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

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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 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: MIC& MBC, organic extract, aqueous extract, anthocyanin extract, pomegranate.

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

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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 superoxide anions, hydroxyl and peroxyl 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), antimutation (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). 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 anymore, the number decreases. 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.

**Materials and methods**

**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 the Microbiology Department of the Food Industry Group of the Faculty of Agriculture of Tehran University. Salmonella typhi (ATCC1609) and Bacillus cereus (ATCC11788) were purchased from the Industrial Research Organization of Iran as the lipophilic ampoule. 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 (by 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.
Methodology

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.

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 in 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) in all three parts of the fruit.

Preparation of the fresh culture (in the logarithmic phase) from microorganisms
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.

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.

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).

Preparing the microbial suspension
A loop filled with each microbial strain under sterile conditions (between two flames of existence) 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 1× 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).

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 1×10^6 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).

**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, a 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:

\[
\text{TAC} = \frac{A \times \text{MW} \times \text{DF} \times 100}{\text{MA}}
\]

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).

**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.

**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:

$$DPPH_{\text{radical-scavenging}}(\%) = \left[1 - \frac{\text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}}\right] \times 100$$

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

<table>
<thead>
<tr>
<th>Dilutions of organic extract of the peel in ppm</th>
<th>1000</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>++</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

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

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.

**Results and Discussion**

In this study, MIC and MBC of the extracts were determined on four microorganisms - E. coli, Staphylococcus aureus, Salmonella typhi, and Bacillus cereus - in 3 replicates, where the average results of 3 replicates were reported as follows.

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. 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 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.

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

<table>
<thead>
<tr>
<th>Type of microorganism</th>
<th>Dilutions of anthocyanin extract of peel in ppm</th>
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<tbody>
<tr>
<td></td>
<td>1000</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>-</td>
</tr>
</tbody>
</table>

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

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.

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

<table>
<thead>
<tr>
<th>Type of microorganism</th>
<th>1000</th>
<th>500</th>
<th>250</th>
<th>125</th>
<th>62.5</th>
<th>31.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

++ shows the high growth of microorganisms, + low growth of microorganisms, and - lack of growth.
(Algurairy et al., 2018). In 2017, the antimicrobial effects of methanol extract, ethanol, and the benzene extract of pomegranate *Pseudomonas fluorescens, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae, Salmonella typhi,* and *Bacillus subtilis* were examined. 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). Abdulbary (2017) reported that pomegranate alcoholic and aqueous extracts could inhibit the growth of *Staphylococcus aureus*.

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.

In 2011, 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).

Total phenolic compounds, total anthocyanin, the highest amounts were found in Anti-oxidant Activities of different parts of Pomegranate were shown in table 1. 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 (Acide, Gabsi, Nebli, and Tounsi). 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.

**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 (Coteet et al., 2011; Dahham et al., 2010). *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

![Fig. 2. DPPH usage between treatment groups](image)

Fig. 2. DPPH usage between treatment groups (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. DPPH: 1,1-Diphenyl-2-picrylhydrazyl)
hydrophobicity of the cell membrane surface is recommended as an active agent (Holley and Patel, 2005). 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 (Negi et al., 2003; Wang et al., 2011).

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