

Antimicrobial activity of Patchouli essential oil (*Pogostemon cablin* (Blanco) Benth.) against skin microbiome

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Abstract: Patchouli Essential Oil (PEO) is derived from the leaves of the patchouli species (*Pogostemon cablin*) and is renowned for its distinct fragrance and therapeutic properties. PEO exhibits potent antibacterial activity against various skin pathogens implicated in acne, dermatitis, dandruff, and other skin infections. This study aims to standardize and determine the potential of PEO to inhibit bacteria and fungi that cause skin problems. Standardization of patchouli essential oil (PEO) by the requirements of the Indonesian National Standards (SNI) for patchouli oil (06-2385-2006) shows that seven test characteristics meet the requirements, including organoleptic, specific gravity, solubility in ethanol, acid number, ester number, optical rotation, and patchouli alcohol content. The antibacterial and antifungal activity test was carried out qualitatively by measuring the diameters of the inhibition zones using the agar disc diffusion method, quantitatively by determining the value of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) using the broth microdilution method and agar diffusion method, respectively. PEO has strong antibacterial and antifungal activity against *Propionibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 1228, and *Malassezia furfur* ATCC 14521 with MIC–MBC values of 312.5 µg/mL, 156.25–312.5 µg/mL, 187.5–375 µg/mL, and 900–>1000 µg/mL respectively. Based on the TLC-Bioautography test, compounds with antibacterial activity are considered sesquiterpenes.

1. INTRODUCTION

Patchouli, a perpetual fragrant herb belonging to the family Lamiaceae, is characterized by its branched and erect growth habit. To thrive, this plant necessitates deep, well-drained, fertile, slightly acidic, and composed of deep loamy soil with a high humus content and nutrients. The essential oil derived from the shade-dried leaves of patchouli is obtained either through steam distillation or hydrodistillation using a Clevenger apparatus (Das, 2015; Donelian *et al.*, 2009). In commercial practice, the oil content of the dried leaves usually 2.5–3.5%. The oil is viscous, red-brown, and has a camphor scent. Patchoulol, also known as patchouli alcohol (C₁₅H₂₆O), constitutes the primary chemical elements contributing to the aromatic essence of the oil. Other

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minor constituents like caryophyllene, α , β -patchoulene, pogostol, and seychellene are also present (Das, 2015).

Based on its multicomponent nature, patchouli essential oil has diverse applications in aromatherapy and exhibits antidepressant, anti-inflammatory, antibacterial, and antioxidant properties (Wei & Shibamoto, 2007; Xian *et al.*, 2011). Several in vitro antimicrobial tests with molecular docking have been carried out using samples of patchouli essential oil against six different types of bacteria, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus proteus*, *Shigella dysenteriae*, *Typhoid bacillus*, and *Staphylococcus aureus*. It was concluded that patchouli essential oil has good antibacterial activity with MIC values of 0.45–7.5 mg/mL and MBC values of 0.8–10 mg/mL (Junren *et al.*, 2021). Apart from its antibacterial properties, patchouli essential oil has antifungal and antiseptic properties, so it is effective in treating various skin conditions such as eczema, dermatitis, psoriasis, and acne (Srivastava *et al.*, 2022).

Skin is an ecosystem in the human body that provides a habitat to various microorganisms that derive nutrition from skin cells and secretions such as sweat and sebum. The fundamental function of the skin is to act as a physical barrier, guarding against potential invasion by poisonous or foreign organisms. The skin serves as a barrier between the inside and the outside body and a habitat for various microorganisms, including bacteria, fungi, viruses, and mites (Eisenstein, 2020). The skin's microorganisms can be beneficial, but they can potentially be harmful to others. The skin has physical and chemical properties that allow selected microorganisms to adapt and inhabit because of the skin's variation characteristics, including skin temperature, humidity, sebaceous gland density, and pH (Carmona-Cruz *et al.*, 2022). The characteristics of the skin and microbiota vary from one part of the body to another. Microorganisms will flourish in a given area of the skin depending on nutrients and moisture availability. *Corynebacterium* and *Staphylococcus* are most frequently found in humid regions. The skin can also hold viruses and fungi, the most prevalent of which is *Malassezia*, associated with dandruff and seborrheic dermatitis (Leung, 2018).

In this paper, the microorganisms discussed are *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Malassezia furfur*, an abundant microorganism in our body. *Propionibacterium acnes* is the primary associated bacterium that causes acne. The fundamental pathophysiological process is believed to encompass the stimulation of sebum production by androgens, changes in keratinization, inflammation, and an imbalance in the skin microbiota. It inhabits sebaceous follicles harboring microcomedones, thus creating an anaerobic and lipid-rich setting for the bacterium. Releasing various enzymes like hyaluronidases, lipases, and proteases leads to local damage and inflammation. (Schommer & Gallo, 2013). *Staphylococcus* sp. are spherical bacteria that group in clusters and are classified as gram-positive. Non-pathogenic *Staphylococcus* sp are identified by their lack of ability to produce coagulase, an enzyme crucial for virulence. The most common coagulase-negative commensal species include *S. epidermidis* and *S. hominis*. Sometimes, these bacteria can lead to healthcare-associated infections in individuals with implanted medical devices like heart valves and intravenous catheters. *S. aureus* has a high affinity for well-differentiated keratinizing cells, particularly those from patients with atopic dermatitis, indicating its preference for binding to these cells (Chiller *et al.*, 2001). *S. aureus* is among the most prevalent bacterial pathogens affecting the human population, responsible for various infections such as infective endocarditis, skin and soft tissue infections, and numerous other ailments (Taylor & Unakal, 2023). Various fungi, such as *Malassezia* sp. and *Candida* sp. have also been recognized as commensal organisms on the human skin. *Malassezia* is associated with the development of conditions such as head and neck dermatitis, seborrheic dermatitis, pityriasis versicolor, and *Malassezia* folliculitis (Kühbacher *et al.*, 2017; Saunte *et al.*, 2020).

The main aim of this study is to standardize and evaluate the effectiveness of PEO against a range of bacterial strains, including common skin pathogens, and determine the critical chemical constituents responsible for the antibacterial properties of PEO.

2. MATERIAL and METHODS

2.1. Essential Oil Standardization

PEO is standardized following those determined by the National Standardization Agency of Indonesia with types of tests including organoleptic examination, determination of specific gravity, refractive index, acid number, ester number, solubility in 70% ethanol, and the specific content of the oil through analysis of the Gas Chromatography-Flame Ionization Detector (GC-FID) instrument.

2.2. Antimicrobial Activity

Based on the Clinical and Laboratory Standards Institute (CLSI) M100 30th edition, performance standards for agar and broth dilution antimicrobial susceptibility tests were carried out. Microbial suspension was prepared to be equal to Mc farland 0.5 turbidity standard. For bacteria, the turbidity of the suspension was measured using UV-Vis spectrophotometry with a wavelength of 625 nm to obtain an absorbance value in the range of 0.08–0.13, which is equivalent to 0.5 Mc Farland or 1×10^8 CFU/mL. For fungi, the turbidity of the suspension was measured using UV-Vis spectrophotometry with a wavelength of 530 nm to obtain an absorbance value in the range of 0.08–0.13, which is equivalent to 0.5 Mc Farland or $1-5 \times 10^6$ CFU/mL.

The bacteria suspension diluted before 10^{-1} , 10^{-2} , and 10^{-3} (1 mL of 10^8 cells /mL) was spread on the surface of a sterile petri dish, and then 15 mL of MHA (Mueller Hinton Agar) was allowed to solidify. Filter paper discs (6 mm in diameter) were soaked in 10 μ L of the essential oil placed on the inoculated plates and allowed to dry for 15 minutes, then incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters. A dilution broth susceptibility assay was used for determination MIC. Stock solutions of the essential oils were prepared in DMSO 1%, Tween 80 1.5%, sterile distilled water, then diluted to several concentrations (%v/v).

The fungi suspension standardized to 0.5 McFarland standard is spread into a sterile petri dish, and then 15 ml of Sabouraud Dextrose Agar (SDA) is allowed to solidify. Filter paper discs were soaked in 10 μ L of the essential oil placed on the inoculated plates and allowed to dry for 15 minutes, then incubated at 32°C for 48–72 hours for *Malassezia fufur*. The diameters of the inhibition zones were measured in millimeters. Dilution broth susceptibility assay was used for determination MIC. Stock solutions of the essential oils were prepared in DMSO 1%, Tween 80 1.5% and sterile distilled water, then diluted to several concentrations (%v/v).

2.3. TLC-Bioautography

A total of 1000 μ L of microbe suspension (5×10^6 CFU/mL) was put into a sterile petri dish, and then 15 mL of sterile agar medium was added, homogenized, and solidified. Samples dissolved in a suitable solvent and spotted on a silica gel 60 F254 TLC plate then developed with a suitable mobile phase, hexane P-ethyl acetate P, (25:1). Once developed, the plate is dried and then placed on the surface of the agar containing the inoculum and left for 10–15 minutes at room temperature. Remove the plate and incubate at $35 \pm 2^\circ\text{C}$ for 16–20 hours. The inhibition zone formed is observed, and the Rf value is determined. The class of compounds from spots present on the TLC plate is determined simultaneously with the agar diffusion TLC-bioautography test to determine the class of antibacterial compounds by visualizing the spots under UV light λ 254 nm and 366 nm, then sprayed with vanillin H₂SO₄ spots.

3. RESULTS and DISCUSSION

3.1. Standardization of Patchouli Essential Oil

Based on the standardization of PEO, it shows in Table 1 that the determination of color, specific gravity, solubility in ethanol, acid number, ester number, optical rotation, and patchouli alcohol content meets the requirements. In contrast, determining the refractive index does not meet the SNI requirements for patchouli oil. The organoleptic properties of the oil include a

woody odor with a sweet and spicy aroma yellow-brown color, as shown in Figure 1. The qualitative quality of patchouli oil can be determined by testing its color following standard specifications. The color intensity of patchouli oil is determined by the amount of color pigment contained, time, and storage location. Iron (Fe) content is one of the causes of the dark color in patchouli oil, but other compounds such as free acids, free fats, dirt, and others can also cause the color of patchouli oil to darken. Therefore, high iron (Fe) content can cause the decomposition of organic matter and affect the quality of patchouli oil (Abram *et al.*, 2021).

Table 1. Standardization of PEO.

Characteristic	Requirement	Result*
	SNI (06-2385-2006)	
Organoleptic	Dark yellow to reddish brown	Yellow to brownish yellow, viscose liquid
Specific gravity (g/mL)	0.950 – 0.975	0.961 ± 0.004
Refractive index**	1.507 – 1.515	1.559 ± 3.606
Solubility in ethanol 90%	1 volume of no more than 10 volumes of 90% ethanol	1 volume no more than 10 volumes of 90% ethanol
Acid value	Max. 8	5.480 ± 0.260
Ester Value	Max. 20	10.660 ± 2.120
Optical rotation	(-)65° to (-)48°	(-)50°
Patchouli alcohol content (GC)	Min. 30%	31.568%

Notes: (*) carried out in triplicate, (**) does not meet the requirements

The specific gravity value of essential oils describes the chemical components contained in the oil. Patchouli oil contains a specific compound called patchouli alcohol ($C_{15}H_{26}O$). Hence, this compound has a relatively long molecular chain, which caused the oil to be dominated by high specific gravity patchouli alcohol components (Aisyah *et al.*, 2020). The constituent components of the essential oil influence the refractive index value. The longer the carbon chain and the more double bonds in the essential oil, the greater the refractive index value (Kuspradini *et al.*, 2021). Essential oils with oxygenated terpene, such as patchouli oil, are more soluble in alcohol than those with non-oxygenated terpene components due to the nonpolar nature of the latter, which lack functional groups, making it challenging to react with alcohol (Aisyah *et al.*, 2020). Acid number is a quality parameter for essential oils to determine the level of free fatty acids in the oil. Determination of the acid number is carried out by titration using a KOH base solution based on the quantity of KOH required to neutralize the free fatty acids present in 1 gram of patchouli oil. Determining the number of esters or bound fatty acids aims to calculate esterified glycerol so that it is a measure of the ester content contained in oil or fat (Handayani *et al.*, 2015). The determination of optical rotation is based on measuring the plane's angle, where polarized light is rotated by an oil layer that is 10 cm thick at a specific temperature. The research-derived patchouli oil exhibits a negative optical rotation, suggesting a higher presence of components that rotate the polarization plane to the left rather than to the right (Lubis *et al.*, 2022).



Figure 1. Visual appearance of Patchouli essential oil.

Analysis of PEO content was obtained based on the results of analysis carried out by PT SIL. This analysis was carried out using a combination of instruments to separate sample components in the form of a gas chromatograph connected to a Flame Ionization Detector (FID) detector as shown in Figure 2. FID is a universal detector that measure compounds containing carbon and hydrogen atoms. The detectors response intensity is determined by the quantity of carbon atoms capable of being oxidized in a molecule. Following passage through a column mixed with a carrier gas, the analyte is combusted in a reaction flame generated by the interaction of hydrogen and oxygen in the air. The current produced by the ionized compound is measured using an electrode (Kronstrand & Jones, 2000). The response produced via FID in the spectrum is the % area under the curve, which is used to calculate the component levels of PEO. Based on the results of the analysis of essential oil content using the GC-FID instrument as shown in Table 2, there are seven main components, namely b-patchoulene (2.794%), caryophyllene (3.354%), alpha guaiene (16.132%), alpha pathchoulene (7.854%), alpha bulnesene (19.115%), pogostole (1.769%), and patchouli alcohol (31.568%). The patchouli alcohol content value obtained shows that the PEO sample has a patchouli alcohol content by SNI requirements.

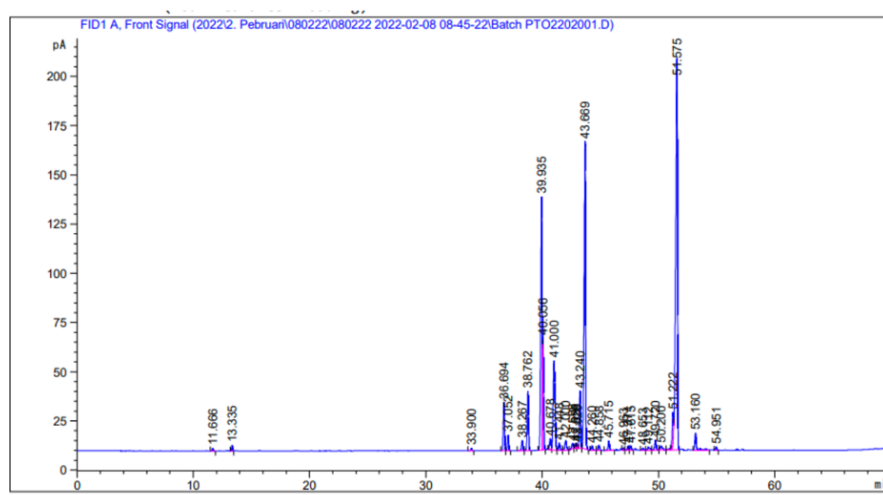


Figure 2. PEO content analysis using GC-FID.

Table 2. PEO content analysis using GC-FID.

Peak	Retention time (minutes)	AUC	%Area	Component
1	36.694	194.114	2.794	B-patchoulene
2	38.762	233.021	3.354	Caryophyllene
3	39.935	1120.660	16.132	A-guaiene
4	41.000	545.622	7.854	A-patchoulene
5	43.669	1327.938	19.115	A-bulnesene
6	51.222	122.938	1.769	Pogostole
7	51.575	2193.061	31.568	Patchouli alcohol

3.2. Standardization of Patchouli Essential Oil

In the in vitro assay, PEO exhibited potent activity against bacteria and fungi on the skin. A greater zone of inhibition suggests that the microbe is more susceptible to the antimicrobial agent present in the disc. As shown in Figure 3 and Table 3, PEO is considered more effective in inhibiting the growth of bacteria than fungi and showed the highest effect relative to its dilutions, except for the *S. epidermidis*, which has an inhibition zone larger at an oil concentration of 5% than it is at a concentration of 10%. The antibacterial activity (MIC) of pure compounds/extracts can be classified into three categories: <400 µg/mL strong effect, 400–800 µg/mL moderate effect, and >800 µg/mL weak impact (Taguri *et al.*, 2006). Based on MIC value as shown in Table 4, *S. aureus* is most susceptible to PEO due to its smallest MIC

value, while *M. furfur* is the most resistant with the largest MIC value. All bacteria tested were gram-positive bacteria. The cell wall of Gram-positive bacteria facilitates the entry of hydrophobic substances like essential oils into the cells, causing harm to both the cell wall and cytoplasm. Gram-positive bacteria feature a cell wall comprising approximately 90% peptidoglycan and a thin teichoic acid layer. Peptidoglycan regulates the passage of molecules into the cells, while teichoic acid forms an essential part of the peptidoglycan structure. Gram-positive bacteria's cell wall contains a higher proportion of the amino acid alanine, which aids in maintaining the stability of proteins in hydrophobic bacteria. Compared to Gram-negative bacteria, this allows for a stronger binding capacity with non-polar compounds such as essential oils. *M. furfur* is a type of yeast, not a bacterium, and it is often more resistant to certain treatments than bacteria. *Malassezia* has rigid cell wall components β -(1,6)-glucans, glycolipids, and glycoproteins, which can make them more resistant to some antibiotics (Vijaya Chandra *et al.*, 2021). The specific antibacterial mechanism of PEO has not yet been explained. Nevertheless, the intricate blend of terpenoids, phenolic acids, flavonoids, and phenylpropanoids in PEO has altered the potential mechanisms by which it inhibits microbial growth and reproduction (Maurya *et al.*, 2021).

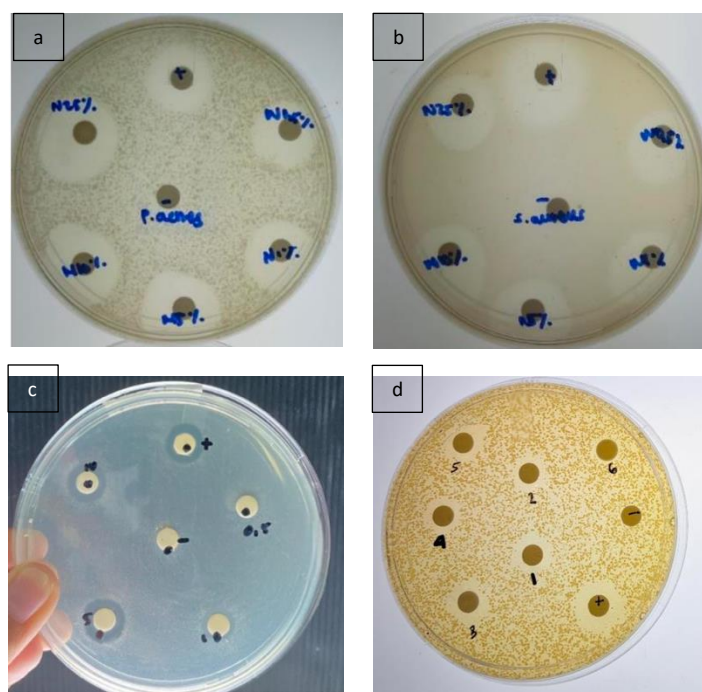


Figure 3. Disc diffusion test results of PEO against (a) *Propionibacterium acnes* ATCC 11827. (b) *Staphylococcus aureus* ATCC 6538. (c) *Staphylococcus epidermidis* ATCC 12228 (d) *Malassezia furfur* ATCC 14521.

Table 3. Disc diffusion test results of PEO for skin microbe in several concentrations (%v/v).

Bacteria	Diameters of the inhibition zones (mm)***					
	25%	10%	5%	1%	0.5%	Positive control*
<i>Propionibacterium acnes</i> ATCC 11827	21.82	21.34	18.01	16.84	16.21	17.10
<i>Staphylococcus aureus</i> ATCC 6538	20.82	19.92	19.46	18.46	16.65	21.05
<i>Staphylococcus epidermidis</i> ATCC 12228	-	10.95	11.40	0.00	0.00	14.18
Fungi	Diameters of the inhibition zones (mm)***					Positive control**
	25%	12.5%	6.25%	3.125%		
<i>Malassezia furfur</i> ATCC 14521	11.48	9.43	8.95	7.02		14.42

Note: (*) concentration of control (tetracycline HCl) 30 μ g/mL, (**) concentration of control (ketoconazole) 10 μ g/mL, (***) carried out in duplicate

Table 4. MIC, MBC, and MFC value of PEO.

Strain of microorganism	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	Category*
<i>Propionibacterium acnes</i> ATCC 11827	312.5	312.5	Strong
<i>Staphylococcus aureus</i> ATCC 6538	156.25	312.5	Strong
<i>Staphylococcus epidermidis</i> ATCC 12228	187.5	187.5	Strong
<i>Malassezia furfur</i> ATCC 14521	970	>3000	Weak

3.3. TLC-Bioautography

Determination of the chromatogram profile of PEO was carried out to see the content of compounds in the oil, especially compounds in the terpenoid group, namely monoterpenoids and sesquiterpenoids, using vanillin sulfate spotting. Based on the monitoring results of PEO, it can be seen that the compound can be observed under UV light λ 254 nm and visible light after being sprayed with visible spots of 10% vanillin sulfate in ethanol at R_f 0.43; 0.33; and 0.17, which is purplish blue color so it is included in the group of terpenoid compounds. Based on the TLC-bioautography results in Figure 4, PEO showed an inhibitory area for antimicrobial compounds against *Propionibacterium acnes* ATCC 11827, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Malassezia furfur* by the R_f obtained on the terpenoid compound spots on the PEO chromatogram profile. Terpenoids in PEO significantly impact the fatty acids in the plasma membrane, changing the permeability, dynamicity, and leakage of cytoplasmic components (Maurya et al., 2021).

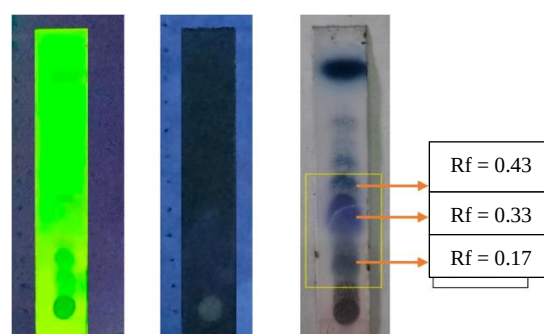


Figure 4. Chromatogram pattern of PEO, stationary phase TLC silica gel 60 F254, mobile phase hexane-ethyl acetate (25:1) in (a) 254 nm UV light (b) 366 nm UV light (c) appearance of vanillin H₂SO₄ spots.

4. CONCLUSION

In conclusion, the efficacy of PEO, derived from patchouli leaf, as an antimicrobial agent against the skin microbiome at a relatively low concentration suggests its importance as a key component of *P. cablin*. This indicates the potential of PEO as a novel antibacterial agent. Future investigations may concentrate on the formulation of pharmaceuticals, identification of molecular targets, and understanding the mechanisms of action of PEO, all of which are crucial for its future use in clinical application.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Aqila Hafizha Hanun: Analysis, Writing, and Editing original draft. **Miftahul Silvani** and **Andra Wahdini:** Analysis. **Elin Julianti** and **Afrillia Nuryanti Garmana:** Supervision and Methodology. **Muhamad Insanu:** Supervision, Methodology, and Funding.

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