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Research Article

Larvacide Activity of Bungur Plants (*Lagerstroemia loudonii* T. & B.)

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ABSTRACT

The prevalence of DHF, especially in Indonesia, is still high. Dengue Hemorrhagic Fever (DHF) is a disease transmitted by the *Aedes aegypti* species mosquito vector. Therefore, reducing the incidence of DHF requires efforts to break the chain of transmission by inhibiting the growth of the *Aedes aegypti* mosquito vector. One of the plants that can inhibit the growth of *A. aegypti* mosquito larvae is the bungur plant. This study aimed to determine the larvicidal activity of ethanol extract of leaves, bark, stems, and fruit of bungur (*Lagerstroemia loudonii* T. & B.) against *A. aegypti* larvae. Extraction process used maceration method with 96% ethanol solvent. Phytochemical screening results showed that the ethanol extract of bungur leaves and fruit contains alkaloids, flavonoids, saponins, quinones, tannins, polyphenols, monoterpenoids, and sesquiterpenoids as well as steroids and triterpenoids, whereas in the ethanol extract bungur bark and stems contains alkaloids, flavonoids, saponins, quinones, tannins, polyphenols, monoterpenoids, and sesquiterpenoids. LC_{50} values of ethanol extract of leaves, bark, stems, and bungur fruit were 374.64 ± 11.88 µg/mL, 396.70 ± 3.99 µg/mL, 425.80 ± 8.15 µg/mL, and 312.54 ± 2.24 µg/mL, consecutively. The results showed that the ethanol extract of the leaves, bark, stems, and fruit of bungur could inhibit the growth of *A. aegypti* larvae. Ethanol extract of bungur fruit has the best larvicidal activity compared to other test extracts.

Keywords: Larvacide, Bungur (*Lagerstroemia loudonii* T. & B.), *Aedes aegypti*, LC_{50}

I. INTRODUCTION

Weather changes trigger various diseases that can attack the community, one of Dengue Hemorrhagic Fever (DHF). Mosquitoes cause this disease with the type of *Aedes aegypti*. In the rainy season, with high rainfall can cause an increase in mosquito growth. *A. aegypti* mosquito is the primary vector that transmits viruses that cause dengue fever. The virus is

transmitted to humans through the bite of Aedes mosquitoes that get the virus when eating infected human blood. DHF is found in many tropical and sub-tropical regions. Dengue fever is still one of Indonesia's prominent public health problems (Achmadi et al., 2010).

Larvae eradication is one way to control the vector that causes dengue fever. The use of insecticides as larvicides is the most common method used by the community to control these vectors' growth. The insecticide that is often used in Indonesia is Temephos (Daniel, 2008). The use of synthetic chemical pesticides has begun to be abandoned and turned to natural pesticides. The development of natural insecticides, readily available, and safe for the human body and the surrounding environment is becoming increasingly popular (Ndione et al., 2007).

Indonesia has various types of plants that are a source of natural insecticides that are useful for mosquito control. There have been many studies reported on plants that have potential as larvicides. Ethanol extract of castor leaves (*Ricinus communis* L.) had a larvacidal effect on *A. aegypti* larvae (Wahyu et al., 2015). The earring herbaceous ethyl acetate extract (*Acalypha indica* L.) had an LC_{50} value of 72.4435 µg / mL (Dina et al., 2015). Hexane and ethanol extract from various parts of avocado plants (*Persea americana* Mill.) Have the potential as larvicides. Hexane extract from seeds had LC_{50} and LC_{90} values of 9.82 mg/L and 22.19 mg/L, while ethanol extract from seeds with LC_{50} value of 16.48 mg/L and LC_{90} 45.77 mg/L. Stem bark ethanol extract had an LC_{50} value of 10.35 mg/L and LC_{90} 26.29 mg/L (Torres et al, 2014). There are still many other studies on plants that have activities as natural insecticides.

Bungur (*Lagerstroemia*) is one type of plant widely grown in Indonesia and can be found in teak forests, heterogeneous forests, and is often found as ornamental plants or protective trees on the roadside (Dalimartha, 2003). Bungur plants belong to the family *Lythraceae* (Cronquist, 1982) and have synonyms of *L. regi*onal Roxb., *L. flos-reginae* Retz., *L. loudonii* T. & B., and *Adambea glabra* Lamk (Hutapea, et al. 1993) Researchers who are interested in this plant and want to prove the activity of bungur plants scientifically. Research has been carried out on bungur plants with Lagerstroemia genera including *L. speciosa, L. loudonii,* and *L. macrocarpa*. On the leaves of *L. speciosa, L. loudonii,* and *L. macrocarpa,* there are alkaloids, flavonoids, and steroids. In *L. speciosa* stem bark there are flavonoid compounds (Hui Huanng et al., 2013) In the part of *L. loudonii* fruit there are ursolic acid and oleanolic acid compounds (Sirimethawong et al., 2013).

Tests on the activity of bungur plants have also been widely reported, such as testing of cytotoxic activity by using the Brine Shrimp Lethality Test (BSLT) method on fungus with *L. speciosa* type which produces LC_{50} values on leaves and stem methanol extracts of 9.602 µg/mL and 72.06 µg/mL (Nasrin et al. 2012). So far, there has been no development of research on toxicity tests on mosquito larvae using bungur plants. From the research that has been done, on the leaf parts of the plant type *L. speciosa* has been isolated and characterization of 14 compounds namely four triterpenes, eight elagat acids, one coumarin and one neolignan. In the part of the fungus seeds *L. speciosa*, the total phenolic acid content of methanol extract measured by the Folin-Ciocalteau method obtained levels of 325 ± 0.01 µg equivalent to gallic acid per mg of extract. Simultaneously, the antioxidant activity was tested using the DPPH method indicating an IC_{50} value of 9.63 ± 0.20 µg / mL (Junaid et al., 2012). The standard extract (GlucosolTM) from the leaves of *L. speciosa* had been tested for its activity as an antidiabetic which is performed on patients with type II diabetes mellitus with daily doses of 32 mg and 48 mg for two weeks showing a significant decrease in blood glucose levels (Judy et al., 2003). The phytochemical analysis of *L. loudonii* has been carried out on the stem bark, which shows alkaloid, flavonoid, polyphenol, tannin, and quinone content, saponin, and monoterpenoid and sesquiterpenoid compounds (Pratiwi, 2016). The enzyme inhibition activity of α-glucosidase extract and fraction (water, ethyl acetate and n-hexane) has been carried out and the stem bark of *L. loudonii* in vitro showed the results were able to inhibit the activity of the α-glucosidase enzyme with IC_{50} values of 240.53 µg/mL (ethanol extract), respectively, 186.11 µg/mL (water etract), 79.48 µg/mL (ethyl acetate extract) and 113.10 µg/mL (hexane extract) (Shaleh, 2016).

Based on the data above, research on the potential of ethanol extract of leaves, bark, stems and bungur fruit (*Lagerstroemia loudonii* T. & B.) as larvicides will be carried out for bioactive tracing which is toxic from natural materials against *Aedes aegypti* mosquito larvae. Toxicity is determined by looking at the value of Lethal Concentration 50 (LC_{50}), which is calculated using probit analysis (Harmita and Radji, 2008).

II. METHOD

A. Plant Material

This study's plant materials were leaves, bark, stems, and bungur fruit (*Lagerstroemia loudonii* T. & B.). was collected from the medicinal plant garden,

School of Pharmacy Institut Teknologi Bandung (ITB). The plants were taxonomically identified School of Life Science and Technology, Institut Teknologi Bandung (ITB).

B. Chemicals

The chemicals used in this study were distilled water, 96% ethanol technical grade, toluene technical grade, ammonia, chloroform technical grade, magnesium powder, amyl alcohol, potassium hydroxide, ether, vanillin sulfate were purchased from Merck (Jakarta, Indonesia). 1% Temephos (Abate) were purchased from Basf Indonesia

C. Animal Test

The test animals used in this study were phase III or IV instar *A. aegypti* mosquito larvae (inclusion criteria) from School of Life Sciences and Technology, Institut Teknologi Bandung (ITB), Bandung, West Java, Indonesia.

D. Tools

The tools used in this study were analytic scales (Mettler Toledo, Hong Kong), macerators, dryer cabinets, ovens (Memert, Germany), rotary evaporators (Heidoph), electric stoves, furnaces, desiccators, water baths (Labcompanion, South Korea).

E. Determination of Moisture Levels of Distillation Method

The leaves, bark, stems, and fruit r (*L. loudonii* simplicia powder were weighed as much as 5 g were added into the dried flask. Added approximately 200 mL of P toluene which had been saturated before with water into the flask. The cooling tube and the receiving tube that had been cleaned and dried, were connected to the flask, then the flask was heated for 15 minutes carefully. After the toluene boils were distilled with approximately two drops/second, most of the water was distilled. Then the refining speed was increased to 4 drops/second. After all the water was distilled, washed the inside of the cooler with toluene, while cleaning with a tube brush connected to copper wire and moistened with toluene. Continued refining for 5 minutes. The receiving tube was left to room temperature. After the water and toluene were entirely separated, the volume of water was read. If there was a water droplet attached to the receiving tube wall, rubbed it with rubber tied to a copper wire and moistened with toluene until dropped of a water drop (Anonim, 2000).

F. Phytochemical Screening

The phytochemical screening phase of the ethanol extract of leaves, bark, stems, and fruit of *L. loudonii* against

includes examined alkaloids, flavonoids, quinones, tannins, polyphenols, saponins, steroids and triterpenes, monoterpenoids and sesquiterpenoids with Tube reagent method.

G. Extraction

Weighed each of 200 g of simplicia leaves, bark, stems, and fruit of *L. loudonii*, put it into the maserator and added 1 L 96% ethanol, then macerated for 24 hours. The maceration results were accommodated; the residue was macerated three times again. The macerated filtrate was collected, then evaporated using a rotary evaporator and concentrated using a thick extract using a water bath with yield of 12 % w/w.

H. Preparation of Test Animals

Prepared plastic trays that had been filled with distilled water, then put *A. aegypti* eggs into it for 4 - 6 days until the eggs developed into III or IV instars.

I. Preparation of Test Solution

The test samples used for larvicidal activity test were ethanol extract of leaves, barks, stems and fruits of *L. loudonii*. Each sample was made with a 1% stock solution obtained by weighing 200 mg of each thick extract on a watch glass that had been pre-treated and then added 20 mL of ethanol solvent. The 1% stock solution obtained had a 10000 µg/mL concentration, which was then dispersed into a stock solution with a concentration of 1000 µg/mL. The stock solution then diluted into various concentrations of 250, 300, 350, 400, 450 and 500 µg/mL. Each concentration was made in 100 mL with distilled water (WHO, 2005). For controls used negative controls and positive controls were made without the addition of test samples. Where negative controls were made only with the addition of 1 mL of ethanol made in 100 mL of distilled water, while the positive control was used Temephos 1% (Abate) which was made by stock solution (1 µg/mL) and diluted into various concentrations of 0.005, 0.01, 0.015, 0.02, 0.025, and 0.03 µg/mL made in 100 mL of distilled water.

J. Larvacide inhibition Activity Testing

The stock solution (1000 µg / mL) of ethanol extract of leaves, barks, stems and fruits of *L. Loudonii*. The pipette solution was put into a plastic cup and added with several distilled water. A total of 25 *A. aegypti* larvae that had reached III or IV instar were put into the plastic glass, and distilled water was added to 100 mL to obtain the treatment group's concentration to 250, 300, 350, 400, 450 and 500 µg mL. For negative control 1 mL of ethanol was added into a plastic cup and added with several distilled water. A total of 25 instar III or IV *A. aegypti* larvae were put into

the plastic cup, and distilled water was added back to 100 mL. As for the positive control, from the stock solution (1 µg/mL) pipette was 0.5, 1, 1.5, 2, 2.5 and 3 mL to make a concentration of 0.005, 0.01, 0.015, 0.02, 0.025 and 0.03 µg/mL. The pipette solution was put into a plastic cup and added with several distilled water. A total of 25 instar III or IV *A. aegypti* larvae were inserted into the plastic cup and distilled water was added to 100 mL so that the positive control concentrations were 0.005, 0.01, 0.015, 0.02, 0.025 and 0.03 µg/mL. The test was carried out three times and repeated at room temperature 25-28 ° C. After 24 hours of testing, the number of dead and living mosquito larvae from each plastic cup was calculated.

K. Data Analysis

After 24 hours of testing, the number of dead and living mosquito larvae from each plastic cup was calculated. The results obtained were recorded in the table. Mortality from the treatment group was corrected using the Abbott formula:

$$\text{Mortality (\%)} = (X-Y) / X * 100\%$$

Note:
X = Larvae that are still alive in the control
Y = The surviving larvae on the test substance.

Lethal Concentration 50 (LC_{50}) value was obtained by calculating using the probit analysis method. LC_{50} was calculated by converting per cent (%) of death into probit values used Finney tables and linear regression of the logarithm of concentration. To determine the significance of larvicidal activity, the data was processed using the SPSS program

III. RESULT AND DISCUSSION

Examination of the characteristics performed on raw material is the determination of water content by azeotropic distillation method. The determination of water content aims to determine the water content in raw material because water is an excellent medium for microorganisms' growth. The results of the determination of water content can be seen in Table I.

Table I. Determination of Water Content

Test	Results (%v/b)			
	Leave	Bark	Stem	Fruit
Water Content	6±0.28	3.2±0.56	4.3±0.14	8±0.28

In Indonesian Herbal Pharmacopoeia, it is stated that an excellent raw material has a water content requirement of less than 10% so that it can reduce the risk of microorganism growth during storage. According to Indonesian

Herbal Pharmacopoeia, the water content of each raw material meets the water content requirements, which is less than 10% v / b. In the determination of water content by azeotrope distillation method, two solvents that have boiling points that are not much different are used, the solvents used were toluene and water. Toluene saturation is carried out so that the water in the raw material is not bound to the toluene used during the distillation to produce accurate water content determination.

In the manufacture of leaf extracts, bark, stems and fruit of bungur, extracts were carried out using the cold method, namely maceration using 96% ethanol. The selection of this maceration method is expected to keep the content of secondary metabolites contained in the sample not damaged by heating because the sample's activity is inseparable from the secondary metabolites contained therein maceration method is simple. The solvent used were ethanol solvent because it is universal, so it can dissolve almost all the secondary metabolites contained in raw material which are polar and nonpolar. Ethanol solvents were quickly evaporated and can inhibit the growth of microorganisms so that the extract obtained can last a long time in storage and is not toxic. The maceration process was carried out for 24 hours. This process was repeated three times, hoping that the search process can take place properly and optimally. The extract obtained was concentrated in a rotary evaporator until a concentrated extract was obtained, which was then evaporated further until a thick extract was obtained. From 200 grams of each raw material obtained thick ethanol extracts of leaves, bark, stems and fruit of bungur 22.31 grams, 48.14 grams, 10.43 grams and 10.72 grams, with per cent extract yield respectively 11.15 %, 24.07 %, 5.22 % and 5.36%.

Phytochemical screening of the extract of leaf, bark, stem and fruit of bungur was carried out to determine secondary metabolites such as alkaloids, flavonoids, saponins, quinones, tannins, polyphenols, monoterpenoids and sesquiterpenoids as well as steroids and triterpenoids. Phytochemical screening results for raw material are shown in Table II. The results of this study are in line with what was reported by Hui Huang (2013).

Larvicidal activity test of ethanol extract of leaves, bark, stems, and bungur fruit against *A. aegypti* larvae instar III or IV. This activity test aims to determine the toxic properties of ethanol extract of leaves, bark, stems, and bungur fruit on the mortality of test larvae within 24 hours, which is determined by calculating Lethal's value Concentration 50 (LC_{50}). In this study, larvicidal activity testing was

carried out based on guidelines from WHO (2005). Data analysis was performed using probit analysis to obtain LC_{50} values. In the study results, there were no deaths in test larvae in the negative control group, whereas in positive controls (Temephos 1%) and each treatment group showed larval test mortality with larvacide effect at different concentrations. The LC_{50} value of the test and comparison extract is shown in Table III.

Table II. Results of phytochemical screening of ethanol extract

Secondary Metabolites	Leave	Stem	Bark	Fruit
Alkaloid	+	+	+	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Kuinon	+	+	+	+
Tanin	+	+	+	+
Polyphenol	+	+	+	+
Monoterpen and Seskuiterpen	+	+	+	+
Steroid and Triterpenoid	+	-	-	+

(+) indicates the class of compound tested
(-) indicates no class of compound tested

Table III. Value of LC_{50} test and comparative extract

No.	Sample	LC_{50} (µg/mL)
1	Ethanol Extract of leaves	374.64 ± 11.88^{a}
2	Ethanol Extract of Bark	396.70 ± 3.99^{a}
3	Ethanol Extract of Stem	425.80 ± 8.15^{b}
4	Ethanol Extract of Fruit	312.54 ± 2.24^{c}
5	Temephos 1%	0.01 ± 0.01^{d}

It was found that the ethanol extract of bungur fruit showed the best results compared to other test extracts because the ethanol extract of bungur fruit was able to kill 50% of the test larva population within 24 hours at a concentration of 312,54 ± 2,24 µg/mL. Based on the LC_{50} values obtained, ethanol extract of leaves (374.64 µg/mL), barks (396.70 µg/mL), stems (425.80 µg/mL) and bungur fruits (312.54 µg/mL) can be included in the toxic category, where an extract can be said to be toxic if it has an LC_{50} value <1000 µg/mL (Meyer, 1982). The smaller the LC_{50} value, the higher the toxicity of an extract. Ethanol extract of leaves, bark, stems, and bungur fruit had larvicide activity much lower than the positive control (Temephos 1%) which was able to kill 50% of the test larvae

population within 24 hours at a concentration of 0.01 ± 0.01 µg/mL. Temephos has anti-cholinesterase properties that work by binding to cholinesterase enzymes, causing continuous muscle contractions that cause death in larvae (Ridha and Nisa, 2011).

The possibility of toxic compounds such as alkaloids, saponins, and flavonoids in extracts that cause death in *A. aegypti* larvae. These compounds act as stomach poisons that can interfere with the digestive system in the larvae. Alkaloid compounds are salts to degrade the cell membrane of the digestive tract and interfere with the nervous system in the larvae. Alkaloids can also cause colour changes in the larvae's body to become more transparent and body movements of the larvae that slow down when stimulated by touch and always bend the body (Cania and Setyanimgrum, 2013). Flavonoid compounds are plant defence compounds that can inhibit eating insects and are also toxic to insects to be used as insecticides (Redha Abdi, 2010). Flavonoids can weaken the respiratory, nervous system and cause death in larvae (Dinata, 2008). Saponin is a terpenoid compound that has the activity of binding to free sterols in the digestive system so that with a decrease in the number of free sterols will affect skin change in insects. Saponin compounds can damage cell membranes and disrupt the process of insect metabolism (Cheeke, 2004). Other compounds that play a role in the death of larvae are tannin compounds which belong to the polyphenol group which can interfere with insects in food scaling because tannins will bind proteins in the pollution system which are needed by insects for growth so that the process of protein absorption in the digestive system becomes disrupted (Yunita, Suprapti and Hidayat, 2009).

IV. CONCLUSION

Ethanol extracts of leaves, bark, stems, and bungur fruit (*Lagerstroemia loudonii* T. & B.) had larvicidal activity with LC50 values of 374.64 ± 11.88 µg/mL respectively, 396.70 ± 3.99 µg/mL, 425.80 ± 8,15 µg/mL, and 312.54 ± 2,24 µg/mL. Ethanol extract of bungur fruit has better and significant larvicidal activity compared to other test extracts.

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