Anti-bacterial Profiling of *Launaea sarmentosa* (Willd.) and *Bruguiera Cylindrical* (L.): Two Distinct Ethno Medicinal Plants of Bangladesh

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**Abstract**

**Aim:** The present study was commenced to investigate the methanolic extracts of *Launaea sarmentosa* (Willd.) and *Bruguiera cylindical* (L.) for antibacterial properties. *Launaea sarmentosa* (Willd.) is a plant of Asteraceae family while *Bruguiera cylindical* (L.) belongs to the family of Rhizophoraceae.

**Materials and Method:** The study was performed by disc diffusion method.

**Results:** Crude methanolic extracts of both *Launaea sarmentosa* (Willd.) and *Bruguiera cylindical* (L.) at various concentration were used for antibacterial screening. The methanolic extracts of *Launaea sarmentosa* (Willd.) produced good antibacterial activities against gram negative (−ve) bacteria and were resistant against gram positive (+ve) bacteria whereas crude methanolic extracts of *Bruguiera cylindical* (L.) were resistant against both gram positive (+ve) and gram negative (−ve) bacteria.

**Conclusion:** Further work especially bioassay-guided fractionation may be avouched in order to isolate and characterize the antibacterial active constituents responsible for the antibacterial property.

**Keywords:** *Launaea sarmentosa* (Willd.); *Bruguiera cylindical* (L.); Disc diffusion; Antibacterial

**Introduction**

Nature always acts as a great source of rescue for human being by providing different remedies from its plants, animals, and other sources to cure all ailments of mankind. Many species of plants containing substances of medicinal value are comprised from the plant kingdom, which are yet to be explored. Medicinal plants are moving from brink to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals [1]. The leaves, stems, barks, flowers and underground parts of medicinal plants are most often used for traditional medicines. Disease-treating formulations and treatment were based on medicinal plants from ancient time, which practiced by the traditional physicians [2]. Microorganisms are responsible for several infectious diseases throughout the world, especially in developing countries and become the major causes of mortality and morbidity in immune-compromised patients [3]. Microorganism infections include; metabolism related infections, GIT infections, urinary tract infection, skin infections and surgical wound infections. Problematical use of antibiotics has increased the advancing of resistance of pathogens against antibiotics has become a tough issue within the recent years [4-9]. Unfortunately antibiotic resistance has become a great concern of treating infectious disease globally which offers great challenges for clinicians and pharmaceutical industry [10]. Many of our currently used antibiotics have become less active against a wide range of pathogen due to emergences of drug resistance. On the other hand, newly discovered drug possess many unwanted side effect. So the analysis of medicinal plants to explore antibacterial agents will be a fruitful task in generating new way of treatment [11,12].

Utilization of plants as natural antioxidants and within the treatment of diseases caused by microbic infection has been limited due to lack of scientific data to support the native information [13]. It is a well-known consequence that almost all of the antibacterial agents which have high degree of antibacterial potential are provoked from natural product [14]. So it is badly need to analyze the natural products in order to explore new antibacterial agent to combat against these condition [15-16]. That is why our present study was undertaken.
Launaea sarmentosa (Willd.) and Bruguiera cylindrical (L.) both are coastal plants. Launaea sarmentosa (Willd.) is locally known as vortashak which are available in Bangladesh, South Africa, coastal Madagascar, Seychelles, Mauritius, India, Sri Lanka, Malaysia, Philippines, Indonesia, Queensland, Australia and Papua New Guinea. The goal of the present study was to assess the antibacterial potentials of crude methanolic extracts of both Launaea sarmentosa (Willd.) and Bruguiera cylindrical (L.) [17-19].

Materials and Methods

Drug and reagents

Standard drug Ampicillin and Cefoxitin was purchased from Beximco Pharmaceuticals limited, Tongi, Bangladesh. Other reagents and materials required during culture preparation and for conduction of this full experiment were provided from pharmaceutical microbiology department of Noakhali Science and Technology University.

Collection of plant materials

For this present investigation the whole plants of Launaea sarmentosa (Willd.) were collected from Darianagar, Cox’s Bazar, Bangladesh and the leaves of Bruguiera cylindrical (L.) were collected from surrounding area of Sonadia deep, Cox’s Bazar on May 2015. The accession number of Launaea sarmentosa (Willd.) and Bruguiera cylindrical (L.) are DACB-38312 and DACB-38325, which were confirmed by the National Herbarium Institute, Mirpur, Dhaka, Bangladesh.

Preparation of plants extracts

After collection of whole plants of Launaea sarmentosa (Willd.) and leaves of Bruguiera cylindrical (L.) plant materials were properly washed, chopped and then finally air dried for some days. To obtained coarse powder from chopped pieces, grinding process were followed by oven dry for 24 hours at possible low temperature. Then in an air-tight flat bottomed container, about 500 g of coarse powder were suspended with 1500 ml of 80% methanol, with continual shake and stirrer at regular interval of time for a period of 15 days at room temperature. Then coarse filtration of slurry was carried through a clean white cotton material and finally with a Whatman No.1 filter paper. Finally, a weight of 5.2 gm of plant extract was obtained by using a rotary evaporator at low temperature and pressure.

Anti-Bacterial Activity

Due to the rapid emergence of resistance to antibacterial agents, screening of antimicrobial agent from a natural source has taken importance all over the world. The present study was conducted by standardized single-disc diffusion method with no modification. This method was described by Bayer et al., 1966 and become the most acceptable method of screening plant extract for antibacterial activity [20].

Test micro-organisms

For carrying out antibacterial potential of plants methanolic extracts, several strains of both gram positive (+ve) and gram negative (-ve) bacteria were collected as pure cultures from the Institute of Chittagong veterinary and animal sciences university.

Preparation of medium

Nutrient agar medium (DIFCO) was used to prepare fresh cultures and to test the sensitivity of the materials against micro-organism. Fixed amount of nutrient agar was mixed with distilled water in a conical flask to prepare the required volume of 1000 ml. The contents were heated in a water bath with continuous stirring to ensure perfect dissolution and NaOH or HCl was used to maintain the pH (7.2-7.6) at 25°C. Then a screw cap test tube was filled with 10 ml and 5 ml of the medium to prepare plates and slants respectively. The capping and subsequent sterilization of test tubes were done by autoclaving at 15 lbs/sq. inch pressure for 20 minutes at 121°C. Finally these slants were used to prepare fresh culture of bacteria which in turn used for sensitivity studies.

Preparation of subculture

With the help of a transfer loop, the test organisms were transferred to the agar slants in an aseptic condition under laminar air cabinet to have fresh pure cultures. To ensure optimum growth, inoculated strains were then incubated for 24 hours at 37°C. Finally sensitivity test was conducted by these fresh cultures of bacterial strains.

Preparation of the test plates

Under aseptic condition, with the help of a sterilized transfer loop, the test organisms were transferred from the subculture to the test tubes containing about 5 ml of melted and sterilized agar medium. To obtain uniform suspension of the organisms, the test tubes were shaken by rotation and then the suspension was dispelled to the sterilized petri dishes without any delay. To obtain homogenous distribution of the test organisms in the media, the petri dishes were rotated clockwise and anticlockwise for several times.

Discs preparation

Three different types of discs namely standard, blank and sample disc were used to conduct antimicrobial screening. A positive control for the test procedure was designed through a standard disc, where standard antibiotic agents were employed against test micro-organism and the reference drug for standard disc preparation was Kanamycin (30 μg/disc). Blank discs were designed as negative control, which assures that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves. The test sample was prepared by dissolving 10 mg of dried crude extracts in 10 ml solvent to make the concentration of 1 mg/ml. Sterilized
metrical (BBL, Cockeysville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood and soaked with solutions of test samples and dried, so that the final volume is 400 μl/disc.

**Diffusion and incubation**

After that, sample, standard antibiotic and control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test microorganisms by using a sterile forceps. The discs were placed 15 mm far from the edge of the plates and from each other to avoid overlapping of the zone of inhibition. Then plates were freeze-dried at 4°C for about 24 hours to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. In order to achieve optimal microbial growth plates were then inverted and incubated at 37°C for 24 hours.

**Determination of antibacterial activity by the zone of inhibition**

The antibacterial potency of the test agents is measured by a clear zone of inhibition, an area given by the test materials by preventing the growth of microbes surrounding the discs. Then antibacterial activity of incubated test materials was confirmed by measuring the diameter of the zones of inhibition in millimeter and centimeter with a transparent scale.

**Statistical analysis**

The present data were represented as Mean ± SD, while one way analysis of variance (ANOVA) followed by Dunnett’s t-test, using SPSS software of version 10, was considered for analyzing the significance level of calculated values (P<0.05).

### Table 1 Antibacterial activities of *Launaea sarmentosa* (Willd.) against both gram positive (+ve) and gram negative (-ve) bacteria.

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>Zones of inhibition (cm) of Extracts</th>
<th>Zones of inhibition (cm) of Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Gram Positive (+ve)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gram Negative (-ve)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>–</td>
<td>1.5±0.047*</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>–</td>
<td>–</td>
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<td></td>
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</tbody>
</table>

Here, (+++) = highly active; (++) = moderately active; (+) = slightly active; (-) = No activity against microorganism. ***** = P<0.001, *** = P<0.01, * = P<0.05 (one way ANOVA tests followed by Dunnetts t-tests on each group P<0.05 were considered statistically significant).

### Table 2 Antibacterial activities of *Bruguiera cylindrical* (L.) against both gram positive (+ve) and gram negative (-ve) bacteria.

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>Zones of inhibition (cm) of Extracts</th>
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<tr>
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<tr>
<td>Gram Negative (-ve)</td>
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<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
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### Results

From the experiment it is exhibited that the methanolic extracts of *Launaea sarmentosa* (Willd.) produced moderate activity against the gram negative (-ve) bacteria while it was resistant against gram positive (+ve) bacteria. On the other side the crude methanolic extracts of *Bruguiera cylindrical* (L.) were resistant against both gram positive (+ve) and gram negative (-ve) bacteria. The overviews of the results are shown in Tables 1 and 2.
Discussions

At different parts of the world medicinal plants are being increasingly reported for their antibacterial properties. Nikaido told that hydrophilic outer membrane of the Gram negative bacteria allows small hydrophilic molecules to pass through the outer membrane which consist of lipopolysaccharide molecules [21]. Furthermore, lipophilic macromolecules have property of passing this outer membrane. Any solute having antibacterial activity is capable of permeating outer membrane of the microorganisms [21]. Crude methanolic extracts used in this study have confined solubility in water; it can penetrate the outer membrane of gram negative bacteria and causing disturbed metabolism, cellular function, loss of cellular constituents and finally leads to death. In other previous studies similar results have also been reported [22,23]. Antibacterial agents are showing their activity against microorganisms by inhibiting cell wall synthesis [24,25], they accumulate in plasma membrane of bacterial and causing depletion of energy [26]. Through interfering in the permeability of cell membrane, antibacterial agents changes the structure and function of key cellular constituents, resulting in mutation, cell damage, and finally leads to death [27]. The medicinal properties of the *Launaea sarmentosa* (Willd.) lie in a several chemical group such as tannins, flavonoids, alkaloids and phenolic compound [19]. On the other hand, this phytochemical constituents are absent in the plant of *Bruguiera cylindrica* (L.). Many parts of the *Launaea sarmentosa* (Willd.) plant especially leaves possess antibacterial properties due to presence of tannins and flavonoids [28, 29]. This Plant also synthesize huge amount of aromatic compound among which phenols or their oxygen-substituted derivatives are predominant [30]. These compounds provide protection against microbes for the plant [31]. This is great to *Launaea sarmentosa* (Willd.) plant extract showed to have phytochemicals responsible for antibacterial effects [32]. May be is that why the crude extracts of *Launaea sarmentosa* (Willd.) surprisingly showed significant antibacterial activity against Gram negative (-ve) bacteria while the crude methanolic extracts of *Bruguiera cylindrica* (L.) were resistant against both gram positive (+ve) and gram negative (-ve) bacteria due to absence of this phytochemical constituents.

Conclusion

From the above experiment it is terminated that the methanolic extracts of *Launaea sarmentosa* (Willd.) revealed moderate antibacterial activities against gram negative (-ve) bacteria and were resistant against gram positive (+ve) bacteria. On the other hand, the crude methanolic extracts of *Bruguiera cylindrica* (L.) were resistant against both gram positive (+ve) and gram negative (-ve) bacteria. In order to sequestrate and characterize the antibacterial active constituents responsible for the antibacterial properties, ulterior work especially bioassay-guided fractionation may be confessed.

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