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Antifungal Activity of Tea Tree Essential Oil (*Melaleuca Alternifolia*) Against *Trichophyton Mentagrophytes*

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Abstract

Dermatophytosis is a superficial skin disease in animals that affects hair follicles, nails, and keratin tissues caused by dermatophytes. *Trichophyton mentagrophytes* is one of the zoophilic dermatophyte genera. The conducting of commercial drug therapy has the potential to induce resistance, systemic toxicity, and teratogenic effects that need to be looked out for. Tea tree essential oil (*Melaleuca alternifolia*) is an alternative in preventing dermatophyte growth due to the presence of a main antifungal component, which is terpinene. This research aims to understand the antifungal activities of tea tree essential oil against dermatophytosis caused by *T. mentagrophytes* through a zone of inhibition test. Determination of the inhibition zone diameter of tea tree essential oil consisted of various concentrations 5, 10, 20, 40, 60, 80, and 100% by well diffusion method which utilizes Sabouraud Dextrose Agar (SDA). The zone of inhibition diameter is measured every 24, 48, and 72 hours using a vernier scale. The measurement results were calculated statistically. The statistical data analysis result shows a difference in the zone of inhibition between concentrations with a significance level of <0,05. The zone of inhibition diameter corresponds to the increase in tea tree essential oil concentration. The results of the average zone of inhibition at 72 hours incubation time exhibit a decrease in the zone of inhibition diameter when compared to the results of 48-hour and 24-hour incubations. These results present that tea tree essential oil has in vitro antifungal potential against *T. mentagrophytes*.

Keywords: antifungal activity; dermatophytosis; tea tree essential oil; *Trichophyton mentagrophytes*

Introduction

Dermatophytosis is a skin disease in animals that affects hair follicles, nails, and keratin tissues caused by dermatophytes. The dermatophytes develop a ring-shaped lesion known as a ringworm as a result of inflammation, with papules forming a ring around the edges of the lesions (Samanta, 2015). *Trichophyton mentagrophytes* is one of the zoophilic dermatophyte genera. Dermatophytosis causes nonspecific signs such as hair loss, erythema, and scaling. Dermatophytosis can be diagnosed using Wood's lamp, direct examination of the hair or scales of the lesion, skin biopsy, dermoscopy, Polymerase Chain Reaction (PCR) test, the colonies can also be examined macroscopically on Sabouraud Dextrose Agar (SDA) media, and microscopically using a 20% KOH solution to show branching and septa hyphae (Moriello, 2014; Yamada *et al*., 2019).

T. mentagrophytes is an example of a zoophilic dermatophyte with various genotypes which are commonly found in rodents, rabbits, cats, dogs and other animals (Kupsch *et al*., 2019). Domestic cats have a higher prevalence of dermatophytes than domestic dogs, though data on this subject may vary across country. Data from different countries varies due to regional variables in temperature, climate, relative humidity, and rainfall (Minnat and Khalf, 2019). Dermatophyte epidemiology is tightly tied to its surroundings; most dermatophyte infections in the house are associated with human or pet interaction (Mattei *et al*., 2014; Soedarmanto *et al*., 2020). The treatment of choice for superficial fungal infections is systemic and

topical medications. Commercial drug therapy has the potential to cause resistance, systemic toxicity, teratogenic effects, and drug interactions that need to be looked out for (Roana *et al*., 2021; Sofariah *et al.*, 2021).

Tea tree essential oil of Melaleuca alternifolia, is one of the most well-known oils. It is commonly used as an antiseptic to treat skin infections and has recently gained a lot of attention for its antifungal effects. It is utilized as a local formulation for dermatological disorders due to its lipophilic nature, which improves skin penetration (Santana-Gálveza *et al*., 2019; Tullio *et al*., 2019). The selection of tea tree essential oil in this study as an alternative in the treatment of dermatophytosis is expected to be a safer topical therapy alternative than commercial topical and oral therapy from the azole group. Along with helping to cure resistant microorganisms, natural compounds can also help to reduce the toxicities of common antifungals that are dose-related (Danielli *et al*., 2018; Scalas *et al*., 2018). Tea tree essential oil (*Melaleuca alternifolia*) could inhibit the growth of dermatophytosis because it contains the main antifungal component, terpinen, largely terpinen-4-ol and α-terpinen, which can inhibit the development of dermatophytes such as *T. mentagrophytes* (Brun *et al*., 2019; Paduch *et al*., 2007). Despite having the potential to treat skin infections, research should be conducted on tea tree (*M. alternifolia*) essential oil to understand the antifungal activities of tea tree essential oil against dermatophytosis caused by *T. mentagrophytes* through a zone of inhibition test (Marcos-Tejedor *et al*., 2021).

Material and Method

Research on antifungal inhibition test against *T. mentagrophytes* was conducted at the Prof. Soeparwi Animal Hospital and Pharmacology Laboratory, Department of Pharmacology, Faculty of Veterinary Medicine, Gadjah Mada University. This research was conducted from August 2021 to February 2022.

Young Living Essential Oil tea tree dilution was carried out with various concentrations of 5, 10, 20, 40, 60, 80, and 100%. V-6 vegetable oil™ is used to dilute the solution. Young Living Essential Oil® as a carrier oil for the topical application of tea tree essential oil. Multilevel dilution formulations were carried out to test tea tree essential oil with different strengths of active compound content. The activity of tea tree essential oil was compared to determine whether well diffusion could test oils with different profiles.

A 100 µl Sebazole® Virbac was given as the positive control. Sebazole shampoo® contains 35 mg/mL econazole nitrate, 19.4 mg/mL sulphur as sodium thiosulfate, 10 mg/mL sodium salicylate, and 5 mg/mL chloroxylenol as a topical treatment for cases of dermatophytosis caused by dermatophytes in animals.

The well diffusion method was carried out using SDA medium. Dermatophyte isolates were taken using a sterile cotton swab, and the swab was carried out slowly on the entire surface of the agar. Two wells were made into the media and perforated using a 100-1000 µl micropipette tip with a wellbore diameter of 0.9 mm. Determination of the inhibition zone diameter of tea tree essential oil consisted of a variety of concentrations (5,

Table 1. Multilevel dilution formulation of tea tree essential oil

No	Dilution	Formulation
1	5%	Tea tree essential oil 5 µl + Vegetable oil 95 µl
2	10%	Tea tree essential oil 10 µl + Vegetable oil 90 µl
3	20%	Tea tree essential oil 20 µl + Vegetable oil 80 µl
4	40%	Tea tree essential oil 40 µl + Vegetable oil 60 µl
5	60%	Tea tree essential oil 60 µl + Vegetable oil 40 µl
6	80%	Tea tree essential oil 0 µl + Vegetable oil 20 µl
7	100%	Tea tree essential oil 100 µl + Vegetable oil 0 µl

10, 20, 40, 60, 80, and 100%) by well diffusion method using Sabouraud Dextrose Agar (SDA). The inhibition zone diameter is measured every 24, 48, and 72 hours using a vernier scale.

The diameter measurement results are entered into the inhibition zone measurement formula:

$$Inhibition\ zone = \frac{(\mathrm{VD} - \mathrm{DW}) + (\mathrm{HD} + \mathrm{DW})}{2}$$

Note:
VD : vertical diameter (mm)
DW : diameter of the well (mm)
HD : horizontal diameter (mm)

Davis and Stout (1971) classified the inhibition zone diameter of antifungal responses for inhibitory activity are categorized into four categories; if the inhibition zone diameter is greater than 20 mm, the category is very strong; if it is between 11-20 mm, the category is strong; if it is between 5-10 mm, the category is medium; and if it is less than 5 mm, the category is weak.

Data were analyzed statistically with one way parametric One-Way Analysis of Variance (ANOVA) test on normally distributed data and Kruskal Wallis nonparametric test on non-normally distributed data to determine whether there was a significant effect of giving various concentrations of tea tree essential oil on diameter. zone of inhibition of *T. mentagrophytes*.

Result and Discussion

Antifungal activity test of tea tree essential oil against *T. mentagrophytes* was carried out at seven concentrations, namely 5, 10, 20, 40, 60, 80, and 100%, as well as a negative control using V-6 vegetable oil™ and a positive control using Sebazole®. The results of measurements of the inhibition zone for 24 hours showed the activity of tea tree essential oil against *T. mentagrophytes* with various results. The results of the antifungal test of tea tree essential oil at 24 hours are shown in Figure 1.

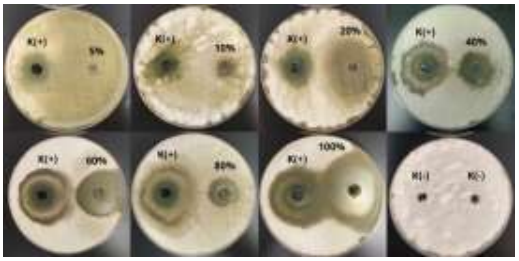

Figure 1. Antifungal test results of tea tree essential oil at 24 hours showed the activity of tea tree essential oil against *T. mentagrophytes* with various results

The conclusion of the data on the mean diameter of the inhibition zone and the standard deviation of tea tree essential oil testing on the growth of *T. mentagrophytes* at 24 hours can be seen in Table 2. The response to antifungal inhibition is interpreted based on Davis and Stout (1971). Based on the results of testing the antifungal activity of tea tree essential oil on the growth of *T. mentagrophytes*, it can be concluded that very strong activity was seen at concentrations of 20, 40, 60, 80, and 100%, while at concentrations of 5 and 10%, it can be interpreted into the strong category. The inhibition zone produced by the positive control can be interpreted as a very strong category and has a higher mean value than the

Table 2. Mean diameter of the inhibition zone (mm) and standard deviation (mm) of tea tree essential oil on the growth of *T. mentagrophytes* at 24 hours

No	Tea Tree Essential Oil Concentration (%)	Mean Diameter of Inhibition Zone (mm) and Standard Deviation (mm) 24 hours of incubation	Interpretation
1	100	27,60 ± 6,06	Very strong
2	80	27,20 ± 1,60	Very strong
3	60	22,65 ± 2,89	Very strong
4	40	22,35 ± 2,32	Very strong
5	20	20,25 ± 1,43	Very strong
6	10	19,35 ± 1,52	Strong
7	5	19,11 ± 4,53	Strong
8	Positive Control	29,25 ± 2,78	Very strong
9	Negative Control	00,00 ± 00,00	Weak

100% concentration, while the inhibition zone shown by the negative control has a weak strength.

Seven concentrations of tea tree essential oil were tested for antifungal effectiveness against *T. mentagrophytes*, together with a negative control using V-6 vegetable oil™ and a positive control using Sebazole®. Tea tree essential oil's antifungal effectiveness against *T. mentagrophytes* was demonstrated by measurements of the inhibitory zone over a 48-hour period, with varying results. Figure 2 displays the outcomes of the tea tree's antifungal test.

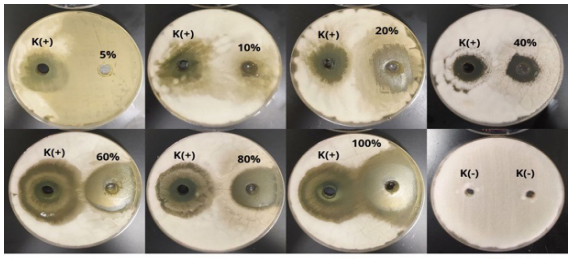

Figure 2. Antifungal test results of tea tree essential oil at 48 hours showed the activity of tea tree essential oil against *T. mentagrophytes* with various results

The results of the tea tree oil experiment on the growth of *T. mentagrophytes* after 48 hours are shown in Table 3, along with the mean inhibition zone diameter and the standard deviation. Davis and Stout (1971) are used to interpret the response to antifungal inhibition. Based on the results of testing the antifungal activity of tea tree essential oil on the growth of *T. mentagrophytes*, it can be concluded that at concentrations of 60, 80, and 100%, very strong activity was observed, while concentrations of 5, 10, 20, and 40% can be classified as strong. The inhibition zone produced by the positive control is regarded as a very strong category, with a decrease in average value when compared to the 100% concentration, whereas the inhibition zone produced by the negative control is interpreted as a weak category.

Tea tree essential oil was tested for antifungal efficacy against *T. mentagrophytes* at seven concentrations, as well as a negative control using V-6 vegetable oil™ and a positive control using Sebazole®. The findings of 72-hour inhibition zone measurements revealed that tea tree essential oil has antifungal action against *T. mentagrophytes* with variable outcomes. Figure 3 displays the outcomes of the tea tree essential oil's antifungal test after 72 hours.

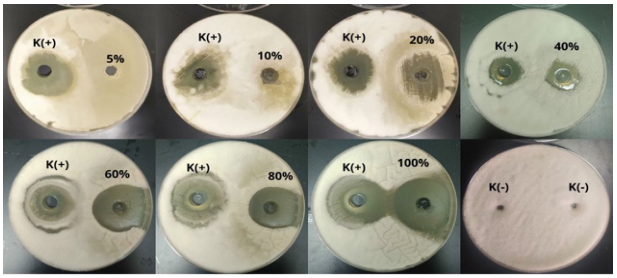

Figure 3. Antifungal test results of tea tree essential oil at 24 hours showed the activity of tea tree essential oil against *T. mentagrophytes* with various results

Table 4 summarizes the results of the data on the mean inhibition zone diameter and the standard deviation of tea tree essential oil testing on the growth of *T. mentagrophytes* after 72 hours. The response to antifungal inhibition is interpreted based on Davis and Stout (1971). Based on the results of testing the antifungal activity of tea tree essential oil on the growth of *T. mentagrophytes*,

Table 3. Mean diameter of the inhibition zone (mm) and standard deviation (mm) of tea tree essential oil on the growth of *T. mentagrophytes* at 48 hours

No	Tea Tree Essential Oil Concentration (%)	Mean Diameter of Inhibition Zone (mm) and Standard Deviation (mm) 48 hours of incubation	Interpretation
1	100	28,25 ± 5,13	Very strong
2	80	27,60 ± 1,29	Very strong
3	60	20,70 ± 2,43	Very strong
4	40	19,65 ± 2,99	Strong
5	20	19,79 ± 1,82	Strong
6	10	16,92 ± 3,84	Strong
7	5	16,80 ± 4,90	Strong
8	Positive Control	27,40 ± 1,99	Very strong
9	Negative Control	00,00 ± 00,00	Weak

Table 4. Mean diameter of the inhibition zone (mm) and standard deviation (mm) of tea tree essential oil on the growth of *T. mentagrophytes* at 72 hours

No	Tea Tree Essential Oil Concentration (%)	Mean Diameter of Inhibition Zone (mm) and Standard Deviation (mm) 72 hours of incubation	Interpretation
1	100	28,75 ± 6,46	Very strong
2	80	25,55 ± 0,60	Very strong
3	60	20,05 ± 2,76	Very strong
4	40	18,95 ± 2,45	Strong
5	20	18,15 ± 2,67	Strong
6	10	16,90 ± 3,21	Strong
7	5	14,70 ± 3,20	Strong
8	Positive Control	23,20 ± 0,65	Very strong
9	Negative Control	00,00 ± 00,00	Weak

it can be concluded that very strong activity was seen at concentrations of 60, 80, and 100%, while at concentrations of 5, 10, 20, and 40%, can be interpreted into the strong category. The inhibition zone produced by the positive control is interpreted as a very strong category, with a decrease in average value when compared to the 100% concentration, whereas the inhibition zone produced by the negative control has a weak strength.

The analysis of the antifungal activity test for various concentrations of tea tree essential oil at 24 and 48 hours was carried out using the One-Way ANOVA parametric test because the test variables were normally distributed. Analysis using the One-Way ANOVA test shows a significance number <0.05, indicating that there is a difference between the concentrations of the resulting inhibition zones. Analysis of the antifungal activity test for various concentrations of tea tree essential oil at 72 hours was carried out using the non-parametric Kruskal Wallis alternative test because the test variables were not normally distributed, therefore the One-Way ANOVA test requirements were not met. Analysis using the Kruskal Wallis method shows a significance number of <0.05, which means that there is a difference between the concentrations of the resulting inhibition zones.

The minimum effective concentration of tea tree essential oil was determined using a linear regression equation. The analysis using linear regression produces a graph, as shown in Figure 4 showing the relationship between the logarithmic value of the tea tree essential oil concentration (%) (x variable) and the inhibition zone (mm) at the 24th, 48th, and 72nd hour (y variable) is shown on Figure 3. The calculation results of the 24th hour (Figure 4a), give a value = 16 and b = 1.6429 with a coefficient of determination (R^2) = 0.9248.The calculation results of the 48th hour (Figure 4b), give a value = 13.571 and b = 2 with a coefficient of determination (R^2) = 0.8634 The calculation results of the 72nd hour (Figure 4c), give a = 11.714 and b = 2.2143 with a coefficient of determination (R^2) = 0.8931. Based on these result, he two variables are positively correlated and are in a very strong category, where the higher the concentration, the higher the inhibition of fungal growth. This is in accordance with the literature from Mulyono (2015), the value of R^2 > 0.75 – 0.99 indicates that the relationship between the two variables is very strong.

The best inhibition zone determination is measured by contact time. Contact time is the time required for a compound to remain wet on the surface and confirm its efficacy. The contact time between the fungus and tea tree essential oil is important to optimize adequate application (Wróblewska *et al*., 2021). Fungal growth can be inhibited after 24 hours of incubation time. However, the growth of *T. mentagrophytes* started to increase again after 48 and 72 hours, as indicated by a decrease in the inhibition zone. This study proves that tea tree essential oil can inhibit fungi at appropriate concentrations in a short period of time. The antifungal compounds contained in tea tree essential oil can work optimally in certain concentrations.

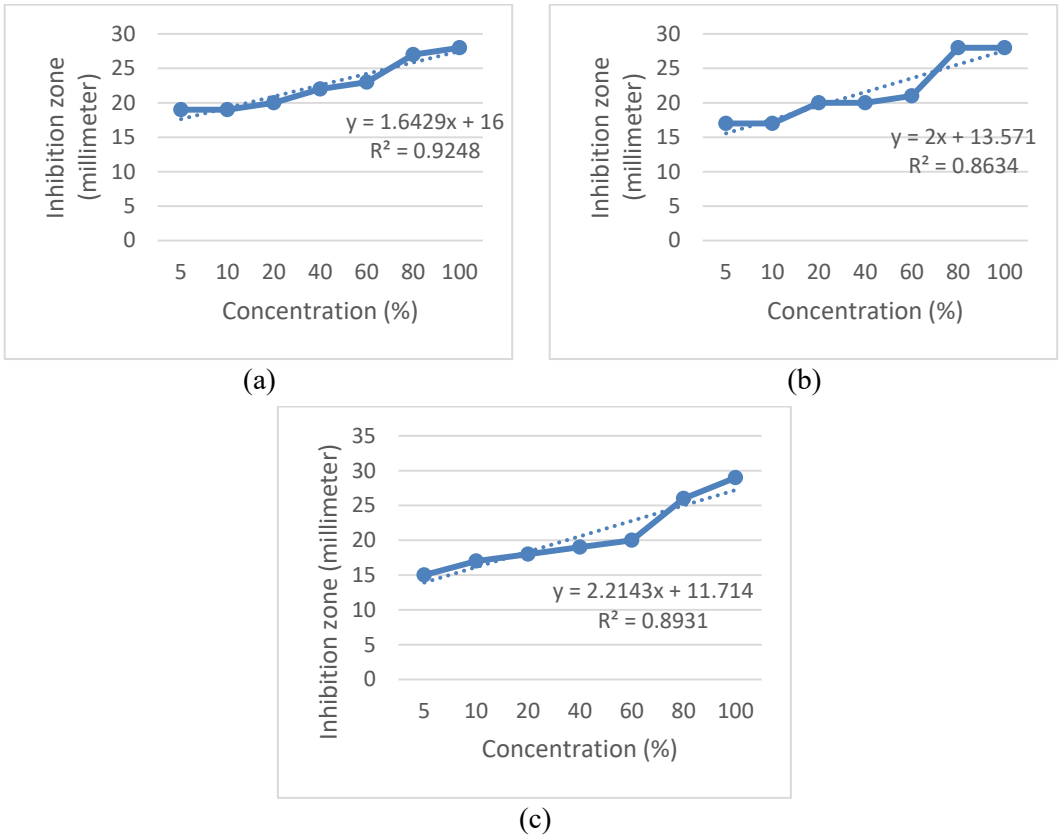

Figure 4. Correlation between the inhibition zone on *T. mentagrophytes* and the concentration of tea tree essential oil at 24 hours (a); 48 hours (b); and 72 hours (c)

According to Melinda *et al* (2019), the large difference in the diameter of the inhibition zone formed at each concentration can be caused by the level of concentration, the amount of active antimicrobial substance contained in tea tree essential oil, and the rate of diffusion of the antimicrobial substance. The diameter of the inhibition zone shows an increase as the concentration of the extract used increases, this can happen because the higher the concentration, the more receptors that work due to the entry of molecules, so that the resulting response increases (Rivera *et al*., 2013).

The average inhibition zone obtained at 72 hours of incubation (Table 4) showed a decrease in the diameter of the inhibition zone by tea tree essential oil against *T. mentagrophytes* when compared to the results of 48 hours of incubation (Table 3) and 24 hours (Table 2). The decrease in the inhibition zone diameter is due to the insufficient amount of antifungal compounds contained in tea tree essential oil, allowing the fungus to continue growing after incubation. Some factors that can affect antifungal activity include external and internal factors such as concentration of antifungal, type of antifungal, condition of the fungus, incubation temperature, contact time, potential of an antifungal substance in the solution tested, and sensitivity of a fungus to antifungal concentrations. There are chemical and physical properties that can also affect the ability of antifungal agents to inhibit fungal growth, such as pH, moisture content, nutrients, and the number of components in them (Fardiaz *et al*., 1992; Nazzaro *et al*., 2017).

The clear zone formed due to the active compound content in tea tree essential oil in the form of terpinen-4-ol (37.7%), which acts as the highest antimicrobial agent in inhibiting the growth of fungi, when compared to the content of tea tree essential oil such as α-terpinen (21.25%), γ-terpinen (10.5%), and 1,8-cineole (5.1%). Terpinen-4-ol and α-terpinen mechanism of action irreversibly damages the cell membrane structure. Damage to the cell membrane and increased membrane permeability result in cytoplasmic fluid leaving the cell. These contents can also damage the mesosoma, located on the folds of the

cell membrane and is involved in the respiratory system and energy production (Brilhante *et al*., 2016; Lestariningrum *et al*., 2011). The content of 1,8-cineole has marginal antifungal activity. However, these components are not primary antifungal components but can damage ion exchange systems in cells and provide a way for other primary components that are more active to invade cells (Carson *et al*., 2006; Lestariningrum *et al*., 2011).

Conclusion

Based on the results, we concluded that tea tree essential oil has in vitro antifungal potential against *T. mentagrophytes*. According to the statistical data analysis, the result shows a difference in the zone of inhibition between concentrations with a significance level of <0,05. The expansion in the diameter of the zone of inhibition is directly proportional to the increase in tea tree essential oil concentration. The results of the average zone of inhibition at an incubation time of 72 hours exhibit a decrease in the diameter of the zone of inhibition when compared to the results of 48-hour and 24-hour incubations.

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