangle rhizome extract content using Agilent Technologies 7890 Gas Chromatograph with Auto Sampler and Mass Selective Detector and Chemstation 5975 data system. Take 100 μL of liquid extract of Bangle rhizome, add 900 μL of Aquadest, Aceton, 70% Ethanol, 96% Ethanol and Methanol pro analysis. Determination of Minimum Inhibitory Concentration (MIC) of Bangle Extract using Acetone, Aquadest, Ethanol 70%, ethanol 96% and Methanol using the microdilution method using a 96 well microwell plate. The microdilution method uses Nutrient Broth (NB) media with Resazurin added to the microwell plate to determine the MIC. First, NB (100 μL) was added to each well, and 100 μL of extract was added

RESULT & DISCUSSION (Times New Roman, 12 pt, Bold, UPPERCASE)

1. Yield Analysis

The maceration method is a method that 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.)

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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.	Etanol 70% Solvent	9,4
4.	Etanol 96% Solvent	6,3
5.	Metanol 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).

2. Phytochemical Screening Test

Phytochemical compounds are secondary metabolite compounds in plants that have certain functions for humans. To find out 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

Based on table 2, 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, whereas in distilled

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water and 70% ethanol there are only four secondary metabolites, namely tannins, triterpenoid alkaloids, and steroids.

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 and 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. while the highest MIC value was obtained in the solvent acetone, Ethanol 96%, Methanol with a value of 0.049 ml/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 a solution of hydrochloric acid and water. The purpose of adding hydrochloric acid and water is to saturate the solution because alkaloids are basic, 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 (2). The difference in color 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 (3). Alkaloids can have anti-diarrheal, anti-diabetic, anti-microbial and anti-malarial properties, but some alkaloid compounds can be toxic (4).

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Saponins are compounds that have hydrophilic and hydrophobic groups (2). The results of the identification of saponin compounds can form foam because they have physical properties that are easily hydrolyzed in water, causing foam when shaken (5). The principle of the saponin test is the hydrolysis reaction of saponin compounds into aglycones (non-sugar compounds) and glycones (sugar compounds) which is characterized by the formation of stable foam (3). Saponin can be efficacious in reducing surface tension so that it can inhibit fungal growth (6). Flavonoids are phenol derivative compounds which are efficacious in reducing cholesterol and lipids because they are antibacterial (5).

Triterpenoid compounds help the body in the process of organic synthesis and restoration of body cells, while steroid compounds show antibacterial, antifungal, antitumor, neurotoxic and anti-inflammatory activity. These two compounds are bioactive compounds that have antibacterial and antioxidant activity through isolation and identification of their activities. Triterpenoid compounds found in plants function as protection to resist insects and microbial attacks (9). Steroid compounds can interact with cell phospholipid membranes which are impermeable to lipophilic compounds, causing membrane integrity to decrease, cell membrane morphology to change, and ultimately causing cell membrane brittleness and lysis (10).

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 and 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. while the highest MIC value was obtained in the solvent acetone, Ethanol 96%, Methanol with a value of 0.049 ml/ML for both fungi.

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