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Biochemical Studies on Pomegranate

Nadia Y. A Attia.[1]; Ibrahim M. Abd El - Aleem[1]; Kareem M.K. El Tobgy[2] and Hala F. Mohamed[2]
1: Department of Biochemistry, Faculty of Agriculture, Moshtohor, Banha University.
2: Department of Medicinal and Aromatic Plants Research, Horticulture Research Institute, Agriculture Research Center.

Abstract

Water, methanol and ethanol were used to extract total phenols, total tannins, anthocyanin, flavonoids and antioxidants activity from pomegranate peels (*Punica granatum* L) by using different methods. The obtained extracts and juice were used to study their antimicrobial effects against some Gram positive bacteria (*Staphylococcus aureus, Listeria monocytogenese, Bacillus megaterium* and *Bacillus cereus)*, some Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumonia* and *Salmonella typi*) and some fungi (*Aspergillus niger* and *Candida albicans*). Also, pomegranate juice and peel water extract were used to study their biological effects against diabetic and hypercholesterolemia in Wister rats. Water extract at 50°C for 20 min showed the highest capacity for extracting total phenols, total tannins, anthocyanin and total flavonoids compounds from dried pomegranate peels. Methanol extract at 50°C for 20 min recorded the highest antioxidant activity. All extracts recorded good inhibition for all the tested microorganisms. Administration of pomegranate juice and peel water extract induced significant decrement in blood serum glucose, triglycerides, total cholesterol, low density lipoprotein (LDL), urea, uric acid and creatinine in diabetic and hypercholesterolemia rats while, high density lipoprotein (HDL) elevated. Hematological parameters showed significant increment in white blood cells (WBC) in the diabetic group and in hypercholesterolemia group administrated with pomegranate juice red blood cells (RBC) and WBC showed significant increment.

Key words: pomegranate – antioxidant – antibacterial – lipid profile – liver functions

Introduction

The extraction of active compounds from plant materials is the first step in the utilization of phytochemicals in the preparation of dietary supplements, food ingredients and pharmaceuticals industries (**Jin and Russell, 2010**). It is generally known that the yield of chemical extraction depends on efficient methods for extraction, type of solvents with varying polarities, extraction time and temperature, as well as on the chemical composition and physical characteristics of the samples. Some previous researchers had reported that higher extraction yields of phenolic compounds were obtained with increasing solvent polarity **(Moure *et al.*, 2000** and **Cheung *et al.*, 2000).** Also, **Wissam *et al.* (2012)** studied the effective extraction of polyphenols and proanthocyanidins from pomegranate peel. They found that the recovery of polyphenols and proanthocyanidins were the highest at 50 °C for 20 min. Water gave the highest extract yield of polyphenols and proanthocyanidins (17.78% and 1.22%). Also, they revealed that two sequential water extractions has the economic and safety merits, and can be used as an environmentally friendly method for producing antioxidants from the pomegranate peel.

Madrigal-Carballo *et al.* (2009) mentioned that tannins were the major phenolics in pomegranate peels, which were more readily dissolved in 50% methanol. A mixture of methanol, ethanol, acetone and water was found to be a better extractant of active phenolics from pomegranate. Also, **Tm *et al.* (2010)** stated that distilled water at 60°C extraction conditions was the best for extracting anthocyanin.

Wang *et al.* (2011) extracted phenols from pomegranate peels by different solvent and temperature conditions. They found the methanol and water gave the highest extract yield of total phenols followed by water and ethanol. Also, they revealed that water extraction, which has the economic and safety merits, can be used as an environmentally friendly method for producing antioxidants from the pomegranate peel. **Hadrich *et al.* (2014)** reported that the methanol and ethanol extracts of pomegranate peels showed the most potent antioxidant activity followed by water and acetone extracts**.**

Al-Zoreky (2009) reported that the methanolic extract of pomegranate fruit induced antibacterial activity against *Listeria monocytogenes*, *S. aureus*, *Escherichia coli* and *Yersinia enterocolitica*, *Candida utilis, Saccharomyces cerevisiae and Aspergillus niger*. **Shaokat *et al.* (2007)** reported that there was little difference between the activities of alcoholic extract and aqueous extract of pomegranate airl against seven bacteria (*Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa* DSM 9027, *Staphylococcus aureus* Cowan 1, *orynebacterium xerosis* UC 9165, *Escherichia coli* DM, *Enterococcus faecalis* A10 and *Micrococcus luteus* LA 2971), and three fungi (*Kluvyeromyces marxianus* A230, *Rhodotorula rubra* MC12 and *Candida albicans* ATCC 1023). Also, they observed that the pomegranate aril extracts had antimicrobial

effects on all microorganisms, giving inhibition zones ranging in size from 13 to 26 mm. **Fawole *et al.* (2013**) studied the antibacterial activities of methanol and aqueous peel extracts of pomegranate against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*). They indicated that the methanolic peel extracts showed strong broad-spectrum activity against Gram-positive and Gram-negative bacteria. None of the aqueous extracts exhibited good antibacterial activity at the highest screening concentration (> 12.5 mg/ml). **Hajoori *et al.* (2014)** reported that *Punica granatum* peel water, ethanol, methanol, acetic acid and petroleum ether extracts had highly significant antimicrobial activity against four gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Staphylococcus aureus*, *Bacillus megaterium)* and six strains of Gram negative bacteria *(Escherichia coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Proteus vulgaris* and *Pseudomonas aeruginosa*). Aqueous, ethanol and methanol extracts were found to be more active towards the microorganisms tested than acetic acid and petroleum ether extracts. *Salmonella typhi* and *Proteus vulgaris* were reported to have significant susceptibility against most of the extracts. Phytochemical analysis of *P. granatum* peel showed the presence of alkaloids, flavanoids, steroids, tannin, glycosides and terpenoids.

Bagri *et al.* (2009) found that the administration of pomegranate aqueous extract at doses of 250 mg/kg and 500 mg/kg for 21 days caused significant reduction in fasting blood glucose, cholesterol, triglycerides and LDL cholesterol in compression with diabetic group induced by sterptozotocin. **Radhika *et al.* (2011)** reported that *Punica granatum* had antidiabetic and hypoglycemic activity of rats treated with alloxan. Administration of crude powder *Punica granatum* reduced the concentration of glucose, triglycerides, cholesterol, LDL cholesterol, vLDL cholesterol and raised the level of HDL cholesterol of both normal group and diabetic treated group. **Osman *et al*. (2012)** concluded that diabetic rats treated with pomegranate peel and juice showed decrement in glucose, alpha amylase, triglycerides, total cholesterol, LDL – cholesterol AST and ALT levels. While, HDL – cholesterol and insulin level elevated. **Bhandary *et al.* (2013)** stated that orally administration of ethanolic extracts of *Punica granatum* whole fruit and seeds (2000 mg/kg body weight) in Swiss albino rats showed that the total cholesterol, LDL and HDL levels recorded moderate non-significant increment while, triglyceride level recorded moderate decrement comparing with control. Total Protein and bilirubin, albumin and serum biomarkers of liver (ALT and AST), kidney (creatnine, uric acid and urea), hematological parameters ((RBC, WBC, Hb, and Platelet Count) did not record any significant alteration.

The aim of this investigation is to study the best method for extracting the antioxidants of pomegranate peel; evaluate pomegranate juice and peel extracts as antimicrobial effects and evaluate pomegranate juice and peel water extract as biological effects on diabetic and hypercholesterolemia rats.

Plant Material

Pomegranate was obtained from Horticulture Research Institute, Agriculture Research Center, Giza, Egypt (August 2013). Pomegranate fruit skins were cleaned, dried and ground to fine powders.

Proximate analyses

Moisture, ash, crude protein, crude fiber and total lipids contents were determined in pomegranate juice and peel according to **A.O.A.C. (2005**). Total hydrolysable carbohydrate was determined according to **Dubois *et al*. (1956).**

Extraction methods

Water, methanol and ethanol were used at 25°C for 24h, at 50°C for 20 min and by using Soxhelt apparatus in addition, boiling water for 5, 10, 20 min were used to identify the most suitable solvent for the extraction of total polyphenols, total tannins, anthocyanin, flavonoids and antioxidant. All extracts were then passed through filter paper and dried in oven at 50˚C.

Chemical composition

Total polyphenols content was estimated by the Folin-Ciocalteu method reported by **Elfalleh *et al.* (2009).** Hydrolysable tannins content was determined by the method of **Çam and Hişil (2010).** Total anthocyanin content was determined according to **Elfalleh *et al.* (2011)** and **Çam *et al.* (2009).** The amount of total flavonoids in the extracts was measured spectrophotometrically by the method of **Djeridane *et al.* (2006).** The scavenging activity on DPPH radical of different extracts was determined according to the method reported by **Okonogi *et al.* (2007**).

Microbial studies

Bacterial and fungal isolates

Clinical isolates of *E .coli* NRRL B/210, *Staph. aeureus* NRRL B/3 B , *Bacillus cereus* NRRL B / G 43 , *Bacillus megtarin* NRRL B/1366 , *Listeria monocytogenase* serotype NRRL Y/477, *Klabsila pneumonia* ATTCC700603 , *Candida albicans* NRRL Y/477, and *Aspergillus niger* NRRL/3 and *Salmonella typhi* ATTC5647006. were obtained from Department of chemistry of Natural and Microbial product, National Research Center, and were kept in the laboratory in the frozen state until used**.**

Antimicrobial activity

Pomegranate juice and peel extracts were sterilized by using finally filter sterilization 0.2 µm filter (Millipore) and stored in sterile vials. The antimicrobial effect of the pomegranate juice and peel extracts were evaluated using disk inhibition zone by the method described by **Orak *et al.* (2011).**

Biological evaluation of water extract and juice.

a. Experimental animals.

A total of 45 of adult's male albino rats (Wister Strain) weighed each of them 200 g approximately were obtained from Organization of Biological Products and Vaccines from Helwan breeding farm, Cairo, Egypt. The rats were housed in stain lasted cages with wire mesh bottoms in a room temperature maintained at 25 ^{o}C ± 2^{o}C. Rats were kept under normal healthy conditions for one week and fed on basal diet. The diet contained Casein 10%, Corn oil 10%, Salt mixture 4%, Vitamin mixture1%, Cellulose 5% and starch 70% (**Reeves *et al* 1993).**

Dosage and administration of decoction: The decoction was administered at a dosage of 3 ml/kg/day of pomegranate juice and 200 mg/kg/day of pomegranate peel water extract (**Abdel Moneim *et al* 2011)**, using a Sondi needle by gastric gavage method **(Iddamaldeniya *et al* 2006).** After that the rats were divided into three main groups 15 rats each. The first main group (control) was divided into three subgroups (5 rats each) the first (control) was fed on basil diet for another 6 weeks. The second group was fed on basil diet and administered of pomegranate juice. The third was fed on basil diet and administered of pomegranate peel water extract.

The second main group was the diabetic group. The rats were injected with a single dose of alloxan solution 150 mg\kg body weight (**Buko *et al* 1996**). After 24hours of alloxan injection, diabetes was confirmed (glucose blood was higher than 180 mg/dl). Rats were left for one week for stabilize diabetes, and then rats were divided into three subgroups. The first (control diabetic) was fed on basil diet for another 6 weeks. The second was fed on basil diet and administered of pomegranate juice. The third was fed on basil diet and administered of pomegranate peel water extract.

The third main group was hypercholesterolemia group. The rats were fed on high fat diet similar to the control diet but differed in more fat content which was 10% sheep fat, 2% cholesterol and 0.25% bile salts and starch 57.75% for 2 weeks **(Abdel-Rahim *et al* 2013)**, then was divided into three subgroups. The first (hypercholesterolemia control) was fed with basil diet for another 6 weeks. The second was fed on basil diet and administered of pomegranate juice. The third was fed on basil diet and administered of pomegranate peel water extract.

Blood sample

At the end of experiment blood was collected in tubes from retro-orbital vein in two separated tubes , one tube with EDTA (ethylene diamine tetra acetic acid) for the determination of hematological parameter and the other was centrifuged at 3000 rpm for 20 min , for serum preparation.

Serum analysis

Serum parameters were determined by enzymatic colorimetric methods, glucose was determined according to the procedure of **Trinder (1969).** Serum triglyceride and total cholesterol were determined according to the methods of **Fossati and Prencipe (1982)** and **Allain *et al*. (1974).** Low density lipoprotein (LDL-cholesterol) and high density lipoprotein (HDL- cholesterol) were determined according to the method of **Tietz *(*1976 a).** Serum total bilirubin, total protein and albumin were determined according to the method of **Walters and Gerarde (1970), Vassault *et al.* (1986)** and **Young *et al.* (1975)**. Alkaline phosphatases (ALP) was determined according to the methods of **Young *et al.* (1972)**. Serum aspartate transferease (AST) and alanine transferease (ALT) activities were measured colorimetrically according to the method of **Tietz (1976 b).** Serum urea, uric acid and creatinine were determined according to **Tietz (1990), Vassault *et al.* (1986) and Tietz (1986).**

Heamatology

The red blood cells (RBC), white blood cells (WBC) counts, and the hemoglobin (Hb) were determined in Mindray 2800 hematology analyzer.

Statistical analysis.

Statistical analysis was done by Duncan's Methods **(SAS, 1996).**

Results and discussion

Chemical composition

Data concerning pomegranate peel and juice chemical composition are shown in Table (1).

Table 1. Chemical composition of pomegranate peel and juice.

Constituents	Pomegranate peel based on dry weight (%)	Pomegranate juice based on fresh weight (%)
Moisture	5.03	86.60
Crude fiber	10.40	-----
Ash	2.15	0.42
Crude protein	2.59	0.13
Total lipid	1.80	0.06
Total carbohydrate	79.08	13.35

Pomegranate peels (dry weight) consist of 5.03 % moisture, 10.40% crude fiber, 2.15% ash,

2.59% crude protein, 1.80% total lipid, 79.08% total carbohydrates.

Data concerning crude fiber and total carbohydrate are in agreement with those reported by **Rowayshed *et al.* (2013**). Data of pomegranate juice show that the juice consisted of 86.6, 0.42, 0.13, 0.06 and 13.35% moisture, ash, crude protein, total lipid and total carbohydrates respectively. Similar results were obtained by **Ramadan *et al.* (2010)** for moisture, ash, protein, and carbohydrate.

Extraction.

Data in Table (2) show the effect of extraction methods on the total phenols, total tannins, anthocyanin, total flavonoids and antioxidants activity.

Table 2 Effect of different extraction methods on total phenols, total tannins, anthocyanin, total flavonoids and antioxidants activity.

Extraction methods	Total phenols g/100g	Total tannins g/100g	Anthocyanin mg/100g	Total flavonoids mg/100g	Antioxidants activity %
Water extract at 25°C for 24h	4.28c ± 0.19	1.36a ± 0.15	73.60b ± 2.69	39.15b ±1.41	78.41c ± 1.55
Methanol extract at 25°C for 24h	4.60c ± 0.29	1.13b± 0.06	49.49c ± 1.68	35.75bc ± 2.26	87.88ab ± 2.5
Ethanol extract at 25°C for 24h	3.38d ±0.28	0.95c± 0.07	41.94e ± 1.6	31.04d ± 1.01	87.21b ± 1.37
Water extract at 50°C for 20 min	6.72a ± 0.28	1.37a ± 0.12	86.19a ± 2.25	43.48a ± 3.72	80.85b ± 1.49
Methanol extract at 50°C for 20 min	5.85b ± 0.21	1.29a ± 0.08	48.30d ± 1.57	38.51bc ± 2.50	90.58a ± 2.86
Ethanol extract at 50°C for 20 min	4.65c ± 0.18	1.16b ± 0.12	38.55g ± 1.47	37.76bc ±	86.39b ± 1.40
Boiling water for 5min	2.761e ± 0.15	0.74d ± 0.01	40.57f ± 1.71	28.87d ± 0.54	74.90d ± 1.42
Boiling water for10 min	1.81f ± 0.15	0.68de ±0.02	37.04h ± 1.75	25.68e ± 2.60	71.04e ± 2.03
Boiling water for 20min	1.23g ± 0.07	0.53f ± 0.05	32.30i ± 1.24	21.31f ± 0.61	60.78f ± 1.39
Water Soxhlet extract	1.02g ± 0.05	0.41g ± 0.02	14.75k ± 1.02	10.26h ± 0.31	55.62g ± 1.20
Methanol Soxhlet extract	1.43g ± 0.07	0.61ef ±0.03	15.63j ± 0.91	17.88g ± 0.68	72.41de ± 2.50
Ethanol Soxhlet extract	1.17g ± 0.04	0.58f ±0.02	15.62j ± 1.20	11.88h ± 1.12	71.61e ± 3.40

a,b,c,……k means within column with different letters differ significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

The obtained results indicate that water extract at 50°C for 20 min followed by methanol extract at 50°C for 20min recorded the highest extraction of total polyphenols. Total tannins recorded the highest value in water extracts at 50°C for 20min and at 25 °C for 24 h followed by methanol extract at 50 °C for 20 min . Water extract at 50 °C for 20 min and at 25 °C for 24 h showed the highest capacity for extracting anthocyanin. Total flavonoids recorded the highest value in water extracts at 50 °C for 20 min and at 25 °C for 24 h followed by methanol, ethanol extracts at 50 °C for 20 min. Antioxidant showed the highest activity in methanol extract at 50 °C for 20 min and at 25°C for 24h followed by ethanol extract at 25°C for 24 h.

Increasing water boiling time caused significant decrement in total polyphenols, total tannins, anthocyanin, total flavonoids and antioxidant activity. Soxhlet extracts showed the lowest extraction efficiency.

It could be conculated that water at 50°C for 20 min was the best solvent for extracting total phenols, total tannin, total anthocyanin and total flavonoids.

The obtained results are in agreement with those obtained by **Noda *et al*. (2002), Hohnov *et al.* (2008), Madrigal-Carballo *et al.* (2009), Wang *et al.* (2011**) and **Orak *et al.* (2012). Al-Rawahi *et al.* (2013)** reported that the polyphenols of the pomegranate are relatively polar compounds, where hydrogen bonds, dipole–dipole, and electrostatic interactions may contribute to their strong solubility in polar solvents, water as the highest polar solvent, as it extracted the highest phenolic compounds followed by methanol and ethanol.

Antimicrobial effects:-

Antimicrobial effects of pomegranate peel extracts and juice:

Effect of pomegranate peel extracts and juice on some Gram positive bacteria:

Table (3) show the antimicrobial effect of various pomegranate peel extracts and juice against some Gram positive bacteria; *Staphylococcus aureus, Listeria monocytogenese, Bacillus megaterium* and *Bacillus cereus*.

Staphylococcus aureus

Results show that ethanol extract at 50 °C for 20 min, boiling water extract for 5 min and methanol extract at 50 °C for 20 min had the highest inhibition activity. Pomegranate juice had no antimicrobial activity.

Listeria monocytogenese

Methanol extract at 50°C for 20 min showed the highest inhibition zone followed by methanol Soxhelt extract. Pomegranate juice had no antimicrobial activity.

Bacillus megaterium:

Methanol extracts at 50°C for 20 min and at 25°C for 24h had the highest inhibition zone 24.33 and 23.17 mm. Pomegranate juice recorded 17.17mm inhibition zone.

Bacillus cereus

Methanol and ethanol extracts at 50°C for 20 min and methanol extract at 25 °C for 24 h showed the highest antimicrobial effect. Water extract at 50°C for 20 min showed 24.50 mm inhibition zone. Pomegranate juice recorded 13.67 mm inhibition zone.

These results are in agreement with those reported by Khan **and Hanee (2011), Dahham *et al.* (2010), Orak *et al.* (2011) and Hajoori *et al.* (2014).**

Effect of pomegranate peel extracts and juice on some Gram negative bacteria

Data present in Table (4) show the effect of various pomegranate peel extracts and juice against some Gram negative bacteria (*Escherichia coli, Klebsiella pneumonia* and *Salmonella typi*).

Escherichia coli

Methanol extract at 50°C for 20 min and boiling water extract for 5 min recorded the highest inhibition zone (21.50 and 20.83mm). Pomegranate juice recorded 13.83mm inhibition zone.

Klebsiella pneumonia

Methanol and ethanol extracts at 50°C for 20 min, recorded the highest inhibition zone (32.00 and 31.33 mm). Pomegranate juice antimicrobial activity was 26.17mm.

Salmonella typhi :

Methanol extracts at 50°C for 20 min and at 25°C for 24h showed the highest inhibition zones and the recorded means were 22.67 and 21.00mm. Pomegranate juice antimicrobial activity recorded 13.83mm.

The obtained results are in agreement with those reported by **Perez and Anesini (1994), Al-Zoreky (2009), Orak *et al.* (2011)** and **Hajoori *et al* (2014)**.

Mean time, **AlFadel *et al* (2014)** reported that pomegranate ethanol extract showed antimicrobial effect while, pomegranate water extract had no antimicrobial activity

Effect of pomegranate peel extracts and juice on some fungi

Data in Table (5) present the effect of pomegranate peel extracts and juice against some fungi (*Aspergillus niger* and *Candida albicans*).

Aspergillus niger :

Methanol extracts at 25°C for 24hr and at 50° C for 20 min had the highest inhibition zones followed by ethanol extract at 50 C° for 20 min Pomegranate juice recorded 12.83mm.

Candida albicans :

Methanol extract at 50°C for 20 min had the highest inhibition zone followed by methanol, ethanol extracts at 25°C for 24hr. Pomegranate juice antimicrobial activity recorded 17.5mm inhibition zone.

The obtained results are in agreement with those reported by **Al Zoreky (2009)** and **Dahham *et al* (2010).**

Biological effects:-

Data concerning the effect of pomegranate juice and peel water extract on blood serum glucose and lipid profile are shown in Table (6).

In diabetic and hypercholesterolemia groups there was significant increment in glucose level comparing with control group. Administration with pomegranate juice and peel water extract caused significant decrement in serum glucose levels in diabetic and hypercholesterolemia groups.

The obtained results are in agreement with those obtained by **Radhika *et al.* (2011)** and **Osman *et al.* (2012)**.

Table 3. Effect of pomegranate peel extracts and juice on some Gram positive bacteria.

Treatments	*Staph. Aureus*			*Listeria monocytogenese*			*Bacillus megaterium*			*Bacillus cereus*		
	Inhibition zone (mm)											
	20µ	40µ	Mean	20µ	40µ	mean	20µ	40µ	mean	20µ	40µ	Mean
Water extract at 25°C for 24h	12.67hi ± 0.58	20.00bd ± 2.89	16.33DE	16.33kl ± 1.8	20.67dh ± 1.80	18.50E	14.33h ± 1.53	24.0b ± 1.00	19.17C	18.33ef ± 3.61	30.00a ± 4.00	24.17BC
Ethanol extract at 25°C for 24h	14.67fh ± 2.31	21.33bc ± 2.08	18.00CD	17.32ik ± 2.31	22.33af ± 4.18	19.80CE	16.0fi ± 1.9	27.0a ± 3.9	21.50B	19.0df ± 1.15	25.67bc ± 0.58	22.33C
Methanol extract at 25°C for 24h	12.0hi ± 1.15	19.00cd ± 2.5	15.50EF	21.00cg ± 3.58	22.00bf ± 3.70	21.50BC	20.33cd ± 2.00	26.0ab ± 2.58	23.17AB	22.0d ± 2.8	28.67ab ± 1.15	25.30B
Water extract at 50°C for 20 min	14.33gi ± 1.5	22.33ab ± 1.15	18.33C	19.67fj ± 2.08	23.00ad ± 2.00	21.33BD	16.33ei ± 3.46	26.67ab ± 1.15	21.50B	21.0de ± 2.52	28.00ac ± 1.15	24.50BC
Ethanol extract at 50°C for 20 min	17.33df ± 1.15	24.67a ± 2.31	21.00A	18.67jk ±2.31	23.33ad ± 2.65	21.00BD	16.67dg ± 4.00	26.00ab ± 4.58	21.83B	21.00de ± 4.81	30.00a ± 2.08	25.50B
Methanol extract at 50°C for 20 min	13.67gi ± 2.7	24.33a ± 2.52	19.02BC	23.67ac ± 4.73	25.00a ± 5.41	24.33A	20.67c ± 1.58	28.00a ± 1.84	24.33A	25.33c ± 2.00	30.33a ± 4.00	27.83A
Boiling water for 5 min	19.00cd ± 1.00	22.67ab ± 3.6	20.83AB	18.00hk ± 1.98	22.67ae ± 1.53	20.33BE	17.69dg ± 1.50	19.00ce ± 2.00	18.33CE	14.33gi ± 1.58	16.33fg ± 2.52	15.33D
Boiling water for10 min	14.00gi ± 1.50	15.67eg ± 0.58	14.83EF	11.33m ± 1.5	17.33ik ± 1.73	14.33F	15.00gi ± 2.00	16.67eh ± 1.00	15.83F	12.67ij ± 1.16	15.00gi ± 1.53	13.33DE
Boiling water for 20 min	12.33hi ± 2.00	15.67eg ± 2.08	14.00F	11.33m ± 1.00	13.00n ±2.90	12.17G	12.0jk ± 0.58	15.00gi ± 2.08	13.50G	12.33ij ± 2.53	15.00gi ± 1.53	13.67DE
Water Soxhlet extract	11.67i ± 2.53	17.33df ± 3.58	14.50EF	14.00lm ± 3.5	17.00jk ± 4.60	15.50F	11.67k ± 2.52	13.67ik ±1.53	12.67G	11.0j ± 1.73	14.67gi ± 1.25	12.83E
Ethanol Soxhlet extract	13.67gi ± 1.7	17.67de ±2.8	15.67EF	17.0jk ± 2.40	21.67bf ± 1.79	19.33DE	15.33fi ± 1.37	18.00cf ± 1.06	16.60EF	12.67ij ± 1.14	16.00fh ± 0.33	14.33DE
Methanol Soxhlet extract	17.33df ± 1.53	19.00cd ± 1.07	18.17CD	20.00ei ± 2.00	24.33ab ± 1.98	22.17B	17.67dg ± 1.53	19.67cd ±2.06	18.67CD	13.00hj ± 1.50	16.00fh ± 1.00	14.50DE
Pomegranate juice	0.00j	0.00j	0.00G	0.00n	0.00n	0.00H	14.67hj ± 2.65	19.67cd ± 3.15	17.17DF	12.67ij ± 1.48	14.67gi ± 1.37	13.67DE
Mean conc.	13.28B	18.44A		16.03B	19.41A		16.1B\	21.49A		16.56B	21.56A	

a,b,…….n means within column with different letters differ significantly ($p \leq 0.05$)from each other means followed by the same letter don't different the 0.05 probability level.

Table 4. ect of pomegranate peel extracts and juice on some Grame negative bacteria.

Treatments	*E. coli*			*Klebsiella pneumonia*			*Salmonella typhi*		
	inhibition zone (mm)								
	20µ	40µ	Mean	20µ	40µ	Mean	20µ	40µ	mean
Water extract at 25°C for 24h	11.73jk ± 1.50	22.67b ± 2.54	17.20D	17.67ln ±2.53	20.67hj ±2.51	19.17F	12.67hi± 1.8	18.33d ±2.89	15.50FG
Ethanol extract at 25°C for 24h	12.67ik ± 1.50	22.00b ± 4.19	17.33D	18.33jm ± 1.50	22.33fh ± 2.89	20.33EF	14.33gh ± 2.31	22.67bc ± 2.08	18.50DE
Methanol extract at 25°C for 24h	13.33hk ± 0.58	22.00b ± 3.80	17.67CD	19.33im ± 2.20	25.67de ± 1.74	22.50D	17.67de ± 1.15	24.33b ±3.15	21.00B
Water extract at 50°C for 20 min	12.67ik ± 1.15	17.33df ± 2.76	15.90F	25.33de ± 4.70	30.00b ± 1.73	27.67B	15.67eg ± 1.16	21.67c ± 2.00	18.67D
Ethanol extract at 50°C for 20 min	15.67fh ± 2.40	23.33b ± 3.51	19.50BC	27.0cd ± 1.15	35.67a ± 2.67	31.33A	16.76df ± 2.15	24.33b ± 2.31	20.53BC
Methanol extract at 50°C for 20 min	14.67fi ± 2.60	28.33a ± 3.00	21.50A	29.67b ± 3.70	34.33a ± 5.8	32.00A	17.33df ± 2.33	28.0a ± 3.50	22.67A
Boiling water for 5min	17.67de ± 3.70	24.00b ± 2.65	20.83AB	20.33hk ± 1.53	23.67eg ± 3.79	22.00DF	17.00df ± 1.00	21.33c ±2.08	19.17CD
Boiling water for10 min	14.67fi ± 2.90	17.67de ±1.56	16.17DE	19.33im ± 1.73	20.33hk ± 3.06	19.83F	13.00hi ± 1.53	15.67eg ± 2.00	14.33GH
Boiling water for 20min	10.67k ± 2.08	13.67gj ± 0.50	12.17G	18.03km ± 4.16	21.33gi ± 4.46	19.67F	11.33i ± 2.10	15.33fg ± 2.08	13.33H
Water Soxhlet extract	12.33ik ± 1.73	14.00gj ± 1.50	13.17FG	15.33n ± 5.77	17.00mn ± 3.7	16.17G	11.67i ±2.53	14.00gh ±0.58	12.83H
Ethanol Soxhlet extract	16.00eh ± 1.80	19.00cd ± 1.15	17.50D	19.67il ± 1.14	24.33ef ± 1.96	22.00DE	15.33fg ± 2.00	18.67d ±3.50	17.00EF
Methanol Soxhlet extract	18.33de ± 1.53	21.67bc ± 2.60	20.02AB	21.33gi ± 1.53	28.33bc ± 2.08	24.83C	17.67de ± 1.50	23.33bc ± 3.87	20.50BC
Pomegranate juice	13.33hk ± 1.82	16.33dg ± 2.50	13.83EF	23.67eg ± 2.08	28.67bc ± 4.8	26.17BC	12.33hi ± 3.00	15.33fg ± 2.31	13.83H
Mean conc.	14.13B	20.15A		21.50B	25.56A		14.82B	20.23A	

a,b,c,……n means within column with different letters different significantly ($p \leq 0.05$) from each other means followed by the same letter don't differz at 0.05 probability level.

Table 5. Effect of pomegranate peel extracts and juice on some fungi

Treatments	*Aspergillus niger*			*Candida albicans*		
	inhibition zone (mm)					
	20µ	40µ	Mean	20µ	40µ	Mean
Water extract at 25°C for 24h	11.0b ± 1.15	21.3dg ±1.52	16.17C	25.67ik ± 0.58	30.0cf ± 1.52	27.83D
Ethanol extract at 25°C for 24h	19.67fi ±2.08	24de ±2.3	21.83B	29.0eh ± 5.72	33.0ab ± 4.70	31.0BC
Methanol extract at 25°C for 24h	22.67df ±0.50	31.76a ± 1.90	27.17A	29.0eh ± 0.76	33.33ab ± 1.67	31.17B
Water extract at 50°C for 20 min	18.67gj ±1.51	23df ± 1.50	20.83B	26.67ji ± 1.50	31.33be ± 2.72	29.00CD
Ethanol extract at 50°C for 20 min	20.67eh ±1.15	30.0ab ± 1.15	25.33A	29.33eh ± 2.30	32.33bc ± 3.40	30.83BC
Methanol extract at 50°C for 20 min	24.33cd ±2.63	26.0bc ± 2.60	25.50A	32.0bd ± 1.00	35.33a ± 2.95	33.67A
Boiling water for 5min	15.33jn ±1.15	17.67hk ±1.52	16.50C	23.33ln ± 1.52	26.33hj ± 1.53	24.33E
Boiling water for10 min	13.33mq ±2.08	15.33jn =± 1.70	14.33CD	18.67op ± 1.57	21.33lo ± 2.33	20.00G
Boiling water for 20min	10.33q ±0.57	12.33nq ± 1.00	11.33E	17.33p ± 2.3	20.33no ± 3.50	18.83GH
Water Soxhlet extract	12.0nq ±0.58	13.33mq ± 2.08	12.67DE	16.33pq ± 3.20	20.67mo ± 1.50	18.50GH
Methanol Soxhlet extract	13.67nq ±2.08	14.67ko ± 2.80	14.17CD	20.67mo ± 2.10	23.67jl ± 2.42	22.17F
Ethanol Soxhlet extract	16.0jm ±1.00	17.0 il ± 5.03	16.50C	23.33km ± 0.57	27.67fi ±3 .13	25.50E
pomegranate juice	11.33oq ±2.40	14.33kp ± 2.12	12.83DE	13.67q ± 1.80	21.33lo± 2.60	17.50H
Mean conc.	16.08B	20.11A		23.38B	27.44A	

a,b,c,……..q means within column with different letters differ significant ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Table 6. Effect of orally intake pomegranate juice and peel water extract on serum glucose and lipid profile.

Groups	Blood serum glucose mg/dl	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Control Basal diet	81.09ce ± 11.00	61.46df ± 4.0	100.30e ± 5.9	39.25f ± 1.60	45.45bd ± 1.12
Basal diet + Pomegranate juice	58.47e ± 7.38	53.62eg ± 3.63	95.84f ± 4.33	35.02eg ± 2.30	47.13bd ± 1.48
Basal diet + Pomegranate peel extract	53.80e ± 13.07	49.37g ± 6.45	87.54fg ± 4.50	32.54fg ± 0.97	49.30a ± 1.62
Diabetic control group + Basal diet	420.10a ± 86.32	91.87b ± 7.69	131.60b ± 7.55	68.24c ± 1.70	29.03g ± 0.97
Diabetic +Basal diet + pomegranate juice	123.80b ± 25.14	63.98de ± 3.28	122.73c ± 5.70	49.77ef ± 0.83	45.86bd ± 1.00
Diabetic + Basal diet + pomegranate peel extract	110.33bc ± 10.46	50.46fg ± 4.28	108.50de ±6.80	48.86eg ± 1.09	46.64bd ± 2.60
Hypercholesterolemia control group + Basal diet	102.00bd ± 16.40	121.65a ± 16.67	157.85a ± 4.78	109.00a ± 3.40	29.77g ± 1.90
Hypercholesterolemia + Basal diet + Pomegranate juice	61.91e ± 9.32	76.42c ± 3.21	137.20b ± 4.95	87.60b ± 2.50	36.05eg ± 1.51
Hypercholesterolemia + Basal diet + Pomegranate peel extract	60.42e ± 9.90	59.61eg ± 5.40	131.66b ± 2.48	69.57c ± 1.03	43.43ce ± 1.33

a,b,c,..g means within column with differ letters different significantly ($p \leq 0.05$)from each other means followed by the same letter don't differ at 0.05 probability level.
Each value represents the mean of 5 rats ± S.E.

Also, data in Table (6) show that administration of pomegranate juice and peel water extract caused significant decrement in triglycerides, total cholesterol and LDL. High density lipoprotein (HDL) showed significant increment in the diabetic group and hypercholesterolemia group administrated with pomegranate water extract comparing with diabetic and hypercholesterolemia control groups.

The obtained results are in agreement with those reported by **Radhika *et al.* (2011) and Abdel-Rahim *et al.* (2013) .**

Data in Table (7) show the effect of orally intake pomegranate peel water extract and juice on serum bilirubin, protein, albumin, AST, ALT and ALP.

Table 7. Effect of orally intake pomegranate juice and peel water extract on liver functions.

Groups	Total Bilirubin (mg/dl)	Total protein (g\l)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Control Basal diet	0.902b ± 0.14	6.95d ± 0.91	2.78e ± 0.26	24.85fg ±2.93	34.09gh ± 1.19	84.06df ±4.6
Basal diet + Pomegranate juice	1.70ab ± 0.13	6.574d ± 0.52	2.95be ± 0.42	22.10g ± 1.80	31.28hi ± 2.59	83.09df ± 2.6
Basal diet + Pomegranate peel extract	1.67ab ± 0.11	6.57d ± 0.75	2.97de ± 0.57	23.54gf ± 1.24	29.97i ± 3.00	77.60f ± 3.62
Diabetic control group + Basal diet	2.10a ± 0.08	6.93d ± 0.91	3.51be ± 0.41	43.53b ± 4.93	66.83b ± 3.19	98.69c ± 5.67
Diabetic +Basal diet + pomegranate juice	1.64ab ± 0.08	6.82d ± 0.27	3.05ce ± 0.43	37.64d ± 3.13	54.15e ± 1.52	87.43de ± 4.25
Diabetic + Basal diet + pomegranate peel extract	1.716ab ± 0.08	6.59d ± 0.75	3.66bd ± 0.65	32.76e ± 1.34	48.26f ± 1.60	79.97ef ± 2.95
Hypercholesterolemia control group + Basal diet	2.53a ± 0.23	9.60a ± 0.42	4.53a ± 1.11	56.26a ± 1.45	77.26a ±4.90	147.30a ± 8.45
Hypercholesterolemia + Basal diet + Pomegranate juice	1.70ab ± 0.27	7.02cd ± 0.57	3.457be ± 0.70	37.12d ± 1.77	59.85cd ± 2.50	114.60b ± 3.43
Hypercholesterolemia + Basal diet + Pomegranate peel extract	1.91ab ± 0.06	7.75bc ± 0.83	3.45be ± 0.33	35.29de ± 1.32	57.17de ± 1.10	112.50b ± 2.60

a,b,c,..f means within column with differ letters different significantly ($p \leq 0.05$) from each other means followed by the same letter don't differ at 0.05 probability level.

Each value represent the mean of 5 rats ± S.E.

Diabetic groups had non significant change in total bilrubin, total protein and albumin and showed significant decrement in AST, ALT and ALP comparing with diabetic control group.

Hypercholesterolemia groups showed significant reduction in total protein, albumin, AST, ALT and ALP comparing with hypercholesterolemia control group.

The obtained results are in agreement with those reported by **Osman *et al.* (2012) and Bhandary *et al.* (2013).**

Kidney functions

Data concerning the effect of orally intake pomegranate juice and peel water extract on kidney functions are shown in Table (8).

Diabetic and hypercholesterolemia groups had significant increment in urea, uric acid and creatinine comparing to control group fed on basal diet. Administration of pomegranate juice and peel water extract showed significant decrement in these parameters comparing with diabetic and hypercholesterolemia control groups .

The obtained results are in agreement with those reported by (**Abdel-Rahim *et al* 2013)** and **Bhandary *et al.* (2013)**.

Effect of pomegranate juice and peel water extract on heamatological Parameters:

Data concerning the effect of orally intake pomegranate juice and peel water extract on the hematological parameters are shown in Table (9). There was significant increment in WBC in diabetic group. While, hypercholesterolemia group showed significant increment in WBC and RBC in the group administrated with pomegranate juice.

The obtained results are in agreement with **Bhandary *et al.* (2013).**

Table 8. Effect of orally intake pomegranate juice and peel water extract on kidney functions.

Groups	Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl
Control Basal diet	50.97fg ± 3.75	2.81de ± 0.15	0.28cd ± 0.10
Basal diet + Pomegranate juice	39.82h ± 1.40	2.35ef ± 0.34	0.15hi ± 0.07
Basal diet + Pomegranate peel extract	40.18h ± 2.80	3.20d ± 0.12	0.13i ± 0.04
Diabetic control group + Basal diet	80.61a ± 6.11	4.64ab ± 0.39	o.37b ± 0.12
Diabetic +Basal diet + pomegranate juice	54.83ef ± 2.80	3.914c ± 0.18	0.24df ± 0.11
Diabetic + Basal diet + pomegranate peel extract	58.6de ± 1.90	3.82c ± 0.29	0.252df ± 0.03
Hypercholesterolemia control group + Basal diet	83.63a ± 5.50	5.05a ± 0.23	0.528a ± 0.18
Hypercholesterolemia + Basal diet + Pomegranate juice	58.5de ± 0.97	4.00c ± 0.16	0.318bc ± 0.11
Hypercholesterolemia + Basal diet + Pomegranate peel extract	46.23gh ± 1.00	4.28bc ± 0.30	0.27ce ± 0.04

a,b,c,.....i means within column with different letters different significantly ($p \leq 0.05$)from each other means followed by the same letter don't differ at 0.05 probability level.

Each value represent the mean of 5 rats ± S.E.

Table 9. Effect of orally intake of pomegranate juice and peel water extract on hematological Parameters.

Groups	RBC ($X10^6$/µl)	WBC ($X10^3$/µl)	Hb (g/dl)
Control Basal diet	6.70ab ± 0.31	15.78eg ± 2.3	14.34ac ± 0.61
Basal diet + Pomegranate juice	6.92ab ± 0.23	19.90b ± 2.07	14.00b ± 0.7
Basal diet + Pomegranate peel extract	7.02ab ± 0.24	19.04b ± 3.11	14.6ab ± 0.45
Diabetic control group + Basal diet	6.54ac ±0.39	14.6g ± 2.4	13.34df ± 1.36
Diabetic +Basal diet + pomegranate juice	6.77ab ± 0.61	16.06ef ± 2.54	13.54cf ± 1.44
Diabetic + Basal diet + pomegranate peel extract	6.75ab ± 0.08	21.36a ± 3.35	13.46cf ± 0.45
Hypercholesterolemia control group + Basal diet	5.76c ± 0.55	17.60cd ± 1.64	12.86ef ± 0.06
Hypercholesterolemia + Basal diet + Pomegranate juice	6.95ab ± 0.31	18.98b ± 2.11	13.46cf ± 0.45
Hypercholesterolemia + Basal diet + Pomegranate peel extract	6.71ac ± 0.16	18.71bc ± 2.00	13.66be ± 0.37

a,b,c,..f means within column with different letters differ significant ($p \leq 0.05$)from each other means followed by the same letter don't differ at 0.05 probability level.

Each value represent the mean of 5 rats ± S.E.

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الملخص

تهدف هذه الدراسة إلي دراسة أفضل طريقه لاستخلاص البولي فينول و التانينات و الانثوسيانين و الفلافونويدات ومضادات الأكسدة من قشور الرمان و قد تم استخدام الماء و الميثانول والايثانول علي درجات حرارة 25م لمده 24ساعة و50م لمده 20 دقيقه وكذلك الاستخلاص بجهاز سوكسلت و الغليان لمده 5و 10و20 دقيقه . أيضا تم دراسة تأثير هذه المستخلصات و عصير الرمان كمضاد للميكروبات وقد تم استخدام بعض الميكروبات الموجبة لجرام مثل

(*Staphylococcus aureus, Listeria monocytogenese, Bacillus megaterium* and *Bacillus cereus)*

والسالبة لجرام مثل

(*Escherichia coli, Klebsiella pneumonia* and *Salmonella typi)*

والفطريات *مثل* (*Aspergillus niger* and *Candida albicans*) *كما تم دراسة تأثير* عصير الرمان والمستخلص المائي للقشور علي الفئران المصابة بارتفاع سكر الدم و الفئران التي تعاني من ارتفاع نسه الكوليستيرول .

أوضحت النتائج أن المستخلص المائي علي درجه حرارة 50م اعلي كفاءه في استخلاص البولي فينول و التانينات و الانثوسيانين و الفلافونويدات بينما كان الميثانول اعلي كفاءه في استخلاص مضادات الأكسدة.

أظهرت جميع المستخلصات تأثير مضاد لجميع الميكروبات موضع الدراسة .

حدث انخفاض في سكر الدم والجلسريدات الثلاثية و الكوليستيرول منخفض الكثافة و اليوريا و حمض اليوريك و الكرياتينين بينما حدث ارتفاع في الكوليستيرول عالي الكثافة في الفئران المصابة بارتفاع سكر الدم و الفئران التي تعاني من ارتفاع نسبه الكوليستيرول. أيضا حدث زيادة في عدد كرات الدم البيضاء في الفئران المصابة بارتفاع سكر الدم.