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# Antimicrobial activity of aqueous and methanolic extracts of pomegranate fruit skin

Ali Sadeghian[1], Ahmad Ghorbani[2,3], Ahmad Mohamadi-Nejad[4], Hassan Rakhshandeh[2,5*]

## Abstract

**Objective:** *Punica granatum*, commonly known as pomegranate, has emerged as a medicinal plant with potential antimicrobial activity. The present study was planned to evaluate this activity against both Gram positive *Staphylococcus aureus* (*S. aureus*) and negative *Pseudomonas aeruginosa* (*P. aeruginosa*) bacteria as well as against pathogenic yeast, *Candida albicans (C. albicans)*.

**Material and Methods:** The aqueous and methanolic extracts of pomegranate fruit skin were prepared using a Soxhalet apparatus. Antimicrobial effect of the extracts was studied and compared with commercial antibiotics using three different methods; agar dilution, cylinder plate, and disk inhibition zone techniques.

**Results:** Both extracts showed good antibacterial activity against *S. aureus* and *P. aeruginosa*. Also the methanolic extract presented strong antifungal effect on *C. albicans*. The antimicrobial activities against *S. aureus*, *P. aeruginosa* and *C. albicans* were comparable with those of cloxacillin, gentamycin and clotrimazole, respectively. The methanolic extract was found to be more effective than aqueous one against all the tested microorganisms.

**Conclusion:** The extracts from pomegranate fruit skin possess strong antimicrobial activity against the tested microorganisms. Therefore this plant could be an important source of new antimicrobial compounds to treat bacterial and fungal infections.

**Keywords:** Pomegranate; *Staphylococcus aureus*; *Pseudomonas aeruginosa*; *Candida albicans*

*1- Microbiology and Virology Research Center, Avicenna Research institute & Department of Medical Bacteriology & Virology, Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I. R. Iran*
*2- Pharmacological Research Center of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I. R. Iran*
*3- Neyshabur School of Medical Sciences, Neyshabur, I. R. Iran*
*4- Department of Radiology, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, I. R. Iran*
*5- Department of Pharmacology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, I. R. Iran*
***Corresponding Author**: Tel: +985118828566; Fax: +985118828567
E-mail: Rakhshandehh@mums.ac.ir

## Introduction

Infectious diseases are still one of the leading causes of death in the world. Although conventional drugs provide effective treatment for some infections, antibiotic resistance continues to grow among key microbial pathogens such as *Staphylococcus aureus (S. aureus*), *Pseudomonas aeruginosa, (P. aeruginosa*), *Streptococcus* spp, and *Enterobacteriaceae* (Bax et al., 2000; Bhavnani and Ballow, 2000). Therefore, the search for new antimicrobial agents is imperative.

Medicinal plants have always been a good source to find new remedies for human health problems. Recently, a wide range of these plants have been screened for antimicrobial property (Martin and Ernst, 2003; Upadhyay et al., 2010). *Punica granatum,* commonly known as pomegranate, has been highlighted in some studies as having this property (Al-Zoreky, 2009; Braga et al., 2005; Perez and Anesini, 1994; Prashanth, 2001). For example, methanolic extract of pomegranate fruit has been shown to induce antibacterial activity against *Listeria monocytogenes*, *S. aureus*, *Escherichia coli* (*E. coli*) and *Yersinia enterocolitica* (Al-Zoreky, 2009). The same activity has been demonstrated for pomegranate against *Klebsiella pneumoniae, Proteus vulgaris* and *Bacillus subtilis* (Prashanth et al., 2001). Although these pieces of evidence implicate pomegranate as antimicrobial therapeutic, some questions still remained to be answered. For example, the antifungal activity of pomegranate and its antibacterial effects on other strains are open questions. Also, more studies are needed to investigate the antimicrobial effects of other types of pomegranate extracts and other parts of this plant. Therefore, in the present study we tested two extracts from pomegranate fruit skin against both Gram positive (*S. aureus*) and negative (*P. aeruginosa*) bacteria as well as against pathogenic yeast, *Candida albicans (C. albicans)*. Moreover, the antimicrobial activities of pomegranate were compared with clotrimazole, gentamycin and cloxacillin.

## Material and Methods

### Drugs and chemicals

Methanol and chloroform were purchased from Merck (Germany). Gentamycin was provided by Darou-Pakhsh Company (Iran). Cloxacillin and clotrimazole were purchased from Pars-Darou Company (Iran).

### Plant and extracts

Pomegranate fruits were collected from Ferdos, Razavi Khorasan province, Iran. The fruit skins were cleaned, dried in the shade and ground to fine powder. Then, two types of extracts were prepared, as described bellow:

1- Aqueous extract of pomegranate: The powder (50 g) was extracted with 600 ml distilled water in a Soxhlet apparatus for 12 h.

2- Methanolic extract of pomegranate: The powder (50 g) was extracted with 600 ml methanol in a Soxhlet apparatus for 12 h. Both extracts were then passed through filter paper and dried in oven at 50˚C.

### Bacterial and fungal isolates

Clinical isolates of *P. aerugirosa* (n=50), *S. aureus* (n=50) and *C. albicans* (n=50) were used in the study. The strains were obtained from patients in Ghaem hospital (Mashhad, Iran) and confirmed using NCCLS guidelines (Baron et al., 1990).

### Antimicrobial activity

The antimicrobial effects of the pomegranate extracts were evaluated using three different methods namely, agar dilution, cylinder plate, and disk inhibition zone.

In agar dilution method, 15 ml of Mueller-Hinton agar medium containing either the plant extracts or the antibiotics

were added to each of the Petri dishes. Concentrations ranging from 2.5 to 40 and 0.31 to 10 mg/ml were used for aqueous and methanolic extracts, respectively. Then, the isolated microorganisms were inoculated on the agar surface.

In cylinder plate method, the test organisms were spread on agar plate and the cylinders punched into the medium. The cylinders were filled with 200 µl of vehicles, antibiotics and plant extracts. Plates were incubated at 37˚C for 24 h. Then, the effects of drugs and extracts were evaluated by measuring diameter of zone of growth inhibition around the cylinders.

In disk inhibition zone method, the Mueller-Hinton agar medium was inoculated with freshly prepared cells of each bacteria and fungi to yield a lawn of growth. After solidification of the agar, a number of sterilized disks were dipped into the solvents (negative controls) or extract solutions and placed on the plates. After incubation at 37˚C for 24 h, the antimicrobial activity was measured as diameter of the inhibition zone formed around the disk. At the same time, a comparison antibiotic control test was made using commercial disks, gentamycin (0.01 mg), cloxacillin (0.005 mg) and clotrimazole (0.008 mg).

## Results

### Antimicrobial activity with agar dilution method

Results for the antimicrobial activity of the extracts in the agar dilution method are shown in Table 1. As expected, the presence of cloxacillin in the culture medium reduced the growth of *S. aureus*. This reduction was happened in 72% of the *S. aureus* isolates, at concentration 0.01 mg/ml of cloxacillin. At the same time, the growth of all *S. aureus* isolates was inhibited by 30 mg/ml of aqueous extract and by 5 mg/ml of methanolic extract. Regarding *P. aeruginosa*, the growth inhibition activity of aqueous extract was 43% at 20 mg/ml which was comparable with that of 0.01 mg/ml of gentamycin (40% inhibition). Also, 0.625 mg/ml of methanolic extract and 0.02 mg/ml of gentamycin demonstrated the same level of activity (50%) against *P. aeruginosa*. A complete growth inhibition was induced by aqueous and methanolic extracts at concentration of 40 and 5 mg/ml, respectively. With the agar dilution method, the aqueous extract showed no activity against *C. albicans*. On the other hand, methanolic extract showed high antifungal activity. At concentration of 5 mg/ml, the inhibitory effect of methanolic extract against *C. albicans* was comparable with that of 0.004 mg/ml of clotrimazole (84% growth inhibition).

### Antimicrobial activity with cylinder plate method

As shown in Table 2, with cylinder plate method, aqueous extract showed moderate response against *P. aeruginosa* and a weak activity on *S. aureus* and *C. albicans*. However, methanolic extract at 40 mg/ml compared favorably with 0.025 mg/ml of cloxacillin against *S. aureus* and with 0.05 mg/ml of gentamycin against *P. aeruginosa*. When *C. albicans* was incubated with 10 mg/ml of methanolic extract, 100% of the inhibition zones had more than 20 mm diameter. A same observation was found with 0.04 mg/ml of clotrimazole (83%)

### Antimicrobial activity with disk inhibition zone method

The methanolic extract, even at high concentration, failed to show considerable antimicrobial activity against *S. aureus* and *P. aeruginosa* (Table3). Regarding *C. albicans*; however, the extract exhibited an acceptable antifungal activity. At the presence of 2.75 mg of methanolic extract, 50% of the inhibition zones had diameter more than 20 mm. This level of antifungal activity was comparable with that of 0.008 mg of clotrimazole.

Table 1**.** Antimicrobial activity of pomegranate extracts against bacterial and fungal isolates tested by agar dilution method.

| | | Growth Inhibition (%) | | |
|---|---|---|---|---|
| **Drug or Extract** | **Concentration (mg/ml)** | ***S. aureus*** | ***P. aeruginosa*** | ***C. albicans*** |
| **Cloxacillin** | 0.0025 | 16 | - | - |
| | 0.005 | 62 | - | - |
| | 0.01 | 72 | - | - |
| **Gentamycin** | 0.005 | - | 30 | - |
| | 0.01 | - | 40 | - |
| | 0.02 | - | 50 | - |
| **Clotrimazole** | 0.001 | - | - | 33 |
| | 0.002 | - | - | 54 |
| | 0.004 | - | - | 84 |
| **AEP** | 2.5 | 0 | - | 0 |
| | 7.5 | 25 | 0 | 0 |
| | 10 | 50 | 22 | 0 |
| | 20 | 76 | 43 | 0 |
| | 25 | 92 | - | - |
| | 30 | 100 | 83 | - |
| | 35 | - | 93 | - |
| | 40 | - | 100 | 0 |
| **MEP** | 0.31 | 0 | 0 | 0 |
| | 0.625 | 62 | 50 | 25 |
| | 1.25 | 75 | 63 | 50 |
| | 2.5 | 88 | 75 | 68 |
| | 5 | 100 | 100 | 84 |
| | 10 | - | - | 100 |

Hyphen sign (-) indicates that the effect was not tested. AEP: aqueous extract of pomegranate; MEP: methanolic extract of pomegranate.

Table 2**.** Antimicrobial activity of pomegranate extracts against bacterial and fungal isolates tested by cylinder plate method.

| | | Relative Percent | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ***S. aureus*** | | | ***P. aeruginosa*** | | | ***C. albicans*** | | |
| **Drug or Concentration** | | **Diameter of inhibition zone (mm)** | | | | | | | | |
| **Extract** | (mg/ml) | <10 | 10-20 | >20 | <10 | 10-20 | >20 | <10 | 10-20 | >20 |
| **Cloxacillin** | 0.025 | 10 | 13 | 77 | - | - | - | - | - | - |
| **Gentamycin** | 0.05 | - | - | - | 25 | 58 | 17 | - | - | - |
| **Clotrimazole** | 0.04 | - | - | - | - | - | - | 0 | 17 | 83 |
| **AEP** | 5 | - | - | - | - | - | - | 85 | 15 | 0 |
| | 10 | 40 | 60 | 0 | - | - | - | 17 | 83 | 0 |
| | 20 | 33 | 67 | 0 | 50 | 50 | 0 | 0 | 83 | 17 |
| | 30 | 17 | 83 | 0 | 33 | 67 | 0 | 0 | 60 | 40 |
| | 40 | 0 | 100 | 0 | 0 | 100 | 0 | - | - | - |
| | 50 | - | - | - | 0 | 60 | 40 | - | - | - |
| **MEP** | 2.5 | - | - | - | - | - | - | 10 | 90 | 0 |
| | 5 | - | - | - | - | - | - | 0 | 50 | 50 |
| | 7.5 | - | - | - | - | - | - | 0 | 15 | 85 |
| | 10 | 0 | 100 | 0 | 67 | 33 | 0 | 0 | 0 | 100 |
| | 20 | 0 | 75 | 25 | 33 | 47 | 20 | - | - | - |
| | 30 | 0 | 22 | 78 | 0 | 55 | 45 | - | - | - |
| | 40 | 0 | 10 | 90 | 0 | 27 | 73 | - | - | - |

Hyphen sign (-) indicates that the effect was not tested. AEP: aqueous extract of pomegranate; MEP: methanolic extract of pomegranate.

Table 3**.** Antimicrobial activity of pomegranate extract against bacterial and fungal isolates tested by disk inhibition zone method.

| | | Relative Percent | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *S. aureus* | | | *P. aeruginosa* | | | *C. albicans* | | |
| **Drug or Concentration** | | **Diameter of inhibition zone (mm)** | | | | | | | | |
| **Extract** | **(mg/ml)** | <10 | 10-20 | >20 | <10 | 10-20 | >20 | <10 | 10-20 | >20 |
| **Cloxacillin** | 0.005 | 13 | 56 | 31 | - | - | - | - | - | - |
| **Gentamycin** | 0.01 | - | - | - | 20 | 64 | 16 | - | - | - |
| **Clotrimazole** | 0.008 | - | - | - | - | - | - | 6 | 41 | 53 |
| **MEP** | 1.3 | 87 | 13 | 0 | - | - | - | 25 | 75 | 0 |
| | 1.6 | 63 | 37 | 0 | 100 | 0 | 0 | 0 | 100 | 0 |
| | 1.9 | 41 | 59 | 0 | 83 | 17 | 0 | 0 | 75 | 25 |
| | 2.25 | 22 | 78 | 0 | 32 | 68 | 0 | - | - | - |
| | 2.75 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 50 | 50 |

Hyphen sign (-) indicates that the effect was not tested. MEP: methanolic extract of pomegranate.

## Discussion

Increase of antibiotic resistance as well as undesirable side effects of synthetic drugs have triggered immense interest in the search for new antimicrobial agents of plant origin. In the present study, extracts of pomegranate fruit skin have been tested against two bacteria (*S. aureus* and *P. aeruginosa*) as well as against pathogenic yeast, *C. albicans*. The *S. aureus* is responsible for a wide variety of diseases, including pneumonia, skin and soft tissue infections, and diabetic foot infections (Shorr, 2007). Similarly, *P. aeruginosa* is a common pathogen associated with burn wound infections, keratitis, and respiratory tract infections (Marquart et al., 2005). Under the conditions employed here, especially in agar dilution method, the bacteria were found to be sensitive to both aqueous and methanolic extracts. These findings are in accordance with the observations of McCarrell et al. who found that aqueous macerated extract of pomegranate rind inhibits growth of *S. aureus* and *P. aeruginosa* (McCarrell, 2008). Similarly, Al-Zoreky has reported that metanolic extract of pomegranate fruit peels is a potent inhibitor for *S. aureus, Listeria monocytogenes*, *E. coli* and *Yersinia enterocolitica* (Al-Zoreky, 2009). In another study, Perez and coworkers showed that boiling water extracts of pomegranate fruit pericarp induces antibacterial activity against *Salmonella typhi* (Perez and Anesini, 1994). The same observation was reported by Prashanth and colleagues (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*), and found activity against all isolates.

The growth inhibition activity of aqueous extract was started at lower concentration for *S. aureus* than for *P. aeruginosa*. Therefore, the extract may be more effective for Gram positive than for Gram negative bacteria. Also, in the case of test bacteria, the antibacterial activity of methanolic extract was started at lower concentration, as compared with aqueous one. So, it is reasonable to assume that the principal chemical constituents with antimicrobial activity were concentrated in the alcoholic fraction. This was in agreement with Ahmad et al. who found alcohol as a better solvent for extraction of antimicrobial active substances compared to water and hexan (Ahmad et al., 1998).

Regarding *C. albicans*, only the methanolic extract could present antifungal activity. This activity, which was observed with all the tested methods,

was strong and comparable with that of clotrimazole. Also, the antifungal activity of methanolic extract was started at lower concentration when compared with its antibacterial effects. Therefore, this type of pomegranate extract may be more specific for fungal than for bacterial infection. Although a significant inhibitory effect of pomegranate water extract on *C. albicans* has been reported by other investigators (Tayel and El-Tras, 2010), in our study the extract did not induce such effect. The explanation for this discrepancy may lie in the method of extraction.

The active phytocompounds responsible for antimicrobial activity of pomegranate remained to be exactly elucidated. However, ellagitannin punicalagin is thought to be the primary constituent involved in the antimicrobial effects (Machado et al., 2002).

In conclusion, the extracts from pomegranate fruit skin possess strong antimicrobial activity against fungi and against both the Gram positive and negative bacteria. Further isolation and purification of the extracts are required to determine the active components responsible for their activity. Although our results support the idea that pomegranate extracts are candidate for treatment of infectious diseases, clinical trials will be required to confirm its antimicrobial action and general safety.

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