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Screening and Potential Analysis of Methanolic Leaf Extract of Mangrove Plants at East Coast Sumatera as Repellent against *Aedes aegypti*

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Abstract

Dengue fever is an infection transmitted through *Aedes aegypti*, which has become a public health problem. The use of natural repellent is very beneficial because of its nature as an insecticide, environmentally friendly, and is not harmful to the community. Based on the benefits obtained, it is deemed necessary to find natural repellent from mangroves to resist the bite of *Aedes aegypti* as a cause of dengue fever. In this study, potential screening and analysis of mangrove extracts as repellent were carried out, which consisted of several stages. Firstly, various methanolic leaf extracts from mangrove plants were carried out and then tested the repellent effectiveness of *Aedes aegypti*, in which all research activities were carried out in the laboratory. The results of repellent test of methanolic leaf extracts from several mangrove at dosage of 100% which was able to resist mosquito bites above 80% per hour are *Sonneratia alba* leaf extract is resist up to 2 hours as much as 85,8%, *Avicennia marina* leaf extract is resist for 3 hours as much as 82,5%, *Avicennia alba* leaf extract for 1 hour resist as much as 80,5%, *Rhizophora apiculata* leaf extract is resist for 2 hours as much as 80,3%, and *Rhizophora stylosa* leaf extract is resist for 1 hours as much as 81,3%. While the leaf extracts from *Sonneratia caseolaris*, *Acanthus ilicifolius* L, *Nypa fructicans*, *Hisbiscus tiliaceus*, *Crimum aciaticum*, *Melastoma candidum*, dan *Bruguiera gymnorrhiza* just able to resist a bite of *Aedes aegypti* mosquito under 80%. The results of this study showed that methanolic leaf extracts from *Sonneratia alba* and *Avicennia marina* were repellent. *Sonneratia alba* and *Avicennia marina* leaves indicate having one or more active compounds as a new natural repellent candidate.

Keywords: mangrove, repellent, *Aedes aegypti*, dengue fever

INTRODUCTION

The presence of mosquitoes is an indication of poor sanitation in a particular place or region. Mosquitoes are one of vector diseases in humans, such as malaria, filarial diseases, yellow fever, dengue fever, and West Nile virus [1,2]. Preventive efforts that can be done to prevent mosquito breeding generally use three methods (drain, bury, and clean places that become mosquito nests). In addition, mosquito repellent can also be used to resist mosquito bites. The use of mosquito repellent is considered a practical and economical action. Considering the use of synthetic repellent is quite dangerous, it is necessary to substitute the ingredients that are safer to use and environmentally friendly, one of which is by utilizing mosquito repellent.

The incidence of dengue disease has increased dramatically throughout the world in recent years. More than 2.5 billion people or more than 40% of the population in the world today are at risk of developing dengue disease. WHO estimates there are 50-100 million dengue infections worldwide every year [3]. Dengue is transmitted by the bite of a female *Aedes aegypti* which infected with the Dengue virus. *Aedes aegypti* is the main vector of Dengue virus [4-8]. One of the prevention efforts against dengue virus transmission is to avoid vectors bites. Replication can reduce exposure to mosquito bites that may be infected with dengue virus [6-11]. The repellent that is widely used by the public is synthetic repellents. An example of synthetic repellent is N,N-diethyl-meta-toluamide (DEET) which is used to resist mosquitoes. Many reports regarding DEET toxicity, ranging from mild effects, such as urticaria and skin eruptions, to severe reactions, such as toxic encephalopathy [12]. This led to the need for research and development of natural repellent derived from plant derivatives.

There are several plants commonly used by the community as natural repellent including legundi (*Vitex trifolia* L.), neem (*Azadirachta indica*), and jeringau (*A. calamus* L.) [13,14]. The results of previous tests on the bioactivity of several mangrove extracts indicate the activity as repellent. This efficacy can be an early indicator of its potential as repellent. From several previous studies also manifested that compounds which are active as antimalarial are also active as repellent. This bioactivity indicates that the plant has a new active compound as a repellent candidate. To be able to be used with efficacy that is proven experimentally, in this study, screened the activity as a repellent against *Aedes aegypti* using several mangrove extracts. The results of this study are expected to be able to get the types of mangrove and fraction extracts that are repellent, at the same time know the class of compounds as the new repellent candidates.

MATERIALS AND METHODS

Materials

The materials used in this study were the leaves of mangroves, namely *Acanthus ilicifolius* L., *Sonneratia alba*, *Nypa fructicans*, *Sonneratia caseolaris*, *Hisbiscus tiliaceus*, *Crimum aciaticum*, *Melastoma candidum*, *Avicennia marina*, *Avicennia alba*, *Rhizophora stylosa*, *Rhizophora apiculata*, and *Bruguiera gymnorrhiza*. These leaves were collected from a Mangrove forest in Muara Sabak (Tanjung Jabung Timur, Jambi) and identified in Department of Biology, Faculty of Science and Technology, University of Jambi. The vector used in this study was *Aedes aegypti* was obtained from Jambi Health Research and Development Center.

All chemicals used were of analytical grade or pro analysis. Aluminum tri chloride, methanol, hydrochloric acid (HCl), glacial acetic acid,potassium acetate, sodium acetate, ferric chloride hexahydrate, Dragendorff's reagent, potassium iodide, iodine were purchased from Sigma–Aldrich. Ethanol, sodium hydroxide (NaOH) were purchased from Merck.

Extraction of plant material

The materials used in this study were leaf extracts of mangrove plants, namely *Acanthus ilicifolius L.*, *Sonneratia alba*, *Nypa fructicans*, *Caseolaris Sonneratia*, *Hisbiscus tiliaceus*, *Crimum aciaticum*, *Melastoma candidum*, *Avicennia marina*, *Avicennia alba*, *Rhizophora stylosa*, *Rhizophora apiculata*, and

Bruguiera gymnorrhiza. The preparation process of extract begins with the manufacture of dried simplicia powder, to a certain degree of fineness. The solvent used in the extraction process is methanol. The ingredients are taken from good quality leaves, then the ingredients are washed thoroughly with water and oven for 48 hours at 50^{o}C. the dried material is then mashed and sifted with mesh no. 20. Then the powder is taken as much as 500 grams, macerated with methanol and filtered. The methanol extract obtained was concentrated, then suspended in an aqueous solution so that in 100 ml of solvent contains 1 gram of fraction (1000 ppm) called a stock solution, which is used in testing [15,16].

Repellent Test Against *Aedes aegypti*

Each type of extract is tested against *Aedes aegypti*, to determine the dose or concentration of extracts based on a series of measurements. Tests in both treatment and control were carried out 3 (three) times and each replication contained 25 *Aedes aegypti*. each cage was filled with 25 female mosquitoes in a hungry state, both hands were put in cages alternately (starting with the left wrist given 10 cc of extract and right hand as a control). Both hands were exposed to *Aedes aegypti* for 5 minutes per hour and carried out for 6 hours [17,18]. The results of this study on the extract are said to be effective if the resistance to mosquito bites is more than 80%, and is declared ineffective if the repulsion is less than 80% [3].

Data Analysis

The research data in the form of extract protection power against mosquito bites obtained from the formula: DP = (KP) / K x 100%, where K = number of mosquitoes that landed on the control hand, P = number of mosquitoes that landed on the hand given, and DP = repulsion against mosquito bites. The calculation of the protection power will be tested statistically, namely the t-test to determine the significant differences between all extracts [3].

Phytochemical test

Phytochemical test were conducted to determine bioactive components contained in potential repellent activity methanolic leaf extract for each treatment. The phytochemical test consisted of alkaloid, flavonoid, saponin, triterpenoid, steroid, tanins, glycosides, quinone and phenolic test. This test method is based on Harborne [19,20].

RESULTS AND DISCUSSIONS

Research has been done on some methanolic leaf extracts of mangrove plants as repellent against *Aedes aegypti*, with the following results: *Sonneratia alba* leaf extract is able to resist up to 2 hours as much as 85.8%. *Avicennia marina* leaf extract was able to refuse for 3 hours as much as 82.5%, *Avicennia alba* leaf extract was able to refuse for 1 hour as much as 80.5%, *Rhizophora apiculata* leaf extract was able to resist for 2 hours as much as 80.3%. *Rhizophora stylosa* leaf extract was able to resist for 4 hours as much as 72.4%. *Sonneratia caseolaris* leaf extract was able to resist for 1 hour as much as 70.6%, while leaf extract of *Acanthus ilicifolius* L., *Nypa fructicans, Hisbiscus tiliaceus, Crimum aciaticum, Melastoma candidum,* and *Bruguiera gymnorrhiza* were only able to resist the bite of Aedes aegypti below 70%. Data can be seen in Table 1.

Dengue is transmitted by the bite of a female *Aedes aegypti* that was infected by the Dengue virus. *Aedes aegypti* is the main vector of Dengue virus [4-10, 21]. One of the prevention efforts against dengue virus transmission is to avoid vector's bites. Replication can reduce exposure to mosquito bites that may be infected with dengue virus (Tomlinson, 1986). The repellent that is widely used by the public is synthetic repellents. An example of synthetic repellent is N,N-diethyl-meta-toluamide (DEET) which is used to resist mosquitoes. Many reports regarding DEET toxicity, ranging from mild effects, such as urticaria and skin eruptions, to severe reactions, such as toxic encephalopathy [12, 22, 23]. This led to the need for research and development of natural repellent derived from plant derivatives. Mosquitoes are insects that are harmful to humans, and can transmit the disease to more than 700 million people each year.

In addition, the use of insecticides in the government's efforts to overcome vector borne diseases is still a priority. The use of insecticides in the health sector, especially those derived from chemicals are still widely used in Indonesia. As for various active insecticidal ingredients that are currently still on the market, as a method of controlling insect infectious diseases, among others, are Organophosphat, Organochlorin, Carbamat and Pyrethroid. Indonesia is a tropical region and there are thousands of types of plants that can be utilized, especially in the field of human health. One of the benefits of plants is their use as pesticides. The use of plants as pesticides has long been known in the world of agriculture, especially to repel insects and pests on agriculture. Plants as these pesticides contain secondary metabolites.

Table 1. Repellent Activity of Various Mangrove against *Aedes Aegypti*

No	Extract dosage 100%	Repellent activity against *Aedes aegypti* per hour of observation (%)					
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
1	*Sonneratia alba* leaf	90.2	85.8	78.2	67.8	51.8	35.9
2	*Avicennia marina* leaf	91.8	87.7	82.5	75.3	66.5	47.4
3	*Avicennia alba* leaf	80.5	77.5	63.7	55.6	48.4	31.9
4	*Rhizophora apiculata* leaf	83.7	80.3	71.1	68.5	53.2	40.7
5	*Rhizophora stylosa* leaf	81.3	77.4	75.5	72.4	65.7	39.8
6	*Sonneratia caseolaris* leaf	70.6	67.5	58.8	46.3	35.5	24.7
7	*Acanthus ilicifolius* leaf	67.1	50.7	41.6	30.7	25.1	14.2
8	*Nypa fructicans* leaf	63.8	45.1	32.4	23.6	17.7	13.1
9	*Hisbiscus tiliaceus* leaf	59.4	42.4	36.2	28.4	22.3	18.7
10	*Crimum aciaticum* leaf	57.2	52.6	48.3	32.1	27.2	23.4
11	*Melastoma candidum* leaf	60.73	48.3	38.2	32.5	29.4	25.4
12	*Bruguiera gymnorrhiza* leaf	56.8	42.6	40.7	35.5	26.7	20.3

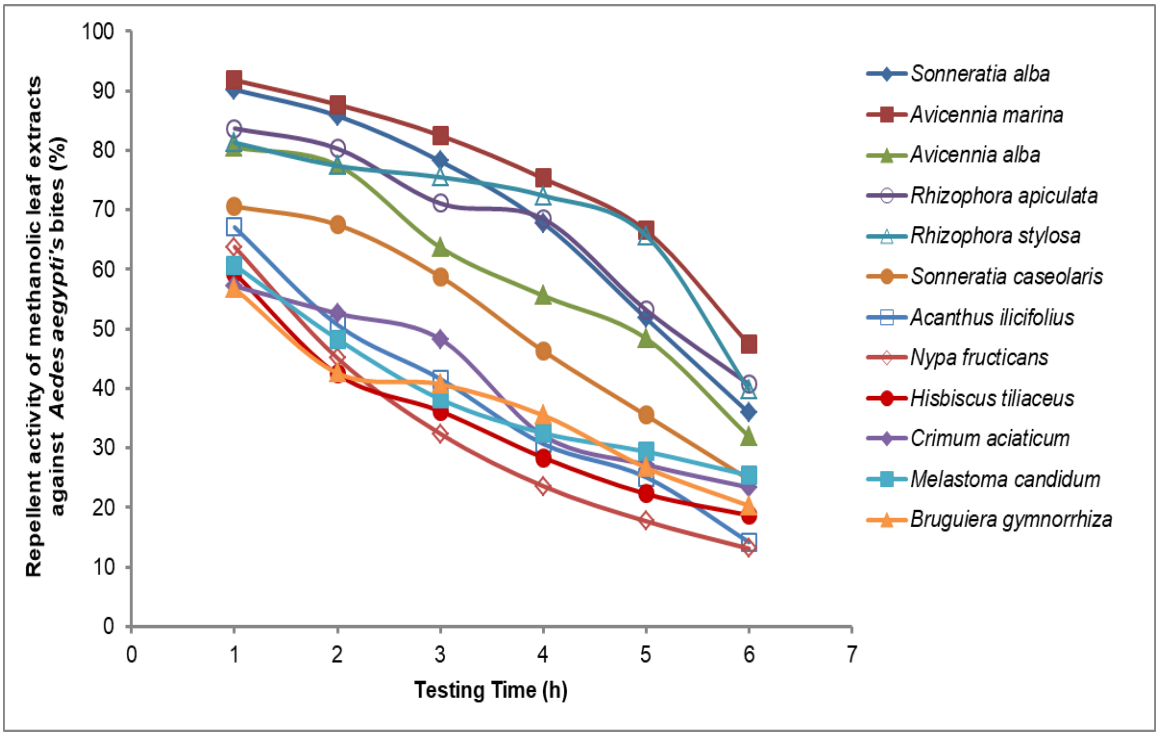

Figure 1. Repellent activity of methanolic leaf extracts of Mangrove plants against *Aedes aegypti*'s bites

Methanolic leaf extracts of *Sonneratia alba* and *Avicennia marina* provide the best activity as a repellent. Based on phytochemical test data (Table 2), the major constituents in these plants are alkaloids, flavonoids and terpenoids.

Table 2. Phytochemical screening of methanolic leaf extracts of *Sonneratia alba* and *Avicennia marina*

Test	Methanolic Leaf Extracts of *Sonneratia alba*	Methanolic Leaf Extracts of *Avicennia marina*
Alkaloid	++	++
Flavonoids	++	++
Terpenoids	++	++
Phenolic	+	+
Steroids	-	+
Tanins	+	+
Saponins	+	-
Glycosides	+	-
Quinone	+	-

+ = presence, − = absence

Secondary metabolites are metabolites that are not essential for organism growth and are found in unique or different forms between one species and another [15,24,25]. Chemical compounds that are the result of secondary metabolites in plants are very diverse and can be classified in several classes of natural compounds, namely Flavonoids, Alkaloids, Steroids, Coumarin, and Terpenoids [15,24,25].

Several types of mangrove from the east coast of Sumatra have been tested as repellent against the *Aedes aegypti* in a study on "Screening and Potential Analysis of Methanolic Leaf Extracts Mangrove Plants at East Coast Sumatera as Repellent against *Aedes aegypti*" as follows: *Sonneratia alb*a leaf extract can resist up to 2 hours as much as 85.8%. Likewise, *Avicennia marina* leaf extract was able to refuse for 3 hours as much as 82.5%. Ethnopharmacologically, people living on the east coast of Sumatra, especially the east coast area of Jambi, are accustomed to rubbing their skin with leaves of *Sonneratia alba* and *Avicennia marina* before entering the forest to be protected from insect attacks.

The repulsion of mosquito bites on the leaves extracts of *Sonneratia alba* and *Avicennia marina* are likely due to the active substances they contain, namely terpenoid group compounds as insect repellent. Besides that, *Sonneratia alba* and *Avicennia marina* produce an aroma that is not fragrant because it contains compounds from essential oils so that insects are not preferred. The leaves of *Sonneratia alba* and *Avicennia marina* feel bitter, sometimes used as traditional medicine, as a relief of malaria fever [12,23-25].

Avicennia alba leaf extract was able to refuse for 1 hour as much as 80.5%, and *Rhizophora apiculata* leaf extract was able to resist for 2 hours as much as 80.3%. The repulsion caused by leaves extracts of *Avicennia alba* and *Rhizophora apiculata* are probably due to the presence of alkaloids, because in agriculture it is used as a pesticide. Besides that, the active ingredients in the leaves of *Avicennia alba* and *Rhizophora apiculata* include alkaloids and terpenoids which have been known to have pharmacological properties, such as antimicrobials and antioxidants.

Rhizophora stylosa leaf extract was able to resist for 4 hours as much as 72.4%. *Sonneratia caseolaris* leaf extract was able to resist for 1 hour as much as 70.6%, while leaves extract of *Acanthus ilicifolius* L, *Nypa fructicans, Hisbiscus tiliaceus, Crimum aciaticum, Melastoma candidum,* and *Bruguiera gymnorrhiza* were only able to resist bites of *Aedes aegypti* below 70%. The lack of repelability of extracts of these plants against *Aedes aegypti* due to the secondary metabolites contained in the

leaves of these plants are not from the group of alkaloid, terpenoid, and essential oils or less.

The results of this study manifested that leaves extracts of mangrove, namely *Sonneratia alba* and *Avicennia marina* were repellent. The leaves of *Sonneratia alba* and *Avicennia marina* indicate having a class of compounds as a new natural repellent candidate.

The ability of different types of plants to resist mosquito bites varies depending on the content of active substances in the plant itself, some are able to repel because of the stinging smell, so that mosquitoes are not liked. The test was carried out for 6 hours, apparently there was a significant difference between each plant extract to refuse mosquito bites ($P < 0.05$) with the t-test. Future prospects need further research on the separation of substances in leaves extract of *Sonneratia alba* and *Avicennia marina*, in order to find out what substances play a role in refusing of mosquito bites. Repellent activity of methanolic leaf extract of Mangrove plants against *Aedes aegypti's* bites can be seen also in figure 1.

In general, secondary metabolites are a natural product. These secondary metabolites although not very important for the existence of an individual, but these compounds play a role in the survival of a species. These secondary metabolites are usually formed because these plants must adapt to their living environment and also to self-defense from attacks by other species [15-19]. The dominant chemical content of the leaves of *Sonneratia alba* and *Avicennia marina* are alkaloids, flavonoids and terpenoids. It is possible that this alkaloid functions as a repellent. According to Harborne (1987) alkaloids are insect repellent. In addition, terpenoids can also function as repellent [19].

The leaves of *Sonneratia alba* and *Avicennia marina* are believed by the rural community to refuse the perching of *Aedes aegypti* mosquitoes, so it can be used as a repellent. This research has proven repellent activity of methanolic leaf extracts of *Sonneratia alba* and *Avicennia marina* against *Aedes aegypti* mosquitoes, and identified terpenoids in methanolic leaf extracts. Repellent activity test from these plant extracts was conducted by observing the time total of refuse of mosquitoes perch on surface of hand. In this test, the hand is not inserted into the cage continuously, but with a certain time interval. Continuous monitoring will cause mosquitoes to fatigue and induce blockade from chemoreceptor antenna. It will cause mosquitoes to refuse to bite. During hand testing should not be added test extracts, no washed and no fight if there are mosquitoes that will perch. Identification of results showed that methanolic leaf extracts *Sonneratia alba* and *Avicennia marina* contained terpenoid active compounds. The repellent activity of methanolic leaf extracts is lower than DEET (data not showed). This is due to the active compound responsible is predicted a terpenoid group compound. This group compounds are relatively easier to evaporate and quickly disappear from application site.

CONCLUSIONS

Based on the results of this study exhibited that 100% of methanolic leaf extracts of mangrove plants that were able to resist the bite of *Aedes aegypti* per hour above 80% were as follows: *Sonneratia alba* leaf extract was able to resist up to 2 hours as much as 85.8%, *Avicennia marina* leaf extract was able to resist for 3 hours as much as 82.5%, *Avicennia alba* leaf extract was able to refuse for 1 hour as much as 80.5%, and *Rhizophora apiculata* leaf extract was able to resist for 2 hours as much as 80.3%. The results of this study manifested that methanolic leaf extracts of *Sonneratia alba* and *Avicennia marina* had activity as repellent. *Sonneratia alba* and *Avicennia marina* leaves indicate having one or more active compounds as a new natural repellent candidate.

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