In-vitro antibacterial activity of Pogostemon benghalensis (N. Burman) Kuntz. Lamiaceae plant from Melghat (M.S.) India

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Abstract
Medicinal plants are the wealthy source of antibacterial agents and curative. The selected plant Pogostemon benghalensis (N. Burman) Kuntz, belongs to family lamiaceae. It is found throughout the tropical and sub-tropical region of India. It is annual, aromatic, undershrub, most common in Melghat forming large patches along the river banks. The plant is used as folk medicine, predominantly in the treatment of intestinal disorder and intermittent fever. This paper deals with the investigation on in-vitro antibacterial activity of crude leaf extract of P. benghalensis against selected pathogenic bacterial strains. All the tested bacteria were found to be highly susceptible to the crude extract of P. benghalensis. The most effective activity was observed in methanolic extract with maximum zone of inhibition ranging from 12mm and 9mm against B. subtilis and S. typhi respectively. The aqueous extract shows comparatively less inhibition ranging from 9mm and 8mm in B. subtilis.

Keywords: Antibacterial activity, Pogostemon benghalensis, Lamiaceae family, Melghat

1. Introduction
In recent years drug resistant to human pathogenic bacteria has been commonly and widely reported in literature [1, 2]. Because of the side effects and resistant that pathogenic microorganisms build against antibiotics, many scientist have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines [3]. The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world [4]. It has been reported that higher plants have shown to be a potential source for the new antimicrobials agents [5]. Antimicrobials may have significant clinical value in treatment of resistant microbial strains [6]. The antimicrobial activity of plant extracts had given us the basis of many applications in pharmaceuticals, alternative medicine and also in natural therapies. Wild plants can be selected for biological screening based on ethano-medical uses, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [7-11]. Lamiaceae members are well known for their medicinal properties. Pogostemon benghalensis (N. Burman) Kuntz. was selected for the study strictly on the basis of its ethanobotanical uses confirmed from the traditional healers of study area. The present paper deals with its in vitro antibacterial activity against some pathogenic strains of bacteria.

2. Material and Methods
The plants were collected from Chikhaldara forest, Melghat region of Amravati district (MS). The collected plants was identified taxonomically by local taxonomist and using floras [12, 13]. Fresh plant material was washed under water, air dried and then homogenized to make fine powder. This powder was packed in air tight polythene bags until further use.

2.1 Preparation of crude extracts
10 gm of air dried plant powder of each plant was mixed with distilled water and methanol and heated slowly for 2 hrs upto boiling. The boiled decoction was then filtered through 8 layered muslin cloths and centrifuged 5000g for 10 min and collected the supernatant. The above procedure was repeated twice. After 6 hours the supernatant collected at an interval of every 2hrs, was pooled together and concentrated to make the final volume [14]. Then, the extract was filter sterilized and stored at 4°C for further use.

2.2 Screening for antibacterial activity
The antibacterial assay performed by agar disc diffusion method [15] all the microbial media used in this experiment were obtained from (Himedia Laboratories, Mumbai). Overnight culture were prepared by inoculating approximately in 2ml nutrient broth with 2-3 colonies of each organism taken from nutrient agar. Broths were incubated overnight 35°C with shaking. Inocula were prepared by diluting overnight bacterial cultures approximately 10 cells per ml in sterile saline. The suspension of tested bacterial strains (0.1ml of cells per ml) was spread on Muller Hinton agar plates [16]. Filter paper discs (6 mm diameter) were impregnated in 20 microliter of the plant extract and dried aseptically. The disc are placed on the bacterial lawn of agar plates (0.1ml of cells per ml) was spread on Muller-Hinton agar plates [16]. Filter paper discs (6 mm diameter) were impregnated in 20 microliter of the plant extract and dried aseptically. The disc are placed on the bacterial lawn of agar plates and incubated at 37°C for 24 hrs. The diameter of the inhibition zones were measured using a scale in millimeters.

3. Results and Discussion
Plant derived antimicrobial agents has great perspectives in medicine and pharmaceutical industries. In the investigation, methanolic and aqueous extract of P. benghalensis were tested for its antibacterial activity against few pathogenic bacterial strains like Bacillus subtilis, Staphylococcus aureus, E. coli, Klebsiella pneumonia and S. typhi. The results are presented in table-1.
Table 1: Inhibitory effect of crude leaf extracts of *P. benghalensis* against various pathogen.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Methanolic extract</th>
<th>Aqueous extract</th>
<th>Streptomycin</th>
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<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12</td>
<td>9</td>
<td>17</td>
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<tr>
<td><em>S. aureus</em></td>
<td>8</td>
<td>7</td>
<td>15</td>
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<tr>
<td><em>E. coli</em></td>
<td>7</td>
<td>5</td>
<td>19</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>7</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>9</td>
<td>8</td>
<td>15</td>
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</tbody>
</table>

3.1 Values are mean inhibition zone (mm) of triplicate analysis

All the tested bacteria were found to be highly susceptible to the crude extract of *P. benghalensis*. However, our study revealed a remarkable antibacterial activity. The most effective activity was observed in methanolic extract with maximum zone of inhibition ranging from 12mm and 9mm against *B. subtilis* and *S. typhi* respectively. The aqueous extract shows comparatively less inhibition ranging from 9mm and 8mm in *B. subtilis*. Though both the extract were found effective, the highest zone of inhibition and the effectiveness is the major consideration in the case of antibacterial activity. When, the activity of crude extracts was compared with positive control, it was found to be significant and this confirms that the selected plant has antibacterial potential. There are several reports indicating the antimicrobial potential of various medicinal plants [17, 18]. However the antibacterial activity against pathogenic bacterial strains was reported by very few workers [19]. The result indicate that the leaves of *P. benghalensis* has a potential broad spectrum antibacterial activity and these extract either individually or in combination can be used as formulation to treat the infectious diseases caused by the test organism.

4. References