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Effectiveness Comparison of Bitter Melon Fruit (*Momordica charantia* L.) Extract with 2% Ketoconazole in Inhibiting *Pityrosporum ovale* Growth *In Vitro*

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Effectiveness Comparison of Bitter Melon Fruit (*Momordica charantia* L.) Extract with 2% Ketoconazole in Inhibiting *Pityrosporum ovale* Growth *In Vitro*

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Abstract. Ketoconazole has long been used as an antifungal therapy. One of fungi that causes dandruff is *P.ovale*. Bitter melon fruit contains antioxidants which can be used as an antifungal. The similarity of antifungal between bitter melon fruit and ketoconazole, made the researchers want to compare the effectiveness of bitter melon fruit extract with 2% ketoconazole in inhibiting *P. ovale* growth. A laboratory experiment study with Post-test only controls group design, at Microbiology Laboratory, Medical Faculty of Universitas Muhammadiyah Semarang using *P.ovale* pure isolates. Determination of sample size is done using two proportions formula. Determination of whether or not *P.ovale* is done macroscopically and microscopically. Sample analysis using the chi-square test. From 30 media given 100% bitter melon extract, 66.7% results did not found in *P.ovale* growth (20 plates). While 30 media were given 2% ketoconazole, 56.7% did not find *P.ovale* growth (17 plates). Chi-square test showed $p = 0.595$ which means there was no difference in effectiveness between giving 100% bitter melon fruit extract and 2% ketoconazole to inhibit *P. ovale* growth. The effectiveness of 100% bitter melon fruit (*Momordica charantia* L.) extract equal to 2% ketoconazole in inhibiting *P.ovale* in vitro growth.

Keywords *Pityrosporum ovale*, bitter melon extract, 2% ketoconazole, dandruff

1. Introduction

Dandruff or seborrheic dermatitis is still a problem for more than 70% of the Indonesian population [10] and ketoconazole is one of drugs as dandruff therapy [3]. Ketoconazole is an antifungal class of azole derived from imidazole synthesis. This drug works to inhibit the action of the p450 cytochrome enzyme on the fungal cell membrane, and it can disrupt the synthesis of ergosterol which is an important component of the fungal cell membrane [10].

Cause of seborrheic dermatitis is *Pityrosporum ovale* (*P. ovale*) [11]. *Pityrosporum ovale* is a non-dermatophyte yeast fungus that can infect human skin, *Pityrosporum ovale* has dysmorphic, lipophilic, saprophytic, unipolar, and is a normal flora of human skin [8].

Temperature, high humidity levels in tropical climates such as Indonesia make microorganisms grow well such as fungi [1]. Indonesia is a tropical country with various traditional medicine, bitter melon fruit (pare) used as an antifungal. The content of bitter melon fruit as antifungal is flavonoid and saponin [12]. Flavonoids synthesized by plants in response to microbial infections, flavonoids



proved *in vitro* to be effective as an antifungal. Saponins have an antifungal effect with increased cell membrane permeability through the breakdown of fat layers on cell membranes [6, 7, 9].

The similarity of antifungal between bitter melon fruit and ketoconazole, made the researchers want to compare the effectiveness of bitter melon fruit extract with 2% ketoconazole in inhibiting *P. ovale* growth.

2. Methods

An experimental laboratory, posttest only control group design conducted at Microbiology Laboratory, Medical Faculty, University of Muhammadiyah Semarang (Unimus). Determination of sample size used two proportions formula. Study used *P. ovale* pure isolates with Sabouraud dextrose Agar (SDA) and olive oil growth media.

Ketoconazole 2% was prepared by smoothing 200 mg of ketoconazole and adding 100 ml of sterile aquadest. Bitter melon extraction was done by drying bitter melon fruit in oven at 50°C for \pm 2 days, blending afterward. The soxhletation method used ethanol solvents with a ratio of 1: 1 between bitter melon fruit and ethanol (500gram: 500ml). Perfect extraction marked with liquid in colorless soxhlet flask. Extraction was evaporated using electromantherl at temperature of 60°C and it results of extracting bitter melon fruit 100%. the research flow can be seen in figure 1.

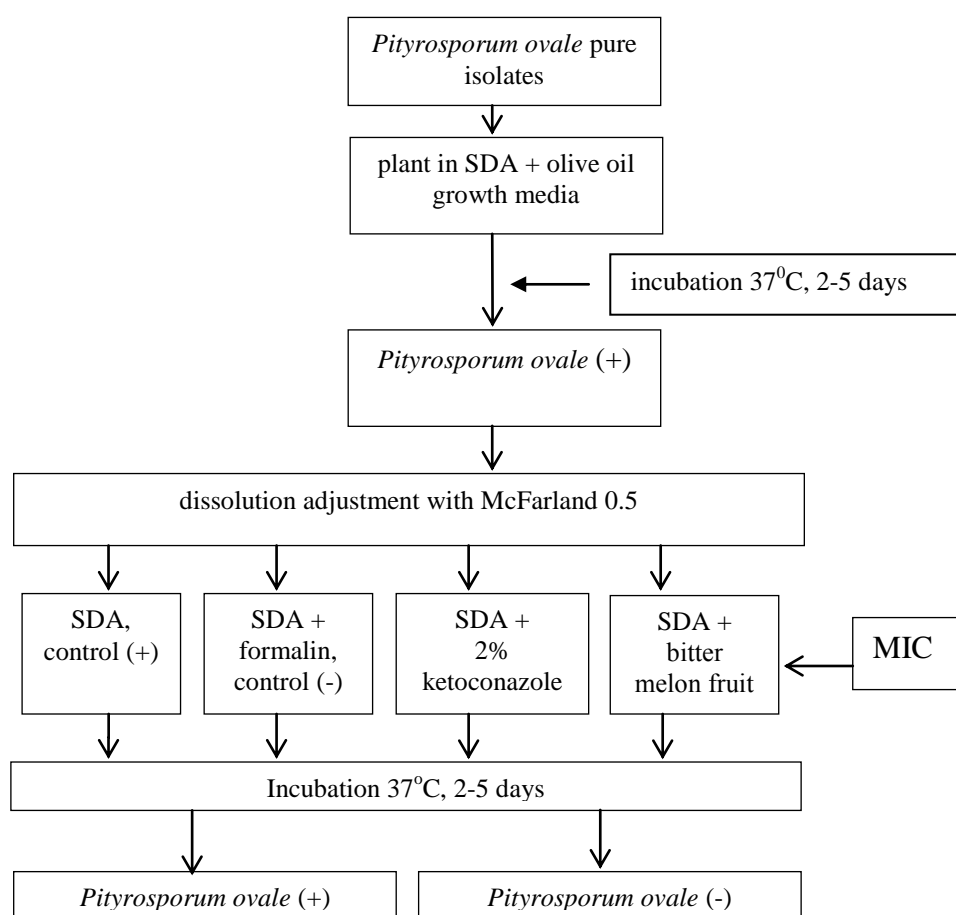


Figure 1. Research flow

Determination concentration of bitter melon extract was carried out through minimum inhibitory concentration (MIC) test by conducting a preliminary test on one study sample with a concentration of bitter melon extract 100%, 75%, 50%, 25%, 5% with 4 repetitions at each

concentration. Observations of *P. ovale* growth were carried out in incubators for 2-5 days at 37°C with the lowest concentration results which did not appear to be *P. ovale* colonies.

Determination of presence or absence *P. ovale* is carried out macroscopic observations if it found yellowish white / cream / light brown colony and white fiber. Microscopic observations if obtained \geq ten yeast cell images are per field of view in 5 fields of view 100x magnification of objective lenses. Sample analysis using the chi-square test.

The research was conducted after obtaining ethical approval No.129 / III / 2018 / Bioetic Commission by the Medical Research Bioethics Commission / Health Faculty of Medicine, Sultan Agung Islamic University, Semarang.

3. Result

From table 1. *Pityrosporum ovale* was not found in 100% bitter melon fruit extract. From the analyzed effectiveness of bitter melon extract (*Momordica charantia* L.) 100% equal to 2% ketoconazole in inhibiting *P. ovale* growth (Table 2).

Table 1. MIC of bitter melon fruit extract on *P. ovale*

Concentration of bitter melon fruit extract	Macroscopic				Microscopic				Conclusion
	C*1	C*2	C*3	C*4	C*1	C*2	C*3	C*4	
100%	-	-	-	-	-	-	-	-	-
75%	+	+	+	+	+	+	+	+	+
50%	+	+	+	+	+	+	+	+	+
25%	+	+	+	+	+	+	+	+	+
5%	+	+	+	+	+	+	+	+	+

*C = petri dish

Table 2. *P. ovale* growth between 100% bitter melon extract with 2% ketoconazole

Comparasion	<i>P. ovale</i> growth		N (%)	P value
	Positive n(%)	Negative n(%)		
100% bitter melon extract	10 (33,3)	20 (66,7)	30 (100)	0,595
2% ketoconazole	13 (43,3)	17 (56,7)	30 (100)	
Total	23 (38,3)	37 (61,7)	60 (100)	

4. Discussion

From the analysis, it was found that 100% bitter melon and 2% ketoconazole extract had the same effectiveness in inhibiting *P. ovale* growth. This is similar to the previous study which stated that *P. ovale* growth can be inhibited by bitter melon extract from a 100% variant of eel pare/ pare eel (*Trichosanthes anguina* L.) in vitro. The effectiveness of eel bitter melon extract was equal to 1% zinc pyrithione in inhibiting *P. ovale* growth [6].

These results are supported by the theory of action mechanism of flavonoid and saponin in inhibiting fungi cell membranes. Bitter melon fruit contains flavonoids and saponins which function as antifungi [12]. Flavonoids inhibit *P. ovale* growth through transpeptidase activity by forming bonds between dissolved proteins and fungi cell walls (mannoprotein layer, chitin, β - (1,3) and β - (1, 6) glucan) [2, 6]. Peptidoglycan disorders cause disruption of cell wall formation. Damage member and cell walls cause important components of microbes such as proteins and other fragments contained in the cytoplasm. This event causes lysis of fungal cells [6, 7].

Membrane damage depends on the lipophilic properties of a flavonoid [2, 6], more lipophilic can increase the ability to damage cell membranes [6,7].

When cell membranes begin to break down, saponins work through the formation of cholesterol complexes in the cell membrane [2, 6]. The increased permeability of cell membranes occurs due to the process of breaking down the fat layer on the membrane causing membrane instability and resulting cell hemolysis [6, 13].

Different with saponins and flavonoids, ketoconazole inhibits *P. ovale* growth through mechanism of ergosterol blocking in ergosterol synthesis pathway [4, 5]. Ketoconazole blocks ergosterol by preventing the mitelation of lanosterol which is one of the precursors of ergosterol. When this is inhibited, there will be a reduction in the amount of ergosterol in the fungal cell membrane, cell membrane becomes unstable, and causes cell death [5, 12].

5. Conclusion

The effectiveness of 100% bitter melon fruit (*Momordica charantia* L.) extract equal to 2% ketoconazole in inhibiting *P. ovale* in vitro growth. Needs other research about the effectiveness of bitter melon extract in inhibiting other types of fungi or comparison of effectiveness of bitter melon fruit extract with other type of bitter melon in inhibiting *P. ovale* activity.

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