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Research Article

Chemical characterization, antimicrobial and mosquito larvicidal activities of the essential oil of *Camellia pleurocarpa* (Gagnep.) Sealy from Vietnam

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Abstract
Camellia pleurocarpa (Gagnep.) Sealy, a species of yellow camellia indigenous to Vietnam, was investigated for its essential oil properties in this study. Hydrodistillation of its leaves yielded a complex mixture rich in sesquiterpenes and diterpenes. Chemical analysis identified 50 constituents, with notable compounds including spathulenol (13.26%), phytol (9.94%), and α-selinene (5.34%). The essential oil exhibited significant antimicrobial activity against three Gram-positive bacteria *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*; one Gram-negative bacterium *Pseudomonas aeruginosa*; and one yeast *Candida albicans*, with minimum inhibitory concentration (MIC) values ranging from 16 to 32 µg/mL and half-maximal inhibitory concentration (IC_{50}) values ranging from 7.26 to 16.23 µg/mL. Furthermore, it demonstrated larvicidal potential against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes, with LC_{50} values ranging from 13.49 to 28.95 µg/mL and LC_{90} values ranging from 28.29 to 53.24 µg/mL. These findings underscore the promising antimicrobial and mosquito larvicidal properties of *C. pleurocarpa* essential oil, suggesting its potential as a natural alternative in combating microbial infections and controlling mosquito-borne diseases. This study represents the first comprehensive report on the chemical compositions and bioactivities of *C. pleurocarpa* essential oil.

Keywords
Camellia pleurocarpa, Theaceae, Gram-positive bacteria, *Aedes aegypti*, *Culex quinquefasciatus*

INTRODUCTION

In recent years, there has been a surge in interest surrounding essential oils due to their diverse applications across various domains[1]. These oils, derived from aromatic plants through distillation or extraction methods, encapsulate the potent essence of botanicals, endowing them with a myriad of therapeutic properties. Their growing popularity stems from perceived efficacy in addressing health and wellness concerns, ranging from stress relief to immune support[1].

The antimicrobial properties of essential oils have garnered considerable interest due to their efficacy against a wide range of pathogens, including bacteria, fungi, and viruses[2]. This broad-spectrum activity positions essential oils as promising alternatives or adjuncts to traditional antimicrobial agents, especially given the escalating issue of antibiotic resistance[2]. Their multifaceted mechanisms of action, combined with a low potential for resistance development, make essential oils an innovative and valuable strategy in the fight against resistant pathogens[2]. Moreover, their natural origin and established safety profile enhance their appeal, particularly within integrative medicine and complementary health practices. Thus, essential oils not only offer a viable option for augmenting

conventional antimicrobial therapies but also represent an important area of ongoing research aimed at optimizing their application in clinical settings[2].

In addition, essential oils have extended their utility beyond human health, finding relevance in pest management strategies[3]. With escalating global concerns over vector-borne diseases such as malaria and dengue fever, there is a growing need for effective and environmentally friendly solutions to repel insect vectors[4]. Essential oils, with their complex compositions comprising various bioactive compounds, have emerged as promising candidates in this regard, exhibiting repellent properties against a wide array of arthropods, including mosquitoes[3].

The genus *Camellia*, belonging to the family Theaceae, consists of evergreen trees and shrubs celebrated for their stunning flowers, which have earned them global admiration[5]. With around 250 species, primarily found in Asia, particularly in China, Japan, Korea, and Southeast Asia, Camellias showcase remarkable adaptability across various habitats, from mountainous regions to subtropical forests[5]. Botanically, they are recognized by their lustrous, dark green leaves and vibrant blooms, spanning from pure white to deep crimson, often displaying a symmetrical petal arrangement that enhances their charm. Beyond their botanical allure, Camellias carry profound cultural significance, notably in Asian societies like China and Japan, where they symbolize longevity, purity, and beauty. Embedded in traditional rituals, ceremonies, and art, Camellias reflect centuries-old cultural values. Moreover, Camellias have garnered attention for their pharmacological potential, with various parts of the plant, such as leaves and seeds, being used in traditional medicine[6,7]. These plant parts are commonly utilized as stimulants, diuretics to promote urine excretion, and astringents to control bleeding and aid wound healing[6,7]. Traditional applications of Camellias also include the treatment of flatulence, regulation of body temperature and blood sugar levels, promotion of digestion, and enhancement of cognitive function[6,7]. Research into their medicinal properties has unveiled promising avenues, particularly in anti-inflammatory, antioxidant, and cardiovascular health[7-9].

Camellia pleurocarpa (Gagnep.) Sealy is a species of the *Camellia* genus, distinguished by its unique characteristics and ecological significance[10]. This species is a small tree, reaching heights of 4 to 10 meters, and is found in Vietnam[10]. Besides, this is an endangered species in the Vietnam Red Data Book[11]. It typically thrives at altitudes of 500 meters in tropical evergreen rainy-season forests. Traditionally, the branches, leaves, and flowers of *C. pleurocarpa* have been used to treat rheumatism, and women consume them for nourishment after childbirth. Despite the extensive research conducted on the chemical composition and biological activities of essential oils from various *Camellia* species[12-18], there remains a notable gap in the literature regarding the essential oil of *C. pleurocarpa*. Addressing this gap is crucial, particularly given the growing interest in discovering new natural products with significant biological activities. In light of this, and as part of our ongoing research on the chemical composition and biological activities of essential oils from Vietnamese plants[19-24], we have undertaken the first comprehensive study on the essential oil of *C. pleurocarpa*. This study aims to provide a detailed analysis of its chemical composition, as well as an evaluation of its antimicrobial properties and mosquito larvicidal activity. The specific objectives of this study are: (1) to analyze the chemical composition of the essential oil from *C. pleurocarpa* leaves collected in Vietnam; (2) to evaluate the antimicrobial activity of the essential oil against seven different strains of microorganisms using the broth microdilution method; and (3) to test the mosquito larvicidal activity of the essential oil against *Aedes aegypti* and *Culex quinquefasciatus* larvae.

Materials and Methods

Plant material

Leaves of *C. pleurocarpa* were collected during a field trip in August 2023 in Pu Luong Nature Reserve, Thanh Hoa province, Vietnam, at the coordinates 20°24'13" N, 105°08'35" E, and an elevation of 427 meters above sea level. A co-

author, Assoc. Prof. Dr. Le Thi Huong from Vinh University, Vietnam authenticated and identified this plant specimen. A voucher specimen with code LTH32 was deposited in the herbarium of that university. A total of 6 kilograms of fresh leaves were collected and quickly transported to the laboratory for essential oil extraction.

Essential oil extraction

Two kilograms of fresh leaves were used to extract essential oils using the hydrodistillation method. The leaves were blended and placed in a 10-liter round-bottom flask containing 6 liters of distilled water. The flask was subjected to hydrodistillation using a Clevenger-type apparatus for 4 h under normal pressure according to the Vietnamese Pharmacopoeia[25], as described previously[22,23]. The extraction was repeated three times. The obtained essential oil was dried with anhydrous sodium sulfate and then placed in clean glass vials for storage at 4°C until analysis.

Essential oil analysis

Essential oil samples of *C. pleurocarpa* were analyzed using gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques, as previously described[21,24].

For the GC-FID analysis, an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with an FID and fitted with an HP-5ms column (30 m x 0.25 mm, film thickness 0.25 µm) was utilized. Hydrogen served as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set to 250°C, while the detector temperature was maintained at 260°C. The column temperature was programmed from an initial temperature of 60°C with a 2-min hold, then ramped at 4°C/min until reaching 220°C, which was held for 10 min. A split ratio of 10:1 was applied, and a volume of 1.0 µL of the sample was injected. The inlet pressure was regulated at 6.1 kPa to ensure consistent performance of the chromatographic system. Quantitative analysis of the essential oil components was carried out by normalizing the peak areas obtained from the FID chromatograms without applying corrective factors. The relative percentage of each compound was determined by comparing its peak area to the total peak area of all detected compounds.

Furthermore, GC-MS analysis was conducted using a mass spectrometer HP 5973 MSD under the same equipment, column, and chromatographic conditions utilized for GC-FID analysis. Helium served as the carrier gas at a flow rate of 1 mL/min. The mass spectrometer operated with an ionization voltage of 70 eV and an emission current of 40 mA. The acquisition scan mass range spanned from 35 to 350 atomic mass units (amu) at a sampling rate of 1.0 scan/s. Compound identification relied on co-injection with authentic standards (Sigma-Aldrich, USA), retention index (RI) comparison, and mass spectral analysis[26,27].

Antimicrobial assay

The antimicrobial activity of essential oil extracted from *C. pleurocarpa* was assessed against various microorganisms, including three strains of Gram-positive bacteria (*Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579), three strains of Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13076), and one strain of yeast (*Candida albicans* ATCC 10231). These microorganisms were sourced from the laboratory stock of the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology in Hanoi, Vietnam. The minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) values were determined using the broth microdilution assay method, as previously described[21,24].

For the experiments, Mueller-Hinton Agar (MHA) and Sabouraud Agar (SA) were used as the testing media for bacteria and fungi, respectively. Essential oil solutions were prepared in 1% dimethylsulfoxide and then diluted in sterile distilled water in micro-test tubes. These diluted solutions were subsequently transferred to 96-well microtiter plates.

Before testing, bacterial cultures were standardized to 5×10^5 CFU/mL in Mueller-

Hinton broth, while fungal cultures were adjusted to 1×10^3 CFU/mL in Sabouraud dextrose broth. The negative control consisted of sterile distilled water, while positive controls included streptomycin as the antibacterial standard and cycloheximide as the antifungal standard.

Incubation was carried out for 24 h at 37°C for bacteria and 30°C for fungi to promote microbial growth. Following incubation, microbial growth was assessed by measuring optical densities at 600 nm using a Spectramax 190-microplate reader. The MIC value was determined as the lowest concentration at which no visible growth of the microorganism occurred.

To determine IC_{50} values, the extent of microbial growth inhibition was quantified by measuring the turbidity of the cultures at 600 nm at specific time intervals (0, 6, 12, 18, and 24 h) using an EPOCH2C spectrophotometer and Rawdata computer software. The IC_{50} value, representing the concentration of the essential oil that caused 50% inhibition of microbial growth, was calculated by plotting the percentage of inhibition against the logarithm of the essential oil concentrations. This was done using a non-linear regression model to fit the dose-response curve.

Mosquito rearing and larvicidal assay

The mosquito larvicidal activity of the essential oil extracted from *C. pleurocarpa* was evaluated against *A. aegypti* and *C. quinquefasciatus* larvae, following previously described methods[21,24]. These mosquito species identification was conducted by Dr. Nguyen Huy Hung, utilizing morphological characteristics and standard taxonomic keys to ensure accuracy[28-30]. Larvae were collected in their early stages and reared at 25±2°C, 65-75% relative humidity, under a 12:12 h light/dark cycle in the Laboratory of Duy Tan University, Da Nang, Vietnam. To maintain species purity, the larvae were bred for two generations before experimentation, using dechlorinated tap water as breeding water, supplemented with a mixture of ground fish food (TetraMin® Tropical Flakes) as larval nourishment. Third-instar larvae were used in this experiment, as they are more resilient to environmental stresses and chemical treatments than younger larvae, providing a more consistent and reliable assessment of the essential oil's larvicidal activity.

The essential oil was dissolved in ethanol (Sigma-Aldrich) to create a 1% stock solution. Twenty larvae at the third instar stage, aged 4-5 days post-hatching, were placed into 300 mL beakers along with various dilutions of the essential oil stock solution to achieve final concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. These concentrations were chosen based on preliminary studies and existing literature to cover a range of doses likely to elicit measurable mortality, allowing for accurate calculation of LC_{50} and LC_{90} values[21]. The experiments were conducted at room temperature (25±2°C) with four replicates. Larvae were not provided food during the treatment to ensure that the mortality observed was solely due to the effects of the essential oil. Permethrin served as the positive control, while ethanol served as the negative control. Larval mortality was recorded after 24 and 48 h. LC_{50} and LC_{90} values, along with their 95% confidence intervals, were calculated using log-probit analysis performed with Minitab® 19.2020.1 software (State College, PA, USA). Corrected mortality was calculated using Abbott's formula, given below, to account for any mortality observed in the control groups.

$$\text{Corrected mortality (\%)} = \frac{\text{test mortality} - \text{control mortality}}{1 - \text{control mortality}} \times 100\%$$

Results and Discussion

Chemical composition of *C. pleurocarpa* essential oil

The hydrodistillation of *C. pleurocarpa* leaves yielded an essential oil at a rate of 0.12% ± 0.01 (v/w). Analysis via GC-FID and GC-MS revealed a complex chemical profile comprising 50 identified constituents (Table 1 and Fig. 1). Notably, the oil exhibited a significant abundance of sesquiterpene hydrocarbons (38.19%), oxygenated sesquiterpenes (30.15%), and diterpenes (10.78%). Among the major compounds identified in the essential oil of *C. pleurocarpa*, spathulenol (13.26%), phytol (9.94%), and α-selinene (5.34%) were

Table 1. Chemical composition of *Camellia pleurocarpa* essential oil

S. no.	Compound name [a]	RT [b]	RI [c]	RI [d]	Area (%)
1	Safrole	21.93	1298	1285	3.15
2	α-Cubebene	23.98	1360	1345	0.29
3	1,2-Dihydro-1,1,6-trimethyl-naphthalene	24.19	1366	1357	0.28
4	α-Copaene	24.94	1389	1374	0.62
5	*cis*-β-Elemene	25.40	1403	1389	3.25
6	α-Cedrene	26.27	1430	1410	1.21
7	(*E*)-Caryophyllene	26.44	1436	1417	3.34
8	β-Gurjunene	26.71	1444	1431	0.55
9	Aromadendrene	27.06	1455	1439	3.33
10	(*Z*)-β-Farnesene	27.23	1461	1440	1.26
11	α-Humulene	27.54	1471	1452	2.41
12	9-*epi*-(*E*)-Caryophyllene	27.77	1478	1464	0.28
13	β-Chamigrene	28.13	1489	1476	1.28
14	*ar*-Curcumene	28.19	1491	1479	1.51
15	α-Amorphene	28.24	1493	1483	0.38
16	(*E*)-β-Ionone	28.35	1496	1483	0.46
17	Germacrene D	28.36	1497	1484	0.57
18	β-Selinene	28.57	1503	1489	3.43
19	Asaricin	28.67	1507	1495	2.15
20	α-Selinene	28.83	1512	1498	5.34
21	β-Bisabolene	28.98	1517	1505	0.21
22	β-Curcumene	29.07	1520	1514	0.22
23	β-Sesquiphellandrene	29.48	1534	1521	3.09
24	δ-Cadinene	29.55	1536	1522	1.94
25	*cis*-Calamenene	29.59	1538	1528	1.11
26	*trans*-Cadina-1,4-diene	29.88	1547	1533	0.34
27	α-Cadinene	30.04	1552	1537	0.25
28	α-Calacorene	30.24	1559	1544	0.84
29	(*E*)-Nerolidol	30.56	1570	1561	1.30
30	β-Calacorene	30.82	1579	1564	0.71
31	Spathulenol	31.36	1597	1577	13.26
32	Caryophyllene oxide	31.56	1604	1582	4.17
33	Clovenol	31.67	1607	1685	0.27
34	Cubeban-11-ol	31.83	1613	1595	0.91
35	*epi*-Cedrol	32.18	1625	1618	1.83
36	1-*epi*-Cubenol	32.77	1646	1627	0.95
37	γ-Eudesmol	32.89	1650	1630	0.64
38	*epi*-α-Cadinol	33.18	1661	1638	0.94
39	α-Muurolol	33.23	1663	1644	0.47
40	Eudesma-4(15),7-dien-1β-ol	33.33	1666	1647	1.08
41	α-Cadinol	33.53	1673	1652	3.56
42	*neo*-Intermedeol	33.61	1676	1658	0.77
43	(Z)-Heptadec-8-ene	33.74	1681	1673	0.58
44	Cadalene	34.10	1693	1680	0.43
45	*n*-Heptadecane	34.29	1700	1700	0.22

Table 1 ***cont***.

S. no.	Compound name [a]	RT [b]	RI [c]	RI [d]	Area (%)
46	Pentadecanal	34.77	1718	1710	0.69
47	Benzyl benzoate	36.51	1782	1759	0.24
48	6,10,14-Trimethylpentadecan-2-one	38.23	1848	1841	1.88
49	Isophytol	40.82	1951	1942	0.84
50	Phytol	44.77	2118	2112	9.94
	Sesquiterpene hydrocarbons (S. No. 2, 4-15, 17, 18, 20-28, 30, 44)				38.19
	Oxygenated sesquiterpenes (S. No. 29, 31-42)				30.15
	Diterpenes (S. No. 49, 50)				10.78
	Others (S. No. 1, 3, 16, 19, 43, 45-48)				9.65
	Total identified				88.77

[a]Elution order on HP-5ms column; [b]Retention time (min); [c]Retention indices on HP-5ms column; [d]Literature retention indices

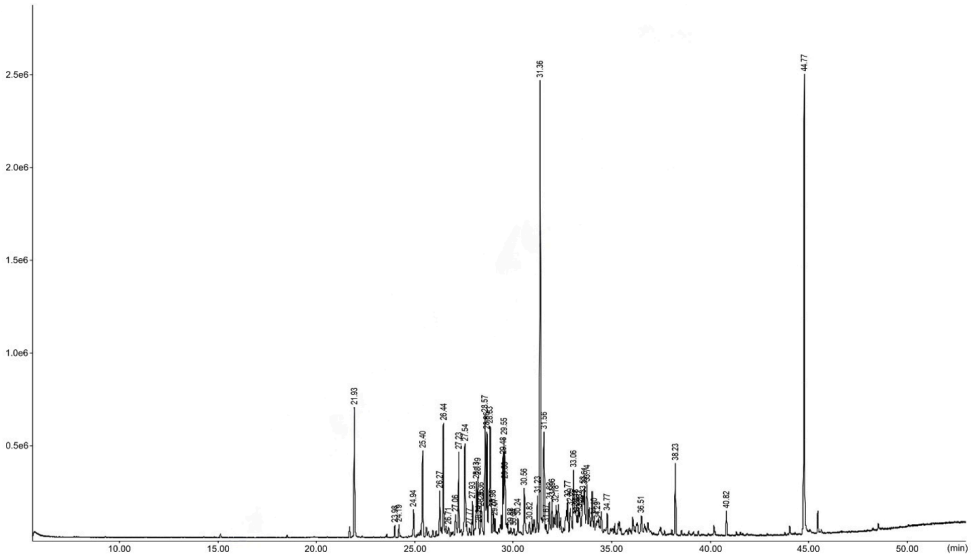

Figure 1. GC chromatogram of *Camellia pleurocarpa* essential oil

prominent. Additionally, substantial quantities of caryophyllene oxide (4.17%), α-cadinol (3.56%), β-selinene (3.43%), (*E*)-caryophyllene (3.34%), aromadendrene (3.33%), *cis*-β-elemene (3.25%), safrole (3.15%), β-sesquiphellandrene (3.09%), α-humulene (2.41%), and asaricin (2.15%) were detected.

This study marks the initial exploration into the chemical composition of essential oil extracted from *C. pleurocarpa*, precluding direct comparisons with analogous samples from the same species. However, extensive examinations of essential oils from other *Camellia* species have been documented, including *C. sinensis*[12,13], *C. tunghinensis*[14], *C. euphlebia*[14], *C. japonica*[15,16], *C. nitidissima*[14,17] and *C. longii*[18] (Table 2). For instance, essential oil from *C. sinensis* exhibited predominant compounds such as nonadecane (18.7%), heneicosane (12.2%), dibutyl phthalate (5.0%), and tricosane (4.9%)[12]. Conversely, *C. tunghinensis* essential oil showcased major constituents like *n*-hexanal (17.2%), 2-pentylfuran (10.6%), phytone (7.5%), and geranylacetone (5.0%)[14]. Notably, phytol (58%), geranylacetone (5.6%), and *n*-hexanal (3.3%) were abundant in the essential oil of *C. euphlebia*[14]. Distinct chemical profiles were observed in *C. japonica* essential oil, with hexamethylcyclotrisiloxane

Table 2. Major components (> 5%) identified from *Camellia* essential oils

Camellia species	Collection location	Plant part	Major components (%)	Reference
C. sinensis	Zhejiang, China	flower	nonadecane (18.7%), heneicosane (12.2%), dibutyl phthalate (5.0%)	12
C. tunghinensis	Guangxi, China	leaf	*n*-hexanal (17.2%), 2-pentylfuran (10.6%), phytone (7.5%), geranylacetone (5.0%)	14
C. euphlebia	Guangxi, China	leaf	phytol (58%), geranylacetone (5.6%)	14
C. nitidissima	Guangxi, China	leaf	linalool (35.8%), phytol (7.9%), geranylacetone (7.3%), methyl salicylate (6.8%)	14
C. japonica	Nonsan, South Korea	seed	hexamethylcyclotrisiloxane (42.36%), octamethylcyclotetrasiloxane (23.28%), decamethylcyclopentasiloxane (5.81%)	15
C. nitidissima	Guangxi, China	leaf	linalool (35.8%), phytol (7.9%), *cis*-geranyl acetone (7.3%), methyl salicylate (6.8%)	17
C. nitidissima	Guangxi, China	flower	α-eudesmol (34.3%), γ-eudesmol (31.5%), linalool (11.1%)	17
C. longi	Lam Dong, Vietnam	flower	α-eudesmol (16.1%), (*E*)-nerolidol (13.0%), β-eudesmol (8.9%), τ-cadinol (6.5%), γ-eudesmol (5.8%)	18

(42.36%) and octamethylcyclotetrasiloxane (23.28%) being predominant[15]. In *C. nitidissima*, significant amounts of linalool (35.8%), phytol (7.9%), *cis*-geranyl acetone (7.3%), and methyl salicylate (6.8%) were found in leaf essential oil, while α-eudesmol (34.3%), γ-eudesmol (31.5%) and linalool (11.1%) dominated in flower essential oil[17]. In another study, the essential oil of *C. longii* mainly consisted of α-eudesmol (16.1%), (*E*)-nerolidol (13.0%), β-eudesmol (8.9%), τ-cadinol (6.5%), and γ-eudesmol (5.8%)[18]. Interestingly, compounds such as (*E*)-nerolidol, γ-eudesmol, and τ-cadinol, which are prevalent in other *Camellia* species, were found in low quantities in the essential oil of *C. pleurocarpa*. Moreover, compounds like linalool, hexamethylcyclotrisiloxane, octamethylcyclotetrasiloxane, and nonadecane were absent. A significant observation is that the essential oil of *C. pleurocarpa* notably lacks monoterpenoids, a feature that distinguishes it from many other *Camellia* essential oils. Monoterpenoids are typically common in essential oils, contributing to their aroma and biological activities[21]. Essential oils devoid of monoterpenoids have been documented in various studies[31-34]. These variations in chemical composition among *Camellia* species' essential oils can be attributed to factors such as plant age, environmental conditions, selection of plant organs, and harvesting times[35-37]. These factors influence plant biosynthesis pathways, ultimately leading to diverse chemical compositions and content, thus contributing to the development of distinct chemotypes[38].

Antimicrobial activity of *C. pleurocarpa* essential oil

The antimicrobial effects of *C. pleurocarpa* essential oil were assessed against seven test microorganisms, comprising three Gram-positive bacteria, three Gram-negative bacteria, and one yeast. The potency of the essential oil was quantitatively evaluated using MIC and IC_{50} values, as presented in Table 3. The essential oil demonstrated activity against all three Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. cereus*), one Gram-negative bacterium (*P.*

Table 3. Antimicrobial activity of *Camellia pleurocarpa* essential oil

Microorganisms	Essential oil		Streptomycin*	Cycloheximide*
	MIC	IC_{50}	MIC	MIC
Enterococcus faecalis ATCC 299212	32	16.23	32	–
Staphylococcus aureus ATCC 25923	16	7.26	32	–
Bacillus cereus ATCC 14579	16	7.78	32	–
Escherichia coli ATCC 25922	–	–	32	–
Pseudomonas aeruginosa ATCC 27853	32	14.37	32	–
Salmonella enterica ATCC 13076	–	–	256	–
*Candida albican*s ATCC 10231	16	8.45	–	32

MIC: Minimum inhibitory concentration (µg/mL); IC_{50}: Half-maximal inhibitory concentration (µg/mL); *Positive control

aeruginosa), and one yeast (*C. albicans*), with MIC values ranging from 16 to 32 µg/mL and IC_{50} values ranging from 7.26 to 16.23 µg/mL. Specifically, the essential oil showed remarkable activity against *S. aureus*, *B. cereus*, and *C. albicans* with an MIC value of 16 µg/mL, while IC_{50} values were 7.26, 7.78, and 8.45 µg/mL, respectively. Furthermore, the essential oil was effective against *E. faecalis* and *P. aeruginosa* with an MIC value of 32 µg/mL, while the IC_{50} values were 16.23 and 14.37 µg/mL, respectively. However, this essential oil did not exhibit activity against the Gram-negative bacteria *E. coli* and *S. enterica*. The positive control, streptomycin, used against gram-positive and gram-negative bacteria, showed activity with an MIC value of 32 µg/mL against *E. faecalis*, *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*. Streptomycin exhibited a higher MIC value of 256 µg/mL against *S. enterica*. Cycloheximide, the standard antifungal drug, demonstrated activity with an MIC value of 32 µg/mL against *C. albicans*. It is noteworthy that the antimicrobial activity of the essential oil was comparable to or even superior to that of the positive control. Previous research has demonstrated that essential oils exhibit strong antimicrobial effects when their MIC values are below 100 µg/mL[39,40]. According to this criterion, the *C. pleurocarpa* essential oil in our study exhibited strong antimicrobial activity. Overall, this antimicrobial data, along with results reported for other *Camellia* plants in the literature, reveal that *C. pleurocarpa* essential oil exhibited higher potency against comparable microorganisms[14,17].

The observed antimicrobial activity of *C. pleurocarpa* essential oil can be attributed to its chemical composition, particularly the presence of bioactive compounds such as sesquiterpenes and diterpenes[41,42]. Spathulenol[43,44], phytol[45], α-selinene[46], caryophyllene oxide[47] and α-cadinol[48] have been reported to possess antimicrobial properties in various studies. These compounds may disrupt microbial cell membranes, inhibit essential microbial enzymes, or interfere with microbial cell processes, leading to microbial growth inhibition[49]. The significant presence of sesquiterpene hydrocarbons and oxygenated sesquiterpenes in the essential oil suggests a broad spectrum of antimicrobial activity, as these compounds have been reported to exhibit potent antimicrobial effects against both Gram-positive and Gram-negative bacteria, as well as fungi. The synergistic interactions between the major and minor components of the essential oil could enhance its antimicrobial activity[50]. The diverse chemical profile of the essential oil contributes to its multifaceted antimicrobial activity, targeting various microbial species through different mechanisms of action. However, the lack of activity against Gram-negative bacteria such as *E. coli* and *S. enterica* could be attributed to the presence of an outer membrane in these bacteria, which acts as a barrier to hydrophobic compounds like essential oils[51]. Additionally, differences

in cell wall structure and composition between Gram-positive and Gram-negative bacteria may influence susceptibility to antimicrobial agents[52].

Mosquito larvicidal activity of *C. pleurocarpa* essential oil

The mosquito larvicidal potential of *C. pleurocarpa* essential oil was assessed against *A. aegypti* and *C. quinquefasciatus* larvae, as detailed in Tables 4 and 5. After 24 h of exposure, the essential oil demonstrated significant activity against *A. aegypti*, with LC_{50} and LC_{90} values of 26.42 µg/mL and 35.05 µg/mL, respectively. In contrast, the LC_{50} and LC_{90} values for *C. quinquefasciatus* were 28.95 µg/mL and 53.24 µg/mL, respectively. Following 48 h of exposure, the LC_{50} and LC_{90} values for *A. aegypti* decreased to 19.82 µg/mL and 28.29 µg/mL, indicating increased larvicidal effectiveness over time. For *C. quinquefasciatus*, the LC_{50} and LC_{90} values dropped to 13.49 µg/mL and 28.89 µg/mL, respectively, demonstrating a marked improvement in larvicidal activity with prolonged exposure. Permethrin, the positive control, exhibited much higher potency against both mosquito species, with LC_{50} values of 0.00064 µg/mL for *A. aegypti* and 0.01618 µg/mL for *C. quinquefasciatus*, and corresponding LC_{90} values of 0.00248 µg/mL and 0.0290261 µg/mL, respectively. The slopes, R^2 values, and distribution types for each analysis are presented in Tables 4 and 5. Fig. 2 illustrates the mortality rates of *A. aegypti* and *C. quinquefasciatus* larvae following exposure to *C. pleurocarpa* essential oil. The results revealed 100% mortality for *A. aegypti* larvae at a concentration of 50 µg/mL after 24 h, while *C. quinquefasciatus* larvae reached 100% mortality at the same concentration after 48 h.

When comparing the susceptibility of the two mosquito species, distinct sensitivity patterns to the essential oil were observed. After 24 h, *A. aegypti* exhibited greater sensitivity, with lower LC_{50} and LC_{90} values compared to *C. quinquefasciatus*. However, after 48 h, *C. quinquefasciatus* showed increased sensitivity, as indicated by lower LC_{50} and LC_{90} values compared to *A. aegypti*. This variation in sensitivity over

Table 4. Mosquito larvicidal activity of *Camellia pleurocarpa* essential oil against *Aedes aegypti* larvae (µg/mL)

Time	Sample	LC_{50} (95% limits)	LC_{90} (95% limits)	χ^2	p	Slope	R^2	Distribution
24 h	Essential oil	26.42 (25.12–28.17)	35.05 (31.79–42.69)	2.474	0.649	0.2955	0.7713	Loglogistic
	Permethrin*	0.00064 (0.00054–0.00074)	0.00248 (0.00197–0.00337)	13.4559	0.009	4236.0	64.9	Loglogistic
48 h	Essential oil	19.82 (18.38–21.23)	28.29 (26.07–31.54)	0.502	0.973	0.2799	0.6684	Loglogistic

*Positive control

Table 5. Mosquito larvicidal activity of *Camellia pleurocarpa* essential oil against *Culex quinquefasciatus* larvae (µg/mL)

Time	Sample	LC_{50} (95% limits)	LC_{90} (95% limits)	χ^2	p	Slope	R^2	Distribution
24 h	Essential oil	28.95 (26.26–31.95)	53.24 (46.49–64.08)	3.196	0.526	0.2259	0.8450	Loglogistic
	Permethrin*	0.01618 (0.01471–0.0178086)	0.0290261 (0.0254368–0.0348442)	7.33992	0.062	356.0	79.11	Loglogistic
48 h	Essential oil	13.49 (12.09–15.05)	28.89 (24.82–35.36)	6.019	0.198	0.1904	0.6327	Loglogistic

*Positive control

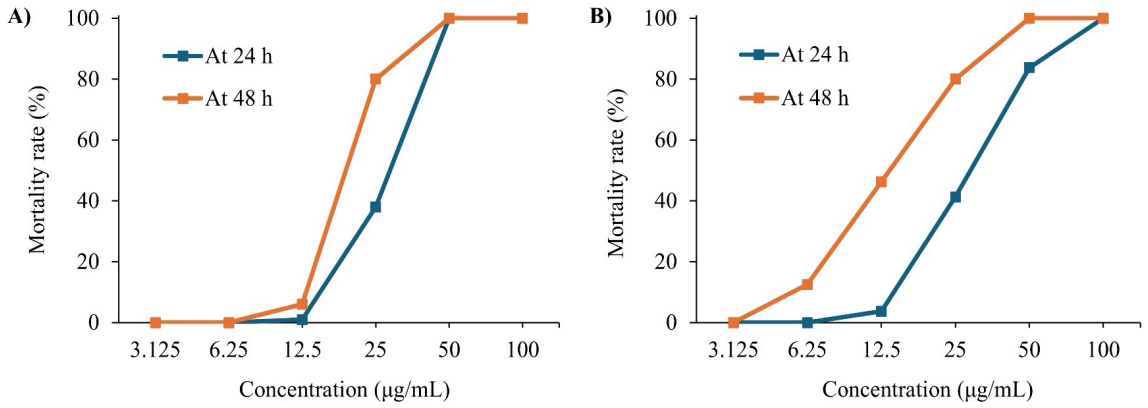

Figure 2. Mortality rate of *Aedes aegypti* (A) and *Culex quinquefasciatus* (B) larvae after exposure to *Camellia pleurocarpa* essential oil

time highlights the impact of exposure duration on the larvicidal effectiveness of the essential oil. Additionally, this difference in sensitivity could be attributed to species-specific physiological and metabolic responses to the active compounds in the essential oil[53,54]. Since this study represents the first investigation of essential oils from the genus *Camellia* on mosquito larvicidal activity, direct comparison with other studies within the same genus is not feasible. However, previous research has suggested that essential oils with LC_{50} values below 100 µg/mL exhibit significant larvicidal activity[53,54]. Therefore, based on this criterion, we can conclude that *C. pleurocarpa* essential oil demonstrated significant larvicidal potential against *A. aegypti* and *C. quinquefasciatus* larvae.

The diverse chemical composition of *C. pleurocarpa* essential oil provides a rich source of bioactive compounds with potential applications across various fields, including insect control. The observed larvicidal activity against *A. aegypti* and *C. quinquefasciatus* larvae underscores the importance of exploring natural plant-based alternatives for mosquito control, especially in light of growing concerns regarding insecticide resistance and environmental safety[55]. The presence of sesquiterpene hydrocarbons, such as α-selinene and β-selinene, is particularly notable due to their recognized insecticidal properties, as they have been reported to exert toxicity against insect pests by disrupting their nervous system or interfering with key physiological processes[56-58]. Additionally, oxygenated sesquiterpenes like spathulenol and caryophyllene oxide are known for their insecticidal and repellent activities, further contributing to the observed larvicidal effects[59,60]. Phytol, a diterpene alcohol, may also play a significant role in the larvicidal activity of the essential oil[59]. While diterpenes have been less studied in the context of insect control compared to sesquiterpenes, they have demonstrated insecticidal properties against various insect species[61]. Phytol could potentially act as a synergist, enhancing the efficacy of other active compounds present in the essential oil. The observed differences in LC_{50} and LC_{90} values between the two mosquito species could be attributed to variations in their susceptibility to the constituents of the essential oil[21]. *A. aegypti* and *C. quinquefasciatus* possess distinct physiological and behavioral characteristics, which may influence their response to chemical stimuli[62].

Conclusions

In conclusion, *C. pleurocarpa* essential oil emerges as a promising candidate for various applications in healthcare and vector control. Its diverse chemical composition, featuring a plethora of bioactive compounds, contributes to its potent antimicrobial activity against a wide spectrum of pathogens. Additionally, its demonstrated efficacy as a larvicide against mosquito vectors highlights its potential in combating mosquito-

borne diseases. The environmentally friendly nature of *C. pleurocarpa* essential oil makes it an attractive alternative to synthetic antimicrobial agents and mosquito larvicides. Further research into its mechanisms of action, safety profile, and practical applications is essential to unlock its full potential in disease control strategies.

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Conflicts of interest

The authors declare no conflict of interest.

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