

ISOLASI DAN AKTIVITAS LARVACIDA MINYAK ATSIRI AKAR (*Curcuma mangga*, Val.) TERHADAP LARVA *Aedes Aegypti* DAN PROFIL GC-MS NYA

ISOLATION AND LARVACIDAL ACTIVITY OF ESSENTIAL OIL MANGO TURMERIC RHIZOMES (*Curcuma mangga*, Val.) AGAINST *Aedes Aegypti* LARVAE AND GC-MS PROFILE

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Abstract

Curcuma mangga, Val has been recognized as a traditional drug since a long time ago. The active ingredients of this plant especially the volatile oil contains monoterpenes and sesquiterpenes. This research was aimed to study about the larvacide's activity of the volatile oil *Curcuma mangga*, Val rhizome and also to analyze its chemical compounds using the GC-MS. The volatile oil was isolated from *Curcuma mangga*, Val with steam and water distillation. The test method for larvacide was done by dissolving the substances into water and added by Tween 20 10 % v/v to the volatile oil. The concentration of the volatile oil of *Curcuma mangga*, Val rhizome were 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, and 350 ppm. The concentration of positive control "abate" were 0,01 ppm; 0,025 ppm; 0,05 ppm; 0,1 ppm; 0,5 ppm while as the negative control was Tween 20 solution. The perception time of larvae's mortality was 24 hours. The chemical compound of *Curcuma mangga*, Val can be analysed by the GC-MS. Data of larvae's mortality were used to estimate the values of LC_{50} with the probit analysis method. This study showed that the volatile oil of *Curcuma mangga*, Val. have clear-brass colour, bitter taste, typically aromatic like mango, rendement equal to $(1,23 \pm 0,029)\%$ v/b and refractive index 1,4881. The value of LC_{50} for the volatile oil of *Curcuma mangga*, Val. rhizome is $(216,17 \pm 12,51)$ ppm while abate equal to $(0,072 \pm 0,024)$ ppm. This finding indicates that abate were more potent to larvae of *Aedes aegypti*. The GC-MS shows 30 peaks chromatogram and six peaks which indicates the possibility of alpha-pinene, camphene, beta-pinene, beta-myrcene, eukalyptole, ar-turmerone presence.

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

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, since the treatment for killing the dengue virus has not been found yet. The 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. In addition, the presence of insecticides in human blood will accumulates in the long periods and become source of killing disease and carcinogenic (Anonim, 1999).

Because of the fact above, it would be required the intensive study 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 eco friendly.

One of the natural ingredients that are widely used in the community is mango turmeric, such as anti-inflammatory, anticancer, and lowering cholesterol. The chemical constituents in mango turmeric contains volatile oil, starch, tannin, sugar, resins, kurdion, curcumin. The 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).

Some previous studies have proved the larvacidal activity of essential oils. The essential oils of 41 plants were evaluated for their effects against third-instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. At first, the oils were surveyed against *A. aegypti* using a 50-ppm solution. Thirteen oils from 41 plants (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum and sandalwood) induced 100% mortality after 24 h, or even after shorter periods. The best oils were tested against third-instar larvae of the three mosquito species in concentrations of 1, 10, 50, 100 and 500 ppm. The lethal concentration 50 values of these oils ranged between 1 and 101.3 ppm against *A. aegypti*, between 9.7 and 101.4 ppm

for *A. stephensi* and between 1 and 50.2 ppm for *C. Quinquefasciatus* (Amer & Mehlhorn, 2006).

Other research also proved that some terpene compounds such as farnesol, farnesat acid and 9-oxo-2 decanoat can inhibit skin turnover of *Aedes aegypti* larvae. While the essential oils of neem seed can cause the mortality of instar IV larvae of *Aedes aegypti* and *Culex quinquefasciatus* and inhibit the development of the pupa into adulthood (Nugroho, 1997). It is possible that the possibility of essential oils of mango turmeric has larvacide activity. So far, the larvacide activity of *Curcuma mangga*, Val essential oil have not been examined yet. Based on the evidence above, it is necessary to examine about the larvaside activity of essential oil's mango turmeric rhizome against larvae of *Aedes aegypti*.

METHODS

A. Tools and Materials

The materials used in this study were : 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, and Tween 20 solution.

The tools used in this study were : a set of tools isolation of essential oils, ABBE refractometer, Stahl distillation, petri dishes, flacon, range in size of pipette volume, pipette drops, timekeeper, and liquid chromatography

mass spectroscopy GC-MS Shimadzu QP 5000 with computer systems.

B. Research Procedures

1. Plant Determination.

Determinations of *Curcuma mangga*, Val 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 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 \pm 6-8 hours, so the water vapor and volatile oil would flowed through the coolant to condense. There were two liquid layers from the condensation, the essential oil (top) and water (bottom). After the distillation process was complete, the essential oils were obtained by separating water. If there was still water in the oil then added anhydrous Na_2SO_4 and measured the volume, collect in the dark, and tightly sealed to against light.

4. The determination of volatile oil content.

The volatile oil's concentration of mango turmeric rhizome was defined by stahl distillation. Rhizome that has been chopped, then weighed as much as 10 grams and incorporated into the stahl distillation flask and add water until completely submerged. 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 was performed using Abbe refractometer. The prism was cleaned with acetone and dried, then the essential oil was dripped and the screw rotated such that the dark areas and bright areas were 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. 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. The percentage of response of the preparation was calculated using average number of cumulative larvae's mortality. The percentage of response then converted into probit values corresponding to the probit table.

$$\text{Percent of death} = \frac{\text{average mortality in the test group} \times 100\%}{\text{The number of test animals}}$$

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 is unnessecar I. 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 LC_{50} is the antilog n.

The value of LC_{50} was obtained by plotting the number of 5 as "y" value in equation (Mursyidi, 1982). The value of LC_{50} 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 were:

1b-2b-3b-4b-12b-14b-17b-18b-19b-20b-21b-22b-23b-24b-25b-26b-27a-28b-29b-30b-31a-32a-33a-34b-333b-334b-335a-336a-337b-338a-339b-340a

(Zingiberaceae)

1a-2b-6b-7a (Curcuma)

1b-4a-5b (*Curcuma mangga*, 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 mangga*, Val.)

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

The steam distillation of the 10 grams mango turmeric rhizomes have produced a rendement average yield of $1.23 \pm 0.029\%$ 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 °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. 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.

Table I. The LC₅₀ Value of Control Treatment (Abate) for 24 Hour

Treatment	Linear Regression Equation	"r" calculational	"r" theoretical	LC ₅₀
1.	$Y = 3,5216x + 9,262$	0,9869	0,8783	0,06
2.	$Y = 2,9900x + 8,357$	0,9591	0,8783	0,08
3.	$Y = 4,2525x + 10,359$	0,9736	0,8783	0,05
4.	$Y = 2,0138x + 6,907$	0,9961	0,81114	0,11
5.	$Y = 3,5216x + 9,352$	0,9999	0,8783	0,06
$\bar{x} = 0,072 \pm 0,024$				

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 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. The value of LC₅₀ at each positive control treatment (abate) for 24 hours is showed in Table I.

The LC₅₀ of each treatment was calculated on average, the value was obtained by (0.072 ± 0.024) ppm and designated as LC₅₀ abate. The value of LC₅₀ at each manggo turmeric essential oil for 24 hours is showed in Table II.

Table II. The LC₅₀ Value of m-Manggo Turmeric Essential Oil s Treatment

Treatment	Linear Regression Equation	"r" calculational	"r" theoretical	LC ₅₀
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
5.	$Y = 8,4246x - 14,629$	0,9993	0,8114	213,80
$\bar{x} = 216,17 \pm 12,51$				

Table III. The Component Analysis Result of Essential Oil Mango Turmeric Rhizomes is Shown

Peak to-	Time retention	% relative concentration	Estimated component
1	3,575	2,92	Alpha-pinene
2	3,775	0,27	Camphene
3	4,308	17,92	Beta-pinene
4	4,792	57,69	Beta-myrcene
6	5,433	2,98	Eucalyptol
24	15,817	2,75	Ar-turmerone

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*.

This study found that that abate has LC_{50} of 0.072 ppm which smaller than the LC_{50} essential oils of mango turmeric rhizome. Abate, as potent larvacide, can cause larval mortality due to organophosphate insecticides containing phosphorothioic 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 benefit as a balancing regulator of acetylcholine in the central nervous system. If the enzyme is 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 through spasms, followed by fainting and deathing (Isman, 2006).

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. This study found that 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.

The component analysis result of essential oil mango turmeric rhizomes is showed in Table III.

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.

The application of this study in real field indicate that the plant-based compounds such as alpha-pinene, beta-pinene and beta-myrcene may be an effective alternative to conventional synthetic insecticides for the control of *Aedes aegypti* larvae (Isman, 2006). The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn will increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme, they could lead to development of new classes of possible safer insect control agents.

Furthermore, it is needed to do some studies such as mode of action and synergism with the biocides under field condition.

CONCLUSION

It is concluded that the LC_{50} of mango turmeric rhizome essential oil was (12.51 ± 216.17) ppm and the LC_{50} of abate was (0.072 ± 0.024) ppm. This

means that the larvaside activity of abate greater than mango turmeric rhizome. Furthermore, the GC-MS analysis found that the essential oil's mango turmeric rhizomes (*Curcuma mangga*, Val.) contained alpha-pinene, camphene, beta-pinene, beta-myrcene, eucalyptol, ar-turmerone.

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