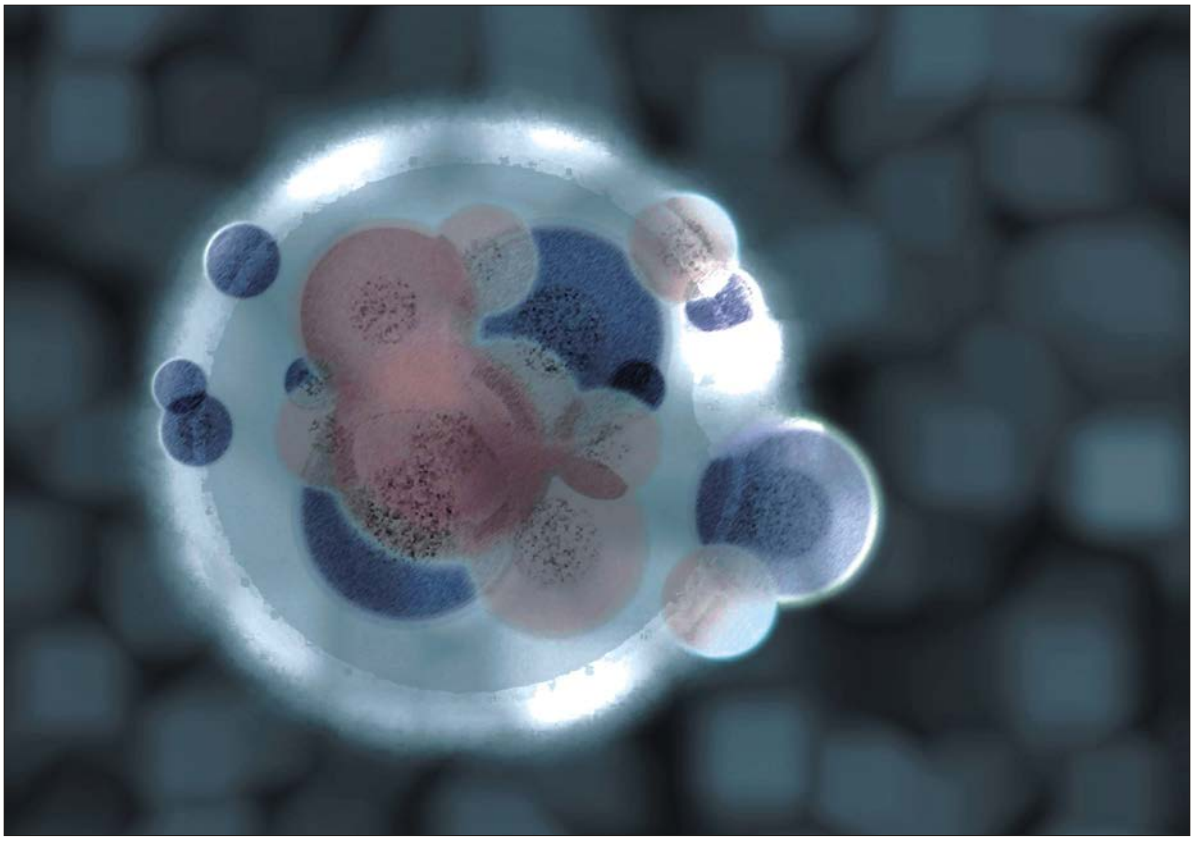

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Antioxidant Activity, Reducing Power and Total Phenolic Content of Iranian Olive Cultivar

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Abstract: Antioxidant activity of methanolic (50%) extracts of olive pulp (*Olea europaea* L.) was investigated. Total antioxidant activity, phenolic contents and reducing power in six Iranian olive cultivars were determined. The highest antioxidant activity (28.699 mmol Fe^{II}/100 g dry plant), total phenolic contents (2997 mg gallic acid/100 g dry plant) and reducing power (8.331 g Vitamin E/100 g dry plant) were detected in Mishen and the lowest in Conservalina. A linear positive relationship existed between the antioxidant activity, total phenolic compounds ($r^2 = 0.976$) and reducing power of the tested olive pulp ($r^2 = 0.848$). Iranian olives possess relatively high antioxidant activity due to contribution of phenolic compounds. The present study shows that Iranian olive cultivars are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses.

Key words: FRAP value, olive, folin ciocalteau

INTRODUCTION

The Mediterranean diet rich in fruit, vegetables and grains as well as olive oil and olive has been associated with a lower risk of coronary heart disease and cancer (De Lorgeril and Salen, 2007; Trichopoulou, 2007; Dalvi *et al*., 2007). The positive role of olive oil has been related to its fatty acid composition and the presence of phenolic compounds (Bendini *et al*., 2007). Polyphenolic compounds influence the sensorial properties of olive fruits and virgin olive oils and are important markers for studying fruit characteristics of different cultivars and for controlling oil production process (Romani *et al*., 1999; Pinelli *et al*., 2003). An understanding of phenolic composition and factors influencing them is critical for designing various products and their storage conditions (Servili *et al*., 2004). Olive oil contains a considerable amount of polyphenols that have a great effect on both the stability and the sensory and nutritional characteristics of the product (Vacca *et al*., 2006). The presence of phenolic compounds with antioxidant activity is of particular importance which is correlated with oil resistance to the development of rancidity (Silva *et al*., 2006; Del Carlo *et al*., 2004; Dinnella *et al*., 2007; Oliveras-López *et al*., 2007). Polyphenols of olea fruits show both autoprotective and nutritional-therapeutic effects. They play an important role in human nutrition as preventative against several diseases (Fitó *et al*., 2007). Compounds with a phenolic structure affect both the taste, in particular the positive bitterness organoleptic attribute and the oxidative stability of the olive oil (Garcia-Mesa and Mateos, 2007). Phenolics are therefore the compounds chiefly responsible for the flavor of olive oil and to a large extent determine the degree of consumer preference for this highly appreciated product (Antoun and Tsimidou, 1997). The amount of these minor components in virgin olive oil depends on agronomical and technological factors such as the olive cultivar (Criado *et al*., 2007). It is known that some single phenolic compound increase the resistance of LDL against oxidation *in vitro*, but the single phenolic compound approach fails to account for the interactions among them and does not take into consideration that some phenolic compounds are correlated with each other (Romani *et al*., 2004). In this respect, it is important to note that the mixture of phenols may exert different activity in comparison with the single phenols, because they may cooperate and modify biological activity (Reaven and

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Witztum, 1996). Therefore individual olive oils, which differ qualitatively and quantitatively in phenolic contents, could have different biological activity. At present, little is known about the antioxidant effect of the total olive extract and the relationship between antioxidant activity and its global phenolic content (Owen *et al*., 2004; Visioli *et al*., 2005). In addition no studies have been conducted to investigate the antioxidant activity of Iranian olive extract. In the present study, the polyphenol content and the antioxidant activity were measured on 6 different cultivar mainly cultivated in Iran. The aim of the research was to test the possibility to use these indexes in the prediction of oil oxidative stability and to evaluate the antioxidant power of the phenolic compounds of olive extract. Furthermore, the contributions of polyphenolic compounds to the antioxidant activity were investigated.

MATERIALS AND METHODS

Materials: All solvents/chemicals used were of analytical grade and obtained from Merck Company (Darmstadt, Germany). Double-distilled deionized water was used for the preparation of aqueous solutions.

Olive cultivars: A total of 30 olives (*Olea europaea* L.) which is five from six cultivars including Conservalina, Blaidy, Amphisis, Shengeh, Cronaiky and Mishen were donated from the agricultural research centers of Saveh in the Markazi province. The olive pulps were dried, sealed in a plastic bottle and stored at -21°C until used.

Sample preparation: Approximately 1 g of dry ground pulp from 6 olive cultivars was extracted for 15 min with 5 mL of 50% methanol at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 10000 g for 15 min and the supernatant was decanted. The residues were extracted under identical conditions for further 6 times. The supernatants were combined and washed with petroleum ether to remove oils, then filtered and diluted to 100 mL with 50% methanol.

Measurement of total antioxidant activity: The FRAP (Ferric reducing antioxidant power assay) procedure described by Benzie and Strain was followed (Benzie and Strain, 1976). The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to it's ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 mL of a (10 mmol L^{-1}) TPTZ (2, 4, 6- tripyridyl- s- triazine) solution in 40 mmol L^{-1}HCL plus 5 mL of (20 mmol L^{-1}) $FeCl_3$ and 50 mL of (0.3 mmol L^{-1}) Acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C. Aliquots of 100 μL sample were mixed with 3 mL FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. For construction of calibration curve 5 concentrations of $FeSO_4 \cdot 7H_2O$ (1000, 750, 500, 250 and 125 mmol L^{-1}) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol L^{-1} $FeSO_4$. Four concentration of vitamin C (0.0625, 0.125, 0.250 and 0.500 mmol L^{-1}) and five other concentration of vitamin E (10, 25, 50, 100 and 200 mg L^{-1}) were used for construction of two other calibration curve and the total antioxidant activity were also reported as vitamin E and C equivalent (Table 1).

Total phenolic compounds analysis: Total phenolics were determined calorimetrically using Folin-Ciocalteau reagent as described by Velioglu *et al.* (1998) with slight modifications. The extract (200 μL) was mixed with 1.5 mL of Folin-Ciocalteau reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22°C, for 5 min. A 1.5 mL sodium bicarbonate solution (60 g L^{-1}) was added to the mixture. After 90 min at 22°C, absorbance was measured at 725 nm using a UV-visible spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a know concentrations of gallic acid standard (25 to 150 μg mL^{-1} of 50% methanol). The concentrations are expressed as mg of gallic acid equivalents per 100 g of dry weight. The total phenolic assay of olive pulp was measured five times for each cultivar and the results are shown in Table 2.

Reducing power: A spectrophotometric method (Ferreira *et al*., 2007) was used for the measurement of reducing power. 2.5 mL of extracts were mixed with 2.5 mL

Table 1: FRAP value, vitamin E and C equivalent antioxidant activity in Iranian olive cultivars

Cultivars	FRAP value mmol Fe^{II}/100 g	Vitamin C mmol/100 g	Vitamin E g/100 g
Conservalina	7.930±0.787	4.478±0.475	2.369±0.236
Blaidy	19.929±1.225	11.986±0.739	5.970±0.368
Amphisis	26.104±2.019	15.710±1.218	7.823±0.606
Shengeh	19.962±0.434	12.339±1.334	6.146±0.664
Cronaiky	20.515±2.210	12.006±0.262	5.980±0.130
Mishen	28.699±1.792	17.276±0.081	8.602±0.538

Table 2: Total phenolic and reducing power content in Iranian olive cultivar

Cultivars	Total phenol g GA/100 g	Reducing power g vitamin E/100 g
Conservalina	0.888±0.043	3.161±0.164
Blaidy	1.889±0.096	6.668±0.534
Amphisis	2.664±0.135	7.293±0.284
Shengeh	2.121±0.082	7.725±0.574
Cronaiky	1.841±0.308	5.545±0.312
Mishen	2.997±0.361	8.331±0.434

phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide (10 mg mL^{-1}). The mixture was incubated at 50°C for 20 min, then rapidly cooled, mixed with 2.5 mL of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and then ferric chloride (0.5 mL, 0.1%) was added and allowed to stand for 10 min. The absorbance was read spectrophotometrically at 700 nm. Five concentration of vitamin E (10, 25, 50, 100 and 200 mg mL^{-1}) were used for construction of calibration curve and the reducing power activity were reported as Vitamin E equivalent per 100 g dry sample (Table 2).

Imprecision: The within-run Coefficient of Variation (CV) was estimated by assaying the FRAP, phenol and reducing power values of olive pulp cultivars three times in the same analytical run. The between run CV was obtained by estimating five and six replicates of each olive pulp in a further analytical run.

Statistical analysis: Three replicates of each sample were used for statistical analysis and the values are reported as mean±SD. Correlation analyses of antioxidant activity versus the total phenolic content and reducing power were carried out using the correlation and regression program in SPSS 11.5 program. Data were subjected to analysis of variance and means were compared by Tukey posthoc multicomparison tests. Differences at $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the antioxidant potential of olive pulp extract and to utilize it as a substitute for synthetic antioxidants. The antioxidant effects of olive pulp extracts have been evaluated using *in vitro* FRAP method (Benzie and Strain, 1976). The FRAP assay treats the antioxidants contained in the sample as reductants in a redox-linked colorimetric reaction and the value reflects the reducing power of the antioxidants. The procedure is relatively simple and easy to standardize. Thus, it has been used frequently in the assessment of antioxidant activity of various fruits and vegetables and some biological samples. Olive oil is one of the Iranians most important oils, but no literature was found reporting the antioxidant activity of it in Iran. In the present study, the FRAP value of olive pulp methanolic extraction of six cultivars was determined in an attempt to make a systematic comparison among their antioxidant activities and identify the cultivar with high antioxidant power for further studies. The study also attempts to quantify the total phenolic compounds present in olive pulp extract. The FRAP value, vitamin C and E equivalent in six olive pulp extract is shown in Table 1. As it can be seen, the FRAP value range from minimum 8.611 to maximum 30.251 mmol Fe^{II}/100 g dry plant in Conservalina and Michen cultivatrs respectively. The results showed that there was no significant statistical differences between Michen and Amphisis but these two cultivars had more potential antioxidant activity than the others ($p<0.001$). Also it can be seen that Bleidy, Shengeh and Cronaiky had the same antioxidant activity with no significant difference with each other and Conservalina had the less FRAP value. In previous studies the antioxidant activity and presence of polyphenol compounds on the *Olea europaea* were investigated. We tried to correlate the FRAP values obtained in this study with the others data (Goamez-Rico *et al*., 2006; Franconi *et al*., 2006; Del Carlo *et al*., 2004; Carrasco-Pancorbo *et al*., 2005; Morello *et al*., 2005). Boskou *et al*. (2006) reported the antioxidant capacity and phenolic profile of table olives from the Greek market with DPPH and Folin-Ciocalteau assay. In 2001, McDonald *et al*. (2001) worked on the phenolic content and antioxidant activity of olive extract too. There are many reports describing the antioxidant activity of olive, but the result varies depending on the assay method. In this paper the total phenolic assay of olive pulp was also investigated. The amount of total phenoics varied in different olive cultivars and ranged from 888 mg GA/100 g dry plant equivalents in pulp of Conservalina to 2997 mg GA/100 g dry plant in Mishen cultivar. The highest total phenolic levels were detected in Michen and Amphisis and the lowest in Conservalina. Boskou *et al*. (2006) reported the total phenolic content of olive pulp ranging from 82 to 171 mg caffeic acid per 100 g. In another paper, McDonald *et al*. (2001) reported the total phenol content based on mg GA per 100 g original dried olive powder ranging from 200 to 590 in different liquid chromatographic fraction, which the sum is 1760 mg GA per 100 g dry olive powder in all fractions. Like antioxidant activity the highest reducing power were detected in Michen (8.331 g vitamin E/100 g dry plant) and the lowest in Conservalina (3.161 g vitamin E/100 g dry plant). The correlation between total antioxidant activity, total phenolic contents and reducing power results of Iranian olive pulp had a correlation coefficient of $R^2 = 0.976$ and 0.848, respectively (Fig. 1, 2). This result suggests that 97% of the antioxidant capacity of Iranian olive pulp results from the contribution of phenolic compounds. Also, it can be concluded that antioxidant activity of pulp extracts is not limited to phenolics. Activity may also come from the presence of other antioxidant secondary metabolites, such as volatile oils carotenoids and vitamins, which in this case contributed to 3% of the

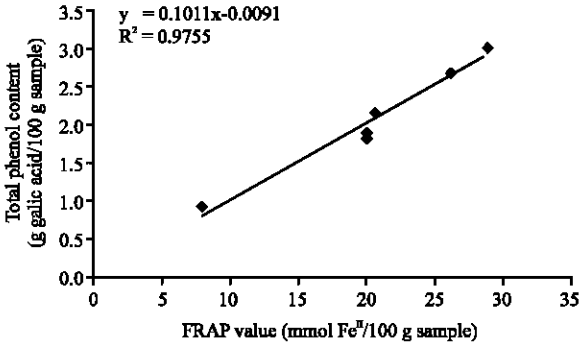

Fig. 1: Relationship between total phenolic content and antioxidant activity of Iranian olive cultivar

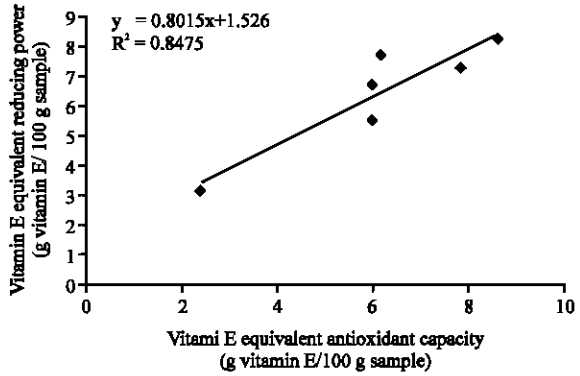

Fig. 2: Relationship between Vitamin E equivalent antioxidant capacity and reducing power in Iranian olive cultivar

antioxidant capacity. On the other hand, about 85% of the antioxidant activity of phenolics is due to their redox properties, which allow them to act as reducing agents, hydrogen donors and the others may also have a metal chelating potential. The present study showed that Iranian olive pulp, which are often present in Iranian dishes, are strong radical scavengers and can be considered as good sources of natural antioxidants for side dishes, medicinal and commercial uses. However, due to the diversity and complexity of the natural mixtures of phenolic compounds, it is rather difficult to characterize every compound and assess or compare their antioxidant activities.

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