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ANTIBACTERIAL PROPERTIES OF *PUNICA GRANATUM* PEELS

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ABSTRACT: The main aim of this study is that due to increasing concerns about the development of antimicrobial resistance among pathogenic bacteria, so alternative strategies are sought that do not use antibiotics to reduce pathogenic bacteria from foods and patients. Plants have been in use for thousands of years to conserve food and treat health diseases. The pericarp (peels) of *Punica granatum* has been commonly employed as a crude drug in Indian traditional medicine for treatment of diarrhea as well as for use as an antihelminthic, diuretic, stomachic, cardiotonic. Antibacterial properties of *Punica granatum* pericarp (peels) extracts (hot aqueous, methanolic and ethanolic) were evaluated against *E.coli, P.aeruginosa and S.aureus* using agar well diffusion method. Hot aqueous, methanolic and ethanolic extracts of *Punica granatum* pericap show an average inhibitory zone diameter of 23.3, 22.3 and 24.5mm respectively which indicates that ethanolic extract shows best result having ZOI greater than that of the standard antibiotic Tetracycline (20.1mm). Ehanolic extract of *Punica Granatum* has lowest MIC of 1.45 µg/ml showing that it is most effective as compared to MICs of other extracts.

Key Words: Antibacterial Properties, Antibiogrm analysis, Minimum inhibitory concentration.

Abbreviations: MIC= Minimum inhibitory concentration, *E. Coli* = *Escherachia coli*, *P. aeruginosa*= *Pseudomonas aeruginosa*, *S. aureus* = *Staphyllococus aureus*, µg/ml = micrograms per microliters, mm = millimeter, ZOI= Zone of inhibition.

INTRODUCTION

An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi, protozoans, etc. On the basis of mode of action, antimicrobials are classified into two broad categories as Microbicidal that kill microbes without leaving any option for their survival and Microbistatic that cease all the metabolic activities of microbes that are important for their survival so they are called as growth inhibitors of microbes. The history of antimicrobials begins with the observation of Pasteur and Joubert who discovered that one type of microbe could prevent the growth of other. That growth inhibition was due to secretion of a compound that later got called as Antibiotic. Nowadays the term antibiotics is not confined to secretions of microbes only but also includes all those synthetic drugs that help body to get rid of any bacterial infection. The discovery of antimicrobials like Penicillin and Tetracycline paved way for better health of people in the world by curing diseases like Gonorrhea, Strep throat and Pneumonia.

Why Herbal Antimicrobials?

The widespread use of commercially available antimicrobials led to the consequence of emergence of antimicrobial resistant pathogens that ultimately led to the threat to global public health. Since 1980 the introduction of new antimicrobials has declined due to the huge expense of developing and testing new drugs. All commercially available antibiotics with prolonged use may have negative effect on human health because they kill gut flora, so human beings need to take probiotics to replace the killed gut flora. All the above points make a clear way for herbal antimicrobials. The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The use of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. Peels of some plants as *Punica granatum* (having antibacterial properties) which are generally treated as wastes are true antibiotics as they are available for no cost, have no side effects and the most important benefit is that antibiotic resistant pathogens will be easily killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and resistant to them.

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 [2-5]. All the compounds of pomegranate peels are reported to have therapeutic properties. Extracts of peels of pomegranate show antibacterial property against bacterial strains of *E. coli, P. aeruginosa and S. aureus*. The aim of the present study was to decipher the antibacterial properties of pericarp (peels that are considered as wastes) of *Punica granatum*, so that they could be used as efficient antimicrobials in near future.

MATERIALS AND METHODS

Plant Materials

Fruit pericarp of pomegranate was collected from a juice shop (Vijay Juice Corner, Gomtinagar, Lucknow). Peels were then cut into smaller pieces and then first washed with tap water followed by washing with distilled water. It was than dried under sunlight until water droplets got completely evaporated. Pericarp was then kept in hot air oven for 3-4 days so that it could get dried. Dried pericarp was then taken for grinding by the help of mixer grinder. Then powdered form of plant sample was than used throughout the study.

TEST ORGANISMS:

Microbial strains of *Staphylococcus aureus* (Gram positive), *Escherichia coli, Pseudomonas aeruginosa* (Gram negative) were provided by MRD Life Sciences which they availed from IMTECH Chandigarh. They were subcultured and used throughout the studies.

EXTRACTION PROCEDURE:

The powdered pericarp was dissolved in different solvents. The solvents used were non polar as well as polar (methanol, ethanol and water). Polar-5 gm of ground Pericarp was added to 50ml of hot boiling water and left in hot water bath for an hour at 70^{o}C so that secondary metabolites got completely extracted. The extract was then filtered with the help of whatman no-1 filter paper and kept in hot air oven for drying. Then dried extract was dissolved in double volume of DMSO (dimethyl solphoxide) so that concentration of sample gets 500 mg/ml.Similarly the ethenolic and methenolic extraction was carried out wherein 5 gm of dried powder was added to 50 ml of 80% methanol and 70% ethanol and kept in dark for 3-4 days , filtered and the filterate was dried to get the antimicrobial which was further dissolved in double volume of the extract thus making the final volume of the extract to 500 mg/ml.

SCREENING OF THE EXTRACTS FOR ANTIBACTERIAL ACTIVITY

Antibacterial activity was assessed by Agar well diffusion method of Kirby Bauer wherein Nutrient agar plates were prepared and were spreaded with 20ul of the available pathogenic cultures. Wells of 8 mm diameter were bored using sterile borer. Wells were loaded with antimicrobial, tetracycline as standard and distilled water as control and were incubated at 37^{o}C for 24 hours.

MINIMUM INHIBITORY CONCENTRATION

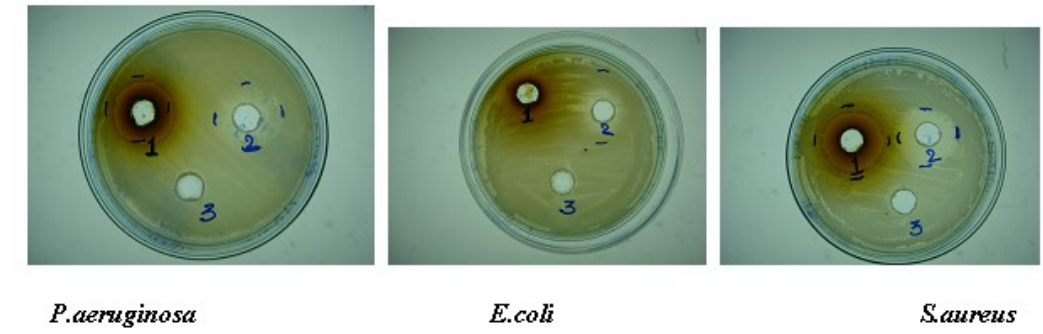
MIC of the antimicrobial extracts was also determined using broth serial dilution technique wherein the antimicrobial was diluted serially in a series of test tubes containing nutrient broth and they were loaded with the respective pathogen against which MIC was to be calculated. The tubes were incubated and than growth of the pathogen was detected using spectrophotometer at 600 nm. Concentaration in the tube where growth increased drastically was stated as Minimum inhibitory concentration.

RESULTS

The development of drug resistance in human pathogens against commonly used antibiotics necessitated a search for new antimicrobials of mainly plant origin. The antibacterial screening of various extracts of *Punica granatum* showed good results as illustrated in the Table -1 and Figures 1-3.

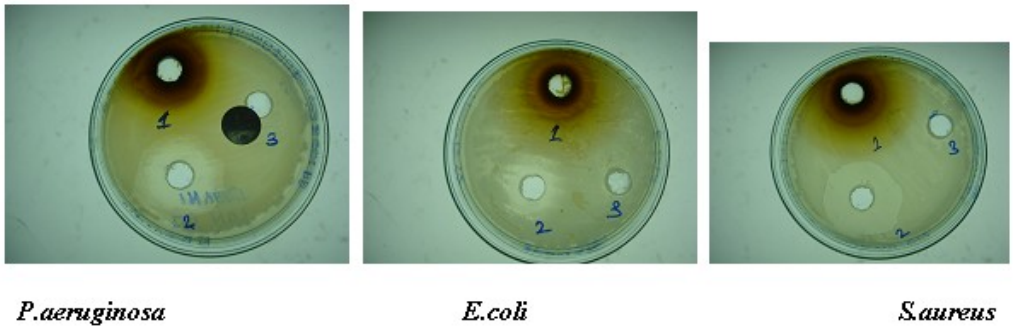
Table- 1: Antibacterial Screening of *Punica granatum.*

Test Organisms	HOT AQUEOUS EXTRACT		METHANOLIC EXTRACT		ETHANOLIC EXTRACT	
	ZOI BY Sample (In mm)	ZOI BY Tetracycline (In mm)	ZOI BY Sample (In mm)	ZOI BY Tetracycline (In mm)	ZOI BY Sample (In mm)	ZOI BY Tetracycline (In mm)
S.aureus	25.5	22	22.5	24	25.5	23
E.coli	22.5	26.5	21	25	22.5	19.5
P.aeruginosa	22	22	23.5	25	25.5	21.5



Note: Well Diameter= 8mm: 1= Sample, 2= Tetracycline, 3= distilled water

Figure1: Antibiogam of hot aqueous extract against *P.aeruginosa, E.coli, S.aureus* respectively



Note: Well Diameter= 8mm: 1= Sample, 2= Tetracycline, 3= distilled water

Figure 2: Antibiogam of methanolic extract against *P.aeruginosa, E.coli, S.aureus* respectively

P.aeruginosa *E.coli* *S.aureus*

Note: Well Diameter= 8mm: 1= Sample, 2= Tetracycline, 3= distilled water

Figure 3: Antibiogam of ethanolic extract against *P.aeruginosa, E.coli, S.aureus* respectively

DISCUSSION

Nearly 80% of the world populations depends on the traditional medicine for primary health care, mainly including the use of natural products [6]. Researchers have extensively studied the biological properties of *Punica granatum* and their results showed that this plant is ethno medically valuable [7]. *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 [8-10]. In the current study the hot aqueous, methanolic and ethanolic extracts of *Punica granatum* showed Zone of inhibition of atleast 22mm against *P.aeruginosa* which was greater than that of Tetracycline 21 , 21mm against *E.coli* which was a little lesser than that of Standard (25mm) and 22.5mm against *E.coli* which was greater than that of standard Tetracycline (19.5mm) respectively. The antibacterial activity of peels of *Punica granatum* may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against both gram+ve and gram –ve bacteria. Ethanolic extracts exhibited higher degree of antibacterial activity as compared to that of other extracts tested against bacteria that cause gut infection, stomachache, diarrhea. [11] reported that *P.granatum* contains large amount of tannins (25%) and antibacterial activity may be indicative of presence of secondary metabolites. The ethanolic extract of *P. granatum* showed some extent of antibacterial activity against *E. coli* [12] and *S. aureus* [13].

CONCLUSION:

In the present study an attempt has been made to decipher the antimicrobial activity of peels of *Punica granatum* (which are generally treated as wastes). Peels of *Punica granatum* are reported to have polyphenols, tannins, flavonoids and anthocyanins (Cyanidins, delphinidins) as bioactive compounds in previous studies. All the three extracts have antibacterial activity against bacterial strains (*E. coli, P. aeruginosa, S. aureus*).

After further purification and characterization of the active metabolites present in *Punica granatum* followed by a detailed study of toxicity and pharmacological effects of the compound, the peel extracts of pomegranate may be used as remedy against various diseases without any side effects.

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