PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF A FEW MEDICINAL PLANTS AGAINST *XANTHOMONAS CAMPESTRIS*

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Summary

The methanol extracts of leaves of eight different medicinal plants, *Leucas aspera, Calotropis gigentia, Ocimum sanctum, Adathoda vasica, Hyptis suaveolens, Teprosia purpurea, Cleome gynandra* and *Cleome viscosa* belonging to different families were used for the investigation of antibacterial studies. In antibacterial screening performed by disc diffusion method against a phytopathogenic bacteria namely *Xanthomonas campestris,* it was found that the methanol extracts of all the plant samples showed significant activity against the tested bacteria. The methanol extracts of *O.sanctum, A.vasica* and *H.suaveolens* exhibited clear zone of inhibition against the tested micro organism. Among these three samples, the MIC value of *A.vasica*, determined by serial dilution technique, was found to be 32µg/ml against *X.campestris*. Phytochemical analysis of methanol extract of all the plant samples revealed that antibacterial activity is due to the presence of steroids, flavonoids and phenolic compounds. The results suggest that *A.vasica* and *H.suaveolens* are potential plants for the management of phyto pathogenic bacteria, *X.campestris* which is known to cause harmful diseases on many plants and economic important crops.

Key words: MIC, antibacterial activity, *Xanthomonas campestris,* phytochemical screening.

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Introduction

Xanthomonas is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. The hosts of this genus include atleast 124 monocotyledonous and 268 dicotyledonous plants, among which the rice bacterial blight, cabbage black rot disease, and

citrus blight disease are the most serious diseases, which cause a big economic impact on agricultural production every year. Chemical control has been proved efficient and economical in controlling blight disease. However, increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (14, 6). Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (10). 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 (9).

Many of the reported tropical plants came under scrutiny, leading to extraction and characterization of their active constituents, which accounted for various uses by man. The most important of these constituents are alkaloids, terpenoids, steroids, phenols, saponins and tannins (1). (4) observed that there is strong need to investigate the chemical composition of many plants to determine their ability to be used as fungicides or insecticides. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities observed by many researchers (2, 15). Plants are considered as rich sources of bioactive chemicals and there may be an alternative source of insect control agents (16). Pathogen control strategies, especially those that are effective, cheap and environmentally non-hazardous are needed. Hence based on the above objectives, this study was focused to analyze the antibacterial activity and phytochemical constituents of the selected medicinal plants against the phytopathogenic bacteria *Xanthomonas campestris*.

Materials and Methods

Collection of plant materials: Fresh plant/ 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 and then powdered using the blender and stored in air tight bottles.

Table 1: Medicinal plant species selected for antibacterial activity

Plant species	Family	Common names	Parts used
Leucas aspera (Willd) spreug.	Lamiaceae	Common Leucas	Leaves
Calotropis gigantia (L)W.T.Aiton	Asclepiadaceae	King's crown	Leaves
Ocimum sanctum L.	Lamiaceae	Holy basil	Leaves
Adathoda vasica Nees.	Acanthaceae	Malabar nut	Leaves
Hyptis suaveolens (L.) Piot	Lamiaceae	American mint	Leaves
Teprosia purpurea (L.) pers.	Fabaceae	Wild indigo	Leaves
Cleome gynandra L.	Capparaceae	Wild spider flower	Leaves
Cleome viscosa L.	Capparaceae	Asian spider flower	Leaves

Methanol extraction: 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^{o}C (8).

Phytochemical analysis: Phytochemical analysis of methanol extracts of the selected plants was conducted following the procedure of (5).

Bacterial strains: *Xanthomonas campestris* (MTCC No. 2286) was procured from the Institute of Microbial Technology (IMTECH), India and was used to examine the antibacterial activity. The microorganisms were maintained at 4^{o}C on nutrient agar slants.

Antibacterial assay: The antibacterial activity assay was performed by agar disc diffusion method (3). Muller Hinton agar medium was seeded with 100µl of inoculum (1× 10^{8} 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 and water) were used as positive and negative control. The plates were then incubated at 37^{o}C for 24 h to allow maximum growth of the microorganisms (3). 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.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of the crude methanol extracts of *O. sanctum, A. vasica* and *H. suaveolens,* against *X.campestris* was determined by using serial dilution technique (12). 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 1×10^{6} cells/ml) followed by incubation at 37^{o}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 (12). 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

Phytochemical analysis

The preliminary phytochemical analysis of the methanol extracts of the selected plants showed the presence of steroids, triterpinoids, reducing sugars, sugars, alkaloids, phenolic compounds, flavonoids and tannins (Table 2).

Table 2: Preliminary phytochemical tests for methanol extracts of selected medicinal plants

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Steroids	+	+	+	+	+	+	+	+
Triterpinoids	–	+	+	+	+	+	–	–
Reducing sugars	+	–	+	+	+	+	–	–
Sugars	+	–	–	–	–	–	–	–
Alkaloids	+	+	+	+	+	+	+	+
Phenolic compounds	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	–	+
Catechins	–	+	–	–	–	–	–	–
Saponins	–	–	+	+	+	–	–	–
Tannins	–	+	+	+	+	+	–	–
Anthroquinones	–	–	+	+	+	–	–	–
Amino acids	–	–	+	+	+	–	–	–

1. *Leucas aspera,* 2. *Calotropis gigentia,* 3. *Ocimum sanctum,* 4. *Adathoda vasica,* 5. *Hyptis suaveolens,* 6. *Teprosia purpurea,* 7. *Cleome gynandra* 8. *Cleome viscosa*

Antibacterial activity assay

The ANOVA analysis of the data revealed that among the eight plants *A.vasica* ($p<0.005$) showed highly significant activity against the tested pathogens (Table 3). Tukey HSD analysis of the data revealed that *X.campestris* was highly susceptible. Antibacterial activity of methanol extract of *O.sanctum* and *H.suaveolens* was highly significant when compared to Kanamycin and Neomycin.

Table 3: Antibacterial activity of leaves extracts of some medicinal plants

Plant samples	Extracts (100µg/ml)	*Xanthomonas campestris* (inhibition zone in mm)
L. aspera	Methanol	14.00±0.85
C. gigantia	Methanol	10.00±0.47
O. sanctum	Methanol	16.00±0.47
A. vasica	Methanol	24.33±0.47
H. suaveolens	Methanol	20.30±0.85
T. purpurea	Methanol	14.66±0.57
C. gynandra	Methanol	10.33±0.57
C. viscosa	Methanol	7.60±0.47
Kanamycin(30µg/ml)	Antibiotic	15.00±0.85
Neomycin (10µg/ml)	Antibiotic	16.33±0.47
Control aqueous	Blank	0.00±0.00
Control methanol	Blank	0.00±0.00

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

Minimum Inhibitory Concentration (MIC)

The MIC of *O.sanctum* was 128µg/ml against *X. campestris.* Then the MIC values of *A.vasica* were 32µg/ml against the tested microorganism. Similarly the MIC values of *H.suaveolens* were 64µg/ml against *X.campestris.* Hence it is concluded that the extracts of *O. sanctum, A. vasica* and *H. suaveolens* showed inhibition of bacterial growth even at low concentrations (Table 4). Among these three plants, the MIC value of *A.vasica* is the lowest against *X.campestris*. Hence *A.vasica* shows significant ($p<0.005$) bactericidal activity compared to other plants. According to the results of antibacterial assay, the methanol extracts of *A. vasica* and *H. suaveolens* might be used as antibacterial agents against *X.campestris* which affects plants.

(7) 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. **(**13) 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 enable 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 (11).

Table 4: MIC Values of three plant extracts (µg/ml) against the tested bacteria

Plant samples	***Xanthomonas campestris***
O. sanctum	128.00±0.00µg/ml
A. vasica	32.00±0.00µg/ml
H. suaveolens	64.00±0.00µg/ml

Results are mean from three sets of experiments, each set in triplicate ± SD, $p < 0.005$.

Conclusions

The results of the present investigation is successful in identifying the phytochemical compounds and 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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