

A Visionary *In-vitro* Analysis for Anti-Bacterial Potential Using Seeds of a Deciduous Shrub *Punica Granatum*

¹Hemant Kr. Sharma, ³Yunus Parvez, ³Harshit K. Shrivastava,
²Shashank Kr. Singhm and ³Anjali Sharma

¹Cytogene Research & Development, LLP, Indira Nagar, Lucknow, U.P., India

²Department of Biotechnology, Delhi Technological University, New Delhi, India

³Department of Biosciences, Faculty of Applied Science, Integral University, Lucknow, India

Abstract: *Punica granatum* i.e., pomegranate plays a very important role in Indian fruit market. From very ancient times pomegranate has been used for many medicinal purposes to cure many short term as well as long term disorders. The current study proves that *Punica granatum* is having a very strong anti-bacterial activity. For the present study firstly, we have used granatum seeds, pericarp (peel) as well as juice to evaluate the antibacterial activity of the fruits against enteric bacteria i.e. *E. coli*. For the detection of antibacterial activity agar gel diffusion method was used. As a result zone of inhibition was only shown by seed. On the other hand, zone of inhibition was not observed in juice and peel. So it was concluded that, the granatum seeds persists the anti-bacterial property. Secondly, solvent extract of the pomegranate peel i.e. water, ethanol and methanol extracts were prepared and antibacterial activity was tested against enteric bacteria *E.coli* by the method of agar diffusion method. As a result, methanol and ethanol extract showed almost same zone of inhibition (21mm) while water extract showed comparatively less zone of inhibition (13mm) with respect to control ofloxacin (32mm) at 150ppm.

Key words: *Punica granatum* • Deciduous • Zone of Inhibition • Ofloxacin • Agar Diffusion

INTRODUCTION

Punica granatum Linn. (Pomegranate) is a member of family Punicaceae which is a deciduous spreading shrub or small tree and has thorns with it. This plant is found all over India. Pomegranate peel is an inedible part obtained during processing of Pomegranate juice. Pomegranate peel is a rich source of tannins, flavonoids, polyphenols and some anthocyanins as Delphinidins, Cyanidins, etc. [1]. Antioxidant and antibacterial properties of pomegranate peel in in-vitro model systems have been reported [2,3, 4]. The compounds of pomegranate peels are reported to have therapeutic properties.

The objectives of present study were to evaluate the antibacterial activity of different parts of pomegranate fruit. During the experiment different parts of pomegranate i.e. pericarp, juice and seeds were separated. They were grounded into fine paste by incorporating water.

The mixture was filtered and the supernatant was used to detect the antibacterial activity by the agar diffusion method. As a result pericarp and fruit juice did not showed any zone of inhibition where as seed showed clear zone of inhibition which was measured out to be 17mm in comparison to zone of inhibition of ofloxacin which was measured to be 32mm.

Further the pericarp of the fruit was peeled out. It was sundried to exclude moisture or water content. The pericarp was grounded into fine powder with the help of mixer grinder. Solvent extracts of the powdered peel was prepared in water, methanol and ethanol.

The antibacterial activity of the three solvent extracts were detected by using agar diffusion method in which methanol and ethanol extract showed almost equal zone of inhibition(21mm) whereas water extract showed lesser zone of inhibition(13mm) as compared to zone of inhibition of ofloxacin(32mm) at 150ppm.

MATERIALS AND METHODS

Collection of Sample: Pomegranate was collected from local market in Lucknow. The fruit was first washed with tap water followed by washing with distilled water.

The fruit was cut to separate out its peel, juice and seed. The peel and seed were grinded by incorporating distilled water with the help of mixer grinder. The fruit juice, seed and peel extract were then filtered by whatmann filter paper no. 1 and the filtrate were collected which were used to study antibacterial property.

In the other section of the study fruit peels were cut into smaller pieces and then first washed with tap water followed by distilled water. It was then dried under sunlight until water droplets got completely evaporated. Pericarp or peel was then completely dried in microwave. Dried pericarp was then taken for grinding by the help of mixer grinder. The powdered pericarp was used throughout the study.

Model Organisms: Anti-bacterial property of *Punica granatum* was tested against enteric bacteria *Escherichia coli*.

Media Preparation

Nutrient Agar media: Nutrient agar media was found to be suitable for the growth of bacteria.

Nutrient Agar 28 gm, in 1000 ml distilled water.

Nutrient agar media preparation must be performed in the following order:

- 2.8 gm of Nutrient agar dissolved in 100ml distilled water.
- The media was sterilized at 15 lbs (121°C) for 15 minutes in an autoclave.
- In the laminar flow poured the media in Petri dish, amount of nutrient agar is 25 ml in each Petri dish.
- Leave the Petri dish in laminar air flow for 2-3 hrs for solidification.
- Solidified media is prepared for spreading/streaking, take *E. coli* culture and spread / streak on the plate surface.
- The plate was kept in the incubator for overnight at 37°C temperature.
- After overnight incubation, colonies were well developed in the Petri dish.
- *E. coli* plates were prepared. It is used when necessary.



Fig. A: Powdered Pericarp

Nutrient Broth: Preparation of Nutrient broth in 100 ml.

Nutrient broth performed in the following order.

- 3gm of Nutrient Broth was taken and dissolved in 100ml Distilled Water.
- Autoclaved it at 121°C for 15 minutes.
- Cool the nutrient broth at room temperature.
- One colony isolated from the master plate.
- This colony inoculated in the nutrient broth by inoculated loop.
- Left the prepared flask in the Incubator for overnight at 37° C.
- Nutrient broth culture is prepared for culturing.

Methodology

Extraction Procedure: The powdered pericarp was dissolved in different solvents. The solvents used were methanol, ethanol and water. 5gm of ground pericarp was added to 50ml of hot boiling water and left in hot water bath for an hour at 70 degree Celsius so that secondary metabolites got completely extracted. The extract was then filtered with the help of whatman filter paper no. 1 and kept in microwave for drying. Similarly ethanolic and methanolic extraction were carried out where 5gm of dried powder was added to 50ml of 80% methanol and 70% methanol and kept in dark for 3 to 4 days. The solvent extracts were filtered and filtrate was used to get the antibacterial activity.

Screening of the Fruit Part for Antibacterial Activity:

Antibacterial activity was assessed by agar well diffusion method by Kirby Bauer where in nutrient agar plates were prepared and were spreaded with 20µl of *E. coli* broth culture. Wells of 8mm diameter were bored. Wells were loaded with ofloxacin as standard and distilled water as negative control and the filtrate of fruit parts i.e. fruit juice, peel and seed extract.



Fig. B: Solvent Extracts of Powdered Pericarp

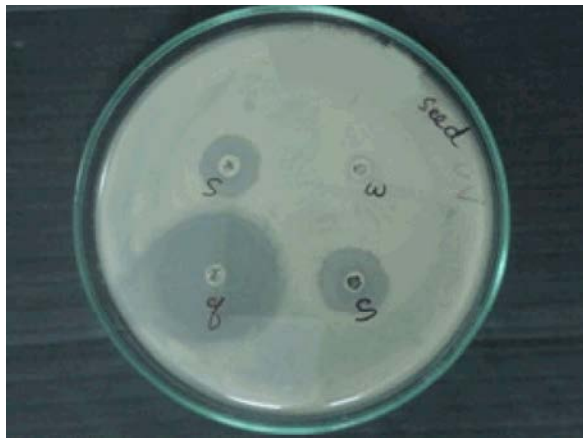


Fig. C: Antibacterial Sensitivity Of Seed Part By Kirby Bauer Method

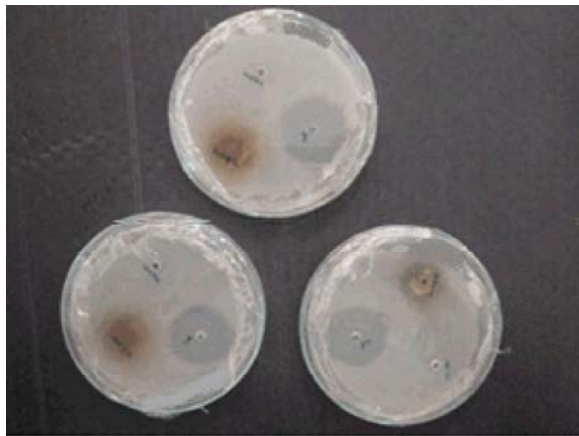


Fig. D: Antibacterial Sensitivity of Solvent Extracts By Kirby Bauer Method.

Screening of the Extracts for Antibacterial Activity: Antibacterial activity was assessed by agar well diffusion method of Kirby bauer where nutrient agar plates were prepared and were spreaded with 20 μ l E.coli broth culture in 100mm petridish. Wells of 8mm diameter were bored. Wells were loaded with ofloxacin as standard, distilled water as negative control and solvent extract of fruit pericarp.

RESULTS

The development of drug resistance in many microorganisms against commonly used antibiotics laid the basis for search of new antimicrobials of plant origin. As in this study pomegranate was screened for antibacterial activity. The results of the study are observed as follows:



Fig. E: Inhibition Zone by Water Extract of Pericarp



Fig. F: Inhibition zone by Ethanol Extract Of Pericarp

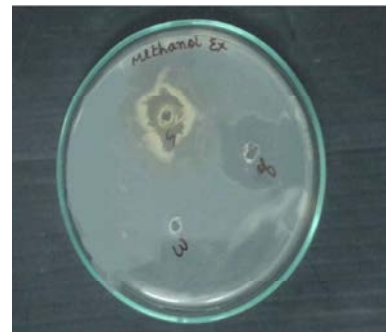


Fig. G: Inhibition Zone by Methanol Extract of Pericarp



Fig. H: Inhibition Zone by Seed

Table A: Antibacterial Activity Of Fruit Parts

Test Organism	Sr. No.	Parts of Fruit	Inhibition Zone of Ofloxacin (+VE Control)	Inhibition Zone of Fruit Part Without Solvent Extract
<i>E. coli</i>	1	Juice	32mm	-
<i>E. coli</i>	2	Seed	32mm	17mm
<i>E. coli</i>	3	Peel (pericarp)	32mm	-

Table B: Antibacterial Activity Of Solvent Extracts Of Fruit Pericarp

Hot aqueous extract of pericarp		Methanolic extract of pericarp		Ethanol extract of pericarp	
Inhibition Zone by Ofloxacin (+VE control)	Inhibition Zone by Sample	Inhibition Zone by Ofloxacin (+VE control)	Inhibition Zone by Sample	Inhibition Zone by Ofloxacin (+VE control)	Inhibition Zone by Sample
31mm	13mm	33mm	21mm	32mm	21mm

DISCUSSION

Nearly 80% of the world populations depends on the traditional medicine for primary health care, mainly including the use of natural products [Sandhaya b et al]. Researchers have extensively studied the biological properties of Punica granatum and their results showed that this plant is ethno medically valuable [Shibumon g and Benny P J (2010)]. Punica granatum peel extracts are currently used for treatment of respiratory diseases and in the preparation of therapeutic formulae. The tannin rich ellagitannins and phenolic acids of Punica granatum have antibacterial, antifungal and antiprotozoal activity (Surasak L. et al., Prashanth D. et al., Vasconcelos L.C.D. et al).

In the current study the hot aqueous extract of pericarp showed zone of inhibition of 13mm, methanolic and ethanolic extracts of Punica granatum showed almost equal Zone of inhibition of 21mm against enteric bacteria *E. coli*.

In the other section of the study the seed of pomegranate exhibited clear zone of inhibition of 17mm on the other hand the other parts of the fruit i.e. pericarp and juice did not showed any zone of inhibition. broad spectrum antimicrobial compounds that act against both gram +ve and gram -ve bacteria. Ethanolic extracts and Methanolic extracts exhibited higher degree of antibacterial activity as compared to that of hot aqueous extracts tested against bacteria that cause gut infection, stomachache, diarrhea. [Lortheeranuwat a et al] reported that *P. granatum* contains large amount of tannins (25%) and antibacterial activity may be indicative of presence of secondary metabolites.

CONCLUSION

Plant extracts are complex and isolating bioactive compound is challenging. Only a few extractants have generally been used for extracting antibacterial compounds from plants. In the present study, the organic solvents proved to be much better than that of aqueous extracts in inhibiting enteric bacteria *E. coli*. On the other hand seed of pomegranate inhibited the enteric bacteria *E. coli*. Hence it can be concluded that seed part of pomegranate have anti-bacterial properties.

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