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

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ABSTRACT

Dengue Hemorrhagic Fever (DHF) caused by Aedes aegypti is a pestilent disease. Many people commonly use larvicide from the hazardous synthetic materials for preventing this disease. In this study, larvical activity of ethanol extract and essential oil of Curcuma mangga rhizome was investigated. Test solutions prepared by diluting each extract and essential oil in order to obtain 100 µg/mL, 50 µg/mL, 10 µg/mL, 5 µg/mL, 1 µg/mL and 0.5 µg/mL concentrations in 100 ml water. Larvae Aedes aegypti instar 3-4 were used in this study. The results of larvical test showed that the extract of Curcuma mangga rhizome was not significantly performed the larvical activity, whereas the essential oil of Curcuma mangga significantly performed the larvical activity with the values of LC₅₀ as 61.025 µg/mL.

Key words: Curcuma mangga, larvical, Aedes aegypti

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 Aedes aegypti 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 chemical methods. The most commonly used controlling method by the public is the chemical method, such as the use of temephos as larvicide (Brown, 1975).

Temephos 1% (Abate®) is a larvicide designated as part of a program to eradicate Larvae Aedes aegypti in Indonesia that has been used for 30 years. This long period of usage has triggered a drug resistance. Resistance of Larvae Aedes aegypti 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 Aedes aegypti against temephos has been reported in Surabaya (Soegijanto, 2006). Interest in 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 insecticides to replace the use of chemical insecticides.

Some of the natural compounds found in plants known to have larvicidal activity 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 Aedes aegypti 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 larvical activity. Curcuma Mangga (Curcuma mangga Val.) is also an example of a plant that comes from the Zingiberacea family. Curcuma Mangga is 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 *Aedes aegypti*.

**MATERIALS AND METHODS**

**Plants:**

Simplicia and Curcuma mangga rhizome obtained 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 *Aedes aegypti* Instar 3-4 hatched from the eggs of *Aedes aegypti* 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 collection of 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 consecutively in 24 hours each. All of the macerates were 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 extract was 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, polyphenols, 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 Aedes aegypti* 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 larvacidal test.

Larvacidal test protocol refers to the WHO (2005). Around 25 larvae instar 3-4 were 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.

\[ Pr = \frac{P_o - P_c}{100 - P_c} \]

Description :
\( Pr = \% \) corrected mortality
\( P_o = \% \) mortality in treatment
\( P_c = \% \) 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 \textit{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 \textit{(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 rhizomes was 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>
<thead>
<tr>
<th>No.</th>
<th>Concentration(µg/mL)</th>
<th>Average Mortality of Larvae Aedesaegypti</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>4.00 ± 1.00</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>5.00 ± 0.50</td>
</tr>
</tbody>
</table>

Data of Larvacidal activity of Curcuma manggarhizome against \textit{Larvae Aedesaegypti} presented in Table 2.

<table>
<thead>
<tr>
<th>Test Materials</th>
<th>( LC_{50} ) (µg/mL)</th>
<th>( LC_{90} ) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract of Curcuma mangga rhizome</td>
<td>499.40</td>
<td>6175.10</td>
</tr>
<tr>
<td>Essential oil of Curcuma mangga rhizome</td>
<td>61.025</td>
<td>87.604</td>
</tr>
</tbody>
</table>

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 Aedes aegypti* 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 manggarhizome were greater than 100 µg/mL, therefore the extract of curcuma manggarhizome was affirmed to have no significant activity against *Larvae Aedes aegypti*. The test results of essential oils of Curcuma manggarhizome against *Larvae Aedes aegypti* 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 manggarhizome 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 oil of Curcuma manggarhizome have larvacidal activity against *Aedes aegypti*. Further tests using the essential oils with lower range of concentration shall be needed to obtain a more accurate value of *LC*$_{50}$.

**CONCLUSIONS AND RECOMMENDATIONS**

Results of the larvacidal activity 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 manggarhizome showed significant activities because it has a value of *LC*$_{50}$ less than 100 µg/mL, i.e. 61.025 µg/mL. It is recommended to perform further research on active compounds which act as larvicide from Curcuma Mangga Rhizome, and it is advisable to formulate into a usable dosage form.

**REFERENCE**


