

## Screening Antibacterial Properties of Plant Extracts against *Xanthomonas axonopodis pv. citri*: An *In Vitro* Studies

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### ABSTRACT

Citrus canker is one of the most devastating disease caused by bacterium *Xanthomonas axonopodis pv. citri*. This work was formulated to control this disease by plant extracts. It is reported that may plants possess antimicrobial properties by nature. Hence two weed plants i.e *Parthenium hysterophorus* and *Euphorbia hirta* was selected for the work. The aqueous leaf extracts of both the weeds were prepared in six different concentrations and tested for production of zone of inhibition against *Xac* by disc diffusion method on nutrient agar. The results showed that the zone of inhibition increases with increase in concentration of both the leaf extracts. Among the two weed leaf extracts the maximum mean inhibition (15.13) was shown by *P. hysterophorus* at 64µl/ml and there was no inhibition produced by low concentration of *Euphorbia hirta*. Hence from this work it showed that leaf extracts of parthenium showed more inhibition to citrus canker pathogen. It may be concluded that this weed can be used to control the disease and thereby control the weed population.

**Keywords--** Antibacterial, Leaf Extracts, Inhibition Zone, Citrus Canker and Weeds.

### I. INTRODUCTION

Citrus canker is a disease affecting citrus crops caused by the bacterium *Xanthomonas axonopodis*. This disease is not harmful to humans but canker significantly affects the vitality of citrus trees. Citrus canker is characterized by erumpent lesions on fruit, foliage and young stem of susceptible cultivars of citrus (Tim R. Gottwald *et al.*, 2002). The lesions ooze bacterial cells that, when dispersed by windblown rain, can spread to other plants. Hence it is very important to manage this disease.

Plants produce numerous secondary plant metabolites that are insignificant for growth and developmental process act against microbial pathogens on the basis of their toxic nature (Schafer *et al.*, 2009). To reduce the use of unnatural chemicals in food, several alternative methods such as use of plant extracts which produce a wide variety of secondary metabolites in response. It has been reported that plant extracts extracted with various solvents and essential oils are found to be rich in antioxidants and bioactive compounds. (Debjani Choudhry *et al.*, 2018) these are many antimicrobial

compounds in weed, which are effective against harmful microorganisms in plants and humans. *Parthenium hysterophorus* is one of the most common weeds that are found in both agricultural and waste lands, have antimicrobial property and serve as an important biocontrol agent against plant pathogens.

*Euphorbia hirta* is a hairy herb that grows in open grassland, roadsides and pathways. It is used in traditional herbal medicine. It is found to possess galactogenic, antimicrobial, antioxidant, antifeedant, larvicidal, repellent and antifeedant activities against *Plutella xylostella*. Hence the present work was done to test the antibacterial properties of *Parthenium hysterophorus* and *Euphorbia hirta* against *Xanthomonas axonopodis pv. citri* that causes citrus canker by disc diffusion method of different concentration of aqueous extracts of the two weeds.

### II. MATERIALS AND METHODS

#### Isolation of Phytopathogen

The citrus canker affected fruits were collected from local markets. The area of showing symptoms of canker were cut out and surface sterilized with 70% ethanol. The suspensions were prepared by grinding it in pestle and mortar. The suspensions were serially diluted up to 10<sup>-6</sup> times and the bacteria was isolated on Nutrient agar medium (Hi media).

Following the procedures of pour plate method for bacterial isolation, 10µl of the sample were plated on nutrient agar in sterile petri plates. Isolation was done in triplicates the plate were allowed to solidify and incubated for a period of 24 hrs at 27±1°C. The bacteria isolated were subjected to microscopic examinations, for morphology and biochemical test like gram staining, VP test, citrate utilization test, MR test, indole production test were conducted. (R.R. Jadhav *et al.*, 2018)

#### Collection of Plant Samples and Preparation of Extracts

Leaves of *Parthenium hysterophorus* and *Euphorbia hirta* were collected from agricultural lands. All the collected leaves were washed and shade dried and made into fine powder. 30g of each plant material was macerated in 50ml of distilled water in a pestle and mortar. It was then filtered through a muslin cloth and then centrifuged at 6000 rpm for 10 minutes. The finally obtained supernatant was stored separately in bottles at

4°C for the further use. The from the final extracts, concentration of 2, 4, 8, 16, 32 and 64 µl/ml was prepared for both *Parthenium hysterophorus* and *Euphorbia hirta*.

#### Determination of Antibacterial Assay

Antibacterial activity of the two weeds leaf extracts was done on nutrient agar by disc diffusion method. The *Xanthomonas axonopodis* pv. citri were streaked and spread on solidified nutrient agar and the incubated overnight. The disc were soaked in different concentration of both the leaf extracts. The procedures of disc diffusion method were followed. The disc soaked with extracts were placed on the incubated plates. Three replication of each treatment were repeated. The plates were incubated at 37°C for 24 hrs and the antibacterial activity was evaluated by quantifying inhibition zones of bacterial growth if any around the discs. One plate serves as a control and the results were interpreted.

### III. EXPERIMENTAL RESULTS

#### Confirmation of the Isolated Pathogen

The bacterial colonies were visible after 24 hours of incubation. The visual observation was done to identify the colony morphology. The colony were observed to be yellow in colour. The colonies were medium to small and circular in shape with even margins the isolated *Xac* were found to be positive for citrate utilization and negative for gram staining, VP test, MR test and indole production test.

#### Effect of *Parthenium Hysterophorus* Leaf Extracts Against *Xanthomonas axonopodis* pv. citri

After incubation of 24 hrs, the results were interpreted. The zone of inhibition was measured in mm and interpreted in table 1. It is seen that leaf extracts of *Parthenium* showed inhibitory effect against citrus canker pathogen. It is seen that the zone of inhibition increases with increase in extracts concentration of *parthenium*. The maximum mean of 15.13 mm was recorded in concentration of 64µl/ml.

**Table 1: Effect of different conc. of *P. hysterophorus* leaf extracts against *Xac*.**

Conc of <i>P.hysterophorus</i> extracts (µl/ml)	Zone of inhibition (mm)			Mean
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
2	6.30	6.00	6.90	6.40
4	6.90	7.30	7.80	7.33
8	8.00	8.60	9.20	8.60
16	11.20	11.00	11.60	11.26
32	13.20	12.70	13.00	12.96
64	15.60	15.00	14.70	15.13
Control	-	-	-	-

#### Effect of *Euphorbia Hirta* Leaf Extracts against *Xanthomonas axonopodis* pv. Citri

All the incubated plates were observed for zone of inhibition. The diameter was measured in mm and recorded in table 2. The results revealed that there was no

inhibition produced by low concentration (2µl/ml) concentration of *Euphorbia hirta* leaf extracts. The mean inhibition was more in concentration of 64 µl/ml (11.76 mm). The zone of inhibition produced by *E. hirta* leaf extracts were less when compared to *P. hysterophorus*.

Table.2. Effect of different conc. of *E. hirta* leaf extracts against *Xac*.

Conc of <i>E. hirta</i> extracts (µl/ml)	Zone of inhibition (mm)			Mean
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	
2	-	-	-	-
4	-	6.00	-	2.00
8	7.10	-	-	2.36
16	9.20	8.90	9.00	9.03
32	10.30	10.00	10.90	10.40
64	11.70	12.00	11.60	11.76
Control	-	-	-	-

#### IV. DISCUSSION

It is said that natural based compounds in plants have enormous therapeutical potential as they can be used as antimicrobial agent without any side effects that are often associated with synthetic antimicrobials (Muhammad Ashfaq *et al.*, 2013). The leaf extracts of *P.hysterophorous* presented a better inhibitory effect on the test organisms (Madan *et al.*, 2011) reported the antimicrobial activity of *P. hysterophorous* against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Sharma and Bhutani (1988) also confirmed *P. hysterophorous* as well known plant used for various medicinal prurpose and providing active extract against pathogens. The knowledge of chemical constituents of plants is important for synthesis of complex chemical substance. The results were also similar to findings of Deepak Sharma *et al.*, 2009, reported that aqueous extracts of *Helianthus annus* and *Parthenium hysterophorus* were found to be almost inactive against all organisms tested. Hence from this finding it can be said that leaf extracts of Parthenium weed can be used as a foliar spray to control *Xanthomonas axonopodis pv.citri*: citrus canker pathogen.

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