

Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents

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Abstract: The aim of this study was to find plant extract with antimicrobial and antioxidant properties that could be potentially used as natural preservative in foods. Pomegranate peel extract (PPE) was evaluated their antimicrobial activity (by the agar diffusion method against the growth of *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Saccharomyces cerevisiae*) and antioxidant effect (by determine the oxidative stability of sunflower oil containing PPE). Also, to clarify the hepatoprotective effect of PPE on levels of serum lipid fractions, liver enzymes and kidney functions, hypercholesterolemic rat groups were fed on basal diet containing PPE (at levels 400 or 800 mg/kg rat body weight) and the remaining groups were positive and negative control. The results showed that PPE had high polyphenolic content (867 mg/g) and was effective against the growth of tested microorganisms and giving inhibition zones ranged from 9.6 to 25.7 mm. The antioxidant activity of PPE at levels 400 and 800 ppm were 41.5 and 63.4%, respectively while BHT at level 200 ppm was 43.9%. The biological study exhibited that feeding hypercholesterolemic rats on basal diet containing PPE at different levels obviously reduced ($P < 0.05$) the levels of TC, TG and TL in rats serum by rates from 41.1-52.1% as compared to (C-) group (28.6-30.2%). Also, results indicated that the lowest levels of ALT, AST, ALP, uric acid, urea and creatinine were found in hypercholesterolemic rat group fed on basal diet containing PPE at level 800 mg/kg between tested rat groups. It could be concluded that, PPE exhibited strong antimicrobial effect and high antioxidant activity. In addition it enhanced liver and kidney functions. Consequently this extract might be used as multifunctional preservative in foods.

Key words: Pomegranate peel % Polyphenols % Antimicrobial % Antioxidant % Liver enzymes % Kidney functions

INTRODUCTION

Numerous food products require protection against microbial spoilage during their shelf life. The growing demand of consumers for safe and natural products has resulted in thorough investigations from food authorities and researchers to assess the feasibility of mild preservation techniques and to improve the microbial quality and safety of products, with maintaining their good nutritional and organoleptic properties [1].

The use of synthetic antioxidants in food has been decreased due to their suspected action as promoters of carcinogenesis, as well for the general consumer rejection of synthetic food additives [2]. Several studies indicated that the use of synthetic antioxidants has begun to be restricted because of their health risks and toxicity [3]. Therefore, the importance of replacing synthetic antioxidants with natural ingredients from spices and

other plant materials has greatly increased. The pomegranate is one of the important dietary sources of antioxidant phenolics [4]. Pomegranate peel is recognized for its many health-promoting qualities and apparent wound-healing properties [5], antimicrobial activity [6], anticancer property [7], antiatherosclerotic and antioxidative capacities [8]. This antioxidant capacity has been mainly attributed to the water-soluble polyphenols, anthocyanins and hydrolysable tannins.

There have been some investigations of the antimicrobial effects and antioxidant activities of several herbs on variety of foods. However, there is paucity of information regarding the inhibitory effects of pomegranate peel extract on food borne pathogens and lipid oxidation. Therefore, the objectives of the present study were to evaluate the ability of pomegranate peel extract to inhibit some microorganisms growing and determine their antioxidant activity. Also, investigating

the effect of this extract on serum lipid fractions, liver and kidney functions through rat feeding trials was another target.

MATERIALS AND METHODS

Materials: Ripened and freshly harvested pomegranate fruits (season October 2009) were obtained from local market. Freshly refined sunflower oil without synthetic antioxidant was obtained from Egypt oils and soap Company, Zagazig, Sharkia, Egypt. Butylated hydroxytoluene (BHT) and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, Mo). *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria), *Aspergillus niger* as mold and *Saccharomyces cerevisiae* as yeast were obtained from Microbiology Department, Sciences Faculty, Al-Azhar University, Cairo, Egypt. Kits for total cholesterol, triglycerides, total lipids, ALT, AST, ALP, uric acid, urea and creatinine were obtained from Biodiagnostic Co., Egypt.

Methods

Preparation of Pomegranate Peel Extract (PPE): Pomegranate fruits were washed by distilled water then peeled and their edible portions were carefully separated. The peels were air dried in a ventilated oven at 40°C for 48 h and ground to a fine powder and passed through a 24-mesh sieve. 100g powdered sample was extracted with 800ml ethanol at room temperature for 24 h in shaking water bath as described by Shan *et al.* [9]. The mixture filtered through a Whatman No. 2 filter paper for removal of peel particles. The extract was freeze-dried and stored in refrigerator at 4°C until use.

Determination of Polyphenolic Compounds: HPLC technique was used for separation and estimation of polyphenolic compounds in PPE according to the method described by Carballo *et al.* [10]. While, total polyphenols were determined colorimetrically using Folin-Ciocalteu reagent according to the method described by Singleton *et al.* [11]. The absorbance was measured by an automated UV-VIS spectrophotometer at 750 nm.

Determination of Antimicrobial Activity: The agar diffusion method was used to study the effect of PPE on growth of *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Saccharomyces cerevisiae* by measuring of the diameter of the inhibition zone [12]. 10ml

sterile assay agar was added to each of two Petri dishes with slow shaking. Warmed agar was inoculated with 0.5ml active bacterial culture (1×10^5 cfu/ml) and allowed to harden in a refrigerator at 4°C for 1 h. After wards, four equidistant holes were made in the agar using sterile cork borers and 100µl PPE (at concentrations 300, 600 or 900 µg/ml) was added on the top of inoculated agar layer then dried at 25°C for 30 min. Plates were kept at 4°C for 1 h then incubated at 37°C for 24-48 h [13]. At the end of this period, inhibition zones formed on medium were accurately measured in mm.

Determination of Antioxidant Efficiency: Different concentrations of PPE (400 and 800 ppm) or BHT (200 ppm) were individually added to 50g sun flower oil to study their antioxidant efficiency. The designation of induction period by rancimat 679 (Metrom Ltd. CH.9100 Herisau, Switzerland) was taken as a tool to compare the effectiveness of pomegranate peel extract and synthetic antioxidant on oxidative stability of oil according to the method described by Mendez *et al.* [14].

Biological Investigation: Twenty four adult male white albino rats (weighing 100 - 120g) were housed individually in mesh-bottom stainless steel cages in a controlled environment. All rats were fed on basal diet, prepared as described by Reeves *et al.* [15], for 7 days. Diet and deionized water were supplied *ad libitum* throughout the study. After wards, rats were randomly divided into four groups as follows:

Group 1: Rats fed on hypercholesterolemic diet, prepared as described by Galal [16], for 7 weeks (C+).

Group 2: Rats fed on hypercholesterolemic diet for 3 weeks followed by basal diet for 4 weeks (C-).

Group 3: Rats fed on hypercholesterolemic diet for 3 weeks followed by basal diet containing PPE at level 400 mg/kg rat body weight for 4 weeks.

Group 4: Rats fed on hypercholesterolemic diet for 3 weeks followed by basal diet containing PPE at level 800 mg/kg rat body weight for 4 weeks.

Biological investigation was carried out at the animal house of Sciences Faculty, Al-Azhar University, Cairo, Egypt.

Blood Sampling: Blood samples were obtained from orbital venous plexus (after 0, 4 and 8 weeks) by means of fine capillary glass tubes. The samples were placed in dry and clean centrifuge tubes and allowed to clot for 1-2 h at 37°C. The serum was separated by centrifugation at 3000 r.p.m. for 10 min. The supernatant serum was then pipette into epindorff tubes and immediately frozen at -70°C until use. At the end of experimental period, rats were sacrificed by decapitation after an overnight fast. The separated serum used for assay of blood serum parameters.

Determination of Serum Lipid Fractions: Total cholesterol, triglycerides and total lipids in blood serum were determined according to the methods described by Allain *et al.* [17], Fossati and Prencipe [18] and Zollner and Kirsch [19], respectively.

Liver Function Tests: Serum alanine amino transferase (ALT), aspartic amino transferase (AST) and alkaline phosphatase (ALP) were measured by ultraviolet spectrometry method according to Oser [20].

Kidney Function Tests: Uric acid, urea and creatinine in blood serum were determined by enzymatic colorimetric methods according to Barham and Trinder [21], Fawcett and Scott [22] and Bartels and Bohmer [23], respectively. Statistical Analysis: The data were statistically analyzed by analysis of variance (ANOVA) and least significance difference (LSD) at a significance of probability 5% [24].

RESULTS

Polyphenolic Composition of PPE: Table 1 shows that total polyphenols content is relatively high in PPE (867 mg/g). Also, the same table indicates that the polyphenolic composition of PPE is characterized by a highly proportion of punicalagin (296 mg/g) in comparison to the remaining compounds.

Antimicrobial Activity of PPE: Pomegranate peel extract showed various degrees of inhibition against the growth of investigated microorganisms as shown in Table 2. Data showed that there were significant differences ($P < 0.05$) among inhibition effects on the tested microorganisms. Hence, the inhibitory effect of PPE increased by increasing their concentrations and was inhibition zones ranged from 9.6 to 25.7 mm. Also, from the same table, it is clear that the highest inhibition was obtained for *E. coli*, while the lowest was for *S. cerevisiae*.

Effect of PPE on Oxidative Stability of Sunflower Oil: Results presented in Table 3 indicated that the induction period of control oil sample (without antioxidant) is 8.2 h. Increasing of PPE level from 400 to 800 ppm in sunflower oil obviously increased the induction period from 11.6 to 13.4 h with antioxidant activity reached to 63.4%. While, antioxidant activity of sunflower oil containing 200 ppm BHT was 43.9%.

Table 1: Polyphenolic composition and TP of PPE

Polyphenolic compounds (mg/g)				TP (mg/g)	
Punicalagin	296	Delphinidin	34	Cinnamic acid	42
Punicalin	15	Pelargonidin	21	Coumaric acid	32
Ellagic acid	18	Quercetin	40	Ferulic acid	28
Gallic acid	71	Kaempferol	62	Sinapic acid	17
Cyanidin	26	Luteolin	33	Caffeic acid	11
					867

Table 2: Effect of PPE on growth of tested microorganisms

Sample Concentration ($\mu\text{g/ml}$)	Diameter of inhibition zone (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>A. niger</i>	<i>S. cerevisiae</i>
PPE 300	11.9 ^c	18.8 ^c	10.3 ^c	9.6 ^c
PPE 600	16.2 ^b	22.1 ^b	12.8 ^b	11.2 ^b
PPE 900	19.6 ^a	25.7 ^a	15.2 ^a	13.0 ^a

^{a, b, c} means in each column with different superscripts are different significantly ($p < 0.05$)

Table 3: Effects of PPE and BHT on oxidative stability of sunflower oil

Items	Control	BHT (200 PPM)	PPE (ppm)	
			400	800
Induction period (h)	8.2	11.8	11.6	13.4
Antioxidant activity* (%)	-	43.9	41.5	63.4

*AA% = Induction period of sample - Induction period of control / Induction period of control x 100

Table 4: Effect of PPE on levels of TC, TG and TL of tested rats

Parameter (mg/dl)	Period (week)	C+	C-	PPE (mg/kg)	
				400	800
TC	0	84.38			
	4	213.56			
	8	258.41 ^a	181.83 ^b	140.69 ^{bc}	123.85 ^c
TG	0	92.21			
	4	191.07			
	8	234.12 ^a	163.41 ^b	137.94 ^{bc}	124.06 ^c
TL	0	192.51			
	4	426.46			
	8	519.89 ^a	371.00 ^b	306.25 ^{bc}	271.34 ^c

^{a, b, and c} means in each row with different superscripts are different significantly (p! 0.05)

Table 5: Effect of PPE on liver enzyme activities of tested rats

Parameter (mg/dl)	Period (week)	C+	C-	PPE (mg/kg)	
				400	800
ALT (GPT)	0	12.38			
	4	18.79			
	8	25.53 ^a	16.86 ^b	14.08 ^{bc}	12.96 ^c
AST (GOT)	0	20.02			
	4	31.97			
	8	44.68 ^a	26.87 ^b	23.04 ^{bc}	20.89 ^c
ALP	0	61.19			
	4	92.76			
	8	127.03 ^a	80.59 ^b	67.11 ^{bc}	61.34 ^c

^{a, b, and c} means in each row with different superscripts are different significantly (p! 0.05)

Table 6: Effect of PPE on levels of uric acid, urea and creatinine of tested rats

Parameter (mg/dl)	Period (week)	C+	C-	PPE (mg/kg)	
				400	800
Uric acid	0	3.72			
	4	5.76			
	8	7.43 ^a	4.87 ^b	4.23 ^{bc}	3.98 ^c
Urea	0	15.43			
	4	22.59			
	8	33.12 ^a	21.63 ^b	18.09 ^{bc}	16.78 ^c
Creatinine	0	0.51			
	4	0.89			
	8	1.27 ^a	0.80 ^b	0.74 ^{bc}	0.66 ^c

^{a, b, and c} means in each row with different superscripts are different significantly (p! 0.05)

Effect of PPE on Serum Lipid Fractions: The levels of total cholesterol, triglycerides and total lipids of rat groups fed on different diets are presented in Table 4. Data indicated that feeding rats on hypercholesterolemic diet for 3 weeks significantly increased (P<0.05) levels of TC, TG and TL from 84.38, 92.21 and 192.51 mg/dl to 213.56, 191.07 and 426.46 mg/dl, respectively.

From the same table, it could be also noticed that feeding the same rats on basal diet containing 400 or 800 mg/kg PPE for another 4 weeks obviously decreased (P<0.05) levels of TC, TG and TL by different rates as shown in Fig. 1.

Effect of PPE on Liver Enzyme Activities: Results present in Table 5 and Fig. 2 show serum ALT, AST and ALP levels initially and after feeding rats on different diets. After feeding on hypercholesterolemic diet for 3 weeks, these levels significantly increased (P<0.05) from 12.38, 20.02 and 61.19 mg/dl to 18.79, 31.97 and 92.76 mg/dl, respectively. At the end of experimental period, these levels obviously decreased (P<0.05) to 12.96, 20.89 and 61.34 mg/dl, respectively after feeding rats on basal diet containing 800 mg/kg PPE, while it were 16.86, 26.87 and 80.59 mg/dl in (C-) group, which fed on basal diet only, respectively.

DISCUSSION

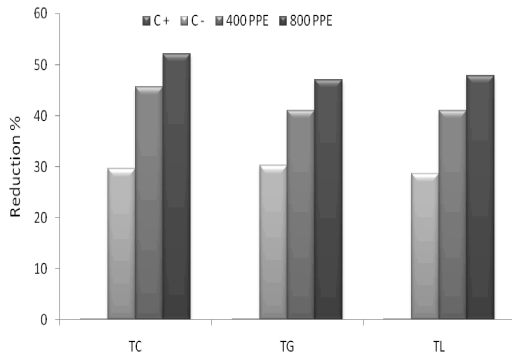


Fig. 1: Reduction % of TC, TG and TL in tested rats serum

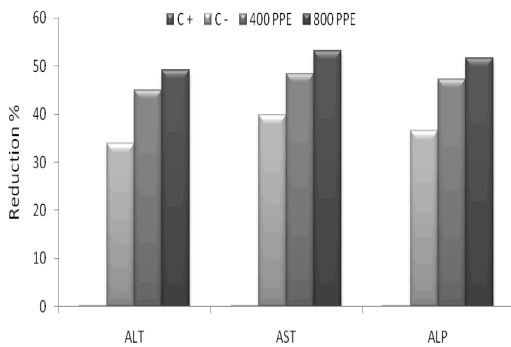


Fig. 2: Reduction % of ALT, AST and ALP in tested rats serum

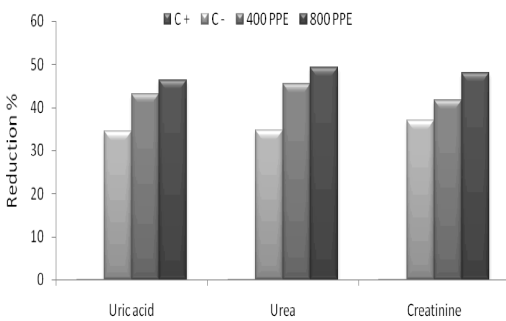


Fig. 3: Reduction % of uric acid and urea in tested rats serum

Effect of PPE on Kidney Functions: Results given in Table 6 and fig. 3 indicated that levels of uric acid, urea and creatinine in the investigated rats at zero time were 3.72, 15.43 and 0.51 mg/dl, respectively. Feeding rats on hypercholesterolemic diet for 3 weeks significantly increased ($P < 0.05$) these levels to 5.76, 22.59 and 0.89 mg/dl, respectively. Feeding these rats on basal diet containing PPE at different levels exhibited extremely lower values of uric acid, urea and creatinine as compared with (C-) group.

In the last few years, an increased attention has been focused on the industrial wastes, especially those containing residual phenols from the plant raw material used. Pomegranate peel is one of the important dietary sources of antioxidant phenolics.

Qualitative analysis of polyphenolic composition indicated that PPE had high amount of total polyphenols (867 mg/g). This result is in agreement with Carballo *et al.* [10] who studied the physical and chemical characteristics of 19 pomegranate cultivars and found that the amount of TP varied between 354 and 783 mg/g.

Pomegranate peel extract was active and effective against the growth of tested microorganisms. Whereas, the inhibition zones ranged from 9.6 to 25.7 mm depend on type of microorganism. These results provide evidence for the presence of antimicrobial phenolic compounds in PPE. These compounds can degrade the cell wall, disrupt the cytoplasmic membrane, damage membrane proteins and interfere with membrane-integrated enzymes, which may eventually lead to cell death [25].

Regarding the antioxidant activity, it could be noticed that addition of PPE at different levels to sunflower oil samples enhanced their oxidative stability. The greatest effect was found at level 800 ppm peel extract as compared to synthetic antioxidant. Generally, PPE exhibit good antioxidant capacity and is effective scavenger of several reactive oxygen species, due to its high levels of polyphenolic compounds [26].

Feeding rat trails exhibited that PPE at level 800 mg/kg rat body weight was able to reduce the levels of TC, TG and TL in hypercholesterolemic rat's serum by rates 52, 47 and 48%, respectively as compared with (C-) group (29, 30 and 28%, respectively).

The current study found a marked effect of PPE on rat liver functions that decreased ALT, AST and ALP by 1 to 1.5-fold compare to (C+) group. These results are in agreement with Toklu *et al.* [27], who studied the effect of chronic administration of PPE on liver fibrosis induced by bile duct ligation in rats and found that serum ALT and AST were significantly decreased by PPE treatment. These indicate that pomegranate peel preserved the structural integrity of the hepatocellular membrane and liver cell architecture which is confirmed by histopathological studies [28, 29].

Regarding the levels of uric acid, urea and creatinine, it could be noticed that lower levels were found in hypercholesterolemic rat group fed on basal diet containing 800 mg/kg PPE than other groups.

It could be concluded that, PPE contained high levels of phenolic compounds, consequently it exhibited effective antimicrobial properties and high antioxidant activity. In addition it enhanced liver and kidney functions. Therefore this extract might be used as multifunctional preservative in foods.

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