Evaluation of the chemical compositions, larvicidal and antimicrobial efficacies of *Zingiber castaneum* and *Zingiber nitens* essential oils

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Abstract

In this paper, we report the chemical constituents, larvicidal and antimicrobial activities of essential oils from *Zingiber castaneum* Škorničk. & Q.B. Nguyễn and *Zingiber nitens* M.F. Newman growing in Vietnam. The main constituents of *Z. castaneum* leaf were bicyclogermacrene (24.8%), germacrene D (12.9%), cis- β -elemene (11.2%) and β -pinene (10.3%), while the pseudo-stem contained bicyclogermacrene (15.8%), cis- β -elemene (9.8%) and germacrene D (9.2%). The significant compound of the rhizome oil was sabinene (22.9%), along with α -pinene (7.8%), β -pinene (6.5%), bornyl acetate (6.1%) and γ -terpinene (5.5%). However, β -pinene (45.8%), α -pinene (10.7%) and bicyclogermacrene (7.8%) were the dominant compounds in the leaf oil of *Z. nitens*. Terpinen-4-ol (77.9%) occurred as the compound occurring in higher amount in the rhizome oil. The rhizome oil of *Z. castaneum* exhibited 100% mortality towards *Ae. aegypti* (concentration, 200 $\mu\text{g/mL}$; 24 h and 48 h) and *Ae. albopictus* (concentration, 100 $\mu\text{g/mL}$; 24 h and 48 h). However, mortality of 81.3% was observed against *Cx. quinquefasciatus* at 48 h (concentration 100 $\mu\text{g/mL}$). The leaf also exhibited 100% mortality against *Ae. aegypti* (concentration, 100 $\mu\text{g/mL}$; 24 h and 48 h) and *Cx. quinquefasciatus* (concentration, 150 $\mu\text{g/mL}$; 24 h and 48 h). The rhizome oil displayed a minimum lethal concentration LC 50 of 121.43 $\mu\text{g/mL}$ and 110.31 $\mu\text{g/mL}$ against *Ae. aegypti* respectively at 24 h and 48 h while values of 49.85 $\mu\text{g/mL}$ and 43.93 $\mu\text{g/mL}$ at 24 h and 48 h were observed against *Ae. albopictus* respectively. In addition, LC 50 values of 88.86 $\mu\text{g/mL}$ and 48.08 $\mu\text{g/mL}$ were recorded respectively against *Cx. quinquefasciatus*. Also, the leaf oil displayed significant larvicidal activity against *Ae. aegypti* with LC 50 of 39.30 $\mu\text{g/mL}$ (24 h) and 31.78 $\mu\text{g/mL}$ (48 h) while LC 50 values of 84.97 $\mu\text{g/mL}$ (24 h) and 47.40 $\mu\text{g/mL}$ (48 h) were recorded respectively against *Cx. quinquefasciatus*. The leaf oil of *Z. nitens* exhibited 100% mortality against *Ae. aegypti* at 24 h and 48 h period (concentration 50 $\mu\text{g/mL}$) while the rhizome oil displayed maximum mortality at concentration of 100 $\mu\text{g/mL}$. The rhizome oil attained only mortality of 93% against *Cx. quinquefasciatus* at test period. No significant mortality was recorded against *Cx. quinquefasciatus* by the leaf oil. The LC 50 values of 17.58 $\mu\text{g/mL}$ (24 h) and 15.12 $\mu\text{g/mL}$ (48 h) were displayed by the leaf oil against *Ae. aegypti* while values of 29.60 $\mu\text{g/mL}$ (24 h) and 26.21 (48 h) were exhibited by the rhizome oil. Only the rhizome oil was toxic against *Cx. quinquefasciatus* with LC 50 of 64.18 $\mu\text{g/mL}$ (24 h) and 59.06 $\mu\text{g/mL}$ (48 h). The pseudo-stem oil of *Z. castaneum* inhibited the growth of *Pseudomonas aeruginosa* (ATCC 25923) with minimum inhibitory concentration (MIC) of 12.5 $\mu\text{g/mL}$, while all other tested samples recorded MIC of 50 $\mu\text{g/mL}$. However, only the pseudo-stem oil of *Z. castaneum* displayed antimicrobial activity against *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112) with MIC of 50 $\mu\text{g/mL}$.

Background

The *Zingiber* species are noted for their economic importance mainly due to volatile and non-volatile constituents and the various biological activities they exhibited. *Zingiber nitens* M.F. Newman is a new species in flora of Vietnam [1]. It is a forming herb 0.5-1.5 m tall with the rhizome being 1 cm in diameter. The leafy shoots composed of about 12 leaves and leaf sheaths are dark brownish green, especially lower ones. The flowers are white at base, pale yellow at apex while the lobes are also pale yellow. There

is no record of the biological activities as well as non-volatile secondary metabolites from this species. Recently, the chemical constituents of essential oils from *Z. nitens* were reported by us [2]. The main constituents of the leaf oil were δ -elemene (17.0 %), β -pinene (12.8 %) and β -elemene (8.8 %) while the stem oil comprised mainly of δ -elemene (20.1 %), germacrene D (8.6 %) and bicyclogermacrene (8.1 %) with β -pinene (21.0 %), δ -elemene (12.8 %) and bornyl acetate (11.8 %) making up the compositions of the root oil [2].

Zingiber castaneum Škorničk. & Q.B. Nguyễn is easily recognized among other terminally flowering species by its upright inflorescence with reflex bracts. The plant is also a rhizomatous herb forming small clumps. The creeping aromatic rhizome which grows up to 1.5 cm in diameter is externally light brown and internally cream white [3]. The translucent light green leaves are glabrous. Flowering starts in July and extends to September. It was found growing in Ninh Bình Province [3]. A recent report [4] identified β -pinene (30.6%), α -pinene (9.5%), β -caryophyllene (9.4%) and bicycloelemene (9.1%) as the main constituents of *Z. castaneum* leaf oil. The compounds occurring in higher quantity in the stem oil were β -caryophyllene (14.7%), δ -cadinene (9.8%), bicycloelemene (8.4%) and α -cubebene (7.8%) while large amount of camphene (15.1%), 1,8-cineole (13.6%), linalool (11.3%) and δ -3-carene (8.5%) were present in the root oil, with (*E*)-nerolidol (23.2%), (*Z*)-9-octadecenamide (17.3%) and β -caryophyllene (10.8%) occurring in of the fruit oil [4].

Till moment, nothing is known about their medicinal uses as well as the biological effects of essential oils from both species. In the present paper, the compounds identified in the essential oils from various parts of both *Z. nitens* and *Z. castaneum* grown in Vietnam were reported. In addition, the larvicidal and antimicrobial efficacies of the essential oils were also described. Previously, the chemical compounds identified in the essential oils of some other *Zingiber* plant grown in Vietnam [5-7], their larvicidal potentials [6-8] and antimicrobial activities [7] were reported by us. Although, terpene compounds were prominent in these essential oils, the identities of the compounds differ from one species to another [2, 4-8].

Vietnam is classified as a hyperendemic dengue country with present throughout the year and dengue fever epidemics have increased in frequency [9]. Mosquitoes have been and continue to be the most deadly creatures on earth. *Aedes albopictus* (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world [10]. Besides it's 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). *Culex quinquefasciatus* Say, commonly known as the southern house mosquito, is a medium-sized brown mosquito that exists throughout the tropics. It is a vector of many pathogens of humans, domestic and wild animals. Viruses transmitted by this species include lymphatic filariasis, West Nile virus, St. Louis encephalitis virus, Western equine encephalitis virus and Zika virus [11]. The yellow fever mosquito, *Aedes aegypti* (Linn), has been a nuisance species for centuries. *Aedes aegypti* is the primary vector of yellow fever, a disease that is prevalent in tropical South America and Africa, and

often emerges in temperate regions during summer months. All four dengue viruses are spread primarily through the bite of an infected *Aedes* species (*Ae. aegypti* and *Ae. albopictus*) mosquito [12].

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. For these reasons, alternative natural insecticides and repellents are now very appreciated by consumers. Essential oils of aromatic plants are considered among the most promising alternative to synthetic chemicals [13]. Essential oils are generally recognized as environmental friendly, easily biodegradable, minimally toxic to mammals and have shown repellent activities against different mosquito species.

As part of our ongoing research aimed at the identification of the chemical constituents, larvicidal and antimicrobial potentials of essential oils from plant (especially *Zingiber* species) grown in Vietnam [2, 4-8], we have obtained essential oils from *Z. nitens* and *Z. castaneum* and hereby report the compounds present therein. In addition, we examined their mosquito larvicidal and antimicrobial activities and present the report for the first time. The authors are aware that there were no previous investigations on the larvicidal and antimicrobial activities of the studied essential oils.

Results And Discussion

Chemical composition of the essential oils

The average yields of the essential oils of *Z. castaneum* were 0.22%, 0.18% and 0.31% (v/w, $\pm$ 0.01, leaf, pseudo-stem and rhizome respectively). The main class of compounds in the leaf oil of *Z. castaneum* were monoterpene hydrocarbons (23.5%) and sesquiterpene hydrocarbons (64.9%). The oil was characterised by abundance of bicyclogermacrene (24.8%), germacrene D (12.9%), *cis*-b-elemene (11.2%), *b*-pinene (10.3%) and d-elemene (6.5%). The contents of bicyclogermacrene, germacrene D and *cis*-b-elemene in the present study are much higher than reported in the previous analysis [4]. High contents of monoterpene hydrocarbons (10.1%), sesquiterpene hydrocarbons (66.2%) and oxygenated sesquiterpenes (16.5%) were present in the pseudo-stem. The major compounds include bicyclogermacrene (15.8%), *cis*-b-elemene (9.8%) and germacrene D (9.2%). There are significant amounts of α -humulene (7.5%), d-elemene (5.4%) and α -Zingiberene (4.6%). Likewise, bicyclogermacrene, germacrene D and *cis*-b-elemene were detected in the present analysed sample in amount higher than reported previously [4]. Zerumbone present in the previous analysed sample was not detected in the present study. The rhizome oil contained monoterpene hydrocarbons (77.4%) and sesquiterpene hydrocarbons (14.4%) with sabinene (22.9%), α -pinene (7.8%), *b*-pinene (6.5%), bornyl acetate (6.1%) and g-terpinene (5.5%) making up the major constituents. This is the first report on the chemical constituents of rhizome oil of *Z. castaneum*.

The essential oils from the leaf and rhizome of *Z. nitens* were obtained in yields of 0.27% and 0.54% (v/w) respectively. From the GC and GC/MS analysis, it was discovered that monoterpene hydrocarbons (59.0%) and sesquiterpene hydrocarbons (36.3%) constitute the bulk of the oil sample (Table 1). The oil

features large percentage of b-pinene (45.8%), a-pinene (10.7%), bicyclogermacrene (7.8%) and a-Zingiberene (6.4%). The main constituent of the leaf oil in the previous analysis [2] such as δ -elemene and β -elemene (8.8 %) were identified in lower quantity in the present study. The dominant classes of compounds in the rhizome oil of *Z. nitens* were mainly monoterpene hydrocarbons (10.2%) and oxygenated monoterpenes (86.5%). Terpinen-4-ol (77.9%) occurred as the compound occurring in higher amount in the rhizome oil. No sesquiterpene compounds could be identified in the oil. All other compounds occurred in much lower quantity. This is the first report on the volatile constituents of the rhizome of *Z. nitens*.

Table 1. Compounds identified in the essential oils of *Z. castaneum* and *Z. nitens*

1. <i>castaneum</i>		<i>Z. nitens</i>						
Sr. No	Compounds	RI (Cal.)	RI (Lit.)	L	PS	Rh	L	Rh
1	Tricyclene	928	921	-	-	0.4	-	-
2	a-Thujene	930	926	-	-	0.5	-	-
3	a-Pinene	939	932	9.6	2.6	7.8	10.7	-
4	a-Fenchene	952	948	-	-	0.9	-	-
5	Camphene	955	952	0.5	1.0	21.2	0.1	-
6	Sabinene	979	972	1.7	1.2	22.9	1.6	1.1
7	b-Pinene	985	978	10.3	3.3	6.5	45.8	0.6
8	Myrcene	992	988	0.3	0.2	2.7	0.5	0.3
9	a-Phellandrene	1011	1009	-	-	0.3	-	-
10	d-3-Carene	1016	1017	-	-	-	0.2	-
11	a-Terpinene	1022	1024	0.1	0.2	3.2	-	1.2
12	o-Cymene	1030	1030	0.2	0.3	1.0	-	1.4
13	Limonene	1034	1032	0.5	0.4	3.3	1.5	-
14	b-Phellandrene	1036	1034	-	-	0.4	0.2	0.2
15	1,8-Cineole	1038	1036	-	-	0.3	0.2	0.3
16	g-Terpinene	1064	1062	0.2	0.4	5.5	0.2	4.6

17	<i>cis</i> -Sabinene hydrate	1074	1073	-	-	-	-	0.5
18	Terpinolene	1095	1094	0.1	0.5	1.8	-	0.8
19	<i>trans</i> -Sabinene hydrate	1106	1110	-	-	-	-	0.6
20	1-Octen-3-yl acetate	1110	1112	-	0.3	-	-	-
21	<i>cis</i> -p-Menth-2-el-1-ol	1130	1130	-	-	-	-	2.0
22	<i>trans</i> -p-Menth-2-el-1-ol	1148	1148	-	-	-	-	1.6
23	Borneol	1178	1177	-	-	0.4	-	-
24	Terpinen-4-ol	1187	1188	0.1	-	4.0	-	77.9
25	α -Terpineol	1200	1200	-	-	-	-	1.9
26	<i>cis</i> -Piperitol	1205	1207	-	-	-	-	0.6
27	<i>trans</i> -Piperitol	1217	1218	-	-	-	-	1.1
28	Fenchyl acetate	1228	1229	0.1	0.3	3.6	-	-
29	2-Decanal	1265	1264	-	0.2	-	-	-
30	Bornyl acetate	1294	1297	0.2	0.5	6.1	-	-
31	Bicycloelemene	1345	1343	-	0.5	-	-	-
32	d-Elemene	1348	1350	6.5	5.4	0.2	0.4	-
33	α -Copaene	1390	1391	0.3	0.4	-	-	-
34	b-Bourbonene	1400	1401	-	-	-	0.2	-
35	<i>cis</i> -b-Elemene	1405	1407	11.2	9.8	0.6	2.8	-
36	<i>cis</i> -Thujopsene	1425	1422	-	-	-	0.1	-
37	b-Caryophyllene	1437	1437	0.4	1.7	0.7	1.2	-
38	g-Elemene	1445	1445	0.4	0.8	-	-	-
39	<i>allo</i> -Aromadendrene	1457	1457	0.1	0.4	-	0.2	-
40	(<i>Z</i>)-b-Farnesene	1461	1465	-	-	0.3	0.5	-

41	α -Humulene	1472	1475	0.8	7.5	0.3	0.3	-
42	9- <i>epi</i> -(<i>E</i>)-Caryophyllene	1479	1480	2.2	2.0	-	1.2	-
43	β -Chamigrene	1490	1489	-	0.6	-	-	-
44	Valencene	1491	1491	0.4	-	-	0.5	-
45	α -Curcumene	1493	1494	0.4	1.6	-	1.4	-
46	Germacrene D	1499	1500	12.9	9.2	0.4	4.7	-
47	Aristolochene	1502	1502	-	-	-	1.8	-
48	α -Zingiberene	1505	1506	1.1	4.6	0.5	6.4	-
49	γ -Amorphene	1510	1508	-	-	-	0.3	-
50	(<i>E,E</i>)- α -Farnesene	1513	1511	-	-	-	1.8	-
51	Bicyclogermacrene	1516	1517	24.8	15.8	0.6	7.0	-
52	β -Bisabolene	1518	1520	0.2	1.3	0.1	1.3	-
53	γ -Cadinene	1531	1530	0.3	0.3	-	0.2	-
54	β -Sesqui[hellandrene	1536	1535	0.2	1.1	-	2.6	-
55	7- <i>epi</i> - α -Selinene	1537	1537	-	-	0.2	-	-
56	δ -Cadinene	1538	1540	1.2	1.3	-	0.6	-
57	Elemol	1565	1563	-	0.2	-	-	-
58	(<i>E</i>)-Nerolidol	1571	1571	0.2	0.5	-	0.2	-
59	Germacrene B	1578	1580	1.3	1.6	-	-	-
60	Germacrene-D-4-ol	1595	1594	2.4	1.8	-	0.2	-
61	Spathulenol	1599	1600	1.2	2.0	-	0.7	-
62	Caryophyllene oxide	1605	1606	-	0.6	0.3	0.4	-
63	Viridiflorol	1606	1608	0.2	-	-	0.1	-
64	Guaiol	1615	1618	-	0.4	-	-	-

65	Zingiberenol	1624	1626	-	1.0	-	0.3	-
66	Ledol	1626	1628	0.3	-	-	-	-
67	Humulene epoxide II	1632	1632	-	0.6	-	-	-
68	α -Acorenol	1644	1644	-	0.3	-	0.1	-
69	Alismol	1648	1650	1.9	-	0.2	-	-
70	1- <i>epi</i> -Cubenol	1649	1652	-	3.2	-	-	-
71	Isospathulenol	1658	1660	-	-	-	0.1	-
72	<i>epi</i> - α -Cadinol	1660	1662	0.4	-	-	0.1	-
73	<i>epi</i> - α -Muurolol	1662	1664	0.4	1.1	-	0.2	-
74	α -Cadinol	1675	1676	0.8	1.6	0.2	0.5	-
75	α -Turmerone	1682	1680	0.4	2.9	1.5	0.5	-
76	Curlone	1716	1720	-	1.0	0.4	-	-
77	Phytol	2120	2119	-	-	-	0.1	-
Total							96.8	94.1
98.3	98.9	96.7	Monoterpene hydrocarbons (Sr. No. 1-14,16,18)			23.5	10.1	77.4
59.0	10.2	Oxygenated monoterpenes (Sr. No. 15,17,19,21-28,30)			0.4	0.8	14.4	
0.2	86.5	Sesquiterpene hydrocarbons (Sr. No. 31-56,59)			64.9	66.2	3.9	
36.3	-	Oxygenated sesquiterpenes (Sr. No. 57,58,60-76)			8.0	16.5	2.6	
3.3	-	Diterpenes (Sr. No. 77)				-	-	
-	0.1	-	Non-terpenes (Sr. No. 20, 29)			-	0.5	-
-	-							

^a Elution order on HP-5MS column; RI (Cal.) Retention indices on HP-5MS column; RI (Lit.) Literature retention indices; Sr. No, Serial Number; - Not identified; L Leaf, PS Pseudo-stem, Rh Rhizome

Mortality of the essential oils against vector mosquitoes

The rhizome oil of *Z. castaneum* exhibited potent mortality (100%) against *Ae. albopictus* at concentration of 100 μ g/mL under the test period of 24 h and 48 h (Table 2). However, this maximum

toxicity could only be observed at concentration of 200 µg/mL against *Ae. aegypti* at the same period. On the other hand, the essential oil was less toxic towards *Cx. quinquefasciatus* achieving mortality of 81.3% at concentration of 100 µg/mL over the same period. The rate of susceptibility of the vectors towards the rhizome oil of *Z. castaneum* was *Ae. albopictus* > *Ae. aegypti* > *Cx. quinquefasciatus*. The leaf oil of *Z. castaneum* also exhibited 100% mortality against *Ae. aegypti* (concentration, 100 µg/mL; 24 h and 48 h) and *Cx. quinquefasciatus* (concentration, 150 µg/mL; 24 h and 48 h). The leaf oil of *Z. nitens* displayed mortality of 100% against *Ae. aegypti* at concentrations of 50 µg/mL at both 24 h and 48 h test period (Table 3). On the other hand, the rhizome oil achieved the same mortality rate only at concentration of 100 µg/mL over the same test period. However, the rhizome oil only exhibited mortality of 92.5% against *Cx. quinquefasciatus* at concentration of 100 µg/mL in the same period. The leaf oil was more toxic towards *Ae. aegypti* than the rhizome oil. There was no mortality in the EtOH used as control for all the tested oil samples. The percentage mortality was dependent on the concentration of the tested oil samples. Thus, higher inhibition of mosquito larvae was observed as concentration increases.

Table 2: Mortality and larvicidal action of *Z. castaneum* rhizome oil

	Concentration (µg/mL)					
	12.5	25	50	100	150	200
<i>Ae. Aegypti</i>						
Leaf						
24 h	5.0 ±.816	15.0 ±.000	53.75 ± 3.304	100.0 ±.000	n.d	n.d
48 h	10.0 ±.816	22.5 ± 1.291	62.5 ± 2.517	100.0 ±.000	n.d	n.d
Rhizome						
24 h	0	0	0	10.0 ±2.708	75.0 ±2.582	100.0±.000
48 h	0	0	13.7 ±1.708	15.0 ±2. 160	64.0 ±2.944	100.0 ±.000
<i>Ae. Albopictus</i>						
Rhizome						
24 h	0	5.0 ±1.258	40.0 ±4.243	100.0 ±.000	n.d	n.d
48 h	0	15.0 ±3.367	48.7 ±1.104	100.0 ±.000	n.d	n.d
<i>Cx. quinquefasciatus</i>						

Leaf						
24 h	0	0	13.75 ±0.000	57.0 ±3.916	100.0 ±0.000	n.d
48 h	0	10.0 ±0.000	42.5 ± 2.646	84.3 ± 1.258	100.0 ±0.000	n.d
Rhizome						
24 h	0	0	3.70 ±.500	55.0 ±3.916	n.d	n.d
48 h	0	10.0 ±1.000	42.5 ±2.6446	81.3 ±1.258	n.d	n.d

Minimum lethal concentration (µg/mL)

	LC ₅₀	LC ₉₀	Regression equation	χ^2	<i>P</i>
<i>Ae. Aegypti</i>					
Leaf					
24 h	39.30	89.94	y = -5.683 + 3.564x	8.472	0.001
48 h	31.78	80.37	y = -4.778 + 3.181x	9.943	0.001
Rhizome					
24 h	121.43	145.28	y = -6.525 +0.054x	9.512	0.001
48 h	110.31	125.33	y = -9.445 +0.086x	2.497	0.001
<i>Ae. Albopictus</i>					
Rhizome					
24 h	49.85	43.93	y = -2.921 +0.059x	6.468	0.001
48 h	71.71	68.12	y = -2.327 +0.053x	7.571	0.001
<i>Cx. quinquefasciatus</i>					
Leaf					
24 h	84.97	141.45	y = -11.172 + 5.791x	7.458	0.001
48 h	47.40	92.29	y = -7.423 +4.429x	6.914	0.001
Rhizome					

24 h	88.86	117.68	$y = -3.952 + 0.044x$	8.502	0.001
48 h	48.08	72.13	$y = -2.562 + 0.053x$	6.871	0.001

^a n =4; ^bno mortality in the EtOH used as control; n.d, not determined

Larvicidal tests

From Table 2, the *Z. castaneum* rhizome oil exhibited larvicidal action towards *Ae. albopictus* with LC50 values of 49.85 mg/mL and LC90 71.71 mg/mL at 24 h while LC50 values of 43.93 mg/mL and LC90 of 68.12 mg/mL were obtained at 48 h. Moreover, the oil sample also showed potential larvicides towards *Cx. quinquefasciatus* with LC50 values of 88.86 mg/mL and LC90 of 117.68 mg/mL at 24 h while LC50 values of 48.08 mg/mL and LC90 of 72.13 mg/mL were obtained at 48 h. In addition, larvicidal activity was also recorded against *Ae. aegypti* having LC50 and LC90 values of 121.43 mg/mL and 145.28 mg/mL (24 h) as well as 110.31 mg/mL and 125.33 mg/mL (48 h). In addition, the leaf oil displayed significant larvicidal activity against *Ae. aegypti* with LC50 of 39.30 µg/mL (24 h) and 31.78 µg/mL (48 h) while LC50 values of 84.97 µg/mL (24 h) and 47.40 µg/mL (48 h) were recorded respectively against *Cx. quinquefasciatus*.

As could be seen in Table 3, the leaf oil of *Z. nitens* displayed larvicidal activity against *Ae. aegypti* more than the rhizome oil at both 24 h and 48 h. The oil recorded LC50 values of 17.58 mg/mL and LC90 23.25 mg/mL at 24 h while LC50 values of 15.12 mg/mL and LC90 of 18.70 mg/mL were established at 48 h. In the same vein, LC50 values of 29.60 mg/mL and LC90 of 37.60 mg/mL at 24 h as well as LC50 values of 26.32 mg/mL and LC90 of 36.92 mg/mL at 48 h were displayed by the rhizome oil towards *Ae. aegypti*. Only the rhizome oil of *Z. nitens* exhibited larvicidal action towards *Cx. quinquefasciatus* with LC50 values of 64.18 mg/mL and LC90 of 92.68 mg/mL at 24 h with LC50 values of 59.06 mg/mL and LC90 of 84.31 mg/mL at 48 h. Permethrin, the standard drug used as control displayed larvicidal activity at much lower values. These findings showed that the concentrations of test substances affected degree of toxicity and mortality rates.

Table 3: Mortality and larvicidal action of *Z. nitens* leaf and rhizome oil

Mortality (%)^a

Concentration (µg/mL)

12.5 25 50 100

Ae. Aegypti

Leaf

24 h	12.5 ±.816	76.3 ±3.862	100.0 ±.000	100.0 ±.000
48 h	15.0 ±.957	82.5 ±3.317	100.0 ±.000	100.0 ±.000

Rhizome

24 h	0	17.5 ±1.291	83.7 ± 2.872	100.0 ±.000
48 h	5.0 ±.816	35.0 ±2.651	90.0 ± 2.309	100.0 ±.000

Cx. quinquefasciatus

Rhizome

24 h	0	6.3 ±.500	15.0 ±.000	92.5 ±1.291
48 h	0	6.3 ±.500	15.0 ±.000	92.5 ±1.291

Minimum lethal concentration (µg/mL)

	LC ₅₀	LC ₉₀	Regression equation	χ^2	<i>P</i>
<i>Ae. Aegypti</i>					
Leaf					
24 h	17.58	23.25	y = -3.979 + 0.226x	9.343	0.001
48 h	15.12	18.70	y = -5.407 + 0.358x	2.095	0.036
Rhizome					
24 h	29.60	37.60	y = -5.688 +0.192x	2.012	0.044
48 h	26.32	36.92	y = -2.990 +0.593x	5.938	0.001
<i>Cx. quinquefasciatus</i>					
Rhizome					
24 h	64.18	92.68	y = -2.887 +0.045x	5.363	0.001

48 h	59.06	84.31	$y = -2.998 + 0.051x$	5.963	0.001
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^a n= 4; ^bno mortality in the EtOH used as control

Overall results in this study showed that essential oils hydrodistilled from the leaf and rhizome of *Z. castaneum* and *Z. nitens* exhibited good mortality and larvicidal activity on *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae. The observed larvicidal action of *Z. castaneum* and *Z. nitens* in this study was comparable with findings from *Zingiber* plants analysed for their larvicidal activity from Vietnam and other parts of the world. The essential oil of *Z. collinsii* from Vietnam displayed larvicidal action against *Ae. albopictus* (LC₅₀ = 25.51 µg/mL; LC₉₀ = 40.22 µg/mL) and *Cx. quinquefasciatus* (LC₅₀ = 50.11 µg/mL and LC₉₀ = 71.53 µg/mL) after 24 h [6]. *Z. zerumbet* oil showed potent larvicidal activity against *Cx. quinquefasciatus* with LC₅₀ of 33.28 mg/mL and 21.81 mg/mL respectively after 24 h and 48 h test period. Moreover, the oil exhibited significant larvicidal action against *Ae. albopictus* within the 24 h and 48 h tested period having LC₅₀ of 55.75 µg/mL and 36.22 µg/mL respectively [7]. The 24 h mosquito larvicidal activity of the rhizome oil of *Z. montanum* from Vietnam [8] was *Ae. albopictus* (LC₅₀ = 35.17 µg/mL; LC₉₀ = 56.02 µg/mL), *Ae. aegypti* (LC₅₀ = 32.20 µg/mL; LC₉₀ = 45.64 µg/mL) and *Cx. quinquefasciatus* (LC₅₀ = 31.12 µg/mL; LC₉₀ = 52.25 µg/mL). The essential oil of *Z. zerumbet* from Malaysia displayed lower larvicidal action against *Ae. aegypti* with LC₅₀ of 102.6 mg/mL [14] while the rhizome oil from Thailand also showed larvicidal action against *Ae. aegypti* with LC₅₀ and LC₉₀ values of 48.92 and 62.2 mg/mL respectively [15]. Likewise, *Z. cernuum* was toxic towards *Ae. aegypti* (LC₅₀ = 44.88 µg/mL), *Ae. albopictus* (LC₅₀ = 55.84 µg/mL) and *Cx. quinquefasciatus* (LC₅₀ = 48.44 µg/mL) after 24 h [16]. *Zingiber officinale* was shown to have larvicidal activity against *Cx. quinquefasciatus* with a LC₅₀ value of 50.78 ppm [17]. The essential oils from the rhizome of *Z. nimmonii* demonstrated significant larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*, with LC₅₀ values of 37.6 and 48.1 µg/mL, respectively. [18]. Moreover, essential oils from *Zingiber* plants have also demonstrated potential insecticidal and larvicidal activities against other insect pests. For example, *Z. officinale* demonstrated action against *Cx. tritaeniorhynchus* and *Anopheles subpictus* with the LC₅₀ and LC₉₀ values as 98.83, 57.98 ppm and 186.55, 104.23 ppm, respectively [19].

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 products [21-22]. For example, considered products showing LC₅₀ ≤ 50 mg/L to be active, 50 mg/L < LC₅₀ ≤ 100 mg/L to be moderately active, 100 mg/L < LC₅₀ ≤ 750 mg/L to be effective, and LC₅₀ > 750 mg/L to be inactive [21]. Likewise, considered compounds with LC₅₀ < 100 mg/L to exhibit a significant larvicidal effect [22]. 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 oils of *Z. castaneum* and *Z. nitens*

had promising effects, according to the criterion established previously [21, 22]. In summary, *Z. castaneum* and *Z. nitens* essential oils from Vietnam revealed important toxicity and larvicidal properties on *Ae. Albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae and stands as a promising tool to manage the phenomenon of insecticides resistant vectors in malaria endemic regions.

The variations in toxicity of essential oils against different species of mosquitoes are common, due to qualitative and quantitative variations of chemical constituents. Interestingly, the active larvicidal compounds in these works, including, α -pinene, β -pinene, sabinene, limonene, p-cymene, 1,8-cineole, terpinen-4-ol, β -caryophyllene, bicyclogermacrene and germacrene [23-25]. The isolation and purification of active compound which could be responsible for the larvicidal activity against mosquito vectors of would be an important step in the development of novel mosquitocidal agents. Production of larvicides from the locally available plants, could be a new acceptable alternative to employ which may lead to decreasing dependence on imported synthetic insecticides and be beneficial for developing countries such as Vietnam.

Antimicrobial test

The essential oil from pseudo-stem of *Z. castaneum* showed stronger inhibitory effect on *P. aeruginosa* with MIC of 12.5 μ g/mL and MIC value of 50 μ g/mL against *A. niger* and *F. oxysporum*. In addition, all the other tested oil samples inhibited the growth of *P. aeruginosa* with MIC value of 50 μ g/mL (Table 4). In this test experiment, activity was presumed to occur with MIC $\leq$ 50 μ g/mL while MIC > 50 μ g/mL is considered inactive towards the tested microorganism. No previous information exists on the antimicrobial activity of essential oils from *Z. castaneum* and *Z. nitens*. The present data represent the first report on the antimicrobial action of the studied essential oils.

Table 4: Antimicrobial activity of *Z. castaneum* and *Z. nitens* essential oils

Organisms	Minimum inhibitory concentration (MIC: mg/mL)				
	<i>Z. castaneum</i> L	<i>Z. castaneum</i> PS	<i>Z. castaneum</i> R	<i>Z. nitens</i> L	<i>Z. nitens</i> L
1. <i>coli</i>	>50	>50	>50	>50	>50
2. <i>eruginosa</i>	50 12.5	50	50	50	
3. <i>subtillis</i>	>50 >50	>50	>50	>50	
4. <i>aureus</i>	>50 >50	>50	>50	>50	
5. <i>niger</i>	>50 50	>50	>50	>50	
6. <i>oxysporum</i>	>50 50	>50	>50	>50	
7. <i>cerevisiae</i>	>50 >50	>50	>50	>50	

8. *albicans* >50 >50 >50 >50

L, Leaf; PS Pseudo-stem; R Rhizome; ≤50 Active; >50 No activity

The observed antimicrobial results of *Z. castaneum* and *Z. nitens* oils were in agreement with information that *Zingiber* oil samples possess antimicrobial action. The antimicrobial activity of essential oils of some *Zingiber* species was reported. The essential oil of *Z. zerumbet* was shown to inhibit the growth of *A. niger* [26] as well as *C. albicans*, *S. aureus*, *Salmonella typhi*, several species of *Trichophyton*, *Streptococcus mutans* [27]. *Z. officinale* and *Z. zerumbet* essential oils were considered potential therapeutic agents against bacterial several infections [28]. Essential oil of *Z. officinale* was efficient against three positive strains of bacteria (*S. aureus*, *B. cereus* and *L. monocytogenes*), with a minimum concentration to inhibit *B. cereus* and *L. monocytogenes* of 6.25 mg/mL [29]. The observed antimicrobial activity of *Z. castaneum* and *Z. nitens* essential oils can be related to the compounds present in it. For example, essential oil constituents such as α-pinene, β-pinene, sabinene, 1,8-cineole, terpinen-4-ol, β-caryophyllene, bicyclogermacrene and germacrene were previously reported to inhibit significantly the growth and cell viability of potential infectious of broad spectrum microorganisms [7, 14].

Materials And Methods

Plants collection

All samples of *Z. castaneum* and *Z. nitens* were collected from Pu Hoat Nature Reserve, Nghệ An Province, Vietnam, in August 2018. Botanical identification was accomplished at Botany Museum, Nghệ An College of Economics, Vietnam, where voucher specimens, LTH741 and LTH 750 respectively, were deposited for future references.

Preparation of samples

In the course of preparation for hydrodistillation process, the leaf, pseudo-stem and rhizome were air-dried (22°C) under laboratory shade for two weeks to reduce the moisture contents. Moreover, unwanted materials were also removed by handpicking. Afterwards, samples were pulverized to coarse powder using a locally made grinder.

Hydrodistillation procedure

A total of 1000 g of each of the pulverized samples were used for the experiment at different time. Known weight of samples was separately and carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Essential oils were obtained by hydrodistillation which was carried out in an all glass Clevenger-type distillation unit designed according to Vietnamese Pharmacopoeia [30] as described previously [2,4-8]. The distillation time was 3 h and conducted at normal pressure. The volatile

oils distilled over water and were collected by running through the tap in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4°C) until the moment of analyses as described previously [2,4-8].

Gas chromatography (GC) analysis

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 mm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature at 250°C, detector temperature 260°C, column temperature programmed from 40°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 of diluted oil in hexane (1: 10) injected was 1.0 mL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were determined on normalized percentages.

Gas chromatography-Mass spectrometry (GC/MS) experiment

An Agilent Technologies HP 6890N Plus Chromatograph fitted with capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 mm) and interfaced with a mass spectrometer HP 5973 MSD was used for this experiment, under the same conditions as those used for gas chromatography analysis as described previously [2,4-7]. The GC conditions were the same as described above with He (1 mL/min) as carrier gas. The MS 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.

Identification of the components of the oils

The identification of constituents from the GC/MS spectra of *Z.nitens* and *Z. castaneum* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C₄-C₄₀), under identical experimental conditions. In some cases, co-injection with known compounds or standards (Sigma-Aldrich, St. Louis, MO, USA) under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition [31] and with those in the literature as described previously [2, 4-8].

Larvicidal activity

Mosquito larvae

Adults of *Culex quinquefasciatus*, *Aedes aegypti* 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 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. nitens* and *Z. castaneum* was evaluated according to WHO protocol [32] with slight modifications. For the assay, aliquots of the essential oils from both samples 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 $25 \pm 2^\circ\text{C}$. The larvicidal test was conducted with four replicates under five concentrations (200, 100, 50, 25 and 12.5 $\mu\text{g/mL}$).

The mortality rate was calculated according to the formula

$$\text{Mc} = (\text{Mo}) / (\text{Mt}) \times 100$$

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 [33] to obtain LC_{50} values, LC_{90} values, 95% confidence limits, and chi square values using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

Antimicrobial activity assay

Microbes

Eight standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the oil samples. The Gram negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram positive strains were *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (ATCC 9763) and *Fusarium oxysporum* (ATCC 48112). Two strains of yeast, *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 16404) were also used for the experiment. Testing media included Mueller-Hinton Agar (MHA) used for bacteria and Sabouraud Agar (SA) used for fungi.

Microdilution broth susceptibility assay

The Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay [34, 35]. For the assays, the essential oil was diluted with DMSO and loaded into the microtiter plate with each of the microbial strains. The plate was then incubated overnight at 37°C . One hundred microlitre of microbial culture of an approximate inoculum size of 1.0×10^6 CFU/mL was added to all well and incubated at 37°C for 24 h. The last row, containing only the serial dilutions of

sample without microorganisms, was used as a negative control. Sterile distilled water and DMSO served as a positive control. The MIC values were determined as the lowest concentration of the test sample that completely inhibits the growth of microorganisms.

Conclusion

Assessment of larvicidal efficacy demonstrated that the rhizome oils of *Z. castaneum* and *Z. nitens*, and the leaf oil of *Z. nitens* were toxic against susceptible and resistant *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae at reasonable LC₅₀, and LC₉₀ levels. Moreover, the pseudo-stem of *Z. castaneum* inhibited the growth of *P. aeruginosa*, *A. niger* and *F. oxysporum* with MIC comparable to other results. In conclusion, we have documented the promising larvicidal and antimicrobial potentials of essential oils from *Z. castaneum* and *Z. nitens* from Vietnam, which could be considered as a potentially alternative source for developing novel formulation for controlling diseases.

Declarations

Authors' contribution

LTH and IAO made a significant contribution to Conceptualization, LTH, TTH, NTB and NTV contributed towards experimental work, formal analysis and data curation. LTH and IAO contributed to interpretation of data and draft of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All the main experimental and characterization data have been presented in the form of tables.

Consent for publication

We the all authors consent to publication.

Ethics approval and consent to participate

Not applicable

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