In vitro antimicrobial agents of fruit peel extracts of *Punica granatum* L. and *Citrus sinensis* against many bacterial and fungal genera

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Abstract

Recently, natural products have been evaluated as sources of antimicrobial agents with efficacies against a variety of microorganisms. This study described the antibacterial and antifungal activities of fruit peel extracts of *Punica granatum* L. and *Citrus sinensis* on the selected bacteria and fungi including *Acidobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* and the fungal isolates used are *Fusarium graminearum* and *Candida albicans*. The peel extract of *Punica granatum* L. has shown highest antimicrobial activity compared to the peel extract of *Citrus sinensis*. Among the selected bacterial and fungal cultures, the highest antibacterial activity was recorded against *Acidobacter* sp. and *Pseudomonas aeruginosa* and among fungi high activity against *Fusarium graminearum* was recorded.

Keywords: antimicrobial agents, *Punica granatum*, *Citrus sinensis*

Introduction

The antimicrobial activity of plants had been received attention many years ago as one of the most effective mechanism for the control of microorganisms. Many studies have been attempted to evaluate the antibacterial activity of some plant extracts including: pomegranate (*Punica granatum* L.) fruit pericarp peels. The extracts of *Punica granatum* (Pomegranate) were shown to have promising antibacterial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Ashebir et al., 1999). *Punica granatum* peel is traditionally used to treat genital infections, mastitis, acne, folliculitis, piles, allergic dermatitis, tympanitis, scalds and also as an antioxidant (Baker et al., 1998). The constituents of *P. granatum* include gallicatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which have therapeutic properties (Bandow et al., 2003). The antibacterial activity of *Citrus sinensis* was evaluated on bacteria strains like *Escherichia coli*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *S. aureus* (Uchechi et al., 2010).

Peels of *Citrus* sp. and *Punica* sp. were commonly used for this purpose (Jayaprakasha et al., 2001; Prashanth et al., 2001; Kirbaslari et al., 2009; Ekwenye and Edeha, 2010). This work has been carried out to evaluate the antibacterial and antifungal activity of fruit peel extracts of *Citrus sinensis* and *Punica granatum* on some pathogenic bacteria including *Acidobacter* sp., *P. aeruginosa*, *S. aureus* and *Klebsiella pneumonia* and the fungal isolates used are *Fusarium graminearum* and *Candida albicans*.

Material and Methods

Preparation of the Plant extract

Firstly fresh fruit peels of *Citrus sinensis* and *Punica...
Granatum (500 g) were obtained (in order to prepare fresh extraction) from a public market in the city of Baghdad, Iraq. The peels were chopped and shade-dried at room temperature for 2 weeks and then grounded to a coarse powder for ease of extraction of active compounds. The powdered plant material (100 g) was packed into a Soxhlet apparatus and extracted with methanol for 4 h. The extract was filtered and the solvent was evaporated under reduced pressure using rotary vacuum evaporator, lyophilized into dry powder and kept in desiccator (Rehman et al., 2007; Dhiman et al., 2012). The following concentrations were prepared: 125, 250, 500, 1000 μg/ml of the crude extract for antimicrobial sensitivity testing.

Microorganisms and culture

Four bacterial strains and three isolate of fungi were procured from College of Science, Department of Biology. The isolates used were Acidobacter sp., Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumonia. The fungal isolates used were Fusarium graminearum and Candida albicans. The bacterial isolates were cultured on nutrient agar medium at 37°C and fungal isolates on potato dextrose agar medium at 28°C.

Screening of the extracts for antibacterial activity

Antibacterial activity was assessed by Agar well diffusion method of Kirby Bauer where in Nutrient agar plates were prepared and were spread with 20 μl of the available pathogenic cultures. Wells of 8 mm diameter were bored using sterile borer. Wells were loaded with different concentrations of the fruit peels extract of Punica granatum L. and Citrus sinensis (125, 250, 500, and 1000) μg/ml and distilled water as control and were incubated at 37°C for 24 h). While antifungal activity was assessed by mixing concentrations with medium and 5 mm disc of each fungus obtained from pure cultures were transferred at the centre of sterile petridishes (in triplicates) and incubated at 28°C for 6 days and then diameter of colony of fungus (mm) was measured.

Results and Discussion

Antibacterial activity assay of fruit peels extract of Punica granatum L. and fruit peels extract of Citrus sinensis were done on 4 isolates from all of (Staphylococcus aureus, Acidobacter sp., Pseudomonas aeruginosa, Klebsiella pneumonia) and Candida albicans. The results obtained demonstrated that fruit peels extract of Punica granatum L. was the most effective against bacteria and Candida albicans than the fruit peels extract of Citrus sinensis at concentrations (250, 500, 1000) μg/ml of Punica granatum L. extract showed closely related results and had effects on all bacterial isolates as well as the most effective one was (1000) μg/ml as shown in (Table 1).

While 1000 μg/ml of Citrus sinensis extract was the most effective (Table 2). The antibacterial activity of Punica granatum L. is due to the presence of tannin, mucilage and resin in their fruit peels. Whereas fruit peels of Citrus sinensis contain essential oils, acids and bitter resin (Al-Rawi and Chakravarty, 1988). Singh et al. (2002) also reported that extracts of Punica granatum peel in different concentrations were effective against S. epidermidis, S. aureus, S. mutans, S. sanguinis and S. salivarius. It is demonstrated that this antibacterial activity may be related to the presence of hydrolysable tannins and polyphenolics in the pomegranate extract specifically punicalagin and gallic acid (Kim et al., 2002; Reddy et al., 2007).

Ekwenye et al. (2010) found that the antibacterial activity of alcoholic and water extracts of Citrus sinensis had low potential for bacteria strains like Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus.
### Table 1. The antimicrobial activity fruit peels extract of *Punica granatum* L

<table>
<thead>
<tr>
<th>Isolates</th>
<th>125 µg/ml</th>
<th>250 µg/ml</th>
<th>500 µg/ml</th>
<th>1000 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidobacter sp.</td>
<td>1.5</td>
<td>2.0</td>
<td>6.0</td>
<td>10.0</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>2.0</td>
<td>3.0</td>
<td>8.0</td>
<td>11.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.0</td>
<td>2.0</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>0.0</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

### Table 2. The antimicrobial activity fruit peels extract of *Citrus sinensis*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>125 µg/ml</th>
<th>250 µg/ml</th>
<th>500 µg/ml</th>
<th>1000 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidobacter sp.</td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2.0</td>
<td>3.5</td>
<td>5.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em></td>
<td>0.0</td>
<td>0.0</td>
<td>2.0</td>
<td>4.5</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Results of the present study showed that the fruit peels extract of *Punica granatum* L. and *Citrus sinensis* against *Fusarium graminearum* were more effective especially at high concentrations (500, 1000) µg/ml (Figs. 1 and 2). These results were in agreement with many studies that revealed the antimicrobial agent of the fruit peels extract of *Punica granatum* L. and *Citrus sinensis* against many bacterial and fungal genera (Jayaprakasha et al., 2001; Prashanth et al., 2001; Kirbyslari et al., 2009; Ekwenye and Edeha, 2010).

**Conclusion**

From these results it can be concluded that the fruit peels of *P. granatum* are highly significant effect against *P. aeruginosa* and *Acidobacter sp.* than fruit peels of *C. sinensis* (P < 0.01). *Fusarium graminearum* was high sensitivity against the fruit peels extract of *Punica granatum* L. and *Citrus sinensis* that is due to its cell wall structure (Vasconcelos et al., 2003).

**Fig. 1.** The antimicrobial activity fruit peels extract of *Punica granatum* L. against *Fusarium graminearum* (Diameter of colony in mm)

**Fig. 2.** The antimicrobial activity fruit peels extract of *Citrus sinensis* against *Fusarium graminearum* (Diameter of colony in mm)

**References**


