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Larvicidal Activity Of *Curcuma heyneana* Val. & v. Zijp Rhizome Against *Aedes Aegypti* Larvae.

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) caused by *Aedes aegypti* mosquito is an acute disease. Many people commonly use larvicide from the hazardous synthetic materials for preventing this disease. The aim of this study was to investigate the larvicidal activity of ethanol extract and essential oil from *Curcuma heyneana* Val & v. Zijp rhizome against *Aedes aegypti* larvae. Larvicidal test was demonstrated by observing the larvae mortality after 24 hours of treatment. The larvicidal test data was evaluated using probit analysis in order to determine the values of LC_{50} and LC_{90}. The essential oil compounds from *Curcuma heyneana* were evaluated by using GCMS (gas chromatography-mass spectroscopy). The results demonstrated that the extract from *Curcuma heyneana* revealed no significant activity against *Aedes aegypti* larvae, whereas the essential oil tested demonstrated significantly larvicidal activity with LC_{50} and LC_{90} values of 35.33 µg/ml and 86.02 µg/ml, respectively (LC_{50}<100 µg/ml). The analysis of essential oil from *Curcuma heyneana* rhizome using GC-MS showed at least 84 compounds separated and 3 major compounds were 11H-[1]Benzopyrano[4,3-b]indol-6-one type 1 (11.47%), 11H-[1]Benzopyrano[4,3-b]indol-6-one type 3 (8.11%) and Isocurcumenol type 1 (7.68%). It was observed that the isolated essential oil from *Curcuma heyneana* possessed remarkable larvicidal properties.

Keywords: *Curcuma heyneana* Val & v. Zijp., Larvicidal, *Aedes aegypti*, essential oil

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INTRODUCTION

Indonesia is a country periodically attacked by an epidemic of dengue fever. Dengue Hemorrhagic Fever (DHF) is an acute disease caused by four dengue virus antigens and spread to a person through a mosquito's bite (White, 2004). *Aedes aegypti* is the major vector of dengue disease (David and Ananthakrishnan, 2004). This time, one dengue vaccine has been licensed, Dengvaxia® (CYD-TDV), developed by Sanofi Pasteur. It was first licensed in Mexico in December 2015 for use in individuals 9-45 years of age living in endemic areas (WHO, 2016). But in fact, the best prevention in handling the vector is how to control the mosquito breeding and how to stop the disease from spreading (Indonesian Ministry of Health, 2010; White, 2004).

A person can get dengue fever four times in his or her lifetime, although a person never gets the same dengue infection twice. Infection with one type of dengue fever provides immunity for the rest of a person's life. Each dengue strain is unique, so that a person can be potentially infected once by each strain of the virus. According to the American Mosquito Control Association, most of mosquitoes develop in the same way. There are four stages in the mosquito's life cycle: egg, larva, pupa, and adult. *Aedes aegypti* experienced a complete metamorphosis with the growth stages of egg, larva, pupa, and adult. *Aedes aegypti* lay they eggs on damp soil that will be flooded by water. Egg are laid one at a time or attached together to form rafts, which float on the surface water. Most eggs hatch into larvae within forty-eight hours. Water is necessary part of mosquito's life so the larva lives in the water and comes to the surface to breathe. Larva look like small centipedes. As long as the water in stagnant though, like in barrels, the larvae can and will survive (Ross et al., 1991; White, 2004).

Due to scanty rain fall and irregular supply of water people in desert develop habit of storing water in domestic containers and this habit leads to create protective conditions for mosquitoes to breed in fresh water and this leads to increase number of domestic breeding sites. To control of these domestic breeding sites, we require effective bio-larvicide which is easily accessible, economic, available throughout the year, non-toxic to non-targeted population and also known to local population. One of the actions in controlling the growth of larvae is the use of insecticide, called larvicide (Singhi and Purohit, 2013; Borror et al., 1992).

Temephos is a group of organophosphate insecticide that is used to control mosquito larvae. Temephos potentially causes excessive stimulation on the nervous system that can cause nausea, dizziness, and confusion (O'Neil, 1989). Temephos is an organophosphate insecticide which has been in use over last 30 years. Recently resistance of *Aedes aegypti* towards organophosphate insecticide has been reported. Water use practices also limits the effectiveness of a temephos-based *Aedes aegypti* larval control program. Due to increasing vector resistance against temephos more attention has been given on the development of plant based biocide. Under the Integrated Mosquito Management (IMM) more emphasis was given on the application of alternative strategies in mosquito control. One of the most effective alternative approaches is to explore the bio-larvicide of botanic origin as a simple and sustainable method (Singhi and Purohit, 2013).

Curcuma heyneana Val. & V. Zijp. (Zingiberaceae) is herbaceous plant that grows in Java. It contains saponins, flavonoids, tannins, curcumin, and essential oils (Indonesian Ministry of Health, 1989; Indonesian Ministry of Health, 2001). The Volatile oils contains 1,8-cineole/limonene (14.2%), isocurcumenol (7.4%), eudesmol (4.7%), curcumanolides A, B (13.1%), dehydrocurdione (10.2%), and curcumenone (2.3%) (Zwaving and Bos, 1992). Based on research that has been executed by Sukari et al. (2010), petroleum ether extract of *Curcuma heyneana* can be potential source of natural larvicides against larvae of *Aedes aegypti*. Therefore, the potential activity of ethanol extract and essential oil from *Curcuma heyneana* against *Aedes aegypti* larvae need further investigation.

MATERIALS AND METHODS

Materials

The plant material of *Curcuma heyneana* rhizome was obtained from the Research Institute for Medicinal and Aromatic Plants, in Lembang, West Java, Indonesia. 3rd-4th instar larvae of *Aedes aegypti* were obtained from Research and Development of Animal Sourced Disease Eradication in Ciamis District, West Java, Indonesia. The chemicals used were ethanol, distilled water, and liquid paraffin.

Method

Preparation and Determination of Plant Material

Curcuma heyneana plant was determined in the laboratory of plant taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. The material was obtained in dried rhizome form. The fresh rhizome had been sorted, cleaned, peeled, and chopped.

Extraction

One hundred sixty gram of dried rhizome was macerated with 1 L of 95% ethanol for 24 hours. Liquid extract was collected and the pulp was macerated in two times with 1.2 L of ethanol for 24 hours. The whole liquid extracts were combined, and then the solvent was evaporated using a rotary evaporator. Obtained viscous extract was evaporated over the water bath to obtain the extracts with constant weight.

Essential Oils Isolation

Two hundred gram of dried rhizomes were chopped and distilled in 1 L flask which had previously been given a boiling stone, then put distilled water approximately ¾ of flask until the material is fully submerged. After that, the flask was coupled to the distillation apparatus. Distillation was executed for approximately 6 hours and performed in 15 times. The oil content was collected, preserved in a sealed sample tube, and stored under refrigeration until analysis.

Larvicidal Activity Testing

Larvicidal activity testing referred to WHO protocol (2005) for laboratory and field testing of mosquito larvicides. The test solution was prepared by diluting each extract and essential oil until the test concentrations obtained were 100 µg/mL, 50 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL, and 0.5 µg/mL.

Batches of 25 third or fourth instar larvae were transferred by means of strainers to small disposable test cups, each containing 100 mL of water. Small, unhealthy or damaged larvae should be removed and replaced. The depth of the water in the cups or vessels should remain between 5 cm and 10 cm; deeper levels may cause undue mortality.

The appropriate volume of dilution was added to 100 mL of water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four replicates were set up for each concentration and an equal number of controls are set up simultaneously with tap water. The test containers were held at 25-28 °C.

After 24 h exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that cannot be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapables of rising to the surface or not showing the characteristic diving reaction when the water was disturbed. The results were recorded on the form provided, where the LC_{50} and LC_{90} values, and slope and heterogeneity analysis are also noted.

Larvae that had pupated during the test period would negate the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded and repeated. If the control mortality was between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott's formula:

$$Mortality\ (\%) = \frac{X - Y}{X} 100,$$

where *X* = percentage survival in the untreated control and *Y* = percentage survival in the treated sample.

Data Analysis

Data of LC_{50} and LC_{90} values were calculated from a log dosage–probit mortality regression line using Polo-PC software programs, copyright from LeOra Software (1987). Standard deviation or confidence intervals of the means of LC_{50} values were calculated and recorded. A test series was valid if the relative standard deviation (or coefficient of variation) was less than 25% or if confidence limits of LC_{50} overlap (significant level at $p < 0.05$). The potency of the chemical against the larvae of a particular vector and strain could then be compared with the LC_{50} or LC_{90} values of other insecticides.

Essential Oil Analysis Using GC-MS

Distilled Essential oil was dissolved in hexane and approximately 0.5 ml of it was analyzed using gas chromatography linked to mass spectrometry (GC-MS). The instrument used was Ion Trap (Varian Saturn 2000) GC-MS. The column used was the VF-17MS (Varian) 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 1.3 ml/min and a pressure of 8.1 Psi. The temperature of injector was 230 °C. Programming began with a temperature of 50 °C for three minutes, then the temperature increased at 150 °C with 5 °C per minute, and then increased for 3 °C until 250 °C in every minute. The temperature of 250 °C was maintained for 3.67 minutes. The identification of the compounds was evaluated by comparing the compound analyzed with those reported in NIST, Curcuma, and Willey libraries data of the peaks.

RESULTS AND DISCUSSION

Preparation and Determination of Plant Material

The result of determination showed that the plant used in this research was *Curcuma heyneana* Val & v. Zijp species from Zingiberaceae family. *Curcuma heyneana* rhizome was flat sliced rhizomes, light, round to oval, branched or irregular; the slice was 1-4 mm thick; 2-5 long; 0.5-4 cm width; margin was either wavy or wrinkled, brownish; center part was whitish yellow; there was leaf node and root scar; clear border between cortex and central cylinder; slender cortex, less than 3 mm width; wide central cylinder; fracture quite even, whitish yellow. It had characteristic odor, slightly bitter and spicy followed by chelate. Based the observations, the plant description was in accordance with the reference (Indonesian Ministry of Health, 2012).

Extraction

The ethanol extract obtained from 160 g of rhizome was as much as 36.74 g with a yield of 22.96%. Ethanol extract from *Curcuma heyneana* rhizome extract had characteristics in viscous shaped, dark brown color, distinctive smell and bitter taste.

Essential Oils Isolation

The essential oil obtained from 2.077 g of fresh rhizome was as much as 8.2 mL with a yield of 0.39% v/w. The essential oil from *Curcuma heyneana* rhizome had characteristic in liquid form, clear yellow color, distinctive smell and bitter taste.

Larvicidal Activity Testing

The ethanol extract and the essential oils were subjected to laboratory bioassay study against *Aedes aegypti*. The extract from *Curcuma heyneana* rhizome demonstrated that the mortality average of larvae for all concentration were the same and no significant larvae mortality for 24 hours. This causes the LC_{50} and LC_{90} values cannot be calculated because of the slope of the curve was 0.00 ± 0.224. In addition, the LC_{50} and LC_{90} values were greater than 100 µg/mL, so the extract revealed no significant activity against *Aedes aegypti* larvae. The thing to note is that the plant extract is considered to have a significant larvicidal activity against *Aedes aegypti* larvae if the LC_{50} value is less than 100 µg/mL (Kiran et al., 2007). Nevertheless, the essential oils tested demonstrated significant larvicidal activity (LC_{50}<100 µg/ml) (Table 1 and Table 2).

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Table 1. Mortality average of *Aedes aegypti* larvae toward extract and essential oil from *Curcuma heyneana* for 24 hours

Concentration (µg/mL)	Mortality average of *Aedes aegypti* larvae	
	Sample	
	Extract	Essential oils
0	1.00 ± 1.00	0,00 ± 0.00
0.5	1.00 ± 1.00	0,00 ± 0.00
1	1.00 ± 1.00	1,00 ± 1.00
5	1.00 ± 1.00	2,00 ± 0.00
10	1.00 ± 1.00	4,00 ± 3.00
50	1.00 ± 1.00	23,00 ± 3.00
100	1.00 ± 1.00	25,00 ± 0.00

Tabel 2. Results of Larvicidal Activity Testing of extract and essential oil from *Curcuma heyneana* rhizome against *Aedes aegypti* larvae for 24 hours

Sample	LC_{50} (µg/mL)	LC_{90} (µg/mL)
Extract of *Curcuma heyneana*	-	-
Essential Oil of *Curcuma heyneana*	16.54	54.77

The larvicidal test of essential oils from *Curcuma heyneana* rhizome against *Aedes aegypti* larvae demonstrated that larvae mortality increased in concentration dependent manner for 24 hours. The larvae were incapacitated and subsequently settled at the bottom of the cups with abnormal wagging at higher concentrations and later died slowly. With the result that the essential oils demonstrated the effect of significant mortality, with LC_{50} value less than 100 µg/mL. This suggests that the essential oil has a significant larvicidal activity against *Aedes aegypti* larvae.

Further test using essential oils with a smaller concentration range was demonstrated to get a more accurate of LC_{50} value (Table 3). Based on these data, it can be demonstrated that their larvae mortality increases in concentration dependent manner for 24 hours. The results of LC_{50} and LC_{90} values of essential oils tested using Probit analysis were 35.33 µg/mL and 86.02 µg/mL, respectively. It demonstrated that essential oils from *Curcuma heyneana* rhizome had significant larvicidal activity against *Aedes aegypti* because the LC_{50} value of the essential oils was less than 100 µg/mL. Earlier authors reported that the larvicidal activity of essential oils of various medicinal and aromatic plants with LC_{50} values ranged between 9.7 µg/mL and 101.4 µg/mL against the larvae of *Aedes aegypti* (Amer et al., 2006).

Tabel 3. Mortality average of *Aedes aegypti* larvae toward essential oil from *Curcuma heyneana* for 24 hours

Concentration (µg/mL)	Mortality average of *Aedes aegypti* larvae
	Essential oils
0	0.00 ± 0.00
10	2.00 ± 1.00
20	5.00 ± 1.00
40	12.00 ± 2.00
60	16.00 ± 1.00
80	24.00 ± 1.00
100	25.00 ± 0.00

Essential Oil Analysis Using GC-MS

The GC-MS analysis was performed to identify the essential oils from *Curcuma heyneana* rhizome. The analysis results of the essential oil using gas chromatography (GC) is shown in Figure 1. To identify the chemical

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structure of each peak in the chromatogram, GC-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 (Table 4).

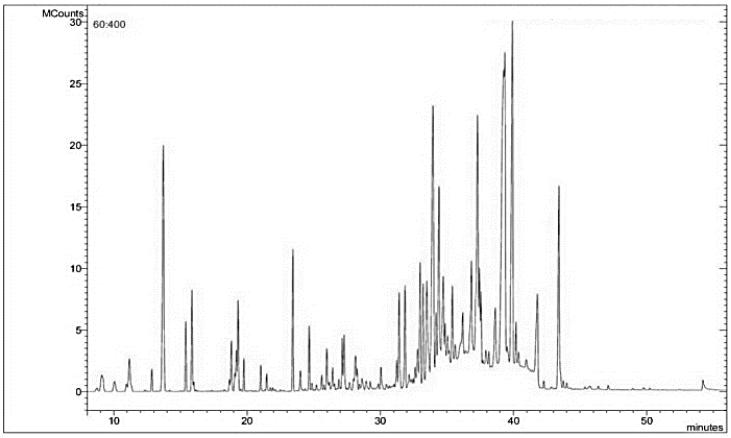

Figure 1. GC chromatogram of Essential oil from *Curcuma heyneana* Val & v. Zijp Rhizome

Table 4. Compounds of essential oil from *Curcuma heyneana* Val & v. Zijp rhizome

No	Time Retention	Compound	Molecular Formula	Molecule Weight	Percentage (%)
1	9.095	- (-) alpha-Pinene	$C_{10}H_{16}$	136	0.58
2	10.051	Camphene	$C_{10}H_{16}$	136	0.32
3	10.948	Sabinene	$C_{10}H_{16}$	136	0.16
4	11.169	2-beta-Pinene	$C_{10}H_{16}$	136	0.95
5	12.851	dl-Limonene	$C_{10}H_{16}$	136	0.35
6	13.713	1,8-Cineole	$C_{10}H_{18}O$	154	5.41
7	15.406	1-Pentatriacontanol	$C_{35}H_{72}O$	508	0.92
8	15.864	Linalyl Isobutyrate	$C_{14}H_{24}O_2$	224	1.56
9	16.000	3-Octyne, 2-methyl	C_9H_{16}	124	0.05
10	18.680	exo-methyl-Camphenilol	$C_{10}H_{18}O$	154	0.18
11	19.081	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	$C_{10}H_{18}O$	154	0.26
12	19.824	Borneol	$C_{10}H_{18}O$	154	0.81
13	20.196	1-Borneol	$C_{10}H_{18}O$	154	0.65
14	20.328	Camphor	$C_{10}H_{16}O$	152	1.32
15	20.762	alpha-Terpinenyl Acetate	$C_{12}H_{20}O_2$	196	0.39
16	22.032	1-Heptadecene	$C_{17}H_{34}$	238	0.31
17	22.474	Unknown**		204	0.24
18	23.441	beta- Elemene	$C1_5H_{24}$	204	1.94
19	24.004	alpha-Gurjunene type 1	$C_{15}H_{24}$	204	0.30
20	24.666	Caryophyllene	$C_{15}H_{24}$	204	0.89
21	24.873	gamma-Elemene	$C_{15}H_{24}$	204	0.11
22	25.210	alpha-Gurjunene type 2	$C_{15}H_{24}$	204	0.09
23	25.609	alpha-Gurjunene type 3	$C_{15}H_{24}$	204	0.20
24	25.778	Benzene, 2,4-bis(1,1-dimethylethyl)-1-methoxy	$C_{15}H_{24}O$	220	0.07
25	25.988	alpha-Humulene	$C_{15}H_{24}$	204	0.58
26	26.099	delta-Guaiene	$C_{15}H_{24}$	204	0.07
27	26.191	alpha-Gurjunene type 4	$C_{15}H_{24}$	204	0.10
28	26.428	alpha-selinene type 1	$C_{15}H_{24}$	204	0.35
29	26.566	gamma-Cadinene	$C_{15}H_{24}$	204	0.09
30	26.885	D-Germacrene	$C_{15}H_{24}$	204	0.13

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No	Time Retention	Compound	Molecular Formula	Molecule Weight	Percentage (%)
31	27.144	beta-Selinene type 1	$C_{15}H_{24}$	204	0.72
32	27.290	alpha-Selinene type 2	$C_{15}H_{24}$	204	0.89
33	27.688	alpha-Gurjunene type 5	$C_{15}H_{24}$	204	0.14
34	27.994	beta-Selinene type 2	$C_{15}H_{24}$	204	0.17
35	28.149	Torreyol	$C_{15}H_{26}O$	222	0.75
36	28.263	beta-Cadinene	$C_{15}H_{24}$	204	0.27
37	28.434	Curzerene	$C_{15}H_{20}O$	216	0.10
38	28.635	Seychellene	$C_{15}H_{24}$	204	0.26
39	28.948	Selina-3,7(11)-diene	$C_{15}H_{24}$	204	0.19
40	29.246	1s, cis-Calamenene	$C_{15}H_{22}$	204	0.16
41	30.052	gamma-Gurjunene	$C_{15}H_{24}$	204	0.38
42	30.456	3-methyl-1,2-dimethoxybenzene	$C_9H_{12}O_2$	152	0.09
43	31.240	beta-Guaiene type 4	$C_{15}H_{24}$	204	0.47
44	31.419	Agarospirol	$C_{15}H_{26}O$	222	1.57
45	31.860	(-)-Caryophyllene oxyde	$C_{15}H_{24}O$	220	1.64
46	32.173	alpha-Eudesmol	$C_{15}H_{26}O$	222	0.18
47	32.346	beta-Guaiene type 1	$C_{15}H_{24}$	204	0.10
48	32.469	Cis-1,4-dimethyladamantane	$C_{12}H_{20}$	164	0.07
49	32.623	gamma-Himachalene type 1	$C_{15}H_{24}$	204	0.28
50	32.808	beta-Guaiene type 2	$C_{15}H_{24}$	204	0.71
51	32.997	(-)-alpha-Selinene	$C_{15}H_{24}$	204	1.97
52	33.220	Selina-3,7(11)-diene type 1	$C_{15}H_{24}$	204	1.50
53	33.493	beta-Guaiene type 5	$C_{15}H_{24}$	204	2.55
54	**33.956**	**Isocurcumenol type 1**	**$C_{15}H_{22}O_2$**	**234**	**7.68***
55	34.195	Selina-3,7(11)-diene type 2	$C_{15}H_{24}$	204	1.11
56	34.414	beta-Eudesmol	$C_{15}H_{26}O$	222	3.73
57	34.507	beta-Guaiene type 3	$C_{15}H_{24}$	204	0.20
58	34.727	Curzerenone	-	-	2.33
59	34.862	Spathulenol type 1	$C_{15}H_{24}O$	220	0.68
60	35.060	alpha-Santalol	$C_{15}H_{24}O$	220	0.68
61	35.175	gamma-Himachalene type 2	$C_{15}H_{24}$	204	0.21
62	35.412	Spathulenol type 2	$C_{15}H_{24}O$	220	1.21
63	35.627	Spathulenol type 3	$C_{15}H_{24}O$	220	0.28
64	36.191	Unknown**			1.18
65	36.847	9-beta-Acetoxy-3,5-alpha.,8-trimethyltricyclo [6.3.1.0(1,5)]dodec-3-ene	$C_{17}H_{26}O_2$	262	2.24
66	37.305	Unknown**			6.23
67	37.447	Ledenalkohol	$C_{15}H_{24}O$	220	1.39
68	37.561	1-Naphthalenamine, 4-bromotype 1	$C_{15}H_{24}O$	220	0.92
69	37.926	Curdione	$C_{15}H_{24}O_2$	236	0.28
70	38.149	1-Naphthalenamine, 4-bromo- type 2	$C_{15}H_{24}O$	220	0.21
71	38.633	Unknown**			1.52
72	**39.263**	**11H- [1] Benzopyrano[4,3-b] indol-6-one type 1**	**$C_{15}H_9NO_2$**	**235**	**11.47***
73	39.356	11H- [1] Benzopyrano[4,3-b] indol-6-one type 2	$C_{15}H_9NO_2$	235	5.47
74	39.533	Unknown**			0.14
75	**39.923**	**11H- [1] Benzopyrano[4,3-b] indol-6-one type 3**	**$C_{15}H_9NO_2$**	**235**	**8.11***
76	40.187	Naphthalene, 1,2,3,4-tetrahydro-1-methyl-8-(1-methylethyl)-	$C_{14}H_{20}$	188	0.63
77	40.381	3,8-alpha-Furanether	$C_{15}H_{20}O_2$	232	0.27
78	40.965	Unknown**			0.19
79	41.799	Unknown**			2.41

No	Time Retention	Compound	Molecular Formula	Molecule Weight	Percentage (%)
80	42.277	Tetraisopropylidene-cyclobutane	$C_{16}H_{24}$	216	0.11
81	43.415	Isocurcumenol type 2	$C_{15}H_{22}O_2$	234	4.21
82	43.749	Benzofuran, 7-cyclohexyl-2,3-dihydro-2-methyl-	$C_{15}H_{20}O$	216	0.10
83	43.999	Cyclohexanone, 2-[(4-methoxyphenyl) methylene]-	$C_{15}H_{22}O_2$	234	0.08
84	54.227	Di(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	370	0.22

Note: * The three major compounds; ** unidentified compounds

At least a total of 84 essential oil compounds were separated by using gas chromatography and 77 compounds were identified by mass spectrometry. The three major compounds were 11H-[1] Benzopyrano [4,3-b]indol-6-one type 1 (11.47%), 11H-[1] Benzopyrano [4,3-b]indol-6-one type 3 (8.11%) and Isocurcumenol type 1 (7.68%). It was observed that the isolated essential oil from *Curcuma heyneana* possessed remarkable larvicidal properties. The essential oil generally consists of complex mixtures of monoterpenes, phenols and sesquiterpenes. According to the research that has been demonstrated by Zhu et al. (2008), β-eudesmol (3.73%) was the ingredient compound which had larvicidal activity against *Aedes aegypti*.

CONCLUSION

The results showed that the extract from *Curcuma heyneana* was not significantly demonstrated the larvicidal activity, whereas the essential oil significantly demonstrated the larvicidal activity with LC_{50} and LC_{90} values of 35.33 µg/ml and 86.02 µg/ml, respectively (LC_{50}<100 µg/ml).

The analysis results of essential oil from *Curcuma heyneana* rhizome using GC-MS showed at least 84 compounds separated and three major compounds were 11H-[1] Benzopyrano [4,3-b] indol-6-one type 1 (11.47%), 11H-[1] Benzopyrano [4,3-b] indol-6-one type 3 (8.11%) and Isocurcumenol type 1 (7.68%). Further studies are need to identify the active compounds from the essential oils that have larvicidal activity and devise the formulation using the oil and the compounds of this plant for use as larvicides in *Aedes aegypti* mosquito control programs.

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