Antibacterial activity of plant extracts against *Xanthomon asaxonopodis* Pv. *Punicae* causing Bacterial blight of Pomegranate (*Punica granatum* L.)

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Abstract- Pomegranate (*Punica granatum* L.) is an ancient fruit, belonging to the smallest botanical family punicaceae. It is the important fruit crop cultivated throughout the world. The crop is affected by 'Bacterial blight' caused by *Xanthomonas axonopodis* pv. *punicae,* which is responsible for the failure of crop. It results in the dropping of leaves as well as fruits. It is very hard to manage the disease with chemicals as well as antibiotics and farmers suffer from heavy economic losses. In the present study, aqueous, and Methanol extracts of Five medicinally useful plants were used against *Xanthomonas axonopodis* pv. *punicae* in *vitro*. The plant extracts showed antibacterial activity and caused inhibition of growth of *Xanthomonas axonopodis* pv. *Punicae*. Among them, Karanj (*Pongamia* pinnata L.) and Tulsi (*Ocimum sanctum* L.)caused maximum inhibition of the test bacterium. In the plant extracts showed antibacterial activity and caused inhibition of growth of *Xanthomonas axonopodis* pv. *Punicae.*

Key Words- Bacterial blight, Antibacterial, Pomegranate, *Xanthomonas axonopodis* pv. *Punicae.*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is belonging to the smallest botanical family punicaceae. The Pomegranate (*Punica granatum* L.) is an ancient fruit crop of India. Pomegranate has been associated with high nutritional value, many health benefits and its entire plant has great economic and therapeutic value. Pomegranate is also well known for various medicinal properties and nutritive values. (Julie Jurenka, 2008). It is native to Iran. It is the important fruit crop cultivated throughout the world. India is largest pomegranate producer in the world sharing about 36 per cent of the world's production and above 30 per cent of the international Trade. (Gargade and Kadam, 2015). The average of total production is 8 lakh tons per annum in India (Pawar*et al.*, 2014). The crop is affected by 'Bacterial blight' caused by *Xanthomonas axonopodis* pv. *punicae,* which is responsible for the failure of crop.

Studies were conducted in different parts of the world identified *Xanthomonas auxonopodis* Pv. *Punicae* as the causal organism of bacterial blight, which is a gram negative short rod bacterium. The pathogen infects all the cultivated varieties nevertheless of age of the plants. The infection appears as yellowish water soaked circular spots on the plant part and later converted to irregular injuries. In advanced stages of infection, tissue necrosis occurs on the leaves and twigs. In the case of fruits the disease develops into cracks and later the fruit became completely back and dries off. This is one of the most damaging diseases of pomegranate. Due to bacterial blight of pomegranate, the yield loss was noted up to 90 per cent (Chowdappa*et al.*, 2018). It results in the falling of leaves as well as fruits. It is very hard to manage the infection with chemicals as well as antibiotics and farmers suffer from heavy economic losses.

The pathogen can infect in any stage of growth of the plant. The damage is observed on fruits which develop black oily spots later become completely black cracking and dries off. In advanced stage of infection tissue necrosis occurs on leaves and twigs. Use of chemicals in agriculture causes several opposing and environmental dangers(Shanthi,2011).The primary disease management includes spraying bleaching powder, farmyard manure, urea, Bordeaux mixture to control bacterial blight of pomegranate (Yenjerappa *et al.*,2014).Regular use of chemicals in agriculture land causes killing of flora and fauna of the soil, increase in development of resistance in plant pathogen against chemicals and residual toxicity remains in plant and animals. To overcome this problem, there is growing interest worldwide in the utilization of sustainable material for pathogen control (Madhiazhagan *et. al.*, 2002).

In the present study, aqueous, and Methanol extracts of Five medicinally useful plants were used against *Xanthomonas axonopodis* pv. *punicae* in vitro. The plant extracts showed antibacterial activity and caused inhibition of growth of *Xanthomonas axonopodis* pv. *Punicae*.

Aims and Objectives-

To isolate pathogenic bacteria from affected fruit samples and to screen the ability of different plant extracts to inhibit the pathogens and control pathogenic bacteria causing bacterial blight to pomegranate.

Materials and Method-

Collection of diseased plant parts of Pomegranate plant

Disease infected Pomegranate fruits were collected from field located at village Rajuri Tal- Rahata, district Ahmednagar, Maharashtra, India.

Isolation and Identification of pathogen from lesions on diseased fruit

The disease infected portion of fruits were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one minute and washed three times with sterile distilled water and mixed gently with sterile scalpel in sterile saline. The presence bacteria in fruit lesion was confirmed by performing ooze test. The suspension was serially diluted and plated on sterile Petri plate with Nutrient Glucose Agar medium with composition Beef extract-0.3%,peptone-0.5%,glucose-0.25%, agar-2%, pH 6.8 (Mondal*et al.*, 2009).The inoculated plates were incubated at 30°C for72 hours. After the incubation typical mucoid yellow color colonies were selected and screened for morphological and bio-chemical characteristics according to Bergy's manual of determinative Bacteriology and identified as *Xanthomonas*.

Preparation of aqueous plant extracts

Leaves of the medicinal plants such as Pongamia (*Pongamia pinnata* L.), Periwinkle (*Cathranthus roseus* (L.) G. Don.), Tulsi (*Ocimum santum*L.), Shatavari (*Asparagus racemosus* Willd.), Adulsa (*Adhatoda vasica* Nees.) were collected from different sites of sonai, Tal- Newasa, Ahmednagar (M.S.) The plant material was wash tap water remove soil and dust particles and then dry in shady place temperature (25 ± 2°C). Plants leaves was crush in mixture and to make fine powder. Fifty grams of leaves pawder was crush in 50ml of sterile distilled water using mortal and pestle. The extracts was filter double layered cheese cloth, and then through Whatman filter paper No.1. The Filter extracts centrifuged at 5000 rpm for 20 minutes supernatant was stored in sterilized bottle and labeled properly. Finally the filtrate was passed through syringe filter of 0.2 µm pore size for sterilization. This filtrate served as 100 per cent standard solution. Filtrate was diluted to 5 per cent and 10 per cent concentration using sterile distilled water. The standard solution was stored at 4°C for further use (Kulshresthaet. *al.*, 2015).

Preparation of methanol plant extract

Leaves of the plants were thoroughly washed and dried under shade at the room temperature (20 ± 2°C). The dried leaves were then ground to a fine powder in an electric grinder. Stock solutions of the extract were prepared by adding ground leaf powder

to 200 ml of each solvent (w/v, 1 g/ 10 ml). Methanol solvents were used for extraction. Prepared extracts were then shaken for 6 hours for homogenous mixing of ground leaf powder in the solvent. After that each extract was passed through Whatman filter paper no.1. Final filtrate was then concentrated to 10 per cent crude extract on a mini rotary evaporator under vacuum at 20°C and was utilized for the experiments (Digvijay*et. al.*, 2014).

In vitro* Antimicrobial activities of the extract against *Xanthomonas axonopodis pv. punicae

Forty-eight hours old bacterial culture of *Xanthomonas axonopodis* pv. *Punicae* was seeded into the nutrient agar medium at lukewarm temperature (40°C), mixed well and poured into sterile Petri plates. Wells of 4 mm diameter was prepared using a cork borer and plant extracts (0.02 ml) were filled in wells. Three replicates were maintained for each extract at 5 per cent and 10 per cent separately. Plates containing nutrient agar with bacterial suspension without any leaf extract were maintained as control. All these Petri plates were incubated at room temperature (28 + 2^{0}C) for 48 hrs. (Bonyadi*et. al.*, 2009).

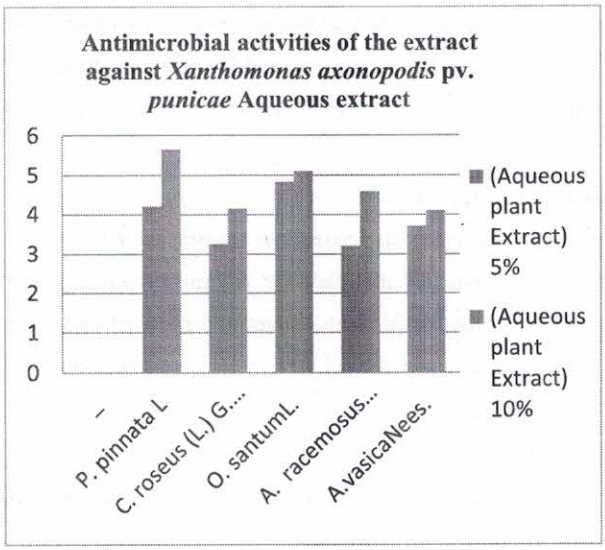
RESULTS AND DISCUSSION

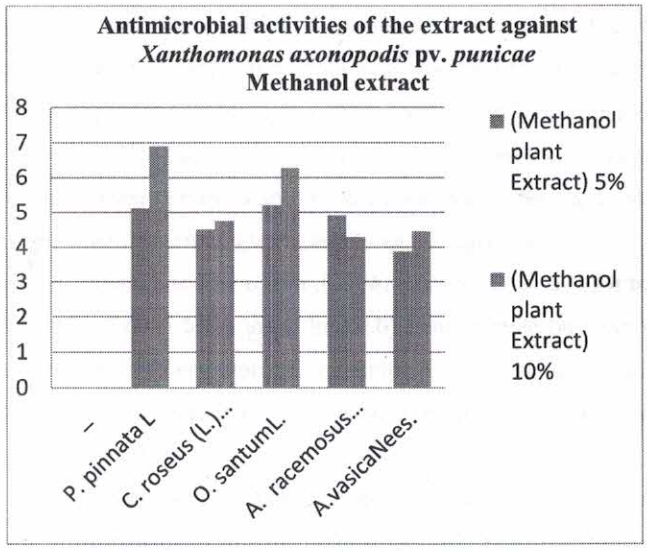
The results of the present study shows that all the plant extract inhibited the pathogen. Higher concentration of aqueous and methanol plant extract show high inhibited compared lower concentration in all three replication. Among the plant extract in case of 5% concentration maximum inhibition zone 4.83 cm in aqueous and 5.20 cm in methanol extract of *O. santum* L. In case of 10% concentration *P. pinnata* L exhibited maximum inhibition 5.66 cm in aqueous and 6.90 cm in methanol extract. *C. roseus (L.) G. Don. and A. vasica* Nees both plants Shows lesser inhibition zone formed. (Table No. 01).

Table No. 01: *In vitro* Antimicrobial activities of the extract against *Xanthomonas axonopodis* pv. *punicae*

Sr. No.	Common Name	Botanical Name	Inhibition Zone (cm)* (Aqueous plant Extract)		Inhibition Zone (cm)* (Methanol plant Extract)	
			5%	10%	5%	10%
1	Control (Sterile Distilled Water)	–	0.00	0.00	0.00	0.00
2	Pongamia	*Pongamia pinnata* L	4.20	5.66	5.12	6.90
3	Periwinkle	*Cathranthus roseus* (L.) G. Don.	3.25	4.15	4.50	4.75
4	Tulsi	*Ocimum santum*L.	4.83	5.10	5.20	6.27
5	Shatavari	*Asparagus racemosus* Willd.	3.20	4.59	4.90	4.28
6	Adulasa	*Adhatoda vasica* Nees.	3.70	4.10	3.88	4.45

*- Mean of three replications.





CONCLUSION

Bacterial blight of Pomegranate is a major disease of the Pomegranate crop that affects around 50% of the production. The present study to find the ecofriendly method of controlling bacterial blight of pomegranate. Pongamia and Tulsi are show that the maximum inhibition of the *Xanthomonas axonopodis* pv. *Punicae* bacteria so these both plant are useful for the controlling of the infection.

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