

Full Length Research Paper

Investigation of antimicrobial activity of *Punica granatum* L. fruit peel ash used for protection against skin infections as folk remedies especially after male circumcision

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Antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial and antimutagenic effects of *Punica granatum* (PG) have already been supported by different studies. PG has a rich history of traditional use in medicine. For centuries, the barks, leaves, flowers, fruits and seeds of this plant have been used to heal several diseases. However, in South Anatolia, Turkey, some people employ the ashes of PG fruit peel as a protective against skin infections, especially after male circumcision. There have been no reports about the antimicrobial activity of PG fruit peel ash as far as the current literature is concerned. The aim of this study was to investigate the antimicrobial activity of ashes of PG fruit peels. The ethanol and a solvent cocktail (dH₂O:ethanol:methanol:aceton:CH₂Cl₂ (1:2.5:2.5:2:2)) extracts of PG fruit peel and peel ashes were tested against *Candida albicans* ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa*, *Salmonella enterica* Serotype Typhimurium SL 1344 and *Shigella flexneri*. The results indicated that both PG fruit peel and peel ashes showed antimicrobial activity against all microorganisms tested. In conclusion, since PG fruit peel ashes showed promising degrees of antimicrobial activity against the tested microorganisms, these preliminary data should be supported by further large-scale studies.

Key words: *Punica granatum*, pomegranate, fruit peel ash, antimicrobial activity, disc diffusion test, male circumcision.

INTRODUCTION

Pomegranate, *Punica granatum* L. (PG) (Lythraceae), is a fruit-bearing deciduous shrub or small tree that has a rich history of traditional use in medicine (Orak et al., 2011). Antibacterial, antifungal, antioxidant, antitumor, antiviral, antimalarial and antimutagenic effects of *P. granatum* (PG) have already been supported by different studies (Ahmad and Beg, 2001; Al-Zoreky, 2009; Mathabe et al., 2006; Panichayupakaranant et al., 2010; Prashanth et al., 2001). Several researchers reported that it is used for prevention and treatment of some of the cancer types such as lung cancer (Khan and Mukhtar, 2007) and prostate cancer (Malik and Mukhtar, 2006). It is also reported that the aqueous decoction is used against dysentery, stomatitis and diarrhea (Lansky et al.,

2004). For centuries, the barks, leaves, flowers, fruits, and seeds of this plant have been used to heal several diseases (Jayaprakasha et al., 2006). PG fruit peels are usually used according to their astringent properties and the seeds are used especially for stimulating fertility and for beauty (Aslam et al., 2006; Gözekçi et al., 2011).

According to the previous studies, it is known that PG fruits are rich sources of polyphenolic compounds (Lansky and Newman, 2007) and the fruit peels contains wide variety of compounds such as gallotannins, ellagic acid, gallagic acid, punicalins, punicalagins, ellagitannins etc. (Mavlyanov et al., 1997; Reddy et al., 2007; Zahin et al., 2010).

As it is mentioned before, pomegranates have been

used extensively in the folk medicine of many cultures (Longtin, 2003). According to Lansky and Newman, (2007) scientific investigations conducted in last years are mostly based on some of the traditional ethnomedical uses of the pomegranate.

In South Anatolia, Turkey, some people employ the ashes of *PG* fruit peel ash as a protective against skin infections, especially after male circumcision. It is also thought to be an accelerator in the wound healing process. Some local peritomists also use pulverised *PG* fruit peel instead of peel ash (Anonymous).

Male circumcision is the surgical removal of some of the foreskin from the penis, which could be undertaken due to the religious, cultural, social or medical reasons (WHO, 2007). Although infant circumcision is very widespread nowadays, the circumcision is usually carried out on boys aged between 7 to 12 years, and it is thought to be the passport to manhood with the surrounding rituals (WHO, 2010). The aim of this study was to investigate the antimicrobial activity of ashes of *PG* fruit peels and compare the results with the antimicrobial activity of *PG* fruit peels.

MATERIALS AND METHODS

PG fruits used in this study were obtained from a local manufacturer in Adana-Kozan, South Anatolia, Turkey. All solvents used in the study were purchased from Sigma-Aldrich, Germany.

Extraction procedure

After *PG* fruits washed extensively, fruits were peeled and half of the *PG* fruit peels were burned at 400 °C as described in Altuner et al. (2010) and the other half was pulverised by using mortar and pestle. Peel ash and pulverised peel were extracted by using ethanol and solvent cocktail (dH₂O:ethanol:methanol:aceton:CH₂Cl₂ (1:2.5:2.5:2:2)) (Altuner and Akata, 2010) by shaking at room temperature for 3 days (Bayar et al., 2010). Extracts were filtrated after 3 days by using Whatman No 1 filter paper. Filtrates were evaporated by a rotary evaporator at 30 °C and pulverised by a freeze-dryer. The residue was used to prepare 300 mg.ml⁻¹ extracts.

Microorganisms

Candida albicans ATCC 26555, *Escherichia coli* ETEC LM 63083, *Pseudomonas aeruginosa* (clinical isolate), *Salmonella enterica* serotype Typhimurium SL 1344 and *Shigella flexneri* (clinical isolate) were used in the study (Kastamonu University, Department of Biology, Botanical Research Laboratory Culture Collection).

Preparation of inocula

All bacterial strains were incubated in atmospheric air at 37 °C for 24 h and *C. albicans* at 27 °C for 48 h. Inocula were prepared by transferring morphologically similar colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard having approximately 10⁸ cfu.ml⁻¹ for bacteria and 10⁷ cfu. ml⁻¹ for *C. albicans* (Hammer et al., 1999). Mueller-

Hinton Agar (Merck, Germany) medium is used for bacteria, where *C. albicans* strain was plated on Sabouraud Dextrose Agar (Merck, Germany).

Disk diffusion method

Disc diffusion test was performed as described previously by Andrews (2007). The culture medium was poured into 120 mm sterile Petri dish to give a mean depth of 4.0 ± 0.5 mm (Altuner and Çetin, 2009). 10, 20 and 30 µl aliquots of each extract was applied on sterile paper discs of 6 mm diameter end up with 3, 6 and 9 mg.µl⁻¹ sample on each disc (Mahasneh and El-Oqlah, 1999). To get rid of any residual solvent which might interfere with the results, discs were left to dry overnight at 30 °C in sterile conditions (Silici and Koc, 2006). The surface of the plates was inoculated using previously prepared inocula containing saline suspension of microorganisms. Inoculated plates were then left to dry for 5 min at room temperature before applying the discs. Discs were firmly applied to the surface of the plate which had an even contact with the agar. Plates were incubated and inhibition zone diameters were expressed in millimetres.

Controls

All extraction solvents and empty sterile discs were used as negative controls.

Statistics

The data determined as the mean of three parallel studies. All values given here are mean values of these three parallel studies. The statistical analysis was performed using a non-parametric method Kruskal-Wallis one-way analysis of variance. A value of p < 0.05 was considered statistically significant.

RESULTS

The diameter of the inhibition zones recorded as the diameter of the zones in millimetres for the samples are given in Table 1. No activity was observed for the negative controls; solvents and empty sterile discs. Table 1 clearly shows that all extracts were presented antimicrobial activity against all microorganisms tested. Especially the antimicrobial activities of both *PG* peel and *PG* peel ash extracts on *Shigella flexneri* are very noteworthy. According to the results it could be concluded that antimicrobial activities of *PG* peel ash extracts were almost higher than antimicrobial activities of all *PG* peel extracts. The highest activity observed was in *S. flexneri*, where the lowest activity was in *P. aeruginosa*.

DISCUSSION

Some researchers studied the antimicrobial activities of *PG* extracts previously. An activity against *E. coli* and *S. aureus* was identified by Mathabe et al. (2006) and Al-Zoreky (2009). Orak et al. (2011) also presented an antimicrobial activity against *E. coli*, *S. aureus*, *S. enteritidis* and *A. parasiticus*. In addition to these, Tayel

Table 1. Disc diffusion test results (inhibition zones in mm).

Bacteria	<i>Punica granatum</i> peel extract			<i>Punica granatum</i> peel ash extract		
	10 µl	20 µl	30 µl	10 µl	20 µl	30 µl
<i>C. albicans</i>	10	17	20	12	18	20
<i>E. coli</i>	-	12	14	8	12	16
<i>P. aeruginosa</i>	-	-	12	-	-	13
<i>S. enterica</i>	-	9	13	-	9	14
<i>S. flexneri</i>	45	50	56	47	51	58

“-”: no activity observed.

and El-Tras (2009) and Endo et al. (2010) reported that punicalagin isolated from *PG* peels possessed strong activity against *C. albicans* and *C. parapsilosis*. Duman et al. (2009) observed an activity against *Bacillus megaterium*, *P. aeruginosa*, *S. aureus*, *Corynebacterium xerosis*, *Micrococcus luteus*, *Enterococcus faecalis*, *Kluyveromyces marxianus*, *Rhodotorula rubra* and *C. albicans*.

As previous studies identified antimicrobial activity of *PG*, it was not a surprise to observe an antimicrobial activity against *C. albicans*, *E. coli*, *P. aeruginosa*, *S. enterica* and *S. flexneri* in this study. But the very surprising point is the antimicrobial activity of *PG* peel ash extracts and their activity which were higher than *PG* peel extracts.

According to American Academy of Pediatrics (1999), there are some risks related to circumcision, which are bleeding, infection, redness around the surgery site and injury to the penis. As it is stated, bleeding and infection are two of the risks related to circumcision and should be taken into account during the healing process after the circumcision. *PG* peel and *PG* peel ash can be used against bleeding and infection. They can be used against bleeding because of the tannins in their nature. Tannins are astringent substances that shrink or constrict body tissues which can help to shrink both blood vessels and the skin and stop bleeding. With this study and previous studies, it is obvious that they can fight against several infections.

Although the healing powers of plants have been used for hundreds of years, plant ashes have mostly been ignored for this purpose. There have been no reports about the antimicrobial activity of *PG* fruit peel ash as far as the current literature is concerned. This study is the very first one which presents the opportunity to use *PG* fruit peel ash as protective against skin infections and it can also prevent bleeding that could occur after male circumcision. But these results should be supported by further large-scale studies.

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