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Antimicrobial activity of mangrove plant (*Lumnitzera littorea*)

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ABSTRACT

Objective: To investigate the antimicrobial activities of *n*-hexane, ethyl acetate and methanol extracts of the leaves of *Lumnitzera littorea* (*L. littorea*) against six human pathogenic microbes. **Methods:** The antimicrobial activity was evaluated using disc diffusion and microdilution methods. **Results:** The antimicrobial activities of the crude extracts were increased with increasing the concentration. It is clear that *n*-hexane extract was the most effective extract. Additionally, Gram positive *Bacillus cereus (B. cereus)* appear to be the most sensitive strain while *Pseudomonas aeruginosa (P. aeruginosa)* and the yeast strains (*Candida albicans (C. albicans*) and *Cryptococcus neoformans (C. neoformans)*) appear to be resistance to the tested concentrations since no inhibition zone was observed. The inhibition of microbial growth at concentration as low as 0.04 mg/mL indicated the potent antimicrobial activity of *L. littorea* extracts. **Conclusions:** The obtained results are considered sufficient for further study to isolate the compounds responsible for the activity and suggesting the possibility of finding potent antibacterial agents from *L. littorea* extracts.

1. Introduction

Infectious diseases represent a serious public health problem and they remain the leading cause of death throughout the world[1-3]. Currently, the problems of microbial drug resistance, an increase of opportunistic infections and the toxicity effect of continued use of several antimicrobial drugs[4] have necessitated a search for new antimicrobial drugs from other sources including natural sources like plants which are the good sources of novel antimicrobial chemotherapeutic agents. Furthermore, plants have been a major source for drug development[5-7]. Plant extracts and products are used in the treatment of infectious disease[8-10].

Lumnitzera littorea (L. littorea)(locally known as Teruntum merah) is small mangrove tree (up to 8 m tall) belongs to the Combretaceae family. It is distributed throughout the back-mangrove of East coast of Africa to Southeast Asia, Australia and Polynesia[11]. However, Teruntum merah has been known traditionally as an important remedy for sprue[11]. Up to date, there are no study has been conducted on the evaluation of the antimicrobial activity of this plant. Therefore, this study aims to investigate the antimicrobial activities of *L. littorea* extracts against six human pathogenic microbes 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). The efficacy of *n*-hexane, ethyl acetate and methanol extracts from the leaves of *L. littorea* were also investigated and described.

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2. Materials and methods

2.1. Plant collection

L. littorea was collected from Matang Mangrove Reserved Park in Perak in August 2010. The Voucher of the specimen was deposited in the Department of Biomedical science, IIUM. The taxonomic identification of this plant was done by Matang Mangrove Forest Reserve (Perak, Malaysia).

2.2. Plant preparation and extraction

The fresh plant was washed under running tap water and dried in a warm room for 3 to 5 d. The samples were grinded into fine powder and extracted by Soxhlet with *n*-hexane, ethyl acetate and methanol successively to get *n*-hexane, ethyl acetate and methanol extracts. Then, all the crude was kept at -20 ℃ until further use.

2.3. Samples preparation

A sample of 100 mg from each extract was dissolved in 1 mL DMSO. The extract was then sterilized by filtration through sterile syringe filter (0.2 μm 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 (*S. aureus* ATCC25923, *B. cereus* ATCC11778), two Gram-negative (*P. aeruginosa* ATCC27853, *E. coli* ATCC35218) and two fungal strains (*C. albicans* ATCC10231 and *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*[12] with some modification. Briefly, inoculum containing 10^7 CFU/mL was spread on Mueller-Hinton agar plates for bacteria and 10^4 CFU/mL was spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (1 or 1.5 mg), standard antibiotics (30 μg of chloramphenicol or 100 μg of amphotericin B) or negative control (DMSO) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 ℃ for 24 h for the bacteria and at room temperature (18±2) ℃ for 24-48 h for yeasts strains. Each sample was tested in duplicate and the zone of inhibition was measured as millimeter diameter.

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. 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 solvent control and the test microorganism (10^7 CFU/mL). Each sample was tested in triplicate and the observation was recorded by naked eyes after 24 h incubation periods at 37 ℃.

Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations equal to and higher than the MIC on agar plates. The lowest concentration of the extract or the standard antibiotic required to completely destroy test microorganisms (no growth on the agar plate) after incubation at 37 ℃ for 24 h was reported as MBC.

3. Results

The antimicrobial activity of *L. littorea* extracts are shown in Table 1. Generally, the results showed that the antimicrobial activities of the crude extracts were increased with increasing the concentration. Although the antimicrobial activity of the extracts tested is variable, two Gram-positive bacteria (*S. aureus* and *B. cereus*) and only gram negative (*E. coli*) were inhibited by the extracts. While, *P. aeruginosa* and the yeast strains appear to be resistance to the tested concentrations since no inhibition zone was observed.

Quantitative analyses on the antimicrobial properties were obtained through the determination of bacteriostatic and bactericidal concentrations of *L. littorea* extracts. Table 2 shows the MIC and MBC of the extracts that produce inhibition zone more than 12 mm. The results of inhibition zone were reflected in lower MIC values. The MIC and MBC values for bacterial strains, which sensitive to the extracts, were in the range of 0.04-1.11 mg/mL and 0.04-10 mg/mL, respectively. Furthermore, in most cases, the MBC values were higher than the MIC values, except for *n*-hexane extract against *B. cereus* (MIC = MBC). According to the disc diffusion results, MIC and MBC values, it is clear that *n*-hexane extract was the most effective extract (Table 1 and 2). Additionally, Gram positive *B. cereus* appears to be the most sensitive strain with inhibition zone of 19 mm (1.5 mg/disc) and the MIC value is 0.04 mg/mL). The inhibition of microbial growth at concentration as low as 0.04 mg/mL indicated the potent antimicrobial activity of *L. littorea* extracts.

4. Discussion

Traditionally, plants were known as the main sources for drugs. Interest in this area continues and many new potent drugs have been isolated. Tropical and sub-tropical areas of the world are rich with many plant species which have

Table 1

Antimicrobial activity of *L. littorea* extracts.

Microorganisms	Zone of inhibition (mm)							
	n-hexane		Ethyl acetate		Methanol		Positive control	Negative control
	1 mg	1.5 mg	1 mg	1.5 mg	1 mg	1.5 mg		
S. aureus	14.5	14.5	11.0	16.0	13.5	17.0	21.0	0.0
B. cereus	16.0	19.0	9.0	9.0	9.0	9.5	27.0	0.0
E. coli	11.5	13.5	13.5	16.5	9.5	11.0	30.5	0.0
P. aeuroginosa	0.0	0.0	0.0	0.0	0.0	0.0	30.5	0.0
C. albicans	0.0	0.0	0.0	0.0	0.0	0.0	17.0	0.0
C. neoformans	0.0	0.0	0.0	0.0	0.0	0.0	15.0	0.0

Positive control: tetracycline (100 μg) or nystatin (100 unite); Negative control: DMSO.

Table 2

MIC and MBC of *L. littorea* extracts and standard antibiotic.

Extracts	*S. aureus*		*B. cereus*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
n-Hexane	1.11	>10.00	0.04	0.04	0.04	0.37
Ethyl acetate	0.37	>10.00	–	–	0.37	>10.00
Methanol	1.11	10.00	–	–	–	–
Tetracycline (μg/mL)	30.00	–	2.00	–	30.00	–

All data were expressed as (mg/mL) except for tetracycline (μg/mL); –: not determined.

effective properties, such as antimicrobial, antiviral and antifungal. Many medicine plant extracts have been known to possess antimicrobial effects. Mangroves possess novel biologically active compounds. The extracts from different mangrove plants and mangrove associates have been reported to possess inhibition action against human and plant pathogens[13-22].

In this report, three different polarity extracts have been tested for antimicrobial activity. With respect to the inhibition panel and the MIC and MBC concentrations, *n*-hexane extract of *L. littorea* was the most effective extract. The methanol and dichloromethane extracts of *L. littorea* also demonstrated antimicrobial effect, although they were lower than the antimicrobial effects of the *n*-hexane extract. The presence of the activity in *n*-hexane, dichloromethane and methanol extracts might be represented by existence of more than one active compound. Chemical analysis of the species belongs to the genus *Lumnitzera* have shown the presence of various bioactive ingredients including alkaloids, steroids, triterpenoids and flavonoids[14,23]. On the other hand, it is interesting to note that the plant extracts showed bacteriostatic and bactericidal actions against *S. aureus*, *B. cereus* and *E. coli*. This suggests that they may possess remarkable therapeutic action in the treatment of infectious disease caused by these species.

The obtained results suggesting the possibility of finding potent antibacterial agents from *L. littorea* extracts and considered sufficient to isolate the compounds responsible for the activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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