ISOLATION AND LARVACIDAL ACTIVITY OF ESSENTIAL OIL MANGO TURMERIC RHIZOMES (Curcuma mangga, Val.) AGAINST LARVAE OF Aedes aegypti MOSQUITOES ALSO GAS CHROMATOGRAM-MASS SPECTROMETRY PROFILE

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ISOLATION AND LARVACIDAL ACTIVITY OF ESSENTIAL OIL MANGO TURMERIC RHIZOMES (*Curcuma mangga*, Val.) AGAINST LARVAE OF *Aedes aegypti* MOSQUITOES ALSO GAS CHROMATOGRAM-MASS SPECTROMETRY PROFILE

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Abstract

Background. *Curcuma mangga.,Val has been recognized by society as traditional drug for a long time. The active ingredience of this plant especially the volatile oil contains monoterpenes and sesquiterpenes. This research aim to know about the larvicide's activity from the volatile oil of Curcuma mangga., Val rhizome and also to analyse its chemical compounds using the GC-MS.*

Method. *The volatile oil was isolated from Curcuma mangga, Val with steam and water distillation. The test method for larvicide was done by dissolving the substances into water and added Tween 20 10 % v/v to the the volatile oil. The consentration of the volatile oil of Curcuma mangga,Val rhizome used by these are 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm. The concentration of positive control "abate" are 0,01 ppm; 0,025 ppm; 0,05 ppm; 0,1 ppm; 0,5 ppm while as the negative control is Tween 20 solution. Larvae of Aedes aegypti amount of 10 larvaes for the each consentration. However, the perception time during 24 hours.*

Result. *Data of larvae's died were used to estimate the values of LC 50 with the probit analysis method. The chemical compound of Curcuma mangga., Val can be analysed by the GC-MS. The results of research showed that the volatile oil of Curcuma mangga, Val. have clear-brass colour, bitter taste, typically aromatic like mango, rendemen equal to (1,23 ± 0,029)% v/b and refractive index 1,4881. The value of LC50 for the volatile oil of Curcuma mangga, Val. rhizome is (216,17 ±12,51) ppm while abate equal to (0,072 ± 0,024) ppm. This matter indicate that abate more potent to larvae of Aedes aegypti. The result of analyse the component of the volatile oil of Curcuma mangga, Val., by the GC-MS obtained of 30 peak chromatogram and six peak which has identified showed the possibility the existence of alpha-pinene, camphene, beta-pinene, beta-myrcene, eukalyptole, ar-turmerone.*

Key words : *larvacidal activity, GC-MS, Curcuma mangga, Aedes aygypti mosquitoes*

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INTRODUCTION

Dengue fever is a health problem in Indonesia, because the Indonesian geographical conditions are very supportive for mosquitoes to live and breed. Some researches show that Dengue Hemorrhagic Fever (DHF) has been found in the all provinces in Indonesia. Two hundred cities have reported an Extraordinary Events. The number of incidence increased from 0.005 per 100,000 population in 1969 and jumped drastically to 627 per 100,000 population (Satari and Meiliasari, 2004).

Dengue virus as a cause of hemorrhagic fever can only be transmitted by mosquitoes. *Aedes aegypti* mosquitoes are the most often caused outbreaks of dengue. (Satari and Meiliasari, 2004). One of the methods for disease prevention is patient care in the form of vector control. It was caused that the killing dengue virus agent have not been found yet until now. Vector eradication has carried out with or without insecticide. Controlling with insecticides both against adult and larval mosquitoes should use "organophosphate" to avoid environmental pollution. But over the last 40 years, the chemical agents have been used extensively. As a result, *Aedes aegypti* and the other dengue vector in some countries have become resistant to common insecticides, including temephos, malathion, fenthion, permethrin, propoxur and fenithroin (Anonymous, 1999). In addition, the presence of insecticides in human blood accumulates in the long periods and become source of killing disease and carcinogenic.

Departing from the fact above, it would require the intensive research to find the appropriate method, which is more effective in view of the environment, biology and chemistry, particularly looking at the safety, efficacy and also environmental friendly.

One of the natural ingredients that are widely used in the community is mango turmeric, such as anti-inflammatory, anticancer, lowering cholesterol and etc. (Anonymous, 2004). Chemical constituents in mango turmeric contains volatile oil, starch, tannin, sugar, resins, kurdion, curcumin. Mango turmeric essential oil-containing components such as monoterpenes (97, 46%). Monoterpenes that contains in the essential oil of mango turmeric, are myrcene (84.61%, phelladrene (6.63%) and trans-ocimene (3.85%) (Makboon, et al., 2004).

Based on research carried out have proved the larvacidal activity of essential oils. Some terpene compounds such as farnesol, farnesenat acid and 9-oxo-2 decanoat can inhibit skin turnover of *Aedes aegypti* larvae. While the essential oils of neem seed cause mortality of instar IV larvae of *Aedes aegypt*i and Culex quinquefasciatus and inhibit the development of the pupa into adulthood (Nugroho, 1997). So the possibility of essential oils of mango turmeric has larvasida activity. Based on this evidence, it is necessary to examine about the larvaside activity of essential oil's mango turmeric rhizome against larvae of *Aedes aegypti.*

RESEARCH METHODS

A. Tools and Materials

The materials were used in this study : turmeric rhizome mango (from the subdistrict Minggir, Kulonprogo), *Aedes aegypti* mosquito larvae instar IV (from the laboratory of Parasitology Faculty of Medicine Gadjah Mada University), distilled water, essential oil of mango turmeric rhizome , sodium anhydrous sulfate pro analysis, Abate 1G, Tween 20 solution.

The tools were used in this study: a set of tools isolation of essential oils, ABBE refractometer, Stahl distillation, petri dishes, flacon, range in size of pipette volume, pipette drops, timekeeper, liquid chromatography mass spectroscopy GC-MS Shimadzu QP 5000 with computer systems.

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Research Procedures

1. Plant Determination.

Determinations were carried out using the book Flora of Java written by Backer and Van den Brick, 1968).

2. Materials Collection and Preparation

The mango turmeric rhizome was taken from the sub-district Minggir, Kulonprogo, Yogyakarta in January 2005. Then the rhizome was collected and washed from the groins which may be attached. Afterthat the rhizome was sliced transversely with a thickness of 2 mm to 4 mm. The slices are then dried in the mattress. The dried rhizomes was ready distilled.

3. Isolation the essential oil of mango turmeric rhizomes

The resulting of simplicia was put in the basket-shaped, then inserted into the steamer. The boiler was filled with water to a surface, which closely from the bottom of the distillation. The process of distillation was performed for ± 6-8 hours, so the water vapor and volatile oil would flowed through the coolant to condense. From the condensation is forming two liquid layers, the essential oil (top) and water (bottom). After the distillation process is complete, so that the essential oils were obtained by separating water. If there is still water in the oil then added anhydrous Na_2SO_4 and then measured the volume and collect in the dark, tightly sealed to against light.

4. The determination of volatile oil content.

The volatile oil's concentration of mango turmeric rhizome was done by stahl distillation. Rhizome that has chopped, then weighed as much as 10 grams and incorporated into the stahl distillation flask and add water until completely submerged. Rhizome simmer until the distillate is obtained. The rendement of essential oils was calculated on the dry rhizome in % v / b. The determination was done by 3 times replication.

5. The essential oil's physical properties of mango turmeric rhizome

a. The organoleptic test.

The examination was conducted on the color, smell and taste.

b. The refractive index test.

The examination of refractive index using Abbe refractometer. Prism is cleaned with acetone and dried, then the essential oil is dripped and the screw rotated such that the dark areas and bright areas are divided into two equal parts horizontally which can be viewed through a telescope. By looking at the scale, we could read the refractive index's value of the essential oils. After finishing, the prism was cleaned.

6. The larvasidal activity test.

The instar IV larvae of *Aedes aegypti* mosquito was obtained from the laboratory of Parasitology Gadjah Mada University. For about 30 pot ointment were used to place media with variation of concentration for each sample test and the number of 10 larvae with 5 times replication. The larval mortality was observed at minute -10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 270, 300 to 24 hours observations by calculating the percentage of larval mortality. The concentration of each sample as follows:

a. Essential oil concentration (ppm): 100, 150, 200, 250, 300, 350

b. Abate concentration (ppm): 0.5, 0.1; 0.05, 0.025; 0.01

7. Analysis of data.

The data was obtained by calculating the cumulative number of dead larvae in each pot ointment for 24 hours observation. Then calculated the average number of cumulative of dead larvae to determine the percentage of response (larvae mortality) of the test preparation. The percentage of response then converted into probit values corresponding to the probit table.

Percent of death =

$$\frac{\textit{average mortality in the test group x 100 \%}}{\textit{The number of test animals}}$$

Furthermore, the concentration's data of the sample is transformed in the form of logarithms and the percentage of concentration was converted to probit value, then made a linear regression curve equation between the logarithm of the concentration as "x" value and the probit as "y" values.

The value of LC50 was obtained by plotting the number of 5 as "y" value in equation (Mursyidi, 1982). The value of LC50 are set with 95% confidence limit (Meyer et al., 1982).

8. Gas Chromatography-Mass Spectrometry

The GC-MS instrument is a combination of two tools, namely gas chromatography (GC17A) and mass spectrometry (QP-5000). The tool is operated entirely by computer.

RESULTS AND DISCUSSION

A. Results of Plant Determination.

The results of mango turmeric rhizome plants determination as follows:

1b-2b-3b-4b-12b-14b-17b-18b-19b-20b-21b-22 b-23b-24b-25b-26b-27a-28b-29b-30b-31a-32a-33a-34b-333b-334b-335a-336a-337b-338a-339 b-340a (Zingiberaceae)
1a-2b-6b-7a (Curcuma)
1b-4a-5b (*Curcuma mango*, Val.) (Backer and Van den Brink, 1968)

Based on the plant's determination above, we can conclude that the plants used in this study have proved as mango turmeric plant (*Curcuma mango*, Val.)

B. The isolation of essential oil's mango turmeric rhizome

The steam distillation of the 10 grams mango turmeric rhizomes have produced an rendement average yield of 1.23 ± 0.029% v / b. The organoleptic of essential oil's mango turmeric rhizome was clear yellowish color, slightly bitter taste, and smell like a typical aromatic mangoes. While the results of measurements of the refractive index from the essential oil of mango turmeric rhizome was performed by the ABBE refractometer is 1.4881 at a temperature of 22.3 ^{o}C.

C. The larvacidal activity test.

The instar IV larvae of *Aedes aegypti* mosquito in this study were obtained from the Medical Parasitology Laboratory of Gadjah Mada University. Because of the essential oil's of mango turmeric was not soluble in water as test medium, so it needs to be added tween 20 10% v / v of oil's volume. Proved that tween 20 as a negative control was not related with the mortality of larvae of *Aedes aegypti.* Whereas the positive control used is abate.

The early stages of this study was to determine the lowest concentration of the test preparation which causes 100% mortality of test animals and the highest concentration which not causes of 100% mortality of test animals. Furthermore, it was made of the concentration levels of concentration are sorted from the lowest to the highest concentration set as the test preparation. The concentration of each test preparation are as follows:
Essential oil concentration (ppm) : 100: 150: 200: 250: 300: 350
Abate concentration (ppm): 0.5, 0.1; 0.05, 0.025; 0.01

In this study, the number of larvae used as much as 10 larvae and 5 times replication for each treatment with a volume of 50 ml of test media. The cumulative's number of larval mortality was observed at minute 10, 20, 30, 40, 60, 90, 120, 150, 180, 210, 240, 270, 300 and the last 24 hours . The larval mortality is

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characterized by increased of motor activity, reduced, and died quietly. The percentage of cumulative mortality was obtained by the probit value using probit table. Then from these data was made linear regression equation between log concentration (x) with the probit (y) is given by y = bx + a. LC50 was obtained by plotting the probit value "5" into the equation, antilog x is the LC50.

Prior to further calculations using the regression equation, is necessary to determine whether there is a significant linear correlation between two variables. This can be done by calculating the correlation coefficient r or correlation test. The value of "r" theoretical (criticism) was calculated at 95% probability level. If the "r" value was calculated from a series of data is smaller than the "r" theoretical then there is no significant relationship between variables x and y, so no need for further regression analysis. But if the "r" value was greater than the "r" theoretical, then there is a significant correlation between x and y. It means that regression analysis can be carried further.

Furthermore, the number of 5 from the y-axis was drawn a straight line which intersecting the linear line to the eye point and then from that point on a straight line was drawn down the x- axis to obtain a cut point. The LC50 is the antilog n.

The LC_{50} of each treatment was calculated on average, the value was obtained by (0.072 ± 0.024) ppm and designated as LC_{50} abate.

The value of LC_{50} at each treatment was calculated on average, the value was obtained at (216.17 ± 12.51) ppm and set as LC_{50} of mango turmeric essential oils against mosquito larvae of Aedes aegypti.

From the results, it can be seen that abate has a LC_{50} of 0.072 ppm was smaller than the LC_{50} essensial oils of mango turmeric rhizome. Abate where it is known as a potent larvasida. Abate can cause larval mortality due to organophosphate insecticides containing phosphorotioic acid, o, o'-(thiodi-4, 1 - phenylene) bis (o, o'-dimethyl) phosphorothioate. Organophosphate class of this pesticides can bind to the enzyme cholinesterase In the body, this cholinesterase enzyme have benefits a balancing regulator of acetylcholine in the central nervous system. If the enzyme is

Table I. The value of LC $_{50}$ at each positive control treatment (abate) for 24 hours

Treatment	Linear Regression Equation	"r" calculatical	"r" theoritical	LC50
1.	Y = 3,5216x + 9,262	0,9869	0,8783	0,06
2.	Y= 2,9900x + 8,357	0,9591	0,8783	0,08

Table II. The value of LC_{50} at each mango turmeric essential oil for 24 hours

Treatment	Linear Regression Equation	"r" calculatical	"r" theoritical	LC50
1.	Y= 7,8072x - 13,316	0,9627	0,8114	221,82
2.	Y = 7,0849x - 11, 743	0,9989	0,7545	230,67
3.	Y = 7,2110x - 11,539	0,9977	0,8114	196,79
4.	Y = 8,6223x - 15,159	0,9920	0,8114	217,77

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bound by these pesticides, the concentration of acetylcholine becomes uncontrolled and also affect several other neurotransmitters. Thus the nerve activity becomes disturbed, resulting in uncontrolled muscle movements. Finally there is a thorough spasms, followed by fainting and deathing. However, it turns out that mango turmeric rhizome essential oils also have larvacide activity against mosquito larvae of *Aedes aegypti*. This can cause by the presence of toxic compounds contained in essential oil or may be affected by emulsion's medium test that reduce the larvae's ability to get oxygen. From the observation's result of larval mortality was not preceded by a state of seizures as abate, only that the larvae begins to be inactive and dead silently.

D. The component analysis of essential oil mango turmeric rhizome with GCMS.

Table III. The component analysis result of essential oil mango turmeric rhizomes was seen in below

No.	Peak to-	Time retention	% relative concentration	Estimated component
1.	1	3,575	2,92	Alpha-pinene
2.	2	3,775	0,27	Camphene
3.	3	4,308	17,92	Beta-pinene
4.	4	4,792	57,69	Beta-myrcene
5.	6	5,433	2,98	Eucalyptol
6.	24	15,817	2,75	Ar-turmerone

The possibility of compounds that can cause mortality in larvae of Aedes aegypti is the alpha-pinene, beta-pinene and beta-myrcene. According to Duke (1992) these compounds can act as an insecticide. The existence of these insecticides agent into the body of *Aedes aegypti* larvae can result in impaired metabolism that allows the emergence of toxic effects of seizures, paralysis, faint and death. In addition, the influence of test media in the form of an emulsion can reduce the chance of larvae to obtain oxygen. Though, oxygen supply is essential for energy production in the body of *Aedes aegypti* larvae. Finally, the larvae eventually die. But do not close the possibility of other toxic chemical agent other than that detected in the essential oil of mango turmeric rhizome that acts as a larvaside.

CONCLUSION

From the research we can conclude:

1. The LC50 of mango turmeric rhizome essential oil (12.51 ± 216.17) ppm and the LC50 of abate by (0.072 ± 0.024) ppm. This means that the larvaside activity of abate greater than mango turmeric rhizome.

2. The GC-MS analysis of essential oil's mango turmeric rhizomes (*Curcuma mangga*, Val.) was estimated to contain the compounds alpha-pinene, camphene, beta-pinene, beta-myrcene, eucalyptol, ar-turmerone.

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