

POTENTIAL STUDY OF MANGROVE (*Avicennia marina*) as *Aedes aegypti* LARVICIDE AGENT

STUDI POTENSI MANGROVE (*Avicennia marina*) SEBAGAI AGEN LARVASIDA *Aedes aegypti*

Irene Devina E^{1*}, Mahmiah², Veryl Hasan³

¹Magister Program of Fisheries and Marine Biotechnology, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya, East Java, 60115. Indonesia

²Department of Oceanography, Faculty of Marine and Technology, Hang Tuah University, Surabaya, Indonesia

³Department of Marine, Faculty of Fisheries and Marine Science, Universitas Airlangga, Surabaya. Indonesia

*Correspondence author email: irededevina2002@gmail.com

Abstract

Aedes aegypti is the main vector that transmits the dengue virus, which causes dengue hemorrhagic fever (DHF). One preventive measure is to exterminate the larvae *A. aegypti* using environmentally friendly alternative materials. Mangrove *Avicennia marina* contains bioactive compounds that have the potential to act as natural larvicides. This study used a completely randomized design (CRD) with five concentration variations (10%, 20%, 30%, 40%, and 50%) and three replications, using a total of 550 test larvae *A. aegypti*. The aim of the study was to identify the active ingredients and effective concentration of leaf extract *A. marina* as a larvicide against *A. aegypti*. The method included sample preparation, maceration extraction with 70% methanol, phytochemical characterization, FTIR and GC-MS analysis, and larvicidal test. The results showed that the methanol extract of leaves *A. marina* has the potential as a natural larvicide based on the content of secondary metabolites, namely alkaloids, phenolics, terpenoids and steroids, which is indicated by the LC₅₀ value by 9.42%.

Keywords: *Avicennia marina*, *Aedes aegypti*, leaves, secondary metabolites, larvicide

Abstrak

Aedes aegypti merupakan vektor utama yang mentransmisikan virus dengue penyebab demam berdarah dengue (DBD). Salah satu upaya pencegahan adalah pemusnahan larva *A. aegypti* menggunakan bahan alternatif ramah lingkungan. Mangrove *Avicennia marina* memiliki senyawa bioaktif yang berpotensi sebagai larvasida alami. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 5 variasi konsentrasi (10%, 20%, 30%, 40%, 50%) dan 3 kali pengulangan, menggunakan total 550 larva uji *A. aegypti*. Tujuan penelitian adalah untuk mengidentifikasi bahan aktif dan konsentrasi efektif ekstrak daun *A. marina* sebagai larvasida terhadap *A. aegypti*. Metode meliputi preparasi sampel, ekstraksi maserasi dengan metanol 70%, karakterisasi fitokimia, analisis FTIR dan GC-MS, serta uji larvasida. Hasil penelitian menunjukkan bahwa ekstrak metanol daun *A. marina* berpotensi sebagai larvasida alami berdasarkan kandungan metabolit sekunder yaitu alkaloid, fenolat, terpenoid, dan steroid, yang ditunjukkan dengan nilai LC₅₀ sebesar 9,42%.

Kata Kunci: *Avicennia marina*, *Aedes aegypti*, daun, metabolit sekunder, larvasida

INTRODUCTION

One way to prevent dengue fever is to destroy the larvae *A. aegypti*. Alternative materials other than chemicals that can be used as larvicides are urgently needed. Synthetic insecticides require caution because their use can cause environmental pollution, the death of various other living creatures, and cause larvae to become resistant (Makkiah et al., 2019). The use of synthetic insecticides can also cause gene mutations in species. Natural insecticides have been proven to provide a significant contribution as a new alternative in efforts to reduce the

number of diseases caused by mosquito vectors (Riyadiet al., 2018). One alternative to natural insecticides is mangrove plants. Mangrove plants are one of the coastal plants that have many benefits. Mangroves have high economic and ecological value. The economic functions of mangroves include providing wood, leaves as raw materials for medicines and others. Ecological functions as a provider of nutrients for aquatic biota, spawning and nursery grounds for various types of biota, and as a barrier against abrasion, hurricanes, and tsunamis.

Mangroves are a typical coastal ecosystem influenced by tides. Furthermore, mangrove soil also has a high salt and water content, abundant sulfide acid, low oxygen content, and other coarse materials derived from the breakdown of organisms. The extreme environmental conditions in the mangrove ecosystem cause mangroves to produce various bioactive compounds that function as a defense mechanism for the environment. Many bioactive compounds have toxicological, pharmacological, and ecological properties (Kokpol et al., 1990 in Mahmiah et al., 2017).

Biolarvicide consists of active components of secondary metabolites such as saponins, terpenoids, alkaloids, and flavonoids in every mangrove ecosystem *A. marina*. This mangrove is known to contain secondary metabolites in the form of saponins, terpenoids, alkaloids and flavonoids (Eriani et al., 2019). One of the types of mangrove *Avicennia* sp. *Avicennia marina* is one of the types of mangroves that fall into the major mangrove category, so *A. marina* easily found in every mangrove ecosystem *A. marina*. This mangrove is known to contain secondary metabolites in the form of saponins, terpenoids, alkaloids and flavonoids (Eriani et al., 2019).

A. marina has bioactive compounds such as saponins, which can eradicate mosquito larvae in a similar way to Abate powder. In several studies conducted by Mukhraini (2016) in Eriani et al. (2019) explains that saponins can degrade cell membranes to enter the nerves and damage cells and can also disrupt the nervous system of larvae by inhibiting the work of the acetylcholinesterase enzyme in larvae.

MATERIALS AND METHODS

Materials and Sampling Locations

Leaf *A. marina* was taken from the mangrove ecosystem around the Medokan Ayu area, Surabaya, East Java. The leaves obtained were wet sorted to separate the leaves from the twigs, then dry sorted at 38°C. After drying, the material was ground to produce 1,800 g of simple powder. The chemicals used included 70% methanol, 96% ethanol, dimethyl sulfoxide (DMSO), ethyl acetate, n-hexane, concentrated HCl, Dragendroff's reagent, FeCl₃, and p.a. methanol. Larvae *A. aegypti* instar III was obtained from the Laboratory of the Institute of Tropical Disease (ITD), Airlangga University, Surabaya.

Extraction

Solvent optimization was determined through mini-maceration using n-hexane, ethanol, and methanol. Based on the optimization results, 70% methanol was chosen as the solvent because it produced an extract with the most complete secondary metabolite content. 1,000 g of simplicia powder was macerated for 3 x 24

hours using 70% methanol, resulting in 8,075 ml of liquid extract. After the evaporation process using *rotary evaporator*, a thick extract of 197 g was obtained with a yield of 19.7%.

Phytochemical Characterization, FTIR, and GC-MS

Phytochemical screening was carried out to identify the groups of secondary metabolite compounds in the thick leaf extract *A. marina*. Identification of the content was carried out using specific tests for alkaloids (Dragendroff's reagent), steroids, triterpenoids, saponins, and phenolics. Further characterization used a Fourier Transform Infrared Spectrophotometer (FTIR) and Gas Chromatography-Mass Spectroscopy (GC-MS) to identify functional groups and compound profiles qualitatively (Eswaraiah et al., 2018).

Larvicide Test

The larvicidal test began with optimizing the DMSO concentration as a solvent that is non-toxic to the larvae, followed by optimizing the extract concentration in the range of 1%–25%. Based on the results of the preliminary test, the main larvicidal test was conducted at four concentration variations (10%, 20%, 40%, and 50%), with a 1% abate as a positive control and a 1.25% DMSO as a negative control. Each treatment used 25 larvae *A. aegypti* instar III with 3 replications, so that the total number of larvae used was 550. Observations of larval mortality were carried out at 24 and 48 hours after treatment (Kristanti et al., 2008). The percentage of larval mortality was calculated using the formula:

$$\% \text{ deaths} = (A - B) / C \times 100\%$$

Description:

A = number of dead larvae in the test solution;

B = number of dead larvae in the blank;

C = initial number of larvae.

Data analysis used linear regression to obtain the LC₅₀ value with the equation $y = bx + a$ (Susilawati et al., 2021).

RESULTS

Solvent Optimization

The results of solvent optimization using the mini maceration technique are presented in (Table 1) methanol produced a dark green extract, ethanol produced a green color, and n-hexane produced a yellow color. Based on these results, 70% methanol was chosen as the extraction solvent for the next stage because it produced the most concentrated extract color indicating a more complete secondary metabolite content.

Table 1. Results of solvent optimization on leaves

<i>A. marina</i>		
No	Solvent	Color
1	Ethanol	Green
2	Methanol	Dark green
3	N-Hexane	Yellow

Phytochemical Screening

Leaf *A. marina* obtained a weight of 1,000 g of simplicia. The weight of 1,000 g of simplicia was taken for maceration for 3x24 hours using 70% methanol and obtained a liquid extract of 8,075 ml. After being thickened, it obtained 197 g and produced a yield of 19.7%. Leaf extract *A. marina*. The phytochemical screening test was carried out to identify the content of secondary metabolite compounds. The results of the phytochemical screening test are presented in (Table 2).

Table 2. Results of Phytochemical Screening of Extracts *A. marina*

No	Metabolite Seconds	Results
1	Alkaloid	+
2	Steroid	+
3	Triterpenoid	+
4	Saponin	+
5	Phenolics	+

Phytochemical screening results of methanol extract of leaves *A. marina* showed positive results in all secondary metabolite tests performed. The presence of alkaloids, steroids, triterpenoids, saponins, and phenolics was

confirmed through specific tests for each compound group.

FTIR Characterization

FTIR spectrum of leaf extract *A. marina* displays several characteristic absorption bands as in (Figure 1). In region I (4,000–2,500 cm^{-1}), wave number 3,358.28 cm^{-1} shows the presence of the N-H functional group (predicted alkaloid group). In region III (2,000–1,500 cm^{-1}), wave number 2,947.50 cm^{-1} shows the C=O carbonyl group. Wave number 1,517.52 cm^{-1} identified as aromatic C=C stretching vibration, which is strengthened by the absorption band in the fingerprint region of 1,017.75 cm^{-1} . These spectral data as a whole support the results of phytochemical screening which confirm the presence of alkaloids, terpenoids, saponins, and phenolics in the leaf extract *A. marina*.



Figure 1. FTIR spectrum of leaf extract *A. marina*

Table 3. Results of GC-MS analysis of leaf extracts *A. marina*

Pk #	RT	% Area	Library	Group Prediction
1	2.822	4.86	N, N'-Diethyl-1,3-[2-13C] propanediamine	Alkaloid
2	3.817	4.97	Methyloxirane	Saponin
3	5.405	1.41	6,6-diethyldihydro-1,3,3- trimethyl-3-Heptene	Saponin
4	5.895	1.99	2-(2-Isobutenyl)-3-methylfuran	Phenolics
5	6.251	0.55	2-Nonadecanol	Saponin
6	6.975	45	N,N-Dimethyl-2- methoxyethylamine Oxirane	Alkaloid
7	7.278	23.85	4-Dimethylamino-3-Methyl- 1,2-Di Phenyl-2-Butyl Propionate	Alkaloid
8	7.777	1.3	N, N-Dimethylpro Panolamine	Alkaloid
9	8.575	2.9	Methylpropylamine	Alkaloid
10		0.9	2-Dodecanone (CAS)	Steroid
11	9.358	0.34	Benzeneethanol (CAS)	Phenolics
12	10.702	0.54	TRANS-CYCLOPENTENE- 3,5-DIOL	Steroid
13	12.151	1.83	Cyclobutanone	Saponin
14	21.001	6.72	4-vinyl-2-methoxy-phenol	Phenolics
15	21.096	2.85	2-N-Propoxyamphetamine	Alkaloid

Analisis GC-MS

GC-MS analysis identified 15 compound peaks in the leaf extract *A. marina* with various predicted groups (Table 3). Alkaloid compounds dominated the chromatogram profile, with two largest peaks at RT 6.975 minutes (45.00% area) namely N,N-Dimethyl-2-methoxyethylamine Oxirane, and RT 7.278 minutes (23.85% area) namely 4-Dimethylamino-3-Methyl-1,2-Di Phenyl-2-Butyl Propionate. Saponin groups were identified at RT 3.817; 5.405; 6.251; and 12.151 minutes, phenolics at RT 5.895; 9.358; and 21.001 minutes, and steroids at RT 9.890 and 10.702 minutes.

Larvicide Test

Results of observations on the number of larval deaths *A. aegypti*. The results of each treatment concentration are presented in Table 4. The trend in larval mortality showed an increase with increasing extract concentration and exposure time. In the 48-hour observation, the 50% concentration resulted in the highest number of deaths (replication 1: 19 larvae). The positive control of 1% abate showed the highest mortality in the first repetition of the 24-hour observation (18 larvae) and 48-hour observation (21 larvae), while the negative control of 1.25% DMSO did not cause larval mortality in all repetitions and observation times. Based on linear regression analysis, the LC value was obtained⁵⁰by 9.42%.

Table 4. Results of Larvicide Test Observations *A. aegypti* leaf extract *A. marina*

Concentration	24 hours			48 hours		
	1	2	3	1	2	3
10	0	0	3	0	12	8
20	1	7	3	8	14	20
40	9	11	7	8	17	14
50	6	8	4	19	9	7
Control (+)	18	1	0	21	0	0
Control (-)	0	0	0	0	0	0

DISCUSSION

Solvent Optimization and Phytochemical Screening

Solvent optimization results showed that methanol produced a dark green extract, while ethanol produced a green color and n-hexane produced a yellow color. The darker color intensity of the methanol extract indicates a richer secondary metabolite content, which was confirmed by phytochemical screening results. This is in line with the study of Sanjeev and Sushil (2022) which confirmed that methanol extract consistently showed the highest toxicity to mosquito larvae compared to acetone and n-

hexane extracts, because methanol is able to attract polar and semi-polar compounds more effectively.

The results of phytochemical screening that detected alkaloids, steroids, triterpenoids, saponins, and phenolics showed differences with Eriani *et al* (2019) on leaves *A. marina* in Riau that did not detect steroids. This difference is most likely due to variations in secondary metabolite content between populations influenced by local environmental conditions, such as salinity levels, light intensity, and soil composition (Supriatna *et al.*, 2019). Friis *et al.* (2021) confirmed that genetic diversity *A. marina*. Geographical differences between locations contribute to variations in the expression of bioactive compounds, so it is normal to find differences in phytochemical profiles between populations.

Spectroscopic Characterization and Identification of Dominant Compounds

FTIR spectrum data showing the N-H group at 3,358.28 cm⁻¹ and aromatic C=C at 1,517.52 cm⁻¹ strongly indicates the presence of aromatic alkaloids in the extract. GC-MS analysis identified 15 compound peaks in the leaf extract *A. marina*. The dominance of alkaloids in the GC-MS profile (total >75% of the chromatogram area) is consistent with Karthi's report *et al* (2020) who identified alkaloids as the main active compounds in *A. marina* and relate it to biological activity against mosquito vectors. The compound profiles identified by GC-MS, including amine and oxirane derivatives, provide insight into diverse toxicity mechanisms against mosquito larvae.

Larvicide Test and Mechanism of Action of Secondary Metabolites as Larvicides

Larval mortality *A. aegypti* increased with increasing extract concentration and exposure duration. Based on linear regression analysis, the LC₅₀ value was obtained of 9.42%, indicating a fairly strong larvicidal potential. This indicates that the higher the concentration and the longer the duration, the higher the larval mortality rate (Hoedjo and Zulhasril, 2016). Karthi *et al* (2020) reported the LC₅₀ value acetone extract *A. marina* against larvae *Aedes aegypti* of 0.164 mg/ml. The difference in LC₅₀ values. This can be caused by differences in solvent types, extraction methods, and the geographical origin of the plants which affect the composition and concentration of active compounds.

Larvicidal activity of leaf extract *A. marina* can be explained through the combined working mechanism of several groups of secondary metabolites. Kumara *et al.* (2021) explains that saponins work by irritating the digestive tract of larvae *A. aegypti*. Alkaloids are gastrotoxic and inhibit the larvae's feeding capacity, while flavonoids act as respiratory

inhibitors or respiratory toxins that can block the larvae's respiratory tract. The dominant alkaloid in this extract works by inhibiting the enzyme acetylcholinesterase, thus causing the accumulation of acetylcholine in the larvae's neuromuscular junctions and leading to death due to nervous system disorders (Mukhraini, 2016 in Eriani *et al.*, 2019). Terpenoids and steroids contribute through disruption of larval cell membranes, while phenolics act as toxic compounds that damage the integrity of biological membranes. The synergy between these secondary metabolites is an advantage of botanical larvicides compared to single chemical insecticides that are more susceptible to resistance (Kumara *et al.*, 2021; Makkiah *et al.*, 2019).

CONCLUSION

Methanol extract of leaves *Avicennia marina* has the potential to be a natural larvicide against larvae *Aedes aegypti* based on the identified secondary metabolite content, including alkaloids, phenolics, terpenoids, and steroids, with LC₅₀ values by 9.42%.

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