Study on the comparative antibacterial activity of *Polyalthia longifolia* (Debdaru) leaf extracts to some selective pathogenic bacterial strains

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**Abstract**

The increasing social and economic implications caused by pathogenic bacteria means there is a constant striving to develop near antibacterial agents. The present study was carried out *in vitro* to determine antibacterial activity of *Polyalthia longifolia* (Debdaru) leaf extracts with hexane, methanol and chloroform against six tested pathogenic bacteria viz. *Bacillus subtilis*, *Sarcina lutea*, *Xanthomonas compestris*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas* sp. Using agar disc diffusion method and MIC determination test. The zone of inhibition against the tested bacteria was found ranging from 21.00 to 44.20mm. The highest zone of inhibition produced by the hexane, methanol and chloroform extracts of *Polyalthia longifolia* at a concentration of 500μg/10μl against pathogenic bacteria i.e. *Sarcina lutea* were found 41.80mm, 44.20mm and 43.50mm respectively. The MIC values of all extracts against six tested bacteria were almost 15.625 μg/ 10μl. The plant leaf extracts can be used in the treatment of infectious diseases caused by them. Extensive investigation is needed to isolate the secondary metabolites from the extracts in order to test specific compounds for antimicrobial activity and the underlying mechanisms.

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Introduction

Antimicrobial activity of medicinal plant has become a global concern nowadays. This problem is of great issue especially in developing countries because infectious diseases are one of the major causes of mortality in these countries. There is a continuous and urgent need to discover new antimicrobial compounds for new infectious diseases. Therefore, researchers are increasingly turning their attention to traditional medicine and searching for new leads to develop better drugs against microbial infections (Parekh and Chanda, 2007). Although hundreds of plants species have been tested for antimicrobial properties, the vast majority have not yet been adequately examined (Balandrin et al., 1985).

Polyalthia longifolia is a member of the Annonaceae family and is large genus of shrubs and trees distributed in many tropical countries around the world. It is commonly known as False Ashoka, the Buddha tree, Indian Fir tree, Ashoka or Devadaru in sanskrit, Debdaru in Bengali, Deodar in Hindi, Asopalav (Gujarati), Glodogantiang (Indonesian),devdar in Marathi and Nettilinkam in Tamil(Singh and Karthikeyan, 2000; Kirtikar and Basum, 1995).Traditionally the plant has been used in Bangladesh and India for several medicinal purposes. Literature survey revealed that various parts of the plant possess different biological activities. The similar variety of the plant i.e. Polyalthia longifolia var. Pedula has been used in traditional system of medicinthe treatment of fever, skin diseases, mouth ulcers, hypertension, helminthiasis, gonorrhea, uterine ailments, leucorrhoea and menorrhagia (Wu et al., 1990; Rosakutty et al., 2000;Sing and Pandey, 1998).The plants containing clerodane diterpenoids and alkaloids have found to be active against a wide variety of microorganisms (Faiz et al., 2008).

The plant extract and isolated compounds were studied for various biological activities like antibacterial activity, antileishmanial activity, antiinflammatory activity, antineurotoxicity, hepatoprotective activity, antiulcer activity, hypotensive activity, cytotoxicity, antifungal activity (Marthanda et al., 2005; Misra et al., 2010; Tanna et al., 2009; Shih et al., 2009; Malairajan et al., 2008; Saleem et al., 2005; Stevigny et al., 2005; Nair and Chanda, 2006a, b).The methanol extract of Polyalthia longifolia exhibited nontoxic and antibacterial (Vijaya et al., 1995). It has been reported that the chloroform extract of Polyalthia longifolia leaf part was tested for antibacterial activity (Santos et al., 1995; Annapurna et al., 1983).

Further screening of this medicinal plant may result in the discovery of novel effective compounds. By considering the medicinal importance for microbial infections, the leaf of Polyalthia longifolia was screened against some pathogenic bacterial strains. The present study was an attempt to investigate the antibacterial activity of Polyalthia longifolia leaf extracts against gram positive bacteria Bacillus subtilis, Sarcina lutea, and gram negative bacteria Xanthomonas campestris, Escherichia coli, Klebsiella pneumonia and Pseudomonas sp. However, widespread exploration is desired to isolate the secondary metabolites from the extracts in order to test specific compounds for antimicrobial activity.

Materials and methods

Plant materials

Healthy, disease free, mature Polyalthia longifolia leaf was collected directly from local region of Kushtia and Jhenidah in Bangladesh. The leaves were cleaned with rinsed water. After cutting into small pieces, they were air dried in room temperature. After 7 days, dried leaves were pulverized into a fine powder by blender machine.

Solvent extraction

30gm powdered sample of Polyalthia longifolia leaf was sequentially extracted with solvents namely hexane, methanol and chloroform and also with water by Soxhlet apparatus for 36 hours (Singh et al., 2012).The crude extracts were then filtered through Whatman No.1 filter paper and all the crude extracts were concentrated under reduced pressure. All the extracts were stored in refrigerator until use.
Test microorganisms
Antibacterial activity of *Polyalthia longifolia* leaf extracts was investigated against four gram negative (*Xanthomonas campestris*, *Pseudomonas sp.*, *Escherichia coli* and *Klebsiella pneumonia*) and two gram positive (*Bacillus subtilis* and *Sarcinolautea*) registered bacterial isolates, which were obtained from the microbial type culture collection of Microbiology Laboratory of the Biotechnology and Genetic Engineering Department, Islamic University, Kushtia, Bangladesh.

Screening the extracts for antimicrobial activity
The disc diffusion method was used to determine the growth inhibition of bacteria by plant extracts (Nostro et al., 2000). In the disc diffusion method, the discs were placed aseptically over the bacterial culture on nutrients agar plates and incubated at 37°C for 24hrs. After inoculation for 24 hrs, the zones of inhibition around the discs were measured by millimetre scale. The experiment replicated three times to confirm the reproducible results. Sterile blank paper discs were impregnated with only sterile solvent (hexane, methanol and chloroform) used as negative control each time. In this study, six bacterial strains were inoculated into nutrient broth and shaken on a shaker machine about 24hrs at 37°C for incubation. After 24hrs, 25µl of inoculums from the prepared culture was transferred to plate count agar media containing petri dishes. The inoculums were spread on the surface of the media with a sterile spreader in laminar air flow. Paper discs embedded within a plant extract were placed on previously inoculated plates and were incubated at 35±0.1°C for 24hrs. After incubation the zones of growth inhibition around disks were measured in mm. Antibacterial activity studies were carried out for each test strains in triplicate and average measurement were calculated. Six microorganisms were tested in the study to determine the antibacterial effect of crude extracts (hexane, methanol and chloroform) of *Polyalthia longifolia* leaf. In antibacterial screening, plate count agar was used as a culture media. Standard amoxicillin, tetracycline and ciprofloxacin were used as positive control for comparison of the antibacterial activity.

Determination of minimum inhibitory concentration (MIC)
The minimum inhibitory concentration (MIC) values were determined according to methods described by Shahidi, 2004 and Kabir et al., 2005 by broth dilution assay. Hexane, methanol and chloroform extracts were serially diluted to concentrations ranging from 500µg/10µl to 15.625 µg/10µl in hexane, methanol and chloroform solvent against six tested pathogenic microorganisms. For preparing 500 µg / 10µl to 15.625 µg / 10µl, 1ml of the solvent was added to each of the six screw capped test tube. 1ml of the having 500 µg / 10µl extracts was added to the first test tube containing 1ml of respective solvent and mixed well and then 1ml of this solution was transferred to the second test tube containing 1ml of the same solvent. After mixing well, 1ml of this solution was transferred to the third test tube. This process of serial dilution was continued up to the last test tube. Finally, the concentration of the last test tube was 15.625 µg / 10µl.The plates were incubated at 37 °C for 24 hour. The lowest concentration of all extracts that produced no visible bacterial growth was recorded as the MIC.

Data analysis
All data were measured average value of three replicates and standard error (±). Results were subjected to ANOVA using Statistical Packages for Social Sciences (SPSS version 20.0) and the means separated using least significant differences (LSD) at 5% level of significance.

Results
In the present study, the antimicrobial activity of three extracts against two gram positive and four gram negative bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.
**Antibacterial activity**

All the extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains. The antimicrobial activities of *Polyalthia longifolia* leaf extracts are compared with standard antibiotics such as amoxicillin, ciprofloxacin and tetracycline, which were used as positive controls. Results of the antimicrobial activity obtained using the disc diffusion assay is summarized in Table 1, 2 and 3 and Figure 1. Diameter of the inhibition zone of different organic extract include 6 mm disc were tested at a concentrations of 500 µg/10µl.

### Table 1. Activity of Methanol extract of *Polyalthia longifolia* leaf against different bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Zone of inhibition (mm)</th>
<th>Control</th>
<th>Methanol extract con. of <em>P. longifolia</em> leaf (µg/10µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A-10</td>
<td>C-5</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td></td>
<td>28.90±0.42</td>
<td>32.95±0.07</td>
</tr>
<tr>
<td><em>S. lutea</em></td>
<td></td>
<td>30.10±0.28</td>
<td>32.85±0.07</td>
</tr>
<tr>
<td><em>X. campestris</em></td>
<td></td>
<td>16.85±0.07</td>
<td>28.15±0.07</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>14.00±0.14</td>
<td>27.40±0.14</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>21.05±0.07</td>
<td>32.00±0.00</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td></td>
<td>13.15±0.21</td>
<td>28.75±0.35</td>
</tr>
</tbody>
</table>

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc). (*) indicates significance value P<0.005

### Table 2. Activity of Chloroform extract of *Polyalthia longifolia* leaf against different bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Zone of inhibition (mm)</th>
<th>Control</th>
<th>Chloroform extract con. of <em>P. longifolia</em> leaf (µg/10µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A-10</td>
<td>C-5</td>
</tr>
<tr>
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<td></td>
<td>28.90±0.42</td>
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<tr>
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<td></td>
<td>30.10±0.28</td>
<td>32.85±0.07</td>
</tr>
<tr>
<td><em>X. campestris</em></td>
<td></td>
<td>16.85±0.07</td>
<td>28.15±0.07</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>14.00±0.14</td>
<td>27.40±0.14</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>21.05±0.07</td>
<td>32.00±0.00</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td></td>
<td>13.15±0.21</td>
<td>28.75±0.35</td>
</tr>
</tbody>
</table>

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc). (*) indicates significance value P<0.005

**Measurement of zone of inhibition**

According to concentration (500 µg/10µl) the zone of inhibition for methanol extract was *B. subtilis* (34.10±0.00), *S. Lutea* (44.20±0.14), *X. campestris* (31.30±0.14), *E. Coli* (36.00±0.00), *K. pneumoniae* (30.00±0.00), *Pseudomonas* sp.(30.00±0.00); for chloroform extract was *B. subtilis* (32.25±0.35), *S. Lutea* (34.85±0.21), *X. campestris* (31.30±0.14), *E. Coli* (36.00±0.00), *K. pneumoniae* (30.00±0.00), *Pseudomonas* sp.(32.25±0.35). The highest zone of inhibition to *Sarcina leutia* for methanol extract at concentration of 500 µg/10µl was 44.20±0.14 mm (p<0.005). The lowest zone of inhibition to *Pseudomonas* sp. for chloroform *Polyalthia*...
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gifoilia leaf extract at concentration of 500 μg/10μl was 27.40±0.56 mm (p<0.005).

Table 3. Activity of Hexane extract of Polyalthia longifolia leaf against different bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Zone of inhibition (mm)</th>
<th>Control A-10</th>
<th>C-5</th>
<th>T-30</th>
<th>Hexane extract con. of P. longifolia leaf (μg/10μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td></td>
<td>28.9±0.42</td>
<td>32.9±0.07</td>
<td>31.0±0.00</td>
<td>34.8±0.21</td>
</tr>
<tr>
<td>S. lutea</td>
<td></td>
<td>30.1±0.28</td>
<td>32.8±0.07</td>
<td>31.3±0.14</td>
<td>41.8±0.42</td>
</tr>
<tr>
<td>X. campestris</td>
<td></td>
<td>16.8±0.07</td>
<td>28.1±0.07</td>
<td>21.1±0.21</td>
<td>37.9±0.14</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td>14.0±0.14</td>
<td>27.4±0.14</td>
<td>20.1±0.00</td>
<td>32.3±0.35</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td></td>
<td>21.0±0.07</td>
<td>32.0±0.00</td>
<td>25.2±0.28</td>
<td>35.0±0.00</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td></td>
<td>13.1±0.21</td>
<td>28.7±0.35</td>
<td>20.2±0.42</td>
<td>31.2±0.35</td>
</tr>
</tbody>
</table>

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (10 µg/disc); (C-5): Ciprofloxacin (5 µg/disc); (T-30): Tetracycline (30 µg/disc)

Table 4. Minimum inhibitory concentration (MIC) Polyalthia longifolia leaf in different solvents.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Minimum inhibitory concentration (MIC)</th>
<th>Organic extracts of P. longifera leaf (μg/10μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Methanol</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td></td>
<td>15.625</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td></td>
<td>15.625</td>
</tr>
<tr>
<td>X. campestris</td>
<td></td>
<td>31.250</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td>15.625</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td></td>
<td>15.625</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td></td>
<td>15.625</td>
</tr>
</tbody>
</table>

MIC of different organic extracts (values in μg /10μl)

Minimum inhibitory concentration (MIC)
Minimum inhibitory concentration was observed with all bacterial strains against three different leaf extracts of Polyalthia longifolia (Table 4). Almost all organic extracts showed MIC value at a concentration of 62.5μg/10μl.

Discussion
Our present work was designed to perform the study on comparative antibacterial activity of different leaf extracts of P. Longifolia against some selective gram positive and gram negative pathogenic bacterial strains. All of the organic extracts of P. Longifolia exhibited greater extent of antibacterial activities.

The antibacterial activities of medicinal plants are attributed due to the presence of flavonoids, tannins and steroidal alkaloids (Fewell et al., 1993; Barnabas et al., 1988; Burapedjo et al., 1995). These reports and presence of flavonoids, tannins and steroidal alkaloids in different extract of Polyalthia longifolia confirm its potential against all selected pathogens.

The plant extracts were justified with standard antibiotics i.e. amoxicillin (10 µg/disc), ciprofloxacin (5 µg/disc), and tetracycline (30 µg/disc) and negative control (only solvent absorbing disc). The negative control showed no activity against all tested bacteria. The standard antibiotics showed significant antimicrobial activity against all tested gram positive and gram negative bacterial strains.
This study suggests that the leaf extract of *Polyalthia longifolia* have a broad spectrum of antibacterial activity, although the degree of susceptibility could differ between different organisms. The antibacterial activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually or in combination. Plant extracts obtained more activity than commercial antibiotics possibly because the plant active substances were soluble in organic solvents (Boer et al., 2005). Our results indicates the potential usefulness of *Polyalthia longifolia* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of bacterial diseases. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove costly in case of prolonged treatment. Therefore, there is needed to develop new antibacterial agents which can satisfy the present demand.

### Conclusion

The extracts of *Polyalthia longifolia* (Debdaru) leaf were found to be effective antibacterial agent. This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity with the required minimum inhibitory concentration (MIC). Further studies should undertake to identify the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

### Acknowledgements

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### References


