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Journal of Advances in Biological and Basic Research 01[01] 2015
www.asdpub.com/index.php/jabbr
e-ISSN-2454-6097

Original Article

Utilization from leaves of olive and pomegranate as a source of bioactive components

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Keywords:

Olive,
Pomegranate,
Phenolic compounds,
Antioxidants,
Phytochemistry,

Abstract

Crude juices of olive and pomegranate leaves were obtained by hydraulic press. The level of polyphenolic compounds in the (olive and pomegranate) juice were510.00 and 722.00ppm. Aliquots of the concentrated olive and pomegranate juice leaves, represent 200, 400, 800 and 1600ppm and butylated hydroxy toluene (BHT, 200ppm) were investigated by Rancimat method at 100ºC and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. These compounds were administrated to rats daily for 6 weeks by stomach tube. The liver (Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities) and kidney (bilirubin, uric acid and creatinine) function tests and serum contents (total lipids, total cholesterol and low and high-density lipoproteins) were measured to assess the safety limits of the phenolic compounds in the olive and pomegranate juice leaves. The data of the aforementioned measurements indicates that the administration of olive and pomegranate juice leaves did not cause any changes in liver and kidney functions. On the contrary, BHT at 200ppm induced significant increases in the enzyme activities and the serum levels of total lipids, uric acid and creatinine.

1. Introduction

Reactive oxygen species (Ros) readily attack and induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA. The oxidative damage is a crucial etiological factor implicated in several chronic human diseases, namely cardiovascular diseases, rheumatism, diabetes mellitus and cancer[1]. Based on growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is warranted. Antioxidants are chemical substances that reduce or prevent oxidation. They have the ability to counteract the damaging effects of free radicals in tissues and thus are believed to protect against cancer, arteriosclerosis, heart disease and several other diseases [2]. Many studies have shown that phenolic compounds display antioxidant activity as a result of their capacity to scavenge free radicals[3]. Phenolic compounds can also act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system[4]. These compounds are known to act as antioxidant not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates.

Probably the most important natural phenolic are flavonoids because of their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties [5]. In fact, flavonoids have been reported as antioxidants, scavengers of a wide range of reactive oxygen species and inhibitors of lipid peroxidation [6]. These compounds which are widely distributed across the plant kingdom represent the most abundant antioxidants in the diet and they have gained tremendous interest as potential therapeutic agents against a wide variety of diseases, most of which involve oxidant damage. The unusually wide pharmacological spectrum of flavonoids was originally thought to result from their antioxidant activity; however, recent studies suggest that various flavonoids may use other protective mechanisms are well.

Flavonoids have also been shown to be highly effective scavenging of most types of oxidizing molecules, including singlet oxygen and other various free radicals that are probably involved in several diseases. On the other hand, numerous studies have shown structure-activity relationships governing antioxidant capacities of flavonoids [7,8]. Food chemists have proposed the replacement of synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), with natural ones because the synthetic antioxidants are suspected to be carcinogenic [9]. In summary, natural antioxidants are useful in the food industry as preservatives to increase the life of food products preventing the loss of their sensory and nutritional value. Epidemiology studies have shown that the traditional Mediterranean diet is associated with low incidence of cardiovascular disease and certain cancers [10]. These beneficial effects on human health have been attributed to the presence in the Mediterranean diet of antioxidants such as phenolic compounds, carotenoids, and tocopherols that play an important role in disease prevention [11]. The olive leaf (*Oleaceae*) has been widely used in folk medicine for several thousand years in European Mediterranean islands and countries. Historically, olive leaf has been used as a remedy for fever and other diseases such as malaria [12,13]. Olive foods such as olive oil and olive leaf in the Mediterranean diet are the primary source of phenolic compounds, which are also important markers for evaluating the quality of olive-based food products. The major active components in olive leaf are known to be oleuropein and its derivatives such as hydroxytyrosol and tyrosol, as well as caffeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diometin-7-glucoside [14-16]. Also, pomegranate is one of the important and oldest fruits of tropical and subtropical regions, which originated in the Middle East. It is widely reported that pomegranate exhibits antivirus, antioxidant, anticancer, antiproliferative activities [17,18]. For centuries, the barks, leave, flowers fruits and seeds of this plant have been used to ameliorate some diseases [19]. The leaves have been widely used by traditional medicine in America,

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Asia, Africa and Europe for the treatment of different types of diseases [20]. The phytochemistry of pomegranate has been widely studied by some researchers and this fruit is found to be a rich source of polyphenolic compounds[19].

The present study was entailed on the direct use of juice obtained by pressing olive and pomegranate leaves without recourse to extraction and fractionation of the total polyphenols. Total phenolic content, electron donating ability by DPPH and antioxidant activity by Rancimat method. Also, identification of phenolic compounds in juice (olive by HPLC and the study effect of crude leaf juice (olive and pomegranate) on rat serum constituents.

2. Materials & Methods

2.1 Source of leaves

The ripe olive and pomegranate leaves were collected during the year 2014 from the Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt.

2.2 Source of sunflower oil

Refined sunflower oil was obtained from Savola Oil .The oil acid and peroxide values were 0.30 mg KoH g^{-1} and 0.60 meq./ kg^{-1}, respectively.

2.3 Solvents, reagents and kits

All solvents used throughout the whole work were analytical grade and distilled before use. Caffeic acid (98%) and folin-Ciocalteau reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Gerbsaure Chemical Co. Ltd., Germany, respectively. Alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, creatinine, and uric acid kits obtained from Borhringer Ingelheim GmbH, Ingelheim, Germany.

2.4 Experimental animals

Albino male rats, 50 days old with an average weight of 60-70g were obtained from the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

*2.*5 Preparation of crude leaves juice

Leaves were cleaned and remove seeds them pressed by hydraulic laboratory press. The resultant crude juice was concentrated using freeze dryer (Labconco Corporation, Kansas, City, Mo, USA) and kept in a brown bottle at 5ºC until use.

2.6 Determination of total polyphenolic

The levels of total polyphenols of fresh crude juice were determined according to the method of Gutfinger (1981) [21]. Caffeic acid was served as a standard compound for the preparation of the calibration curve.

2.7 Phenolic fraction

Phenolic facrtion was isolated by solid phase extraction and analyzed by reversed-phase HPLC using a diode array UV detector. A Hewlett-Packard series 1,100 liquid chromatographic system (Waldbronn, Germany) equipped with diode array detector and a lichrosorb Rp18 column (4.00 mmid C250 mm, particle size 5mm, Merck, Darmstdt) used. Elution was performed at a flow rate of 1.00 ml/min with mobile phase of water/acetic acid (98:2 v/v, solvent A) and methanol/ acetonitril (50:50, v/v, solvent B), starting with 5% B then increase to levels of 30 % at 25 min, 40% at 35 min., 52 % at 40 min.; 70% at 50 min., 100 % at 55 min., and kept at this stage for 5 min. Quantification of phenolic compounds was carried out at wave length of 280nm using *P*-hydroxybenzoic acid as an internal standard.

2.8 Oxidation system

Different concentrations of phenolic compounds from juice (200, 400, 800 and 1600ppm) and BHT (200 ppm) were individually add to sunflower oil to study their antioxidant behavior. The designation of an induction period, measured by using a Rancimat Instrument, was taken as a tool to compare the effectiveness of the phenolic compounds on sunflower oil stability.

2.9 Designation of induction period by Rancimat

Rancimat method was used to evaluate oxidative stability, because it is fast and reliable [22]. Stability was expressed as the oxidation induction time (hr) measured with the Rancimat 679 apparatus (Metrohm Co. Switzerland) using an oil sample of 5.0 g warmed to 100 ± 2ºC, and an air flow of 20 l/hr. the time taken to reach a fixed level of conductivity was measured.

2.10 DPPH free radical-scavenging activity

The DPPH free radical scavenging assay was carried out, as previously reported by Cheel et al[23]., (2007) with some modification. The crude juice from olive and pomegranate at various concentrations (200, 400, 800 and 1600ppm) were added to a 0.06nm DPPH solution in ethanol and reaction mixture was shaken vigorously. After incubation for 30 min at room temperature, the absorbance at 517nm was recorded spectorphotometrically. Vitamin E was used as a reference as the test compound. A control solution, without the tested compound, was prepared in the same manner as the assay mixture. All the analysis was done in triplicate. The degree of discolorisation indicates the free radical scavenging efficiency of the substances. The antioxidant activity was calculated as an inhibitory effect (IE %) of the DPPH radical formation as follows:

IE% = 100 X ($A_{517cntrol}$-$A_{517sample}$ /$A_{517cntrol}$), and expressed ad IC50. The IC50 value was defined as the concentration (in μg/ml) of the compound required to scavenge the DPPH radical by 50%.

2.11 Nutrition experiments

A total of eighty albino male rats were raised in the animal house of faculty of Agriculture, Cairo University, Giza, Egypt. The animals were fed on a basal diet for 7 days as an adaptation period. The basal diet was formulated according to A. O. A. C. (2005) method and consisted of casein (15%), corn oil (10%), cellulose (5%), salt mixture (4%), vitamin mixture (1%) and starch (65%). Water was available *ad libitum*. Known volumes of the concentrated crude sidr (fruit and leaf) juice were dissolved in a mixture of distilled water and Tween 20 (12:1, V/V) to obtain polyphenols concentrations of 200, 400, 800 and 1600ppm. BHT solution (200ppm) was prepared exactly as mentioned above for juice (olive and pomegrante). The rats divided into ten groups and each group comprised eight rats. The first group presents the control rats, second group was given BHT at 200ppm, third, fourth, fifths and sixth were given 200, 400, 800 and 1600 of polyphenols from olive leaf juice. Seventh, eighth, ninth and tenth groups were given 200, 400, 800 and 1600ppm of polyphenols from pomegrante leaf juice. Each rat group was stomach ingested by gavage daily (1ml) from the crude juice (olive and pomegrante) and BHT solution for 6 weeks.

2.12 Blood samples

Blood samples were taken at the start of the experiment and after 1, 2, 3, 4, 5, and 6 weeks of the administration of the crude sidr juice (olive and pomegrante) and BHT. The blood samples were obtained from orbital plexus venous by means of fine capillary glass tubes according to the method outlined by Schermer [24](1967). It was not possible to collect 10 ml blood from a single rat, hence the blood of eight rats in each group was pooled. The blood samples were placed in dry and clean centrifuge tubes and allowed to clot for 1-2 h at room temperature. Sera were then removed using a Pasteur pipette and centrifuged for 20 min at 110 rpm. The clean supernatant Sera were then kept frozen until analysis.

2.13 Sera analysis

Alanine aminotransferase ALT (E. C. 2. 6. 1. 2), Aspartate aminotransferase AST (E. C. 2. 6. 1. 1) and AP (E. C. 3. 1. 3. 1) activities were measured according to the methods described by Kachmar & Moss [25] (1976), Bergmryer & Harder [26] (1986) and Varley et al[27]., (1980), respectively. Types of bilirubin (total and direct), urea and acid were determined according to the methods described by Fawcett & Scott [28] (1960), Dounas et al[29]., (1987) and Barham & Trinder [30](1972), respectively. The levels of serum cholesterol, low and high density lipoproteins and total lipids were determined according to the methods outlined by Roechlau et al[31]., (1974), Assmann[32], (1979) & Frings & Dunn [33] (1979). Each determination was carried out in triplicate and the mean values are presented in the text.

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2.14 Statistical analysis

Data were collected and expressed and expressed as the mean ± saturated deviation of three independent experiments and analysis for statistical significant from control, using the Dunnett test (SPSS 11.5 Statistics Software; SPSS, Chicago, IL).

3. Results & Discusssion

3.1 Polyphenols content

There is currently great interest world-wide in finding new and safe antioxidant from natural sources to prevent oxidative rancidity of foods and so the present study focused on extracts of olive and pomegranate containing polyphenols, which do not have an undesirable odor when inhaled through the nose or an undesirable and tongue taste. Although, in this publication, the active principal is assumed to be polyphenolic in origin the occurrence of other unidentified active principles is not excluded. Thus though reference is always made to the polyphenolic material as being antioxidant, the case is that we have proved it unequivocally. The total polyphenols contents obtained are shown in Table 1. The concentration of polyphenols in the different plant is as follows: olive > pomegranate. It can be seen from Table 1 that pomegranate contain as low total polyphenols as olive.

3.2 Phenolic compounds

Identification of phenolic compounds by HPLC technique was used to identify the major phenolic compounds in the leaf juice (olive and pomegranate). The identification was based on comparisons on the chromatographic retention time and UV absorbance spectra of compounds in olive and pomegranate samples with those of authentic standards. Data of the HPLC analysis of leaf juice samples (olive and pomegranate) are given in Table 2. Data show that the phenolic compounds of leaf juice (olive and pomegranate) were made up of 13 compounds. The main phenolic compounds of olive leaves are (tyrosol, hydroxytyrosol, *P*-hydroxybenzoic acid and tannic acid), while the main phenolic compounds of pomegranate are (tyrosol, hydroxytyrosol, *P*-hydroxybezoic acid and vanillic acid).

3.3 The antioxidant activity of olive and pomegranate phenolic compounds:

The antioxidant activities of phenolic compounds from the leaf juice (olive and pomegranate) were assessed by the Rancimat method. This method the induction period for the onset of oxidative rancidity in sunflower oil at 100ºC. In the present study, simple model systems comprising sunflower oil with phenolic compounds were used to assess oxidation behavior. An experiment was performed with sunflower and BHT (200ppm) to compare the antioxidant efficiency of the phenolic compounds from olive and pomegranate with the most commonly used synthetic antioxidant material. It has been reported that synthetic antioxidants (BHT, BHA and PG) are added at concentrations of 100- 400ppm to fats and oils to suppress the development of peroxides during food storage[34]. Therefore, the phenolic compounds were added to sunflower oil at concentrations of 200, 400, 800 and 1600ppm. Table 3 shows the olive and pomegranate leaves on the oxidative rancidity of sunflower oil. The results illustrate that all the polyphenols of olive and pomegranate juice and added at various concentrations to the test system, exhibited antioxidant activity. However, statistical analysis showed that had no significant differences of phenolic compounds for both plant olive and pomegranate leaves on sunflower oil stability.

3.4 Scavenging capacity of DPPH free radicals

The changes in scavenging capacity of the phenolic compounds antioxidant measured with the hydrophobic DPPH free radical is shown in Table 4. Four levels of the concentrations of leaf juice phenolic compounds (olive and pomegranate) were used with a very high scavenging capacity of 40.00 after only 10 min. In all cases the scavenging capacity did not increase after the first 10 min of incubation. The reaction of BHT with DPPH was similar to phenolic compounds with DPPH; the scavenging capacities were similar.

3.5 Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and phosphatase activities (AP)

Table 5, 6 &7 show the activities of ALT, AST and AP for control rats and the values were slightly increased during the whole experiment (6 weeks). The administration of BHT at 200 ppm to experimental rats induced significant increases in serum ALT and AST activities after 3 weeks from the commencement and towards the end of the experiment. In the mean time, BHT induced significant increase in serum AP activity after only 1 week from the beginning of the experiment. The administration of polyphenols in crude leaf juice (olive and pomegranate) at 200, 400 800 and 1600 ppm did not cause any significant changes in enzyme activities compared with the control experiment. Baron (1987) mentioned that the rise in the activities of ALS, AST AND AP in rat serum is a sign of hepatocelluor damage. This case has been found in rats administered only BHT and not with polyphenolic of leaf juice (olive and pomegranate).

3.6 Serum total lipids

The results (Table 8) show that there was no significant increase in the total lipids for control rats throughout the whole experiment. The administration of BHT at 200 ppm caused significant and gradual increase in serum total lipids. The rise in total lipids at the end of the experiment was approximately 1.1 greater than that at the beginning of the experiment. The administration of polyphenolic compounds at 200, 400, 800 and 1600 ppm present in crude leaf juice (olive and pomegranate) induced non-significant rise in rat serum total lipids. In other words, the several of phenolic compounds possessed the same function as that noticed with control rats.

3.7 Serum total cholesterol and low-density lipoproteins cholesterol

Tables (9 &10) show the levels of serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) of control rats. Rats administered BHT (200 ppm) and phenolic compounds (200, 400, 800 and 1600ppm) of crude leaf juice (olive and pomegranate). The results for the control rats and rats administered BHT (200ppm), indicated that there were no significant increase in total cholesterol and LDL-C levels during the entire experiment. Also, administration of polyphenols of leaves (olive and pomegranate) juice at various concentrations exhibited non-significant increases in the total cholesterol and LDL-C.

3.8 Serum high-density lipoprotein cholesterol

The results (Table 11) for the rats of the control and BHT group indicated that there was no significant rise in the levels of high-density lipoprotein cholesterol (HDL-C) throughout the whole experiment. Also, crude leaf juice (olive and pomegranate) polyphenols at various concentrations induced non-significant rise in rat serum HDL-C.

3.9 Serum total bilirubin

Table 12 shows the effect of leaf juice polyphenolic compounds (olive and pomegranate) at various levels (200, 400, 800 and 1600 ppm) and BHT (200 ppm) on the levels of rat serum total those administered various concentrations of phenolic compounds in leaf juice (olive and pomegranate), did not show any changes in the levels of total bilirubin. Conversely, BHT 200 ppm caused significant and gradual increases in total bilirubin levels during the entire experiment.

3.10 Serum uric acid

The data (Table 13) for the control rats and rats administered various concentrations of crude leaf juice (olive and pomegranate) phenolic compounds showed non-significant changes in the levels of uric acid during the entire experimental period. On the contrary, BHT at 200ppm exhibited gradual increases on the levels of rat serum uric acid. It is worth noting that a significant increase in uric acid levels occurred at the second week and towards the end of experiment.

3.11 Serum creatinine

The data (Table 14) for control rat group and the polyphenols rat groups indicate that there were no significant

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changes of serum creatinine levels during the entire experiment. In contrast, BHT at 200ppm caused increases in the levels of rat serum creatinine and the increase took place from the second week of the experiment. In generals, liver and kidney functions of rats administered crude leaf juice (olive and pomegranate) did not cause any adverse effects in liver and kidney functions. Consequently, one would suggest adding crude leaf juice (olive and pomegranate) to increase their shelf-life of oils without any deleterious effect on human health. In general, the data for biochemical measurements demonstrate that the polyphenolic compounds of crude leaf juice (olive and pomegranate) are quite safe for human health. Conversely, the results made it clear that BHT as a synthetic antioxidant has to be abandoned from use.

Table 1: Phenolic contents as caffeic acid of leaves (Olive and pomegranate)

	Phenolic contents (ppm)
Olive leaves	510.00 ± 10.90
Pomegranate leaves	722.00 ± 12.15

Mean value ± standard deviation (SD).

Table 2: Phenolic compounds (ppm) of leaves (olive and pomegranate)

	Leaves	
	Olive	Pomegranate
Quercetin	3.00±0.01	1.50±0.001
Caffeic acid	7.30±0.91	8.30±0.82
Gallic acid	5.20±0.52	2.10±0.02
Apigenin	2.50±0.01	5.30±0.46
Chlorogenic acid	6.20±0.63	1.42±0.01
Ferulic acid	5.77±0.60	6.50±0.56
Cinamic acid	10.32±1.01	9.22±0.98
P-coumaric acid	9.50±0.91	6.30±0.67
P- hydroxy benzoic acid	19.07±2.53	18.50±2.00
Tannic acid	12.11±1.55	10.30±1.30
Hydroxy tyrosol	22.30±3.00	25.60±4.31
Vanillic acid	9.30±0.95	16.70±1.91
Tyrosol	25.50±4.50	31.20±5.81

Mean value ± standard deviation (SD).

Table 3: Effect of polyphenols obtained from leaves (olive and pomegranate) on sunflower oil oxidative stability.

System	Induction period (hr)
Sunflower oil (control, C)	7.50±2.00
C+BHT (200PPM)	11.30±3.15
Polyphenols of olive leaves(POL):	
C+POL (200ppm)	10.50±2.95
C+POL (400ppm)	11.70±3.20
C+POL (800ppm)	14.30±5.13
C+POL (1600ppm)	18.50±7.50
Polyphenols of pomegranate leaves (PPL)	
C+PPL (200ppm)	10.60±2.55
C+PPL (400ppm)	11.50±3.25
C+PPL (800ppm)	15.00±6.00
C+PPL (1600ppm)	18.70±7.19

Mean value ± standard deviation (SD).

Table 4: Scavenging capacity of DPPH free radicals by polyphenols leaves (olive and pomegranate) and BHT

System	Scavenging capacity (%)
Control	40.00±2.30
BHT (200ppm)	60.00±3.50
POL (200ppm)	52.00±2.90
POL (400ppm)	63.00±3.70
POL (800ppm)	75.00±5.51
POL (1600ppm)	93.00±7.30
PPL (200ppm)	54.00±2.95
PPL (400ppm)	67.00±3.85
PPL (800ppm)	78.00±5.18
PPL (1600ppm)	95.00±7.63

Mean value ± standard deviation (SD).

Table 5: Influence of leaves (olive and pomegranate) juice and BHT on the activity of serum alanine aminotransferase of rats (IU L^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	11.40±0.30	11.50±0.11	11.60±0.31	10.411.65±	11.70±0.40	11.73±0.71	11.52±0.01
BHT (200ppm)	11.40±0.30	12.80±0.41	13.50±0.13	17.00±0.61	20.22±0.41	22.30±0.62	25.50±0.04
POL (200ppm)	11.40±0.30	11.60±0.10	11.65±0.11	11.72±0.03	11.84±0.43	11.90±0.57	12.01±0.31
POL (400ppm)	11.40±0.30	11.66±0.33	11.73±0.31	11.90±0.46	12.00±0.19	12.05±0.35	12.21±0.41
POL (800ppm)	11.40±0.30	11.56±0.16	11.80±0.01	11.85±0.12	11.93±0.32	12.00±0.22	12.05±0.25
POL(1600ppm)	11.40±0.30	11.55±0.22	11.70±0.09	11.90±0.17	12.01±0.23	12.15±0.22	12.25±0.51
PPL (200ppm)	11.40±0.30	11.61±0.29	11.62±0.16	11.73±0.03	11.89±0.13	11.95±0.46	12.01±0.39
PPL (400ppm)	11.40±0.30	11.50±0.16	11.69±0.23	11.81±0.25	11.92±0.31	12.00±0.97	12.19±0.51
PPL (800ppm)	11.40±0.30	11.53±0.19	11.71±0.03	11.80±0.17	11.95±0.37	12.03±0.42	12.20±0.54
PPL(1600ppm)	11.40±0.30	11.67±0.06	11.83±0.20	11.83±0.20	11.92±0.39	11.98±0.43	12.17±0.13

Mean value ± standard deviation (SD).

Table 6: Influence of leaves (olive and pomegranate) juice and BHT on the activity of serum aspartate aminotransferase of rats (IU L^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	11.61±0.34	11.71±0.29	11.95±0.11	12.00±0.11	12.11±0.50	12.20±0.31	12.30±0.21
BHT (200ppm)	11.61±0.34	11.90±0.61	12.93±0.17	13.85±0.21	15.93±0.26	22.13±0.31	23.15±0.19
POL (200ppm)	11.61±0.34	11.63±0.21	11.72±0.20	11.75±0.61	11.70±0.19	11.83±0.23	11.85±0.51
POL (400ppm)	11.61±0.34	11.65±0.32	11.70±0.63	11.82±0.09	11.91±0.21	11.99±0.22	12.01±0.57
POL (800ppm)	11.61±0.34	11.70±0.51	11.75±0.04	11.93±0.11	11.95±0.19	12.00±0.59	12.11±0.65
POL(1600ppm)	11.61±0.34	11.82±0.63	11.85±0.63	11.94±0.19	11.99±0.12	12.03±0.05	12.15±0.32
PPL (200ppm)	11.61±0.34	11.79±0.59	11.79±0.06	11.83±0.01	11.89±0.19	11.93±0.34	12.00±0.53
PPL (400ppm)	11.61±0.34	11.75±0.56	11.79±0.11	11.82±0.60	11.90±0.29	11.93±0.31	11.99±0.45
PPL (800ppm)	11.61±0.34	11.79±0.41	11.83±0.21	11.92±0.05	11.95±0.01	12.05±0.04	12.22±0.43
PPL(1600ppm)	11.61±0.34	11.75±0.71	11.83±0.21	11.90±0.19	11.94±0.21	11.99±0.01	12.15±0.32

Mean value ± standard deviation (SD).

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Table 7: Influence of leaves (olive and pomegranate) juice and BHT on the activity of alkaline phosphatase of rats (IU L^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	65.00±0.46	64.93±0.13	65.03±0.13	65.06±0.13	65.19±0.29	65.20±0.31	65.31±0.07
BHT (200ppm)	65.00±0.46	65.50±0.12	65.90±0.51	67.20±0.01	71.30±0.36	73.20±0.56	80.02±0.16
POL (200ppm)	65.00±0.46	65.03±0.30	65.12±0.39	65.19±0.15	65.31±0.23	65.23±0.66	65.41±0.23
POL (400ppm)	65.00±0.46	65.13±0.52	65.20±0.24	65.22±0.20	65.35±0.19	65.41±0.75	65.31±0.66
POL (800ppm)	65.00±0.46	65.00±0.01	65.09±0.19	65.14±0.52	65.29±0.64	65.25±0.18	65.46±0.31
POL(1600ppm)	65.00±0.46	65.04±0.50	65.19±0.35	65.22±0.01	65.49±0.19	65.31±0.70	65.33±0.39
PPL (200ppm)	65.00±0.46	65.13±0.09	65.25±0.61	65.29±0.61	65.31±0.25	65.36±0.01	65.29±0.41
PPL (400ppm)	65.00±0.46	65.05±0.19	65.13±0.72	65.17±0.19	65.16±0.39	65.22±0.49	65.25±0.53
PPL (800ppm)	65.00±0.46	65.13±0.22	65.16±0.64	65.10±0.34	65.31±0.46	65.22±0.05	65.36±0.41
PPL(1600ppm)	65.00±0.46	65.23±0.31	65.23±0.31	65.32±0.29	65.40±0.55	65.41±0.33	65.39±0.18

Mean value ± standard deviation (SD).

Table 8: Influence of leaves (olive and pomegranate) juice BHT on the levels of serum total lipids of rats (mg dl^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	288.00±0.83	288.50±0.15	288.61±0.31	288.71±0.90	288.90±.11	288.95±0.45	290.00±0.76
BHT (200ppm)	288.00±0.83	290.00±0.90	292.00±1.04	295.30±2.50	299.70±0.09	303.00±0.13	310.20±0.82
POL (200ppm)	288.00±0.83	288.10±1.03	288.30±2.03	288.51±0.80	288.90±0.42	288.85±1.09	290.15±2.00
POL (400ppm)	288.00±0.83	288.40±0.60	288.55±1.19	288.67±0.89	288.95±2.01	290.00±0.35	290.30±1.06
POL (800ppm)	288.00±0.83	288.20±0.19	288.35±1.11	288.61±0.95	288.75±0.46	288.95±0.56	290.10±0.66
POL(1600ppm)	288.00±0.83	288.29±0.49	288.50±2.30	288.78±1.22	288.30±0.67	288.50±0.19	288.11±0.76
PPL (200ppm)	288.00±0.83	288.00±0.57	288.30±1.01	288.60±0.97	288.13±0.95	288.90±0.89	288.30±0.73
PPL (400ppm)	288.00±0.83	288.19±1.03	288.35±2.00	288.45±2.11	288.52±0.81	288.55±0.59	288.90±1.01
PPL (800ppm)	288.00±0.83	288.11±0.77	288.49±1.00	288.56±0.66	288.11±0.89	288.90±1.17	290.00±0.90
PPL(1600ppm)	288.00±0.83	288.01±1.00	288.19±1.00	288.50±0.66	288.90±1.90	289.00±2.05	289.50±0.79

Mean value ± standard deviation (SD).

Table 9: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum total cholesterol of rats (mg dl^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	160.00±0.14	160.50±0.29	161.20±0.30	160.30±0.01	160.22±0.19	162.00±0.82	161.90±0.39
BHT (200ppm)	160.00±0.14	160.20±0.19	160.00±0.34	161.50±0.45	161.90±0.72	161.50±0.21	162.00±0.31
POL (200ppm)	160.00±0.14	160.30±0.01	160.80±0.42	161.20±0.41	161.90±0.81	162.03±0.61	162.00±0.13
POL (400ppm)	160.00±0.14	160.33±0.19	160.45±0.03	160.55±0.80	161.31±0.39	162.13±0.18	161.00±0.09
POL (800ppm)	160.00±0.14	160.55±0.23	160.90±0.75	161.00±0.42	161.20±0.31	161.33±0.01	161.70±0.61
POL(1600ppm)	160.00±0.14	160.00±0.21	160.31±0.19	161.00±0.22	161.50±0.69	161.72±0.39	161.49±0.11
PPL (200ppm)	160.00±0.14	160.09±0.59	161.00±0.30	161.50±0.19	161.00±0.09	161.31±0.41	161.50±0.40
PPL (400ppm)	160.00±0.14	160.15±0.19	160.22±0.39	160.52±0.82	160.75±0.13	160.90±0.31	160.99±0.12
PPL (800ppm)	160.00±0.14	160.29±0.22	160.56±0.39	160.75±0.45	160.90±0.56	161.00±0.61	161.20±0.65
PPL(1600ppm)	160.00±0.14	160.00±0.29	160.55±0.34	161.20±0.01	161.35±0.01	161.00±0.19	161.00±0.41

Mean value ± standard deviation (SD).

Table 10: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum HDL-cholesterol of rats (mg dl^{-1})

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	40.13±0.52	40.33±0.22	40.50±0.35	41.00±0.09	41.31±0.14	41.60±0.67	42.00±0.31
BHT (200ppm)	40.13±0.52	40.00±0.60	40.50±0.05	40.39±0.35	41.22±0.19	41.50±0.01	41.00±0.30
POL (200ppm)	40.13±0.52	41.00±0.70	41.39±0.19	40.71±0.27	40.79±0.42	41.30±0.18	41.39±0.37
POL (400ppm)	40.13±0.52	40.59±0.64	40.75±0.55	41.03±0.67	41.90±0.01	42.00±0.63	42.10±0.13
POL (800ppm)	40.13±0.52	40.60±0.13	40.90±0.63	40.85±0.72	41.30±0.94	41.50±0.13	42.00±0.29
POL(1600ppm)	40.13±0.52	40.00±0.60	40.81±0.33	40.90±0.01	40.85±0.72	41.00±0.19	41.35±0.18
PPL (200ppm)	40.13±0.52	40.05±0.67	40.19±0.82	40.71±0.13	41.30±0.84	41.73±0.35	42.00±0.60
PPL (400ppm)	40.13±0.52	40.50±0.19	40.66±0.01	40.75±0.04	40.91±0.42	41.00±0.13	41.30±0.19
PPL (800ppm)	40.13±0.52	40.39±0.72	40.69±0.89	40.18±0.32	41.09±0.43	41.33±0.84	41.90±0.93
PPL(1600ppm)	40.13±0.52	40.80±0.61	41.00±0.22	41.35±0.81	41.69±0.11	41.69±0.59	42.00±0.19

Mean value ± standard deviation (SD).

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Table 11: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum LDL-cholesterol of rats (mg dl-1)

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	100.60±1.06	100.71±0.83	100.39±0.29	100.81±2.04	101.00±0.91	101.20±0.19	101.50±0.56
BHT (200ppm)	100.60±1.06	101.00±0.78	101.30±0.35	101.34±0.49	100.90±0.85	101.50±0.95	101.66±1.09
POL (200ppm)	100.60±1.06	100.33±0.88	101.11±2.03	101.19±0.50	101.50±0.59	101.90±0.19	102.00±0.89
POL (400ppm)	100.60±1.06	100.90±0.67	101.00±0.52	101.50±0.64	101.90±0.66	101.95±0.23	102.01±0.85
POL (800ppm)	100.60±1.06	100.75±0.52	100.85±0.39	100.95±0.25	101.00±1.03	101.50±0.14	102.00±0.45
POL(1600ppm)	100.60±1.06	100.90±0.18	101.11±0.38	101.19±0.91	101.50±0.55	101.90±0.71	102.00±0.79
PPL (200ppm)	100.60±1.06	100.65±0.13	100.75±1.05	100.80±1.00	100.95±2.03	101.95±0.19	102.15±0.89
PPL (400ppm)	100.60±1.06	100.75±0.39	101.00±0.95	101.35±0.90	101.45±0.85	101.90±1.16	102.60±0.81
PPL (800ppm)	100.60±1.06	100.90±1.00	100.95±0.89	101.20±0.72	101.31±0.33	101.22±0.89	101.55±0.19
PPL(1600ppm)	100.60±1.06	100.63±0.90	100.63±0.90	100.83±0.41	101.09±0.42	101.29±0.30	101.39±0.42

Mean value ± standard deviation (SD).

Table 12: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum total bilirubin of rats (mg dl-1)

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	1.00±0.01	1.01±0.01	1.01±0.02	1.02±0.01	1.01±0.03	1.00±0.01	1.02±0.01
BHT (200ppm)	1.00±0.01	1.01±0.01	1.07±0.003	1.23±0.01	1.33±0.01	1.39±0.02	1.43±0.02
POL (200ppm)	1.00±0.01	1.01±0.01	1.01±0.02	1.02±0.02	1.01±0.02	1.02±0.01	1.01±0.03
POL (400ppm)	1.00±0.01	1.01±0.03	1.00±0.01	1.01±0.04	1.01±0.01	1.02±0.02	1.02±0.01
POL (800ppm)	1.00±0.01	1.01±0.02	1.00±0.04	1.01±0.003	1.01±0.003	1.02±0.02	1.02±0.01
POL(1600ppm)	1.00±0.01	1.01±0.01	1.01±0.01	1.02±0.02	1.02±0.02	1.01±0.02	1.01±0.02
PPL (200ppm)	1.00±0.01	1.01±0.01	1.01±0.01	1.02±0.01	1.02±0.01	1.01±0.01	1.01±0.02
PPL (400ppm)	1.00±0.01	1.00±0.02	1.01±0.02	1.02±0.03	1.01±0.01	1.01±0.03	1.02±0.03
PPL (800ppm)	1.00±0.01	1.00±0.01	1.01±0.02	1.00±0.02	1.01±0.02	1.01±0.01	1.01±0.01
PPL(1600ppm)	1.00±0.01	1.01±0.01	1.02±0.01	1.02±0.01	1.01±0.01	1.01±0.02	1.02±0.02

Mean value ± standard deviation (SD).

Table 13: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum urea of rats (mg dl-1)

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	30.00±0.30	30.19±0.42	30.18±0.13	30.11±0.15	30.00±0.17	29.19±0.17	29.50±0.43
BHT (200ppm)	30.00±0.30	33.85±0.10	39.00±0.31	45.00±0.13	52.00±0.04	62.00±0.31	69.50±0.31
POL (200ppm)	30.00±0.30	30.00±0.60	30.00±0.32	29.15±0.33	29.50±0.13	30.11±0.41	30.19±0.41
POL (400ppm)	30.00±0.30	30.00±0.90	30.01±0.04	29.50±0.10	29.90±0.41	29.85±0.69	30.00±0.13
POL (800ppm)	30.00±0.30	30.00±0.72	30.00±0.19	30.00±0.66	30.00±0.01	29.81±0.19	29.00±0.51
POL(1600ppm)	30.00±0.30	29.85±0.22	29.90±0.25	30.00±0.32	29.00±0.39	30.00±0.34	30.00±0.21
PPL (200ppm)	30.00±0.30	29.00±0.33	29.50±0.41	29.20±0.19	29.11±0.22	30.00±0.25	30.00±0.68
PPL (400ppm)	30.00±0.30	30.12±0.29	30.00±0.31	30.01±0.39	29.80±0.35	29.50±0.22	29.00±0.01
PPL (800ppm)	30.00±0.30	30.27±0.22	30.20±0.23	30.15±0.14	30.30±0.04	30.00±0.05	30.00±0.16
PPL(1600ppm)	30.00±0.30	29.00±0.33	29.00±0.69	29.50±0.01	29.55±0.10	29.00±0.42	29.00±0.42

Mean value ± standard deviation (SD).

Table 14: Influence of leaves (olive and pomegranate) juice and BHT on the levels of serum creatinine of rats (mgdl-1)

Concentration of phenolic compounds	Blood withdrawal period (week)						
	0	1	2	3	4	5	6
Control rats	0.88±0.10	0.88±0.03	0.87±0.09	0.87±0.04	0.88±0.04	0.088±0.11	0.88±0.19
BHT (200ppm)	0.88±0.10	1.01±0.30	1.29±0.03	1.48±0.01	1.60±0.02	1.80±0.01	1.91±0.01
POL (200ppm)	0.88±0.10	0.87±0.10	0.86±0.41	0.86±0.08	0.85±0.07	0.85±0.13	0.85±0.14
POL (400ppm)	0.88±0.10	0.87±0.10	0.86±0.07	0.84±0.06	0.85±0.13	0.85±0.00	0.85±0.12
POL (800ppm)	0.88±0.10	0.88±0.19	0.88±0.09	0.86±0.01	0.83±0.03	0.80±0.10	0.80±0.10
POL (1600ppm)	0.88±0.10	0.85±0.20	0.85±0.22	0.83±0.09	0.83±0.09	0.82±0.07	0.80±0.02
PPL (200ppm)	0.88±0.10	0.88±0.02	0.87±0.10	0.85±0.08	0.85±0.08	0.85±0.07	0.85±0.10
PPL (400ppm)	0.88±0.10	0.85±0.10	0.85±0.30	0.84±0.14	0.84±0.12	0.84±0.10	0.84±0.12
PPL (800ppm)	0.88±0.10	0.86±0.09	0.85±0.42	0.82±0.09	0.82±0.12	0.81±0.11	0.80±0.11
PPL(1600ppm)	0.88±0.10	0.85±0.10	0.84±0.01	0.82±0.01	0.82±0.30	0.80±0.11	0.80±0.11

Mean value ± standard deviation (SD).

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