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# Antifungal Activity Test of the Lavender Essential Oil (*Lavandula Angustifolia)* against *Trichophyton Mentagrophytes*

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**Abstract**

Dermatophytosis is a zoonotic skin disease generally found in pets and humans caused by fungal colonization of dermatophytes, such as *Trichophyton mentagrophytes*. Treatment for dermatophytosis is still a challenge due to the antifungal resistance and toxicities of antifungal drugs. Lavender essential oil (*Lavandula angustifolia*) contains active compounds such as *linalyl acetate*, *linalool*, *cineole*, *camphor*, and *lavandulyl acetate* which are the active chemicals that inhibit the growth of fungi. This research aims to determine antifungal activity test of lavender essential oil in inhibiting the growth of the fungus *T. mentagrophytes*. The antifungal activity of lavender essential oil was investigated using agar well diffusion method which utilizes *Sabouraud Dextrose Agar* (SDA) as a medium with a variety of lavender essential oil concentrations (5, 10, 20, 40, 60, 80, and 100%). The agar is incubated and the inhibition zone is measured every 24, 48, and 72 hours. The results were descriptively analyzed based on the inhibition zone formed around the agar well. The results show that lavender essential oil at the concentrations of 5, 10, 20, 40, 60, 80, and 100% were able to inhibit the growth of *T. mentagrophytes*. This result concludes that lavender essential oil at the concentration of 5% is considered as the effective concentration for dermatophytosis treatment because it has the least level of toxicity.

**Keywords:** antifungal activity test, dermatophytosis, lavender essential oil, *Trichophyton mentagrophytes*

## Introduction

Dermatophytosis is a skin ailment that commonly afflicts animals and poses a zoonotic threat to humans (Bond, 2010). This disease is caused by colonization of dermatophyte fungi that attack tissues containing keratin such as the stratum corneum of the skin, nails, and hair (Rosita & Kurniati, 2008). According to Bond (2010), afflicted animals will exhibit lesions that manifest as a combination of alopecia, papules, erythema, crusts, and scaling. Dermatophytosis symptoms in dogs and cats are characterized by baldness and hair loss that forms rings, hence the popular term "ringworm." Three genera, namely Epidermophyton, Trichophyton, and Microsporum, are responsible for causing dermatophytosis (Outerbridge, 2006).

*Trichophyton mentagrophytes* is a species of dermatophyte fungi that is widespread worldwide. This zoophilic species can infect humans and domestic animals such as cats, dogs, sheep, cows, horses, pigs, rodents, and monkeys. *T. mentagrophytes* can infect tissues such as hair, nails, or skin and subsequently develop into cylindrical lesions with smooth-walled macroconidia and characteristic microconidia.

Modern dermatophyte treatment has significantly developed, with cure rates reaching 80-90%. Effective treatment requires the regular use of drugs for the recommended period of time. The use of lavender essential oil is intended to provide a safer treatment alternative to the azole group. Essential oil is a natural extract derived from plants, particularly flowers, leaves, wood, seeds, or flower buds (Harahap et al., 2019).

Lavender essential oil has been reported to be active in fighting many species of fungi such as *Candida albicans*, *Aspergillus strains*, and *Cryptococcus neoformans*. The high content of 1,8-cineole, fenchone, and trans-α-necrodyl

acetate indicates antifungal activity against dermatophytes, yeast strains, and *Aspergillus*, with MIC ranging from 0.16 to 0.31 µL/mL. The content of carvacrol and (Z)-ocimene is effective in combating dermatophytes and *Cryptococcus neoformans*, with MIC and MLC values of 0.16 µL/mL and 0.32 µL/mL. The antifungal mechanism of lavender essential oil acts through the cytoplasmic cell membrane and causes disruption and ultimately cell death in fungal cells (Salehi et al., 2018). The purpose of this study was to determine the effectiveness of lavender essential oil against *Trichophyton mentagrophytes.*

## Material and Method

The research was conducted at the Prof. Soeparwi Animal Hospital and Pharmacology Laboratory, Department of Pharmacology, Faculty of Veterinary Medicine, Gadjah Mada University, this research used to investigate the antifungal inhibition properties of Young Living Essential Oil lavender against *T. mentagrophytes*. The study was carried out from August 2021 to February 2022. The lavender essential oil was diluted with V-6 vegetable oil™ to various concentrations of 5%, 10%, 20%, 40%, 60%, 80%, and 100%. Different dilution formulations were used to test lavender essential oil with varying active compound content. The efficacy of lavender essential oil was evaluated using well diffusion testing, and its activity was compared to oils with different profiles. Lavender essential oil was applied topically using Young Living Essential Oil® as a carrier oil (Buckle, 2003).

Table 1. Multilevel dilution formulation of Lavender essential oil

| Concentration | Lavender Essential Oil (µl) | Vegetable oil (µl) |
|---|---|---|
| 100% | 100 | - |
| 80% | 80 | 20 |
| 60% | 60 | 40 |
| 40% | 40 | 60 |
| 20% | 20 | 80 |
| 10% | 10 | 90 |
| 5% | 5 | 95 |

Note: Lavender essential oil is mixed with vegetable oil according to the ratio in Table1.

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

The testing was carried out using the agar well diffusion method in SDA medium. A cotton swab was dipped in *T. mentagrophytes* suspension and spread evenly onto Sabouraud Dextrose Agar (SDA) medium. This process was repeated twice, with the swab being rotated by 60º each time to ensure even distribution (Scorzoni et al., 2007). Two wells with a diameter of 0.9 mm were made in each plate using a 68-75 µl micropipette tip. Lavender essential oil was prepared at concentrations of 100, 80, 60, 40, 20, 10, and 5% and added to the wells using a 100 µl micropipette, alongside a positive control of Sebazole® shampoo and a negative control of vegetable oil. The positive and negative controls were also added to the wells in 100 µl quantities. The plates were then incubated at 27°C for 24, 48, and 72 hours in an incubation chamber (Tahir et al., 2016). The effectiveness of the antifungal activity of lavender essential oil was determined based on the clear zone (inhibition zone) that appeared around the wells, which was measured in millimeters using a vernier caliper every day for 3 days.

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

$$ROW = \frac{absolute\ organ\ wt.}{b.\ wt.\ of\ the\ animal\ on\ sacrifice\ day}\ x\ 100$$

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

The antifungal response inhibition zone diameter is classified into four categories based on its inhibitory activity. If the diameter of the inhibition zone is more than 20 mm, it is classified as very strong. If it is between 11-20 mm, it is classified as strong. If the diameter is between 5-10 mm, it is classified as medium, and if it is less than 5 mm, it is classified as weak (Davis and Stour, 1971).

The data was analyzed using statistical methods. One-Way Analysis of Variance (ANOVA) was used for normally distributed data, while the non-parametric Kruskal Wallis test was used for non-normally distributed data. The aim was to determine if there was a significant impact of administering different concentrations of lavender essential oil on the diameter of the inhibition zone against *T. mentagrophytes*.

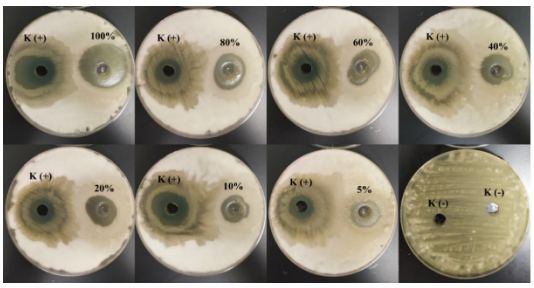
## Result and Discussion

Based on the results of the study, lavender essential oil tested in vitro has been shown to have the ability to inhibit the growth of *T. mentagrophytes* fungus. This is evidenced by the formation of inhibition zones around wells that have been treated with lavender essential oil at various concentrations, as shown in Table 2.

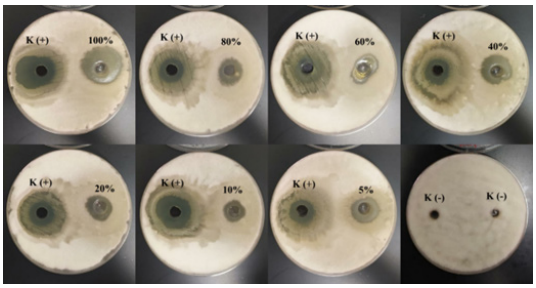
Table 2. Mean diameter of the inhibition zone (mm) and standard deviation (mm) of lavender essential oil on the growth of *T. mentagrophytes*

[1], A

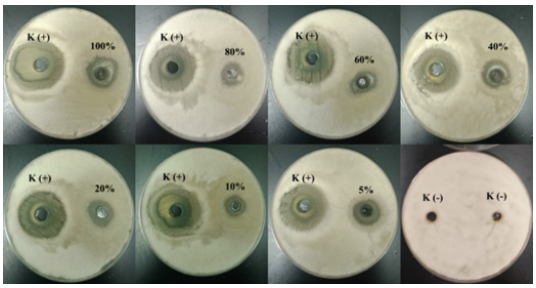
| Mean and Standard Deviation of Inhibition Zone of Lavender Essential Oil (mm) | | | | |
|---|---|---|---|---|
| Treatment | Day 1 | Day 2 | Day 3 | Mean |
| Control (+) | 29,25 ± 2,78 | 27,40 ± 1,99 | 23,20 ± 0,65 | 26,62 ± 3,21 |
| 100% | 27,90 ± 2,48 | 25,40 ± 3,38 | 22,65 ± 3,67 | 25,32 ± 3,71 |
| 80% | 24,50 ± 2,77 | 22,25 ± 1,59 | 20,40 ± 2,41 | 22,38 ± 2,75 |
| 60% | 22,95 ± 1,55 | 19,75 ± 1,92 | 18,90 ± 1,77 | 20,53 ± 2,43 |
| 40% | 22,35 ± 1,93 | 19,30 ± 4,43 | 17,75 ± 3,18 | 19,8 ± 3,67 |
| 20% | 19,15 ± 3,10 | 14,60 ± 2,30 | 12,48 ± 5,29 | 15,41 ± 4,53 |
| 10% | 18,3 ± 1,85 | 14,20 ± 2,15 | 12,40 ± 2,04 | 14,97 ± 3,16 |
| 5% | 15,70 ± 2,18 | 13,75 ± 1,72 | 12,10 ± 1,59 | 13,85 ± 2,29 |
| Control (-) | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |




(a)




(b)




(c)

Figure 1. (a) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the first day on Sabouraud Dextrose Agar (SDA) media. (b) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the second day on Sabouraud Dextrose Agar (SDA) media. (c) Inhibition zone formed by the antifungal activity of lavender essential oil against *T. mentagrophytes* on the third day on Sabouraud Dextrose Agar (SDA) media. K (+) = positive control; 100% = 100% concentration; 80% = 80% concentration; 60% = 60% concentration; 40% = 40% concentration; 20% = 20% concentration; 10% = 10% concentration; 5% = 5% concentration; K (-) = negative control.

The inhibition zones that appeared on Sabouraud Dextrose Agar (SDA) media due to the antifungal activity of lavender essential oil against *T. mentagrophytes* on the first, second, and third day can be seen in Figure 1.

Table 2 displays the mean results obtained from the measurement of the inhibitory zone of lavender essential oil on the growth of *T. mentagrophytes*. The table demonstrates that no inhibitory zone was formed in the negative control, whereas the positive control produced the largest mean diameter of the inhibitory zone. The lavender essential oil at different concentrations exhibited varying mean diameter of the inhibitory zone. The mean inhibitory zone diameters of lavender essential oil at concentrations of 100, 80, 60, 40, 20, 10, and 5% were 25.32 ± 3.71; 22.38 ± 2.75; 20.53 ± 2.43; 19.8 ± 3.67; 15.41 ± 4.53; 14.97 ± 3.16; and 13.85 ± 2.29 mm, respectively, while that of the positive control was 26.62 ± 3.10 mm. The results of the mean data indicate that lavender essential oil possesses antifungal activity against *T. mentagrophytes* and continues to retain its capability to inhibit the growth of *T. mentagrophytes* at a concentration of 5%.

The correlation results between the inhibitory zone against *T. mentagrophytes* fungi and the concentration of lavender essential oil at 5, 10, 20, 40, 60, 80, and 100% on the first day, second day, and third day can be seen in Figure 2.

Figure 2 depicts the antifungal activity of lavender essential oil against *T. mentagrophytes* at various concentrations. The figure illustrates differences in antifungal activity across several variations of lavender essential oil concentrations, namely 5%, 10%, 20%, 40%, 60%, 80%, and 100%, as well as positive and negative controls. The positive control exhibited an average inhibition zone of 29.25 mm on the first day, 27.40 mm on the second day, and 23.30 mm on the third day, while the negative control showed an average inhibition zone of 0 mm. As shown in Figure 2, the average inhibition zone increased as the concentration of lavender essential oil increased, with respective values on the first day of 15.70 mm, 18.30 mm, 19.15 mm, 22.35 mm, 22.95 mm, 24.50 mm, and 27.90 mm for 5%, 10%, 20%, 40%, 60%, 80%, and 100% concentrations, respectively. On the second day, the respective values were 13.75 mm, 14.20 mm, 14.60 mm, 19.30 mm, 19.75 mm, 22.25 mm, and 25.40 mm, while on the third day, they were 12.1 mm, 12.4 mm, 12.48 mm, 17.75 mm, 18.9 mm, 20.40 mm, and 22.65 mm.

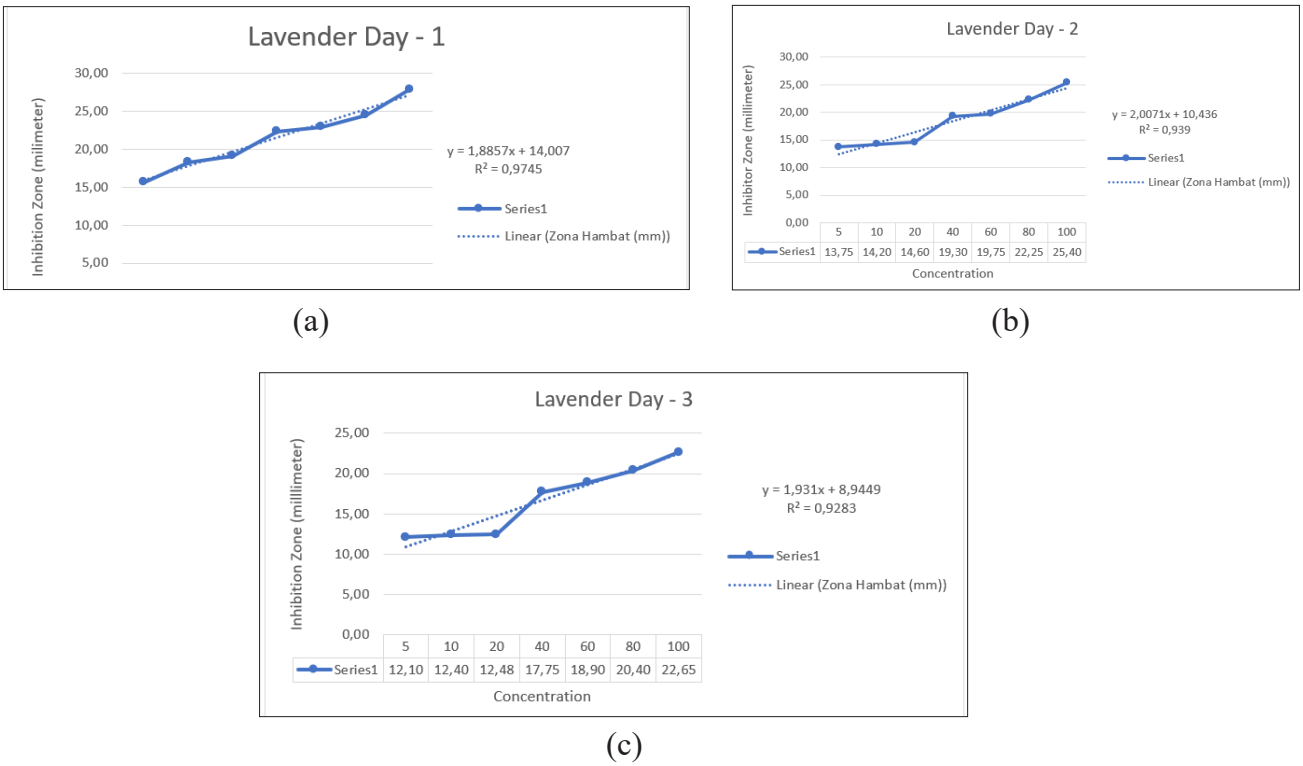



Figure 2. Correlation between the inhibition zone on *T. mentagrophytes* and the concentration of lavender essential oil at 24 hours (a); 48 hours (b); and 72 hours (c)

**Table 3.** Behavioral and physical observations in mice given daily oral doses (28 days) of Euphorbia hirta ethanolic leaf extract.

| Observations | Control, n=5 | *Euphorbia hirta* leaf extract | |
|---|---|---|---|
| | | 500 mg/kg, n=5 | 1000 mg/kg, n=5 |
| Skin color | n | n | n |
| Piloerection | - | - | - |
| Alopecia | - | - | - |
| Tachypnea | - | - | - |
| Stool | n | w, 3/5 | w, 3/5 |
| Lacrimation | - | - | +, 3/5 |
| Retching | - | - | - |
| Corneal Opacity | - | - | +, 3/5 |
| Vocalization | - | - | - |
| M. membrane | n | n | n |
| Palpebral opening | n | n | n |
| Exophthalmos | - | - | - |
| Tremor | - | - | - |
| Staggering gait | - | - | - |
| Grip | n | N | n |
| Opisthotonus | - | - | - |
| Alertness | n | N | n |
| Touch response | n | n | n |
| Morbidity | - | - | - |
| Mortality | - | - | - |

*Legend:* (n) normal; (-) absent; (+) present, (w) watery

[1], A

Figure 2 shows the relationship between x variable, which is the logarithm of lavender essential oil concentration (%), and y variable, which is the inhibition zone (mm). The calculation results on the first day yielded values of a = 14.007 and b = 1.8857 with an $R^2$ value of 0.9745, indicating a strong positive correlation between the two variables. On the second day, the calculation resulted in values of a = 10.436 and b = 2.0071 with an $R^2$ value of 0.939, indicating a strong positive correlation between the two variables. The calculation on the third day yielded values of a = 8.9449 and b = 1.931 with an $R^2$ value of 0.9283, indicating a strong positive correlation between the two variables. The inhibitory effect of lavender essential oil on the growth of *T. mentagrophytes* increases with increasing concentration of lavender essential oil, in accordance with Sugiyono (2012) statement that an R2 value of > 0.80 - 1.000 indicates a very strong relationship between the two variables.

The analysis of the antifungal activity test at various concentrations of essential oils on the first day was conducted using Kruskal-Wallis

**Table 4**. Body weights of mice given daily oral doses of Euphorbia hirta ethanolic leaf extract for 28 days.

| Treatment | Mean Body Weight (grams ± SD) | | | | | % Difference in weight |
|---|---|---|---|---|---|---|
| | D0 | D7 | D14 | D21 | D28 | |
| Control | 21.82 ±4.03 | 23.91 ± 2.72 | 24.88 ± 2.32 | 25.94 ± 2.42 | 26.16 ± 1.73 | +19.89 |
| 500 mg/kg | 20.91 ±1.63 | 20.85 ± 1.96 | 20.22 ± 1.62 | 19.96 ± 1.65 | 20.44 ± 2.39 | -2.25 |
| 1000 mg/kg | 22.28 ± 2.59 | 21.47 ± 2.03 | 19.38 ± 4.73 | 20.47 ± 3.32 | 20.17 ± 3.55 | -9.47 |
| *p*-values, ns | 0.94 | 0.42 | 0.69 | 0.52 | 0.26 | |

*Legend:* Values are expressed as mean ± SD; mean values within a column are not significantly different; ns – not significant, p>0.0.5

statistical test and One Way ANOVA test. The result of Kruskal-Wallis statistical test on the first day showed a significance value of 0.000, where the significance value is smaller compared to p (0.05), and the result of One Way ANOVA statistical test on the second and third days obtained a significance value of 0.000, where the significance value is smaller compared to p (0.05). Thus, the interpretation is that H0 is rejected and H1 is accepted, so it can be concluded that there is a significant difference in the antifungal potential of lavender essential oil at each concentration given.

The linear data shows a difference in the ability to inhibit the growth of fungi at each concentration. There is a decrease in the diameter of the inhibition zone from the lowest concentration to the highest concentration. The difference in the diameter of the inhibition zone can be caused by different active compound contents in each concentration.

According to Pedraza Chaverri et al. (2008), higher concentrations of lavender essential oil will result in larger zones of inhibition. When a solution has a high concentration, it will also contain higher levels of active ingredients. The numerous active compounds found in lavender essential oil increase its antifungal potential, as evidenced by the larger diameter of the zones of inhibition. The active compounds in lavender essential oil with antifungal potential are linalyl acetate, linalool, cineole, camphor, and lavandulyl acetate (Erland & Mahmoud, 2016). Linalyl acetate actively inhibits microorganism growth and inhibits the extension of fungal hyphae, while linalool has potent fungicidal activity. Linalool inhibits fungal growth by modulating the mevalonate pathway, altering intermediary molecules at the cellular level in eukaryotic cells, disrupting the cell membrane, and modulating functions related to cell permeability and signaling by creating deformities and holes in the cell wall (D'Auria et al., 2005; Rehab & Zeinab, 2016; Salehi et al., 2018).

Cineole in lavender essential oil has strong fungicidal properties. According to D'Auria et al. (2005), cineole has higher antifungal activity than camphor. Cineole works by altering the properties of fungal cell walls and triggering compensatory transcriptional responses to cell wall damage, thereby inhibiting fungal cell survival. Although cineole and linalool are minor components of lavender essential oil, they can synergize to inhibit microbe growth, according to Burt (2004).

The topical use of lavender essential oil requires knowledge of its properties and how to use it, especially its dosage and toxicity level. Lavender essential oil used topically affects the central nervous system and lymphatic circulation system as soon as it enters the dermis layer and circulates to every cell in the body (Koensoemardiyah, 2010). The effects of topical lavender essential oil exposure on cats may include hypersalivation, depression, lethargy, ataxia, weakness, tremors, muscle fasciculations, paresis, and abdominal discomfort (Bates, 2018).

Based on the data analysis, lavender essential oil with a concentration of 5% has effective antifungal properties against *T. mentagrophytes* and the lowest toxicity level, making it a safe and economical herbal remedy for treating dermatophytosis fungal infections such as *T. mentagrophytes*.

## Conclusion

The research has demonstrated that lavender essential oil exhibits antifungal properties against *T. mentagrophytes* in vitro. We conducted statistical analysis which revealed a noteworthy distinction in the size of the inhibition zone between various concentrations of lavender essential oil, with a significance level of less than 0.05. Additionally, we observed a proportional increase in the diameter of the inhibition zone as the concentration of lavender essential oil increased. However, it should be noted that the diameter of the inhibition zone decreased after a 72-hour incubation period compared to the results obtained after 48 hours and 24 hours of incubation.

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