

**EVALUATION OF ANTI MICROBIAL ACTIVITY OF PLANT EXTRACTS AGAINST BACTERIAL BLIGHT CAUSING XANTHOMONAS AXONOPODIS IN POMEGRANATE**

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**ABSTRACT**

Bacterial blight of pomegranate caused by *Xanthomonas axonopodis* is a major biotic constraint in India. Many studies were carried out to find alternative methods using safe and natural plant extracts to the existing chemical control methods. In the present study 25 plant extracts with 3 solvents namely Hexane, Chloroform, and Methanol were evaluated for their antibacterial activity against *Xanthomonas axonopodis* by Well Diffusion method and MIC (Minimum Inhibitory Concentration) which was calculated by Broth Dilution method using antibiotic Streptomycin as standard. Among 25 plants extracts *Spheranthus indicus*, *piper Beetle*, *Tribulus terrestris*, have shown antibacterial activity from which Chloroform extract of *Spheranthus indicus* has shown highest activity against the pathogen with the inhibition zone of 15mm diameter at 10mg/ml concentration, and with MIC value of 0.8mg/ml. promising for the further study of their plant products

**KEYWORDS:** Pomegranate, Bacterial Blight, *Xanthomonas axonopodis*, plant extracts, Anti bacterial activity.**INTRODUCTION**

Pomegranate (*Punica granatum*) belongs to the family *Punicaceae* is an ancient fruit crop of India and also regarded as the fruit of paradise. It is native to Iran but extensively cultivated in Mediterranean regions specially in Spain, Morocco, Egypt, Japan, USA, USSR, Bulgaria, and Southern Italy. It is regarded as the "vital cash crop" of an Indian farmer and is grown in an area of 1.3 hectare with a production of 11.0 lakh tonns (Jadhav and Sharma, 2009).

The fruits of Pomegranate is known to possess pharmaceutical and therapeutic properties. The fruit has a wide consumer market and high economical value for its attractive, juicy, sweet, acidic and refreshing arils, nutritional and medicinal properties and is a good source of carbohydrates and minerals such as Calcium Iron and Sulphur. It is rich in Vitamin 'C' with Citric acid as the predominant organic acid (Malhotra et al., 1983). The loss in the production of yield is very high annually due to microbial infections and pests along with several physical and chemical constraints. Among the disease infecting pomegranate, the bacterial disease popularly known as "bacterial blight" caused by *Xanthomonas axonopodis Punicae* (Hingorani and Singh) (Vauterin et al.,) is a major biological production constraint. Chand and Kishun (1991) noticed that the epidemic of bacterial blight of Pomegranate is causing 60to 80% losses at Indian Institute of Horticultural Research (IIHR) experimental plots. Now it is learnt that, bacterial blight of Pomegranate is a wide spread major production

constraint. The disease prevailed in all the seasons with varying degree of severity. Studies on cultural, morphological, physiological and biochemical features of the pathogen are of immense use in understanding the nature of pathogen. Not much work has been done on this aspect but felt necessary to do so.

The pathogen can infect at any stage of growth in the life of plant. The damage is observed on fruits which develop black oily spots later become completely black, then splits and dries off. In advanced stage of infection, tissue necrosis occurs on leaves and twigs. In the case of fruits the disease develops into cracks (Yenjeerappa 2011) and later the fruit becomes completely black and dries off. Spraying various chemical formulation of antibiotics like Streptomycin, Copper Oxychloride, Bordax mixture, Bromopol to control the disease produces limited success (Mondal K.K and Mani. C 2012). Regular use of chemicals in agricultural land causes killing of natural and useful flora and fauna of the soil, increase in development of resistance in plant pathogen against the chemicals and residual toxicity remains in plant and animals. To overcome these problems there is a worldwide growing interest in the development and utilization of harmless, economical, and eco friendly material for pathogen control (Madhiashagan et al., 2002).

In order to reduce the use of the harmful chemical pesticides plant extracts are widely screened for its antimicrobial activity against several pathogenic micro

organisms with the aim of drug development (Britto A.J.D., Gracelin D.H.S and Isabastian S.R., 2011). Plant disease management measures are eco-friendly, effective and economical in this way in comparison to chemical or physical methods.

## MATERIAL AND METHODS

### Isolation and identification of pathogen

Fruits samples infected with bacteria are collected from orchard of Solapur, and identified by the National Research Centre on Pomegranate, Solapur, Maharashtra, India. The isolated bacteria in fruit lesions were confirmed by performing Ooze test. Ooze test is performed by cutting 1cm of the infected portion of the fruit and the outer surface was removed and sterilized with 0.1% Mercuric Chloride solution for 1 minute and later washed 3 times with sterile distilled water. Then the infected portion was squeezed gently with a sterile scalpel into a sterile saline by placing in a petriplate. Later the suspension is inoculated into a sterile petriplate with yeast extract medium (Yenjeerappa S.T. 2009) at pH 6.5 and were incubated at 30°C for 48hrs. After the incubation typical mucoid yellow color colonies were selected and screened for morphological and biochemical characteristics according to Bergy's manual of determinative Bacteriology and identified as *Xanthomonas*.

### Preparation of Plant extracts

The 25 plant selected for the study were mostly weeds. These were chosen so that it might reduce the cost of the plant material during industrial production of the pesticide in case significant activity is found in them.

### Collection of plant material

25 Healthy plants (table-1) were collected based on the information from literature and through field observation. The plant material was collected from Visakhapatnam District, Andhra Pradesh, India. Taxonomic identification of the plants was done by the reference to the flora of Visakhapatnam by Venkateswarlu et al (1972) as well as comparison with the authenticated herbarium specimens available in the Department of Botany, Andhra University. The collected material was washed thoroughly under running tap water and finally with sterile distilled water. Then the materials are air dried on sterile blotter under shade (M.K.Khoka 2012).

### Solvent Extraction of Plant Material

The completely shade dried plant material was coarsely powdered using electric blender. Then it was allowed to Soxhlet extraction separately and successively with Hexane, Chloroform and Methanol solvents. Later the Soxhlet material was subjected to distillation to remove the solvent. After distillation different extracts obtained were concentrated with Rotary evaporator and brought to complete dryness over a water bath to yield the crude extract and preserved at 4°C for further use (Raghavendra et al., 2006).

### Anti Microbial activity of the plant extracts

The anti bacterial activity of plant extracts was determined by Agar Well Diffusion method (Schaad et al., 1984) to screen for antibacterial activity of the Hexane, Chloroform, and Methanol solvents of 25 plant extracts. Dimethyl sulfoxide (DMSO) is used as solvent to dissolve the crude extract of plant.

### Preparation of media and plates for Agar diffusion method

In order to determine the antibacterial activity of plant extracts Agar Well Diffusion method was performed using Yeast Extract Agar as nutrient medium. 100ml of Yeast Extract Agar medium was taken and autoclaved at 121°C at 15lb pressure. The medium was removed and brought to 40°C. 1ml of the tested bacterial suspension of *Xanthomonas axonopodis* (0.1ml of 0.5 Mc Farland standards) was added to the Yeast extract agar nutrient medium and mixed well then it is poured into petriplates and allowed to cool under strict aseptic conditions. After the solidification of media, wells were made in each petriplate with the help of cup borer of 4mm diameter. The wells of each plate were well marked with permanent marker. To determine the potential of plant extracts different concentrations ranging 500mg/ml, 250mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, to 10mg/ml, were diluted in DMSO solution and 40ul of plant extract was poured into each well according to the markings on the petriplates.

### Minimum Inhibitory Concentration (MIC)

In order to determine minimal inhibitory concentration (MIC) bacterial isolates were diluted in 1ml Yeast extract Broth to get 0.5Mc Farland 0.1ml turbidity. 1ml of various concentrations of *Sphaeranthus indicus* extract ranging 10mg/ml, 9mg/ml, 8mg/ml, 7mg/ml, 6mg/ml, and 5mg/ml, were added into respective test tubes marked. Containing 0.9ml of Yeast Extract Broth each according to the concentrations into each tube 50ul of bacterial suspension was added. One tube of 10ml Yeast Extract Broth with no plant extract is taken as test control, and one tube with 10ml of Yeast Extract broth were taken as blank. One tube with 9ml of broth is taken and 1ml of Streptomycin (5mg/ml Conc) was added as standard. Then the tubes were incubated at 30°C for 24hrs. After incubation MIC was evaluated by observing the tubes for the lowest concentration of the plant extract that is inhibiting the visible growth of bacteria. The turbidity indicating the growth of the pathogen was measured by using Spectrophotometer. The MIC value of *Sphaeranthus indicus* was obtained at 0.8mg/ml conc.

## RESULTS

After incubation of the plates for 24 hrs at 30°C results were observed and noted. The results of the present study shows that the Chloroformic extract of *Sphaeranthus indicus* exhibited highest activity against the tested pathogen *Xanthomonas axonopodis* with 15mm diameter of inhibition zone at 10mg/ml conc, where as methanolic and hexane extracts showed no activity. Methanolic

extract of *Piper Beetel* showed moderate activity with 15mm diameter of inhibition zone at 100mg/ml conc. The other solvent extracts of same plant showed no activity. The methanolic extract of *Tribulus terrestris* showed less activity at 100mg/ml conc with 10mm diameter of inhibition zone. No antibacterial activity was observed for other solvents .Plant Extracts with 3

solvents of remaining plants also did not show any antimicrobial activity. Antibiotic Streptomycin of conc 5mg/ml is used as standard with the inhibition zone of 16mm diameter. The results of antibacterial activity of different solvents of *Spheranthus indicus*, *Beetel wine*, and *Tribulus terrestris* along with standard antibiotic Streptomycin are summarized in (Table -2).

**Table 1: Description of the plant used**

S.No	Botanical name	Family	local/ vernacular name	parts used	Uses
1	Areva lenata (L.)	Amaranthaceae	Pinducura, pindichettu	Whole plant	The plant used for the treatment of snake bite, this plant is used for food for people. In the whole plant only the leaves are edible
2	Antigonum leptopus(Hook. & Arn0	Polygonaceae	batani	Leaves	In some parts of the world the tubes and flowers are consumed as food
3	Aistolopia aciolata	Aristolochiaceae	Gaadidagadapaaku	Leaves	This plant is used for dermatitis , allergic disorders, leprosy, Leaves are anti inflammatory
4	Blumea mollis	Astaraceae	Kukka pogaku	Leaves, stem	This plant is for traditional and herbal medicine
5	Croton bonplandianum	Euphorbiaceae	Ban Tulasi	Whole plant	Leaves are used to reduce cough, Seed parts applied locally on czema, and ring worm to cure, Latex is used to heal cuts and wounds
6	Chloroxylon Swietenia(Rox)DC	Rutaceae	bella	Leaves	Various pats are also used in the treatment of Snake bites, Common cold and cough, ophthalmic infection and cataract, astringent, itches headache
7	Cassia tora L. syn)	Caesiulpinaceae	Chinnakasinta	Whole plant	It is very useful in treating skin diseases like ring worm, the leaves and seeds of cassia tora are useful in leprosy, constipation and cough
8	Eclipata alba (L.) Hassk	Astaraceae	Guntaglagaraku	Whole plant	It is used in ayurveda and siddha for the teatment of kapha and vata imbalance. A preparation obtained from leaf juice oiled with sesame or coconut oil is for anointing the head to render the hair lack and lixuriant.
9	Euphoria hirta	Euphorbiaceae	Nanabola	Whole plant	Euphoria hirta (Asthma her) medicinal her which is used in the various countries for treatment of diseases. It is used for relieving toothache, severe headache, rheumatism. The latex of the plant is used to remove thorns from the skin.
10	Hyptis suaveoleus(L.)Poit	Lamiaceae	Seematulasi	Whole plant	The essential oils of this plant use known for its antioxidant and anti microbial activities.
11	Jotropha carcus(L.)	Euphorbiaceae	Nepalam	Leaves	The leaves, seeds and oil is used to treat ulcer, tumour, scaies, wound, skin diseases, seeds are used for bio-fuel.
12	Leucas aspera	Lamiaceae	Tummichettu	Whole plant	Leucas aspera is reported to have antifungal, anti oxidant and cytotoxic activity. It is used in the traditional medicine. It is a herb that has the ability to reduce fevers. This plant is used commonly as an insecticide.
13	Phyllanthus amarus	Phyllanthaceae	Nela usiri	Whole plant	This is used in Indian Ayurvedic system of medicine which is used for problems of the stomach, liver, kidney.
14	Piper Beetle	Piperaceae	Tamalapaku	Leaf	This plant is Antiseptic, aphordisiac, expectorant, bronchitis, impotential rheumatism acts synergistically upon central nerves system, wounds diphtheria and

					carminative
15	<i>Ricinus communis</i>	Euphorbiaceae	Amudam	Leaves	It is a harmless purgative and very effective in treating heumatic and skin disorders. Castor leaves are used as an external application tooils and swelling , The root of plant has been used for relieving liver disorders and different forms of inflammation
16	<i>Rhinchosia minima</i> (L.)DC	Fabaceae	Adavi	Leaves	This plant leaves are used for the treatment for wounds
17	<i>Spheranthus indicus</i> Linn.	Astaraceae	Bodasaram	Wholeplant	According to Ayurveda, thisher ishot, laxative, digetile tonic, fattening altrnative, it used to treat insanity, tueculosis, indigestion, The plant is used as a soil fertility indication.
18	<i>Sida cordifolia</i>	Malvaceae	Chittamutti	Whole plant	It cures diarrrohea, Leaves are cooked and eaten in cases of bleeding piles, Juice of the whole plant used to cure rhematism
19	Tagetus	Tagetus erecta	Marigold/Banathi	Whole plant	It is used in the treatment of minor burns, When is used in the form of ointments or a tincture, It is also used in the treatment of allergic reactions, One of the medicinal use of marigold promote blood.
20	<i>Thespeciapapulnia</i> (L.)sol.	Malvaceae	Gangaraavi	Bark & Leaves	Bark is used to treat skin diseases. Leaves are applied to inflamed and swollen joints. Young fruit secrets a yellow stocky sap used to treat ringworm and other skin diseases, roots used tonic.
21	<i>Triumfeta pantanda</i> (L.)	Malvaceae	Chirusitrika	Leaves	The crushed leaf is applied in dressings for treatment of goiter.
22	<i>Tribulus teristris</i>	Zygophyllaceae	Palleru	Whole plant	It is best known for its clogging effects on. It is used in tonics in Indian Ayurvedic practice, where it is known y its Sanskrit name "GokshuraSurata". Also used as an aphrodisiac, diuretic and nervier in Ayurveda, unani, or in another medical system in India
23	<i>Tridax procumbens</i> L.	Astaraceae	Gaddi chamanthi	Whole plant	It is used to treats Inflammation, hepatic disorder, wounds, microbial infections, reduce immunity, arthrosclerosis and neurological disorder.
24	<i>Trianthema portulacastrum</i>	Aizoaceae	Amatimada,Galizeru	Whole plant	This plant cures cures bronchitis , piles, and heart diseases
25	<i>Vernonia cinerea</i> (L.)Less.	Astaraceae	Sahadevi	Whole plant	This plant is used for the Ayurvedic medicine, and It has been used as essential oil.

**Table 2: Antibacterial activity of plant extracts on growth of *Xanthomonas axonopodis* pv.**

S.no	Antibacterial activity of plant extracts on growth of <i>Xanthomonas axonopodis</i> pv.																		
	500mg/ml			250mg/ml			100mg/ml			50mg/ml			25mg/ml			10mg/ml			
	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	
<i>Spheranthus indicus</i>	Nil	23	Nil	Nil	22	Nil	Nil	20	Nil	Nil	19	Nil	Nil	17	Nil	Nil	15	nil	
<i>Piper beetel</i>	Nil	Nil	18	Nil	Nil	17	Nil	Nil	15	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Moderate
<i>Tribulus terrestris</i>	Nil	Nil	13	Nil	Nil	12	Nil	Nil	10	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	low

## DISCUSSION

Chloroformic and Hexane extracts *Spheranthus Indicus* has shown significant anti bacterial activity against *S. aureus* and *S.epidermis* at low concentration (Duraipandiyar *et al.*, 2009). The hexane extract of *Spheranthus indicus* has shown highest activity on some capsicum pathogens. Methanol extract of *Spheranthus*

*indicus* showed significant anti fungal activity against *F.oxysporum* (Dewa *et al.*, 2012) Sohbat Bahraminejad *et al.*, (2011) used roots of *Tribulus terrestris* Methanolic extract against *Rhizoctonia solani*, *Fusarium oxysporum*, and *Candida sativus*, respectively. *Piper beetle* moderate activity against tested pathogen *Xanthomonas axonopodis* where as Zain *et al.*, (2012) reported that

Methanolic extract of *Piper betle* leaf has been possess antifungal activity against *Fusarium oxysporum*. Jaya vijaya (2003) showed that the leaf extract of *piper beetle* exhibited a strong antifungal activity against *Fusarium oxysporum* with MIC by 3mg/ml on malt extract agar medium.

### CONCLUSION

Bacterial blight of Pomegranate is a major disease of the Pomegranate crop that affects around 50% of the production. The current scenario forces to find an eco friendly and effective method to control the disease. The present study highlights the activities of Chloroform, Methanol and Hexane extracts of *Spheranthus indicus*, *Piper Beetle*, and *Tribulus terrestris* against the pathogen *Xanthomonas axonopodis* among which the crude Chloroform extract of *Spheranthus indicus* showed significant activity inhibiting *Xanthomonas axonopodis* at lower concentrations, when compared with the standard drug Streptomycin. These results cane further extrapolated to find an economical ecofriendly and effective method to treat bacterial blight of Pomegranate.

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