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Preliminary *in-vitro* screening of some mangrove plant extracts for antibacterial compounds against clinical isolates of bacteria

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Abstract
Twenty-four aqueous and ethanol plant extracts obtained from mature leaves, tender leaves, shoot and bark of *Avicennia marina, Bruguiera gymnorhiza* and *Rhizophora mucronata* were screened for antibacterial activity against clinical isolates of *Pseudomonas* sp., *Escherechia coli, Staphylococcus* sp., *Klebsiella* sp., *Acinetobactor* sp. and *Shigella* sp. Ethanol extracts were found to be more effective than aqueous extracts on certain clinical strains. Both aqueous and ethanolic extracts of *A. marina* showed the highest antibacterial activity. Moreover, sequential extraction of ground materials of *A. marina* in petroleum ether, chloroform, ethyl acetate, ethanol and water showed an inhibitory effect on certain strains of bacteria namely *Shigella* sp. and *Pseudomonas* sp. Soxhlet extracts of mature leaves, tender leaves and bark of *A. marina* in chloroform, ethyl acetate and ethanol showed the most clear antibacterial activity against *Shigella* sp.

Introduction
Traditional medicine plays a significant role in the health care system of developing countries, especially in Asia including Sri Lanka. Some hospital-acquired infections may be resistant to antibiotics and the cost of treating these infections has become a burden to national health service. Therefore, proper measures must be taken to deal with this problem. Studies are underway for extracting biologically active natural products that may have potential application for different purposes (eg. antimicrobial agents of *Echinacea purpurea* and *E. angustifolia* (Briskin 2000), phytomedicine of *Piper methysticum* for treatment for anxiety, nervous tension. Numerous medicines derived from mangroves (ashes or bark infusions) can be applied for skin disorders, sores including leprosy, asthma and rheumatic disorders (Field 1995, Bandaranayake 1998). Scientific studies on the antibacterial activity of mangrove plants have not been reported in Sri Lanka. Therefore, it is worthwhile launching a research project to carry out the screening of mangrove plants for antibacterial activity, isolation and chemical characterization of the natural products responsible for the inhibitory effect on pathogenic microorganisms. Thus the aim of the research project was to screen of some selected mangrove plants for antibacterial activity.

Materials and methods
About 0.3 g of the plant materials (bark, mature and tender leaf and shoot) of each species (*A. marina*, *R. mucronata* and *B. gymnorhiza*) were crushed with one milliliter of sterilized distilled water and 95% ethanol separately using a sterilized mortar and pestle. Crushed material, which was mixed with sterilized distilled water and 95% ethanol separately in 1.5 ml Eppendorf tube was centrifuged at 10,000 rpm for 2 minutes. These crude extracts were tested *in vitro* against clinical bacterial isolates namely, *E. coli, Shigella, Acinetobactor* and *Klebsiella* (obtained from the Faculty of Medicine, University of Ruhuna), *Staphylococcus* from infected urine, *E. coli* strain from infected

blood and *Pseudomonas* from a wound (obtained from the General Hospital, Matara) for antibacterial activity by agar diffusion technique (Castillo 1998).

Antibacterial activity was visualized as a clear zone on Petri plates. Quantitative measurements were determined by measuring the size of the inhibitory zone. For sequential extraction, 50 g of each fresh plant material of *A. marina* was ground separately. Then the sequential extraction of ground bark and leaf materials was carried out in a Soxhlet extractor using 300 ml of petroleum ether, chloroform, ethyl acetate, ethanol and water as solvents. The extraction time period for each solvent was 3 hours. Each concentrated extract (by evaporation) was tested against each bacterial strain. Appropriate controls without plant materials were performed.

Results
Some of the ethanolic and aqueous extracts of leaf, shoot and bark of *A. marina*, *R. mucronata* and *B. sexangula* exhibited antibacterial activity against the growth of bacteria used in this experiment (Table 1). Furthermore it shows that the degree of antibacterial activity of these plant extracts was not similar. Almost all crude ethanolic extracts showed better inhibitory activity against bacteria than the aqueous extracts (Table 1). None of the aqueous extracts showed any inhibitory effect on *E. coli* (Table 1). Mature leaf extracts were found to be more effective against all the tested bacteria in comparison to other extracts. Comparatively *A. marina* was found to have the highest activity for all bacterial strains followed by *B. gymnorhiza*. Therefore, *A. marina* was selected as the test plant for further experiments.

Table 1. Degree of growth inhibition measured in millimetres (1-8) from the edge of the well to the edge of the inhibition zone of *Staphylococcus* sp., *E.coli, Shigella* sp. and *Pseudomonas* sp. by plant extracts of *A. marina, B. gymnorhiza* and *R. mucronata.* (ML- mature leaves, TL- tender leaves, B- bark S-shoot and (--) no inhibition)

Plant material		Bacterial strain							
		Obtained from the faculty of Medicine				Obtained from the General hospital, Matara			
		E. coli		***Shigella* sp.**		***Pseudomonas* sp.**		***Staphylococcus* sp.**	
		W	E	W	E	W	E	W	E
A. marina	ML	--	5	4	8	3	6	3	1
	B	--	4	1	3	--	2	--	5
	TL	--	6	--	7	2	4	2	5
	S	--	5	1	5	2	2	--	3
B. gymnorhiza	ML	--	3	--	3	2	2	--	--
	B	--	4	--	4	2	4	--	4
	TL	--	--	--	--	--	2	--	--
	S	--	1	--	1	2	3	--	--
R. mucronata	ML	--	1	--	1	1	2	--	--
	B	--	1	2	4	2	5	--	3
	TL	--	--	--	--	--	--	--	--
	S	--	1	--	1	1	--	--	--

According to the results, the highest inhibitory activity was given for *Shigella* sp. tested with the ethyl acetate extracts of mature leaves, tender leaves and bark obtained from *A. marina*. The compounds extracted in petroleum ether from the leaves of *A. marina* showed a moderate inhibitory effect against bacteria (data not shown). Soxhlet extracts of mature leaf of *A. marina* using chloroform, ethyl acetate, ethanol exhibited the highest inhibitory activity on *Shigella* (Figure 1). Neither *Acinetobactor* nor *Klebsiella* gave any inhibition by any tested plant extract. Therefore, these two bacterial strains are the most resistant to all types of extracts under study, while *Shigella* was the most sensitive organism for those. Soxhlet ethyl acetate and ethanol fractions were found to have the most active components against all test organisms.

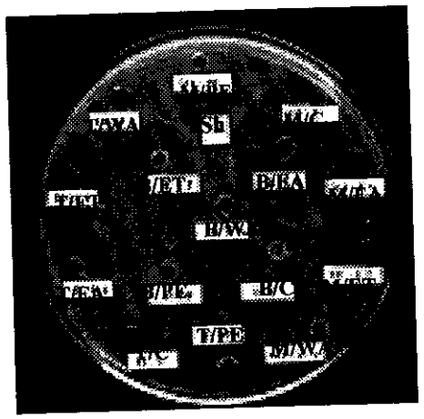

Figure 1. Inhibition of growth of *Shigella* sp. (Sh) by sequential Soxhlet plant extracts of mature leaves (ML), tender leaves (TL) and bark (B) of A. marina obtained from petroleum ether (PE), chloroform (C), ethyl acetate (EA), ethanol (ET) and water (WA)

Discussion

A. marina, *R. mucronata* and *B. gymnorhiza* were selected as test plants because there are reports that people living in coastal areas use them against microbial infections (Bandaranayake, 1998). As some mangrove plant extracts can inhibit pathogenic bacterial growth, they can be used as medicines to cure bacterial infections. Effect of different extracts of *A. marina*, *R. mucronata* and *B. gymnorhiza* on some selected microorganisms have been studied and the results clearly indicated that the tested bacterial strains were sensitive to the many crude extracts of bark, leaf (mature and tender) and shoot of these plants. Therefore, these extracts must have active ingredient(s) that can inhibit the bacterial growth. Compounds in wells can diffuse through agar medium and can exert inhibitory effect on bacterial growth in the diffused area. As no clear zones were seen in control proves that solvents could not influence bacterial growth.

As *A. marina* showed best results, further studies were conducted only with *A. marina* extracts. However, only mature leaves, tender leaves and bark extractions were used for further tests, as shoot extraction did not show clear inhibition. Probably the presence of low concentration of the active ingredient(s) in this extract could be the reason. As no inhibition was given for *Klebsiella* sp. and *Acenitobactor* sp., it can be assumed that those compounds extracted in solvents could not play a role in inhibiting the growth of these strains. However, some pathogenic strains obtained from blood, urine and a wound showed considerable sensitivity towards the plant extracts, and the discovery of novel anti-bacterial compounds from these plants is promising.

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