



Antioxidant activity and inhibitory effect of cultivars of Olive (*Olea europaea*) against lipid peroxidation in mice liver

[Fare karaciğeri lipid peroksidasyonuna karşı kültür zeytin'in Olive (*Olea europaea*) antioksidant ve inhibisyon etkileri]

Abdul Khaliq¹,
Syed Dilnawaz Ahmad²,
Syed Mubashar Sabir¹,
Asmatullah Khan³

¹University of Poonch, Rawalakot Azad Kashmir
Pakistan

²University of Azad Jammu and Kashmir,
Muzaffarabad, AK Pakistan

³University of Poonch, Department of Eastern
Medicine and Surgery, Rawalakot Azad Kashmir,
Pakistan

Correspondence Address
[Yazışma Adresi]

Mubashar Sabir, PhD.

University of Poonch Rawalakot,
Department of Chemistry, Azad Kashmir, Pakistan
Phone: +92 058 23442250
Fax: +92 058 24960007
E-mail: mubashersabir@yahoo.com

Translated by [Çeviri] Dr. Ebru Saatçi

ABSTRACT

Objective: The aim of this study is to investigate the *in vitro* antioxidant activity, the total phenolic and flavonoid contents and the possible protective effects of seven cultivars of olive on lipid peroxidation induced by iron in mice liver.

Methods: Antioxidant activity was evaluated by using different assays, including thiobarbituric acid reactive species (TBARS), total antioxidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and metal ion-chelating activities.

Results: The aqueous extracts of fruit showed inhibition against thiobarbituric acid reactive species (TBARS) induced by pro-oxidant (10 μ M FeSO₄) in mice liver. The order of the antioxidant activity among different cultivars on lipid peroxidation assay is Maurino>Mission>Picholine>Ascolano>Carolea>Itrana>Pedolina. Moreover, the free radical scavenging activities of the extracts was evaluated by the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (IC₅₀, 85.9 μ g/ml (Ascolano), 82.1 μ g/ml (Mission), Pedolino and Carolea >200 μ g/ml, 85.1 μ g/ml (Picholine), 143.6 μ g/ml (Maurino) and 150 μ g/ml (Itrana). Principal component analysis showed that phenolics and flavonoids contributed to the antioxidant activity of olives.

Conclusion: The oxidative stress in liver can be potentially managed/prevented by the dietary intake of olive fruit.

Key Words: Antioxidant activity, olive, lipid peroxidation, phenolics, flavonoids

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Bu çalışmanın amacı, fare karaciğerinde demir ile indüklenen lipid peroksidasyonu üzerine, yedi farklı kültür zeytininin total fenolik ve flavanoidlerinin *in vitro* antioksidant aktivitelerini incelemektir.

Metod: Antioksidant aktiviteler, tiyobarbitürik asit reaktif türler (TBARS), total antioksidant, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radikali ve metal iyon şelatlama aktiviteleri gibi farklı metotlarla incelenmiştir.

Bulgular: Meyve ekstraktları fare karaciğerinde, pro-oksidant (10 μ M FeSO₄) ile indüklenen TBARS üzerine inhibisyon etkisi göstermiştir. Lipid peroksidasyonu üzerine farklı kültürlerin antioksidant aktiviteleri sırasıyla Maurino>Mission>Picholine>Ascolano>Carolea>Itrana>Pedolina'dır. Bunlardan başka, ekstraktların serbest radikal yakalama aktiviteleri 2,2-diphenyl-1-picrylhydrazyl (DPPH) radikali ile ölçülmüştür. Buna göre türe göre IC₅₀'ler 85.9 μ g/ml (Ascolano), 82.1 μ g/ml (Mission), Pedolino and Carolea >200 μ g/ml, 85.1 μ g/ml (Picholine), 143.6 μ g/ml (Maurino) and 150 μ g/ml (Itrana) olarak bulunmuştur. Zeytindeki fenoliklerin ve flavanoidlerin antioksidant aktivitesi gösterdiği bulunmuştur.

Sonuç: Diyetel zeytin alımıyla karaciğer oksidatif stressi potansiyel olarak önlendi.

Anahtar Kelimeler: Antioksidant aktivite, zeytin, lipid peroksidasyonu, fenolikler, flavanoidler

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Introduction

During metabolism, reactive oxygen species (ROS) are generated spontaneously in cells and are implicated in the aetiology of different degenerative diseases, like heart diseases, stroke, rheumatoid arthritis, diabetes and cancer [1,2]. A number of studies have shown that the use of polyphenolic compounds found in tea, fruits and vegetables is associated with low risk of these diseases [3]. Consequently, there is a great deal of interest in edible plants that contain antioxidants and health-promoting phytochemicals as potential therapeutic agents. One of such plant is Olive (*Olea europaea* L.) which belongs to the family *Oleaceae* and is native to tropical and warm temperate regions of the world. Olive is also considered as multipurpose crop with great yield potential. The tree is famous for its fruit and is also called the olive, is commercially important in the Mediterranean region as an important source of olive oil. The olive is typically distributed in the coastal areas of the eastern Mediterranean Basin, the adjoining coastal areas of southeastern Europe, western Asia and northern Africa as well as northern Iran at the south end of the Caspian Sea. The olive tree possesses medicinal and nutritional values. Over the centuries, extracts obtained from leaves of olive have been used for promoting health and used in preservation. Similarly, olive is a famous folk remedy to treat fever and some tropical diseases such as malaria [4]. Economically, the fruit of olive is an important as it yields nutritious edible oil with potential medicinal functions [5]. The health benefits of olive oil are mainly due to the presence of high content of monounsaturated fatty acid and different bioactives which includes tocopherols, carotenoids, phospholipids and phenolics, with multiple biological activities [6,7].

Antioxidant activity is used to measure a compound to reduce the pro-oxidants or reactive species of pathologic significance [8]. Much attention has been focused on natural antioxidants capable of inhibiting lipid peroxidation which is mediated in several pathological conditions such as atherosclerosis, cancer and aging [9]. Ferric reducing antioxidant power (FRAP), total phenolic assay by using the Folin-Ciocalteu reagent, total flavonoid content and DPPH radical scavenging activity are the common methods used to evaluate the antioxidant properties [10]. The demands of natural antioxidants are high for application as nutraceuticals and as food additives because of consumer preferences [10]. Since antioxidants from plant source are safe and easily available, olive fruits was subjected to determine and quantify various antioxidant activities. This would be the first scientific study to evaluate the antioxidant activity of these cultivars from Pakistan and these cultivars are also widely used in world. Hence, the objective of this study is to investigate the antioxidant and inhibitory effect of seven important cultivars of olive on Fe(II) induced lipid peroxidation in mice liver *in vitro* to find out the better cultivar for cultivation and

phytotherapy. The DPPH radical activity, total antioxidant activity, phenolic and flavonoid contents of these cultivars were also determined.

Materials and Methods

Thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid were purchased from Sigma Aldrich (St. Louis, MO, USA). Ferrous sulphate was obtained from Biochemicals (Lahore). All other reagents used in this study were of analytical grade.

Preparation of the fruit extracts

The fruits of different cultivars of olive which include Maurino, Mission, Picholine, Ascolano, Carolea, Itrana and Pedolino were collected at the same harvesting time from the field area of Pakistan Agricultural Research Council, Islamabad Pakistan and authenticated.

Fruits were washed and dried in hot air at 40°C and ground to a fine powder in mill. Ground material (5 g) was extracted with hot water (250 ml) for 30 minutes followed by filtration through whatman No.1 filter paper. The obtained residues were re-extracted under the same conditions. The combined filtrates were evaporated in rotary evaporator below 40°C. The extracts obtained after evaporation of solvent was weighed to determine the yield and stored at -20°C. The extract at a final concentration (1 mg/ml) was then serially diluted to obtain the desired concentration of plant for the experiment.

Test animals

All animal studies were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. Male BALB/c mice (2.0–2.5 months and 24–30 g), were purchased from National institute of health Islamabad, were used for *in vitro* studies. The animals were kept in separate cages with continuous access to food and water in a room with controlled temperature (22±3°C) and on a 12 h light/dark cycle with lights turned on at 7:00 a.m.

Production of TBARS from liver tissues

Production of TBARS was determined using a modified method [11]. The mice were anaesthetised with chloroform, sacrificed by decapitation and the liver was quickly removed and placed on ice. One gram of liver tissues were homogenised in cold 100 mM Tris buffer pH 7.4 (1:10 w/v) and centrifuged. The homogenates (100 µl) were incubated with or without 50 µl of the various freshly prepared oxidant (iron) and different concentrations of the fruit extracts together with an appropriate volume of deionised water to give a total volume of 300 µl at 37°C for 1 h. The color reaction was carried out by adding 200, 500 µl each of the 8.1% Sodium dodecyl sulphate (SDS), acetic acid (pH 3.4) and 0.6% TBA respectively. The reaction mixtures, including those of serial dilutions of 0.03 mM standard MDA were incubated at 97°C for 1 h. The absorbance was read after cooling the tubes at 532 nm in

a spectrophotometer.

Antioxidant activity by DPPH radical scavenging

The antioxidant activities of the plant extracts were measured using the stable DPPH radical according to the method of Hatano et al. [12]. Briefly 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations (25-200 µg/ml) of aqueous extracts. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm. The capacity to scavenge the DPPH radical was calculated using the following equation: (%) scavenging = $[(A_0 - A_1)/A_0] \times 100$, where, A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample itself. The IC_{50} values (extract concentration that cause 50% scavenging) were determined from the graph of scavenging effect percentage against the extract concentration. All determinations were carried out in triplicate.

Total antioxidant assay

The assay was based on the reduction of molybdenum, Mo (VI)-Mo (V) by the extracts and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [13]. The extracts at concentration (100 µg/ml) were mixed with 3 ml of the reagent solution (0.6 M H_2SO_4 , 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 mins. The mixture was cooled to room temperature and the absorbance of the solution was measured at 695 nm.

Metal chelating activity

The Fe(II) chelating ability of the aqueous extracts were determined using a modified method [14].

Determination of phenolics

The total phenolic content as gallic acid equivalent was determined by the method of Singleton et al. [15]. The aqueous extract (0.5 ml) was added to 2.5 ml, 10% Folin-Ciocalteu's reagent (v/v) and 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated at 45°C for 40 minutes and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard phenol. The mean of three readings was used and the total phenolic content was expressed as milligrams of gallic acid equivalents/g extract.

Determination of flavonoid

Flavonoids were expressed as quercetin equivalents. Quercetin was used to make the calibration curve [0.04, 0.02, 0.0025 and 0.00125 mg/ml in 80% ethanol (v/v)]. The standard solutions or extracts (0.5 ml) were mixed with 1.5 ml of 95% ethanol (v/v), 0.1 ml of 10% aluminium chloride (w/v), 0.1 ml of 1 mol/l sodium acetate and 2.8 ml of water. The volume of 10% aluminium chloride was substituted by the same volume of distilled water in the blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The mean of three readings was used and the

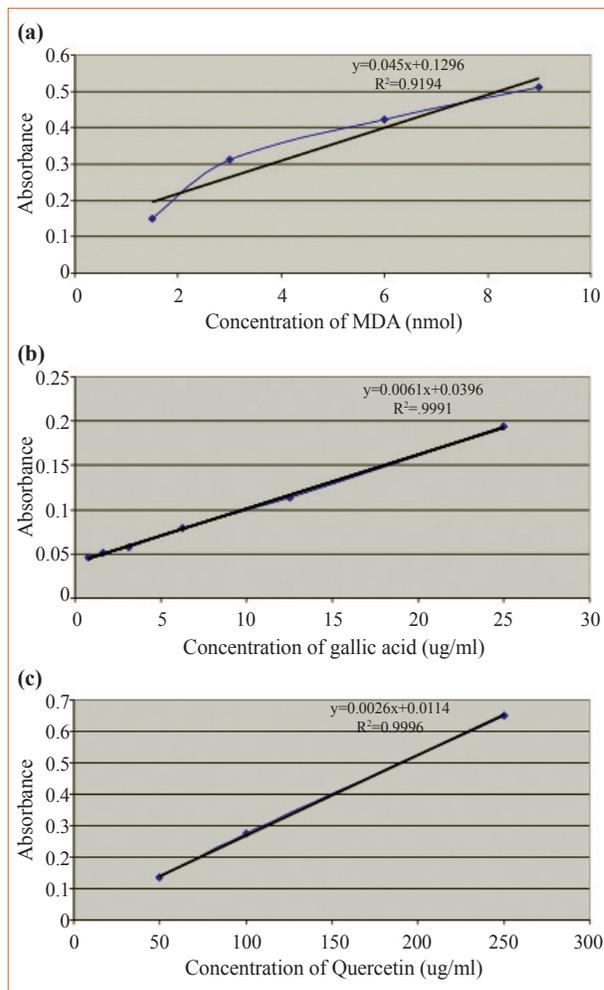


Figure 1. Standard curves used for estimation of TBARS, phenolics and flavonoids (a) standard curve of malondialdehyde (b) standard curve of phenolics (c) standard curve of flavonoids.

total flavonoid content was expressed as milligrams of quercetin equivalents/g of extract by the method of Kosalac et al. [16].

Statistical analysis

The results were expressed as means \pm standard deviation. The data was analyzed by one way ANOVA and different group means were compared by Duncan's multiple range (DMR) test where necessary. $p < 0.05$ was considered significant in all cases. The main variance in data was detected using Principal component analysis. The software Package Statistica (version, 4.5) was used for the analysis of data.

Results

Inhibition of lipid peroxidation using iron as pro-oxidant in mice liver

Lipid peroxidation was measured in terms of MDA (Figure 1a). Lipid peroxidation in mice liver homogenate was induced with iron and the potential antioxidant effect of aqueous extract of different cultivars of olive was de-

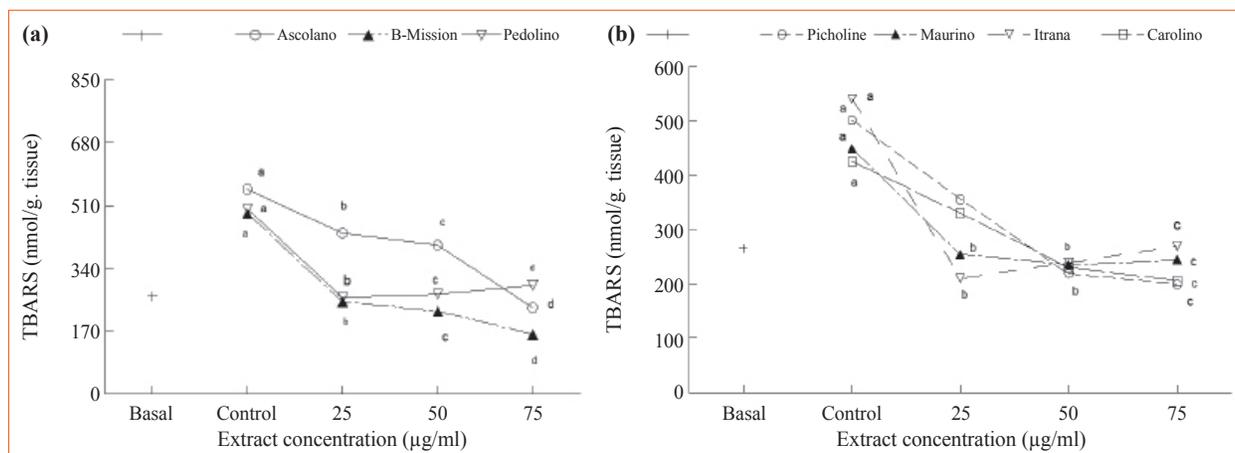


Figure 2. The inhibitory effect of aqueous extracts of olive against lipid peroxidation in mice liver. **(a)** antioxidant activities of Ascolano, Mission and Pedolino on iron (10 μM) induced lipid peroxidation in liver. The results are means of three separate experiments \pm SD. Values in figures followed by different letters are significantly ($p<0.05$) different from each other by DMR test. **(b)** antioxidant activity of Picholine, Maurino, Itrana and Carolea. Basal includes the tissue without prooxidant. Control includes the tissue with prooxidant, FeSO_4 (10 μM). The results are means of three separate experiments \pm SD. Values in figures followed by different letters are significantly ($p<0.05$) different from each other by DMR test.

terminated. Figure 2 shows the antioxidant effect of plant extracts in mice liver. Here, the TBARS was induced with 10 μM iron. The results revealed that treatment with Fe(II) caused a significant ($p<0.05$) increase in thiobarbituric acid reactive substances (TBARS) compared to the basal. Separate controls were used for different cultivars of olive (Figure 2). Treatment with different concentrations of olives caused a marked decrease in lipid peroxidation (Figure 2). The cultivars, Mission and Maurino significantly reduced ($p<0.05$) the lipid peroxidation upto 50 $\mu\text{g/ml}$, beyond which there is less inhibition of lipid peroxidation. In order to compare the antioxidant activity among different cultivars IC_{50} values (extract concentration that cause 50% inhibition) were calculated (Table 1). Lower the IC_{50} value higher is the antioxidant activity of plant extract. Maurino and Mission showed the lowest and almost similar IC_{50} values (Table 1). Similarly, Picholine and Ascolano showed the similar IC_{50} values. Itrana showed the highest IC_{50} value and thus antioxidant activ-

ity of this cultivar is the least. The order of antioxidant activity among these genotypes are Maurino>Mission>Picholine>Ascolano>Carolea>Itrana>Pedolina.

Antioxidant activity by DPPH radical scavenging activity

The free radical scavenging activities of extracts was measured by the ability to scavenge DPPH radical. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. Now DPPH radical has been widely used in assessment of radical scavenging activity because of its ease and convenience. Five different concentrations (25, 50, 100, 150 and 200 $\mu\text{g/ml}$) of extracts were used to study the free radical scavenging activity. The scavenging activities of the fruits on DPPH radical were high for all extracts. Pedolino and Carolea displayed less than 50% scavenging of DPPH radical at tested concentrations (Figure 3). Therefore, it was not possible to calculate their IC_{50} values and hence their IC_{50} values are higher than 200 $\mu\text{g/ml}$. The order of antioxidant activity among cultivars was Mission >Picholine>Ascolano>Maurino>Itrana.

Total antioxidant activity by phosphomolybdenum assay

The total antioxidant activity of different cultivars was expressed as ascorbic acid equivalent is shown in Figure 4. All the extracts showed their reducing abilities expresses as $\mu\text{g/ml}$ of ascorbic acid equivalent. There was a statistically significant ($p<0.05$) difference among the antioxidant activity of all cultivars. However, the order of their reactivity was Mission>Ascolano>Itrana>Maurino>Carolea>Picholine.

Total phenolic content

The total phenolic content was expressed as gallic acid equivalent are shown in Figure 1b. The extracts showed high phenolic content (128.8-164.3 mg/g) and there was a non significant difference ($p<0.05$) among different geno-

Table 1. IC_{50} values of different genotypes of olive

Genotypes	IC_{50} ($\mu\text{g/ml}$) for DPPH assay	IC_{50} ($\mu\text{g/ml}$) for TBARS assay
Ascolano	85.9 ^a	64.87 ^a
Mission	82.1 ^b	44.71 ^b
Pedolino	>200 ^c	>200 ^c
Picholine	85.1 ^{ad}	62.18 ^d
Maurino	143.6 ^e	44.1 ^{bf}
Itrana	150 ^f	75 ^g
Carolea	>200 ^{cg}	73.28 ^h

Values in figures followed by different letters are significantly ($p<0.05$) different from each other by DMR test.

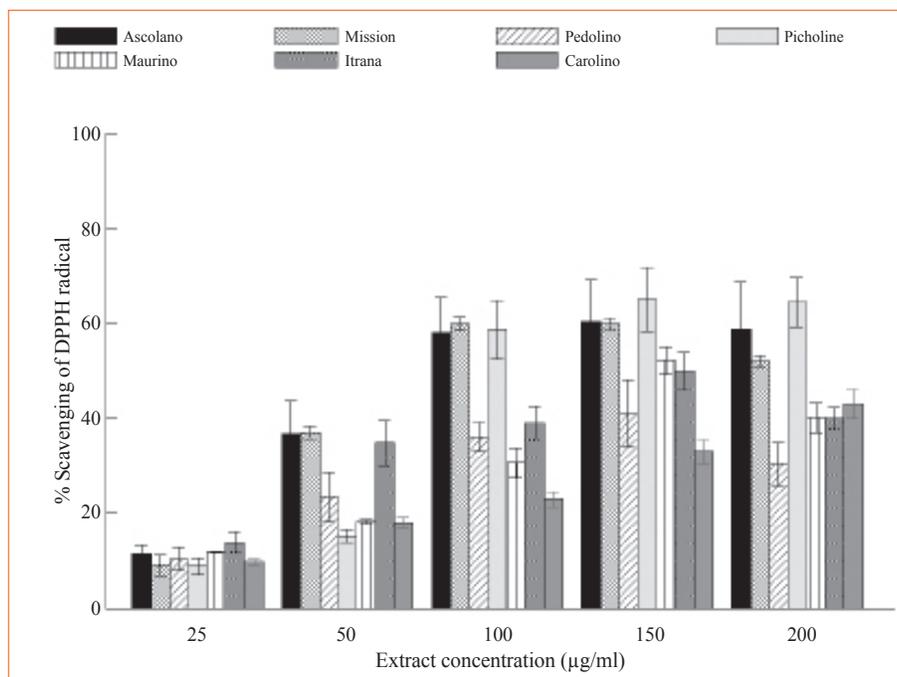


Figure 3. DPPH radical scavenging activity of different cultivars of olive. Values are means±SD (n=3).

types (Figure 5). The highest phenolic content 164.3 ± 3.3 mg/g was detected in Maurino, whereas, Pedolino showed the least phenolic content 126.5 ± 3.8 mg/g. Picholine and Carolea contained the same amount of phenolics.

Total flavonoid content

The total flavonoid content was expressed as quercetin equivalent are shown in Figure 1c. There was a statistically significant difference ($p<0.05$) among different cultivars. The flavonoid content was found in the range of 0.365-8.3 mg/g (Figure 6). Mission contained the highest amount of flavonoid (8.3 ± 0.98 mg/g) whereas, Pedolino contained the least amount (0.365 ± 0.03 mg/g) of flavonoid.

Descriptive statistics

Descriptive statistics including the minimum, maximum, mean and standard deviation are shown in Table 2. Mission olives exhibited the highest value of total flavonoid content (TFC), total phenolic content (TPC), total antioxidant activity (TAA), whilst Pedolino olives exhibited the lowest value of these parameters. The spread around the mean was small for all parameters except for TAA which exhibited large type of fluctuation with most of olives. In general, all types of fruits showed reasonable difference between the maximum and minimum values. TAA and TPC exhibited the highest contribution in discriminating the types of olives, whilst TFC showed less contribution in explaining the variation among seven types of olive.

Principal component analysis

Principal component analysis (PCA) was performed to understand six parameters, namely, DPPH free radical scavenging activity, total antioxidant activity (TAA), lipid

peroxidation (TBARS), total phenolic content and total flavonoids (Figure 7). The most significant component, total antioxidant activity showed the largest variation of 60.87%, DPPH activity showed 22.93% and TBARS showed 12.16%. While, total phenolic and flavonoid contents showed 3.65% and 0.3183% variation respectively. All the parameters were shown to be highly loaded on factor 1 (PC1). DPPH free radical scavenging and TBARS were found to be similarly loaded, which indicates that these two properties are closely related to antioxidant activity. Phenolic content was highly loaded on PC1, which suggests that phenolic compounds are good antioxidants. Total antioxidant activity was similarly loaded on flavonoids, which indicates that flavonoids are major contributors for total antioxidant activity. In general, flavonoid compounds have greater antioxidant potentials than phenolic acids [17,18]. Plant cultivars were loaded on both PC1 and PC2. Maurino showed the highest variation and is loaded on PC2. Whereas, Mission, Ascolano and Picholino showed significant variation and are loaded on PC1. Three cultivars namely Itrana, Carolea and Pedolino showed negative correlation.

Discussion

Oxidative stress is now recognised to be associated with more than 200 diseases, as well as with the normal aging process [19]. There is a strong correlation between thiobarbituric acid-reactive substances (TBARS) as a maker of oxidative stress and products that reflect oxidative damage to DNA [20]. It is known that metal-catalysed generation of ROS results in an attack not only on DNA and proteins, but also on other cellular components involv-

Table 2. Descriptive statistics including minimum, maximum, mean and standard deviation (sd) for selected parameters of the olives

Type of olive	Parameter	Minimum	Maximum	Mean	SD
Ascolano	TFC	4.38	4.42	4.40	0.02
	TPC	130	140	135.05	5
	TAA	210	220	215.16	5.1
Mission	TFC	7.9	8.7	8.3	0.04
	TPC	127	130.5	134	3.5
	TAA	348	360	354	6
Pedolino	TFC	0.1	0.6	0.355	0.25
	TPC	122	128	125.5	3.12
	TAA	89	96.2	92.87	3.63
Picholine	TFC	4.1	7.2	5.73	1.55
	TPC	124.3	132	128.36	3.86
	TAA	125	137	130.8	6
Maurino	TFC	4.3	5.2	4.78	0.452
	TPC	155	170	163.11	7.57
	TAA	148	155	151.5	3.5
Itrana	TFC	4.8	5.25	5.1	0.25
	TPC	128	130.7	133	3.2
	TAA	159	168	163.8	4.25
Carolea	TFC	0.4	0.8	0.57	0.2
	TPC	125	131	128.3	3.3
	TAA	129	133	131.16	2.1

TFC: Total flavonoid content; TPC: Total phenolic content; TAA: Total antioxidant activity.

ing polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation [21]. Naturally occurring antioxidants have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress [22]. Increases in the formation of TBARS in iron(II) sulphate (10 μ M)-induced oxidative stress, as compared to the normal, suggest possible damage of tissues with an overload of iron. Free iron in the cytosol and mitochondria can cause considerable oxidative damage by increasing superoxide production, which can react with Fe(III) to regenerate Fe(II) that participates in the Fenton reaction [23]. Iron overload results in the formation of lipid peroxidation products, which have been demonstrated in a number of tissues, including the liver and kidneys [24]. Storage of iron in the liver leads to liver cirrhosis. Rats overloaded with iron showed toxic effects, such as hepatocellular hypertrophy, cardiomyopathy, pancreatic atrophy, splenic white pulp atrophy and hemosiderosis in the

liver, heart, pancreas and endocrine glands, respectively [25]. The possible mechanisms of iron toxicity include free radical-mediated peroxidative reactions, which are readily catalysed by iron. The protections offered by the aqueous extracts of olive suggest that they may be useful to ameliorate the oxidative stress in the mice liver. The decrease in the Fe(II) induced lipid peroxidation in the mice liver homogenates in the presence of the extracts could be as result of the ability of the extracts to chelate Fe(II) and/or scavenge free radicals produced by the Fe(II) catalyzed production of reactive oxygen species (ROS) in the mice liver. The cultivars, Maurino and Mission possessed strong antioxidant activity and showed comparatively higher ability to reduce the TBARS and thus can be utilized against potential overload of iron in liver disease. Our results are consistent to the results of Vaseleios et al. [26] where the antioxidant activity of different olive fruit extracts is dependent on the genotypes.

Antioxidants are substances that neutralize free radicals

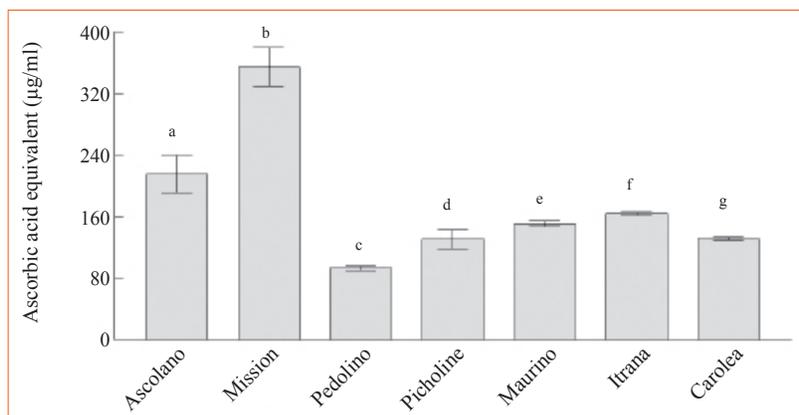


Figure 4. Total antioxidant activity of different cultivars of olive at 100 µg/ml measured by phosphomolybdenum reduction assay. Values in figure followed by different letter are significantly ($p < 0.05$) different from control by DMR test. Results are means±SD (n=3).

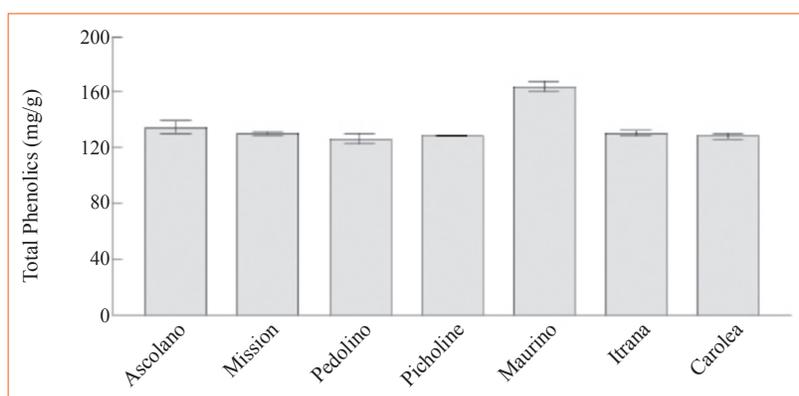


Figure 5. Total phenolic content among different cultivars of olive. The values are not significantly ($p > 0.05$) from each other by DMR test. Results are means±SD (n=3).

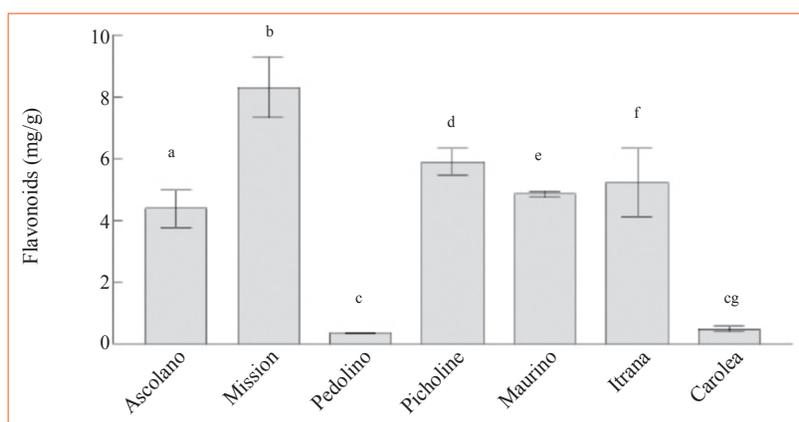


Figure 6. Total flavonoid content among different cultivars of olive. Values in figure followed by different letter are significantly ($p < 0.05$) different from control by DMR test. Results are means±SD (n=3).

and their negative effects. They act at different stages (prevention, interception and repair) and by different mechanisms: reducing agents by donating hydrogen, quenching singlet oxygen, acting as chelators and trapping free radicals [27]. Research studies report that the fruits, vegetables, grains and other plants are an important

source of polyphenols, bioactive compounds that have been found to provide a very strong antioxidant and free radical scavenging activity. Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases [1]. DPPH[•] is considered to

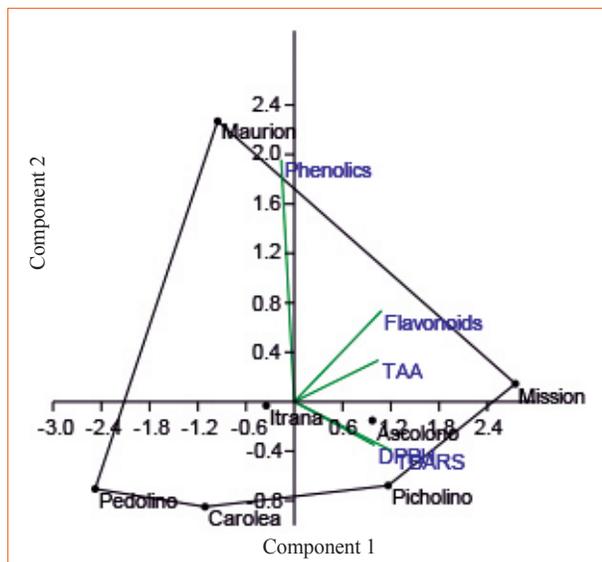


Figure 7. Principal component analysis of DPPH, TBARS (thio-barbituric acid reactive substances), TAA (total antioxidant activity), flavonoids and phenolics for seven cultivars of olive.

be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid autoxidation. Antioxidants react with DPPH, reducing the number of DPPH free radicals to the number of their available hydroxyl groups. Therefore, the absorption at 517 nm is proportional to the amount of residual DPPH. It is visually noticeable as a discoloration from purple to yellow. The high DPPH radical scavenging activity of genotypes suggests their use in diseases arising from free radical attack. Mission cultivar showed the highest DPPH radical scavenging activity and hence can be considered as a source of potent antioxidant activity. The high antioxidant activity of Mission is in agreement to Obied et al. [28] who reported that the high antioxidant potential is due to the lipophilic nature of B-Mission extract. The antioxidant activity of these olive genotypes are relatively higher than those collected from Egypt. Qusti et al. [29] reported the IC_{50} values of 0.69 mg/ml and 1.24 mg/ml in black and green olives from Egypt.

In the phosphomolybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the plant extracts demonstrated electron-donating capacity showing their ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products [30]. Allhorn et al. [31] reported that the reducing property can be a novel antioxidation defense mechanism, possibly through the ability of the antioxidant compound to reduce transition metals. Mission displayed the highest reducing ability in Phosphomolybdenum assay.

Plant-derived polyphenolic flavonoids are well known to exhibit antioxidant activity through a variety of mechanisms including scavenging of ROS, inhibiting lipid peroxidation and chelating metal ions [20]. Hence their

mechanism of action is multiple; it includes the inhibition of enzymes involved in ROS generation, chelating of trace metals such as free iron and copper, and the ability to reduce highly oxidizing free radicals by hydrogen donation, thus protecting us from serious diseases such as heart, strokes and even cancers [32]. Olive genotypes were found to be rich in phenolic and flavonoids. The high antioxidant activity of Maurino and Mission in this study is due to their high phenolic and flavonoid contents. The high phenolic content among these genotypes are in agreement to the results of Qusti et al. [29] where the phenolic content was in the range of 84.08-144.47 mg/g. Several phenolics and flavonoids are found in olive fruit which includes hydroxytyrosol, luteolin 7-*O*-glucoside, oleuropein, rutin, apigenin 7-*O*-glucoside and luteolin, 5-*O*-caffeoylquinic acid, verbascoside, quercetin 3-*O*-rhamnoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside.

Conclusion

From the results of this study, we conclude the high efficacy of the crude aqueous extract of different genotypes of olive, in free radical scavenging, inhibition of reactive oxygen species and lipid peroxidation which may be associated with their high medicinal use as a functional food and effectiveness in treatment of different diseases amongst which the liver disease is the most important. However, more detailed *in vivo* studies are required to evaluate the antioxidant and hepatoprotective activity of olive genotypes.

Conflict of Interest

There are no conflicts of interest among the authors.

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