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# Phytochemical Analysis and Antibacterial Activity of *Punica granatum* L. Rind Extracts on Common Enteric Pathogens

**Priti Uchgaonkar* and Neha Dahiya**

Department of Biotechnology and Bioinformatics, Plot No. 50, Sector 15, Pad. Dr. D. Y. Patil University, C.B.D. Belapur, Navi Mumbai - 400 614, India.

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**The present study was undertaken with an objective to find out the antibacterial activity of *Punica granatum* rind extracts against organisms causing enteric infections. Aqueous, methanolic and acetone extracts of pomegranate rind were prepared and their activity was studied on pathogenic strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Shigella flexneri* using well diffusion method. All the three extracts showed varying levels of antibacterial activity. Acetone extracts exhibited highest antibacterial activity on all the pathogenic strains as compared to aqueous and methanolic extracts. Phytochemical analysis of all the extracts revealed that the antimicrobial activity is due to the presence of phenols and related compounds.**

**Key words:** *Punica granatum*, Enteric infections, Antibacterial activity, Phytochemicals.

Infectious diseases are major causes of morbidity and mortality in the developing worlds accounting for about 5.8 million deaths each year in infants and in children[1,2]. Most of the pathogens causing enteric infections have developed resistance to commonly prescribed antibiotics. The constant development of drug resistance in human pathogens has resulted in the advancement of "alternative medicine" which includes a search for new antimicrobial substances from natural sources like plants and microbes[3].

* To whom all correspondence should be addressed.

*Ayurveda* is one of the most important forms of alternative medicine available in India. It involves the use of plants and plant products which are valuable sources of natural products used for maintaining human health[4]. The use of vegetables and fruits like garlic, pomegranate, and white radish with known antimicrobial activity can be of great significance in the treatment of infections[5]. Different parts of plants, herbs, and spices have been used for prevention of infections due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active constituents found in many fruit juices and fruit peels[6].

*Punica granatum* L. (Pomegranate) belonging to family *Puniaceae* has long been esteemed as food and medicine and is a diet in convalescence for a person suffering from diarrhea[7]. It is used in *Siddha*, *Ayurveda* and *Unani* medicine especially for treatment of gastrointestinal diseases. Pomegranate has been

considered important since prehistoric times as an agency of longevity. Hindu physicians use flowers and rind of fruit combined with aromatics such as clove, cinnamon, pepper etc as a bowel astringent in diarrhea[8]. In addition to its ancient uses, pomegranate is used in several systems of medicine for a variety of ailments. Pomegranate juice and peel provide protection against hepatotoxicity, Methicillin Resistant *Staphylococcus aureus*, and genital *Herpes* virus[9, 10]. It is used as an astringent, and in dysentery, colic pain, dyspepsia, inflammation, and bleeding disorders including piles[11].

Therefore, the aim of the present work was to investigate the antimicrobial activity of pomegranate rind on enteric pathogens along with an analysis of its phytochemical constituents.

## MATERIAL AND METHODS

### Plant material

Fresh pomegranate fruits were collected from the local markets of Vashi, Navi Mumbai. The fresh fruits were thoroughly washed with water and cleaned. The rind was separated from the fruit and shade dried at room temperature. The completely dried rind was finely powdered and used for solvent extraction.

### Preparation of extracts

### Preparation of aqueous extracts

15g of powder was macerated with 50ml of sterile distilled water using mortar and pestle for 15 minutes. The macerate obtained was filtered through double layered muslin cloth followed by centrifugation at 3500rpm for 30 minutes. The clear supernatant was considered as aqueous extract and stored in clean dry at 5° C until further use[12].

### Preparation of solvent extracts

50g of shade dried powder was filled in the thimble and extracted successively with 200ml of methanol using a soxhlet extractor for 48hrs. The solvent extracted was concentrated under reduced pressure using a rotary evaporator and stored in clean dry vials at 5° C until further use. The acetone extract was prepared in the above manner using acetone as the extracting solvent. All the extracts prepared were subjected to phytochemical analysis and antimicrobial activity assay[13].

### Phytochemical analysis

Phytochemical tests for bioactive constituents were carried out on small portions of all the three extracts using standard phytochemical procedures. Benedicts tests was used for reducing sugars, frothing test for saponins, ferric chloride solution test for tannins, chloroform - sulphuric acid test for triterpenes, NaOH-HCl test for flavonoids and 4-AAP test was used for phenols[14].

### Antimicrobial activity assay

Antimicrobial activity assay was conducted against pathogenic strains of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Shigella flexneri*. These cultures in their pure form were obtained from MGM pathology laboratories, Vashi. Sterile Nutrient agar medium was used as basal media for the growth of these bacteria. Nutrient agar plates were prepared by pour plate method using 20ml of nutrient medium. The sterile molten medium was cooled to 45° C and mixed thoroughly with 1.0 ml of growth culture of concerned test organism (1x $10^8$ cells / ml). This was then poured into sterile petri plates and allowed to solidify. Wells of 6mm size were punched with sterile cork borer and 100 µl of each of the extract was added aseptically to each well. The plates were incubated overnight at 37° C and observed for zones of inhibition. Respective pure solvents (sterile distilled water, methanol and acetone) served as negative control[15].

## RESULTS AND DISCUSSION

### Phytochemical analysis

The results of qualitative phytochemical analysis of *Punica granatum* rind extracts are presented in Table 1. All the extracts reveal the presence of tannins. Tannins have been found to form irreversible complexes with proline rich proteins resulting in inhibition of protein synthesis[16]. Herbs that exhibit the presence of tannins as their main component are astringent in nature and used for treatment of intestinal disorders[17] Along with tannins, phenols and triterpenes were present in methanolic and acetone extracts whereas flavonoids were present in aqueous and acetone extracts. Reducing sugars and saponins were found exclusively in aqueous extracts and acetone extracts respectively. The

presence of these phytochemicals attribute for the antibacterial activity of *Punica granatum* rind extracts.

**Antimicrobial activity assay**

Antibacterial activity in the extracts of *Punica granatum* fruit rind was evaluated against enteric organisms known to cause gastrointestinal infections. Three different solvents namely methanol, acetone and distilled water were used for preparing extracts. The antibacterial potency of these extracts was assessed by the presence or absence of inhibition zones and zone diameters (mm). Of the three extracts selected for study, acetone extracts exhibited maximum antibacterial activity against all the test organisms (Table 2). All the phytochemicals considered in the present study were found present in acetone extracts. This could be the reason for acetone extract exhibiting highest antimicrobial activity as compared to aqueous and methanolic extracts. Aqueous extracts were found to be effective against Gram positive organisms like *Staphylococcus aureus* whereas no inhibition was observed against Gram negative organisms like *Escherichia coli* and *Salmonella typhi*. A small zone of inhibition was observed against *Shigella flexneri* using aqeuous extract. Methanolic extracts gave best results against *Shigella flexneri* as compared to acetone extracts.

The results of the present study reveal the fact that organic solvent extracts (methanolic and acetone extracts) exhibit greater antimicrobial activity as compared to aqueous extracts. These observations can be rationalized in terms of polarity of the compounds being extracted by each solvent and by their ability to diffuse in different media used for essay[18]. Preliminary results of this study indicate that *Punica granatum* rind has high potential of antimicrobial activity. This study could lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

**Table 1.** Phytochemical analysis of *Punica granatum* rind extracts

| Phytochemical to be tested | Test used | Aqueous extract | Methanolic extract | Acetone extract |
|---|---|---|---|---|
| Reducing sugars | Benedicts test | + | - | - |
| Tannins | Ferric chloride test | + | + | + |
| Saponins | Frothing test | - | - | + |
| Triterpenes | chloroform - sulphuric acid test | - | + | + |
| Phenolic compounds | 4-AAP test | - | + | + |
| Flavonoids | NaOH - HCl test | + | - | + |

**Key:** +: present, -: absent

**Table 2.** Antimicrobial activity of *Punica granatum* rind extracts

| Test organisms | Zone of inhibition diameter (mm) | | |
|---|---|---|---|
| | Aqueous extract | Methanolic extract | Acetone extract |
| *Staphylococcus aureus* | 12.0 | 10.0 | 20.0 |
| *Escherichia coli* | 0.0 | 7.0 | 10.0 |
| *Salmonella typhi* | 0.0 | 5.0 | 15.0 |
| *Shigella flexneri* | 5.0 | 25.0 | 20.0 |

Results presented are the average values of triplicates.

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