

Phytochemicals and Larvicidal Activity of *Sonneratia alba* Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali

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Abstract

Dengue is an endemic disease with a high incidence in almost all Southeast Asian countries, including Indonesia. This infectious disease is caused by a virus transmitted by the bite of *Aedes aegypti* mosquito as the main vector. Effective mosquito vector control is a crucial step in stopping the spread of this virus. Of the several methods available, the use of larvicides is considered one of the most successful treatments in reducing the number of mosquito vectors. However, widely used synthetic larvicides can have undesirable side effects on the environment and non-target organisms including human health. The aim of this study was to discover a new biolarvicide from natural materials that is relatively safer. In this study, the biolarvicidal activity of mangrove plant species that are commonly found in Ngurah Rai Mangrove Forest Bali was tested, namely *Sonneratia alba*. Mangrove root simplicia was extracted by reflux method using three different solvents namely methanol, chloroform, and n-hexane. The content of compounds in the extract were analyzed using GC/MS method. The larvicidal activity of the extracts were tested on *A. aegypti* instar III/IV larvae with concentrations of 0.1, 1, 10, 100, and 1000 ppm for 24 hours. Subsequently, the average value of larval mortality was used to calculate the LC50 of each extract. The results showed that *S. alba* methanol extract provided the best larvicidal activity compared to chloroform and n-hexane extracts, with mortality of 69.33% at a concentration of 1000 ppm and LC50 of 1265 ppm. GC-MS analysis showed that the methanol extract of *S. alba* contained five dominant compounds namely Methyl 2-hydroxy-eicosanoate (19.55%); 4H-1-Benzopyran-4-one, 3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy (16.48%); 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (10.06%); Benzamide, N-[4-(2-naphthyl)-2-thiazolyl]- (9.40%); and 2,3-Dihydro-3,5-bis(3-methoxyphenyl)-1H-inden-1-one (6.52%). The results of this study provide a preliminary result on larvicide activity from mangrove *S. alba* in order to develop bio larvicides from nature which is safer for human health and environment.

Keywords: larvicidal activity; roots; *Sonneratia alba*.

INTRODUCTION

Dengue is an infectious disease caused by the dengue virus (DENV). This virus has four serotypes, namely DENV 1, DENV 2, DENV 3, and DENV 4 (Gupta et al., 2021). Dengue is a mosquito-borne virus with the main vector being the female *Aedes aegypti*, which is also a vector of yellow fever, chikungunya, and Zika (WHO, 2020). DENV is estimated to infect 50-100 million people worldwide annually, causing an economic burden for both governments and individuals (Guo et al., 2017). This disease has caused health burden to the community, mainly because limited diagnostic tool available for the early phase of infections, no specific therapy to cure the infections, and limited availability for effective and efficient vector control systems (Cucunawangsih & Lugito, 2017). In the last 50 years, Indonesia has shown an increase in the incidence of dengue hemorrhagic fever, where Bali and Borneo (Kalimantan) have been the areas with the highest incidence in recent years (Harapan et al., 2019).

To date, no antiviral therapy is available for dengue other than supportive therapy (WHO, 2020). CYD-TDV vaccine, the first and only dengue vaccine legally distributed in 2015 has failed to meet safety and efficacy aspects (Wellekens et al., 2020). Therefore, the current focus is to prevent transmission of the virus from mosquitoes to humans through vector control methods (Jing & Wang, 2019). One of the most common methods used to control dengue vectors is the use of the organophosphate compound temephos (Abate[®]) (George et al., 2015). Unfortunately, long-term use of synthetic larvicides has been shown to cause resistance in vector organisms, as well as to cause unwanted effects on non-targets, including human health and the environment (Pavela et al., 2019). This has led to intensive research to find natural larvicides that are safer and more effective since the last few decades (Pavela et al., 2019).

Secondary metabolites derived from plants can be used as an alternative bio larvicides which are safer for humans and the environment, relatively inexpensive and

easy to obtain, biodegradable, as well as exhibit broad spectrum activity and specific targets against different species of mosquito vectors (Ghosh et al., 2012). Mangrove plants are a potential source that can be explored to find new bio larvicidal compounds. Harsh environmental conditions such as high salinity and low oxygen levels make mangrove plants adapt, one of which is by synthesizing unique secondary metabolites (Dahibhate et al., 2019). Of the various parts of mangrove plants, roots are one of the important and interesting parts to study because they are directly exposed to fluctuating oxygen and salinity levels (Reef & Lovelock, 2014). A phytochemical study of mangrove plant *Sonneratia alba* from Musi River Estuary, South Sumatera, Indonesia have shown that root extract of this species is rich in compounds such as flavonoids, triterpenoids, steroids, tannins, and phenols (Rahmania et al., 2018). Secondary metabolites such as flavonoids, triterpene, phenolic acid, and others were found to have larvicidal activity (de Souza Wuilida et al., 2019). Therefore, in this study we aimed to screen for larvicidal activity of *S. alba* root extracts by using three different solvents: n-hexane, chloroform, and methanol, against *Aedes aegypti* instar III/IV larvae. This research is expected to accelerate the discovery and development of natural larvicides that are effective and safe for human health and the environment in order to create effective and sustainable vector control management.

MATERIALS AND METHODS

Materials

Sonneratia alba root was collected from Simbar Segara area of Ngurah Rai Mangrove Forest (-8.72547397637542, 115.2002679693129) (Figure 1), Bali, Indonesia, on November 2022 during low tide. Solvents methanol, chloroform, and n-hexane were purchased from Merck-Supelco, Germany. Mosquito

eggs were collected in West Denpasar area using ovitraps. Temephos (Abate[®]) was used as a positive control.



Figure 1. Simbar Segara area, Ngurah Rai Mangrove Forest, Denpasar, Bali.

Methods

Sample preparation

Root samples of *S. alba* (Figure 2) were taken from five plants at different locations in Simbar Segara area of Ngurah Rai Mangrove Forest Bali. Sampling was carried out on Thursday, 3 November 2022 at 11.00 to 13.00 WITA during low tide. The collected roots were then washed under running water, cut into smaller sizes, and oven-dried at 50°C until the moisture content was <10%. The dry ingredients were chopped and blended into a coarse powder and then stored at room temperature until further processing. Sample determination was carried out at Characterization Laboratory, “Eka Karya” Botanical Garden, Baturiti, Tabanan, Bali-Indonesia.



Figure 2. *Sonneratia alba* tree (A), root part (B), and root samples (C).

Extraction

For each solvent (methanol, chloroform, or n-hexane), 100 grams of dry powder was refluxed 2x2.5 hours using 500 ml solvent at 50-55°C. The reflux results were filtered using Whatmann No.1 filter paper and evaporated using rotary evaporator at 50°C. The extract was stored in a sealed and airtight vial at 4°C until used.

Larvae maintenance

Aedes aegypti mosquito eggs were collected using a simple ovitrap (Djiappi-Tchamen et al., 2022) in the West Denpasar area. Mosquito eggs were kept in plastic clips covered with silica gel until they were hatched. Eggs were hatched by placing filter papers containing mosquito eggs in a tray filled with water to a depth of ± 2 cm. The room was maintained at an optimum temperature of $27 \pm 2^\circ\text{C}$, humidity of $75 \pm 10\%$ and with dim lighting. The larvae of *A. aegypti* mosquitoes in instar III/IV phase aged 3-6 days with a size of 4-6 mm were used as samples in this study.

Larvicidal activity testing

Larvicidal bioactivity testing was carried out based on the method described by World Health Organization WHO, 2005) with a slight modification. Stock solutions (10,000 ppm) of each extract were prepared by dissolving 1000 mg of the extract in 100 mL of distilled water and 0.1% dimethyl sulfoxide (DMSO). Stock solutions were used to prepare working solutions with concentrations of 0.1, 1, 10, 100, and 1000 ppm. A total of 1 mg/L temephos (Abate®) was used as a positive control, while 0.01% DMSO was used as a negative control.

A total of 20 instar III/IV larvae were transferred into 100 mL of each working and control solutions, then incubated for 24 hours. The number of dead larvae was counted after 24 hours of exposure. Mortality rates were corrected using the Abbott formula (Abbott, 1925) and percentage mortality was calculated based on the average of three repetitions.

Phytochemical screening

Compounds identification of the extracts were carried out using gas chromatography coupled to mass spectrometry (GC-MS) at the Forensic Laboratory, Bali Regional Police Station. Each component was identified by comparing between the mass spectrum and the retention time of the sample with a standard.

Data analysis

The LC50 and LC90 values were calculated using Probit analysis with Statistical Package for the Social Sciences (SPSS) software version 25.

RESULTS AND DISCUSSION

Larvicides are crucial in *A. aegypti* control program for killing mosquitoes' larvae. However, long-term use of synthetic larvicides have serious negative impact on the human health, environment, as well as increase the risk of resistance in vector organism (Pavela et al., 2019). In the contrary, some medicinal plants have larvicidal properties that have allowed them to be used as safer alternatives (Al-Rashidi et al., 2022; Aljameeli, 2023). Mangrove plants with their unique secondary metabolites are one of the most potential alternatives to synthetic larvicides. Some studies have shown that mangrove plants such as *Rhizophora mucronata*, *Avicennia marina*, and *Excoecaria agallocha* show the potential to be utilized as biolarvicides (Ali et al., 2012; Karthi et al., 2020; Pradeepa et al., 2015). In this study, larvicidal activity of mangrove plant *Sonneratia alba* was examined using three different solvents namely methanol, chloroform, and n-hexane.

The larvicidal activity testing results with three repetitions showed that the methanol extract of *S. alba* showed the most prominent result compared to chloroform and n-hexane extracts with percentage of mortality of 69.33% at a concentration of 1000 ppm (Table 1). This result indicated that different solvent polarity can affect the bioactivity of extracts from the same plant sample because of different active biochemicals extracted by each solvent. In addition, Probit analysis results show that the LC50 of *S. alba* methanol extract is 1265 ppm. This result is lower than the positive control, which showed 100% mortality rate at only 1 ppm. A previous study conducted by Karthi et al (2020) showed that acetone extract of *R. mucronata* leaves displayed an effective efficacy against *A. aegypti* larvae with LC50 of 0.11 ppm. A study by Ali et al (2012) reported that ethanol-water mixture extract of *A. marina* demonstrates larvicidal activity against *A. aegypti* larvae with LC50 of 135.8 ppm. Such large discrepancies that were obtained in this study compared to previous studies could possibly happened because the bioactive compounds in the methanol extract of *S. alba* present in low quantity. Moreover, the larvicidal activity of plant extracts can varies greatly depends on the plant species, plant part used, age of plant part, solvent, and vector species (Ghosh et al., 2012). To the best of our knowledge, this is the first study reported larvicide potential from *S. alba* root. Previous study showed that methanol, chloroform, and n-hexane extracts of *S. alba* roots have no antibacterial activity against *Staphylococcus aureus*, but n-hexane extract displayed activity against *Streptococcus mutans* (Wijaya & Indraningrat, 2021).

Table 1. Larvicidal activity screening of *S. alba* root extracts.

Solvents	Concentration (ppm)	Number of Larvae (n)	Mortality			Average \pm SD	% Mortality
			R1	R2	R3		
Methanol	0.1	25	0	0	0	0.00	0.00
	1	25	0	0	0	0.00	0.00
	10	25	0	0	0	0.00	0.00
	100	25	4	1	5	3.33 \pm 2.08	13.33
	1000	25	18	15	19	17.33 \pm 2.08	69.33
Chloroform	0.1	25	0	0	0	0.00	0.00
	1	25	0	0	0	0.00	0.00
	10	25	0	0	0	0.00	0.00
	100	25	0	0	0	0.00	0.00
	1000	25	4	2	2	2.67 \pm 1.15	10.67
N-hexane	0.1	25	0	0	0	0.00	0.00
	1	25	0	0	0	0.00	0.00
	10	25	0	0	0	0.00	0.00
	100	25	0	1	0	0.33 \pm 0.58	1.33
	1000	25	2	5	3	3.33 \pm 1.53	13.33
Positive control	1	25	25	25	25	100 \pm 0.00	100.00
Negative control	-	25	0	0	0	0.00	0.00

Note: R = repetition

The results showed that there were five main compounds contained in the methanol extract of *S. alba*, namely Methyl 2-hydroxy-eicosanoate (19.55%); 4H-1-Benzopyran-4-one, 3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy (16.48%); 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (10.06%); Benzamide, N-[4-(2-naphthyl)-2-thiazolyl]- (9.40%); and 2,3-Dihydro-3,5-bis(3-methoxyphenyl)-1H-inden-1-one (6.52%) (Table 2). Meanwhile, the GC-MS analysis of chloroform extract showed the five dominant compounds

were Stigmasterol (57.91%); Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)- (6.28%); 1H-Indole, 5-methyl-2-phenyl- (4.66%); Nonane, 2,2,4,4,6,8,8-heptamethyl- (3.53%); and Cyclic octaatomic sulfur (3.04%) (Table 3). In addition, the five dominant compounds found in the n-hexane extract were Stigmasterol (32.61%); Stigmast-4-en-3-one (29.37%); Campesterol (8.20%); 9,19-Cyclolanost-24-en-3-ol, (3.beta.)- (5.70%); and 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)- (4.80%) (Table 4).

Table 2. GC-MS analysis results of methanol extract of *S. alba*.

No	Compounds	RT	%area
1	1,2,3-Benzenetriol	12.138	5.17
2	Propanoic acid, 3-hydroxy-	13.519	2.00
3	3-Hydroxy-4-methoxybenzoic acid	14.576	1.01
4	2,3,4,5-Tetrafluoroanisole	14.678	1.62
5	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	16.625	10.06
6	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	17.394	0.98
7	trans-Sinapyl alcohol	19.203	2.92
8	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl) cyclohex-2-enone	24.200	1.79
9	Orcinol, monoacetate	24.682	2.62
10	1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl- ATAU Nitroscanate	24.740	3.43
11	1-Octadecene	26.087	2.25
12	1-Hexacosene	26.164	0.69
13	1-Hexacosene	26.253	0.62
14	Tricosane	26.425	1.87
15	9H-Xanthen-9-one, 1-hydroxy-3,5,6-trimethoxy	26.581	1.50
16	1-Hexacosene	27.087	0.90
17	Methyl 2-hydroxy-eicosanoate	27.516	19.55
18	1-Hexacosene	27.803	2.89
19	4H-1-Benzopyran-4-one, 3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy	28.408	16.48
20	Benzamide, N-[4-(2-naphthyl)-2-thiazolyl]-	28.458	9.40
21	2,3-Dihydro-3,5-bis(3-methoxyphenyl)-1H-inden-1-one	28.793	6.52
22	1-Hexacosene	29.043	5.73

Note: RT = retention time

Table 3. GC-MS analysis results of chloroform extract of *S. alba*.

No	Compounds	RT	%area
1	Fumaric acid, 2-dimethylaminoethyl hexadecyl ester	5.992	1.11
2	9,19-Cyclolanost-6-ene-3,7-diol, diacetate	8.609	0.52
3	d-Ribose, 2-deoxy-bis(thioheptyl)-dithioacetal	8.648	0.74
4	Nonane, 2,2,4,4,6,8,8-heptamethyl-	11.400	3.53
5	4,25-Secoobscurinervan-4-one-22-ethyl-15,16-dimethoxy-, (22.alpha.)	14.083	0.88
6	3(2H)-Furanone, dihydro-2,2-dimethyl-5-phenyl-	16.797	0.80
7	Octadecane	17.251	0.51
8	Docosane	18.275	1.00
9	Heptacosane	19.249	2.04
10	Cyclic octaatomic sulfur	19.650	3.04
11	Heptacosane	20.183	1.92
12	Eicosane	21.073	0.89
13	17-Pentatriacontene	21.923	0.73
14	17-Pentatriacontene	22.754	0.46
15	Octatriacontyl pentafluoropropionate	23.190	2.28
16	17-Pentatriacontene	23.548	1.64
17	1H-Indole, 5-methyl-2-phenyl-	23.938	4.04
18	1H-Indole, 5-methyl-2-phenyl-	24.036	4.66
19	1-Nonadecene	24.091	1.43
20	17-Pentatriacontene	24.175	1.02
21	3,5,7-Triazatricyclo[6.3.0.0(3,7)]undec-11-ene-4,6-dione, 2,2-diphenyl-5-methyl-	24.205	1.39
22	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	29.026	6.28
23	Stigmasterol	29.343	57.91
24	5-Fluoro-3-trifluoromethylbenzoic acid, nonadecyl ester	29.652	1.17

Note: RT = retention time

Table 4. GC-MS analysis results of n-hexane extract of *S. alba*.

No	Compounds	RT	%area
1	2,4-Di-tert-butylphenol	13.999	1.21
2	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	23.611	5.70
3	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, acetate, (E,E)-	23.705	4.80
4	7,11-Hexadecadienal	23.786	4.48
5	Bis(2-ethylhexyl) phthalate	23.941	3.91
6	Stigmast-4-en-3-one	25.483	29.37
7	21-Hydroxyprogesterone, trifluoroacetate	25.602	2.36
8	17-Pentatriacontene	27.827	1.21
9	Campesterol	29.017	8.20
10	Stigmasterol	29.342	32.61
11	Stigmasterol	29.437	6.15

Note: RT = retention time

Methyl 2-hydroxy-eicosanoate is a compound that majorly present in methanol extract of *S. alba* leaves. This compound is fatty acid methyl esters that previously found in oil palm roots, aerial parts of *Ipomoea eriocarpa*, and *Terminalia benzoe* (Alexander, Dayou, Abdullah, & Chong, 2017; Das & Himaja, 2014; El-Rafie, Mohammed, Hamed, Ibrahim, & Abou Zeid, 2016). However, its pharmacological activity data is still limited. Other major compounds in methanol extract are still poorly studied as well.

Stigmasterol was abundant in both chloroform and n-hexane extracts. Stigmasterol is a phytosterol that belong to tetracyclic triterpenes class and is known to exhibit

some pharmacological property such as anticancer, antiinflammation, antioxidant, antibacterial, antifungal, and antiparasitic (Bakrim et al., 2022; Morgan et al., 2021). In addition, a study conducted by Gade et al (2017) indicates that acetylcholinesterase inhibitory activity of stigmasterol and 1-haxacosanol is accountable for larvicidal activity of *Chromolaena odorata* (Gade et al., 2017). However, percent mortality of chloroform and n-hexane extracts against *A. aegypti* larvae in this study are only 10.67% and 13.33% respectively at concentration of 1000 ppm. It could be that the amount of stigmasterol in both extracts was not sufficient to give strong larvicidal effect.

CONCLUSIONS

In conclusion, methanol extract of *S. alba* root displayed the highest larvicidal activity compared to chloroform and n-hexane extracts, with 69.33% mortality value at 1000 ppm and LC50 value of 1265 ppm. Five main compounds found in the methanol extract were Methyl 2-hydroxy-eicosanoate; 4H-1-Benzopyran-4-one, 3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; Benzamide, N-[4-(2-naphthyl)-2-thiazolyl]-; and 2,3-Dihydro-3,5-bis(3-methoxyphenyl)-1H-inden-1-one. To the best of our knowledge, this is the first report on larvicidal activity from *S. alba* root extract. However, further studies are required to determine the exact larvicide compound(s) from *S. alba* root extract.

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