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EVALUATION OF POMEGRANATE PEEL AND ITS BEVERAGES AS ANTIBACTERIAL AND ANTICANCER AGENT

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ABSTRACT

Pomegranate (*Punica granatum*) peel is an important source of bioactive compounds against a varied number of maladies. The main target of the present investigation was to determine the chemical composition, total phenols, flavonoids, and antioxidant activity of pomegranate peel (PGP) as well as the potential biological activities of aqueous peel extract. Antibacterial activity was measured using 20% and 40% aqueous extracts of pomegranate peel (PGPa) using well diffusion plate method against *Helicobacter pylori and Campylobacter jujini* which are the most common bacteria causing gastric infections worldwide. Pomegranate peel showed high content of total phenols (198.33 mg Gallic acid/g) and flavonoids, (46.86 mg Quercetin/g) with strong antioxidant activity (95.96%). Inhibition percentages of 40% aqueous extracts were 79 and 45.6 % for *Campylobacter jujini and Helicobacter pylori* at 100 µl/well, respectively. Thus, minimum inhibitory concentration (MIC) of aqueous extract was 125 and 250 µg/ml for *Campylobacter jujini and Helicobacter pylori*, respectively. Morphological studies by scanning electron microscopy using 20 % of aqueous pomegranate peel (PGPa) showed segmentation, elongation and enlargement of *Campylobacter jujini* bacterial cells. Antitumor activity of pomegranate peel against intestinal cancer cell lines (Caco-2 cells) was shown at various concentrations of extracts with IC_{50} 120 ±1.30 µg/ml. Sensory evaluation of pomegranate peel beverage at series concentrations from 0.5 to 2.5 % was done to evaluate the impact of its application in the beverage production. This study suggests the possibility that PGP beverage can be used for dietary therapy to treat microbial infections.

Key words**:** *Pomegranate peel, pomegranate beverages, Helicobacter pylori, Campylobacter jujini, Scanning electron microscopy, antitumor activity.*

1. INTRODUCTION

At the present time, fruit wastes and by products are disposed often at a cost to the manufacturer. Therefore, using these wastes in a suitable form as a source of polyphenols may be of considerable economic benefit to food processors. One of these plants is *Punica granatum* (Punicaceae), commonly called pomegranate, which is an edible fruit cultivated in Mediterranean countries. Pomegranate peel (PGP) contributes about 50% of the whole weight of fruit, which contains bioactive compounds such as phenolic components, tannins, pro-anthocyanidins components, flavonoids (Li *et al*., 2006) and complex polysaccharides (Jahfar *et al.*, 2003). *P. granatum* has been widely used as a traditional medicine in America, Asia, Africa and Europe for the treatment of different types of diseases, to treat gastrointestinal diseases, parasitic diseases, and has anti-oxidant properties (Rabah *et al.*, 2015). The anti-inflammatory, antioxidant, and antimicrobial characteristics of PG could be advantageous in the treatment of stomach ulcers. Braga *et al*. (2005) showed that PG extract can be a potential antibacterial therapeutic agent due to its ability to inhibit enterotoxin.

Foodborne diseases are a serious and global problem, mainly observed in infants, young children, the elderly and the immunocompromised. Due to lack of side effects compared to synthetic (antibiotic) drugs, new trends in treatment have relied on plants for medication. From ancient

times, plants have been proved to be a powerful therapeutic agents for the treatment of various human diseases. It has been observed that pomegranate, exhibited strong antimicrobial activity against some groups of G^+ve and G^-ve pathogens (Kossah *et al.*, 2011). Substantial portion of the cancer burden worldwide is attributable to microbial pathogens. In the case of bacterial infection, several events (*e.g.* the establishment of chronic inflammation, as well as the production of genotoxins or bacterial products that interfere with regulation of cell cycle progression and apoptosis), in association with host genetic factors, may contribute to the acquisition of the mutter phenotype (Guerra *et al.*, 2011). Gastrointestinal diseases affect most people at some stage in their life. Two major causative factors are *Helicobacter pylori* and *Campylobacter jejuni* and a major predisposing factor for peptic ulcer disease (PUD) and gastric carcinoma (Kusters *et al.*, 2006). All *Campylobacter jejuni* species produce a genotoxin, which induces DNA double strand breaks, could lead to an increased risk of cancer especially in the gastro-intestinal tract (Brauner *et al.*, 2010). In this regard, using of pomegranate has a considerable anticarcinogenic effect which is mainly due to ellagic acid, that induces apoptosis in human colon cancer cell line *via* the intrinsic pathway with release of cytochrome C into the cytosol (Kasimsetty *et al.*, 2010).

Based on these facts, the aim target of the present study was to investigate the chemical composition, bioactive compounds and antioxidant activity of waste from pomegranate peel concerning the application of natural antimicrobials. Also, to examine the beverage from PGP to be used in the treatment of gastrointestinal microbial infections.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Pomegranate peel

The fresh and mature pomegranate (*Punica granatum* L.) as a source of pomegranate peel was obtained from the Horticultural Research Institute, Agriculture Research Center, Giza, Egypt.

2.1.2. Chemicals and reagent

All chemicals, including Folin - Ciocaltea's reagent, DPPH (1, 1-Diphenyl-2-picryl-hydrazyl), standards of phenolic, flavonoids and all others reagents used in the study (analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1.3. Bacterial culture

The *H. pylori* bacterial strain (ATCC 43504) and the *C. jejuni* bacterial strain (ATCC 700819) were obtained from Regional Center of Mycology and Biotechnology, (RCMB) Cairo, Egypt and pre-cultured on agar plates containing brain heart infusion (Oxoid) supplemented with 5% (v/v) sterile horse blood (Oxoid). The plates were incubated at 37 ºC in an incubator under microaerophilic conditions for 24 - 48 h.

2.1.4. Cell lines and cell cultures

The human intestinal cancer cell lines (Caco-2 cells) were obtained from RCMB Cairo, Egypt.

2.2. Methods

2.2.1. Technological method

2.2.1.1.Preparation of Pomegranate peel Powder (PGP)

Pomegranate peels were washed and spread as a thin layer in the dryer after stabilizing by heated air at the desired temperature 50 ºC. The peels were crushed using pestle and mortar followed by high speed laboratory blender, then sieved with 20-30 mesh to obtain finely divided powder (Senadeera, *et al*., 2003).

2.2.1.2. Preparation of Pomegranate aqueous extracts (PGPa)

Pomegranate peels powder PGP at concentration 20 and 40 gm were soaked in 100 ml boiling distilled water then stored in a vacuum flask for 12 h. The crude extract was filtered through muslin followed by Whatman No. 1 filter paper the filtrate was made up to 100 ml using distilled water and transferred to clean and dried bottle, and stored at 4°C until use (Vaishnavi *et al*., 2007).

2.2.1.3. Preparation of Pomegranate peel beverage

The hot beverage of pomegranate peel was prepared at different concentrations by mixing pomegranate peel powder at (0.5, 1.0, 1.5, 2.0 and 2.5 gm) with 100 ml water, then 5 g of sugar was added.

2.2.2. Chemical methods

2.2.2.1. Chemical Composition of pomegranate peel Powder

Moisture, crude protein, ash, crude fat and dietary fiber were determined by the standard

procedures of the A.O.A.C (2005). Carbohydrates were calculated by difference.

2.2.2.2.Determination of total phenolic compounds

The total phenolic compounds of pomegranate peels and its beverage were determined calorimetrically using Folin-Ciocalteu reagent according to the method described by Singleton *et al*. (1999). The absorbance of the mixture was measured at 725 nm against blank using spectrophotometer (Jenway 6705 uv /vis). Gallic acid was used as a standard.

2.2.2.3. Determination of total flavonoids

Total flavonoids of pomegranate peels and its beverage were determined according to the methods of Zhishen *et al.* (1999). Previous extract (0.4 ml) was added to 4 ml of H_2O. Then 0.3 ml of 5% $NaNO_2$ was added. After five min, 0.3 ml of 10 % $AlCl_3$ was added. After six min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The color was measured at 510 nm against a blank reagent using spectrophotometer (Jenway 6705 uv /vis). Quercetin was used as standard.

2.2.2.4. Determination of antioxidant activity

The antioxidant activity of pomegranate peels and its beverage were determined based on the radical scavenging ability in reacting with a stable DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) free radical according to Brand-Williams *et al*. (1995). DPPH (2.4 mg) in 100 ml methanol was prepared and 3.9 ml of this solution was added to 0.1 ml of sample extract. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min in the dark. Then the absorbance was measured at 515 nm using spectrophotometer (Jenway 6705 uv/vis). The radical scavenging percentage was calculated by the following equation:

Scavenging activity (%) = 1- (absorbance of extract /absorbance of control) × 100%

2.2.2.5. Identification of phenolic acids and flavonoids compounds

Phenolic and flavonoid compounds were fractionated using HPLC according to the method of Goupy *et al*. (1999) and Mattila *et al*. (2000). The methanolic extract of pomegranate peels powder and its beverage (2%) were injected into Agilent HPLC (Series 1200) 5HC-C18, 250 x 4.6 mm and ultraviolet detector which adjusted at 280 nm for phenolic acids and 330 nm for flavonoids. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min. Column temperature was maintained at 35°C. The fractionated phenolic and flavonoids were identified comparison of its retention time compared with those of automatic areas.

2.2.3. Evaluation of antitumor activity

Pomegranate peels was tested for any cytotoxic effect against human intestinal tumor cell line (Caco-2 cells). The cell suspension was prepared in complete growth Roswell Park Memorial Institute medium (RPMI) supplemented with 50 µg/ml gentamycin. The aliquots of 100 µl of cell suspension 1×10^5 were added to each well on a 96-well tissue culture plate. The blank wells contained complete RPMI medium in place of cell suspension. The cells were incubated for 24 h at 37 ºC in a humidified incubator with 5% CO_2. After the formation of a complete monolayer cell sheet in each well of the plate, PGP was added. Serial two- fold dilutions of the sample ranging from 7.81 µg/ml to 500 µg /ml was added into a 96- well tissue culture plate using multi channel pipette (eppendorff, Germany). After treatment (24 h), the culture supernatant was replaced by fresh medium. Then the cells in each well were incubated at 37 °C with 100 µl of MTT solution (5 mg/ml) for 4 h. After the end of incubation, the MTT solution was removed, then 100 µl of DMSO was added to each well. The absorbance was detected at 570 nm using a microplate ELISA reader (SunRise TECAN, Inc, USA). The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments .

Data Calculations: The percentage of cell viability was calculated using the Microsoft Excel®; according to the following equation:

% of cell viability = [1 − (ODt/ODc)] × 100%,

where ODt is the mean optical density of wells treated with the tested compound and ODc is the mean optical density of untreated cells. Cisplatin was used as positive control. The absorbance is proportional to the number of surviving cells in the culture plate. The tested sample was also compared using the IC_{50} value, *i.e.*, the concentration of an individual compound leading to 50% cell death that was estimated from graphical plots of viable cells vs compound concentrations.

2.2.4. Microbiological methods

2.2.4.1. Antibacterial activity

The antibacterial activity of pomegranate peel and its aqueous extract PGPa against *H. pylori* (ATCC 43504) and *C. jejuni* (ATCC 700819) strains were carried out by well agar diffusion method. Diluted bacterial culture (100 µl, 10^6 CFU/ml) of the bacterial strains was spread on the surface of BHI (Brain Heart Infusion) medium. Aqueous extract PGPa was used at 25, 50, 75, and 100 µl/ ml of each well and distilled water as a negative control. After incubation at 37 ^oC in a microaerophilic atmosphere for 24 h. The diameters of the growth-free zones around the disks were measured and subtracted from the diameter of well diffusion, giving the sizes of the inhibition zones beyond the well (Valgas *et al*., 2007).

2.2.4.2. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of pomegranate peel extracted PGPa as antibacterial activity against *Campylobacter juijuni and H. pylori* were determined. The minimum inhibitory concentrations (MIC) were determined by dilution method (Abhijeet *et al*., 2010), using two-fold serial dilution of the purified PGPa starting with the concentrations of the aqueous extract achieved was the following 500, 250 ,125, 62.5, 31.25, 16, 8, 4, 2, 1 µg /ml. respectively. Petri dishes containing 20 ml of BHI medium were seeded with bacterial suspension. Wells were made on agar using a sterile cork porer. Into the wells, 100 µl of aqueous extracts were added and incubated at 37 ºC under microaerophilic conditions for 48 h. Two petri dishes were prepared for each antibacterial agent. The lowest concentration of the aqueous extract of pomegranate peel that produced visible inhibition zone around the well was regarded as MIC.

2.2.4.3. Scanning electron microscopy

Sample (*Campylobacter jujini* cells treated with 20% of PGPa) was fixed by gluteraldhyed 2.5% and dehydrated by serial dilution of ethanol with agitation using automatic tissue process (Leica EMTP, Leica Microsystem; Austria), then samples were dried using Co_2 critical point dryer (Model: Audosamdri-185, Tousimis; Rockville, Maryland ,USA). Samples were coated by gold sputter coater (SPI-Modeule, USA). The samples were observed by scanning electron microscopy (Model: JSM-55500LV; JEOL LTD- Japan) by using high vaccum mode at the Regional Center of Mycology and Biotechnology, (RCMB) Cairo, Egypt.

2.2.5. Sensory evaluation

The hot beverage extract of the pomegranate peel was prepared at different concentrations by soaking pomegranate peel powder (0.5, 1.0, 1.5, 2.0 and 2.5 g) 100 ml water, then 5 g of sugar was added .The evaluation was carried out by 10 trained panelists of Special Food and Nutrition, FTRI, ARC, Giza, Egypt according to the method of Sensory Evaluation of Mirghani *et al*., (2012).

2.2.6. Statistical analysis

The data of the present study were subjected to analysis of variance and the Fisher's least significant difference test, SAS (1996) in order to compare the mean values of the investigated parameters at significance levels of $P \geq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Chemical composition, total phenol, total flavonoids and antioxidant activity

Chemical composition, total phenol, flavonoids and antioxidant activity of pomegranate peel powder (PGP) are presented in Table (1). Results indicated that the pomegranate peel powder (PGP) contained high amount of fiber and carbohydrate amounted in (15.94 and 68.86 %), respectively. Moreover, low protein content was found in PGP (4.35%). These results are in agreement with Sayed-Ahmed (2014) who reported that pomegranate peel (PGP) had a high content of fiber and carbohydrate.

It could be noticed that PGP had high content of total phenolic and total flavonoids (Table 1). In addition, high free radical scavenging activity is recorded in peels 95.96%. In this respect, Singh and Immanuel (2014) stated that PGP is considered as a good source of bioactive compounds due to the higher content of polyphenols and flavonoids. The use of plants for treating various diseases including food born disease is used in many countries under the name of Folk medicine. The use of plants for curing diseases was important as it is already proven by seeing the problems associated with synthetic antibiotics (Khan and Hanee, 2011).

Table (1): Chemical composition of pomegranate peel (dry weight).

Constituents	Pomegranate peel (PGP)
Protein (%)	**4.35± 0.29**
Oil (%)	**4.16± 0.38**
Ash (%)	**5.69±0.35**
Fiber (%)	**15.94±0.97**
Carbohydrate (%)	**68.86±1.25**
Total phenol (mg GAE/gm)	**198.33 ±2.51**
Total Flavonoid (mg Que/gm)	**46.63±1.96**
Antioxidant activity (%)	**95.96± 0.21**

Values are mean of three replicates ±SD.

3.2. Antibacterial activity of pomegranate peel aqueous extract (PGPa) against *Campylobacter jujini* and *H. pylori.*

Results in Table (2) showed the effect of pomegranate peel aqueous extract on human bacterial pathogens *Campylobacter jujini and H. pylori* which are the most common cause of gastric infections worldwide. Two different concentrations of aqueous extract (20 % and 40 % w/v) were used. Because 20% optimum conc. which have antibacterial activity against *Campylobacter jujini and H. pylori.* For each concentration series of dilutions were used *i.e.* 25, 50, 75 and 100 µl/ml. The results indicated that increasing the concentration of extract led an to increasing percentage of inhibition to 79 % against *Campylobacter jujini* and 45. 6 % against *H.pylori* at concentration of 100 µl/ml of pomegranate peel aqueous extract for (40%,w/v). The minimum inhibitory concentration of PGPa was 125 and 250 µg/ml for *Campylobacter jujini and H. pylori,* respectively. Meanwhile, the level of resistance to amoxicillin (30µg/ml) among both *C. jejuni* and *H.pylori* was 35 and 20%, respectively.

Our finding indicates that the antimicrobial of PGPa showed higher effect when compared with Amoxicillin by using 20% extract the inhibition percent by about 54% and 43% respectively with *Campylobacter* and *H.pylori.* While 40% extract showed inhibitory effects by about 79% and 45.6% resensitivity against selected strain.These result may be attributed to the high content of phytochemical and phenolic compounds in pomegranate peels hot aqueous extract.

Our data agree with Moghaddam (2011) which showed the pomegranate rind extracts had highly inhibitory activity against *H. pylori*. Pomegranate peels are considered waste parts or by products obtained during juice processing and characterized by the significant presence of polyphenols such as ellagitannins, ellagic acid, gallic acid and flavonoids, associated with biological properties

Table (2): Antibacterial activity of pomegranate peel (PGP) aqueous extract against *Campylobacter jujini* ***and*** *H.pylori*

Concentration µl/ml	Inhibition%			
	Campylobacter jujini		*H.pylori*	
	20%	**40 %**	**20%**	**40 %**
25	**14**	**19**	**7.6**	**11.4**
50	**27**	**47.5**	**19**	**21.6**
75	**42**	**66**	**32.5**	**31.3**
100	**54**	**79**	**43**	**45.6**
MIC at 20% (µg/ml)	**125**		**250**	
(AMC) amoxicillin (30µg/ml)	**35**		**20**	

such as antioxidant and antimicrobial agents. Sun Hee *et al.*, (2013), confirmed the inhibitory effects of pomegranate peel aqueous extracts on different bacterial isolates may be referred to the effect of flavonoids (*e.g*:7-*O*-butylnaringenin). Additionally, some phenolic compounds possess antimicrobial activities against various pathogenic bacteria, including *C. jejuni* (Klancnik *et al.*, 2012).

3.3. Effect of pomegranate peel aqueous extract (20%) on *Campylobacter jujini* morphology by using SEM.

Scanning electron microscopy (SEM) was used to further investigate the mechanisms by which pomegranate peel aqueous extract PGPa at concentration 20% which have optimum concentration antibacterial activity against target organism (*C. jejuni)* cells. The SEM image in Fig. (1 A and B) illustrates untreated cells, meanwhile Fig. (1 C, E and D) illustrates treated cells with 20% of pomegranate peel aqueous extract (PGPa). The Spiral-shape of *C. jejuni* cells exhibits a dramatic change from spiral to coccoid forms after treatment with PGPa. The SEM image in Fig. (1 C, E and D) illustrates the dominance of coccoid forms in treated cells and shows the formation of irregular cell surfaces as the cells showed rough surface.

These coccoid cells remained intact and possessed sheathed polar flagella. Also, the treated cells exposed pore formation and local rupture in cell membranes may be due to the presence of phenolic compounds. Metamorphous cells of broken cell walls. The obtained data are in agreement with Borges *et al*. (2013). Also, He and chen (2010) reported a similar transformation in morphology when *C. jejuni* cells were exposed to different environmental stresses, including oxidative stress.

The present results indicate that the mechanisms of detectable cell damages might be depending on the presence of different kinds of flavonoids. The synergistic antimicrobial activity of phenolic compounds is associated with the alteration in membrane permeability and antibiotic accumulation in *C. jejuni*.

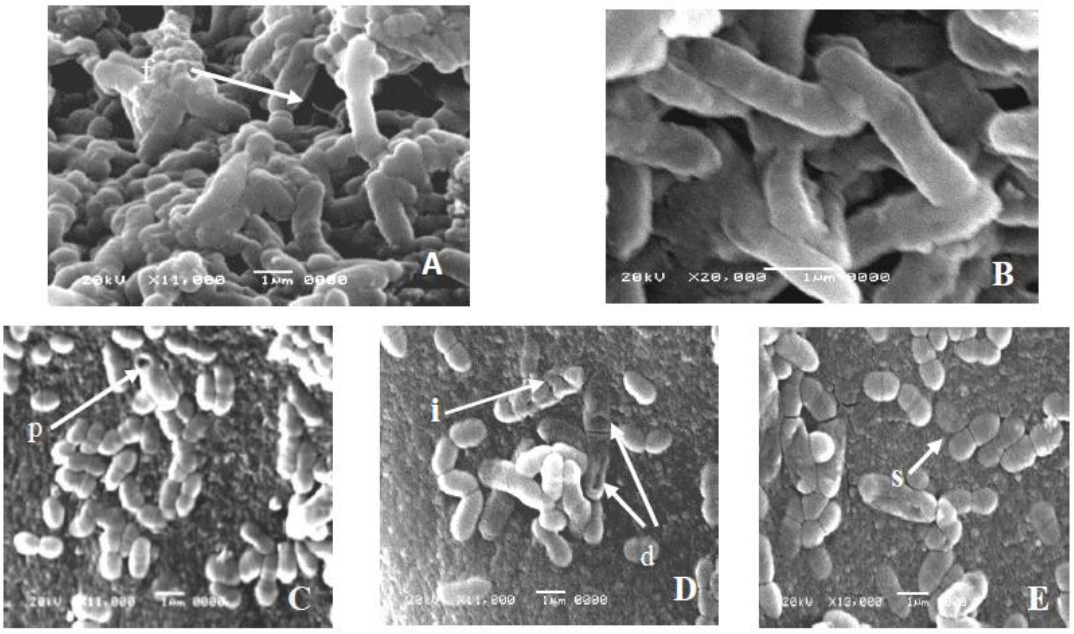

Fig. (1): SEM micrographs of *Campylobacter jujini* untreated with (PGP) pomegranate peel extract. (A, B) control, (C, D and E) treated with 20 % aqueous extract (PGPa): (f) flagella, (p) pore formation, (i) irregular shape, (d) damage.

In this context, Borges *et al*. (2013) reported that gallic acid relatively at high concentrations *i.e*., >100 µg ml^{-1} increases membrane permeability in Gram-negative and positive bacteria, such as *E. coli*, *Pseudomonas aeruginosa*, and *Listeria monocytogenes.* Similar results were obtained by Sun Hee *et al.* (2013) who tested the antimicrobial effects of flavonoid on *H. pylori*. Generally, PGPa extract possess the strongest antibacterial properties against *C. jejuni* due to their high content of phenolic and flavonoid compounds.

3.4. Evaluation of the antitumor activity.

Effect of pomegranate peel aqueous extract (PGPa) against human intestinal tumor cell line (Caco-2 cells) was evaluated. Cytotoxicity affecting the Caco-2 cell line provides preliminary information about toxicity on intestinal cancer cells. The current results indicating that increasing the concentration of extract affect cell lines in the range of from 125 to 500 µg/ml leads to decrease cell population in the cell lines. Results illustrated in Table (3) clear that (PGPa) exhibited high inhibitory activity even at low concentrations. As the inhibitory activity of (PGPa) at 250 µg/ml reached 62.19% (Caco-2). By increasing the concentration to 500 µg/ml the inhibitory activity reached 70.25%. In the present study, the anticancer activity of aqueous peel extract of pomegranate against Caco-2 cell line revealed growth inhibition starting at a concentration of 62.5 µg/ml and IC_{50} 120±1.30.

Table (3): Evaluation of antitumor Activity of pomegranate peel (µg/ml) against Caco-2 cell line.

Concentration (µg/ml)	Inhibitory %
500	**70.25±1.39**
250	**62.19±1.98**
125	**51.48±2.36**
62.5	**34.62±3.14**
31.25	**25.8±0.38**
15.6	**17.55±0.17**
7.8	**8.28±0.36**
3.9	**3.11±0.13**
0	**0**
***IC_{50}**	**120±1.30**

***IC_{50}: value of the concentration an individual compound leading to 50% cell death**

Fig. 2 (a and b) illustrated the morphological changes of Caco-2 cells untrated and treated tumer cell with PGPa clearly observed. The untreated

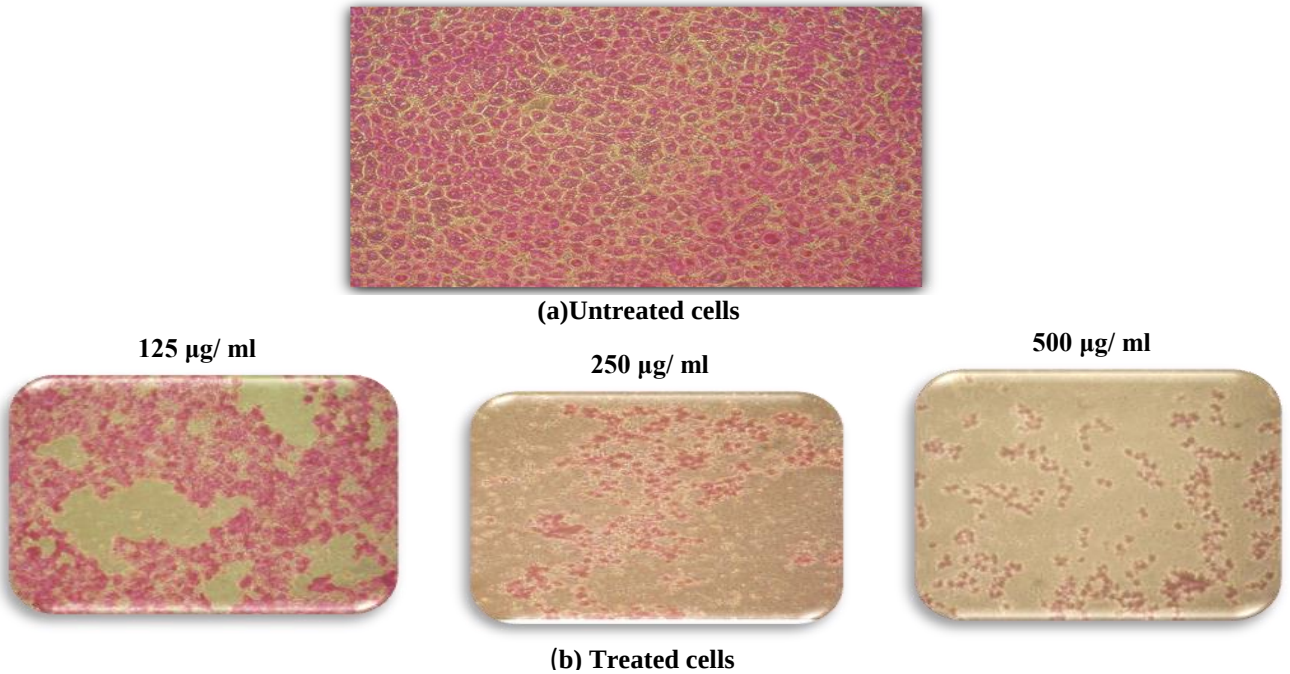

Fig. (2): Effect of PGP on carcinoma cell line (Caco-2): (a) untreated cell; (b) treated cell of different concentration of PGP.

tumer cells showed adherent growth and completely homogenous layer a regular shape (Fig. 2a), meanwhile (Fig. 2b) the tumor cell was clearly decreased as a result of treatment with different concentration.

In agreement with that of Rather *et al.* (2010) who found that the anticancer activity of extract from pomegranate peel against cell lines caused growth inhibition at a concentration 50 ug/ml. While Larrosa *et al.*, (2006) showed that pomegranate treated Caco-2 cells arrested in the S phase of the cell cycle, down-regulation of cyclins A and B1 and upregulation of cyclin E.

Vicinanza *et al.* (2013), indicated that both ellagic acid and urolithin A contribute to the mechanism of anticancer action of pomegranate products, however, urolithin was less effective in inhibiting cancer cell proliferation. While Sadik and Asker (2014) tested the extracts of *Punica granatum* for their antitumor activity against Ehrlich ascites carcinoma cells (EACC) and found that the viability of EACC was greatly increased by water extract followed by ethanol and methanol extracts. This effect may be due to the interaction between extract compounds and proteins located on the membrane of cells.

3.5. Sensory evaluation of pomegranate peel beverage.

The results (Table 4) show that beverage samples from pomegranate peel powder concentration up to 2% had high scores in all their sensory attributes. However, taste score decreased significantly at 2.5% concentration level. This might be related to the slight bitterness of taste due to increasing polyphenols at the highest level of pomegranate peel powder. Data of taste, flavor, odor and overall acceptability showed no significant difference between beverage samples prepared at concentration 1, 1.5 and 2%. Beverage samples prepared with pomegranate peel powder had high total score except in case of 2.5 % concentration level which significantly decreased than the other concentration.

3.6. Bioactive compound content in pomegranate peel beverage

The total amount of phenols, flavonoids content as bioactive compound and antioxidant activity of pomegranate peel beverage are illustrated in Fig (3). It could be noticed that the amount of phenols and flavonoids of PGP beverage were increased by increasing the amount of pomegranate peel powder. Also, it could be attributed to the higher total phenol and flavonoid contents of pomegranate peel beverage prepared at 2% (w/v) with recoded 19.208 and 17.55 mg/ml. Fig. (3) illustrated pomegranate peel beverages are good source of bioactive compounds which agrees with Nuamsetti *et al.* (2012) who reported that the phenolic content of hot aqueous extracts of the peels was 166.83 mg/100 ml.

DPPH radical scavenging activity assay assessed the ability of the beverage to donate hydrogen or to scavenge free radicals. DPPH radical is a stable free radical and when it reacts with an antioxidant compound which can donate hydrogen, it is reduced to diphenyl-picrylhydrazine. Regarding antioxidant activity, results showed a positive relationship between pomegranate peel powder as a beverage and the antioxidant activity. The potent antioxidant activities of pomegranates are attributed to its polyphenols.

Table (4): Sensory Evaluation of pomegranate peel beverage.

Samples	Taste (10)	Flavor (10)	Odor (10)	Color (10)	After taste (10)	Overall Acceptability (10)
0.5%	8.70 ± 0.47^{b}	9.14 ± 0.39^{b}	9.05 ± 0.48^{ab}	9.06 ± 0.39^{ab}	8.64 ± 0.39^{b}	8.87 ± 0.56^{b}
1.0%	9.23 ± 0.52^{a}	9.51 ± 0.41^{a}	9.45 ± 0.33^{a}	9.42 ± 0.53^{a}	9.26 ± 0.46^{a}	9.27 ± 0.38^{a}
1.5%	9.34 ± 0.56^{a}	9.56 ± 0.37^{a}	9.49 ± 0.32^{a}	9.52 ± 0.46^{a}	9.31 ± 0.52^{a}	9.35 ± 0.34^{a}
2.0%	8.75 ± 0.45^{ab}	9.25 ± 0.46^{a}	9.34 ± 0.44^{a}	8.79 ± 0.47^{b}	8.85 ± 0.57^{b}	8.96 ± 0.51^{ab}
2.5%	6.48 ± 0.69^{c}	8.07 ± 0.38^{c}	8.61 ± 0.36^{b}	8.14 ± 0.51^{c}	7.82 ± 0.35^{c}	7.55 ± 0.49^{c}

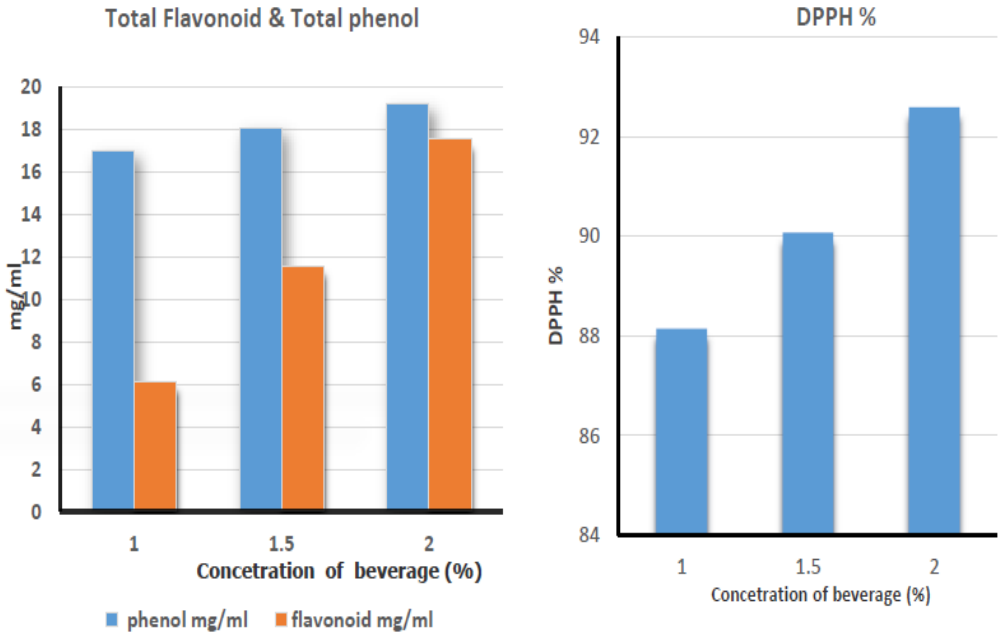

Fig (3): Total phenols and total flavonoids as bioactive compound content and antioxidant activity of PGP Beverage.

The antioxidant activity in the present study showed similar trend with those reported by Bharani and Namasivayam (2016).

3.7. Identification and fractionation of phenols and flavonoids of pomegranate peel powder and its beverage.

High-performance liquid chromatography (HPLC) was used for the identification and quantitative analysis of polyphenolic compounds of pomegranate peel. In Table (5) the current study several phenols were separated quantitatively. In general, the composition of polyphenolic substances in PGP was higher than that of PGP beverage. PGP had 7 components were most common types can be stated as Ellagic, Catechein, gallic, Chlorogenic, Epi-catechine, Protocatchoic and Caffeine with amounts of (1491.85, 531.55, 209.916, 187.35, 99.302, 68.014 and 56.63 mg/100g) which constituted about 95.9% of the total phenols. However, the phenolic compounds: caffeic acid, iso-ferulic, ferulic acid and coumarin were present as minor constituents. Thus, the presence of phenols confirms the antimicrobial activity of pomegranate, in agreement with (Gibbons, 2005) who successfully evaluated dietary polyphenols as chemopreventive and therapeutic agents to their direct antimicrobial action and antibiotic modulation activity.

Additionally, Yi *et al.* (2014) reported that this has been linked to their chemical structure and their ability to form complexes with bacterial membranes and proteins. Several studies have shown the antibacterial activity of polyphenols against *H. pylori* (Ankolekar *et al.*, 2011). Besides, Elango *et al.* (2011) evaluated the anticarcinogenic activity of pomegranate peel extract against A549 tumor cells and confirmed that the actions was due to the presence of phenolic components which proposed the presence of high amount of Gallic acid with its anticarcinogenic activities.

Table (5): HPLC analysis for Identification of phenols of pomegranate peel powder and its beverage.

Phenols	PGP (mg/100g)	PGP Beverage 2% (mg/100 ml)
Caffeine	56.63	1.234
iso-feruilc	4.488	0.106
Caffeic	23.07	0.521
p- coumaric	9.82	0.213
Ferulic	22.86	0.509
Chlorogenic	187.35	4.091
Epi-catechine	99.302	2.157
Catechol	138.42	3.948
Catechein	531.55	9.979
Protocatchoic	68.014	2.416
Ellagic	1491.85	28.844
coumarin	13.01	0.352
Gallic	209.916	5.085
Total	2756.964	59.455

Meanwhile, in the present study several flavonoids have been separated quantitatively. Table (6) illustrated the most common types can be stated as Hesperidin, Hespertin, Naringin, Rutin and Qurectrin with amounts 121.13, 44.267, 72.26, 56.95 and 28.21 mg/100g respectively.

Table (6): HPLC analysis for identification of flavonoid of pomegranate peel powder and its beverage.

Flavonoid	PGP (mg/100g)	PGP Beverage 2% (mg/100 ml)
Rhamentin	5.26	0.7992
Kampferol	3.00	0.684
Hespertin	44.267	2.335
Narengenin	3.03	0.143
Qurectin	3.65	0.157
Qurectrin	28.21	1.033
Rutin	56.95	3.727
Hesperidin	121.13	6.634
Naringin	72.26	4.083
Apigenin	3.00	0.151
Total	335.1	27.746

Ferrazzano *et al.* (2011) reported many natural flavonoids as possessing various pharmacological properties. In addition to their antioxidant activity, flavonoids also show good antibacterial activity against both gram-positive and gram-negative isolates (Coppo and Marchese, 2014). Some researchers established that flavonoids particularly quercetin and its glycoside derivatives are the main compounds responsible for the antioxidant properties (Silvia *et al*., 2011). These classes of compounds possess a broad spectrum of biological activities including radical scavenging properties (Balasnram *et al*., 2006). In general, this point needs further research to elucidate the effect of individual phenolic compound and its concentration on the antioxidant phenomenon.

Conclusion

The findings of the present study suggest that pomegranate peels and its beverage are rich in polyphenolic content and antioxidant activity with antibacterial alternatives as a better substitute in place of synthetic antioxidants and antibiotics. In addition, bioactive compounds from pomegranate peel beverage can be used as a dietary adjuvant for controlling human infections with *Campylobacter*.

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تقييم قشر الرمان ومشروبه كمضاد للبكتريا ومضاد للسرطان

ابتهال العدوى الخولاني

قسم بحوث الاغذية الخاصة - معهد بحوث تكنولوجيا الاغذية - مركز البحوث الزراعية - الجيزة- مصر

ملخص

تعتبر قشور الرمان مصدر مهم للمركبات النشطة بيولوجيا المضادة لعدد كبير من الأمراض اخطرها السرطان. لذلك يهدف البحث إلى تقدير التركيب الكيماوي، الفينولات الكلية، الفلافونويدات، النشاط المضاد للأكسدة لقشر الرمان، وتقديرالنشاط البيولوجي المحتمل للمستخلص المائي لقشر الرمان. كما تم قياس النشاط المضاد للبكتيريا باستخدام 20٪ و40٪ من المستخلصات المائية باستخدام طريقة well diffusion ضد الميكروبات الآتية *Campylobacter jujini, Helicobacter pylori* المسببة للالتهابات والقرح المعدية في جميع أنحاء العالم. أظهرت النتائج أن قشورالرمان بها نسبة عالية من الفينولات الكلية (198.33) mg Gallic acid /gm) والفلافونويدات الكلية (46.86 mg Quercetin/gm) مع نشاط مضاد للأكسدة عالية (95.96٪). كانت نسب تثبيط النمو الميكروبى للمستخلصات المائية بتركيز 40٪ (79% و 45.6٪) ضد كلا من *Helicobacter pylori* , *Campylobacter jujini* في 100 ميكرولتر/well على التوالي. وكذالك كان الحد الأدنى للتثبيط (MIC) للمستخلص المائي 125 و 250 µg/ml ضد كل من بكتريا *Campylobacter jujini* و *Helicobacter pylori*، على التوالي. كذلك تم دراسة التغيرات المورفولوجية باستخدام الميكرسكوب الإلكتروني عند تركيز 20٪ من مستخلص قشر الرمان المائي (PGPa) على ميكروب *Campylobacter jujini* حيث اظهرت نتيجة الفحص تجزئة واستطالة وتوسيع الخلايا البكتيرية.

تم قياس النشاط المضاد للأورام من قشر الرمان ضد الخلايا السرطانية من القولون Caco-2 أعدّ المستخلص من تركيزات مختلفة وتبين ان النشاط IC_{50} 120±1.30. كما أجرى تقييم حسي للمستخلص المائى لقشور الرمان (PGPa) كمشروب عند تركيزات تتراوح من (0.5 إلى 2.5٪). اثبتت النتائج المتحصل عليها في هذه الدراسة إمكانية استخدام مشروب قشور الرمان في علاج العدوي الميكروبية للانسان وخاصه لميكروبي *Campylobacter jujini* , *Helicobacter pylori*.

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