Mosquito Larvicidal Activity of the Essential Oil of *Zingiber collinsii* against *Aedes albopictus* and *Culex quinquefasciatus*

Le T. Huong^{1*}, Trinh T. Huong^{2,3}, Nguyen T.T. Huong^{2,4}, Nguyen H. Hung⁵,
Pham T.T. Dat^{6,7}, Ngo X. Luong³, and Isiaka A. Ogunwande^{8*}

¹ School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, VIETNAM

² Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, VIETNAM

³ Faculty of Natural Science, Hong Duc University, 565 Quang Trung, Đông Vệ, Thanh Hóa City, Thanh Hóa Province, VIETNAM

⁴ Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, VIETNAM

⁵ Center for Advanced Chemistry, Institute of Research and Development, Duy Tan University, 03 Quang Trung, Da Nang, VIETNAM

⁶ Department of Biotechnology, Nong Lam University, Ho Chi Minh City, Vietnam

⁷ Center of Scientific Research and Practice, Tran Van On, Phu Hoa, Thu Dau Mot, Binh Duong province, VIETNAM

⁸ 7, University Drive, Aleku Area, Osogbo, NIGERIA

Abstract: The chemical composition and larvicidal activity of essential oils from the leaves and rhizomes of *Zingiber collinsii* Mood & Theilade (Zingiberaceae) were reported. The main compounds in the leaf oil were α -pinene (25.6%), β -caryophyllene (16.8%), β -pinene (16.1%) and bicyclogermacrene (6.9%) while the rhizome oil consist mainly of camphene (22.5%), β -pinene (16.3%), α -pinene (9.0%) and humulene oxide II (9.0%). The rhizome oil demonstrated larvicidal effects towards fourth instar larvae of mosquito vectors. The highest mortality (100%) was observed at 24 h exposure against *Aedes albopictus* (concentration 100 μ g/mL) and 48 h (concentration of 50 and 100 μ g/mL), while the highest mortality (100%) was observed for *Culex quinquefasciatus* at 24 h and 48 h at concentration of 100 μ g/mL. The 24 h mosquito larvicidal activity of the rhizome oil against *Ae. albopictus* were $LC_{50} = 25.51$ μ g/mL; $LC_{90} = 40.22$ μ g/mL and towards *Cx. quinquefasciatus* with $LC_{50} = 50.11$ μ g/mL and $LC_{90} = 71.53$ μ g/mL. However, the 48 h larvicidal activity were $LC_{50} = 20.03$ μ g/mL and $LC_{90} = 24.51$ μ g/mL (*Ae. albopictus*), as well as $LC_{50} = 36.18$ μ g/mL and $LC_{90} = 55.11$ μ g/mL (*Cx. quinquefasciatus*). On the other hand, no appreciable mortality and larvicidal activity was observed for the leaf oil. The larvicidal activity of the essential oils of *Z. collinsii* was being reported for the first time.

Key words: *Zingiber collinsii*, essential oil composition, monoterpenes, sesquiterpenes, larvicidal activity

1 Introduction

Vietnam is classified as a hyperendemic dengue country with present throughout the year and dengue fever epidemics have increased in frequency¹. Mosquitoes have been and continue to be the most deadly creatures on earth. *Culex quinquefasciatus* Say (Diptera: Culicidae), the southern house mosquito, is a vector of lymphatic filariasis and other arboviruses including West Nile virus, St. Louis encephalitis virus and Zika virus^{2,3}. The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world^{4,5}. Besides its aggressive daytime biting behaviour,

the medical importance of *Ae. albopictus* is due to its ability to transmit many human pathogens and parasites (e.g. yellow fever, dengue fever, West Nile, Japanese encephalitis, St. Louis encephalitis, chikungunya viruses, filarial nematodes).

The control of adult mosquitoes commonly relies on the use of synthetic insecticides and repellents, but treatments with such chemicals are expensive, show scarce efficacy and have a strong environmental impact associated to relevant human health risks^{6,7}. For these reasons, alternative natural insecticides and repellents are now very appreciated by consumers. Essential oils of aromatic plants are con-

* Correspondence to: Isiaka A. Ogunwande, 7, University Drive, Aleku Area, Osogbo, NIGERIA; Le T. Huong, School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghệ An Province, VIETNAM

E-mail: isiakaogunwande@gmail.com (IAO); lehuong233@gmail.com (LTH)

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sidered among the most promising alternative to synthetic chemicals⁸). Essential oils are generally recognized as environmental friendly, easily biodegradable, minimally toxic to mammals and have shown repellent activities against different mosquito species.

Zingiber Miller (Zingiberaceae) is distributed in tropical to warm-temperate Asia with the highest diversity in the monsoonal parts of Asia. It is considered the largest genus in subfamily Zingiberoideae with more than 200 names corresponding to approximately 100-150 species⁹. *Zingiber collinsii* Mood & Theilade is a newly described species in the genus. The plant is known to repel insect pests. A chemical investigation of the rhizomes of *Z. collinsii* resulted in the isolation and identification of zerumbone, acoumarin (scopoletin), quercetin, rutin, 5-(hydroxymethyl) furfural, bisdemethoxycurcumin and demethoxycurcumin¹⁰. The monoterpene hydrocarbons, α -pinene (50.2%), β -pinene (23.6%) and limonene (5.3%) were reported previously as the main constituents of the rhizome oil¹¹. Till moment the authors are not aware of any report describing the biological activity of *Z. collinsii* essential oils.

Many essential oils from *Zingiber* plants have been used as insecticides for controlling many insect pests including mosquito vectors. The essential oils of *Z. cassumunar* displayed a high larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*^{12, 13}, while *Z. ottensii* and *Z. zerumbet* have also displayed similar actions¹⁴. The oils of *Z. officinale* exhibited toxicity towards *Ae. albopictus* and *Cx. quinquefasciatus*¹⁵. The larvicidal action of several *Zingiber* essential oils against mosquito vectors of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* have been reported¹⁶. However, such report and activity was never reported for the essential oil of *Z. collinsii*.

In this paper we report the volatile components of the leaves and rhizome oils, as well as the larvicidal action of the rhizome oil against the fourth-instar larvae of vector mosquitoes, *Ae. albopictus* and *Cx. quinquefasciatus*. This is in continuation of our extensive research aimed at identifying the chemical composition and sourcing for potential larvicides from natural sources^{17, 18}.

2 Experimental Procedures

2.1 Plant collection

The leaves and rhizome of *Z. collinsii* were collected from Thượng Nhật commune, Nam Đông District, Thừa Thiên Huế Province (GPS 16° 20' N 107° 35' E), Vietnam in September 2018. Voucher specimens THH 748 was deposited at the Botany Museum, Vinh University, Vietnam.

2.2 Hydrodistillation of the essential oils

Two kilogram of each of the air-dried leaves and rhi-

zomes of *Z. collinsii* was used for the experiment at different time. The samples were carefully and separately introduced into the flask, after which distilled water was added to cover the surface of the sample. Essential oils were obtained by hydrodistillation for 3 h at normal pressure, according to the Vietnamese Pharmacopoeia¹⁹ conducted in Clevenger-type apparatus. The distilled oils were recovered into previous weighed sample bottle through the receiver arm of the distillation unit. The oils were kept under refrigeration till moment of analysis. All the oil samples were light yellow coloured.

2.3 Analysis of the essential oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m $\times$ 0.25 mm, film thickness 0.25 μ m, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4 °C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μ L. Inlet pressure was 6.1 kPa. The relative amounts of individual components were calculated based on the GC peak area (FID response), as previously described^{17, 18}.

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m $\times$ 0.25 mm, film thickness 0.25 μ m) and interfaced with a mass spectrometer HP 5973 MSD was used for the gas chromatography-mass spectrometry (GC/MS analysis). The same conditions described above was also used for GC. The mass spectrum operating conditions were as follows: ionization voltage 70eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

2.4 Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C₆-C₄₀), under identical experimental conditions, co-injection with standards or known essential oil constituents, and by comparing with MS literature data as described previously^{17, 18}.

2.5 Larvicidal activity

2.5.1 Mosquito larvae

Adults of *Culex quinquefasciatus* and *Aedes albopictus* collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16° 03' 14.9"N, 108° 09' 31.2"E). Adult mosquitoes were maintained in entomological cages (40 $\times$ 40 $\times$ 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on mice. Eggs hatching were induced with tap water. Larvae were reared in plastic trays (24 $\times$ 35 $\times$ 5 cm).

The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at $25 \pm 2^\circ\text{C}$, 65-75% relative humidity, and a 12:12 h light: dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University, Vietnam.

2.5.2 Larvicidal test

The larvicidal activity bioassay was performed according to the method proposed by the World Health Organization with slight modifications²⁰. For the assay, aliquots of the essential oil of *Z. collinsii* dissolved in EtOH (1% stock solution) was placed in a 200-mL beaker and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using EtOH was also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were carried out $25 \pm 2^\circ\text{C}$. Each test was conducted with four replicates with concentrations (100, 50, 25 and 12.5 $\mu\text{g/mL}$).

The mortality rate was calculated according to the formula:

$$Mc = (Mo - Mt) / (100 - Mt) \times 100$$

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality

2.5.3 Statistic analysis

The data obtained were subjected to log-probit analysis²¹ to obtain Median lethal concentrations namely LC_{50} values, LC_{90} values, and 95% confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

3 Results

3.1 Chemical constituents

The yields of essential oils were 0.22% and 0.35% (v/w) respectively, for the leaf and rhizome, calculated on a dry weight basis. The samples of oils obtained from the hydro-distillation were light yellow coloured. Thirty-five compounds representing 98.1% of the oil contents were identified in the leaf of *Z. collinsii* (Table 1). Also, monoterpene hydrocarbons (49.0%) and sesquiterpene hydrocarbons (36.3%) represent the main classes of compounds present in the oil. The major constituents of the leaf oil were mostly terpene hydrocarbons represented by α -pinene (25.6%), β -caryophyllene (16.8%) and β -pinene (16.1%). On the other hand, 51 constituents accounting for 97.7% of the total oil content were identified in the rhizome oil. The representative classes of compounds present in the oil were monoterpene hydrocarbons (58.3%), oxygenated sesquiterpenes (21.1%) and oxygenated monoterpenes (10.9%). The significant compounds of the rhizome oil were camphene (22.5%), β -pinene (16.3%), α -pinene (9.0%) and humulene oxide II (9.0%).

3.2 Larvicidal test

The 24 h and 48 h mosquito larvicidal activities of the essential oil were determined against the mosquito vectors at concentrations of 12.5, 25, 50 and 100 $\mu\text{g/mL}$. The percentage mortality as well as the minimum lethal concentration is shown in Table 2. Assessment of larvicidal efficacy demonstrated good larvicide effects by the rhizome oil towards mosquito vectors. The highest mortality (100%) was observed at 24 h exposure for *Ae. albopictus* (concentration 100 $\mu\text{g/mL}$) and 48 h (concentration of 50 and 100 $\mu\text{g/mL}$), while the highest mortality (100%) was observed for *Cx. quinquefasciatus* at 24 h and 48 h at concentration of 100 $\mu\text{g/mL}$. There was no mortality in the EtOH controls. The 24 h mosquito larvicidal activities of the rhizome oil were *Ae. albopictus* ($LC_{50} = 25.51$ $\mu\text{g/mL}$; $LC_{90} = 40.22$ $\mu\text{g/mL}$) and *Cx. quinquefasciatus* ($LC_{50} = 50.11$ $\mu\text{g/mL}$ and $LC_{90} = 71.53$ $\mu\text{g/mL}$). However, the 48 h mosquito larvicidal activities were $LC_{50} = 20.03$ $\mu\text{g/mL}$ and $LC_{90} = 24.51$ $\mu\text{g/mL}$ (*Ae. albopictus*), as well as $LC_{50} = 36.18$ $\mu\text{g/mL}$ and $LC_{90} = 55.11$ $\mu\text{g/mL}$ (*Cx. quinquefasciatus*). Moreover, no significant mortality and larvicidal activity was observed for the leaf oil. Permethrin, the standard drug used as control displayed larvicidal activity against *Cx. quinquefasciatus* with LC_{50} of 2.19 $\mu\text{g/mL}$ and 1.91 $\mu\text{g/mL}$ at 24 h and 48 h respectively. The LC_{50} values of 1.60 $\mu\text{g/mL}$ and 1.12 $\mu\text{g/mL}$ were obtained against *Ae. albopictus* at 24 h and 48 h respectively.

4 Discussion

The constituents of essential oils of *Z. collinsii* leaf are being reported for the first time. The abundance of α -pinene and β -pinene in the rhizome oil was in agreement with a previous report on the same part¹¹. As usual, ubiquitous terpenoids were identified in both essential oils, consistent with most data obtained for the essential of *Zingiber* genus analysed from Vietnam and other parts of the world. However, the identities of these terpene compounds may vary from one species to another depending on several factors such as environmental and climatic conditions, age and nature of the plants, parts of the plant being analysed, handling procedure etc.. For example, recently, β -pinene and β -caryophyllene were identified as the main constituents in the leaf oil and stem oil of *Z. vuquangensis*²², while the root oil contained bornyl acetate, zerumbone and α -humulene, with β -pinene, 1,8-cineole, α -pinene and β -caryophyllene making up the significant compounds of the fruit oil. The same authors reported that the leaf oil *Z. castaneum* was dominated by β -pinene, α -pinene, β -caryophyllene and bicycloelemene, while identifying larger amounts of β -caryophyllene, δ -cadinene and bicycloelemene in the stem²². However, while camphene, 1,8-cineole and linalool constituted the bulk of the root oil,

Table 1 Constituents of essential oils from *Z. collinsii*.

S/N	Compound ^a	RI ^b	RI ^c	Percent composition	
				Leaf	Rhizome
1	Tricyclene	928	921	—	0.8
2	α -Thujene	930	921	—	0.1
3	α -Pinene	939	932	25.6	9.0
4	Camphene	956	946	1.7	22.5
5	Sabinene	976	956	0.6	0.5
6	β -Pinene	984	978	16.1	16.3
7	β -Myrcene	993	988	1.2	0.9
8	α -Phellandrene	1010	1004	—	0.5
9	δ -3-Carene	1016	1008	—	0.8
10	Limonene	1035	1030	1.2	4.1
11	β -Phellandrene	1036	1032	0.3	2.5
12	1,8-Cineole	1038	1034	—	0.6
13	γ -Terpinene	1064	1056	—	0.1
14	Terpinolene	1095	1098	0.3	0.3
15	Linalool	1104	1100	1.0	1.7
16	Perillene	1105	1104	2.0	—
17	<i>n</i> -Nonanol	1106	1110	1.2	—
18	Camphor	1155	1148	—	0.4
19	Pinocarvone	1173	1170	—	0.1
20	Borneol	1178	1180	—	1.8
21	Terpinen-4-ol	1187	1189	—	0.2
22	Cryptone	1197	1193	—	0.2
23	Myrtenal	1208	1210	—	0.2
24	Neral	1247	1248	0.3	—
25	Cumaldehyde	1251	1254	—	0.3
26	Geranial	1275	1278	0.5	—
27	Bornyl acetate	1294	1293	—	5.2
28	Isobornyl acetate	1297	1297	—	0.2
29	2-Undecanol	1304	1300	0.5	—
30	<i>cis</i> - β -Elemene	1404	1404	4.9	0.5
31	β -Caryophyllene	1419	1417	16.8	2.3
32	β -Gurjunene	1436	1434	—	0.1
33	Aromadendrene	1455	1452	0.6	—
34	α -Humulene	1457	1454	2.0	1.3
35	9- <i>epi</i> -(<i>E</i>)-caryophyllene	1479	1476	0.7	0.3
36	β -Chamigrene	1480	1482	0.5	0.2
37	Germacrene D	1485	1484	0.6	0.1
38	Aristolochene	1502	1502	0.4	—
39	β -Selinene	1505	1505	1.6	0.3
40	(<i>E</i> , <i>E</i>)- α -Farnesene	1513	1508	—	0.7

Table 1 Continued.

S/N	Compound ^a	RI ^b	RI ^c	Percent composition	
				Leaf	Rhizome
41	Bicyclogermacrene	1514	1510	6.9	1.4
42	β-Bisabolene	1518	1512	0.9	–
43	δ-Cadinene	1537	1535	0.5	0.2
44	Elemol	1565	1561	–	0.1
45	(<i>E</i>)-Nerolidol	1571	1568	0.5	–
46	Germacrene D-4-ol	1595	1594	–	0.7
47	Spathulenol	1599	1590	1.1	1.0
48	Caryophyllene oxide	1605	1598	1.7	3.2
49	Humulene oxide I	1621	1618	–	0.6
50	10- <i>epi</i> -γ-Eudesmol	1643	1644	–	0.2
51	Humulene oxide II	1653	1650	–	9.0
52	α-Murolol	1662	1660	–	0.8
53	δ-cadinol	1665	1664	–	0.1
54	α-Eudesmol	1674	1672	0.5	2.4
55	ar-Turmerone	1678	1676	1.6	0.2
56	neo-Intermedeol	1680	1678	–	2.1
57	Intermedeol	1684	1685	–	0.2
58	14-Hydroxy-9- <i>epi</i> -(<i>E</i>)-Caryophyllene	1691	1690	–	0.2
59	Curlone	1716	1720	0.6	0.1
60	Z-Lanceol	1794	1790	0.9	0.1
61	6,10,14-Trimethylpentadecan-2-one	1848	1846	0.7	–
62	Phytol	2125	2124	1.6	–
Total				98.1	97.7
Monoterpene hydrocarbons (Sr. No. 1-11, 13, 14, 16)				49	58.3
Oxygenated monoterpenes (Sr. No. 12, 15, 18-28)				1.8	10.9
Sesquiterpene hydrocarbons (Sr. No. 30-43)				36.3	7.4
Oxygenated sesquiterpenes (Sr. No. 44-61)				7.6	21.1
Diterpenes (Sr. No. 62)				1.6	–
Non-terpenes (Sr. No. 17, 29)				1.7	–

^a Elution order on HP-5MS column; ^b Retention indices on HP-5MS column; ^c Literature retention indices; Sr. No., Serial Number; – Not identified

(*E*)-nerolidol, (*Z*)-9-octadecenamide and β-caryophyllene were the main constituents in the fruit oil²²⁾. The main constituents of the leaves oil of *Z. nudicarpum* were α-cedrol (14.8%), β-eudesmol (13.8%), β-pinene (11.7%) while β-pinene (27.6%), α-pinene (8.5%) were obtained in the root with (*E*)-nerolidol (30.0%), β-caryophyllene (9.4%) and sabinene (8.5%) making up the composition of the fruit oil²³⁾.

The larvicidal activity was found to be concentration dependent. Thus, total and complete inhibition of mosquito larvae was observed in the 100 mg/L compared to other

concentrations. The larvicidal activity of some *Zingiber* oils has been documented in the literature. For example, *Z. zerumbet* showed the most effective extract against *Cx. quinquefasciatus* larvae with LC₅₀ = 49.28 mg/L and LC₉₀ = 83.87 mg/L while *Z. officinale* var. *rubrum*, *Z. montanum* and *Z. spectabile* displayed larvicidal action against *Ae. albopictus* with LC₅₀ = 96.86, 99.04 and 93.35 mg/L; LC₉₀ = 168.65, 153.77, and 168.65 mg/L respectively¹⁶⁾. The volatile oil from *Z. officinalis* was shown to have larvicidal activity against *Cx. quinquefasciatus* with a LC₅₀ value of 50.78 ppm. The essential oils from the rhizome of *Z. nim-*

Table 2 Larvicidal activity of *Z. collinsii* rhizome oil.

Concentration ($\mu\text{g/mL}$)	Mortality (%) ^a			
	<i>Ae. albopictus</i>		<i>Cx. quinquefasciatus</i>	
	24 h	48 h	24 h	48 h
12.5	0	1.2 $\pm$.500	0	4.6 $\pm$.957
25	67.5 $\pm$ 1.732	73.7 $\pm$.500	7.5 $\pm$ 1.291	16.3 $\pm$ 1.893
50	70.0 $\pm$ 1.891	100.0 $\pm$.000	38.7 $\pm$ 2.217	66.3 $\pm$ 2.500
100	100.0 $\pm$.000	100.0 $\pm$.000	100.0 $\pm$.000	100.0 $\pm$.000
Minimum lethal concentration $\mu\text{g/mL}$				
Treatment time	<i>Ae. albopictus</i>		<i>Cx. quinquefasciatus</i>	
	24 h	48 h	24 h	48 h
LC ₅₀	25.51 (23.329-28.095)	20.03 (18.619-21.262)	50.11 (46.103-55.617)	36.18 (33.274-38.486)
LC ₉₀	40.22 (36.568-45.312)	24.51 (23.257-26.057)	71.53 (64.230-83.322)	55.11 (50.496-61.409)
Regression Equation	$y = -1.91 + 0.075x$	$y = -5.73 + 0.286x$	$y = -3.00 + 0.600x$	$y = -2.45 + 0.680x$
χ^2	11.34	8.73	7.61	10.78
<i>P</i>	< 0.001	< 0.001	< 0.001	< 0.001

^a (n= 4); There was no mortality in the EtOH controls

monii demonstrated significant larvicidal activity against *Cx. quinquefasciatus* LC₅₀ value 48.1 $\mu\text{g/mL}$ ²⁴⁾. The larvicidal activity of hydrolates of *Z. officinale* was found promising with LC₅₀ of at 15.8 (%v/v) respectively against *Ae. albopictus* and 21.8 (%v/v) against *Cx. quinquefasciatus*¹⁵⁾.

Since the WHO has not established a standard criterion for determining the larvicidal activity of natural products, several authors have developed individual criteria to characterize the potency of mosquito larvicides developed from natural products²⁵⁻²⁷⁾. For example, Komalamisra *et al.*²⁶⁾ considered products showing LC₅₀ $\leq$ 50 mg/L to be active, 50 mg/L < LC₅₀ $\leq$ 100 mg/L to be moderately active, 100 mg/L < LC₅₀ $\leq$ 750 mg/L to be effective, and LC₅₀ > 750 mg/L to be inactive. Kiran *et al.*²⁷⁾ considered compounds with LC₅₀ < 100 mg/L to exhibit a significant larvicidal effect. It should be stressed that these criteria must be directly correlated with the time of exposure and the origin of larvae, which are variables that can alter the LC₅₀ values. The results obtained in this study showed that the essential oil of *Z. collinsii* rhizome had promising effects, according to the criterion established previously²⁶⁾, exhibiting LC₅₀ values of 25.51 $\mu\text{g/mL}$ and 50.11 $\mu\text{g/mL}$ respectively against *Ae. albopictus* and *Cx. quinquefasciatus* after 24 h of exposure. Overall results in this study showed that essential oils of *Z. collinsii* exhibited good mortality and larvicidal activity on *Ae. albopictus* and *Cx. quinquefasciatus* larvae comparable to many other *Zingiber* essential oils screened for mosquito larvicidal activity.

The larvicidal activity of *Z. collinsii* is likely caused by the wide variety of phytochemicals and volatile composites present in the oil. The observed mosquito larvicidal activities of the essential oils may be due to synergistic actions of the major compounds namely α -pinene, β -pinene, β -caryophyllene, bicyclogermacrene and humulene epoxide II. At times, minor compounds present in the oil sample may impact synergistic effect on the observed activities. Compounds such as α -pinene^{18, 28-30)}, β -pinene^{18, 28-30)}, β -caryophyllene¹⁸⁾, bicyclogermacrene³¹⁾, and humulene epoxide II²⁵⁾, seem to play an important role in increasing the potential toxicity of essential oils. These compounds have previously shown to demonstrate larvicidal activities against mosquito vectors.

5 Conclusion

In this study, the chemical composition of essential oil of the leaf (realized for the first time in Vietnam) and rhizome of *Z. collinsii* by GC-MS were evaluated. This allowed the identification of 62 compounds in both oil samples. The major compounds are: α -pinene, β -pinene, camphene, β -caryophyllene and humulene oxide II. The rhizome oil exhibited larvicidal property against larvae of *Ae. albopictus* and *Cx. quinquefasciatus* after 24 h of exposure.

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