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Potential of Leaf Extracts *Sonneratia alba* and *Avicennia alba* as a Biolarvacide of *Aedes aegypti* Mosquito

Potensi Ekstrak Daun Sonneratia alba *dan* Avicennia alba *sebagai Biolarvasida Nyamuk* Aedes aegypti

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ABSTRAK

Penggunaan larvasida sintetik secara terus menerus sebagai upaya pengendalian populasi nyamuk vektor penyakit Demam Berdarah Dengue (DBD) dapat menyebabkan dampak negatif pada lingkungan dan manusia. Senyawa bioaktif dari hewan dan tumbuhan dapat sebagai alternatif larvasida alami (biolarvasida). Tumbuhan mangrove spesies Sonneratia alba *dan* Avicennia alba *dikenal memiliki beberapa jenis bioaktif sehingga potensial sebagai biolarvasida. Penelitian ini bertujuan untuk mengetahui potensi ekstrak daun* S. alba *dan* A. alba *sebagai biolarvasida terhadap larva nyamuk Aedes aegypti. Metode penelitian eksperimental murni dilakukan dengan post-test-only group design. Uji toksisitas larvasida ekstrak daun* S. alba *dan* A. alba *terhadap larva* Ae. aegypti *untuk mendapatkan nilai* LC_{50} *dilakukan dengan perlakuan konsentrasi ekstrak sebesar 500 ppm, 1000 ppm, dan 1500 ppm. Larvasida sintetik (Abate) digunakan sebagai kontrol positif dan akuades sebagai kontrol negatif. Analisis data dilakukan secara deskriptif dan statistik (analisis probit). Hasil penelitian menunjukkan bahwa ekstrak daun* S. alba *dan* A. alba *berpotensi sebagai biolarvasida dimana berdasarkan nilai* LC_{50} *ekstrak* A. alba *(*LC_{50}*: 1053 ppm) lebih potensial sebagai biolarvasida dibandingkan dengan ekstrak* S. alba *(*LC_{50}*: 14112 ppm). Kerusakan fisik dan perubahan gerak larva nyamuk menunjukkan bahwa toksisitas ekstrak daun* A. alba *bersifat akut, sedangkan daun* S. alba *bersifat kronis.*

Kata kunci*: Aedes aegypti, biolarvasida, mangrove*

ABSTRACT

The continuous use of synthetic larvacides as an effort to control the vector mosquito population of Dengue Hemorrhagic Fever (DHF) can cause negative impacts on the environment and humans. Bioactive compounds from animals and plants can be used as an alternative to natural larvacides (biolarvacides). Mangrove species *Sonneratia alba* and *Avicennia alba* are known to have several bioactive types, so they are potential as biolarvacides. This study aimed to determine the potential of *S. alba* and *A. alba* leaf extracts as biolarvacides against *Aedes aegypti* mosquito larvae. This research was true experimental with a post-test-only group design. The larvicidal toxicity test of the leaf extracts of *S. alba* and *A. alba* against *Ae. aegypti* larvae to obtain the LC_{50} value was carried out by treating the extract concentrations at 500 ppm, 1,000 ppm, and 1,500 ppm. Synthetic larvicide (abate) was used as a positive control and Aquadest as a negative control. Data analysis was carried out descriptively and statistically (probit analysis). The results showed that the leaf extracts of *S. alba* and *A. alba* had potential as biolarvacides. Based on the LC_{50} value, the extracts of *A. alba* (LC_{50}: 1,053 ppm) were more potent as biolarvacides than the extracts of *S. alba* (LC_{50}: 14,112 ppm). Physical damage and behavioral changes in mosquito larvae movement indicated that the toxicity of *A. alba* leaf extract was acute while that of *S. alba* leaf extract was chronic.

Keywords: *Aedes aegypti*, biolarvacide, mangrove

INTRODUCTION

The case of dengue hemorrhagic fever (DHF) in Indonesia during 2022 continues to increase. It is because dengue fever spreads quickly and can infect both children and adults. The DHF vaccine is still being developed, but a safe and effective vaccine has not yet been found. Therefore, eradicating the disease is focused on controlling the vector mosquito population, namely the *Aedes aegypti mosquito*.[1] One of the practical efforts to control the vector mosquito population is to use larvacides.

The most widely used larvacides are synthetic because they are more effective, inexpensive, easy, and practical. Long-term use of these larvacides can result in mosquito larvae resistance, water pollution, and human poisoning.[2] As a result, it is critical to learn about alternative natural larvacides in order to avoid causing mosquito larvae resistance and polluting the environment. Natural larvacides (biolarvacides) can be derived from bioactive compounds such as animal bioactives and plants, among others.[1,3] Plant bioactive compounds with the potential to act as biolarvacides are flavonoids, alkaloids, saponins, tannins, and essential oils.[4] Some of these bioactive compounds can be found in mangrove plants due to their living in extreme areas, which requires them to have self-defense by producing bioactive compounds.

Several mangrove species have a high abundance and wide distribution in Indonesia, including *Sonneratia alba* and *Avicennia alba*. These two species are mostly found in the coastal areas of Segara Anakan, Cilacap.[5] This mangrove plant lives in coastal areas with extreme conditions, so it has bioactive compounds to defend against predators and damage to body tissues[7]. Some of the bioactive compounds in the leaves of the two mangrove species are phenols, flavonoids, steroids, triterpenoids, saponins, tannins, and alkaloids.[8,9] These compounds have potential as biolarvacides. To determine the possibility of the potential *S. alba and A. alba* as a biolarvicide against *Ae. aegypti* mosquitoes, it is necessary to do a toxicity test to determine the value of LC_{50} (*lethal concentration 50*) and its toxicity properties.

METHODS

This research was conducted in June 2022 at the Marine Biotechnology Laboratory, Jenderal Soedirman University. The materials tested as treatments were three series of concentrations (500, 1,000, and 1,500 parts per million) of leaf extract of *S. alba* and *A. alba* against *Ae. aegypti* mosquito larvae, which were extracted using the *Pressurized Liquid Extraction* (PLE) method. The PLE method was carried out as follows: mangrove leaf powder weighing up to 20 grams was heated in a pressure cooker with distilled water at a 1:4 w/v ratio. The mangrove leaf extract was heated for about 30 minutes at 1.1 bar pressure and 1210 °C temperature. The extract obtained was filtered using filter paper. The filtration results are heated in a pressure cooker with the same solvent concentration and time. The results of the first and second filtrations are combined in a container for evaporation. Furthermore, the positive control was given synthetic larvacides (abate: 100 ppm), while the negative control was given distilled water. This study used a pure experimental method with a post-test-only group design and three repetitions of each treatment.

The tools and materials used in this research are used for preparing *Ae. aegypti* mosquito larvae, preparing test extracts, and screening phytochemical and toxicity tests of leaf extracts of *S. alba* and *A. alba* against mosquito larvae of *Ae. aegypti*.

Preparation of mosquito larvae for testing begins with rearing and sorting until the third instar stage of mosquito larvae is obtained. Furthermore, the larvae were acclimatized for one hour to obtain healthy (actively moving and looking agile with movements forming a figure eight or *zig-zag*) and adaptive larvae.

Preparation for mangrove extracts starts with leaf sampling in the coastal areas of Segara Anakan, Cilacap. Mangrove leaf sampling was carried out using the Purposive Random Sampling method. Sampling was carried out at low tide to make it easier to use a knife to take part in the form of mangrove leaves that are protected from any disease. The leaves are then cleaned and dried for further extraction. The extraction process used the PLE method with distilled water as a solvent.

The test extracts from the leaves of *S. alba* and *A. alba* were prepared in three concentration series, namely 500, 1,000, and 1,500 ppm. This determination was made based on the results of the literature study and preliminary tests. The positive control was made from 100 ppm Abate solution, and the negative control was made from distilled water.

Screening for phytochemicals was carried out on the leaf extracts of *S. alba* and *A. alba* to determine the presence of a group of bioactive compounds using color visualization analysis techniques. The group of bioactive compounds detected included flavonoids, alkaloids, tannins, saponins, quinones, steroids, and triterpenoids.

Toxicity tests were carried out on each treatment series of extract concentrations (500 ppm, 1,000 ppm, and 1,500 ppm) and control (positive and negative) using ten mosquito larvae for each treatment. The test was carried out for 72 hours. Observations were made on the number of mosquito larvae deaths, behavioral responses, and physical damage to mosquito larvae at 6, 12, 24, 48, and 72 hours of exposure. The test larvae's mortality percentage data was analyzed using probit analysis to obtain the value LC_{50}.

RESULTS

The results showed that the leaf extracts of *S. alba* and *A. alba* could kill *Ae. aegypti* mosquito larvae. At the same concentration of extract treatment, it was shown that *A. alba* leaf extract could kill *Ae. aegypti* mosquito larvae more than *S. alba* leaf extract (Table 1).

Table 1. The Average Percentage of Mortality in *Ae. aegypti* mosquito larvae

The average percentage of mortality *in Ae. aegypti* mosquito larvae (%)											
Species mangrove		*Sonneratia alba*					*Avicennia alba*				
Exposure time (hours)		6	12	24	48	72	6	12	24	48	72
Extract concentration (ppm)	500	6.7	20	23.3	26.7	36.7	6.7	16.7	20	26.7	43.3
	1,000	3.3	6.7	10	20	23.3	6.7	20	26.7	33.3	46.6
	1,500	10	23.3	26.7	33.3	46.7	6.7	23.3	30	36.7	56.7
		Range: 3.3-46.7					Range: 6.7-56.7				

Table 1 shows that in the highest concentration series (1,500 ppm), the extract of *S. alba* was only able to kill 46.7% of the maximum test mosquito larvae, while *A. alba* killed as much as 56.7% for observation for about 72 hours. The positive control treatment caused the death of all test larvae at 12 hours of observation, while the negative control did not show death in the test larvae. That indicates that the death of the test larvae is a pure effect of the treatment with mangrove leaf extract, which has bioactive compounds (Table 2). Probit analysis was carried out on the mortality data for each extract treatment of the two mangrove species to find the LC_{50} value (Figure 1).

Table 2. Results of Phytochemical Screening of *Sonneratia alba* and *Avicennia alba* Leaf Extracts

Bioactive Compound		*Sonneratia alba*	*Avicennia alba*
Alkaloid	Dragendroff	-	-
	Wagener	-	-
	Mayer	-	-
Flavonoid	-	+	+
Steroid	-	+	+
Triterpenoid	-	-	-
Saponin	-	+	+
Tannin	-	+	+

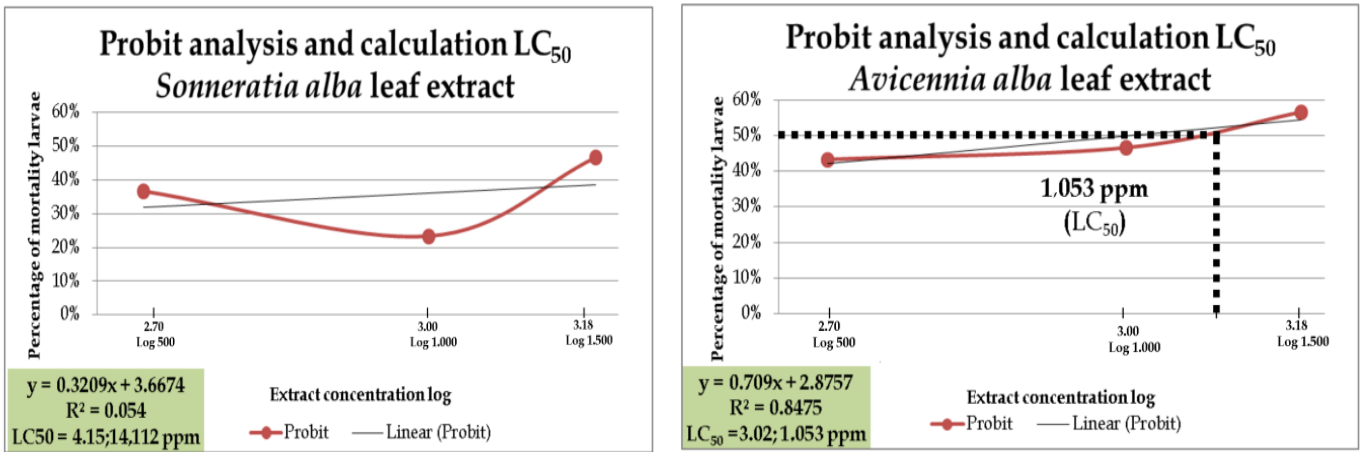

Figure 1. Probit Analysis and Calculation of LC_{50}.

Figure 1 shows that the LC_{50} value based on probit analysis showed that the LC_{50} value was achieved in the toxicity test of *A. alba* leaf extract at 1,053 ppm. In contrast, in the toxicity test on the *S. alba* leaf extract, the LC_{50} value was achieved at a higher concentration than the tested concentration, which is 14,112 ppm. That shows that *A. alba* leaf extract is more effective in killing *Ae. aegypti* mosquito larvae compared to *S. alba* extract because it takes less *A. alba* extract to kill mosquito larvae than *S. alba* extract. The higher the LC_{50} value, the more ingredients are needed, so the extract is more inefficient and uneconomical if used as a source of biolarvacides.[10]

The observations on *Ae. aegypti* mosquito larvae after exposure to *S. alba* leaf extract and *A. alba* for 72 hours caused changes in behavior in the form of movements that were initially fast but became slower, eventually leading to death. Physical damage due to exposure to the test extract can be seen from the onset of discoloration, ecdysis on the larval body, shrinkage of the body, swelling of the organs, and ending with death (Table 2).

Table 2. Changes in Behavior and Physical Damage of Larvae During the Study

Mangrove Species	Observation	Exposure time (Hours)					Information
		6	12	24	48	72	
S. alba	Movement responses	move fast	move fast	Weak motion	Weak motion	Slow motion	Chronic
	Physical damage	 (a)	 (b)	 (c)	 (d)		
A. alba	Behavior responses	move fast	Weak motion	Slow motion	Slow motion	Slow motion	Acute
	Physical damage	 (a)	 (b) (c)	 (d)			
Control (+)	Behavior responses	-	-	-	-	-	-
	Physical damage	 (e)	-	-	-	-	
Control (-)	Behavior responses	move fast	move fast	move fast	move fast	move fast	-
	Physical damage	 (a)	 (a)	 (a)	 (a)	 (a)	

Description: (a). initial conditions; (b). discoloration; (c). ecdysis; (d). disabled; (e). dead

Table 2 shows that the behavioral responses that occur in the treatment of *S. alba* leaf extract at 6–12 hours of observation are that the larvae movement is still fast. At 24 to 48 hours of observation, the larval movement began to weaken, and at 72 hours of observation, the larval movement began to slow down. The treatment with *A. alba* extract showed that the movement of the larvae weakened after 24 hours of observation. Observations for 48 hours showed that the movement of the larvae slowed down until they did not move, and eventually, many deaths occurred. Positive control treatment indicated that death had occurred since the first observation, and negative control showed no change in larval behavior.

DISCUSSION

The results also showed that the leaf extracts of *S. alba* and *A. alba* tested had higher potency than in previous research. which stated that the extract of *S. alba* mangrove leaves prepared with the methanol solvent maceration method had an LC_{50} value against *Ae. aegypti* mosquito larvae of 14,415 ppm.[7] Another study stated that *Rhizophora stylosa* leaf extract obtained *with the* physical extraction method (*ultrasonic*) had an LC_{50} value of 2,885 ppm for *Ae. aegypti* mosquito larvae.[1]

The potential for biolarvacides in the mangrove extracts of *S. alba* and *A. alba* may be due to the influence of the bioactive compounds in the two tested mangrove leaf extracts. Several bioactive compounds that can cause the death of mosquito larvae are flavonoids, alkaloids, saponins, and tannin groups.[4] Based on screening results, the phytochemicals that have been carried out show that the leaf extracts of *S. alba* and *A. alba* positively contain bioactive compounds from the flavonoid, steroid, saponin, and tannin

groups. That shows that the two mangrove species have the potential to be bioleptics because they can kill the larvae of the *Ae. aegypti* mosquito.

Physical damage to the treatment of *S. alba* extract in the form of changes in the body color of mosquito larvae occurred after 6 hours of observation. At 12 hours of observation, there was an ecdysis process, and after 24 hours, there were defects in the abdomen and a siphon of mosquito larvae. Observations for 48 hours showed that death occurred with physical damage to the peritrophic membrane, respiratory system, and anal papillae. Changes in the color of the larvae due to treatment with *A. alba* extract also occurred after 6 hours of observation. Observation for 12 hours showed the process of ecdysis and damage to the abdomen. At 24 hours, observation showed the death of larvae with damage to the subperitrophic epithelium, endoperitrophic epithelium, peritrophic membrane, and intestine. The positive control treatment showed physical damage to the abdomen, while the negative control showed no physical damage to the larvae.

Based on this, it can be stated that the toxicity properties of the leaf extract of *S. alba* were chronic, while those of the *A. alba extract* were acute. That is because, in the treatment of *S. alba* leaf extract, death occurred during a long exposure time (48 hours) at a high concentration of mangrove extract (LC_{50}: 14,112 ppm), while in the treatment of *A. alba* leaf extract, death occurred during a short exposure (24 hours) with a low extract concentration (LC_{50}: 1,053 ppm).

The physical damage to *Ae. aegypti larvae that* occurred might be due to the presence of bioactive compounds in the leaf extracts of *S. alba* and *A. alba.* This matter is evidenced by observations, which showed that in the negative control treatment, the mosquito larvae did not experience any changes in behavior or physical damage at all during the test. The difference in the brownish-green color of larvae in the test of S. alba extract and yellowish green in the test of A. alba extract is due to physical damage in the form of color changes that occur in the bodies of mosquito larvae.

The ecdysis process in *Ae. aegypti* larvae indirectly indicates that the tested *S. alba* and *A. alba* leaf extracts have affected the physiological performance of *Ae. aegypti* mosquito larvae. The presence of steroid compounds may play a role in preventing larvae from producing the hormone ecdysone, which initiates the process of ecdysis, or skin turnover. The process of ecdysis is one form of larval adaptation when the environment is stressed.[11]

The digestive tracts of mosquito larvae that were damaged indicated that the extract had affected the work of the organs. The saponin compound's content can damage the outermost cell membrane, allowing toxic substances from the test extract to enter the larvae's body more easily.[12] Saponin compounds can also damage or deform the digestive wall of *Aedes aegypti* larvae.[13] That can be seen in the toxicity test of the two leaf extracts, but the most severe defects occurred in the treatment of *A. alba* extract, which was thought to contain more saponins than *S. alba* extract.

The death of mosquito larvae due to the treatment with *S. alba* leaf extract was thought to be because the *S. alba* leaf extract may have more flavonoid compounds than the *A. alba* extract. The flavonoid bioactive compounds in the extract are toxic to the larval respiratory system by damaging the siphon membrane and anal papilla.[13] Flavonoid compounds also cause weakness in the nerves and damage the respiratory system, which will cause the larvae to be unable to breathe and eventually die.[14] The bioactive compounds from the tannin group in the two extracts could disrupt the larval muscles' performance, affecting the larvae's behavior from active to passive.[13] The tannin group can also affect the absorption of protein in the midgut of larvae, so that the larvae will lack nutrition and end up in death.[14] Steroid compounds can stimulate ecdysis in larvae.[11] Based on this, it can be stated that the leaf extract of *A. alba* is more effective at killing *Ae. aegypti* larvae than *S. alba* leaf extract.

Differences in the bioactive content of *S. alba* leaf extract and *A. alba* are probably caused by differences in leaf anatomy in the thickness of

the mesophyll tissue. Bioactive compounds in plant tissues are a by-product of plant metabolism, accumulating in vacuole organelles.[15] These organelles are abundant in the mesophyll tissue, especially the spongy tissue, which functions as a storage place for metabolic products and by-products.[16] Based on this, it can be assumed that the thicker the spongy tissue of a leaf, the more bioactive compounds it contains. Based on the results of this study, it was found that the potential as a larvicidal bioactive compound of *A. alba* was superior to that of *S. alba*. That is related to the anatomical condition of *S. alba* leaves, which are thicker than *A. alba leaves*, but that does not mean that *S. alba* leaves can produce more bioactive compounds. The higher quantity of bioactive compounds in *A. alba* leaves was caused by spongy tissue consisting of two to three layers in the mesophyll tissue, whereas *S. alba* leaves only had one layer of spongy tissue in the mesophyll tissue. Several bioactive compounds, such as alkaloids, phenolic compounds, tannins, lipids, and flavonoids, are stored in sponge tissue.[16] The research results show that the more layers of sponges on a leaf, the more potential there is for bioactive compounds such as biolarvacides.

CONCLUSION

The research results concluded that the leaf extracts of *S. alba* and *A. alba had* potential as biolarvacides, where the potency of *A. alba* was higher than that of *S. alba.* The LC_{50} value of *A. alba* leaf extract was reached at a concentration of 1,053 ppm, which is a lower concentration than the *S. alba* extract, which was estimated to be reached at a concentration of 14,112 ppm. The toxicity properties seen from responses in behavior and physical damage to mosquito larvae showed that *A. alba* leaf extract was acute. In contrast, *S. alba* leaf extract was chronically active against *Ae. aegypti* mosquito larvae. *A. alba* leaf extract is more effective at killing *Ae. aegypti* larvae than *S. alba* leaf extract.

RECOMMENDATION

In the following study, it is necessary to purify and quantitatively analyze the bioactives contained in the leaf extracts of *S. alba* and *A. alba.* Furthermore, the pure compound was tested for toxicity against *Ae. aegypti* mosquito larvae to know precisely the groups of compounds that have the most role as biolarvacides against *Ae. aegypti* mosquito larvae. It is also necessary to test the toxicity of these compounds to get an effective dosage and test them on the other mosquito vector species.

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AUTHOR CONTRIBUTION

The main contributors in this article are MTH, who contribute as conceptors, data analysts, and article writers. The co-contributors are BM and SPMW, who contributed to data collection and article correction.

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