(Olea europaea L.) Pomace and Their IngredientsTota Phenolic Contents and Antioxident Activities of Olive

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Abstract

In this study, the determination of total phenolic and antioxidant activity of the wastes of *Olea europaea* L. wastes was determined obtained from the process of pressing and extraction of oil from the fruits of *O. europaea* L. which are peels, seeds and pomace (peels and seeds). Five solvents where used in the extraction process to identify the best method for the extraction. The results of the present study showed that the type of solvent in the extraction process and the selected part of the waste have important role in determining of phenolic compounds as well as antioxidant activity of the pomace, peels and seeds. Hydroethanolic extracts (50% water) was the best in phenolic compounds extraction comparison with other solvents (water at room temperature, boiling distilled water and hydrochloric acid diluted with distilled water by (1% and 5%). In contrast, extracts from the pomace, peels and seeds using 5% hydrochloric acid had higher antioxidant activity through their ability to inhibit the activity of the synthetic free radical DPPH (2.2-diphenyl-1-pcriylhydrazal) when compared with other solvents. The extracts of peels showed significantly higher ($P \leq 0.05$) amounts of the phenolic compounds and the antiradical activity against DPPH than those prepared from the pomace and seeds. [DOI: 10.22401/JNUS.21.2.16]

Keywords**:** Total phenolic content, ntioxidant activity, olive pomace, olive seeds. olive peels.

Introduction

Fruit and vegetable residues contain active substances are formed and its secondary products in large quantities during the manufacturing process and thus may affect the environment, so this problem should be reduced by utiliying these wastes in various manufacturing processes such as pharmaceutical industry [١]. Olive oil processing has been recognized as one of the most problematic in terms of environmental pollution, as it, next to olive oil (20%), produces huge amounts of two waste matrices known as pomace (30%) and wastewater (50%). Interestingly, both of them are in turn valorised by several pharmaceutical and food industries mainly due to their high phenolic content [٢].

Trees of *Olea europaea* L. belong to the Tribe Oleae and family Oleaceae, comprising around 600 specious and some 25 genera, including *Olea* – which contains an economically important European olive tree known as *Olea europaea* L. Phoenicians were the first who introduced it to the western regions, first Greek islands and later to the Mediterranean Basin (Spain, Italy etc.). Olive fruits are of great nutritional importance, It contains carbohydrate (19%), protein (1.6%), mineral salts (1.5%), cellulose (5.8%) and various vitamins A, B and C, and many minerals such as iron, potassium, magnesium, manganese and copper, as well as its high

content of oil (15-20%) [3,4,5]. Other important constituents of olives are various phenolic compounds. the major phenolic compounds present in olive fruits are anthocyanins (cyaniding and delphinidin glucosides), flavonols (mainly quercetin-3-rutinoside), flavons (luteolin and apigenin glucosides), phenolic acid (hydroxybenzoic hydroxycinnamic and other), phenolic alcohols (tyrosol and hydroxytyroso), secoiridoids (oleuropein, demethyloleuropein and ligstroside) and verbascoside, hydroxycinnamic acid derivative [٦]. These phenolic compounds, especially oleuropein, have been associated with a reduced incidence of hypertension, cardiovascular disease, diabetes and hyperlipidemia due its antioxidant activity and its anti-hypertensive, anti-inflammatory, hypoglycemia and hypocholesterol properties[٧]. Recent research showed olive leaf extracts contain polyphenols such as oleuropein and

hydroxytyrosol could reverse the chronic inflammation and oxidative stress that induce cardiovascular, hepatic and metabolic symptoms in a rat model of diet-induced obesity and diabetes without changing blood pressure [٨,٩].

Free radicals are biological products that generate as a result of oxidation in the body and are very harmful molecules that cause a serious damage which represents itself in the form of protein denaturation, lipid peroxidation, and oxidative DNA [10,11] which are associated with a wide range of diseases and illnesses including s'Alzheimer, Parkinson's, diabetes, rheumatic diseases and motor neuron diseases [١٢].

The objective of the present study was to determine the total phenolic content (TPC) and antioxidant activities of the extracts prepared from the olive pomace and their ingredients (peels and seeds) using five different solvent.

Material and Methods

samples used in the study

Olive fruits were obtained from gardens of Diyala University,College of Engineering in October 2016, using 10 kg of olive fruit, washed well with tap water to remove the dust, then squeezed with an electric stirrer. Juice was collected in continues and was not used in the study. The remaining waste were divided in to three groups seeds, peels and pomace as it (peels and seeds). After that, the waste was dried and crushed by an electric mill until it was turned into soft powder.

Preparation of aqueous extracts

For determination of total phenolic content (TPC), 100 mg of pomace, peels and seeds powders were weighed. Each was placed in plastic tubes containing 10 ml of selected solvents (cold distilled water, boiled distilled water, 50% diluted ethanol and 1% and 5% hydrochloric acid) to obtain a concentration of 10 $mg.mL^{-1}$ for each solvent used in the study. In the case of free radicals experiment, 10 mL of selected solvents (cold distilled water, boiled distilled water, 50% diluted ethanol and 1% and 5% hydrochloric acid) were added to different quautities of used powders included 10, 25, 50 and 100 mg to obtain concentrations of 1, 2.5, 5 and 10 $mg.mL^{-1}$, respectively.

The five extracts mentioned above were left for two days at room temperature, then centrifuged at 3000 rpm. the solutions was filtered and collected it to used in all experiments.

Determination of Total Phenolic Contents

The amount of phenolics in the water extracts of the selected materials was determined by mixing 10 µl of each extract with 200 µL of sodium carbonate solution diluted by 2% in the micro-plate drillings containing 96 well microplate and allowed to react For five minutes at room temperature, and then 10 microliters of Folin reagent solution (50 %) was added then left the plate to react again for 30 min at 30c. The optical density was measured at 490 nm and calibrated with a water solution of Gallic acid of concentrations ranging from 100 to 1600 Mm. The amount of phenolic substances in the water extracts in mg of Gallic acid was represented by the following equation :

$Y = 0.0008 X + 0.0596$ [١٣].

Y: represents the optical density.

X: represents the value to be found.

Scavenging of 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH)

This assay was performed using a previously described method [١٣] with some minor modifications [١٤]. 20 µl of water extracts were mixed with 200 µl of 0.2 DPPH prepared in 96% absolute alcohol. Micro-plates were also used for this purpose and incubated at room temperature for half an hour. The optical density was measured at 490 nm using the ELISA assay. The free radical activity was calculated as a percentage using the following equation:

$B / A*100$

$A – B / A * 100$

A: absorbance of control incubation.

B: absorbance of extract**.**

Statistical Analysis

All measurements were performed in triplicates. The results were expressed as mean ± SEM and analyzed using SPSS version 21.0 [١٥].

Results and Discussion

Total phenolic contents (TPC) of the extracts prepared from olive residues (pomace, peels and seeds).

The results of the present study showed that the type of the solvent used in the extraction plays an important role in determining the total phenolic contents **(**TPC) of pomaces and their ingredients (peels and seeds). Similarly, the results of the previous studies have shown that the extraction process of phenolic compounds is affected largely by the polarity of the solvents used and the solubility of the phenolic compounds in these solvents [16, 17]. Accordingly, it is not easy to find the suitable solvent for the extraction of the phenolic compounds from all the samples [١٨] as some compounds could be extracted more easily by using alkaline solvents [19], while the extraction of some other phenolic compounds could be achieved more easily by using acidic solvents [19,20]. This may explain the lower quantities of phenolic compounds extracted from the seeds of the two date cultivars in comparison with those obtained from the entire pomaces and flesh due to the low solubility of the seeds in the solvents used.

The lowest value of phenolics found in the olive residue was in 1% diluted hydrochloric acid. The results showed that the content of the olive peels of phenolic material was higher than that of the pomace and seeds and significant differences (P≤ 0.05) Table (1). The diluted ethanol extract with distilled water (50%) showed higher content than the rest of the solvents used in the study (distilled water at room temperature, boiling distilled water and hydrochloric acid diluted with distilled water by 1% and 5%) in its ability to extract phenolics from olive residues (pomace, peels and seeds). The highest value of extracted phenolics from olive residue was after using ethanol solution (50%) reached 101.3 mg in the peels extract and 93.8 mg in the pomace extract and 93.4 mg in the seed extract. Conversely, the study showed by [٢١] in order to determine the total phenolic content in tomato and their components (peels and seeds) exceeded the boiling distilled water solution on the other solvents used in the study (distilled water at room temperature, distilled water containing hydrochloric acid 1% and 5% as well as the diluted ethyl alcohol solution with distilled water by 50%) in its ability to extract the phenolics from the residues in tomato. In another similar study by [٢٢] who extracted phenolic compounds from the residues of two varieties of grapes (Des Anz and Black Density) using four types of solvents in the extraction process: water at room temperature, boiling distilled water, 5% dilute hydrochloric acid and ethanol 50%. it was observed that the hydrochloric acid solution (diluted with distilled water 5%) higher than the rest of the solvents in its ability to extract and release the phenolics from grapes.

Table (1)

The total phenolic content of extracts prepared from olive oil residues using five different solvents, Eech values represents the Mean ± Standard error (SE) of three replicates.

Total phenolic content (mg GAE/g dry weight)			
Solvent	**Pomace Mean± SE**	**Peels Mean± SE**	**Seeds Mean± SE**
Cold water	$11.7^{de} \pm 1.2$	$24.2^{de} \pm 2.1$	$3.4^{de} \pm 2.3$
Boiling water	$19.2^{de} \pm 5.7$	$23.8^{de} \pm 3.2$	$38.1^{bc} \pm 2.8$
HCL (1%)	$6.7^{de} \pm 1.9$	$24.6^{de} \pm 2.9$	$2.5^{e} \pm 1.1$
HCL (5%)	$27.1^{cd} \pm 4.6$	$63.4^{b} \pm 5.8$	$25.0^{de} \pm 2.3$
Ethanol (50%)	$93.8^{a} \pm 4.9$	$101.3^{a} \pm 3.2$	$93.4^{a} \pm 3.2$
The averages with different characters were significantly different from each other at a significant level (P ≤ 0.05).			

Antioxidant activity (Ability to inhibit the activity of free radicals)

Tables (2, 3 and 4) show the ability of the extracts prepared from the pomace, peels and seeds of olive to scavenge the activity of the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and their ability based on the solvent type, selected part of the residues and the concentration of the extract.

The results showed that the extracts prepared in distilled water acidified with 5% HCl aqueous solution were significantly ($P< 0.05$) higher at scavenging the activity of DPPH-radical than the extracts of the same products but using other solvents (distilled water at room temperature, boiling distilled water, hydrochloric acid Diluted with 1% distilled water and ethanol alcohol 50%). Similarly, [٢٣] evaluated the capacity of the extracts, prepared from the peels and seeds of Iraqi sweet oranges, to scavenge the activity of the synthetic DPPH-radical and reported that the highest free radical scavenging activity, expressed as the percentage inhibition, was found in both peels and seeds prepared in 5% HCl aqueous solutions, followed by those prepared in boiling water, 50% ethanol, and distilled water only. Similarly, the study showed by [٢٢] ,That antioxidant efficacy and the ability to inhibit the fr.ee radical (DPPH) in two varieties of grapes, showed the results of the extracts prepared in hydrochloric acid better than the rest of the extracts. Another study evaluated the antioxidant efficacy of extracts from Japanese rice bran extracted by boiling distilled water, acetone and methanol, that the results showed the boiling distilled water extract significantly exceeded the acetone and methanol extract [٢٤] While other studies have demonstrated that alcohol extract has the ability to remove the free radical [25,26,27,28].

Table (2)

Effect of different concentrations of olive pomace extracts in inhibiting the free radical (DPPH) using five different solvent.

% Inhibition of DPPH radical				
Solvent	**Concentrations of extracts ($mg.ml^{-1}$) Mean ± SE**			
	10	**25**	**50**	**100**
Cold water	$^{*}11.1^{g} \pm 2.6$	$70.0^{ab} \pm 0.5$	$59.3^{cde} \pm 5.6$	$65.9^{abc} \pm 0.3$
Boiling water	$14.2^{g} \pm 0.9$	$70.9^{ab} \pm 0.0$	$56.6^{de} \pm 4.7$	$64.2^{bcd} \pm 2.2$
HCL (1%)	$40.2^{f} \pm 3.2$	$67.7^{ab} \pm 1.4$	$69.2^{ab} \pm 2.8$	$62.4^{bcd} \pm 2.6$
HCL (5%)	$75.6^{a} \pm 1.5$	$68.2^{abc} \pm 2.3$	$72.6^{a} \pm 1.2$	$68.7^{ab} \pm 0.9$
Ethanol (50%)	$12.9^{g} \pm 0.6$	$71.7^{ab} \pm 0.9$	$64.2^{bcd} \pm 0.5$	$54.5^{e} \pm 1.4$
The averages with different characters were significantly different from each other at a significant level ($P \leq 0.05$)				

Table (3)

Effect of different concentrations of olive peels extracts in inhibiting the free radical (DPPH) using five different solvents.

% Inhibition of DPPH radical				
Solvents	**Concentrations of extracts (mg/ml) Mean ± SE**			
	10	**25**	**50**	**100**
Cold water	$^{*}23.1^{gf} \pm 2.6$	$70.3^{a} \pm 0.9$	$64.5^{a} \pm 1.4$	$40.4^{e} \pm 1.0$
Boiling water	$20.3^{g} \pm 0.4$	$64.8^{a} \pm 2.1$	$61.7^{ab} \pm 2.5$	$56.9^{bc} \pm 1.9$
HCL (1%)	$41.4^{e} \pm 1.5$	$61.7^{ab} \pm 2.1$	$66.3^{a} \pm 0.2$	$65.9^{a} \pm 1.8$
HCL (5%)	$70.8^{a} \pm 1.4$	$69.2^{ab} \pm 0.5$	$71.3^{a} \pm 2.2$	$71.8^{a} \pm 0.9$
Ethanol (50%)	$22.6^{f} \pm 0.6$	$68.8^{a} \pm 0.3$	$55.3^{cd} \pm 0.4$	$50.0^{a} \pm 1.0$
The averages with different characters were significantly different from each other at a significant level ($P \leq 0.05$)				

Table (4)
Effect of different concentrations of olive seeds extracts in inhibiting the free radical (DPPH) using five different solvents.

% Inhibition of DPPH radical				
Solvents	**Concentrations of extracts (mg/ml) Mean ± SE**			
	10	**25**	**50**	**100**
Cold water	$3.0^{f} \pm 0.1$	$70.7^{ab} \pm 0.1$	$64.4^{c} \pm 2.2$	$64.3^{c} \pm 1.2$
Boiling water	$2.0^{f} \pm 1.7$	$71.4^{ab} \pm 0.3$	$68.9^{bc} \pm 0.2$	$58.2^{a} \pm 0.6$
HCL (1%)	$39.4^{e} \pm 0.9$	$72.5^{ab} \pm 0.7$	$68.9^{bc} \pm 1.1$	$69.7^{bc} \pm 0.7$
HCL (5%)	$75.4^{a} \pm 0.8$	$74.8^{a} \pm 0.2$	$68.9^{bc} \pm 3.1$	$68.7^{bc} \pm 1.0$
Ethanol (50%)	$4.9^{f} \pm 0.5$	$71.6^{ab} \pm 0.6$	$73.9^{bc} \pm 0.1$	$67.7^{a} \pm 1.4$
The averages with different characters were significantly different from each other at a significant level ($P \leq 0.05$).				

Conclusion

The results of the present study showed that the determination of phenolic compounds and antioxidant activities was dependent on the extracting solvent used and selected part of the residues and that the olive residues (generated from the extraction process of olive oil) could be considered as a potential inexpensive source of natural antioxidants and may be used as alternative to antioxidants in pharmaceutical and food formulations.

Reference

[1] Baska, R., Shrisakthi, S., Sathyapiya B., Sathyarapriya, R., Nithya, R and Podagodi.(2011). Antioxidant potential of peel extracts of banana varieties (Musa sapientum). Food and Nutrition Science, 2:1128-1133.

[2] Klen, T.J. (2014). Olive fruit phenols in olive oil processing: the fate and antioxidan potential. University of Nova Gorica.

[3] Kiritsakis, A.K.(1998).Olive oil from the tree to the table (2nd ed). Trumbull:food and Nutrition Press, Inc.

[4] Boudhrioua, N., Bahloul, N., Ben Slimen, L and Kechaou, N.(2009).Comparison on the total phenols content and the color of fresh and infrared dried olive leaves Industrial Crops and products, 29(2-3).412-419.

[5] Salvador, M.D., Fregapane, G.(2010). Major and Minor lipid Constituents of Cornicabra Virgin Olive Oil and the influence of corp season changes. InV. R. Preedy and R. R. Watson (Eds.), Olives and Olive Oil in Health and Disease Prevention. San Diega: Academic Press, pp.241.

[6] Boskou, D. (2006). sources of natural phenolic antioxidants. Trends in food science and Technology, 17(9):505-512.

[7] El, S.n and Karakaya, S.(2009).olive tree (*Olea europaea*) leaves .potential beneficial effects on human health.Nutrition reviews, 67(11):633-638.

[8] Poudyal. H., Campbell, F and Brown, L.(2010).Olive leaf extract attenuates cardiac, hepatic and metabolic change in high carbohydrate, high fat-fed rats. Journal of Nutrition, 140(5):946-953.

[9] Kinsella, J. E., Frankel, E., German, B. and Kanner, J. (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. Food Technology, 47: 85–89.

[10] Williams, G. M. and Jeffrey, A. M. (2000). Oxidative DNA damage: Endogenous and chemically induced. Regulatory Toxicology and Pharmacology, 32: 283–292.

[11] Bjelakovic, G.; Nikolova, D.; Gluud, L.; Simonetti, RG and Gluud, C. (2008). Antioxidant supplements for prevention of mortality in healthy participants and patinents with various diseases. Cochrane Data base of systematic Reviews, 16(2): 200-240.

[12] Molan, A.L., Flanagan, J., Wei, W. and Moughan, P.J. (2009). Selenium containing green tea has higher antioxidant and prebiotic activities than regular green tea. Food Chemistry, 114: 829-835.

[13] Benzie, I. F. F. and Strain, J. J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology, 299: 15–27.

[14] Elliott, A.C., and Woodward, W. A. (2007). Statistical analysis quick reference guidebook: With SPSS Examples. Sage Publication Pve. Ltv.p 259.

[15] Alothman, M.; Bhat, R. and Karim, A.A. (2009). Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvent. Food Chemistry, 115: 785-788.

[16] Sulaiman, S.F., Sajak, A.A.B., Supriatno, K.I.O. and Seow, E.M. (2011). Effect of solvents in extracting polyphenolic and antioxidant of selected raw vegetables. Journal of Food Composition and analysis, 24: 506-515.

[17] Kchaou, W., Abbes, F., Bleker, C., Attia, H. and Besbes, S. (2013). Effect of extraction solvent on phenolic contents and antioxidant activities of Tunisian date varieties (*Phoenix dactylifera* L.). Industrial Crop and Products, 45: 262-269.

[18] Verma, B., Hucl, P. and Chibbar, R. N. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. Food Chemistry, 116: 947-954.

[19] Choi, Y., Jeong, H.S. and Lee, J. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chemistry, 103: 130-138.

[20] Al-obaidy, D. A. A. (2016). Study the Total Phenolic Contents and antioxidant activity to extract of tomato pomace using the *Musca domestica* L. larvae as an Experimental Model. Msc thesis, Diyala University, Sciences College, Biology Department.

[21] Al-kaylani, M. O. B. (2015). Evaluation of the antioxidant and the activity against the viability of various stages of the domestic fly *Musca domestica* L. for the water extracts of grape pomace *Vitis vinifera* L. Msc thesis, Diyala University, Sciences College, Zoology Department.

[22] Molan, A. L., Ismail, M. H. and Nsaif, R.H. (2016). Phenolic contents and antioxidant activity of peels and seeds of orange (*Citrus sinensis*) cultivated in Iraq. World Journal of Pharmaceutical Sciences, 5: 473-482.

[23] Okai, K. H., Kanbara, K., Sugita, A., Matsumoto, Cand Okai, N.A. (2004). Potent antioxidative and antigenotoxic activity in aqueous extract of Japanse Rice Bran-association with peroxidase activity . phytother.Res, 18:628-633.

[24] Qsman, M.F. and Taha, E.A. (2009). Antioxidant activity of water extract of propolis from different regons in kafrelshikh govern orate. Food Technology Departmant, Economic Entomology Department, Face Agriculture, Kafrelsheikh University Eygypt.

[25] Marinova, G. and Batchvarov (2011). Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulgarian Journal of Agricultural Science, 17(1): 11-24.

[26] Khalaf, H.H., Sharoba, A.M., Elsadani, R.A., El Nashaby, F.M. and El Shiemy, S.M. (2014). Antioxidant properties of some extracts. Journal Food and Dairy Science, Mansoura University, 5(4): 247-263.

[27] Shekhar, T.C. and Anju, G. (2014). Antioxidant Activity by DPPH Radiacl Scavenging Methode of Agreatum Conyzoides Linn Leaves. American Journal of Ethano Medicine, 1(4): 244-249.