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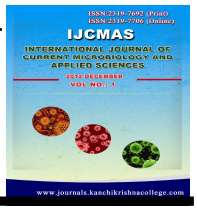


**Original Research Article**

# *In-vitro* antibacterial activity of certain plant extracts against plant disease causing bacteria isolated from citrus plant

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**Keywords**

*Xanthomonas campestris*; Antimicrobial activity; Medicinal plants; Citrus canker.

**A B S T R A C T**

A total of 30 samples were collected Kancheepuram district, Tamilnadu that includes citrus fruit, leaves and stem for screening the plant pathogenic bacteria with reference to *Xanthomonas campestris*. The bacterial isolates from the samples for the present study were confirmed as *X.campestris*. Among the 30 plant samples 20 isolates showed Positive for *X.campestris*. The bacterial isolates from the citrus plant samples for the present study were confirmed by using conventional tests. The leaves of four plant species, *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum*, *Plumbago zeylanica* were collected and subjected to different solvent extractions that include ethanol, acetone, benzene, petroleum ether and alcohol extraction at different concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm. Five plant pathogenic bacteria of *X. campestris* pv. *citri* (Xcc 539, 540, 541, 542 and 543) and one *X. campestris* bacterial isolate from citrus plants by the agar disc diffusion method. *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum*, *Plumbago zeylanica* at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm showed significant activity against five plant pathogenic bacteria and one bacterial isolate. Bacteriomycin has been used as standard antibiotic reference. Zone of inhibition for all the solvent extracts in different concentrations for each of the test bacteria were recorded. The efficacy of growth inhibition of the plant extracts at each concentration showed that at 5000 ppm, the extract of *Aegle marmelos* and *Ocimum canum* showed largest inhibition zone in the acetone and alcoholic plant extracts. There is a scope to use plant extracts of the leaves of *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum*, *Plumbago zeylanica* plant extracts against plant pathogenic bacteria.

## Introduction

Citrus canker is one of the most feared of citrus diseases, affecting all types of important citrus crops. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic conditions. The disease is endemic in India, Japan and other South- East Asian countries, from where it has spread to all other citrus producing continents except Europe. Generally canker does not occur in arid citrus growing areas and has been eradicated from some areas. However, widespread occurrence of the disease in many areas is a continuous threat to citriculture especially in canker free areas. Intensive research on citrus canker is being carried out throughout the world which has been reviewed by Rossetti (1977), Civerolo (1981, 1984), Chand and Pal (1982), Schoulties *et al* (1987), Stall and Civerolo (1991) and Goto (1992). However, all these reviews are either brief, restricted to one country, or by now out of date. This work aims to present an overview of

citrus canker worldwide with special reference to India.

Citrus bacterial canker (CBC) is an economically important disease in many tropical and subtropical countries. Several pathotypes have been described within the genus *Xanthomonas* primarily distinguished by their geographical origin and host range in addition to certain genotypic characteristics (Brunings and Gabriel, 2003).

Owing to technology evolution, chemical substance is a necessary part in agricultural production processes, because they are used to inhibit plant pathogens which are important enemies of agricultural products. However, chemical substances not only afford damage to environment, but they provide direct effect to human as well. Hence, mitigation of chemical substances using is the best path to solve these problems. The medicinal plant extracts is one of the interesting ways to substitute chemical substances for inhibition of plant pathogens which does not render wicked problems to environment and living being in the world.

Traditional medicines play an important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. The subcontinent is rich in medicinal plants and is one of the richest countries in the world as regards genetic resources of medicinal plants. Moreover the agro climatic conditions are conducive for introducing and domesticating new exotic plant varieties (Krishnaraju *et al*., 2005). Several plants have been used in Plant diseases (Premanathan *et al.*, 2000). The rational design of novel drugs from traditional medicine offers new prospects in modern plant disease management.

Bacteria cause severe damage to stored food commodities, plants and fruits. Among different species of bacteria, *Xanthomonas* sp. and fungi *Aspergillus* sp. *Fusarium* sp. *and Penicillium* sp. are associated with heavy loss of grains, fruits, vegetables and other plant products during picking, transit and storage rendering them unfit for human consumption by producing mycotoxins and affecting their nutritive value (Miller, 1995; Galvano *et al*., 2001). Many plant pathogens, which cause severe damage to plants especially citrus, were generally managed by synthetic chemicals, which were considered both efficient and effective. The continuous use of these synthetic bactericides and fungicides started unraveling nonbiodegradability and known to have residual toxicity to cause pollution (Pimentel and Levitan, 1986). Pesticide pollution of soil and water bodies is well documented (Nostro *et al*., 2000). Hence in recent time application of plant metabolites for plant disease management has become important viable component of Integrated Pest Management, as plant metabolites are eco-friendly.

Plants have been formed the basis of natural pesticides, that make excellent leads for new pesticide development (Newman *et al*., 2000). The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management Traditionally plants have been well exploited by man for the treatment of human diseases, Ayurveda is a good example, but not much information is available on the exploitation of plant wealth for the management of plant diseases, especially against phytopathogenic bacteria and fungi. Hence the present study is carried out to isolate, identify the plant pathogen from citrus canker disease and subjected to antimicrobial activity by using medicinal plants.

## Materials and Methods

### Sample collection

Citrus leaves, stem and fruit samples were collected from different areas. Samples were taken from infected citrus plant leaf, stem and fruit which show early stage of the disease symptom to avoid some saprophytic microorganisms that grow in tissues killed by the primary pathogen (Takahashi, 1984). The vascular citrus canker bacteria like *Xanthomonas campestris* ooze out from the diseased tissue. Therefore, ooze/exudate samples were taken in the field using toothpick and then suspended in sterilized distilled water in half filled screw-capped vials according to Takahashi (1984). Each sample was labeled and all information including date, location, and part of plant name were recorded.

The sample for analysis of bacterial angular leaf spot on plants with symptoms can be generally leaves with young water soaked spots when possible, or dry spots and with or without exudates. In the case of suspected systemic infections it is necessary to analyse tissue from the crown of the plants.

**Sample preparation**

The samples are prepared using a general procedure valid for isolation and identification of *Xanthomonas* sp. Select plants with young symptoms (water soaked spots in the lower part of the leaves). Externally disinfect the parts to analyse with 70% ethanol aseptically excise 0.1 g from each leaf containing water soaked spots. If the plants show vascular symptoms cut the roots and the leaves keeping the mortar and pestle, carefully rinse the cut plants under tap water and then disinfect them by immersion for 1 min in 70% ethanol and rinse each plant separately three times in sterile distilled water. Take 0.1 g per sample from the leaves or mortar and pestle and add 9 ml of PBS.

Bacteria were isolated from aerial part (fruits, leaves, and stem) of citrus exhibiting characteristic symptoms of bacterial diseases from different locations. Isolation was performed by cutting surface-disinfested plant materials into small pieces from the margins of spots with a sterile razor blade. The cut plant tissue was inserted into and soaked in a test tube containing 2 ml sterile water. Loop full of the resulting aqueous suspension was streaked onto standard medium. Plates were incubated at 27°C for 2-5 days.

**Isolation and Preservation**

After incubation, a loopful of the suspension was streaked on YPSA plates (Yeast extract, 5g; Peptone, 10g; Sucrose, 20g; Agar, 12-15g in 1liter distilled water with pH 7.4 and autoclaved at 121o C for 15 minutes). The plates were incubated at 28°C for 48-72 hours according to Schaad (1988). Bacterial colonies from each plate were further sub cultured and pure bacterial colonies were transferred to YPSA slants incubated at 28°C for 48-72 hours and preserved at 4°C for further work.

**Morphological and Biochemical Tests**

**Gram staining reaction**

The Gram-reaction of each isolate was determined following the staining procedure in Schaad (1988). First, thinly spread bacterial smear was prepared on a clean slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for one minute and washed in tap water for few seconds. It was again flooded with iodine solution for one minute and washed and blot-dried. It was then decolorized with 95% ethyl alcohol by applying drop by drop until no more color flows from the smear and washed and blot dried. Finally slides were counter stained for about 10 seconds with safranin, washed and examined under microscope using oil immersion objective. Isolates that appeared pink, Gram negative bacteria were subjected for further tests.

**Growth on Asparagine medium**

All Gram-negative isolates were allowed to grow on Asparagine medium (Asparagine, 0.5g; KH2PO2, 0.1g; MgSO4.7H2O, 0.2g; KNO3, 0.5g; CaCl2, 0.1g; NaCl, 0.1g and agar, 12-15g (for plates) in 1 liter distilled water with pH 7 and autoclaved at 121°C for 15 minutes) at 28°C for 48-72 hours with out any other carbon and nitrogen sources (Dye and Bradbury, 1984). This is used as a diagnostic test for *Xanthomonas* because they are not able to grow on it while others like yellow Enterobacteriaceae and many Pseudomonads can grow on it. The growth of the bacteria on Asparagine agar plates and broth was recorded and those isolates that were unable to grow on the medium were taken for further tests. In all cases, uninoculated medium was taken as negative control. Isolates that grew on Asparagine but which formerly showed yellowish mucoid growth and found grown alone on YPSA medium initially were included in further tests.

**Growth on Nutrient agar with 5% Glucose**

Each isolate was streaked on nutrient agar with 5% glucose (Nutrient agar, 23g; 5% Glucose in 1 liter distilled water with PH 7 and autoclaved at 121°C for 15 minutes) and incubated at 28°C for 48-72 hours. Mucoid and yellow colony growth on this medium is one of the characteristics that differentiate *Xanthomonas campestris* from other *Xanthomonas* species (Dye and Bradbury, 1984). Therefore the growth and colony color of each isolate was recorded.

**Presence of Xanthomonadin pigment**

Each isolate was streaked on nutrient agar and incubated at 28°C for 48 hours. About 2-3 loop full colonies of each bacterial isolate were transferred to 3 ml of ethanol in test tubes and were placed in boiling water bath until the pigment was removed. The suspension was then centrifuged at 13,000 rpm for 15 minutes to remove cell debris. The supernatant was decanted and the methanol was allowed to evaporate by keeping the ethanol extract in 50-60°C water bath until the optical density of the pigment extract reaches 0.4 at 443 nm. 5µl of

each extract was spotted on a precoated, silica gel plates and a total 25µl was spotted. The silica gel plates were developed in ethanol solvent. The solvent was allowed to move approximately 10 cm. and the yellow spots were marked with a pencil while wet. A yellow spot with an average Rf value of 0.45 was taken as positive for the presence of the pigment (Schaad, 1988).

**Salt Tolerance**

Isolates were inoculated to nutrient broth with 0%, 1%, 2%, 3%, 4% and 5% NaCl concentration to evaluate their salt tolerance. Inoculated salt free (0%) nutrient broth was used as positive control and uninoculated broth of each salt concentration was used as negative control and the presence or absence of growth was recorded.

**Antibacterial activity against plant pathogenic bacteria**

**Selection of plants**

Based on the literature of plants were selected on the presence of different groups of phytochemical compounds. They are,

| Plants selected | Major class of phytochemical(s) present |
|---|---|
| *Aegle marmelos* | Terpenoid |
| *Aristolochia indica* | Polyphenol |
| *Ocimum canum* | Terpenoid |
| *Plumbago zeylanica* | Quninone |

**Collection of selected plants**

The leaves of the plants above listed were collected largely in polythene bags and immediately transported to the laboratory for processing.

**Preparation of crude extract**
**Solvent extraction**
The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the flowers of individual plants were powdered using a mixer grinder. A known quantity of leaves powder (50 g) of each plant was taken in five 250 ml conical flask and added with 100 ml of ethanol (85%), acetone(85%), petroleum ether(85%), benzene(85%) and alcohol (85%) separately (Karuppusamy *et al.,* 2001, Prakash *et al.,* 2006a, 2006b).

Solvent was used for the extraction of phytochemicals because it has the ability to dissolve the phytochemical compounds like tannins, polyphenols, flavonols, terpenoids and alkaloids (Silva *et al*., 1997). The solvents-leaves powder mixtures were kept at room temperature for 48 hours and rapidly stirred using glass rod every 8 hours.

After 48 hours, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaves powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (Crude extract) obtained for each plant was transferred to screw cap bottles and stored under refrigerated (4°C) condition till use.

**Preparation of stock solution**

By using digital electronic balance, 200 mg of each ethanol, acetone, petroleum ether, benzene and alcohol crude extract was carefully taken in a standard measuring flask and 5ml of ethanol was added to dissolve the ethanolic crude extract, 5ml of acetone was added to dissolve the acetone crude extract, 5ml of petroleum ether was added to dissolve the petroleum ether crude extract, 5ml of benzene was added to dissolve the benzene crude extract and 5 ml of alcohol was added to dissolve the alcohol crude extracts separately. One or two drops of emulsifier (Triton-X100) were added to completely dissolve all the crude extract. Then it was made up to 200 ml by adding distilled water. This forms the stock solution of 1000 ppm (i.e., 1mg/ml). For 2000 ppm, 400 mg of crude extract dissolve and made up to 200 ml of distilled water (i.e., 2mg/ml). For 3000 ppm, 600 mg of crude extract dissolve and made up to 200 ml of distilled water (i.e., 3mg/ml). For 4000 ppm, 800 mg of crude extract dissolve and made up to 200 ml of distilled water (i.e., 4mg/ml). For 5000 ppm, 1g of crude extract dissolves and made up to 200 ml of distilled water (i.e., 5mg/ml).

For the antimicrobial assay using individual plant extract, the stock solution of 1000, 2000, 3000, 4000 and 5000 ppm was directly used.

**Antimicrobial assay**
**Microorganisms used**

Five selected plant pathogenic strains namely *Xanthomonas campestris* pv. citri. 539, 540, 541, 542 and 543 maintained in the Laboratory of Department of Microbiology, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kanchipuram which were originally obtained from

the Microbial Type Culture Collection (MTTC) of Institute of Microbial Technology (IMTECH), Chandigarh were used for the present study.

**Kirby Bauer Disc Diffusion Technique**

This technique was used to test the sensitivity of selected test organisms to the ethanolic extracts individually and in combination as described above (Bauer *et al*., 1966).

**Preparation of antimicrobial disc using crude extracts**

Discs of 5 mm in diameter from a sheet of filter paper were punched out, placed in petridishes allowing a distance of 2-4 mm between each disc and sterilized in a hot air oven at 160ºC for 1 hour. After allowing the disc to cool, 20 µl (0.02 ml) of each test solution was added on to each disc and then the discs were dried at 37ºC in an incubator for one hour (Cheesbrough, 1984). For control set, the discs were added with distilled water (200 ml) containing 5ml of appropriate + 2 drops of emulsifier at 20µl/disc. Preparation of plates:

The Petri plates of 100mm diameter with Trypticase Soy Agar (TSA) were swabbed with broth culture of each test bacteria in separate plates by using sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Four discs of each extract were placed in squares. Control sets with a standard antibiotic Bacteriomycin (1000, 2000, 3000, 4000 and 5000 ppm) were simultaneously maintained. Also the discs without plant extract (discs prepared using 200 ml distilled water + 5 ml appropriate + one or two drops of emulsifier) were also maintained as another set of control for each test organism. The plates were then incubated at 37ºC for 24hours and the zone of inhibition (IZ) was measured in diameter (cm) around the discs and recorded. The assays were performed with three replicates.

## Results

**Morphology and biochemical characteristics of bacterial isolate**

A total of 30 samples of citrus plant parts include leaf, stem and fruit were collected for the study from different places of Kancheepuram town, Tamilnadu. There were 30 samples collected in this present study for the identification of citrus canker causing *Xanthomonas campestris* bacteria.

The phenotypic characteristics of bacterial isolate from citrus plants indicated in Table 2. Preliminary identification of the isolates was performed by conventional tests.

Additional biochemical tests were performed as previously described to confirm speciation, including production of acid in the presence of Lactose, mannitol, fructose, glucose, mannose and sucrose (Table.1).

Among the 30 samples 20 isolates showed Positive for *Xanthomonas campestris* (isolate.1). The highest isolates were obtained in citrus fruits and leaves samples. Results on each sample showed positive for three samples and their distribution of isolates were tabulated in Table-2.

Among the 20 isolates bacterial isolate.1 were subjected to antimicrobial sensitivity test. Different solvents extracts of the plant leaves includes *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum* and *Plumbago zeylanica* showed antimicrobial activity against bacterial isolate.1 and plant pathogenic bacterial strain (Table.3-6).

The results revealed that the ethanolic extract of *Aegle marmelos* at all concentrations inhibited *X.campestris strains* and *Aristolochia indica*, *Ocimum canum* and *Plumbago zeylanica* at 1000, 2000, 3000, 4000, and 5000 ppm inhibited the plant pathogenic bacteria. The ethanolic extracts of *Aegle marmelos* and *Ocimum canum* at 4000 and 5000 ppm showed an inhibition effect against *X. campestris* strains and low inhibition zone showed at 1000 and 2000 ppm (Table.3-6).

Antibacterial reference standard, Bacteriomycin had equal effect on *X. campestris* bacterial isolate and *X. campestris* strains tested (Table.7).

**Antibacterial test by agar disc diffusion method**

In the present study, the extracts of *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum*, *Plumbago zeylanica* at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm were tested against Five plant pathogenic bacteria of *X. campestris* pv. *citri* (*Xcc* 539, 540, 541, 542, 543 and 544) and one *X. campestris* bacterial isolate from citrus plants by the agar disc diffusion method.

Data on the effects of plant extracts on *Xcc 539* are given in Figure 1 and Table 1. In *Xcc* 539, the results revealed that the leaf extracts of four plants in different solvents and all concentrations were most

effective and could inhibit *Xcc* 539. The leaf extracts of *Aegle marmelos* and *Ocimum canum* also showed an inhibition effect against *Xcc* 539 at 4000 and 5000 ppm but low inhibition zone showed at 1000 and 2000 ppm. The efficacy of crude extracts varied according to the concentration levels.

The efficacy of growth inhibition of the plant extracts at each concentration showed that at 5000 ppm, the *Aegle marmelos* and *Ocimum canum* ethanolic extracts showed the largest inhibition zone (2.8 cm); At 5,000 ppm, the *Ocimum canum* ethanolic extract showed the largest inhibition zone (2.6 cm).

The growth inhibition of these plant extracts against *Xcc* 539 compared to bacteriomycin (positive control) at 5,000 ppm revealed that four plant extracts were not significantly different from bacteriomycin. Plant extracts, namely *Aegle marmelos*, *Ocimum canum and Plumbago zeylanica* at 5000 ppm, *Ocimum canum* at 4000 ppm and *Aegle marmelos* at 3000 ppm showed higher inhibition effect than bacteriomycin.

Data on the effects of plant extracts on *Xcc* 543 are given in Table 5. In *Xcc* 543, the results revealed that the ethanolic extract of *Aegle marmelos* at all concentrations inhibited *Xcc 543* and *Aristolochia indica*, *Ocimum canum* and *Plumbago zeylanica* at 1000, 2000, 3000, 4000, and 5000 ppm inhibited this plant pathogenic bacteria.

Ethanolic and alcoholic extracts of *Aegle marmelos* and *Ocimum canum* at 5000, showed a highest inhibition effect against this plant pathogenic bacteria. *Aegle marmelos* and *Ocimum canum* ethanolic extracts inhibited *Xcc* 543 at 1000 ppm but low inhibition at other solvents. The efficacy of crude extracts varied according to the concentration levels and different solvents.

The efficacy of growth inhibition of the plant extracts at each concentration showed that at 5000 ppm, the *Aegle marmelos* and *Ocimum canum* ethanolic extracts showed the largest inhibition zone (2.9 cm); At 5000 ppm, the *Aristolochia indica* alcohol extract showed the highest inhibition zone (2.6 cm); At 3,000 ppm, the ethanolic and actone extracts of *Plumbago zeylanica* showed the highest inhibition zone (2.6 cm) and At 1,000 ppm, the ethanolic and petroleum ether extracts *Aegle marmelis* and *Ocimum canum* showed a highest inhibition zone of 1.9 cm but the other extracts such as and *Aristolochia indica and Plumbago zeylanica* moderate inhibition of the growth of Xcc 543.

The growth inhibition of these plant extracts against *Xcc* 543 compared to bacteriomycin (positive control) at 5000 ppm showed that eight plant extracts, (petroleum ether and benzene extracts of *Aegle marmelos* and *Ocimum canum* at 3000ppm; ethanolic and alcoholic extracts of *Aegle marmelos* and *Ocimum canum* at 4000ppm; ethanolic and alcoholic extracts of *Aegle marmelos* and *Ocimum canum* at 5000ppm), were equal amount of inhibition occurred when compared to bacteriomycin.

Data on the effects of plant extracts on *Xcc* 544 are given in Figure 5 and Table 5. In *Xcc* 543, the results revealed that the ethanolic extract of *Aegle marmelos* at all concentrations inhibited *Xcc 544* and *Aristolochia indica*, *Ocimum canum* and *Plumbago zeylanica* at 1000, 2000, 3000, 4000, and 5000 ppm inhibited this plant pathogenic bacteria.

Extracts of *Aegle marmelos* and *Ocimum canum* at 5000 ppm showed a highest inhibition effect against this isolate. Acetone and alcoholic extracts of *Ocimum canum* inhibited *X. campestris* bacterial isolate at 5000 ppm but low at 1000, 2000 and 3000 ppm. The efficacy of crude extracts varied according to the concentration levels and different solvents.

The efficacy of growth inhibition of the plant extracts at each concentration showed that at 5000 ppm, the ethanolic extracts of *Aegle marmelos* and *Ocimum canum* showed the largest inhibition zone (2.9 cm); At 4000 ppm, the *Aegle marmelos* and *Ocimum canum* showed the highest inhibition zone (2.7 cm); At 3000 ppm, the petroleum ether, acetone and alcohol *Ocimum canum* extract showed the highest inhibition zone (2.5 cm) and At 1000 ppm, the ethanolic extracts of *Ocimum canum* showed an inhibition zone of 2.2 cm but the other extracts such as *Aegle marmelos indica* and *Plumbago zeylanica* showed low inhibition on *Xcc* bacterial isolate.

**Table 1.** Antibacterial activity of four plant extracts in different solvents against *X. campestris* pv. *citri* 540 by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 1.9a | 2.2 | 2.3 | 2.5 | 2.8 |
| | Acetone | 1.6d | 1.9 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.9a | 2.1 | 2.2 | 2.4 | 2.6 |
| | Benzene | 1.7b | 2.0 | 2.2 | 2.4 | 2.7 |
| | Alcohol | 1.8 | 2.2 | 2.3 | 2.5 | 2.6 |
| *Aristolochia indica* | Ethanol | 1.7b | 1.9 | 2.0 | 2.2 | 2.3 |
| | Acetone | 1.6d | 1.8 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.5 | 1.9 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.4 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.3 |
| *Ocimum canum* | Ethanol | 1.8 | 2.0 | 2.2 | 2.3 | 2.6 |
| | Acetone | 1.6 | 1.9 | 2.0 | 2.1 | 2.5 |
| | Petroleum ether | 1.7b | 2.0 | 2.1 | 2.2 | 2.6 |
| | Benzene | 1.8 | 1.9 | 2.2 | 2.2 | 2.4 |
| | Alcohol | 1.4 | 1.8 | 2.0 | 2.1 | 2.2 |
| *Plumbago zeylanica* | Ethanol | 1.5 | 1.8 | 1.9 | 2.1 | 2.2 |
| | Acetone | 1.4 | 1.8 | 1.9 | 2.0 | 2.2 |
| | Petroleum ether | 1.5 | 1.7 | 2.0 | 2.2 | 2.4 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.0 | 2.1 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.2 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

**Table 2.** Antibacterial activity of four plant extracts in different solvents against X. campestris pv. *citri* 540 by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 2.2 | 2.4 | 2.5 | 2.7 | 2.9 |
| | Acetone | 2.0 | 2.1 | 2.5 | 2.6 | 2.8 |
| | Petroleum ether | 2.1 | 2.2 | 2.4 | 2.5 | 2.7 |
| | Benzene | 1.9 | 2.3 | 2.4 | 2.5 | 2.7 |
| | Alcohol | 2.0 | 2.4 | 2.5 | 2.6 | 2.8 |
| *Aristolochia indica* | Ethanol | 1.5 | 1.7 | 1.9 | 2.2 | 2.5 |
| | Acetone | 1.4 | 1.8 | 1.9 | 2.0 | 2.3 |
| | Petroleum ether | 1.5 | 1.7 | 2.0 | 2.1 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.8 | 2.0 | 2.1 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.5 |
| *Ocimum canum* | Ethanol | 1.9 | 2.2 | 2.3 | 2.4 | 2.8 |
| | Acetone | 1.7 | 1.8 | 1.9 | 2.1 | 2.4 |
| | Petroleum ether | 1.9 | 2.1 | 2.2 | 2.4 | 2.6 |
| | Benzene | 1.8 | 2.1 | 2.2 | 2.5 | 2.8 |
| | Alcohol | 1.8 | 2.2 | 2.3 | 2.4 | 2.7 |
| *Plumbago zeylanica* | Ethanol | 1.6 | 1.9 | 2.2 | 2.4 | 2.6 |
| | Acetone | 1.5 | 1.8 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.5 | 1.7 | 2.0 | 2.2 | 2.5 |
| | Benzene | 1.6 | 1.7 | 2.2 | 2.4 | 2.6 |
| | Alcohol | 1.6 | 1.9 | 2.2 | 2.3 | 2.5 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

**Table 3.** Antibacterial activity of four plant extracts in different solvents against *X. campestris* pv. *citri* 541 by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 2.0 | 2.2 | 2.4 | 2.7 | 2.9 |
| | Acetone | 1.9 | 2.0 | 2.2 | 2.6 | 2.8 |
| | Petroleum ether | 1.9 | 2.2 | 2.4 | 2.7 | 2.9 |
| | Benzene | 2.0 | 2.2 | 2.5 | 2.6 | 2.8 |
| | Alcohol | 2.0 | 2.2 | 2.4 | 2.7 | 2.9 |
| *Aristolochia indica* | Ethanol | 1.7 | 1.9 | 2.2 | 2.4 | 2.5 |
| | Acetone | 1.5 | 1.8 | 2.1 | 2.3 | 2.4 |
| | Petroleum ether | 1.5 | 1.7 | 2.0 | 2.2 | 2.5 |
| | Benzene | 1.6 | 1.7 | 2.2 | 2.4 | 2.6 |
| | Alcohol | 1.6 | 1.9 | 2.2 | 2.3 | 2.5 |
| *Ocimum canum* | Ethanol | 2.2 | 2.4 | 2.5 | 2.6 | 2.9 |
| | Acetone | 2.0 | 2.1 | 2.5 | 2.6 | 2.8 |
| | Petroleum ether | 2.1 | 2.2 | 2.3 | 2.4 | 2.9 |
| | Benzene | 1.9 | 2.2 | 2.4 | 2.6 | 2.8 |
| | Alcohol | 2.0 | 2.3 | 2.4 | 2.5 | 2.9 |
| *Plumbago zeylanica* | Ethanol | 1.9 | 2.2 | 2.3 | 2.4 | 2.6 |
| | Acetone | 1.8 | 2.0 | 2.1 | 2.2 | 2.4 |
| | Petroleum ether | 1.9 | 2.1 | 2.2 | 2.4 | 2.5 |
| | Benzene | 1.8 | 2.2 | 2.3 | 2.5 | 2.6 |
| | Alcohol | 1.8 | 2.2 | 2.4 | 2.5 | 2.6 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

**Table 4.** Antibacterial activity of four plant extracts in different solvents against *X. campestris* pv. *citri* 542 by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 2.1 | 2.2 | 2.1 | 2.6 | 2.9 |
| | Acetone | 2.0 | 2.2 | 2.2 | 2.4 | 2.6 |
| | Petroleum ether | 2.0 | 2.2 | 2.4 | 2.5 | 2.8 |
| | Benzene | 2.0 | 2.1 | 2.2 | 2.5 | 2.6 |
| | Alcohol | 2.0 | 2.1 | 2.4 | 2.5 | 2.6 |
| *Aristolochia indica* | Ethanol | 1.8 | 1.9 | 2.1 | 2.2 | 2.4 |
| | Acetone | 1.8 | 1.9 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.5 | 1.9 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.3 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.2 |
| *Ocimum canum* | Ethanol | 1.9 | 2.2 | 2.4 | 2.6 | 2.8 |
| | Acetone | 1.6 | 1.9 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.9 | 2.1 | 2.2 | 2.4 | 2.5 |
| | Benzene | 1.7 | 2.0 | 2.1 | 2.2 | 2.6 |
| | Alcohol | 1.8 | 2.2 | 2.2 | 2.4 | 2.6 |
| *Plumbago zeylanica* | Ethanol | 1.7 | 1.9 | 2.0 | 2.2 | 2.4 |
| | Acetone | 1.6 | 1.8 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.5 | 1.9 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.2 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.4 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

**Table 5.** Antibacterial activity of four plant extracts in different solvents against *X. campestris* pv. *citri* 543 by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 1.9 | 2.2 | 2.3 | 2.6 | 2.8 |
| | Acetone | 1.6 | 1.9 | 2.2 | 2.3 | 2.5 |
| | Petroleum ether | 1.9 | 2.1 | 2.4 | 2.5 | 2.6 |
| | Benzene | 1.7 | 2.0 | 2.2 | 2.3 | 2.6 |
| | Alcohol | 1.8 | 2.2 | 2.3 | 2.5 | 2.8 |
| *Aristolochia indica* | Ethanol | 1.7 | 1.9 | 2.0 | 2.4 | 2.5 |
| | Acetone | 1.6 | 1.7 | 1.9 | 2.2 | 2.4 |
| | Petroleum ether | 1.5 | 1.8 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.4 |
| | Alcohol | 1.4 | 1.8 | 2.2 | 2.4 | 2.6 |
| *Ocimum canum* | Ethanol | 1.9 | 2.2 | 2.4 | 2.5 | 2.8 |
| | Acetone | 1.6 | 1.9 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.9 | 2.1 | 2.2 | 2.4 | 2.6 |
| | Benzene | 1.7 | 2.0 | 2.1 | 2.4 | 2.6 |
| | Alcohol | 1.8 | 2.2 | 2.4 | 2.6 | 2.8 |
| *Plumbago zeylanica* | Ethanol | 1.7 | 1.9 | 2.0 | 2.2 | 2.6 |
| | Acetone | 1.6 | 1.8 | 2.0 | 2.1 | 2.6 |
| | Petroleum ether | 1.5 | 1.9 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.5 |
| | Alcohol | 1.4 | 1.8 | 1.9 | 2.1 | 2.4 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

**Table 6.** Antibacterial activity of four plant extracts in different solvents against bacterial isolate *X. campestris* by agar disc diffusion method.

| PLANT EXTRACTS USED | SOLVENTS USED | MEAN INHIBITION ZONE DIAMETER (CM)[1] | | | | |
|---|---|---|---|---|---|---|
| | | CONCENTRATION (PPM) | | | | |
| | | 1000 | 2000 | 3000 | 4000 | 5000 |
| *Aegle marmelos* | Ethanol | 2.0 | 2.2 | 2.3 | 2.7 | 2.9 |
| | Acetone | 1.8 | 1.9 | 2.2 | 2.4 | 2.5 |
| | Petroleum ether | 2.0 | 2.1 | 2.4 | 2.5 | 2.6 |
| | Benzene | 1.9 | 2.0 | 2.2 | 2.4 | 2.6 |
| | Alcohol | 2.1 | 2.2 | 2.4 | 2.5 | 2.9 |
| *Aristolochia indica* | Ethanol | 1.9 | 2.2 | 2.3 | 2.4 | 2.6 |
| | Acetone | 1.8 | 2.0 | 2.1 | 2.2 | 2.4 |
| | Petroleum ether | 1.9 | 2.1 | 2.2 | 2.4 | 2.5 |
| | Benzene | 1.8 | 2.2 | 2.3 | 2.5 | 2.6 |
| | Alcohol | 1.9 | 2.2 | 2.4 | 2.5 | 2.6 |
| *Ocimum canum* | Ethanol | 2.2 | 2.4 | 2.5 | 2.7 | 2.9 |
| | Acetone | 2.0 | 2.1 | 2.5 | 2.6 | 2.8 |
| | Petroleum ether | 2.1 | 2.2 | 2.4 | 2.5 | 2.7 |
| | Benzene | 2.0 | 2.3 | 2.4 | 2.5 | 2.7 |
| | Alcohol | 2.1 | 2.4 | 2.5 | 2.6 | 2.9 |
| *Plumbago zeylanica* | Ethanol | 1.8 | 1.9 | 2.1 | 2.2 | 2.4 |
| | Acetone | 1.7 | 1.9 | 2.0 | 2.1 | 2.4 |
| | Petroleum ether | 1.6 | 1.9 | 2.0 | 2.2 | 2.3 |
| | Benzene | 1.6 | 1.7 | 1.9 | 2.2 | 2.3 |
| | Alcohol | 1.5 | 1.8 | 1.9 | 2.1 | 2.2 |

(Values are mean of three replicates)
[1]Means with the same letter superscript is not significantly different at 5% level by't' test

The growth inhibition of these plant extracts against *Xcc* bacterial isolate compared to bacteriomycin (positive control) at 5000 ppm showed that eighteen plant extracts, (peterolum ether and alcoholic extracts of *Aegle marmelos* and *Ocimum canum* at 1000 ppm; ethanolic and alcoholic extracts of *Aristolochia indica* at 2000 ppm; peterolum ether, benzene and alcoholic extracts of *Aegle marmelos, Aristolochia indica* and *Ocimum canum* at 3000 ppm and ethanolic extracts of *Aegle marmelos* and *Ocimum canum* at 4000 ppm; ethanolic and alcoholic extracts of *Aegle marmelos* and *Ocimum canum* at 5000 ppm), were equal amount of inhibition occurred when compared to standard.

All isolates were found tolerant to NaCl 1-5%. The reaction of most of the isolates to indole production, nitrate reduction, gelatin liquefaction and catalase tests are found similar with Eshetu (1981) and Gizachew (2000) studies and are also similar with general characteristic of *Xanthomonas campestris* described in Dye and Bradbury (1984). With regard to starch hydrolysis, differences in reaction among isolates were observed among isolates in this work, where all the isolates used in his work did not hydrolyze starch. Dye and Bradbury (1984) described the characteristics of *Xanthomonas campestris*, like starch neither hydrolysis with neither positive nor negative. This indicates the possibility of finding pathovars and strains as positive or negative for that specific test within the species. This ensures the presence of variation among isolates of given population.

The results of this study are encouraging since the 85% of solvents include ethanol, acetone, petroleum ether, benzene and alcohol extracts of four plants in different concentrations like 1000, 2000, 3000, 4000 and 5000 ppm all showed antibacterial activity against five isolates of *X. campestris pv. citri* strains and *X. campestris bacterial isolate* from Citrus plants by the agar disc diffusion method. The diameters of inhibition zones increased with the increase of concentration levels of plant extracts. Similar antibacterial properties of these plants have been reported in many studies, but most studied the antibacterial activity of the extracts against human pathogens. The various crude extracts of *Aegle marmelos, Aristolochia indica*, *Ocimum canum, Plumbago zeylanica* at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm showed significant activity against five plant pathogenic bacteria and one bacterial isolate as well as positive Bacteria (Perumal *et al.,* 1999; Okunade, 2002, Karuppusamy *et al.,* 2001; Prakash *et al.,* 2006a, 2006b).

In the present study, the extracts of *Aegle marmelos*, *Aristolochia indica*, *Ocimum canum*, *Plumbago zeylanica* at concentrations of 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm were tested against Five plant pathogenic bacteria of *X. campestris* pv. *citri* (*Xcc 5*39, 540, 541, 542 and 543) and one *X. campestris* bacterial isolate from citrus plants by the agar disc diffusion method.

**Table 7.** Antibacterial activity reference standard against *X. campestris* strains and isolate by agar disc diffusion method.

| BACTERIA | ZONE OF INHIBITION IN CM | | | | |
|---|---|---|---|---|---|
| | 1000 | 2000 | 3000 | 4000 | 5000 |
| *X. campestris 539* | 2.0 | 2.2 | 2.4 | 2.6 | 2.8 |
| *X. campestris 540* | 2.1 | 2.2 | 2.3 | 2.5 | 2.9 |
| *X. campestris 541* | 2.2 | 2.2 | 2.4 | 2.5 | 2.8 |
| *X. campestris 542* | 2.0 | 2.3 | 2.5 | 2.6 | 2.9 |
| *X. campestris 543* | 2.0 | 2.3 | 2.4 | 2.6 | 2.8 |
| *X. campestris* (Bacterial isolate from citrus plants) | 2.1 | 2.2 | 2.4 | 2.7 | 2.9 |

The present study is an important step in developing plant based pesticides which are ecofriendly for the management of the plant pathogenic bacteria and development of commercial formulation of botanicals. It is important observation that all the biomolecules are polar in nature with their solubility more to ethanol, petroleum ether, acetone, benzene and alcholol.

In conclusion, Citrus canker continues to be the cause of worldwide concern as a potentially hazardous threat to citriculture. There is a wide range of physiological, biochemical, serological, molecular and pathogenic variation among strains of bacteria associated with citrus canker. Moreover new strains are originating regularly as a result of mutation. In the present study inhibition of *x. campestris* bacterial isolate and *X.campestris* strains by some of the solvent plant extracts in different concentrations similar to the standard antibiotic (Bacteriomycin) shows that plant extracts can serve as a good alternative to chemical bactericides considering their safety, degradable qualities and cost effectiveness.

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