Biolarvicidal activity of *Rhizophora stylosa* leaf extract against *Aedes* sp.

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Abstract. The research focus on biolarvicidal activity of *Rhizophora Stylosa* leaf extract against *Aedes sp*. *R. stylosa* leaf was collected from Gampong Pande, Banda Aceh. This research was conducted at laboratory of Marine Chemistry and Fisheries Biotechnology, Marine and Fisheries Faculty, Universitas Syiah Kuala from Desember 2020 to March 2021. The objective of this study was to figure out the secondary metabolites content in the methanol extract of the mangrove leaf and its biolarvicidal activity against *Aedes sp.* larvae. The secondary metabolites were screened out using phytochemical screening. The extract was tested for its biolarvicidal activity against third instar larvae of the *Aedes sp.* mosquito at incubation’s time of 48 hours. The biolarvicidal activity is expressed in LC₅₀ which was determined by probit analysis using SPSS. The results of qualitative test showed that the extract contained of flavonoids, saponins, triterpenoids and tannins. Based on the results of probit analysis, the LC₅₀ value of the extract against *Aedes sp.* was 858.89 ppm.

1. Introduction

Dengue viral infections are one of the most important mosquito borne diseases in the world. Annually, 100 million cases of dengue fever occur worldwide [1]. Dengue is known as an urban disease and the number of cases reported keeps increasing yearly. The mosquito species from *Aedes sp.* was responsible for the transmission of dengue in some countries [2]. According to [3], the control of mosquitoes at the larval stage is extremely essential to prevent the disease outbreak, and the common method used to control the larvae is through the application of chemical. The use of synthetic chemicals has not been sufficiently effective in controlling Aedes spp., largely because of selective pressure for the development of resistant mosquito strains. However, bioprospecting for natural products can be a viable alternative to break the vector cycle without promoting resistance to conventional insecticides [4].

Some researches had been conducted to figure out the responsible secondary metabolites from natural product against Aedes sp. larvae. It had been reported that secondary metabolites such flavonoids, tannins, and saponins from natural product had the high biolarvicidal activity against mosquito larvae [5].

Mangrove is well known as coastal plant and widely found in Banda Aceh coastal area, Indonesia. Some mangroves, such as *Rhizophora mucronata* [6], *Sonneratia alba* [7], *Avicennia marina* [8], *Xylocarpus granatum* [9] and other coastal plant such *Ipomoea pes-caprae* [10] had been studied and
contained of secondary metabolites responsible as biolarvicidal agent against Aedes sp. larvae. However, the different part of plant showed the different toxicity on bioassay. Toxicity refers to the harmful effect on organism. The toxicity assessment of any compound that is prone to be used in direct contact with bioassay [11].

Thought that methanol is effective to attract various compound with a large range of polarity, and leaf of mangrove known rich of tannins [12]. Further, regarding to the previous study [13] that revealed the ethyl acetate extract from Rhizopora stylosa bark was effective as biolarvicide against Aedes aegypti, therefore this study focused on biolarvicidal activity of methanol extract from R. stylosa leaf against Aedes sp. Briefly, the objective of the research was to determine LC50 value of the R. stylosa leaf extract.

2. Material and Methods

2.1. Material

Samples used in this study were Rhizopora stylosa leaf and Aedes sp. larvae for bioassay. The reagents were methanol 99% GR, aquadest, Mercury (II) chloride powder, Potassium iodide GR, Hydrochloric Acid 37 % GR, Chloroform, anhydrous acetic acid 96% GR, buthanol 99% GR, Sulfuric acid 97 % GR, Iron (III) chloride GR and Magnesium powder. Equipments used were rotary evaporator (The BUCHI’s R-100 rotary evaporator, V-100 vacuum pump, Chiller F-100/ F-105), tubes (5 ml), spatula, analytical ballance (AS 520.X2 PLUS Analytical Balance), beaker glass (pyrex 50 ml), grinder, pipettes (200 – 1000 μl) and measured glassware (pyrex 100 ml)

2.2. Sample preparation and Extraction

Rhizopora stylosa leaf was collected from Gampong Pande, Banda Aceh, Indonesia. Leaves were chopped, air dried, and grinded to the powder. Leaf of R. stylosa was macerated according to the method of [14] using methanol during 24 h and repeated three times, then filtrated using Whatmann paper no. 42 and evaporated at 40 °C using rotary evaporator. The bioassay sample in this study was the third instar larvae of Aedes sp. obtained from Parasitology Laboratory, Veterinary Faculty, Universitas Syiah Kuala.

2.3. Phytochemical screening

Phytochemical screening of R. stylosa leaf extract was carried to figure out the secondary metabolites content in the extract. Methods used as followed the methods by [14,15,16] using Wagner reagent for alkaloids, Mg powder and HCl reagent for flavonoids, aquadest for saponins, Lieberman Burchad reagent for triterpenoids and steroid, last FeCl₃ reagent for tannins screening.

2.4. Biolarvicidal activity

The method of this larvicidal activity based on the method of [13]. The extract of R. stylosa was treated against Aedes sp. by treating the larvae with 8 concentrations of extract: 0, 200, 400, 600, 800, 1000, 1200 and 1400 ppm with 2 repetitions. The extract solutions were diluted with aquadest and stirred using magnetic stirrer. The bioassay was 10 individuals of Aedes sp. larvae (instar III). The larvae were treated in 50 ml extract solution using beaker glass as container within 48 h of observation. The mortality value then was measured as follow the formula as mentioned in point 2.5, while the toxicity level was categorized according to [13] as shown in Table 1.

2.5. Mortality and LC₅₀ measurement

Mortality percentage was measured using the the following formula $P (%) = \frac{x}{y} \times 100\%$, where as $P$ (%) is mortality percentage, $x$ (ind) is the number mortal larvae, and $y$ (ind) is the amount of initial observed larvae. The value of LC₅₀ was determined using probit analysis method statistically [8].
Table 1. Toxicity category of LC_{50} (mg/L) value \[13\]

| Toxicity Category | LC_{50} (mg/L) |
|-------------------|----------------|
| Non toxic         | > 10000        |
| Low               | 1000 – 10000   |
| Moderate          | 100 – 1000     |
| Toxic             | 1 – 100        |
| High toxic        | < 1            |

3. Result and Discussion

3.1. Phytochemical Screening

*Rhizophora stylosa* leaf extract was screened by phytochemical methods and known contained of flavonoids, saponins, triterpenoids and tannins. The result and reagents used for phytochemical screening as shown in Table 2.

Table 2. Secondary metabolites screening of *Rhizophora stylosa* leaf extract

| Secondary Metabolites | Reagents            | Result  |
|-----------------------|---------------------|---------|
| Alkaloids             | Wagner              | -       |
| Flavonoids            | Mg + HCl            | Positive|
| Saponins              | Aquades            | Positive|
| Triterpenoids         | Liebermann Burchard | Positive|
| Steroids              | Liebermann Burchard | -       |
| Tannins               | FeCl_{3}            | Positive|

*Rhizophora stylosa* identified according to \[17\] as sample in this study was widely found in Gampong Pande, Banda Aceh, Indonesia. To figure out the secondary metabolites content in the leaf of the mangrove, the methanol extract of the sample was screened out using phytochemical screening test. It was confirmed that leaf of *R. stylosa* contained of secondary metabolites; flavonoids, saponins, triterpenoids, and tannins (Table 2), as found in its bark except alkaloids \[13\], and in leaf, fruit and root of *Avicennia marina* except alkaloids, fenolics and glycosides \[8\]. Based on the study from \[5\], secondary metabolites such as flavonoids, tannins, and saponins from natural product had the high biolarvicidal activity against mosquito’s larvae. However, it had been had proven that ethyl acetate extract from *R. stylosa* bark was effective as biolarvicide against *Aedes aegypti* \[13\]. Hence in this study, the methanol extract from leaf of *R. stylosa* also effective as biolarvicidal agent against *Aedes* sp.

Those biolarvicidal agents; flavonoids, tannins, saponins and triterpenoids play the important role in larvae mortality. According to \[18\] and \[19\], flavonoids cause the demage of the larvae respiratory system, while tannins known as enzyme inhibitor, especially protease in degrading the amino acids \[20\] and saponins can cause the irritation of larvae digestive tract mucosa \[21\].

3.2 Mortality of Aedes sp. and LC_{50} of the leaf extract

*Rhizophora stylosa* leaf extract found effective as larvacidal agent against third instar larvae of *Aedes* sp. (after incubating during 48 hours). The mortality of larvae increased as the concentration of the extract increased as shown in Figure 1. It was obtained that the optimum concentration of treatment was at 1200 ppm with the mortality percentage was 95%.

Based on probit analysis, the LC_{50} value of *R. stylosa* leaf extract was 858.89 ppm. This value is lower than the LC_{50} value of *R. stylosa* bark extract (951.2 ppm), studied by \[13\]. Despite, the different part of plant showed the different toxicity on bioassay, but both parts of *R. stylosa* have the similar category of toxicity based on the toxicity level (100 – 1000 ppm), categorized as moderate (Table 1) \[13\]. Those LC_{50} values are better than other extract of other mangroves; bark of *Rhizophora mucronata* (78819.5 ppm) and all part of *Sonneratia alba* (>1000 ppm), where as categorized as non toxic and low
toxic, respectively [6,7]. While the extract from root of *Rhizophora apiculata* (6.85 ppm) and from leaf (2.72 ppm), fruit (1.58 ppm) and root (1.23 ppm) of *Avicennia marina*, whereas categorized as toxic [8, 22].

**Figure 1.** Mortality percentage of *Aedes* sp. larvae treated by *R. stylosa* leaf extract

4. Conclusion

Based on this study, it was obtained that *Rhizophora stylosa* leaf extract contained of flavonoids, saponins, triterpenoids and tannins. The optimum concentration of the leaf extract was 1200 ppm and the LC$_{50}$ value was 858.877 ppm. It was concluded that the toxicity of the extract against *Aedes* sp. has a moderate toxicity.

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