BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 10, Number 2, October 2021 | Pages: 117-121 | DOI: 10.14421/biomedich.2021.102.117-121 ISSN 2540-9328 (online)

Antibacterial Activity of Mangrove Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali

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Manuscript received: 08 October 2021. Revision accepted: 15 October, 2021. Published: 21 October, 2021.

Abstract

The increasing rate of antimicrobial resistance in the past decades has motivated the search for novel antibacterial compounds to overcome infectious diseases. Among diverse natural sources, mangrove ecosystems offer untapped sources of biological active compounds for future antibacterial medicine. This research was aimed to evaluate antibacterial activities of crude extracts of four dominant mangrove plants from the Ngurah Rai Mangrove Forest namely *Rhizophora mucronata*, *Avicennia marina*, *Rhizophora apiculata*, and *Sonneratia alba*. Roots of these four plants were extracted using methanol, chloroform, and n-hexane. These crude extracts were tested against two Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*) and two Gram negative bacteria (*Escherichia coli* dan *Klebsiella pneumoniae*) by *disc difussion assay*. We found that 3 mg/mL of N-hexane crude extracts from *R. apiculata* yielded the highest zone of inhibition of 8.64 mm against *S. aureus*. While, 3 mg/mL of chloroform crude extract of *R. apiculata* yielded the highest inhibition of 19.83 mm against *S. mutans*. Unfortunately, no zone of inhibition was observed when crude extracts were tested against Gram negative indicator strains. Our results indicate that the root crude extracts of *R. apiculata* yielded the highest zone of inhibition against Gram positive indicator strains compared to root crude extracts of *R. mucronata*, *S. alba*, and *A. marina*. Further research is required to determine the antibacterial activities of the mangrove crude extracts against other bacterial indicator strains to determine their spectrum of activities.

Keywords: mangrove; roots, antibacterial; *Rhizopora apiculata*.

INTRODUCTION

Antimicrobial substances play a crucial role during prevention and medication of infectious diseases, post-operation infections, infections after chemotherapy or infections for diabetic patients, late stage of kidney diseases and patients with rheumatoid arthritis (Ventola, 2015). However, incidence rate of antimicrobial resistance has increased dramatically in the past decades (Ventola, 2015), which cause difficulty to treat infectious diseases, increase cost for medication, and lead to higher mortality rate (World Health Organization, 2020). World Health Organization reported approximately 700,000 mortality every year until 2014 due to antimicrobial resistance and it is predicted the casualties of antimicrobial resistance reached 10 million in 2050 (Departemen Kesehatan Republik Indonesia, 2016).

Immediate actions are required to overcome the increasing rate of antimicrobial resistance such as a better diagnose and prescription of antibiotics, optimization of therapy regimen, and prevention of infectious diseases (Ventola, 2015). In addition, the search for novel antimicrobial compounds are urgently needed since many antibiotics on the market are no longer effective to eradicate bacterial infections (Ventola, 2015). Among natural resources, mangrove ecosystems are the ideal habitat to search for novel antimicrobial activities. Mangrove refers to ecosystem where seawater and fresh water dissolve, which characterize by plants that can tolerate high salinity and limited oxygen (Friess, 2016). A number of studies showed that mangrove plants are rich of various of active compounds such as alkaloid, steroid, terpenoid, saponin, tannin, flavonoid dan poliphenol (Dahibhate et al., 2018; Gouda et al., 2015; Patra & Mohanta, 2014). Furthermore, diversity of these secondary metabolites led to broad biological activities such as antibacterial, antifungal, antioxidant, antiviral, and anticancer, which overall could potentially be developed as the source of new drugs (Patra & Thatoi, 2011; Saranraj, 2015).

A study conducted in Kalimantan showed antibacterial activities of root of mangrove plant of *Rhizophora apiculata* (Usman, 2018). However, a similar study from mangrove plants found in Bali have not been conducted. The Ngurah Rai Mangrove Forest is the biggest mangrove ecosystem in Bali which has more than 16 different mangrove spesies and the four dominant species are *Rhizophora mucronata*, *Avicennia marina*, *Rhizophora apiculata* and *Sonneratia alba* (Lugina et al., 2017). To date, antibacterial properties of these four dominant mangrove species are rather unexplored. Therefore, we aimed to screen for antibacterial activities of crude extract of the root of these four mangrove species by using three different solvents: methanol, chloroform and n-hexane against Gram negative and positive indicator strains. It is expected antibacterial activities of these crude extracts will shed light to explore antibacterial active compounds from root of mangrove plants.

MATERIALS AND METHODS

Materials

Roots of the four dominant mangrove plants: *Rhizophora mucronata*, *Avicennia marina*, *Rhizophora apiculata* and *Sonneratia alba* were collected from the Ngurah Rai Mangrove Forest (8º43'40.4886" S, 115º11'42.80313" E), Bali, Indonesia, on August 2019 during the low tide. The species determination was carried out in Eka Karya Botanical Garden, Bedugul, Tabanan, Bali, Indonesia. The solvents (methanol, chloroform, and n-hexane) were purchased from Merck, Germany.

Methods

Sample extraction

For each of mangrove species, 50 g of each dry powder of mangrove root were macerated twice using 250 mL of individual solvent (methanol, chloroform, or n-hexane) at room temperature for 24 hours and filtered using Whatmann filter paper No. 1. The extracts were then evaporated using *rotary evaporator* (IKA®, RV8) at 50°C, 90 rpm and stored in closed and air tight vial at 4°C until further used.

Antibacterial screening

From each mangrove root extract, three level of concentrations were prepared: 1 mg/mL, 3 mg/mL and 5 mg/mL. Subsequently, each crude extract was exposed against bacterial indicator strains Gram positive (*Staphylococcus aureus* dan *Streptococcus mutans*) and Gram negative (*Escherichia coli* dan *Klebsiella pneumoniae*) by *disc diffusion assay*. One hundred microlitre of bacterial indicator strains were spotted on Luria Bertani agar and spread using a sterile spreader. Thirty microlitre of crude extracts were spotted on a 6 mm paper disc and air dried for 30 minutes. Paper discs in triplicates containing crude extracts were placed on agar media containing bacterial indicator strains. Ampicillin and Streptomycin were used as positive controls, while the solvents, methanol, chloroform, and n-hexane were used as negative controls. Antibacterial activities were calculated based on average zone of inhibition (ZOI) that were formed from triplicate disc.

RESULTS AND DISCUSSION

The results showed that antibacterial activities were displayed by some extracts against Gram positive strains, *S. aureus* and *S. Mutans* (Table 1), but none against Gram negative strains, *E. coli* dan *K. pneumoniae*. Among the four mangrove species, only *R. apiculata* exhibited antibacterial properties against both Gram positive indicator strains. The concentration of 3 mg/ml N-hexane extract showed the highest ZOI of 8.64 ± 0.13 mm against *S. aureus* while 3 mg/ml chloroform extract yielded the highest ZOI of 19.83 ± 0.63 mm against *S. mutans*. A study by Seepana and co-workers (2016) also showed that methanol, ethanol, and hexane extract of *R. apiculata* roots is active against *Salmonela typhi* and *S. aureus*. Their study also showed that methanol, hexane, and ethanol *R. apiculata* root extracts have no activity against *E. coli*. However, there are activities against other Gram-negative bacteria *Salmonella typhi*, shown by methanol, hexane, and ethanol extracts of *R. apiculata* root. Ethanolic extract of *R. apiculata* root also displays activity against *K. pneumonia* (Seepana et al. 2016).

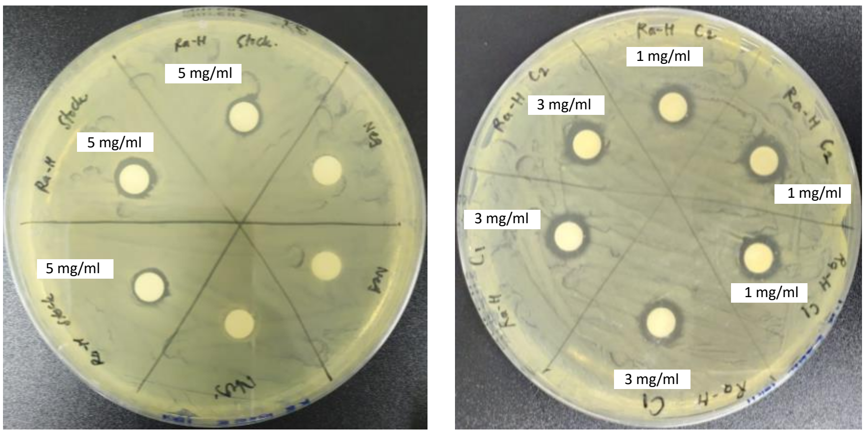

Figure 1. Antimicrobial activity of *R. apiculata* N-hexane extracts in triplicates against *S. aureus.* The concentration of 3 mg/ml N-hexane extract showed the highest ZOI (8.64 ± 0.13 mm).

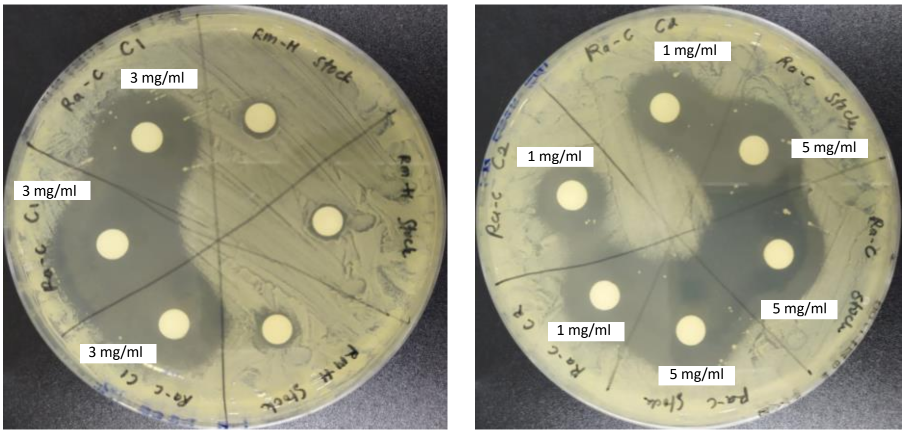

Figure 2. Antimicrobial activity of *R. apiculata* chloroform extracts in triplicates against *S. mutans*. The concentration of 3 mg/ml chloroform extract yielded the highest ZOI of 19.83 ± 0.63 mm against *S. mutans.*

Table 1. Antibacterial activities of mangrove root extracts.

Bacterial Strains	**Samples**	**Solvents**	**Concentration**	**ZOI (mm)**
Staphylococcus aureus	*Rhizopora apiculata*	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	8.05 ± 0.64
			3 mg/ml	8.64 ± 0.13
			5 mg/ml	7.84 ± 0.61
	Rhizopora mucronata	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
	Sonneratia alba	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
	Avicennia marina	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
	Positive controls	Ampicillin	10 μg	19.20 ± 0.98
		Streptomycin	10 μg	15.54 ± 0.22
	Negative controls	Methanol	-	-
		Chloroform	-	-
		N-hexane	-	-

Bacterial Strains	Samples	Solvents	Concentration	ZOI (mm)
Streptococcus mutans	*Rhizopora apiculata*	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	13.98 ± 1.49
			3 mg/ml	19.83 ± 0.63
			5 mg/ml	15.92 ± 0.68
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
	Rhizopora mucronata	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	7.32 ± 0.43
	Sonneratia alba	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	7.39 ± 0.21
			5 mg/ml	7.96 ± 0.25
	Avicennia marina	Methanol	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		Chloroform	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
		N-hexane	1 mg/ml	-
			3 mg/ml	-
			5 mg/ml	-
	Positive controls	Ampicillin	10 µg	31.72 ± 3.03
		Streptomycin	10 µg	20.76 ± 1.49
	Negative controls	Methanol	-	-
		Chloroform	-	-
		N-hexane	-	-

In addition, other Rhizopora species namely *R. mucronata* also displayed antibacterial activity in which 5 mg/ml n-hexane extract showed ZOI of 7.32 ± 0.43 mm against *S. mutans*. Rhizopora species including *R. apiculata* and *R. mucronata* contain many medically important secondary metabolites such as essential oils, glycosides, alkaloids, phenolic compounds, and tannins (Habib et al., 2018). Tannins is known to have antimicrobial properties against both Gram-positive and Gram-negative bacteria. This compound passes the bacterial cell wall and intrudes the cell metabolism, resulted in bacterial death (Kaczmarek, 2020).

Furthermore, N-hexane extract of *S. alba* root showed activity against *S. mutans* in which the concentration of 3 mg/ml and 5 mg/ml yielded ZOI of 7.39 ± 0.21 and 7.96 ± 0.25 mm respectively. Although study on *S. alba* root extract is still limited, there are a number of studies focusing on leaves and bark of this plant. A study by Saad and co-workers (2012) showed that methanolic extract of *S. alba* leaves antibacterial activities were observed against Gram-negative bacteria *E. coli* (17,5 mm), as well as Gram-positive bacteria namely *S. aureus* (12.5 mm) and *Bacillus cereus* (12.5 mm) (Saad et al., 2012).

Our result indicate that overall, crude extract of mangrove root displayed much lower antibacterial activities compared to positive control ampicillin (10 µg) dan streptomycin (10 µg). However, zone of inhibition *R. apiculata* crude extracts using n-hexane (3 mg/L) were comparable to that of streptomycin. Therefore, *R. apiculata* could potentially contain novel antibacterial compounds. However, composition of active compounds in the crude extracts need to be confirmed. It is expected that once this active compound has been identified, it can be isolated and further developed as new antibiotics drugs.

CONCLUSION

In conclusion, crude extracts of root *R. apiculata* displayed the highest antibacterial activities compared to other crude extracts of different mangrove plants. We found that extraction using n-hexane yielded the highest inhibition zones compared to methanol and chloroform. Further research is required to elucidate the active compounds in n-hexane.

***Acknowledgements*:** We would like to acknowledged financial support from UP2M of Faculty of Medicines and Health Sciences under grant no. 417/Unwar/FKIK/UP2M/KP-02/VII/2019.

***Conflicts of Interest*:** The authors declare that there are no conflicts of interest.

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