

## Antimicrobial activity of methanolic and ethanolic crude extracts of carp, peel and seed of *Punica granatum* L.

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### Abstract

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*Punica granatum* L. (pomegranate) is an ancient fruit that is widely consumed as fresh fruit and juice. *In vitro* and *in vivo* studies have demonstrated that this fruit possesses antioxidant, antidiabetic, hypolipidemic, antibacterial, anti-inflammatory, and antiviral activities. In this study, the antimicrobial activities of two types of cultivars (sour and sweet) were tested. Therefore, methanolic and ethanolic extracts of different fruit parts (carp, peel, and seed) of *Punica granatum* L. were prepared and tested against four Gram-negative bacteria species, three Gram-positive bacteria species, and three fungal species using agar diffusion method as well as broth dilution method. The maximum value of the inhibition zone (40 mm) was obtained by ethanolic peel crude extract of sweet *P. granatum* against the Gram-positive *Micrococcus luteus*. The minimum inhibition zone (10 mm) was obtained, however, by an ethanolic crude extract of *P. granatum* against *Salmonella typhimurium*. The minimal inhibitory concentrations (MIC) of *P. granatum* crude extracts showed that methanolic peel crude of sour *P. granatum* had the lowest MIC value (10 µg/ml) against *Serratia marcescens*. In contrast, the highest MIC value (360 µg/ml) was in case of methanolic seed crude of *P. granatum* against *Micrococcus luteus*.

**Keywords:** *Punica granatum*; carp, peel; seed; antimicrobial; methanolic, ethanolic

### Introduction

For a long time, plants have played an important role in the treatment of diseases. Until recently, more than 80% of pharmaceutical drugs are derived from traditional medicines (WHO, 2000). The active compounds are, in most cases, secondary metabolites (like steroids, alkaloids, phenols, and tannins). These compounds are present in certain taxa (Balandrin et al., 1985) and have a substantial curing potential

of certain diseases (Iwu et al., 1999; Dzotam et al., 2018). Because of the considerable use of commercial drugs as an antimicrobial for the treatment of infectious diseases, uncontrolled acquired resistance and multiple drug-resistances of pathogenic bacteria became a challenging concern to health care, hospital, environment, and research (Evans 2002; Flora et al., 2019; Pallavali et al., 2019).

*Punica granatum* L. (pomegranate) is a small tree that produces fruit-bearing growing between three and seven

meters tall. The native land of pomegranate is Northern India and Iran. It has been cultivated widely throughout our region, including Iraq, Lebanon, Egypt, Saudi Arabia, Palestine, and Jordan (Abd Al-razaq, 2013). The fruit contains several parts: seed, peel, leaf, flower, and pericarp. Each part has an interesting pharmaceutical activity. For instance, the fruit has taken considerable attention because it's high antioxidant activity (Gil et al., 2000).

Additionally, juice, peel, and oil were found to be weak estrogenic and used in the treatment of menopausal symptoms (Larrosa et al., 2009). Pomegranate showed anticancer activities by using several parts (juice, peel, and oil), including interference with tumor cell proliferation, invasion, and angiogenesis, which may be associated with anti-inflammatory effects pomegranate. More than one application of treatment (as anticancer and chronic inflammation treatment that plays a necessary role in etiology) suggested because of pharmacological actions showed by all parts of pomegranate (Ephraim and Newman, 2007). The purpose of this study is to evaluate the antimicrobial activity of *P. granatum* L. cultivars (sour and sweet) growing in Jordan and to investigate the variation in the antimicrobial activity of different parts (carp, peel, and seed).

## Materials and Methods

### Plant material

*P. granatum* L. fruits were collected from Kufrsoum village – Irbid (32°42'3.65"N, 35°47'39.42"E) during the month of October. This village is well known for producing this type of fruits at the national level. Fruits were collected and grouped into two groups according to their taste as either sour or sweet.

### Tested bacterial species

Different bacterial species were tested with respect to their susceptibility to extracts of *P. granatum*. The tested bacteria include three Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, and *Micrococcus luteus* ATCC 9341) and five Gram-negative bacteria (*Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, *Serratia marcescens* ATCC 27117, *Escherichia coli* ATCC 25922, *Escherichia coli* DH10B), and one isolate of *Escherichia coli* isolated from a clinical sample was provided by King Abdullah University Hospital/ microbiology department. All strains except the clinical sample were provided from the laboratory of microbiology and biotechnology, Department of Biological Sciences, Yarmouk University-Jordan.

### Tested fungal species

Three fungal species were tested with respect to their susceptibility to extracts of *P. granatum*, including *Aspergillus niger*, *Penicillium digitatum*, and *Candida albicans*. These strains were also provided from the laboratory of microbiology and biotechnology, Department of Biological Sciences, Yarmouk University-Jordan.

### Extraction process

Three parts (carp, seed, and peel) of *P. granatum* were dried in the shade for two weeks. Then, they were ground to powder in liquid nitrogen. The powders were extracted with both absolute ethanol and methanol by soaking for 48 h. The solvents have been removed using a rotary evaporator under reduced pressure at temperatures below 45°C. The resulting crude extracts were stored at -20°C until used. Serial dilutions of extracts were prepared in distilled water (Machado et al., 2002).

### Antibacterial assay

#### Agar-well diffusion method

The different bacterial strains were inoculated into the nutrient broth, and on nutrient agar media, the resulting pure colonies were used to prepare bacterial suspensions. A loop full of the tested bacteria was used to inoculate nutrient broth and was incubated for 24 h at 30°C. The bacterial suspension turbidity was adjusted to 0.5 McFarland standards. One hundred microliters of each test organism were inoculated into solidified agar plates.

The antibacterial activity was investigated by the agar-well diffusion method (Allen et al., 1991). Four hundred  $\mu\text{g mL}^{-1}$  of each plant extract have been used for this test to evaluate antibacterial activity. The bacterial suspension (100  $\mu\text{l}$ ) was then smeared on agar plates with sterile glass-rod, 8 mm in diameter were created in the medium using a sterile cork borer. Each crude plant extract (400  $\mu\text{g mL}^{-1}$  concentration) were investigated.

Autoclaved distilled water was used as a negative control for the extracts, whereas standard antibiotics such as ampicillin (250  $\mu\text{g gmL}^{-1}$ ) were included as positive controls. The inoculated agar plates were incubated at 30°C for 48 h. Then, the diameter of the inhibition zone around each well was measured in millimeters (Ndukwe et al., 2006).

#### Minimum inhibitory concentration (MIC)

The tested bacterial strains were cultured in 10 ml of nutrient broth tube and incubated for 24 h at 30°C. After incubation, 10  $\mu\text{l}$  of each tube was added into a separate well of sterile 96 well micro-plate. The different volume of extracts was added to the different well. Also, the nutrient broth was

added into well up to 100  $\mu\text{l}$  final volume to get a concentration range from 0.1 to 0.001 mg mL<sup>-1</sup>. The microplate was incubated for 24 h at 30°C, and then 10  $\mu\text{l}$  of each well was inoculated onto a nutrient agar plate and incubated at 30°C for 24 h to verify the viability of bacterial cells (Pendleton et al., 2012).

### Antifungal activity assay

#### The agar well diffusion method

Each isolate of tested fungus was spread on the surface of complete media (Sucrose 30 g, Ammonium tartrate 5 g, Ammonium nitrate 1 g, Potassium phosphate (monobasic) 1 g, Magnesium sulfate (7 H<sub>2</sub>O) 0.48 g, Sodium chloride 1 g, Calcium chloride (2 H<sub>2</sub>O) 0.13 g Yeast extract 1 g) (CM) plate by completely streaking of an aliquot of 100  $\mu\text{l}$  spore suspension (1x10<sup>8</sup> spores mL<sup>-1</sup>) in radial patterns. Wells of 8 mm diameter were made in the medium, and each was filled with a particular concentration of crude extract. Control drug, benomyl 50%, and sterile distilled water have been used as a positive and negative control, respectively. The cultured plates were incubated for 3-5 days at 25°C  $\pm$ 1°. Inhibition zone diameter was measured in two directions at right angles to each other. Experiments were carried out in triplicates per treatment (Ndukwe et al., 2006).

#### Amended agar method

Different concentrations ( $\mu\text{g mL}^{-1}$ ) of the crude extracts from the *P. granatum* were amended on the surface of solidified complete media (CM) plates. Twenty microliters (20  $\mu\text{l}$ ) of conidiospore suspension (1x10<sup>8</sup> spores mL<sup>-1</sup>) from each of the isolates were placed and left as drops on the surface of the amended media and incubated at room temperature for three hours until the liquid became completely absorbed.

Along with each treatment, benomyl 50% was used as a positive control, 200  $\mu\text{l}$  of sterile distilled water was used as negative controls. The inoculated plates were incubated for 3-5 days at 25°C.

Colony diameter was determined by measuring the average radial growth of each tested isolate. The diameters of the growing colonies were measured in two directions, right angles to each other.

## Results

### Antimicrobial activity of methanolic extract of sour *P. granatum*

*In vitro* antimicrobial activities of methanolic extract of sour *P. granatum* parts (carp, peel, and seed) are presented in Figure 1. Methanolic peel extract showed the highest inhibitory effect against all studied microorganisms, while

the methanolic seed extract obtained from sour *P. granatum* showed the lowest inhibitory effect.

The presented data showed that the largest inhibition zone was observed in the carp methanolic extract obtained from the sour *P. granatum* against *Staphylococcus aureus* ATCC 29213, and the peel methanolic extract obtained from the sour *P. granatum* against *Pseudomonas aeruginosa* ATCC 27853, (Figure 1). However, the lowest inhibition zone was observed in the seed methanolic extract obtained from the sour *P. granatum* against *Salmonella typhimurium* ATCC 13311 and *Bacillus cereus* ATCC 11778 species. Moreover, the results showed that Gram negative bacteria (*Pseudomonas aeruginosa* ATCC 27853 and *Serratia marcescens* ATCC 27117) were more sensitive than Gram positive bacteria in regard to a methanolic extract obtained from the sour *P. granatum* (Figure 1). On the other hand, all *P. granatum* methanolic and ethanolic extracts showed no inhibitory effect against all *Escherichia coli* strains used in this study.

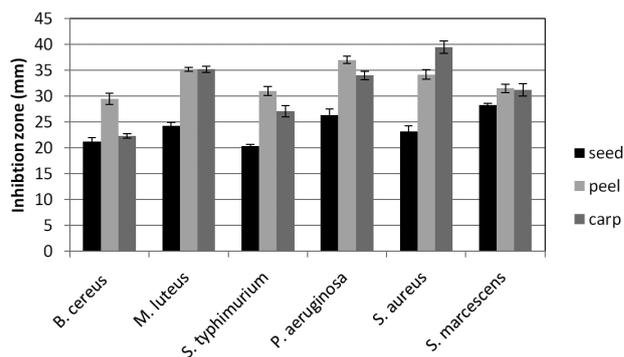
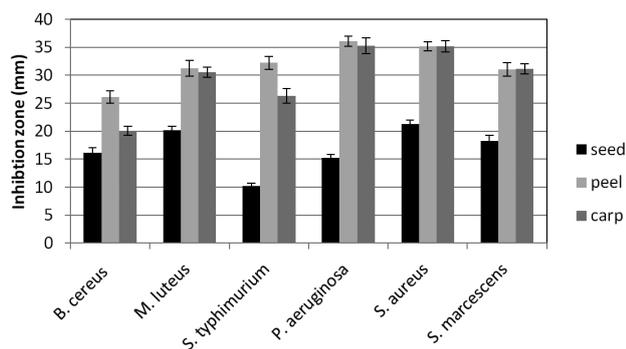


Fig. 1. Antibacterial activity of methanolic crude extracts of carp, peel, and the seed of sour *P. granatum*

### Antimicrobial activity of ethanolic extract of sour *P. granatum*.

*In vitro* antimicrobial activities of the ethanolic extract obtained from the sour *P. granatum* (carp, peel, and seed) results) revealed that the ethanolic peel extract showed the highest inhibitory effect against the studied microorganisms, while ethanolic seed extract obtained from sour *P. granatum* showed the lowest inhibitory effect (Figure 2).

On the other hand, the largest inhibition zone was observed in the peel and carp ethanolic extract obtained from the sour *P. granatum* against *Pseudomonas aeruginosa* ATCC 27853. However, the lowest inhibition zone was observed in the seed methanolic extract obtained from the sour *P. granatum* against *Salmonella typhimurium* ATCC 13311 and *Pseudomonas aeruginosa* ATCC 27853 species. Moreover, the results showed that Gram negative bacteria (*Pseudomonas aerugino-*



**Fig. 2. Antimicrobial activity of ethanolic crude extracts of carp, peel, and the seed of sour *P. granatum***

*sa* ATCC 27853 and *Serratia marcescens* ATCC 27117) were more sensitive than Gram positive bacteria in regard to the ethanolic extract of the sour *P. granatum* (Figure 2).

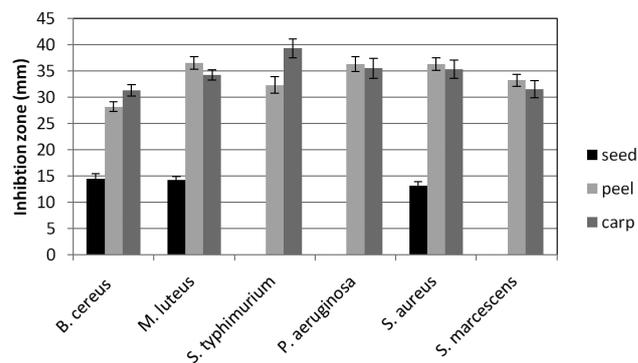
#### Antimicrobial activity of methanolic extract of sweet *P. granatum*

*In vitro* antimicrobial activities of methanolic extract of sweet *P. granatum* (carp, peel, and seed) results figure (3) show that methanolic peel extract obtained from sweet *P. granatum* demonstrate the best inhibitory effect against the studied microorganisms. In contrast, methanolic seed extract derived from sweet *P. granatum* showed the lowest inhibitory effect. However, methanolic seed extract derived from the sweet *P. granatum* showed no inhibitory effect against Gram negative bacteria.

On the other hand, the maximum inhibition zone was observed in the carp methanolic extract obtained from the sweet *P. granatum* against *Salmonella typhimurium* ATCC 13311 and peel methanolic extract obtained from sweet *P. granatum* against *Micrococcus luteus* ATCC 9341. However, the lowest inhibition zone was observed in the seed methanolic extract obtained from the sweet *P. granatum* against *Micrococcus luteus* ATCC 9341 and *Bacillus cereus* ATCC 11778 species. Moreover, results showed that Gram negative bacteria were more sensitive than Gram positive bacteria in regards to the methanolic (carp and peel) extracts obtained from the sweet *P. granatum* (Figure 3).

#### Antimicrobial activity of ethanolic extracts of sweet *P. granatum*.

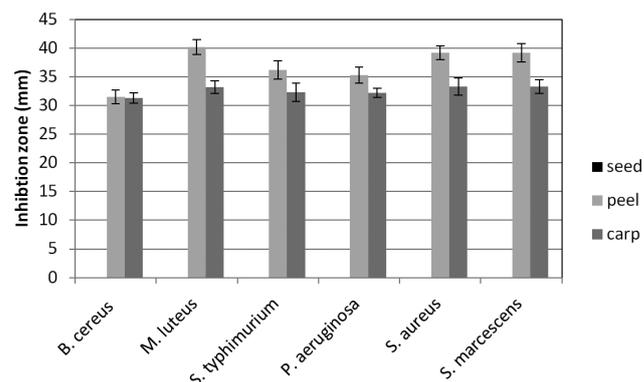
*In vitro* antimicrobial activities of the ethanolic extract obtained from the sweet *P. granatum* (carp, peel, and seed) results showed that the ethanolic peel extract derived from the sweet *P. granatum* led to the highest inhibitory effect against the tested microorganisms. In contrast, the ethanolic



**Fig. 3. Antimicrobial activity of methanolic crude extracts of carp, peel, and the seed of sweet *P. granatum***

seed extract obtained from the sweet *P. granatum* showed no inhibitory effect.

On the other hand, the largest inhibition zone was detected in the peel ethanolic extract obtained from the sweet *P. granatum* against *Pseudomonas aeruginosa* ATCC 27853 and *Serratia marcescens* ATCC 27117. However, the lowest inhibition zone was observed in the carp and peel ethanolic extract obtained from the sweet *P. granatum* against *Bacillus cereus* ATCC 11778. Moreover, the results showed that Gram positive bacteria (*Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 29213) were more sensitive than Gram negative bacteria in regard to the ethanolic extract obtained from the sweet *P. granatum* (Figure 4).



**Fig. 4. Antimicrobial activity of ethanolic crude extracts of carp, peel, and the seed of sweet *P. granatum***

#### Minimal inhibitory concentrations (MIC)

##### Methanolic crude extract of sour and sweet *P. granatum*

The minimal inhibitory concentrations (MIC) of the methanolic crude extract obtained from sour and sweet *P. granatum* were determined against different bacterial species. The results indicate that different extracts of sour and sweet *P.*

**Table 1. Minimal inhibitory concentration (MIC) [ $\mu\text{g mL}^{-1}$ ] of the methanolic crude extract of carp, peel, and the seed of sour and sweet *P. granatum***

Bacterial strain	Carp		Peel		Seed	
	Sour	Sweet	Sour	Sweet	Sour	Sweet
<i>Bacillus cereus</i> (ATCC 11778)	80	60	80	70	90	340
<i>Micrococcus luteus</i> (ATCC 9341)	40	30	30	35	80	360
<i>Salmonella typhimurium</i> (ATCC 13311)	60	80	80	70	90	NT
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	80	15	60	20	70	NT
<i>Staphylococcus aureus</i> (ATCC 29213)	30	30	40	20	80	260
<i>Serratia marcescens</i> (ATCC 27117)	15	15	10	25	50	NT

*granatum* showed variation in the values of minimal inhibitory concentrations (MIC) (Table 1). Methanolic peel crude extract showed the most potent inhibition against *Serratia marcescens* ATCC 27117 (MIC  $10 \mu\text{g mL}^{-1}$ ), and the methanolic carp crude extract exert the most potent inhibition against *Serratia marcescens* ATCC 27117 (MIC  $15 \mu\text{g mL}^{-1}$ ). In contrast, the lowest inhibition was obtained by seed crude extract against *Micrococcus luteus* ATCC 9341 (MIC  $360 \mu\text{g mL}^{-1}$ ).

Furthermore, the methanolic crude extract of the carp of the sweet *P. granatum* displayed almost the same MIC value against *Micrococcus luteus* ATCC 9341 ( $30 \mu\text{g mL}^{-1}$ ) and *Serratia marcescens* ATCC 27117 ( $10 \mu\text{g mL}^{-1}$ ). Moreover, the methanolic crude extract of the peel of the sweet *P. granatum* showed a similar MIC value ( $20 \mu\text{g mL}^{-1}$ ) against *Serratia marcescens* ATCC 27117 and *Staphylococcus aureus* ATCC 29213, and identical values ( $70 \mu\text{g mL}^{-1}$ ) against *Salmonella typhimurium* ATCC 13311 and *Bacillus cereus* ATCC 11778.

Furthermore, the methanolic crude extract obtained from the carp of the sour *P. granatum* demonstrated the same value of MIC against *Pseudomonas aeruginosa* ATCC 27853 ( $80 \mu\text{g mL}^{-1}$ ) and *Bacillus cereus* ATCC 11778. Moreover, the methanolic crude extract of the peel from sour and sweet *P. granatum* showed the same MIC values against *Salmonella typhimurium* ATCC 13311 ( $80 \mu\text{g mL}^{-1}$ ) and *Bacillus cereus* ATCC 11778. However, the methanolic crude extract of the seed from sour and sweet *P. granatum* showed the same

MIC values ( $80 \mu\text{g mL}^{-1}$ ) against *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 29213, and the same MIC value ( $90 \mu\text{g mL}^{-1}$ ) against *Salmonella typhimurium* ATCC 13311 and *Bacillus cereus* ATCC 11778 (Table 1).

#### **Ethanolic crude extract of the sour and sweet *P. granatum***

The minimal inhibitory concentrations (MIC) of the ethanolic crude extract obtained from sour and sweet *P. granatum* were determined against different bacterial species. Results indicate that the various extracts of the sour and sweet *P. granatum* vary in their Minimal inhibitory concentrations (MIC). Table 2 showed that the peel crude extract from the sweet *P. granatum* had the most potent inhibition against *Micrococcus luteus* ATCC 9341 (MIC  $10 \mu\text{g mL}^{-1}$ ). In contrast, the weakest activity was obtained from the seed crude extract from the sour *P. granatum* tested against *Salmonella typhimurium* ATCC 13311 (MIC  $240 \mu\text{g mL}^{-1}$ ).

Furthermore, seed extract from sour *P. granatum* showed almost the same MIC value against *Bacillus cereus* ATCC 11778 ( $70 \mu\text{g mL}^{-1}$ ) and *Micrococcus luteus* ATCC 9341. Moreover, the sour ethanolic crude extract of the carp showed the same MIC value against *Bacillus cereus* ATCC 11778 ( $70 \mu\text{g mL}^{-1}$ ) and *Salmonella typhimurium* ATCC 13311. Furthermore, the sour ethanolic crude extract of the peel showed identical MIC values ( $40 \mu\text{g mL}^{-1}$ ) against *Pseudomonas aeruginosa* ATCC 27853 and *Micrococcus luteus* ATCC 9341.

**Table 2. Minimal inhibitory concentration (MIC) [ $\mu\text{g mL}^{-1}$ ] of the ethanolic crude extract of carp, peel, and the seed of sour and sweet *P. granatum***

Bacterial strain	Carp		Peel		Seed	
	Sour	Sweet	Sour	Sweet	Sour	Sweet
<i>Bacillus cereus</i> (ATCC 11778)	70	80	80	80	90	NT
<i>Micrococcus luteus</i> (ATCC 9341)	30	20	40	10	90	NT
<i>Salmonella typhimurium</i> (ATCC 13311)	70	90	50	80	240	NT
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	50	70	40	55	150	NT
<i>Staphylococcus aureus</i> (ATCC 29213)	20	60	15	40	80	NT
<i>Serratia marcescens</i> (ATCC 27117)	25	30	20	15	60	NT

Moreover, sweet ethanolic crude extract of the peel demonstrated identical MIC values ( $80\mu\text{g. mL}^{-1}$ ) against *Salmonella typhimurium* ATCC 13311 and *Bacillus cereus* ATCC 11778.

#### Antifungal activity assay

The crude extracts and their liquid fractions were tested against three fungal species; *Candida albicans*, *Penicillium digitatum* and *Aspergillus niger* to evaluate their antifungal activity, results show no antifungal activity against the studied fungi.

#### Discussion

This study is interested in assessing the antimicrobial activity of sour and sweet pomegranate (*P. granatum* L.) cultivars, collected from Kufirsoum village – Irbid. The variation in antimicrobial activity of different parts (carp, peel, and seed) is also evaluated. Some studies have already shown that some of the common pomegranate cultivars have possessed antibacterial activity against several pathogenic and antibiotic-resistant pathogens. Prashanth and his colleagues (2001) reported that methanolic extracts of *P. granatum* fruit rind have activity against *S. aureus*, *E. coli*, *Bacillus subtilis*, and *Salmonella typhimurium*. This agrees with the findings of our study since methanolic extract of *P. granatum* revealed high activity against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Micrococcus luteus* ATCC 9341, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853 and *Serratia marcescens* ATCC 27117. In contrast, no activity against all strains of *Escherichia coli* was observed in his study. In addition, Al-Zoreky (2009) reported that the 80% methanolic extract of pomegranate peels was a potent inhibitor for *S. aureus* and *E. coli*. But in this study, methanolic extract of *P. granatum* have good activity against *Staphylococcus aureus* ATCC 29213, but no activity against *Escherichia coli* was observed.

This study showed that ethanolic peel extract of *P. granatum* has an inhibitory effect against *Salmonella typhimurium* ATCC 13311 and other Gram negative bacteria. This agrees with Choi *et al.* (2011), who investigated the *in vitro* antimicrobial activity of pomegranate peel ethanolic extract against sixteen strains of *Salmonella*. Several bacterial strains as *E. coli*, *S. aureus*, *Enterobacter* sp., *Bacillus* sp., and *Micrococcus* sp. were strongly inhibited by pomegranate extraction using *in vitro* antibacterial evaluation (Melendez and Capriles, 2006) that provide evidence for the presence of antibacterial compounds in *P. granatum*. The findings of this study confirm the effectiveness of pomegranate fruit in antimicrobial activity. Negi and Jayaprakasha (2003) extract-

ed pomegranate peels with different solvents and assayed them for antibacterial activity. Acetone, methanolic, and water extracts were evaluated against both Gram-positive and Gram-negative bacteria. The acetone extract showed the highest antibacterial activity, followed by methanolic and water extract. Results obtained in our study showed that methanolic extracts have high antibacterial activity.

On the other hand, minimal inhibitory concentration (MIC) was examined. Different extracts of *P. granatum* varied in their MIC values. Methanolic carp and peel crude extracts of *P. granatum* show the most potent inhibition against *Serratia marcescens* ATCC 27117 (MIC  $10\mu\text{g mL}^{-1}$ ). In contrast, the lowest inhibition was observed in methanolic seed crude extract of *P. granatum* against *Micrococcus luteus* ATCC 9341 (MIC  $400\mu\text{g mL}^{-1}$ ). Similar results were obtained by Sajjad *et al.* (2015), who evaluated the antibacterial activity of *P. granatum* peels extract. They found that methanolic extract of *P. granatum* has the maximum activity against *S. aureus* (MIC  $12.5\mu\text{g mL}^{-1}$ ).

The result showed no antifungal activity by all extracts of *P. granatum* against all tested fungal species. Similar results were obtained by Abdollahzadeh *et al.* (2010), who evaluated antifungal activity of *P. granatum* and they found that no activity of methanolic extract of *P. granatum* against *A. viscosus* and *C. albicans*. However, Siham *et al.* (2007) showed that *P. granatum* peels possess antifungal activity against *Candida albicans*, *Candida tropicalis*, and *Aspergillus niger*.

In general, carp and seed methanolic extract (either sour or sweet) showed higher antibacterial activity against tested bacteria than ethanolic extract. In contrast, peel ethanolic extract showed higher antibacterial activity against tested bacteria than methanolic extract.

In conclusion, the studied *P. granatum* plant extracts have excellent potential as an antibacterial activity that may be of use for development of pharmaceutical for the therapy of infections. The results of the study support the traditional medicinal systems of this plant. In general, the result showed the various antimicrobial activity of crude extracts species against tested bacteria. Such as dissimilarity between antimicrobial activities of extracts sour and sweet *P. granatum*. This study revealed that all Gram positive and Gram negative (except *E. coli*) bacteria were significantly inhibited using methanolic and ethanolic extracts of all parts of sour *P. granatum*. And this study showed that crude extracts of carp and peel of sweet *P. granatum* have antibacterial activity against all tested bacteria (except *E. coli*), but no activity by seed extract of sweet *P. granatum*. The result showed no antifungal activity by all extracts of *P. granatum* against all tested fungal species. This inhibitory potential can be used for the treatment of some infectious diseases caused by the

pathogenic agents. Carp, peel, and seed extracts of *P. granatum* May provide excellent sources for novel bioactive natural products that may serve as new pharmaceutical agents to address unmet therapeutic needs.

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