Investigation on Antimicrobial Activity of *Punica granatum* Linn. Acetone Peel Extract

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**Abstract**

The present study evaluated the antibacterial activity of *Punica granatum* Linn. acetone peel extracts against human pathogenic bacterial strains and antifungal activity was evaluated against plant pathogenic fungus *Pyricularia oryzae*. Different concentrations (10, 20 and 30 mg/mL) of the acetone peel extracts were tested against pathogenic bacterial strains namely *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Antibacterial activity was measured using diffusion disc plates on agar. Inhibition diameters were measured after incubation for 24 to 48 h at 37°C. Blanks of solvent processed in the same way were also tested for antibacterial activity. The antifungal activity was carried out by using five different concentrations (50, 100, 150, 200 and 250 mg/mL) of *P. granatum* acetone peel extract against the test plant pathogenic fungus *Pyricularia oryzae*. Acetone peel extract of *Punica granatum* showed significant activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *B. cereus* in all the three concentrations tested. Maximum inhibitory zone was found against *Bacillus cereus* (35±0.40 mm) where as minimum inhibitory effect was recorded against *B. subtilis* (7±0.15 mm) at 10 mg/mL concentration. It was noted that acetone peel extract inhibited the growth of test fungi with increase in the concentration of the extract. Maximum zone of inhibition (mycelial dia of 11±0.20 mm) was found in 250 mg/mL concentration whereas, lowest inhibition (mycelial dia of 69±0.20 mm) was found in 50 mg/mL concentration. The present study showed interesting antimicrobial activity and further characterization may be carried out to test acetone peel extracts for various therapeutic and pharmacological activities.

**Keywords:** *Punica granatum* Linn., acetone peel extract, antimicrobial activity, agar disc diffusion.

**Introduction**

Pomegranate (*Punica granatum* L.) is an ancient, beloved plant and fruit. Pomegranates are native to central Asia, but since the pomegranate tree is highly adaptive to a wide range of climates and soil conditions, it is grown in many different geographical regions including the Mediterranean basin, Asia, and California. Recent scientific findings corroborate traditional usage of the pomegranate as a medical remedy and indicate that pomegranate tissues of the fruit, flowers, bark and leaves contain bioactive phytochemicals that are antimicrobial, reduce blood pressure, and act against serious diseases such as diabetes and cancer. These findings have led to a higher awareness of the public to the benefits of the pomegranate fruit and consequently to a prominent increase in the consumption of its fruit and juice. 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. Peels of some plants as *Punica granatum* 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 become resistant to them (Khan and Haneel, 2011). Dahham *et al.* (2010) evaluated the antibacterial and antifungal activities of pomegranate peel extract (rind), seed extract, juice and whole fruit on the selected bacteria and fungi. The peel extract has shown highest antimicrobial activity compared to other extracts. Pai *et al.* (2011) investigated the antibacterial activity of Pomegranate rind extracts (alcoholic and aqueous) against various enteric pathogens. Most significant inhibitory effect was seen against *Shigella flexneri* and *Aeromonas hydrophila*. Tayel and El-Tras (2010) screened the antibacterial activity of pomegranate peel extract against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* and found significant inhibition compared to other investigators. In a study by Bhadbhade *et al.* (2011), ethanol and water extracts of pomegranate demonstrated inhibitory effects against *S. mutans* and *P. gingivalis*. 

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Chaitra et al. (2012) investigated on antimicrobial properties of leaf extracts of Punica granatum L. The methanolic extract inhibited Staphylococcus aureus, Bacillus cereus, Salmonella typhi and Proteus mirabilis, whereas, the chloroform, ethyl acetate and aqueous extracts exhibited moderate inhibitory effect against the test bacteria. Nitave and Patil (2014) evaluated the antibacterial and antifungal activity of ethanolic extract of Punica granatum peel on selected bacterial gram positive and gram negative and fungal cultures and found that the ethanol extracts of peel of Punica granatum may be utilized as a potential source of antimicrobial and antifungal agents. Keeping the above facts in mind this study evaluated the antimicrobial activity of acetone peel extract of P. granatum.

Materials and methods
Collection of plant material: Fresh fruits of Punica granatum were collected Tirupattur, Tamil Nadu, India and transported to laboratory (Fig. 1). The fruits were washed with running tap water, rinsed well in distilled water and exposed to drying at room temperature for about 5 min in open air.

Extraction of acetone peel extract of P. granatum for antimicrobial activity: Peel powder (0.2 g) of Punica granatum was ground well and extracted using 30 mL of 75% acetone, filtered, pellet was discarded and the supernatant is dried in a petri plate. Six mL of N-butanol and 3 mL of distilled water were mixed well and allowed to stand for 20 min until separation. Finally the acetone layer was separated using micropipette and used for further analysis.

Antibacterial activity of acetone P. granatum peel extract: Different concentrations (10, 20 and 30 mg/mL) of the acetone peel extracts were tested against pathogenic bacterial strains namely Bacillus cereus, B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. The bacterial cultures were grown in Mueller Hinton Agar and Broth (Himedia) (Lopez et al., 2001). Antibacterial activity was measured using diffusion disc plates on agar (Erturk et al., 2003). About 0.1 mL of each culture of bacteria was spread on agar plate surfaces. All bacterial strains were grown in Mueller Hinton Broth for 24 h at 37°C and plated on Mueller Hinton Agar for agar diffusion experiments. Paper discs (6 mm in dia) were placed on the agar medium to load different concentrations of peel extracts of Punica granatum (10, 20 and 30 mg/mL). Inhibition diameters were measured after incubation for 24 to 48 h at 37°C. Blanks of solvent processed in the same way were also tested for antibacterial activity.

Antifungal activity of acetone P. granatum peel extract: The antifungal activity was carried out by using five different concentrations (50, 100, 150, 200 and 250 mg/mL) of P. granatum acetone peel extract against the test plant pathogenic fungus Pyricularia oryzae.

Results and discussion
The use of plants for treating diseases is as old as the human civilization. The use of plants for curing diseases was inevitable as it is already proven by seeing the problems associated with synthetic antibiotics (Khan and Hanee, 2011). Different concentrations (10, 20 and 30 mg/mL) of the acetone peel extracts were tested against pathogenic bacterial strains namely Bacillus cereus, B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Antibacterial activity was measured using diffusion disc plates on agar. Table 1 lists the antibacterial activity of acetone peel extract of P. granatum. Acetone peel extract of Punica granatum showed significant activity against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and B. cereus in all the three concentrations tested. Maximum inhibitory zone was found against Bacillus cereus (35±0.40 mm) where as minimum inhibitory effect was recorded against Bacillus subtilis (7±0.15 mm) at 10 mg/mL concentration (Fig. 2). The present findings coincide with the recent research reports.

Fig. 1. Punica granatum in its natural habitat.

Each concentrations of about 0.5 mL were aseptically poured into the petriplate followed by the addition of 9.5 mL of melted PDA and were swirled gently to achieve thorough mixing of the contents. After the solidification of the media, one inoculum disc of the test fungus was aseptically inoculated upside down at the centre of the petriplate and incubated at 25±2°C for 7 d. Inhibition was assessed in terms of the colony diameter.
Table 1. Antibacterial activity of acetone peel extract of *P. granatum.*

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Zone of inhibition (mm)</th>
<th>Control (Solvent alone)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg/mL</td>
<td>20 mg/mL</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>8±0.20</td>
<td>10±0.10</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>7±0.15</td>
<td>7±0.20</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>25±0.25</td>
<td>28±0.32</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12±0.25</td>
<td>26±0.32</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>23±0.25</td>
<td>29±0.35</td>
</tr>
</tbody>
</table>

Table 2. Antifungal activity of acetone peel extract of *P. granatum.*

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Mycelial Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>69±0.20</td>
</tr>
<tr>
<td>100</td>
<td>56±0.15</td>
</tr>
<tr>
<td>150</td>
<td>47±0.10</td>
</tr>
<tr>
<td>200</td>
<td>23±0.15</td>
</tr>
<tr>
<td>250</td>
<td>11±0.20</td>
</tr>
</tbody>
</table>

Pai *et al.* (2011) investigated the antibacterial activity of Pomegranate rind extracts (alcoholic and aqueous) against various enteric pathogens namely *Vibrio cholerae,* Enterotoxigenic *E. coli,* Enteropathogenic *E. coli,* Enterotoxigenic *E. coli,* Salmonella and *Shigella* species along with few strains of Candida were used in the study. The results obtained were encouraging as the ethanolic extract showed greater zones of inhibition against the various enteric pathogens tested in comparison with the aqueous extract. Most significant inhibitory effect was seen against *Shigella flexneri* and *Aeromonas hydrophila.* The activities observed could be due to the presence of some of the secondary metabolites like, alkaloids, anthraquinones, sterols, glycosides, saponins, terpenes and flavonoids detected in the plant (Egharevba and Kunle, 2010). Ashok Kumar and Vijayalakshmi (2013) evaluated *in vitro* antimicrobial activity against bacterial strains *Staphylococcus aureus,* *Bacillus circulans,* *Klebsiella pneumoniae,* *Vibrio vulnificus* and *Salmonella typhi.* *Punica granatum* peel extracts showed higher antimicrobial potential, *Staphylococcus aureus* growth was completely inhibited at lesser concentration by the extracts. Parashar *et al.* (2014) evaluated the antimicrobial activity of various extracts prepared from pomegranate fruit peels and found that 80% methanolic extract of peels was a potent inhibitor for *Yersinia enterocolitica,* *Listeria monocytogenes,* *Staphylococcus aureus* and *Escherichia coli.*

The antifungal activity was carried out by using five different concentrations (50, 100, 150, 200 and 250 mg/mL) of *P. granatum* acetone peel extract against the test plant pathogenic fungus *Pyricularia oryzae.* It was noted that acetone peel extract inhibited the growth of test fungi with increase in the concentration of the extract. Maximum zone of inhibition (mycelial dia of 11±0.20 mm) was found in 250 mg/mL concentration whereas, lowest inhibition (mycelial dia of 69±0.20 mm) was found in 50 mg/mL (Table 2 and Fig. 3).

The present study coincides with the findings of Chaitra *et al.* (2012) who investigated methanolic extract of *P. granatum* and demonstrated antifungal activity against *Aspergillus niger,* *A. flavus,* *Trichophyton rubrum,* *Candida albicans* and *Cryptococcus* sp. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties. In a similar study, Ashok Kumar and Vijayalakshmi (2013) evaluated *P. granatum* peel extract against fungal strains *Candida albicans,* *Cryptococcus neoformans* and *Candida tropicalis* which also showed good inhibition.

**Conclusion**

The present study showed interesting preliminary antibacterial activity of *Punica granatum* Linn. acetone peel extracts against human pathogenic bacterial strains and antifungal activity against plant pathogenic fungus *Pyricularia oryzae.* Further characterization may be carried out to test the peel extracts for various therapeutic and pharmacological activity.
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References