

Antibacterial investigation of *Punica granatum* (Pomegranate Rind) extract against different plant pathogenic bacteria

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ABSTRACT

The antibacterial investigation of *Punica granatum* (Pomegranate rind) extract was performed against *Agrobacterium tumefaciens*, *Erwinia chrysanthemi*, *Pseudomonas pisi*, *Pseudomonas solanacearum*, *Xanthomonas malvacearum* and isolated plant pathogenic bacteria as test organisms. This screening of antibacterial activity of *Punica granatum* rind extract was determined by agar- well diffusion method.

Among them, *Agrobacterium tumefaciens* (23mm) and *Pseudomonas pisi* (22mm) are found to be more sensitive and *Xanthomonas malvacearum* (18mm) is found to be less sensitive. And among the isolated ones all are found to be the highly sensitive but bacteria isolated from Cotton Leaves (33mm), Tuwar Seeds (32mm) and Lemon Leaves(30mm) are found to be the more sensitive. And bacteria isolated from Tuwar Leaves (29mm) and Cotton Bolls (29mm) are found to be the less sensitive against *Punica granatum* rind extract.

Thus, results obtained indicate that, *Punica granatum* fruit rind posse's potent antibacterial activity as compare with standard antibiotic Streptomycin against all the tested plant pathogenic bacteria. This antibacterial activity was due to the presence of bioactive phytochemical.

Key words: *Punica granatum* rind extract: Antibacterial Activity, Plant Pathogenic bacteria.

INTRODUCTION

***Punica granatum* (Pomegranate)**

Punica granatum is a shrub or small tree; it belongs to the family Punicaceae and species *Punica granatum*. This shrub mainly contain tannin, which occurs in all parts of tree, particularly in fruit rind (up to 26% in dried rind), stem bark (10-25%), root bark (28%) and leaves (11%). The fruit and the bark of the stem and root have been widely used as tanning material.

It is native of Iron but now cultivated throughout India. It is found growing wild in the warm

valleys and outer hills of the Himalayas between 900 and 1800m.

The use of plant extracts for their antibacterial action has been the subject of research by many workers.

MATERIAL AND METHODS

Following materials and methods were used in this investigation.

Preparation of Extract (Test extract)

For the preparation of rind extract,

completely dried rind was taken and grinded to form fine powder. Then only one gram of fine powder was taken in sterile china dish and 15 ml. of sterile distilled water was added to it and mixed properly. Then mixture was used as pomegranate rind extract for antibacterial activity.

Preparation of Antibiotic Streptomycin Solution (Control)

For the preparation of Antibiotic Streptomycin solution, weighed 1g of antibiotic and dissolved in 10ml sterile distilled water and mixed properly. After that pipetted 1ml of solution by sterilized pipette and added to 99ml sterile distilled water and mixed properly. Now from that 100ml, pipetted 0.1ml solution by using micropipette with sterile tips for antibacterial testing as control.

Media Used For Maintenance and Testing of Microorganisms

All the microorganisms used in this study were maintained and tested on following mediums.

Nutrient agar medium was used for *E.chrysanthemi*, *P.pisi* and *P.solanacearum*. Yeast-Extract- Beef- Extract medium was used for *Agrobacterium tumefaciens*. And Malt-Extract-Glucose-Yeast-Extract-Peptone medium was used for *Xanthomonas malvacearum*. Slants were prepared of used medium and kept in refrigerator. Subculturing was made after every fifteen days. All the mediums were prepared as per the composition given in NCIM catalogue.

Method of isolation of plant pathogenic bacteria from (Diseased part) of host plant.

Isolation of bacteria from diseased part of plant has been done by Serial dilution, Spread plate method. The aim behind to perform all these methods is to isolate the pure culture of bacteria. Dubey R.C. and Maheshwari D.K., A Textbook of Microbiology.

Antibacterial testing (Kirby-Bauer) Agar-Well Diffusion Method

For antibacterial testing agar-well diffusion method was used. For this purpose, a nutrient agar was prepared as per the composition and sterilized. Before of this inoculums of standard

and test organism were prepared by inoculating a loopful of culture in a five ml nutrient broth (HIMEDIA, MUMBAI, INDIA) and incubated at 37°C for 24 hrs, until a moderate turbidity was developed. This over night broth culture adjusted to yield approximately 1.0×10^8 cfu/ml for bacteria. Then in sterilized Petri dishes 0.5ml of broth culture were taken by micropipette with sterile tips aseptically in vicinity of burner. Then pour, sterilized and cooled to 45°C of molten form of nutrient agar of volume 20ml in sterilized Petri dishes and mixed thoroughly with broth culture and allowed to solidify. After proper solidification, make a well in the center of agar plate by flamed cork-borer of (8mm diameter). Then by using micropipette with sterile tips place, prepared extract in a well in fixed volume of 0.1ml, to fill the well completely. Then place the Petri dishes in straight position in incubator at 37°C for 24 hrs. for bacteria. Similarly place the Petri dish of positive control i.e. standard antibiotic streptomycin for incubation at 37°C for 24 hrs. After completion of incubation period of 24 hrs. the zone of inhibition were measured in (mm) diameter. All the tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameter (mm) produced by the plant extracts.

RESULTS AND DISCUSSION

The results obtained in the experiments are shown in table-1 and Graph-1 *Punica granatum* rind extract has shown the antibacterial activity against all the tested plant pathogenic bacteria. Prepared dilution of dried rind has shown zone of growth inhibition. This shows the sensitivity of the bacterium on nutrient agar plates.

Among the standard strains of bacteria *Agrobacterium tumefaciens* (23mm) and *Pseudomonas pisi* (22mm) are found to be more sensitive and *Xanthomonas malvacearum* (18mm) is found to be less sensitive. And among the isolated, organism from Cotton Leaves (CL33mm), Tuwar Seeds (TuS 32mm) and Lemon Leaves (LL30mm) are found to be the most sensitive one and from Cotton Bolls (CB 29mm) and Tuwar Leaves (TuL 29mm) are less sensitive.

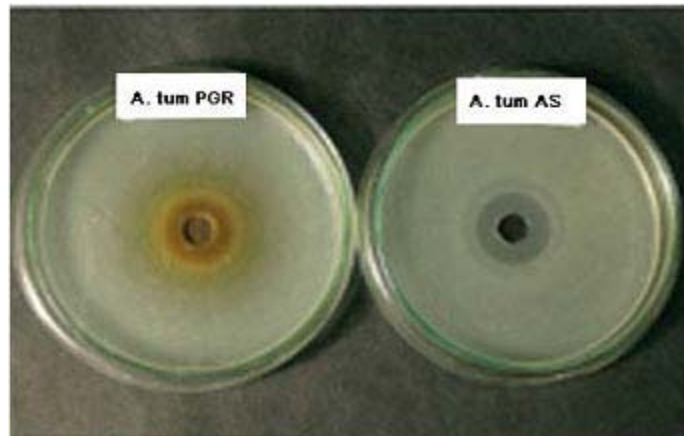


Plate 1: Effect of *Punica granatum* rind extract on plant pathogenic bacteria *Agrobacterium tumefaciens*

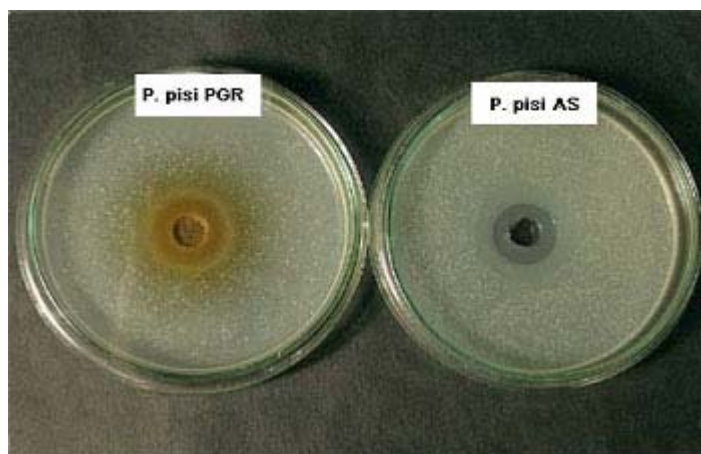


Plate 2: Effect of *Punica granatum* rind extract on plant pathogenic bacteria *Pseudomonas pisi*

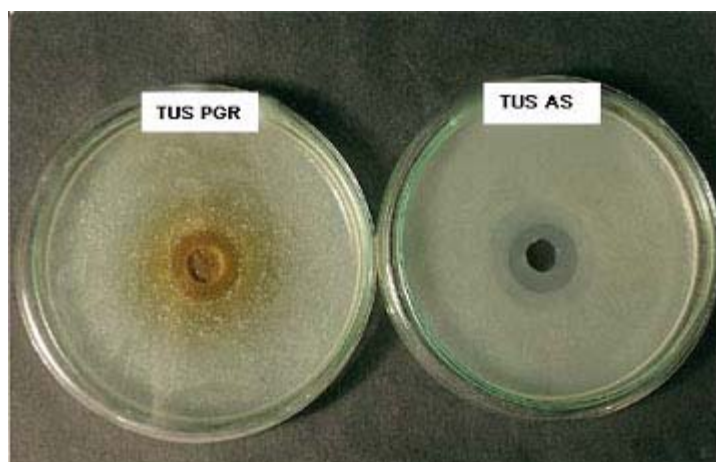


Plate 3: Effect of *Punica granatum* rind extract on bacteria isolated from Tuwar Seeds (TuS).

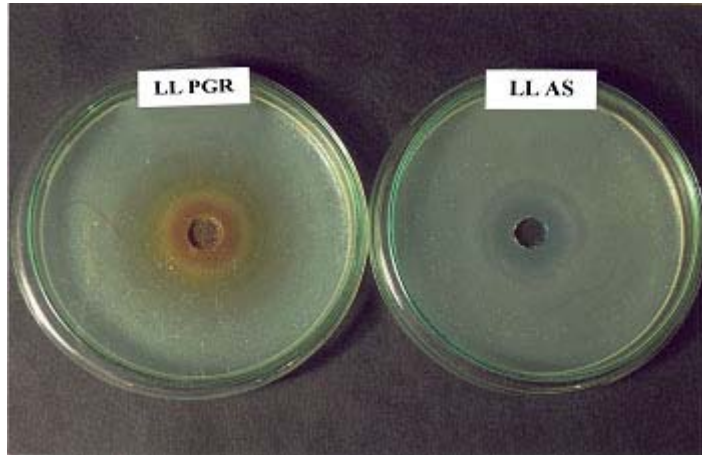


Plate 4: Effect of *Punica granatum* rind extract on bacteria isolated from Lemon Leaves (LL)

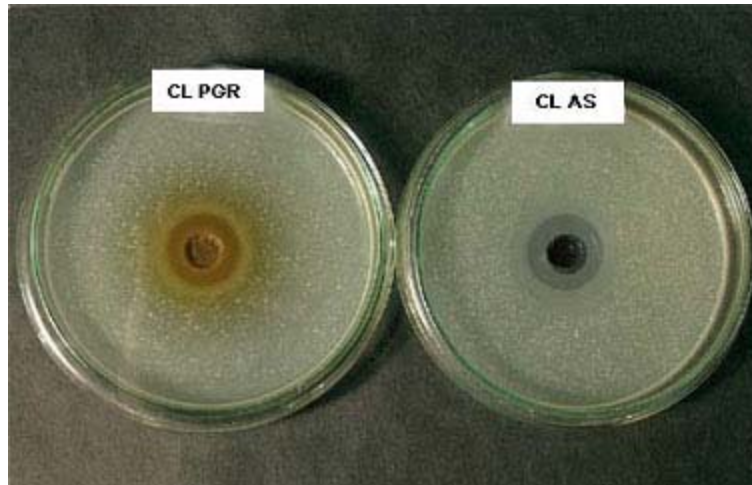
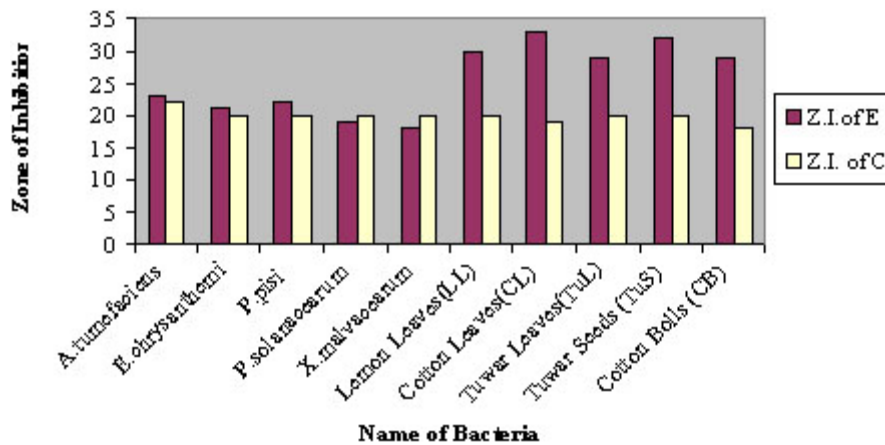


Plate 5: Effect of *Punica granatum* rind extract on bacteria isolated from Cotton Leaves (CL)



Graph 1: Effect of *Punica granatum* rind extract on plant pathogenic bacteria

Table 1: Effect of *Punica granatum* (Pomegranate) rind extract against different plant pathogenic bacteria

S. No.	Name of Bacteria	Zone of Inhibition of rind extract (mm)	Zone of Inhibition of Control (mm)
1.	<i>A. tumefaciens</i> (NCIM 2232)	23	22
2.	<i>E. chrysanthemi</i> (NCIM 5213)	21	20
3.	<i>P. pisi</i> (NCIM 2204)	22	20
4.	<i>P. solanacearum</i> (NCIM 5103)	19	20
5.	<i>X. malvacearum</i> (NCIM 2310)	18	20
6.	Lemon Leaves (LL)	30	20
7.	Cotton Leaves (CL)	33	19
8.	Tuwar Leaves (TuL)	29	20
9.	Tuwar Seeds (TuS)	32	20
10.	Cotton Bolls (CB)	29	18

CONCLUSION

Results reported here contribute to the knowledge of the antibacterial efficacy of dried *Punica granatum* rind extract. It has been reported that the fully natural rind extract affects the tested bacteria and exerts complete bactericidal activity against some of the tested plant pathogenic bacteria; these are *Agrobacterium tumefaciens*, *Pseudomonas pisi* and *Erwinia chrysanthemi*. And it act as bacteriostatic against *Pseudomonas solanacearum* and *Xanthomonas malvacearum*.

This allows the conclusion that antibacterial properties of rind extract is found to be the potent against Crown – gall disease, caused by *Agrobacterium tumefaciens* in Potato, Tobacco, and Tomato etc. Stem – blights disease caused by

Pseudomonas pisi in, Beans and Soft – rot disease caused by *Erwinia chrysanthemi* in fleshy vegetables respectively. As well as it is found to be the potent against organism isolated from Cotton Leaves which cause the disease Angular – leaf spot and from Tuwar Seeds which cause the disease Blight in seeds.

So that, to get rid from all these hazards we can suggest the use of rind extract to control the bacterial diseases of plants. This investigation is complete new one as there is no literature available related to this. So, the results obtained of the effectiveness of *Punica granatum* rind extract against test plant pathogenic bacteria has not correlated with the previous investigation. Further phytochemical investigation required to identify the active compound.

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