A. John De Britto*, P. Benjamin Jeya Rathna Kumar and D. Herin Sheeba Gracelin

INTRODUCTION
Pathovars of Xanthomonas are known to cause diseases on several vegetable and cash crops and are reported to have developed resistance to ampicillin, penicillin and streptomycin \[1\]. Aeromonas hydrophila is one of the causative agents for diarrhoeal infections in children and immunocompromised patients. These are ubiquitous water borne organisms and have gained importance as human pathogens causing gastrointestinal and extra intestinal infections \[2\]. To control the disease causing pathogens, number of synthetic pesticides and antibiotics are used by the farmers. But pesticides cause environmental pollution and many unwanted effects in human. Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of pathogenic microorganisms \[3\].

The medicinal plants are the important biological source whose parts (leaves, seeds, stem, roots, fruits, foliage etc.) extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals \[4\]. Plant extracts are highly efficient against microbial infections. It is estimated that around 70,000 plant species, from lichens to tall trees, have been used at one time or other for medicinal purposes \[5\]. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties \[6\]. The antimicrobial efficacy tribute to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, hence due the presence of maximum numbers of the phytochemicals and inhibitory effects, *A. precatorius* plant might be used as antibacterial agent to control the plant and animal pathogens.

Key words: *A. precatorius*, phytochemical analysis, antibacterial activity.

ABSTRACT
*A. precatorius* L. is a medicinal plant. The medicinal properties of the plants are determined by the presence of secondary metabolites (phytochemical constituents) in it. In the present study the phytochemical analysis and antibacterial activity in the methanol extract of five parts (leaves, stem, flowers, seed coat and seeds) of *A. precatorius* was carried out to analyses the presence of phytochemicals and inhibitory effect of the plant against plant (*Xanthomonas campestris*) and animal pathogenic bacteria (*Aeromonas hydrophila*). The result of the phytochemical analysis showed the presence of phenolic compounds, tannins, alkaloids, flavonoids and steroids in the selected plant. The methanol extract of seeds showed significant inhibition (p<0.005) in antibacterial studies. Hence due the presence of maximum numbers of the phytochemicals and inhibitory effects, *A. precatorius* plant might be used as antibacterial agent to control the plant and animal pathogens.

MATERIALS AND METHODS
Collection of plant materials
Fresh plant and plant parts were collected randomly from the region of Tirunelveli, India. Fresh plant material was washed; shade dried and then powdered using the blender and stored in air tight bottles.

Extraction of plant materials
10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C \[7\].

Phytochemical analysis
Phytochemical analysis of methanol extracts of different parts of *A. precatorius* was conducted following the standard procedure \[8\].

Bacterial strains
*Aeromonas hydrophila* (MTCC No. 646), *Xanthomonas campestris* (MTCC No. 2286) were procured from the Institute of Microbial Technology (IMTECH), India and were used to examine the antibacterial activity. The microorganisms were maintained at 4°C on nutrient agar slants.

Antibacterial assay
The antibacterial activity of methanol extracts of different parts of *A. precatorius* was tested by disc diffusion method \[9\]. Muller Hinton agar medium was seeded with 100µl of inoculum (1×10⁵ CFU/ml). The impregnated discs containing the test sample (100µg/ml) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Kanamycin 30µg/disc, Neomycin 10µg/disc) and blank discs (impregnated with solvent) were used as positive and negative control. The plates were then incubated at 37°C for 24 h to allow maximum growth of the microorganisms. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and mean of the three experiments was recorded.

Minimum Inhibitory Concentration (MIC)
The MIC of the aqueous and methanol extracts of different parts of the
selected plant was determined by serial dilution technique as described by [10]. 1 mg/ml of the sample solutions of all the extracts were prepared using Dimethyl Sulfoxide (DMSO). In this technique a large number of test tubes were used and each of the test tubes was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (inoculum contains 1×10⁶ cells/ml) followed by incubation at 37°C for 24 hours to allow the growth of the bacteria. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC). Another three test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth observed was only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth. Experiments were done in triplicate and repeated twice.

Statistical analysis
All data were expressed as mean ± SD. Statistical analyses were evaluated by one-way ANOVA followed by Tukey HSD test. Values with P < 0.005 were considered statistically significant.

RESULT AND DISCUSSION

Phytochemical analysis
The preliminary phytochemical analysis of the leaves, stem, flowers, seed coats and seeds of A. precatorius showed the presence of steroids, triterpenoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins (Table 1).

Table 1: Phytochemical analysis of methanol extracts of selected plant parts

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Leaves</th>
<th>Stem</th>
<th>Flowers</th>
<th>Seed coat</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sugars</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catechins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthroquinones</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Antibacterial activity assay
The ANOVA analysis of the data revealed that among the five parts of A. precatorius (p<0.005) the seeds showed highly significant activity against the tested pathogens (Table 2) when compared to the other four parts. Tukey HSD analysis of the data revealed that A. hydrophila was highly susceptible than X. campestris. Antibacterial activity of methanol extract of seeds of A. precatorius was highly significant when compared to Kanamycin and Neomycin.

Table 2: Antibacterial activity of different parts of selected plant (zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Samples</th>
<th>X. campestris</th>
<th>A. hydrophila</th>
<th>Neomycin</th>
<th>Kanamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>17.00±0.00</td>
<td>20.30±0.47</td>
<td>17.00±0.82</td>
<td>08.00±1.60</td>
</tr>
<tr>
<td>Stem</td>
<td>08.00±0.82</td>
<td>11.60±1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowers</td>
<td>10.30±0.47</td>
<td>12.45±0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed coats</td>
<td>09.23±0.45</td>
<td>13.25±0.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seeds</td>
<td>18.35±0.47</td>
<td>21.45±0.92</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data given are mean of three replicates ± standard error. P < 0.005

Minimum Inhibitory Concentration (MIC)
The MIC values of the methanol extracts of leaves of the selected plant were 32µg/ml and 64µg/ml against A. hydrophila and X. campestris respectively. Then the MIC value of stem was 124µg/ml against the two microorganisms. Similarly the MIC values of flowers and seed coats of A. precatorius were 64µg/ml. The MIC values of the seeds were 16µg/ml and 8µg/ml against X. campestris and A. hydrophila respectively. Hence it is concluded that the methanol extracts of seeds and leaves of A. precatorius showed inhibition of bacterial growth even at low concentrations (Table 3). Among these five parts, the MIC value of seeds of the selected plant is the lowest against the two selected pathogens. Hence the seeds show significant (p<0.005) bactericidal activity compared to other parts.

Anti-phyo pathogenic activity of crude and methanol extract of leaves, stem bark, seed and dry fruit of Terminalia thorellii, against four phyto pathogens was reported by [11]. In the present study the antibacterial activity of methanol extracts of different parts of A. precatorius was tested against two bacteria. The similar work was carried out by [12] in Datura metel. For that plant the leaves and flowers showed significant inhibitory effects. But in the present work, the seeds of the selected plant exhibited potential inhibition against the selected bacteria. [13]reported the biocontrol of Xanthomonas campestris using the methanol extracts of different parts of Azadiracta indica. Hence to achieve the successful results in bio control of pathogens and screen the potential medicinal properties of A. precatorius, the present work was carried out. The investigation provides successful positive results.

CONCLUSIONS
Hence this study suggests that A. precatorius plant (seeds and leaves) extract has significant antibacterial activity, which might be helpful in preventing or slowing the process of various plant diseases. The results of the present study also indicate that the plant parts possess many phytochemicals which could be responsible for the observed antibacterial activities of the plant.

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