ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF POUZOLZIA ZEYLANICA (L.) BENN

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Abstract

The current study was to investigate the presence of Anti-Bacterial activity on the ethanol extract of Pouzolzia zeylanica (L) Benn which was tested by using cup plate method. The present investigation reveals that ethanol extracts of concentration 1000 mg/ml showing Anti-Bacterial activity against both gram positive and gram negative organisms such as Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, pseudomonas aeruginosa, Escherichia coli, Shigella dysentariae, Salmonella typhi. The extract showed very good antibacterial activity against Staphylococcus aureus and Escherichia coli. Due to these promising results, further in vivo studies over Pouzolzia zeylanica(L) Benn must be conducted.

Key words: Pouzolzia zeylanica (L) Benn, Anti-Bacterial activity, Cup plate method.

Introduction:

Active principles isolated from higher plants is appears to be one of the important alternative approaches to enclose antibacterial activity and hence antibiotic resistance and the management of diseases caused by microbial attack. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics. Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. From this view point, the ethanol extract of Pouzolzia zeylanica(L) Benn were screened for antibacterial potential against important seven human pathogenic bacteria.

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Volume 2, Issue 1, January-February 2012
habit; stem erect or prostrate, 15-30 cm long. Leaves 2-3.8 cm long, ovate or ovate-lanceolate, obtuse, acute or acuminate, entire. Flowers minute, in small auxiliary androgynous clusters. Its duration is perennial which means it will grow year after year. This species has been used medicinally in Malaysia and Indonesia. Leaves are anthelmintic and vulnerary; used as a cicatrizant for gangrenous ulcers, in syphilis and gonorrhoea. Leaf juice is used as galactagogue. Poultice of the herb is applied to sores, boils and to relieve stomachache. Previous phytochemical investigation of the plant revealed the presence of chemical constituents such as flavones, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and their salts. As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antimicrobial activity of *Pouzolzia zeylanica*(L) Benn against different Gram-positive and Gram-negative bacteria.

**Materials and Methods**

**Plant material**

The whole plant of *Pouzolzia zeylanica* (L.) Benn. was collected from Hathazari, Chittagong, Bangladesh in the month of August 2011, after selection of plants suitable herbarium sheet for plant with some general information were prepared and send to Bangladesh Council of Scientific & Industrial Research (BCSIR), Baluchara, Chittagong for identification. They identified and provided us the scientific name of the plants.

**Extraction**

After collection, the plant was cut into small pieces and air dried for several days. The plant materials were then ground into coarse powder. The dried and ground plant powder (138gm) were soaked in ethanol (750ml) in an air tight, clean flat bottomed container for 7 days at room temperature with occasional stirring and shaking. The extract was then filtered first through a fresh cotton plug and finally with a whatman filters paper. The filtrate (ethanol extract) obtained was evaporated under ceiling fan until dried. It rendered a greenish color concentrate paste. The weight of the crude extract was 8.202 gm and the yield value of powdered plant material of *Pouzolzia zeylanica* (L.) Benn was 5.94%. The concentrated paste was designated as crude extract or ethanolic extract.

**Evaluation of Anti-Bacterial activity**

The following media were used for our antibacterial studies.

**Nutrient agar for bacteria**

Beef extract-0.3%, Sodium chloride-0.5%, Peptone-0.5%, Agar-2.0%, pH-7.2-7.4.

**Bacterial Cultures**

To investigate the antibacterial activity both the Gm (+) and Gm (-) species were selected. For the evaluation, the Gram positive and Gram negative of clinical isolates: *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae* were selected respectively. All the clinical species were supplied by the
Faculty of Microbiology, Chittagong University, Chittagong, Bangladesh. All the test strains were maintained on nutrient agar slopes and were sub-cultured. These bacteria served as test pathogens for antibacterial activity assay.

**Sterilization**
Sterilization of the media, water, etc., was carried out by autoclaving at 15 lbs/inch$^2$ and 121°C for 20 minutes. The glassware like syringes, Petri dishes, pipettes, empty test-tubes were sterilized by dry heat in an oven at a temperature of 160°C for one hour.
The sterilized medium was cooled to 40°C and poured into the Petri dishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

**Chemicals used for Anti-Bacterial Assay**
Amoxicillin was used as reference standard and Di-methyl Sulphoxide (DMSO) as solvent (because of high polarity nature).

**Assay method**
All the experimentation was done in aseptic area under laminar air-flow unit. The Cylinder Plate Method or Cup Plate Method was adopted for the study. $^5$

**Cylinder Plate Method or Cup Plate Method**
In cup plate method, the antibacterial substance diffuses from the cup through a solidified agar layer in a Petri dish or a plate to an extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antibacterial substance. The antibacterial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.
The Ethanol extract of *Pouzolzia zeylanica* (L.) Benn was screened for antibacterial activity against a wide spectrum of micro-organisms and the activity was compared with appropriate reference standards (amoxicillin for both gram-positive and gram-negative organisms). Micro-organisms were grown in nutrient agar medium. Dimethyl sulphoxide (DMSO) was used as control and as drug vehicles for the plant extracts.

**Preparation of test and standard solutions**
Initially 10mg samples were weighed accurately and dissolved in 10ml DMSO to get a concentration of 1000μg/ml. The stock solution of reference standards (amoxicillin) was prepared at a concentration of 25μg/ml by using sterile water. Antibacterial activity was screened by adding 0.05 ml/50 μl stock solutions to each cup by micropipette.

**Evaluation of antibacterial activity:**

**Determination of Zone of Inhibition by Cup Plate Method:**
The cylinder plate assay of drug potency is based on measurement of the diameter of zone of inhibition of bacterial growth surrounding cylinders (cups), containing various dilutions of test compounds.
A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each extract and reference standards were added
to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of dimethyl sulphoxide which was used as a vehicle. The diameter of the zone of inhibition was measured and recorded.

**Measuring the Zone of Inhibition**
The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. Zone of inhibition was measured using plastic scale. Then the values were mentioned in table-1.

**Results and Discussion**

**Antibacterial assay**
The ethanol extract of whole plant of *Pouzolzia zeylanica*(L) Benn (1000 μg/disc) were screened for antibacterial activity against seven gram positive & gram negative bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Salmonella typhi*) (Table-1) by disc diffusion method. The ethanol extract exhibited antibacterial activity in compared with the standard drug amoxicillin listed in Table 1. The ethanol extract of *Pouzolzia zeylanica*(L) Benn showed highest antibacterial activity against *Staphylococcus aureus, Escherichia coli*.

**Conclusion:**
From the present study, it can be concluded that ethanol extract of *Pouzolzia zeylanica*(L) Benn are good anti-Bacterial agents and are so effective against Gram positive and Gram negative microorganisms.

**Acknowledgement**
Authors are grateful to the authority of BGC Trust University Bangladesh Chittagong for extending to the cordial support to perform these investigations.

**References**
Table-1: Antibacterial activity of the ethanol extract of EEPZ, standard and control test

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>EEPZ</td>
</tr>
<tr>
<td></td>
<td>1000 μg/disc</td>
</tr>
<tr>
<td><strong>Gram Positive Species</strong></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>11.5</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>14</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>35.75</td>
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<tr>
<td><strong>Gram Negative Species</strong></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15.7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>26.75</td>
</tr>
<tr>
<td>Shigella dysentariae</td>
<td>19.25</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>15.75</td>
</tr>
</tbody>
</table>

**EEPZ =** ethanol extract of *Pouzolzia zeylanica (L) Benn*, **S =** Standard (Amoxicillin) & **C =** Control