

ANTIFUNGAL TEST OF THE ETHANOL EXTRACT OF BROTOWALI STEM (*Tinospora crispa*) ON THE GROWTH OF *Trichophyton rubrum* IN VITRO

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ABSTRACT

Background: *Trichophyton rubrum* is an infectious dermatophyte fungus which is the most common cause of dermatophytosis. Fungal resistance and the side effects of therapy are problems of antifungal agents. Phytochemical test of brotowali stem extract (*Tinospora crispa*) consist of flavonoids, phenols and triterpenoids which have antifungal effects. This study aimed to examine the effectiveness of brotowali stem extract (*Tinospora crispa*) as antifungal agent towards the growth of *Trichophyton rubrum* in vitro using agar well diffusion method.

Subjects and Method: This was an experimental study using brotowali stem extract with concentration of 10%, 12,5%, 25%, 30%, 40%, 50%, 60%, 75%, and 100%. The dependent variable was *Trichophyton rubrum* growth. The independent variable was brotowali stem extract (*Tinospora crispa*). The data were obtained from the inhibition zone showed in agar well diffusion method in Sabouraud Dextrose Agar media. The data were analyzed using Kruskal-Wallis test.

Results: The average diameter of inhibition zone of each variance (10%, 12,5%, 25%, 30%, 40%, 50%, 60%, 75%, and 100%) were 2.167 mm, 6.367 mm, 7.0 mm, 10.67 mm, 11.9 mm, 13.07 mm, 15.8 mm, 17.96 mm dan 17.13 mm, respectively, and they were statistically significant ($p = 0.001$).

Conclusion: Brotowali stem extract has weak antifungal effectiveness at concentration 10%, 12,5%, 25%, intermediate antifungal effectiveness at concentration 30% and strong antifungal effectiveness at concentration 40%, 50%, 60%, 75% and 100%.

Keywords: antifungal, brotowali stem, well diffusion, *Trichophyton rubrum*

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BACKGROUND

Dermatophytosis skin disorders often occur in tropical countries, such as Indonesia. High temperature and humidity can promote mold growth. Dermatophytosis is also known to be contagious and cause chronic infection in healthy individuals. Dermatophytosis spread throughout the world with different prevalence in each country. As many as 20% of people suffer from *Tinea corporis* infection which is the most dominant followed by *Tinea cruris* and *Tinea pedis* based on a study of the incidence of dermatophyte infection (Dewi et al., 2019).

Indonesia's health profile in 2010 describes skin and subcutaneous tissue diseases as the third of the ten diseases with the highest frequency in Indonesia hospitals for outpatients, with 192,414 visits. This means that skin disease in Indonesia is still very dominant (Data and Information Center, 2011).

The incidence of skin diseases caused by fungi in Prof. Dr. RD Kandou hospital, Manado, from January to December 2012, showed that there were 65 cases and increased from January to December 2013 to 153 cases (Dewi et al., 2019).

Trichophyton rubrum (*T. rubrum*) is the most common causative agent of dermatophytosis worldwide, the location of the lesions is mainly on the feet, skin and between human nails (Kadhim et al., 2015). This fungus can infect human skin and nails through the degradation of keratin. Keratin is a fibrous protein which is the main structural component of human skin and nails. The mechanism of *Trichophyton rubrum* is to attack through the stratum corneum, the outermost layer of the epidermis, to degrade keratin (White et al., 2015).

Dermatophytosis treatment can be given topically or systemically. Azole class of drugs can be used in cases of dermatophytosis; however, these drugs have some quite dangerous effects, which can cause liver toxicity, especially in long-term use (Widaty et al., 2016). Antifungal agents such as griseofulvin, azole derivatives and allylamine which are used as therapeutic options are less useful in the treatment of dermatophytosis as a result of the development of fungal resistance, long treatment duration and the presence of therapeutic side effects, so additional options of antifungal therapy with therapeutic potential are needed (De Oliveira Pereira et al., 2015).

Brotowali (*Tinospora crispa*) is a nutritious plant that grows in tropical areas, one of which is in Indonesia. In Asian countries such as the Philippines, Thailand, Malaysia, Indonesia, India, China and Vietnam, *Brotowali* is used for rheumatism, as a medicine for fever, cholera, jaundice, appetite stimulant and is also used as an antiparasitic both in animals and humans. *Brotowali* is also used in modern medicine for the treatment of type II diabetes. People in Indonesia usually use *Brotowali* stalks by boiling them to reduce glucose levels and reduce fever (Wiranto et al., 2019).

Brotowali stem contains flavone, triterpenoid, diterpenoid, cis-clerodane type furan

diterpenoid compounds, alkaloids, lignans and sterols based on phytopharmaceutical studies (Ahmad et al., 2016). The ethanol extract of the *Brotowali* stem contains compounds that have anti-fungal properties, namely flavonoids, triterpenoids, phenolic compounds, fatty acids and alkaloid (Warsinah et al., 2015). Based on the background that has been described, the authors intend to test the effectiveness of the ethanol extract of *Brotowali* stem (*Tinospora crispa*) on the growth of fungus *Trichophyton rubrum* in vitro.

SUBJECTS AND METHOD

1. Study Design

This was an experimental study with a post-test control group design only. This study used *Brotowali* (*Tinospora crispa*) stem extract with various concentrations which were tested for its effectiveness on the growth of *Trichophyton rubrum*, then compared with a control group comparison.

2. Study Materials

a. Test Materials

Brotowali stem extract processed at the Indonesian Spice and Medicinal Crops Study Institute (BALITTRO) Bogor by maceration method and 96% ethanol solvent.

b. Chemicals and medium

0.9% sterile NaCl, tablet ketoconazole 200 mg, alcohol, and media Sabouroud Dextrose Agar (SDA).

c. Pure Culture

The pure culture of this study was *Trichophyton rubrum* obtained from the Department of Parasitology, Faculty of Medicine of UPN Veteran Jakarta.

d. Tools

Copper cylinder 6mm, autoclave, incubator, sterile stirrer, beaker glass, burner lamp, tweezers, sterile petri dish, sterile cotton swab, syringe, handsocon, mask, test tube, test tube rack, erlenmeyer tube, digital

calipers, wipes, matches, label paper, barns, permanent markers.

3. Work Procedure

The tools are first sterilized by autoclave with a temperature of 121 ° C and a pressure of 15 Psi for 15-20 minutes, then the tools are left to stand at room temperature until dry.

The standard of McFarland is an indicator of fungal turbidity which is equivalent to 10⁸ CFU/ ml. How to make the standard McFarland is by mixing 0.05 ml of 1% BaCl₂ solution into a test tube with 9.95 ml of 1% H₂SO₄ and homogenizing

Brotowali stem extract is processed in the following steps: simplicial is made into powder, then added with solvent and stirred using a stirrer for ± 3 hours, then let it stand for 24 hours, then the first filtrate is formed with dregs, the dregs are added with solvent and stirred using a stirrer for ± 1 hour, then filtered using filter paper, until a second dregs and filtrate are formed. The first and second filtrate are put together and then rotated until thick/ evaporated by the solvent so that the extract becomes thick. For testing the anti-fungal effect, the *Brotowali* stem extract will be diluted using sterile distilled water to form 8 concentrations, namely 10%, 12.5%, 25%, 30%, 40%, 50%, 60%, 75%, and 100% concentrations are not diluted.

The positive control contained 2% ketoconazole solution which was obtained by crushing 200 mg of ketoconazole and dissolving it with 10 ml of sterile distilled water. The negative control in this study was sterile distilled water.

Mushroom growth medium was prepared by inserting 20 grams of Sabouraud Dextrose Agar (SDA) into erlenmeyer and dissolving it with 500 ml of distilled water. Then it is heated by placing it on a hot plate and then homogenizing it by stirring the solution.

The method used in this test is the well method. The first step is to label the variations in the extract concentration, positive

control, and negative control on the bottom of the SDA media using a permanent marker, next is to make the layer first on the SDA media, then implant a copper cylinder to make a well hole in each SDA media as a place to put the variations *Brotowali* stem extract concentration and positive control and negative control. In SDA media that will be placed with various concentrations of 10%, 12.5%, 25%, 30% and negative control, 4 holes will be made per 1 medium, for a concentration of 40% and 50% 2 holes will be made per 1 medium, for a concentration of 60%, 75%, and 100%, and positive control will be made 1 hole per 1 media. After that, pour the layer second, which is 10 ml of liquid SDA which has been added with suspension *T. rubrum* on each SDA medium. After that, wait 10-15 minutes for the media to harden and the next step is to remove the copper cylinder that makes the wells so that they form wells on the SDA media. The *Brotowali* stem extract that has been diluted into various concentrations, positive control and negative control will be poured into the well according to the respective label. This test is carried out 3 times. After that, all the petri dishes that have been given the extract, positive and negative controls will be incubated for 1 x 24 hours and the diameter of the clear area formed using a digital caliper will be calculated.

4. Data Analysis

The data were analyzed using non-parametric Kruskal-Wallis test and continued with Post Hoc (Mann-Whitney) test. on the grounds that the data obtained from this study were normally distributed but not homogeneous even though the data had been transformed.

RESULTS

Based on the results of experiments that have been carried out, the diameter size of the inhibition zone of each treatment group has been measured using a digital caliper. The

results of the average diameter of the inhi-

bition zone can be seen in Table 1.

Table 1. The average diameter of the inhibition zone of the *Brotowali* stem extract on the growth of *T. rubrum*

The concentration of <i>Brotowali</i> stem extract, negative and positive controls	The average diameter of the inhibition zone on <i>T. rubrum</i> (mm)	Description (antifungal power)
Control (-)	0	No
Control (+)	35.7	Very Strong
10%	2.17	Weak
12.5%	6.37	Weak
25%	7.0	Weak
30%	10.67	Moderate
40%	11.9	Strong
50%	13.07	Strong
60%	15.8	Strong
75 %	17.96	Strong
100%	17.13	Strong

DISCUSSION

Inhibition areas formed on preparations *T. rubrum* showed the antifungal effectiveness of the *Brotowali* stem extract. The table above shows that the negative control group did not have an inhibitory area or had no anti-power against fungal *T. rubrum*. In the positive control (ketoconazole) there is a picture of the inhibition zone with an average of 35.7 mm and is included in the very strong category of anti-fungal power based on the criteria of Davis and Stout (2009).

The average diameter of the inhibition zone produced by the *Brotowali* stem extract in inhibiting the growth of *T. rubrum* with a concentration of 10%, 12.5%, and 25% was included in the weak category (<5 mm), the concentration of 30% was included in the moderate category (5-10 mm), and a concentration of 40%, 50%, 60%, 75% and 100% have an average diameter area of the resistor ranges from 10-20mm, which means included into the strong category (Davis and Stout, 2009).

The data that has been obtained showing that each group concentration had antifungal effectiveness in inhibiting the growth of *T. rubrum*. This study requires a high ex-

tract concentration (96% ethanol solvent) if the percent unit is converted into ppm units compared to the study conducted by Nuryanti et al. (2015), and the inhibition area is directly proportional to the concentration up to a concentration of 75%, meaning that at a concentration of 10%-75% in accordance with study conducted by Nuryanti et al. (2015) regarding the antifungal activity test of ethanol extract (ethanol solvent 70 %) *Brotowali* stems against the fungus *Pityrosporium ovale* and *Trichophyton mentagrophytes* using the disc diffusion method, namely the greater the concentration the greater the inhibition area.

The antifungal effectiveness produced by the *Brotowali* stem extract comes from the secondary metabolite compounds contained therein. Based on the results of phytochemical tests carried out at BALITTRO, the *Brotowali* stem extract was positive for alkaloids, saponins, tannins, phenols, flavonoids, triterpenoids, steroids, and glycosides. According to Warsinah et al. (2015) flavonoids, triterpenoids, phenols and alkaloids are compounds in the ethanol extract of the *Brotowali* stem which are anti-fungal.

The mechanism of flavonoids as antifungal is as an inhibitor of Fatty Acid Synthase (FAS), an anti-fungal target, by down-regulating genes that express FAS (Bitencourt et al., 2014).

Phenolic compounds denature and coagulate proteins, cause the cell cycle to stop during the replication phase, and damage the mitochondria so that fungal cell growth is inhibited (Dewi et al., 2019). Terpenoids interfere with the cytoplasmic membrane and the development of fungal spores (Lutfiyanti et al., 2012). Alkaloid is an alkaline compound with a pH > 7 (Lutfiyanti et al., 2012). *T. rubrum* grows optimally at pH 6 so that when *T. rubrum* placed at pH > 7 growth *T. rubrum* will be depressed (Kadhim and Al-Hamadani, 2015).

Brotowali stem extract has effectiveness in inhibiting the growth of *Trichophyton rubrum* in vitro using the well diffusion method. There were significant differences in almost all treatment groups because the significance value was less than 0.05 (<0.05).

This means that the effectiveness of the antifungal at each concentration has a different inhibitory strength except at a significance value greater than 0.05, which means that there is no significant difference in the effectiveness of the antifungal in that group. The 75% concentration was the most effective in inhibiting the growth of *Trichophyton rubrum* in vitro using the well diffusion method with the highest average diameter of the inhibition area, namely 17.96mm.

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