LARVACIDAL ACTIVITY OF EXTRACT AND ESSENTIAL OIL OF CURCUMA MANGGA RHIZOME (*Curcuma mangga* VAL.) AGAINST LARVAE*Aedesaegypti*

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) causedby Aedesaegypti is a pestilentdisease. Many people commonlyuselarvacidefromthehazardous syntheticmaterials for preventingthis disease.In this study, larvacidal activity of ethanolextractandessential oil of Curcuma mangga rhizome was investigated.Test solutions prepared by diluting each extract and essential oil in order to obtain100 µg/mL, 50 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL and 0.5 µg/mLconcentrations in 100 ml water.Larvae Aedesaegyptiinstar 3-4 were used in this study.The results of larvacidal test showed that the extract of Curcuma mangga rhizome was not significantly performed the larvacidal activity, whereas the essential oil of Curcuma mangga significantly performed the larvacidal activity with the values of LC_{50} as 61.025 µg/mL.

***Key words**: Curcuma mangga, larvacidal, Aedesaegypti*

INTRODUCTION

Dengue fever is a tropical disease that is the most widely reported in more than 100 countries and 2.5 billion people living in endemic areas of dengue. The Indonesian archipelago that consists of thousands of small islands and five major islands are also experiencing a dry season and the rainy season with a transition period generally in September. During this transition period, wells and puddles are found everywhere and are beneficial to the life cycle of the mosquitoes. The increased distribution of*Mosquito Aedesaegypti* populations leading to increased cases of dengue fever (Juniarti et *al.,* 2011).

One effort to break the chain of mosquitoes is by way of vector control using insecticides. The controlling of mosquitoes as the disease vector can use several methods, i.e. the environmental, biological, and chemicalmethods. The most commonly used controlling method by the public is the chemical method, such as the use of temephos as larvacide (Brown, 1975).

Temephos 1% (Abate®) is a larvacidedesignated as part of a program to eradicate Larvae *Aedesaegypti* in Indonesia that has been used for 30 years. This long period of usage has triggered a drug resistance. Resistance of *Larvae Aedesaegypti*against temephos has been reported in several countries such as Brazil, Bolivia, Argentina, Venezuela, Cuba, Caribbean, and Thailand (Felix, 2008). Moreover, the resistance of *Larvae Aedesaegypti*against temephoshas been reported in Surabaya (Soegijanto, 2006).

Interestin discovering, developing and using the insecticides that are natural, readily available, effective, and safe for the human body and the environment had stimulated rationale for doing research. The research is intended as an attempt to obtain natural insecticidesto replace the use of chemical insecticides.

Some of the natural compounds found in plants known to have larvacidalactivity are flavonoids, saponins and tannins. Components of essential oils such as *camphor*(Amer and Mehlhorn, *2006), β-eudesmol,* and *tumerone*are affirmed to cause the death of 100% of larvae of *Aedesaegypti*instar 3 after 24 hours or less (Zhu et al., 2008).

Research conducted by Sofian in 2010 showed that some plants of Zingiberaceaefamily are known to have larvacidal activity. Curcuma Mangga*(Curcuma mangga* Val.) is also an example of a plant that comes from the Zingiberaceae family. Curcuma Manggais rich in chemical compounds such as tannins, saponins and flavonoids (Ahmad, 2009), as

well as having the components of chemical compounds of essential oil i.e. *myrcene, β-osimene, β-pinene and α-pinene* (Gusmaini et al., 2004).

Therefore further research on the larvacidal activity of extract and essential oils of Curcuma mangga rhizome against *Aedesaegypti.*

MATERIALS AND METHODS

Plants:

Simplicia and Curcuma mangga rhizomeobtained from plantation of Research Institute for Medicinal and Aromatic Plants (BALITTRO), Manoko, District Lembang, West Bandung regency.

Chemicals:

Chemicals used in this study consist of 95% ethanol, distilled water, amyl alcohol, ammonia, hydrochloric acid 2 N, gelatin solution 1%, potassium hydroxide 5%, chloroform, magnesium powder, iron (III) chloride, *Dragendorff* reagent,*Liebermann Burchard* reagent,*Mayer* reagent and sulfuric vanillin reagent.

Larvae Aedes aegypti

Larvae Aedesaegypti Instar 3-4 hatched from the eggs *of Aedesaegypti* obtained from Loka Research and Development of Animal Sourced Disease Eradication (LokaLitbangP2B2), Ciamis District.

Methods

Preparation of Materials and Plant Determination

Preparation of materials consists of collectionof material, determination of plants and materials processing. The Manggarhizome plants were then determined in the Laboratory of Plant Taxonomy Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran. Simplicia was obtained in a dry state and already powdered. The fresh rhizome of Curcuma Manggawas sorted well (wet sorting), cleaned, peeled and chopped.

Extraction

A total of 160 grams of simplicia Curcuma manggarhizome was macerated with ± 800 mL of ethanol 95% for 24 hours. The macerat was contained and its pulp was re-macerated twice consecutivelyin 24 hours each. All of the macerateswere combined, then the solvent was evaporated using a rotary evaporator at a temperature of 50 ° C. The formed viscous extract was then evaporated on *water bath* at a temperature of 50 ˚ C until the constant weight of extractwas obtained.

Isolation of Essential Oils

A total of ± 250 g rhizome of Curcuma manggawas chopped and put into 1000 mL round flask which had previously been given a boiling stone. Distilled water was included approximately ¾ of flask until fully submerged rhizomes, then the flask coupled to the distillation equipment. The distillation was performed for approximately 6 hours / day and 15 times for each plant. The content of essential oil is measured in% v / b.

Phytochemical screening

Phytochemical screening includes the test of compounds of alkaloids, polifenolat, tannins, flavonoids, monoterpenoid and sesquiterpenoids, steroids and triterpenoids, quinones, and saponins.

Larvacidal activity test

The test solutions for essential oils was made from dilution of essential oils 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.

Eggs of *Mosquitoes Aedesaegypti* were obtained from the *Mosquitoes*colony bred at the Laboratory of Entomology, LokaLitbang P2B2 Ministry of Health in Ciamis. The eggs were transferred into a plastic tray filled with water and allowed to stand for 4-5 days until the eggs hatched and transformed into mosquito larvae instar 3-4. These larvae will be used for larvacidaltest.

Larvacidal test protocol refers to the WHO (2005). Around 25 larvae instar 3-4were taken directly using pipette from the eggs incubation containers, and then transferred into a 250 mL plastic cup. The plastic cups had contained extracts and oils in concentrations of respectively 100 µg/mL, 50 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL and 0.5 µg/mL, with a volume of water up to 100 mL.they were stored at 25 °C for 24 hours. Each test was repeated three times. Evaluation of larval

mortality after 24 hours was made by counting the number of dead larvae. Larvae were considered to be dead when they gave no response to a given stimulus.

According to the analysis of larvacidal test data by WHO (2005), in case of mortality in the control that exceeds 20%, the experiment has to be repeated, but when the mortality in the control is 5-20%, the percent of mortality in the treatment has to be corrected by *Abbott's* equation.

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

Description :
Pr =% corrected mortality
Po =% mortality in treatment
Pc =% mortality in control
The results of larvacidal test were analyzed using the Probit analysis program to obtain *the* LC_{50} and LC_{90} of extracts and essential oils against *Larvae Aedesaegypti*.

RESULTS AND DISCUSSION

Results from Preparation of Materials and Plant Determination

Simplicia as curcuma mangga was obtained from experimental garden of Manoko, Lembang, then taken to the Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjadjaran for determination. The results showed that the plants used in this study came from the Zingiberaceaefamily with the species of Curcuma Rhizome *(Curcuma mangga* Val.).

Extraction Results

The extract of ethanol obtained from 167.75 g powder of Curcuma mangga rhizomesimplicia was 11.39 g with yield of 6.79%. Characteristicof ethanol extract of Curcuma mangga was thick, dark brown slightly reddish, distinctive smell and bitter.

Results of Essential Oil Isolation

Essential oils obtained from 3,705 g of fresh Curcuma mangga rhizomewas 6.3 mL in a concentration of 0.17% v / b. The color of obtained essential oils of curcuma mangga was pale yellow, has distinctive smell like mango and bitter.

Results of Phytochemical Screening

Results of phytochemical screening on the extract of Curcuma Mangga Rhizome showed the content of compounds of alkaloids, flavonoids, monoterpenoid and sesquiterpenoids, and quinones.

Results of Larvacidal Activity Test

The results of larvacidal activity test of extracts and essential oils of Curcuma mangga rhizome after 24 hours in a large range of concentration are presented in Table 1 below:

Table 1. Average Mortality of *Larvae Aedesaegypti* against Extract and Essential Oil of Curcuma manggarhizome for 24 Hours

No.	Concentration(µg/mL)	Average Mortality of Larvae Aedesaegypti	
		Extract	Essential oil
1	0	0.00 ± 0.00	0.00 ± 0.00
2	0.5	0.00 ± 0.00	0.00 ± 0.00
3	1	0.00 ± 0.00	0.00 ± 0.00
4	5	0.00 ± 0.00	0.00 ± 0.00
5	1.0	1.00 ± 0.00	0.00 ± 0.00
6	5.0	4.00 ± 1.00	6.00 ± 3.00
7	100	5.00 ± 0.50	24.00 ± 2.00

Data of Larvacidal activity of Curcuma manggarhizome against *Larvae Aedesaegypti*is presented in Table 2.

Table 2. Data of Larvacidal activity of Curcuma mangga rhizome against Larvae Aedesaegypti

Test Materials	LC_{50} (µg/mL)	LC_{90} (µg/mL)
Extract of Curcuma mangga rhizome	499.40	6175.10
Essential oil of Curcuma mangga rhizome	61.025	87.604

The results showed that the average mortality of larvae for all concentrations in Extract of Curcuma mangga rhizome is small, where there is no significant larval mortality within 24 hours. Extract of plants shall be considered to have a significant

larvacidal activity against *Larvae Aedesaegypti* when the LC_{50} value is less than 100 µg/mL (Maia, et al., 2007). The values of LC_{50} and LC_{90} obtained from the extract of curcuma manggarhizomewere greater than 100 µg/mL, therefore the extract of curcuma mangga rhizome was affirmed to have no significant activity against *Larvae Aedesaegypti*.

The test results of essential oils of Curcuma mangga rhizome against *Larvae Aedesaegypti* showed an increase of larval mortality consistently with the increase of concentrations within 24 hours. The values of LC_{50} and LC_{90} of the essential oils of Curcuma mangga rhizome were 61.025 µg/mL and 87.604 µg/mL. Therefore, the essential oil of curcuma manggarhizome had significant effect of mortality, with LC_{50} less than 100 µg/mL.This indicated that the essential oilof Curcuma mangga rhizome have larvacidal activity against *Aedesaegypti.*Further tests using the essential oils with lowerrange of concentration shall be needed to obtain a more accurate value of LC_{50}.

CONCLUSIONS AND RECOMMENDATIONS

Results of the larvacidalactivity test showed that there was no significant activity of the extract of Curcuma Mangga because of the value of LC_{50} which is greater than 100 µg/mL, while the essential oils of Curcuma manggashowed significantactivities because it has a value of LC_{50} less than 100 µg/mL, ie 61.025 µg/mL.

It is recommended to perform further research on active compounds which act as larvacide from Curcuma Mangga Rhizome, and it is advisable to formulate into a usable dosage form.

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