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Larvicidal activity of ethanol extract and essential oil from *Zingiber aromaticum* Val. rhizome against *Aedes aegypti* larvae

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Abstract

Aim: Dengue haemorrhagic fever (DHF) transmitted by *Aedes aegypti* is a disease that causes many deaths. Many people commonly use larvicide from hazardous synthetic materials for preventing this disease. In this study, we investigated the larvicidal activity of ethanol extract and essential oil from *Zingiber aromaticum* Val. rhizome against *Aedes aegypti* larvae.

Methods: The larvicidal test was executed by observing the larvae mortality after 24 hours of treatment and evaluated using PROBIT analysis in order to determine the LC_{50} value. The essential oil was analyzed by using gas chromatography-mass spectroscopy (GC-MS).

Results: The results showed that the extract and essential oil from *Z. aromaticum* rhizome represented significant larvicidal activity with LC_{50} values of 16.04 µg/mL and 21.08 µg/mL, respectively. The analysis result of essential oil from *Z. aromaticum* rhizome with GC-MS showed at least 40 peaks separated and at least 21 components identified with the three largest components that were camphor (7.67%), zerumbone (5.29%) and α-pinene (4.90%).

Conslusion: As a conclusion, it was observed that the extract and isolated essential oil from *Z. aromaticum* possessed remarkable larvicidal properties (LC_{50}<100 µg/mL). This study should be continued to search for potentially active compounds from those ethanol extract and essential oil.

Keywords: *Aedes aegypti*, essential oil, ethanol extract, larvicidal activity, *Zingiber aromaticum* Val.

INTRODUCTION

Dengue Haemorrhagic Fever (DHF) is one of the public health problem and endemic disease in some regions in Indonesia. Almost each year, this disease is identified as a terrific incident in many regions especially occurred in the rainy season. In 2011, it was recorded 24.362 cases of this disease with 196 of deaths [1]. DHF is caused by DEN-1, DEN-2, DEN-3 or DEN-4 viruses (Denggi type 1-4) [2]. Dengue virus is transmitted to humans through the bite of *A. aegypti* mosquitoes which infected by the virus. DHF is the most important disease of all arthropod-born viral disease [3]. The symptoms include high fever, haemorrhage, liver enlargement and circulatory failure. This disease increases the vascular permeability and decreases plasma volume [3]. There is no vaccine and medicine for this Dengue. The cure is for supportive reasons in form of taking rest and intravenous fluids injection. However, eradicating mosquito breeding and kill both larvae and mosquitoes are the best preventative action [4].

Common method to control the vector development is using insecticides as larvicides. In Indonesia, Temephos is the common insecticides that have been used since 1976. In 1980, Temephos 1% was appointed to be the massive method for killing *A. aegypti* larvae in Indonesia [4]. Nevertheless, it is not recommended for drinking due to limitation of benefits [5]. Besides that, the high costs of using chemical insecticides and the emergence of resistance are important point to be considered. An interest in developing and using natural, easy-to-obtain, and safe bio pesticides for both the human body and the environment is one of the solutions [6].

Some of natural compounds from plants are known as larvicide due to their activity, that are compound groups of flavonoids, saponins, and tannins. The essential oil components such as camphor, β-eudesmol and tumerone are confirmed lead to about 100% mortality of 3rd instar *A. aegypti* larvae less or after 24 hours [7,8,9].

Z. aromaticum is one of the identified plant which has active chemical components as larvicide [10]. Moreover, some plant extracts from Zingiberaceae family were studied about their larvicidal activity [11]. *Z. aromaticum* contains several active compounds such as β-curcumene, bisabolene, zingiberene, sesquiphellandrene, zerumbone, limonene, camphor, gingerol, zingerone, paradol, hexahydroxy-curcumin and Dihydro-gingerol [10].

Based on paragraphs above, *Z. aromaticum* had potential activity as a larvicide. So that, this study was performed to investigate regarding larvicidal activity of ethanol extract and essential oil from *Z. aromaticum* rhizome against *A. aegypti* larvae, and identify of its compounds using GC-MS analysis.

MATERIALS AND METHODS

Plant Material Preparation

The plant material used in this research was *Z. aromaticum* rhizome obtained from the Research Institute for Medicinal and Aromatic Plants in Lembang, West Java, Indonesia. The plant material was authenticated and determined (number 45/HB/12/2011) in herbarium by senior taxonomist scientist at The Plant Taxonomy Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia. The material was obtained in a dry and pollinated state. Fresh rhizome with good quality was selected (wet sorting), cleaned, peeled and chopped.

A. aegypti Larvae

The 3rd-4th instar larvae of *A. aegypti* were obtained from Unit of Vector Borne Diseases Research and Development, National Institute of Health Research and Development, Ministry of Health of Republic of Indonesia in Ciamis District, West Java, Indonesia. The chemicals used were ethanol, distilled water, and liquid paraffin.

Extraction

As many as 160 g of dried rhizomes were macerated with 1 L of 95% ethanol for 24 hours. The macerate was accommodated and the dreg was macerated again for two consecutive times each with 1,200 mL of ethanol for 24 hours. The whole macerates were combined, then the solvent was vaporized using a rotary evaporator. The viscous extract obtained was evaporated over the water bath until the extract was in constant weight.

Isolation of Essential Oil

Total of 200 g of rhizomes were shredded and put into a round flask that previously had been inserted boiling stone, then added the distilled water approximately ¾ of flask until fully submerged. After that, the flask was coupled to the distillation apparatus. Distillation process was executed for approximately 6 hours as much as 15 times. Essential oil content was calculated in % v/w.

Phytochemical Screening

Phytochemical screening included tests of alkaloid, polyphenol, tannin, flavonoid, monoterpenoid and sesquiterpenoid, steroid and triterpenoid, quinone, and saponin according to the Farnsworth method with some modifications [12].

Larvicidal Activity Test

The test solution was prepared by dilution of each extract and essential oil to obtain the concentration of 100 µg/mL, 50 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL and 0.5 µg/mL. *A. aegypti* mosquito eggs were obtained from mosquito's colonies which bred at Unit of Vector Borne Diseases Research and Development, National Institute of Health Research and Development, Ministry of Health of Republic of Indonesia in Ciamis District, West Java, Indonesia. The eggs were removed into plastic trays filled with water and stand for 4-5 days until they hatch and turn into 3^{rd}-4^{th} instar of mosquito larvae. Those larvae were used for larvicidal testing. The larvicidal test refers to the WHO protocol (2005) [13]. The 3^{rd}-4^{th} instar larvae of 25 heads were taken directly with pipettes from mosquito egg incubation containers, then transferred into 250 mL plastic cup. Then, plastic cups were stored at 25°C for 24 hours. Evaluation of larval mortality after 24 hours was executed by counting the number of dead larvae. Larvae were declared dead if they did not respond to stimuli given.

On the analysis of larvicidal test data according to WHO (2005), if deaths were occurred in controls exceeding for 20%, the experiment should be repeated, but if deaths were occurred in the control for 5-20%, the percent of deaths on the treatment should be corrected by the Abbott's formula, as follows:

$$P_r = \frac{P_o - P_c}{100 - P_c}$$

Information:

Pr = % of deaths corrected

Po = % of deaths on treatment

Pc = % death on control

The results of larvicidal test were analyzed using Probit analysis program to obtain LC_{50} and LC_{90} values from extract and essential oil into *A. aegypti* larvae [13].

Examination of Essential Oils Using Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The distillation essential oil was dissolved with n-hexane solvent. Total of 0.5 µL samples were analyzed using a gas chromatographic tool connected to a mass spectroscopy detector (GC-MS). The instrument used was GCMS-GCMS-QP 5000 Shimadzhu. The column used was a DB-17 capillary column with a length of 30 m and a diameter of 0.25 mm. The carrier gas used was helium with a flow rate of 0.9 mL/min and a pressure of 41.7 kpa. The injector temperature used was 250°C. The programming temperature began with 40°C for two minutes. The temperature of 250°C was maintained for 3.67 minutes. Identification results in component compared in similarity (Similarity Identification) with data banks that already exist on the computer, the data banks NIST, Curcuma and Willey. This examination was conducted in Chemical Research Center Laboratory, Indonesian Institute of Sciences, Bandung, Indonesia.

RESULTS AND DISCUSSION

Plant Preparation Result

The results of determination showed that the plant used in this study was from the Zingiberaceae family with the species of *Z. aromaticum*. The rhizome of this plant was flat sliced, light, branched or irregular; the length was uncertain, 1-2 cm thick; the colour of the surface was light brown to dark brown and the tip was bent. Grate leaves were clearly visible. It had characteristic odour and slightly bitter. Based on the observations, the plant description was in accordance with the reference [14].

Extraction Result

The ethanol extract from 158.5 g of dry powder of *Z. aromaticum* rhizome was 13.21 g with a yield of 8.33%. The characteristics of this extract was a dark brown extract. It had a distictive smell and a bitter taste.

Isolation of Essential Oil Result

Essential oil obtained from 2,500 g of fresh *Z. aromaticum* rhizome was as much as 2.7 mL with a yield of 0.11% (mL/g).

Phytochemical Screening Results

The results of phytochemical screening could be concluded that the extract of *Z. aromaticum* rhizome contained group compounds of monoterpene, sesquiterpene and triterpene. The results are presented in Table 1. The phytochemical screening was performed as a preliminary step to search for potentially active compounds of the plant.

Ultimately, the goal in searching plants for biologically active or medicinally useful compounds should be to isolate the one or more constituents responsible for a particular activity. Hence, with the selection of plant for phytochemical investigation, either on the basis of one or more approaches set forth under phytopharmacological approaches, or through some other avenue, phytochemical screening techniques can be a valuable aid [12].

Table 1: The results of phytochemical screening of extract from *Z. aromaticum* rhizome

Phytochemical screening test	*Z. aromaticum* rhizome extract
Alkaloid	-
Polyphenol	-
Tannin	-
Quinone	-
Flavonoid	-
Monoterpenoid and Sesquiterpenoid	+
Saponin	-
Steroid	-
Triterpenoid	+

Note: (+) Detected; (-) Undetected

Larvicidal Activity Testing Results

The results of larvicidal activity test for extract and essential oil of *Z. aromaticum* rhizome for 24 hours was showed by the mortality average of *A. aegypti* toward both the extract and the essential oil (Table 2). That results were analyzed by using PROBIT analysis in order to calculate the LC_{50} and LC_{90} values of both the extract and the essential oil (Table 3).

Table 2: Mortality average of *A. aegypti* larvae toward extract and essential oil from *Z. aromaticum* for 24 hours

Concentration (µg/mL)	Mortality average of *A. aegypti* larvae	
	Sample	
	Extract	Essential oil
0	0.00 ± 0.00	0.00 ± 0.00
0.5	0.00 ± 0.00	0.00 ± 0.00
1	2.00 ± 1.00	1.00 ± 1.00
5	3.00 ± 1.00	1.00 ± 0.00
10	13.00 ± 1.00	2.00 ± 1.00
50	19.00 ± 1.00	21.00 ± 2.00
100	21.00 ± 1.00	25.00 ± 0.00

Table 3: Results of larvicidal activity testing of extract and essential oil from *Z. aromaticum* rhizome against *A. aegypti* larvae for 24 hours

Sample of *Z. aromaticum*	LC_{50} (µg/mL)	LC_{90} (µg/mL)
Extract	16.04	132.36
Essential oil	21.08	69.69

Based on the results of larvacidal testing above, it could be concluded that the extract of *Z. aromaticum* rhizome showed the existence of larvae mortality increased in accordance with the increase in concentration for 24 hours. The values of LC_{50} and LC_{90} from the PROBIT analysis were 16.04 µg/mL and 132.36 µg/mL, respectively. The LC_{50} value which less than 100 µg/mL indicated that the extract of *Z. aromaticum* rhizome was considered to have a significant larvicidal activity against *A. aegypti* mosquito larvae [15].

The results of the essential oil test of *Z. aromaticum* rhizome against *A. aegypti* larvae showed an increase of larvae mortality in accordance with the increase of its concentration for 24 hours. The values of LC_{50} and LC_{90} from the larvicidal test of the essential oil using PROBIT analysis were 21.08 µg/mL and 69.69 µg/mL, respectively. The LC_{50} value of the essential oil was also considered the same as the extract to have a significant larvicidal activity against *A. aegypti* mosquito larvae [15]. It was observed that the extract and the isolated essential oil from *Z. aromaticum* possessed remarkable larvicidal properties.

Essential Oil Analysis Result Using GC-MS

The results of the essential oil analysis using gas chromatography (GC) is shown in Figure 1. To identify the chemical structure of each peak in the chromatogram, the mass spectroscopy (MS) was used and the resulted of fragmentation pattern of each peak was compared with the pattern of authentic compounds in the NIST, Curcuma, and WILEY libraries. The resulted of essential oil components was shown in Table 4.

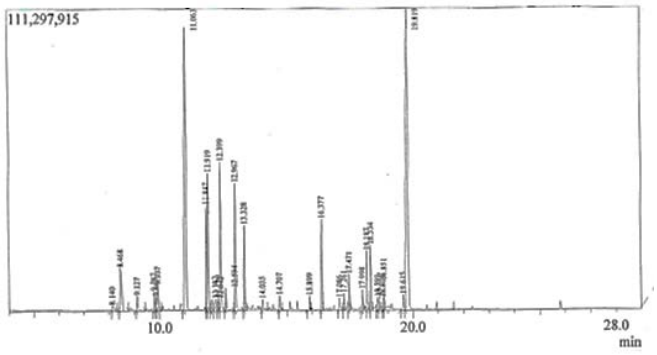

Figure 1: GC chromatogram of essential oil from *Z. aromaticum* rhizome

Based on the GC analysis results of essential oil from *Z. aromaticum* rhizome, it showed at least 21 components of compounds that have been identified from the mass spectrum. These components are mostly classified as sesquiterpenes, in addition there are some belonging to the monoterpene class. The results showed at least 40 peaks separated and at least 21 components identified, i.e. α-pinene, champhene, β-myrcene, limonene, citronella, champhor, 4-terpineol, α-terpineol, β-citronellol, trans-geraniol, citronellyl acetate, trans-caryophyllene, α-humulene, Δ-cadinene, elemol, β-selinene, caryophyllene oxide, β-eudesmol, zerumbone and 1,8-Cineole. In the previous literature, it was known that the essential oil of the *Z. aromaticum* rhizome had champhor and limonene components which become the essential oil compound which had activity as larvicide [10,14]. Both compounds have percentages in the sample respectively of 7.67% and 2.81%. However, the results of the research found other compounds which have larvicidal activity that were 1.8-Cineole and β-Eudesmol with percentage levels in the sample respectively were 2.85% and 2.12% [8,9]. The analysis results of essential oil from *Z. aromaticum* rhizome using GC-MS showed that the three largest components were camphor (7.67%), zerumbone (5.29%), and α-pinene (4.90%).

Table 4: Essential oil compounds from *Z. aromaticum* rhizome using MS analysis

Num.	RT	SI	MW	Name of compound	Composition (%)
1	8.140	98	136	α-Pinene	4.90
2	8.468	97	136	Champene	4.49
3	9.127	96	136	β-Myrcene	3.21
4	9.867	95	136	Limonene	2.81
5	9.937	93	154	1,8-Cineole	2.85
6	11.063	91	154	Linalool	4.69
7	11.847	98	154	Citronella	1.86
8	11.919	97	152	Camphor	7.67
9	12.399	97	154	4-Terpineol	2.14
10	12.594	96	154	α-Terpineol	2.49
11	12.967	98	156	β-Citronellol	2.13
12	13.328	96	154	Trans-Geraniol	2.18
13	14.707	96	198	Citronellyl acetate	2.34
14	15.899	96	204	Trans-Caryophyllene	2.02
15	16.377	98	204	α-Humulene	1.84
16	17.086	90	204	Δ-Cadinene	3.91
17	17.471	95	222	Elemol	1.97
18	18.187	89	204	β-Selinene	1.98
19	18.334	83	220	Caryophyllene oxide	1.91
20	18.851	95	222	β-Eudesmol	2.12
21	19.819	92	218	Zerumbone	5.29

Note: RT (retention time), SI (similarity index), MW (molecule weight)

CONCLUSIONS

The results showed that the extract and essential oil from *Z. aromaticum* rhizome represented significant larvicidal activity with LC_{50} values of 16.04 µg/mL and 21.08 µg/mL, respectively. It was observed that the extract and the isolated essential oil from *Z. aromaticum* possessed remarkable larvicidal properties (LC_{50}<100 µg/mL). The analysis result of essential oil from *Zingiber aromaticum* Val. rhizome with GC-MS showed at least 40 peaks separated and at least 21 components identified with the three largest components were camphor (7.67%), zerumbone (5.29%), and α-pinene (4.90%).

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