

## Antimicrobial Activity of *Sonneratia ovata* Backer

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### ABSTRACT

*Sonneratia*, a genus of mangroves from family Lythraceae, is reported for number of high biological activity including antimicrobial. *Sonneratia ovata* Backer is one of the species which traditionally used by Indonesian people for the treatment of some diseases. In this research, the investigation about biological activity of *S. ovata* Backer as anti-microbial has been performed. The methanol extract of *S. ovata* Backer is highly potential as antimicrobial against gram positive bacteria, *S. aureus*, with  $IC_{50}$  value of 7.7  $\mu\text{g/ml}$  which is higher than of Ampicillin as positive control with  $IC_{50}$  value of 37.8  $\mu\text{g/ml}$ . Furthermore, stigmasterol one of the major compound of *S. ovata* Backer has been isolated from the methanol extract of the stem bark of *S. ovata* Backer. Therefore, *S. ovata* Backer is proven to have high activity as antimicrobial, and highly potential to be a new source of antimicrobial agent.

## 1. Introduction

Pathogenic microbes are one of the causes of number of disease in human, animals, or plants (Purnomo *et al.* 2018). Pathogenic microbes are consist of some kinds of microorganisms, such as bacteria, fungus, and algae. Some diseases caused by pathogenic microbes, specially bacteria are skin infection, pneumonia, endocarditis, and osteomilietis that caused by gram positive bacteria (Bush and Perez 2017). Because of the large number of newly discovered pathogens in last period, as well as the increasingly strong resistance of pathogens to antibiotics which brings pathogens become immune to some well-known antimicrobials (Putri *et al.* 2018; Auwaliyah *et al.* 2019; Ramadhania *et al.* 2019), therefore, many new antimicrobials are needed to inhibit the pathogens growth (Rahman *et al.* 2005).

*Sonneratia* (Lythraceae) is one of the genus of mangroves which only exist in Indomalayan area. *Sonneratia* consists of some species, such as *S. caseolaris*, *S. alba*, *S. apetala*, *S. griffithi*, *S. hainanensis*, *S. lanceolata*, and *S. ovata* (Mao and Foong 2013). Its high ability to adapt in extreme environment with high temperature and salinity, strong wind, and muddy anaerobic soil makes *Sonneratia* become rich of secondary metabolites with unique biological activity and high of medical potential. Traditionally, *Sonneratia*

has been used in treatment of some diseases, such as asthma, hepatitis, hemorrhage, ulcer, and hemmoroid (Bandaranayake 2003). Some reports showed some medical potential of *Sonneratia*, such as *S. caseolaris* has strong antioxidant activity (Bunyapraphatsara 2003), while Saad *et al.* 2012 reported that *S. alba* has positive antimicrobial activity against some microorganisms (Saad *et al.* 2012). *S. griffithi* shows a high activity as antihyperglycemia (Tiwari *et al.* 2008), and *S. caseolaris* shows intestinal  $\alpha$ -glucosidase inhibitory activity (Kaewpiboon *et al.* 2012). One of the species of *Sonneratia* grows naturally in Indonesian coastline, specially in Aru Island, Maluku is *S. ovata* Backer. Morphology of the tree of *S. ovata* Backer is a columnar tree with quadrangular branches high of 7.2 meters, pale brown to grey color, thin and sharp pneumatophores. The leaves are apex obtuse but rounded at the base, glossy on the upper surface but satiny lower surface. The fruits of *S. ovata* Backer are berry-like with seeds and sized 4-6 cm (Goutham-Bharathi *et al.* 2012).

Research about bioactivity of *S. ovata* Backer have been reported (Khumaidah *et al.* 2018). There are several compounds have been successfully isolated from the extract of *S. ovata* Backer and examined for the activity. Four compounds were isolated by Nguyen *et al.* 2015. There are three new fenolic compounds; sonnerfenolic A, sonnerfenolic B, and sonnerfenolic C, and one cerebroside called sonnercerebroside. Sonnercerebroside showed high cytotoxic activity against MCF-7 cancer cell with  $IC_{50}$  value of  $112.8 \pm 9.4 \mu\text{m}$  (Nguyen *et al.* 2015).

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*S. ovata* Backer, which traditionally called *Manggustang Pante*, has been used as a folk medicine to treat diseases for over decades by drink the water extract of *S. ovata* Backer. That ethnobotanical report indicates that *S. ovata* Backer has high biological activity which can lead to further investigation, specially for the antimicrobial activity.

## 2. Materials and Methods

### 2.1. Extraction

The stem bark of *S. ovata* Backer was collected from Aru Islands, Maluku. The plant was dried and powdered to form a 1.5 kg of sample. Extraction was conducted by soaking sample in seven liters of methanol within three days at room temperature. Solvent then was evaporated to give 153 grams of methanol extract.

### 2.2. Isolation

Crude methanol extract of the stem bark of *S. ovata* Backer then was partitioned with butanol and water (1:1). The butanol phase was collected and evaporated, then was subjected to the silica gel column chromatography. Column chromatography was eluted with mixture of *n*-hexane:ethyl acetate (7:3) to gain five fractions. Thin layer chromatography was used to combine the fractions with the same profile. Fraction with one clear spot on TLC chromatogram then purified for NMR examination.

### 2.3. Bacteria Preparation

The antimicrobial assay using gram positive and negative bacteria, *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*. The bacteria were obtained from the collection of Microorganism Laboratory, Department of Chemistry, ITS, then were inoculated in Nutrient Broth and incubated for 24, 20, 24, and 21 hours, respectively. Each of the bacteria in Nutrient Broth then was diluted until final concentration of  $1 \times 10^4$  CFU/ml.

### 2.4. Antimicrobial Assay

Antimicrobial assay was done by adapting method of Arias *et al.* 2004 with modification. A 50  $\mu$ l of each of gram positive and negative bacteria with concentration of  $1 \times 10^4$  CFU/ml was added to 5  $\mu$ l sample in 445

$\mu$ l Nutrient Broth. The mixture was then incubated for 18 hours at 37°C. The antimicrobial activity then calculated on 96 micro-well plate at 630 nm using spectrophotometer. Positive and negative controls were Ampicillin and DMSO, respectively (Arias *et al.* 2004).

## 3. Results

### 3.1. Isolation of Stigmasterol from *S. ovata* Stem Bark

The methanol extract of the stem bark of *S. ovata* Backer (3 grams) was partitioned with butanol and water (1:1), two times. The butanol phase were combined and evaporated to yield 0.83 gram of crude butanol phase. The crude butanol phase then diluted in ethyl acetate and subjected to the column chromatography, with *n*-hexane:ethyl acetate (7:3) as the eluent. The fractionation using column chromatography to give five fractions. The profile of the second fraction (Fraction B) was monitored using TLC with cerium sulfate as the coloring agent and yielded one clear spot with Rf value of 0.58. Furthermore, TLC chromatogram profile of fraction B and Stigmasterol which was already identified using <sup>1</sup>H and <sup>13</sup>C-NMR, were examined. For <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 0.91 (s, 3H), 0.98 (s, 3H), 1.06 (s, 3H), 1.19 (s, 3H), 1.23 (s, 3H), 3.20 (tdd, OH, H-3), 4.14 (s, 1H), 4.57 (s, 1H), and 5.24 (m, 1H, H-6) and <sup>13</sup>C-NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 71.95 (C3), 129.40 (C5), 121.87 (C6), 29.08 (C16), 140.88 (C22), 138.47 (C23), 32.04 (C25), and 12.41 (C29).

### 3.2. Antimicrobial Assay

Screening of inhibition against microbial by extract of *S. ovata* Backer showed a significant data where *S. ovata* Backer strongly inhibits growth of *S. aureus* bacteria and slightly inhibits *B. subtilis* bacteria. In addition, extract of *S. ovata* Backer is not active against *E. coli* and *P. aeruginosa* (Table 1). This result indicates a high potential activity of *S. ovata* Backer against *S. aureus* bacteria and lead to the calculation of IC<sub>50</sub> value presented in Figure 1 and summarized in Table 2. On the other side, Stigmasterol as a major compound of *S. ovata* Backer has been previously reported its minimum inhibitory concentration against *S. aureus* and *E. coli* bacteria.

Table 1. Inhibition rate (%) of extract of *S. Ovata* Backer

Sample	Inhibition (%) <sup>1</sup> against bacteria of			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Extract of <i>S. ovata</i> Backer	155.43±13.9	23.8±11.56	NA <sup>2</sup>	NA <sup>2</sup>
Positive control (Ampicillin)	109.13±4.83	86.91±15.8	912.32±1461.9	136.8±54.9

#### 4. Discussion

The TLC chromatogram profile comparison between fraction B of *S. ovata* Backer and Stigmasterol standard shows the same  $R_f$  value of 0.58. This result shows that the fraction B isolated from *S. ovata* Backer is Stigmasterol, which was already identified using  $^{13}\text{C}$  NMR (Table 2). The  $^{13}\text{C}$  NMR spectrum showed signals on the chemical shift of 140.9, 138.5, 129.4, and 121.9 ppm as groups of alkene. Signal 71.9 ppm for  $-\text{C}-\text{OH}$  (C-3), 31.7 ppm for  $-\text{CH}-$  (C-25), 29.1 ppm for  $-\text{CH}_2$  (C-16), and 12.4 ppm for carbon methyl (C-29). From the NMR data above and comparison to the previously NMR data identified by Nayak *et al.* 2015 it is clarified that the compound is Stigmasterol (Figure 2) (Nayak *et al.* 2015).

Screening of inhibition against microbial by extract of *S. ovata* Backer showed a significant data. Extract of *S. ovata* Backer strongly inhibits growth of *S. aureus* bacteria at  $155.43 \pm 13.95\%$ , while Ampicillin as the positive control only inhibits at  $109.13 \pm 4.83\%$ . However, the inhibition rate of extract *S. ovata* Backer against *B. subtilis* bacteria is as low as  $23.8 \pm 11.56\%$  compared to Ampicillin which strongly inhibits at  $86.91 \pm 15.8\%$ . On the other side, extract of *S. ovata* Backer does not inhibit the gram negative bacteria at all, both against *E. coli* or *P. aeruginosa*. This result indicates a high potential

activity of *S. ovata* Backer against *S. aureus* bacteria and lead to the calculation of  $\text{IC}_{50}$  value. Extract of *S. ovata* Backer shows  $\text{IC}_{50}$  value of  $7.7 \mu\text{g/ml}$  and Ampicillin shows  $\text{IC}_{50}$  value of  $37.8 \mu\text{g/ml}$ . The significant result of the inhibitory activity of *S. ovata* Backer against *S. aureus* reveals the fact that *S. ovata* Backer has extremely stronger activity than the common antibacteria, Ampicillin.

The inhibition of gram positive bacteria, *S. aureus* is estimated to be associated with the increasing permeability of bacteria membrane (Greenway and Dyke 1979). Commonly, long chain fatty acid inhibit gram positive bacteria better than gram negative bacteria because of the outer membrane of gram negative bacteria (Nieman 1954). Gram negative bacteria has the outer membrane that prevents fatty acid to reach the inner sitoplasm, while there is no outer membrane owned by gram positive bacteria. The previous report showed that long chain fatty acids are bactericidal for the gram positive bacteria that causing lysis of protoplasts which stabilized osmotically, leakage of absorbing material and protein, both from bacteria and protoplasts, and inhibition in oxygen and amino acid uptake (Galbraith *et al.* 1971).

Stigmasterol, which as major compound of *S. ovata* Backer has also been tested previously for its inhibitory activity against some bacteria. Bacteria

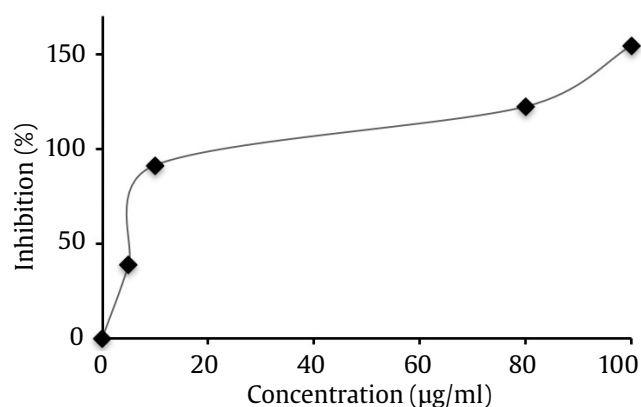


Figure 1. Antimicrobial activity of *S. ovata* extract against *S. aureus*

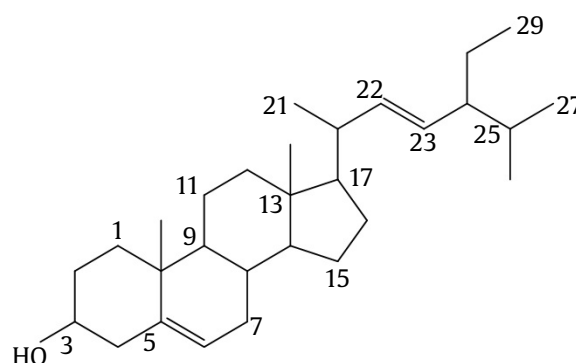


Figure 2. Chemical structure of Stigmasterol

Table 2.  $\text{IC}_{50}$  value of extract of *S. ovata* Backer

Sample	$\text{IC}_{50}$ value ( $\mu\text{g/ml}$ ) against bacteria of			
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Extract of <i>S. ovata</i> Backer	7.7	NT <sup>2</sup>	NA <sup>1</sup>	NA <sup>1</sup>
Positive control (Ampicillin)	37.8	NT <sup>2</sup>	10.28	NT <sup>2</sup>

<sup>1</sup>NA: Not active, <sup>2</sup>NT: Not tested

used in the assay were both gram positif and gram negative bacteria, *S. aureus* and *E. coli*. The minimum inhibitory concentration was determined using broth dilution method, and the MIC was defined as the lowest concentration of the compound inhibiting the visible growth of each micro-organism, resulting the MIC value of 12.5 and 25 µg/ml for the *S. aureus* and *E. coli* bacteria, respectively (Yusuf *et al.* 2018). This report showed that Stigmasterol is strongly inhibit both gram positive and gram negative bacteria that means Stigmasterol is potential as antimicrobial agent.

## 5. Conclusion

Extract of *S. ovata* Backer has high biological activity as antimicrobial agent. The extract of *S. ovata* Backer is highly potential as antimicrobial against gram positive bacteria, *S. aureus* better than Ampicillin. Furthermore, a major compound has been isolated from the methanolic extract of the stem bark of *S. ovata* Backer and recognized as Stigmasterol. Stigmasterol has also performed a high inhibitory activity against *S. aureus*. Therefore, extract of *S. ovata* Backer is highly potential as a new antimicrobial against *S. aureus*. Further isolation from the extract of *S. ovata* Backer and other biological activity assay, both on extract and compounds are needed to enrich the reports of *S. ovata* Backer.

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