Int.J.Curr.Microbiol.App.Sci (2017) *6*(12): 3802-3807

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 12 (2017) pp. 3802-3807**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.612.437

Antibacterial Activity of *Punica granatum* (Pomegranate) Fruit Peel Extract against Pathogenic and Drug Resistance Bacterial Strains

Alka Chaudhary[1] and Siddarth Nandan Rahul[2*]

[1]Department of Microbiology C.C.S. University Meerut- 250110, UP, India
[2]Department of Biotechnology, Agriculture Sciences and Agricultural Informatics, Shobhit University, Meerut- 250110, UP, India
**Corresponding author*

Keywords

Punica Granatum (Pomegranate) fruit peel, Pathogenic bacterial strains, Antimicrobial activity, Disc diffusion method, Minimum Inhibitory Concentration.

Article Info

Accepted:
28 October 2017
Available Online:
10 December 2017

A B S T R A C T

The Botanical name of Pomegranate is *Punica granatum* and it is a bearing fruit shrub, belonging to the family Lythraceae. *Punica granatum* originated in the region of northern India. Every part of the *Punica granatum* is useful for our body such as seeds and fibers which provide vitamins-C, vitamins-K, Folate. Seeds of *Punica granatum* contain oil acids such as Punica acid, Palmitic acid, stearic acid and Oleic acid. *Punica granatum* peel also shows Antioxidant and Antibacterial activity. Antibacterial activities of peel extract were studied against some bacterial strains as- *Pseudomonas fluorescens Pseudomonas aeruginosa Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhi*, and *Bacillus subtilis* (all are gram negative and also pathogenic to the human). The *Punica granatum* peel extract were prepared in three organic solvents- Methanol, Ethanol and Benzene. All extract showed the degree of effectiveness against all bacterial strains but the Methanol extract showed the maximum growth inhibition 85.71% against *Klebsiella pneumoniae* bacteria at 100µl/ml extract concentration. *Klebsiella pneumoniae* is pathogenic and cause disease Pneumonia and can progression in to serve bacterial infection to bloodstream infection, wound infection, urinary tract infection and meningitis. It is also resistance for some antibiotics such as Lincomycin and Oleandomycin. The present study depict that he *Punica granatum* fruit peel extract (which is waste in industry) showed good Antibacterial activity against given pathogenic and some drug resistance bacterial strains.

Introduction

Medicinal plants have always been a good source to find new remedies for human health problems. Recently a wide range of these plants have been screened for antimicrobial property (Martin and Ernst 2003, Upadhyay *et al.*, 2010).

Punica granatum commonly called as Pomegranate, recently describe as nature power fruit, is a plant used in folkloric medicine for the treatment of various diseases (Adbel moneim *et al.*, 2011; Ajay kumar *et al.*, 2005) widely cultivated in Mediterranean region. Pomegranate has strong antioxidant and anti-inflammatory properties recent studies have demonstrated its anticancer activity in several human cancers (Adhami and Mukhtar, 2007; Longtin, 2003). In addition pomegranate peel extract with an abundance of flavonoid and tannins has been show to have a high antioxidant activity (Abdel moneim *et al.*, 2011).

Pomegranate (*Punica granatum* L.) is the fruit of a tree belonging to the family Punicaceae. It is native from Iran to the Himalayas in northern India and has been cultivated and naturalized over the entire Mediterranean region since ancient times (Jurenka, 2008 and Meerts *et al.,* 2009). The ripe fruit contains many arils separated by a white, membranous pericarp (Jurenka, 2008). Studies show that pomegranate juice (PJ) has potent antioxidant activity (ability to scavenge free radicals), significantly higher than more commonly consumed fruit juices such as grape, cranberry, grapefruit, and orange (Azadzoi *et al.,* 2005; Basu 2009; Guet *et al.,* 2008).

This activity has been attributed to antioxidant properties of polyphenols, including ellagitannins (hydrolyzable tannins) and anthocyanins (condensed tannins) (Cowan, 1999). Punicalagins are the major ellagitannins in the whole fruit and can be hydrolyzed to ellagic acid and other smaller polyphenols in vivo (Jurenka, 2008; Lansky, 2007). Pomegranate has been used in traditional medicine for the treatment of dysentery, diarrhea, helminthiasis, and respiratory pathologies (Lansky 2005–Sánchez-Lamar, 2008). Currently there is considerable interest toward evaluating plant sources for alternative treatments against pathogenic bacteria which are now showing resistant to many drugs. Pomegranate (*Punica granatum*) peel is an inedible part obtained during processing of pomegranate juice which is completely waste in the industry. In the present investigation we have tested various extract of *Punica granatum* against pathogenic and drug resistant bacterial strains.

Materials and Methods

Preparation of plant material

The plant material (fruit part) was collected from the Cotton Research Station D.M. road Bulandshahr region UP (India) during the starting of winter season. Collected plant material was clean up and crushed into powder form using pestle mortal.

Extract preparation

Fruit peel extract were prepared by immersing 1gm of powder in 5ml with four different organic solvents as Methanol, Ethanol and Benzene for 24 hours after filtration the extract were evaporated by the help of rotator evaporator. For stock solution each extract was re-dissolved with 5ml DMSO (dimethyl sulphoxide).

Test microbial strains

All tested bacterial strains viz., *Pseudomonas aeruginosa* (MTCC 162), *Pseudomonas fluorescens* (MTCC 254), *Proteus vulgaris* (MTCC 123), *Bacillus subtilis* (MTCC 251), *Klebsiella pneumonia* (MTCC 140), *and Shigella flexneri* (MTCC 182), *Salmonella typhi* (MTCC 326) were collected from MTCC (microbial type culture collection) IMTECH Chandigarh. These microorganisms were maintained on nutrient agar media (NAM) at 30^{o}C for further investigation.

Antibacterial screening

The antimicrobial screening of the bacterial strains were carried out disc diffusion method (Grover and Moore 1962)**.** The plant extracts of 0.1ml were mixed in 0.9 ml of pre sterilized nutrient broth and then added 0.1ml bacterial culture suspension. In control sets, DMSO (in place of the plant extract) was used in the medium in appropriate amount. Culture tubes were incubated for 24 hours at 30^{o}C.

After incubation, sterile disc of 6mm (Himedia) were dip in to the broth (treated as well as control separately), disc were

aseptically inoculated on the agar surface of the nutrient agar medium in plates. Inoculated Petriplates were incubated at 30°C and the observations were recorded after 24 hours. Percentage of bacterial growth inhibition (BGI %) was calculated per formula.

$$BGI\ (\%) = \frac{dc - dt}{dc} \times 100$$

Where,

dc= diameter of control
dt= diameter of test

Results and Discussion

Some bacterial strains were drug resistance for some antibiotic *viz.*, *Klebsiella pneumoniae* was resistance for many drugs as Lincomycin and Oleandomycin. *Pseudomonas aeruginosa* was resistance for many drugs as Tobramycin, Lincomycin and Oleandomycin (Table 1). The result of antibacterial activity of Pomegranate (*Punica granatum*) fruit peel was determined by disc diffusion method. All the extract of Pomegranate showed the antibacterial activity against all the pathogenic bacterial strains. The Methanol extract of Pomegranate fruit peel showed maximum growth inhibition 85.71% against *Klebsiella pneumoniae* and also showed minimum growth inhibition - 10.29% against *Bacillus subtilis*. The Ethanol extract of Pomegranate fruit peel showed maximum growth inhibition 77.45% against *Pseudomonas aeruginosa* and also showed minimum growth inhibition 40.07% against *Pseudomonas fluorescens*. The Benzene extract of Pomegranate fruit peel showed maximum growth inhibition 82.35% against *Pseudomonas aeruginosa* and also showed minimum growth inhibition 55.93% against *Salmonella typhi* (Table 2). The MIC value of Methanol extract against *Klebsiella pneumoniae*, in Ethanol extract against *Pseudomonas aeruginosa*, and in Benzene extract against *Pseudomonas aeruginosa* showed in Table 3.

Fig.1 Effect of different extracts on bacterial growth

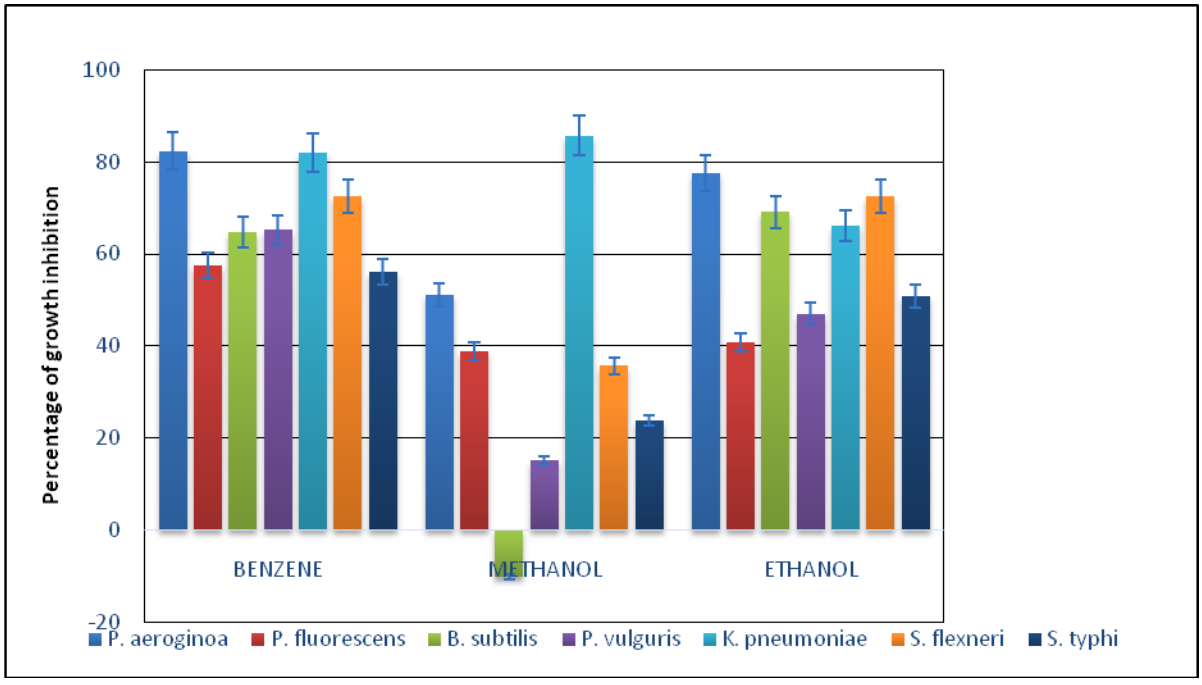

Table.1 Zone of inhibition by different drugs against bacterial strains (Sahrawat and Shahi, 2013)

Antibiotics	Zone of drug against bacterial strains (mm)						
	Proteus vulgaris	***Pseudomonas aeruginosa***	***Pseudomonas fluorescens***	***Bacillus subtilis***	***Klebsiella pneumoniae***	***Shigella flexneri***	***Salmonella typhi***
Tobramycin	21	-	-	14.2	23	19.5	10.0
Cephaloridine	17.5	12.02	-	-	14.5	-	8.50
Kanamycin	22.5	10.0	11.5	15.6	12.5	16.5	7.50
Lincomycin	-	-	-	-	-	-	-
Norfloxacin	13	-	-	17.5	18.5	18.5	17.5
Oleandomycin	-	-	-	-	-	-	-

Table.2 Antibacterial screening of different extract of Pomegranate fruit peel against pathogenic and drug resistance bacterial strains by Disc diffusion method

Pathogens	Percentage of growth inhibition at 100µl/ml		
	Methanol	**Ethanol**	**Benzene**
Pseudomonas aeruginosa	41.17%	48.52%	-32.03%
Pseudomonas fluorescens	1.85%	25.92%	40.74%
Bacillus subtilis	42.15%	62.74%	67.64%
Proteus vulgaris	25.07%	0%	-28.07%
Klebsiella pneumoniae	61.65%	80.04%	81.20%
Bacillus subtilis	-10.09	69.11	64.70
Shigella flexneri	35.63	72.41	72.41

Table.3 Minimum inhibitory concentration of Pomegranate fruit peel against bacterial strains

Bacterial strains	MIC against pathogens in µl/ml		
	Methanol	**Ethanol**	**Benzene**
Klebsiella pneumoniae	1.56×10^{-6}	-	-
Pseudomonas aeruginosa	-	12.5×10^{-3}	-
Pseudomonas aeruginosa	-	-	6.25×10^{-4}

As the present study on the antimicrobial activity of Pomegranate extract on the several human pathogenic bacteria (Fig. 1). The study shows that the various extract of Pomegranate can successfully control these kinds of bacteria as the other researchers have revealed that pomegranate peel is a rich source of tannins, flavonoids and other phenolic compounds (Li *et al.*, 2006). Antioxidant and antibacterial properties of pomegranate peel in in-vitro model systems have been reported (Negi and Jayaprakasha, 2003; Reddy *et al.*, 2007; Opara *et al.*, 2009; Alzoreky 2009). As the study says that Benzene extract of Pomegranate fruit peel can inhibit the population of both *Pseudomonas aeruginosa* and *Salmonella typhi* respectively which is already shown by many scientist like Lansky, 2005; Al-Zoreky, 2009; Gould *et al.*, 2009; Machado *et al.*, 2003; Opara *et al.*, 2008 in their study which was on antimicrobial activity of pomegranate has been documented with different extracts and the potential therapeutic applications of the antimicrobial

properties of pomegranate components have been investigated in human and murine models (Jurenka, 2008; Basu, 2009; Aviram *et al.*, 2008; Betanzos *et al.*, 2011; Sumner *et al.*, 2005; Rock *et al.*, 2008; Rosenblat *et al.*, 2006; Shukla *et al.*, 2008). However, FPJ antimicrobial activity has not been explored in spite of its high content of antioxidants.

Overall it can concluded that Pomegranate (*Punica granatum* L.) peel is a good source of antibacterial compounds against pathogenic and drug resistance bacterial strains and it can be use as drug against pathogens. The study reveals that different extraction with different solvents has antimicrobial activity and it should be further investigated.

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How to cite this article:

Alka Chaudhary and Siddarth Nandan Rahul. 2017. Antibacterial Activity of *Punica granatum* (Pomegranate) Fruit Peel Extract against Pathogenic and Drug Resistance Bacterial Strains. *Int.J.Curr.Microbiol.App.Sci.* 6(12): 3802-3807. doi: https://doi.org/10.20546/ijcmas.2017.612.437