



Original research

Comparing total anthocyanins, total phenolics and antioxidant activities of extracts (aqueous, organic and anthocyanin) obtained from pomegranate (peel, juice, and seed) and antimicrobial activity of peel extracts on the four pathogenic bacteria

Hoda Parseh^{a,*}, Alireza Shahablavasani^b^aDepartment of Food Science and Engineering, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran^bInnovative Technologies in Functional Food Production Research Center, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran

ABSTRACT

Nowadays, consumers are highly worried about using chemical preservatives in foods. Thus they tend to use natural and safe food products with healthful benefits. Pomegranate and its peel can have such a role. This study aimed to determine the antioxidant activity, total phenolics, and flavonoids properties of different parts of pomegranate extracts. Three types of extracts were prepared with different solvent (water extract, organic and anthocyanin extract). The total phenolic and antioxidant activities were highest in peels, intermediate in juice and lowest in seeds and total anthocyanin was highest in juices. The organic extracts have the highest antioxidant activity. Then we examined the antimicrobial activity of peel extracts (organic, aqueous and anthocyanin extracts) and determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on two gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and two gram-negative bacteria *E. coli* and *Salmonella typhi* using liquid dilution susceptibility testing method. Thus, 62.5 ppm MIC of the organic and anthocyanin extracts of the peel was useful in *Staphylococcus aureus* and *Salmonella typhi* bacteria. Furthermore, organic and anthocyanin extracts of the peel at a concentration of 125 ppm had bactericidal effects on *Staphylococcus aureus* and *E. coli* bacteria and *Bacillus cereus* and *Salmonella typhi* at a concentration of 250 ppm. Thus, one can state that pomegranate peel extracts have high antibacterial effects because of high phenolic compounds and high levels of anthocyanin with high antioxidant activities.

Keywords: Organic extract, Aqueous extract, Anthocyanin extract, Pomegranate

Received 1 November 2017; Received 3 March 2018; Accepted 18 March 2018

1. Introduction

Foodborne diseases are caused by consuming food with pathogenic bacteria, which has been a significant concern for public health. In the statistics reported in America in 1993-1997 determined that almost 75% of the cause of the diseases was connected with pathogenic bacteria (Olsen et al., 2000). The use of natural products and natural antibacterial compounds has been of great significance (Conner, 1993). Capital due to the presence of food microorganisms. Therefore, today, consumers are so concerned about the use of chemical preservatives in foods and have a tendency towards safe, natural food products with beneficial effects (Wu et al., 2009). Plants and fruits have plenty of antioxidants and polyphenolic compounds widely used in the food industry due to health benefits and

antimicrobial effects (Wu et al., 2008). Pomegranate is a native fruit of the tropical areas, whose origin is Iran, according to most experts and has spread to other parts of the world from Iran (Mousavinejad et al., 2009). Pomegranate is a product with a wide variety of secondary metabolites, such as alkaloids, tannins and phenolic compounds such as phenolic acids and their derivatives, and flavonoids (such as anthocyanin) (Mousavinejad et al., 2009). These compounds can provide protection against hepatotoxicity, exhibit estrogen-like activity, reduce systolic blood pressure, and decrease low-density lipoprotein (LDL) susceptibility to aggregation and retention (Naz et al., 2007). The flower, seed, and peel extracts of pomegranate also have a potent antioxidant activity (Schubert et al., 1999; Singh et al., 2002; Kaur et al., 2006). Here, pomegranate peel extract has more ability and capacity to inhibit or suppress

*Corresponding author.

E-mail address: Parseh.H@gmail.com (H. Parseh).

superoxide anions, hydroxyl and peroxy radicals and can limit the oxidation of low-density lipoproteins by pomegranate peel extract compared to other parts of the body, such as the inner fruit (Li et al., 2006). Studies have shown that pomegranate peel extract has antibacterial (Al-Zoreky., 2009; Danae L., 2009), antiviral (Martos et al., 2010), antimutagenesis (Lansky, 2007) and antioxidant properties and can be used as a natural preservative in the food and nutraceutical industries (Ghasemian et al., 2006; Singh et al., 2002).

Minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent with an inhibitory effect on the growth of a particular microorganism, meaning that microorganism is present in the environment but cannot reproduce. The reduction in the number of microorganisms under these conditions is not because of bactericidal effects of the essential oil; however, due to the microorganisms reaching death phase and as it does not multiply any more, the number decreases. Minimum bactericidal concentration (MBC) is the lowest concentration of the antimicrobial agent, leading to the death of microorganisms, so no living microorganisms should be present in a medium containing MBC (Aussalahet al., 2007).

The purpose of this study was to evaluate the antimicrobial properties of the parts of extracts from pomegranate peels including organic, aqueous and anthocyanin extracts and to determine MIC and MBC of two types of gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus* and two types of gram-negative bacteria *E. coli* and *Salmonella typhi*. Antioxidant activity, total phenolics, and total anthocyanins of different parts of pomegranate extract.

2. Material and Methods

2.1. Materials

The pomegranate used for this study (Saveh sub-acid cultivar) was prepared in December 2011 from the Agricultural and Horticultural Research Center of Saveh. Microbial strains of *E. coli* O157: H7 (ATCC35218) and *Staphylococcus aureus* (PTCC1431) were prepared from Department of Food Science and Technology, University of Tehran, Iran. *Salmonella typhi* (ATCC1609) and *Bacillus cereus* (ATCC11788) were purchased from the Industrial Research Organization of Iran. Mueller Hinton Agar and Mueller Hinton Broth media were used to conducting the experiments by MAST Company of Britain. Broth Nutrition medium Microbial, MRS Broth, nutrient agar, and MRS agar (Merck Co., Germany) were used for fresh culture and activation of microbial strains. Methanol, aqueous, acetone and acetic acid HPLC grade extracts were used by Merck Co. Germany.

2.2. Specimen preparation

Fruits first were washed and cleaned, then their peel was separated by a knife and using a press juicer, their juice was extracted, and seeds were separated. Peels and seed first were utterly dried in an oven at 60°C and next powdered with a mill. Finally, they were smoothed using a 1 mm sieve.

2.3. Extraction method

Powders of peel, seed, and juice of pomegranate were weighed as 25 grams and poured into a matte glass container with a lid and

mixed with a special solvent, and then placed on a magnetic shaker for 24 hours. Then, they were separated by centrifugation for 5 minutes with 5000 rpm of solvent, and the residue was mixed with a new solvent and placed on a magnetic shaker for 24 hours. Later on, the residue was separated from scum and solvent by centrifuge. This solvent was mixed with the previous solvent. Extraction of the aqueous extract was done using water/methanol solvent at a ratio of 85/15 vol/vol, according to Seeram et al. (2004) method. Organic extract was made using acetone/methanol/ water dilution at a ratio of 40/40/20 vol/vol/ vol using the method of Neto et al. (2006); also, the extraction of anthocyanin was done by the method of Wu et al. (2006) using methanol/water/ acetic acid (85, 14.5, 0.5 vol/vol/ vol) in all three parts of the fruit.

2.4. Preparation of preculture

Each bacterial strain was cultured on the culture medium the day before the MBC and MIC testing so that after a nocturnal incubation, the microorganisms are in a logarithmic phase while preparing the microbial suspension.

2.5. Preparing the different concentrations of extract

Different concentrations of the extracts were prepared by emulsifying a certain value of them and using a solvent specific to each of them.

2.6. Preparing 1 McFarland Suspension:

The standard suspension 1 McFarland was prepared by adding 0.1 mL of the aqueous solution of 1.175 grams of barium chloride, slowly by continuous mixing to 9.9 mL of sulfuric acid%1 (Aussalahet al., 2007). The turbidity by this suspension created a cell density of approximately 3×10^8 cells per milliliter, and then the turbidity was measured using a spectrophotometer set (CECIL 2502-Instruments Cambridge England Serial No. 125-624) at a wavelength of 625 nm (Barnon and Fineg, 1990).

2.7. Preparing the microbial suspension

A loop filled with each microbial strain under sterile conditions was added to 25 ml of Mueller Hinton Broth culture to prepare a microbial concentration suspension. Then, until the equalization of its optical density (OD) with 1 McFarland solution, it was diluted using a liquid culture medium (MHB). To get 10^6 microorganisms per mL, it was mixed with MHB liquid medium under sterile conditions at a ratio of 1: 500 (Barnon and Fineg, 1990).

2.8. Evaluation of the antimicrobial activity of essential oils using dilution-susceptibility test method in a liquid medium

Six levels of concentration of each extract (31.25, 62.5, 125, 250, 500, 1000 ppm) were prepared for experiments using liquid-dilution susceptibility test. One milliliter of standard inoculum liquid, whose preparation method was mentioned in the previous section (containing 10^6 microorganism per milliliter), was added to six test tubes containing equal volume (one milliliter) of dilutions prepared from the extract. An antimicrobial test tube was considered as a growth control (positive control), so the control contains one

milliliter of microbial suspension and one milliliter of distilled water. Adding the microbial suspension to the dilutions of the extracts will dilute the microbial suspension and the concentration of the antimicrobial agent, which will be considered in the preparation of the specimens. A negative control containing all components except microbial suspension of growth was considered for preparing each dilution of the antimicrobial agent. After these steps, the control tube containing no antimicrobial agent (control) 0.5 ml was taken to another test tube. Then, 0.5 mL of broth medium was added to it, and 0.001 ml of this mixture was cultured on an autosampler containing MHA immediately using an autosampler to count the number of colonies grown after one night of incubation. The number of emerging colonies should be 250-300 (Barnon and Fineg, 1990). These operations were performed separately for each extract and three microorganisms in three replicates. After one-night incubation at 37°C, the surface culture was performed for each of the test tubes on a solid culture medium Mueller Hinton Agar, and then the plates were inoculation to see growth or non-growth of microorganisms at one night (Barnon and Fineg, 1990).

2.9. Anthocyanin determination

The total anthocyanin content (TAC) of the pomegranate juice was determined using the pH differential method with two buffer systems. Sample preparation was conducted as described for color measurement. The pH of potassium chloride buffer was 1.0 (0.025 M), and sodium acetate buffer was pH 4.5 (0.4 M) (Lako et al., 2007). Briefly, 1 ml sample was mixed with 24 ml of corresponding buffers and read against water as a blank at 510 and 700 nm. Absorbance (A) was calculated as:

$$A = (A_{510} - A_{700}) \text{ pH } 1.0 - (A_{510} - A_{700}) \text{ pH } 4.5$$

The total anthocyanin content of each sample (mg cyanidin-3-glucoside/100 ml) was calculated as:

$$TAC = \frac{A \times MW \times DF \times 100}{MA}$$

Where MW is molecular weight of cyanidin-3- glucoside (4,492), DF is the dilution factor (25), and MA is the molar extinction coefficient of cyanidin-3-glucoside (26,900) (Cam et al., 2009).

2.10. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu reagents with analytical grade Gallic acid as the standard. 0.1 mL of extract diluted 10-fold with distilled water was added to distilled water (6 mL) and Folin–Ciocalteu phenol reagents (0.5 mL). After 4 minutes, 20% sodium carbonate (1.5 mL) was added to the mixture; then it reached to 10ml with distilled water. After being kept in total darkness for 2 h, the absorbance was measured at 765 nm using a spectrophotometer (CEILE- 2, UK.). Amounts of TP were calculated using gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) g/g of dry plant matter.

2.11. Determination of antioxidant activities

The scavenging activity on DPPH radical of different extracts was determined following the method reported by Okonogi et al. (2007). A test solution of different concentrations was prepared from a stock solution of methanolic and aqueous extracts. DPPH was

dissolved in methanol and mixed with an aliquot of 100 µl of each dilution. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. The radical scavenging activity was calculated as % inhibition by the following formula:

$$\text{DPPH}_{\text{radical-scavenging}} (\%) = [1 - \text{ABS}_{\text{sample}} / \text{ABS}_{\text{control}}] \times 100$$

The effective concentration at 50% (EC50) values calculated denotes the effective concentration of a sample required to decrease the absorbance at 517 nm by 50%. All measurements were performed in triplicate.

3. Results and Discussion

Table 1 shown MIC and MBC of organic extract of peel on four microorganisms of *E. coli*, *S. aureus*, *S. typhi*, and *B. cereus*. The results of experiments on an organic extract of peel at six concentrations tested showed that this extract was nine on average in the plates at *S. aureus* at a concentration of 62.5 in the colonies grown; showing the inhibitory effect of this extract against the tested bacteria, and this concentration is introduced as MIC. Also, at the concentration from 125 to 1000 ppm, no growth was observed in the bacteria, and the concentration 125 ppm was introduced as MBC. Similarly, according to the results for *E. coli* at a concentration of 62.5 ppm, on average, ten colonies were grown, reported as MIC, and the concentration of 125 ppm is the MBC. Concerning *Salmonella typhi* and *Bacillus cereus* bacteria, MIC was 125 ppm, and MBC was 250 ppm. As the results show, the organic extract of the peel has bactericidal and inhibitory effects on all four microorganisms, showing the great effect of this extract and susceptibility of the bacteria to this extract is, respectively, *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, and *salmonella typhi*. The most susceptible bacteria are reported against the organic extract of *Staphylococcus aureus* and the most resistant bacterium as *Salmonella typhi*. One can state that gram-positive bacteria are more susceptible than gram-negative ones.

The results of experiments on peel anthocyanin extract at six concentration showed that this extract had an inhibitory effect on *Staphylococcus aureus* at a concentration of 62.5 (Table 2). This concentration was introduced as MIC and from a concentration of 1000 to 125 ppm, there was no bacterial growth - the concentration of 125 ppm was introduced as the MBC. Thus, according to the results for *E. coli*, MIC was 62.5 ppm and MBC 125 ppm. Concerning *Salmonella typhi* and *Bacillus cereus*, 125 ppm was reported as MIC and 250 ppm as MBC. As the results show, the anthocyanin extract of the peel has inhibitory and bactericidal effects on all four microorganisms, showing the great effect of this extract

Table 1. The results of antimicrobial effect of organic extract of peel on test microorganisms

Type of microorganism	Dilutions of organic extract of the peel (ppm)					
	1000	500	250	125	62.5	31.25
<i>Staphylococcus aureus</i>	-	-	-	-	9	++
<i>Escherichia coli</i>	-	-	-	-	10	+
<i>Salmonella typhi</i>	-	-	-	20	+	+
<i>Bacillus cereus</i>	-	-	-	10	+	++

++ shows the high growth of microorganisms, + low growth of microorganisms, and - lack of growth

Table 2: The results of antimicrobial effect of anthocyanin extract on test microorganisms

Type of microorganism	Dilutions of anthocyanin extract of the peel (ppm)					
	1000	500	250	125	62.5	31.25
<i>Staphylococcus aureus</i>	-	-	-	-	4	+
<i>Escherichia coli</i>	-	-	-	-	7	+
<i>Salmonella typhi</i>	-	-	-	8	+	+
<i>Bacillus cereus</i>	-	-	-	5	+	++

++ shows the high growth of microorganisms, + low growth of microorganisms, and - lack of growth

and the susceptibility of the bacteria to this extract is, respectively, *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, and *salmonella typhi*. The most susceptible bacteria reported against the organic extract of the peel are *Staphylococcus aureus* and the most resistant bacterium as *Salmonella typhi*.

According to the results, the aqueous extract of the peel on the microorganism *E. coli* was MIC and MBC at 125 ppm and against the *Staphylococcus aureus* MIC and MBC at 250 ppm. This extract had an inhibitory effect on *Salmonella typhi* and *Bacillus cereus* microorganisms at a concentration of 250 ppm and bactericidal effects at a concentration of 500 ppm. One should note that according to the test method and the selection of concentration levels, the inhibitory and bactericidal effects of the aqueous extract of the peel on the *Staphylococcus aureus* and *E. coli* are consistent. This shows that MIC and MBC effects of this extract are likely to be close on *Staphylococcus aureus* and *E. coli* or cannot be separated from each other under test conditions. Previously, the effects of different concentrations of pomegranate peel extract on controlling *S. aureus*, *E. coli*, *Salmonella enterica*, *Shigella sonnei*, *Enterococcus faecalis*, and *Bacillus subtilis* have been investigated (Rosas-Burgos et al., 2017). Coteet et al. (2011) examined the antibacterial effect of aqueous, organic, and anthocyanin extracts of *Cornus mas* on seven gram-positive and gram-negative bacteria. Their results showed that organic and anthocyanin extracts contained high levels of phenolic and anthocyanin compounds and showed higher antibacterial activity compared to the aqueous extract.

Dahham et al. (2010) studied the antimicrobial activity of aqueous and methanol extracts of different parts of the pomegranate on seven gram-positive and gram-negative bacteria using disk diffusion method. Their results were as follows; methanol extracts showed significantly higher activity compared to aqueous extract, organic extract of the peel showed inhibitory effect on all bacteria and the peel extract showed a significantly higher anti-bacterial effect compared to aqueous and nucleus extracts. Gram-positive bacteria, especially *Staphylococcus aureus*, showed more susceptibility compared to gram-negative bacteria.

Table 3. The results of antimicrobial effect of aqueous extract of peel on test microorganisms

Type of microorganism	Dilutions of organic extract of the peel (ppm)					
	1000	500	250	125	62.5	31.25
<i>Staphylococcus aureus</i>	-	-	-	+	++	++
<i>Escherichia coli</i>	-	-	-	-	+	++
<i>Salmonella typhi</i>	-	-	19	+	++	++
<i>Bacillus cereus</i>	-	-	12	+	++	++

++ shows the high growth of microorganisms, + low growth of microorganisms, and - lack of growth

Zoreky (2009) tested antibacterial activity of pomegranate peel's extract on *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli* and *Salmonella enteritis*, and reported MIC at a range of 0.5 to 4 mg/mL, where the highest value, 4, belonged to *Salmonella*, showing more resistance of it to the extract compared to other bacteria and the most susceptible bacteria was *Bacillus subtilis*.

Sadeghian et al. (2011) examined the antimicrobial effect of aqueous and methanol extracts of pomegranate peel on gram-positive bacteria *Staphylococcus aureus* and gram-negative *Pseudomonas aeruginosa* and *Candida albicans* yeast. Both types of extract showed good antimicrobial activity, and it was found that the methanol extract was more effective than the aqueous extract. The aqueous and methanol extracts showed a complete growth inhibitory concentration at concentrations of 40 and 5 mg/ml, and the methanol extract at a concentration of 5 mg/mL contained inhibitory effects on the growth of *Candida*, whereas the aqueous extract did not have an activity on this yeast. Moreover, gram-positive bacteria were more susceptible than gram-negative bacteria.

This study investigating the effect of pomegranate ethanol extract on *Staphylococcus aureus* showed that antimicrobial activity of pomegranate peel extract is comparable with commercial antibiotics like clindamycin, chloramphenicol, gentamicin, and vancomycin (Algurairy et al., 2018). The antimicrobial effects of methanol extract, ethanol, and the benzene extract of pomegranate were examined on *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsiella pneumonia*, *Salmonella typhi*, and *Bacillus subtilis*. All the extracts were effective in controlling the growth of these species. However, maximum growth inhibition (85.71%) of *Klebsiella pneumoniae* was obtained by the methanol extract at concentrations of 100 µl/ml (Chaudhary and Rahul, 2017). Nik Fallah et al. (2014) examined the effects of different concentrations of pomegranate peel extract, pomegranate seed extract, and the mix of them on *Streptococcus mutans* and *Lactobacillus acidophilus* under laboratory conditions. In this study, pomegranate peel extract had an inhibitory effect on bacterial growth. Pomegranate seed extract showed no effects on the growth of these two bacteria. However, the combination of extracts of pomegranate seed and peel had a more significant inhibitory effect on *L. acidophilus*. In contrast, the highest inhibitory effect on the growth of *S. mutans* was obtained by the pomegranate peel extract. The effect of the combination of pomegranate peel extract, metal salts, and vitamin C on different bacteria was investigated. In this study, the combination of pomegranate peel extract with ZnSO₄ inhibited the growth of *Bacillus subtilis*, *Staphylococcus spp.* moreover, *Brucella spp.* compared with other compounds. Also, the combination of pomegranate peel and vitamin C inhibited more growth against *E. coli* and *B. indicus* than other compounds (Yehia et al., 2011). Ferrazzano et al. (2017) examined the effect of pomegranate peel extract and juice on *Streptococcus mutans* and *Rothiadentocariosa* and reported that extract of the peel effectively reduced the growth and survival of both bacteria. Pomegranate juice extract showed high and moderate inhibition against *S. mutans* and *R. dentocariosa*, respectively.

As the results show, the extracts extracted from peel have an excellent antimicrobial effect, which can be attributed to phenolic compounds, and it was shown that generally, there is a direct relationship between these phenolic compounds and the antioxidant activity and antimicrobial properties (Ghasemzadeh et al., 2010).

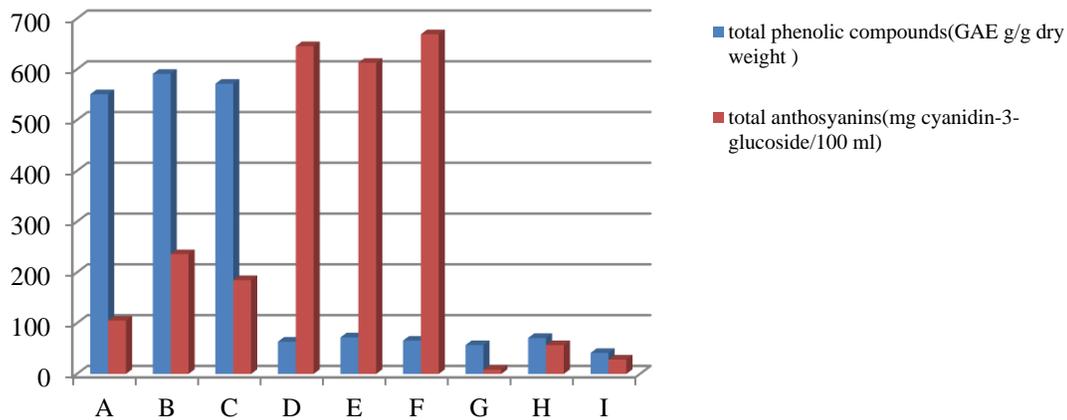


Fig. 1. Total phenolic compounds and total anthocyanidin properties of different parts of pomegranate (A: aqueous extract of pomegranate peel; B: organic extract of pomegranate peel; C: anthocyanin extract of pomegranate peel D: aqueous extract of pomegranate juice; E: organic extract of pomegranate juice; F: Anthocyanin extract of pomegranate juice; G: aqueous extract of pomegranate seed; H: organic extract of pomegranate seed; I: anthocyanin extract of pomegranate seed)

Table 4. Total phenolic compounds, Total anthocyanin and anti-oxidant activities of different parts of pomegranate

Group	A	B	C	D	E	F	G	H	I
Total phenolic	551±28	591±18	572±31	64±3	72±4	66±5	57±6	71±8	41±2
Total Anthocyanin	105±15	236±75	185±144	646±119	612±23	669±7	8±3	57±5	28±14
DPPH	0.0049 ±0.0015	0.0030 ±0.0007	0.0034 ±0.0008	0.5020 ±0.127	0.3900 ±0.0850	0.3520 ±0.1020	1.155 ±0.2550	0.1990 ±0.1260	0.6640 ±0.0890

A: aqueous extract of pomegranate peel; B: organic extract of pomegranate peel; C: anthocyanin extract of pomegranate peel; D: aqueous extract of pomegranate juice; E: organic extract of pomegranate juice; F: anthocyanin extract of pomegranate juice; G: aqueous extract of pomegranate seed; H: organic extract of pomegranate seed; I: anthocyanin extract of pomegranate seed

Total phenolic compounds, total anthocyanin, the highest amounts were found in anti-oxidant activities of different parts of pomegranate were shown in table 4. According to our results, the highest amount of total phenolic compounds and highest anti-oxidant activity were found in group B, C, and A, respectively. Also, about total anthocyanin highest amounts were group F, D, and E, respectively. A positive relationship between antioxidant activities and total phenolic contents was observed, the high level of total phenolic and flavonoid in pomegranate indicated high antioxidant activities. This correlation was also reported in previous studies on other plants (Ghasemzadeh et al., 2010; Hasna et al., 2009; Praven et al., 2007). Todaro et al. (2016) determined anthocyanin and total polyphenol contents as well as antioxidant activities of diverse sicilian and spanish pomegranate accessions using the Folin-Ciocalteu reagent. Based on the results, the anthocyanin and polyphenol contents were correlated with their antioxidant activities.

Abid et al. (2017) studied the total phenol contents and antioxidant activities of water, ethanol, and acetone extracts from four Tunisian pomegranate peels. They detected 24 compounds in pomegranate peel. The acetone fraction of acid ecotype was rich in ellagitannins, a group of phenolics that could be responsible for demonstrated antioxidant properties. Hmid et al. (2017) studied phenolic compounds and antioxidant activities of eighteen pomegranate cultivars in Morocco. They evaluated total polyphenols, total flavonoid, and total anthocyanin content. Phenolic compounds were identified as gallic, chlorogenic, caffeic, ferulic, ellagic acids, catechin, epicatechin, phloridzin, quercetin,

and rutin. pomegranate juice showed a high amount of polyphenols and antioxidant activity significantly, but some differences were found among the cultivars. The results revealed that the flavonoids are among the constituents involved in the antioxidant activity of pomegranate juice.

4. Conclusions

Overall, the results showed that *Staphylococcus aureus* was more susceptible than the extracts of other pathogens as *Salmonella typhi*, *E. coli* O175: H7 and *Bacillus cereus* and the results showed that *Salmonella typhi* was the most resistant microbial in this test. *Staphylococcus* and *Bacillus* are gram-positive bacteria, but *Salmonella* and *E. coli* are gram-negative bacteria. It appears that the cause of more resistance of gram-negative bacteria to essential plant oils is perhaps the more complexity of the dual cell membrane of these organisms compared to the single glycoprotein/techoic acid membrane of gram-positive bacteria. Furthermore, it seems that the resistance of microbial cells depends on the rate and degree of solubility of the antimicrobial agents in the lipid section of the cell membrane. Although this issue cannot be a complete explanation for the difference in susceptibility of gram-positive and negative bacteria, the difference in hydrophobicity of the cell membrane surface is recommended as an active agent. Also, the result obtained in this study showed the pomegranate was rich in phenol and flavonoid compounds with potentially high antioxidant activities. The total phenolic and antioxidant activities were highest in peels, intermediate in juice and lowest in seeds and total anthocyanin was highest in juices. The organic

extracts have the highest antioxidant activity, and the water extracts have the lowest antioxidant activity. Antioxidant activities were highly correlated with total phenolic. This result was also reported in previous studies.

Acknowledgments

Gratitude is expressed to the Council for Research at the Transport Phenomena Laboratory, Department of Agricultural Engineering and Technology, University of Tehran, Karaj, Iran.

References

- Abid, M., Yaich, H., Cheikhrouhou, S., Khemakhem, I., Bouaziz, M., Attia, H., & Ayadi, M. A. (2017). Antioxidant properties and phenolic profile characterization by LC-MS/MS of selected Tunisian pomegranate peels. *Journal of food science and technology*, 54(9), 2890-2901.
- Algurairy, A. T. M. (2018). Assessing the antibacterial activity of pomegranate against *Staphylococcus aureus* obtained from wound infections. *Research journal of pharmaceutical biological and chemical sciences*, 9(4), 1602-1606.
- Baron, J. O., & Finegold, M. (1990). Diagnostic microbiology 8th edition. The CV Company, New York, 435-433.
- Çam, M., Hışıl, Y., & Durmaz, G. (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food chemistry*, 112(3), 721-726.
- Chaudhar, A., & Rahul, S. N (2017). Antibacterial activity of *Punica granatum* (Pomegranate) fruit peel extract against pathogenic and drug resistance bacterial strains. *International journal of current microbiology applied science*, 6(12), 3802-3807.
- Conner, H. T., Dumas, D. H., & Nelson, L. L. (1993). *U.S. Patent No. 5,176,794*. Washington, DC: U.S. Patent and Trademark Office.
- Côté, J., Caillet, S., Dussault, D., Sylvain, J. F., & Lacroix, M. (2011). Effect of juice processing on cranberry antibacterial properties. *Food research international*, 44(9), 2922-2929.
- Dahham, S. S., Ali, M. N., Tabassum, H., & Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences*, 9(3), 273-281.
- Ferrazzano, G. F., Scioscia, E., Sateriale, D., Pastore, G., Colicchio, R., Pagliuca, C., & Scaglione, E. (2017). In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic bacteria. *BioMed research international*, 2017.
- Ghasemian, A., Mehrabian, S., & Majd, A. (2006). Peel extracts of two Iranian cultivars of pomegranate (*Punica granatum*) have antioxidant and antimutagenic activities. *Pakistan journal of biological science*, 7, 1402-405.
- Ghasemzadeh, A., Jaafar, H. Z., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale Roscoe*). *Molecules*, 15(6), 4324-4333.
- Hmid, I., Elothmani, D., Hanine, H., Oukabli, A., & Mehinagic, E. (2017). Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. *Arabian Journal of Chemistry*, 10, S2675-S2684.
- Holley, R. A., & Patel, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food microbiology*, 22(4), 273-292.
- Kaur, G., Jabbar, Z., Athar, M., & Alam, M. S. (2006). *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and chemical toxicology*, 44(7), 984-993.
- Lako, J., Trenery, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry*, 101(4), 1727-1741.
- Lansky, E. P., & Newman, R. A. (2007). *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of ethnopharmacology*, 109(2), 177-206.
- Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food chemistry*, 96(2), 254-260.
- Lucas, D. L., & Were, L. M. (2009). Anti-*Listeria monocytogenes* activity of heat-treated lyophilized pomegranate juice in media and in ground top round beef. *Journal of food protection*, 72(12), 2508-2516.
- Mousavinejad, G., Emam-Djomeh, Z., Rezaei, K., & Khodaparast, M. H. H. (2009). Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. *Food Chemistry*, 115(4), 1274-1278.
- Naz, S., Siddiqi, R., Ahmad, S., Rasool, S. A., & Sayeed, S. A. (2007). Antibacterial activity directed isolation of compounds from *Punica granatum*. *Journal of food science*, 72(9), M341-M345.
- Negi, P. S., & Jayaprakasha, G. K. (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. *Journal of food science*, 68(4), 1473-1477.
- Neto, C. C., Krueger, C. G., Lamoureaux, T. L., Kondo, M., Vaisberg, A. J., Hurta, R. A., & Reed, J. D. (2006). MALDI-TOF MS characterization of proanthocyanidins from cranberry fruit (*Vaccinium macrocarpon*) that inhibit tumor cell growth and matrix metalloproteinase expression in vitro. *Journal of the science of food and agriculture*, 86(1), 18-25.
- Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., & Chowwanapoonpohn, S. (2007). Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. *Food chemistry*, 103(3), 839-846.
- Olsen, S. J., MacKinnon, L. C., Goulding, J. S., Bean, N. H., & Slutsker, L. (2000). Surveillance for foodborne-disease outbreaks, United States, 1993-1997.
- Osman, H., Rahim, A., Isa, N., & Bakhr, N. (2009). Antioxidant activity and phenolic content of *Paederia foetida* and *Syzygium aqueum*. *Molecules*, 14(3), 970-978.
- Oussalah, M., Caillet, S., Saucier, L., & Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food control*, 18(5), 414-420.
- Praven, K., Ramamoorthy, A., & Awang, B. (2007). Anti-oxidant activity, total phenolic and flavonoid content *Morinda citrifolia* fruit. *Journal of engineering science*, 2, 70-80.
- Rosas-Burgos, E. C., Burgos-Hernández, A., Noguera-Artiaga, L., Kačániová, M., Hernández-García, F., Cárdenas-López, J. L., & Carbonell-Barrachina, Á. A. (2017). Antimicrobial activity of pomegranate peel extracts as affected by cultivar. *Journal of the science of food and agriculture*, 97(3), 802-810.
- Sadeghian, A., Ghorbani, A., Mohamadi-Nejad, A., & Rakhshandeh, H. (2011). Antimicrobial activity of aqueous and methanolic extracts of pomegranate fruit skin. *Avicenna Journal of Phytomedicine*, 1(2), 67-73.
- Schubert, S. Y., Lansky, E. P., & Neeman, I. (1999). Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of ethnopharmacology*, 66(1), 11-17.
- Seeram, N. P., Adams, L. S., Hardy, M. L., & Heber, D. (2004). Total cranberry extract versus its phytochemical constituents: antiproliferative and synergistic effects against human tumor cell lines. *Journal of agricultural and food chemistry*, 52(9), 2512-2517.
- Singh, R. P., Chidambara Murthy, K. N., & Jayaprakasha, G. K. (2002). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of agricultural and food chemistry*, 50(1), 81-86.
- Todaro, A., Cavallaro, R., La Malfa, S., Continella, A., Gentile, A., Fischer, U., & Spagna, G. (2016). Anthocyanin profile and antioxidant activity of freshly squeezed pomegranate (*Punica granatum* L.) juices of sicilian and spanish provenances. *Italian journal of food science*, 28(3), 464-479.
- Viuda-Martos, M., Fernández-López, J., & Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: a review. *Comprehensive reviews in food science and food safety*, 9(6), 635-654.
- Wu, V. C. H., Qiu, X., Bushway, A., & Harper, L. (2008). Antibacterial effects of American cranberry (*Vaccinium macrocarpon*) concentrate on foodborne pathogens. *LWT-Food Science and Technology*, 41(10), 1834-1841.