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Effectiveness of Bitter melon (*Momordica charantia L*) Extract on Growth of *Trichophyton rubrum*: In Vitro Study

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Abstract

Background: *Trichophyton rubrum* is a fungus that most often causes superficial fungal infections. The development of herbal ingredients as antifungal therapy is carried out to find alternative therapies for fungal infections. Bitter melon contains various compounds that have the potential to be used as antifungals, including flavonoids and saponins.

Objective: The aim of this study was to determine the effect of bitter melon extract (*Momordica charantia L*) on the growth of *Trichophyton rubrum*.

Methods: This study used bitter melon extract at concentrations of 5%, 25%, 50%, 75%, and 100%. Ketoconazole concentrations of 2% were used as a positive control. Pure cultures of *Trichophyton rubrum* were diluted using physiological NaCl. Fungal growth was observed microscopically and macroscopically.

Results: *Trichophyton rubrum* grew at a concentration of 5%, while at concentrations of 25%, 50%, 75%, and 100%, there was no *Trichophyton rubrum* growth. Bitter melon extract at a concentration of 25% had a similar effect as Ketoconazole 2%.

Conclusion: Bitter melon extract at concentrations of 25%, 50%, 75%, and 100% had a similar effect to 2% ketoconazole in inhibiting growth of *Trichophyton rubrum*.

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INTRODUCTION

Indonesia is a country with a tropical climate that has relatively high temperatures and humidity. High temperatures and high humidity are places where plants and microorganisms grow. One of the microorganisms that can reproduce in tropical climates is a fungus.[1] Skin type and individual hygiene are factors that encourage the development of fungi.[2] Fungi can grow and reproduce in an environment of 25-28°C. Dermatophytes are a group of fungi that can form molecules that bind to keratin and use it as a source of nutrients to form colonies.[3,4] Three types of Dermatophytes as fungi that cause skin diseases include *Epidermophyton, Microsporum, and Trichophyton*.[5] *Trichophyton rubrum* is the most common cause of superficial fungal infections.[6] Dermatophytes attack keratinized tissues (such as skin, hair, and nails)[7], attack layers of skin from the stratum corneum to the stratum basalis.[8] *Trichophyton rubrum* can cause tinea pedis, tinea cruris, and tinea corporis.[9]

The incidence of diseases caused by fungi in Indonesia ranges from 2.93% - 27.6%.[10,11] Data for 2012 at RSUP Prof. Dr. R. D. Kandou Manado found 1.61% cases of dermatophytosis,[12] while in 2013, there were 3.7% cases of dermatophytosis of the total cases of skin diseases.[13] Based on the location found, 35.3% of tinea cruris, tinea pedis 32.7%, tinea capitis 7.2%, then tinea unguium or onychomycosis 5.3%, and tinea corporis 2.6%. In addition, there is also a combination location of tinea corporis et cruris 17%, and no data were found regarding tinea barbae and tinea imbrikata.[14]

The development of medicine in Indonesia has begun to target traditional or natural medicine because it is considered safer and does not cause side effects.[5] One of the plants known to Indonesian people and widely used is.[6] Bitter melon/ pare (*Momordica charantia L*) can be found in most parts of Indonesia. Indonesians have long used bitter melon as a daily food, and have long been trusted and used as a traditional medicine to treat various diseases.[7]

Bitter melon is one of the fruits that contain flavonoids and saponins that can function as antibacterial and antifungal.[9] Flavonoids are the largest group of phenolic compounds found in nature. Flavonoids are effective in vitro as antifungal agents. Flavonoids can bind to soluble proteins and cell walls, and the high lipophilicity of flavonoids further damages the membrane. Flavonoids can interfere with peptidoglycan transpeptidase activity and interfere with cell wall formation.[15] Saponins have antibacterial and antifungal properties. Saponins disrupt the lipid layer of the membrane, increase the permeability of cell membranes, disrupt cell membranes, and induce cytolysis.[16] There are still many skin diseases caused by dermatophytes, especially *Trichophyton rubrum*. Potential for chemical compounds in bitter melon as antifungal, the researchers wanted to know the effectiveness of bitter melon extract on the growth of *Trichophyton rubrum*.

METHODS

This type of research is true experimental research conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Semarang. The independent variable was bitter melon extract, and the dependent variable was the growth of *Trichophyton rubrum*. Sample calculation using Federer formula. Grouping is divided into 7, including 1). Bitter

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melon extract at a concentration of 5%, 2). Bitter melon extract at a concentration of 25%, 3). Bitter melon extract at a concentration of 50%, 4). Bitter melon extract at a concentration of 75%, 5). Bitter melon extract at 100% concentration, 6) positive control using 2% ketoconazole, and 7)—negative control without using standard drugs or bitter melon extract.

A solution of 2% ketoconazole is obtained by smoothing 0.1 g of 200 mg ketoconazole, then put in a test tube and adding 5 ml of sterile distilled water. To make bitter melon extract using the soxhletation method by washing and cutting bitter melon into small pieces and drying in an oven at 50^0C for two days. Pieces of dried bitter melon fruit in a blender to produce powder preparations. Extraction using the soxhlet tool. Enter 440 ml of ethanol solvent in a soxhlet flask, and put the bitter melon powder in a filter paper bag into a siphon tube. Do the soxhletation process so that bitter melon powder is extracted perfectly. Extraction results were then evaporated with a rotary evaporator at a temperature of 60°C and concentrated on a water bath at a temperature of 60^0C. Extraction results are ready to use in a concentration of 100%.

The distribution of concentrations used for the study included 5%, 25%, 50%, 75%, and 100%. The concentration determination of bitter melon extract was determined by the minimum inhibitory level test (MIC) in a preliminary test. A phytochemical test of the bitter melon extract was carried out to determine the content of saponins, flavonoids, alkaloids, triterpenoids, and polyphenols.

Growth of *Trichophyton rubrum* using Saboraund Dextrose Agar (SDA) media, Pure culture of *Trichophyton rubrum* was diluted using physiological Nacl and homogenized until the turbidity color was the same as Mc Farland 1. The MIC growth test of *Trichophyton rubrum* was carried out in groups given bitter melon extract concentrations of 5%, 25%, 50%, 75%, and 100%, and a positive control group; treatment was repeated four times. *Trichophyton rubrum* was grown in an incubator at 37^0C for 2-5 days.

Growth of *Trichophyton rubrum* was seen through macroscopic examination with the results: there were white colonies stacked in the middle and maroon colored at the edges and bottom, and microscopic examination showed fine hyphae on a 400x magnification microscope with Lactophenol Cotton Blue (LPCB) staining. The results are said to be inhibited if no white colonies are piled up in the middle and the maroon at the edges is cherry red, either macroscopically or microscopically. Research data were tabulated and analyzed by descriptive analysis and the Kruskal-Wallis test.

RESULTS

Bitter Melon Extract Analysis

The results of a phytochemical test of the bitter melon extract are shown in Table 1. Phytochemical screening of bitter melon extract identified saponins, flavonoids, alkaloids, triterpenoids, and polyphenols. Saponins content was identified from the presence of foam, and flavonoid content was identified from the extract solution, which turned red. Alkaloid content was identified as orange on the addition of dragendrof and white on the addition of Mayer. Triterpenoids appear orange in color with the addition of ether, anhydrous acetic acid, and H2SO4, while polyphenols turn

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blackish-green when the bitter melon extract is added with FeCl3.

Minimum Inhibitory Level Test of Bitter Melon Extract

Minimum inhibitory level of bitter melon extract in inhibiting the growth of *Trichophyton rubrum* was determined at a minimum concentration that could inhibit the growth of *Trichophyton rubrum*. The concentrations used consisted of 5%, 25%, 50%, 75%, and 100%, with four repetitions per concentration. This study also included a positive standard, namely 2% ketoconazole which was also tested in 4 replications. The results of MIC determination are shown in Table 2.

Based on Table 2, growth of *Trichophyton rubrum* was not found in the group with 25%, 50%, 75%, 100% bitter melon extract, and 2% ketoconazole treatment. The staining results showed that microspores appeared in a petri dish containing 5% bitter melon extract (figure 1). The microscopic examination of hyphae and conidia of *Trichophyton rubrum* found in 5% bitter melon extract can be seen in figure 2. It can be seen that 25% bitter melon extract concentration is a minor concentration, which has similar effectiveness to 2% ketoconazole.

Table 1. Results of phytochemical test of bitter melon extract

Chemical compounds	Phytochemical test reaction	Results
Saponins	Foam is formed for 10 minutes	Saponins (+)
Flavonoids	The solution of amyl alcohol is red	Flavonoids (+)
Alkaloids	Orange solution White solution	Alkaloids (+)
Triterpenoids	Orange solution	Triterpenoids (+)
Polyphenols	Dark green solution	Polyphenols (+)

Table 2. MIC of bitter melon extract on the growth of *Trichophyton rubrum*

Treatment group	Macroscopic				Microscopic				Conclusion
	C1	C2	C3	C4	C1	C2	C3	C4	
5% bitter melon extract	+	+	+	+	+	+	+	+	+
25% bitter melon extract	-	-	-	-	-	-	-	-	-
50% bitter melon extract	-	-	-	-	-	-	-	-	-
75% bitter melon extract	-	-	-	-	-	-	-	-	-
100% bitter melon extract	-	-	-	-	-	-	-	-	-
2% ketoconazole	-	-	-	-	-	-	-	-	-

C: petri dish, +: colony, - : no colony

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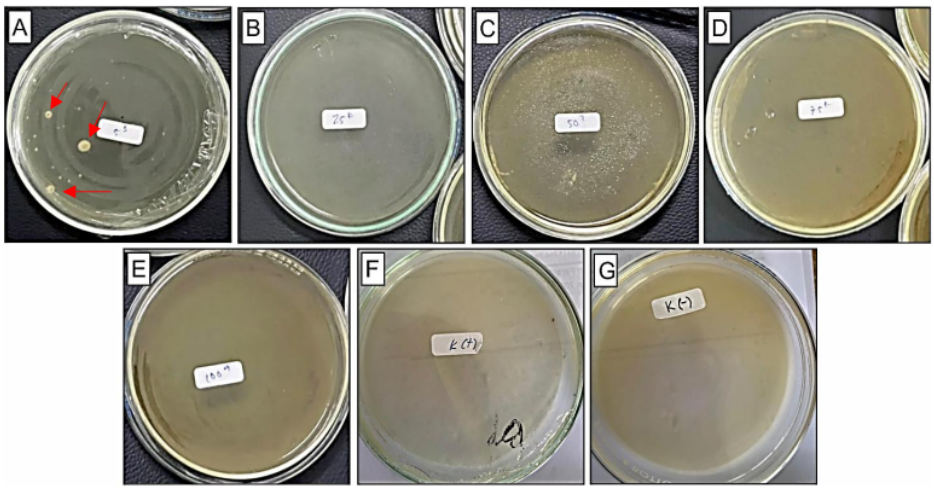

Figure 1. Growth of *Trichophyton rubrum* on SDA medium with 5% bitter melon extract (A), 25% (B), 50% (C), 75% (D), 100% (E), positive control group/ketoconazole (F), and the negative control group (G). Red arrows indicate fungal growth.

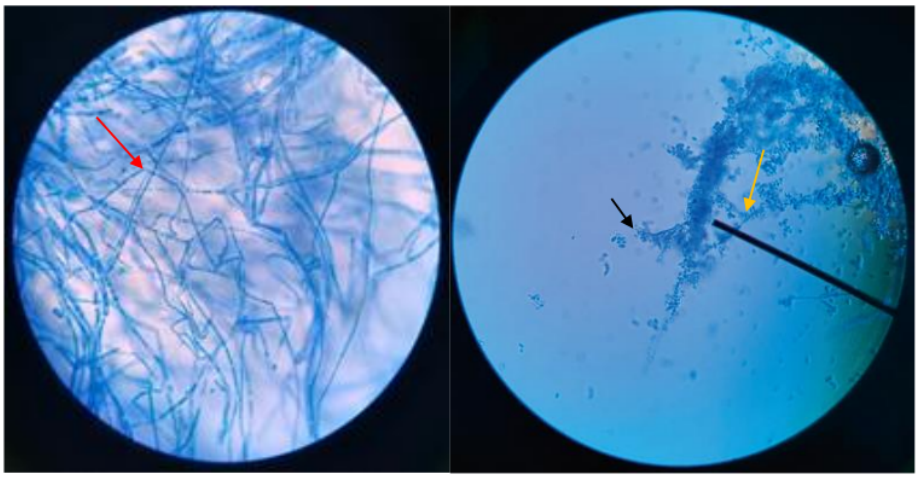

Figure 2. In microscopic observation with Lactophenol staining, Red arrows indicate hyphae, black arrows show tear-drop-shaped pyriform microconidia attached to hyphae tips, yellow arrows indicate smooth, straight hyphae attached to microconidia and bird-on-wire shaped, conidia on conidiospores such as pine trees.

DISCUSSION

The bitter melon extract inhibited the growth of *Trichophyton rubrum* at a concentration of 25%. The growth inhibitory effect of *Trichophyton rubrum* on 25% bitter melon extract concentration was equivalent to 2% ketoconazole. The results of this study confirmed that

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bitter melon has the ability to inhibit fungal growth, as in previous studies, that bitter melon extract inhibited the growth of the fungus *Fusarium oxysporum*.[17] The antifungal test of the bitter melon extract was also effective against the growth of the fungus *Candida albicans*. The MIC was obtained at a concentration of 40%.[18] Comparison of the effectiveness of bitter melon extract as an antifungal compared to Ketoconazole has also been carried out by Mulyono with the results of the effectiveness of bitter melon extract at a concentration of 100% similar to the effectiveness of 2% ketoconazole in inhibiting the growth of *Pityrosparum ovale*.[19]

The positive control used in this study was ketoconazole. This is similar to previous studies.[19] Ketoconazole inhibits fungal growth through ergosterol, a major component of fungal cell membranes.[5] Ketoconazole works by inhibiting 14α-demethirase, a microsomal cytochrome P450 enzyme. Enzyme 14α demethirase is required to convert lanosterol into ergosterol in fungal cell membranes resulting in impaired membrane permeability and membrane-bound enzyme activity and cessation of fungal cell proliferation. The mechanism of action of antifungal flavonoids occurs by denaturing fungal cell wall proteins, thereby damaging fungal cell membranes. Denatured fungal cell wall proteins are susceptible to penetration of other fungal active substances, namely saponins, which weaken cell walls. Saponins break down the lipid layer of the membrane and increase the permeability of fungal cell membranes, causing membrane instability and fungal cell division.[16,20] Mechanism of action of different antifungal agents is shown in Figure 3. The action of flavonoids as antifungals lies in the function of cell walls and the destruction of fungal cell membranes, or the antifungal mechanism of action of flavonoids involves antifungals similar to candins and polyenes. Candins have a mechanism of action that prevents the synthesis of glucan from the cell walls of fungi. Polyenes interact with cell membrane sterols (ergosterol) to form channels along the cell membrane, causing cell leakage (lysis) and fungal death.[6]

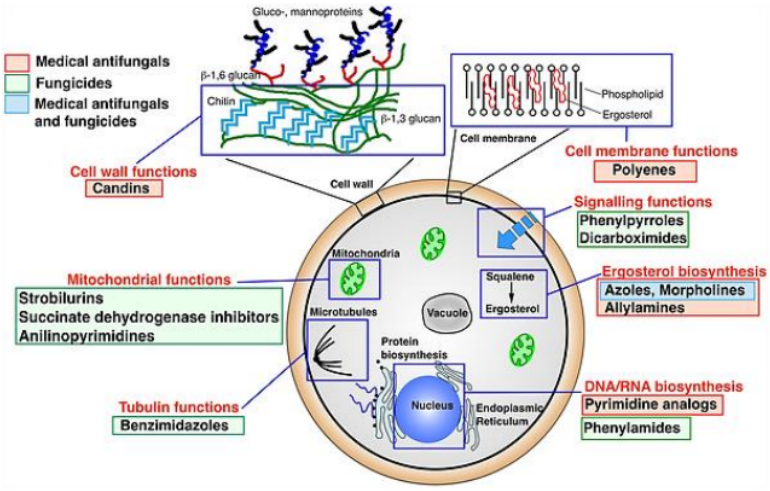

Figure 3. Antifungal Mechanism of Action [21]

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Flavonoids and saponins have different antifungal mechanisms than azoles, but the result is fungal cytolysis, which is effective in suppressing fungal growth.[21] Phytochemical screening of bitter melon extract for this study also detected alkaloids, triterpenoids, and polyphenols. Triterpenoids exert antifungal activity by destroying cell membranes, inhibiting fungal protein synthesis, and suppressing fungal growth. Alkaloids inhibit fungal nucleic acid biosynthesis, so fungi cannot grow until they die. Polyphenols are bactericidal compounds that work by denaturing proteins. Denatured fungal cell wall proteins cause fungal cell walls' fragility and allow active fungal substances' penetration. Protein in the denatured fungal cell wall is the enzyme -1,3 glucan, so the enzyme cannot function, and it causes metabolism and nutrient absorption processes to be disrupted so that the fungus will die.[22]

It is necessary to conduct a similar study in vivo; the optimal dose is between 25%, 50%, 75%, and 100%, with other types of fungi/dermatophytes, with variations in the maturity of bitter melon, as well as calculating the minimum kill rate for comparison of effectiveness.

CONCLUSION

Bitter melon extract at concentrations of 25%, 50%, 75%, and 100% had a similar effect to 2% ketoconazole in inhibiting the growth of *Trichophyton rubrum.*

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