Antibacterial activity of a few medicinal plants against Xanthomonas campestris and Aeromonas hydrophila

A. John De Britto*, D. Herin Sheeba Gracelin and Steena Roshan Sebastian

ABSTRACT

The methanol and aqueous extracts of leaves of six different medicinal plants, Acalypha indica, Aerva lanata, Phyllanthus amarus, Phyllanthus emblica, Cassia auriculata and Caesalpinia pulcherrima, were used for the investigation of antibacterial studies. In antibacterial screening performed by disc diffusion method against two types of bacteria namely Xanthomonas campestris (plant pathogen) and Aeromonas hydrophila (human pathogen), it was found that the methanol extracts of all the plant samples showed significant activity against the two tested bacteria. The methanol extracts of Acalypha indica, Aerva lanata and Phyllanthus amarus exhibited clear zone of inhibition against the tested microorganisms. Among these three samples, the MIC value of Aerva lanata, determined by serial dilution technique, was found to be 32µg/ml and 64µg/ml against Xanthomonas campestris and Aeromonas hydrophila respectively.

Key words: MIC, antibacterial activity, Xanthomonas campestris and Aeromonas hydrophila.

INTRODUCTION

Pathovars of Xanthomonas are known to cause diseases on several vegetable and cash crops (Mandavia et al., 1999). This seriously hinders the management of diseases of crops and agriculture products. 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 (Mahajan and Das, 2003). 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 extraintestinal infections (Agger et al., 1985; Ananthan and Alavandi, 1999; Vila et al., 2003). The six selected plants Acalypha indica L. (Euphorbiaceae), Aerva lanata (L) Juss.ex Schult (Amaranthaceae), Phyllanthus amarus L. (Euphorbiaceae), Phyllanthus emblica L. (Euphorbiaceae), Cassia auriculata L. (Caesalpiniaceae) and Caesalpinia pulcherrima (L)Sw (Caesalpiniaceae) have high medicinal properties and active compounds (Pranithanchai et al., 2009; Rao et al., 2005; Schiebinger, 2004). The antibacterial studies of the above medicinal plants were already investigated against some common human pathogenic bacteria (Anushia et al., 2009). The present study analyses the antibacterial activities of these plants against Xanthomonas campestris (plant pathogenic bacteria) and Aeromonas hydrophila (human pathogenic bacteria).

MATERIALS AND METHODS

Collection of plant materials

Fresh plant and plant parts were collected randomly from the region of Tirunelveli, India. The plants and the parts screened together with their families and common names are given in Table 1. Fresh plant material was washed, shade dried, then powdered using the blender and stored in air tight bottles.

Aqueous extraction

Ten gram of plant powder was added to 100 ml of distilled water and mixed well. After 24 hr the supernatant was collected and concentrated to make the crude extract. It was stored at 4°C (Harbone, 1973).

Methanol extraction

Ten gram of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 24 hr the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 4°C (Harbone, 1973).

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 assay was performed by agar disc diffusion method (Bauer et al., 1966). Muller Hinton agar medium was seeded with 100 µl of inoculum (1× 10^8 CFU/ml). The impregnated discs containing the test sample (100 µg/ml) were placed on the agar medium seeded with tested microorganisms. Standard antibiotic discs (Kanamycin 30 µg/disc, Neomycin 10µg/disc) and blank discs (impregnated with solvent and water) were used as positive and negative control. The plates were then incubated at 37 °C for 24 hr to allow maximum growth of the microorganisms (Bauer et al., 1966). 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 the mean of the three experiments was recorded.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude methanol extracts of A.indica, A.lanata and P.amarus against X.campestris and A.hydrophila were determined by using serial dilution technique (Reiner, 1982). 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 tube was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution were added. These test tubes were then inoculated with the selected organisms (inoculum contains 1×10^6 cells/ml) followed by incubation at 37 °C for 24 hr 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 MIC.

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.

RESULTS AND DISCUSSION

Antibacterial activity assay

Antibacterial activity of aqueous extracts of all the six plants are presented in Table 2. Highly significant antibacterial activity was observed in P.amarus followed by A.lanata and A.indica, respectively against two tested pathogens. Among the two pathogens A.hydrophila was highly susceptible.

The ANOVA analysis of the data revealed that among the six plants, P.amarus (p < 0.005) showed highly significant activity against the tested pathogens (Table 2). Tukey HSD analysis of the data revealed that X.campestris was highly susceptible.

Antibacterial activity of methanol and aqueous extract of P.amarus and A.lanata was highly significant when compared to Kanamycin and Neomycin.

Hence it is concluded that the extracts of A.indica, A.lanata and P.amarus showed inhibition of bacterial growth even at low concentrations. Among these three plants, the MIC value of A.lanata is the lowest against both X.campestris and A.hydrophila. A.lanata shows significant (p<0.005) bactericidal activity compared to other plants. According to the results of antibacterial assay, the methanol extracts of P.amarus and A.lanata might be used as antibacterial agents against X.campestris and A.hydrophila which affect plants and animals respectively.

### Table 1. Medicinal plant species selected for antibacterial activity

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Family</th>
<th>Common name</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acalypha indica L.</td>
<td>Euphorbiacae</td>
<td>Indian acalypha</td>
<td>Leaves</td>
</tr>
<tr>
<td>Aerva lanata (L) Juss. Schult</td>
<td>Amaranthaceae</td>
<td>Mountain knot grass</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Phyllanthus amarus L.</td>
<td>Euphorbiacae</td>
<td>Stone breaker</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Phyllanthus emblica L.</td>
<td>Euphorbiacae</td>
<td>Indian gooseberry</td>
<td>Leaves</td>
</tr>
<tr>
<td>Cassia auriculata L.</td>
<td>Caesalpiniacae</td>
<td>Tanner’s cassia</td>
<td>Leaves</td>
</tr>
<tr>
<td>Caesalpinia pulcherrima (L) Sw.</td>
<td>Caesalpiniacae</td>
<td>Peacock flower</td>
<td>Leaves</td>
</tr>
</tbody>
</table>

The MIC of A.indica was 128 µg/ml against X. campestris and A. hydrophila. Then the MIC values of A.lanata were 32 µg/ml and 64 µg/ml against the above two microorganisms. Similarly the MIC values of P. amarus were 64µg/ml and 128 µg/ml against X.campestris and A. hydrophila respectively. Hence it is concluded that the extracts of A.indica, A.lanata and P. amarus showed inhibition of bacterial growth even at low concentrations. Among these three plants, the MIC value of A.lanata is the lowest against both X.campestris and A. hydrophila. A. lanata shows significant (p<0.005) bactericidal activity compared to other plants. According to the results of antibacterial assay, the methanol extracts of P. amarus and A. lanata might be used as antibacterial agents against X.campestris and A.hydrophila which affect plants and animals respectively.
The bactericidal action of some selected herbal extracts viz., Acalypha indica, Achyranthes aspera, Aloe vera, Azadirachta indica, Datura metel, Hibiscus rosasinensis, Nerium oleander, Ocimum sanctum, Ocimum basilicum, Phyllanthus emblica, Polyalthia longifolia, Piper betle, Punica granatum, Solanum torvum and Solanum trilobatum were tested in vitro against the worth of citrus canker disease causing pathogen, X. axonopodis (Manonmani et al., 2009). Egwaikhide et al. (2008) screened hexane, ethyl acetate and methanolic extracts of dried leaves powder of Eucalyptus globules for basic secondary metabolites and antibacterial activity against Staphylococcus aureus, Staphylococcus faecallis, Bacillus stearothermophilus, Staphylococcus epidermidis, Bacillus cereus, Bacillus polymyxa, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus anthracis, Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens. Ghosh et al. (2008) evaluated the antibacterial potentiality of hot aqueous and methanol solvent extracts of mature leaves of Polyalthia longifolia against six reference bacteria. Highest antibacterial activity was observed against K. pneumoniae in both the extracts followed by E. coli in hot aqueous extract and B. subtilis in methanol extract as evident from MIC values. Shirsat (2008) reported the anti – phytopathogenic activity of crude and methanol extract of leaves, stem bark, seed and dry fruit of Terminalia thorelii, against four phyto pathogens. An important characteristic of plant extracts and their components is their hydrophobicity, which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death (Rastogi and Mehrotra, 2002). The results of the present investigation is successful in identifying the antibacterial activity of selected medicinal plants which will help in further identifying the nature of the bioactive principle and its solubility, isolation and characterization of the active principle responsible for the activity.

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REFERENCES


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