

Mengungkap Seskuiterpen Utama dalam Minyak Atsiri Daun Nilam Menggunakan GC-MS

Unveiling Major Sesquiterpenes in Patchouli Leaf Essential Oil Using GC-MS

Muhammad Taupik¹/Mohamad Adam Mustapa²/Fahmi As Radjak³/A. Mu'thi Andy Suryadi⁴/
Mohamad Aprianto Paneo⁵

^{1,2,3,4,5}Department of Pharmacy, Faculty of Sport and Health, Gorontalo State University

✉ Corresponding author: muhtaupik@ung.ac.id

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*Corresponding author

Muhammad Taupik, Department of Pharmacy, Faculty of Sport and Health, Gorontalo State University, E-Mail: muhtaupik@ung.ac.id

Abstrak

Nilam (*Pogostemon cablin* Benth.) merupakan tanaman tropis yang dikenal luas sebagai sumber utama minyak nilam, minyak atsiri dengan nilai ekonomi dan farmasi yang tinggi. Penelitian ini bertujuan untuk mengidentifikasi kandungan kimia minyak atsiri daun nilam dan menentukan senyawa utamanya. Ekstraksi dilakukan menggunakan pelarut dengan polaritas berbeda, dilanjutkan dengan analisis menggunakan Kromatografi Gas-Spektrometri Massa (KG-MS). Hasil penelitian menunjukkan lima senyawa dominan: Bisiklo[5.3.0]dekana, 2-metilen-5-(1-metilvinil)-8-metil (19,99%), Aromandendren (12,04%), Naftalena, dekahidro-4a-metil-1-metilen-7 (9,20%), Kariofilen (7,29%), dan Valerena-4,7(11)-diena (6,87%). Senyawa-senyawa ini berperan penting dalam aroma dan aktivitas biologis minyak nilam. Metanol, sebagai pelarut polar, menghasilkan rendemen tertinggi (10%), sementara n-heksana menghasilkan rendemen terendah (2%). Temuan ini menegaskan potensi minyak atsiri nilam sebagai sumber alami untuk aplikasi farmasi, kosmetik, dan aromaterapi.

Kata Kunci: Essential oil, *Pogostemon cablin* Benth., Patchouli, GC-MS

Abstract

Patchouli (*Pogostemon cablin* Benth.) is a tropical plant widely recognised as the main source of patchouli oil, an essential oil with high economic and pharmaceutical value. This study aimed to identify the chemical constituents of patchouli leaf essential oil and determine its major compounds. Extraction was performed using solvents of different polarities, followed by analysis with Gas Chromatography–Mass Spectrometry (GC-MS). The results revealed five dominant compounds: Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl (19.99%), Aromandendrene (12.04%), Naphthalene, decahydro-4a-methyl-1-methylene-7 (9.20%), Caryophyllene (7.29%), and Valerena-4,7(11)-diene (6.87%). These compounds play an important role in both the aroma and biological activities of patchouli oil. Methanol, as a polar solvent, produced the highest yield (10%), while n-hexane yielded the lowest (2%). The findings confirm the potential of patchouli essential oil as a natural source for pharmaceutical, cosmetic, and aromatherapy applications.

Keywords: Essential oil, *Pogostemon cablin* Benth., Patchouli, GC-MS

BACKGROUND

Traditional medicine in Indonesia is the result of the integration of local knowledge, culture, and empirical practices that have been passed down from generation to generation. The utilisation of medicinal plants is very diverse, along with Indonesia's biological wealth which has great potential to be developed as a source of pharmaceutical, food and cosmetic raw materials. One of the secondary metabolites that is widely researched is essential oil, because it is volatile, has a distinctive aroma, and has biological activities such as antioxidants, anti-inflammatory, and antimicrobial (Capellades et al 2020).

The demand for essential oils has increased rapidly in recent years, as the cosmetic, perfume, food, pharmaceutical, and aromatherapy industries have grown. Indonesia is one of the world's major producers of essential oils, with leading commodities such as patchouli, citronella, nutmeg, cloves, and eucalyptus (Daniati, E et al, 2021). Among these commodities, patchouli (*Pogostemon cablin* Benth.) has strategic value because it produces patchouli oil, which is the main ingredient in the global perfume and cosmetics industry.

Patchouli oil is mainly obtained from the leaves of 6-8 month old plants through a distillation process. The main contents are patchouli alcohol (30-35%), α -patchoulene, α -bulnesene, and norpatchoulene. Patchouli alcohol is reported to play an important role as a dominant compound with antioxidant, antiseptic, and anti-inflammatory

activities (Das, G et al, 2021) In addition, variations in the chemical composition of patchouli oil can be influenced by environmental factors, extraction methods, and the type of solvent used (Edim, M, 2021)

Although patchouli oil has been widely utilised, scientific data regarding the identification of its bioactive compounds, especially from the Gorontalo region, is still limited. Therefore, this study aims to identify the chemical components of patchouli leaf essential oil using the Gas Chromatography-Mass Spectrometry (GC-MS) method, so as to provide scientific information that supports the wider use of patchouli oil in the pharmaceutical, cosmetic, and aromatherapy fields.

METHODS

Instruments and Materials

This study used a blender (Philips, Indonesia) for simplisia grinding, analytical scales (Ohaus Pioneer PA214, USA) for sample weighing, measuring cups, beakers, and separating funnels (Pyrex, USA), and a glass maceration vessel. The extraction process was carried out at the Laboratory of Pharmaceutical Chemistry, Department of Pharmacy, Gorontalo State University. Solvent evaporation was performed using a rotary evaporator (Buchi R-210, Switzerland). The main material was fresh patchouli (*Pogostemon cablin* Benth.) leaves obtained from Marisa region, Pohuwato Regency, Gorontalo Province. The chemicals used consisted of methanol (p.a., Merck, Germany), n-hexane (p.a., Merck, Germany), ethyl acetate (p.a., Merck, Germany), 70% and 96% ethanol (Brataco, Indonesia), ammonia (p.a., Merck, Germany), and distilled water. Identification of essential oil compounds was conducted using Gas Chromatography–Mass Spectrometry (GC-MS, Agilent Technologies 7890A GC System with 5975C MSD detector, USA) at the Integrated Laboratory, Universitas Padjadjaran.

Plant Determination

The botanical determination of the patchouli plant was carried out at the Laboratory of Pharmacy, Gorontalo State University. Based on morphological examination and comparison with taxonomic references, the sample was confirmed as *Pogostemon cablin* Benth. (Lamiaceae). The determination certificate was issued under number B/86/UN47.17/LABFARM/TA/00.03/2025.

Simplisia Preparation

The patchouli leaves selected were fresh green old leaves, not yellowed, and free of mould. Picking was done in the morning at 09.00-11.00 WITA. After harvesting, the leaves were washed using running water, drained, chopped, and dried in the shade without direct exposure to sunlight. The dried leaves were ground with a blender to a fine powder, sieved, and stored in a closed container at room temperature.

Extract Preparation

Extraction was carried out by multistage maceration method using solvents with different polarities, namely n-hexane (non-polar), ethyl acetate (semi-polar), and methanol (polar). The maceration process lasted for three times twenty-four hours with occasional stirring. The filtrate from maceration was filtered using Whatman No. 42 filter paper, while the residue was re-soaked with fresh solvent until saturated. The filtrate of each solvent was evaporated with a rotary evaporator at low pressure to obtain a thick extract.

Determination of yield

The extract yield is calculated by comparing the weight of the thick extract obtained with the initial weight of the simplisia, then expressed in per cent (% w/b). This calculation is used to assess the efficiency of the extraction process [2],[13].

GC-MS Analysis

Analysis of chemical compounds was performed using GC-MS with an HP-5MS capillary column (30 m × 0.25 mm). The injector temperature was set at 250 °C with helium carrier gas at a flow rate of 1 mL/min. The temperature programme was started from 50 °C for five minutes, then increased gradually by 10 °C per minute to 280 °C and maintained for fifteen minutes. The detected compounds were identified based on retention time and mass spectra, then compared with the NIST library and previous research reports (Eiska, L.R. et al, 2021). GC-MS method was chosen because of its sensitivity and accuracy in the analysis of volatile compounds in essential oils (Fachrudin., 2016)

RESULTS AND DISCUSSION

Characteristics of Patchouli Leaf Samples

The samples used in this study were patchouli leaves (*Pogostemon cablin* Benth.) obtained from the Marisa region, Pohuwato Regency, Gorontalo Province. The determination results showed that the samples used were correctly included in the *Pogostemon cablin* Benth species. Patchouli leaves have distinctive morphological characteristics in the form of an oval to oval shape, serrated edges, smooth hairy surface, dark green colour, and a distinctive sharp aroma. Leaf length ranges from 5-10 cm with pinnate leaf bones.

These characteristics are in accordance with the report of Parinduri et al. who explained that patchouli leaf morphology includes an oval to oval shape with a smooth hairy surface and dark green colour. These morphological characteristics become one of the important parameters in botanical identification due to similarities with other species such as *Pogostemon heyneanus* which is often used as a raw material for essential oils (Hashiesh, H et al 2021). Thus, the selection of patchouli leaves as samples in this study can be ensured to have appropriate taxonomic validity.

In addition, agro-climatic factors in the Pohuwato region are thought to contribute to the quality of secondary metabolites in patchouli leaves. As reported in previous studies, environmental conditions such as rainfall, humidity,

light intensity, and soil type affect essential oil content and variations in bioactive compounds produced. This is important because variations in chemical composition are often found in patchouli oil from various producing regions in Indonesia, including Aceh, North Sumatra, and Java (Kakanopas, P, 2022)

Extraction Results and Yield

The multistage extraction carried out on patchouli leaf simplisia resulted in differences in colour, consistency, and yield in each type of solvent. The n-hexane extract was light yellow to brown in colour with a yield of 2% (10 g of 500 g dry simplisia). The ethyl acetate extract produced a golden brown colour with a yield of 3% (15 g), while the methanol extract gave a dark reddish brown colour with a yield of 6% (30 g). In addition, total extraction with methanol solvent gave the highest yield, which was 10% (50 g). These results indicate that methanol as a polar solvent is more effective in extracting secondary metabolites of patchouli leaves than semi-polar or non-polar solvents. **Table 1** presents data on the calculation of patchouli leaf extract yield based on the type of solvent used.

Table 1. Patchouli leaf extract yield based on solvent type

Solvent	Extract Weight (g)	Yield (%)	Extract Colour
n-Hexane	10	2	Light yellow - brown
Ethyl acetate	15	3	Golden brown
Methanol fraction	30	6	Dark brown - reddish
Total methanol	50	10	Deep dark brown

These results are consistent with the report of Daniati et al. which shows that polar solvents such as methanol are better able to dissolve secondary metabolite compounds, including essential oils, than non-polar n-hexane. Other studies also confirm that differences in solvent polarity greatly affect the effectiveness of extraction and the amount of compounds extracted from plant simplisia (Khanifah, 2021)

In general, the high yield in polar solvents indicates that most of the secondary metabolites of patchouli leaves are polar to semi-polar. This condition supports the report of Van Beek & Joulain which states that the essential oil content of patchouli besides patchouli alcohol also includes various polar and semi-polar sesquiterpenes, so that the use of methanol can increase extraction efficiency. Thus, this data confirms that methanol is the best solvent to obtain patchouli leaf extract with maximum results.

Essential Oil Compound Profile Based on GC-MS Analysis

Analysis of the chemical composition of patchouli leaf essential oil was conducted using Gas Chromatography-Mass Spectrometry (GC-MS, Agilent Technologies 7890A GC System with 5975C MSD detector, USA). This method allows the separation of volatile compounds based on differences in retention time, then identifies the molecular structure through mass spectra. The analysis results are displayed in the form of a chromatogram with several peaks representing volatile compounds in essential oils. From the analysis, five dominant compounds with the highest percentage of area were detected. (Kouao, T, et al 2023)

Table 2. Results of GC-MS analysis of patchouli leaf essential oil

No.	Retention Time (minutes)	Compound Name	Area ($\times 10^8$)	Area (%)
1	10,999	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl	1,44	19,99
2	10,945	Aromandendrene	8,65	12,04
3	10,818	Naphthalene, decahydro-4a-methyl-1-methylene-7	6,61	9,20
4	10,548	Caryophyllene	5,24	7,29
5	10,268	Valerene-4,7(11)-diene	4,93	6,87

The results in **Table 2** and **Figure 1** show that the compound with the highest concentration is Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl (19.99%), followed by Aromandendrene (12.04%), Naphthalene, decahydro-4a-methyl-1-methylene-7 (9.20%), Caryophyllene (7.29%), and Valerene-4,7(11)-diene (6.87%). These compounds generally belong to the sesquiterpene class, which is known to play a role in providing the distinctive aroma and biological activity of patchouli essential oil (Parinduri, W et al 2023)

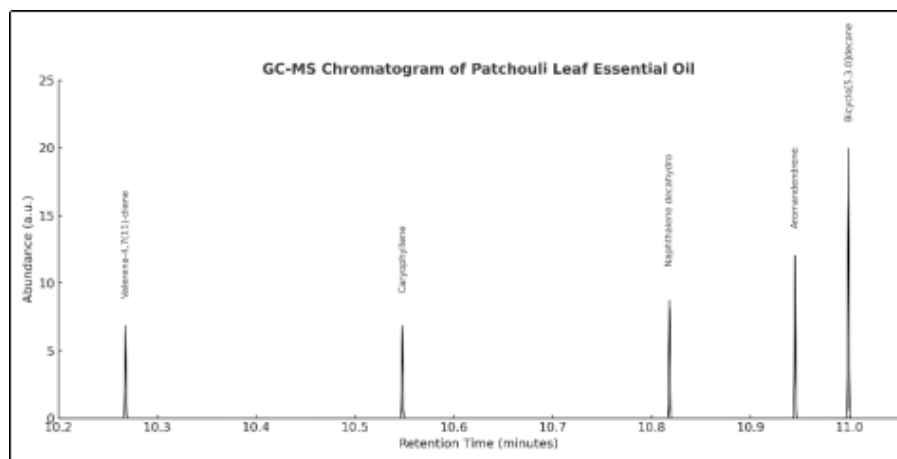


Figure 1. GC-MS chromatogram of patchouli (*Pogostemon cablin* Benth.) leaf essential oil showing five dominant compounds.

The dominance of sesquiterpene compounds in patchouli oil is in accordance with the reports of Van Beek & Joulain and Variyana et al. which states that the main content of patchouli oil comes from the sesquiterpene group, especially patchouli alcohol, α -bulnesene, and α -patchoulene. Differences in relative concentrations between compounds are most likely influenced by environmental factors, harvest age, and extraction conditions.

The GC-MS method has proven to be very effective for the analysis of volatile compounds because it is able to separate peaks with high accuracy and compare mass spectra with the NIST library. This supports the utilisation of GC-MS as a standard method in essential oil analysis, including patchouli oil, due to its sensitivity and accuracy in identifying complex compounds (Prévost, S, 2023).

Main Compounds of Patchouli Leaf Essential Oil

GC-MS analysis of patchouli (*Pogostemon cablin* Benth.) leaf essential oil identified five main compounds with the highest relative concentrations, namely *Bicyclo[5.3.0]decane*, *2-methylene-5-(1-methylvinyl)-8-methyl, Aromandendrene*, *Naphthalene, decahydro-4a-methyl-1-methylene-7*, *Caryophyllene*, and *Valerene-4,7(11)-diene*. These compounds belong to the sesquiterpene group which are known to play an important role in the aroma and biological activity of essential oils (Rambe, R. et al 2021)

Bicyclo [5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl

This compound is the dominant component with a concentration of 19.99%. Its bicarocyclic structure provides molecular stability as well as a sharp characteristic aroma. Based on the mass spectrum, this compound shows a main peak at m/z 93, with distinctive fragments at m/z 41, 55, 67, 107, and 204 that match the characteristics of sesquiterpenes [6]. The presence of this compound was also reported by Fachrudin[6] in hydrodistilled patchouli leaf essential oil, which confirmed that this compound is an important component in determining the quality of patchouli oil.

Aromandendrene

Aromandendrene was detected with a percentage of 12.04% and a retention time of 10.945 minutes. This compound belongs to the group of tricyclic sesquiterpenes with the molecular formula $C_{15}H_{24}$. Its biological activities include anti-inflammatory, analgesic, antioxidant, antibacterial, as well as natural insecticidal potential. These results are in accordance with the reports of Van Beek & Joulain and Zhao et al. who found aromandendrene as a typical component in patchouli essential oil and other plants from the Lamiaceae family.

Naphthalene, decahydro-4a-methyl-1-methylene-7

This compound accounts for 9.20% of the total essential oil components with a mass spectrum displaying a base peak at m/z 122. This fully hydrogenated naphthalene structure contributes to the volatility and characteristic aroma of patchouli oil. A similar compound was also reported by Daniati et al. who found concentrations close to 8.64% in patchouli oil from Aceh. Apart from being aroma-determining compounds, these naphthalene derivatives are also known to have potential antibacterial and antioxidant activities (Sari, N. M et al, 2023)

Caryophyllene

Caryophyllene was detected with a concentration of 7.29%. This compound is a cyclic sesquiterpene with the molecular formula $C_{15}H_{24}$ and is widely known for its pharmacological activities as anti-inflammatory, antimicrobial, as well as its potential as a selective ligand on CB2 receptors. These findings are in line with the studies of Variyana et al. [17] and Kouao et al. [10], which reported caryophyllene as one of the important components in essential oils of various species. The content of caryophyllene in patchouli oil confirms the pharmacological added value of this commodity.

Valerene-4,7(11)-diene

This compound was found at a concentration of 6.87%. Valerene-4,7(11)-diene has a sesquiterpene structure with cyclohexane and cyclobutane rings, giving it volatile properties and a characteristic aroma. Mass spectrum analysis showed a base peak at m/z 161, consistent with the report of Das et al. This compound is reported to have

sedative and neuroprotective effects, making it relevant for the development of aromatherapy and pharmaceutical products.

Overall, these five major compounds contribute to the aroma quality of patchouli oil while strengthening its potential in the pharmaceutical, cosmetic, and aromatherapy fields. The predominance of sesquiterpenes in the results of this study also confirms conformity with international literature regarding the chemical profile of patchouli oil.

Pharmacological Implications and Comparison with Previous Studies

The results of GC-MS analysis show that patchouli (*Pogostemon cablin* Benth.) leaf essential oil is rich in sesquiterpene compounds, especially Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl, Aromandendrene, Naphthalene, decahydro-4a-methyl-1-methylene-7, Caryophyllene, and Valerena-4,7(11)-diene. The presence of these compounds makes an important contribution not only to the characteristic aroma of patchouli oil, but also to its biological activities.

Pharmacologically, the sesquiterpene derivative bicyclo[5.3.0]decane is known to have antioxidant activity which supports its use in the cosmetic industry. Aromandendrene is reported to be anti-inflammatory, analgesic, antibacterial, and acts as a potential natural insecticide in pest control. Hydrogenated naphthalene derivatives contribute to antibacterial and antioxidant properties which are relevant for natural antiseptic applications. Meanwhile, caryophyllene is known as a CB2 receptor selective ligand with anti-inflammatory and analgesic activities so it may play a role in pain and inflammation therapy. Meanwhile, valerene-4,7(11)-diene is reported to have sedative and neuroprotective effects which supports its potential as an aromatherapy agent and support for sleep disorder therapy.

This finding is consistent with the results of Van Beek & Joulain who reported the dominance of sesquiterpenes in patchouli oil from various regions, especially patchouli alcohol, α -bulnesene, and α -patchoulene. However, the results of this study showed a difference in proportion, where the compound bicyclo[5.3.0]decane was more prominent than patchouli alcohol. This difference can be caused by environmental factors, harvest age, and variations in extraction methods, as reported by Daniati et al. and Suarantika et al.

In addition, these results also support the study of Variyana et al. who found that the use of a combination of water and steam distillation methods affected the chemical composition of patchouli oil, especially the sesquiterpene content. Thus, the composition of patchouli oil from Gorontalo analysed in this study confirms that local agro-climatic conditions can produce different chemical profiles compared to other patchouli producing regions in Indonesia.

From a pharmacological perspective, the presence of these compounds strengthens the potential of patchouli oil as a pharmaceutical, cosmetic, and aromatherapy raw material. The antioxidant, antibacterial, anti-inflammatory, and sedative activities of the dominant compounds in patchouli oil indicate a great opportunity to be developed as a multifunctional natural ingredient. This is in line with the global trend in utilising essential oils as an alternative to nature-based products with higher safety than synthetic materials

Research Limitations

This study has several limitations that need to be considered in interpreting the results. The patchouli (*Pogostemon cablin* Benth.) leaf samples used only came from one location and one harvest season, so they do not represent variations between regions or seasons. The extraction method applied is multistage maceration, not distillation, so the profile of the compounds obtained may differ from pure essential oil. The identification of compounds by GC-MS is also still limited to NIST library matching without verification using authentic standards or calculation of retention indices, so the accuracy of identification and quantification is relatively not optimal. In addition, this study did not include analytical replication or method validation, so data precision and reliability of results still need to be improved

CONCLUSION

GC-MS analysis of patchouli (*Pogostemon cablin* Benth.) leaf essential oil successfully identified five dominant sesquiterpene compounds: Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl (19.99%), Aromandendrene (12.04%), Naphthalene, decahydro-4a-methyl-1-methylene-7 (9.20%), Caryophyllene (7.29%), and Valerena-4,7(11)-diene (6.87%). Methanol was found to be the most effective solvent, yielding 10% extract compared with lower yields from non-polar solvents. These findings confirm the potential of patchouli essential oil as a multifunctional natural resource with applications in pharmaceuticals, cosmetics, and aromatherapy. Further research is recommended to optimise extraction methods, validate GC-MS analysis, and evaluate direct pharmacological activities to strengthen its scientific and industrial utilisation

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