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A Study on Biofilms Inhibition and Preservative Activity of Pomegranate Peel Methanol Extract (PPME)

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ABSTRACT

Objectives: Microorganisms with biofilms are associated with chronic human infections (highly resistant to antimicrobial agents). Pomegranate is used in treatment of several diseases, as its peels contain phenolic compounds and hydrolysable tannins. We aimed to study the effect of PPME on inhibition of biofilms and as a natural preservative in food industrial application. **Methods:** Pomegranate peel was extracted with methanol. E. coli clinical isolates were identified by standard microbiological procedures. Antibiotic susceptibility testing was performed using disc diffusion method. Antibacterial activity of PPME was tested using agar well diffusion assay. Minimum Inhibitory Concentration (MIC) was evaluated according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) agar dilution method. Biofilms detection was done by Tube method. Total phenolic content (TPC) in PPME was determined using spectrophotometric method. PPME ability to scavenge (1, 1diphenyl-2-picryl hydrazyl) DPPH free radicals was assessed by the standard method. Total antioxidant capacity of PPME was evaluated by phosphormolybdenum method. Total Phenolic Content (TPC) for cooked patties was freshly analyzed at different concentrations (1-0.25mg/ml). **Results:** Tested isolates were multidrug resistant. High antibacterial activity of PPME was found. MIC of PPME was found to be 10mg/ml. Tested isolates were biofilms producers, PPME largely affected on biofilm forming ability. TPC for treated group was (2785.19-349.22 µg/kg): for untreated group was 1251-226 µg/kg). DPPH radical scavenging activity was 38.12-5.45%. Thiobarbituric Acid Reactive Substances (TBARS) values were measured for samples from (0 -9 days) of storage period which was 0.522-1.3,0.859-1.816mg malonladehyde/kg meat for treated, untreated group respectively. AOA% was 31.49-66.92 from (0 – 9) days of storage. **Conclusions:** PPME was able to inhibit biofilm formation in tested isolates and can be promising in inhibiting contamination caused by biofilm forming bacteria in food industry.

Keywords: *Antibacterial activity; Antioxidant activity; Biofilm; Escherichia coli; Pomegranate peel extract.*

INTRODUCTION

According to Qur'an, the fruits like grapes, date, fig, olive and pomegranate are gifts and heavenly fruits of Allah. Pomegranate is one of the medicinal plants used in medicine for treatment of several disease, which was one of the oldest fruits that have not changed much through the history of man and regarded as an important source of phenolic compounds, including hydrolysable tannins, which possess high antioxidant activity[1].

Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit[2-4].

Polyphenols, flavonoids, condensed and hydrolysable tannins extracted from fruits, vegetables, herbs and spices have been explored as potential agents for treating or preventing a wide range of infections[5-7].

The antimicrobial mechanisms of phenolic compounds involve the reaction of phenolics with microbial cell membrane proteins and/or protein sulfhydryl groups that yield bacterial death due to membrane protein precipitation and inhibition of enzymes such as glycosyltransferases[8,9].

Pathogen adhesion to the host tissue is regarded as an important initiating step in many types of infection because it helps the bacteria to resist the defense mechanism in the body[10] and biofilms formation (a slimy layer with embedded micro colonies) is most important and widespread mode for increase pathogenicity of the microorganism and helps bacteria to resist the surrounding environment condition and antibiotic concentration[11].

Biofilms are a self-protection growth pattern of bacteria, which are different from planktonic cells. They have been of considerable interest in food hygiene since biofilms may contain spoilage and pathogenic bacteria which increases post-processing contamination and risk to public health. In addition, biofilms cells are more resistant to cleaning and disinfection processes in the food industry. Biofilms formation is a complex process in which genetic mechanisms and numerous factors such as the properties of substratum and bacterial cell surfaces are involved. In order to further understand the intricate mechanisms behind biofilms formation, various techniques including physical, chemical and molecular methods have been used to establish the possible model of biofilms formation in food industry. Therefore, the importance of bacterial biofilms in food safety control and biofilms formation mechanisms will be discussed in this paper. The objective of all efforts is to provide new insights for developing biofilms-free food-processing systems[12]. *E. coli* has been an important gram-negative model organism for in vitro analysis of biofilms formation on biotic surfaces[13,14].

E. coli is part of the normal flora in the intestine in humans and animals. Nevertheless, it is the most frequent cause of community and hospital acquired urinary tract infections and blood stream infection at all ages, also associated with intra-abdominal infections such as peritonitis, and with skin and soft tissue infections, meningitis in neonates and one of the leading causative agents of food borne infections worldwide[15].

Food-borne pathogens cause a considerable public health burden and challenge. They cause illnesses and deaths in all populations, particularly in groups at risk such as infants, children, elderly and immune-compromised persons. Diarrheal diseases, almost all of which are caused by food-borne or waterborne microbial pathogens, are leading causes of illness and death in less developed countries, killing an estimated 1.9 million people annually at the global level. Even in developed countries, it is estimated that up to one third of the population is affected by microbiological food-borne diseases each year. The majority of the pathogens causing this significant disease burden are now considered to be zoonotic. The occurrence of some of these zoonotic pathogens seems to have increased significantly over recent years. The most important source of food-borne disease is raw or improperly cooked food (meat and poultry, raw eggs, unpasteurized milk, shellfish and rice). Food handlers play a major role in ensuring food safety throughout the chain of food production. The most commonly recognized food borne infections are those caused by bacteria (*Campylobacter spp., Salmonella spp., E. Coli O157:H7, L. monocytogenes*); viruses (Hepatitis A virus, Hepatitis E virus, Rotavirus); mycotoxins; marine biotoxins and parasites (*Taenia solium, Taenia saginata, Echinococcus spp., Trich. spiralis, Fasciola, Cryptosporidium parvum, Entamoeba histolytica, Toxoplasma gondii*)[16].

Infections with *E. coli* usually originate from the person affected (auto-infection), but strains with a particular resistance or disease-causing properties can also be transmitted from animals, through the food chain or between individuals[15].

Antioxidants are added to different meat products to prevent lipid oxidation, retard development of off-flavors, and improve color stability. In the food industry, they can be divided into natural and synthetic antioxidants. BHA (butylated hydroxyanisole), PG (propyl gallate), and TBHQ (tert-butylhydroquinone) are examples of synthetic antioxidants; while ingredients obtained from natural sources which exhibit antioxidative potential in a food model system are considered as natural antioxidants. These antioxidants play a very important role in the food industry. However, synthetic antioxidants have been identified as toxicological and carcinogenic agents in some studies [17-20].

Thus, the food industry now chooses natural products over synthetic ones. Consequently, the food market is demanding natural antioxidants, free of synthetic additives and still orientated to diminish the oxidation processes in high-fat meat and meat products. Antioxidants vary widely in chemical structure and have varied mechanisms of action. The key mechanism is their reaction with free radicals to form relatively stable inactive products[21]. Thus, antioxidants delay lipid oxidation by scavenging free radicals which are generated in the initiation phase, propagation phase, or during the breakdown of the hydroperoxides. The level needed for such antioxidants to be effective in a given product corresponds to the concentration necessary to inhibit all chain reactions started by the initiation process. As long as the concentration of the antioxidants

is above this threshold level the total number of free radicals is kept at a constant low level. Subsequently, the antioxidant is gradually depleted and when its level is finally below the threshold level, radicals escape from the reaction with the antioxidant and the concentration of hydroperoxides increases. The high level of hydroperoxides further increases the concentration of radicals, and the remaining antioxidant molecules are used up completely. When all the antioxidants are consumed, the oxidative processes accelerate, and the increase in the production of secondary oxidation products leads to the progressing deterioration of the meat product. Based on their mode of action, antioxidants inhibit or prevent oxidation; they are again classified into 2 groups. The 1st group is primary antioxidants, which react directly with lipid radicals and convert them into relatively stable products; these are also called as chain-breaking antioxidative compounds. The 2nd group is secondary antioxidants, which can reduce the rate of oxidation by different mechanism of action.

Thus, it was aimed in this present work to evaluate the effect of PPME on inhibition of biofilms formation as one of important food industrial application as natural preserver due to its high antioxidant capacity. Hence, this study is of high importance in inhibiting contamination caused by biofilm forming bacteria in food industry.

MATERIALS AND METHODS

Pomegranate Peel Methanol Extract (PPME) Preparation

The fine powdered of Pomegranate peel was extracted with methanol (10%w/v) at room temperature for (48 hours). Then the extract was filtered through Wattman No.1. Rot-evaporation was performed on the methanol extract to evaporate the methanol for further studies[22].

Bacterial isolates

Twenty one clinically isolated samples were kindly supplied from Dr. Mohamed Abdel-Moaty, Mohamed, Abdel-All and El-Hendawy[23] after being identified (Isolates that were gram-negative, lactose-fermenting, non-swarming, indole positive, oxidase negative, producing acid slant/acid butt reaction with or without gas on triple sugar iron medium test, citrate negative and urease negative identified as *E. coli*[24]).

Susceptibility testing

Identified *E.coli* isolates were tested for their susceptibility to antibiotics discs purchased from Oxoid (Imipenem, Ceftriaxone, Cefotaxime, Vancomycin, Amoxicillin ,Clindamycin, Ciprofloxacin, Gentamycin ,Norfloxacin, Chloramphenicol ,Doxycycline and Nitrofurantoin) according to Bauer, Kirby, Sherris and Turck[25].Susceptibility testing was done on Mueller–Hinton agar (Oxoid)using McFarland 0.5 from overnight cultures. Inhibition zone diameters were interpreted according to EUCAST 2011 and The Clinical & Laboratory Standards Institute (CLSI) 2009–11 guidelines.

Determination of Antimicrobial Activity:

The agar well diffusion method was used to study the effect of PPME on growth of identified *E.coli* isolates by measuring of the diameter of the inhibition zone of well filled with 100µl PPME at concentration 300mg/ml , distilled water was used as a negative control[26].

Determination of minimum inhibitory concentration (MIC) of PPME

The MIC was evaluated according to EUCAST[27] agar dilution method in Mueller Hinton agar medium. PPME was dissolved in distilled water, and diluted by two-fold serial dilutions ranging from 40-0.025mg/ml. To 19 ml of agar medium, each dilution were added swirled carefully, then poured in Petri dishes and then leave to solidify. Subsequently, 2µl of each bacterial strain (10^4 CFU/ml) were inoculated on the Mueller Hinton agar surface. MIC was defined as the lowest antibiotic concentration, showing no visible bacterial growth after incubation time (37°C for 24h).

Tube method

It was the qualitative method used for biofilms detection before and after treatment with PPME[28],[29].A loopful of test organisms was inoculated in 10 mL of trypticase soy broth(Oxoid) supplemented with 1% glucose in test tubes. After incubation at 37°C for 24 hr., tubes were decanted and washed with phosphate buffer saline (pH 7.3) and then leave to dry then stained with crystal violet (0.1%). Wash excess stain was with deionized water. Tubes were dried in inverted position. The scoring for tube method was done according to the results of the control strains. Biofilms formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilms formed was scored as 1-weak/none, 2-moderate and 3-high/strong. The experiment was performed in triplicate and repeated three times.

Total phenolic content of PPME

The concentration of phenolics in plant extracts was determined using spectrophotometric method[30]. Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of Folin-Ciocalteu's reagent and 2.5 ml 20 % Sodiun carbonate (Na_2CO_3). The samples were

thereafter incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm, the blank is all of the added reagents except the sample. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract).

DPPH radical scavenging activity for PPME

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method[31], adopted with suitable modifications[32]. The stock solution of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250 and 125 µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanolic solution of DPPH in concentration of 11.8 mg/ml. After 30 min incubation in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. Control simple contained all the reagents except the extract. Percentage inhibition was calculated using (Equation 1), whilst IC_{50} values were estimated from the percentage inhibition versus concentration plot, using a non-linear regression algorithm, data were presented as mean values ± standard deviation (n = 3).

Equation 2:

% inhibition= A (blank)-A (sample) / A (Blank) × 100

Evaluation of antioxidant capacity for PPME by phosphor molybdenum method

The total antioxidant capacity of PPME was evaluated by the method of Prieto et al., (1999). An aliquot of 0.1 ml of sample (100 µg) solution was combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95° C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank in spectrophotometer. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. Ascorbic acid have been used as standard antioxidant and total antioxidant capacities of the extracts were expressed as mg/g equivalents of ascorbic acid[33].

Preparation of meat samples:

Two 100 grams of beef meat were weighted and one of them played as control (without any addition of the extract) and the other one was treated with 10 ml PPME[34].10 ml on 2%(w/v) Sodium chloride was added to control group instead of PPME, complete homogenization of these samples was occurred and cooking of these patties was done at hot air oven up to internal heat temperature reached to 80ºC. After cooling to room temperature, the obtained patties were stored at refrigerator for 9-days. Total phenolic content for cooked patties was analyzed freshly while TBARS values (lipid per oxidation) were measured at the obtained patties samples for 0, 3, 6 and 9 days of storage period and antioxidant activity (AOA%)was calculated.

Total phenolic content for cooked meat patties

Total phenolic content for cooked meat patties were estimated by Folin–Ciocalteus (F–C) assay[35]. Five grams meat was blended with 25 ml boiled distilled water and extracted for 1 h. Suitable aliquots of extracts were taken in different test tubes and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F–C (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance was recorded at 725 nm after 40 min. The amount of total phenolics was calculated as ascoric acid equivalent from the calibration curve using standard Ascorbic acid solution (0.1 mg/ml). During storage period total phenolics were measured at an interval of 3 days. The average decrease in total phenolics from 0^{th} day to 9^{th} day was also calculated arithmetically and expressed in percentage.

Thiobarbituric Acid Reactive Substances (TBARS)

The method published by Maraschiello, Sarraga and Garcia Regueiro[36] was used and modified by Byun, Lee, Jo and Yook[37] briefly meat aliquot (0.5 g) was weighed, added of 10 mL of 0.4M perchloric acid and samples were vigorously mixed (1 min). Then aliquot (2.5 mL) of 25 % TCA was added. The samples were stored for 15 min at 4°C, and then they were mixed and centrifuged (5 min, 4000 rpm, at 4°C). Supernatant aliquots (3.5 mL) were added to 1.5 mL of 0.8 % TBA and incubated for 30 min at 70°C. After incubation, the absorbance at 538nm was measured against a blank consisting of 2.5 mL of H_2O, 1 mL 25% aqueous TCA, and 1.5 mL 0.8 % TBA. The absorbance was converted to TBAR values using 1, 1, 3, 3- tetraethoxypropane to prepare a standard curve. Triplicates of 10 g samples were prepared for each treatment. TBA value is expressed as mg MDA/ kg meat.

Statistical analysis

The data are expressed as mean ± SD. Statistical comparisons were performed by One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. The results were considered statistically significant if the p value was less than 0.05.

RESULTS

Susceptibility of E. coli isolates to different antibiotics

All isolates were sensitive to Imipenem, while all were resistant to Ceftriaxone, Cefotaxime, Vancomycin, Amoxicillin and Clindamycin. Results for Ciprofloxacin showed that 28.5% of isolates were sensitive, while 71.5 were resistant, but for Gentamycin 90.5% were sensitive, 4.5% intermediate while 4.5% were resistant. Norfloxacin showed that 14.3% were sensitive while 85.7% were resistant. Chloramphenicol 57.14% were sensitive 4.5% intermediate while 38.1% were resistant, opposite to Doxycycline 14.3% were sensitive, 85.7% were resistant and Nitrofurantoin results were 76.2% sensitive,4.5% intermediate while 19.3% were resistant, Figure 1.

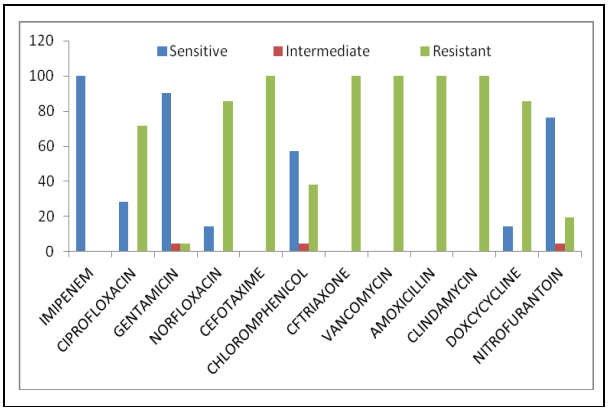

Figure 1. Susceptibility of *E.coli* isolates to different antibiotics

Antibacterial activity of PPME against E. coli isolates:

PPME showed various degrees of inhibition against the growth of investigated *E. coli* isolates, as shown in Figure 2 , the antibacterial activity of PPME against *E. coli* isolates ranged from 14 to 16.6 mm inhibition zones. It is clear that the highest inhibition was obtained for isolate 8.

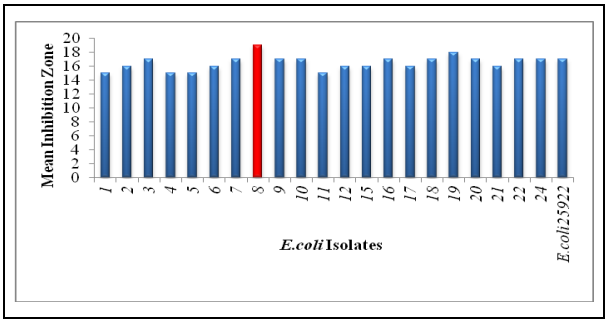

Figure 2. Error! Reference source not found.

MIC of PPME against E. coli isolates

An experiment was done for the determination of MIC of PPME against *E.coli* isolates under investigation. Serial dilutions (0.025-40 mg/ml) were made from the extract, and the results were found to be 10mg/ml for all isolates.

Tube method was used for biofilms detection (A) before and (B) after treatment with PPME

Biofilms formation was considered positive when a visible film lined the wall and the bottom of the tube. The amount of biofilms formed was scored as 1-weak/none, 2-moderate and 3-high/strong, Figure 3 and Table 1.

Total phenolic content (TPC) of PPME

Result of TPC of methanolic pomegranate peels extract was 30.9 mg GAE/g.

DPPH radical scavenging activity for PPME:

DPPH radical scavenging activity assay assessed the ability of the extract to donate hydrogen or to scavenge free radicals. DPPH radical is stable free radical and when it reacts with an antioxidant compound which can donate hydrogen it is reduced to diphenylpicrylhydrazine. Initially the solution was deep violet in color was due to the reduction of DPPH with antioxidant compounds present in the peels of pomegranate. The reduction was determined by the decrease in absorbance at 517 nm, Table 2. Maximum antioxidant activity of 38.12% was found in 1mg/ml of methanolic pomegranate peels extract and minimum antioxidant activity of 5.45% was found in 0.125mg/ml.

Table 1: The amount of biofilms formed was scored as 1-weak/none, 2-moderate and 3-high/strong

E. coli isolates	Biofilms formation before treatment	Biofilms formation after treatment
1	Weak/none	Weak/none
2	Moderate	Weak/none
3	Moderate	Weak/none
4	High	Moderate
5	High	Moderate
6	Moderate	Moderate
7	High	Weak/none
8	Weak/none	Weak/none
9	Moderate	Moderate
10	Moderate	Moderate
11	Moderate	Moderate
12	Moderate	Weak/none
15	High	Moderate
16	Moderate	Weak/none
17	High	Weak/none
18	Weak/none	Weak/none
19	High	Weak/none
20	Weak/none	Weak/none
21	High	Weak/none
22	High	Weak/none
24	Moderate	Weak/none

Evaluation of antioxidant capacity for PPME by phosphosmolybdenum method

The total antioxidant capacities of PPME was measured spectrophotometrically through phospho-molybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyzed and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm[33] . A high absorbance value of the sample indicates its strong antioxidant activity. The total antioxidant activity of PPME at 1mg/ml was 420µM/l ascorbic acid.

Total phenolic content for cooked meat patties:

Total phenolic content of PPME were illustrated in Table 3. Of treated meat patties was significantly (P<0.05) higher in control (untreated) meat patties.

Table 2. DPPH percentage of different concentrations of PPME

Used concentrations(mg/ml)	DPPH (%)
1	38.12
0.5	31.18
0.25	12.71
0.125	5.45

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS values and anti-oxidative activity (AOA %) of the cooked beef patties: Effect of PPME on thiobarbituric acid reactive substances (TBARS) values in cooked beef patties were shown in Table 4. All the treated meat patties significantly (P<0.05) reduced the TBARS values throughout storage compared to the control sample. The TBARS values significantly (P < 0.05) increased in control and a gradually increase was noticed also for all treated patties samples throughout the storage period, Table 5.

At the 9th day of storage the increase in control sample was the highest relative to all treated samples. AOA% in PPME patties increased (P< 0.05) up to the 9th day of storage.

Table 3. Total phenolic content for cooked meat patties

Concentration of PPME (mg/ml)	Total phenolic content (µg/kg) via Gallic acid	
	Untreated meat patties(control)	Treated meat patties
1	1251	2758.19
0.5	998.41	1190.56
0.25	618.89	723.43
0.125	226.2	349.22

Table 4: Thiobarbituric Acid Reactive Substances (TBARS) on cocked meat patties

Group of used patties	TRABS values (mg malonaldehyde/kg meat) via st. curve Refrigerated storage at 4ºC±1) days			
	0	3	6	9
Control (Untreated meat patties)	0.859	1.111	1.279	1.816
Treated meat patties	0.522	0.833	1.126	1.300

DISCUSSION

Food-borne pathogens cause a considerable public health burden and challenge. They cause illnesses and deaths in all populations, particularly in groups at risk such as infants, children, elderly and immune-compromised persons. According to WHO[15] the high percentage of resistance to 3rd generation cephalosporin reported for *E. coli* means that treatment of severe infections likely to be caused by this bacteria in many settings must rely on Carpaenems, the last resort to treat severe community and hospital acquired infections, our results comply with this as we recorded resistance in all isolates to Ceftriaxone, Cefotaxime, while all were sensitive to Imipenem. Also WHO[15] reported that *E. coli* with high resistance to fluoroquinolone meaning limitations to available oral treatment for conditions which are common in the community, such as urinary tract infections, in the same pathway our results reported that 28.5% of isolates were sensitive Ciprofloxacin, while 71.5% were resistant.

Industrial scale extraction of phenolic compounds from Pomegranate peels is carried out by using solvents such as methanol, ethanol, acetone, chloroform and ethyl acetate. Polar solvents have greater anti-oxidant extraction capability compared to non-polar solvents. The use of different solvents other than water for peel phenolic extraction are reported to yield different phenolic content ratios and associated antioxidant activity[3,4].

PPME was active against the growth of tested *E. coli* isolates, where inhibition zones ranged from 14 to 16.6 mm. These results provide evidence for the presence of antimicrobial compounds in PPME, and that agree with the results of Ibrahium[38], which showed that Pomegranate peel extract had high polyphenolic content (867 mg/g) and was effective against the growth of *S. aureus*, *E. coli*, *A. niger* and *S. cerevisiae* and giving inhibition zones ranged from 9.6 to 25.7 mm.

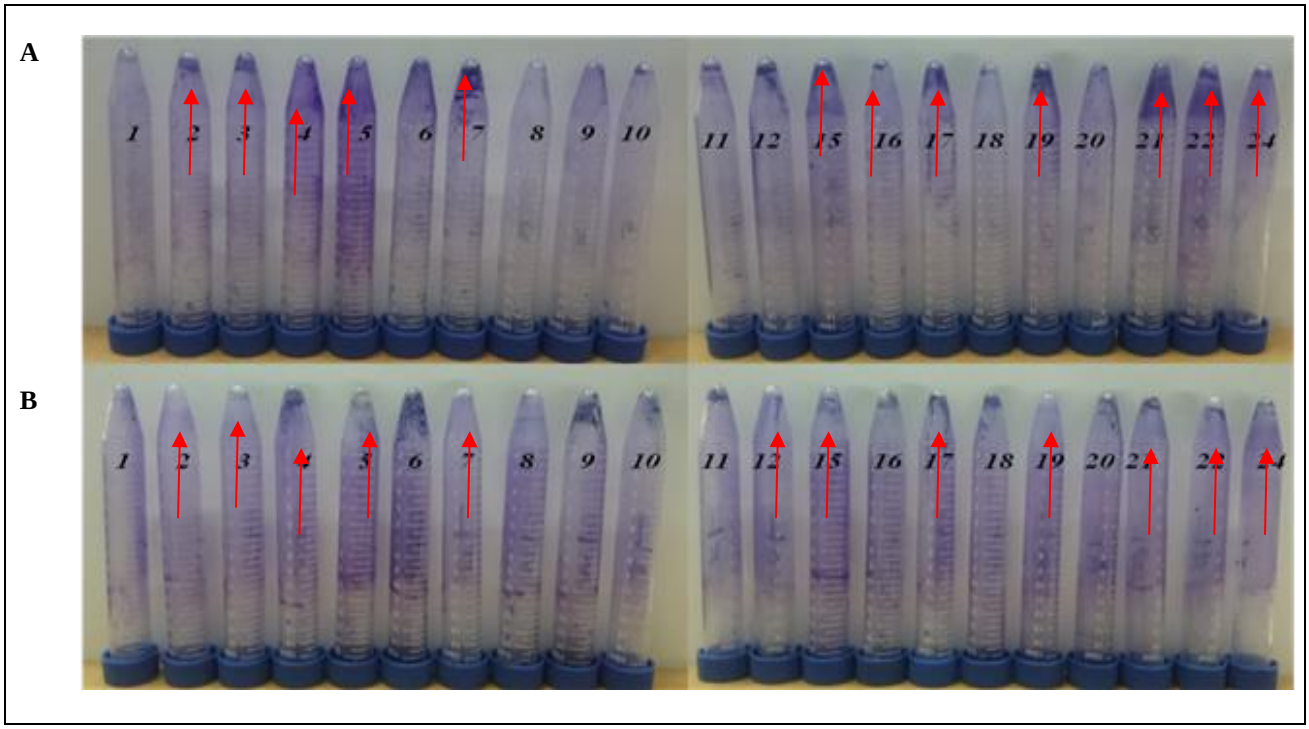

Figure 3. Biofilm detection (A) before and (B) after treatment with PPME. Red arrow point to biofilm formation before and after treatment.

Also reported results[39] showed clearly that pomegranate peel extracts were active against *S. aureus* and *E. coli* bacteria in comparison to ciprofloxacin as a positive control and the distilled water as a negative control. The alcohol solvent could be considered as the best one among the three solvents alcohol, acetone and distilled water which were used in their study. Also they found that when bacteria were treated with alcohol extract of pomegranate peel it came to be transferred from high biofilms producer to producer only, and that comply with our results.

The antibacterial activity was attributed of pomegranate peel extracts due to the presence of the broad spectrum antimicrobial compounds[40].

It was also reported[41] that methanol extract of *Punica granatum* MIC for *E. coli* isolates were between 0.64-2.56 mg/ml while in this study, results reported to be 10 mg/ml.

Pomegranate peel extracts contain compound such as tannins that can interact with macromolecules, including carbohydrates and proteins, which made these compounds as promising anti-adhesive and anti-biofilms[42].

Biofilms forming isolates from symptomatic UTI showed mixed drug resistance pattern[43], which agree with these results.

In the present study, total phenolic content was higher than in similar Egyptian study which the extract had 6.2 mg GAE/g dry solids[44], while in another studies in India the total phenolic content was 124.3 mg GAE/g dry solids[45,46]. Also, Yamani cultivar was found to have high total phenolics (91.2 mg GAE/g dry solids)[47].This variation of total phenolics could associated with the difference in cultivars, methods of extraction and environmental conditions such as relative humidity and temperature of extracts. It was suggested that the high amounts of bioactive compounds in an edible part which could be used for different purposes in the food industry such as enrichment or development of new products[48].

High antioxidant activity of pomegranate is due to the higher content of polyphenols. The results were more or less similar to Negi, Jayaprakasha and Jena[49] and Jayaprakasha, Singh and Sakariah[50] for pomegranate peel.

Table 5: The antioxidant activity of cooked beef patties as affected by addition of PPME as antioxidants stored at 4+1°C for 9 days

Group of used patties	Antioxidant activity (%)Refrigerated storage at 4°C±1) days			
	0	3	6	9
Treated with PPME	31.49	37.19	55.47	66.92

A high absorbance value of the sample indicates its strong antioxidant activity. The total antioxidant activity of PPME at 1mg/ml was 420µM/l ascorbic acid. Previously, Jayaprakasha, Girennavar and Patil[51] indicated that the total antioxidant activity of citrus was due to the presence of phenolics and flavonoids. The total antioxidant capacity in the present investigation may be attributed to total phenolic and flavonoid contents.

Phenolic compounds, secondary metabolites products by the plants, are generally responsible for the antioxidant activity of many fruits and vegetables[52].

They are important molecules contribute to antioxidant and pharmacological properties[53]. Most pomegranate fruit parts are known to contain higher polyphenolic compounds [54]. Phenolics present in pomegranate fruit peels may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product and to terminate free radical chain reaction. Protein precipitable phenolics, is a feature that comes from the chemical property (protein binding) of the polyphenolic compounds present in the extract of pomegranate fruit juice[55]. Pomegranate by-products (rind and seeds) have substantial amount of phenolic compounds and significant free radical scavenging activity[56]. Previous studies have demonstrated that pomegranate peel extracts exhibit markedly higher antioxidant capacity than the pulp, juice, and seed extracts[57-60].Therefore, there is a growing interest in the potential use of pomegranate peels as natural food preservatives and natural pharmaceuticals properties of pomegranate peel extracts[61-63]. There is widespread concern over hydrolysable tannins in pomegranate peels[64].

The large amount of phenolics contained in PPME may cause its strong antioxidant ability as stated by Li, Yang, Wei, Xu and Cheng[58] . Also, a significant relation between phenolic content and antioxidant effect of pomegranate peel extract has been reported by Negi and Jayaprakasha[3]. The total antioxidant capacity or activity has been generally recognized as a tool to test the antioxidant potential of a pure compound or a food extract[65]. Antioxidant activity of a food could be a useful index to predict oxidative stability[66]. Data on the antioxidant activity of cooked beef patties as affected by addition of PPME as antioxidants stored at 4+1°C for 9 days were depicted in Table 4. Within the tested samples, a significant difference between the AOA percent as a result of adding the PPME during storage for 9 days was observed. Thus using natural plant extracts to inhibit contamination by biofilms forming bacteria in food industry.

The pomegranate peel phenolics may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminate free radical chain reactions[49,50].

CONCLUSION

It could conclude from the obtained findings that PPME is of high importance in inhibiting contamination caused by biofilm forming bacteria in food industry.

Conflict of Interest

The authors declare there is no conflict of interest.

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