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ORIGINAL RESEARCH

Chemical Composition, Antioxidant, and Mosquito Larvicidal Activity of Essential Oils from *Hyptis capitata* Jacq

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Background: Mortality and morbidity associated with vector-borne diseases, particularly those caused by mosquitoes, are increasing and new means of controlling them, including bio-larvicides, are needed. Malaria is a serious threat in many countries of Africa and Asia, and eco-friendly vector preventing measures are very much essential. Plant-derived larvicides are of great importance in this context. *Hyptis capitata* is an aromatic medicinal plant which is widely distributed in tropical countries. The aim of the present study is to examine the chemical composition, antioxidant and mosquito larvicidal effects of essential oils of this plant, extracted by hydro-distillation.

Methods: Chemical compositions of essential oils were analyzed using gas chromatography–mass spectrometry (GC-MS). Antioxidant activity was tested by the 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) assay and the mosquito larvicidal activity was checked against the fourth instar larvae of the malarial vector *Anopheles stephensi*. Fingerlings of *Oreochromis mossambicus* were used as a bio-model for toxicity studies.

Results: A total of 48 constituents, inclusive of 44 (94.67%) from inflorescence and 19 (97.09%) from leaf oil were identified; δ-cadinene (14.68%) and linalool (6.99%) were the major constituents of the inflorescence oil, while leaf oil contained 1-octen-3-ol (34.08%), methyl linoleate (17.2%), and germacrene D (11.16%). Antioxidant analysis showed an effective concentration (EC_{50}) value of 22.76 µg/mL for leaf oil and 26.18 µg/mL for the inflorescence oil, corresponding to 17.57 µg/mL of ascorbic acid. Both oils showed a respectable larvicidal effect and the lethal concentrations (LC_{50}) are 39.08 µg/mL and 33.19 µg/mL for the inflorescence and leaf oil, respectively. Notably, both the inflorescence and leaf oils are not very toxic to fish with respect to the concentrations tested.

Conclusion: This study showed that the essential oils extracted from the leaves and inflorescences of *H. capitata* are effective antioxidants and can act as inexpensive mosquito larvicidal agents.

Keywords: *Hyptis capitata*, *Anopheles stephensi*, antioxidant, larvicidal activity, essential oil

Introduction

The genus *Hyptis* belongs to the family of Lamiaceae, distributed in tropical and subtropical regions. Based on the morphological and molecular evidence, it encompasses 148 species.[1] The pharmacological importance of this genus in treating respiratory disorders, nasal congestion, fever, liver diseases, gastrointestinal malfunctions, skin infections,[2] and even human immunodeficiency virus[3] is well-known. *Hyptis suaveolens* (L) Poit is the first species in the family, reported in 1952, describing its essential oil composition.[4] Phytochemical analysis of this genus revealed the presence of many bioactive compounds. However, only 10% of members were elaborately studied and further research needs to be conducted to know the diversity of the genus and medicinal importance of the constituents contained in them.[2] Studies done so far show that terpenes, terpenoids, lignans, and flavonoids are commonly present in this genus and

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the chemical compositions vary greatly among species, depending on geographic locations and genetic and climatic factors.[5]

Hyptis capitata Jacq., commonly known as knobweed, is an aromatic medicinal plant, native to central America and naturalized in tropical countries. *Hyptis capitata* and *H. suaveolens* are invasive plants that dominate over and subdue the growth of native plant species.[6] It is an annual shrub which grows to a maximum height of 200 cm, characterized by aromatic globose head inflorescence with lanceolate leaves with toothed margins which are 8–14 cm long. This plant is also used by tribes for curing fever and asthma.[7] Pharmacological research has validated its antimicrobial, antioxidant, and anti-cancer properties.[7] For instance, ursolic acid is an anti-cancer compound predominant in this plant,[8] and another report describe borneol as the major constituent in its essential oil.[9] However, further characterization of this essential oil has not been conducted so far.[2]

Vector-borne diseases contribute to more than 17% of all infectious diseases, causing over 700,000 deaths annually, and can be inflicted by parasites, bacteria, or viruses.[10] More than half a dozen vectors pose serious threats to mankind, out of which mosquitoes are the most dreadful, spreading several viral diseases (Chikungunya, dengue, Rift valley fever, yellow fever, Zika, Japanese encephalitis, and West Nile fever) and a couple of notable parasitic diseases (malaria and lymphatic filariasis).[10] Among all, malaria remains a major public health issue, especially in Asia and Africa. As per the World Malaria Report, 241 million cases of malaria and 627,000 deaths occurred globally in 2020.[11] According to a previous world malaria report by WHO in 2019, India is the most affected country in Southeast Asia. The geographical position and climatic factors of the country are ideal for malarial parasites and vectors.[12] In the urban slum regions of the country, *A. stephensi* is the primary vector, which has recently been reported as a predominant insecticide-resistant variety.[12,13] In such a situation of severe resistance, controlling the vectors with synthetic pesticides is not advisable, as they are toxic to humans, plants, and animals; for instance, organophosphates and pyrethroid larvicides.[14]

The current trend of curbing the mosquito menace is focused on the investigation of natural substances which can be used to control vectors in the larval stage itself. Bio-larvicides (from plants, bacteria, algae, lichen, and fungi) have received a lot of attention because they are biodegradable and safer. An extensive literature survey indicated that, over the last 5 years, 52 compounds derived from plants (70%), bacteria (17%), and fungi (13%) have been evaluated for their mosquito larvicidal activity.[15]

In this context, the screening of essential oils from plants as insecticides and larvicidal agents have gained attention.[16] Essential oils are plant-derived volatile lipophilic liquids and odoriferous substances. They are deemed as biotic and abiotic stress alleviators in plants, but are toxic to pathogens. However, the toxicity of these oils depends on their major constituents, which may have individual or synergistic activity.[17] The aim of this work is to investigate the chemical composition, antioxidant, and mosquito larvicidal activities of essential oils from the leaves and inflorescence of *H. capitata*.

Materials and Methods

Extraction of Essential Oils

The leaves and matured inflorescence of *H. capitata* were collected from Kollam district (9.0927° N, 76.8612° E), Kerala, India. The plant was taxonomically confirmed by Dr. Mathew Dan, Plant Genetic Resource Division, Jawaharlal Nehru Tropical Botanical Garden and Research Institute, India, and specimens were deposited in the Department of Botany, St. Stephen's College (Voucher No. RM 0021) for future reference. For the extraction of essential oils, 150 g of fresh leaves or inflorescence were subjected to hydro-distillation in a Clevenger-type apparatus for 3 h. The oil sample extracted in 10 mL of diethyl ether (Et_2O) was dehydrated over anhydrous sodium sulfate and was kept in a refrigerator for further analysis. The percentage yield of oil was calculated on the basis of fresh weight of the plant materials used for extraction.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of essential oils was carried out with a Shimadzu QP 2020 NX equipped with SH-Rxi-5Sil MS, (1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane, 30 m x 0.25 mm, i.d., 0.25 µm film thickness, Restek, USA) capillary column and flame ionization detector (FID 2030); 0.1 µL essential oil diluted with Et_2O was injected in the split mode (split

ratio: 50:1). Helium of 99.9% purity was used as the carrier gas, at a flow rate of 1.4 mL/minute. The oven had an initial temperature of 60°C for 2 minutes, which was then increased to 200°C for 2 minutes at the rate of 5°C/minute, then increased to 220°C at the rate of 3°C/minute. Finally, the temperature was increased to 250°C at the rate of 6°C/minute, and the total run time was 1 hour; the detector and injection port temperatures were 240°C. The electron impact ionization (EI) mode was 70 eV; the mass, m/z was scanned for a range of 20–550. Identification of individual constituents in essential oil were carried out with the aid of the spectral libraries of NIST and WILEY as well as the literature,[18] on the basis of a retention index (RI) calculated with n-alkanes C9–C25. Quantification of individual constituents was done on the basis of the relative area of the peak percentage (FID response), without applying a correction factor.

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate) Radical Scavenging Activity

The free radical scavenging activity of essential oils was evaluated as reported elsewhere.[19] For this, 0.1 mL aliquots of essential oils (dissolved in dimethyl sulfoxide) were added to 3.9 mL of 0.2 Mm DPPH in methanol (MeOH). The mixture was shaken vigorously and incubated in dark conditions at room temperature for 30 minutes. A decrease in the absorbance was monitored at 515 nm (Agilent Cary 60 UV-Vis Spectrophotometer) against MeOH as the control and ascorbic acid as the standard. Radical scavenging activity was measured as the percentage of DPPH decolorization by using the following equation and the experiment was performed in triplicate.

$$\%\ \text{Inhibition} = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}}\right] \times 100$$

The inhibitory concentration (IC_{50}) of the essential oil needed to scavenge 50% of DPPH was calculated from the plot of concentration versus percentage of inhibition.

Mosquito Larvicidal Assay

The larvicidal assay was performed as per the procedure of WHO.[20] Aliquots of different concentrations of essential oils ranging from 20–120 µg/mL were prepared from 1% stock solution by serial dilution with water. The control was a blend of DMSO and water. Twenty larvae in the early fourth instar stage were selected to study the larvicidal activity, with five replicates corresponding to each concentration. The mortality rate was calculated after 24 hours and the lethal concentrations, LC_{50} and LC_{90} in 95% confidence limits of upper and lower confidence levels were calculated by probit analysis.

Ichthyotoxic Assay

Ichthyotoxic activity was evaluated to study the effects of essential oils against non-target organisms. Fingerlings (1.5–2.0 cm) of *Oreochromis mossambicus* were used as a bio-model as it has been used for many toxicological studies of plant extracts.[21,22] Five fingerlings were introduced in each experimental and control glass bowls containing 200 mL water dissolved with chosen concentration (20–160 µg/mL) of the oil. Immediate reflex changes and mortality were observed continuously for 6 hours and at 1-hour intervals for the next 12 hours. After 24 hours of exposure, the number of dead and live fishes were counted.[21]

Data Analysis

The data of bioassays were described as mean standard deviation (SD) using SPSS for Windows version 25 (Statistical Package for Social Services, Chicago, IL).

Results

Analysis of Essential Oil of Leaves and Inflorescence

Essential oils from the inflorescence and leaves of *H. capitata* were obtained with a yield of 0.30% and 0.15%, respectively. A total of 48 components, representing 44 (94.67%) from inflorescence oil and 19 (97.09%) from leaf oil were identified and the data are given in Table 1. Gas chromatographic profiles of the essential oils of inflorescence and leaf are depicted in Supplementary Figure S1. The major constituents of inflorescence oil were δ-cadinene (14.68%),

Table 1 Composition of Essential Oils of Inflorescence and Leaf of *H. capitata*

S. No.	Compounds	RI	RI^L	Content %	
				Leaf Oil	Inflorescence Oil
1.	α-Pinene	931.86	932	–	0.4
2.	1-Octen-3-ol	975.60	974	34.08	6.46
3.	3-Octanol	995.74	988	–	0.19
4.	Benzene acetaldehyde	1,039.20	1,036	–	0.54
5.	n-Octanol	1,067.89	1,063	–	0.65
6.	Linalool	1,098.58	1,095	–	6.99
7.	n-Nonanal	1,103.17	1,100	–	0.87
8.	Hexenylbutanoate	1,190	1,193	–	0.24
9.	n-Decanal	1,203.97	1,201	–	3.53
10.	Hexenyl 2-methyl butanoate	1,228.50	1,232	–	0.4
11.	Hexyl-2 methyl butanoate	1,233.87	1,233	–	1.29
12.	Hexenylisovalerate	1,240.18	1,241	–	0.35
13.	Methyl citronellate	1,265.65	1,257	2.57	0.89
14.	n-Decanol	1,270.56	1,266	–	0.3
15.	Longicyclene	1,365.80	1,371	–	0.88
16.	α-Copaene	1,372.17	1,374	2.07	3.05
17.	β-Bourbonene	1,379.71	1,387	0.88	–
18.	β-Caryophyllene	1,414.56	1,417	5.24	3.48
19.	n-Octyl2-methylbutanoate	1,431.06	1,431	–	4.53
20.	Prenyl limonene	1,444.41	1,443	0.97	3.16
21.	α-Humulene	1,449.75	1,452	0.74	1.33
22.	Muurola-4 (14), 5-diene	1,467.23	1,465	–	1.91
23.	β-Acoradiene	1,471.35	1,474	2.63	5.21
24.	α-Neocallitropsene	1,474.51	1,474	–	2.96
25.	Germacrene D	1,475.48	1,480	11.16	–
26.	γ-Himachalene	1,482.76	1,481	–	1.15
27.	β-Selinene	1,487.37	1,489	1.08	1.18
28.	β-Guaiene	1,490.04	1,492	1.34	0.41
29.	δ-Selinene	1,493.68	1,493	1.9	3.21
30.	α-Muurolene	1,497.81	1,500	1.19	1.62
31.	δ-Amorphene	1,507.41	1,511	2.48	3.61
32.	δ-Cadinene	1,513.55	1,522	7.24	14.68

(*Continued*)

Table 1 (Continued).

S. No.	Compounds	RI	RI^L	Content %	
				Leaf Oil	Inflorescence Oil
33.	Cubebol	1,517.39	1,514	–	1.57
34.	Zonarene	1,526.85	1,528	–	1.6
35.	Bisabolene	1,530.94	1,528	–	1.61
36.	Nerolidol	1,535.03	1,531	–	0.61
37.	Laciniatafuranone	1,551.91	1,548	1.79	–
38.	Germacrene B	1,557.28	1,559	–	0.83
39.	Epi-Cubenol	1,621.27	1,618	–	0.37
40.	Eudesmol	1,627.12	1,630	–	0.46
41.	Sesquilavandulol	1,632.44	1,631	–	0.45
42.	Cadinol	1,635.37	1,638	–	2.12
43.	Cadin 4-en-7-ol	1,639.89	1,635	–	0.54
44.	α-Cadinol	1,648.4	1,652	–	2.03
45.	Pogostol	1,659.84	1,651	1.6	2.66
46.	Farnesyl acetone	1,904.34	1,913	–	1.01
47.	Geranyl linalool	2,017.19	2,026	0.93	–
48.	Methyl linoleate	2,103.37	2,095	17.2	3.34
Class compositions					
Monoterpene hydrocarbons					0.4
Oxygenated monoterpenes					10.82
Sesquiterpene hydrocarbons				38.92	51.88
Oxygenated sesquiterpenes				1.6	10.81
Oxygenated diterpenes				0.93	
Aliphatics Alcohols				34.08	7.3
Aldehyde and ketone					2.42
Esters				19.77	11.04
Others				1.79	
Total				**97.09**	**94.67**

Abbreviations: RI, retention index; RI^L, RI from literature; –, not detected.

linalool (6.99%), 1-octen-3-ol (6.46%), β-acoradiene (5.21), n-octyl2- ethylbutanoate (4.53%), δ-amorphene (3.61%), n-decanal (3.53%), β-caryophyllene (3.48%), methyl linoleate (3.34%), δ-selinene (3.21%), prenyl limonene (3.16%), and α-copaene (3.05%). Major constituents of leaf oil were 1-octen-3-ol (34.08%), methyl linoleate (17.2%), germacrene D (11.16%), δ-cadinene (7.24%), and β-caryophyllene (5.24%). This shows that variations in essential oil compositions occur with the plant part used. The amount of δ-cadinene (14.68%), β-acoradiene (5.21), δ-amorphene (3.61%), δ-selinene (3.21%), prenyl limonene (3.16%), α-copaene (3.05%), pogostol (2.66%), α-muurolene (1.62%), α-humulene

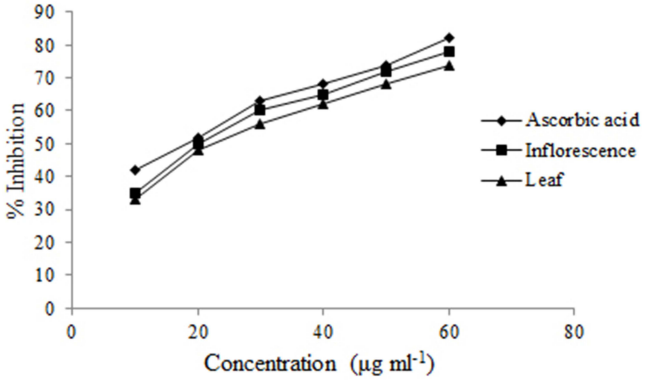

Figure 1 Free radical scavenging activity (in %) of essential oils from *H. capitata* by DPPH assay.

(1.33%), and β-selinen (1.18%) were found to be more in the inflorescence. However, 1-octen-3-ol (34.08%), methyl linoleate (17.2%), β-caryophyllene (5.24%), methyl citronellate (2.57%), and β-guaiene (1.34%) were higher in leaves.

Antioxidant Activity

The radical scavenging activity of both oils was examined with reference to the well-known antioxidant, ascorbic acid, and the result is presented in Figure 1. The percentage of inhibition was higher in the case of inflorescence oil; but the difference was not that significant. The EC_{50} value represents the amount of essential oil, needed for a 50% inhibition of the initial concentration of DPPH radical. Results show that inflorescence oil has a lower EC_{50} (22.76 µg/mL) than the leaf oil (26.18 µg/mL), and the lower the value the better is the inhibition efficiency; however the former is only moderately efficient compared to ascorbic acid, with EC_{50} = 17.57 µg/mL.

Table 2 Larvicidal Activity of the Essential Oils from Leaf and the Inflorescence of *H. capitata* Against *A. stephensi*

Source of Essential Oil	Concentration (µg/mL)	% of Mortality at 24 h±SD	LC_{50} µg/mL (LCL-UCL)	LC_{90} µg/mL (LCL-UCL)	X^2
Inflorescence	Control	0±0	33.19 (28.74–37.00)	81.69 (76.70–87.89)	49.66
	20	42±2.73			
	40	54±4.18			
	60	71±4.18			
	80	87±2.73			
	100	100±0			
Leaves	Control	0±0	39.08 (35.26–42.51)	87.06 (82.56–92.41)	59.85
	20	37±2.73			
	40	47±2.73			
	60	67±4.47			
	80	83±2.73			
	100	97±4.47			
	120	100±0			

Abbreviations: SD, standard deviations; LCL & UCL, lower confidence limits and upper confidence limits; X^2, chi-square test.

Table 3 Ichthyotoxic Activity of Essential Oils from Leaf and the Inflorescence of *H. capitata*

Source of Essential Oil	Concentration (µg/mL)	% of Mortality at 24 h±SD	LC_{50} µg/mL (LCL-UCL)	LC_{90} µg/mL (LCL-UCL)	X^2
Inflorescence	Control	0±0	183.80 (167.21 −221.77)	258.21 (220.43–364.23)	100.82
	80	0±0			
	100	6.6±11.54			
	120	13.3±11.54			
	140	26.66±11.54			
	160	33.33±11.54			
Leaves	Control	0±0	200.57 (177.72–339.10)	271.77 (220.63–623.27)	91.04
	80	0±0			
	100	0±0			
	120	6.66±11.54			
	140	13.33±11.54			
	160	26.66±11.54			

Abbreviations: SD, standard deviations; CL & UCL, lower confidence limits and upper confidence limits; X^2, chi-square test.

Mosquito Larvicidal Activity

The larvicidal activity of essential oils distilled from the leaves and inflorescence of *H. capitata* is presented in Table 2. The oils showed potent larvicidal activity against the fourth-instar larvae of *A. stephensi*. The 24h LC_{50} value (the concentration required to kill 50% of the larvae) of inflorescence oil was 33.19 µg/mL and its LC_{90} value was 81.69 µg/mL, whereas the corresponding values for leaf oil were 39.08 and 87.06 µg/mL, respectively. When the dosage was decreased, there occurred a corresponding reduction in mortality rate.

Ichthyotoxic Activity

In the present study, the in vitro ichthyotoxic activity was considered as the ability of essential oils to slay the fish in respective concentrations. The results showed that the oils only produced toxicity at higher concentrations (Table 3). The LC_{50} and LC_{90} values of inflorescence oil after 24 hours were 183.80 and 258.21 µg/mL, respectively. The corresponding LC_{50} and LC_{90} values of leaf oil were 200.57 and 271.77 µg/mL, respectively.

Discussion

Composition of Essential Oil of Leaves and Inflorescence

Sesquiterpene hydrocarbons (38.92%), aliphatic alcohols (34.08%), and esters (19.77%) were the predominant compounds present in the essential oils from leaf, whereas in inflorescence sesquiterpene hydrocarbons (51.88%), oxygenated monoterpenes (10.82%), oxygenated sesquiterpenes (10.81%), aliphatic alcohols (7.3%), and esters (11.04%) were dominant. This result is in agreement with a couple of previous reports which showed that essential oils of *Hyptis* spp. contain sesquiterpenes and monoterpenes; however, the compositions greatly depend on geographical regions and methods of extraction.[2,17] Most interestingly, 1-octen-3-ol and δ-cadinene were the major components In the leaf oil and inflorescence oil, respectively. 1-Octen-3-ol is an eight carbon alcohol conferring aroma to edible mushrooms and is an economically important aromatic component of several food items and beverages. This compound has already been reported in several species of *Hyptis*; but not in higher amounts.[2,17] In fact the constituents of essential oil from

inflorescence were 2.5-fold when compared to essential oils of leaf, and were also more aromatic. Based on an earlier study, sesquiterpenes γ-cadinenes and caryophyllene, monoterpenes, α-pinenes, and terpinols are common constituents of genus *Hyptis*.[2] Cadinenes are bicyclic sesquiterpenes, grouped into four subclasses. In *H. floribunda*, γ-cadinene (17.9%) and δ-cadinene (10.7%) were found to be major compounds; however, in *H. glomerata*, only γ-cadinene (14%) was the predominant component.[17]

Comparison of Antioxidant Potentials

A low antioxidant activity can be correlated to the absence of phenolic constituents in the oil.[2] Also, cadinene derivatives were reported to have only moderate antioxidant responses.[18] However, synergistic effects from a number of other components like alcohols, ethers, ketones, aldehydes, and monoterpenes would had contributed significantly to the total antioxidant properties.[19] The lamiaceae family is reputed for their aromatic members and they are used in various traditional medicines. Among the members, thyme, basil, oregano, rosemary, sage, and lemon balm are the most common species with regard to their biological properties.[23] Earlier reports on antioxidant properties of *Hyptis* spp. showed widely differing responses, in comparison to other species, *H. brevipes*, *H. rhomboidea*, and *H. suaveolens*.[17] Nine species of the genus *Hyptis* are reported in the literature in correlation to their impressive antioxidant activities.[2,24–26] For instance, the methanolic extract of *H. suaveolens* exhibited potent antioxidant activity as determined by 2.20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, 2.2-diphenyl-1-picrylhydrazyl, and ferric reducing antioxidant power assays.[25] A recent study further demonstrated the higher antioxidant activity of ethanol-water extract of *H. suaveolens*.[27] Moreover, several studies reported that most of the species of plants belonging to the aromatic family Lamiaceae are rich sources of polyphenolic compounds, including vitamin C, vitamin E, quercetin, isorhamnetin, and kaempferol with antioxidant activity.[25,28,29]

Mosquito Larvicidal Activity

The LC_{50} values found from the present study are lower than the effective concentration (50 µg/mL) reported earlier.[20] Similar results were reported in the earlier works done in several species of the genus *Hyptis*. For instance, a study reported the larvicidal activity of methanolic leaf extract of *H. capitata* against *Culex quinquefasciatus* exhibited larval mortality of 93.3±11.54% at a concentration of 25 mg/mL ($P<0.05$) and the LC_{50} was found to be 11.15 mg/mL.[30] A previous study on essential oils from *H. suaveolens* showed insecticidal activity against *A. albopictus* larvae at the highest concentration range of 450–400 µg/mL and there were no significant differences in larval mortality, which ranged between 98.33% and 93.33%; terpinolene was found to be the most effective single component.[31] Besides, a recent study reported that the hexane extract of *H. suaveolens* possesses potential larvicidal effects (LC_{50}=272.5 mg/L).[32] Likewise, larvicidal potency of volatile oil obtained from *H. spicigera* against the *A. gambiae* and *C. quinquefasciatus* demonstrated that they were susceptible and the corresponding concentrations were 45.18 and 53.87 µg/mL, respectively. The chemical analysis of the oil showed that the major constituents are β-caryophyllene (25.7%), caryophyllene oxide (11.56%), sabinene (9.60%), 2-carene (8.78%), α-pinene (6.52%), and 1-octen-3-ol (4.91%).[33]

The larvicidal potentials of several phytochemicals have been previously identified and successfully utilized in mosquito control programs, and are being treated as perfect substitutes for synthetic insecticides.[12] Constituents of essential oils from Lamiaceae members are aromatic and correspond to a variety of compounds with enhanced larvicidal activitives.[21] This may be attributed to the presence of individual constituents with specific activity or due to a synergistic effect caused by diverse types of functional groups present in several bioactives.[22] Earlier studies on essential oils also reported the stronger larvicidal activity of some of the major compounds which are also found in the present study, such as δ-cadinene,[23] linalool,[24] germacrene D,[25] and β-caryophyllene.[26] Another major compound, 1-octen-3-ol, is a known mosquito attractant and was previously evaluated as a control measure in the case of mosquito larvae, by incorporating in the formulation of an insecticide.[30] In this study, the larvicidal assay showed that essential oil of *H. capitata* is very effective against larvae of *A. stephensi*. It can be envisaged that constituents of essential oils can interrupt the total physiological functions leading to the inhibition of enzymes and nerve receptors, ultimately causing tissue damage in the midgut.[12]

Ichthyotoxic Activity

It is to be noted that both the inflorescence and leaf oils are not very toxic to the fishes with respect to the concentrations tested (ie, 5-times higher than the LC_{50} concentration of larvicidal). The ichthyotoxic activity exhibited by the essential oil may be due to the presence of sesquiterpenes.[34] In general, plant-based essential oils have not shown any toxicity, even to the homoeothermic animals, and they are known as "generally recognized as safe" as per the reports of Environmental Protection Agency and Food and Drug Administration in the USA.[35] However, further in-depth studies are required to figure out the mechanism of action of both oils and their extent of toxicity in different aquatic organisms.

Even though most of the members are aromatic plants, studies on chemical constituents and biological activity are limited in number. Only five *Hyptis* spp. (*H. suaveolens*, *H. martiusii*,[12] *H. pectinata*, *H. fruticosa*, and *H. alata*[28]) have been studied so far in connection with their mosquito larvicidal activity and the results suggest that essential oils from these members are excellent sources of larvicidal phytochemicals.

Conclusions

To the best of our knowledge, this is the first detailed report on the essential oil content and the bioactivity of *H. capitata*. The GC-MS analyses have identified the presence of δ-cadinene (14.68%), linalool (6.99%), 1-octen-3-ol (6.46%), and β-acoradiene (5.21) in the inflorescence oil; 1-octen-3-ol (34.08%), methyl linoleate (17.2%), germacrene D (11.16%), δ-cadinene (7.24%), and β-caryophyllene (5.24%) in the leaf oil. Both types of essential oils studied showed remarkable antioxidant properties, particularly the leaf oil with a lower EC_{50} value of 22.76 µg/mL. The larvicidal potential against the fourth instar larvae of the malaria vector, *A. stephensi*, is noteworthy in the case of leaf oil with a LC_{50} value of 33.19 µg/mL. The ichthyotoxic activity with regard to LC_{50} values of both types of essential oils are 5-times higher than the larvicidal concentrations, indicating a lower extent of toxicity. The present findings would substantiate the further utilization of this plant as a source of medicines and also as a larvicide in mosquito control programs.

Ethical Approval

No ethical approval was required for this study because it does not involve any human/animal subjects.

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Disclosure

The authors declare that they have no conflicts of interest in relation to this work.

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