Phytochemical Screening And Antibacterial Activity Of *Punica granatum* Fruit Rind Extracts

Hagir G. Abd Elaleem, Alsheikh A. Albasheer, Khadiga G. Abd Elaleem

**ABSTRACT**

**Background:** The present study was carried out at National Center for Research, Medicinal and Aromatic Plant Research Institute (MAPRI), Sudan. **Objective:** the objective of the study was to investigate the presence of various phytochemicals substance in the ethanolic and aqueous chloroform extracts of *Punica granatum* fruit rind and to evaluate the antibacterial activity of different extracts against gram positive bacteria (Staphylococcus aureus) and gram negative bacteria (Pseudomonas aeruginosa, Proteus vulgaris and Escherichia coli). **Results:** Natural products have been evaluated as sources of antimicrobial agents with efficacies against bacteria. This study described the antibacterial activities of pomegranate fruit rind on the selected bacteria. The phytochemical screening exhibited the presence of different component like Triterpenoids & Steroids, Alkaloids, Tannins, Saponins and Glycosides. Methanol extract exhibited highly pronounced activity (39,34, 32 and 31) mm inhibition zone against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Proteus vulgaris respectively. Chloroform extract obtained lowest activity against Proteus vulgaris, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli respectively. **Conclusion:** The various fruit rind extracts of *Punica granatum* have revealed Triterpenoids, Steroids, Flavonoids, Tannins, Saponins, Alkaloids and Glycosides. Methanol extract of pomegranate fruit rind showed highly antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris respectively, compared to methanol extract, chloroform and water extract expressed moderate effect to the same bacteria although chlorophorm extract obtained lowest activity against Escherichia coli, also consider as active antibacterial effects (more than 15 mm). Further research is required to isolate and characterize the active antibacterial molecules in pomegranate fruit rind. The extracts from pomegranate fruit skin possess strong antimicrobial activity against the bacteria under study, therefore this plant could be an important source of high antibacterial compounds. The results of present study nourish the traditional usage of *Punica granatum* plant fruit rind extracts which possess compounds with antibacterial potential that can be used as anti-bacterial agents as new drugs for the therapy of infectious diseases caused by the studied bacteria. Besides having high antibacterial activity, pomegranate fruit also have high flavonoid constitute and can be used as antioxidant resource.

**Key words:** Phytochemical, *Punica granatum*, extracts, chloroform, Aqueous, Pseudomonas aeruginosa, Staphylococcus aureus.

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**INTRODUCTION**

Medical efficiency of many medicinal plants has been proven by many traditional reports (Bharat and Parabia, 2010; Bouzouina et al., 2016; Kada., et al 2016 and Ehssan et al., 2016). The pomegranate, *Punica...
grannum is the predominant member of Punicaceae family. The pomegranate tree typically grows 12-pommeled-shaped, and crowned by the pointed calyx. The fruit contains numerous seeds separated by white, membranous pericarp and each is surrounded by small amounts of acidulous, red juice. Punica granatum L., is a native shrub of Asia and Mediterranean Europe that has a rich history of traditional use in medicine. For centuries, the barks, leaves, flowers, fruits, and seeds of this plant have been used medicines (Jayaprakasha et al., 2006). The potential curative properties of pomegranate are wide ranging and include medication and prevention of cancer cardiovascular disease, diabetes (Barathikannan et al., 2016). Other potential applications include infant brain ischemia, Alzheimer’s disease, male infertility, arthritis, and fatness, (Lad and Frawley, 1986; Caceres et al., 1987; Saxena and Vikram, 2004; Schubert et al., 1999; Lansky and Newman, 2007 and Katz et al., 2007). It is widely reported that pomegranate exhibits antiviral, antioxidant, anticancer, and antiproliferative activities (Bayizit et al., 2016; Kasliwal and Quadri, 2016; Faria et al., 2007; Adhami and Mukhtar, 2006). The medicinal value of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and tannins, (Simoes et al., 2009; Rangasamy and Namaskivayam, 2014). The present study was designed to investigate the presence of various phytochemical substances in the ethanolic, aqueous and chloroform extracts of Punica granatum fruit rind and to evaluate the antibacterial activity of different extracts against gram positive bacteria (staphylococcus aureus) and gram negative bacteria (Pseudomonas aeruginosa, Proteus vulgaris and Escherichia coli).

**Methodology:**

The methanol, chloroform and aqueous extracts of pomegranate fruit skin were prepared. Antibacterial activity of extracts was studied using Cup-plate method(Kavanagh, 1972)

**Plant and extracts:**

Pomegranate fruits were purchased from local market Khartoum, Sudan. The fruit skins were cleaned, dried in the shade, then three types of extracts were prepared as described below:

**Ethanolic and chloroform extract:**

The fruit rind of P. granatum was dried in hot air oven at 50 C for a week. The dried plant material was powdered using mixer grinder, and subjected to soxhlet extraction with ethanol and chloroform for 24 hours. The mixture was evaporated to dryness in a rotary flash evaporator and stored in refrigerators. The condensed extracts were used for preliminary screening of phytochemicals.

**Aqueous extract:**

The fruit rind powder was boiled in distilled water for about 25 minutes, kept in room temperature overnight and filtered. The filtrate was evaporated to dryness in a hot air oven and stored in a refrigerator. The condensed extracts were used for preliminary screening of phytochemicals.

**Preliminary phytochemical screening:**

The fruit rind extract was taken in concentration of 1mg/ml, tests were carried out for phytochemical screening according to the method described by Kumar et al., 2009.

**Test for Steroids and Triterpenoids:**

Liebermann Burchard test:

Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

**Test for Glycosides:**

Keller Killiani Test

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

**Test for Saponins:**

Foam Test – Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.
Test for Alkaloids:

*Hager's Test:*  
Test solution was treated with few drops of Hager’s reagent (saturated picric acid solution). Formation of yellow precipitate would show a positive result for the presence of alkaloids.

Test for Flavonoids:

*Alkaline reagent Test:*  
Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Lead acetate solution Test:

Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

Test for Tannins:

*Gelatin Test:*  
Test solution when treated with gelatin solution would give white precipitate indicating the presence of tannins.

Isolates Bacteria:

Clinical isolates of Pseudomonas aeruginosa ATCC27852, Staphylococcus aureus ATCC25923, Escherichia coli ATCC 2592 and Proteus vulgaris ATCC7380 were used in this study.

Antibacterial activity assay:

The assay for antibacterial activity for all extracts was tested by Cup-plate method (Kavanagh, 1972; Parekh and Channda, 2007a; b). Bacterial suspensions were cultured in peptone water for 24 h at 37 C° and 2ml of this culture was spread over Mueller – Hinton agar in Petri dishes. Wells (5mm diameter) were cut in agar plates and were filled with different extracts. The plates incubated at room temperature for 24 hour. The resulting zone of inhibition was measured after 24 h. Each treatment of isolates and antibacterial agent was repeated three times. Inhibition zones in mm (including disc diameter) around discs were measured. The antimicrobial activity was expressed as the diameter of inhibition zones produced by the extracts against test microorganisms. The isolate which showed a clear zone of inhibition more than 15 mm including the 5mm well size was considered sensitive and those with less than 15 mm as resistant.

Results:

The various fruit rind extracts of *Punica granatum* have revealed include Triterpenoids, Steroids, Flavonoids, Tannins, Saponins, alkaloids and Glycosides. The results of preliminary phytochemical analysis are shown in Table 1. Triterpenoids & Steroids, Flavonoids and tannins and Saponins were abundant in the Methanol extract.

<table>
<thead>
<tr>
<th>Chemical tests</th>
<th>Extract type</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Triterpenoids &amp; Steroids</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Test for Alkaloids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Test for Flavonoids</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Test for Tannins</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Test for Saponins</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Test for Glycosides</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

+= indicates presence of phytochemicals and -= indicates absence of phytochemicals. +++= shows high concentration. ++= shows moderate concentration.

The antimicrobial efficacy of *Punica granatum* fruit rind against different bacterial strains was evaluated by determination of the zones of inhibition. The results of the antibacterial activity of the all extracts were presented in Table 2.

methanol extract of fruit rind contains more constituents (47 g) followed by chloroform and water (20 g and 17g) respectively. The fruit rind of *Punica granatum* were analyzed against different bacterial pathogens namely Staphylococcus, Proteus vulgaris, Pseudomonas aeruginosa and Escherichia coli. All extracts showed
potential activity against the selected bacteria. Among the pathogens selected for this study, one was found to be gram positive and the other three were gram negative. After 24 hour the minimum inhibitory zone was measured. The zone of inhibition showed by *Punica granatum* fruit rind extract against all test bacteria ranged from (39-17 mm). A highest activity (39 mm) inhibition zone, was seen against Staphylococcus aureus with methanol extract, this finding in agree with (Kumar et al., 2013; Dahham et al., 2010; Sadeghian et al., 2011). Lowest activity (17 mm) was measured against Escherichia coli with Chlorophorm extract, also consider as active effects (more than (15 mm)). In general, the methanol extract showed highest activity against all bacteria under study as shown in Table 2, (Figure 1) and Figures (2,3,4 and 5). The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols.

<table>
<thead>
<tr>
<th>Solvent extract</th>
<th>Plant weight/g</th>
<th>Yield/g</th>
<th>Staphylococcus aureus</th>
<th>Proteus vulgaris</th>
<th>Pseudomonas aeruginosa</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (mm)</td>
<td>100</td>
<td>47</td>
<td>39</td>
<td>31</td>
<td>34</td>
<td>32</td>
</tr>
<tr>
<td>Chlorophorm (mm)</td>
<td>100</td>
<td>17</td>
<td>20</td>
<td>27</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Water (mm)</td>
<td>100</td>
<td>20</td>
<td>20</td>
<td>26</td>
<td>26</td>
<td>27</td>
</tr>
</tbody>
</table>

Diameter of Inhibition zone in mm
Less than 15 mm: Resistant
More than 15 mm: Susceptible

Fig. 1: Effect of different pomegranate fruit rind extracts on the bacterial cultures.

Fig. 2: Methanol and chloroform extracts against Staphylococcus aureus.

Fig. 3: Methanol and chloroform extracts against Pseudomonas aeruginosa.

Fig. 4: Methanol and chloroform extracts against Escherichia coli.

Fig. 5: Methanol and chloroform extracts against Proteus vulgaris.

Fig. (2,3,4 and 5): Sensitivity test of pomegranate fruit rind methanol and chloroform extracts against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris respectively.
Discussion:

Increase of antibiotic resistance as well as complication side effects of synthetic drugs have execute interest in the search for new antimicrobial agents of plant origin. Methanol extract of Punica granatum fruit rind contains more constituents (47 g) followed by chloroform and water (20g and 17g) respectively, this finding is in agreement with (Shahaby et al., 2016). The phytochemical screening in the present study has revealed the presence of triterpenoids, steroids, flavonoids, tannins, alkaloid, glycoside and saponin in the Punica granatum fruit rind with different solvent extract. Since fruit rind methanol extract contains more constituents it can be considered for further investigation. Triterpenoids & Steroids, Flavonoids and tannins and Saponins were abundant in the methanol extract. Further the presence of different phyto-contents may be responsible for the therapeutic properties of pomegranate. Methanol extract showed the highest activity against all bacteria in compare to aqueous and chloroform extract. P. granatum L. fruit rind extract were effective against all testing bacteria, previous studies obtained that Punica granatum peel extracts in different concentrations were effective against different bacterial strain (Naziri.,2012; Khaleel et al., 2016; Shahaby et al., 2016), also this result disagree with (Khaleel et al., 2016), reported that, the antibacterial activity with different solvent extract using n-hexane, methanol and ethyl acetate, demonstrate that, only ethyl acetate extract possessed antibacterial activity tested on the plant pathogenic bacteria, this may be due to difference on bacteria species and extract concentration.

Highest inhibition zone showed by methanol extract against Staphylococcus aureus (39 mm), this finding is in agree with (Kumar and Vijayalakshmi, 2013), they found that Punica granatum peel have potential effective against Staphylococcus aureus at lowest concentration. Followed by Pseudomonas aeruginosa (34 mm), and also agree with finding of (Sadeghian et al., 2013), they found that punica granatum methanol and water fruit extract is highly effective against gram positive Staphylococcus aureus (S. aureus) and negative Pseudomonas aeruginosa (P. aeruginosa). Followed by Escherichia coli (32 mm) and Proteus vulgaris with (31 mm) inhibition zone. Aqueous and chloroform extract exhibited moderate antibacterial activity inhibition zone compare to methanol extract, maximum inhibition zone induce by chloroform was (27mm) with Proteus vulgaris and minimum is (17mm) with Escherichia coli, lowest antibacterial activity was obtained by chloroform extract against Escherichia coli (17 mm). The result in agree with (Prashanth et al., 2001). They tested a number of extracts of pomegranate against a range of bacteria (S. aureus, E. coli, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis and Salmonella typhi), found antibacterial activity against all bacteria isolates under study.

Conclusion:

In the present study, we found that most of the biologically active phytochemicals were present in the methanol, aqueous and chloroform extracts of Punica granatum fruit rind. The various fruit rind extracts of Punica granatum have revealed Triterpenoids, Steroids, Flavonoids, Tannins, Saponins, alkaloids and Glycosides. The results of preliminary phytochemical analysis prevent that, Triterpenoids & Steroids, Flavonoids, tannins and Saponins were abundant in the methanol extract.

All extracts showed bactericidal activity with the selected bacteria. Methanol extract of pomegranate fruit rind showed highly antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris respectively, chloroform and water extract expressed moderate effect to the same bacteria, although chloroform extract obtained lowest activity against Escherichia coli, however consider as active antibacterial effects (more than 15 mm). In general, the methanol extract showed highest activity against all bacteria under study, this finding may be due to present of Triterpenoids & Steroids, Flavonoids, tannins and Saponins in abundant amount in the methanol extract. Further research is required to isolate and characterize the active antibacterial molecules in pomegranate fruit rind in order to develop pharmacognical treatment from pomegranate fruit rind extract. The extracts from pomegranate fruit skin possess strong antibacterial activity against the bacteria under study. Therefore this plant could be an important source of high antibacterial compounds. Besides having high antibacterial activity, pomegranate fruit also have high flavonoid constitute and can be used as antioxidant. The results of present study nourish the traditional usage of materials Punica granatum plant fruit rind extracts which possess compounds with antibacterial potential that can be used as anti-bacterial agents as new drugs for the therapy of infectious diseases caused by the studied bacteria.

REFERENCES


