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Effectiveness of *Allium sativum* L. Ethanol Extract on the Growth of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*

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Abstract: Treatment of *Trichophyton* sp. and *Candida* sp. infections can do with various antifungal drugs, one of which is *Allium sativum* L. This herb contains Allicin, flavonoids, tannins, saponins and essential oils that function as antifungals. The research objective was to compare the effectiveness of *Allium sativum* L. ethanol extract on the growth of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*. Type of experimental research with the design of the post-test only control group design. The independent variable was the concentration of ethanol extract of *Allium sativum* L. and the dependent variable growth of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*. The statistical test used was Mann-Whitney. Antifungal activity test was carried out by liquid dilution method with a concentration of 2.5, 5, 7.5, 10, 12.5, 14, 15, 16, 17.5, 18, 20, 22, 24, 26 and 28 mg/mL the results showed that Minimum Inhibitory Concentration (MIC) against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis* were 17.5, 5, 16 and 20 mg/mL Minimum Bactericidal Concentration (MBC) results against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis* are 20, 7.5, 18 and 22 mg/mL. Based on the Mann-Whitney *Trichophyton rubrum* and *Trichophyton mentagrophytes* test, a synergistic value of 0.037 ($p<0.05$) obtained at a concentration of 7.5 mg/mL up to 15 mg/mL. *Candida albicans* and *Candida tropicalis* a significance value of 0.037 ($p<0.05$) at a concentration of 18 mg/mL.

Key words: *Allium sativum* L., *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans*, *Candida tropicalis*, antifungals

INTRODUCTION

Fungal infections in humans can be either dermatophytosis or candidiasis (Brooks *et al*., 2007). Dermatophytes consist of three genera including *Microsporum*, *Trichophyton* and *Epidermophyton* with the most common causative species *Trichophyton rubrum* and *Trichophyton mentagrophytes*. *Candida albicans* is the most pathogenic species in causing candidiasis in addition to *Candida tropicalis*.

Infection due to dermatophyte fungi can found throughout the world with an estimated 20-25% of the world's population infected with dermatophyte fungi. These dermatophytes fungi can grow well in tropical countries that have high temperatures and humidity such as Indonesia.

Fungal infections can treat by administering antifungal drugs through topical or systemic pathways but some unwanted side effects in the administration of synthetic antifungal medicines found, so, a good antifungal from herbs is needed.

Herbaceous plants that grow in Indonesia can function as antilarval, antifungal and antibacterial. Among these plants are *Anredera cordifolia* (Dwiyanti *et al*., 2015), lime (Dwiyanti and Lutpiatina, 2018), *Cananga odorata* (Lamk). Hook Nuryati *et al*. (2015), *Carica papaya* Linn (Swastika *et al*., 2016), *Syzygium polyanthum* (Dwiyanti *et al*., 2017), *Kaempferia galanga* L. *Jatropha curcas* (Wiratni *et al*., 2017) as. Also, herbal plants that are widely used by society are *Allium sativum* L. *Allium sativum* L. has benefits as an antifungal.

Allicin is the main component that acts to give the aroma of *Allium sativum* L. and functions as an active substance that inhibits various species of fungi and bacteria. Allicin in *Allium sativum* L. becomes one of the

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polar components produced by extraction using ethanol solvents. Other active compounds which are polar components with antifungal properties in *Allium sativum* L. are saponins, flavonoids, tannins and essential oils. Polar compounds will be interested and their effectiveness can be tested as an antifungal against fungal growth using ethanol solvents.

The Aala *et al*. (2012) study showed the pure antimicrobial effect of allicin on ten *Trichophyton rubrum* isolates with Minimum Inhibitory Concentration (MIC) ranging from 0.78-12.5 μg/mL. Gholib's study of the effectiveness of *Allium sativum* L. ethanol extract on *Trichophyton mentagrophytes* showed a MIC of 0.75%.

Andayani and Kurniawan's research (2013) showed that the ethanol extract of *Allium sativum* L. at a concentration of 40, 60, 80 and 100% could inhibit the growth of fungi *Candida albicans* with the diameter of the inhibition zone of each concentration was 11.6, 14.8, 18.6 and 21.4 mm. Another study conducted by Rambet stated that MIC pure juice of *Allium sativum* L. on the growth of *Candida albicans* obtained at a concentration of 50%. The results of other studies by Diba and Alizadeh, MIC of water and ethanol extract of *Allium sativum* L. against *Candida tropicalis* were 9.55 and 10.90 mg/mL.

Previous research showed that *Allium sativum* L. inhibited the growth of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis* but there was no difference in inhibition of ethanol extract from the four fungi. This study aims to compare the effectiveness of *Allium sativum* L. ethanol extract with a concentration of 2.5, 5, 7.5, 10, 12.5, 14, 15, 16, 17.5, 18, 20, 22, 24, 26 and 28 mg/mL and *Trichophyton rubrum* , *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*.

MATERIALS AND METHODS

The type of research conducted is experiment with the design of posttest-only control group design. The ingredients used are *Allium sativum* L. tubers which are fresh, dense not wrinkled and do not show black parts. The independent variable in this study was the variation in the concentration of *Allium sativum* L. ethanol extract. The dependent variable in this study was the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis* based on the determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Determination test of *Allium sativum* L. was carried out at FMIPA Laboratory, Lambung Mangkurat University, Banjarmasin Indonesia.

Allium sativum L. bulbs 100 g were crushed and macerated with 100 mL 70% ethanol for 3 days. Maserati concentrated by evaporating ethanol using a 50°C waterbath until thick extract formed. Thick extract equals 1000 mg/mL. The extract made a concentration of 200 mg/mL with TSB thinners. Furthermore, it is further diluted to obtain a concentration of 2.5-28 mg/mL.

Determination of MIC by adding to 1 mL of a solution of various concentrations with 1 mL of fungal suspension, so that, the final concentration of the solution becomes half the initial concentration of 2.5, 5, 7.5, 10, 12, 5, 14, 15, 16, 17.5, 18, 20, 22, 24, 26 and 28 mg/mL. Repetition is done 3 times. The incubation temperature of 37°C for 5 days (for *Trichophyton* sp.) 1 day (for *Candida* sp.) read the results by looking at tubes containing the lowest levels of extract but still able to inhibit the growth of fungi marked with clear colored solutions expressed as MIC values.

Determination of MBC by taking MIC suspension at each concentration of 20 μL then spread on SDA plate with five repetitions. It is incubated 5 days at 28°C (for *Trichophyton* sp.) 1 day at 37°C (for *Candida* sp.). Colonies that grow in SDA calculated.

RESULTS AND DISCUSSION

The results of the study can be seen in Table 1-6. The Mann-Whitney test was used to compare the variations in concentration with the growth of *Trichophyton rubrum* and *Trichophyton mentagrophyte*. The results of the Mann-Whitney test at a concentration of 7.5 mg/mL up to 15 mg/mL obtained a significance of 0.037 ($p<0.05$). So that, there are differences in the effectiveness of *Allium sativum* L. ethanol extract on the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes* at these concentrations.

The Mann-Whitney test was carried out to compare the effect of *Allium sativum* L. ethanol extract on variations in concentration on *Candida albicans* and

Table 1: Results of MIC of *Allium sativum* L. ethanol extract on growth *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Concentration of ethanol extract of *Allium sativum* L. (mg/mL)	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
2, 5	Turbid	Turbid
5	Turbid	Clear
7, 5	Turbid	Clear
10	Turbid	Clear
12, 5	Turbid	Clear
15	Turbid	Clear
17, 5	Clear	Clear
20	Clear	Clear

Table 2: Results of MBC of *Allium sativum* L. ethanol extract on growth *Trichophyton rubrum* and *Trichophyton mentagrophytes*

Concentration of ethanol extract of *Allium sativum* L. (mg/mL)	*Trichophyton rubrum*	*Trichophyton mentagrophytes*
2, 5	663	15
5	587	9
7, 5	470	0
10	351	0
12, 5	193	0
15	81	0
17, 5	7	0
20	0	0

Table 3: Mann-Whitney test results comparison of the concentrations of ethanol extract of *Allium sativum* L. to the growth of *Trichophyton rubrum* and *Trichophyton mentagrophytes*

	Concentration of *Allium sativum* L. ethanol extract against *T. mentagrophytes* (mg/mL)							
Variables	2.5	5	7.5	10	12.5	15	17.5	20
2, 5	0.05							
5		0.05						
7, 5			0.037*					
10				0.037*				
12, 5					0.037*			
15						0.037*		
17, 5							0.121	
20								1.000

(*) there is a difference ($p<0.05$)

Table 4: Results of MIC of *Allium sativum* L. ethanol extract on growth *Candida albicans* and *Candida tropicalis*

Konsentrasi Ekstrak etanol *Allium sativum* L. (mg/mL)	*Candida albicans*	*Candida tropicalis*
14	Turbid	Turbid
16	Clear	Turbid
18	Clear	Turbid
20	Clear	Clear
22	Clear	Clear
24	Clear	Clear
26	Clear	Clear
28	Clear	Clear

Candida tropicalis. Based on the results of statistical tests, at a concentration of 18 mg/mL a significance value of 0.037 ($p<0.05$) can be stated that there is a difference between *Candida albicans* and *Candida tropicalis*.

Essential oils to appropriately extracted. Josling (2005) mentions that Allicin is considered the main component responsible for inhibiting fungal growth. Tanin produces antifungal effects by inhibiting chitin synthesis in the formation of cell walls in fungi and damaging cell membranes. Also, according to Gholib, the components of active substances that have antifungal properties in *Allium sativum* L. are saponins, flavonoids and essential oils that work by influencing the permeability of fungal membranes and synthesis of nucleic acids, so that, the fungus cannot reproduce accurately.

The data in Table 1 shows the MICs of *Trichophyton rubrum* and *Trichophyton mentagrophytes* were 17.5 and 5 mg/mL. Different results from Rahmah's research (2013) showed that the MIC of ethanol extract of

Table 5: Results of MBC of *Allium sativum* L. ethanol extract on growth *Candida albicans* and *Candida tropicalis*

Konsentrasi Ekstrak etanol *Allium sativum* L. (mg/mL)	*Candida albicans*	*Candida tropicalis*
14	25	209
16	5	13
18	0	4
20	0	1
22	0	0
24	0	0
26	0	0
28	0	0

Table 6: Mann-Whitney test results comparison of the concentrations of ethanol extract of *Allium sativum* L. to the growth of *Candida albicans* dan *Candida tropicalis*

	Concentration of *Allium sativum* L. ethanol extract against *Candida albicans* (mg/mL)							
Variables	14	16	18	20	22	24	26	28
14	0.050							
16		0.127						
18			0.037*					
20				0.121				
22					1.000			
24						1.000		
26							1.000	
28								1.000

(*) there is a difference ($p<0.05$)

Allium sativum L. against *Trichophyton rubrum* isolates at concentrations of 4-8%. Ghalib's study (2010) on the ethanol extract of *Allium sativum* L. against *Trichophyton mentagrophytes* showed MIC at a level of 0.75%. The results of this study obtained a higher MIC value but the research conducted by (Gholib) and (Rahmah) showed that the same thing with this study, namely *Trichophyton rubrum* has a MIC value higher than *Trichophyton mentagrophytes*.

Based results on Table 4. MIC, *Candida albicans* was obtained at a concentration of 16 mg/mL while *Candida tropicalis* found at a level of 20 mg/mL. These results are different from the study by Fei *et al*. (2008) showing that MIC occurs at a concentration of 20 mg/mL for *Candida albicans*. Another research by Diba and Alizadeh, showed that MIC *Candida tropicalis* happened at a level of 10.90 mg/mL. There were differences in the MIC values between *Candida albicans* and *Candida tropicalis* in this study with higher *Candida tropicalis* concentrations than *Candida albicans*.

This difference in the results of research on *Candida* can cause by the extraction process and technique carried out using ethanol. Fei *et al*. (2008) used aqua as a solvent. Ethanol used because it is capable of dissolving almost all substances, both polar, semipolar and nonpolar. Another study by Diba and Alizadeh, used Soxhlet extraction techniques while this study used maceration techniques. The Soxhlet extraction method can easily filter more active compounds than the maceration method.

In addition to having the ability to inhibit *Allium sativum* L. it also can kill *Trichophyton rubrum* and *Trichophyton mentagrophytes*, this shown result in the MBC test. Based on Table 2 the effects of growth and calculation of the number of colonies on SDA media showed that MBC fungi *Trichophyton rubrum* was 20 mg/mL and the fungus *Trichophyton mentagrophyes* was 7.5 mg/mL.

The concentration of *Allium sativum* L. ethanol extract has a different MBC value for the fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes* even though this fungus originates from the same genus, *Trichophyton*. This is due to differences in the cell wall structure of each fungus, Yue *et al*. (2015) states that *Trichophyton rubrum* has longitudinal hyphae growth with two cell wall layers whose outer layer has high-density particles whereas in *Trichophyton mentagrophytes* according to Pock-Steen and Kobayasi (1970) Mushroom hyphae have two layers of cell walls with thin outer layers. Different conditions of the cell walls of *Trichophyton rubrum* and *Trichophyton mentagrophytes* affect the ability of the antimicrobial substances *Allium sativum* L. to penetrate the cell wall to be able to kill fungi which cause the MBC value between *Trichophyton rubrum* and *Trichophyton mentagrophytes* not the same. Based on the work process of antimicrobial substances in influencing fungal growth, Josling (2005) mentions that antimicrobial Allicin substances in *Allium sativum* L. react with microorganisms by penetrating cell walls and can interfere with the biochemical balance in them.

Based on Table 5 shows the MBC value of *Candida albicans* is 18 mg/mL and *Candida tropicalis* is 22 mg/mL. This indicates that there is a difference in the effectiveness of *Allium sativum* L. ethanol extract on the growth of *Candida albicans* and *Candida tropicalis* with a higher killing power of *Candida albicans* than *Candida tropicalis*. This difference can occur because in phylogeny *Candida tropicalis* is a close relative of *Candida albicans*, so that, the pathogenicity is quite high such as *Candida albicans*. Also, according to research by Fei *et al*. (2008), *Candida tropicalis* is only able to produce occasional pseudohypha while *Candida albicans* can switch to hyphae, so that, allicin in *Allium sativum* L. more suppresses hyphae growth in *Candida albicans* than *Candida tropicalis*.

CONCLUSION

Conclusion there are differences in the effectiveness of *Allium sativum* L. ethanol extract on the growth of fungi *Trichophyton rubrum* and *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*.

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SIGNIFICANCE STATEMENT

Active compounds which are polar components with antifungal properties in *Allium sativum* L. are Allicin, saponins, flavonoids, tannins and essential oils. Polar compounds will be interested in using ethanol solvents. Previous research showed that *Allium sativum* L. inhibited the growth of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis* but there was no difference in inhibition of ethanol extract with the same method of the four fungi. The method used in the study was Minimal Concentration Inhibition (MIC) and Minimum Bactericidal Concentration (MBC). The aim of the study was to compare the effectiveness of *Allium sativum* L. ethanol extract with a concentration of 2.5, 5, 7.5, 10, 12.5, 14, 15, 16, 17.5, 18, 20, 22, 24, 26 and 28 mg/mL against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*. This study provides information about the comparison of the effectiveness of garlic ethanol extract (*Allium sativum* L.) which can be used as a basis for alternative treatments for *Trichophyton rubrum* infections, *Trichophyton mentagrophytes*, *Candida albicans* and *Candida tropicalis*.

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