Chemical Composition and Antibacterial Activity of (*Litsea cubeba* (Lour.) Pers.) Essential Oil

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Abstract
The (*Litsea cubeba* (Lour.) Pers.) is widely used to treat intestinal diseases, abdominal pain, helminth infections, digestion, stimulates digestive enzyme secretion, enhances appetite, reduces bloating, and indigestion. However, the chemical composition and biological properties of the essential oil of (*Litsea cubeba* (Lour.) Pers.) collected in Thanh Hoa, Vietnam, have not been investigated. Gas chromatography-mass spectrometry (GC-MS) was used to identify the chemical composition of (*Litsea cubeba* (Lour.) Pers.) essential oil. The agar disc diffusion method was used to evaluate the antioxidant and antibacterial activities of the essential oil. All parts of the plant — leaves, flowers, and bark — contain essential oils, among which the bark has the highest content. The main constituents include Z-citral (32.9%), sabinen (14.2%), linalool (9.5%), limonen (9.2%) and α-pinen (6.0). In the antibacterial test, *Litsea cubeba* essential oil demonstrated strong antibacterial activity against *E. coli*, with an inhibition resistance of 86.80%. Our results indicate that the essential oil of (*Litsea cubeba* (Lour.) Pers.) collected in Thanh Hoa, Vietnam, has great potential in the pharmaceutical industry.

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Keywords: Thanh Hoa, *Litsea Cubeba* (Lour.) Pers, Essential Oil, Z-Citral, Antibacterial.

1. Introduction

Litsea cubeba (Lour.) Pers. is a deciduous shrub or small tree belonging to the Lauraceae family. The plant is characterized by slender, round branches that are smooth or covered with fine hairs. The bark of young branches is greenish-yellow, turning brown and smooth as the plant matures. The leaves are alternate, leathery, and aromatic, with a lanceolate, obovate, or narrowly elliptic blade measuring 7–11 × 1.4–2.4 cm. The leaf apex is acute, the base cuneate; the upper surface is dark green, the lower surface lighter, both glabrous. There are 6–7 pairs of lateral veins, and the petiole is 6–12 mm long and smooth.

The inflorescences are solitary umbels, unisexual, with a common peduncle 2–3 cm long and umbel stalks 3–6 mm long. Each spherical umbel (approximately 3 mm in diameter) bears 4–6 flowers. Male flowers are small, with six ovate tepals about 2 mm in length. The fruit is nearly spherical, 4–5 mm in diameter, smooth, green when young and turning black upon ripening, borne on a fruit stalk 2–4 mm long [1, 2].

The species grows either scattered or in small clusters within secondary forests or regenerating forests after shifting cultivation, typically at altitudes ranging from 100 to 1, 500 m. The flowering season occurs from February to March, and fruiting takes place between July and August [3]. Propagation is usually achieved through seeds sown in spring. Fruits are harvested during summer (May–June), while roots and leaves can be collected throughout the year. In Vietnam, *L. cubeba* is widely distributed across various provinces, including Lao Cai (Sa Pa), Son La (Thuan Chau, Song Ma, Moc Chau), Hoa Binh (Da Bac), Ha Giang (Vi Xuyen), Cao Bang (Nguyen Binh), Lang Son (Huu Lung), Bac Kan (Ba Be), Thai Nguyen, Quang Ninh (Yen Tu), Phu Tho, Vinh Phuc (Tam Dao), Hanoi (Ba Vi), Ninh Binh (Cuc Phuong), Nghe An (Quy Chau), Ha Tinh (Vu Quang), Quang Binh (Dong Hoi), Quang Tri, Thua Thien–Hue (Bach Ma), Da Nang (Tourane), Khanh Hoa (Nha Trang), Dak Lak, Kon Tum (Dak Glei, Sa Thay), Gia Lai (Kon Ha Nung), and Lam Dong (Da Lat). Beyond Vietnam, the species is also found in India, China, and Malaysia

[4].

In traditional medicine, parts such as the leaves, buds, and bark of the (*Litsea cubeba* (Lour.) Pers.) are considered to have high medicinal value and have been used for a long time in folk remedies. Specifically, *Litsea cubeba* leaf water helps support digestion, stimulates digestive enzyme secretion, enhances appetite, reduces bloating, indigestion, and treats conditions like diarrhea and dysentery [5, 6]. Additionally, extracts from the leaves and buds of the *Litsea cubeba* tree are used for antiseptic purposes, to treat open wounds, abscesses, and skin inflammation. The *Litsea cubeba* leaves applied to the skin help reduce inflammation, promote healing, and have anti-aging effects due to their natural antioxidant, antibacterial compounds [7]. Several traditional remedies also report benefits in supporting the treatment of diabetes, infections, joint pain, and weight management by regulating fat metabolism [8, 9].

Several publications report the chemical composition of *Litsea cubeba*, especially that of the essential oil [10]. However, studies have also shown that the raw material from different regions and different extraction methods also greatly affect the content of *Litsea cubeba*. Therefore, when testing its chemical activity, it is possible to discover different active constituents according to the above factors. This study aims to analyze and characterize the chemical composition and biological properties *Litsea cubeba* essential oil collected in Thanh Hoa, Vietnam, in support of its potential exploitation and effective utilization. Determination of the chemical composition of *Litsea cubeba* essential oil was conducted using gas chromatography-mass spectrometry (GC-MS). The agar disc diffusion method was used to evaluate the antibacterial activities.

Material and Methods

Chemicals

Butylated hydroxytoluene (BHT), C7–C30 straight-chain hydrocarbons, reference chemicals for identification, Tween 80, and DPPH were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical analytical grades, the culture media, and standard antibiotic discs were procured from Merck (Darmstadt, Germany) and Oxoid Ltd. (Basingstoke, Hampshire, UK), respectively.

Plant Material

The (*Litsea cubeba* (Lour.) Pers.) were collected from Thanh Hoa, Vietnam, in January 2022. A voucher specimen (No. MT-TH-01) was prepared and deposited at the Faculty of Natural Science and Technology, Hong Duc University, Thanh Hoa Provinve, Vietnam.

Fig 1: The fresh leaves of (*Litsea cubeba* (Lour.) Pers.) collected in Thanh Hoa Province.

Extraction of Essential Oil

Collected leaves were cleaned, cut into small pieces, and subjected to steam distillation for 3.5 hours by a Clevenger-type apparatus. The essential oil obtained was then dehydrated using anhydrous sodium sulfate and stored in a sealed vial at 10 °C in the dark prior to subsequent experiments.

Analysis of Essential Oil by GC-MS

To analyze the composition of the essential oil from the leaves of *Litsea cubeba*, a Trace GC Ultra-ITQ900 system (Thermo Fisher Scientific, MA, USA) was used. Data were interpreted by MassFinder 4.0 software. The separation was performed on a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC operational parameters included injector temperature 250 °C, detector temperature 260 °C, oven temperature program 60 to 260 °C at a heating rate of 4 °C/min, carrier gas helium at a flow rate of 1.0 mL/min, and sample injection volume 1 µL in split mode with a split ratio of 1:10. The mass spectrometer was operated in electron ionization (EI) mode with the following parameter values: ionization energy 70 eV, interface temperature 280 °C, ion source temperature 230 °C, MS quadrupole temperature 200 °C, and scan range 35–650 amu [10]. The retention indices of the essential oil constituents were determined using an HP-5 MS column and standard C7–C30 straight-chain hydrocarbon reference standards (Sigma-Aldrich Chemical Company, USA). The mass spectra and retention indices of individual compounds were identified by comparing them with those in GC-MS libraries (National Institute of Standards and Technology-NIST 08 and Wiley 09th version) and/or with published data. The relative percentages of the identified compounds were calculated based on GC peak areas without applying correction factors.

Antimicrobial Activity

The agar disc diffusion method was used to evaluate the antibacterial activity of the essential oil against the Gram-negative bacterium *E. coli* (ATCC 25922). A liquid culture of *E. coli* (10^7 CFU/mL) was evenly spread on solidified agar in Petri dishes. Circular filter paper discs (6 mm diameter) were placed at the center of each dish, and 40 µL of the essential oil (extracted by steam distillation and dissolved in 10% dimethyl sulfoxide, DMSO) was applied to the discs. DMSO (10%) served as a negative control. The plates were sealed and incubated at 37 °C, and the diameters of the inhibition zones around the discs were measured to assess antibacterial activity. All experiments were conducted in triplicate for accuracy. The minimum inhibitory concentration (MIC) of the essential oil was determined by the broth microdilution method, as described by Hanh *et al.* (2023). The essential oil was serially diluted two-fold with ethanol in a 96-well plate to achieve concentrations ranging from 1.0 to 10.0 mg/mL, and then 20 µL of bacterial suspension (pH 7.4–7.6) was added to each well. The plates were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of essential oil that visibly inhibited bacterial growth. Each assay was performed in triplicate to ensure result reliability.

Statistical Analysis

All experiments were carried out in triplicate. Analysis of variance (ANOVA) and Statistica 5.5 software (StatSoft Inc., Tulsa, OK, USA) were utilized to analyze the results. The results are presented as the mean ± standard deviation (SD).

Results and Discussion

Investigation of Factors Affecting Essential Oil Yield

Effect of Distillation Time

Table 1: Effect of distillation time on essential oil yield.

Distillation time (h)	1h30	2.0	2.5	3.0	3.5	4.0
Essential oil content (%)	0.027	0.092	0.157	0.231	0.247	0.185

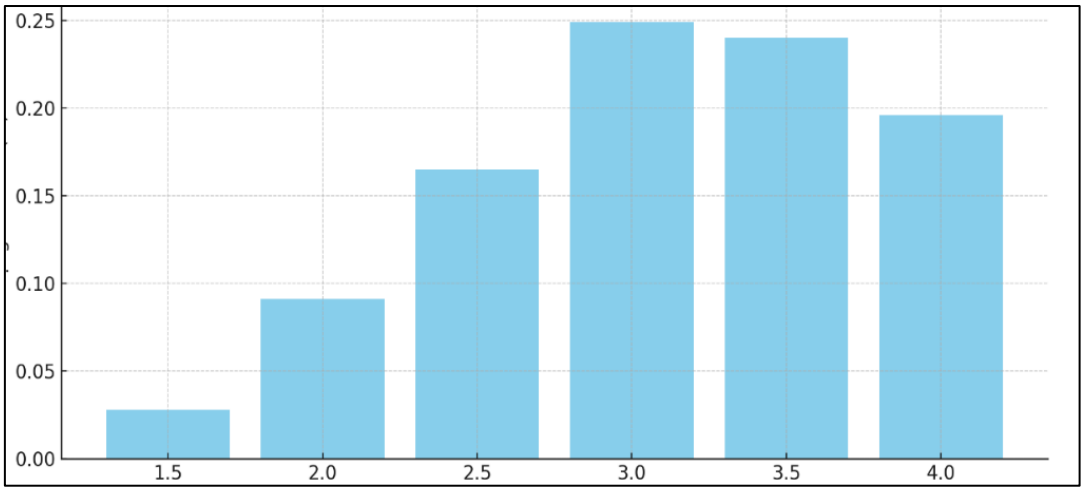


Fig 2: Effect of distillation time on essential oil yield.

The essential oil content increased gradually from 1.5 to 3.5 hours and reached the highest value at 3.5 hours (0.247%), followed by a slight decrease thereafter. This finding indicates that 3.5 hours is the optimal distillation duration to obtain the maximum essential oil yield. Prolonged distillation beyond this point may cause losses due to evaporation or thermal degradation of volatile compounds. Therefore, the optimal condition for obtaining the highest essential oil yield was determined to be a distillation time of 3.5 hours**.**

Effect of Raw Material-to-Water Ratio

The amount of essential oil obtained varied with the ratio of raw material to water. The maximum yield (0.247%) was achieved at a ratio of 1/12 (g/mL), after which it decreased at higher dilution levels (1/14). This ratio indicates that the amount of water was sufficient to release essential oil effectively without excessive dilution or reduction in vapor pressure during distillation. Consequently, the optimal condition for achieving the highest essential oil yield was a raw material-to-water ratio of 1/12 (g/mL).

Table 2: Effect of raw material-to-water ratio on essential oil yield.

Raw material/water ratio (g/mL)	1/8	1/10	1/12	1/14
Essential oil content (%)	0.121	0.138	0.247	0.212

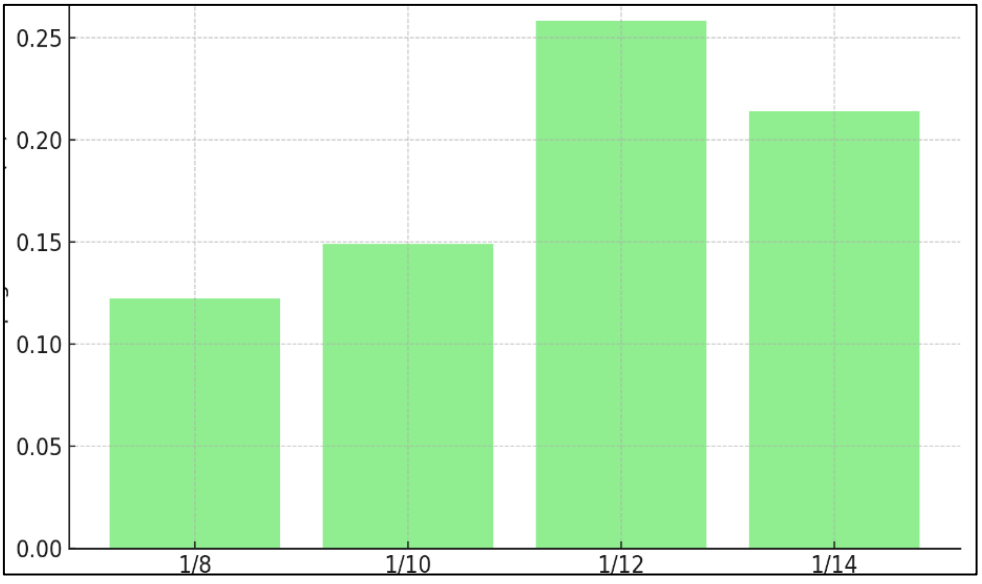
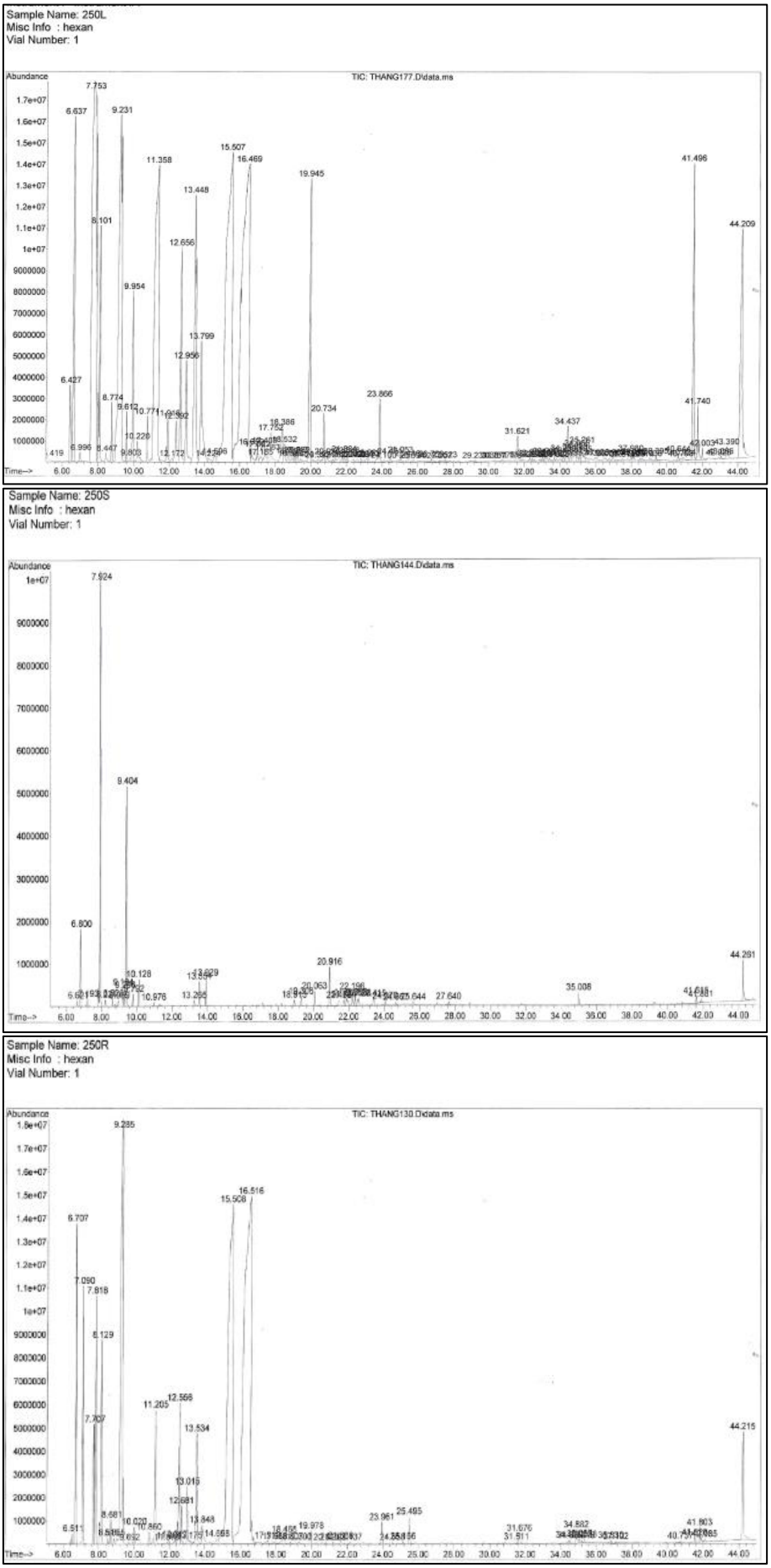


Fig 3: Effect of raw material-to-water ratio on essential oil yield.

Essential oil was extracted from the leaves and stems of *Litsea cubeba* via hydrodistillation, with a 3.0% yield (w/w, based on fresh weight). The total ion chromatogram obtained from GC-MS analysis is shown in Figure 4 and Table 3.



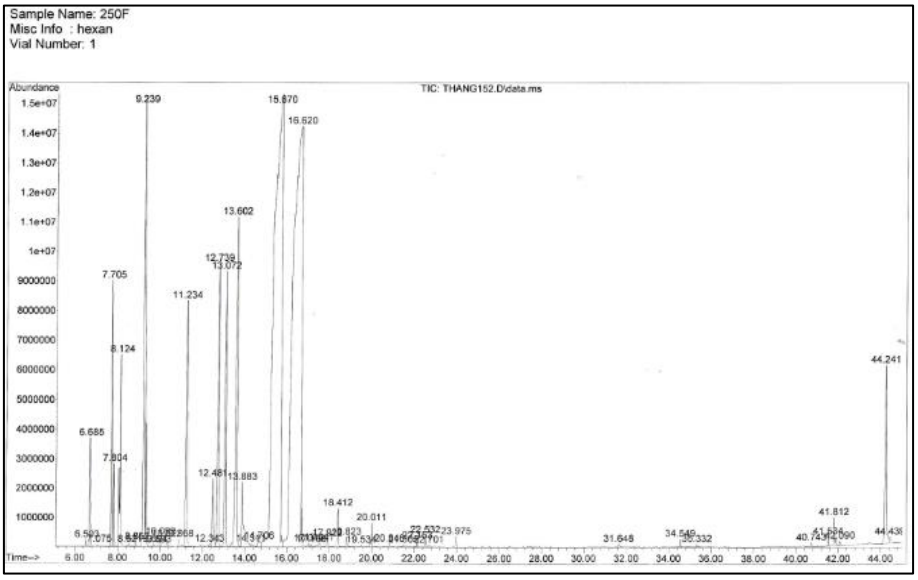


Fig 4: GC-MS total ion chromatogram of *Litsea cubeba* essential oil.

All parts of the plant—including leaves, flowers, and bark—contain essential oils, among which the fruits have the highest oil content, ranging from 0.1% to 3%. The essential oil is light and aromatic. Experimental results indicated that the physicochemical parameters were consistent with both the standard reference values and those reported in previous studies. The specific gravity of the oil, which is lighter than water at 20 °C, ranged from 0.8400 to 0.9600 g/mL, while the refractive index values were between 1.450 and 1.560. These parameters qualitatively reflect the good quality of the *Litsea cubeba* essential oil obtained from all samples, with minimal variation among them [12].

The main constituents of the essential oils from different parts of the plant are as follows (Table 3).

Table 3. Chemical compositions of the essential oil of *Litsea cubeba*

Litsea cubeba	**Leaves**	Z-citral (32.9%), sabinen (14.2%), linalool (9.5%) and limonen (9.2%)
	Bark	Z-citral (53.2%), sabinen (10.2%) and limonen (13.2%)
	Fruits	Z-citral (66.1%) and limonen (7.0%)
	Roots	Z-citral (59.9%) and limonen (13.6%)
	Branches	Z-citral (38.1%), sabinen (17.6%) and α-pinen (6.0%)

Compared with previous studies, the main components in the essential oil of *Litsea cubeba* fruits are primarily citral (a mixture of neral and geranial), accounting for 55%–75%. Other constituents present in significant amounts include citronellal, linalool, limonene, camphor, and methyl heptenone [7, 8, 10].

The active compounds - D-limonene (1-Methyl-4-(prop-1-en-2-yl)cyclohex-1-ene), β-citronellal (3, 7-dimethyloct-6-enal), sulcatone (6-Methylhept-5-en-2-one), and β-linalool (3, 7-dimethylocta-1, 6-dien-3-ol) — found in the experimentally obtained *Litsea cubeba* essential oil have wide applications in cosmetics, fragrance technology, and the pharmaceutical industry. Notably, many studies have highlighted their insect-repellent and antimicrobial properties in *Litsea cubeba* essential oil as well as in other types of essential oils [11, 12].

The antimicrobial activity of *Litsea cubeba* essential oil against *E. coli* is summarized in Table 4. The essential oil demonstrated strong activity, with an inhibition zone of 26.0±0.1 mm and Resistance of 86.80%. Essential oil at a 2.00 mg/mL concentration. Compared with previous studies, this study shows many similarities [13]. Antibacterial activity of *Litsea cubeba* essential oil against *E. coli* to similar Ciprofloxacin used as a positive control exhibited an inhibition zone of 34.0 mm and resistance of 100%.

These findings highlight the significant antibacterial potential of *Litsea cubeba* essential oil. This suggests that *Litsea cubeba* essential oil could serve as a natural antimicrobial agent, with promising applications in combating bacterial infections.

Table 4. Antibacterial activity of *Litsea cubeba* essential oil against *E. coli*

Bacterial density	Essential oil (mg/mL)	IZD (mm)			Resistance (%)
		1	2	3	
10^6 CFU	2.00	26	27.5	26	86.80
10^6 CFU	1.75	20	21	21	67.25
10^6 CFU	1.50	17	16	17	56.68
10^6 CFU	1.25	11	10	11	43.47
10^6 CFU	1.00	7.5	7	7	34.36
10^6 CFU	0.75	3	2.5	25	11.87
10^6 CFU	0.50	0	0	0	0.00
Ciprofloxacin[a]	1.00	34	34	34	100

[a]Ciprofloxacin: Positive control for antibacterial activity, IZD: inhibition zone diameters.

Conclusion

This study is the first to investigate the essential oil from the leaves of *Litsea cubeba* collected in Thanh Hoa, Vietnam. GC-MS analysis identified a diverse chemical composition. The main chemical constituents of Litsea cubeba essential oil were identified as Z-citral (32.9%), sabinene (14.2%), linalool (9.5%), limonene (9.2%), and α-pinene (6.0%). In the antibacterial test, *Litsea cubeba* essential oil demonstrated strong antibacterial activity against *E. coli*, with an inhibition resistance of 86.80%. All studied compounds were These findings underscore the potential of *Litsea cubeba* essential oil as a natural resource for pharmaceutical applications, particularly ind antibacterial therapies.

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