

Research Article

***Antifungal Effectiveness of Ultrasonic-Assisted Extraction (UAE)-
Extracted Green Tea Leaf (Camellia sinensis) against Malassezia furfur
In Vitro***

Efektivitas Antifungi Ekstrak Daun Teh Hijau (*Camellia sinensis*) dengan Metode
Ultrasonic Assisted Extraction terhadap *Malassezia furfur* secara *In Vitro*

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Abstract

Fungal infections in Indonesia affect 2.89% of the population and have the potential to develop resistance to azole-class antifungals, as seen in Pityriasis versicolor caused by *Malassezia furfur*. Green tea leaf (*Camellia sinensis*) extract offers a safer antifungal alternative containing tannins, flavonoids, alkaloids, and triterpenoids. This experimental study evaluated *C. sinensis* extract effectiveness against *M. furfur* in vitro using a post-test only control group design. Extracts were prepared via Ultrasonic Assisted Extraction (UAE) method at 10%, 15%, 20%, and 25%, with sterile aquadest and 2% ketokonazol as controls. Antifungal activity was assessed using the well diffusion method on Sabouraud Dextrose Agar (SDA) by measuring the inhibition zone after 24 and 48 hours of incubation. Inhibition was generally moderate at 24 hours but weakened at 48 hours, except at 20% and 25% concentrations. Bivariate analysis of 24-hour data using the Kruskal-Wallis's test showed a significant difference in the inhibition of *M. furfur* among green tea leaf extract concentrations ($p < 0.05$), with higher concentrations associated with greater inhibition. Based on post-Hoc analysis, it was found that the 20% concentration was the most effective concentration for inhibiting *M. furfur*.

Keywords: antifungal; green tea leaf extract; *Malassezia furfur*; pityriasis versicolor; ultrasonic assisted extraction

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Introduction

Indonesia is a tropical country with hot and humid temperatures. These conditions support the growth of fungi, which can lead to a relatively high incidence of superficial dermatophytosis due to fungal infections in Indonesia.¹ Overall, around 7.7 million Indonesians or 2.89 percent of the total population, experience fungal infections each year.² Approximately 20–25% of people worldwide experience superficial dermatomycosis or diseases caused by superficial fungal infections of the skin.¹ A common type of dermatomycosis is tinea versicolor.³

Pityriasis versicolor (PV) or tinea versicolor is a chronic superficial skin fungal infection characterized by changes in skin pigmentation and is usually mild and asymptomatic.⁴ PV is a fungal infection caused by the *Malassezia* fungus, which consists of 19 species, including *Malassezia furfur* (*M. furfur*). The prevalence of PV reaches up to 50% in tropical regions, while it is estimated to be around 1–4% in temperate climates and 1% in cold climates.⁵ Many PV patients do not seek medical treatment, making it difficult to determine the exact number of PV cases in Indonesia. The number of PV cases ranges from 8.8% to 38.2%, according to data collected from various teaching hospitals.⁶ Several studies report that 13.33% of construction workers in Sukatani, Depok, are infected with *Malassezia* sp. In the Ciputat area, PV was found in 54.8% of teenagers aged 16–20 years. In Tomang, West Jakarta, 4.8% of productive-age men were infected, while in Pondok Pesantren Cibinong, Bogor, the infection was found in 51.6% of students.⁷

The current management of *Malassezia* infections involves the use of systemic or topical antifungal agents. Side effects such as liver dysfunction, anaphylactic reactions, nausea, vomiting, and headaches can occur during the use of antifungal chemicals.⁸ The increased use of antifungal drugs to treat fungal infections can raise the risk of resistance, making the use of herbal medicines considered safer compared to synthetic drugs.⁹

Green tea leaves are one of the many herbal medicinal plants found in Indonesia.¹⁰ Based on data from the 2023 Indonesian Tea Statistics publication by the Central Bureau

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of Statistics (BPS), tea production in Indonesia in 2023 reached 116.51 thousand tons, with 64.98% of the total coming from West Java.¹¹ Green tea can be consumed as a beverage or food and as an active packaging material that can extend the shelf life of fresh meat.¹² Based on the processing method, tea is classified into several types, including green tea, dark tea, white tea, oolong tea, yellow tea, and black tea.¹³ These various types of tea differ based on the presence or absence of enzymatic fermentation and oxidation of tea polyphenols during processing, which affects the compound content in the tea leaves. Green tea is produced with little or no enzymatic oxidation, allowing most of its polyphenols to remain unoxidized. In contrast, other types of tea undergo varying degrees of enzymatic oxidation during processing (commonly referred to as fermentation), resulting in changes to their polyphenol composition.¹²

Several previous studies have proven that green tea leaf extract (*Camellia sinensis*) has antifungal activity against various types of fungi. Selviani et al.'s research¹⁴ found that green tea leaf extract at a concentration of 20% is very effective in inhibiting the growth of *Candida albicans*. Lestari's research¹⁵ reported that green tea leaf extract at a concentration of 2.5 mg/ml is effective in inhibiting *Aspergillus flavus*. Meanwhile, Paradise et al.'s research¹⁶ reported that green tea leaf extract at a concentration of 25–75% can inhibit *Candida albicans* on the first day, however, its effectiveness decreases on the second day. Until now, no research has specifically assessed the effectiveness of green tea leaf extract against the growth of *M. furfur*.

Green tea leaves contain flavonoids, tannins, alkaloids, and terpenoids that act as antifungal compounds.¹⁷ In the field of health, tea leaves can be utilized either directly or through a more complex extraction process. This extraction serves to enhance the effectiveness of the active compounds found in green tea.¹⁸ Traditional extraction methods such as Soxhlet have many drawbacks, such as high energy consumption, the use of large amounts of solvents, and the risk of damaging thermosensitive compounds. Therefore, the Ultrasound-Assisted Extraction (UAE) method was chosen because it can enhance extraction efficiency, maintain the biological activity of the compounds, and support the principles of green extraction that are more environmentally friendly.¹⁹

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Based on this, this study aims to determine the effectiveness of green tea leaf extract (*Camellia sinensis*) using the UAE method against the inhibition of *M. furfur* growth in vitro.

Methods

The research design used in this experimental study is a post-test-only control group. The research was conducted at the Parasitology Laboratory, Faculty of Medicine, Universitas Pembangunan Nasional "Veteran" Jakarta, located on Jalan RS. Fatmawati Raya, Pondok Labu, Cilandak District, Depok City, West Java. The research took place from August to November 2025.

In this design, the research subjects are divided into two main groups, namely the treatment group that receives the extract and the control group. The *C. sinensis* extract used has concentrations of 10%, 15%, 20%, and 25%. The control group consists of a positive control given 2% ketoconazole and a negative control given aquadest. The positive control is made from 0.2 grams of ketoconazole dissolved in 10 ml of sterile aquadest, resulting in a 2% ketoconazole solution. The negative control consists of 10 ml of aquadest.

The antifungal activity test was conducted using the well diffusion method. Suspension of *M. furfur* adjusted to McFarland standard 0.5 was inoculated onto SDA media. Each well was filled with 100 μ L of the test solution according to the treatment group, then incubated at room temperature. The growth of the fungus was evaluated post-treatment by observing the clear zone as an indicator of fungal growth inhibition around the well area using a calliper at 24 and 48 hours of incubation. The data analysis used for the 24-hour observation results used the Kruskal-Wallis test with the Post Hoc Mann-Whitney test, while the 48-hour results used the One-Way ANOVA test with the Post Hoc Bonferroni test. The statistical significance was set at $p < 0.05$.

Results

Phytochemical tests were conducted to detect the active compound content in green tea leaf extracts. The active compounds found in green tea leaves include alkaloids, saponins, flavonoids, tannins, and triterpenoids. The most dominant active compounds

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found were alkaloids and tannins, while the lowest active compound detected in the test results was triterpenoids (Table 1).

The diameter of the inhibition zone is measured using a calliper. The inhibition zone is measured based on the clear area formed around the well. Measurements were taken at 24 hours and 48 hours after the application of green tea leaf extract on the agar medium (Table 2 dan 3). The addition of extracts with varying concentrations included 10%, 15%, 20%, and 25% on *M. furfur* cultures, yielding research results presented in Figure 1 and 2. Inhibition zones were observed at each concentration and the positive control with ketoconazole.

The largest inhibition zone diameter at 48 hours post-treatment was 5.55 mm at 25%, and the smallest was 3.825 mm at 10% (Table 3). Additionally, no clear zones were observed in the negative control with aquadest, while a clear zone with an average diameter of 17.23 mm was found in the positive control with ketoconazole.

Table 1 Phytochemical Test of Green Tea Leaf Extract (*Camellia sinensis*)

Active Compounds	Result
Alkaloid	+++++
Saponin	++++
Flavonoid	+++
Tanin	+++++
Triterpenoid	+

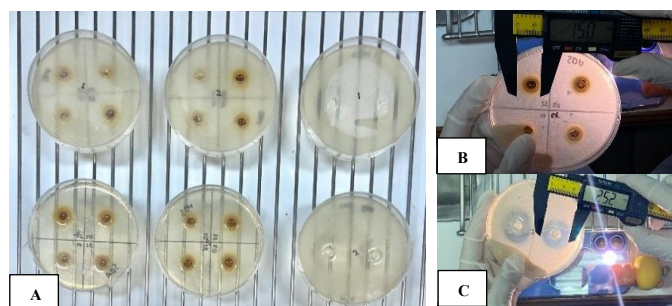


Figure 1 Diameter of the Inhibition Zone at 24 Hours

A) Preparation with concentrations of 10%, 15%, 20%, 25%, and positive control, each with 4 repetitions after being incubated for 24 hours. B) Shows the diameter of the well area and the clear area formed, measured using a caliper at a concentration of 25% after 24 hours. C) The diameter of the well and the clear area formed in the positive control after 24 hours.

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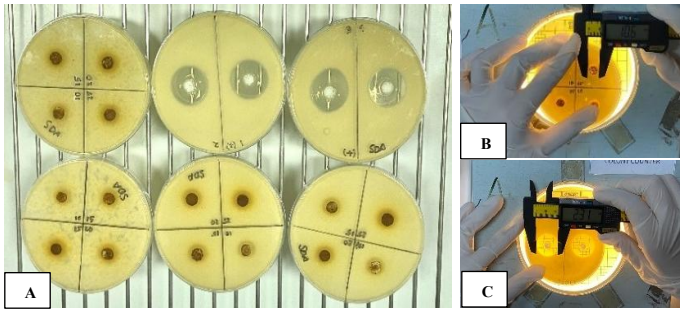


Figure 2 Diameter of the Inhibition Zone at 48 Hours

A) Preparation with concentrations of 10%, 15%, 20%, 25%, and a positive control, each with 4 repetitions after being incubated for 48 hours. B) Shows the total diameter of the wells and the clear area formed, measured using a caliper at a concentration of 10% after 48 hours. C) The Diameter of the wells and the clear area formed in the positive control after 48 hours.

Based on the graph in Figure 3, the effectiveness of green tea leaf extract against *M. furfur* culture increases proportionally with the increase in concentration. Based on the average inhibition zone diameter values at various concentrations, the 25% concentration showed the highest results in inhibiting *M. furfur*, with an inhibition diameter reaching 9.3 mm at 24 hours post-treatment. After 48 hours of treatment, a decrease in the diameter of the inhibition zone was observed at all concentrations (10%, 15%, 20%, and 25%).

Table 2 Diameter of the Treatment Inhibition Zone 24 Hours

Repetition	Inhibition Diameters (mm)					
	10%	15%	20%	25%	Positive Control	Negative Control
1	5.6	10	10.4	7.8	19.1	0
2	6.1	7.1	8.5	9	19.3	0
3	8.1	7	8.3	10.8	19.4	0
4	5.9	7.2	9	9.6	19.2	0
Average	6.425	7.825	9.05	9.30	19.25	0

Table 3 Diameter of the Treatment Inhibition Zone 48 Hours

Repetition	Inhibition Diameters (mm)					
	10%	15%	20%	25%	Positive Control	Negative Control
1	3.8	4.3	4.5	4.9	17.2	0
2	3.6	4.6	5.5	6	17.1	0
3	3.8	5.3	5.4	5.8	17.3	0
4	4.1	4.3	5.2	5.5	17.3	0
Average	3.825	4.625	5.15	5.55	17.225	0

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Based on the Post Hoc test conducted on the 24-hour data (Table 4), a p-value < 0.05 was obtained. This implies that there are significant differences between the treatment groups. However, there were no significant differences in the concentrations of 10% with 15%, 15% with 20%, 15% with 25%, and 20% with 25%, which had a p-value >0.05.

In the Post Hoc test for the 48-hour data (Table 5), a p-value of <0.05 was obtained. This implies that there are significant differences between the treatment groups. However, there is no significant difference at concentrations of 10% with 15%, 15% with 20%, and 20% with 25%, which have a p-value >0.05.

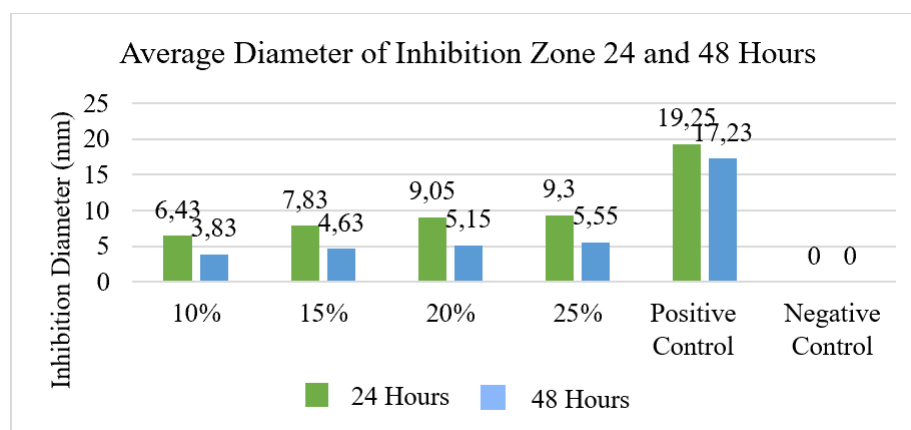


Figure 3 Average Diameter of Inhibition Zone of Green Tea Leaf Extract (*C. sinensis*) at 48 Hours against *M. furfur*

Table 4 Post Hoc Mann Whitney at 24 Hours

Treatment Group	10%	15%	20%	25%	Positive Control	Negative Control
10%	-	0.149*	0.021	0.043	0.021	0.014
15%	0.149*	-	0.149*	0.149*	0.021	0.014
20%	0.021	0.149*	-	0.663*	0.021	0.014
25%	0.043	0.149*	0.663*	-	0.021	0.014
Positive Control	0.021	0.021	0.021	0.021	-	0.014
Negative Control	0.014	0.014	0.014	0.014	0.014	-

* No significant differences

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Table 5 Post Hoc Bonferroni at 48 Hours

Treatment Group	10%	15%	20%	25%	Positive Control	Negative Control
10%	-	0.060*	0.001	0.000	0.000	0.000
15%	0.060*	-	0.665*	0.019	0.000	0.000
20%	0.001	0.665*	-	1.000*	0.000	0.000
25%	0.000	0.019	1.000*	-	0.000	0.000
Positive Control	0.000	0.000	0.000	0.000	-	0.000
Negative Control	0.000	0.000	0.000	0.000	0.000	-

* No significant differences

Discussion

Based on the research results, green tea leaf extract shows effective antifungal activity against the fungus *M. furfur*. Evidence of this activity is shown by the presence of a zone around the wells in the *M. furfur* culture media that have been treated with the treatment groups. Observation of this inhibition zone was conducted at two time periods, namely 24 hours and 48 hours post-treatment. The increase in the concentration of green tea leaf extract has been proven to enlarge the diameter of the inhibition zone. This may be attributed to the higher concentration of secondary metabolites, which contributes to a larger inhibition zone diameter.²⁰ These findings are consistent with previous studies on other fungal species. Previous research has demonstrated that green tea leaf extract also exhibits inhibitory activity against *Candida albicans*, with the antifungal effect increasing in a concentration-dependent manner.¹⁴ The Keben (*Barringtonia asiatica* L.) seed extract with three concentration levels of 5%, 10%, and 15% showed higher antifungal activity against *M. furfur* at a concentration of 15%.⁹ The same pattern was also observed in the tamarind seed extract (*Tamarindus indica* Linn.), which indicated that as the concentration increased to 5%, 10%, 15%, and 20%, the inhibition diameter also became larger.²⁰

In the observations conducted, it was also seen that the diameter of the inhibition zone decreased from the 24-hour to the 48-hour observation, reflecting that the effectiveness of the green tea leaf extract decreased over time. This explains that the bioactive components found in green tea leaf extract have fungistatic properties, not fungicidal. Fungistatic compounds can only inhibit fungal growth, whereas fungicidal compounds can cause fungal death.²¹ This finding is consistent with the research by Paradise et al.¹⁶ which showed that green tea leaf extract exhibited a decrease in the

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diameter of the inhibition zone on the second day compared to the first day in suppressing the growth of *C. albicans*.

The inhibition zone in mm is the clear/translucent area around the well. The inhibition zone is then categorized based on antifungal activity according to the Davis and Stout criteria.¹⁴ These criteria have four categories of antifungal activity based on the clear/translucent area around the well. Categorized as weak if the diameter is <5 mm, moderate if the diameter is 5-10 mm, strong if 11-20 mm, and very strong if the diameter is >20 mm.¹⁴ The antifungal activity categories of green tea leaf extract (*C. sinensis*) against the fungus *M. furfur* based on the Davis and Stout criteria are presented in Table 6.

Based on that classification, the inhibition zones produced by the concentrations of green tea leaf extract (10%, 15%, 20%, and 25%) at the 24-hour measurement fall into the moderate inhibition category (5-10 mm). Meanwhile, the negative control showed no inhibition area. At the 48-hour measurement, the 10% and 15% concentrations dropped to the weak inhibition category (<5 mm), while the 20% and 25% concentrations remained in the moderate category. Meanwhile, the positive control (2% ketoconazole) showed a strong inhibition zone (>10 mm), indicating effectiveness that surpassed all concentrations of green tea leaf extract, both at the 24-hour and 48-hour measurements.

The ability of green tea leaf extract (*C. sinensis*) to inhibit the growth rate of the fungus *M. furfur* is based on the secondary metabolites contained in the extract. The results of the phytochemical screening confirmed the presence of five main compounds in the green tea leaf extract, including saponins, alkaloids, tannins, flavonoids, and steroids. Tannins and alkaloids are the compounds with the highest concentrations, while triterpenoids are the compounds with the lowest concentration in the green tea leaf extract. However, triterpenoids also contribute to antifungal activity.

Tannins act as antifungals by inhibiting the formation of fungal cell wall layers.²² Alkaloids can cause damage to the components that make up the cell's peptidoglycan.²³ Saponins function by reducing the surface tension of the cell membrane, which in turn increases the permeability of the membrane. Flavonoids inhibit fungal cell division by penetrating the fungal cell wall.²³ Triterpenoids could inhibit the activity of the enzyme glucan synthase, which is a component of the fungal cell wall.²⁴

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Table 6 Categories of Antifungal Ability of Green Tea Leaf Extract (*C. sinensis*) against *M. furfur*

Concentration	Average Inhibition Diameter in 24 Hours	Explanation	Average Inhibition Diameter in 48 Hours	Explanation
10%	6.425	Moderate	3.825	Weak
15%	7.825	Moderate	4.625	Weak
20%	9.05	Moderate	5.15	Moderate
25%	9.30	Moderate	5.55	Moderate
Positive Control	19.25	Strong	17.225	Strong
Negative Control	0	-	0	-

Statistical tests show significant differences between treatment groups. The significant differences obtained at each concentration indicate that the increase in concentration results in a significant difference in effectiveness in inhibiting *M. furfur*. In the Post Hoc follow-up test on the 24 and 48-hour data, the 25% concentration, which produced the largest inhibition zone diameter, did not show a significant difference compared to the 20% concentration. The analysis showed that the 20% concentration did not differ significantly from the 15% concentration. Although increasing the concentration to 25% did not produce a further significant improvement, the 20% concentration was considered the optimal concentration because it demonstrated a statistically significant difference compared with the 10% concentration.

Based on the research by Selviani et al.¹⁴, the extract of green tea leaves (*C. sinensis*) in inhibiting the growth of *C. albicans* was tested at concentrations of 10%, 15%, and 20%. The results showed that the 20% concentration provided the most effective inhibition, with an average inhibition zone diameter of 18.84 mm. This indicates that a higher concentration is likely needed to inhibit *M. furfur* compared to *C. albicans*. This is supported by the findings of Ratte et al.²⁵ which indicate that the ethanol extract of *Hibiscus rosa-sinensis* L., rich in alkaloids, flavonoids, phenols, and tannins, can inhibit the growth of *C. albicans*. However, a similar effectiveness is not observed on *M. furfur*. The absence of antifungal activity is due to obstacles in the mechanism of compound attachment to the fungal cell surface or in the mechanism of active compound diffusion into the fungal cell cytoplasm. Effectiveness also depends on other parameters, such as the concentration level, environmental conditions (temperature and pH), microbial density, the potential of an extract in culture, and the sensitivity response of the test

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microbes themselves.²⁶ The thickness of the cell wall of *M. furfur* is one of its distinctive features, representing about 26-37% of the cell volume and mostly consisting of glucan (70%), protein (10%), and lipid (15-20%).²⁷

Based on the Davis and Stout zone inhibition classification and the results of significant difference analysis through the Post Hoc test, it can be concluded that a 20% concentration is the optimum concentration of green tea leaf extract in inhibiting the growth of *M. furfur*. In the 24-hour observation, this concentration produced an average inhibition zone diameter of 9.05 mm, which falls into the moderate inhibition category. Suggestions for future research include testing the effectiveness of green tea leaf extract (*C. sinensis*) on other types of fungi to expand its applications and conducting tests using specific metabolite compounds to achieve the best effectiveness in inhibiting the growth of *M. furfur*.

Conclusion

The results of this study indicate that green tea leaf extract (*Camellia sinensis*) effectively inhibits the growth of *Malassezia furfur*. The increase in the diameter of the clear zone was observed to be in line with the increase in concentration, although no statistically significant differences were found across all variations. A 20% concentration proved to be the most effective, with the largest average inhibition zone at 24 hours, measuring 9.05 mm.

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