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ANALYSIS OF ESSENTIAL OIL COMPOUNDS FROM EUCALYPTUS (*Eucalyptus pellita*) LEAVES AND THEIR BIOACTIVITY AGAINST *Staphylococcus aureus*

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Abstract

Eucalyptus (Eucalyptus pellita) is a plant commonly found in Garut Regency, Indonesia, with its leaves showing potential for essential oil extraction. Hence, this study aimed to determine the yield, analyze the chemical composition, and evaluate the bioactivity of essential oils derived from Eucalyptus pellita leaves against Staphylococcus aureus. The essential oil was extracted utilizing Stahl distillation from leaves collected in Cisarua Village, Samarang District, Garut Regency. The chemical composition was identified through Gas Chromatography-Mass Spectrometry (GC-MS), and its antibacterial activity against S. aureus was assessed using the disc diffusion method. As a result, the average essential oil yield was 0.34%, with compounds identified as α-phellandrene (3.20%), α-terpineol (5.52%), γ-terpinene (7.07%), 1,8-cineole (7.26%), α-terpinyl acetate (13.40%), and limonene (50.47%). The bioactivity test results indicated that the inhibition zone diameter at a 25% concentration of essential oil was categorized as moderate, while concentrations of 50% and 100% demonstrated strong antibacterial activity.

Keywords: *Essential oil; Eucalyptus pellita; Stahl distillation; Staphylococcus aureus; Garut Regency.*

Introduction

Eucalyptus is a plant species that varies in height, ranging from 4.6 meters to 97.5 meters (Khan, Hasan, & Khan, 2020). It comprises many species, including *Eucalyptus pellita*, *Eucalyptus grandis*, *Eucalyptus alba*, *Eucalyptus deglupta*, and *Eucalyptus urophylla*. Each species exhibits unique characteristics and properties (Saputra, 2023). Eucalyptus contains bioactive compounds that act as antioxidants and antibiotics, allowing it to address various illnesses, such as respiratory infections and cancer (Limam et al., 2020).

Eucalyptus plants can be found in Garut Regency, West Java Province, where the locals refer to them as *"Kalites"* or *"Kalices."* The species commonly found in this region is *Eucalyptus pellita*.

Eucalyptus pellita is one species known for its rapid growth compared to others. Its utilization has primarily been limited to its trunk, while its leaves and bark remain as waste (Saputra, 2023). The

leaves, however, hold potential for use in producing essential oil, which is known for its numerous benefits.

Studies have reported that the geographic location of the plant influences the chemical composition of essential oil. For instance, essential oil from *Eucalyptus globulus* leaves in Algeria differs in chemical composition from that in Tanzania. The Algerian oil primarily contains 1,8-cineole, isovaleraldehyde, α-terpineol, α-pinene, and spathulenol (Harkat-Madouri et al., 2015), while the Tanzanian oil predominantly features eucalyptol, α-pinene, γ-terpinene, β-myrcene, and terpinene-4-ol (Almas, Innocent, Machumi, & Kisinza, 2021).

Additionally, the plant species significantly influences its essential oil's chemical composition. For example, essential oils from *Eucalyptus citridora* and *Eucalyptus pellita* sourced from PT. Toba Pulp Lestari, Tbk. exhibit different chemical profiles. The essential oil of *E. citridora* contains α-pinene, 1,5-cyclooctadiene, eucalyptol, α-terpinyl acetate, trans-methyl dihydrojasmonate, and isopropyl (Sitohang, 2019). In contrast, *E. pellita* essential oil is composed of α-pinene, eucalyptol, α-terpineol, α-terpinolene, β-pinene, trans-caryophyllene, and globulol (Sembiring, 2019).

The objectives of this study included determining the yield of essential oil and analyzing the chemical composition of essential oil from *Eucalyptus pellita* leaves. Unlike the study conducted by Sembiring (2019), which used samples from PT. Toba Pulp Lestari, Tbk. in Medan, North Sumatra, the present research focused on samples collected from Cisarua Village, Samarang District, Garut Regency, West Java Province.

Essential oils are known for their antibacterial properties. Various studies have reported the antimicrobial activity of essential oils, demonstrating effectiveness against different microorganisms (Elaissi et al., 2011). For instance, an essential oil derived from citronella leaves exhibits bioactivity against *Escherichia coli* and *Staphylococcus aureus*, containing compounds such as trans-geraniol, beta-citronellol, citronella, cyclohexene, and alpha-amorphene (Sefriyanti, Jayuska, & Alimuddin, 2020).

This study also aimed to examine the bioactivity of essential oil from *Eucalyptus pellita* leaves against *Staphylococcus aureus*, a gram-positive bacterium that causes infections in the skin and invasive tissues (Bartlett & Hulten, 2010). It is also resistant to several antibiotics, including nafcillin, oxacillin, beta-lactamase, vancomycin, and methicillin (Jawetz, Melnick, & Adelberg, 2004). The increasing resistance of bacteria to antibiotics underscores the potential use of bioactive compounds in *Eucalyptus pellita* essential oil sourced from Cibunar Hamlet, Cisarua Village, Garut Regency, West Java Province.

Methodology

Tools and Materials

The tools and materials used in this study included aquadest, eucalyptus (*Eucalyptus pellita*) leaves sourced from Cibunar Hamlet, Cisarua Village, Samarang District, Garut Regency, Tween 80 (technical grade), anhydrous Na_2SO_4 pa (Merck), sterile aquabidest, 70% alcohol, cotton swabs, parafilm, parafilm disks, bacteriological agar (Oxoid), Mueller-Hinton agar (Oxoid), amoxicillin, *Staphylococcus aureus* ATCC 6538 (obtained from the Central Laboratory of Universitas Padjadjaran), various glassware, a blender, a Stahl distillation apparatus, a heating mantle, scissors, vial bottles, aluminum foil, spatulas, dropper pipettes, thermometers, analytical balances, cutters, 2000 mL round-bottom flasks, ice baths, micropipettes, an autoclave, calipers, vortex mixers, paper disks, an incubator, and a Shimadzu TQ8050 GC-MS instrument.

Procedures

Eucalyptus leaves preparation

The preparation began with collecting eucalyptus (*Eucalyptus pellita*) leaves, which were subsequently cleaned and air-dried. The dried leaves were

crushed until pulverized. A sample of 200 grams was weighed for a single distillation.

Essential oil extraction

200 grams of eucalyptus (*Eucalyptus pellita*) leaves were placed in a 2000 mL round-bottom flask. Sufficient aquadest (approximately 1400-1500 mL) was added to fully submerge the sample. The round-bottom flask was then connected to a Stahl distillation apparatus. The mixture was heated to boiling for approximately 5 hours at a temperature not exceeding 100°C, yielding the essential oil. The oil was separated from the distillate and mixed with anhydrous Na_2SO_4. The oil layer was then decanted, and its volume was measured (Sitohang, 2019).

Chemical content identification using GC-MS

A 1 µL sample was injected into the GC-MS using a syringe, with conditions tailored to the equipment. The chromatogram was then analyzed (Sitohang, 2019).

Antibacterial activity testing

The essential oil was prepared and divided into three variations: 1) Pure essential oil (100% concentration); 2) Essential oil mixed with Tween 80 at a 1:1 ratio (50% concentration); and 3) Essential oil mixed with Tween 80 at a 1:3 ratio (25% concentration).

The antibacterial activity of 100%, 50%, and 25% eucalyptus essential oil was tested using the paper disk method. Petri dishes containing agar media were equilibrated to room temperature for 10-15 minutes. Sterilized cotton swabs were dipped into the bacterial suspension and evenly spread on the agar surface, then left to stand for 5 minutes. Sterile paper disks were prepared, and 15 µL of the test suspension (sample) and positive control were dropped onto the disks. The disks were placed on the agar surface previously inoculated with bacteria. The plates were incubated at 37°C for 16-18 hours. The resulting inhibition zones were observed and measured using calipers.

Results and Discussion

Sample preparation

Figure 1. Eucalyptus (*E. pellita*) leaves

Eucalyptus leaves were obtained from eucalyptus *(E. pellita)* trees in Cibunar Hamlet, Cisarua Village, Samarang District, Garut Regency, West Java Province, Indonesia. The leaves were separated from the stems and petioles, cleaned of dust, and dried. The drying process aimed to reduce the moisture content in the leaves. The drying was carried out by air-drying at 25–27°C for 3 to 4 days. The dried samples were then ground using a blender. Grinding the leaves served to reduce their size, thereby increasing the surface area. This enlarged surface area could facilitate greater interaction between the leaves and distilled water during the extraction process (Senduk, Montolalu, & Dotulong, 2020).

Essential oil extraction

The method used for extracting essential oils was water distillation. The distillation apparatus employed in this study was a Stahl distillation unit. Water distillation is advantageous due to its simplicity, as the sample is merely immersed in water and boiled (Pratiwi & Utami, 2018). The water or distilled water (aquadest) used as a solvent must completely submerge the sample during the distillation process to ensure maximum essential oil extraction from the eucalyptus leaves.

The distillate obtained from the extraction process formed two layers. The upper layer was the essential oil, while the lower layer was water. These layers remained separate due to differences in

polarity and density. The essential oil layer, having a lower density than water, floated above the water layer. The extracted essential oil appeared slightly yellow, clear liquid with a distinct and strong aroma. The yield of essential oil obtained from eucalyptus *(E. pellita)* leaves is presented in Table 1 below.

Table 1. Yield of essential oil distillation from eucalyptus *(E. pellita)* leaves

No.	Sample Weight (g)	Essential Oil (mL)	Yield (%)
1	200	0.70	0.35
2	200	0.80	0.40
3	200	0.60	0.30
4	200	0.70	0.35
5	200	0.60	0.30
x̄ (Average)	200	0.68	0.34

The variations in essential oil yield, as shown in Table 1, were affected by the dryness of the leaves. A higher degree of dryness supports oil diffusion from the leaves into the distilled water during distillation. Thus, drier leaves produce more efficient oil extraction (Utomo & Mujiburohman, 2018).

The degree of dryness of eucalyptus leaves is affected by the drying duration. To achieve a high yield, the drying period should be optimized at 3 days. In this study, the leaves were dried for 3–4 days at 25–27°C. However, not all leaves were at their optimum drying period during the distillation process. Leaves exceeding the optimal drying time experienced oil evaporation, reducing the essential oil content in the leaves (Ratnaningsih, Insusanty, & Azwin, 2018).

The average yield of essential oil from the distillation process was 0.34%. This yield was higher than that reported by Anggraini, Khabibi, and Tamin (2019), which ranged between 0.080–0.130%.

Identification of chemical compounds using GC-MS

The essential oil obtained was analyzed using GC-MS (Gas Chromatography-Mass Spectrometry). It comprised gas chromatography (GC) for separation and mass spectrometry (MS) as a detector (Margareta & Wonorahardjo, 2023). Gas chromatography separates compound components based on their boiling points and polarity, followed by mass spectrometry (MS) to identify the chemical compounds. The gas chromatography results for eucalyptus leaf essential oil are shown in the chromatogram in Figure 2.

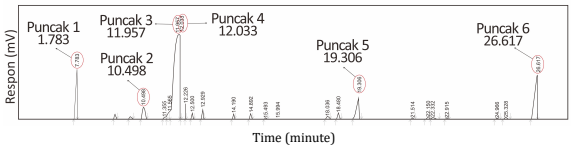

Figure 2. Gas Chromatography (GC) chromatogram of eucalyptus *(E. pellita)* leaf essential oil from Garut Regency

The chromatogram in Figure 2 revealed six main peaks representing the constituent compounds of eucalyptus leaf essential oil. The identity of each peak's compound was determined by analyzing the mass spectrometry results using a reference data library. Further information on the compounds represented by each peak is summarized in Table 2.

Table 2. Main components of eucalyptus *(E. pellita)* leaf essential oil

No.	Retention Time (minutes)	Area (%)	Name
1	7.789	7.07	γ-Terpinene
2	10.489	3.20	α-Phellandrene
3	11.957	50.47	Limonene
4	12.033	7.26	1,8-Cineole
5	19.306	5.52	α-Terpineol
6	26.617	13.40	α-Terpinyl Acetate

Based on Table 2, the retention time for each compound varied. This retention time serves as the identity of a compound, enabling differentiation between one

compound and another (Hashibuan et al., 2022). Differences in retention time among these compounds were caused by variations in their volatility, which were influenced by physical properties, specifically vapor pressure and boiling point. The lower the boiling point, the higher the volatility; conversely, the higher the boiling point, the lower the volatility (Dewi et al., 2018).

The six constituent compounds belonged to the monoterpenoid group, each with a distinct mass spectrum.

Antibacterial activity test

The extracted essential oil was tested for antibacterial bioactivity against *Staphylococcus aureus* using the disc diffusion method. *S. aureus* is a skin pathogen frequently affecting humans. The results of the antibacterial activity test are presented in Figure 3.

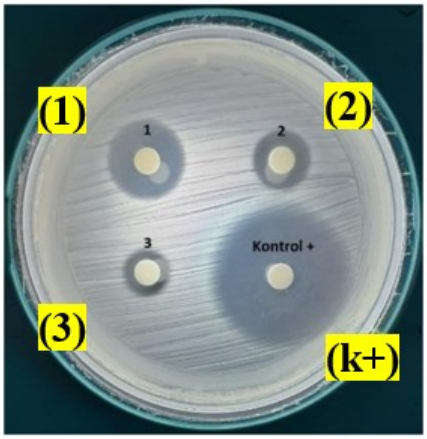

Figure 3. Antibacterial activity test results against *S. aureus:* (1) 100% essential oil sample; (2) 50% essential oil sample; (3) 25% essential oil sample; and (K+) Positive control

Figure 3 displays that the antibacterial test was conducted in duplicate using three essential oil concentrations and one control. The concentrations tested were 100%, 50%, and 25%, with amoxicillin as the positive control. Amoxicillin is a broad-spectrum antibiotic capable of inhibiting both Gram-positive and Gram-negative bacteria (Lestari & Maida, 2019). Tween 80 was used for dilution to prepare the three essential oil concentrations. Tween 80, a nonpolar surfactant, interacted with essential oils via lipophilic groups (Rahayu, Kiromah, & Maretha, 2021). The antibacterial activity of each essential oil concentration against *S. aureus* is detailed in Table 3.

Table 3. Inhibition zone diameter of essential oil

Sample	Essential Oil Concentration (%)	Inhibition Diameter (mm)	Strength
Sample 1	100	17.55	Strong
Sample 2	50	12.05	Strong
Sample 3	25	9.80	Moderate
Amoxicillin	0.01	31.40	Very Strong

The diameter of the inhibition zone is closely related to the strength of antibacterial activity. According to Davis and Stout (1971), an inhibition zone diameter greater than 20 mm is categorized as very strong, a range of 10–20 mm is categorized as strong, a range of 5–10 mm is categorized as moderate, and less than 5 mm is categorized as weak. Based on Table 3, the average inhibition zone diameter at various essential oil concentrations was categorized as strong for essential oil concentrations of 100% and 50%. Meanwhile, a 25% essential oil concentration was categorized as moderate. The higher the essential oil concentration, the larger the clear zone and the stronger the inhibitory effect. The results of this study were consistent with research conducted by Salehi et al. (2019), in which the eucalyptus leaf essential oil demonstrated a good ability to inhibit the growth of *S. aureus* bacteria and had significant potential as a microbiostatic agent.

Conclusion

The extraction of essential oil from eucalyptus (*Eucalyptus pellita*) leaves obtained from Cisarua Village, Samarang Subdistrict, Garut Regency, resulted in an average yield of 0.34%. Analysis using GC-MS identified the chemical compounds in

the oil, including α-phellandrene (3.20%), α-terpineol (5.52%), γ-terpinene (7.07%), 1,8-cineole (7.26%), α-terpinyl acetate (13.40%), and limonene (50.47%). The bioactivity test of the essential oil against *S. aureus* bacteria demonstrated that the inhibitory diameter at a 25% concentration was categorized as moderate. Meanwhile, the inhibitory diameters at 50% and 100% concentrations were categorized as strong.

Suggestions

Future researchers are recommended to conduct anti-inflammatory activity tests on the essential oil from eucalyptus leaves to assess and ensure its safety for human consumption.

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