ABSTRACT

Aims: In the present study, the chemical constituents, antimicrobial and larvicidal potentials of hydrodistilled essential oil from the rhizome of *Zingiber zerumbet* were evaluated.

Study Design: The study was designed in different phases which are: collection of mature rhizomes of *Zingiber zerumbet*, hydrodistillation of essential oils, chemical analysis of the essential oils, determination of the antimicrobial potential and evaluation of larvicidal activity.

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Place and Duration of Study: The study was conducted at School of Natural Science, Vinh University, Vinh City, Nghệ An Province, Vietnam. The duration of the study was between August and December 2018.

Methodology: The rhizomes of Z. zerumbet were collected from Bến En National Park, Thanh Hóa Province, Vietnam, in August 2018. The air-dry sample was subjected to hydrodistillation process using Clevenger-apparatus to obtained essential oils. We evaluated the larvicidal potential of the oil against Culex quinquefasciatus and Aedes albopictus at 24 h and 48 h according to World Health Organisation protocol. The antimicrobial activity (MIC) was determined by microdilution broth susceptibility assay. Statistical analysis was performed using GraphPad Prism (version 7.02). The LC50 values, LC90 values and 95% confidence limits were obtained by using XLSTAT v. 2018.5.

Results: The most abundant compound of the essential oil was zerumbone (51.3%). The essential oil showed mortality of 98.3% (24 h) and 100% (48 h) against the Ae. albopictus at concentration of 100 µg/mL. In the same vein, mortality of 100% was displayed against Cx. quinquefasciatus under the tested time and concentration. The essential oil exhibited larvicidal activity towards Cx. quinquefasciatus showing minimum lethal concentrations, LC50 values of 33.28 µg/mL (24 h) and 21.81 µg/mL (48 h). The LC50 values of 55.75 µg/mL and 36.22 µg/mL at 24 h and 48 h respectively were obtained against Ae. albopictus. The result of the antimicrobial test indicated that Z. zerumbet oil inhibited the growth of Aspergillus niger (ATCC 9763) with MIC of 50.0 µg/mL.

Conclusion: Results demonstrated that the essential oil of Z. zerumbet was effective in the control of tested mosquitoes, Culex quinquefasciatus and Aedes albopictus and the microbe, Aspergillus niger.

Keywords: Zingiber zerumbet; essential oil; zerumbone; antimicrobial activity; larvicidal activity.

1. INTRODUCTION

The plant, Zingiber zerumbet (L.) Smith (family Zingiberaceae), a wild edible ginger species has been widely investigated for the various biological activities which they exhibited [1]. For example, extracts and essential oil from Z. zerumbet oil have been exploited for their antifungal and antymycotoxin efficacy against some microbes such as Aspergillus flavus and Aspergillus ochraceus [2]. Another report showed that the essential oils of Z. zerumbet and zerumbone, a constituent of the oil, through inhalation increases the food consumption and ultimately the body weight in tested animals [3]. Literature information has shown the essential oil derived from Z. zerumbet from all over the world possessed several biological activities such as antimicrobial [1], larvicidal [1], anti-nociceptive [4,5], anti-inflammatory [6] and antioxidant [7]. Moreover, Z. zerumbet oils were toxic, thus exhibiting insecticidal action [8,9]. The various biological activities exhibited by extracts and essential oils of Z. zerumbet from other parts of the world have been documented [1].

The chemical composition of essential oils of Z. zerumbet has been widely studied world over. A survey of literature has shown that zerumbone and its analogues was always the main chemical compound of the essential oil. However, the contents of zerumbone varied from one analysed sample to another in the same region, and from one region to another. The main constituents of the rhizome oil of Z. zerumbet from India [2] were zerumbone (49.8%) and α-caryophyllene (20.1%). Also, zerumbone (74.82%) constituted the bulk of the oil sample analysed from another location in India [10]. Zerumbone (36.12%) and humulene (10.03%) were reported as main compounds of oil sample from Malaysia [5], while another sample from Malaysia [11] contained zerumbone (60.4%) and humulene epoxide II (20.84%) as significant compounds. Likewise zerumbene (46.37% and 39.09%) and caryophyllene (28.01% and 25.81%) were the main constituents obtained from samples analysed in Thailand [7], while the same authors also reported the abundance of zerumbone (11.44% - 45.38%) and terpinen-4-ol (25.47%-31.06%) from another oil sample collected from other region of Thailand. A sample of Z. zerumbet oil collected and analysed also from Thailand [12] contained α-humulene (31.9%) and zerumbone (31.7%) as major constituents. The constituents occurring in higher amount in sample of Z. zerumbet rhizome oil analysed from China [9] were zerumbone (40.2%), α-caryophyllene (8.6%) and humulene epoxide II (7.3%).

However, low amount or total absence of zerumbone has been reported from some essential oils of Z. zerumbet around the world.
For example, the main constituents of the root oil of \textit{Z. zerumbet} from Vietnam [13] were \textit{α}-citral (26.1%), camphene (16.3%) and sabine (14.6%) while the flower oil contained (\textit{E})-nerolidol (34.9%), \textit{β}-caryophyllene (10.2%), linalool (17.1%). (\textit{E})-Nerolidol (21.4%), \textit{α}-pinene, (10.3%) and \textit{β}-pinene (31.4%) make up the compositions of the leaf oil of \textit{Z. zerumbet} from Reunion Island [14]. The contents of zerumbone from sample analysed in Vietnam [13] and Reunion Island [14] were insignificant.

Mosquitoes have been and continue to be the most deadly creatures on earth. \textit{Aedes albopictus} (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world [15]. Apart from the aggressive nature and daytime biting behavior, \textit{Ae. albopictus} has the ability to transmit many human pathogens and parasites such as yellow fever, dengue fever, West Nile, Japanese encephalitis, St. Louis encephalitis, chikungunya viruses and filarial nematodes. \textit{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 [16]. It has been demonstrated that some extracts and essential oils from \textit{Z. zerumbet} were used for the control of the mosquito vectors [10]. The larvicidal and pupicidal activities of rhizome oil of \textit{Z. zerumbet} had been reported [17]. Some authors have recommended that the oil of \textit{Z. zerumbet} may be use as mosquito larvicide [10]. The toxicity of the hexane extract of \textit{Z. zerumbet} against \textit{Cx. quinquefasciatus} had been reported [18]. However, till moment the authors are not aware of any information on the use of \textit{Z. zerumbet} essential oil from Vietnam as possible larvicides.

The aim of the present study was to determine the chemical composition, establish the larvicidal potential and evaluate the antimicrobial activity of essential oil from the rhizome of \textit{Z. zerumbet} growing in Vietnam. Recently, we have reported the chemical composition, antimicrobial and larvicidal activities of essential oils from other \textit{Zingiber} species [19,20] and other plants [21]. This is part of our extensive research directed towards the characterization of the chemical constituents and biological activities of economically important flora of Vietnam.

2. MATERIALS AND METHODS

2.1 Plant Sample
The rhizomes of \textit{Z. zerumbet} were collected from Bén En National Park, Thanh Hôa Province, Vietnam, in August 2018. A voucher specimen, HVC 700, was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Hydrodistillation of Essential Oil
For this experiment, 500 gram each of the air-dried samples was used during separate process. Each plant 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 [22] described in other studies [19-21]. 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 in other studies [19-21].

2.3 Analysis of Essential Oil
Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890 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 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 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were determined on normalized percentages.

An Agilent Technologies HP 7890N Plus Chromatograph fitted with 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 this experiment, under the same conditions as those used for gas chromatography analysis as described previously.
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.

2.3.1 Identification of constituents of essential oil

The identification of constituents from the GC/MS spectra of *Z. zerumbet* was performed on the basis of comparison 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 under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition [23] and with those in the literature as described in other studies [19-21].

2.4 Larvicidal Activity

2.4.1 Mosquito larvae

Adults of *Culex quinquefasciatus* and *Aedes albopictus* collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16°03′14.9″N, 108°09′31.2″E). Adult mosquitoes were maintained in entomological cages (40 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, Vietnam.

2.4.2 Larvicidal test

The larvicidal activity bioassay was performed according to the previously established protocol [24]. For the assay, aliquots of the essential oil of *Z. zerumbet* 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. Each test was conducted with four replicates under four different concentrations (100, 50, 25 and 12.5 μg/mL).

The mortality rate was calculated according to the formula:

\[
\text{Mc} = \frac{\sum \text{Mo}}{\text{Ni}} \times 100
\]

Mo = sum total of mortality in a particular treated group, Ni = number in the treated group, Mc = calculated mortality.

2.5 Antimicrobial Activity

Eight standardized ATCC strains from laboratory stock cultures were used in the evaluation of the antimicrobial activity of the oils of *Z. zerumbet*. The Gram negative strains were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 25923). The Gram positive strains were *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* subsp. *aureus* (ATCC 11632), *Aspergillus niger* (ATCC 11774) 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. The Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay [25,26]. 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 10⁶ 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.

2.6 Statistical Analysis

The data obtained were subjected to log-probit analysis [27] to obtain Median lethal concentrations namely LC₅₀ values, LC₉₀ values, and 95% confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France). Statistical analysis 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.

3. RESULTS AND DISCUSSION

3.1 Chemical Constituents of the Essential Oil

The essential oil of Z. zerumbet was obtained in a yield of 0.65% (±0.01, v/w), based on a dry weight basis. The colour of the oil was light yellow. As could be seen in Table 1, the compounds including their percentages and retention indices deduced on HP-5MS column were indicated. With the aid of GC/MS, 27 compounds accounting for 97.1% of the volatile contents were identified in the rhizome oil. Oxygenated sesquiterpenes (69.0%) and oxygenated monoterpenes (14.5%) were the main classes of compounds discernible in the oil of Z. zerumbet. The contents of the hydrocarbon derivatives were 6.7% and 6.9% respectively for the monoterpenes and sesquiterpenes. Zerumbone (51.3%) was the major compound identified in the oil. Other significant constituents include homulene epoxide (6.4%), homulene epoxide II (5.5%), α-humulene (5.4%), camphe (4.1%) and 1,8-cineole (3.2%). A noteworthy observation was that, zerumbone, the main compound in the present study, was also the main compound of Z. zerumbet reported from other region of the world [2,5,7,9-15]. However, the quantity of this compound, zerumbone, varied from one geo-graphical location to another. The percentage of zerumbone in the present study was larger than amounts reported for oils analysed from India [2], Malaysia [5], Thailand [7,12] and China [9]. However, other analysed samples from Malaysia [11] and India [10] contained higher amount of zerumbone when compared with this study. This variation in the nature of the chemical constituents may be due to several factors which include nature and age of plant, parts of plant being analysed, handling and harvesting procedure as well as the differences in the environmental and climatic conditions between various point of analysis.

3.2 Larvicidal Activity of the Oil

The larvicidal effects of essential oil of Z. zerumbet are summarized in Table 2. These include mortality (%) and minimum lethal concentrations (LC90 and LC50). The results showed that Z. zerumbet oil displayed mortality of 97.5% (24h) and 100% (48 h) against Ae. albopictus when tested at concentration of 100 µg/mL. Also, mortality of 100% was recorded by the oil towards the larvae of Cx. quinquefasciatus after 24 h and at concentration of 100 µg/mL. However, 100% mortality was observed at 48 h when the concentration was 50 µg/mL. The EIOH used as control did not display any mortality towards the mosquito vectors. Thus it can be inferred that the mortality represented by percentage was dependent on the concentration of the tested oil samples, which correlated with the fact that the highest mortality rate was observed at the maximum concentration of 100 µg /L. The essential oil was more susceptible to Cx. quinquefasciatus than Ae. albopictus.

The potential larvicides of Z. zerumbet oil towards Ae. albopictus could be seen in Table 2. The oil showed good larvicidal activity depicted by minimum lethal concentrations (LC90) values of 55.75 µg/mL at 24 h and 36.22 µg/mL at 48 h. The LC90 values recorded against Ae. albopictus were 135.98 µg/mL and 96.18 µg/mL at 24 h and 48 h respectively. Also, the oil exhibited more potent larvicidal action towards Cx. quinquefasciatus than Ae. albopictus. This was feasible by much lower LC50 values of 33.28 µg/mL at 24 h and 21.81 µg/mL at 48 h. Moreover, the LC90 values of 61.97 µg/mL at 24 h and 39.62 µg/mL at 48 h showed by the oil towards Cx. quinquefasciatus larvae were lower than data obtained for Ae. Albopictus. Permethrin, the standard drug used as control recorded higher larvicidal activity at much lower LC50 and LC90 values. In summary, essential oil of Z. zerumbet exhibited good mortality and larvicidal activity against Ae. albopictus and Cx. quinquefasciatus larvae.

Literature reports have shown that extracts and essential oils of Z. zerumbet exhibited larvicidal actions against Ae aegypti and Cx. quinquefasciatus. To the best of our knowledge, the mortality and larvicidal activities of the oils toward Ae. Albopictus were not reported previously. The resultant mortality and larvicidal activities of the studied oil sample are comparable with other data reported for similar activities. In a previous report on the toxicity of Z. zerumbet to insect pests, extracts obtained from hexane, ethyl acetate and methanol showed knock down effect against Cx. quinquefasciatus with values of 100%, 100% and 70% at 16, 15 and 22 min respectively [18]. Essential oils obtained from rhizomes of Z. cassumunar, Z. zerumbet and Z. ottensii from Thailand showed 100%, 100% and 97.6% mortality to Ae. aegypti at 5, 10 and 5 min respectively [28]. Also, oil of Z.
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Ottensii (5 min), Z. zerumbet (10 min) and Z. cassumunar (10 min) caused 100% mortality to Cx. quinquefasciatus [28]. The repellent, ovicidal and deterrent activities of essential oils from Z. cassumunar against Ae. albopictus have been reported [29]. Furthermore, researches have shown the oil of Z. officinale also displayed 100% mortality towards Cx. quinquefasciatus at 120 min [30]. The repellent and deterred biting activities of Z. zerumbet oil towards Ae. aegypti and Cx. quinquefasciatus was reported recently [31].

The LC50 and LC90 values of essential oil hydrodistilled from Z. zerumbet of Thailand origin against Ae. aegypti were 48.88 ppm and 62.17 ppm [12]. Likewise, oil of Z. Zerumbet showed activity against Ae. aegypti with LC50 and LC90 values of 82.05 mg/L and 121.05 mg/L [11]. In addition, LC50 value of 102.6 μg/mL was obtained in analysed oil from Malaysia against Ae. aegypti with LC50 and LC90 values of 82.05 mg/L and 121.05 mg/L [11]. In addition, LC50 value of 102.6 μg/mL was obtained in analysed oil from Malaysia against Ae. aegypti [32]. Z. zerumbet oil from Thailand displayed larvicidal action against Cx. quinquefasciatus with LC50 and LC90 values of 49.28 mg/L and 83.87 mg/L [11]. The LD50 indicating the adulticidal activity of hexane, ethyl acetate and methanol extracts of Z. Zerumbet against Cx. quinquefasciatus were 86.13, 53.83 and 46.61 ppm respectively [18]. Thus the oil of Z. zerumbet from Vietnam displayed higher activity against Cx. quinquefasciatus when compared with similar samples [11,18].

Table 1. Compounds identified in the essential oil of Z. zerumbet

| Sr. No | Compound a | RI b | RI a | Percent composition |
|-------|-----------|------|------|---------------------|
| 1     | α-Pinene  | 938  | 932  | 0.8                 |
| 2     | Camphene  | 954  | 952  | 4.1                 |
| 3     | β-Pinene  | 985  | 980  | 0.1                 |
| 4     | Myrcene   | 991  | 988  | 0.1                 |
| 5     | δ-3-Carene| 1015 | 1014 | 0.5                 |
| 6     | o-Cymene  | 1029 | 1028 | 0.4                 |
| 7     | Limonene  | 1033 | 1030 | 0.7                 |
| 8     | 1,8-Cineole| 1036| 1034| 3.2                 |
| 9     | Fenchone  | 1095 | 1094 | 0.4                 |
| 10    | Linalool  | 1102 | 1100 | 1.7                 |
| 11    | Camphor   | 1154 | 1154 | 6.7                 |
| 12    | Camphene hydrate | 1159 | 1160 | 0.2 |
| 13    | Borneol   | 1176 | 1177 | 0.7                 |
| 14    | Terpinen-4-ol | 1186| 1186| 0.5 |
| 15    | α-Terpineol| 1199| 1189| 0.6 |
| 16    | Borneyl acetate | 1292| 1289| 0.3 |
| 17    | Isobornyl acetate | 1295| 1298| 0.2 |
| 18    | β-Caryophylene | 1436| 1429| 1.2 |
| 19    | α-Humulene | 1470| 1470| 5.4 |
| 20    | β-Selinene | 1502| 1505| 0.3 |
| 21    | Caryophyllene oxide | 1605| 1610| 3.7 |
| 22    | Humulene epoxide I | 1618| 1620| 6.4 |
| 23    | Humulene Epoxide II | 1630| 1632| 5.5 |
| 24    | Humulene epoxide III | 1651| 1652| 0.6 |
| 25    | β-Eudesmol | 1672| 1762| 0.3 |
| 26    | Zerumbone | 1756| 1754| 51.3 |
| 27    | γ-Bicyclohomofarnesal | 1826| 1830| 1.2 |
| Total |           |      |      | 97.1                |

Monoterpene hydrocarbons 6.7
Oxygenated monoterpene s 14.5
Sesquiterpene hydrocarbons 6.9
Oxygenated sesquiterpenes 69.0

a Elution order on HP-5MS column; b Retention indices on HP-5MS column; c Literature retention indices
(See Materials and methods)
Table 2. Percentage mortality and larvicidal potential of *Z. Zerumbet* rhizome oil

| Concentration (μg/mL) |   |   |   |   |
|-----------------------|---|---|---|---|
|                       | 24 h | 48 h | 24 h | 48 h |
| **Ae. albopictus**     |       |       |       |       |
| 12.5                  | 1.25  | 2.50  | 0.00  | 0.00  |
| 25                    | 10.00 | 28.75 | 20.00 | 48.75 |
| 50                    | 35.00 | 51.25 | 65.00 | 100.00|
| 100                   | 97.50 | 100.00| 100.00| 100.00|
| **Cx. quinquefasciatus** |       |       |       |       |
| 12.5                  | 1.25  | 2.50  | 0.00  | 0.00  |
| 25                    | 10.00 | 28.75 | 20.00 | 48.75 |
| 50                    | 35.00 | 51.25 | 65.00 | 100.00|
| 100                   | 97.50 | 100.00| 100.00| 100.00|

Minimum lethal concentrations (μg/mL)

| Parameters          | 24 h    | 48 h    | 24 h    | 48 h    |
|---------------------|---------|---------|---------|---------|
| **Ae. albopictus**   |         |         |         |         |
| LC₅₀                | 55.75 (46.903-73.690) | 36.22 (31.523-43.069) | 33.28 (30.167-36.993) | 21.81 (19.702-24.749) |
| LC₉₀                | 135.98 (95.611-262.589) | 96.18 (72.688-152.586) | 61.97 (53.197-77.206) | 39.62 (32.997-53.485) |
| Regression equation | $y = -5.779 + 3.310x$ | $y = -4.710 + 3.021x$ | $y = -7.224 + 4.746x$ | $y = -6.622 + 4.946x$ |
| $X^2$               | 6.176   | 7.407   | 9.171   | 7.607   |
| $P$                 | 0.000   | 0.000   | 0.000   | 0.000   |
| **Cx. quinquefasciatus** |         |         |         |         |

| Parameters          | 24 h    | 48 h    | 24 h    | 48 h    |
|---------------------|---------|---------|---------|---------|
| LC₅₀                |         |         |         |         |
| LC₉₀                |         |         |         |         |
| Regression equation |         |         |         |         |
| $X^2$               |         |         |         |         |
| $P$                 |         |         |         |         |
The larvicidal action of essential oils of *Z. zerumbet* was comparable to activities reported for other *Zingiber* species. The essential oil under investigation displayed higher larvicidal action than *Z. officinale* var. *rubrum* and *Z. spectabile*. The LC50 values of 96.86 mg/L and 93.35 mg/L displayed by the oils of *Z. officinale* var. *rubrum* and *Z. spectabile* respectively against *Ae. albopictus* [11] were higher than values obtained for *Z. zerumbet* in this study. On the other hand, *Z. collinsii* [19] showed much higher activity against *Ae. albopictus* (LC50 and LC90 of 25.51 and 40.22 μg/mL respectively). This was also the case for *Z. montanum* where LC50 values of 35.17, 32.20 and 31.12 μg/mL were obtained against *Ae. albopictus*, *Ae. aegypti* and *Cx. quinquefasciatus* respectively [20]. The activity of *Z. cernuum* oil [32] towards *Ae. albopictus* (LC50 of 55.84 μg/mL) was similar to that of *Z. zerumbet* in this study. In the same vein, essential oils from the rhizome of *Z. nimmonii* [33] demonstrated higher larvicidal activity against *Ae. aegypti* (LC50 37.6 μg/mL) when compared with *Z. zerumbet* under investigation. The essential oils of *Z. collinsii* from Vietnam [19], *Z. officinale* from Thailand [30], *Z. cernuum* from India [32] and *Z. nimmonii* [33] exhibited lower activity towards *Cx. quinquefasciatus* with LC50 values of 50.11 μg/mL, 50.78 ppm, 48.44 μg/mL and 48.1 μg/mL respectively. The rhizome oils of *Z. officinale* from Malaysia however displayed larvicidal actions against *Ae. aegypti* 4th instar larvae with LC50 value of 197.2 μg/mL [34], while the activities of the hydroalts of *Z. officinale* [35] against *Ae. albopictus* and *Cx. quinquefasciatus* were 15.8% (v/v) and 21.8% (v/v).

Till moment, there have been no established standard criteria for determining the larvicidal activity of natural products and essential oils. By this some authors [36,37] have proposed individual criteria in order to establish the potency of mosquito larvical actions of natural products. In one such criteria [36] products showing LC50 ≤ 50 mg/L were considered 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. According to the criterion established previously [37], the essential oil of *Z. zerumbet* rhizome was considered to be active against *Ae. albopictus* (LC50 55.75 μg/mL) and *Cx. quinquefasciatus* (LC50 33.28 μg/mL). Therefore, this study established the potent mortality and larvicidal activity of essential oils of *Z. zerumbet* against the tested mosquito vectors.

It is well known that there are variations in the toxicity of essential oils against different species of mosquitoes and other insect pests [18]. This was mainly due to differences in the nature and amount of chemical constituents identified in the oil samples. Noteworthy observation was that some constituents of essential oil under investigation were known for their larvicidal activity. The larvicidal activity of some compounds such as α-pinene, β-pinene, 1,8-cineole, β-caryophyllene and zerumbone have been reported [1]. However, it may be proposed that the larvicidal activity of essential oils of *Z. zerumbet* may be due to high content of zerumbone present in it. Zerumbone, was previously reported to displayed larvicidal activity against *Ae. aegypti* larvae with LC50 of 41.75 mg/L and LC90 of 57.66 mg/L [38]. Also, α-pinene and β-pinene were reported to exhibit active larvicidal potential against *Ae. aegypti* with much lower LC50 values of 15.4 and 21.1 ppm respectively [39]. The larvicidal activity of β-caryophyllene (LC50 18.0 ppm) and α-humulene (LC50 5.0 ppm) towards *Ae. aegypti* larvae was also reported previously [40]. Moreover, the activity of some other minor compounds may also be taken into consideration.

**Table 3. Antimicrobial activity of the essential oil**

| Organisms          | MIC (μg/mL) |
|--------------------|-------------|
| *E. coli*          | -           |
| *P. aeruginosa*    | -           |
| *B. subtilis*      | -           |
| *S. aureus subsp. aureus* | - |
| *A. niger*         | 50 ± 0.50   |
| *F. oxysporum*     | -           |
| *S. cerevisiae*    | -           |
| *C. albicans*      | -           |

- No activity

The development of potential larvicides from available non-poisonous plants locally could be an acceptable alternative which can reduce dependence on imported synthetic insecticides. This could be beneficial for developing countries such as Vietnam which is currently suffering from dengue fever epidemics in recent years.

### 3.3 Antimicrobial Activity of the Oil

The data obtained from the antimicrobial study on essential oil *Z. zerumbet* rhizome is shown in Table 3. The data indicated that the oil sample displayed antibacterial activity and thus inhibitory the growth of *A. niger* with MIC of 50 μg/mL. However, the studied *Z. zerumbet* oil did not
inhibit the growth of other tested microorganism. Hence, the oil was highly effective against *A. niger* while showing non-activity against other microorganisms.

The effective antimicrobial action exhibited by *Z. zerumbet* oil towards *A. niger* was noteworthy. *Z. zerumbet* oil previously displayed antimicrobial action against *A. flavus* and *A. ochraceus* with MIC values of 160 and 175 ppm [2]. Therefore, it may be postulated that the essential oil of *Z. zerumbet* oil have good activity against *Aspergillus* species. In addition, the ineffectiveness of the oil of *Z. zerumbet* against other microorganism was in agreement with reports from Malaysia in which no activity was shown towards *A. niger, C. albicans* among others [1]. However, the oils of *Z. zerumbet* from other regions have shown potency against some other microbes [1] contrary to the activity of the investigated oil sample. For example, sample of *Z. zerumbet* oil from Indonesia have proved effective against *E. coli*, *P. aeruginosa* and *Salmonella typhi* with MIC of 1.25, 1.25 and 0.625 mg/mL [41]. The potency of *Z. zerumbet* essential oil as a therapeutic agent against *S. aureus, B. cereus, P. aeruginosa* and *E. coli* has been reported [42].

The observed antimicrobial activity may be attributed to the main compound (zerumbone). The synergistic action of some other minor compounds may also be taken into consideration. It has been reported that zerumbone exhibited a number of biological activities which includes anti-mycological [2], antimicrobial [10] and antifungal [43]. The antimicrobial of several compounds of essential oil of *Z. zerumbet* have also been reported [1].

4. CONCLUSION

This study showed that the rhizome essential oil of *Z. zerumbet* exhibited larvicidal activity against *Ae. albopictus* and *Cx. quinquefasciatus*. In addition, the oil displayed antimicrobial action *A. niger* at reasonable MIC level. The most abundant compound of the oil was zerumbone, which was consistent with majority of analysed samples all over the world. In conclusion, the data presented therein revealed the potentials of essential oils of *Z. zerumbet* from Vietnam as larvicidal and antimicrobial agents.

DISCLAIMER

The plant, chemicals and mosquito larvae used for this research are commonly and predominantly use products in our area of research and country, Vietnam. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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