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Antibacterial activity of pomegranate fruit peels and arils

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ABSTRACT: The in vitro antibacterial activities of different extracts of pomegranate fruit peels and arils (with seeds) were investigated by agar-well diffusion and broth dilution methods against four food-related bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium*). The solvents used as extractants in this study were hot water, 95% ethanol, and acetone. Their total phenolic contents were also evaluated. All pomegranate extracts contained high levels of phenolics and exhibited antibacterial activity against all bacteria tested. The hot-water extract of the peels was the most potent with the minimal inhibitory concentration of 207 mg/ml against *E. coli* and less than 103.6 mg/ml against the other bacteria. Gram-positive bacteria were generally more sensitive to the extracts than Gram-negative ones.

KEYWORDS: minimal inhibitory concentration (MIC), Gram positive bacteria, Gram negative bacteria, total phenolic content

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruits are widely consumed, fresh and in commercial products, such as juices, jams, and wines. Most pomegranate fruit parts are known to possess substantial antioxidant activity[1]. The flower[1], seed oil[2], seed extract, and peel extract[3] of pomegranate also have a potent antioxidant activity. Furthermore, the water-methanol extract of pomegranate peel possesses antimicrobial activity against eleven microorganisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Listeria monocytogenes*, *Candida utilis*, *Saccharomyces cerevisiae*, and *Aspergillus niger*[4]. Braga et al[5] reported that the methanol extracts of *P. granatum* whole fruits were able to inhibit not only the growth of *S. aureus* FRI 722 but also the production of enterotoxins. However, rare studies have reported antibacterial activity of pomegranate arils. In this study, we examined the antibacterial activity of hot-water extract, ethanol extract, and acetone extract of pomegranate fruit peels and arils (with seeds) by agar-well diffusion and broth-dilution method against four food-related bacteria—two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *S. typhimurium*).

MATERIALS AND METHODS

Pomegranate fruit and preparation of the extracts

Pomegranate fruits were purchased from local markets in Bangkok. After opening the fruit, the arils (with seeds) were manually separated from the peels. Collected peels and arils were then rinsed with tap water. These peels and arils were ground separately in a blender. Fifty grams of blended peels or arils were placed in 250-ml Erlenmeyer flasks, followed by adding 100 ml of solvents having an increasing polarity: acetone, 95% ethanol, and hot water. The flasks were then shaken at room temperature for 18 h prior to filtration. The filtrates were concentrated under reduced pressure with a rotary evaporator at 40 °C. These crude extracts were kept at 4 °C until use.

Microorganisms and culture

A total of four food-related bacteria were kindly provided by the Culture Collection of Department of Microbiology, Faculty of Science, King Mongkut's University of Technology Thonburi. They are two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *S. typhimurium*). The strains were maintained at 4 °C in Mueller Hinton agar slants. Before experimental use, cultures from solid media were sub-cultivated in Mueller Hinton broth, incubated for 24 h at 37 °C, and used as the inocula for the determination of antibacterial activity.

Determination of antibacterial activity

The modified agar well-diffusion method[6] was conducted to evaluate the antibacterial activities of the extracts. A freshly grown culture was serially diluted, and 0.1 ml of prepared cells (1.5×10^7 colony forming

units per millilitre, CFU/ml) was aseptically spread onto the surface of Mueller Hinton agar and then left to dry for 30 min. Wells (8 mm in diameter) were made in media using a sterilized stainless steel borer. Each well was filled with 30 µl of the crude extracts. The plates were left at room temperature for 30 min to allow diffusion of materials in media. The controls were prepared using residue obtained by concentrating each solvent under reduced pressure (the same as done with the filtrates) and diluting with 10 ml DMSO. Plates were incubated at 37 °C for 18–24 h. Inhibition zones in mm (including well diameter) around wells were measured. The antibacterial activity was expressed as the diameter of inhibition zones produced by the extracts against test bacteria. All tests were performed in triplicate. The results were expressed as mean ± standard deviation. Statistical significance was calculated by ANOVA, followed by Scheffé's test. The p values of < 0.05 were considered significant.

Determination of MIC

The broth-dilution method[7] was adopted to determine the minimal inhibitory concentration (MIC) of the active extract revealed by the previous well assay. The inoculum of each test bacterium was prepared by diluting the overnight culture of the bacterium in Mueller Hinton broth to a level of 1.5×10^7 CFU/ml. Two millilitres of the extracts diluted in two-fold dilution with DMSO were added to a sterile glass tube containing 0.5 ml Mueller Hinton broth (the concentration of bacteria approximately 1.5×10^7 CFU/ml). The tubes were incubated at 37 °C for 18–24 h. Because of the turbidity and dark colour of the extracts, 0.1 ml of the mixture in the tubes were spread onto the surface of Mueller Hinton agar. Plates were incubated at 37 °C for 18–24 h. The MIC was defined as the lowest concentration (mg/ml) of the extract resulting in bacteria density lower than 300 colonies per plate. The test was conducted twice.

Determination of total phenolic content

The total phenolic content of all extracts was determined using the Folin-Ciocalteu method described previously[8]. Briefly, 0.5 ml of diluted extract was added to a test tube and then mixed with 5 ml of Folin-Ciocalteu reagent (0.2 N). After 8 min, 2 ml of Na_2CO_3 (15%) was added. The reaction mixture was incubated at 50 °C for 15 min before the absorbance (at 760 nm) of mixtures was recorded against a blank. Total phenolic content of the extracts was calculated from standard gallic acid solutions (0–0.1 mg/ml), and expressed as mg gallic acid equivalents (GAE) per 100 g fruit dry weight.

The 100-g fruit dry-weight was calculated from the quantity (ml) of each extract obtained from 50 g of either blended peels or arils (with seeds). Other representative pomegranate fruit peels or arils were weighed, dried for 18 h at 95 °C, and the dry masses were recorded. If X ml of the extract was obtained from 50 g of peels or arils, and Y g of dry masses was obtained from Z g of peel or aril wet weight, then the dry weight of 0.5 ml extract (in grams) equals

$$\frac{(50\text{ g})\,(0.5\text{ ml})\,(Y\text{ g})}{(Z\text{ g})\,(X\text{ ml})}.$$

If 0.5 ml extract has V mg GAE of total phenolic contents, this equals

$$\frac{(V\text{ mg GAE})\,(100\text{ g})\,(Z\text{ g})\,(X\text{ ml})}{(50\text{ g})\,(0.5\text{ ml})\,(Y\text{ g})}$$

(in mg GAE per 100 g fruit dry weight).

RESULTS AND DISCUSSION

Four food-related bacteria were tested for their sensitivity to hot-water extract, ethanol extract, and acetone extract of pomegranate fruit peels and arils (with seeds). The antibacterial potency was initially determined by the agar well-diffusion method. Table 1 presents diameters of inhibition zones (clear zones around wells) exerted by the different extracts towards test bacteria. The solvent residue diluted with DMSO (the control) showed no inhibitory zone. On the other hand, all extracts from pomegranate fruit peels and arils exhibited inhibitory activity against all test bacteria (Table 1), with the highest inhibition zones on ethanol extracts (26.3–34.0 mm inhibition zones for peels and 19.7–24.3 mm inhibition zones for arils). The ethanol extracts of arils have significantly higher antibacterial activity than other aril-extracts. On the other hand, the antibacterial activity of ethanol extract of peels was not significantly different from other extracts of peels. Siri et al[9] found that both water and ethanol extracts of pomegranate fruit peels showed anti-*Aeromonas caviae* activity with more effectiveness in water extracted samples. In contrast, Al-Zoreky[4] reported that only water-methanol extract of peels have marked inhibition (12–20 mm inhibition zones) and the water extract was inactive against eleven microorganisms tested, such as *S. aureus* (2 strains), *B. subtilis*, *E. coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, *Candida utilis*, *Saccharomyces cerevisiae*, and *Aspergillus*

Table 1 Antibacterial activity of different extracts of pomegranate fruit peels and arils determined by agar well-diffusion method.

Microorganism	Inhibition zone (mm)					
	Hot water extract		Ethanol extract		Acetone extract	
	Peel	Aril	Peel	Aril	Peel	Aril
B. subtilis	28.0 ± 1.7^{a}	15.0 ± 0.0^{x}	31.0 ± 1.7^{a}	24.3 ± 0.6^{y}	31.0 ± 1.0^{a}	14.3 ± 1.2^{x}
S. aureus	30.0 ± 0.0^{a}	16.7 ± 1.5^{x}	34.0 ± 1.7^{b}	20.7 ± 1.2^{y}	30.7 ± 1.2^{a}	$19.3 \pm 1.2^{x,y}$
E. coli	27.0 ± 1.0^{a}	14.0 ± 1.0^{x}	29.0 ± 1.0^{a}	23.7 ± 0.6^{y}	30.3 ± 0.6^{a}	15.7 ± 1.2^{x}
S. typhimurium	23.7 ± 0.6^{a}	15.7 ± 1.2^{x}	26.3 ± 1.2^{b}	19.7 ± 0.6^{x}	$24.7 \pm 0.6^{a,b}$	17.0 ± 2.6^{x}

Means and standard deviation for $n = 3$. For each bacteria, the experimental values within each row that have no common superscript are significantly different ($p < 0.05$) according to Scheffé's multiple comparison test. Any two means not marked by the same superscript (for example, a and b for the extracts of peels and x and y for the extracts of arils) are significantly different ($p < 0.05$). Any two means marked by the same superscript (for example, a and a or x and x) are not significantly different.

niger. Meléndez and Capriles[10] found that methanol extract of pomegranate fruit was active against *E. coli*, *S. aureus*, and *B. subtilis* with the diameter of inhibition zone 12, 22, and 12 mm, respectively. The activity of ethanol extracts of peels against *S. aureus*, *B. subtilis* and *E. coli* (29–34 mm inhibition zones, Table 1) was comparable to the study of Ahmad and Beg[6] (10–40 mm inhibition zones), but higher than that of Al-Zoreky[4] (13–17 mm inhibition zones). This may be due to the different extraction method, strain sensitivity, antimicrobial procedures used in the test. The extract seems to be thermostable because the hot-water extract (using boiling water) still retained the activity nearly the same as other extracts. This was also found by Al-Zoreky[4].

Quantitative evaluation of antimicrobial activity of all extracts was carried out against test bacteria by broth dilution techniques. The MIC, in mg/ml, of all extracts is presented in Table 2. It appeared that the hot-water extract of the peels was the most effective against all test bacteria with the MIC values less than 210 mg/ml (Table 2). Moreover, the hot water extract of the arils was also effective against *B. subtilis* and *S. typhimurium* with the MIC value of 105 mg/ml. This may relate to some components in the extract that dissolved well in the water but did not diffuse well through the agar. The opposite was found by Prashanth et al[11] who reported that methanol extracts of peels were more active than water extracts against *E. coli*, *S. aureus*, and *B. subtilis*. The MIC of the methanol extracts was 6–12 mg/ml, while that of the water extracts was 25–50 mg/ml. In this study, the MIC for ethanol extract of peels was 242–500 mg/ml against all test bacteria (Table 2). Voravuthikunchai et al[12] found that the aqueous extract of pomegranate peel was active against *E. coli* O157:H7, MIC/MBC 0.19/0.39 mg/ml. Variations in results among studies on pomegranate fruit peel extracts were recorded with MIC determination. The values for MIC against *S. aureus* ranged from 0.62 to $\geqslant$ 250 mg/ml[4]. The MIC varied from 0.39–25 mg/ml against several strains of *E. coli*[4,11,12].

Table 2 Minimum inhibitory concentration (MIC) of different extracts of pomegranate fruit peels and arils determined by broth dilution method.

Microorganism	MIC (mg/ml)					
	Hot water extract		Ethanol extract		Acetone extract	
	Peel	Aril	Peel	Aril	Peel	Aril
B. subtilis	<104	105	242	194	444	222
S. aureus	<104	840	242	778	444	888
E. coli	207	840	499	778	3500	888
S. typhimurium	<104	105	242	778	<222	1776

The MIC values for test bacteria seemed to correlate with the total phenolic content found in the extracts. The total phenolic content of hot-water extracts of fruit peels was the highest, followed by the ethanol and acetone extracts, respectively (Table 3). This showed that the solvent that is less polar (acetone) extracted less amount of total phenolic compounds. Polyphenols are hydrophilic phytochemicals and thus more hydrophilic extractants are better solvents for their recovery from plant[4]. Different results were found by Negi et al[13] who used sun-dried and powdered pomegranate peels extracted with a Soxhlet extractor. They reported that the phenolic content of acetone, methanol, and water extracts of the peels was 52, 46.2, and 4.8%, respectively. Pomegranate

Table 3 Total phenolic content of different extracts of pomegranate fruit peels and arils.

Pomegranate	Total phenolic content (mg GAE/100 g dry weight)		
	Hot water extract	Ethanol extract	Acetone extract
Peels	166.83	152.65	85.48
Arils (with seeds)	87.32	72.84	64.60

peel contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid, and gallic acid[13]. Hayrapetyan et al[14] found that two pure compounds commonly found in pomegranate-peel extract, namely ellagic acid and gallic acid, did not show considerable inhibition of *L. monocytogenes*. Ahmad and Beg[6] reported that the phytochemical components found in alcoholic extract of pomegranate are alkaloid, flavonoid, glycoside, phenol, and tannin. Li et al[15] reported that phenolic compounds in pomegranate juice are punicalagin isomers, ellagic acid derivatives, and anthocyanins. Machado et al[16] reported the antibacterial activity of punicalagin against *S. aureus* (MRSA strains) with the MIC value of 768 µg/ml.

CONCLUSIONS

Besides having high antioxidant activity, pomegranate arils also have antibacterial activity and may be used as medicine for humans. This reduces the cost and the risk of antibiotic consumption. Furthermore, added-value from the peels which is the byproduct could provide health benefits to humans and may be employed in food preservation and pharmaceutical purposes.

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