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# CHEMICAL COMPOSITION, ANTIMICROBIAL AND MOSQUITO LARVICIDAL ACTIVITIES OF THE ESSENTIAL OIL OF *CHLORANTHUS ERECTUS* COLLECTED IN VIETNAM

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**ABSTRACT.** *Chloranthus erectus*, a subshrub native to South and Southeast Asia, has traditionally been used for its medicinal properties. This study investigated the chemical composition of the essential oil extracted from the aerial parts of *C. erectus* collected in Vietnam and evaluated its antimicrobial and mosquito larvicidal activities. The essential oil was obtained by hydrodistillation with a yield of 0.21% (v/w) and analyzed using gas chromatography coupled with flame ionization detection and mass spectrometry (GC–FID/MS). The major constituents identified were (*E*)-β-ocimene (13.41%), myrcene (12.85%), spathulenol (12.55%), and bicyclogermacrene (12.01%). Antimicrobial activity was assessed by determining the minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration ($IC_{50}$) against selected bacterial and fungal strains. The essential oil showed antimicrobial activity, particularly against *Candida albicans* (MIC: 16 µg/mL; $IC_{50}$: 8.96 µg/mL), as well as against Gram-positive bacteria, including *Bacillus cereus* and *Enterococcus faecalis*. Larvicidal assays demonstrated toxicity against larvae of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*, with lethal concentration ($LC_{50}$ and $LC_{90}$) values below 100 µg/mL after 24 and 48 h of exposure. These results highlight the bioactive potential of *C. erectus* essential oil for pharmaceutical and environmentally friendly mosquito control applications.

***Keywords:*** *Chloranthus erectus, essential oil, antimicrobial activity, Candida albicans, mosquito larvicidal activity*

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## INTRODUCTION

The genus *Chloranthus* (family Chloranthaceae) comprises approximately 14 recognized species of perennial herbs and subshrubs, predominantly distributed across tropical and subtropical regions of Asia [1]. These species are commonly found in countries such as China, Vietnam, Japan, India, and Malaysia, where they typically inhabit forested, humid, and mountainous ecosystems [1]. Many *Chloranthus* species have a long history of use in traditional Asian medicine. Ethnobotanical records document their application in the treatment of various ailments, including inflammation, fever, pain, and infected wounds [1]. These traditional uses have stimulated extensive phytochemical investigations, revealing that *Chloranthus* species are rich sources of structurally diverse secondary metabolites. To date, several classes of bioactive compounds have been identified within the genus, notably sesquiterpenoids, diterpenoids, flavonoids, and lignans [2, 3]. These metabolites exhibit a broad spectrum of biological activities, including anti-inflammatory, antioxidant, antimicrobial, and cytotoxic effects, thereby supporting both their traditional applications and pharmacological potential [2, 3].

Among the less-studied members of the genus is *Chloranthus erectus*, a species native to forested and montane regions of South and Southeast Asia [4]. It occurs in countries such as Vietnam and China, where it has traditionally been used for wound healing and the treatment of skin infections. Despite its ethnopharmacological relevance, the phytochemical and biological profiles of *C. erectus* remain relatively underexplored compared with other *Chloranthus* species. Preliminary investigations have reported the presence of various phytochemical constituents, including alkaloids, flavonoids, terpenoids, saponins, quinones, glycosides, and steroids, some of which have demonstrated antimicrobial, antioxidant, antipyretic, and anti-inflammatory properties [5–8]. However, studies focusing specifically on the essential oil composition of *C. erectus* remain scarce. Essential oils are volatile and aromatic plant-derived compounds that frequently exhibit potent biological activities [9]. In other *Chloranthus* species, essential oils have demonstrated antimicrobial, antioxidant, and anti-inflammatory effects [10–14]. A study conducted in Malaysia reported that germacrone was the major constituent of *C. erectus* essential oil, accounting for 36.62% of the total composition [15]. The oil also exhibited antifungal activity against *Ceratocystis fimbriata* [15].

To date, this Malaysian report remains the only published study describing the essential oil composition of *C. erectus*. Importantly, essential oil composition is strongly influenced by geographic origin, environmental conditions, and genetic variability, often resulting in distinct chemotypes [16, 17]. Therefore, findings obtained from Malaysian populations cannot be assumed to represent plants growing in other ecological regions. To the best of our

knowledge, no previous study has characterized the essential oil composition of *C. erectus* collected in Vietnam, nor has any investigation evaluated its larvicidal activity against mosquito vectors. This represents a significant knowledge gap, particularly given Vietnam's distinct climatic and ecological conditions, which may give rise to a unique chemotype with different biological properties.

The increasing prevalence of antimicrobial resistance (AMR) and the resurgence of mosquito-borne diseases represent major global public health challenges [18, 19]. The overuse and misuse of synthetic antibiotics have accelerated the emergence of multidrug-resistant bacterial and fungal pathogens, thereby compromising treatment efficacy [18]. Concurrently, the extensive use of synthetic insecticides has led to resistance in major mosquito vectors, including *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus*, which transmit diseases such as dengue, chikungunya, Zika, and lymphatic filariasis [19]. Moreover, synthetic agents pose environmental risks due to their persistence and toxicity toward non-target organisms. These challenges underscore the urgent need for alternative and environmentally sustainable solutions. In this context, essential oils have emerged as promising candidates for antimicrobial and vector control applications [20–22]. Due to their complex mixture of bioactive constituents and multifaceted mechanisms of action, essential oils may reduce the likelihood of resistance development [20, 21]. Additionally, many have demonstrated larvicidal activity against disease-vector mosquitoes, offering biodegradable and comparatively safer options for integrated vector management strategies [22].

Given the limited data available on *C. erectus* essential oil, particularly from Vietnam, the present study aimed to investigate its potential as a source of bioactive compounds. Notably, this study provides the first report describing both the chemical composition and the larvicidal activity of *C. erectus* essential oil from a Vietnamese population. The specific objectives were to: (1) characterize the chemical composition of essential oil extracted from the aerial parts of *C. erectus* collected in Vietnam using gas chromatography with flame ionization detection and mass spectrometry (GC–FID/MS); (2) evaluate its antimicrobial activity against selected bacterial and fungal pathogens; and (3) assess, for the first time, its larvicidal activity against larvae of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*.

## RESULTS AND DISCUSSION

### *Chemical composition of Chloranthus erectus essential oil*

The essential oil extracted from *C. erectus* was obtained with a yield of 0.21% ± 0.01 (v/w) and analyzed by GC–FID/MS (Fig. S1). A total of 92.95% of the oil composition was identified, comprising several groups of

compounds (Table 1). Monoterpene hydrocarbons were the predominant class, accounting for 39.63% of the total oil, followed by sesquiterpene hydrocarbons (27.10%), oxygenated sesquiterpenes (19.35%), and oxygenated monoterpenes (5.12%), while other minor constituents contributed 1.75%. The principal components were (*E*)-β-ocimene (13.41%), myrcene (12.85%), spathulenol (12.55%), and bicyclogermacrene (12.01%), which together represented a substantial proportion of the total composition (Table 2). Other notable constituents included cis-β-elemene (4.80%), camphene (3.41%), α-pinene (3.22%), α-phellandrene (2.13%), elemol (2.07%), and bornyl acetate (2.04%). Compound identification was performed by comparison of retention indices (RI), calculated relative to a homologous series of *n*-alkanes on an HP-5ms column, together with mass spectral matching against the NIST library and published data. Although minor RI deviations were observed for certain sesquiterpenes, identification was confirmed by consistent agreement between RI values and MS fragmentation patterns, ensuring the analytical reliability of the reported composition. Overall, the chemical diversity of this essential oil indicates potential applications in the fragrance and pharmaceutical industries, largely attributable to the presence of bioactive terpenes and oxygenated derivatives.

**Table 1.** Distribution of chemical classes in the essential oil of *Chloranthus erectus*

| Chemical class | Percentage (%) |
|---|---|
| Monoterpene hydrocarbons | 39.63 |
| Oxygenated monoterpenes | 5.12 |
| Sesquiterpene hydrocarbons | 27.10 |
| Oxygenated sesquiterpenes | 19.35 |
| Other compounds | 1.75 |
| Total identified | 92.95 |

Notably, the chemical profile observed in this study differs markedly from the only previously reported analysis of *C. erectus* essential oil from Malaysia, where germacrone (36.62%) was the predominant compound [15]. In contrast, germacrone was absent in our sample. Such variation may result from environmental influences, genetic diversity, or differences in extraction and analytical methodologies [16, 17]. Since sesquiterpenes like germacrone are synthesized via the mevalonate pathway, its absence may reflect ecological or seasonal modulation of terpene biosynthesis [23].

**Table 2.** Chemical composition of the essential oil from *Chloranthus erectus*

| Compound name [a] | RT [b] | RI [c] | RI [d] | Area (%) |
|---|---|---|---|---|
| α-Pinene | 9.79 | 939 | 932 | 3.22 |
| Camphene | 10.28 | 955 | 946 | 3.41 |
| Sabinene | 10.98 | 978 | 969 | 0.13 |
| β-Pinene | 11.15 | 984 | 974 | 1.09 |
| Myrcene | 11.41 | 993 | 988 | 12.85 |
| α-Phellandrene | 11.97 | 1010 | 1002 | 2.13 |
| *o*-Cymene | 12.63 | 1029 | 1022 | 1.42 |
| Limonene | 12.78 | 1034 | 1024 | 0.99 |
| β-Phellandrene | 12.83 | 1035 | 1025 | 0.24 |
| (*Z*)-β-Ocimene | 12.93 | 1038 | 1032 | 0.56 |
| (*E*)-β-Ocimene | 13.32 | 1050 | 1044 | 13.41 |
| Terpinolene | 14.84 | 1094 | 1086 | 0.18 |
| Rosefuran | 15.02 | 1099 | 1094 | 0.15 |
| Linalool | 15.13 | 1102 | 1095 | 1.13 |
| Perillene | 15.19 | 1104 | 1102 | 0.31 |
| Linalyl acetate | 20.54 | 1257 | 1254 | 0.71 |
| Geranial | 21.15 | 1275 | 1264 | 0.15 |
| Bornyl acetate | 21.79 | 1294 | 1284 | 2.04 |
| Safrole | 21.93 | 1298 | 1285 | 1.11 |
| δ-Elemene | 23.58 | 1347 | 1335 | 0.35 |
| α-Terpinyl acetate | 23.88 | 1356 | 1346 | 0.63 |
| Cyclosativene | 24.72 | 1382 | 1369 | 0.63 |
| α-Copaene | 24.93 | 1388 | 1374 | 0.54 |
| *cis*-β-Elemene | 25.41 | 1403 | 1385 | 4.80 |
| (*E*)-Caryophyllene | 26.44 | 1436 | 1417 | 0.93 |
| γ-Elemene | 26.72 | 1444 | 1434 | 1.21 |
| Aromadendrene | 27.07 | 1456 | 1439 | 0.33 |
| *cis*-Muurola-3,5-diene | 27.38 | 1465 | 1448 | 0.30 |
| α-Humulene | 27.53 | 1470 | 1452 | 0.29 |
| 9-*epi*-(*E*)-Caryophyllene | 27.76 | 1478 | 1664 | 0.38 |
| *epi*-Zonarene | 28.06 | 1487 | 1480 | 0.26 |
| Valencene | 28.13 | 1489 | 1483 | 0.19 |
| Germacrene D | 28.37 | 1497 | 1484 | 0.65 |
| β-Selinene | 28.56 | 1503 | 1489 | 1.18 |
| Asaricin (= Sarisan) | 28.67 | 1507 | 1495 | 0.64 |
| Bicyclogermacrene | 28.88 | 1514 | 1500 | 12.01 |
| γ-Cadinene | 29.41 | 1531 | 1513 | 0.61 |
| δ-Cadinene | 29.55 | 1536 | 1522 | 0.85 |
| Zonarene | 29.66 | 1540 | 1528 | 0.20 |

| Compound name [a] | RT [b] | RI [c] | RI [d] | Area (%) |
|---|---|---|---|---|
| Elemol | 30.37 | 1564 | 1548 | 2.07 |
| *E*-Nerolidol | 30.57 | 1570 | 1561 | 1.99 |
| Germacrene B | 30.77 | 1577 | 1562 | 1.39 |
| Spathulenol | 31.39 | 1598 | 1577 | 12.55 |
| Caryophyllene oxide | 31.57 | 1604 | 1582 | 0.76 |
| 1-*epi*-Cubenol | 32.76 | 1646 | 1627 | 0.39 |
| γ-Eudesmol | 32.89 | 1650 | 1630 | 0.22 |
| *epi*-α-Cadinol | 33.19 | 1661 | 1638 | 0.28 |
| β-Eudesmol | 33.49 | 1672 | 1649 | 0.55 |
| α-Eudesmol | 33.56 | 1674 | 1652 | 0.54 |
| Unidentified | 42.38 | 2015 | − | 1.57 |

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

Comparative analyses with other species in the *Chloranthus* genus further highlight interspecific metabolic divergence. For instance, *C. serratus* essential oil is dominated by cycloisolongifolene, 8,9-dehydro-9-formyl- (23.3%), 4-hydroxy-β-ionone (11.4%), curzerene (9.6%), and eremanthin (9.4%)-compounds not prevalent in *C. erectus* [11]. Likewise, bornyl acetate, which is a major component in *C. japonicus* (30.98%) and *C. multistachys* (35.99%), is present at a much lower concentration (2.04%) in *C. erectus* [10].

The essential oil profile of *C. elatior* shows partial similarity to *C. erectus*, with both containing bicyclogermacrene (*C. erectus*: 12.01%; *C. elatior*: 11.3%) [13]. However, *C. elatior* is also characterized by high levels of bicycloelemene (11.2%), (*Z*)-β-ocimene (7.8%), and *allo*-ocimene (6.3%) [13], whereas *C. erectus* contains a higher proportion of (*E*)-β-ocimene (13.41%). These differences underscore the influence of genetic and ecological factors on terpene profiles, even among closely related species [16, 17].

Intraspecific variation is also evident within *C. spicatus*, whose essential oil composition varies with geographic origin and plant part analyzed. For example, Vietnamese leaf oil is dominated by α-cadinol (10.0%), bicyclogermacrene (9.1%), and bicycloelemene (8.2%), while stem oil is rich in guaiol (16.9%), bicycloelemene (6.0%), and α-humulene (5.6%) [13]. A separate Vietnamese study by Tesso *et al.* reported (*Z*)-β-ocimene (6.3%), *allo*-aromadendrene (6.2%), sarisane (4.2%), and selina-4(15), 7(11)-diene (6.4%) as dominant components [12]. In contrast, *C. spicatus* from China exhibited a distinctly different profile, with 1,5,5-trimethyl-6-methylenecyclohexene (14.58%) as the most abundant compound, along with bicyclogermacrene (11.12%) and others [14].

Overall, the observed differences in essential oil composition of *C. erectus* and other *Chloranthus* species can be attributed to a combination of genetic, environmental, and methodological factors [16, 17]. The absence of germacrone in *C. erectus* from our study, despite its dominance in the Malaysian sample, highlights the need for further research into the role of ecological conditions in essential oil biosynthesis. Likewise, intraspecific variations, as seen in *C. spicatus* from different geographical regions, suggest that external factors such as climate, soil nutrients, and seasonal fluctuations play a critical role in determining the chemical profile of *Chloranthus* essential oils. These findings underscore the importance of continued investigations into the chemotaxonomic and pharmacological significance of *Chloranthus* essential oils.

### *Antimicrobial activity of Chloranthus erectus essential oil*

The essential oil of *C. erectus* demonstrated antimicrobial activity against several tested bacterial and fungal strains, with minimum inhibitory concentration (MIC) values ranging from 16 to 64 µg/mL and half-maximal inhibitory concentration ($IC_{50}$) values from 8.96 to 24.15 µg/mL (Table 3). The oil inhibited the growth of *Candida albicans* with a MIC of 16 µg/mL and an $IC_{50}$ of 8.96 µg/mL, showing activity comparable to the reference antifungal agent cycloheximide. Among the bacterial strains evaluated, *Bacillus cereus* and *Enterococcus faecalis* showed MIC values of 32 µg/mL, with corresponding $IC_{50}$ values of 9.21 and 15.34 µg/mL, respectively. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inhibited at a MIC of 64 µg/mL, with $IC_{50}$ values of 24.15 and 20.34 µg/mL, respectively. No inhibitory effect was observed against *Escherichia coli* and *Salmonella enterica*, whereas the positive control streptomycin inhibited these strains with MIC values of 256 µg/mL. Overall, the findings indicate that *C. erectus* essential oil displays antimicrobial activity against Gram-positive bacteria and yeast under the tested conditions.

**Table 3.** Antimicrobial activity of the essential oil from *Chloranthus erectus*

| Microorganisms | Essential oil | | Positive control [c] |
|---|---|---|---|
| | MIC [a] | $IC_{50}$ [b] | MIC [a] |
| *Enterococcus faecalis* ATCC 299212 | 32 | 15.34 | 32 |
| *Staphylococcus aureus* ATCC 25923 | 64 | 24.15 | 64 |
| *Bacillus cereus* ATCC 14579 | 32 | 9.21 | 32 |
| *Escherichia coli* ATCC 25922 | – | – | 256 |
| *Pseudomonas aeruginosa* ATCC 27853 | 64 | 20.34 | 256 |
| *Salmonella enterica* ATCC 13076 | – | – | 256 |
| *Candida albicans* ATCC 10231 | 16 | 8.96 | 16 |

[a]Minimum inhibitory concentration (µg/mL). [b]Half-maximal inhibitory concentration (µg/mL).
[c]The positive controls for bacteria and yeast were streptomycin and cycloheximide, respectively.

Compared to previous studies, the antimicrobial potency observed in this work appears more pronounced. In an earlier report, the essential oil of *C. erectus* collected in Malaysia showed antifungal activity against *Ceratocystis fimbriata* but was ineffective against *Colletotrichum* and *Fusarium* species [15]. This discrepancy may reflect differences in chemical composition. The Malaysian sample was dominated by germacrone [15], while the present oil contains high levels of (*E*)-β-ocimene, myrcene, spathulenol, and bicyclogermacrene—compounds with well-established antimicrobial properties [24–27].

Essential oils from other *Chloranthus* species have also demonstrated significant antimicrobial activity. For example, the oil of *C. japonicus* showed strong antibacterial effects against *B. cereus* (MIC = 0.39 mg/mL), while *C. multistachys* was effective against both *B. cereus* and *Candida lipolytica* (MIC = 0.78 mg/mL) [10]. Moreover, the essential oil of *C. spicatus* has been reported to inhibit a broader spectrum of microorganisms, including *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*, suggesting a wider antimicrobial range than that observed for *C. erectus* [14].

The antimicrobial effects of *C. erectus* essential oil can be attributed to both the major and minor constituents, and their potential synergistic interactions [28]. Among the dominant compounds, (*E*)-β-ocimene and myrcene are monoterpene hydrocarbons known to compromise microbial membrane integrity by increasing permeability and inducing intracellular leakage [29, 30]. Spathulenol, an oxygenated sesquiterpene, exerts antimicrobial effects through membrane destabilization and enzyme inhibition [31]. Bicyclogermacrene, a sesquiterpene hydrocarbon, has also been linked to bacteriostatic activity, likely through interference with membrane structure and energy pathways [32]. Although these major components likely contribute significantly to the observed bioactivity, minor constituents such as *cis*-β-elemene, camphene, α-pinene, and bornyl acetate may enhance the overall effect. These compounds may act synergistically by promoting membrane disruption, enhancing solubility or cellular uptake, or inhibiting microbial efflux systems [25]. This complex interaction among components supports the widely accepted notion that essential oils act through multifaceted mechanisms, often exhibiting greater efficacy as whole mixtures than as isolated compounds [33].

The higher susceptibility of Gram-positive bacteria to *C. erectus* essential oil, as compared to Gram-negative strains, can be explained by fundamental differences in cell wall architecture [34]. Gram-positive bacteria possess a thick but porous peptidoglycan layer that facilitates the penetration of lipophilic molecules such as terpenes [34]. In contrast, the outer membrane of Gram-negative bacteria, rich in lipopolysaccharides, acts as a selective barrier that limits the entry of hydrophobic compounds, thereby reducing the

antimicrobial efficacy of essential oils [35]. These differences in membrane architecture likely account for the variation in susceptibility and highlight the importance of targeting membrane structure in the development of plant-based antimicrobial agents.

### *Mosquito larvicidal activity of Chloranthus erectus essential oil*

The insecticidal properties of essential oils have been widely documented, particularly in their effectiveness against mosquito vectors [36]. In this study, the larvicidal activity of *C. erectus* essential oil was assessed against the larvae of three mosquito species: *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* (Table 4). After 24 h of exposure, the essential oil produced $LC_{50}$ values of 50.51 µg/mL for *Ae. aegypti*, 48.36 µg/mL for *Ae. albopictus*, and 45.66 µg/mL for *Cx. quinquefasciatus*, with corresponding $LC_{90}$ values of 67.32 µg/mL, 67.19 µg/mL, and 60.11 µg/mL, respectively. Under the same conditions, the positive control permethrin yielded $LC_{50}$ values of 0.000643 µg/mL (*Ae. aegypti*), 0.0024 µg/mL (*Ae. albopictus*), and 0.0165 µg/mL (*Cx. quinquefasciatus*), and $LC_{90}$ values of 0.00246 µg/mL, 0.0042 µg/mL, and 0.0305 µg/mL, respectively. Following 48 h of exposure, the *C. erectus* essential oil showed reduced lethal concentration values, with $LC_{50}$ values of 42.67 µg/mL for *Ae. aegypti*, 43.14 µg/mL for *Ae. albopictus*, and 41.21 µg/mL for *Cx. quinquefasciatus*, while the corresponding $LC_{90}$ values were 67.02 µg/mL, 60.01 µg/mL, and 64.70 µg/mL, respectively. This time-dependent increase in toxicity aligns with previous findings on botanical larvicides and underscores the cumulative effects of essential oil components upon extended exposure [37–39].

**Table 4.** Mosquito larvicidal activity of the essential oil from *Chloranthus erectus* against *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* (µg/mL)

| Time | Mosquitoes | $LC_{50}$ (95% limits) | $LC_{90}$ (95% limits) | $\chi^2$ | *p* |
|---|---|---|---|---|---|
| 24 h | *Aedes aegypti* | 50.51 (47.90−53.39) | 67.32 (61.47−80.96) | 0.903 | 0.825 |
| | *Aedes albopictus* | 48.36 (45.44−51.24) | 67.19 (61.57−77.87) | 0.122 | 0.941 |
| | *Culex quinquefasciatus* | 45.66 (42.46−48.08) | 60.11 (56.06−68.37) | 0.248 | 0.970 |
| 48 h | *Aedes aegypti* | 42.67 (39.50−45.95) | 67.02 (60.72−76.72) | 0.743 | 0.863 |
| | *Aedes albopictus* | 43.14 (40.09−45.89) | 60.01 (55.62−67.03) | 1.317 | 0.518 |
| | *Culex quinquefasciatus* | 41.21 (38.13−44.42) | 64.70 (58.65−73.89) | 2.582 | 0.461 |

The enhanced toxicity observed over time may be associated with the progressive bioaccumulation of active compounds and their sustained interference with vital larval systems such as the nervous and respiratory systems. Key constituents of *C. erectus* essential oil—including (*E*)-β-ocimene, myrcene, spathulenol, and bicyclogermacrene—have been reported in previous studies to exhibit neurotoxic and cytotoxic effects [40–44]. Based on literature evidence, these compounds may contribute to larval mortality through mechanisms such as disruption of ion channels, inhibition of acetylcholinesterase, and impairment of mitochondrial function [45, 46]. Their lipophilic nature is generally considered to facilitate penetration through the larval cuticle, thereby enhancing bioavailability and potentially prolonging toxic effects [45, 46].

In addition to the individual bioactivity of these compounds, the observed larvicidal efficacy may also be influenced by synergistic interactions among multiple constituents. Previous research has suggested that combinations of monoterpenes and sesquiterpenes can exert greater toxicity than their isolated counterparts [47, 48]. Such synergism has been proposed to involve enhanced membrane permeability, inhibition of detoxifying enzymes, and prolonged persistence of toxic effects [45]. Hence, the larvicidal potential of *C. erectus* essential oil is likely attributable to the collective action of its complex chemical matrix rather than any single dominant component.

Species-specific differences in susceptibility were also apparent. *Cx. quinquefasciatus* showed slightly higher sensitivity, particularly after 24 h of exposure. This variation may reflect inherent differences in larval cuticle composition, metabolic rate, or detoxification capacity, which can influence the uptake and metabolism of xenobiotics [45]. Such interspecies variability emphasizes the need for evaluating botanical insecticides across multiple vector species to better understand their spectrum of activity and potential for targeted control.

Compared to other plant-based larvicides, the essential oil of *C. erectus* demonstrates comparable, if not superior, efficacy. The $LC_{50}$ values reported here fall well within the generally accepted threshold of <100 µg/mL for effective botanical larvicides [47, 48]. Given the limited prior research on the insecticidal potential of this species, these findings offer a novel contribution and highlight *C. erectus* as a promising candidate for mosquito control.

Overall, the data indicate that *C. erectus* essential oil may serve as a promising candidate for the development of eco-friendly larvicidal formulations. The demonstrated time-dependent toxicity, broad-spectrum activity, and synergistic interactions among its constituents support its application in integrated vector management (IVM) strategies. Future studies should aim to isolate and characterize the active principles, investigate their specific mechanisms of action, and evaluate environmental safety to facilitate the development of effective and sustainable mosquito control products.

## CONCLUSIONS

The essential oil extracted from *C. erectus* contained a diverse array of bioactive terpenoids, with (*E*)-β-ocimene, myrcene, spathulenol, and bicyclogermacrene identified as the major constituents. This chemical profile differs markedly from that previously reported for Malaysian populations, in which germacrone was the dominant compound, suggesting the presence of a distinct Vietnamese chemotype. The essential oil showed antimicrobial activity against several tested microorganisms, including Gram-positive bacteria and *C. albicans*, and also displayed larvicidal activity against three mosquito vectors—*Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*.

Importantly, this study represents the first report of the larvicidal activity of *C. erectus* essential oil and the first characterization of its essential oil composition from Vietnam, thereby substantially expanding the current phytochemical and biological knowledge of the species. These findings underscore the potential of *C. erectus* essential oil as a natural source of antimicrobial and insecticidal agents. Further studies are warranted to isolate key bioactive constituents, elucidate their mechanisms of action, and evaluate formulation stability and environmental safety to support potential applications in pharmaceutical development and integrated vector control strategies.

## MATERIALS AND METHODS

### *Plant material*

The aerial parts of *C. erectus* were collected in August 2023 during a field expedition in Pu Luong Nature Reserve, Thanh Hoa province, Vietnam (20°28'43'' N, 105°6'52'' E, elevation: 667 m) (Fig. S2). The identification of the plant was conducted by Assoc. Prof. Dr. Le Thi Huong from Vinh University, Vietnam. A voucher specimen (LTH42L) was deposited at the university's herbarium for future reference. To maintain the integrity of bioactive compounds, the freshly collected plant material was transported directly to the laboratory for extraction.

### *Essential oil extraction*

Essential oil extraction was performed using hydrodistillation in a Clevenger-type apparatus, following standard procedures outlined in the Vietnamese Pharmacopoeia [49] and previous studies [50, 51]. A total of 6 kg of *C. erectus* aerial parts was processed in three independent extractions, each using 2 kg of plant material. The distillation process was conducted at

atmospheric pressure for 4 h. After collection, the essential oil was dried over anhydrous sodium sulfate to remove residual moisture. The oil was then stored in sterilized glass vials at 4°C for up to one month before further analysis.

### *Essential oil analysis*

The chemical composition of the essential oil was analyzed by GC–FID/MS [52]. For GC–FID analysis, an Agilent Technologies HP 7890A Plus gas chromatograph equipped with an HP-5ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a flame ionization detector was employed. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60°C (held for 2 min) to 220°C at a rate of 4°C/min, followed by a final hold of 10 min. The injector and detector temperatures were set at 250°C and 260°C, respectively, with a split ratio of 10:1. Relative percentages of the individual components were calculated by peak area normalization without the use of correction factors and are therefore presented as semi-quantitative values.

GC–MS analysis was performed under identical chromatographic conditions using an HP 5973 mass selective detector. Helium served as the carrier gas at a flow rate of 1.0 mL/min. The mass spectrometer operated in electron ionization mode at 70 eV with an emission current of 40 mA, scanning over a mass range of m/z 35–350 at a rate of 1 scan/s. Retention indices (RI) were calculated relative to a homologous series of *n*-alkanes ($C_8$–$C_{25}$) analyzed under the same experimental conditions. Compound identification was achieved by comparing calculated RI values with published data and by matching mass spectra with those in the NIST and Wiley libraries [53, 54].

### *Antimicrobial assay*

The antimicrobial activity of the essential oil was evaluated against seven microbial strains: three Gram-positive bacteria (*E. faecalis* ATCC 299212, *S. aureus* ATCC 25923, *B. cereus* ATCC 14579), three Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. enterica* ATCC 13076), and one yeast strain (*C. albicans* ATCC 10231). These strains were obtained from the American Type Culture Collection (ATCC, Manassas, USA).

The MIC and $IC_{50}$ values were determined using the broth microdilution method [37, 38]. Bacterial strains were cultivated on Mueller-Hinton Agar, while fungal assays were conducted on Sabouraud Agar. The essential oil was dissolved and serially diluted in dimethyl sulfoxide (DMSO) to obtain the desired test concentrations prior to addition to 96-well microtiter plates. Bacterial suspensions were standardized to $5 \times 10^5$ CFU/mL in Mueller-Hinton broth, while fungal suspensions were adjusted to $1 \times 10^3$ CFU/mL in Sabouraud

dextrose broth. DMSO served as a negative control, while streptomycin and cycloheximide served as positive controls for antibacterial and antifungal assays, respectively. Plates were incubated at 37°C for 24 h (bacteria) and at 30°C for 24 h (fungi), and microbial growth was assessed by measuring optical density at 600 nm using a Spectramax 190 microplate reader. $IC_{50}$ values were determined based on turbidity reduction using an EPOCH2C spectrophotometer.

### *Mosquito larvicidal assay*

The larvicidal activity of the essential oil was tested against third instar larvae of *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* following standardized protocols [37, 38]. Larvae were collected and reared under controlled laboratory conditions at Duy Tan University, Vietnam. A 1% stock solution of the essential oil was prepared in DMSO, followed by serial dilutions to obtain test concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. Each bioassay consisted of twenty larvae placed in 300 mL beakers containing the respective concentrations. Experiments were conducted at room temperature (25°C) with three replicates per concentration. Permethrin served as a positive control, while DMSO acted as a negative control. Larval mortality was recorded at 24 and 48 h post-exposure, and the lethal concentration values ($LC_{50}$ and $LC_{90}$) were calculated using log-probit analysis.

### *Statistical analysis*

All experiments were performed in triplicate. $IC_{50}$ values for antimicrobial assays were derived using non-linear regression modeling of the dose-response curve. $LC_{50}$ and $LC_{90}$ values for the larvicidal assay were determined through log-probit regression analysis with 95% confidence intervals. Data analysis was conducted using Minitab 19.2020.1 (State College, PA, USA) and GraphPad Prism 9.5.1.733 (GraphPad Software Inc., San Diego, CA, USA).

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