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In vitro antimicrobial activity of mangrove plant *Sonneratia alba*

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ABSTRACT

Objective: To investigate the antimicrobial property of mangrove plant *Sonneratia alba* (*S. alba*). **Methods:** The antimicrobial activity was evaluated using disc diffusion and microdilution methods against six microorganisms. Soxhlet apparatus was used for extraction with a series of solvents, *n*-hexane, ethyl acetate and methanol in sequence of increasing polarity. **Results:** Methanol extract appeared to be the most effective extract while *n*-hexane extract showed no activity. The antimicrobial activities were observed against the gram positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*), the gram negative *Escherichia coli* (*E. coli*) and the yeast *Cryptococcus neoformans*. *Pseudomonas aeruginosa* and *Candida albicans* appeared to be not sensitive to the concentrations tested since no inhibition zone was observed. *E. coli* (17.5 mm) appeared to be the most sensitive strain followed by *S. aureus* (12.5 mm) and *B. cereus* (12.5 mm). **Conclusions:** From this study, it can be concluded that *S. alba* exhibits antimicrobial activities against certain microorganisms.

1. Introduction

Nowadays, the indiscriminate use of commercial antimicrobial drugs has caused multiple drug resistance in human pathogenic microorganisms^[1]. In addition to this problem, hypersensitivity, immune-suppression and allergic reactions are sometimes present from the adverse effects of antibiotics on the host^[2]. This situation forced scientist to search for new and effective antimicrobial agents to replace the current regimens^[3]. It is well known that some plants containing active compounds are able to inhibit the microbial growth. Studying plant-based antimicrobial properties provides additional information in developing natural antibiotics and discovering the alternative of antimicrobial drugs for the treatment of infectious disease. Mangroves have been a source of interest for their novel natural products as they contain biologically active antiviral, antibacterial and antifungal compounds^[4].

Sonneratia alba (*S. alba*) belongs to Sonneratiaceae family. In Malaysia, it is known as mangrove apple or

“perepat”. The leaf is rounded, leathery, and opposite, upper and underside of it is similar. They are found from East Africa throughout the Indian subcontinent, Southeast Asia, northern Australia, Borneo and Pacific Islands. Traditionally, *S. alba* ripe fruits are used to expel intestinal parasites while half-ripe fruits are usually applied for coughs treatment^[5]. Members of the Sonneratiaceae family are rich sources of tannins which are known for its antimicrobial activity^[6]. Therefore, the present study was designed to evaluate the antimicrobial activity of *S. alba* extracts against six human pathogenic microbes.

2. Materials and methods

2.1. Plant collection

S. alba was collected from Matang Mangrove Reserve Forest, located in Perak on August 2010. The fresh leaves of *S. alba* were washed thoroughly to remove dirt and soil. The leaves were then stored in the dry room for a week. They were ground into fine particle after drying and kept in closed container before being stored at room temperature until further used. Information of the plant, the place of collection, and date of collection were recorded. The voucher of the specimen was deposited in the Department of Biomedical Science, IIUM, Malaysia. The taxonomy of this

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plant was done by Matang Mangrove Reserve Forest (Perak, Malaysia).

2.2. Extraction

Ground samples of *S. alba* leaves were weighed and transferred into a thimble. Soxhlet apparatus was used for extraction. Ground samples were finely extracted with a series of solvents, *n*-hexane, ethyl acetate and methanol in sequence of increasing polarity using 5 L for each solvent at room temperature. Then, extracts were concentrated to dryness under vacuum and reduced pressure using rotary evaporator to obtain concentrated extracts.

2.3. Samples preparation

A sample of 100 mg from each extract was dissolved in 1 mL dimethyl sulphoxide (DMSO). The extract was then sterilized by filtration through sterile syringe filter with 0.2 μ m pore. Finally the filtered extract was stored as aliquots until it was used.

2.4. Microbial strains

Six reference strains of human pathogens were used in this study including two gram-positive [*Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus*) ATCC11778], two gram-negative [*Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC27853, *Escherichia coli* (*E. coli*) ATCC35218] and two fungal strains [*Candida albicans* (*C. albicans*) ATCC10231 and *Cryptococcus neoformans* (*C. neoformans*) ATCC90112].

2.5. Antimicrobial assay

2.5.1. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the extracts according to Qaralleh *et al* [7] with some modification. Briefly, inoculums containing 10⁹ CFU/mL were spread on Mueller–Hinton agar plates for bacteria and 10⁵ CFU/mL were spread

on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (1.0 and 1.5 mg), standard antibiotics (100 μ g of tetracycline or 100 μ g of nystatin) or negative control (DMSO) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 hours for the bacteria and at room temperature (18–20 °C) for 24–48 hours for fungus strains. Each sample was tested in duplicate and the diameter for the zone of inhibition was measured as mm.

2.5.2. Microdilution method

Minimum inhibitory concentration (MIC) was measured by determining the smallest amount of extract or standard antibiotic needed to inhibit the visible growth of a test microorganism after 24 hours incubation periods at 37 °C. This was done using 96-well plates, the assay plates were filled with Mueller–Hinton broth medium (MHB) containing different concentrations of extracts, tetracycline or negative control (DMSO) and the test microorganisms (10⁹ CFU/mL).

The MIC for fungal strains was performed using 96-well plate. Each well contained potato dextrose broth (PDB), different concentration of extracts, nystatin or negative control (DMSO) and the test fungal strains (10⁵ CFU/mL). Incubation was performed at room temperature (18–20 °C) for 48 hours.

Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations equal to or higher than the MIC on agar plates. The lowest concentration of the extracts or the standard antibiotics required to completely destroy test microorganisms (no growth on the agar plate) after incubation at 37 °C for 24 hours (bacteria) and room temperature at (18–20 °C) for 48 hours (yeasts) was reported as MBC and minimal fungicidal concentration (MFC).

3. Results

The results of disc diffusion method of *S. alba* extracts using different polarity solvents were shown in Table 1. The

Table 1

Antimicrobial activity of different *S. alba* extracts using disc diffusion method.

Microorganisms	Zone of inhibition (mm)						Positive control	Negative control
	<i>n</i> -Hexane		Ethyl acetate		Methanol			
	1.0 mg	1.5 mg	1.0 mg	1.5 mg	1.0 mg	1.5 mg		
<i>S. aureus</i>	0.0	0.0	12.0	14.5	11.5	12.5	23.0	0.0
<i>B. cereus</i>	0.0	0.0	7.5	9.5	11.0	12.5	17.0	0.0
<i>E. coli</i>	0.0	0.0	9.0	12.0	16.0	17.5	23.0	0.0
<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0	0.0	0.0	13.0	0.0
<i>C. albicans</i>	0.0	0.0	0.0	0.0	0.0	0.0	11.0	0.0
<i>C. neoformans</i>	0.0	0.0	10.0	10.5	9.0*	11.0*	15.0	0.0

*: Partial zone of inhibition; Positive control: Tetracycline or Nystatin; Negative control: DMSO.

Table 2

MIC, MBC and MFC of *S. alba* extracts and standard antibiotic (mg/mL).

Extract	<i>S. aureus</i>		<i>B. cereus</i>		<i>E. coli</i>		<i>C. neoformans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
Ethyl acetate	1.110	10.000	1.110	3.330	1.110	3.330	0.370	10.000
Methanol	1.110	3.330	1.110	1.110	3.330	10.000	–	–
Tetracycline	0.010	0.120	0.002	0.120	0.012	1.000	–	–
Nystatin	–	–	–	–	–	–	0.370	0.370

–: not determined.

antimicrobial activity of the crude extracts was tested using two different concentrations. The antimicrobial activity was increased with the increasing concentration. The zones of inhibition at concentrations of 1.0 and 1.5 mg/disc were ranged from 0.0 to 12.0 mm and 0.0 to 17.5 mm, respectively. Both methanol and ethyl acetate extracts showed variable inhibition activity against the strains tested in this study while *n*-hexane extract showed no activity. Clearly, methanol extract appeared to be the most effective extract. Furthermore, the antimicrobial activities of the ethyl acetate and methanol extracts showed inhibition activity against both gram positive strains tested (*S. aureus* and *B. cereus*), only the gram negative *E. coli* and only the yeast *C. neoformans*. *P. aeruginosa* and *C. albicans* appeared to be not sensitive to the concentrations tested since no inhibition zone was observed. Furthermore, *E. coli* (17.5 mm) appeared to be the most sensitive strain followed by *S. aureus* (12.5 mm) and *B. cereus* (12.5 mm).

The MIC values of tetracycline, nystatin and *S. alba* extracts which showed maximum antimicrobial activity were depicted in Table 2. The MIC and MBC values of the extracts tested were (0.370–3.330 mg/mL) and (1.110–10.000 mg/mL), respectively. The using of quantitative test of MIC indicated potent antifungal activity of ethyl acetate extract against the yeast strain *C. neoformans*.

4. Discussion

Researches on use of plants as the source of drugs and dietary supplements are increasing in recent years. Pharmacologists, botanists, microbiologists, and natural-products chemists have offered their approaches to the development of new potent drugs derived from plants for treatment of infectious diseases. Plants have been found *in vitro* to have antimicrobial property as they are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids[8].

From this study, antimicrobial property of the *S. alba* extracts with a series of solvents, *n*-hexane, ethyl acetate and methanol has been tested. It has been observed that both methanol and ethyl acetate extracts showed variable inhibition activity against the strains tested while *n*-hexane extract showed no activity at all.

A number of review and research articles provide the information of the biological activities among the members of Sonneratiaceae family. Mangrove species such as *Sonneratia griffithii* were reported to show remarkable antihyperglycemic activity[9]. The extracts from the leaves, stems, barks and roots of mangrove species such as *Sonneratia apetala* have shown positive result for antioxidant activity test[10]. It has also been tested for plant growth regulators, growth hormone tests on plants and antiviral activity test. *Sonneratia caseolaris* was found to be traditionally used to stop bleeding, check hemorrhages, treat piles and it was also used as sprain poultices. This species was also tested for toxicity against mosquito larvae[11]. Increasing attention is being focused on the use of tannins which are polyphenolic substances as antimicrobial agents or prevention of dental caries. This compound proved to be

rich in the members of Sonneratiaceae family[6].

To our knowledge, this is the first report on antimicrobial property of *S. alba* extracts. It is suggested that, methanol extract of *S. alba* may possess promising therapeutic action in the treatment of infectious diseases caused by the species of *E. coli*, *S. aureus* and *B. cereus*. The discovering of its rich source of tannins which are known for its antimicrobial activity offers a valuable study for discovering the alternative of conservative antimicrobial drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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