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Chemical composition and larvicidal activity of essential oils from *Zingiber montanum* (J. Koenig) Link ex. A. Dietr. against three mosquito vectors

[Composición química y actividad larvicida de aceites esenciales de *Zingiber montanum* (J. Koenig) Link ex. A. Dietr. contra tres vectores de mosquitos]

Le T Huong[1], Trinh T Huong[2,3], Nguyen TT Huong[2,4], Nguyen H Hung[5], Pham TT Dat[6,7], Ngo X Luong[3] & 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 Vietnam Academy of Science and Technology, 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, 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]*Foresight Institute of Research and Translation, 19, Eleyele, Ibadan, Oyo State, Nigeria*
Contactos | Contacts: **Isiaka A OGUNWANDE** - ***E-mail address:*** **isiakaogunwande@gmail.com**

Abstract: The chemical composition and larvicidal activity of essential oils derived from the leaves and rhizomes of *Zingiber montanum* (J. Koenig) Link ex. A. Dietr. were reported. The main compounds in the leaf oil were β-pinene (13.8%), β-phellandrene (11.3%) and α-pinene (7.3%) while the rhizome oil was dominated by sabinene (41.1%), terpinen-4-ol (22.7%) and (E)-nerolidol (14.3%). The minimum lethal concentration (larvicidal activity) LC_{50} of the rhizome oil at 24 h against *Aedes albopictus* was 35.17 µg/mL, while LC_{50} values of 32.20 µg/mL and 31.12 µg/mL were obtained against *Aedes aegypti* and *Culex quinquefasciatus* respectively. At 48 h the oil displayed larvicidal action with LC_{50} values of 23.18 µg/mL, 25.58 µg/mL and 18.99 µg/mL respectively towards *Ae. albopictus*, *Ae. Aegypti* and *Cx. quinquefasciatus*. The leaf oil did not exhibit significant mortality and larvicidal action. The results indicate the potential of rhizome essential oil of *Z. montanum* as a source of larvicidal agent.

Keywords: *Zingiber montanum*; Essential oil; Monoterpnes; Mortality; Larvicidal activity

Resumen: En el presente trabajo se reportan la composición química y actividad larvicida de los aceites esenciales obtenidos de hojas y rizomas de *Zingiber montanum* (J. Koenig) Link ex. A. Dietr. Los principales compuestos en el aceite de hojas fueron β-pineno (13.8%), β-felandrene (11.3%) y α-pineno (7.3%); mientras que los más abundantes en el aceite de rizomas fueron sabineno (41.1%), terpinen-4-ol (22.7%) y (E)-nerolidol (14.3%). La concentración letal mínima (actividad larvicida) LC_{50} del aceite de riomas ante *Aedes albopictus* fue 35.17 µg/mL, mientras que los valores de LC_{50} de 32.20 µg/mL y 31.12 µg/mL fueron obtenidos ante *Aedes aegypti* y *Culex quinquefasciatus* respectivamente. A las 48 horas, el aceite mostró acción larvicida con valores de LC_{50} de 23.18 µg/mL, 25.58 µg/mL y18.99 µg/mL respectivamente, ante *Ae. albopictus*, *Ae. Aegypti* and *Cx. quinquefasciatus*. El aceite de hojas no mostró mortalidad ni acción larvicida significativa. Los resultados indican el potencial del aceite esencial de rizomas de *Z. montanum* como una fuente de agentes larvicidas.

Palabras clave: *Zingiber montanum*; Aceite esencial; Monoterpenos; Mortalidad; Actividad larvicida

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INTRODUCTION

Vietnam is classified as a hyperendemic dengue country with present throughout the year and dengue fever epidemics have increased in frequency. Mosquitoes have been and continue to be the most deadly creatures on earth. *Aedes albopictus*, *Aedes aegypti* and *Culex quinquefasciatus* are the main vectors which transmits several diseases such as dengue fever and other related diseases (Hung *et al*., 2019). Chemical control of these vectors of diseases have an impact on the environment and humans, also burden a high cost. One of the efforts to reduce the negative impact of synthetic insecticide is to find out alternative natural insecticide from plant-based insecticides.

Zingiber Miller (Zingiberaceae) is distributed in tropical and warm-temperate Asia with the highest diversity in monsoonal parts of Asia. It is considered the largest genus in the subfamily Zingiberoideae with more than 200 names corresponding to approximately 100-150 species (Theillade & Mood, 2000). *Zingiber montanum* (J. Koenig) Link ex A.Dietr. (syn. *Zingiber cassumunar* Roxb.) is a rhizome forming perennial herb, with rather stout, leafy stem, up to 2 m high. The rhizome is yellow inside, strongly aromatic. The leaves are lance-shaped, 30-45 cm long, stalkless, velvet-hairy along midrib only on the lower surface with persistent red or purplish-brown colour. The pseudostem is cylindrically erect and enveloped by leafy sheaths reaching up to 1.2-1.8 m high. The purplish-brown flowering stem arises from the root, about 7-15 cm long. The flowers are pale yellow which tube which is about 2.5 cm long (Lim, 2016). The plant is used in ethnomedicine for the treatment of constipation, rheumatism, wounds, asthma, mosquito repellent among others (Singh *et al*., 2015).

The chemical constituents of essential oil from *Z. montanum* have been reported from a different origin. The main monoterpene compounds that featured prominently in the oil consist of α-pinene and β-pinene (Huong *et al*., 2017), sabinene, (*Z*)-ocimene and γ-terpinene (Chaiyana *et al*., 2017; Leelarungrayub *et al*., 2017; Bacha & Adelheid, 2018) and terpinen-4-ol (Vipada & Yingyoong, 2012; Chaiyana *et al*., 2017). The major sesquiterpene constituents comprised mainly caryophyllene (Kamazeri *et al*., 2012), valencene, eudesma-4(14),11-diene and germacrene D (Huong *et al*., 2017), caryophyllene oxide (Bhuiyan *et al*., 2008), 1(10),4-furanodien-6-one and curzerenone (Bordoloi *et al*., 1999). The non-terpene compounds consist of (*E*)-1(3,4-dimethylphenyl)butadiene, (*E*)-(3,4-dimethoxyphenyl) but-l-ene, (*E*)-4-(3,4-dimethoxyphenyl)but-3-ene-l-yl acetate and 2,6,9,9-tetramethyl-2,6,10-cycloundecatrien-1-one (Kamazeri *et al*., 2012; Leelarungrayub *et al*., 2017; Bacha & Adelheid, 2018; Verma *et al*., 2018). There are several other minor constituents which differ from one another depending on the origin of the sample being analyzed. Essential oils from *Z. montanum* have previously displayed antibacterial (Boonyanugomol *et al*., 2017), antioxidant (Manochai *et al*., 2010; Manochai *et al*., 2017) and anesthetic (Khamsopa *et al*., 2018) activities among others.

Previous studies have shown that *Z. montanum* essential oil reduces the biting rate of mosquitoes and displayed repellent and ovicidal actions (Phukerd & Soonwera, 2014) and exhibited mortality and larvicidal action against *Cx. quinquefasciatus, Ae. albipoctus* and *Ae. aegypti* (Phukerd & Soonwera. 2013; Boonyuan *et al*., 2014; Cotchakaew & Soonwera, 2014). A previous report indicated that *Z. montanum* oil displayed larvicidal action against *Ae. aegypti*, *Ae. albopictus* and *Culex quinquefasciatus* with LC_{50} of 84.95, 99.04 and 176.35 mg/L, respectively (Restu *et al*., 2017). However, the larvicidal activity of essential oil from *Z. montanum* grown in Vietnam has not been previously evaluated and reported.

The purpose of this research was to determine the killing power (mortality) and larvicidal activity of the leaf and rhizome essential oils of *Z. montanum* against the fourth-instant larvae of *Ae. albopictus*, *Ae. aegypti* and *Cx quinquefasciatus*. This is in continuation of our extensive research aimed at the characterization of the volatile constituents and biological activities of *Zingiber* species in particular (Huong *et al*., 2019; Huong *et al*., 2020) and the flora of Vietnam in general (Hung *et al*., 2019; Ban *et al*., 2020).

MATERIALS AND METHODS

Plant collection

The leaves and rhizomes of *Z. montanum* were collected from Bình Chuẩn Commune, Pù Huống, Natural Reserve, Nghe An Province, Vietnam, in August 2018. Botanical identical was conducted by Dr. L.T. Huong, Vinh University, Vinh City, Vietnam. A voucher specimen (TTH 734) was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

Hydrodistillation of essential oils

Two kilograms each of air-dried and pulverized sample of leaf and rhizome of *Z. montanum* were used for this experiment. The samples were carefully and separately introduced into 5 L 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 established procedure (Vietnamese Pharmacopoeia, 2009) conducted in the 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 until the moment of analysis. Analysis was done in triplicate.

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 x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV) 250ºC, detector temperature 260ºC, column temperature programmed from 60ºC (2 min hold) to 220ºC (10 min hold) at 4ºC/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response).

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as a carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s. The MS fragmentation patterns were checked with those of other essential oils of known composition.

Identification of the constituents of essential oils

The identification of constituents of essential oils of *Z. montanum* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_6-C_{40}), under identical experimental conditions, co-injection with standards compounds. The mass spectra were compared with available library search (NIST, 2018) as described previously (Ban *et al*., 2020).

Mosquito larvae

Adults of the used mosquitoes were 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 x 40 x 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 (24×35×5 cm). The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at 25 ± 2°C, 65-75% relative humidity, and a 12:12 h light-dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University.

Larvicidal test

Larvicidal activity of the essential oils from *Z. montanum* was evaluated according to an established protocol (WHO, 2005) with slight modifications. For the assay, aliquots of the essential oils 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 at 25 ± 2°C. Each test was conducted with four replicates using four concentrations (100, 50, 25 and 12.5 μg/mL). Permethrin was used as a positive control.

The mortality rate was calculated according to the formula:

$$Mc = Mo/Mt \times 100$$

Mo = number of larvae dead in the treated groups, Mt = number of larvae introduced and Mc = calculated mortality

Statistical analysis

The data obtained were subjected to log-probit analysis (Finney, 2009) to obtain LC_{50} values, LC_{90} values, 95% confidence limits, and chi square values using XLSTAT v. 2018.5 (Addinsoft, Paris, France). Statistical analysis (ANOVA) of the differences between mean values obtained for experimental groups were calculated as a mean of standard deviation (SD) of four independent measurements using Microsoft excel program 2003.

RESULTS AND DISCUSSION

Chemical constituents of the essential oils

The yield of essential oils was 0.18% ±0.01 and 0.31% ± 0.01 (v/w, leaf and rhizome respectively), calculated on a dry weight basis. Both samples of oils obtained from the hydrodistillation were light yellow coloured. As usual, ubiquitous terpenoids were identified in both essential oils, consistent with most data obtained for the essential of *Zingiber* genus analyzed from Vietnam and other parts of the world. Fifty-three compounds representing 87.1% of the oil contents were identified in the leaf of *Z. montanum* (Table No. 1). These comprised of monoterpene hydrocarbons (43.4%), oxygenated monoterpenes (16.4%), sesquiterpene hydrocarbons (9.3%) and oxygenated sesquiterpenes (14.1%). The main constituents of the leaf oil were β-pinene (13.8%), β-phellandrene (11.3%) and α-pinene (7.3%). On the other hand, 27 constituents accounting for 98.8% of the total oil content were identified in the rhizome oil under study. The representative classes of compounds present in the oil were monoterpene hydrocarbons (56.5%), oxygenated monoterpenes (26.3%) and oxygenated sesquiterpenes (14.3%). The significant compounds of the rhizome essential oil were sabinene (41.1%), terpinen-4-ol (22.7%) and (*E*)-nerolidol (14.3%). The main compounds of the leaf oil were identified in much lower amounts in the rhizome oil and vice versa. This seemingly differences and observation may be due to the fact that different plant organs stored different bioactive phytochemical. This may ultimately affect the ethnomedicinal uses as well as biological activities (Feduraev *et al*., 2019). The abundance of α-pinene and β-pinene in the leaf essential oil was in agreement with a previous report on leaf of *Z. montanum* from Vietnam (Huong *et al*., 2017). In addition, sabinene and caryophyllene oxide present in the leaf oil of samples from Bangladesh (Bhuiyan *et al*., 2008) were also identified in the Vietnam sample. However, 1(10),4-furanodien-6-one and curzerenone, the abundant compounds in the leaf oil of sample from India (Bordoloi *et al*., 1999) were conspicuously absent in the present oil sample. The high content of sabinene and terpinen-4-ol in the rhizome was consistent with findings for most of the reports on the volatile contents of this plant part (Vipada & Yingyoong, 2012; Chaiyana *et al*., 2017; Leelarungrayub *et al*., 2017; Bacha & Adelheid, 2018). However, (*E*)-1-(3,4-dimethoxyphenyl)butadiene, commonly reported for most samples in other parts of the world was not identified in the present study. This may be due to several factors such as environmental and climatic conditions, age and nature of the plants, parts of the plant being analyzed, handling procedure etc. (Sharifi-Rad *et al*., 2017).

Table No. 1
Chemical constituents of essential oils from the leaf and rhizome of *Z. montanum*

Sr. No	Rt (min)	Compounds[a]	RI (cal.)	RI (Lit)	Leaf[b]	Rhizome[b]
1.	9.87	α-Thujene	930	921	-	0.4
2.	10.14	α-Pinene	939	932	7.3	1.7
3.	10.63	Camphene	955	954	1.5	0.2
4.	11.34	Sabinene	979	976	1.9	41.1
5.	11.51	β-Pinene	984	978	13.8	2.9
6.	11.74	Myrcene	992	988	1.1	1.3
7.	12.34	α-Phellandrene	1010	1008	0.2	-
8.	12.74	α-Terpinene	1022	1022	-	1.9
9.	13.02	o-Cymene	1030	1028	0.5	0.8
10.	13.11	Limonene	1035	1030	2.0	0.5
11.	13.22	β-Phellandrene	1036	1032	11.3	1.1
12.	13.27	1,8-Cineole	1038	1034	-	0.5
13.	13.31	(*Z*)-β-Ocimene	1039	1036	1.8	-
14.	13.68	(*E*)-β-Ocimene	1049	1044	2.0	0.3
15.	14.17	γ-Terpinene	1064	1056	-	3.4
16.	14.52	*cis*-Sabinene hydrate	1074	1077	-	0.7
17.	15.22	Terpinolene	1095	1094	-	0.6
18.	15.38	Rosefuran	1099	1098	0.2	-
19.	15.53	Linalool	1104	1102	0.2	-
20.	15.63	*trans*-Sabinene hydrate	1106	1106	-	0.6

21.	15.61	Nonanal	1106	1106	0.4	-
22.	15.73	o-Guiacol	1109	1110	0.3	-
23.	16.47	*cis*-p-Menthe-2-en-1-ol	1130	1132	-	0.5
24.	17.10	*trans*-p-Menthe-2-en-1-ol	1148	1148	1.3	0.4
25.	17.18	*trans*-Sabinol	1151	1150	0.5	-
26.	17.31	*cis*-Sabinol	1154	1152	0.2	-
27.	17.87	Camphor	1155	1154	1.8	-
28.	17.97	Pinocarvone	1173	1172	0.3	-
29.	18.48	Terpinen-4-ol	1187	1187	0.5	22.7
30.	18.80	Cryptone	1197	1199	4.2	-
31.	18.94	α-Terpineol	1201	1200	0.1	0.3
32.	19.11	*cis*-Piperitol	1205	1206	-	0.1
33.	19.18	Myrtenal	1208	1210	1.0	-
34.	19.50	*trans*-Piperitol	1217	1212	-	0.2
35.	20.67	Neral	1247	1245	1.5	-
36.	21.02	Cumaldehyde	1251	1248	1.1	-
37.	221.7	Bornyl acetate	1294	1292	0.4	-
38.	22.89	Isobornyl acetate	1297	1297	2.0	-
39.	22.39	Cumin alcohol	1301	1300	0.6	-
40.	23.47	4-hydroxy-Cryptone	1333	1340	0.5	-
41.	24.95	α-Terpinyl acetate	1357	1360	-	0.3
42.	25.80	*cis*-β-Elemene	1404	14105	0.3	-
43.	26.85	β-Caryophyllene	1437	1440	1.3	-
44.	27.42	β-Gurjunene	1450	1452	1.0	-
45.	27.59	(*Z*)-β-Farnesene	1461	1456	1.0	-
46.	27.94	α-Humulene	1472	1472	0.2	-
47.	28.47	3,4-Dimethoxybenzaldehyde	1489	1490	0.7	-
48.	28.83	β-Chamigrene	1500	1498	0.9	-
49.	28.92	Aristolochene	1502	1508	1.3	-
50.	29.02	α-Zingiberene	1504	1510	-	0.1
51.	29.21	α-Selinene	1513	1512	0.5	-
52.	29.36	β-Bisabolene	1518	1520	0.2	-
53.	29.87	β-Sesquiphellandrene	1535	1532	2.3	0.8
54.	29.96	7-*epi*-α-Selinene	1538	1540	0.3	-
55.	30.94	(*E*)-Nerolidol	1571	1571	1.0	14.3
56.	31.77	Spathulenol	1599	1590	1.8	-
57.	31.96	Caryophyllene oxide	1605	1610	2.2	-
58.	32.89	Humulene oxide I	1621	1630	1.1	-
59.	33.05	Humulene oxide II	1633	1634	1.4	-
60.	34.36	Apiole	1690	1700	0.5	-
61.	34.92	Curlone	1716	1712	3.2	-
62.	35.44	Asarone aldehyde	1729	1726	2.1	-
63.	36.89	Benzyl benzoate	1783	1770	0.7	-
64.	38.60	6,10,14-Trimethylpentadecan-2-one	1849	1847	0.6	-
65.	42.10	Phytol	2120	2119	2.4	1.1
Total					**87.1**	**98.8**
Monoterpene hydrocarbons (Sr. No. 1-15, 17)					**43.4**	**56.5**
Oxygenated monoterpenes (Sr. No. 16, 18-20, 23-41)					**16.4**	**26.3**
Sesquiterpene hydrocarbons (Sr. No. 42-46, 48-54)					**9.3**	**0.9**
Oxygenated sesquiterpenes (Sr. 55-59, 61-64)					**14.1**	**14.3**
Diterpenes (Sr. No. 65)					**2.4**	**1.1**
Non-terpeens (Sr. No. 21, 22, 47)					**1.0**	**-**
Phenylpropene (Sr. No. 60)					**0.5**	**-**

Mortality test

The mosquito larvicidal activity of the essential oils was determined against the mosquito vectors at concentrations of 12.5, 25, 50 and 100 µg/mL. The test periods were 24 h and 48 h. The percentage mortality as well as the minimum lethal concentrations is shown in Table No. 2. The rhizome essential oil demonstrated good larvicide activity towards the mosquito vectors. The highest mortality (100%) was obtained at 24 h and 48 h of exposure to *Ae. aegypti* (concentration 50 µg/mL) and *Ae. albopictus* (concentration 100 µg/mL). The highest mortality (100%) was observed towards *Cx. quinquefasciatus* at 48 h (concentration of 50 µg/mL) and at 24 h (concentration of 100 µg/mL). There was no mortality in the EtOH controls. The mortality test was found to be concentration dependent. Thus, the mortality rate was insignificant at lower concentrations of 12.5 µg/mL and 25.0 µg/mL when compared with other higher concentrations. The results in this study revealed that the rhizome essential oil of *Z. montanum* demonstrated potent mortality towards the fourth-instant larvae of *Ae. Albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* comparable with previous studies describing similar activity. The essential oil of *Z. montanum* was reported to reduce the biting rate of *Cx. quinquefasciatus* and *Ae. aegypti* (Cotchakaew & Soonwera, 2014; Phukerd & Soonwera, 2014; Restu *et al*., 2017) and *Ae. albipoctus* (Phukerd & Soonwera, 2013).

Table No. 2
Percentage mortality and larvicidal action of *Z. montanum* essential oil

Mortality (%) [a]						
Concentration (µg/mL)	*Ae. albopictus*		*Ae. aegypti*		*Cx. quinquefasciatus*	
	24 h	48 h	24 h	48 h	24 h	48 h
12.5	0	0	0	2.5 ± 0.577	16.3± 1.258	25 ± 0.816
25	25 ± 4.546	56.3 ± 5.909	25 ± 3.464	47.5± 6.245	25 ± 2.582	56.3 ± 4.992
50	63.7 ± 2.363	96.3 ± 0.957	75 ± 1.633	100 ± 0.000	96.3 ± 0.957	100 ± 0.000
100	100 ± 0.000	100 ± 0.000	100 ± 0.000	100 ± 0.000	100 ± 0.000	100 ± 0.000
Minimum lethal concentration (µg/mL)						
	Ae. albopictus		*Ae. aegypti*		*Cx. quinquefasciatus*	
Parameters	24 h	48 h	24 h	48 h	24 h	48 h
LC_{50}	35.17	23.18	32.20	23.58	31.12	18.99
LC_{90}	56.02	35.12	45.64	31.20	52.25	31.18

Larvicidal test

The rhizome essential oil showed larvicidal efficacy against *Ae. albopictus* with minimum lethal concentrations LC_{50} value of 35.17 µg/mL and LC_{90} value of 56.02 µg/mL at 24 h period. However, LC_{50} of 32.20 µg/mL and LC_{90} of 45.64 µgmL were recorded against *Ae. aegypti* at 24 h. The rhizome oil after 24 h displayed larvicidal action towards *Cx. quinquefasciatus* with LC_{50} of 31.12 µg/mL and LC_{90} of 52.25 µg/mL. However, at 48 h test period, LC_{50} of 23.18 µg/mL and LC_{90} of 35.12 µg/mL were recorded against *Ae. albopictus*. In addition, rhizome oil exhibited a larvicide effect at 48 h towards *A. aegypti* (LC_{50} = 25.58 µg/mL and LC_{90} = 31.20 µg/mL) and *Cx. quinquefasciatus* (LC_{50} = 18.99 µg/mL; LC_{90} = 31.18 µg/mL). Permethrin, the standard drug used as control displayed qualitative larvicidal activity against the three mosquito vectors. The leaf oil displayed no significant mortality and larvicidal action. The results revealed that the rhizome essential oil of *Z. montanum* showed potent larvicidal action against the fourth-instant larvae of *Ae. Albopictus, Ae. aegypti* and *Cx. quinquefasciatus* when compared with similar studies. The rate of susceptibility of the vectors towards the rhizome oil of *Z. montanum* was *Ae. albopictus* < *Cx. quinquefasciatus* < *Ae. aegypti*. From Table No. 3, the model summary indicated that 78.3%, 86.8% and 88.7% of latave of *Ae. albopictus, Cx. quinquefasciatus* and *Ae. aegypti* were killed. The essential oil of *Z. montanum* was reported to showed larvicidal activity towards *Ae. aegypti, Ae. albopictus* and *Cx. quinquefasciatus* and (Restu *et al*., 2017).

Table No. 3
Table of ANOVA

Model summary

	Model	**R**	**R Square**	**Adjusted R square**	**Standard Error of Estimate**
Ae. aegypti	1	.946[a]	.895	.887	2.799
Ae. albopictus	1	·898[a]	.806	.783	3734
Cx. quinquefasciatus	1	.936	.876	.868	2964

ANOVA[b]

Model	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig**
Ae. aegypti					
Regression	934.322	1	934.322	119.263	.000[a]
1 Residual	109.678	14	7.834		
Total	1044.000	15			
Ae. albopictus					
Regression	808.769	1	808.769	58.015	.000[a]
1 Residual	195.169	14	13.941		
Total	1003.938	15			
Cx. quinquefasciatus					
Regression	872.763	1	872.763	99.349	.000[a]
1 Residual	122.987	14	8.785		
Total	995.750	15			

[a] **Predictors: (Constant), Y; Dependent Variable, X**

The larvicidal activity of essential oils from some other *Zingiber* plants grown in Vietnam and other parts of the world has been reported in the literature. For example, the rhizome essential oil of *Z. zerumbet* from Vietnam exhibited larvicidal activity towards *Cx. quinquefasciatus* and *Ae. albopictus* with median lethal concentrations, LC_{50} values of 33.28 µg/mL and 55.75 µg/mL, respectively after 24 h (Huong *et al*., 2019). In addition, the rhizome of *Z. collinsii* from Vietnam also displayed larvicidal activity against *Ae. albopictus* (LC_{50} = 25.51 µg/mL) and *Cx. quinquefasciatus* (LC_{50} = 50.11 µg/mL) after 24 h (Huong *et al*., 2020). Table No. 4 indicates the larvicidal potential of some *Zingiber* essential oils analyzed from Vietnam and other parts of the world. The essential oil of *Z. montanum* in this study exhibited larvicidal activity against *A. aegypti* with LC_{50} value much lower than previously reported *Zingiber* oil samples. On the other hand, the oil displayed activity slightly higher than *Z. collinsii* against *Ae. albopictus* and *Cx. quinquefasciatus*. Variations in toxicity of essential oils against different species of mosquitoes are common due to qualitative and quantitative variations of chemicals constituents (Ammer & Mehlhorn, 2006). This might have been responsible for the observed differences in the larvicidal action of the various *Zingiber* oils towards the different mosquito vectors.

Since the WHO has not established a standard criterion for determining the larvicidal activity of natural products, several authors (Komalamisra *et al*., 2005; Kiran *et al*., 2006; Magalhães *et al*., 2010) have developed individual criteria to characterize the potency of mosquito larvicides developed from natural products. For example, Komalamisra *et al*. (2005) considered products showing $LC_{50} \leq 50$ mg/L to be active, 50 mg/L < $LC_{50} \leq 100$ mg/L to be moderately active, 100 mg/L < $LC_{50} \leq 750$ mg/L to be effective, and $LC_{50} > 750$ mg/L to be inactive. 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 LC_{50} values. The results obtained in this study showed that the essential oil of *Z. montanum* rhizome had promising effects, according to the criterion established previously (Magalhães *et al*., 2010), exhibiting larvicidal activity against *Ae. albopictus* (LC_{50} = 35.17 µg/mL), *Ae. aegypti* (LC_{50} = 32.20 µg/mL) and *Cx. quinquefasciatus* (LC_{50} = 31.12 µg/mL) after 24 h of exposure. Overall results in this study showed that essential oils of *Z. montanum* possessed good mortality and larvicidal activity on the mosquito vectors used in this study.

Table No. 4
Larvicidal activity of essential oils of some *Zingiber* plants

Essential oil	Origin	Parts	LC$_{50}$ 24 h			
			Ae. aegypti	*Ae. albopictus*	*Cx. quinquefasciatus*	References
Z. collinsii	Vietnam	Rhizome	-	25.51 μg/mL	50.11 μg/mL	Huong *et al*., 2020
Z. zerumbet	‘’	‘’	-	55.75 μg/mL	33.28 μg/mL	Huong *et al*., 2019
‘’	Malaysia	‘’	102.6 μg/mL	-	-	Jantan *et al*., 2003
‘’	Thailand	‘’	48.88 ppm	-	-	Sutthanont, *et al*., 2010
‘’	Malaysia	‘’	82.05 mg/L	106.57 mg/L	49.28 mg/L	Restu *et al*., 2017
Z. officinale	Malaysia	‘’	197.2 μg/mL			Jantan *et al*., 2003
Z. cernuum	India	‘’	48.44 μg/mL	55.84 μg/mL	48.44 μg/mL	Rajeswary *et al*., 2018
Z. officinale var. *rubrum*	Malaysia	‘’	120.60 mg/L	96.86 mg/L	130.50 mg/L	Restu *et al*., 2017
Z. spectabile	‘’	‘’	155.93 mg/L	93.35 mg/L	107.78 mg/L	Restu *et al*., 2017
Z. officinale	‘’	‘’	-	15.8% [a]	21.8% [a]	Rabha *et al*., 2016
Z. officinale	Thailand	‘’	-	-	50.78 ppm	Pushpanathan *et al*., 2008
‘’	India	‘’	40.5 mg/mL	-	-	Kalaivani *et al*., 2012
‘’	Brazil	‘’	70.6 mg/mL	-	-	Dias and Moraes, 2013
Z. nimmonii	Thailand	‘’	44.46 μg/mL	-	48.26 μg/mL,	Govindarajan *et al*., 2016
Z. montanum	Malaysia	‘’	84.95 mg/L	99.04 mg/L	176.35 mg/L	Restu *et al*., 2017
‘’	Vietnam	‘’	32.20 μg/mL	35.17 μg/mL	31.12 μg/mL	This study

The larvacidal activity of *Z. montanum* was likely caused by the wide variety of phytochemicals and volatile composites present in the oil. The observed mosquito larvicidal activity of the rhizome essential oil may be due to the synergistic actions of the major compounds or some minor compounds present in the oil. Compounds such as sabinene (Cheng *et al*., 2013), terpinen-4-ol (Govindarajan *et al*., 2015) and (*E*)-nerolidol (Magalhães *et al*., 2010; Hung *et al*., 2019), play an important role in increasing the potential toxicity of essential oils against targeted insect vectors. These compounds have previously demonstrated larvicidal activity against mosquito vectors. Therefore, *Z. montanum* essential oils and their constituents could be developed as control agents against mosquito larvae.

CONCLUSION

In this study, the chemical composition of essential oil of the leaf and rhizome of *Z. montanum* were evaluated by GC and GC-MS. This allowed the identification of ubiquitous mono- and sesquiterpene compounds in both oil samples. The major compounds were: α-pinene, β-pinene, sabinene, terpinen-4-ol and (*E*)-nerolidol. The rhizome oil exhibited larvicidal activity against larvae of *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* after 24 h and 48 h.

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