Antifungal Activity Of Java Acid Leaf Ethanol Extract (Tamarindus Indica L.) On Fungi Growth Malassezia Furfur And Trichophyton Mentagrophytes

Lilis Sugiarti[1]*, Dias Khoerunnisak[2], Ferianto[3]

[1,2]Institut Teknologi Kesehatan Cendekia Utama Kudus

[3]Rumah Sakit Tama Hiyosida byouin, Kanagawaken, Kawasakishi Asaoku Ozenji

*Corresponding Author: lilis_suwarno@yahoo.co.id

Abstract. Fungi are heterotrophic organisms that, if alive, can cause disease in plants and animals, including humans. This research was conducted to determine the potential inhibitory power and Minimum Inhibitory Concentration (MIC) of ethanol extract of tamarind leaves against the fungi Malassezia furfur and Tricophyton mentagrophytes and to determine the correlation between antifungal potential and extract concentration. The extraction method uses the maceration method and fungal testing uses the disc diffusion method and data analysis uses ANOVA, correlation, and regression. The results of the analysis of the diameter of the inhibition zone for Malassezia furfur and Trichophyton mentagrophytes obtained a significant value of 0.000 ($p<0.05$). The optimum inhibitory concentration against Malassezia furfur and Trichophyton mentagrophytes at a concentration of 100 mg/mL is in a strong category. There is a positive relationship between Malassezia furfur and Tricophyton mentagrophyes. The results of linear regression on Malassezia furfur obtained the equation $y=0.1068x+3.8004$, while Trichophyton mentagrophytes obtained the equation $y=0.1258x+3.4978$. The results of the ANOVA obtained from the inhibitory power of the two fungi were not significantly different. The ethanol extract of tamarind leaves has antifungal potential and has a close relationship, the effect of extract concentration on Malassezia furfur is 96.66% and Tricophyton mentagrophytes is 96.5%, the inhibitory power of the two fungi is not significantly different

Keywords: [Ethanol extract of tamarind leaves, Antifungi, Malassezia furfur, Tricophyton mentagrophyten.]

INTRODUCTION

Fungi are cosmopolitan living creatures that can grow anywhere, and are close to human life, both in the air, soil, water, clothes, and even on the human body itself (Hasanah, 2017). Diseases caused by fungal infections can include tinea versicolor (Pityriasis versicolar) which is caused by the fungus Malassezia furfur. Tinea versicolor is a fungal infection characterized by white to reddish-brown spots. Skin that sweats easily, is damp and lack of knowledge about skin health and hygiene are also factors that cause the growth of Malassezia furfur (Alawiyah, Khotimah & Mulyadi, 2016).

*Trichopyton mentagrophytes*which is a fungus from the genus Trichophyton in the dermatophyte group which can cause disease on human skin. This fungus can infect horn tissue such as nails, hair, finger skin, and the stratum corneum which can cause tinea pedis or what is often called athlete's foot (water fleas). Afifah & Nurwaini, 2018).

Indonesia is rich in plants that have the potential to have various benefits as traditional medicinal ingredients. One of the plants that acts as an antimicrobial is tamarind, tamarind leaves can be used as a treatment for various diseases. The active ingredients in tamarind leaves are saponins, tannins, and flavonoids. Flavonoids are phenolic compounds that work as antifungals to inhibit the synthesis of fungal nucleic acids. Tamarind leaf extract has abilities such as antibacterial, antifungal, anti-inflammatory, and antioxidant (Fakhrurrazi, Hakim & Keumala, 2016).

METHODS

Material

The materials used in this research were tamarind leaves (Tamarindus indica L.) taken from Bae Pondok Village, Bae District, Kudus Regency, Central Java, Malassezia furur ATTC 1157 fungus culture, Trichophyton mentagrophytes ATCC 1613 fungus culture obtained from the collection.

Microbiology Laboratory of Dr. Hospital Kariadi Semarang, 70% ethanol, 4% DMSO solvent (Dimethyl Sulfoxide), ketoconazole, distilled water, CYG liquid media (Casein Yeast Glucose), PDA Media (Potato Dextrose Agar), Magnesium, concentrated HCl, FeCl31%.

Tool

Analytical balance, oven, blender, autoclave, Laminar Air Flow (LAF), hotplate and magnetic stirrer, water bath, measuring cup, funnel, brown bottle, micropipette and tip, incubator, test tube, test tube rack, test tube clamp, petri dish, Bunsen burner, tweezers, stir stick, ose needle, flannel cloth, Whatman No.1 paper, caliper and vortex tool, rotary evaporator and Drigalsky spatula.

The Progress of Research on Making Simplicia

The obtained tamarind (Tamarindus indica L.) leaves were then washed and dried in an oven at 40℃ for 2-3 days and then ground to obtain tamarind (Tamarindus indica L.) leaf powder with a degree of fineness of 40 mesh which was then continued with maceration process.

Making Extracts

200 grams of tamarind leaf powder (Tamarindus indica L.) were weighed and then macerated using 1000 mL of 70% ethanol solvent. (Ratio 1: 5) for 24 hours and repeat the maceration process until completely extracted.

Tamarind Leaves Ethanol Free Test

0.1 gram of thick extract of tamarind leaves (Tamarindus indica L.) was put into a test tube, 1 ml of acetic acid and 2 drops of concentrated sulfuric acid were added, then heated with a Bunsen heater.

Phytochemical Screening

1. Flavonoid Identification: 0.5 grams of concentrated extract of tamarind leaves (Tamarindus indica L.) was dissolved in ethanol and 0.1 grams of Mg and 2 drops of concentrated HCl were added. If a blackish-orange color forms, it is positive for containing flavonoids (Harborne, 1987).
2. Tannin Identification: 0.5 gram of concentrated extract is dissolved in 10 mL of distilled water, then filtered and the filtrate is dripped with 3 drops of FeCl31%. If a blackish-green color forms, then it is positive for containing tannin (Harborne, 1987).
3. Saponin Identification: 0.5 grams of the concentrated extract is dissolved in hot distilled water and added with HCl then shaken. If stable foam is formed, it is positive for containing saponin (Harborne, 1987).

Making PDA Media

20 grams of powdered PDA media was dissolved in 1 L of distilled water and then heated on a hot plate until completely dissolved, then the media was sterilized in an autoclave at 121 ˚C for 15 minutes.

Making Mushroom Suspension

One cycle of fungal colonies was inoculated in 10 mL of CYG liquid media and then incubated at 37ºC for 24 hours. Next, cell density was measured using a spectrophotometer by taking 10 mL of the suspension using a pipette to the cuvette limit, after which it was put into the spectrophotometer. Measurements were carried out at a wavelength of 625 nm and an absorbance of 0.08-0.1 to obtain the Mc Farland turbidity standard, namely 108 cfu/mL (Sugiarti & Fitrianingsih, 2018).

Making Extract Concentration for Testing

Preparing the concentration of tamarind leaf extract was carried out by making a dilution series, namely by weighing 400 mg of tamarind leaf extract dissolved in 4 mL of 4% DMSO, added to test tube I (mother solution), from reaction tube I (equivalent to a concentration of 100 mg/mL) take 2 mL and put it into reaction tube II (equivalent to a concentration of 50 mg/mL) then in reaction tube I where 2 mL has been taken, 2 mL of 4% DMSO is added, the next step is to carry out the same graded dilution to reach a concentration of 6.25 mg/mL, then for a concentration of 75 mg/mL this was done by weighing 150 mg of ethanol extract into another test tube, dissolving it with 2 mL of 4% DMSO, then vortexing for 60 seconds.

Setting Up Positive and Negative Controls

The positive control used ketoconazole. In this positive control, 200 mg of ketoconazole was dissolved in 2 mL of sterile distilled water, then dilution was carried out until a ketoconazole

concentration of 20 µg was dissolved in 1 mL of sterile distilled water. Meanwhile, the negative control used 4% DMSO and sterile distilled water, as well as PDA media control (Dewi, 2010).

Antifungal Test

100 µL of the test fungal suspension was taken with a cell density of 108 cfu/mL and put into a sterile petri dish containing solid PDA media, then spread evenly using a Driglosky spatula and waited until the test fungal suspension was completely absorbed in the media. After that, 9 paper discs with a diameter of 6 mm were each dripped with 200 µL of various treatments until everything was absorbed into the paper discs. Each petri dish is given 4-5 paper discs, so it requires 2 petri dishes for treatment. The next step is to place the paper disc on the surface of the media, then the petri dish is incubated at 37ºC for 24 hours. After 24 hours, observe whether there is a clear zone around the disc paper. The diameter of the clear zone formed was measured using a caliper. The presence of a clear area around the paper disc indicates antifungal activity. After that, the diameter of the inhibition zone is calculated.

RESULTS AND DISCUSSION

Tamarind Leaves Ethanol Free Test

The thick extract of tamarind leaves was carried out with an ethanol-free test to find out whether the tamarind leaf extract was truly 70% ethanol-free, namely by esterification. The ethanol-free test on the thick extract of tamarind leaves did not smell of ester. This test aims to ensure that the ethanol used as a solvent does not interfere with the antimicrobial activity of tamarind leaf extract (Sofya, 2019).

Phytochemical Screening of Tamarind Leaf Extract

The ethanol extract of tamarind leaves was positive for containing flavonoids, indicated by the formation of an orange color after being treated with Mg powder and concentrated HCl, tannins were indicated by the formation of a blackish green color, and saponins by the formation of stable foam for 15 minutes. The purpose of adding Mg powder and concentrated HCl is to reduce the benzopyrone core contained in the flavonoid structure so that red or orange flavilium salts are formed (Ergina, Nuryanti & Pursitasari, 2014).

Test of the Antifungal Potential of Tamarind Leaf Ethanol Extract against Fungi*Malassezia furfur*and Tricophyton mentagrophytes

Differences in Antifungal Concentration of Ethanol Extract of Tamarind Leaves on the Diameter of the Inhibitory Zone of the Fungus Malassezia furfur

The results can be seen from the table of the average diameter of the inhibition zone of ethanol extract of tamarind leaves on the growth of Malassezia furfur at a concentration of 100; 75; 50; 25; 12.5; 6.25 mg/mL respectively are 13.8; 12.0; 10.0; 7.2; 5.0; 3.5mm. It can be seen that the greater the concentration of tamarind leaf extract, the greater the diameter of the inhibition zone produced. The average optimal inhibitory concentration for the Malassezia furfur fungus with a concentration of 100 mg/mL can inhibit the growth of fungus with a diameter of 13.8 mm, which means it is in a strong category, while the minimum inhibitory concentration with a concentration of 6.25 mg/mL can inhibit the growth of fungus with a diameter of 3 .5mm. The positive control ketoconazole 20 µg/mL had an inhibition zone diameter against the Malassezia furfur fungus with a diameter of 8.2 mm. The ethanol extract of tamarind leaves at a concentration of 25 mg/mL had an inhibitory power that was not significantly different from the positive control ketoconazole 20 µg/mL, so the ethanol extract of tamarind leaves has great potential to be applied as an antifungal agent. The negative controls used were DMSO and sterile distilled water. The results obtained were an inhibitory zone diameter of 0 mm for both fungi, which means that the two negative controls did not have antifungal properties because they did not have a fungal inhibition zone.

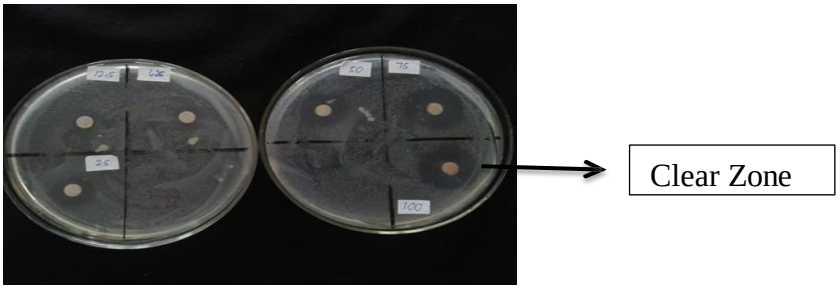

Figure 1
Antifungal Test Results of Ethanol Extract of Tamarind Leaves Against the Fungus Malassezia furfur

Table 1
Results of Inhibitory Zone Diameter of Ethanol Extract of Tamarind Leaves Against Malassezia Furfur Fungus

Concentration	Diameter of inhibition zone of Malassezia furfur fungus (in mm)
6.25mg/mL	3.5 ±1.0a
12.5mg/mL	5.0 ± 0.8b
25mg/mL	7.2 ± 0.8c
50mg/mL	10.0 ± 1.2d
75mg/mL	12.0 ± 1.0e
100mg/Ml	13.8± 1.6f
Positive Control	8.2 ± 0.8c
Negative Control 1	-
Negative Control 2	-

Note: Numbers followed by different letters indicate significant differences.

Relationship between Extract Concentration and Fungal Inhibition Zone Diameter Malassezia furfur

The diameter of the inhibition zone obtained in this study was analyzed using a correlation test which aims to determine the relationship between the 2 variables. The results of the correlation show that the Malassezia furfur fungus has a correlation coefficient between the total concentration and the inhibitory power against the fungus which is 0.965, which shows that there is a relationship between the concentration of tamarind leaf ethanol extract and the diameter of the inhibition zone of the Malassezia furfur fungus. The results of the correlation test can be seen in Table 2 below. The results of the correlation test values show a very strong relationship between the extract concentration and the diameter of the inhibitory zone for the Malassezia furfur fungus, which means that the higher the extract concentration, the larger the inhibitory zone for the Malassezia furfur fungus.

Table 2

Correlation Test Results Between Extract Concentration and Inhibition Zone Diameter

		Concentration	Inhibitory Power
Concentration	Pearson Correlation	1	,965**

	Sig. (2-tailed)		,000
	N	30	30

Effect of Concentration on the Average Diameter of the Fungal Inhibition Zone Malassezia furfur

The effect of the concentration of tamarind leaf ethanol extract can be determined if the correlation test results show a strong level of relationship. The results of the linear regression analysis can be seen in Figure 2 below. Based on the results of the linear regression test, the value of y= 0.1068x+3.8004 is obtained, which means that every increase of 1 mg/mL can increase the diameter of the resistance by 0.1068 mm with an initial diameter of 3.8004 mm. The diameter of the fungal zone is influenced by the extract concentration by 96.66% (R2=0.9666), and the remaining 3.34% is influenced by other factors.

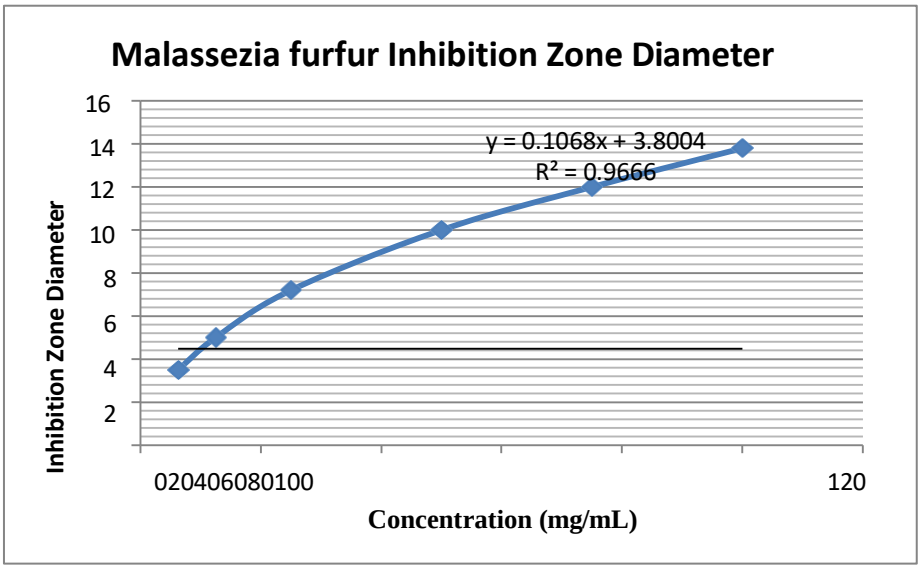

Figure 2
Graph of the Effect of the Concentration of Ethanol Extract of Tamarin Leaves on the Diameter of the Inhibitory Zone of the Fungus Malassezia furfur

Differences in Antifungal Concentration of Ethanol Extract of Tamarind Leaves on the Diameter of the Inhibition Zone of the Fungus Tricophyton mentagrophytes

Diameter of the inhibition zone of ethanol extract of tamarind leaves on the growth of the fungus Tricophyton mentagrophytes at a concentration of 100; 75; 50; 25; 12.5; 6.25 mg/mL respectively are 16.0; 12.4; 10.2; 8.0; 5.2; 3.0 mm.. Results can be seen in the table. The average optimal inhibitory concentration for Tricophyton mentagrophytes fungi with a concentration of 100 mg/mL can inhibit the growth of fungi with a diameter of 16.0 K. A concentration of 6.25 mg/mL can inhibit the growth of fungi with a diameter of 3.0 mm. The positive control ketoconazole 20 µg/mL had an inhibition zone diameter against the fungus Tricophyton mentagrophytes with a diameter of 8.2 mm. The inhibitory power of this positive control was not significantly different from the inhibitory power of the ethanol extract of tamarind leaves at a concentration of 25mg/mL. This shows that the ethanol extract of tamarind leaves has great potential to be developed as a medicine for athlete's foot. The negative controls used were DMSO and sterile distilled water. The results obtained were a diameter of 0 mm for the Tricophyton mentagrophytes fungus, which means that the two negative controls did not have antifungal properties because they did not have antifungal properties. After all, they did not have a fungal inhibition zone.

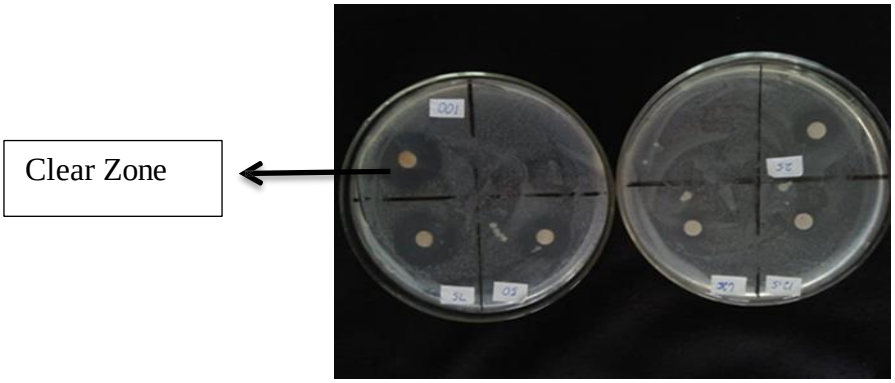

Figure 3
Antifungal Test Results of Ethanol Extract of Tamarind Leaves Against the Fungus Tricophyton mentagrophytes

Table 3
Diameter of the Inhibitory Zone of Ethanol Extract of Tamarind Leaves Against the Fungus Tricophyton mentagrophytes

Treatment	Diameter of the inhibition zone of the fungus Tricophyton mentagrophytes (in mm)
A1	3.0 ± 1.5a
A2	5.2 ± 0.8b
A3	8.0 ± 1.5c
A4	10.2 ± 0.8d
A5	12.4 ± 1.6e
A6	16.0 ± 1.5f
C+	8.2 ± 0.8c
C-1	-
C-2	-

Note: Numbers followed by letters that are not the same indicate significant differences.

Relationship between Extract Concentration and Fungal Inhibition Zone Diameter Trcophyton mentagrophytes

The results of the correlation show that the Tricophyton mentagrophyes fungus has a correlation coefficient between total concentration and inhibitory power against the fungus of 0.958, a value close to 1, indicating a very strong relationship between the extract concentration and the diameter of the fungal inhibition zone and is a unidirectional relationship, so the higher the concentration of ethanol extract. The greater the zone of fungal inhibition in tamarind leaves. The results of the correlation test can be seen in Table 4 below:

Table 4

Correlation Test Results Between the Concentration of Ethanol Extract of Tamarind Leaves and the Diameter of the Inhibition Zone

		Concentration	Inhibitory Power
Concentration	Pearson Correlation	1	,958**
	Sig. (2-tailed)		,000
	N	30	30

Effect of Concentration on the Average Diameter of the Fungal Inhibition Zone Trichophyton mentagrophytes

Based on the results of the linear regression test, the value of y= 0.1258x+3.4978 is obtained, which means that every increase of 1 mg/mL can increase the diameter of the resistance by 0.1258 mm with an initial diameter of 3.4973 mm. The diameter of the fungal zone is influenced by the extract concentration by 96.5% (R2=0.965), and the remaining 3.5% is influenced by other factors. The graph can be seen in Figure 4 below:

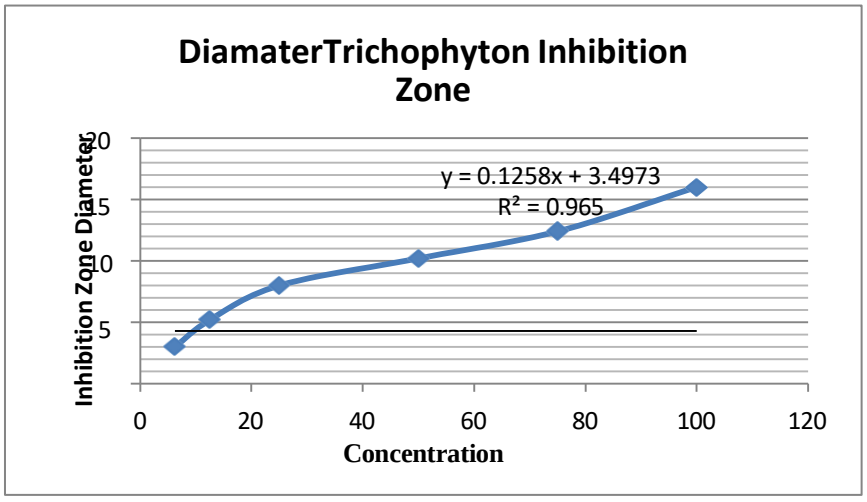

Figure 4
Graph of the Effect of Tamarind Leaf Ethanol Extract Concentration on the Diameter of the Inhibitory Zone

Differences in the Inhibitory Power of Ethanol Extract of Tamarind Leaves Against Malassezia furfur and Tricophyton mentagrophyton

The difference in the inhibitory power of the ethanol extract of tamarind leaves between Malassezia furfur and Tricophyton mentagrophytes can be analyzed using the T-Test. Based on the T-Test results at a concentration of 100mg/mL Malassezia furfur with Tricophyton mentagrophytes, a sig value was obtained. 0.063, concentration of 75mg/mL Malassezia furfur with Tricophyton mentagrophytes obtained a sig value. 0.659, concentration of 50mg/mL Malassezia furfur with Tricophyton mentagrophytes obtained a sig value. 0.771, the concentration of 25 mg/mL Malassezia furfur with Tricophyton mentagrophytes obtained a sig value. 0.347, while at concentrations of 12.5 mg/mL and 6.25mg/mL Malassezia furfur and Tricophyton mentagrophytes a sig value of 1.000 was obtained. From the results obtained, it can be seen in Figure 5 below and it is concluded that the inhibitory power of the ethanol extract of tamarind leaves between Malassezia furfur and Tricophyton mentagrophyton is not significantly different

and the ethanol extract of tamarind leaves can inhibit the growth of Malassezia furfur and Tricophyton mentagrophytes fungi.

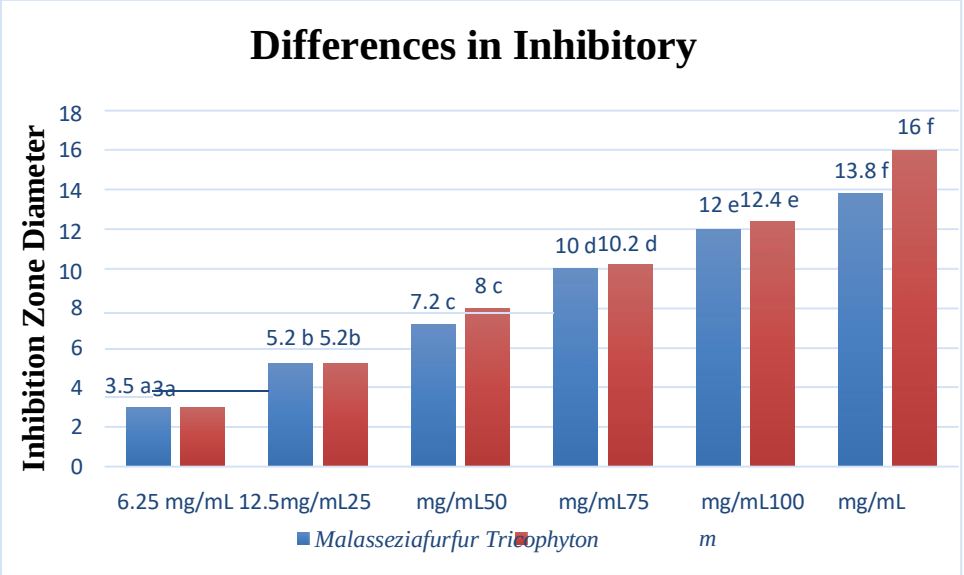

Figure 5
Differences in the Inhibitory Power of Malassezia furfur and Tricophyton mentagrophytes

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that:

1. Ethanol extract from tamarind leaves has antifungal potential against the growth of Malassezia furfur and Tricophyton mentagrophytes fungi.
2. The optimum inhibitory concentration was at a concentration of 100 mg/mL for Malassezia furfur and Tricophyton mentagrophytes in the strong category. Meanwhile, the minimum inhibitory concentration of the two fungi was 6.25 mg/mL.
3. There is an effect of extract concentration on the diameter of the inhibition zone of 96.66 % in Malassezia furfur while 96.5% in Tricophyton mentagrophytes and the rest is influenced by other factors.
4. There was no significant difference in inhibitory power in the ethanol extract of tamarind leaves between Malassezia furfur and Tricophyton mentagrophytes.

REFERENCES

Alawiyah, T., Khotimah, S., & Mulyadi, A. (2016). 'Antifungal activity of blood sea cucumber extract (Holothuria atra Jeager) against the growth of the fungus Malassezia furfur which causes tinea versicolor'. Journal of Pharmaceutical Sciences, Vol. 5(1), p. 59–67.

Alfiah, RR, Khotimah, S., & Turnip, M. (2015). 'Effectiveness of methanol extract of sembung rambat (Mikania micrantha Kunth) leaves on the growth of the fungus Candida albicans'. Protobiont Journal, vol. 4(1), p. 52–57.

Dewi, FK (2010). Antibacterial activity of ethanol extract of noni fruit (Morinda citrifolia L.) against spoilage bacteria in fresh meat. Thesis. Sebelas Maret University, Surakarta.

rgina, Nuryanti, S., & Pursitasari, ID (2014). 'Qualitative test of secondary metabolite compounds in palado leaves (Agave angustifolia) extracted with water and ethanol solvents'. Academic Journal of Chemistry. vol. 3(3), p. 165–172.

Fakhrurrazi. Hakim, FR, and Keumala, NC (2016). 'The effect of tamarind leaves (Tamarindus

Indica Linn) on the growth of Candida Albicans'. Journal Of Syiah Kuala Dentistry Society, Vol. 1(1), p. 29–34.

Harborne, J. B. (1987). Phytochemical Methods. 2nd Edition. ITB : Bandung.

Hasanah, J.B. (2017). 'Getting to know Aspergillosis, a fungal infection of the genus Aspergillus'. Journal of Healthy and Prosperous Families, Vol. 15(2), p. 76-86.

Sofya, WS (2019). 'Test the antibacterial activity of a combination of ethanol extract of guava leaves (Psidium guajava L.) and binohong leaves (Anredera cordifolia Ten) against Staphylococcus aureus ATCC 25923 by dilution'. Journal of Chemical Information and Modeling, 53(9), 1689–1699.

Sugiarti, L., & Fitrianingsih, S. (2018). 'Antibacterial activity of ethanol extract of parijoto leaves (Medinilla speciosa Blume) against the growth of Propionibacterium acnes and Staphylococcus aureus bacteria'. Scholar's Journal of Pharmacy, vol 2(1). p. 60-67.