Antibacterial and antifungal properties of the methanol extract from the stem of *Argyreia argentea*
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**Abstract**

Antibacterial properties of methanol extract of *Argyreia argentea* stem was studied on Gram positive and Gram negative bacteria by disc diffusion method. The extract showed zone of inhibition against Gram positive bacteria (*Bacillus cereus, B. subtilis, B. megaterium* and *Staphylococcus aureus*) and Gram negative bacteria (*E. Coli, Salmonella typhi, S. paratyphi, Pseudomonous sp. (I), Pseudomonous sp. (II) and Shigella sonnei*). In addition, the extract was found effective against some fungi like *Aspergillus flavous, Fusarium equiseti, Altenaria alternate, Aspergillus niger, Colletotrichum corphori*.

**Introduction**

*Argyreia argentea* (family Convolvulaceae) is an evergreen shrub that is mainly found in different districts of Bangladesh (Uddin, 2006). It is widely used by the tribal communities of Chittagong Hill tracts (locally known to Chakma as *bitarak rupar tola ludi*) for the treatment of various diseases (boils, gastric, tumor, marasmus, paralysis and spermatorrhoea). However, a few scientific evaluations of this plant have been documented. This study interests to evaluate the antibacterial and antifungal activity of *A. argentea* methanol extract.

**Materials and Methods**

**Collection of plant**

The stems of *A. argentea* were collected from Chittagong Hill tracts, Bangladesh, in January 2009. The plant was taxonomically identified and authenticated by Bangladesh National Herbarium, Mirpur, Dhaka. The specimen is preserved in Bangladesh National Herbarium under the Plant Accession No. 34198.

**Preparation of crude extract**

The fresh stems of *A. argentea* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces, air dried at room temperature for about 20 days and ground into powder to store in an airtight container. 790 g powder was macerated in 8 L pure methanol (99% Anal-R) for 7 days at room temperature with occasional stirring. Methanol extract, 7 days later, was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50°C through rotatory vacuum evaporator. The concentrated extracts were collected in an eggplant flask and allow to air dry for complete evaporation of methanol. The whole process was repeated three times and finally, 15 g greenish colored, concentrated stem extract was obtained (1.9% w/w) which was kept in refrigerator to 4°C.

**Bacterial strains**

Gram positive (*Bacillus cereus, B. subtilis, B. megaterium* and *Staphylococcus aureus*) and Gram negative (*E. Coli, Salmonella typhi, S. paratyphi, Pseudomonous* sp. (I),
Pseudomonous sp. (II) and Shigella sonnei) bacterial species were used.

Fungal strains

Three human pathogens (Aspergillus fumigates, A. flavous and A. niger) and three plant pathogens (Fusarium equiseti, Altenaria alternate and Colletotrichum corphori) were used.

Preparation of stem extracts solution

A measured amount of 200 mg A. argentea stem extract was dissolved in 2 mL of methanol to give a solution of known concentration (100 pg/mL). Methanol was chosen as solvent because, in addition to the complete dissolution of the crude extracts, it has no inhibitory effect on the cultures.

Preparation of sample discs

The sample discs of about 4 mm in diameter were cut by punching machine from Whatman No. 1 filter paper. The discs were taken in a petri dish and sterilized by autoclave, dried in oven at 180°C.

Standard antibiotic disc

Kanamycin antibiotic disc (Oxoid, England,) with concentrations of 30 pg/disc was used as standard to compare with the sample.

Assay for antibacterial activity

Antibacterial activity of plant extract was determined by disc diffusion method (Bauer et al., 1966). All the test bacterial species were collected from the Research Laboratories of the Department of Microbiology, University of Chittagong. Dried filter paper discs (4 mm in diameter) impregnated with the culture medium. The blocks were placed at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. The inoculated plate was incubated at 25°C. The experiment was repeated for three times. Proper control (PDA) without extracts was also maintained. After five days of incubation the diameter of fungal colony was measured in mm.

The percentage of inhibition of mycelial growth of the test fungus was calculated by the following formula:-

\[ I = \frac{(C-T)}{C} \times 100 \]

Where, I = Percentage of inhibition; C = Diameter of the fungal colony in control; T = Diameter of the fungal colony in treatment.

Results

Table I showed that 1,000 pg/disc of extract exhibited 13, 14, 10 and 15 mm zone of inhibition against Gram positive bacteria Bacillus cereus, B. subtilis, B. megaterium and Staphylococcus aureus respectively, and 14, 13, 10, 14, 12 and 12 mm zone of inhibition against Gram negative bacteria namely E. coli, Salmonella typhi, Salmella paratyphi (Figure 1A), Pseudomonous sp. (I) (Figure 1B), Pseudomonous sp. (II) (Figure 1C) and Shigella sonnei (Figure 1D), respectively. On the other hand, standard antibiotic kanamycin (30 μg/disc) showed more significant antibacterial activity against all tested Gram positive and Gram negative bacteria showing the larger zone of inhibition in every case. This results indicate that A. argentea stem extract has promising antibacterial activity. In the assay of antifungal activity (Table II), A. argentea stem extract inhibited the mycelia growth of Aspergillus flavous, Fusarium equisetii (Figure 2B), Altenaria alternate (Figure 2A) and Colletotrichum corphori with the %inhibition of 44.4, 66.7, 44.4 and 75.6%, respectively, whereas no inhibition was observed against Aspergillus niger (Figure 2C) and Aspergillus fumigates.

Discussion

Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Some of these compounds are toxic to animals,
but others may not be toxic. Indeed, many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in human (Wallace, 2004) because plants are the natural reservoir of many antimicrobial, anti-cancer agents, analgesics, anti-diarrheal, antifungal as well as various therapeutic activities (Lucy and DaSilva, 1999). Acceptance of medicines from such plant origin as an alternative form of healthcare is increasing because they

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
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<tbody>
<tr>
<td>Kanamycin (30 pg/disc mm)</td>
<td>A. argentea extract (1,000 pg/disc mm)</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>28</td>
<td>13</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>32</td>
<td>14</td>
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<td>Bacillus megaterium</td>
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<td>10</td>
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<td>Staphylococcus aureus</td>
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<td>15</td>
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<tr>
<td>E. Coli</td>
<td>30</td>
<td>14</td>
</tr>
<tr>
<td>Salmonella typhae</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Salmonella para typhae</td>
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<td>10</td>
</tr>
<tr>
<td>Pseudomonous Sp. (I)</td>
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<td>14</td>
</tr>
<tr>
<td>Pseudomonous Sp. (II)</td>
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<td>12</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>28</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 1: Zone of inhibition showed by the A. argentea extract (A-C) against the bacterial strains (A) Salmonella para typhae; (B) Pseudomonous Sp. (I); (C) Pseudomonous Sp. (II) and (D) Shigella sonnei (inhibition zone in presence of kanamycin)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Human pathogen</th>
<th>Plant pathogen</th>
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</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>0</td>
<td>66.7</td>
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<tr>
<td>Aspergillus flavus</td>
<td>44.4</td>
<td>66.7</td>
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<tr>
<td>Aspergillus niger</td>
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<tr>
<td>Fusarium equiseti</td>
<td>66.7</td>
<td>72.2</td>
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<tr>
<td>Alternaria alternata</td>
<td>44.4</td>
<td>66.7</td>
</tr>
<tr>
<td>Colletotrichum corphori</td>
<td>75.6</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Table II: In vitro antifungal activities of A. argentea extract

Table I: Diameter of zone of inhibition against bacteria

Figure 2: Percentage of fungal growth inhibition showed by the A. argentea stem extract against the fungal strains (A) Alternaria alternata; (B) Fusarium equiseti; (C) Aspergillus niger. Left column is treated and right column is control.
are serving as promising sources of novel antibiotic prototypes (Rabe and Van Staden, 1997; Koduru et al., 2006). Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have variously been reported to have antimicrobial activity (Okeke et al., 2001; Ebi and Ofoefule, 1997).

In the current study, the results of testing the crude extracts for antimicrobial activities against 10 bacterial and 6 fungal species might be due to the presence of some sorts of bioactive or inhibitory compounds or factors in the extract or synergism by the existence of some compounds or factors in the extract of A. argentea.

**Conclusion**

This demonstrates that the methanol extract of A. argentea stem extract exhibits antibacterial and antifungal effect in experimental models which therefore offer a scientific basis for using this plant as a good source of traditional microbiological references.

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**Conflict of Interest**

Authors declare no conflict of interest

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**References**


