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Original Article

CHARACTERIZATION OF ISOLATED CRYSTALS FROM MANGROVE LEAVES (*AVICENNIA MARINA* AND *SONNERATIA ALBA*) AND THEIR ANTIBACTERIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Objective: The study aimed to characterize the isolated crystal of *Avicennia marina* (*A. marina*) and *Sonneratia alba* (*S. alba)* leaves and identify the antibacterial activity of their isolated crystal.

Methods: Each of the leaves *A. marina* and *S. alba* was extracted using ethanol 96%, then fractionated by liquid-liquid extraction method using n-hexane and ethyl acetate. The crystal of the isolated crystal was found in the water fraction. The isolated crystals were characterized using Fourier Transform-Infrared (FT-IR) and Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) Spectroscopy and identified the antibacterial activity against *Staphylococcus aureus* (*S. aureus*) using the diffusion method.

Results: The FT-IR spectrum showed that the isolated crystal was a polysaccharide structure with vibrations in the O-H, C=O, C-O, C-H, and S=O bonds. The SEM-EDX spectrum revealed the high-level content of carbon and oxygen, with sulfuryl group proposing the sulfated polysaccharide compound. Water fraction of *A. marina* and *S. alba* have inhibition zones 16 mm and 10 mm, respectively. The results showed the water fraction of *A. marina* and *S. alba* have strong and moderate antibacterial activity, respectively. Meanwhile, the antibacterial activity of the isolated crystal was none. The isolated crystal was estimated a sulfated polysaccharide but was not pure. So that the antibacterial activity was not detected.

Conclusion: Even though, the water fraction of *A. marina* and *S. alba* leaves can be developed as antibacterial promising.

Keywords: *Avicennia marina, Sonneratia alba*, Mangroves, *Staphylococcus aureus*, Isolated crystal, Antibacterial activity

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INTRODUCTION

In this decade, a new disease developed so quickly. A disease related to antibacterial infection was challenging to handle because many microorganisms resistant to several antibiotics [1]. *Staphylococcus aureus* was known to cause skin and soft tissue infection, pneumonia, and abscess. The use of antibiotics for this condition was pervasive, thus leading to the emergence and spread of Methicillin-Resistant *Staphylococcus aureus* (MRSA), which is the primary cause of nosocomial infection and a public health problem worldwide [2]. The condition encouraged scientists to find plants with potential antibacterial activity, especially against *S. aureus* [3].

Indonesia has an extensive mangrove ecosystem, which has 3.49 million hectares of mangrove area, or 19 percent of the world's mangrove area. Several provinces in Indonesia that have a lot of mangrove forests are Bali and Nusa Tenggara (34,835 ha), Java (35,911 ha), Sulawesi (118,891 ha), Maluku (221,560 ha), Kalimantan (735,887 ha), Sumatra (666,439 ha), and Papua (1,497,724 ha) [4]. Mangroves are commonly used as living places for shrimp, crabs, and fish [5]. Mangroves have ecological function and pharmacological activities that is beneficial for people's health. Such as antifungal, antiviral, anti-cancer and anti-diabetic [6].

Estimated 54 species of mangrove plants, two of which are *A. marina* and *S. alba.* Both species are widely distributed in Indonesian mangrove forests. *A. marina* was reported to have many pharmacological activities, such as antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory activities [7–11]. Antibacterial were the major pharmacological activities of *A. marina.* In addition, the root of *S. alba* from Bali was also reported to have antibacterial activities against *S. aureus* [12]. Their secondary metabolites influenced the pharmacological activity of mangroves. Many studies reported the secondary metabolites isolated from stems, fruits, seeds, and leaves of *A. marina* and *S. alba,* such as terpenoids, steroids, alkaloids, glycosides, flavonoids, phenols, and tannins group [13-16]. However, the isolated crystals from *A. marina* and *S. alba* have never yet been analyzed for their antibacterial activity. So, it is the first study to identify the antibacterial activity of the isolated crystal of *A. marina* and *S. alba* leaves against *S. aureus.* In addition, this study will characterize the isolated crystal of *A. marina* and *S. alba* using Fourier transform infrared (FT-IR) and Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) spectroscopy.

MATERIALS AND METHODS

Material

The reagents used were obtained from Merck (Darmstadt, Germany), namely ethanol, n-hexane, ethyl acetate, and nutrient agar. The leaves of *A. marina* and *S. alba* were collected from the Buwun Mas area, Sekotong, West Lombok, West Nusa Tenggara, Indonesia. The samples were authenticated by I Gde Merthe (senior lecturer in Plant Taxonomy) at the Silviculture Laboratory, Department of Forestry, Faculty of Agriculture, University of Mataram, Indonesia. The standard test bacteria, *Staphylococcus aureus* ATCC 25923, used in the current study was obtained from the Laboratorium of Microbiology, Department of Pharmacy, Faculty of Medicine, University of Mataram, Indonesia. The spectrum of infrared (IR) was measured using Fourier Transform-Infrared (FT-IR) spectrometer 100 PERKIN ELMER. In addition, the scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) spectrum were determined using SEM-EDX Spectroscopy JEOL JCM-7000 Neoscope™ Benchtop.

Methods

Preparation of sample

The leaves of *Avicennia marina* and *Sonneratia alba* were washed using running water. The samples were air-dried in an open space and covered with a black cloth to prevent direct exposure to sunlight. The dried samples were mashed using a blender and sifted using a sieve of 40 mesh. Each 450 g of *A. marina* and *S. alba* were extracted with ethanol 96% (1:5) using the sonication method at 35 °C for 30 min. Re-extraction was carried out two times. Each filtrate of *A. marina* and *S. alba* was concentrated using a vacuum rotary evaporator at 40 °C to get ethanol extract of *A. marina* and *S. alba*. These extracts were fractioned using the liquid-liquid chromatography method. The fractionated using 3 solvents were n-hexane, ethyl acetate and water fraction. The crystal was obtained when the water fraction was concentrated using vacuum rotary evaporator. The crystal was recrystallized using ethanol. The scheme for the preparation of the *A. marina* can be seen in fig. 1. The preparation of *S. alba* was same with the fig. 1.

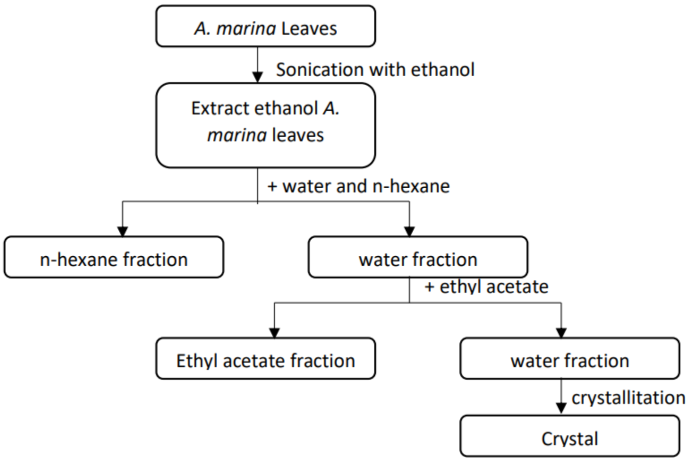


Fig. 1: Preparation of sample

Fourier transform infrared (FT-IR) spectroscopy

Each crystal from *A. marina* and *S. alba* was mixed with KBr (1:9) and measured using the FT-IR spectrometer 100 PERKIN ELMER. The spectrum FT-IR of crystal was scanned in the frequency region of 4000-400 cm^{-1}. The functional groups of crystal were analyzed based on the peak in the spectrum of FT-IR.

Scanning electron microscope-energy dispersive X-ray (SEM-EDX)

SEM-EDX analysis using SEM-EDX Spectroscopy (JEOL JCM-7000 Neoscope™ Benchtop) was conducted to characterize the morphological structure and elemental composition of the isolated crystals (crystal) from *A. marina* and *S. alba.* A field emission scanning electron microscope with high vacuum mode, work distance (WD) of 12.3 mm, and magnification of 1,000x equipped with an EDX spectrometer was used at an accelerating voltage of 15 kV [17].

Antibacterial assay

Antibacterial activity of water fraction and the isolated crystal against *S. aureus* was measured using the agar-disk diffusion method. One ml of inoculum bacterial culture of *S. aureus* was spread on nutrient agar plates. The 100 µl of water fraction and isolated crystal (0.5 and 50% b/v in DMSO 10%) were inserted into 8 mm diameter wells of agar media. The agar disk was incubated at 37 °C for 24 h. The negative and positive control were DMSO 10% and standard antibiotic of ampicillin (0.01%), respectively. The antibacterial activity of samples was determined based on diameters of the bacterial growth inhibition zone.

Statistical analysis

The analysis of the antibacterial activity of crystals from *A. marina, S. alba,* and ampicillin (positive control) was using the Statistical Package for Social Sciences (SPSS, version 16) software with One-Way Analysis of Variance (ANOVA) method followed by Tukey's. If the $p<0.05$, the antibacterial activity of samples is significantly different.

RESULTS AND DISCUSSION

The yield of extract, fraction, and crystal

The yield of leaves ethanol extract of *A. marina* and *S. alba* was 11.06% and 16.03%, respectively. It showed that the compounds in *S. alba* were extracted more in ethanol than compounds in *A. marina*. Meanwhile, the yield of water fraction of *A. marina* and *S. alba* was 47.52% and 37.38%, respectively. The yield of water fraction was almost 50%. It indicated that the compounds in the leaves of A. marina and S. alba tend to be polar. The major polar compounds that spread in mangroves was tannin, flavonoid glycoside, and sulfate polysaccharide [13, 18]. The crystal yield from the water fraction of *A. marina* and *S. alba* was 31.5% and 28%, respectively. Thatoi *et al.* (2016) reported almost all group of compounds was isolated from the leaves of *A. marina.* The compound including the polar compounds such as 10-O-[E-cinnamoyl]-geniposidic acid (glycosides group) and Luteolin 7-O-methylether-3'-O-β-D-glucoside 3 (Flavonoid glycoside). The isolated compounds from *S. alba* such as 28-oic acid, stigmasterol, 12–diene, ß-sitosterol, triterpenoid, lupan-3β-ol, 3β-hydroxy-lup-9(11), and lupeol [19-21].

Fourier transform-infrared (FT-IR) spectrum

The FT-IR spectrum of *A. marina* and *S. alba* leaves showed the same type of vibration (table 1). The FT-IR spectrum frequencies of the two isolated crystals were different but were still in the same range of vibration types. The difference in frequency between the two isolated crystals was estimated because the two isolates were still not pure.

Table 1: Frequency and type of vibration of isolated crystal from *Avicennia marina* and *Sonneratia alba* leaves

Frequency (cm^{-1})		Type of vibration
Avicennia marina	*Sonneratia alba*	
3437.35	3422.21	O-H stretch
1629.70	1637.44	C=O
1404.95	1398.14	C-H bending (CH_2)
1200-950	1200-1000	C-O-C
1238.52	1220.55	S=O
900-800	900-800	C-O-S

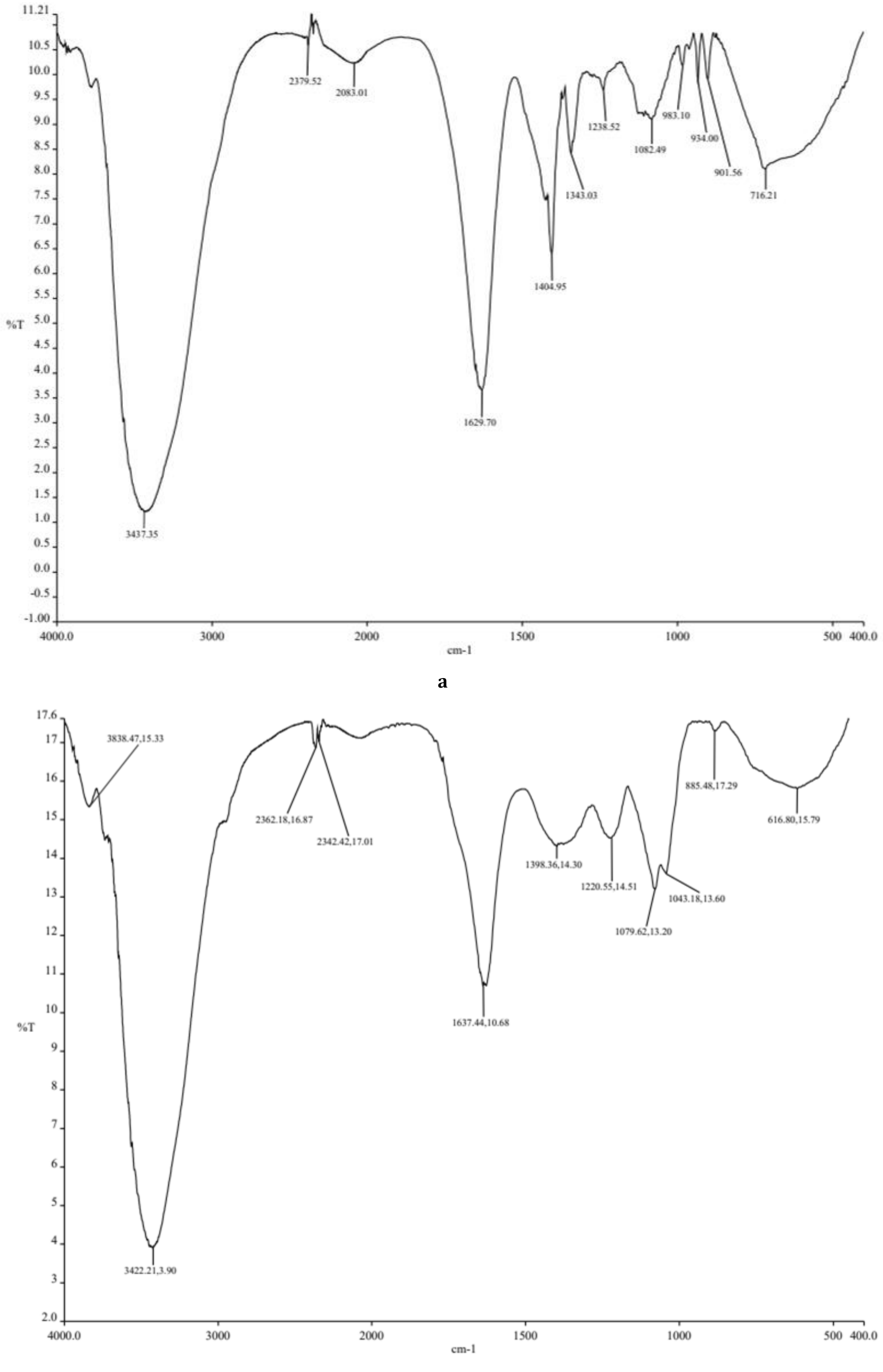


Fig. 2: Spectrum FT-IR of crystal from *Avicennia marina* (a) and *Sonneratia alba* (b)

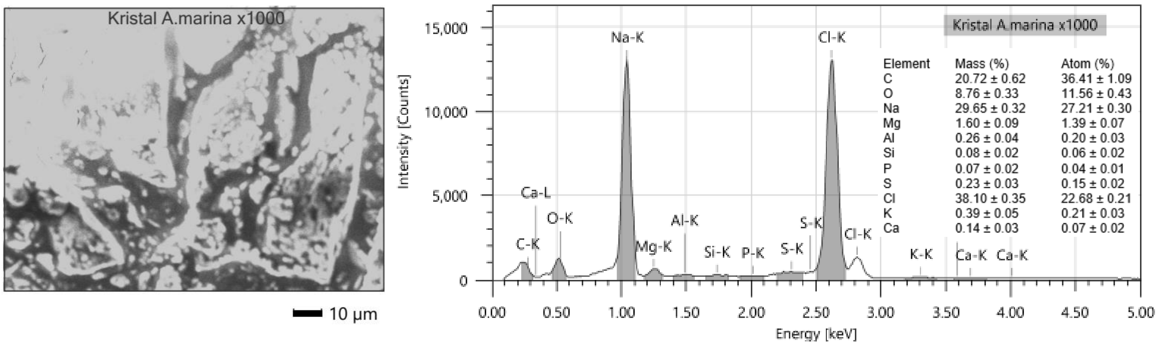
The spectra FT-IR of crystal from *A. marina* was reported in fig. 2A. The peak at frequency 3437.35 cm^{-1} showed the vibration of the O-H stretch. Its peak described the intermolecular or intramolecular OH group in polysaccharides [22]. The absorption at 1629.70 was related to the vibration of the C=O bond. The strong peak at 1404.95 cm^{-1} indicated the presence of C-H bending, especially CH_2 [23]. The peak at spectra FT-IR 1200-950 cm^{-1} confirmed the vibration of C-O-C and C-O-H bonds in polysaccharides [24].

The FT-IR spectra of crystals from *S. alba* leaves are shown in fig. 2B. The strong peak at 3422.21 cm^{-1} performed the O-H stretching vibration [25]. It indicated the presence of polysaccharide chains. The absorption at frequency 1637.44 showed the vibration of C=O, indicating the presence of uronic acid in the polysaccharide structure. The bending vibration of C-H was demonstrated with the peak at 1398.14 cm^{-1}. The absorption peak at 1220.55; 1079.62; and 885.48 cm^{-1} showed the sugar units in crystal from *S. alba*. In addition, the strong peak at 1200-1000 reported the C-O-C vibration from glycosidic in polysaccharide structure [26].

The peak at 1238.52 cm^{-1} in *A. marina* crystal and the peak at 1220,55 cm^{-1} in *S. alba* crystal indicated the vibration of the S=O bond. Vibration at 800-900 cm^{-1} indicated the presence of sulfated polysaccharides [27]. The FT-IR spectra of *A. marina* crystal showed more vibration peaks at 800-900 cm^{-1} than *S. alba* crystal. The C-O-S bond vibration was indicated by the peak at 846 cm^{-1} [28]. The crystal from *A. marina* and *S. alba* was indicated the sulfated polysaccharide.

SEM-EDX spectrum

The crystal surface features of *A. marina* and *S. alba* appear significantly different (fig. 3).



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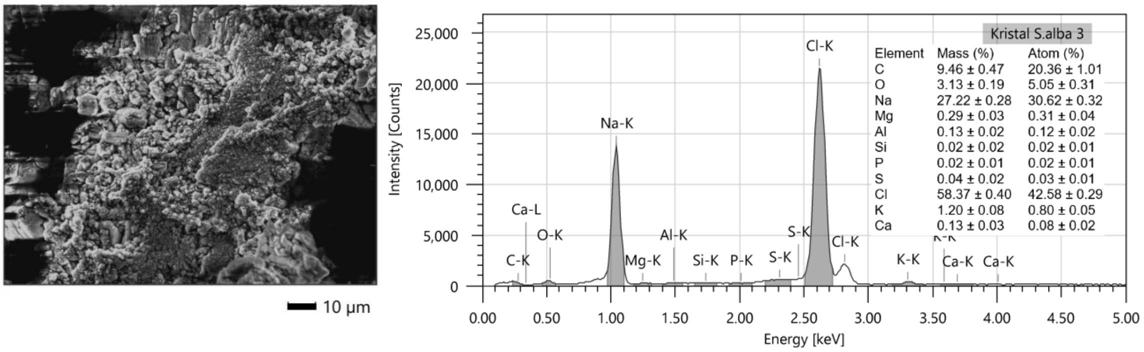

b

Fig. 3: Scanning electron microscope-energy dispersive X-ray (SEM-EDX) spectrum of isolated crystals (crystal) from (a) *A. marina* leaves and (b) *S. alba* leaves

A. marina crystals look flatter, but there are many long gaps like canals. Meanwhile, the *S. Alba* crystals look uneven over the entire surface, and there are gaps whose pattern is similar to that of *A. marina*. Fig. 3 also shows the elemental composition of crystals of *A. marina* and *S. alba* based on SEM-EDX analysis. The SEM-EDX analysis was able to qualitatively identify chemical elements present on the surface of the crystal. The SEM-EDX spectrum showed the presence of oxygen, carbon, sodium, magnesium, aluminum, silicon, phosphorus, sulfur, chlor, potassium and calcium in both crystals. Higher levels of carbon (36.41%), sodium (27.21%), chlor (22.68%) and oxygen (11.56%) were present in *A. marina*, while *S. alba* had higher levels of chlor (42.58%), sodium (30.62%), carbon (20.36%), and oxygen (5.05%) compared to *A. marina*. Both crystal samples contained high carbon and oxygen with sulfuryl groups, which predicted as sulfated polysaccharides. However, the sulfur content of both samples (0.15% and 0.03%) is very low compared to purified fucoidan (9.83%), a sulfated polysaccharide obtained from *Fucus vesiculosus* [29]. The low sulfur detected in the crystals surface is probably because the present of sodium and chlor which may be bound to the sulfated polysaccharide, so the crystals need further purification.

Antibacterial activity

The water fractions of the *A. marina* and *S. alba* were efficiently suppressing the growth of *S. aureus* with variable potency. The water fraction (0.5%) of *A. marina* showed a medium inhibition (6 mm), while *S. alba* is not active (table 2). Water fraction in concentration 50% of *A. marina* and *S. alba* had the maximum zone of inhibition against *S. aureus* (14 mm and 10 mm, respectively), which was categorized as strong activity [30]. However, both crystals of *A. marina* and *S. alba* had none inhibition.

Table 2: Antibacterial activities of water fraction and isolated crystal of mangrove against *Staphylococcus aureus*

Samples	Mean zone Inhibition (mm)	
	Avecennia marina	*Sonneratia alba*
Ampicilin (positive control)	60	61
DMSO (negative control)	0	0
Water fraction 0.5%	6	0
Water fraction 50%	14	10
Crystal 0.5%	0	0
Crystal 50%	0	0

N=3

The phytochemical screening of water fraction of *A. marina* was positively contained some metabolites such as steroids, flavonoids, phenolics, and saponin [31]. These compounds are proposed to be responsible for the antibacterial activity. The isolated crystals which are suggested as sulfated polysaccharides based on FT-IR and EDX spectrum, did not show antibacterial activities due to not pure crystal.

CONCLUSION

The FT-IR spectrum of isolated crystals from *A. marina* and *S. alba* based on the vibration in the O-H, C-H, C=O, C-O, and S=O bands were characterized as sulfated polysaccharides. The SEM-EDX spectrum revealed the high-level content of carbon and oxygen with the sulfuryl group proposing the sulfated polysaccharide compound. The water fraction *A. marina* and *S. alba* leaves showed strong and moderate antibacterial activity, respectively. The antibacterial activity in isolated crystals was not detected. Even though, the water fraction of *A. marina* and *S. alba* leaves can be developed as antibacterial promising, especially against *S. aureus*.

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AUTHORS CONTIBUTIONS

Lina Permatasari interpreted the FT-IR spectrum and isolated the compound. Handa Muliasari interpreted the SEM-EDX spectrum and antibacterial activity. Fania Rahman extracted and Fractionated the Mangrove leaves.

CONFLICT OF INTERESTS

Declared none

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