

Physicochemical properties and antibacterial activity of essential oil fractions obtained from fresh *Litsea angulata* leaves

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Manuscript received: 12 February 2025. Revision accepted: 2 May 2025.

Abstract. Putri AS, Nur M, Kusuma IW, Egra S, Kuspradini H. 2025. Physicochemical properties and antibacterial activity of essential oil fractions obtained from fresh *Litsea angulata* leaves. *Nusantara Bioscience* 17: 96-102. *Litsea angulata* Blume (Lauraceae) is widely grown in Kalimantan Island and is traditionally used as a medicinal plant. This work aimed to analyze the characteristics of the physicochemical from *L. angulata* oil fractions and the potency of its antibacterial activity. *Litsea angulata* fresh leaves were distilled, and their oil fractions were obtained by simple fractional distillation using water and steam distillation methods. The fraction of essential oil is based on the different distillation times. In this experiment, the following distillation periods were tested: 0-60 min, 60-120 min, and 120-180 min. The yield and color determined the physicochemical properties, while the chemical properties, as their compositions, were analyzed by GC-MS. The agar diffusion method was employed to assess antibacterial activity. Two kinds of synthetic standards, including chlorhexidine and chloramphenicol, were used to compare their antibacterial properties. *Streptococcus sobrinus*, *Streptococcus mutans* and *Staphylococcus aureus* were the microorganisms used in this investigation. The results showed fractions yield (range 0.09-1.66%) reached a maximum in 0-60 min, and color clearness. All oil fractions have various colors and chemical components according to their treatments. The diameter inhibition against selected bacteria (range 10.22-15.11 mm) was highest at fraction 0-60 min. This essential oil is potentially developed in the pharmaceutical industry as a natural alternative product.

Keywords: GCMS, *Litsea agulata*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*

INTRODUCTION

Essential oils are high-priced hydrocarbons generated solely by certain plant species. Their applications have a lengthy history in traditional herbal medicine, which lacks empirical validation. In recent decades, there has been a worldwide increase in interest in essential oils. A significant rise in global awareness regarding research-driven technologies that facilitate and sustain health condition management has been seen. As a consequence, the topic of essential oils has garnered increased interest and relevance within the fields of academic research and industrial applications (Oliveira et al. 2021). The global essential oil market is highly promising, with target markets including Japan, the United States, the United Kingdom, and several other European nations. The oils in demand comprise citronella oil, clove-leaf oil, ylang-ylang oil, vetiver oil, sandalwood oil, and patchouli oil. These oils will then be refined to create items suitable for use as perfumes, pharmaceuticals, dental pastes, confections, or flavoring ingredients for food (Sastrohamidjojo 2021).

Essential oils, fragrant essences produced from aromatic herbs, have been utilized in several ways, i.e., aromatherapy,

food preservation and flavoring, perfumery, pharmaceutical, and beauty care products (Naeem et al. 2018; Herman et al. 2019). These organic compounds have a variety of medicinal effects and biotechnological uses (Oliveira et al. 2014). Oil-secreting glands can be observed in several plant structures, including roots, flowers, wood, fruits, barks, seeds, and leaves, with occasional distribution over the entire plant (Naeem et al. 2018). Numerous studies have demonstrated the presence of essential oils' antiviral, antibacterial, antifungal, anti-inflammatory, anticarcinogenic, antimutagenic, and antioxidant properties, among other things. The lactone group in the sesquiterpenoid compound group is known as an active molecule that is toxic to pathogenic bacteria (Shaaban et al. 2012). Currently, around 3000 essential oils have been identified. Among other things, peppermint oil has properties that can cure various ailments, such as relieving headaches, flu, cough, sinusitis, nasal congestion, and other digestive problems (Nayak et al. 2020). Lemongrass oil can reduce anxiety, while also acting as an antibacterial, anti-inflammatory, and antifungal (Gaba et al. 2020). Lavender oil can relieve insomnia, muscle pain, stress, and poor sleep quality. Tea tree and chamomile oils relieve fungal skin infections, dandruff, and

head lice (Sahu et al. 2025). Chamomile oil also relieves anxiety and improves sleep quality (Kazemi et al. 2024). Clove oil helps with acne, strengthens hair roots, improves scalp circulation, and reduces dandruff (Haro-González et al. 2021). Jasmine oil helps with irregular heartbeat, insomnia, and stress (Shah et al. 2022). Nutmeg oil helps stimulate digestion, invigorate the skin, and boost energy (Vuković et al. 2022). The majority of aromatic plant species are members of the following families: Myrtaceae, Lauraceae, Umbelliferae, Labiatae, and Asteraceae (Hassaballa and Alsiddig 2022).

The genus *Litsea* Lam., a member of the family Lauraceae, exhibits a notable prevalence in tropical and subtropical climates. This plant is an important tropical forest vegetation and is known for its essential oil (Jose et al. 2015; Kamle et al. 2019). *Litsea* has various characteristic aromas and is potentially used as a medicinal plant (Li et al. 2024). *Litsea angulata* Blume, belonging to the genus *Litsea* (seed part), is used by Kalimantan local people to treat boils (Kuspradini et al. 2019) examined various components of this species, including bark, branches, and leaves, as possible sources of natural antioxidants and antibacterial agents. This study is limited to the essential oil of *L. angulata*; thus, the purpose of the current research was to evaluate the physicochemical characteristics and antibacterial properties of the essential oil fraction derived from fresh leaves using simple fractional distillation.

MATERIALS AND METHODS

Plant material and research location

The study's raw material was *L. angulata* leaves from Botanical Garden of Universitas Mulawarman (-0.450032°N,

117.216505°E), Samarinda, East Kalimantan, Indonesia (Figure 1). The work experiment was carried out at the Forest Product Chemistry and Renewable Energy Laboratory, Faculty of Forestry, Universitas Mulawarman, using 4,250 g of fresh materials. The collected material was prepared for distillation without any treatment or drying process.

Fractional distillation

The fractional distillation procedure used in this study was simple, using water and steam distillation techniques. The essential oils were fractionated based on different distillation times, as described by the method of Zheljzkov's research (Zheljzkov et al. 2014) with minor adjustments. This experiment treated the distillation periods of 0-60 min (fraction 1), 60-120 min (fraction 2), and 120-180 min (fraction 3). The essential oil fractions were recovered directly and dehydrated using anhydrous sodium sulfate (Na_2SO_4) and stored at room temperature in firmly closed dark vials prior to analysis. Essential oil yield is expressed in percent oil (% v/v).

Physicochemical parameters

Color, refractive index, and chemical composition determined the profile of the physicochemical properties of the achieved fractions.

Color

In this study, the color of the oil fraction was observed by the method of Shabbir et al. (2009).

Refractive index

The refractive index value was determined according to Patty and Loupatty 2016, and measurements were made with the help of a hand refractometer (Atago, Japan).

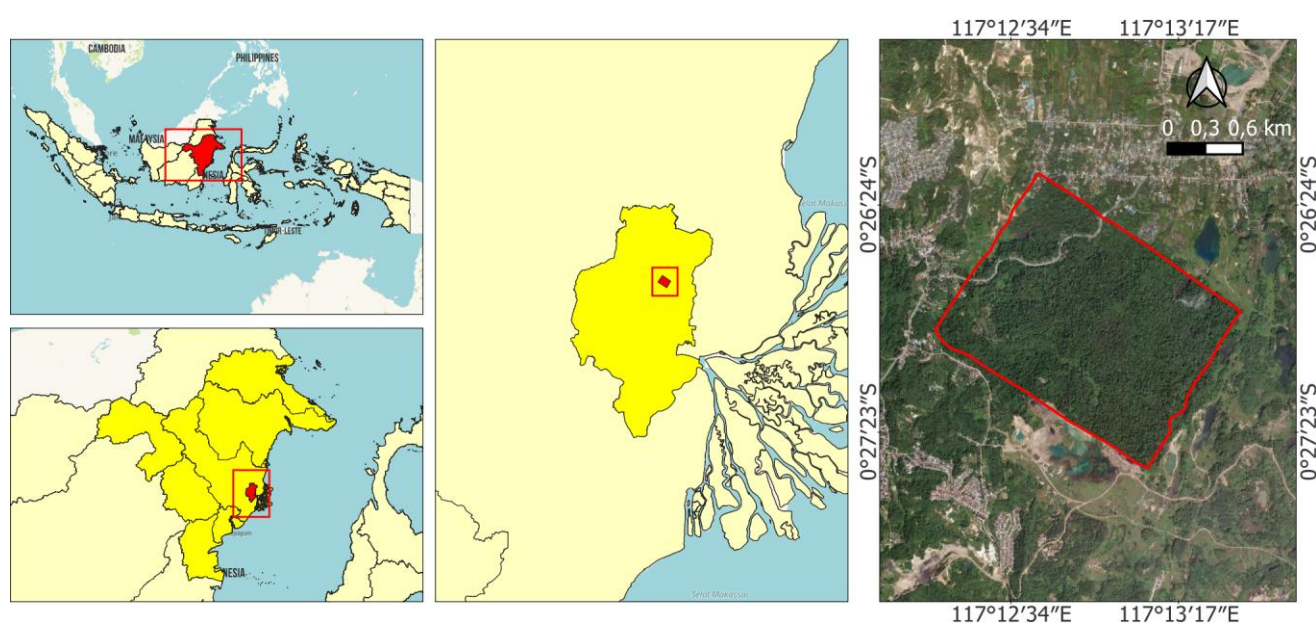


Figure 1. Research sites: Botanical Garden of Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia

Gas Chromatography-Mass Spectrometry (GC-MS)

The identification of essential oil fraction chemical compositions was determined with the application of the GC-MS technique by the modification procedure of Iordache (Iordache et al. 2011). In our studies, we used an RTX-5MS capillary column (Ultra Shimadzu-QP-2010) 30 m × 0.25 mm × 0.25 µm. The following settings were used to collect GC-MS spectra: The injector and detector temperatures were 250 and 280°C; the inlet pressure was 93.5 kpa; split ratio was 200; column flow was 1.44 mL.minute⁻¹; the linear velocity was 49.3 cm.second⁻¹; carrier gas was helium and purge flow 3.0 mL.minute⁻¹. The Kovats retention indices were performed on the basis of a range of alkane standard solutions (C8 to C40) using the measurement of van Den Dool and Kratz 1963. The identities of the compositions were assigned by comparing their mass spectra with those available in the NIST database, and the retention indices, taken from the literature and our own data, confirmed this.

Antibacterial assay

The activity of antibacterial from essential oil fractions was evaluated by the agar well diffusion method (Kuspradini et al. 2018). The bacteria used in this study include *Streptococcus mutans* Clarke, 1924, *Streptococcus sobrinus*, and *Staphylococcus aureus* Rosenbach, 1884. Briefly, the agar plates were inoculated with bacterial suspension, and 20 µL of the samples were loaded into wells of 7 mm diameter. The bacterial incubation time was 18-24 h at 37°C (Su et al. 2015). Chloramphenicol and chlorhexidine were used as standard antibiotics for positive control. A solution containing 40% ethanol was included as a negative control. The essential oil's concentration was measured and converted to mg (mg/20 µL). The test was repeated in triplicate.

Data analysis

GC-MS analyzed the presence of chemical constituents. The chromatographic peak area was used to calculate the percentage components of oil and given as the average of three duplicate analyses. The inhibition zone diameter was measured in mm by a ruler in each replication, and the average values were calculated.

RESULTS AND DISCUSSION

In this work, we meticulously investigated the leaves of *L. angulata*, identifying and obtaining three essential oil fractions through a comprehensive research process that involved simple fractional distillation using water and steam distillation methods. The yields of these oil fractions, a result of our thorough research, are detailed in Table 1. We further conducted a rigorous physicochemical characterization of these oils, including their color, refractive index, and chemical composition. The physico-properties, such as color and refractive index, are also

presented in Table 1. In comparison, Table 2 provides a comprehensive chemical profile of the extracts, instilling confidence in our readers about the chemical constituents of all extracts.

In this case, essential oil yield typically ranged from 0.09 to 1.66%, and fraction 1 gave higher yields than other fractions with a clear color. Distillation time influenced the yield of essential oil produced. The oil was optimally distilled at a distillation time of 0-120 min, while the yield produced was very low at 120-180 min of distillation time. This is also related to the color of essential oils. At the beginning of the distillation time, the color of the essential oil obtained was clear, and after 60 to 180 min was more concentrated.

The refractive index of *L. angulata* ranges from 1.407 to 1.427, with a highest value of 1.427, which was detected in fraction 3. These results are in contrast with the physical characteristics of the essential oil produced by the different distillation periods. The essential oil's refractive index is closely linked to its composition, while the constituents are dependent on the soil nutrients. The longer the chained compositions (sesquiterpenes and oxygenated compounds) that are distilled, the greater the essential oil medium density, making it hard for light to be refracted and resulting in a higher essential oil's refractive index (Arpi et al. 2011).

GC-MS analysis investigated the ingredients of three fractions of *L. angulata* leaves essential oil. More than 15 compounds were identified in each fraction. Table 2 summarizes the appearance of chemical components in samples.

Fraction 1 is composed of essential oil samples extracted from 0-60 min of distillation time, which are characterized by a higher percentage of camphor (62.38%) and followed by α -phellandrene (26.07%). Fraction 2 consists of oils extracted from the distillation periods of 60-120 m. These fraction oils were found to contain camphor, α -phellandrene, *trans*-caryophyllene, and α -humulene, which were detected at 37.14, 23.81, 10.06, and 10.06%, respectively. Fraction 3 is composed of essential oils isolated from 120-180 min of distillation time, which is characterized by a higher content of sesquiterpenes group in the total of 68.76% compounds. In comparison, *trans*-caryophyllene was a dominant component (28.53%) and had a lower content in the monoterpenes group (23.03%). However, in the *L. angulata* oil obtained after 120 min of distillation, α -phellandrene was detected at about 10.21%.

Table 1. The physicochemical profiles of *Litsea angulata* leave essential oil fractions

Samples	Yields (%)	Color	Refractive index
Fraction 1	1.66	Clear	1.407
Fraction 2	0.41	Yellowish	1.414
Fraction 3	0.09	Yellow pale	1.427

Table 2. Chemical constituents of *L. angulata* leave essential oil fractions

Chemical components	Percentage (%)			RI ^{exp}			RI ^{lit}
	Frc 1	Frc 2	Frc 3	Frc 1	Frc 2	Frc 3	
β-Pinene	0.92 ± 0.03	0.87 ± 0.04	0.36 ± 0.01	952	953	953	952
β-Myrcene	0.45 ± 0.01	0.39 ± 0.01	-	962	963	-	962
α-Phellandrene	26.07 ± 0.38	23.81 ± 0.56	10.21 ± 0.28	1002	1003	1002	1001
α-Terpinene	0.60 ± 0.11	-	-	1013	-	-	1014
m-Cymene	2.45 ± 0.10	4.97 ± 0.09	2.84 ± 0.05	1021	1021	1021	-
β-Phellandrene	-	2.33 ± 0.04	1.37 ± 0.04	-	1028	1028	1032
(-)-Limonene	1.77 ± 0.11	-	-	1025	-	-	-
γ-Terpinene	0.39 ± 0.01	0.48 ± 0.01	-	1054	1054	-	-
α-Terpinolene	1.14 ± 0.04	1.61 ± 0.02	-	1084	1084	-	1089
Camphor	62.38 ± 0.64	37.14 ± 0.40	6.69 ± 0.08	1150	1149	1147	1144
(-)-Borneol	0.54 ± 0.07	0.44 ± 0.01	-	1168	1168	-	-
Decanal	-	0.65 ± 0.01	1.14 ± 0.06	-	1192	1192	-
cis-Sabinol	-	-	0.44 ± 0.01	-	-	1200	-
6,6-Dimethyl-2-(3-oxo-butyl)-bicyclo[3.1.1]heptan-3-one	0.51 ± 0.02	1.48 ± 0.01	1.37 ± 0.02	1243	1293	1243	-
Acetic acid 1,3,3-trimethyl-2-oxa-bicyclo[2.2.2]oct-6-yl ester	-	1.89 ± 0.10	1.40 ± 0.04	-	1315	1315	1431
2,6-Heptanedione, 3-methylene-	-	0.89 ± 0.04	0.74 ± 0.00	-	1323	1323	-
α-Copaene	0.44 ± 0.01	1.97 ± 0.04	6.17 ± 0.09	1375	1703	1376	1376
trans-Caryophyllene	1.11 ± 0.03	10.06 ± 0.28	28.53 ± 0.30	1579	1212	1471	-
α-Humulene	-	10.06 ± 0.28	3.59 ± 0.05	-	1832	1766	-
Alloaromadendrene	-	-	0.35 ± 0.00	-	-	1883	-
δ-Cadinene	-	-	1.19 ± 0.04	-	-	1923	1523
(-)-Germacrene A	-	1.35 ± 0.01	7.00 ± 0.10	-	2048	2051	-
β-Bisabolene	-	2.23 ± 0.04	6.62 ± 0.04	-	2088	2091	-
Germacrene D	-	-	0.83 ± 0.03	-	-	1477	-
δ-Cadinene	-	2.15 ± 0.05	4.26 ± 0.01	-	1478	1477	-
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	-	-	0.84 ± 0.01	-	-	1465	-
Nerolidol b (cis or trans)	-	-	0.39 ± 0.01	-	-	1455	-
(-)-Caryophyllene oxide	-	0.77 ± 0.02	3.20 ± 0.13	-	1408	1414	-
Torreyol	-	0.55 ± 0.01	-	-	1558	-	-
α-Cedrol	-	-	0.81 ± 0.03	-	-	1576	-
δ-Cadinol	-	-	2.07 ± 0.00	-	-	1563	-
α-Cadinol	-	-	0.86 ± 0.33	-	-	1550	-
α-Bisabolol	-	-	0.62 ± 0.13	-	-	1523	-
d-Nerolidol	-	-	0.54 ± 0.06	-	-	1970	-
Monoterpenes	96.69	72.65	23.03				
Sesquiterpenes	1.55	20.28	68.76				
Other	0.55	4.26	3.50				

Note: ^aFrc: fraction; ^bRI^{exp}: retention indices of the experiment; ^cRI^{lit}: retention indices of literature

In this case, distillation time affects the chemical constituents and value of the main components identified from the samples. The high and low chemical constituent appearance percentages from monoterpenes and sesquiterpenes groups may be influenced by molecular rearrangement and hydrolysis, which may develop during the distillation process (Wesołowska et al. 2010); hence, to release their active compounds, essential oils should be distilled at the correct time (Schmidt 2020). Gamma terpinene has a boiling point of 181 to 182°C and a KI of about 1085. In the work of Olmedo et al. (2014), where essential oil was fractionated with short-path molecular distillation, it was determined that from this approximate value of KI, a heavier fraction of higher boiling point was constituted, which is what is observed: the increase of fraction 3 from approximately said KI (Olmedo et al. 2014).

A new source of camphor and α-phellandrene was found in this aromatic plant. In our previous project results (Kuspradini et al. 2020), camphor and α-phellandrene were not detected in *L. angulata* leaves crude oil. The quality of chemical compounds found in plants may be affected by environmental circumstances at the time of sampling and growing parameters such as soil type. Ilić et al. (2022) mentioned that the concentration of essential oil depends on the population variations and the climate factors. Based on the results of Goyal et al. 2021, there is a significant difference in the percentage of oil between geographical regions and cultivation altitude. Consequently, more research into environmental or climatic conditions is required for this species.

Camphor and α-phellandrene were two dominant volatile components with the highest concentrations detected in the

L. angulata fresh leaves essential oil. Camphor hydrazone derivatives have anticancer, antitussive, and insecticide activities, and they may be used to treat Leishmaniasis disease (da Silva et al. 2020). Camphor also has various negative side effects when used in high doses, including nausea, vomiting, headache, dizziness, tremors, seizures to death (Santos and Cabot 2015). α -phellandrene is commonly used in fragrances and has a characteristic, slightly green, terpenic, citrus, black pepper-like aroma. Only a few papers reported on the α -phellandrene biological activity, and it has been discovered that it is ineffective as a hypercholesterolemic, hyperlipidemic agent, antibacterial, and antifungal (Işcan et al. 2012). Thangaleela et al. (2022) assessed the function of α -phellandrene across a range of healthcare sectors.

Table 3 demonstrates the antibacterial activity of *L. angulata* essential oil fractions. All fractions could inhibit the growth of the tested microorganisms (*S. mutans*, *S. sobrinus*, and *S. aureus*). This result showed that fraction 1 was active in inhibiting the growth of *S. sobrinus* and *S. aureus* (15.11 and 16.89 mm, respectively). At the same time, the highest diameter against *S. mutans* was found in fraction 2. The strength of antibacterial activity on the oil fractions is presented in Table 3.

From the results presented above, all samples have the bacterial strains' inhibitory activities except the lowest concentration for all fractions against *S. sobrinus*. Fraction 1 showed the best inhibition towards *S. sobrinus* and *S. aureus* at the highest concentration; however, the greatest activity was shown by fraction 2 against *S. mutans*. The role assures the activity indicated by fractions 1 and 2 of the chemical content in the oil, which is rich in monoterpenes. Sesquiterpenes also have a role in bacterial inhibition, as seen in the activity possessed by fraction 3; however, they have not shown the highest activity. The chemical components and the quantity of main compounds in the extracts can affect the inhibitory activity (Qin et al. 2024). The presence of major chemical ingredients in the extract influenced their biological activities.

Literature surveys revealed that various essential oils from a number of plants contained camphor as a main component, demonstrating antifungal properties (Sobhy et

al. 2023). Bouazama et al. (2017) investigated *Lavandula pedunculata* (Mill.) Cav. and *Lavandula dentata* L. from South Morocco, which contained 53.1 and 50.3% of camphor possessing inhibitory activity toward *Pseudomonas aeruginosa* A, *Escherichia coli* E, *S. aureus*, and *Rhodococcus fascians*. *Cinnamomum camphora* (L.) J.Presl, known as a camphor-producing plant, has been assayed as an antibacterial agent, potentially. In line with the research of Chaudhari et al. (2020), cinnamon oil fraction from *C. camphora* showed the inhibition toward *S. mutans* of about 12.51 mm. Previous work stated *C. camphora* oil exhibits its antibacterial potential against *Bacillus subtilis*, *P. aeruginosa*, *E. coli*, *Salmonella typhimurium*, *S. aureus*, and *Chromobacterium violaceum* (Hu et al. 2019; Wang et al. 2019). Moreover, based on Chen et al. (2020) statement that the volatile oil derived from *C. camphora* leaves was tested to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) (the value of MIC 0.8 mg/mL).

As mentioned by Işcan (2017), the monoterpene α -phellandrene showed moderate to good inhibitory efficacy against various bacteria and *Candida* species. According to the findings of Zhang et al. (2017), α -Phellandrene and Nonanal might be effectively employed as biological fungicides to combat *Penicillium cyclopium* in postharvest tomato fruits. These volatile compositions were capable of penetrating cell lipid structures and damaging the fungal cell membrane's integrity. Thangaleela et al. (2022) reviewed that α -Phellandrene exhibited in vitro antimicrobial activities against bacterial strains *Salmonella choleraesuis*, *B. subtilis*, *E. coli*, *Rhodococcus rhodochorus*, *Micrococcus luteus*, *S. aureus*, *Arthrobacter protophormiae*, and fungus *Aspergillus flavus*.

In the case of fraction 3, the appearance of trans-caryophyllene as a sesquiterpenes group also supports, to a considerable degree, the inspected activity. The compound trans-caryophyllene has pharmacological effects as an antibacterial and antifungal. The trans-caryophyllene analgesic property extracted from the volatile oil of *Cordia verbenacea* showed good anti-inflammatory and antiplasmodic efficacy (Fernandes et al. 2007; Leonhardt et al. 2010; Astani et al. 2011).

Table 3. Antibacterial properties of *Litsea angulata* leaf essential oil fractions

Samples	Concentrations (mg/20 μ L)	Diameter of inhibition (mm)		
		<i>Streptococcus mutans</i>	<i>Streptococcus sobrinus</i>	<i>Staphylococcus aureus</i>
Fraction 1	21.50	20.33 \pm 1.45	15.11 \pm 1.02	16.89 \pm 1.02
	10.75	11.33 \pm 1.53	9.22 \pm 0.19	16.78 \pm 1.09
	5.38	8.33 \pm 0.88	0.00 \pm 0.00	14.11 \pm 0.51
Fraction 2	19.30	22.44 \pm 1.35	14.33 \pm 2.19	16.33 \pm 2.33
	9.65	17.67 \pm 1.00	8.33 \pm 0.58	16.33 \pm 1.45
	4.83	8.56 \pm 0.51	0.00 \pm 0.00	13.67 \pm 1.15
Fraction 3	19.10	12.89 \pm 1.84	10.22 \pm 0.69	13.11 \pm 1.92
	9.55	9.44 \pm 0.69	8.11 \pm 0.51	12.89 \pm 0.19
	4.78	7.89 \pm 0.19	0.00 \pm 0.00	11.67 \pm 0.88
K(+) ¹	0.01	23.89 \pm 0.38	27.89 \pm 0.38	19.22 \pm 3.72
K(+) ²	0.01	15.67 \pm 0.58	15.67 \pm 0.33	15.78 \pm 0.19

Note: ^aK(+)¹: chloramphenicol; ^bK(+)²: chlorhexidine

In conclusion, the results reported on the essential oil fraction yield and chemical constituents of *L. angulata* at different distillation periods. We consider that for producing the highest quantity and quality of *L. angulata* essential oil, it is judicious to distill for 0-120 min to gain the optimal and maximum percentage of camphor. This plant was potentially a new source of camphor and α -phellandrene as a monoterpenes group. As shown in the antibacterial properties, the components from the monoterpenes group played a role in this work. It is summarized that the essential oil of *L. angulata*, rich in monoterpenes group components, can be used as a natural alternative product as a medicinal plant to inhibit the growth of *S. mutans*, *S. sobrinus*, and *S. aureus*, offering hope for new treatments.

ACKNOWLEDGEMENTS

This work was funded by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia (grant number: 221/UN17.41/KL/2019). Raymoon Silaban supplied the landscape map of the research location.

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