BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2012. Vol. **9**(1), 229-233

Antibacterial Activity of Methanol Extract of Pomegranate (*Punica granatum* L.) Peels

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(Received: 18 February 2012; accepted: 10 May 2012)

The aim of the present study was to examine the antibacterial activity of methanol extract of pomegranate peels against some Gram positive and negative bacteria. The antibacterial activity was evaluated using both agar-diffusion method and minimum inhibitory concentration (MIC). With the exception of *Streptococcus faecalis* and *Salmonella, a*ll of the bacteria showed sensitivity to methanol extracts of pomegranate peel The Minimum Inhibitory Concentration varied with *Streptococcus salivarius* and *Bacillus subtilis* having the lowest MIC (1mg/ml) while *Streptococcus faecalis and Salmonella* where more resistant, having MICs of 4 and 8 mg/ml respectively.

Key words: Pomegranate peels, *Punica granatum*, Opportunist infections, Antibacterial activity, Methanol extract.

Interest in the study of the therapeutic effects of plants has recently increased significantly (Thuille *et al*., 2003; Alanís, 2005) and large numbers of people around world rely on complementary medicine for their health care needs (Magee, 2005; Duraipandiyan *et al*., 2006). The widespread acceptance of traditional medicine and the increasing development of bacterial resistance to antibiotics has led workers to investigate the potential antibacterial activity of medicinal plants (Scrinivasan *et al*. , 2001; Kumarasamy *et al*., 2002; Ali *et al*., 2001; Masika and Afolayan, 2002; Hamill *et al*., 2003; Shah, 2005). Many plants have been screened for their antimicrobial activity, but relatively few have been found to be sufficiently active (Poyart -Salmeron, 1990; Meng, 2000) and non toxic to humans (Izzo, 2004) for them to be accepted in standard medical practice. Fruits, peels and the roots of pomegranate have been commonly used in herbal remedies by local healers in a number of cultures. Peel of pomegranate (*Punica granatum*) has been used in traditional medicine for the treatment of diarrhea and dysentery (Ahmad and Beg, 2001; Braga *et al*., 2005; Voravuthikunchai *et al*., 2005; Reddy *et al*., 2007), and extract of the bark of this plant have been shown to possess antibacterial activity against Gram-positive bacteria and Gram-negative bacteria (Kadi et al. 2011). Pomegranate peel extracts also show antimicrobial activity against a wide variety of enteric pathogens as well as some food-borne pathogens (Pai *et al*. 2011; Al-Zoreky, 2009), while Abdollahzadeh *et al*. (2011) also demonstrated the antibacterial activity

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of extract of *Punica granatum* L peels against oral pathogens.

The aim of the present study was to determine the antibacterial activity of pomegranate peels extract against a range of Gram positive and negative-gram bacteria.

MATERIALS AND METHODS

Plant material

Pomegranate fruits were collected from the Saada region of Yemen. Fruits were rinsed with distilled water. The peel was then removed manually and then mixed in a blender after which, the samples were freeze dried and stored at -20° C until processed.

Preparation of the extract

The peel extract was prepared essentially as described by Zhenbin *et al,* (2011), i.e. by extraction with methanol in a flask held in a thermostatic water bath shaker (15:1 (w/w) ratio solvent/sample as dry weight) at 40 °C for 4 h. The liquid extract was then filtered through Whatman No. 1 filter paper by vacuum enhanced filtration and the filtrate was air dried in hood at room temperature; any residual moisture was then removed in a vacuum oven at 50 ± 2°C.

Antibacterial activity

Bacteria used

The bacteria used in the study were obtained from the Microbiology Laboratory of the College of Dentistry and Department of Food

Table 1. Antibacterial activity of extract of pomegranate peel by agar-diffusion method at deferent concentrations.

Microorganisms	Concentrations (mg)			
	0.5	1	2	4
Staphylococcus aureus	15* ±0.25	16±0.0	16±0.25	18±0.5
Staphylococcus epidermidis	0	8±0.25	10±0.25	14±0.5
Streptococcus mutans	10±0.25	10±0.5	12±0.0	13±0.25
Streptococcus salivarius	9±0.5	10±0.0	12±0.25	14±0.0
Streptococcus faecalis	0	0	0	0
Bacillus subtilis	8±0.0	10±0.0	12±0.25	14±0.25
Escherichia coli	9±0.5	12±0.25	15±0.0	16±0.5
Salmonella	0	0	0	0

* Inhibition zones in mm (Means of triplicate)

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extract of pomegranate peel.

Microorganism	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus aureus	2	16
Staphylococcus epidermidis	2	8
Streptococcus mutans	2	16
Streptococcus salivarius	1	16
Streptococcus faecalis	4	32
Bacillus subtilis	1	32
Escherichia coli	2	32
Salmonella	8	32

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

Science, King Saud University. The following bacteria were used: Gram-positive bacteria- *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Streptococcus faecalis, Streptococcus salivarius* and *Bacillus subtilis*; Gram-negative bacteria: *Escherichia coli and Salmonella.* All strains were inoculated onto Nutrient Agar and incubated at 37° C for 24 hours.

***In vitro* antibacterial activity of the extract**

A range of concentrations (0.5, 1, 2, 4 mg w/v in methanol) of the pomegranate peel extract was tested against bacteria using the agar-diffusion method. The solutions were sterilized using a syringe filter (0.45 µm) and 50 µl of the various concentrations were transferred onto sterile filter disks (6 mm) and left to allow the ethanol to evaporate. The individual bacteria tested were inoculated, using a swab, onto the surface of Muller Hinton Agar. Disks containing the extracts were then were placed onto the surface of the inoculated media and the petri dishes were incubated at 37° C for 24 h, and any resultant inhibition zones were measured.

Minimum inhibitory concentration (MIC)

The broth media dilution method was used to determine the MIC of the extract of pomegranate peels; the final concentration of the extract was 0.5–32 mg/ml in brain heart infusion broth; all of different concentrations were inoculated with 100 µl of strains and incubated at 37° C for 24 hour. The MIC is defined as the lowest concentration (mg/ml) of the extract resulting in clear broth media while minimum bactericidal concentration (MBC) is defined as the lowest concentration (mg/ml) of the extract resulting in no growth of bacteria after culture on agar media.

RESULTS AND DISCUSSION

The bacteria used here were tested for their sensitivity to methanol extracts of pomegranate peels. The antibacterial properties of the peel extracts were firstly determined by agar-diffusion method. Table 1 shows the diameter of the inhibition zones resulting from exposure of the bacteria to the peel extracts. With the exception of Streptococcus *faecalis* and *Salmonella,* all bacteria were inhibited by the extracts. The inhibition of bacteria was directly related to the concentration of extract, where inhibition zones increasing with increasing extract concentration (0.5-4mg). *Staphylococcus aureus* was the most readily inhibited bacterium followed by *Escherichia coli*, while *Streptococcus faecalis* and *Salmonella* were not inhibited by the extracts. The results are similar to those presented by Al-Zoreky (2009), while the inhibition of bacteria growth reported here for pomegranate peel extracts are greater than those reported by Abdollahzadeh *et al.* (2011) using *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans* and *Streptococcus salivarius*.

The MICs for the methanol extract of pomegranate peels varied with the bacteria used (Table 2). The lowest observed MICs were for *Bacillus subtilis* and *Streptococcus salivarius* (1mg/ml) while *Salmonella* and *Streptococcus faecalis* showed MICs of 4 and 8 mg/ml respectively. The MICS of *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans* and *Escherichia coli* were 2mg/ml. The MIC of the pomegranate extract against *Staphylococcus aureus* was similar to that reported by Al-Zoreky (2009), while the MIC of *Bacillus subtilis* and *Escherichia coli* were higher than that hose reported by this author. The minimum bactericidal concentration (MBC) of methanol extracts of *Staphylococcus epidermidis* was 8mg/ml compared with values of 16-32mg/ml for the other bacteria.

Blansky and Newman (2007) suggested that the inhibitory effect exhibited by the crude extracts of the bark of *Punica granatum* bark can be explained by the presence of tannins that represent 28% of bark constituents, Such high molecular weight compounds have been found *in vitro* to possess a variety of pharmacological properties including being antioxidants, antimicrobial and anti-inflammatory agents (Kadi *et al*.2007). The antimicrobial activity of pomegranate bark may also result from other secondary metabolites such as phenolics and saponins (Kadi et al, 2007). It is likely that similar compound to the above are present in the pomegranate peel and help explain its antibacterial activity (Abdollahadeh *et al*. 2011). The last mentioned authors have suggested that pomegranate peel extracts could be used in the treatment of oral pathogens (Abdollahadeh *et al*.

2011) and have also pointed out that such extracts possess antifungal activity, particularly against *Candida albicans*. The results of the present study confirm the antibacterial activity of pomegranate peel and point to the potential use of such extracts or their purified components in the control of pathogenic bacteria particularly MRSA and other bacteria which are currently resistant to antibiotics.

ACKNOWLEDGEMENTS

This work was supported by King Saud University, Deanship of Scientific Research, College of Science Research Centre.

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