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Total Phenolic Content and Antioxidant Activity of Phyto-Extracts of Some by-Products/Wastes from Food Factories in Egypt

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Total Phenolic Content and Antioxidant Activity of Phyto-Extracts of Some by-Products/Wastes from Food Factories in Egypt

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Abstract:

The present study aims to determine the contents of total phenolic compounds and antioxidant activity in three food processing by-products extracts including eggplant peel extract (EPP), onion skin extract (OSE), pomegranate peel extract (PPE) and their mixture. Also, exploring the relationship(s) between the content of these extracts of phenolic compounds and their antioxidant activities, was in the scope of this investigation.The selected phyto-extracts showed considerable differences in antioxidant activity (AA) and the OSE recorded the highest value (90.61%) followed by ME (89.69), PPE (86.03) and EPE (81.04). Also, the total phenolics content was ranged 98.95 to 298.56 mg GAE/ g. The OSE was exhibited the highest content of total phenolics while EPP recorded the lowest value. Furthermore, when all selected phyto- extracts were included in the statistical analysis, there was a positive and highly significant ($p \leq 0.05$) relationship between total phenolics and antioxidant activity. The highest value was recorded for OSE (r^2= 0.8982, $p \leq 0.05$) followed by PPE (r^2=0.8633, $p \leq 0.01$), EPE (r^2= 0.8471, $p \leq 0.05$) and ME (r^2= 0.7840, $p \leq 0.05$), respectively. The samples also recorded other biological activity i.e. inhibition of low density lipoprotein (LDL) oxidation. In conclusion, we recommended to pay attention in the future to carry out more research in the area of therapeutic nutrition with food processing by-products with the high content of different categories of bioactive compounds and extended their applications in human diets, industrial and medical applications instead of the synthetic antioxidants/chemicals used which have induced healthy hazards and side effects for the human being.

Keywords:

Eggplant peel, onion skin, pomegranate peel, extractive value, β-Carotene Bleaching assay, inhibition of LDL oxidation,

المحتوى الكلى من الفينولات والنشاط المضاد للأكسدة في المستخلصات النباتية لبعض المنتجات الثانوية / المخلفات لمصانع الأغذية في مصر

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مستخلص البحث:

تهدف الدراسة الحالية إلى تقدير المحتوى الكلى من المركبات الفينولية والنشاط المضاد للأكسدة في ثلاثة مستخلصات نباتية مشتقة من النواتج الثانوية/المخلفات لبعض مصانع الأغذية فى مصر. وتشمل المستخلص على مستخلص قشر الباذنجان (EPP) ومستخلص قشر البصل (OSE) ومستخلص قشر الرمان (PPE) وخليطها .أيضًا ، كان استكشاف العلاقة (العلاقات) بين محتوى هذه المستخلصات من المركبات الفينولية وأنشطتها المضادة للأكسدة في نطاق هذا التحقيق ، حيث أظهرت المستخلصات النباتية المختارة اختلافات كبيرة في نشاط مضادات الأكسدة (AA) وسجل OSE أعلى نسبة القيمة (90.61٪) تليهاME (89,69%)، PPE (86.03%) ، EPP (81,04%). كما تراوح محتوى الفينولات الكلي من 98.95 إلى 298.56 مجم / GAE جم .أظهرت OSE أعلى محتوى من إجمالي الفينولات بينما سجلت EPPأقل قيمة .علاوة على ذلك ، عندما تم تضمين جميع المستخلصات النباتية المختارة في التحليل الإحصائي ، كانت هناك علاقة موجبة وذات دلالة عالية ($p\leq 0.05$) بين محتوى الفينولات والنشاط المضاد للأكسدة .تم تسجيل أعلى قيمة لمستخلص قشر لبصل($r^2 = 0.8982$ ، $p\leq 0.05$) ، تليها مستخلص قشر الرمان ($r^2 = 0.8633$ ، $p\leq 0.01$) ، ومستخلص قشر الباذنجان ($r^2 = 0.8471$ ، $p\leq 0.05$) ، ومخلوط المستخلصات ($r^2 = 0.7840$ ، $p\leq 0.05$) على التوالى .سجلت العينات أيضًا نشاطًا بيولوجيًا آخر ، مثل تثبيط أكسدة البروتين الدهني منخفض الكثافة (LDL) . وفى النهاية، أوصينا بالاهتمام في المستقبل بإجراء المزيد من الأبحاث في مجال التغذية العلاجية مع المنتجات الثانوية لتجهيز الأغذية ذات المحتوى العالي من مجموعات مختلفة من المركبات النشطة بيولوجيًا وتوسيع تطبيقاتها في النظم الغذائية البشرية والتطبيقات الصناعية والطبية بدلاً من مضادات الأكسدة / المواد الكيميائية الاصطناعية المستخدمة والتي تسببت في مخاطر صحية وآثار جانبية للإنسان .

الكلمات المفتاحية:

قشر الباذنجان ، قشر البصل ، قشر الرمان ، القيمة الاستخلاصية، اختبار التبييض-β كاروتين ، تثبيط أكسدة البروتينات الدهنية منخفضة الكثافة.

Introduction

Food factory waste is a by-product produced during food processing. It includes peels, seeds, whey, blood, bones, and wastewater from food processing. According to the Food and Agriculture Organization (FAO), nearly a third of the edible parts of food produced for human consumption are lost or wasted globally. The agricultural industry in the Arab world represents a large proportion of waste, estimated at 18.14 million tons per year, and processed fruit and vegetable residues represent about 6.14% of this amount (Ahmed, 2015). The processing of fruits and vegetables generates large amounts of waste such as peels, seeds, stones, meal, etc. Disposal of these materials is usually a problem, exacerbated by legal restrictions. plant waste subject to microbial spoilage; Therefore, drying is necessary before further exploitation. The cost of drying, storage and transportation impose additional economic constraints on the use of waste. Therefore, agro-industrial waste is often used as feed or as fertilizer. However, the demand for feed or fertilizer varies and depends on agricultural production. Moreover, the valuable nutrients found in agro-industrial waste are lost. Thus, new aspects related to the use of these wastes as by-products for further exploitation in the production of food additives or nutritional supplements with high nutritional value have gained increasing attention because they are high-value products and their recovery may be economically beneficial.

Food processing waste is a cheap source of valuable components (bioactive compounds) due to the ease of recovery and recycling of compounds within the food chain as food and functional additives in various products. It can also be the extraction and purification of bioactive compounds and their use as natural antioxidants as alternatives to industrial additives, which studies have proven dangerous to human health (Vasso and Constantina, (2007). Synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be vitamins (C and E), minerals (selenium and copper) and phytonutrients (phenolic compounds, nitrogen compounds and carotenoids) (Hall and Cuppett. 1997). Science the beginning of 20th century, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975 and Ito *et al.*,1983). Thus, the interest in natural antioxidants has increased considerably.

Phenolic compounds are important components of many fruits and vegetables contributing to their colour and sensory properties. Epidemiological studies have demonstrated that the composition of phenol-rich food retards the progression of arteriosclerosis and reduces the incidence of heart diseases by preventing the oxidative stress, that is, lipid peroxidation in arterial macrophages and

in lipoproteins (Aviram *et al., 2004* and Gil *et al., 2003).* Also,, crude extracts of plant parts rich in phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Dewan, 2003; Abo El-Nagaa, 2003 and Elhassaneen, 2004). The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufactures, and consumers as the trend of the future is moving toward functional food with specific health effects (Loliger, 1991). The antioxidant activity of several plant materials has recently been reported (Yen and Duh, 1995; Oomah and Mazza, 1996; Wang *et al*., 1996, Cao *et al*., 1996 and Ghaly, 2004); however, information on the relationship between antioxidant activity and phenolic content of many plant parts such food processing by products is not available. Therefore, the present study aims to determine the contents of total phenolic compounds in three food processing by-products extracts including onion skin, eggplant peel, pomegranate peel and their mixture. Also, exploring the relationship(s) between the content of these extracts of phenolic compounds and their antioxidant activities, will be in the scope of this investigation.

2. Materials and Methods

2.1. Materials

2.1.1. Plant parts

Pomegranate (*Punica granatum*) and eggplant (*Solanum melongena*) fruits were purchased from Minia local markets, Minia City, Egypt. Red onion (*Allium cepa* L.) skin (ROS) was obtained by special arrangements with some fresh onion merchants, met Khorab village, Sinbellaween Center, Dakhlia Governorate, Egypt

2.1.2. Chemicals

All chemicals (except other mention), solvents and buffers were of analytical grade were purchased from El-Ghomhorya Company for Trading Drugs, Chemicals and Medical instruments, Cairo, Egypt.

2.1.3. Equipment's

Absorbance (Abs) and fluorescence (FL) for different assays were measured using Labo-med. Inc., spectrophotometer, CA and Schematzu fluorescence apparatus, Japan, respectively.

2. 2. Methods

2.2.1. Preparation of plant parts extracts

Plant parts extracts were prepared according to the methods of Aly et al., **(**2017) as follow: Eggplant and apple and red onion fruits were peeled manually to obtain eggplant and pomegranate peels, red onion skin. Skin and peels were washed then dried in a hot air oven (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA) at 55 ^{0}C until arriving by the moisture in the final product to about 7%. The dried peels and skin were ground into a fine powder in high mixer speed (Moulinex Egypt, ElAraby Co., Egypt). The material that passed through an 80 mesh sieve was retained for use. Powders obtained were used for different types extracts as follow: A 20 g from dried plant powder plus 180 ml methanol (80%, v/v) were homogenized and transferred to a beaker and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 60 min at room temperature (25±5 ^{0}C). The extract was then separated from the residue by filtration through filter pPPEr (Whatman No. 1). The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of was removed under reduced pressure at 45°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) and the extract could be ready for the basil diet blending purpose.

2.2.2. Chemical analysis

2.2.2.1. Determination of total phenolics

Total phenolics in selected vegetables processing by-product extracts were analyzed spectrophotometrically using Folin-Ciocalteu reagent according to the method of Singleton and Rossi, (1965). In brief, 200 mg of sample was extracted for 2 h with 2 mL of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 mL vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 ^{0}C for 5 min; 0.75 ml of sodium bicarbonate (60g/L) solution was added to the mixture after 90 min at 22 0C, absorbance was measured at 725 nm. Results are expressed as gallic acid and equivalents (GAE).

2.2.2.2. Antioxidant activity (AA)

Antioxidant activity (AA) of selected vegetables processing by-product extracts and standards (α-tocopherol and BHT) was determined according to the BCB assay following a modification of the procedure described by Marco, (1968). For a typical assay, 1mL of β-carotene solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of Tween 20. Each mixture was then dosed with 0.2 mL of 80% MeOH (as

control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal auto-oxidation at 50 ^{0}C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of *β*-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT and α-tocopherol in 80% methanol were used as the control. Antioxidant activity was calculated in four different ways as follow: 1) absorbance was plotted against time, as a knit curve, and the absolute value of slope was expressed as antioxidant value (AOX) (Al-Saikhan *et al.,* 1995), 2) antioxidant activity (AA) was all calculated as percent inhibition relative to control using the equation (Al-Saikhan *et al.,* 1995) AA= ($R_{control}$ - R_{sample}) / $R_{control}$ x 100 where: $R_{control}$ and R_{sample} were the bleaching rates of β-carotene in reactant mixture without antioxidant and with plant part extract, respectively, 3) this method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of Marinova *et al.,* (1994) using the equation [ORR = R_{sample} / $R_{control}$] where: $R_{control}$ and R_{sample} are the same in the previous method, and 4) the antioxidant activity coefficient (AAC) was calculated as described by Mallet *et al.,* (1994) [AAC = (Abs_{S120}- Abs_{C120})/ Abs_{C0}- Abs_{C120}) x 100] where: Abs $_{S\ 120}$ was the absorbance of the antioxidant mixture at time 120 min, Abs $_{C\ 120}$ was the absorbance of the control at time 120 min, Abs $_{C\ 0}$was the absorbance of the control at zero time.

2.2.2.3. β-carotene bleaching (BCB) assay

For *β*-carotene bleaching (BCB) assay, antioxidant activity (AA) against time (every 10 min thereafter for 120 min) for the all tested vegetables processing by-product extracts was measured/constructed according to Marco, (1968). The AA was all calculated as percent inhibition (bleaching rates of *β*-carotene in reactant mixture of plant part extracts) relative to control (bleaching rates of *β*-carotene in reactant mixture of without plant part extracts) such as described by Al-Saikhan *et al.,* (1995).

2.2.2.4. Inhibition of low density lipoprotein (LDL) oxidation

Inhibition of LDL oxidation was determined according to the method of Princen *et al.,* (1992). Adult male white albino rat, Sprague Dawley strain obtained from Research Institute of Ophthalmology, Medical Analysis Department, Giza, Egypt and tThe basal diet prepared according to the following formula as mentioned by AIN, (1993). Serum was collected and diluted by phosphate buffer (50 mM, pH 7.4) to the concentration of 0.6%. Quantities of 5.0 ml diluted serum were mixed with 10 µl DMSO or 10 µl DMSO containing various concentrations of the all tested

vegetables processing by-product extracts. A 20 µl of CuSO4 solution (2.5 mM) was added to initiate the reaction and the absorbance at 234 nm was recorded then was taken every 20 min thereafter for 140 min at room temperature. The final result was expressed by calculation the net area under the curve.

2.3. Statistical Analysis

All measurements were carried out put in triplicates and expressed as mean± standard deviation (SD). Statistical analysis was performed with the Student *t*-test and MINITAB-12 computer program (Minitab Inc., State College, PA).

3. Results and Discussion

3.1. Extractive value of selected plant parts using water and different organic solvents

The extractive value means the quantity which is soluble in water and different organic solvents. It was determined by successive extraction in water and different solvents using a Soxhlet's apparatus. The extractive value makes a valuable test to check the quality of drug/additive/food supplement and any variation in the chemical constituents may cause a change in it. Thus, it helps in the determination of the adulteration and is an index of the purity of the material. The extractive value of selected plant parts using water and different organic solvents are shown in Table (1). The extractive value for the selected plant parts in water and hexane was low (3.46-4.21%) while relatively high in methanol and ethanol (6.09-7.91%). Similar data was recorded by Saleh (2016) and Ali et al., (2017) for other plant parts/phyto-extracts. All of those data confirmed that selected plant parts constituents were found in both lipophilic and hydrophilic phases i.e. accordance with the known rule "like dissolve like". The variation in the extractive values of the selected plant parts may be possible due to the presence of specific compound according to the solubility, soil condition, atmospheric condition and water content of the sample (Saleh, 2016; Aly et al., 2017). All of these data indicated that the methanol/ethanol extracts of the selected plant parts were recommended for the further studies from the practical and economical point of view.

Table 1. Extractive value of selected plant parts using water and different organic solvents

Extract	Mean extract, yield (%) ±SD			
	Water	Methanol	Ethanol	Hexane
Eggplant peel (EPE)	3.69 ± 0.23 ^{b}	6.95 ± 0.37 ^{b}	6.59 ± 0.54 ^{b}	3.61 ± 0.44 ^{b}
Onion skin (OSE)	4.19 ± 0.56 ^{a}	7.99 ± 0.64 ^{a}	7.91 ± 0.96^{a}	4.21 ± 70 ^{a}
Pomegranate peel (PPE)	3.46 ± 0.42 ^{b}	6.27 ± 0.29 ^{b}	6.09 ± 0.47 ^{b}	3.71± 0.60 ^{b}

Each value represents mean ±SD. Means in the same column with different superscript letters are significantly different at p≤ 0.05.

3.2. Antioxidant activities of phyto-extracts

3.2.1. Antioxidant activity (AA)

The antioxidant activities of the selected phyto-extracts and their mixture are shown in Table (2). From such data it could be noticed that the selected phyto-extracts showed considerable differences in antioxidant activity (AA) and the OSE recorded the highest value (90.61%) followed by ME (89.69), PPE (86.03) and EPE (81.04). Such data are similar to that obtained by Aly et al., (2017) who found that OSE showed strong antioxidant activity because of its probably high phenolics content (179.71 ± 21.90 GAE. g^{-1}) while EPE and TPE showed considerable content in both AA (82.44 and 80.98 %) and the total phenolics (71.06 and 41.56 mg GAE. g^{-1}), respectively. Also, Elhassaneen *et al.*, (2016) and Sayed Ahmed, (2016) recorded partially the same date for some food processing by-products including onion skin and eggplant peel.

Table 2. Antioxidant activity of selected phyto-extracts

Extract	Antioxidant value[a] AOX (A/h)	Antioxidant activity[b] AA (%)	Oxidation rate ratio[c] (ORR)	Antioxidant activity coefficient[d] (AAC)
Eggplant peel extract (EPE)	0.107± 0.001	81.04± 7.65 [c]	0.189± 0.020	581.16± 21.50
Onion skin extract (OSE)	0.053± 0.012	90.61± 6.43 [b]	0.093± 0.012	747.53± 33.11
Pomegranate Peel Extract (PPE)	0.079± 0.009	86.03± 3.87 [bc]	0.139± 0.007	667.91± 40.20
Mixture (ME)	0.058± 0.006	89.69± 5.44 [b]	0.103± 0.019	731.53± 46.01
Control	0.565± 0.021	0.00± 0.00	0.998± 0.119	0.00± 0.00
a-tocopherol, 50 mg/L	0.006± 0.002	98.99± 0.96 [a]	0.010± 0.002	893.21± 10.55
BHT, 50 mg/L	0.047± 0.007	91.76± 0.50[b]	0.082± 0.005	767.52± 8.17
Eggplant peel extract (EPE)	0.107± 0.004	81.04± 0.75 [c]	0.189± 0.007	581.16± 6.21

[a] Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).
[b] Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively
[c] Oxidation rate ratio (ORR) = R sample / R control
[d] Antioxidant activity coefficient (AAC) = (Abs S 120 - Abs C 120) / Abs C 0 - Abs C 120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C 0 was the absorbance of the control at zero time.
[e] Each value represents mean ±SD. Means in the same row with different superscript letters are significantly different at $p \leq 0.05$.

3.2.2. *β* -Carotene Bleaching (BCB)

β-Carotene Bleaching (BCB) assay based on measured the ability of an antioxidant to inhibit lipid peroxidation in a model system made of *β*-carotene and linoleic acid subject a rapid discoloration in the absence of an antioxidant. The free linoleic acid radical formed upon the losing of a hydrogen atom from one of its methylene groups attacked the β-carotene molecules, which lost the double bonds and subsequently its characteristic orange color. The absorbance of the reaction medium at 470 nm was monitored on a spectrophotometer by taking measurements at 10 min intervals, and the rate of bleaching of *β*-carotene was calculated by fitting linear regression to data over time according to Marco (1968). Antioxidant activity of selected phyto-extracts and standards/References assayed by the β-carotene bleaching method is shown in Table (3) and Figure (1). From such data it could be noticed that OSE and WE exhibited the lowest decreasing/high stability followed by PPE and EPE, respectively. Comparing to the antioxidants standard used, the values of OSE and ME absorbance's through 120 min are coming well i.e. closing the line of 50 mg/L of α-tocopherol and 100 mg/L of BHT as well as up to the line of 50 mg/L of BHT. Such data confirmed that ME and OSE have high stability when comparing to that most common standards, α-tocopherol and BHT. Such data are similar to that obtained by Aly et al., (2017) who found that OSE and EPE showed strong antioxidant activity assayed by BCB method. Also, several authors found that some food by-products/plant parts extracts including onion skin, eggplant peel recorded highly antioxidant activity through exhibited high antioxidant stability when

comparing with α-tocopherol as the standard (Velioglu *et al*., 1998; Ghaly, 2004 ; Elhassaneen and Abd Elhady, 2014; Elassaneen *et al.,* 2016; Saleh, 2016; Sayed Ahmed, 2016).

Table 3. Antioxidant activity of selected phyto-extracts and standards/References assayed by the β-carotene bleaching method

Phyto-extract	Time (min)												
	0	10	20	30	40	50	60	70	80	90	100	110	12
EPE	0.901	0.829	0.815	0.781	0.772	0.689	0.684	0.650	0.648	0.636	0.621	0.610	0.600
OSE	0.901	0.894	0.877	0.877	0.862	0.827	0.801	0.778	0.773	0.761	0.760	0.758	0.756
PPE	0.901	0.855	0.846	0.846	0.838	0.765	0.739	0.723	0.720	0.709	0.690	0.679	0.665
ME	0.901	0.880	0.870	0.870	0.856	0.827	0.805	0.783	0.767	0.761	0.754	0.746	0.740
Control	0.901	0.617	0.598	0.533	0.501	0.454	0.430	0.374	0.333	0.260	0.231	0.190	0.163
a-Toc, 50 mg/L	0.901	0.898	0.889	0.878	0.878	0.848	0.836	0.840	0.823	0.825	0.813	0.804	0.799
BHT, 50 mg/L	0.901	0.878	0.870	0.866	0.856	0.828	0.802	0.780	0.764	0.752	0.749	0.741	0.737

EPE, eggplant peel extract, OSE, onion skin extract, PPE, pomegranate peel extract, ME, mixture extract of EPE, OSE, and PPE by equal parts, a-Toc, alpha-tocopherol, BHT, butalyted hydroxytoluene

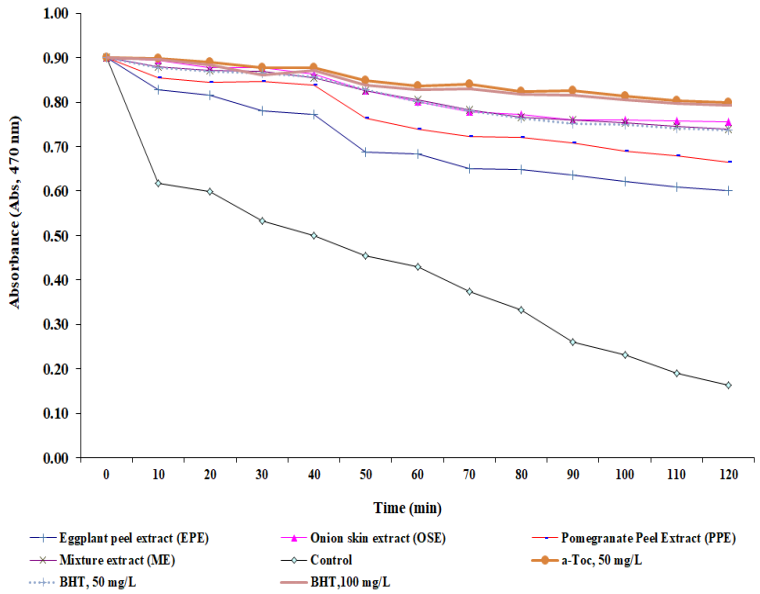


Figure 1. Antioxidant activity of selected phyto-extracts and standards/references assayed by the β-carotene bleaching method
EPE, eggplant peel extract, OSE, onion skin extract, PPE, pomegranate peel extract, ME, mixture extract of EPE, OSE, and PPE by equal parts, a-Toc, alpha-tocopherol, BHT, butalyted hydroxytoluene

3.2.3. Total phenolics content of the selected phyto- extracts

Total phenolics contents of selected phyto-extracts and their mixture are shown in Table (4). From such data it could be noticed that the total phenolics content was ranged 98.95 to 298.56 mg GAE/ g. The OSE was exhibited the highest content of total phenolics while EPP recorded the lowest value. Such data are in accordance partially with that obtained by several authors who reported that many food processing by-products/plant parts including onion skin and eggplant peel rich in their total phenolics contents (Elhassaneen and Abd Elhady, 2014; Elhassaneen *et al.*, 2016 and Sayed Ahmed, 2016). Also, Velioglu *et al*., (1998) determined the total phenolics of 28 plant products and noticed that it was varied from 169 to 10548 mg/100 g of dry product. The big differentiations have been noticed in different plant parts which due to the type, variety and color of the part (Kumar *et al*., 1991; Onyeneho and Hettiarachchy, 1993; Elhassaneen and Abd Elhady, 2014; Aly et al., 2017).

Table 4. Total phenolic contents of selected phyto-extracts

Extracts	Total phenolics (mg GAE. g^{-1})
Eggplant peel extract (EPE)	98.95 ± 10.67^{d}
Onion skin extract (OSE)	268.56 ± 13.67^{a}

Pomegranate Peel Extract (PPE)	133.87 ± 8.09 [c]
Mixture (ME)	169.62 ± 11.54 [b]

Each value represents mean ±SD. Means in the same row with different superscript letters are significantly different at p≤ 0.05.

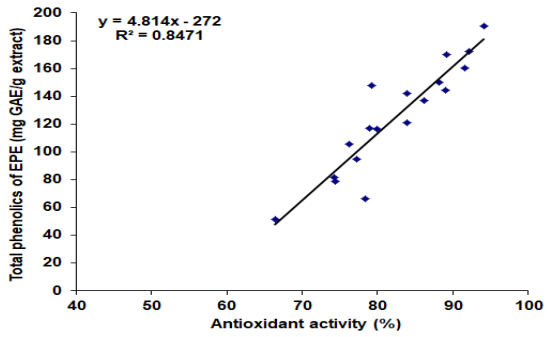
V.2.4. Relationship between total phenolic contents and antioxidant activity of the tested phyto-extracts

Relationship between total phenolic contents and antioxidant activity in tested phyto-extracts are shown in Table (5) and Figure (2). Such data indicated that when all selected phyto- extracts were included in the statistical analysis, there was a positive and highly significant (p≤ 0.05) relationship between total phenolics and antioxidant activity. The highest value was recorded for OSE (r^2= 0.8982, p≤ 0.05) followed by PPE (r^2=0.8633, p≤ 0.01), EPE (r^2= 0.8471, p≤ 0.05) and ME (r^2= 0.7840, p≤ 0.05), respectively. These relationships indicated that phenolic constituents play a major role in the antioxidant activity of the selected phyto-extracts and other some roles were depended on the occurrence of other bioactive compounds beside the phenolics such vitamins (ascorbic acid and tocopherols), sterols, pigments and minerals (Mashal, 2016; Elhassaneen and Abd Elhady, 2014; Sayed Ahmed, 2016). Data of the current study proved the importance of using all selected phyto- extracts as natural antioxidants in different therapeutic applications.

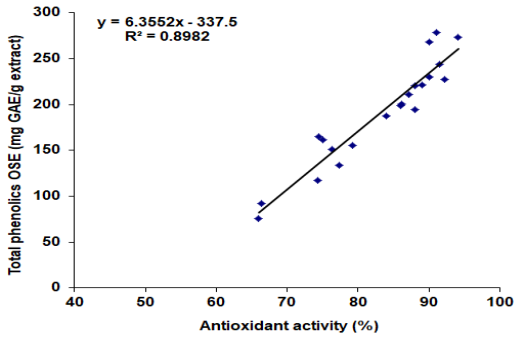
Table 5. Relationship between antioxidant activities (AA) and total phenolic contents of selected phyto-extracts (n=18)

Relationship between antioxidant activities and total phenolic contents		r^2
EPE	Total phenolics (mg GAE. g^{-1}) = 4.814 (Antioxidant activity, %) – 272	0.8471*
OSE	Total phenolics (mg GAE. g^{-1}) = 6.3552 (Antioxidant activity, %) – 337.5	0.8982**
PPE	Total phenolics (mg GAE. g^{-1}) = 2.243 (Antioxidant activity, %) – 62.669	0.8633**
ME	Total phenolics (mg GAE. g^{-1}) = 5.136 (Antioxidant activity, %) – 270.76	0.7840*

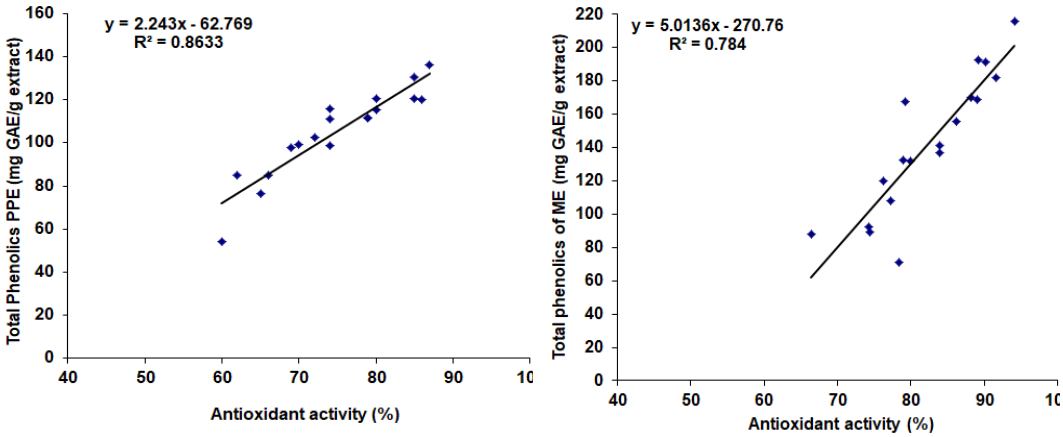
EPE, eggplant peel extract, OSE, onion skin extract, PPE, pomegranate peel extract, ME, mixture extract of EPE, OSE, and PPE by equal parts. * P ≥ 0.05, ** P ≥ 0.01



Eggplant peel extract (EPE)



Onion skin extract (OSE)



Pomegranate peel extract (PPE)

Mixture extract (ME)
Mixture extract of EPE, OSE, and PPE by equal parts

Figure 2. Relationship between antioxidant activities (AA) and total phenolic contents of selected phyto-extracts (n=18)

3.2.5. Inhibition of low density lipoprotein (LDL) oxidation by the selected phyto-extracts

Inhibition of low density lipoprotein (LDL) oxidation by the selected phyto-extracts was shown in Figure (3). Such data indicated that the inhibitive action of the all selected phyto-etracts and their mixture against CuSO4-induced LDL oxidation, as evidenced by decreased conjugated dienes production in a dose-dependent fashion. The EPE, OSE, PPE and their mixture acted more dramatically in protecting LDL against oxidation. Is that means a possibility of those extracts in the prevention of atherosclerosis through inhibiting LDL oxidation. Such effect could be attributed to the high content of different bioactive constituents/antioxidants (phenolics, phenolic acids, vitamins, volatile oil components etc) in the tested phyto-extracts. With this context, Aviram *et al.*, (2000) found that pomegranate juice effectively protect LDL against oxidation *in vitro*, which was attributed to the high levels content of polyphenols and ascorbic. Also, Li *et al.*, (2006) noticed that the pomegranate peel extract acted more efficiency in protecting LDL against oxidation due to its higher content of polyphenolic constituents. Such mechanisms of actions met with the increasing of the levels of glutathione (GSH) and glutathione reductase (GSH-Rd) in liver and lungs as well as increase in inhibition of NADPH-dependent lipid peroxidation (Majid *et al*., 1991). Other studies indicated that phenolic constituents formed a complex reaction with peroxyl radicals and inhibition of the LDL oxidation (Laranjinha *et al*., 1994). Such data are corresponding well with that reported by Aly et al., (2017). Generally, Chisolm and Steinberg, (2000) reported

that LDL oxidation plays a key role in early atherosclerosis. The therogenic effect of oxidized LDL is probably due to its cytotoxic toward arterial cells and stimulates the monocytes to be adhesive to the endothelium which leads to the development of atheromatous plaques (Hong and Cam, 2015). Thus, data of the present study with the other confirmed that the tested phyto-extracts and their mixture could be used as promising agents in atherosclerosis prevention through inhibiting LDL oxidation mechanism.

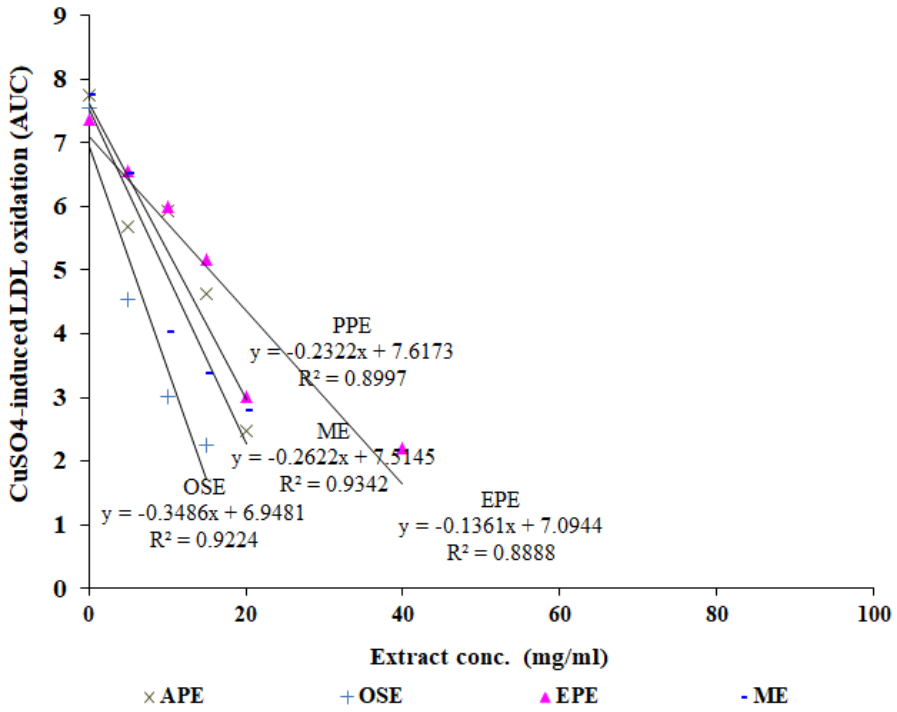


Figure 3. Dose-dependent inhibition of CuSO4-induced LDL oxidation *in vitro* by phyto-extracts. The conjugated dienes formation was monitored kinetically as the absorbance at 234 nm and the result is expressed as the area under the curve (AUC).
EPE, eggplant peel extract, OSE, onion skin extract, PPE, pomegranate peel extract, ME, mixture extract of EPE, OSE and PPE by equal parts.

In conclusion, tested phyto-extracts such eggplant peel extract (EPP), onion skin extract (OSE), pomegranate peel extract (PPE) showed significantly higher antioxidant activities and contained higher phenolics content. The nutritional, industrial and therapeutic effects have been exhibited by such food processing by-products (phto-extracts) and their mixture probably attributed to their highly content of bioactive compounds (phytochemicals), including mainly phenolic

compounds. We recommended to pay attention in the future to carry out more and more research in the area of therapeutic nutrition with food processing by-products with the high content of different categories of bioactive compounds and extended their applications in human diets, industrial and medical applications instead of the synthetic antioxidants/chemicals used which have induced healthy hazards and side effects for the human being.

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