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 µg/mL; 24 h and 48 h) and Ae. albopictus (concentration, 100 µg/mL; 24 h and 48 h). However, mortality of 81.3% was observed against Cx. quinquefasciatus at 48 h (concentration 100 µg/mL). The leaf 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 rhizome oil displayed a minimum lethal concentration LC 50 of 121.43 µg/mL and 110.31 µg/mL against Ae. aegypti respectively at 24 h and 48 h while values of 49.85 µg/mL and 43.93 µg/mL at 24 h and 48 h were observed against Ae. albopictus respectively. In addition, LC 50 values of 88.86 µg/mL and 48.08 µg/mL were recorded respectively against Cx. quinquefasciatus . Also, the leaf oil displayed significant larvicidal activity against Ae. aegypti with LC 50 of 39.30 µg/mL (24 h) and 31.78 µg/mL (48 h) while LC 50 values of 84.97 µg/mL (24 h) and 47.40 µg/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 µg/mL) while the rhizome oil displayed maximum mortality at concentration of 100 µg/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 µg/mL (24 h) and 15.12 µg/mL (48 h) were displayed by the leaf oil against Ae. aegypti while values of 29.60 µg/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 of 64.18 µg/mL (24 h) and 59.06 µg/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 µg/mL, while all other tested samples recorded MIC of 50 µg/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 µg/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, ± 0.01, leaf, pseudo-stem and rhizome respectively. The main class of compounds in the leaf oil of *Z. castaneum* were monoterpenic hydrocarbons (23.5%) and sesquiterpenic 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 monoterpenic hydrocarbons (10.1%), sesquiterpenic 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 *a*-humulene (7.5%), *d*-elemene (5.4%) and *a*-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 monoterpenic hydrocarbons (77.4%) and sesquiterpenic hydrocarbons (14.4%) with sabinene (22.9%), *a*-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 monoterpane hydrocarbons (59.0%) and sesquiterpane 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 monoterpane 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 sesquiterpane 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***

| 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|
| 4  | a-Fenchene     | 952       | 948       | -  | -  | 0.9|
| 5  | Camphene       | 955       | 952       | 0.5| 1.0| 21.2|
| 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  | cis-Sabinene hydrate | 1074 | 1073 | -   | -   | -   |
|     | 0.5  |
| 18  | Terpinolene | 1095 | 1094 | 0.1 | 0.5 | 1.8 |
|     | 0.8  |
| 19  | trans-Sabinene hydrate | 1106 | 1110 | -   | -   | -   |
|     | 0.6  |
|   | Compound                          | 1 | 2   | 3   | 4   |
|---|-----------------------------------|---|-----|-----|-----|
| 20| 1-Octen-3-yl acetate              | 1110| 1112 | -   | 0.3 |
|   |                                    |   |     |     |     |
| 21| cis-p-Menth-2-el-1-ol              | 1130| 1130 | -   | -   |
|   |                                    |   |     |     |     |
| 22| trans-p-Menth-2-el-1-ol            | 1148| 1148 | -   | -   |
|   |                                    |   |     |     |     |
| 23| Borneol                           | 1178| 1177 | -   | 0.4 |
|   |                                    |   |     |     |     |
| 24| Terpinen-4-ol                     | 1187| 1188 | 0.1 | 4.0 |
|   |                                    |   |     |     | 77.9|
| 25| a-Terpineol                       | 1200| 1200 | -   | -   |
|   |                                    |   |     |     | 1.9 |
| 26| cis-Piperitol                      | 1205| 1207 | -   | -   |
|   |                                    |   |     |     | 0.6 |
| 27| trans-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 |
| 33| a-Copaene                          | 1390| 1391 | 0.3 | 0.4 |
|   |                                    |   |     |     |     |

|   |   |   |   |   |   |
|---|---|--|--|--|
| 2  | 0.4|   |   |   |
| 3  | 0.3| 0.4|   |   |
|   | Compound           |   |   |   |   |   |   |
|---|-------------------|---|---|---|---|---|---|
| 34| b-Bourbonene      | 1400 | 1401 | - | - | - |
| 35| cis-b-Elemene     | 1405 | 1407 | 11.2 | 9.8 | 0.6 |
| 36| cis-Thujopsene    | 1425 | 1422 | - | - | - |
| 37| b-Caryophyllene   | 1437 | 1437 | 0.4 | 1.7 | 0.7 |
| 38| g-Elemene         | 1445 | 1445 | 0.4 | 0.8 | - |
| 39| allo-Aromadendrene| 1457 | 1457 | 0.1 | 0.4 | - |
| 40| (Z)-b-Farnesene   | 1461 | 1465 | - | - | 0.3 |
| 41| a-Humulene        | 1472 | 1475 | 0.8 | 7.5 | 0.3 |
| 42| 9-epi-(E)-Caryophyllene| 1479 | 1480 | 2.2 | 2.0 | - |
| 43| b-Chamigrene      | 1490 | 1489 | - | 0.6 | - |
| 44| Valencene         | 1491 | 1491 | 0.4 | - | - |
| 45| ar-Curcumene      | 1493 | 1494 | 0.4 | 1.6 | - | 1.4 |
| 46| Germacrene D      | 1499 | 1500 | 12.9 | 9.2 | 0.4 |
| 47| Aristolochene     | 1502 | 1502 | - | - | - |
|   |    |                  |   |   |   |
|---|---|-----------------|---|---|---|
| 1.8 | - | a-Zingiberene   | 1505 | 1506 | 1.1 | 4.6 | 0.5 |
| 6.4 | - | g-Amorphene     | 1510 | 1508 | - | - | - |
| 0.3 | - | (E,E)-a-Farnesene | 1513 | 1511 | - | - | - |
| 1.8 | - | Bicyclogermacrene | 1516 | 1517 | 24.8 | 15.8 | 0.6 |
| 7.0 | - | b-Bisabolene     | 1518 | 1520 | 0.2 | 1.3 | 0.1 |
| 5.2 | - | g-Cadinene       | 1531 | 1530 | 0.3 | 0.3 | - |
| 0.2 | - | b-Sesquihellandrene | 1536 | 1535 | 0.2 | 1.1 | - |
| 2.6 | - | 7-epi-a-Selinene | 1537 | 1537 | - | - | 0.2 |
| 5.6 | - | d-Cadinene       | 1538 | 1540 | 1.2 | 1.3 | - |
| 0.6 | - | Elemol           | 1565 | 1563 | - | 0.2 | - |
| 5.8 | - | (E)-Nerolidol    | 1571 | 1571 | 0.2 | 0.5 | - |
| 0.2 | - | Germacrene B     | 1578 | 1580 | 1.3 | 1.6 | - |
| 0.2 | - | Germacrene-D-4-ol | 1595 | 1594 | 2.4 | 1.8 | - |
|   | Compound                | m/z | m/z | Retention Time | RRT | Area |
|---|-------------------------|-----|-----|----------------|-----|------|
| 61| Spathulenol             | 1599| 1600| 1.2           | 2.0 | -    |
| 62| Caryophyllene oxide     | 1605| 1606| -             | 0.6 | 0.3  |
| 63| Viridiflorol            | 1606| 1608| 0.2           | -   | -    |
| 64| Guaiol                  | 1615| 1618| -             | 0.4 | -    |
| 65| Zingiberenol            | 1624| 1626| -             | 1.0 | -    |
| 66| Ledol                   | 1626| 1628| 0.3           | -   | -    |
| 67| Humulene epoxide II     | 1632| 1632| -             | 0.6 | -    |
| 68| a-Acorenol              | 1644| 1644| -             | 0.3 | -    |
| 69| Alismol                 | 1648| 1650| 1.9           | -   | 0.2  |
| 70| 1-epi-Cubenol           | 1649| 1652| -             | 3.2 | -    |
| 71| Isospathulenol          | 1658| 1660| -             | -   | -    |
| 72| epi-a-Cadinol           | 1660| 1662| 0.4           | -   | -    |
| 73| epi-a-Muurolol          | 1662| 1664| 0.4           | 1.1 | -    |
| 74| a-Cadinol               | 1675| 1676| 0.8           | 1.6 | 0.2  |
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 µg/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

| Mortality (%) a | Concentration (µg/mL) |
|-----------------|-----------------------|
| 12.5 | 25 | 50 | 100 | 150 |
| 200 |

Ae. Aegypti

Leaf

| 24 h | 48 h |
|------|------|
| 5.0 ± .816 | 10.0 ± .816 |
| 15.0 ± .000 | 22.5 ± 1.291 |
| 53.75 ± 3.304 | 62.5 ± 2.517 |
| 100.0 ± .000 | 100.0 ± .000 |

n.d

Rhizome

| 24 h | 48 h |
|------|------|
| 0 | 15.0 ± 3.367 |
| 0 | 48.7 ± 1.104 |
| 10.0 ± 2.708 | 100.0 ± .000 |
| 75.0 ± 2.582 |
| 13.7 ± 1.708 | 64.0 ± 2.944 |
| 100.0 ± .000 |

Ae. Albopictus

Rhizome

| 24 h | 48 h |
|------|------|
| 0 | 0 |
| 5.0 ± 1.258 | 15.0 ± 1.104 |
| 40.0 ± 4.243 | 100.0 ± .000 |
| n.d | n.d |
| 100.0 ± .000 | 100.0 ± .000 |
**Cx. quinquefasciatus**

Leaf

| Time  | Eggs | Larvae | Adults | LC\textsubscript{50} | LC\textsubscript{90} | Regression equation |
|-------|------|--------|--------|----------------|-----------------|---------------------|
| 24 h  | 0    | 0      | 13.75 ± .000 | 57.0 ± 3.916  | 100.0 ± .000   |                      |
|       | n.d  |        |         |                |                 |                     |
| 48 h  | 0    | 10.0 ± .000 | 42.5 ± 2.646 | 84.3 ± 1.258  | 100.0 ± .000   |                      |
|       | n.d  |        |         |                |                 |                     |

Rhizome

| Time  | Eggs | Larvae | Adults | LC\textsubscript{50} | LC\textsubscript{90} | Regression equation |
|-------|------|--------|--------|----------------|-----------------|---------------------|
| 24 h  | 0    | 0      | 3.70 ± .500  | 55.0 ± 3.916  | n.d             |                     |
|       | n.d  |        |         |                |                 |                     |
| 48 h  | 0    | 10.0 ± 1.000 | 42.5 ± 2.646 | 81.3 ± 1.258  | n.d             |                     |
|       | n.d  |        |         |                |                 |                     |

**Minimum lethal concentration (µg/mL)**

|          | LC\textsubscript{50} | LC\textsubscript{90} | Regression equation |
|----------|-----------------|-----------------|---------------------|
|          | X\textsuperscript{2} | P               |                     |

**Ae. Aegypti**

Leaf

| Time  | Eggs | Larvae | Adults | LC\textsubscript{50} | LC\textsubscript{90} | Regression equation |
|-------|------|--------|--------|----------------|-----------------|---------------------|
| 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

| Time  | Eggs | Larvae | Adults | LC\textsubscript{50} | LC\textsubscript{90} | Regression equation |
|-------|------|--------|--------|----------------|-----------------|---------------------|
| 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 |      |        |         |                |                 |                     |
Ae. Albopictus

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

Cx. quinquefasciatus

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

\(^a n = 4; ^b\)no 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 (%) | 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         |
**Minimum lethal concentration (µg/mL)**

|          | Cx. quinquefasciatus | Ae. Aegypti | Rhizome |
|----------|----------------------|-------------|---------|
|          |                      | Leaf        |         |
|          |                      | Rhizome     |         |
|          | LC₅₀                 | LC₉₀        | Regression equation |
|          | X²                   | P           |         |
| 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 |
| 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                |             |         |         |
| 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                |             |         |         |
| 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                |             |         |         |
Overall results in this study showed that essential oils hydrodisitlled 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* (LC\textsubscript{50} = 25.51 µg/mL; LC\textsubscript{90} = 40.22 µg/mL) and *Cx. quinquefasciatus* (LC\textsubscript{50} = 50.11 µg/mL and LC\textsubscript{90} = 71.53 µg/mL) after 24 h [6]. *Z. zerumbet* oil showed potent larvicidal activity against *Cx. quinquefasciatus* with LC\textsubscript{50} 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 LC\textsubscript{50} 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* (LC\textsubscript{50} = 35.17 µg/mL; LC\textsubscript{90} = 56.02 µg/mL), *Ae. aegypti* (LC\textsubscript{50} = 32.20 µg/mL; LC\textsubscript{90} = 45.64 µg/mL) and *Cx. quinquefasciatus* (LC\textsubscript{50} = 31.12 µg/mL; LC\textsubscript{90} = 52.25 µg/mL). The essential oil of *Z. zerumbet* from Malaysia displayed lower larvicidal action against *Ae. aegypti* with LC\textsubscript{50} of 102.6 mg/mL [14] while the rhizome oil from Thailand also showed larvicidal action against *Ae. aegypti* with LC\textsubscript{50} and LC\textsubscript{90} values of 48.92 and 62.2 mg/mL respectively [15]. Likewise, *Z. cernuum* was toxic towards *Ae. aegypti* (LC\textsubscript{50} = 44.88 µg/mL), *Ae. albopictus* (LC\textsubscript{50} = 55.84 µg/mL) and *Cx. quinquefasciatus* (LC\textsubscript{50} = 48.44 µg/mL) after 24 h [16]. *Zingiber officinale* was shown to have larvicidal activity against *Cx. quinquefasciatus* with a LC\textsubscript{50} 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 LC\textsubscript{50} 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 LC$_{50}$ and LC$_{90}$ 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 LC$_{50}$ ≤ 50 mg/L to be active, 50 mg/L < LC$_{50}$ ≤ 100 mg/L to be moderately active, 100 mg/L < LC$_{50}$ ≤ 750 mg/L to be effective, and LC$_{50}$ > 750 mg/L to be inactive [21]. Likewise, considered compounds with LC$_{50}$ < 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 LC$_{50}$ 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 μg/mL and MIC value of 50 μg/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 μg/mL (Table 4). In this test experiment, activity was presumed to occur with MIC £ 50 μg/mL while MIC > 50 μg/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     | Z. castaneum L | Z. castaneum PS | Z. castaneum R | Z. nitens L | Z. nitens L |
|---------------|----------------|-----------------|----------------|-------------|-------------|
| 1. coli       | >50            | >50             | >50            | >50         | >50         |
| 2. aeruginosa | 50 12.5        | 50              | 50             | 50          | 50          |
| 3. subtillis  | >50 >50        | >50             | >50            | >50         | >50         |
| 4. aureus     | >50 >50        | >50             | >50            | >50         | >50         |
| 5. niger      | >50 50         | >50             | >50            | >50         | >50         |
| 6. oxysporum  | >50 50         | >50             | >50            | >50         | >50         |
| 7. cerevisiae | >50 >50        | >50             | >50            | >50         | >50         |
| 8. albicans   | >50 >50        | >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 inhibited 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, NghệAn 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 hydrodisitillation 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 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 ± 2°C. The larvicidal test was conducted with four replicates under five concentrations (200, 100, 50, 25 and 12.5 μg/mL).

The mortality rate was calculated according to the formula

\[ Mc = \frac{(Mo)}{(Mt)} \times 100 \]

\( Mo \) = 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 [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
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 inoculums size of 1.0 x 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 LC\(_{50}\), and LC\(_{90}\) levels. Moreover, the pseudo-stem of *Z. castanuem* 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
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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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