

¹Department of Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot Azad Kashmir, Pakistan

²Department of Chemistry, University of Poonch, Rawalakot, Azad Kashmir, Pakistan

³University of Azad Jammu and Kashmir, Muzaffarabad, Azad Kashmir, Pakistan

⁴Phytochemical Research Laboratory, Department of Industrial Pharmacy, Federal University of Santa Maria, Santa Maria, Brazil

⁵Department of Biochemistry, University of Baluchistan, Pakistan

⁶Pakistan Agricultural Research Centre, Islamabad, Pakistan

⁷Department of Eastern Medicine, University of Poonch, Rawalakot, Azad Kashmir, Pakistan

Antioxidant activities and phenolic composition of Olive (*Olea europaea*) leaves

Abdul Khaliq¹, Syed Mubashar Sabir^{2*}, Syed Dilnawaz Ahmad³, Aline Augusti Boligon⁴, Margareth Linde Athayde⁴, Abdul Jabbar⁵, Imtiaz Qamar⁶, Asmatullah Khan⁷

(Received October 25, 2014)

Summary

The present study compares the antioxidant activities of leaves of eight cultivars of olive. The aqueous extracts of leaves 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 cultivars on lipid peroxidation assay is Gemlik > Frantio > Doleca-Agogia > Moriolo > Mission > Uslu > Leccino > Carotina. Different varieties of olive showed good antioxidant properties, IC₅₀ values ranged between 22.46 to 198 μ g/ml on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The major phenolic acids, some flavonoid aglycone and glycosides were identified in leaves by high performance liquid chromatography. Ellagic acid (29.80 \pm 0.02 mg/g), caffeic acid (15.73 \pm 0.01 mg/g), gallic acid (15.69 \pm 0.01 mg/g), rutin (34.56 \pm 0.03 mg/g), quercetin (16.41 \pm 0.01 mg/g), epicatechin (11.04 \pm 0.01 mg/g) and quercitrin (15.32 \pm 0.03 mg/g) were predominant in infusion of olive.

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 (HALLIWELL et al., 1992). 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 (HERTOG et al., 1993). 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 (SOLER-RIVAS et al., 2000).

A study has indicated that extract of olive leaves had a capacity to lower blood pressure in animals and increase blood flow in coronary arteries, relieved arrhythmia and prevented intestinal muscle spasms (BENAVENTE-GARCIA et al., 2000), diarrhea, to treat respiratory and urinary tract infections; olive oil and olive leaf extracts are some

of these foodstuffs with recognized medicinal benefits and food preservation properties dating back to the Egyptian empire (MEDINA et al., 2007).

Antioxidant activity is used to measure a compound to reduce the pro-oxidants or reactive species of pathologic significance (SOMOGYI et al., 2004). 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 (FREI, 1994). 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. The demands of natural antioxidants are high for application as nutraceuticals and as food additives because of consumer preferences (KUMAR and CHATOOPADHYAY, 2007). Since antioxidants from plant source are safe and easily available, olive leaves was subjected to determine and quantify various antioxidant activities. There is limited information available on the antioxidant activity and phenolic profile of these olive cultivars. In view of the potential role of olive as dietary source of flavonoids as well as its possible use as functional food, this study was aimed to evaluate the antioxidant and inhibitory effect of eight important cultivars of olive on Fe(II) induced lipid peroxidation and to find out the phenolic profile of olive for their possible use in food and phytotherapy. The DPPH radical activity, total antioxidant activity, phenolic and flavonoid contents of these cultivars were also determined.

Materials and methods

Chemical and reagents

Methanol, formic acid, gallic acid, chlorogenic acid, caffeic acid and ellagic acids purchased from Merck (Darmstadt, Germany). Epicatechin, quercetin, quercitrin, rutin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Thiobarbituric acid (TBA), malonaldehyde-bis-dimethyl acetal (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ammonium molybdate was purchased from Sigma Aldrich (St. Louis, MO, USA). Ferrous sulphate was obtained from Biochemicals (Lahore). All chemical were of analytical grade.

Preparation of the leaves extracts

The leaves of different cultivars of olive which include Doleca-Agogia, Mission, Moriolo, Maurino, Carotina, Leccino, Gemlik, Uslu were collected from three locations, Quetta, Zhoab and Lorallia research stations of Pakistan at the same harvesting time.

Leaves 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

* Corresponding author

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 22-30 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 (OHKAWA et al., 1997). 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 leaves extracts together with an appropriate volume of de-ionised water to give a total volume of 300 µl at 37 °C for 1 h. The colour 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 by scavenging of stable DPPH radical according to the method of HATANO et al. (1998). 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 = [(A₀ - A₁)/A₀] x 100, Where, A₀ is the absorbance of the control reaction and A₁ is the absorbance of the sample itself. The IC₅₀ 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 phosphomolybdenum 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 (PRIETO et al., 1998). The extracts at concentration (100 µg/ml) were mixed with 3 ml of the reagent solution (0.6 M H₂SO₄, 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.

Determination of phenolics

The total phenolic content as gallic acid equivalent was determined by the method of SINGLETON et al. (1999). This method quantifies

various phenolic acids and flavonoids in the extract. 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 min and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was expressed as milligrams of gallic acid equivalents/g extract was calculated using the following linear equation based on calibration curve:

$$Y = 0.0063x + 0.0396 \quad (R^2 = 0.99)$$

Where, Y is the absorbance at 765 nm and x is the total phenolic content of different extracts of olive.

Determination of flavonoids

The total flavonoid content was expressed as quercetin equivalents. A standard curve was prepared from quercetin [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 total flavonoid content was expressed as milligrams of quercetin equivalents/g of extract by the method of KOSALEC et al. (2004). The total flavonoid content was calculated using the following linear equation based on calibration curve:

$$Y = 0.0026x + 0.0114 \quad (R^2 = 0.996)$$

Where, Y is the absorbance at 765 nm and x is the total flavonoid content of different extracts of olive.

Quantification of phenolic compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C₁₈ column (4.6 mm x 150 mm) packed with 5 µm diameter particles; the mobile phase was water containing 1 % formic acid (A) and methanol (B), and the composition gradient was: 13 % of B until 10 min and changed to obtain 20 %, 30 %, 50 %, 60 %, 70 %, 20 % and 10 % B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by PEREIRA et al., (2014) with slight modifications. *Olea europaea* extract and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the *Olea europaea* extract was analyzed at a concentration of 15 mg/mL. The presence of nine phenolic compounds, namely, gallic acid, chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin, quercitrin, quercetin and kaempferol, was investigated. Identification of these compounds was performed by comparing their retention times and UV absorption spectra with those of external standards. The flow rate was 0.7 mL/min, injection volume 50 µl and the wavelength were 254 for gallic acid, 280 for epicatechin, 327 nm for chlorogenic, caffeic and ellagic acids, and 365 nm for quercetin, quercitrin, rutin and kaempferol. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 - 0.300 mg/mL for kaempferol, quercetin, quercitrin, rutin and epicatechin; and 0.050 - 0.450 mg/mL for ellagic, gallic, caffeic and chlorogenic acids. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 12754x + 1197.3 (r = 0.9998); chlorogenic acid: Y = 11864x + 1267.5 (r = 0.9999); caffeic acid: Y = 12693x + 1308.1 (r = 0.9995); ellagic acid: Y = 13172x + 1246.9 (r = 0.9998); epicatechin: Y = 11849x + 1287.6 (r = 0.9993); quercitrin: Y = 12581x + 1317.4

($r = 0.9999$); rutin: $Y = 13028x + 1267.7$ ($r = 0.9998$); quercetin: $Y = 11896x + 1197.5$ ($r = 0.9994$) and kaempferol: $Y = 11679x + 1265.8$ ($r = 0.9999$). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and $10 \sigma/S$, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve (BOLIGON et al., 2013).

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) and Turkey test where necessary. $P < 0.05$ was considered significant in all cases. The software Package Statistica (version, 4.5) was used for analysis of data.

Results

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

Lipid peroxidation (an index of oxidative stress) in mice liver homogenate was induced with iron and the potential antioxidant effect of aqueous extract of different cultivars of olive was determined. Fig. 1 shows the antioxidant effect of different cultivars of olive in mice liver. Here, the TBARS was induced with $10 \mu\text{M}$ 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 (Fig. 1). Treatment with different concentrations of olives caused a marked decrease in lipid peroxidation (Fig. 1). All the cultivars were found to be capable of reducing the lipid peroxidation almost comparable to the basal level. In order to compare the antioxidant activity among different cultivars IC_{50} values (Concentration that cause 50 % inhibition) were calculated (Tab. 1). Thus the order of antioxidant activity is Gemlik $>$ Frantio $>$ Doleca-Agogia $>$ Moriolo $>$ Mission $>$ Uslu $>$ Leccino $>$ Carotina (Tab. 1).

DPPH radical scavenging activity

The free radical scavenging activities of extracts was measured by the ability to scavenge DPPH radical. 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 analyze the free radical scavenging activity. All the cultivars showed their abilities to scavenge the DPPH radical which were evaluated in terms of their IC_{50} values (Fig. 2a). All the cultivars showed their ability to reduce the DPPH. However, their order of reactivity is (IC_{50} , $22.46 \mu\text{g/ml}$ (Leccino), $23.5 \mu\text{g/ml}$ (Gemlik), $29.64 \mu\text{g/ml}$ (Doleca-Agogia), $46.34 \mu\text{g/ml}$ (Moriolo), $82.1 \mu\text{g/ml}$ (Mission) and $115.5 \mu\text{g/ml}$ (Uslu), $196 \mu\text{g/ml}$ (Frantio) and $198 \mu\text{g/ml}$ (Carotina) (Tab. 1).

Total antioxidant activity by phosphomolybdenum assay

The total antioxidant activity of different cultivars was expressed as ascorbic acid equivalent is shown in Fig. 3. There was a statistically significant ($P < 0.05$) difference among the antioxidant activity of all cultivars. However, the order of their reactivity was Dolece-Agogia $>$ Moriolo $>$ Leccino $>$ Gemlik $>$ Mission $>$ Picholino $>$ Frantio $>$ Carotina (Fig. 2b).

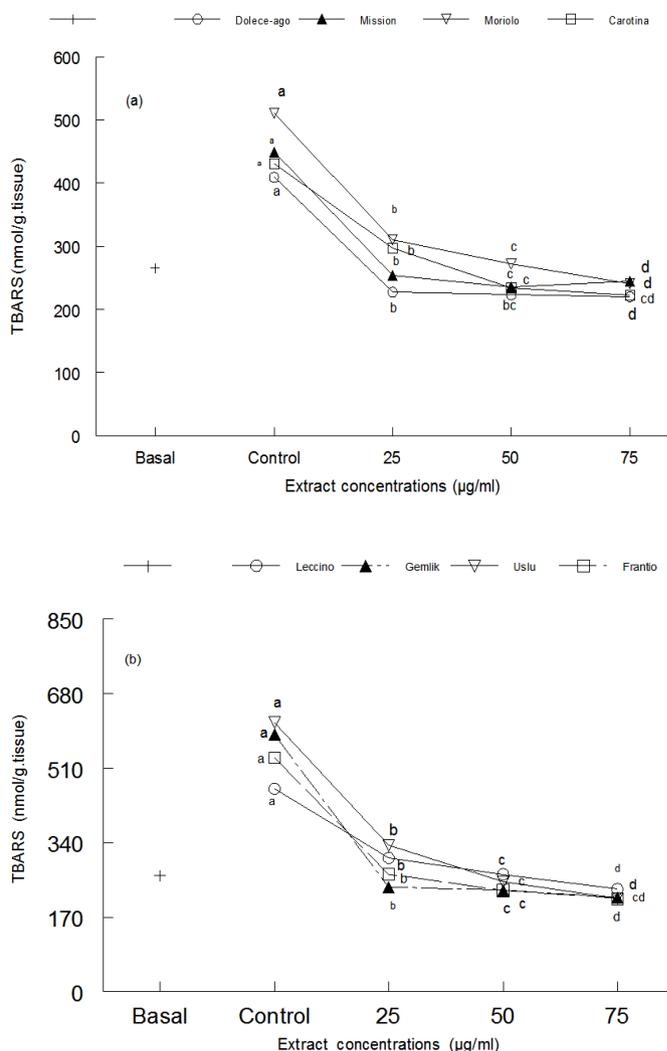


Fig. 1: The inhibitory effect of aqueous extract of eight cultivars of olive on iron sulphate induced lipid peroxidation in mice liver. The results are means of three separate experiments \pm SD. $P < 0.05$ is significantly different from control by DMR test. All the values are significantly different ($P < 0.05$) from control by DMR test.

Tab. 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
Doleca-Agogia	29.64	28.4
Mission	82.1	44.71
Moriolo	46.34	32.05
Carotina	198	64
Leccino	22.46	59.32
Gemlik	23.5	21.18
Uslu	115.5	57.42
Frantio	196	26

Total phenolics and flavonoid contents

The total phenolic content was expressed as gallic acid equivalent, while, flavonoid content as quercetin equivalent (Fig. 3). There was a statistically significant difference ($P < 0.05$) among different cultivars. The phenolic content was found in the range of $109.6 \pm$

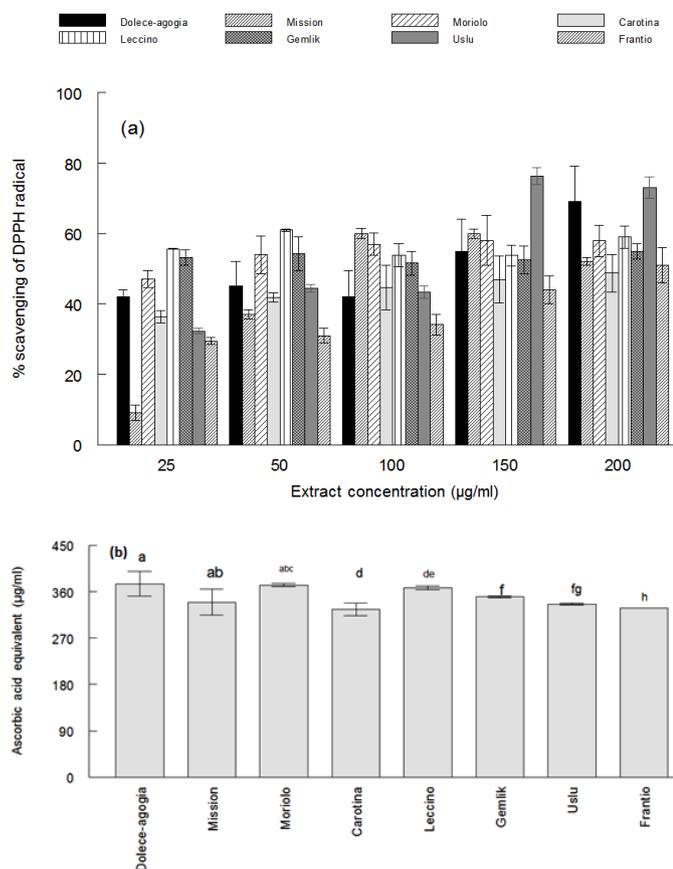


Fig. 2: Antioxidant activities of olive cultivars (a). DPPH radical scavenging activity of different cultivars of olive. Values are means \pm SD (n=3). (b). total antioxidant activity of different cultivars of olive at 100 μ g/ml measured by phosphomolybdenum reduction assay. Results are means \pm SD (n=3). Values in figure followed by different letter are significantly ($P < 0.05$) different from control by DMR test.

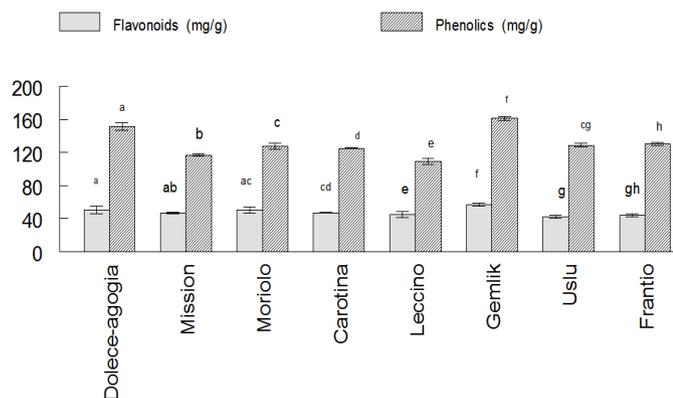


Fig. 3: Total phenolic and flavonoid contents among different cultivars of olive. Values in figure followed by different letter are significantly ($P < 0.05$) different from control by DMR test. Results are means \pm SD (n=3).

3.68 to 161 \pm 1.9 mg/g. Gemlik cultivar which showed the highest antioxidant activity showed the highest phenolic content. While, leccino contained the lowest amount of phenolics (Fig. 3). The flavonoid content was found between 42 \pm 1.97 to 57 mg/g. Gemlik contained the highest amount of flavonoid. Whereas, Uslu contained the least amount of flavonoid (Fig. 3).

Content of phenolics, flavonoids and HPLC characterization of phenolic compounds

HPLC fingerprinting of *Olea europaea* was also acquired (Fig. 4). The samples of *Olea europaea* (Gemlik) displayed the highest antioxidant activity, therefore, its phenolic profile was determined. The compounds were identified based on the spectral characteristics and retention times compared with authentic standards. *Olea europaea* extract revealed the presence of gallic acid ($t_R = 11.73$ min; peak 1), chlorogenic acid ($t_R = 21.09$ min; peak 2), caffeic acid ($t_R = 25.01$ min; peak 3), ellagic acid ($t_R = 31.65$; peak 4), epicatechin ($t_R = 34.81$ min; peak 5), rutin ($t_R = 40.25$ min; peak 6), quercitrin ($t_R = 45.19$ min; peak 7), quercitrin ($t_R = 50.97$ min; peak 8) and kaempferol ($t_R = 58.36$ min; peak 9) (Fig. 4, Tab. 2).

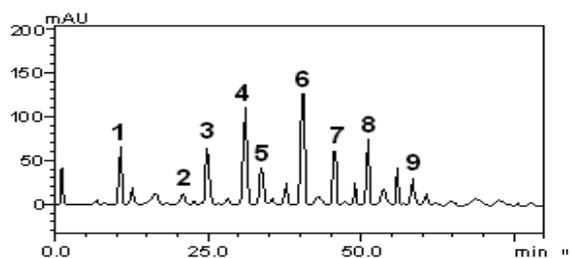


Fig. 4: Representative high performance liquid chromatography profile of *Olea europaea* extract, detection UV was at 327 nm. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), ellagic acid (peak 4), epicatechin (peak 5), rutin (peak 6), quercitrin (peak 7), quercetin (peak 8) and kaempferol (peak 9).

Tab. 2: Phenolics and flavonoids composition of *Olea europaea* extract

Compounds	<i>Olea europaea</i>		LOD mg/mL	LOQ mg/mL
	mg/g	%		
Gallic acid	15.69 \pm 0.01 a	1.56	0.017	0.058
Chlorogenic acid	3.17 \pm 0.03 b	0.31	0.025	0.083
Caffeic acid	15.73 \pm 0.01 a	1.57	0.031	0.107
Ellagic acid	29.80 \pm 0.02 c	2.98	0.029	0.096
Epicatechin	11.04 \pm 0.01 d	1.10	0.016	0.054
Rutin	34.56 \pm 0.03 e	3.45	0.035	0.115
Quercitrin	15.32 \pm 0.03 a	1.53	0.007	0.025
Quercetin	16.41 \pm 0.01 a	1.64	0.012	0.039
Kaempferol	7.38 \pm 0.02 f	0.73	0.009	0.028

Results are expressed as mean \pm standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

Discussion

Oxidative stress is now recognised to be associated with more than 200 diseases, as well as with the normal aging process (GHASANFARI et al., 2006). 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 (CHEN et al., 2005). 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 involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation.¹⁷ 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 (FRAGA and OTEIZA, 2002). Iron overload results in the formation of lipid peroxidation products, which have been demonstrated in a number of tissues, including the liver and kidneys (HOUGLUM et al., 1990). 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, Gemlik, Frantio and Dolece-AgogiaB-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.

DPPH radical scavenging activity is usually performed as a measure of electron denoting capacity of antioxidant (BAUMANN et al., 1997). Antioxidants are substances that neutralize free radicals 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 (DEVASAGAYAM et al., 2004). DPPH• is considered to be a model of a stable lipophilic radical. A chain reaction of lipophilic radicals is initiated by lipid auto-oxidation. 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 these cultivars suggests their use in diseases arising from free radical attack.

The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (PRIETO et al., 1999). The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherols and carotenoids. Dolece-Agogia showed the highest reducing activity on phosphomolybdenum assay whereas, Carotina showed the least.

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 (SHAHIDI, 1997). *In vitro* experimental systems have also shown that flavonoids possess anti-inflammatory, antiallergic, antiviral and anticarcinogenic properties (MIDDLETON, 1998). The hot water extracts of olive showed high content of phenolics and flavonoids. Ellagic acid, caffeic acid, gallic acid, rutin, quercetin, epicatechin and quercitrin were predominant in infusion of olive.

This is a detailed phytochemical study which reports different phenolics and flavonoids in olive leaves. Earlier studies have shown that the main phenolic compounds in olive leaves are oleuropein, verbascoside, ligestroside, tyrosol and hydroxytyrosol (CATURLA et al., 2005). The observed antioxidant activity and protecting ability of olive against lipid peroxidation is due to the phenolic acids (ellagic acid, gallic acid, chlorogenic acid and caffeic acid) and flavonoids (rutin, quercitrin, epicatechin, and quercetin and kaempferol). The exogenous antioxidants from olive extracts may act directly or indirectly with the internal antioxidant system for synergetic effects to protect several diseases linked to free radicals such as heart diseases, neurodisorders and other stress related disorders.

From the results of this study, we conclude a high efficacy of the crude aqueous extract of eight cultivars of olive, in free radical scavenging, inhibition of reactive oxygen species and lipid peroxidation which may be associated with its 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 activity and bioavailability of olive.

Conflict of Interest

None declared.

References

- BAUMANN, J., WURN, G., BRUCHLAUSEN, F.V., 1979: Prostaglandin synthase inhibiting O₂-radical scavenging properties of some flavonoids and related phenolic compounds. *Arch. Pharmacol.* 313, 330-337.
- BENAVENTE-GARCIA, O., CASTILLO, J., LORENTE, J., ORTUNO, A., DEL RIO, J.A., 2000: Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chem.* 68, 457-462.
- BOLIGON, A.A., KUBIÇA, T.F., MARIO, D.N., BRUM, T.F., PIANA, M., WEIBLEN, R., LOVATO, L., ALVES, S.H., SANTOS, R.C.V., ALVES, C.F.S., ATHAYDE, M.L., 2013: Antimicrobial and antiviral activity-guided fractionation from *Scutia buxifolia* Reissek extracts. *Acta Physiol. Plant.* 35, 2229-2239.
- CATURLA, N., PÉREZ-FONS, L., ESTEPA, A., MICOL, V., 2005: Differential effects of oleuropein, a biophenol from *Olea europaea*, on anionic and zwitterionic phospholipid model membranes. *Chem. Phys. Lipids* 137, 2-17.
- CHEN, H.J., WU, C.F., HUANG, J.L., 2005: Measurement of urinary excretion of 5-hydroxymethyluracil in human by GC/NICI/MS: Correlation with cigarette smoking, urinary TBARS and etheno DNA adduct. *Toxicol. Lett.* 155, 403-410.
- DEVASAGAYAM, T.P.A., TILAK, J.C., BOLOOR, K.K., SANE, K.S., GHASKADBI, S.S., LELE, R.D., 2004: Free radicals and antioxidants in human health: current status and future prospects. *J. Assoc. Physicians India.* 52, 794-804.
- DORMAN, H.J.D., KOSAR, M., KAHLOS, K., HOLM, Y., HILTUNEN, R., 2003: Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agri. Food Chem.* 51, 4563-4569.
- FRAGA, C.G., OTEIZA, P.I., 2002: Iron toxicity and antioxidant nutrients. *Toxicology* 80, 23-32.
- FREI, B., 1994: Non enzymatic defence system. In natural antioxidants in human health and disease. In: Briviba, K., Sies, H. (eds.). Academic Press, London, UK.
- GHASANFARI, G., MINAIE, B., YASA, N., LEILU, A.N., AZADEH, M., 2006: Biochemical and histopathological evidences for beneficial effects of *Satureja Khuzestanica* Jamzad essential oil on the mouse model of inflammatory bowel diseases. *Toxicol. Mech. Methods* 16, 365-372.
- HALLIWELL, B., GUTTERIDGE, J.M.C., CROSS, C.E., 1992: Free radicals, antioxidants and human disease: Where are we now? *J. Lab. Clin. Med.* 119, 598-620.
- HATANO, T., KAGAWA, H., YASUHARA, T., OKUDA, T., 1998: Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. *Chem. Pharm. Bull.* 36, 2090-2097.
- HERTOG, M.G.L., HOLLMAN P.C.H., VAN, D.P.B., 1993: Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juice. *J. Agri. Food Chem.* 41, 1242-1246.
- HOUGLUM, K., FILIP, M., WITZTUM, J.L., CHOJKIER, M., 1990: Malondialdehyde and 4-hydroxynonenal protein adducts in plasma and liver of rats with iron overload. *J. Clin. Inves.* 86, 1991-1998.
- KARADAG, A., OZCEKIK, B., SANER, S., 2009: Review of methods to determine antioxidant capacities. *Food Anal. Methods* 2, 41-60.
- KOSALEC, I., BAKMAZ, M., PEPELINIAK, S., VLADIMIR-KNEZEVIC, S., 2004: Quantitative analysis of the flavonoids in raw propolis from northern Croatia. *Acta Pharm.* 54, 65-72.
- KUMAR, A., CHATTOPADHYAY, S., 2007: DNA damage protecting activity and antioxidant potential of pudina extract. *Food Chem.* 100, 1377-1384.
- MEDINA, E., ROMERO, C., BRENES, M., GARCIA, P., DE CASTRO, A., GARCIA, A., 2008: Profile of anti-lactic acid bacteria compounds during the storage of olive which are not Treated with alkali. *Eur. Food Res. Tech.* 228, 133-138.

- MIDDLETON, E.J., 1998: Effect of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol.* 439, 175-82.
- MYRIAM, B.S., HAFEDH, A., MANEF, A., 2012: Study of phenolic composition and biological activities assessment of olive leaves from different varieties grown in Tunisia. *Med. Chem.* 2, 107-111.
- OBEID, H.K., BEDGOOD, JR. D.R., PRENZLER, P.D., ROBARDS, K., 2007: Bio screening of Australian olive mill waste extracts: Biophenol content, antioxidant, antimicrobial and molluscicidal activities. *Food Chem. Toxicol.* 45, 1238-1248.
- OHKAWA, H., OHISHI, N., YAGI, K., 1997: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351-358.
- PEREIRA, R.P., BOLIGON, A.A., APPEL, A.S., FACHINETTO, R., CERON, C.S., TANUS-SANTOS, J.E., ATHAYDE, M.L., ROCHA, J.B.T., 2014: Chemical composition, antioxidant and anticholinesterase activity of *Melissa officinalis*. *Ind. Crop. Prod.* 53, 34-45.
- PRIETO, P., PINEDA, M., AIGUEL, M., 1999: Spectrophotometer Quantization of antioxidant capacity through the formation of Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Anal. Biochem.* 269, 337-341.
- SHACTER, E., 2000: Protein oxidative damage. *Methods Enzymol.* 319, 428-436.
- SHAHIDI, F., 1997: Natural antioxidants: an overview. In: Shahidi, F. (ed.), *Natural antioxidants: Chemistry, health effects and applications*, 1-7. Champaign, IL: AOCS Press.
- SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M., 1999: Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu's reagent. *Methods Enzymol.* 299, 152-178.
- SOMOGYI, A., ROSTA, K., PUSZTAI, P., TULASSAY, Z., NAGY, G., 2007: Antioxidant measurements. *Physiol. Meas.* 28, 41-55.
- SOLER-RIVAS, C., ESPIN, J.C., WICHERS, H.J., 2000: Oleuropein and related compounds. *J. Sci. Food Agri.* 80, 1013-1023.

Address of the corresponding author:
E-Mail: mubashersabir@yahoo.com