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Antibacterial activity of aqueous and ethanol extracts of mangrove species collected from Southern Sri Lanka

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Abstract

Objective: To study the antibacterial activity of mangrove extracts against clinical isolates of *Shigella* sp., *Pseudomonas* sp. and antibiotic resistant bacteria, *Staphylococcus aureus* and *Proteus* sp.

Methods: Crud aqueous and ethanol extracts of mature leaves, tender leaves, bark and shoot of mangroves species (*Avicennia marina*, *Bruguiera gymnorhiza, B. sexangula, Rhizophora mucronata, R. apiculata, Derris trifoliata, Exoecaria agallocha,* and *Lumnitzera racemosa*) were tested for antibacterial activity using agar diffusion method.

Results: Both aqueous and ethanol extracts showed considerable activity against all tested bacterial species. However, all the ethanol extracts showed higher inhibition activity than those of aqueous extracts. For both aqueous and ethanol extracts, inhibition zones were larger for non- antibiotic resistant bacteria *Shigella* sp. and *Pseudomonas sp.* than antibiotic resistant *S. aureus* than *and Proteus* sp. Charcoal treated plant extracts of *Avicennia marina*, *Bruguiera gymnorhiza* and *Rhizophora mucronata* were able to inhibit both clinical isolates of bacterial species more than those of untreated plant extracts. The most clear antibacterial activity was shown by almost plant extracts of *Lumnitzera racemosa* for both bacterial strains. Mature, young leaf and bark extracts of *Exoecaria agallocha* also showed considerable antibacterial activity while *Derris trifoliata* did not show relatively good antibacterial activity.

Conclusion: Potential antibacterial activity of both aqueous and ethanol of mangroves suggests, these extracts could be used as an alternative source for treatment of infections caused by these pathogenic bacteria.

Key Words :Bioassay, Antibatcterial activity, Mangroves, Inhibition, Ethanol extracts.

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INTRODUCTION

Bacteria, fungi, viruses and other microorganisms are potentially pathogenic to human. Antibiotic resistant bacteria outbreaks have been reported in hospitals throughout the world.[1] Therefore, discovery of new antibiotics to combat such diseases is very important and essential[2]. Tropical forests are the hot spots for the plant diversity in the world.[3] Plants are a gift of nature providing food, forage, timber, cloths, cosmetics, firewood, charcoal, building materials, tannin, flavors and etc. Also they supply limitless drugs and medicines to cure infectious diseases including chronic diseases. [4,5] A large number of plants on the earth's surfaces and their products has been extensively used throughout the history and world as drugs and remedies to treat human diseases.[6] According to World Health Organization (WHO), more than 80% of the world population depends on the traditional medicine for their primary healthcare needs. In developing countries, including Sri Lanka, traditional medicine plays a significant role. Native and low-income people like farmers and small isolate villagers use folkloric medicine for the treatment of general infectious diseases.[7] One such resource is folk medicines and systematic screening of these may result in the discovery of novel effective medicines. Investigation of different extracts obtained from traditional medicinal plants is being done to examine the potential sources of new antimicrobial agents. Numerous studies have done to assess the antimicrobial activity. [8]

In Sri Lanka, there is a rich mangrove ecosystem. The total cover of mangroves in Sri Lanka has been estimated as 100 km^2. [9] The dwellers in the coastal areas in the tropical and sub - tropical countries are being used some mangrove species to cure some diseases.[10] Although there are large number of mangrove species found in Sri Lanka, attention has not been focused on scientific studying the antimicrobial activity to examine their usefulness in medicine. The extraction of novel natural chemical compounds from mangrove in addition to already known medicinal plants is in its infancy.[10] Extracts and chemicals from mangroves have pharmaceutical value.[10] Extracts and chemicals from mangroves are used mainly in folkloric medicine and

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these practices are being continued.[10,11] Considering the above aspects an attempt has been made to determine the effectiveness of activity of aqueous and ethanol extracts of different extracts obtained from different parts of the mangrove plant against clinical isolates as well as antibiotic resistant bacterial strains.

MATERIALS AND METHODS

Preparation of plant extracts by grinding method

In this study, mangrove plants were selected based on their medicinal properties. Mature and tender leaves, shoots and barks of *Avicennia marina*, *Bruguiera gymnorhiza, B. sexangula, Rhizophora mucronata, R. apiculata, Derris trifoliata, Exoecaria agallocha,* and *Lumnitzera racemosa* were used to obtain the aqueous and ethanol *extracts.* Amounts of 0.3g of mature leaves, tender leaves, shoot and bark were weighted and crushed with one milliliter of sterilized distilled water and 95% ethanol separately using a sterilized mortar and pestle. Crushed material was centrifuged in 1.5 ml Eppendorf tube at 10,000 rpm for 2 minutes in a microcentrifuge. Supernatant was transferred into new vial.

Preparation of inoculums

The clinical isolates of *Shigella* sp. and *Pseudomonas* sp. and two antibiotic resistant *Staphylococcus aureus* and *Proteus* sp. were used as the test organisms. *Staphylococcus aureus* was resistance to Ceftazidime (15 µg/ml), Gentanicin (10 µg/ml), Kanamycin (50 µg/ml) and Ticarcillin/Clavulanic acid while *Proteus* sp. was resistant to Gentamicin (10 µg/ml) and Kanamycin (50 µg/ml). Stock cultures were maintained at 4^0 C slopes of nutrient agar (peptone 5 g, beef extract 3 g, NaCl 8 g, agar 18 g, deionized water 1000 ml). Active cultures for experiment were prepared by transferring a loop full of cells from the stock cultures to sterilized test tubes containing nutrient agar incubating overnight at 37^0 C. Cultures were dilutes with nutrient agar broth to achieve optical densities corresponding to $2 X10^6$ colony forming units (CFU/ml).

Antibacterial susceptibility test

Agar diffusion method was used to screen the antibacterial activity. Hundred micro liters (~$2 X10^6$ CFU/ml) of overnight cultures of each bacterial strain was added to Petri dishes (143 mm diameter) containing solid nutrient agar, spread uniformly and allowed to dry for 5 minutes. Wells were made using a cork borer (size-3) on the solidified medium. Prepared wells were filled with 50 µl of each extract obtained from the grinding method. For control, ethanol and water were used instead of plant extracts. Plates were incubated overnight at room temperature. Size of inhibition zone was measured using a metric ruler from the edge of the well to the edge of the inhibition zone.

Testing of charcoal treated and untreated plant extracts for antibacterial activity

A small amount of activated charcoal was added into ethanol extracts into 1.5 ml of Eppendorf tubes and incubated at 40^0 C for 10 minutes in a water bath, centrifuged at 10, 000 rpm for 2 minutes. Supernatant was transferred into a new vial. Inoculation was made by adding 100 µl (~$2 X10^6$ CFU/ml) of over-night cultures of *bacterial* species into sterilized Petri dishes containing nutrient agar. After drying, wells were loaded with 25 µl of charcoal treated plant extracts. This procedure was repeated with untreated plant extracts. Plates were incubated overnight at room temperature. Inhibitory zones were measured using a metric ruler.

RESULTS AND DISCUSSION

Antimicrobial is a diverse group of naturally occurring or laboratory-synthesized chemicals,at very low concentrations, are able to kill or inhibit the growth of microorganisms. Salvarsan was the first example of an antimicrobial drug synthesized in the laboratory. [12] Antibiotics are a subset of antimicrobials that are naturally produced by the biosynthetic processes of molds and bacteria. Today, antimicrobials are routinely prescribed for so many bacterial diseases. Unfortunately, the overuse and misuse of these life-saving drugs, coupled with the bacterial world's amazing ability to adapt under selective pressure, had led to an increase in the number of organisms that are resistant to the effects of antimicrobials. [13] In this study, clinical bacteria as well as antibiotic resistant bacteria were used to study the effect of mangrove plant extracts on their growth.

The development of drug resistance limits the usefulness of all known antimicrobials. In addition to, there are so many reasons for development of resistance. Sensitive bacteria can acquire antibiotic resistance. Acquisition of antimicrobial resistance can also occur through spontaneous mutation and through DNA transfer.[12] In response to the increased resistance, the pharmaceutical industry is searching to develop new antimicrobial drugs. Recently, the acceptances of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researches to investigate the antimicrobial activity of medicinal plants.[13,14,15]

Primarily plant extracts of mature leaves, tender leaves, bark and shoot of *A. marina, B. gymnorhiza, and R. mucronata were used* to test of antimicrobial activity against pathogenic species of *Shigella* and *Pseudomonas and* antibiotic resistance bacteria: *Staphylococcus aureus* and *Proteus* sp. (Figure 1) Since growth of both

pathogenic and antibiotic resistant species were inhibited by water and ethanol obtained by grinding method, plant extracts of *B. sexangula, D. trifoliata, E. agallocha, L. racemosa* and *R. apiculata* were used to test of antimicrobial activity against antibiotic resistance bacteria: *Staphylococcus aureus* and *Proteus* sp.

In total sixty aqueous and ethanol extracts of different mangrove plant parts were checked for the growth of bacterial strains. All ethanol extracts exhibited more inhibition than aqueous extracts against all bacterial strains tested. Therefore, ethanol extracts were more effective against all bacterial species than aqueous extracts. According to the results shown in the Figure 1a, 1b 1c and 1d, some plant extracts exhibited growth inhibition for both strains. Furthermore it shows that the degree of antibacterial activity of these plant extracts was not similar.

Plant extracts of mature leaf and tender leaf of *A. marina* strongly inhibited the bacterial growth than extracts of shoot and bark of the same plant. None of the aqueous extracts of *A. marina* showed any inhibitory effect on *Proteus* sp. The mature leaf extracts were found to be more effective against *Shigella* sp. and *Pseudomonas sp.* in comparison to tender leaf extracts. Bark extracts of *B. gymnorhiza* and *R. mucronata* showed the highest antibacterial activity compared to the other extracts of the same plant species. Shoot extracts of all species showed less antibacterial activity compared to the other extracts. Bark extracts of *B. gymnorhiza*, and *R. mucronata* exhibited more antibacterial activity against all bacterial species than bark extracts of *A. marina*.

According to the results (Figure 1a-1d), almost all plant extracts exhibited more inhibition for *Staphylococcus aureus* than *Proteus* sp. These results agree with the previous results. [16] Plant extracts of *L. racemosa* (Figure 1a) exhibited more pronounced inhibition against both bacterial species than other plant extracts of *E. agallocha*, *B. sexangula* and *D. trifoliata*. The highest antibacterial activity was exhibited for *Staphylococcus aureus* by ethanol extracts of bark and tender leaf of *L. racemosa* and aqueous extract of shoot of *L. racemosa.* Ethanol extract of mature leaf of *L. racemosa* and aqueous extract of tender leaf of *L. racemosa* showed the second most efficient antibacterial activity against *S. aureus.* It shows that extractable chemical compounds in *L. racemosa* were more effective against bacteria (Figure 1a).

When growth inhibition of *Proteus* sp. is considered, the highest antibacterial activity was exhibited by ethanol extract of tender leaf of *E. agallocha* (Figure 1b). The second most efficient antibacterial activity was shown by ethanol extract of bark of *B. sexangula,* aqueous extract of mature leaf of *E. agallocha*, ethanol extracts of bark and tender leaf of *L. racemosa* against *Proteus* sp. Any extract of *A. marina* and *D. trifoliata* did not show antibacterial activity against *Proteus* sp. (Figure 1d). Probably, *Proteus* sp. may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decreased intracellular drug accumulation[17] against

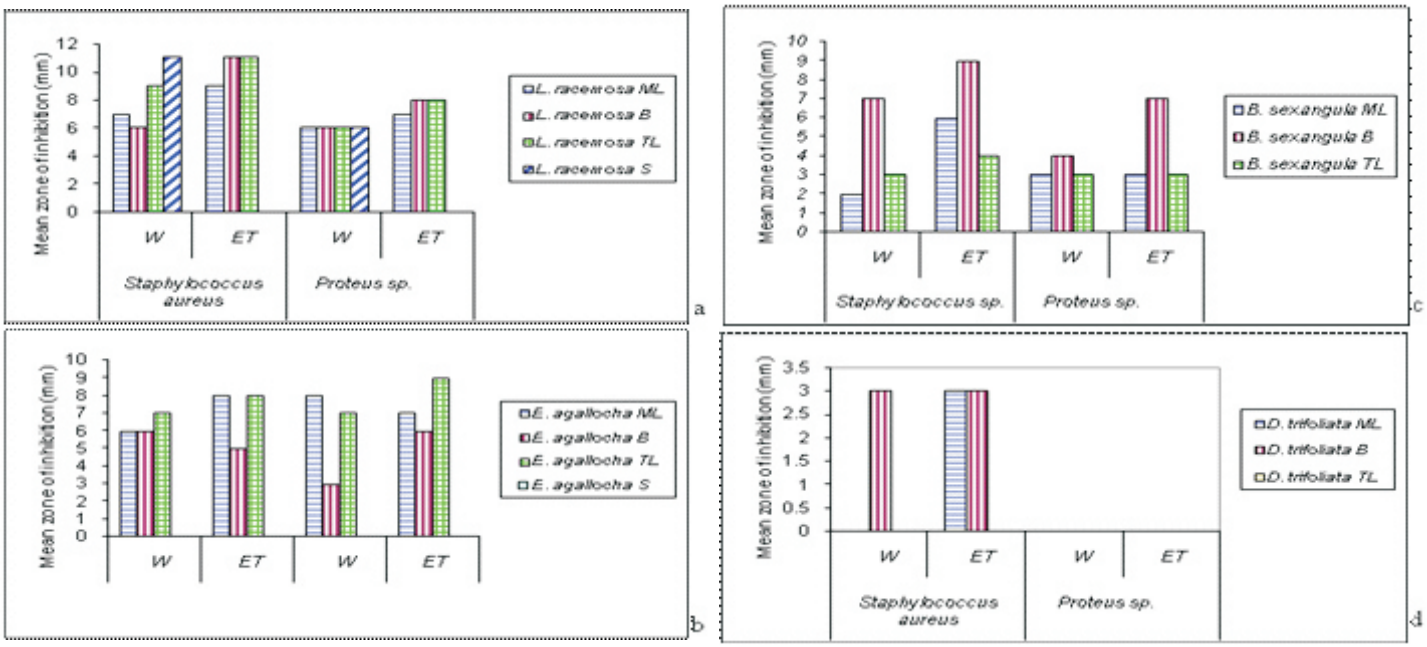

Figure 1: **Mean zone of growth inhibition of *Staphylococcus aureus* and *Proteus* by extracts of *L. racemosa*, *E. agallocha*, *B. sexangula* and *D. trifoliate*. ML- mature leaves, TL- tender leaves, B- bark, S- shoot, W- water, E- ethanol.**

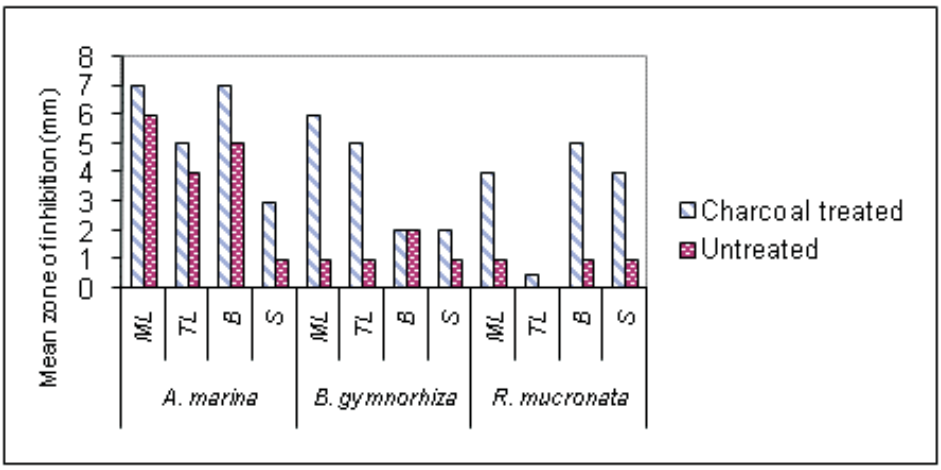

Figure 2. Comparison of mean zone of growth inhibition of *Staphylococcus aureus* using charcoal treated and untreated plant extracts of A. *marina, B. gymnorhiza* and *R. mucronata*. ML-mature leaves, TL-tender leaves, B- bark, S-shoot.

mangrove plant extracts. Also components, which are present in plant extracts, may be not sufficient to show antibacterial activity against *Proteus* sp. As well as, none of the plant extracts of tender leaf of *D. trifoliata* and shoot of *E. agalocha* were able to inhibit both bacterial strains.

Both water and ethanol are high polar solvents. Therefore, only polar compounds are extracted into water and ethanol. There may be non-polar or less polar compounds, which are not extracted into water or ethanol. But these components may have an antibacterial activity. Therefore, non-polar solvents can be used in addition to water and ethanol[16]. The result indicates that the ethanol extracts had more efficient antibacterial compounds than those of water extracts. No clear zones were seen in controls. It proves solvents; water and ethanol could not influence on bacterial growth.

Degree of inhibition of *Staphylococcus aureus* sp. was compared between charcoal treated and untreated ethanol extracts of mature leaf, tender leaf and bark of *A. marina*, *B. gymnorhiza* and *R. mucronata* using agar diffusion technique. Charcoal treated extracts strongly inhibited the bacterial growth than untreated plant extracts (Figure 2). To see whether there is an influence of pigments in plant extracts on its effectiveness; extracts were checked with and without pigments of the extracts against bacterial growth. To remove pigments of the extracts, activated charcoal was used. For the comparison of the activity of pigments present in plant extracts, only *Staphylococcus aureus* sp. was used. As ethanol extracts were most effective against all bacterial species, only ethanol extracts obtained by grinding method were used. Charcoal treated plant extracts showed more inhibition on bacterial growth while charcoal untreated extracts showed less inhibition. Pigments of plant extracts may have resulted by chlorophyll pigments or other compound that possess a colour. Therefore, we can assume that they may contribute to the reduction of bacterial growth and may increase the survival ability of bacteria.

Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins[16]. Plant saponins have interesting biological activities.*A.officinalis* produces pharmacologically significant steroidal saponins and sapogenins.[9] Many types of flavonoids, which are present in mangrove plants, have the role of therapeutic agents. The majority of natural products used in medicine today are alkaloids. Alkaloids are also considered as an active bactericidal component. Several biological activities such as antibacterial an antiseptic are reported for many types of tannin.[9] *Avicennia* spp. are used for skin diseases, boils and wounds and blood purifier, *Bruguiera* spp. are used for eye diseases while *Rhizophora* spp. are used for diarrhea and tuberculosis.[9]

CONCLUSION

Mangrove plant species of *Avcennia marina*, *Bruguiera gymnorhiza, B. sexangula, Rhizophora mucronata, R. apiculata, Exoecaria agallocha,* and *Lumnitzera racemosa* can be used to produce novel medicines for bacterial infections. Presence of different chemical compounds (e.g. Alkaloids, saponins, flavonoids and etc.) in mangrove plant extracts and their relative amounts will be determined using chemical tests. For more successful separations of chemical components in plant extracts column chromatography and other analytical methods will be conducted. Further, it is

intended to extent the testing of antimicrobial activity against few other bacterial strains and fungal isolates.

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