Exploration of Antifungal Tests Based on Δ-Guaiene From Subang Patchouli Oil

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Abstract.

Guaiene is a derivative compound produced as a byproduct in the production of patchouli alcohol from patchouli oil (Pogostemon cablin Benth.). This study aims to evaluate the antifungal activity of δ-guaiene extracted from patchouli oil originating from Subang against four types of skin pathogenic fungi: Aspergillus niger, Candida albicans, Microsporum gypseum, and Trichophyton mentagrophytes. The method used was a laboratory experiment, with the main parameters observed including Inhibition Zone Diameter (IZD), Minimum Inhibitory Concentration (MIC), and Minimum Fungicidal Concentration (MFC). The results showed that δ-guaiene inhibited the growth of C. albicans at a concentration of 40%, with an IZD of 0.15 mm. Against A. niger, the compound demonstrated an inhibition zone of 1.4 mm at a 20% concentration and had an MFC of 20%. For T. mentagrophytes, an IZD of 0.4 mm was observed, with both MIC and MFC at 20%. Activity against M. gypseum showed an IZD of 0.25 mm, with MIC and MFC also at 20%. These findings suggest that δ-guaiene from patchouli oil has potential as a candidate antifungal agent derived from local resources, supporting the development of natural bioactive compounds and sustainability.

Keywords: *Antifungal activity; δ-guaiene; patchouli oil and Subang.*

I. INTRODUCTION

Patchouli oil (*Pogostemon cablin* Benth.) is one of Indonesia's leading essential oils, widely used in the perfume, cosmetics, and pharmaceutical industries due to its function as a natural fixative. In 2008, Indonesia was recorded as the world's largest producer of patchouli oil (Baser & Buchbauer, 2010). The oil has been designated a national strategic commodity to enhance the competitiveness of the essential oil industry (Gunawan, 2009). Unfortunately, most of Indonesia's patchouli oil exports are still in the form of raw materials rather than value-added derivatives.The main composition of patchouli oil includes approximately fifteen compounds, with dominant components such as patchouli alcohol, δ-guaiene, α-guaiene, seychellene, and α-patchoulene (Aisyah et al., 2012). These compounds are classified into two main groups: oxygenated hydrocarbons and sesquiterpene hydrocarbons. Sesquiterpenes such as α-guaiene and δ-guaiene contribute nearly half of the oil's weight and play a significant role in its biological activity, including antifungal, anti-inflammatory, and antioxidant properties (Srivastava et al., 2022).Specifically, δ-guaiene is a sesquiterpene hydrocarbon with the chemical formula $C_{15}H_{24}$. This compound has been reported to exhibit important pharmacological activities, such as antagonism of the platelet-activating factor (PAF) receptor and inhibition of the arachidonic acid pathway, which may influence platelet aggregation (Srivastava et al., 2022). In addition, according to Chakrapani et al. (2013), various compounds found in patchouli oil demonstrate antimicrobial, anti-inflammatory, antimutagenic, and cytotoxic activities, supporting its potential as a raw material for natural pharmaceutical products.

The development of value-added products from patchouli oil—such as isolated δ-guaiene—can significantly increase the economic value of local commodities. Vacuum fractional distillation has been successfully applied to isolate δ-guaiene fractions from patchouli oil (Amrullah et al., 2017; Sidabutar et al., 2020). However, research evaluating the biological potential of this fraction, particularly against dermatophytic fungi that commonly infect humans, remains limited.A previous study by Nurjanah et al. (2025) reported that α-guaiene exhibited antifungal activity against fungi such as *Candida albicans*, *Aspergillus niger*, *Microsporum gypseum*, and *Trichophyton mentagrophytes*. The results indicated that each fungal species showed varying sensitivity to the compound, with *T. mentagrophytes* demonstrating the

highest resistance. However, the antifungal activity of δ-guaiene against similar pathogenic fungi remains underexplored.Developing derivative products from patchouli oil, such as δ-guaiene isolates, not only increases economic value but also creates new business opportunities in downstream industries. This natural resource-based innovation has the potential to drive local economic growth, particularly in patchouli-producing regions such as Subang and its surroundings.

By strengthening research-based processing capabilities and downstream industrial involvement, farmers and small-to-medium enterprises (SMEs) can participate directly in the high-value product supply chain, thus generating employment and promoting regional economic development.The sustainable bioeconomy approach to patchouli oil utilization aligns with national development goals that emphasize innovation and sustainability. In addition to contributing to the health sector through the antifungal potential of δ-guaiene, diversification of patchouli-based products also promotes the substitution of imported active ingredients in pharmaceuticals and cosmetics with locally sourced materials. This shift can reduce dependency on foreign products and strengthen domestic industrial independence, ultimately improving community welfare through increased national income and more equitable economic distribution.Furthermore, collaboration between research institutions, universities, and industry stakeholders is key to accelerating technology transfer and commercialization of research outcomes. These activities can be facilitated through business incubation schemes, technical assistance, and access to financing—particularly for SMEs in agriculture and aromatic plant processing sectors. In this way, innovations derived from patchouli oil can move beyond academic discourse and become real drivers of inclusive and sustainable economic growth.

As part of efforts toward sustainable agricultural development, patchouli cultivation should adopt environmentally friendly practices. The use of organic fertilizers, soil and water conservation techniques, and appropriate crop rotation can enhance productivity without damaging ecosystems. Moreover, partnerships among farmers, cooperatives, and industry players can promote the adoption of sustainable agriculture standards, including certifications such as organic or fair trade. This approach not only strengthens the economic resilience of farmers but also elevates Indonesia's position in the global market as a producer of essential oils that upholds environmental and social responsibility. Consequently, the development of patchouli-based products will bring long-term benefits to the environment, society, and the national economy.Based on this background, this study aims to investigate the antifungal activity of δ-guaiene isolated from patchouli oil against pathogenic fungi, namely *Aspergillus niger*, *Candida albicans*, *Microsporum gypseum*, and *Trichophyton mentagrophytes*. The results of this study are expected to provide a scientific foundation for the development of patchouli-based products from Subang, support the utilization of local resources, and contribute to the achievement of the Sustainable Development Goals (SDGs) through innovation in the health sector and the green industry.

II. METHODS

This study employed an experimental approach to determine the antifungal activity of δ-guaiene compounds isolated from patchouli oil obtained through fractional distillation. The antifungal assay was conducted against four types of skin pathogenic fungi: Aspergillus niger, Candida albicans, Microsporum gypseum, and Trichophyton mentagrophytes. The evaluation was based on three primary parameters: Inhibition Zone Diameter (IZD), Minimum Inhibitory Concentration (MIC), and Minimum Fungicidal Concentration (MFC).A Completely Randomized Design (CRD) was applied for the statistical analysis of the IZD data. The CRD included two factors: the first factor was five different concentrations of patchouli oil fractions containing the highest δ-guaiene content, and the second factor was the type of fungal species. Meanwhile, the MIC and MFC data were analyzed descriptively. All treatments for each assay were performed in duplicate, and the IZD results were further analyzed using Duncan's Multiple Range Test to determine statistically significant differences among treatments.

Test fungi were cultured in appropriate media according to their growth characteristics and incubated at 27°C for varying durations: C. albicans for 48 hours, A. niger for 72 hours, T. mentagrophytes for 96 hours, and M. gypseum for 120 hours. Ketoconazole served as the positive control, while n-hexane was used

as the negative control. Throughout the antifungal testing process, fungal growth changes were observed after incubation to assess the effectiveness of the treatments.The research was conducted in three main stages. The first stage involved fungal identification through microscopic observations to confirm the species based on morphological characteristics such as hyphae, conidia, and vesicles. For example, A. niger was identified by the presence of filamentous structures, septate hyphae, and vesicles, consistent with the descriptions provided by Paramita (2021). The second stage was the antifungal assay of δ-guaiene derived from patchouli oil fractions. The IZD was measured using the well diffusion method to determine the inhibition ability of each fraction concentration. MIC and MFC values were obtained using the microdilution method, with concentration ranges adjusted based on five vacuum-distilled patchouli oil fractions.

The detailed procedures for measuring IZD, MIC, and MFC are outlined below:

A. Disk Diffusion Method (IZD)

1. Prepare serial concentrations of the test sample.
2. Dissolve the test sample using a suitable solvent (e.g., DMSO or ethanol).
3. Sterilize the PDA medium by autoclaving at 121 °C for 15 minutes.
4. Pour the sterilized PDA medium into sterile Petri dishes and allow it to solidify.
5. Prepare fungal inoculum by dissolving fungal colonies in 0.85% NaCl.
6. Standardize the turbidity of fungal suspension using McFarland standard 1 (OD measured with spectrophotometer).
7. Inoculate the fungal suspension evenly onto the surface of PDA medium using a sterile cotton swab.
8. Make wells on the PDA surface using a sterile borer (6 mm diameter).
9. Add different concentrations of the test sample into each well.
10. Use ketoconazole as positive control and solvent (e.g., DMSO) as negative control.
11. Incubate the Petri dishes at 27 °C for 48–72 hours.
12. Measure the diameter of inhibition zones formed around the wells.
13. Record results for antifungal activity evaluation.

B. Minimum Inhibitory Concentration (MIC)

1. Prepare serial dilutions of the test sample in liquid PDA or Sabouraud broth.
2. Inoculate fungal suspension into each test tube or well (depending on microdilution or macrodilution method).
3. Incubate all tubes or wells at 27 °C for 48–72 hours.
4. Observe for turbidity or fungal growth.
5. Determine MIC as the lowest concentration of the sample that inhibits visible growth of the fungus.

C. Minimum Fungicidal Concentration (MFC)

1. From the MIC test, take an aliquot from tubes or wells showing no visible fungal growth.
2. Streak onto a fresh PDA medium in Petri dishes.
3. Incubate at 27 °C for 48–72 hours.
4. Observe for fungal colony growth.
5. Determine MFC as the lowest concentration of the sample that shows no fungal growth on PDA, indicating fungicidal activity.

The third stage was data analysis. The Inhibition Zone Diameter (IZD) data obtained from the tests were statistically analyzed using a two-factor Completely Randomized Design (CRD) to determine the effects of δ-guaiene fraction concentrations and fungal species on the inhibition zone diameter. Meanwhile, the MIC and MFC data were analyzed descriptively to identify the lowest concentration ranges capable of effectively inhibiting or killing fungal growth. The results of this analysis are expected to provide quantitative information on the antifungal potential of δ-guaiene as a candidate for natural active ingredients.

III. RESULT AND DISCUSSION

A. Inhibition Zone Diameter (IZD)

The antifungal activity test was conducted using the well diffusion method on four fungal species: Candida albicans, Aspergillus niger, Trichophyton mentagrophytes, and Microsporum gypseum. The

inhibition zone diameters (IZDs) formed were observed and measured quantitatively. The IZD data were then analyzed using a Completely Randomized Design (CRD), followed by Duncan's multiple range test to determine significant differences among treatments. This method differs from the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) tests, which were evaluated descriptively.The results of the antifungal activity test using the well diffusion method indicate that δ-guaiene, a compound found in patchouli oil, exhibits antifungal properties. Statistical analysis showed that both the concentration of δ-guaiene and the type of fungi had a highly significant effect on IZD. The concentrations of patchouli oil and the solvent n-hexane used in the test can be found in Appendix 10. The average IZD values from various concentrations for each fungal species are presented in Table 1.

Table 1. Average Inhibition Zone Diameter (IZD) (mm) on *Candida albicans, Aspergillus niger, Trichophyton mentagrophytes*

Fungal Species	Replicate	20%	40%	60%	80%	100%
Candida albicans	1	0	0.05 ± 0.16	0	0.10 ± 0.32	0.05 ± 0.16
	2	0	0.25 ± 0.35	0.30 ± 0.35	0	0.05 ± 0.16
Aspergillus niger	1	2.85 ± 1.29	1.85 ± 0.97	2.85 ± 1.06	1.15 ± 0.24	3.55 ± 2.63
	2	2.15 ± 1.38	2.60 ± 0.97	1.30 ± 0.48	2.45 ± 1.07	1.90 ± 0.39
T. mentagrophytes	1	1.65 ± 0.47	1.50 ± 0.53	1.00 ± 0	1.20 ± 0.26	0.40 ± 0.21
	2	1.90 ± 0.32	1.05 ± 0.16	0.90 ± 0.21	0.05 ± 0.16	0.20 ± 0.26
Microsporum gypseum	1	1.10 ± 0.32	1.10 ± 0.21	2.30 ± 0.48	0.10 ± 0.21	0.25 ± 0.26
	2	1.15 ± 0.24	0.85 ± 0.24	0.75 ± 0.26	0.84 ± 0.25	0.75 ± 0.26

Table 2. Average Inhibition Zone Diameter (IZD) of δ-Guaiene Against C. *albicans, A. niger, T. mentagrophytes,* and *M. gypseum*

Code	Treatment Description	Mean IZD (mm) ± SD
A	20% δ-guaiene, *C. albicans*	0.00 ± 0.00^{a}
B	40% δ-guaiene, *C. albicans*	0.28 ± 0.26^{ad}
C	60% δ-guaiene, *C. albicans*	0.15 ± 0.18^{ae}
D	80% δ-guaiene, *C. albicans*	0.05 ± 0.16^{ab}
E	100% δ-guaiene, *C. albicans*	0.05 ± 0.16^{ae}
F	20% δ-guaiene, *A. niger*	2.50 ± 1.34^{k}
G	40% δ-guaiene, *A. niger*	2.23 ± 0.97^{jk}
H	60% δ-guaiene, *A. niger*	2.08 ± 0.77^{jk}
I	80% δ-guaiene, *A. niger*	1.80 ± 0.66^{gk}
J	100% δ-guaiene, *A. niger*	2.77 ± 1.51^{k}
K	20% δ-guaiene, *T. mentagrophytes*	1.78 ± 0.42^{gk}
L	40% δ-guaiene, *T. mentagrophytes*	1.28 ± 0.35^{k}
M	60% δ-guaiene, *T. mentagrophytes*	0.95 ± 0.11^{aj}
N	80% δ-guaiene, *T. mentagrophytes*	0.63 ± 0.21^{ai}
O	100% δ-guaiene, *T. mentagrophytes*	0.30 ± 0.24^{af}
P	20% δ-guaiene, *M. gypseum*	1.13 ± 0.28^{ak}
Q	40% δ-guaiene, *M. gypseum*	0.98 ± 0.23^{ak}
R	60% δ-guaiene, *M. gypseum*	1.53 ± 0.37^{fk}
S	80% δ-guaiene, *M. gypseum*	0.47 ± 0.23^{ag}
T	100% δ-guaiene, *M. gypseum*	$0.500.26^{ah}$

Note: Values represent the mean of two replicates. Treatments with the same lowercase letters in the superscript within the same column are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Based on Duncan's test at a 5% significance level, the Inhibitory Zone Diameter (IZD) values for *Candida albicans* and *Microsporum gypseum* at concentrations ranging from 20% to 100% were not significantly different, except for *M. gypseum* at 60%, which showed a significant difference. At the minimum concentration of 20%, δ-guaiene was already capable of inhibiting the growth of C. albicans and M. gypseum, although the 60% concentration demonstrated a distinctly stronger inhibitory effect. This suggests that while lower concentrations may exhibit initial antifungal activity, higher concentrations can produce more noticeable effects.Furthermore, the IZD values for *Aspergillus niger* and *M. gypseum* at concentrations of 20% to 60% also showed no significant difference, reinforcing the pattern that δ-guaiene's

antifungal effectiveness varies not only by concentration but also by fungal species. The appearance of clear inhibition zones in the test plates indicates the antifungal activity of δ-guaiene. The larger the inhibition zone, the greater the measured IZD value, and thus, the stronger the antifungal effect.

In the case of *C. albicans*, no inhibition zone was observed at the 20% concentration, resulting in an IZD of 0 mm. This may be due to the limited diffusion ability of δ-guaiene when mixed with a high proportion of n-hexane, which could dilute the active compound to levels too low to exert antifungal effects (Wisnianti, 2024). Therefore, at low concentrations, δ-guaiene may not demonstrate measurable antifungal activity against certain fungi. The highest inhibition zone was recorded for *A. niger* at a 100% δ-guaiene concentration, with an IZD of 2.77 mm. This is presumably due to the higher δ-guaiene content in pure patchouli oil, which allows for more active compounds to be available in inhibiting fungal growth. According to Pelczar and Chan (1988), increasing the concentration of an extract generally enhances the number of active antimicrobial compounds, thereby improving its inhibitory effect.The most notable IZD values for each fungus were as follows: C. albicans showed the highest inhibition at 40% concentration (0.28 mm), A. niger at 100% concentration (2.77 mm), T. mentagrophytes at 20% concentration (1.78 mm), and M. gypseum at 60% concentration (1.53 mm). However, based on classification by Morales (2003), all the IZD values observed in this study fall into the category of low antifungal activity.

B. Minimum Inhibitory Concentration (MIC)

The antifungal activity assay using the Minimum Inhibitory Concentration (MIC) method was conducted by microdilution to determine the lowest concentration of the antimicrobial agent capable of inhibiting fungal growth after incubation (Kowalska et al., 2021). The patchouli oil was diluted in n-hexane at various concentrations, and the details of the test concentrations can be found in Appendix 11. The MIC test results are summarized in Table 3.

Table 3. MIC Results on *Candida albicans, Aspergillus niger, Trichophyton mentagrophytes,* and *Microsporum gypseum*

Fungi	Replicate	Concentration (%)	2.5	5	10	15	20	40	MIC (%)
Candida albicans	A	Growth	+	+	+	-	-	–	15
	B	Growth	+	+	+	+	-	–	20
	D	Growth	+	+	+	-	-	–	15
	E	Growth	+	+	-	-	-	–	10
Aspergillus niger	A	Growth	+	+	-	-	-	–	10
	B	Growth	+	+	+	+	-	–	15
	D	Growth	+	+	+	-	-	–	15
	E	Growth	+	+	+	+	-	–	20
T. mentagrophytes	A	Growth	+	+	+	+	-	–	20
	B	Growth	+	+	+	+	-	–	20
M. gypseum	A	Growth	+	+	-	-	-	–	10
	B	Growth	+	+	-	-	-	–	10

Note: (+) = fungal growth observed, (−) = no fungal growth observed

The MIC results presented in Table 1 reflect the minimum concentrations at which fungal growth was completely inhibited, as observed through the absence of turbidity in the test wells compared to the control. For *Candida albicans*, the MIC ranged between 10% to 20%, depending on the replicate, with most inhibition clearly observed at 15%. For *Aspergillus niger*, the MIC was recorded at 15%. The MIC for *Trichophyton mentagrophytes* was observed at 20%, and for *Microsporum gypseum*, complete inhibition occurred at a concentration of 10%.The clarity of the well medium served as the primary indicator of antifungal activity. A clear well indicated that fungal growth had been successfully inhibited by the δ-guaiene component in patchouli oil. These results confirm that patchouli oil demonstrates varying levels of antifungal potency depending on both the fungal species and concentration tested.

C. Minimum Fungicidal Concentration (MFC)

The analysis results indicated that both the concentration of δ-guaiene and the type of fungus significantly affected the Minimum Fungicidal Concentration (MFC), as shown in Table 4.

Table 4. MFC Results on *Candida albicans, Aspergillus niger, Trichophyton mentagrophytes*, and *Microsporum gypseum.*

Fungi	Replicate	2.5%	5%	10%	20%	40%
C. albicans	A	+	+	+	+	+
	B	+	+	+	+	+
	D	+	+	+	+	+
	E	+	+	+	+	+
A. niger	A	+	+	+	+	+
	B	+	+	+	+	+
	D	+	+	+	+	+
	E	+	+	+	+	+
T. mentagrophytes	A	+	+	+	+	–
	B	+	+	+	+	–
	D	+	+	+	+	–
	E	+	+	+	+	–
M. gypseum	A	+	+	+	–	–
	B	+	+	+	–	–
	D	+	+	+	–	–
	E	+	+	+	–	–

Note: "+" indicates growth inhibition observed; "–" indicates no inhibition.

The MFC test results showed no inhibition zones for *C. albicans* and *A. niger*, consistent with findings by Aisyah (2021), who compared patchouli oil with citronella and nutmeg seed oils. The inhibition zones for citronella and nutmeg seed oils against *C. albicans* and A. niger were 22 mm and 38.28 mm for citronella, and 8.38 mm and 9.12 mm for nutmeg seed oil, respectively.Hydrophobic molecules in patchouli oil, such as β-caryophyllene, α-guaiene, δ-guaiene, 1H-3a,7-methanoazulene, and patchouli alcohol, are thought to attack ergosterol in fungal cell membranes, particularly in *C. albicans.* This results in altered membrane permeability and subsequent membrane damage, leading to leakage of intracellular contents and eventual cell death (Jothi et. al., 2023).

Thus, the antifungal activity of patchouli oil depends heavily on its chemical composition and the concentration of active compounds.The mechanism of δ-guaiene's antifungal activity may vary across fungal species. In A. niger, it likely works by delaying spore germination and inhibiting mycelial growth. Higher concentrations of essential oil components lead to stronger inhibition (Zahaf et. al., 2017). According to Dewi (2021), essential oil compounds can inhibit A. niger growth by thinning the hyphal cell walls, suppressing up to 80% of fungal proliferation.In *T. mentagrophytes*, δ-guaiene is thought to inhibit hyphal development, while in *M. gypseum*, inhibition likely occurs due to mitochondrial membrane destruction and cell wall disruption, causing morphological changes to fungal cells (Maulani et al., 2022).In conclusion, antifungal activity varied between fungal species, which may be attributed to differences in protein composition among *C. albicans, A. niger, T. mentagrophytes*, and *M. gypseum*.

IV. CONCLUSION

δ-Guaiene, a compound derived from patchouli oil with a purity of 47%, has shown antifungal properties against several fungal species, including *Candida albicans, Aspergillus niger, Trichophyton mentagrophytes*, and *Microsporum gypseum*. This compound's ability to inhibit the growth of these fungi suggests its potential as a natural antifungal agent. The antifungal activity of δ-Guaiene was evaluated based on the diameter of the inhibition zone, which reflects the compound's efficacy in suppressing fungal growth at varying concentrations.For *C. albicans*, δ-Guaiene exhibited a modest antifungal effect, with an inhibition zone diameter of 0.15 mm at a minimum concentration of 40%. This indicates a relatively weak antifungal activity against this yeast, which is a common pathogen in human infections. Despite its weak activity, the results demonstrate that δ-Guaiene may still hold some potential for use in antifungal formulations, especially when combined with other compounds to enhance its efficacy.

In contrast, *A. niger* showed a more noticeable inhibition with a diameter of 1.4 mm at a minimum concentration of 20%. Although this inhibition is still categorized as weak, the lower concentration required for activity suggests that δ-Guaiene has a somewhat higher potency against this fungal species. The

minimum inhibitory concentration (MIC) for *A. niger* was determined to be 20%, indicating that δ-Guaiene could potentially serve as a low-concentration antifungal agent in controlling this particular mold.Similarly, both *T. mentagrophytes* and *M. gypseum* were inhibited by δ-Guaiene, although their response was also weak. For T. mentagrophytes, the inhibition zone diameter was 0.4 mm at a minimum concentration of 20%, while *M. gypseum* exhibited a 0.25 mm inhibition at the same concentration. The minimum inhibitory concentration and minimum fungicidal concentration for both fungi were also found to be at 20%. These results suggest that δ-Guaiene's antifungal activity is relatively consistent across different fungal species, though its effect remains limited and would likely require further optimization for practical applications in antifungal treatments

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