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Evaluation of Bioactive Compounds in Pomegranate Fruit Parts as an Attempt for Their Application as an Active Edible Film

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Abstract: Pomegranate fruit contains high content of phytochemical constituents which have many health benefits. Peel and rind are wastes of pomegranate fruit processing, represent up to 50% of its weight. This study aimed to evaluate the ethanolic extract of pomegranate fruit parts: arils, rind and peel as sources of bioactive compounds as well as their antioxidant and antimicrobial activities for their application as an active edible film. Results clearly demonstrated that peel extract (PE) had the highest content of total phenolics and flavonoids (342 mg GAE /g and 82.33mg catechol /g, respectively) followed by rind extract (RE) containing 213.00 mg GAE/g and 70.50 mg catechol /g, respectively, and finally arils extract (AE) (108.22 mg GAE /g and 55.58 mg catechol/g), respectively. Results indicated that total anthocyanins content was concentrated in PE (15.24mg Cynidian-3-glycoside/g) and AE (11.04 mg Cynidian-3-glycoside/g), while RE (6.51 mg Cynidian-3-glycoside/g) had the lowest value. Peel extract exhibited the highest antioxidant activity followed by RE and were significantly higher than that of AE. These results were confirmed with the DPPH and $ABTS^{+}$ assays. Consequently, PE followed by RE had higher antimicrobial activity against several pathogenic strains than AE and can be used as natural preservative for food. Peel extract and RE were incorporated into pectin film at concentration of 15 mg/ml to develop an active edible film. Pectin film without the tested fruit parts extract was used as the control film. The obtained results revealed that the film prepared from pectin with PE and RE was successfully developed and considers as an active edible film with antioxidant and antimicrobial properties.

Keywords: Pomegranate, Ethanolic Extract, Antioxidant and Antimicrobial Activity, Active Edible Film

1. Introduction

Pomegranate tree is considered as a medicinal plant and the fruit is better known as nature's power fruit recognized worldwide for pleasant taste and excellent health benefits [1]. Fruits of pomegranate are considered one of the most important deciduous shrubs grown successfully in Egypt, not only for local consumption but also for exportation [2]. An increasing demand of pomegranate for industrial processing to make juice, jams, syrup, sauce, flavouring, colouring agents and nutraceuticals is needed, in addition, to the growing demand for fresh consumption, where the rind or peel and seeds of the fruits are discarded [3]. Pomegranate possesses enormous antioxidants activity [4] and is rich in bioactive compounds, mainly polyphenols and anthocyanins, with known health benefits, and is effective in prevention of atherosclerosis, low-density lipoprotein oxidation, prevention of cancer, treatment of cardiovascular diseases and dental conditions, and protection from ultraviolet radiation [5].

The nutraceutical properties of pomegranate fruit are not limited to the edible part: in fact the non-edible fractions of

fruit and tree (i.e. peel, seeds, flowers, bark, buds and leaves), although considered as waste, contain even higher amounts of specific nutritionally valuable and biologically active components as compared to the edible fruit [6-8]. Pomegranate peel represents about 40–50% of the total fruit weight [9] and this may lead to various environmental problems [10]. It is produced as by product in huge amounts by the food industry and it is an important source of bioactive compounds [11]. Pomegranate peel has been known for many years for its health benefit, including antibacterial activity. The high level of bioactive compounds in the peel as well as the reported health benefits to date make these desirable by-products as functional ingredients in food, nutraceuticals and pharmaceutics [12]. Pomegranate rind is rich in tannins and polyphenols that demonstrate remarkable antimicrobial activity [13].

Edible coatings and edible films belong to the modern food protection system; over the past few years, interest in the use of edible coatings for perishable foods has considerably increased due to their advantages and potential applications [14]. Edible active films can function either by releasing the active agents into the surrounding atmosphere or through the absorption of components that can deteriorate the quality of food such as oxygen, moisture and free radicals [15]. Active agents with antimicrobial properties may help to extend the shelf life and maintain quality and food safety by increasing the lag phase and slowing the growth phase of microorganisms [16].

The objectives of the present research were to determine and identify the major phytochemical compounds found in the ethanolic extracts of pomegranate fruit parts (arils, rind and peel). The antioxidant and antimicrobial activities of each extracts as well as pectin-based edible films containing ethanolic extracts were also studied.

2. Materials and Methods

2.1. Materials

Pomegranate (*Punica granatum* L.) fruits variety "Wonderful" were obtained from local markets in Alexandria, Egypt. The fruits were selected on the basis of size uniformity, maturity stage “based on skin color and firmness "and absence of physical damage.

2.2. Chemicals

All chemicals used were of analytical grade, and were purchased from El-Gomhouria Co. for Chemical and Medical Requisites, Alexandria, Egypt. Chemicals used in HPLC were purchased from Bio-Rad chemical Co., CA, and U.S.A.

2.3. Methods

2.3.1. Preparation of Pomegranate Fruit Parts

Figure 1 shows pomegranate fruit parts, while Figure 2 shows the outline of preparing pomegranate fruit parts powder arils (A), rind (R) and peel (P) of pomegranate fruits. The fruits were washed with tap water, drained, cut manually to quarters and separated to arils, peel (leathery skin) and rind (spongy mesocarp), all cut into small pieces using a sharp knife and dried in an air oven at 50ºC for 24 hr. Then were ground using an electrical mill (SEB 21260), sieved to obtained particle size of 60 mesh and kept in low density polyethylene bags and stored at 5°C.

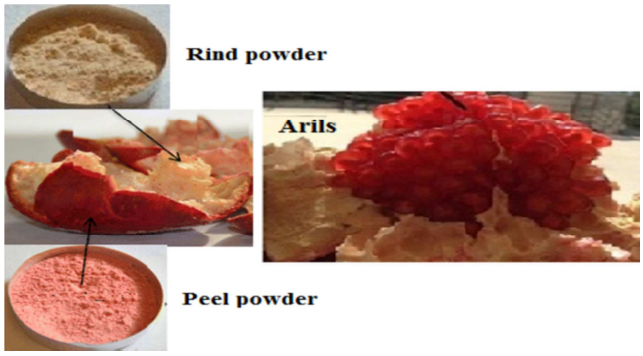

Figure 1. *Pomegranate fruit parts.*

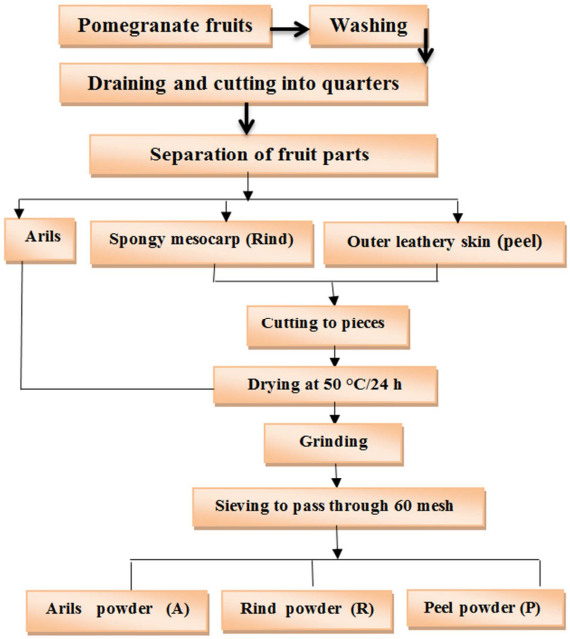

Figure 2. *Flow sheet for preparing pomegranate fruit parts powder arils (A), rind (R) and peel (p).*

2.3.2. Ethanolic Extraction of Dried Powder of Pomegranate Fruit Parts

Dried powder of A, R and P were extracted by ethanol 80% (1:5 w/v) at room temperature (25°C ± 2) for 24 hr. The mixture of ethanol with each of them was filtered, and the rough particles were removed by strainer. Then, the residue was reextracted using the same conditions. The collected extracts were concentrated in a rotary evaporator (40°C at 700 mmHg). The entire procedure was performed in the dark. The concentrated ethanolic extract for each powder was subjected to chemical analysis.

2.3.3. Preparation of Pectin Based Edible Films Containing Pomegranate Peel and Rind Ethanolic Extracts

According to Norajit *et al.* [17], with some modification,

pectin–based edible film was prepared by casting in 2 stages:

i. Dissolving 2 g pectin in 100 ml of distilled water, and glycerol (0.5 g/g of pectin) and calcium chloride (0.01g/g of pectin) were added to the suspension. The suspension was then heated to 70°C under stirring, until all the solids were dissolved and a homogeneous suspension was achieved. The suspension was cooled to 40°C, then the fruit part extract were added at the tested concentration (15 mg/ml) according preliminary study**.** A control treatment without the tested fruit parts extract was prepared for comparison.
ii. The film-forming solution was casted on an acrylic-coated plate and then dried for 10-12 h in an oven at about 35°C to obtain a constant weight. Then, the film could be easily removed from the plate. Before analysis, the films were conditioned in desiccators under 58% RH, at 25°C, for 48 h.

2.4. Analytical Methods

2.4.1. Total Phenolics Content

Total phenolic contents of extracts were determined using the method developed by Abirami *et al*. [18]. One and half milliliters of Folin–Ciocalteu's reagent (diluted 10 times) and 1.2 ml of Na2CO3 (7.5% w/v) were added to 300 µl of sample extract. Mixtures were shaken and kept at room temperature for 30 min before measuring absorbance at 765 nm using a spectrophotometer (Pg T80+, England), testes were carried out in triplicate. Total phenol content (TPC) was expressed as Gallic acid equivalent (GAE) in mg/g plant material or extract.

2.4.2. Total Flavonoids Content

The total flavonoids content of extracts were determined according to Barros *et al*. [19]. Shortly, 0.5 ml of sample extract was mixed with 2 ml of distilled water followed by addition of 150 µl of NaNO2 (5%) solution. After 6 min, 150 µl of AlCl3 (10% w/v) was added and allowed to stand for another 6 min before 2 ml of NaOH (4% w/v) was added. The mixture was brought to 5 ml with distilled water. Then the mixture was allowed to stand for 15 min at room temperature. The absorbance was measured at 510 nm using a spectrophotometer (Pg T80+, England). A calibration curve of Rutin was prepared and total flavonoids content were determined.

2.4.3. Determination of Total Anthocyanins Content (TA)

TA content was determined by pH differential method using two buffer systems: potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M) according to Elfalleh *et al*. [20]. Methanolic extract were mixed with 3.6 ml of corresponding buffers and read against water as a blank at 510 and 700 nm. Absorbance (A) was calculated using this formula A = [(A510 –A700) pH1.0 - (A510 –A700) pH4.5] with a molar extinction coefficient of 29600. Results were expressed as mg of cyanidin-3-glucoside equivalents per g dw (mg CGE/g dw).

2.4.4. HPLC Determination of Phenolic and Flavonoid Compounds

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A); 12-14 min (80% A) and 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35°C.

2.4.5. Scavenging Activity by DPPH Assay

Scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined according to the procedure based on Brand-Williams *et al*. [21]. Two milliliters of 0.15 mM DPPH were added to 1 ml of extracts in different dilutions. A control was prepared by adding 2 ml of DPPH to 1 ml of methanol. The contents of the tubes were mixed and allowed to stand for 30 min, and absorbance was measured at 517 nm using a spectrophotometer (Pg T80+, England). The results were expressed as% radical scavenging activity according to equation (1).

$$\text{Radical scavenging activity\%} = (A_{control} - A_{sample})/A_{control} \times 100 \quad (1)$$

Where:

$A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound).

A_{sample} is the absorbance with the test compound.

2.4.6. Determination of Radical $ABTS^+$ Scavenging Activity

The stock solutions of $ABTS^+$ reagent was prepared according to Hwang and Do Thi [22] by reacting equal quantities of a 7 mM aqueous solution of ABTS* with 2.45 mM potassium persulfate for 16 h at room temperature (25°C) in the dark. The working solution was then prepared by diluting 1 mL ABTS* solution with 60 mL of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0± 0.02 units at 734 nm using the spectrophotometer. Extracts (50 µL) were allowed to react with 4.95 mL of the $ABTS^+$ solution for 1 h in a dark condition. Then the absorbance was read at 734 nm using the spectrophotometer.% inhibition of the $ABTS^+$ free radical was calculated by equation (2)

$$\text{Inhibition (\%)} = 100 \times [(A_{control} - A_{sample}) / A_{control}] \quad (2)$$

Where:

$A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound).

A_{sample} is the absorbance with the test compound.

The standard curve was prepared using Trolox. The results were expressed as mg Trolox equivalents (TE)/g sample). Additional dilution was needed if the $ABTS^+$ value measured was over the linear range of the standard.

2.4.7. Antimicrobial Activity of the Ethanolic Extracts of Pomegranate Fruit Parts and Pectin Based Edible Films

i. Microorganisms and culture conditions

Ten pathogenic strains used to scan samples were; four Gram-positive strains; *Streptococcus dysgalactiae* subsp. *Equisimilis, Streptococcus mutans*EMCC1815, *Bacillus subtilis* DB100 and *Clostridium botulinum* ATCC3584*,* five Gram-negative strains; *Proteus hauseri*EMCC1227, *Escherichia coli* ATCC 25922, *Escherichia coli* BA12296, *Klebsiella pneumoniae* EMCC 1637 and *Pseudomonas marginalis*EMCC1271, in addition to one tested yeast strain; *Candida albicans*ATCCMYA-2876. All strains were obtained from Microbiological Resources Centres (MERCIN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The test was held and the strains were maintained in 60% glycerol/ LB culture at -80^{o}C by the Department of Food Technology, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Borg El-Arab, Alexandria, Egypt.

ii. Determination of minimum inhibitory concentration (MIC)

To examine antimicrobial activity of sample extracts, agar well diffusion assay was used against pathogenic bacteria and yeast as described by Hamad *et al.* [23]**.** The bacterial strains were grown in nutrient broth at 37°C, while, yeast strain was grown on Potato Dextrose Agar (PDA) at 29°C for 24 h.

Briefly, 100 µL of overnight activated culture of each pathogen strain (10^6 CFU/mL) were aseptically spread over nutrient agar plates. About 100 µL of 100% extract was transferred into each agar well individually. The plates were then incubated at 37°C for 18 h and the formed clear zones (if found) were measured and recorded. A set of 5 concentrations of sample extracts (50, 25, 12.5, 6.25 and 3.1 mL / 100 mL), were examined to determine the minimum inhibitory concentration (MIC) of each against a specific pathogenic strain [24]. The zones of inhibition were calculated by measuring the diameter of the inhibition zone around the well (mm), including the well diameter. The readings were taken in three different fixed directions in all duplicates and the average values were tabulated.

2.5. Statistical Analysis

Data were statistically analyzed by a general linear model procedure of the Fisher's protected least-significant difference (PLSD) test using SAS, 2004 (SAS Institute, Inc ,. Cary, NC) [25]. This test combines ANOVA with a comparison of differences between the means of the treatments at the significance level of $P \leq 0.05$.

3. Results and Discussion

3.1. Bioactive Compounds

Total phenolics, total flavonoids and total anthocyanins content of the ethnolic extracts of pomegranate fruit parts (PE, RE and AE) are shown in Table 1.

Values of total phenolics and flavonoids in the ethanolic extract of pomegranate fruit parts can be arranged as follow: PE had the highest values (342 mg GAE /g dw) of phenolics followed by RE (213.00 mg GAE /g dw), while, AE had the lowest value (108.22 mg GAE /g dw). Also, flavonoids content of PE, RE and AE had the same trend and their values were 82. 33, 70.50 and 55.58 mg catechol /g dw, respectively. These results revealed that PE had the highest content of total phenolics and flavonoids followed by RE and finally AE. Orak *et al*. [26] reported that pomegranate peel, seed and juice contained considerable amounts of phenolic compounds. Mphahlele *et al*. [12] stated that these compounds are mainly concentrated in fruit peels and mesocarp. Statistically, the obtained data of total phenolics and flavonoids showed high significant variation between the three tested extracts.

Table 1. *Total phenolics, flavonoids and anthocyanins content of ethanolic extract of pomegranate fruit parts (dw).*

Component	Fruit part extract*		
	AE	RE	PE
Total phenolics (mg GAE /g)	108.22^{c}±0.30	213.00^{b}±0.33	342.00^{a}±1.0
Total flavonoids (mg catechol /g)	55.58^{c}±0.12	70.50^{b}±0.38	82.33^{a}±0.25
Total anthocyanins mg Cynidian-3-glycoside/g	11.04^{b}±0.09	6.51^{c}±0.36	15.24^{a}±0.24

*AE: Arils extract RE: Rind extract PE: Peel extract.
The experimental values within each row that have no common superscript are significantly different ($p \leq 0.05$).

The data of total phenolics for PE in the present study are compatible with that mentioned by Derakhshan *et al*. [27] and higher than that mentioned by Rosas-Burgas *et al*. [28] and Sumaiya *et al*. [29]. The results of flavonoid obtained in the present study are higher than that found by Farag *et al* [30], Souleman *et al*. [31] and Sumaiya *et al*. [29]**.** These variations may be affected by many factors, such as cultivar source, growing and climatic conditions as well as extraction methods.

Total anthocyanins content in the ethanolic extract of the pomegranate fruit parts were 15.24, 11.04 and 6.51 mg Cynidian-3-glycoside /g dw for PE, AE and RE, respectively. These results showed that PE had the highest values followed by AE, while, RE had the lowest value. These results indicated that total anthocyanins content was concentrated in fruit peels and arils. From the statistical point of view, it can be noted high significant differences among the three values. Zhu *et al*. [32] confirmed that total anthocyanins content was higher in peel than that found in fruit juice. Hou *et al*. [33] stated that pomegranate fruit is rich in anthocyanin and it has many health benefits' such as antioxidant ability, anti-inflammatory, anti-cancer and other physiological functions.

3.2. Identification of Phenolics and Flavonoids in the Ethanolic Extracts of Pomegranate Fruit Parts

Several phenolic and flavonid compounds were identified in ethanolic extracts of the three pomegranate fruit parts (AE, RE and PE) using HPLC separation as shown in Table 2. The

number of unknown compounds detected in AE, RE and PE were 14, 19 and 20, respectively, according to the identified standard. Meanwhile, the identified phenolic and flavonoids compounds in PE, RE and AE extracts were 11, 9 and 6 respectively. These results have not been consistent with previous studies [31, 34-37]. These variation may be due to many reasons, such as, cultivar, agriculture process, solvent used, conditions of extraction and identification methods [37].

Table 2. *Identification of phenolics and flavonoids in the ethanolic extract of pomegranate fruit parts (µg /g dw).*

Compounds	Fruit part extract *		
Phenolics	**AE**	**RE**	**PE**
Gallic acid	28.519	278.44	6041.1
Caffeic acid	ND	ND	1220.37
Coumaric acid	ND	5.148	4.05
Vanillin	ND	ND	20.25
Cinnamic acid	43.22	1.829	0.7
Propyl gallate	3.01	4.55	6.35
Flavonoids			
Catechin	ND	963.36	864.325
Rutin	ND	7.425	24.15
Naringenin	24.38	58.85	1830.52
Querectin	6.89	14.36	0.7
4.7-Dihydroxyiso flavone	2.30	0.83	2.6
Un known	14	19	20

*ND: Not detected.
*AE: Arils extract RE: Rind extract PE: Peel extract.

The results in Table 2 shows that, PE had six identified phenolic compounds. They were gallic acid (6041.1 µg/g dw) that was the predominant followed by caffeic acid (1220.37 µg/g dw), while, the remaining four compounds were relatively low in their concentrations. These results are in accordance with that found by Souleman and Ibrahim [31] who found that gallic acid and caffeic acid were the most predominant compounds in pomegranate cultivars. RE had four identified compounds, where gallic acid (278.44 µg/g dw) was also the predominant, while, AE had 3 identified compounds where cinnamic acid (43.22 µg/g dw) was the most predominate.

Five flavonoids compounds were identified in PE and RE, while, only three compounds were identified in AE. These compounds in PE can be arranged in descending order according to their concentration (µg/g dw) as follows: naringenin (1830.52), catechin (864.33), rutin (24.15), 4.7-dihydroxyiso flavone (2.6) and finally querectin (0.7). The same previous compounds had different ranking according to their concentrations (µg/g dw) in RE as follows: catechin (963.36), naringenin (58.85), querectin (14.36), rutin (7.43) and finally 4.7- dihydroxyiso flavones (0.83). AE had only three identified compounds, where naringenin (24.38 µg/g dw) had the highest concentration followed by 4.7-dihydroxyiso flavones (2.3 µg/g dw).

Generally, PE had the highest concentration of phenolic and flavonoid compounds followed by RE, while AE had the lowest one except for cinnamic acid. Also, it was observed that PE was characterized by a high proportion of galic acid, caffeic acid, naringenin and catachin, while, RE was characterized by a high proportion of galic acid and catechin in comparison to the remaining compounds.

3.3. Antioxidant Activity

Table 3 shows the antioxidant capabilities of ethanolic extracts of pomegranate fruit parts (PE, RE and AE) as assessed by DPPH and ABTS radical scavenging.

The radical-scavenging activity on DPPH was expressed as IC_{50}. This value was the concentration of the extract required inhibiting 50% of the initial DPPH free radical. Table 3 revealed that PE exhibited the highest activity followed by RE and AE. The mean values were 1.9, 2.64 and 3.81 µg/ ml, respectively. The lower IC_{50} value means stronger scavenging DPPH free radicals. In general the antioxidant activity of PE was significantly higher than that of the other parts. This is consistent with [26].

Table 3. *Antioxidant activity of the ethanolic extract of pomegranate fruit parts.*

Antioxidant activity	Fruit part extract*		
	AE	**RE**	**PE**
IC_{50} (mg/ml)	4.81^{a}±0.16	2.64^{b}±0.23	1.90^{c}±0.02
ABTS mg Trolox Equiv/g	20.33^{c}±0.36	261.34^{b}±0.92	321.29^{a}±0.24

*AE: Arils extract RE: Rind extract PE: Peel extract.
The experimental values within each row that have no common superscript are significantly different ($p \leq 0.05$).

The $ABTS^{+}$ method was used to confirm the results from the DPPH test since it is based on a similar antioxidant mechanism and the results are shown in Table 3. The ethanolic extracts of pomegranate fruit parts showed that $ABTS^{+}$ activities of PE and RE were 312.29 and 261.34 mg TE/g dw, respectively. These values were significantly higher than that of arils being 20.33 mg TE/g. These results were correlated well with the findings of Jalal *et al*. [38] who reported significantly higher $ABTS^{+}$ activities of pomegranate peel.

The results clearly confirmed that PE contained more antioxidants followed by RE while AE had the lowest activity which are confirmed with the DPPH and $ABTS^{+}$ assays. The results implied that bioactive compounds from the peel might be potential resources for the development of antioxidant function dietary food. In general, antioxidant activities of PE were higher than that of RE and AE. This fact is consistent with previous studies which indicated high antioxidant activity of pomegranate peel comparing with the other parts [26, 39].

3.4. Antimicrobial Activity of Ethanolic Extract of Pomegranate Fruit Parts (AE, RE and PE)

The antimicrobial activity of ethanolic extracts of pomegranate fruit parts (AE, RE and PE) were investigated against some pathogenic strains by disc diffusion method. Figures 3, 4 and 5 show antimicrobial activity using various concentrations (3.5, 7.5, 15, 30, 62.5, 125 and 250 mg / ml) of ethanolic extracts. Results revealed that ethanolic extracts

of all pomegranate fruit parts can successfully control or inhibit the visible growth of the tested types of pathogenic strains and possessed an inhibitory effect. It was observed that antimicrobial activity differed according to the tested pomegranate part, its concentration and the types of pathogenic strains. PE had the highest inhibitory activity followed by RE, while, AE had the lowest. Increasing the concentration of any fruit part extract caused increase in the inhibition zone**.** These results are in agreement with several studies [40, 41].

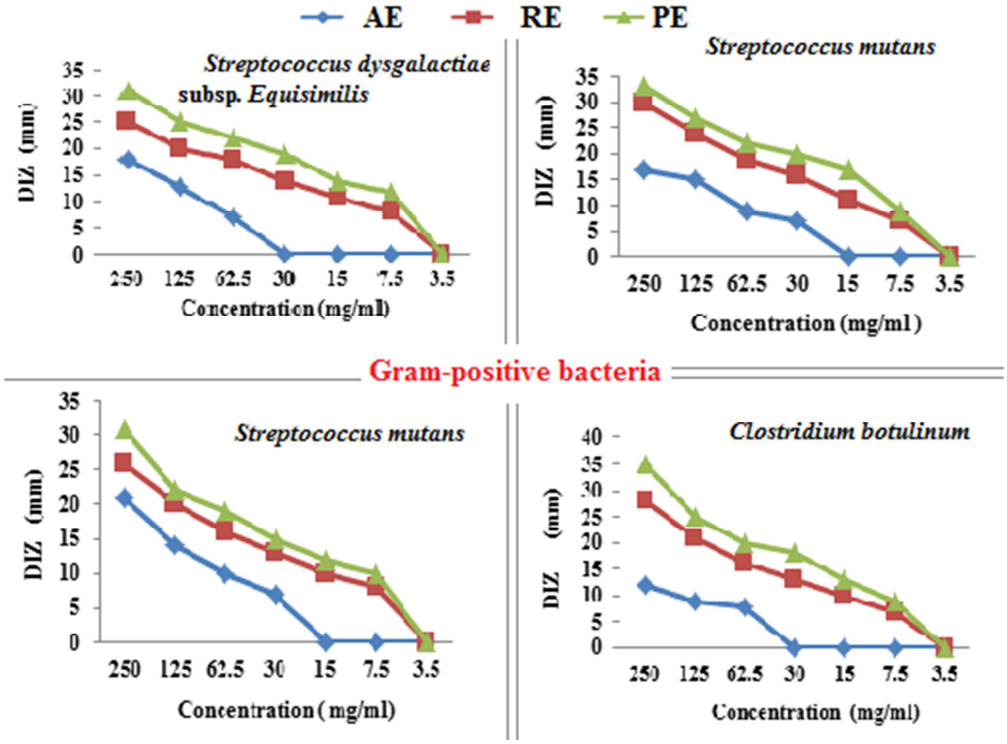

Figure 3. *Antimicrobial activity of pomegranate fruit parts ethanolic extracts against Gram-positive bacteria.*

DIZ: Diameter inhibition zone.
AE: Arils extract. RE: Rind extract. PE: Peel extract.

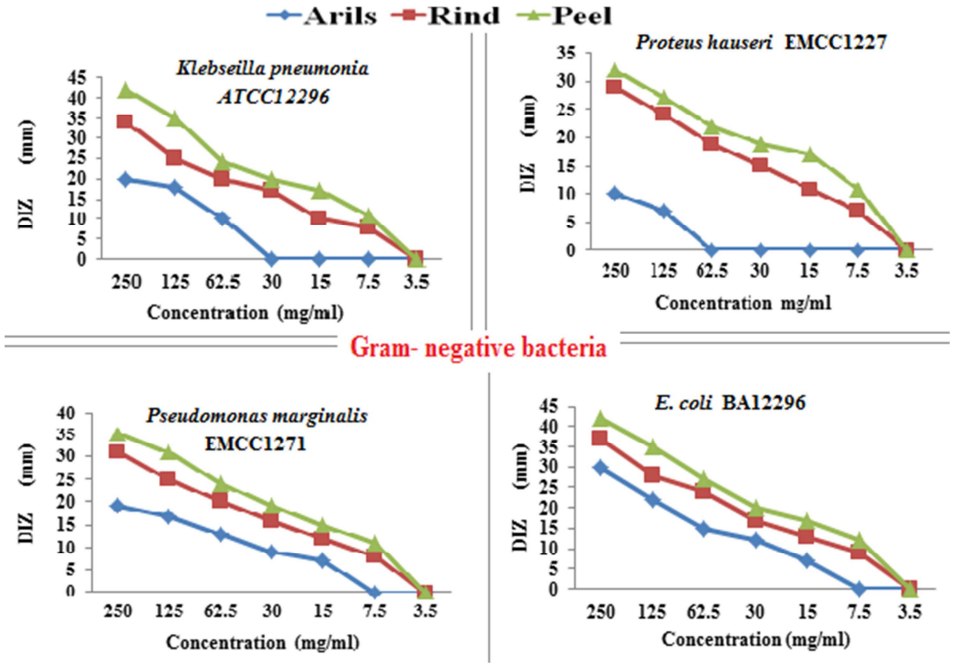

Figure 4. *Antimicrobial activity of pomegranate fruit parts ethanolic extracts against Gram- negative bacteria.*

DIZ: Diameter inhibition zone.
AE: Arils extract. RE: Rind extract. PE: Peel extract.

This could be due to either quantitative or qualitative difference in the phytochemical arrangement of these parts, as well as total antioxidant activity (Table 1). Generally, comparing the sensitivity of pathogenic strains tested showed that Gram- negative bacteria were more sensitive to pomegranate extract parts used than Gram-positive bacteria. Diameter inhibition zone (DIZ) for Gram-negative ranged between 30 to 7 mm for AE, 37 to 6 mm for RE and 42 to 8 mm for PE, while DIZ for Gram- positive ranged between 21 to 7 mm for AE, 30 to 7 mm for RE and 35 to 9 for PE.

Table 4. *The MIC of the ethanolic extracts of pomegranate fruit parts and its diameter inhibition zone.*

Pathogenic strains	AE		RE		PE	
	MIC	DIZ	MIC	DIZ	MIC	DIZ
Gram-positive bacteria						
Streptococcus dysgalactiae sub sp. equisimilis	62.5	7	7.5	8	7.5	12
Streptococcus mutans EMCC1815	30	7	7.5	5	7.5	9
Bacillus subtilis DB100	30	7	7.5	7	7.5	10
Clostridium botulinum ATCC3584	62.5	8	7.5	7	7.5	9
Gram-negative bacteria						
Proteus hauseri EMCC1227	62.5	9	7.5	6	7.5	8
Escherichia coli ATCC25922	125	7	7.5	7	7.5	11
Escherichia coli BA12296	15	7	7.5	9	7.5	12
Klebseilla pneumonia ATCC12296	62.5	10	7.5	8	7.5	11
Pseudomonas marginalis EMCC1271	15	7	7.5	8	7.5	11
Yeast						
Candida albicans ATCCMYA2876	62.5	7	7.5	6	7.5	9

MIC: Minimum Inhibition Concentration (mg/ml).
DIZ: diameter inhibition zone (MM).
AE: Arils extract RE: Rind extract PE: Peel extract.

Minimum inhibitory concentration (MIC) of ethanolic extracts and it's diameter of inhibition zone (DIZ) are presented in Table 4. In general, PE and RE were more effective even at a concentration of MIC 7.5 mg/ml for all tested strains than AE that had 15, 30 and 62.5 mg/ml MIC.

Khan and Hanee [42] reported that the antibacterial activity of peels of pomegranate may be indicative of presence of metabolic toxins or broad spectrum antimicrobial compounds that act against both Gram- positive and Gram - negative bacteria.

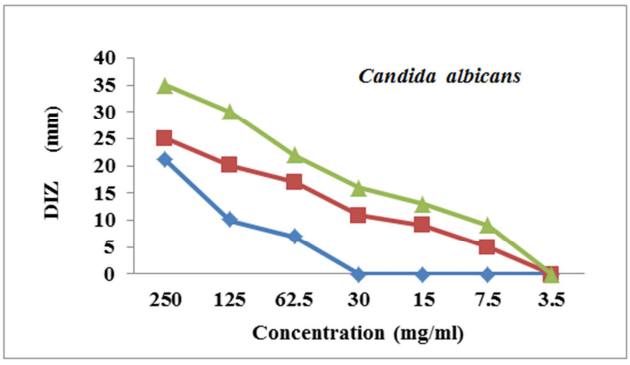

Figure 5. *Antimicrobial activity of pomegranate fruit parts ethanolic extract against Yeast (Candida albicans ATCCMYA 2876).*

DIZ: Diameter inhibition zone.
AE: Arils extract. RE: Rind extract. PE: Peel extract.

Figure 5 shows the highest activity against *Candida albicans* (ATCCMYA 2876) was recorded for PE that had inhibition zone ranged between 9 to 35 mm at concentration 7.5 to 250 mg/ml followed by RE that had inhibition zone ranged between 6 to 25 mm at concentration 7.5 to 250 mg/ml. The lowest inhibition zone ranged between 7 to 22 mm at concentration 62.5 to 250 mg/ml was observed for the AE. These results are in agreement with those previously studies [43, 44].

The obtained results revealed that all the ethanolic extracts of pomegranate parts especially PE is effective for all tested pathogenic strains. Ali *et al*. [45] reported that the possible mechanism of action of pomegranate parts, especially the peel against microorganisms can be related with the phenolic toxicity that interacts to sulfhydryl groups of proteins in microorganisms.

The differences in microbial activity of the ethanolic extract of pomegranate fruit parts may be due either to the quantitative or qualitative differences in phytochemical arrangement of these parts, as well as total antioxidant activity (Table 1). Previous studies by Duman [46] revealed that antimicrobial activity of pomegranate fruit extracts correlated to phytonutrient properties, such as total phenolic and anthocyanin compounds as well as the antioxidant power.

It can be concluded that pomegranate (*Punica granatum* L.) fruit parts, especially PE are as a potential source of antimicrobial activity against several pathogenic strains and it can be used as natural preservative for food.

3.5. Antioxidant Activity of Pectin –Based Edible Films

Table 5 shows the theoretical and measured phenolic, flavonoid content and antioxidant activity in the prepared films. The results revealed that films containing PE higher total phenolic, total flavonoid content and antioxidant activity than that containing RE. There is a decrease in the values between theoretical and measured phenolic and flavonoid content. This may be due to occurrence losses during film preparation.

Table 5. *Total phenolics, total flavonoids content and antioxidant activity of prepared films.*

Films	Total phenolics (mg GAE/g)		Total flavonoids (mg catechol/g)		IC_{50} µg/ml
	Theoretical	Measured	Theoretical	Measured	
Control	-	-	-	-	-
Based RE	$97.98^{b} \pm 0.35$	$71.98^{b} \pm 0.22$	$32.43^{b} \pm 0.12$	$23.1^{b} \pm 0.11$	$48.3^{a} \pm 0.9$
Based PE	$157.32^{a} \pm 0.45$	$131.2^{a} \pm 0.47$	$37.87^{a} \pm 0.17$	$28.8^{a} \pm 0.14$	$37.7^{b} \pm 0.4$

The experimental values within each row that have no common superscript are significantly different ($p \leq 0.05$).

Control: pectin only EP: ethanolic extract of peel ER: ethanolic extract of rind.

IC_{50}: the concentration required for inhibiting 50% of the initial DPPH free radical.

These losses may have stemmed during the shaking, drying method, compounds may be leached and oxidation [47]. Table 5 shows that films containing PE showed higher radical scavenging activities than that containing RE. The IC50 values were 37.7 and 48.3 µg/ml, respectively, while, the control film did not have any antioxidant activity. Figure 6 shows the radical-scavenging activity of different concentration of pectin films incorporated with the RE and PE.

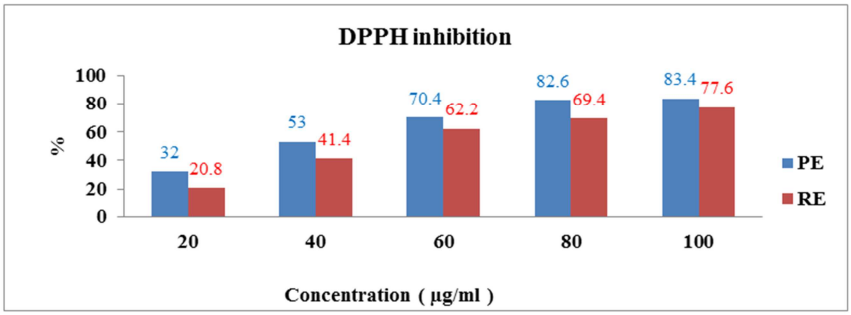

Figure 6. *DPPH inhibition of pectin edible films based PE and RE.*

The high antioxidant activities of films with PE may be attributed to its high contents of total phenolic and total flavonoids. The results clearly indicated that pomegranate PE appeared as a richer in natural antioxidants than the other parts. Pectin–based edible films gained antioxidant activity by the addition of pomegranate RE and PE and their antioxidant properties enhancement was dependent on the concentration used.

3.6. Antimicrobial Activity of Pectin –Based Edible Films

Table 6 shows the antimicrobial activity of pectin –based edible films incorporated with ethanolic extracts of pomegranate PE and RE at concentration two fold of MIC (15 mg/ml). The results showed remarkable antimicrobial activities. The control film did not show any antimicrobial activity against tested pathogenic strains, while pectin–based edible films incorporated with PE and RE exhibited pronounced inhibition zones against most of the tested pathogenic strains. The films containing PE caused the wider inhibition zone compared to RE.

Table 6. *Antimicrobial activity of pectin –based edible films.*

Pathogenic strain	Inhibition zone diameter (mm)**		
	Edible film with		
	Control	RE	PE
Gram-positive bacteria			
Streptococcus dysgalactiae subsp. *equisimilis*	-	25	35
Streptococcus mutans EMCC1815	-	35	43
Bacillus subtilis DB100	-	-	27
Gram-negative bacteria			
Proteus hauseri EMCC1227	-	25	30
Escherichia coli ATCC25922	-	36	40
Klebseilla pneumonia ATCC12296	-	-	-
Pseudomonas marginalis EMCC1271	-	-	-
Yeast			
*Candida albicans*ATCCMYA2876	-	-	-
Fungi			
Aspergillus flavus EMCC 274	-	-	-
Aspergillus parasiticus EMCC 886^{T}	-	17	25

Inhibition zone diameter (mm).
(-): No inhibiting zone formed.

The three tested Gram-positive bacteria were more sensitive to pectin–films based PE than RE. The films with RE had no effect on the growth of *Bacillus subtilis* DB100, in contrast, films with PE caused 27 mm inhibition zone. As for the tested Gram-negative bacteria, films with RE and PE had no effect on the growth of *Klebseilla pneumonia* ATCC12296 and *Pseudomonas marginalis* EMCC1271, while, *Proteus hauseri* EMCC1227 and *Escherichia coli* ATCC25922 strains were sensitive to the two extracts. The antimicrobial activity of PE was stronger than RE.

The addition of RE and PE to pectin –based edible films had no effect on the growth of the tested yeast strain (*Candida albicans* ATCCMYA2876). Two fungi strains were tested, one of them *Aspergillus parasiticus* EMCC 886T was high sensitive to pectin film based PE than RE, while, the other strain *Aspergillus flavus* EMCC 274 was resistant to the prepared films.

As it can be seen in Table 6, incorporated pectin film with PE and RE has shown antimicrobial activity against some of the tested pathogenic strains, while, ineffectiveness against the other strains. These variation may be due to the interaction of bioactive components in the pectin film matrix that make it difficult to release from the film or possibility of degradation of active compounds during film preparation [47].

4. Conclusion

As a conclusion of the previous mentioned data and discussion, ethanolic extracts of pomegranate fruit peel and rind had higher amounts of bioactive compounds as well as higher antioxidant activity. The results clearly confirmed that PE contained more antioxidants followed by RE while AE had the lowest activity which are confirmed with the DPPH and $ABTS^{+}$ assays. The result revealed that ethanolic extracts of all pomegranate fruit parts can successfully control or inhibit the visible growth of the tested types of pathogenic strains and possessed an inhibitory effect. It was observed that antimicrobial activity differed according to the tested pomegranate part, its concentration and the types of pathogenic strains. The minimum inhibitory concentration (MIC) was 7.5 mg/ ml for all tested strains than AE that varied from 15-62.5 mg/ml. The differences in microbial activity may be due either to the quantitative or qualitative differences in the phytochemical arrangement of these parts, as well as total antioxidant activity. The incorporation of PE and RE into pectin film resulted in film with good antioxidant and antimicrobial properties and considers as an active edible film and thus it can extend the shelf life of food products.

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