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Zingiber zerumbet Rhizome Essential Oil: Chemical Composition, Antimicrobial and Mosquito Larvicidal Activities

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Authors' contributions

This work was carried out in collaboration among all authors. Author LTH designed the study and wrote the protocol. Authors HVC, NTGA and NTV performed the statistical analysis while authors LTH, NHH and NTHT managed the analyses of the study. Authors IAO and AOG wrote the first and final drafts of the manuscript. Authors IAO and AOG managed the literature searches. All authors read and approved the final manuscript.

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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 LC_{50} values, LC_{90} 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, $LC_{50,}$ values of 33.28 µg/mL (24 h) and 21.81 µg/mL (48 h). The LC_{50} 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 Zingiberacea), 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 antimycotoxin 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 zerumbone (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 *Z. zerumbet* from Vietnam [13] were α-citral (26.1%), camphene (16.3%) and sabinene (14.6%) while the flower oil contained (*E*)-nerolidol (34.9%), *β*-caryophyllene (10.2%), linalool (17.1%). (*E*)-Nerolidol (21.4%), *α*-pinene, (10.3%) and *β*-pinene (31.4%) make up the compositions of the leaf oil of *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. *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, *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. *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 *Z. zerumbet* were used for the control of the mosquito vectors [10]. The larvicidal and pupicidal activities of rhizome oil of *Z. zerumbet* had been reported [17]. Some authors have recommended that the oil of *Z. zerumbet* may be use as mosquito larvicide [10]. The toxicity of the hexane extract of *Z. zerumbet* against *Cx. quinquefasciatus* had been reported [18]. However, till moment the authors are not aware of any information on the use of *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 *Z. zerumbet* growing in Vietnam. Recently, we have reported the chemical composition, antimicrobial and larvicidal activities of essential oils from other *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 *Z. zerumbet* were collected from Bến En National Park, Thanh Hóa 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^{o}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^{o}C, detector temperature 260^{o}C, column temperature programmed from 40^{o}C (2 min hold) to 220^{o}C (10 min hold) at 4^{o}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

[17-19]. 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 (C_4-C_{40}), 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:

$$Mc = \Sigma Mo/Nt \times 100$$

Mo = sum total of mortality in a particular treated group, Nt = 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 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. 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^{o}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^{o}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 LC_{50} values, LC_{90} 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 humulene epoxide I (6.4%), humulene epoxide II (5.5%), α-humulene (5.4%), camphene (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 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 (LC_{50} and LC_{90}). 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 EtOH used as control did not displayed 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 (LC_{50}) values of 55.75 µg/mL at 24 h and 36.22 µg/mL at 48 h. The LC_{90} 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 LC_{50} values of 33.28 µg/mL at 24 h and 21.81 µg/mL at 48 h. Moreover, the LC_{90} values of 61.97 µg/mL at 24 h and 39.62 µg/mL at 48 h shown 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 LC_{50} and LC_{90} 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.*

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. officnale* 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 LC_{50} and LC_{90} 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 LC_{50} and LC_{90} values of 82.05 mg/L and 121.05 mg/L [11]. In addition, LC_{50} value of 102.6 µg/mL was obtained in analysed oil from Malaysia against *Ae. aegypti* [32]. *Z. zerumbet* oil from Thailand displayed larvicdial action against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 49.28 mg/L and 83.87 mg/L [11]. The LD_{50} 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	Bornyl 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 monoterpenes				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)	**Mortality (%)**			
	Ae. albopictus		***Cx. quinquefasciatus***	
	24 h	**48 h**	**24 h**	**48 h**
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	***Ae. albopictus***		***Cx. quinquefasciatus***	
	24 h	**48 h**	**24 h**	**48 h**
LC_{50}	55.75 (46.903-73.690	36.22 (31.523-43.069)	33.28 (30.167-36.993	21.81 (19.702-24.749
LC_{90}	135.98 (95.611-262.589)	96.18 (72.688-152.586)	61.97 953.197-77.206	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	0.00	0.000

The larvicidal action of essential oils of *Z. zerumbet* was comparable to activities reported for other *Zinigiber* species. The essential oil under investigation displayed higher larvicidal action than *Z. officinale* var. *rubrum* and *Z. spectabile.* The LC_{50} 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* (LC_{50} and LC_{90} of 25.51 and 40.22 µg/mL respectively). This was also the case for *Z. montanum* where LC_{50} 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* (LC_{50} 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* (LC_{50} 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 LC_{50} 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 LC_{50} value of 197.2 µg/mL [34], while the activities of the hydrolates 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 larvicidal actions of natural products. In one such criteria [36] products showing $LC_{50} \leq 50$ mg/L were considered to be active, 50 mg/L < $LC_{50} \leq 100$ mg/L to be moderately active, 100 mg/L < $LC_{50} \leq 750$ mg/L to be effective, and $LC_{50} > 750$ mg/L to be inactive. According to the criterion established previously [37], the essential oil of *Z. zerumbet* rhizome was considered to be active against *Ae. albopictus* (LC_{50} 55.75 µg/mL) and *Cx. quinquefasciatus* (LC_{50} 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 larvicdial 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 LC_{50} of 41.75 mg/L and LC_{90} 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 LC_{50} values of 15.4 and 21.1 ppm respectively [39]. The larvicidal activity of β-caryophyllene (LC_{50} 18.0 ppm) and α-humulene (LC_{50} 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 studued *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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