# 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 micro organisms. 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 gasterointestinal 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 $^{o}$ 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 $^{o}$ 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 $^{o}$ C on nutrient agar slants.

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

| Plant species | Family | Common name | Part used |
|---|---|---|---|
| *Acalypha indica* L. | Euphorbiaceae | Indian acalypha | Leaves |
| *Aerva lanata* (L) Juss. Schult | Amaranthaceae | Mountain knot grass | Whole plant |
| *Phyllanthus amarus* L. | Euphorbiaceae | Stone breaker | Whole plant |
| *Phyllanthus emblica* L. | Euphorbiaceae | Indian gooseberry | Leaves |
| *Cassia auriculata* L. | Caesalpiniaceae | Tanner's cassia | Leaves |
| *Caesalpinia pulcherrima* (L) Sw. | Caesalpiniaceae | Peacock flower | Leaves |

**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 ($1\times 10^8$ 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 $1\times10^6$ 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. Three more test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth was observed only in test tubes (solution content was cloudy) containing medium and inoculum and the other two were clear showing no growth (Reiner, 1982). 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.

**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.

**Minimum Inhibitory Concentration (MIC)**

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.

**Table 2.** Antibacterial activity of leaves extracts of some medicinal plants

| Plant samples | Extracts (100µg/ml) | *Xanthomonas campestris* (inhibition zone in mm) | *Aeromonas hydrophila* (inhibition zone in mm) |
|---|---|---|---|
| *Acalypha indica* L. | Aqueous | 9.00±1.00 | 0.00±0.00 |
| | Methanol | 11.00±1.00 | 11.00±0.82 |
| *Aerva lanata* (L) Juss. Schult | Aqueous | 10.33±0.57 | 11.33±1.15 |
| | Methanol | 11.66±1.52 | 14.66±0.57 |
| *Phyllanthus amarus* L. | Aqueous | 14.00±1.00 | 17.00±1.00 |
| | Methanol | 19.33±0.57 | 17.33±0.57 |
| *Phyllanthus emblica* L. | Aqueous | 5.33±0.57 | 0.00±0.00 |
| | Methanol | 5.00±1.00 | 8.00±0.82 |
| *Cassia auriculata* L. | Aqueous | 0.00±0.00 | 0.00±0.00 |
| | Methanol | 7.33±0.47 | 6.33±0.47 |
| *Caesalpinia pulcherrima* (L) Sw. | Aqueous | 0.00±0.00 | 0.00±0.00 |
| | Methanol | 7.60±0.47 | 8.66±0.47 |
| Kanamycin(30µg/ml) | Antibiotic | 15.00±0.85 | 12.66±0.47 |
| Neomycin (10µg/ml) | Antibiotic | 16.33±0.47 | 15.66±0.47 |
| Control aqueous | Blank | 0.00±0.00 | 0.00±0.00 |
| Control methanol | Blank | 0.00±0.00 | 0.00±0.00 |

Data given are mean of three replicates ± standard error, $p < 0.005$

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

Plant Molecular Biology Research Unit, Post Graduate and Research Department of Plant Biology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India, Phone: 0091- 462- 4264374, Fax: 0091- 462- 2561765, *Corresponding author E-mail: bjohnde@yahoo.co.in

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