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Chemical Composition of *Curcuma aromatica* Salisb. Rhizome Essential Oils and Evaluation of Larvicidal Activity against *Aedes aegypti*

Thành phần hóa học của tinh dầu thân rễ *Curcuma aromatica* Salisb. và đánh giá hoạt tính diệt ấu trùng đối với *Aedes aegypti*

Bui Van Nguyen[a], Vo Thi Kim Hau[b], Pham Duc Thinh[c], Hoang Van Trung[d], Nguyen Huy Hung[e, f], Vu Thi Hien[g*]
Bùi Văn Nguyên[a], Võ Thị Kim Hậu[b], Phạm Đức Thịnh[c], Hoàng Văn Trung[d], Nguyễn Huy Hùng[e, f], Vũ Thị Hiền[g*]

[a]*University of Khanh Hoa, 01 Nguyen Chanh Street, Nha Trang, Khanh Hoa*
[a]*Trường Đại học Khánh Hòa, số 01 Nguyễn Chánh, phường Nha Trang, tỉnh Khánh Hòa*
[b]*Graduate University of Science and Technology (GUST), VAST, 18 Hoang Quoc Viet Street, Nghia Do, Hanoi, Vietnam*
[b]*Học viện Khoa học và Công nghệ (GUST), Viện Hàn lâm Khoa học và Công nghệ Việt Nam (VAST), số 18 đường Hoàng Quốc Việt, Nghĩa Đô, Hà Nội, Việt Nam*
[c]*Institute of Oceanography, 01 Cau Da, Nha Trang, Khanh Hoa*
[c]*Viện Hải dương học, số 01, Cầu Đá, phường Nha Trang, tỉnh Khánh Hòa*
[d]*Vinh University, 182 Le Duan, Truong Vinh, Nghe An*
[d]*Trường Đại học Vinh, số 182 Lê Duẩn, phường Trường Vinh, tỉnh Nghệ An*
[e]*Faculty of Pharmacy, College of Medicine and Pharmacy, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam*
[e]*Khoa Dược, Trường Y Dược, Đại học Duy Tân, 03 Quang Trung, Đà Nẵng, Việt Nam*
[f]*Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, Vietnam*
[f]*Trung tâm Hóa tiên tiến, Viện Nghiên cứu và Phát triển, Đại học Duy Tân, 03 Quang Trung, Đà Nẵng*
[g]*Ho Chi Minh City University of Natural Resources and Environment, 236B Le Van Sy Street, Tan Son Hoa Ward, Ho Chi Minh City, Vietnam*
[g]*Trường Đại học Tài nguyên và Môi trường Thành phố Hồ Chí Minh, 236B đường Lê Văn Sỹ, phường Tân Sơn Hòa, Thành phố Hồ Chí Minh, Việt Nam*

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Abstract

This study characterized the chemical composition of two rhizome essential oils of *Curcuma aromatica* Salisb. (N1 and N2) and evaluated the larvicidal activity of N2 against *Aedes aegypti*. The oils were obtained by steam distillation and analyzed by gas chromatography–mass spectrometry (GC-MS). The reported constituents accounted for 93.24% of N1 and 95.00% of N2. N1 was characterized by ethyl *p*-methoxycinnamate (14.25%), curzerene (9.97%), (*E*)-ethyl cinnamate (7.30%), and *trans*-β-elemenone (5.53%), whereas N2 was characterized by methyl isocostate (22.36%), curzerene (11.51%), and β-elemenone (6.55%); both oils also contained several abundant unidentified constituents. Larvicidal activity was evaluated against third- to fourth-instar *Ae. aegypti* larvae using 25 larvae per replicate and four replicates per concentration. N2 exhibited LC_{50} values of 20.13 µg/mL (95% CI: 18.65–21.51) at 24 h and 17.18 µg/mL (95% CI: 15.91–18.65) at 48 h, while the corresponding LC_{90} values were 27.78 µg/mL (95% CI: 25.77–30.72) and 21.77

[*]*Corresponding author:* Vu Thi Hien
Email: vthien@hcmunre.edu.vn

µg/mL (95% CI: 19.91–24.55), respectively. The decrease in lethal concentrations with increasing exposure time indicates an exposure-time-related larvicidal effect. These results demonstrate marked chemical variation between the two *C. aromatica* oils and notable larvicidal activity of N2. Confirmation of the major tentatively identified constituents, characterization of the abundant unidentified peaks, and further evaluation of formulation performance and non-target safety are warranted.

Keywords: Aedes aegypti, Curcuma aromatica, essential oil, GC-MS, larvicidal activity

Tóm tắt

Nghiên cứu này xác định thành phần hóa học của hai mẫu tinh dầu thân rễ nghệ trắng (*Curcuma aromatica* Salisb.), ký hiệu N1 và N2, đồng thời đánh giá hoạt tính diệt ấu trùng Aedes aegypti của mẫu N2. Tinh dầu được thu bằng phương pháp chưng cất lôi cuốn hơi nước và phân tích bằng sắc ký khí ghép khối phổ (GC–MS). Tổng tỷ lệ các cấu tử ghi nhận đạt 93,24% ở N1 và 95,00% ở N2. N1 được đặc trưng bởi ethyl p-methoxycinnamate (14,25%), curzerene (9,97%), (E)-ethyl cinnamate (7,30%) và trans-β-elemenone (5,53%), trong khi N2 được đặc trưng bởi methyl isocostate (22,36%), curzerene (11,51%) và β-elemenone (6,55%); cả hai mẫu tinh dầu đều chứa một số cấu tử chưa định danh với hàm lượng đáng kể. Hoạt tính diệt ấu trùng được đánh giá trên ấu trùng *Ae. aegypti* tuổi III–IV, với 25 ấu trùng cho mỗi lần lặp và bốn lần lặp ở mỗi nồng độ. N2 có giá trị LC_{50} là 20,13 µg/mL (KTC 95%: 18,65–21,51) sau 24 giờ và 17,18 µg/mL (KTC 95%: 15,91–18,65) sau 48 giờ; các giá trị LC_{90} tương ứng là 27,78 µg/mL (KTC 95%: 25,77–30,72) và 21,77 µg/mL (KTC 95%: 19,91–24,55). Sự giảm các nồng độ gây chết khi thời gian phơi nhiễm tăng cho thấy hoạt tính diệt ấu trùng có liên quan đến thời gian phơi nhiễm. Kết quả cho thấy sự khác biệt rõ rệt về thành phần hóa học giữa hai mẫu tinh dầu *C. aromatica* và hoạt tính diệt ấu trùng đáng chú ý của N2. Cần tiếp tục xác nhận các cấu tử chính được định danh tạm thời, xác định các pic chưa định danh có hàm lượng cao, đồng thời đánh giá đặc tính của dạng bào chế và tính an toàn đối với các sinh vật không mục tiêu.

Từ khóa: Aedes aegypti, *Curcuma aromatica*, tinh dầu, GC-MS, hoạt tính diệt ấu trùng

1. Introduction

Aedes aegypti is a major vector of dengue, Zika, chikungunya, and other arboviral diseases. Because its immature stages develop in relatively confined aquatic habitats, larval control remains an important component of integrated vector management. Conventional larvicides can provide rapid suppression, but repeated reliance on synthetic insecticides is constrained by the evolution of resistance and by concerns over environmental persistence and effects on non-target organisms. These limitations have stimulated interest in biodegradable plant-derived products, particularly essential oils, as complementary sources of mosquito-control agents.

Essential oils are complex mixtures of volatile secondary metabolites, mainly terpenoids and phenylpropanoids, and their biological effects often reflect the combined action of multiple constituents. The family Zingiberaceae is especially rich in aromatic species, and several Curcuma taxa produce rhizome oils with pronounced chemical diversity. In *C. aromatica*, this variability is well documented. Xiang et al. identified 78 constituents in rhizome essential oils collected from 12 habitats in China, with substantial differences in the proportions of 8,9-dehydro-9-formyl-cycloisolongifolene, germacrone, ar-turmerone, turmerone, ermanthin, β-sesquiphellandrene, and ar-curcumene [6]. Chai et al. reported a contrasting oil dominated by 1,8-cineole (53.86%) and neocurdione (9.89%) [1]. Such variation indicates that chemical characterization of individual *C. aromatica* sources is essential before their biological activity can be meaningfully compared.

Vietnamese *C. aromatica* material also appears chemically heterogeneous. Rhizome essential oil collected in Dien Bien was reported to contain α-zingiberene (17.85%), β-sesquiphellandrene (13.28%), and *ar*-curcumene (9.45%) [4]. In another study, Vietnamese *Curcuma* accessions cultivated in North Alabama showed C. aromatica oils rich in curzerenone, germacrone, and 1,8-cineole [3]. These reports collectively suggest that

provenance, growing environment, harvest stage, and analytical conditions may strongly influence the volatile profile.

C. aromatica also has documented anti-mosquito potential. Choochote et al. reported larvicidal activity of the rhizome volatile oil against fourth-instar *Ae. aegypti*, with an LC_{50} of 36.30 ppm, together with adulticidal and repellent effects [2]. However, quantitative larvicidal data linked to GC-MS profiles of Vietnamese *C. aromatica* oils remain limited. The present study therefore aimed to characterize and compare two *C. aromatica* rhizome essential oils, designated N1 and N2, and to quantify the larvicidal activity of N2 against *Ae. aegypti* by estimating LC_{50} and LC_{90} values after 24 and 48 h of exposure.

2. Materials and methods

2.1. Plant material and essential oil isolation

Fresh rhizomes of *Curcuma aromatica* Salisb. were collected from Buôn Nhang, Krông Jing Commune, M'Đrắk District, Đắk Lắk Province, Vietnam (12°45′39″N, 108°42′06″E) in December 2024. The plant material was identified by Dr. Pham Van The, a botanist at Van Lang University, Vietnam, based on diagnostic morphological characteristics observed in photographs and plant specimens provided for taxonomic examination. No voucher specimen was deposited in an herbarium.

The rhizomes used to obtain the two essential-oil samples (N1 and N2) were collected from the same location during the same sampling period but differed in post-harvest handling and the time elapsed before distillation. In particular, the rhizomes used for N2 were stored under refrigerated conditions after collection prior to distillation. Fresh rhizomes were cleaned and processed before steam distillation. The essential oil was separated, dried, and stored in tightly closed containers until GC-MS analysis and larvicidal testing. Steam distillation of 10 kg of fresh *C. aromatica* rhizomes yielded approximately 15 mL of essential oil, corresponding to an oil yield of approximately 0.15% (v/w, mL/100 g fresh weight).

2.2. GC-MS analysis

Chemical composition was analyzed using an Agilent Technologies GC-MS system coupled to an Agilent 5977B mass selective detector and fitted with an HP-5MS UI capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injection volume was 1 µL with a split ratio of 25:1. The oven was held at 60°C for 3 min, programmed to 180°C at 3°C/min, then to 240°C at 5°C/min and held for 5 min. The injector temperature was 300°C. The quadrupole, ion-source, and transfer-line (Aux-2) temperatures were 150, 230, and 250°C, respectively. Mass spectra were acquired by electron ionization at 70 eV over m/z 50-550 at two scans/s. For GC-MS analysis, 1 µL of the essential oil was diluted with 1 mL of dichloromethane (CH_2Cl_2), and 1 µL of the resulting solution was injected into the GC-MS system.

Constituents were assigned on the basis of mass-spectral matching together with retention-index (RI) information and reference data. Relative composition was expressed as percentage peak area without response-factor correction. Peaks lacking sufficient evidence for a confident assignment were retained as unknown compounds rather than assigned speculative identities.

2.3. Mosquito rearing and larval selection

Aedes aegypti was maintained under laboratory conditions at 25-28°C with a 12:12 h light:dark photoperiod. Healthy third- to fourth-instar larvae were selected for bioassays. Each

experimental unit consisted of 25 larvae in a cup containing 150 mL of distilled water, with a water depth of approximately 5-10 cm [5].

2.4. Larvicidal bioassay

N2 was evaluated at 12.5, 25, 50, and 100 µg/mL in the concentration–response assay used for probit analysis. Each concentration was tested in four replicates (R1-R4), with 25 larvae per replicate. Ethanol served as the solvent control and permethrin as the positive control. Mortality was recorded after 24 and 48 h of exposure. Where required, observed mortality was corrected using Abbott's formula [5].

2.5. Statistical analysis

Concentration-mortality data were analyzed by log-dose-probit regression using SPSS version 22. LC_{50} and LC_{90} values and their 95% confidence intervals (CIs) were estimated for 24- and 48-h exposure periods. Model fit was evaluated using the chi-square (χ^2) goodness-of-fit statistic; $p > 0.05$ was interpreted as no significant evidence of lack of fit.

3. Results

3.1. Essential oil yield

Steam distillation of 10 kg of fresh *C. aromatica* rhizomes yielded approximately 15 mL of essential oil, corresponding to an oil yield of approximately 0.15% (v/w, mL/100 g fresh weight).

3.2. Chemical composition of N1 and N2

GC-MS revealed complex and clearly differentiated chemical profiles for N1 and N2 (Table 1). The reported constituents accounted for 93.24% of N1 and 95.00% of N2, with 39 and 42 peaks/constituents reported, respectively. Both oils contained camphene, 1,8-cineole, 2-bornanone, isoborneol, β-elemene, β-caryophyllene, γ-elemene, curzerene, α-farnesene, δ-cadinene, viridiflorol, and several oxygenated sesquiterpenes, but their relative abundances differed substantially.

N1 was characterized by ethyl *p*-methoxycinnamate (14.25%), curzerene (9.97%), (*E*)-ethyl cinnamate (7.30%), *trans*-β-elemenone (5.53%), and β-elemene (3.75%). Importantly, unidentified constituents also represented a substantial fraction of the oil: unknown compound 4 (RI 1792) accounted for 15.54% and unknown compound 1 (RI 1753) for 9.54%. Thus, the most abundant peak in N1 remained unidentified.

N2 showed a markedly different profile. Methyl isocostate was reported as the most abundant identified constituent (22.36%), followed by curzerene (11.51%), β-elemenone (6.55%), β-elemene (3.62%), and 2-bornanone (2.84%). Unidentified constituents were again prominent, particularly unknown compound 1 (RI 1753; 11.83%), unknown compound 2 (6.09%), and unknown compound 3 (4.93%).

Curzerene and β-elemene occurred at broadly comparable levels in N1 and N2 (9.97 vs. 11.51% and 3.75 vs. 3.62%, respectively), whereas several other constituents differed sharply. (*E*)-Ethyl cinnamate decreased from 7.30% in N1 to 0.84% in N2; ethyl p-methoxycinnamate (14.25%) was reported only in N1, while methyl isocostate (22.36%) was reported only in N2. These differences distinguish the two samples chemically, although replicate GC-MS analyses would be required to determine whether they represent reproducible sample-specific profiles.

Table 1. Chemical composition of *C. aromatica* essential oils N1 and N2

No.	Compound	Experimental RI*	N1 (%)	N2 (%)
1	Camphene	950	0.16	0.17
2	1,8-Cineole	1032	0.21	0.20
3	2-Bornanone	1144	2.66	2.84
4	Isoborneol	1156	1.02	1.10
5	endo-Borneol	1165	0.18	-
6	δ-Elemene	1337	0.49	0.57
7	β-Elemene	1390	3.75	3.62
8	β-Caryophyllene	1416	0.22	0.19
9	γ-Elemene	1432	1.18	1.10
10	α-Caryophyllene	1451	0.14	0.13
11	(*E*)-Ethyl cinnamate	1465 / 1463	7.30	0.84
12	β-Chamigrene	1473 / 1478	0.10	0.94
13	Germacrene D	1478 / 1483	0.99	0.46
14	β-Eudesmene	1483	0.50	-
15	α-Selinene	1491	0.42	0.34
16	Curzerene	1495	9.97	11.51
17	α-Farnesene	1507 / 1506	1.61	1.46
18	δ-Cadinene	1521	0.19	0.17
19	Epiglobulol	1584	0.58	-
20	Viridiflorol	1591	0.55	0.52
21	*cis*-β-Elemenone	1597	0.69	-
22	*trans*-β-Elemenone	1602	5.53	-
23	Di-epi-1,10-cubenol	1612	0.41	0.46
24	*E*-Farnesene epoxide	1626	0.82	0.69
25	*Iso*-spathulenol	1635	0.20	-
26	*tau*-Cadinol	1640 / 1641	0.16	0.14
27	α-Eudesmol	1651	0.62	0.67
28	Pogostole	1659	0.18	0.19
29	Intermedeol	1666	0.21	-
30	Cedr-8-en-13-ol	1686	0.22	0.23
31	Germacrone	1691	1.16	-
32	Unknown compound 1	1753	9.54	11.83
33	Ethyl *p*-methoxycinnamate	1757	14.25	-
34	Unknown compound 2	1763 / 1755	4.49	6.09
35	Unknown compound 3	1774 / 1762	1.90	4.93
36	Unknown compound 4	1792 / 1768	15.54	0.52
37	Unknown compound 5	1847 / 1774	1.93	2.01
38	Unknown compound 6	1851 / 1847	2.02	2.58
39	Unknown compound 7	1873 / 1850	1.15	2.76
40	Borneol	1165	-	0.16
41	Bornyl acetate	1383	-	0.12
42	Guaia-6.9-diene	1447	-	0.09
43	Globulol	1584	-	0.59
44	β-Elemenone	1601	-	6.55
45	Selin-6-en-4α-ol	1634	-	0.20

No.	Compound	Experimental RI*	N1 (%)	N2 (%)
46	Ylangenol	1666	-	0.26
47	(*E,E*)-Germacrone	1691	-	1.63
48	α-trans-Bergamotenol	1699	-	0.46
49	β-Santalol	1716	-	1.52
50	Methyl isocostate	1791	-	22.36
51	Unknown compound 8	1873	-	1.80
	Total reported constituents		93.24	95.00

* When two RI values are provided, the first and second values correspond to N1 and N2, respectively.

3.3. Larvicidal activity of N2

N2 exhibited concentration- and exposure-time-related larvicidal activity against Ae. aegypti. At 24 h, mortality was 0%, 81%, 100%, and 100% at 12.5, 25, 50, and 100 µg/mL, respectively. At 48 h, mortality at 25 µg/mL increased to 97%, while complete mortality was maintained at 50 and 100 µg/mL. Probit estimates are summarized in Table 2. No mortality was observed in the ethanol solvent control. For the permethrin positive control, the 24-h LC_{50} and LC_{90} values were 0.00638 µg/mL (95% CI: 0.00548–0.00744) and 0.0232 µg/mL (95% CI: 0.0182–0.0318), respectively. The goodness-of-fit test for the permethrin concentration–mortality model yielded χ^2 = 8.868 and p = 0.031.

Table 2. LC_{50} and LC_{90} values of N2 essential oil against *Aedes aegypti*

Exposure	LC_{50} (µg/mL; 95% CI)	LC_{90} (µg/mL; 95% CI)	χ^2	p
24 h	20.13 (18.65–21.51)	27.78 (25.77–30.72)	0.268	0.874
48 h	17.18 (15.91–18.65)	21.77 (19.91–24.55)	0.014	0.993

At 24 h, the estimated LC_{50} and LC_{90} values for N2 were 20.13 µg/mL (95% CI: 18.65–21.51) and 27.78 µg/mL (95% CI: 25.77–30.72), respectively. At 48 h, these values decreased to 17.18 µg/mL (95% CI: 15.91–18.65) and 21.77 µg/mL (95% CI: 19.91–24.55), respectively. Thus, extending the exposure period from 24 to 48 h reduced the estimated LC_{50} and LC_{90} values by approximately 14.7% and 21.6%, respectively. The goodness-of-fit tests for N2 were non-significant at both observation times (24 h: χ^2 = 0.268, p = 0.874; 48 h: χ^2 = 0.014, p = 0.993), indicating no significant evidence of lack of fit of the probit models.

4. Discussion

4.1. Variation in chemical composition

The most striking chemical finding was the pronounced divergence between N1 and N2 despite their assignment to the same species. This observation is consistent with the broad intraspecific variability reported for *C. aromatica*. Xiang et al. identified 78 constituents across rhizome oils from 12 Chinese habitats and found substantial differences in both composition and bioactivity; dominant compounds included 8,9-dehydro-9-formyl-cycloisolongifolene, germacrone, ar-turmerone, turmerone, ermanthin, β-sesquiphyllandrene, and *ar*-curcumene [6]. By contrast, Chai et al. reported an oil dominated by 1,8-cineole (53.86%) and neocurdione (9.89%) [1]. In the present samples, 1,8-cineole was only 0.20-0.21%, emphasizing how strongly C. aromatica volatile profiles may vary among plant sources.

The present profiles also differ from other Vietnamese-origin material. C. aromatica rhizome oil collected in Dien Bien was reported to contain α-zingiberene (17.85%), β-sesquiphellandrene (13.28%), and ar-curcumene

(9.45%) [4], whereas Vietnamese accessions cultivated in North Alabama contained curzerenone (14.7-18.6%), germacrone (10.7-14.7%), and 1,8-cineole (5.2-11.7%) [3]. N1 instead contained substantial ethyl p-methoxycinnamate and (*E*)-ethyl cinnamate, while N2 was reported to contain a high proportion of methyl isocostate. Geographic origin, genotype, developmental stage, harvest season, post-harvest handling, and analytical procedures are all plausible contributors to this variation. However, because the present study did not manipulate these factors experimentally, their individual contributions cannot be resolved.

The large unidentified fraction deserves particular attention. The most abundant N1 peak (RI 1792; 15.54%) was unidentified, and N2 contained an unidentified peak at RI 1753 representing 11.83%. Consequently, the current data support the description of N1 and N2 as chemically distinct samples, but not the formal designation of chemotypes. Likewise, the reported identification of methyl isocostate at 22.36% in N2 and ethyl *p*-methoxycinnamate at 14.25% in N1 should be regarded as requiring analytical confirmation because these assignments strongly influence interpretation of the oils. Confirmation using authentic standards where available, retention indices on columns of complementary polarity, and/or higher-resolution structural methods would substantially strengthen the chemical conclusions.

4.2. Larvicidal activity against Aedes aegypti

N2 showed notable larvicidal activity against *Ae. aegypti*, with a 24-h LC_{50} of 20.13 µg/mL. Choochote et al. reported a 24-h LC_{50} of 36.30 ppm for *C. aromatica* volatile oil against fourth-instar *Ae. aegypti* [2]. In dilute aqueous systems, ppm is numerically close to µg/mL, so N2 appears more active on a nominal concentration basis. This comparison should nevertheless be interpreted cautiously because differences in mosquito strain, larval age, essential-oil composition, solvent system, exposure conditions, and statistical design can materially affect LC estimates. The key result of the present study is therefore the internally derived concentration-response relationship rather than superiority over a historical oil.

The decrease in LC_{50} from 20.13 µg/mL at 24 h to 17.18 µg/mL at 48 h, together with the corresponding decline in LC_{90} from 27.78 to 21.77 µg/mL, indicates that mortality continued to accumulate with exposure time. This temporal pattern is consistent with the raw observations at intermediate concentrations and suggests that the toxic effect is not restricted to an immediate acute response. From a screening perspective, such activity is encouraging; however, laboratory potency alone does not establish practical larvicidal performance. Essential oils are hydrophobic and volatile, and their apparent efficacy can change substantially with dispersion, organic matter, temperature, light exposure, and storage.

4.3. Relationship between composition and activity

The chemical basis of N2 activity cannot be assigned from the present experiment. N2 contained curzerene (11.51%), β-elemenone (6.55%), β-elemene (3.62%), 2-bornanone (2.84%), and several oxygenated sesquiterpenes, but the bioassay evaluated the complete oil rather than isolated constituents. Moreover, a substantial fraction of N2 remained unidentified. Essential-oil toxicity may result from additive, synergistic, or antagonistic interactions among major and minor constituents; therefore, attributing activity to a single compound would exceed the evidence available here. Bioassay-guided fractionation, testing of purified major constituents, and reconstructed-mixture experiments would be needed to establish causal composition-activity relationships.

The study has several strengths, including direct GC-MS comparison of two C. aromatica samples and quantitative estimation of N2 larvicidal potency with 95% CIs and satisfactory probit goodness-of-fit statistics. It also has important limitations. First, only N2 was subjected to larvicidal testing, preventing a direct test of whether the chemical differences between N1 and N2 translate into biological differences. Second, the GC-MS data appear to derive from single analytical profiles rather than replicate compositional analyses, so between-run analytical variability cannot be assessed. Third, several abundant peaks remain unidentified or require confirmation.

4.4. Significance and limitations of the study

Future work should therefore prioritize confirmation of the major chemical assignments and unknown peaks, simultaneous testing of N1 and N2 against the same Ae. aegypti cohort, and evaluation of the principal fractions or compounds responsible for activity. Formulation studies are also warranted because improved aqueous dispersion may increase reproducibility and persistence of essential-oil larvicides. Before practical application is considered, residual activity, environmental stability, and toxicity toward representative non-target aquatic organisms should be assessed under semi-field conditions.

5. Conclusions

Two *C. aromatica* Salisb. rhizome essential oils, N1 and N2, showed markedly different GC-MS profiles. The reported constituents represented 93.24% and 95.00% of the respective oils. N1 was characterized by ethyl *p*-methoxycinnamate, curzerene, (*E*)-ethyl cinnamate, and *trans*-β-elemenone, whereas N2 was reported to contain high proportions of methyl isocostate, curzerene, and β-elemenone. Several abundant peaks in both samples remained unidentified. N2 displayed substantial larvicidal activity against *Ae. aegypti*, with LC_{50} values of 20.13 µg/mL at 24 h and 17.18 µg/mL at 48 h. These findings support *C. aromatica* as a source of mosquito-larvicidal essential oil, while also highlighting the need for rigorous confirmation of its major constituents and chemical variability. Comparative bioassays of multiple chemically characterized samples, identification of the active fractions, formulation studies, and non-target safety testing are required before practical application can be considered.

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