

Delignification Effect of Aceh Patchouli Leaves (*Pogostemon cablin* Benth.) by *Trichoderma viride* on the Quality of Patchouli Oil

Michael Anthony Thongiratama¹, Azminah², Theresia Desy Askitosari¹,
Poppy Hartatie Hardjo^{1*}

¹Master's Program in Biotechnology, Faculty of Biotechnology, University of Surabaya, East Java, Indonesia

²Department of Pharmacy, Faculty of Pharmacy, University of Surabaya, Surabaya, East Java, Indonesia

*Corresponding author: poppy_hardjo@staff.ubaya.ac.id

Submitted: 28 September 2025 Accepted: 10 June 2026 Published: 29 August 2026

ABSTRACT

Aceh patchouli (*Pogostemon cablin* Benth.) is an aromatic plant that produces essential oil, with patchouli alcohol (PA) as the primary compound determining its quality. This study aimed to investigate the effects of delignification using the fungus *Trichoderma viride* on PA content, other chemical components in patchouli oil, and the functional group characteristics of the oil. A factorial experimental design was employed, with the first factor being leaf delignification (control without delignification, natural delignification, and delignification by *T. viride* for 5 days), and the second factor being the extraction method (hydrodistillation and steam distillation). PA content and the composition of volatile compounds were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS), while Fourier-Transform Infrared (FTIR) spectroscopy was used to characterize functional groups. This study provides a novel integrated approach by combining biological delignification using *T. viride* with different distillation techniques and spectroscopic–multivariate analysis to systematically evaluate changes in patchouli oil composition and extraction efficiency. The results indicated that delignification by *T. viride* significantly increased PA content, with the highest concentration reaching 61.12%, compared to 37.96% in the control treatment. The levels of other compounds, such as caryophyllene, decreased following delignification, indicating a shift in chemical composition. Delignification treatment had a significant effect on PA content, whereas the distillation method showed no significant difference. FTIR analysis revealed consistent major functional groups, including O–H and C–H vibrations, suggesting that the primary chemical functionalities of the oil were retained. However, these results should be interpreted cautiously due to the complex mixture nature of essential oils and the overlapping of spectral bands. Overall, *T. viride* demonstrated potential as an effective and environmentally friendly biological pretreatment agent to enhance patchouli oil quality by improving PA yield without evident alteration of its dominant functional groups.

Keywords: distillation; FTIR; GC-MS; patchouli alcohol

INTRODUCTION

Aceh patchouli (*Pogostemon cablin* Benth.) is a high-value essential oil crop, with patchouli alcohol (PA) as the principal compound determining oil quality (Swamy, 2015). Indonesia supplies approximately 85% of the global patchouli oil market; however, the PA content of Indonesian patchouli oil remains relatively low (<30%) (Tribun Manado, 2024). According to SNI 06-2385-2006, the minimum PA requirement is

30%, while the international standard requires at least 40% (Abduh *et al.*, 2023).

PA exhibits various biological activities, including antiviral, anti-inflammatory, anticancer, and antioxidant properties (Yu *et al.*, 2019; Swamy, 2015). Besides PA, patchouli oil contains compounds such as α -guaiene, β -caryophyllene, spathulenol, α -bulnesene, and β -patchoulene, which contribute to its distinctive spicy-woody aroma and provide pharmacological benefits such as antimicrobial, antioxidant, and

chemosensitizing effects (Nurjanah *et al.*, 2022; Cardoso *et al.*, 2021; do Vale *et al.*, 2019). PA is synthesized through the mevalonate pathway in glandular trichomes on the leaves, and its content is influenced by light intensity, soil type, harvest age, and postharvest handling (Swamy, 2015; Fatharani *et al.*, 2023; Yuliani *et al.*, 2021).

Trichoderma spp. are widely recognized as fungi valued both as biocontrol agents against plant pathogens and as contributors to biodegradation processes (Rimanti *et al.*, 2024). Among them, *Trichoderma viride*, a member of the Hypocreaceae family, is particularly effective in cellulose and hemicellulose hydrolysis, with a delignification capacity of up to 56%, thereby enhancing enzymatic biomass degradation (Ghorbani *et al.*, 2015). The enzymatic activity of *T. viride* involves the production of ligninolytic and cellulolytic enzymes that contribute to delignification. These enzymes can break down complex bonds in patchouli leaves, including β -(1,4)-glycosidic linkages in cellulose, thus loosening the lignocellulosic structure (Akbar *et al.*, 2014). This process may improve the extraction efficiency of active compounds such as PA without altering their chemical structure, making *T. viride* a promising biological pretreatment agent for improving patchouli oil quality.

Distillation is a separation process based on differences in boiling points, widely applied in industries such as petroleum, alcohol, and essential oils (Uwar & Soselissa, 2022). The principle involves heating a mixture until the more volatile components evaporate, followed by condensation to recover purified liquids. The process consists of two main stages: evaporation and condensation. In solid-liquid mixtures, separation occurs relatively easily, whereas in liquid-liquid mixtures, vapor pressure interactions can hinder complete separation (Saxena & Chandwankar, 2020). One commonly used method for extracting essential oils is hydrodistillation, in which the raw material is heated with water in a distillation flask. The resulting vapor carries the essential oil to a condenser, where it is cooled and separated from the water (Santosa *et al.*, 2023). This method has the advantage of preserving heat-sensitive volatiles, as the boiling temperature of the water-plant mixture is lower than in dry distillation.

The success of hydrodistillation is influenced by the quality of the raw material, including leaf dryness, pretreatments such as delignification, and the leaf-to-stem ratio. Mild

delignification can facilitate essential oil release by disrupting leaf cellular structures, allowing the oil to be more easily extracted. The optimal distillation duration for patchouli leaves ranges from 5 to 8 hours to achieve maximum yield without degrading volatile components (Fitri *et al.*, 2017). Another widely used technique is steam distillation, which separates compounds at lower temperatures than their normal boiling points, thereby preventing thermal degradation (Nadliroh & Fauzi, 2021). This process can be performed by passing steam directly through the material or by heating it with a solvent. In essential oil extraction, steam distillation is particularly effective because it leverages differences in volatility between essential oil constituents and nonvolatile substances (Battaglia, 2019).

Steam distillation offers the advantage of producing essential oils with high purity, but its efficiency depends on process parameters such as temperature, pressure, and distillation time (Daryono *et al.*, 2016). Previous studies have shown that careful adjustment of these parameters can increase PA yield in Aceh patchouli oil (Ermaya *et al.*, 2019). Furthermore, the freshness and moisture content of the raw materials are critical factors influencing the final quality of essential oils.

Therefore, this study aimed to evaluate the effectiveness of delignification using *Trichoderma viride* and to compare hydrodistillation and steam distillation in terms of PA content, the presence of other compounds, and the chemical functional groups of PA, with the goal of producing high-quality patchouli oil that meets international standards.

MATERIALS AND METHODS

This study was conducted from December 2024 to June 2025 at the following locations: the Plant Biotechnology Laboratory, the Microorganism Biotechnology Laboratory, and the Greenhouse of the Faculty of Biotechnology, University of Surabaya, as well as the Research Laboratory of the Faculty of Pharmacy, University of Surabaya. The equipment used included beakers with capacities of 50–1000 mL (Pyrex), 10–100 mL measuring cylinders (Iwaki), glass spatulas, a microwave, an autoclave, a Bunsen burner, a digital balance, a laminar Air Flow (LAF) cabinet, a lighter, a metal spoon, tweezers, an oven, culture bottles, 100–1000 μ L micropipettes, black cloth, a ruler, a scalpel, Petri

dishes, inoculating loops, a drying cabinet, a vacuum desiccator, a Gas Chromatography–Mass Spectrometry (GC–MS) system (Shimadzu), a Fourier transform infrared (FTIR) spectrometer (Agilent), a steam distillation apparatus, a hydrodistillation apparatus, and cuvettes.

The materials included Aceh patchouli (*Pogostemon cablin* Benth.) leaves of the Sidikalang variety, 96% ethanol, aluminum foil, *Trichoderma viride* culture, 0.85% NaCl solution, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), and anhydrous sodium sulfate (Na_2SO_4). The experimental variables consisted of (1) controlled variables, namely the moisture content of patchouli leaves (10%) and drying temperature (60°C using a drying cabinet); (2) independent variables, namely delignification treatment (control without delignification, natural delignification, and delignification with *T. viride* for 5 days) and distillation method (hydrodistillation and steam distillation); and (3) dependent variables, namely patchouli oil yield, PA content, other volatile components, and PA functional groups.

The experiment was arranged in a randomized complete block design (RCBD) with two factors: delignification treatment (three levels) and distillation method (two levels), with 10 replicates per treatment. Randomization was applied to all experimental units within each block to minimize environmental variability. Blocking was based on incubation batch to control variations in environmental conditions. Delignification treatment was performed on 100 g of dried patchouli leaves for each group: control (no delignification), natural delignification, and delignification with *T. viride*. Natural delignification was defined as lignin degradation occurring without the addition of an external microbial inoculum, relying on indigenous microorganisms present on the plant material under controlled incubation conditions.

For the fungal treatment, the leaves were soaked in a suspension of *T. viride* hyphae, with an inoculum concentration standardized to approximately 10^6 – 10^7 spores/mL (determined using a haemocytometer), incubated at 27°C for 5 days, then dried and ground into powder. During incubation, environmental parameters were controlled: the pH was maintained at 5.5–6.0, the moisture content was maintained at 60–70% (w/w), and samples were incubated under aseptic conditions to prevent microbial

contamination. Periodic monitoring was conducted to ensure consistency.

Patchouli oil extraction was carried out using two distillation methods. In hydrodistillation, 100 g of powdered leaves were placed into a 1 L flask, filled with distilled water to two-thirds of its volume, and distilled using a Clevenger apparatus for 8 hours or until no further oil droplets were observed to prevent thermal degradation of volatile compounds. In steam distillation, 100 g of dried leaves were placed in the distillation chamber and treated with steam from a boiler at 100°C for 6–8 h, which was considered an optimal duration based on essential oil extraction efficiency.

In both methods, the resulting oil was separated using a separatory funnel, dried with Na_2SO_4 , and stored in dark bottles at 5°C. PA content was analyzed using GC–MS with automatic injection, programmed heating from 80°C to 280°C, and compound identification by comparison with the instrument's mass spectral library. GC–MS analysis was performed with an injection volume of 1 μL and a split ratio of 1:10, using helium as the carrier gas at a constant flow rate of 1 mL/min.

All analyses were conducted in triplicate to ensure reproducibility, and instrument calibration was carried out prior to measurement. Compound separation was achieved using a Restek Rtx-5MS GC column, a semipolar capillary column consisting of 5% diphenyl and 95% dimethyl polysiloxane (60 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness). The column exhibited high thermal stability, with a maximum operating temperature of 350°C and a minimum bleed temperature of 330°C, making it suitable for the analysis of volatile and semi-volatile compounds. Functional group characterization was performed using FTIR spectroscopy (Cary 630) equipped with an Attenuated Total Reflection (ATR) accessory over a wavelength range of 4000–500 cm^{-1} to compare spectral profiles among treatments.

FTIR measurements were performed with 32 scans per sample and a resolution of 4 cm^{-1} , and background correction prior to each measurement to ensure spectral accuracy and consistency. GC–MS and FTIR data were further analyzed using multivariate approaches with Minitab software, applying Principal Component Analysis (PCA) to determine grouping patterns based on physicochemical properties and Cluster Analysis to classify treatments according to similarity in characteristics. Prior to multivariate

analysis, the data were tested for normality and homogeneity, and significant differences among treatments were evaluated using ANOVA followed by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Patchouli oil yield was influenced by delignification treatment. The highest yield (2.5%) was obtained from delignification with *Trichoderma viride* (Table 1), whereas no significant difference was observed between water distillation and steam distillation.

Biological delignification using *Trichoderma viride* has the potential to enhance extraction efficiency, as ligninolytic enzymes such as laccase and manganese peroxidase can degrade the lignin matrix in the cell walls of Aceh patchouli leaves, thereby facilitating the release of essential oil compounds (Saini *et al.*, 2015; Kumari *et al.*, 2018).

FTIR spectroscopy was employed to identify potential changes in chemical composition and functional groups in patchouli leaves subjected to the delignification treatments.

Figure 1 presents the FTIR spectra of leaves subjected to delignification with *T. viride*, natural delignification (consortium of native microorganisms), and the control without delignification. The overlapping absorption bands across treatments indicated no detectable differences in the infrared spectra, suggesting that delignification did not alter the chemical composition or functional groups of the various compound components in patchouli leaves.

At a wavenumber of approximately 3298 cm^{-1} , an absorption band was observed, indicating the presence of $-\text{OH}$ groups from alcohols, while the band at 1000–1100 cm^{-1} corresponds to the C–O stretching of secondary alcohols or esters. Delignification with *T. viride* produced a lower infrared absorption intensity than the other treatments. This suggests that enzymatic activity of the fungus may have degraded or transformed polar groups, thereby facilitating the extraction of essential oil (Muharam, 2017). A more detailed interpretation of the FTIR spectra in Figure 1 is presented in Table 2.

Table 1. Average Yield of Patchouli Oil (%) from 100 g of Patchouli Leaves Based on Delignification Treatment and Extraction Method

Sample	Extraction Method	Total Yield (%)
Control	Water Distillation	1.33±0.02 ^a
	Steam Distillation	1.47±0.01 ^a
Delignification with <i>T. viride</i> (5 days)	Water Distillation	2.28±0.08 ^{bc}
	Steam Distillation	2.5±0.04 ^c
Natural Delignification (5 days)	Water Distillation	2.05±0.03 ^b
	Steam Distillation	2.15±0.05 ^b

Note: Values in the same column followed by different letters indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $\alpha = 5\%$.

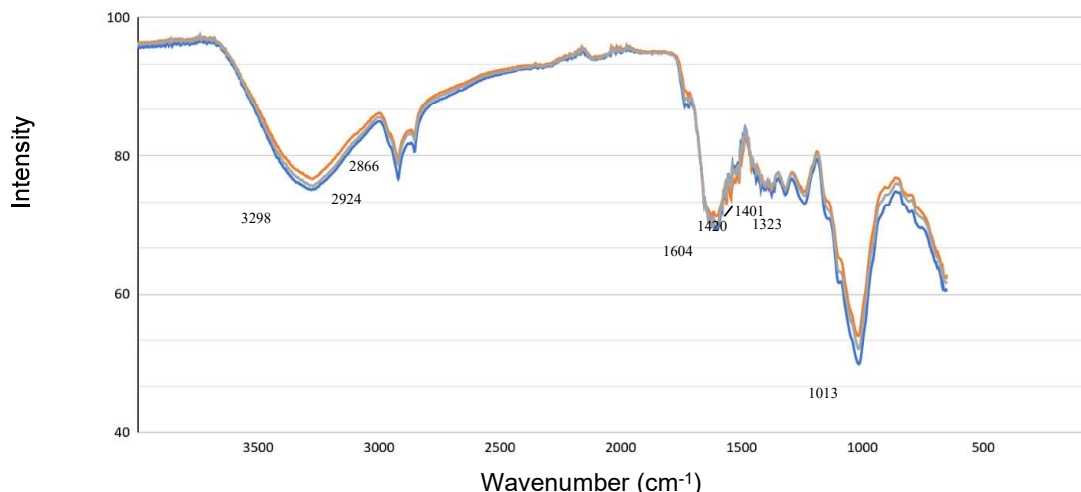


Figure 1. FTIR spectra of patchouli leaves under different delignification treatments.

Note: — *T. viride* delignification, — Natural delignification (without *T. viride*), — Control (no delignification)

Table 2. Identification of Functional Groups in Patchouli Leaf Extract Compounds Based on FTIR Spectra

Wavenumber (cm ⁻¹)	Bond/Mode	Functional Group
3298	–OH	Alcohol group
2924	–CH ₂ , –CH ₃	Alkyl group
2866	C–H	Aliphatic bond
1604	C=C	Aromatic C=C bond
1420	–CH ₃ , –CH ₂	Alkyl group
1401	–CH ₂	Alkyl group
1377	–CH ₃	Methyl group
1323	O–H bending	Alcohol or phenol
1013	–OH	Alcohol

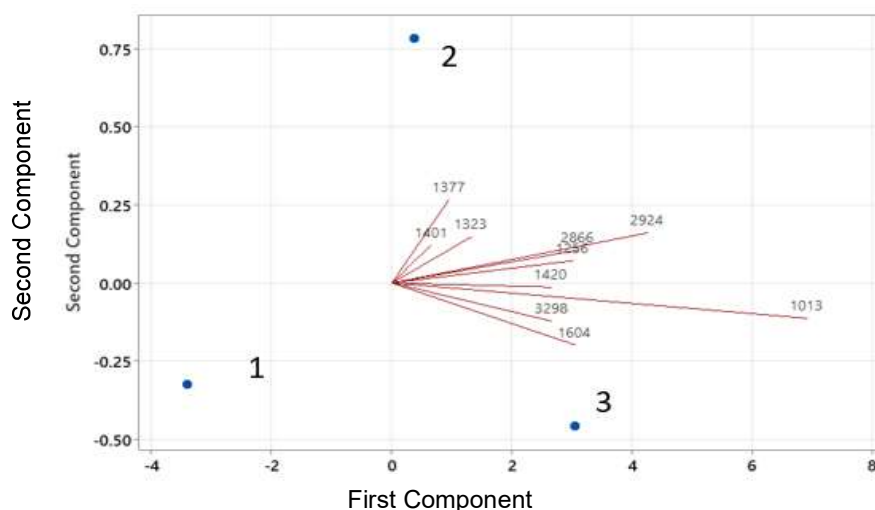


Figure 2. Principal Component Score Plot (PC1 vs. PC2) of Aceh Patchouli Leaves

Note: 1 = Control leaves (no delignification); 2 = Leaves treated with natural microbial consortium; 3 = Leaves treated with *Trichoderma viride*

The overlaid FTIR spectra in Figure 1 revealed the presence of various functional groups associated with the major constituents of essential oil, including compounds associated with patchouli alcohol (PA). However, because the distilled oil remained a multicomponent mixture rather than a purified PA standard, peak assignments were therefore interpreted as general functional groups rather than as direct confirmation of a single compound. The band at 1013 cm⁻¹ is more appropriately attributed to C–O stretching vibrations of alcohol-containing compounds, which is consistent with oxygenated sesquiterpenes such as PA. At 1323 cm⁻¹, an O–H bending vibration was observed, indicative of alcohols or phenols.

The peaks at 1377, 1401, and 1420 cm⁻¹ indicated –CH₃ (methyl) and –CH₂– (methylene) groups, which are part of the aliphatic hydrocarbon chain in sesquiterpenes, including

patchouli alcohol. The band at 1604 cm⁻¹ represents aromatic C=C stretching, likely arising from minor phenolic components or lignin degradation products in the leaves during extraction or fermentation (Table 2) (Zhou *et al.*, 2010).

The absorptions at 2866 and 2924 cm⁻¹ correspond to aliphatic C–H stretching vibrations, commonly associated with saturated hydrocarbon chains (alkyl groups) such as –CH₂– and –CH₃ within sesquiterpenoids. Meanwhile, the broad peak at 3298 cm⁻¹ is characteristic of free –OH groups not strongly involved in hydrogen bonding, further supporting the presence of alcohol groups consistent with patchouli alcohol as the major constituent of patchouli oil (Table 2). Overall, FTIR results indicated that delignification treatments mainly affected relative absorption intensity rather than introducing new functional groups, suggesting

preservation of the principal chemical framework of patchouli metabolites.

The PCA score plot in Figure 2 demonstrated clear separation among patchouli leaf treatments along the first principal component (PC1), indicating that the applied delignification methods significantly influenced the chemical characteristics of the samples. PCA is a multivariate statistical technique used to reduce data dimensionality while preserving the most important variance, allowing visualization of relationships among samples and variables (Jolliffe & Cadima, 2016).

Samples treated with *Trichoderma viride* were distinctly separated from both control and naturally delignified samples, suggesting that biological delignification using *T. viride* led to substantial modification of the chemical composition. This observation is consistent with previous studies reporting that *Trichoderma* spp. produce ligninolytic enzymes such as cellulases and hemicellulases, which contribute to the breakdown of lignocellulosic structures and facilitate the release of bioactive compounds (Harman *et al.*, 2004).

The loading plot further indicated that specific wavenumbers, particularly 1013 cm^{-1} (C–O alcohol), 2924 cm^{-1} (aliphatic C–H), and 1256 cm^{-1} (C–O ester/secondary alcohol), contributed most strongly to the variation along PC1. These functional groups are closely related to the presence of patchouli alcohol and other oxygenated compounds, which are key determinants of patchouli oil quality. In contrast, wavenumbers such as 1377 cm^{-1} , 1323 cm^{-1} , and 1401 cm^{-1} showed lower contributions, indicating relatively smaller contributions to treatment differentiation (Larkin, 2017).

Furthermore, cluster analysis supported the PCA findings by grouping samples based on similarities in their chemical profiles. Hierarchical clustering using Euclidean distance and Ward's method revealed distinct clusters corresponding to the treatments, supporting the finding that delignification affected the chemical characteristics of the samples. Cluster analysis is commonly used alongside PCA to validate grouping patterns and improve interpretation of multivariate data.

Overall, these results suggest that the differences among treatments were primarily associated with variations in alcohol, alkyl, and

ester functional groups. The *T. viride* treatment exhibited the most pronounced differences, indicating that biological delignification can modify the chemical profile of patchouli leaves and potentially increase patchouli alcohol content. This finding highlights the importance of pretreatment methods in improving essential oil extraction efficiency and quality.

The score plot analysis revealed clear separation among patchouli leaf treatments based on their FTIR spectra. The wavenumber at 1013 cm^{-1} (C–O alcohol) contributed most strongly to PC1, followed by 2924 cm^{-1} ($-\text{CH}_2/-\text{CH}_3$), 1256 cm^{-1} (C–O ester/secondary alcohol), and 2866 cm^{-1} (aliphatic C–H). In contrast, the bands at 1377 cm^{-1} , 1323 cm^{-1} , and 1401 cm^{-1} showed smaller contributions because of their relatively uniform intensities across all samples (Figure 2).

Overall, these results indicate that the main differences among treatments were driven by alcohol, alkyl, and aromatic groups, with the *Trichoderma viride* treatment exhibiting the most significant changes. This finding suggests that delignification with *T. viride* can modify the chemical profile of patchouli leaves and enhance patchouli alcohol content (Muharam *et al.*, 2017).

GC-MS analysis (Figure 3) further showed that patchouli oil from different treatments exhibited relatively similar compound profiles across treatments, particularly within the retention-time range of 18–26 min. This interval corresponded to the elution of oxygenated sesquiterpenes such as patchouli alcohol, the major constituent of patchouli oil (Nurjanah *et al.*, 2020).

Patchouli alcohol (PA) was detected at a retention time of 24.554 min, consistent with literature values and exhibited the largest peak area among the detected compounds, suggesting that neither the control nor the biological treatments caused substantial degradation. The chromatogram profiles (Figure 3) were similar across all treatments, indicating no major differences in chromatographic patterns.

Quantitative GC-MS analysis further confirmed the presence of PA as the predominant compound, supporting the potential of *Trichoderma viride* as a biological pretreatment agent for optimizing patchouli oil extraction.

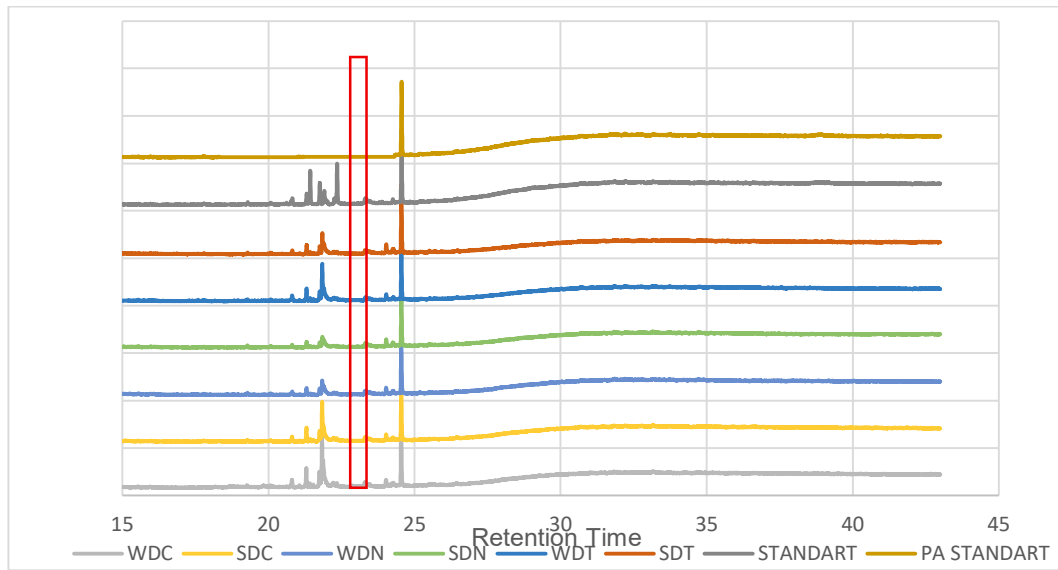


Figure 3. Overlaid GC-MS chromatograms of patchouli oil from different treatments

Note: WDC = Water distillation control; SDC = Steam distillation control; WDN = Water distillation with natural delignification; SDN = Steam distillation with natural delignification; WDT = Water distillation with *T. viride* treatment; SDT = Steam distillation with *T. viride* treatment.

Table 3. Selected Compounds Detected by GC-MS Across All Delignification and Distillation Treatments

Compound Name	RT	SI	Treatments (Peak Area)					
			WDC	SDC	WDN	SDN	WDT	SDT
Methanoazulene	20.815	91	242,837.67	203,091.33	226,486.67	187,694	145,193	NA
Caryophyllene	21.307	97	672,133.67	535,807	753,592.5	269,401	464,478	468,612.7
α -Guaiene	21.428	95	134,306.67	101,850	NA	NA	NA	NA
Seychellene	21.755	92	531,095.33	422,040.67	443,999.67	279,306	371,191	356,897
Azulene	21.95	92	110,467.33	100,608.5	NA	NA	187,880	NA
Patchouli alcohol	24.554	95	1,787,655.33	2,096,270.33	2,136,615	2,147,152.67	2,403,391.67	2,455,755

Note: RT = Retention Time; SI = Similarity Index; WDC = Water distillation control; SDC = Steam distillation control; WDN = Water distillation with natural delignification; SDN = Steam distillation with natural delignification; WDT = Water distillation with *T. viride* treatment; SDT = Steam distillation with *T. viride* treatment.

GC-MS analysis of patchouli oil (Table 3) identified six major compounds consistently detected across treatments, namely patchouli alcohol (PA), caryophyllene, seychellene, α -guaiene, azulene, and methanoazulene. The relative abundance of these compounds varied depending on the delignification treatment and distillation method, indicating that both pretreatment and extraction conditions influenced the chemical profile of patchouli oil.

Patchouli alcohol (PA) was the dominant compound in all treatments and showed the highest peak area in the SDT treatment (2,403,891.67), followed by other treatments with lower values, such as SDC (2,096,270.33). When expressed as relative composition (Table 4), PA increased from 37.96% in the control to 61.12%

after *Trichoderma viride* treatment, indicating a substantial enhancement in oil quality. This result is consistent with previous findings that biological pretreatment may improve essential oil yield and composition by disrupting lignocellulosic structures and facilitating the release of intracellular metabolites (Swamy & Sinniah, 2015; Zhao *et al.*, 2020).

Although the primary focus of this study was the enhancement of patchouli alcohol (PA) as the main quality indicator of patchouli oil, the GC-MS results also revealed that several other compounds experienced increases under certain treatments. In particular, methanoazulene and azulene showed higher abundance in selected treatment combinations, indicating that the delignification process influenced not only PA but

also other volatile constituents. These findings suggest that biological pretreatment contributed to a broader modification of the chemical profile, while the increase in PA remained the most significant and economically relevant outcome. Thus, improvements in oil quality were associated mainly with PA enrichment, accompanied by supportive changes in several minor compounds.

Caryophyllene exhibited the highest peak area in the WDC treatment (672,133.67), but its concentration decreased in biologically treated samples. In relative terms, this decrease suggests a shift in chemical composition rather than complete degradation, which may be attributed to changes in extraction efficiency or transformation into minor compounds. Similar trends have been reported where sesquiterpenes are reduced following biological or thermal treatments (Ismail *et al.*, 2020).

Seychellene showed a pattern similar to caryophyllene, with higher abundance in untreated samples and reduced levels after delignification. This reduction may indicate structural transformation or reduced volatility under treatment conditions, although specific reaction pathways were not directly confirmed in this study.

α -Guaiene was detected only in the control samples (WDC and SDC) and was absent in treated samples. This suggests that the compound is sensitive to processing conditions, particularly pretreatment and distillation, leading to reduced detectability in GC-MS analysis. Previous studies have also reported that certain sesquiterpenes are unstable under thermal or oxidative conditions (Zhao *et al.*, 2020).

Azulene was detected in limited treatments (WDC, SDC, and WDT), while absent in others, including SDT. Its inconsistent presence indicates sensitivity to both temperature and processing conditions. Similarly, methanoazulene was detected in most treatments except SDT, with the highest peak area observed in WDC (242,837.67). The absence of these compounds in certain treatments may reflect changes in volatility or transformation into undetected derivatives, rather than complete degradation.

Overall, the variation in compound profiles among treatments indicates that delignification and distillation methods significantly influence both the qualitative and quantitative composition of patchouli oil. Quantitative comparison between

peak area (Table 3) and percentage composition (Table 4) confirms that treatments not only affected absolute compound abundance but also altered the relative distribution of components within the oil.

The increase in PA content following *T. viride* treatment was consistent with previous reports highlighting the role of biological pretreatment in enhancing essential oil quality through improved accessibility of plant secondary metabolites (Narayanasamy *et al.*, 2012; Hardjo *et al.*, 2024). However, further studies involving direct enzyme activity assays are required to confirm the underlying mechanisms.

Differences in compound profiles among treatments can be attributed to pretreatment effects such as delignification or fermentation, which influence the release of active metabolites from plant tissue. Previous studies have shown that biological treatments, such as using fungi like *T. viride*, can enhance the availability of secondary metabolites. Although enzymatic activity was not directly measured in this study, the increase in secondary metabolites may be associated with the action of lignocellulose-degrading enzymes produced by *Trichoderma* spp. These enzymes catalyze the hydrolysis of β (1,4)-glycosidic linkages in cellulose, thereby leading to depolymerization of the polysaccharide matrix. Consequently, the disruption of the rigid and highly ordered crystalline structure of the lignocellulosic cell wall enhances porosity and reduces structural recalcitrance. This structural modification facilitates the release of intracellular essential oil components localized within the vacuoles, thereby improving mass transfer and increasing extraction efficiency during subsequent distillation (Zhao *et al.*, 2020).

As shown in Table 4, delignification with *T. viride* for five days significantly increased PA content to 61.12%, well above the SNI minimum standard (30%) and higher than the control without delignification (37.96%). This increase is associated with ligninolytic enzymes such as laccase, lignin peroxidase, and manganese peroxidase, which degrade cell wall lignin and facilitate the release of key secondary metabolites during distillation (Narayanasamy *et al.*, 2012; Hardjo *et al.*, 2024).

Therefore, biological delignification using *Trichoderma viride* may serve as an environmentally friendly approach to enhance the quality of patchouli oil.

Table 4. Effect of Delignification Treatments on Patchouli Alcohol (PA) Content in Aceh Patchouli Oil

Treatment	PA Content (%)
Control (no delignification)	37.96 ± 5.67 ^a
Natural delignification (5 days)	48.50 ± 6.11 ^{ab}
Delignification with <i>T. viride</i> (5 days)	61.12 ± 11.22 ^b

CONCLUSION

Biological delignification of *Pogostemon cablin* leaves using *Trichoderma viride* significantly increased patchouli alcohol (PA) content, reaching a maximum of 61.12%, while causing a reduction in some minor compounds, such as methanoazulene, caryophyllene, and α -guaiene. Delignification plays a key role in degrading the lignin matrix of trichome cell walls in *P. cablin* leaves, facilitating more efficient extraction of secondary metabolites, particularly PA, during distillation. Qualitative analyses using FTIR and GC–MS indicated that delignification primarily affects the quantitative aspect of extraction, enhancing the yield of target compounds without evident changes in the major functional groups detected by FTIR.

ACKNOWLEDGEMENTS

This research was funded by the Directorate General of Research and Development of the Ministry of Higher Education, Science, and Technology under the 2025 Master's Thesis Research Scheme (Contract No. 001/SP-Lit/LPPM-01/Diktisaintek/FTB/V/2025) (on behalf of Dr. Popy Hartatie Hardjo).

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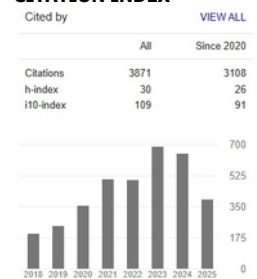
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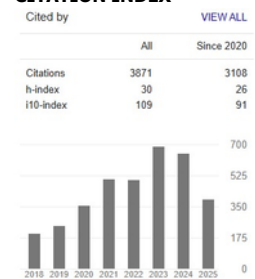
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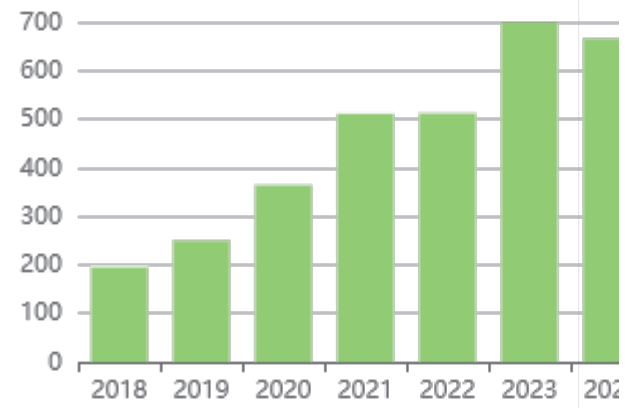
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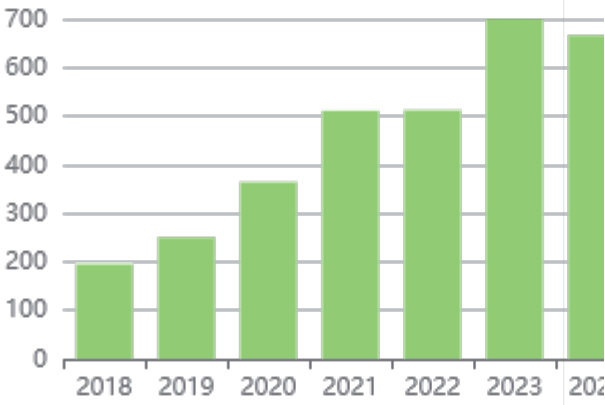
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