Oil, protein, antioxidants and free radical scavenging activity of stone from wild olive trees (*Olea europaea* L.*)*

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Abstract: The wild olive trees or oleaster (var. *sylvestris*) and the cultivated olive trees (var. *europaea*) constitute the two botanical varieties of *Olea europaea* L. from Mediterranean. In this study, a partial chemical profile was conducted including the total lipids, the fatty acid profiles, soluble proteins, polyphenols, flavanoids contents and antioxidants activities of stone from six oleaster trees. The comparison was made by two olive cultivars cultivated in the same region. The oleaster and cultivar stones were richer in oil content having an average of 8.99 and 7.38 % dry weight basis (DW), respectively. Qualitatively, all studied oils have the same fatty acids profile with the oleic acid C18:1n-9 as the major fatty acid. The oleaster stone oils were richer in monounsaturated fatty acids having an average of 64.87%. They, also, richer in protein content with an average of 198.86 mg/g DW.The globulin is the major fraction, followed by the albumin, the prolamin and the glutemin fractions. The oleaster stone extracts contain polyphenols, flavonoids with an average of 151.14 and 11.91 mg gallic acid equivalent/100g of DW, respectively. The studied extracts showed antioxidant activity using the free radical scavenging activity determined by DPPH and ABTS. The unexploited oleaster stone seems to be a source of oil with good fatty acids balance, in protein and antioxidants metabolites and would be useful for the formulation of supplements and/or pharmaceutical ingredients.

Keywords: *Olea europaea*, wild olive, protein, oil, phenolics, antioxidant.

INTRODUCTION

The olive trees have been used widely in folk medicine in Mediterranean islands and countries such as Spain, Italy, France, Greece, Morrocco, Tunisia, Turkey, etc. Olive oil is commonly used in food, as a frying medium, and in cosmetic formulations, as an emollient. It is a combination of monounsaturated and polyunsaturated fats with low saturated fat levels. It is appreciated for its stability and good characteristics and is considered as most useful edible oil in the World due to its nutriment contents and beneficial effects (Visioli *et al*., 1997). *Olea europaea* L. includes cultivated (Var. *europaea*) and wild or oleaster (Var. *sylvestris*) as two botanical varieties in Mediterranean basin (Green, 2002). The *Olea europaea* L. spreads in the Mediterranean basin where it is indigenous and in other regions with a Mediterranean climate where it has been introduced (Costa, 1998). Lipid oxidation has been one of the main interests of the scientific community for centuries. Although, there are no conclusive results on the safety of these substances, worldwide interest has arisen for the recovery and exploitation of antioxidants from natural sources (Loliger *et al*., 1996). Recent research has focused on antioxidant compounds derived from leaves and fruit of olive trees, numerous fruits and vegetables, as well as aromatic plants and spices (Gkanatsiou *et al*., 2007; Elfalleh *et al*., 2009, Nasri *et al*., 2011, Ebrahimzadeh *et al*., 2010). In traditional medicine, the olive tree is used as a diuretic, hypotensive, emollient, febrifuge and tonic, for urinary and bladder infections and for headaches (Hutchings *et al*., 1996). The hypotensive and hypoglycemic effects of olive leaves from Mediterranean *Olea europaea* have been well documented (Ribeiro *et al*., 1986; Cherif *et al*., 1996). Moreover, in this case of *Olea* uses, little is known on the wild olive trees or oleaster from Mediterranean basin, Var *sylvestris*. Traditional medicine and medication therapy that derives largely from herbal medicine constitute a highly significant component of modern medical care for most of the world's population. The importance of traditional medicine has come to the attention of the pharmaceutical industry and the medical research community. There is concurrently a renewed recognition of the potential of natural products for new drug discovery (De Vos, 2010). Recently, plant based medicinals have been found to be effective in the treatment of cancer, HIV, and malaria, so much so that some pharmaceutical companies have directed research in this area (Fabricant Buenz *et al*., 2001; Gertsch, 2009).

Several historical reports (Camps-Fabrer, 1997) have pointed out that the oleaster trees were native in Tunisia. The molecular diversity revealed that a few cultivars are issued from the Tunisian oleaster trees based on assignation and admixture analyses (Breton *et al*., 2006). Using nuclear and choroplastic SSR markers, it has been reported that the olive in Tunisia has probably three geographical origins (Hannachi *et al*., 2010). Therefore, the oleaster trees were important genetic resources deserving to be known not only its genetic characterization but also its technological potentialities

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such as phytochimical composition. The oleaster trees dispread in Tunisia in natural and agro-ecosystems (Hannachi *et al*., 2008). The oleaster trees constitute an unexploited material in Tunisia.

Despite the large amount of olive oils produced and their confirmed medicinal values, little is known on the chemical composition of stone from oleaster trees, particularly in Tunisia. The recognition of the benefits of phytotherapy in particular has led to increasing research into plant products in order to isolate bioactive compounds.

The objective of this study is to carry out on chemical profiling of stone from wild olive trees (oleasters). The oil content, fatty acid composition, soluble proteins, the polyphenols, flavonoids and the antioxidants activities were analysed. The comparison was made by two main olive cultivar cultivated in the same region.

MATERIAL AND METHODS

Material

Olives were picked at maturity time from two cultivars Zarrazi and Chemlali cultivated in situ and six oleaster trees in Tunisian south (Gabès province, southern Tunisia, 33°40‘N, 10°15‘E). Pulps were separated from their stones. The obtained stones were used to further analyses.

Chemicals

All solvents were of reagent grade without any further purification. Gallic acid, rutin and Folin-Ciocalteu’s phenol reagent were purchased from Sigma Chemical Co. (USA). The analytical reagent grade methanol was obtained from Lab-Scan (Labscan Ltd, Dublin, Ireland). The water used in sampling was prepared with a Millipore Simplicity (Millipore S.A.S., Molsheim, France). All chemicals used in antioxidant activity assays were of chromatography grade and were purchased from Sigma Chemical Co. (Poole, Dorset). Spectrophotometric measurements were performed on Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan).

Lipid extraction and fatty acid analysis

Lipids were extracted by the method of Soxhlet as described previously (Nasri and Triki, 2007). Fats were transmethylated using boron trifluoride in methanol (Metcalfe *et al*., 1966). The fatty acid methyl esters formed by transmethylation were analyzed on a Hewlett Packard Model 5890 gas chromatograph (Palo Alto, CA, USA) fitted with a CPSIL-88 column (100 m × 0.25 mm i.d., film thickness 0.20 µm; Varian, Les Ulis, France). Hydrogen was used as a carrier gas (inlet pressure 210 kPa). The oven temperature was held at 60°C for 15 min, increased to 85°C at 3°C/min and then to 190°C at 20°C/min, and finally held at 190°C. The injector and the flame ionization detector were maintained at 250°C and 280°C, respectively. Fatty acid methyl esters were identified by comparison with standards. The data were computed using the Galaxie software (Varian, France) and reported as a percentage of the total fatty acids. All analyses were made in triplicate.

Storage proteins extraction from olive stones

In order to extract all classes of storage proteins of olive stone, we adopted a fractionation protocol of the various categories of proteins basing on their solubility differences as described previously by Nasri and Triki (2007) and later by Elfalleh *et al*. (2010). The extraction procedure is based on solubility differences of proteins in various solvents (Osborn, 1924). First, a milled sample (500 mg dry weight of stone) was extracted with distilled water (10 ml). The suspension was stirred at laboratory temperature for 20 min and then centrifuged at 10000 rpm for 15 mn. The filtrated supernatant was used as the extract 1 (albumin fraction). The remaining insoluble sample was mixed with 10 ml of aqueous NaCl 5% (w/v) solution. The extraction procedure was repeated, and the extract 2 was collected (globulin fraction). After following extractions with aqueous 70% (v/v) ethanol and aqueous 0.2% NaOH solution the extract 3 (prolamin fraction) and the extract 4 (glutelin fraction) were obtained.

Storage protein determination using Bradford assay

The protein content of each sample was quantified using the method described by Bradford (1976). 100 mg Coomassie Brilliant Blue G-250 (Sigma-Adrich Co) was dissolved in 50 ml ethanol (95%) and 100 ml of phosphoric acid (85%) was added. The solution was diluted, filtered, and used as the color reagent for protein quantization. Standard solutions of reagent grade BSA (Equitech-Bio, Inc., Kerriville, TX) were prepared containing 0-400 µg protein. Samples were covered with parafilm mixed, and then incubated for 5 min before absorbance measurement at 595 nm in spectrophotometer (Anthelie Advanced, Microbeam, S.A). All standard and sample unknowns possessed the same solution matrix. The protein content of each sample was determined by fitting a least squares regression curve of the quantity of standard protein concentration vs.photometric absorbance.

Determination of total polyphenols content

Total phenols were estimated, in triplicate by the Folin-Ciocalteu method described previously (Nasri *et al*., 2011). From each sample, 0.5 ml of methanolic solution to 0.5 ml of Folin- Ciocalteu (Prolabo) reagent was added. After, 4 ml of sodium carbonate 1M solution was added to the mixture solution. The tubes were laid for 5 min in a water bath at 45°C and then put in a cold water bath. Absorbance was measured at 765 nm, using a Shimadzu 1600-UV spectrophotometer. Total phenolic contents were expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100g DW).

Determination of total flavonoids content

Total flavonoids were measured spectrophotometrically, in triplicate, following the method described previously (Elfalleh *et al*., 2009). This method based on the formation of a complex flavonoid–aluminium, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. 1 ml of methanolic extract was mixed with 1 ml of 2% AlCl3 methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a Shimadzu 1600-UV spectrophotometer. Total flavonoid contents were expressed as rutin equivalents in mg per 100 g dry weight (mg RE/100 g DW).

DPPH radical scavenging activity

The scavenging activity on DPPH radical of methanolic extracts was determined, in triplicate, following the method reported previously by Okonogi *et al*. (2007). A methanolic test solution of deferent concentrations prepared from a stock solution of stone extracts (1 mg of dry powder per ml). DPPH (100 µM) was dissolved in ethanol and mixed with an aliquot of 100 µl of each dilution. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

ABTS radical scavenging activity

The total antioxidant activity values were estimated in triplicate by the Trolox equivalent antioxidant capacity (TEAC) test described previously (Nasri *et al*., 2011). In this test, we measured the relative capacity of antioxidants to scavenge the $ABTS^{\cdot+}$ radical compared to the antioxidant potency of Trolox which is used as a standard. $ABTS^{\cdot+}$ was generated by oxidation of ABTS with potassium persulfate. The $ABTS^{\cdot+}$ radical generated by mixing 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$ in the dark for 24 h, at room temperature. Before usage, the $ABTS^{\cdot+}$ solution was diluted with ethanol to get an absorbance of 0.700±0.020 at 734 nm. 25 µl of antioxidant sample or Trolox standard was added to 1 ml of the diluted $ABTS^{\cdot+}$ solution. The reaction mixture was homogenised for 20 s and then the absorbance was recorded at 734 nm for 5 min. The final TEAC value of the antioxidant compound was calculated by comparing $ABTS^{\cdot+}$ decolourisation with Trolox, which gives a useful indication of the antioxidant potential of the stone extracts.

Statistical and chemometric methods

Analysis of variance (ANOVA) was performed using Statistica software (version 8), to evaluate the significance of differences between individual chemical contents of stones at the level of $p < 0.05$.

RESULTS

Oil content and fatty acids composition of stone

The oil content was expressed in percent of dry weight basis (DW). The oil content from oleaster stones varied to 8.04 (OlGab4) from 9.43 % (OlGab6) with an average of 8.99%. The stones of Zarrazi and Chemlali, olive cultivars, have 7.54 and 7.23%, respectively (table1). The oil contents differs significantly ($P < 0.05$). Qualitatively, all studied oils have the same fatty acids profile; whereas, quantitatively, the ANOVA showed some significant differences (table1). The major fatty acid is the oleic acid (C18:1n-9), followed by linoleic (C18:2n-6) and palmitic acids (C16:0) in all studied oils. The oils extracted from oleaster stone were rich in monounsaturated fatty acids (MUFA), 64.87%. The two olive cultivars have an average of 63.96% MUFA. The polyunsaturated fatty acids (PUFA) have an average of 17.34 and 17.80% in stone oils from oleaster and cultivars, respectively. The MUFA and PUFA showed significant difference within all studied oils.

The stone oils had high unsaturated fatty acids (UFA) and lower saturated fatty acids (SFA) contents. The UFA have an average of 82.28% in oleaster stone and 81.76% in cultivar ones. The oleaster and cultivar stone oils have an average of 17.34 and 17.80% of SFA, respectively.

Protein content of olive stone

The contents of storage proteins extracted from oleaster stone varied to 186.07 (OlGab5) from 204.25 (OlGab2) mg/g DW. The stone of Zarrazi and Chemlali have protein contents 138.07 and 144.68 mg/g DW, respectively. The globulin is the major fraction, followed by albumin, prolamin and glutelin fractions in all studied stones. The olive stone from oleasters were richer in protein content compared to the cultivars ones (table 2).

Antioxidants contents of stone extracts

The polyphenols and flavonoids contents were reported in table3. The polyphenols content ranged from 123.20 (OlGab4) to 178.77 (OlGab1) mg GAE/100 g DW with an average of 151.44 mg GAE/100 g DW. The polyphenols content were 761.89 and 212.19 mg GAE/100 g DW, respectively, in Zarrazi and Chemlali stone extracts. The flavonoids contents have an average of 11.91 and 17.73 mg RE/100 g DW in oleaster and cultivar stones, respectively. The differences were significant ($P < 0.05$) of polyphenols and flavonoids contents from stones extracts.

Antioxidant activities: ABTS and DPPH scavenging capacity

The antioxidant activities of the extracts from stones were evaluated as trolox equivalent antioxidant capacity (TEAC) calculated from DPPH•- and ABTS•+ scavenging capacity (table 3).

Table 1: Oil content and fatty acids composition of oil extracted from six oleasters and the two olive cultivar Zarrazi and Chemlali

	Oil %(DW)	14:00	16:00	16:1n-9	16:1n-7	17:00	18:00	18:1n-9	18:1n-7	18:2n-6
OlGab1	9.41±0.01	0.12±0.04	12.33±0.06	0.15±0.08	0.50±0.01		3.10±0.01	63.65±0.02	1.50±0.00	15.93±0.02
OlGab2	8.27±0.05	0.12±0.03	12.01±0.08	0.09±0.01	0.48±0.04	0.09±0.00	3.52±0.01	61.97±0.14	1.45±0.02	17.80±0.01
OlGab3	9.42±0.71	0.12±0.01	12.33±0.15	0.09±0.04	0.50±0.01	0.08±0.01	3.06±0.08	63.60±1.47	1.50±0.28	15.93±1.41
OlGab4	8.04±0.44	0.13±0.01	12.16±0.21	0.08±0.06	0.50±0.02	0.09±0.01	3.06±0.22	61.97±0.28	1.45±0.16	15.93±0.01
OlGab5	9.40±0.28	0.12±0.01	12.01±0.03	0.10±0.01	0.50±0.16	0.09±0.00	3.09±0.04	63.61±0.05	1.45±0.26	17.80±0.02
OlGab6	9.43±0.28	0.12±0.03	12.32±1.36	0.10±0.03	0.50±0.04	0.08±0.00	3.52±0.13	61.97±0.34	1.50±0.14	17.80±0.02
Mean oleasters	8.99±0.29	0.12±0.02	12.19±0.32	0.10±0.04	0.49±0.04	0.08±0.00	3.22±0.08	62.80±0.38	1.47±0.14	16.86±0.25
Chemlali	7.23±0.04	0.08±0.01	10.29±0.11	0.09±0.01	0.56±0.01	0.08±0.01	4.36±0.00	64.39±0.01	0.98±0.02	14.76±0.04
Zarrazi	7.54±0.05	0.09±0.00	9.65±0.01	0. 20±0.00	0.10±0.00	0.09±0.00	4.46±0.01	60.73±0.02	0.89±0.02	20.05±0.01
Mean cultivars	7.38±0.05	0.08±0.00	9.97±0.06	0.14±0.00	0.33±0.00	0.08±0.00	4.41±0.01	62.56±0.02	0.93±0.02	17.40±0.02
F	16.27*	1.38	9.13*	2.40	11.92*	4.44*	73.89*	10.50*	5.24*	22.71*
P < 0.05	0.0004	0.33	0.00	0.12	0.0001	0.04	0.0001	0.002	0.02	0.0001

	20:00	20:1n-9	18:3n-3	22:00	C24:0	SFA	MUFA	PUFA	UFA
OlGab1	0.58±0.01	0.39±0.01	0.50±0.00	0.40±0.07	0.17±0.00	13.60±0.05	65.79±0.11	16.43±0.02	82.21±0.13
OlGab2	0.61±0.00	0.44±0.01	0.46±0.00	0.34±0.01	0.18±0.01	13.34±0.10	63.98±0.07	18.26±0.01	82.24±0.08
OlGab3	0.58±0.03	0.40±0.01	0.52±0.01	0.40±0.03	0.18±0.01	13.67±0.10	65.68±1.73	16.44±1.41	82.12±3.14
OlGab4	0.57±0.01	0.38±0.02	0.47±0.01	0.40±0.17	0.18±0.02	13.52±0.02	63.99±0.20	16.40±0.02	80.39±0.22
OlGab5	0.62±0.01	0.36±0.03	0.47±0.03	0.39±0.04	0.17±0.03	13.39±0.11	65.69±0.36	18.27±0.05	83.95±0.41
OlGab6	0.58±0.08	0.37±0.01	0.48±0.03	0.43±0.02	0.17±0.01	13.69±1.27	64.07±0.18	18.28±0.01	82.35±0.19
Mean oleasters	0.59±0.02	0.39±0.02	0.48±0.01	0.39±0.06	0.17±0.01	13.53±0.28	64.87±0.44	17.34±0.25	82.21±0.69
Chemlali	0.67±0.00	0.40±0.00	0.34±0.00	0.32±0.04	0.16±0.01	11.59±0.13	66.01±0.02	15.10±0.04	81.10±0.01
Zarrazi	0.74±0.00	0.88±0.01	0.45±0.00	0.32±0.01	0.16±0.01	11.04±0.00	61.91±0.00	20.50±0.01	82.41±0.01
Mean cultivars	0.71±0.00	0.64±0.00	0.40±0.00	0.32±0.02	0.16±0.01	11.31±0.07	63.96±0.01	17.80±0.02	81.76±0.01
F	0.76	245.65*	24.37*	6.57*	0.76	10.45*	10.00	22.83*	1.70
P < 0.05	0.64	0.0001	0.0001	0.01	0.63	0.00	0.0001	0.0001	0.24

Each value is the mean±Standard deviation. F: test F. * significant difference at $p < 0.05$

The free radical scavenging activity determined by DPPH varied from 6.12 (OlGab5) to 8.37 mM TEAC (OlGab4) with an average of 6.88 mM TEAC in oleaster stone extracts. The DPPH values have an average of 12.14 mM TEAC in stone from two studied cultivars. The ABTS values have an average of 0.48 and 0.61 mM TEAC in oleaster and cultivar stone extracts, respectively. The ABTS values have an average of 0.48 and 0.61 mM TEAC in oleaster and cultivar stone extracts, respectively.

DISCUSSION

The main source of vegetable fats in the Mediterranean diet is olive oil. The composition of this oil differs from other vegetable oils that are currently consumed in many countries. Olive oil contains high amounts of oleic acid and a smaller amount of linoleic acid. The oleic acid level is one of the parameters characterizing olive oil. As expected, our results showed that the oleic acid is the major fatty acids in stone oleaster oil as the olive cultivars stone oils. Consequently, stone from oleasters would be a source of essential fatty acids required for human health.

It has been reported that the fatty acids composition differences are mainly due to location distribution since the chemical composition of crops varies with the crop cultivars, soil and climatic conditions of the area other than genetic control (Breene *et al*., 2007; Hannachi *et al*., 2007). The PUFA are now well documented to have protective effects against lipid peroxidation (Kratz *et al*., 2002). The oleaster stone oils as the cultivated stone oils were rich in unsaturated fatty acids. The high level of unsaturated fatty acids makes oils desirable in terms of nutrition and this criterion used to valorise new vegetable oils (Nehdi, 2011).

The seed storage proteins are important because they ensure feeding the germinating embryo which enables it to attain the autotrophy. They are also important for the human and animal nutrition providing more than the half of daily protein requirement (Cheftel *et al*., 1985). These proteins were initially classified by Osborne (1924) according to their solubility properties into albumins (water soluble), globulins (saline soluble), prolamins (alcohol soluble) and glutelins (residue). Most of the physiologically active proteins (enzymes) are found in the albumin or globulin groups. Nutritionally, the albumins and globulins have a very good amino acid balance. They

Table 2: Contents of stone soluble proteins and compositions in proteinic classes mg/g DW

	Albumin	Globulin	Prolamin	Glutelin	Total prot
OlGab1	64.88 ± 21.21	97.09 ± 0.05	23.93 ± 0.11	13.61 ± 0.27	199.50 ± 20.90
OlGab2	51.23 ± 34.58	84.25 ± 5.37	30.25 ± 0.82	20.34 ± 1.57	186.07 ± 26.82
OlGab3	65.22 ± 9.26	97.51 ± 0.87	23.76 ± 1.22	13.62 ± 0.12	200.09 ± 11.23
OlGab4	70.40 ± 13.61	87.94 ± 0.05	26.14 ± 6.63	16.99 ± 6.10	201.46 ± 26.39
OlGab5	67.18 ± 36.88	95.50 ± 3.88	26.36 ± 5.18	15.22 ± 0.12	204.25 ± 27.94
OlGab6	74.27 ± 18.17	84.25 ± 12.45	29.58 ± 0.93	13.68 ± 1.45	201.77 ± 28.24
Mean oleasters	65.5 3 ± 22.29	91.09 ± 3.78	26.67 ± 2.48	15.57 ± 1.60	198.86 ± 23.59
Chemlali	28.73 ± 0.11	67.20 ± 0.32	44.89 ± 0.05	3.86 ± 0.05	144.68 ± 0.42
Zarrazi	27.11 ± 0.48	89.29 ± 0.05	19.54 ± 0.05	3.04 ± 0.16	138.98 ± 0.32
Mean cultivars	27.92 ± 0.29	78.24 ± 0.19	32.21 ± 0.05	3.45 ± 0.11	141.83 ± 0.37
F	1.54	7.91*	12.55*	14.01*	3.28
P	0.28	0.005	0.001	0.001	0.06

Each value is the mean ± Standard deviation. F: test F. * significant difference at $p < 0.05$

are relatively richer in lysine, tryptophan, and methionine. Globulins, prolamins and glutelins as storage reserves, are not present systematically in the seeds of all plant species (Bewley and Black, 1983). In the present study, we noted that the globulin is the major protein fraction constituent for all oleaster stones, followed by the albumin, prolamin and glutelin (table 2). The differences between studied stones suggested that the genotype is the major factors to influence the protein fractions constituents which are in agreement with those for most legumes (Vasconcelos *et al*., 2010). In contrast, it has been reported that protein fractions varied according several factors as in *vignera* genus, the extracting method, the cultivars and also the genetic and environment factors (Adsule *et al*., 1986). Indeed, its richness in oil, the oleaster stones were, also, richer in protein with globulins as the major fraction.

It is well known that fruit and vegetables play a key role against numerous cancer and cardiovascular disease (WCRF/AICR, 2007). This protective effect has generally been attributed to different antioxidant constituents, such as flavonoids and phenolic acids.

Polyphenols are important antioxidants that protect the oil against oxygen radicals at the cellular level and due to self oxidation along long shelf storage. Phenols improve olive oil quality due to both organoleptic effect and namely for its sharp bitter taste. Generally, the oxidative stability of olive oil was mainly correlated with the concentration of total phenols (Tura *et al*., 2007). In this study we noted the richness of unexploited oleaster stone in natural antioxidants as polyphenols and flavonoids, hence, the oleaster stones subject of this study constitute a new edible oil source characterized by an important natural antioxidant substance. The research conducted on the chemical composition of cultivated olive oil highlights that the polyphenols are remarkably variable according to the variety, the agronomic conditions, the state of ripeness, and the technology of conservation (Krichene *et al*., 2007). The stone is richer on polyphenols and flavonoids and it is seemed to improve the organoleptic quality and oxidative stability of the olive oil, although the olive seed contributes to the olive oil aroma during the virgin olive oil extraction (Luaces *et al*., 2003).

Free radical scavenging activity, determined by DPPH and ABTS were usually used in several plant extracts (Elfalleh *et al*., 2009; Gourine *et al*., 2010; Nasri *et al*., 2011).

Considering that multiple reaction characteristics and mechanisms involved in the estimation of the total antioxidants, no single method could accurately reflect all the antioxidants in a mixed system due to the complex nature of phytochemicals (Chu *et al*., 2000). Currently, the ABTS method was used to confirm the results obtained on the DPPH test since both methods are based on a similar antioxidant mechanism and the extracts used in both tests were methanol-soluble.

The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential. In addition, synergism between the antioxidants in the mixture makes the antioxidant capacity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants (Djeridane *et al*., 2006).

Several components of fruits and vegetables have been demonstrated to enclose high antioxidant activity, e.g. ascorbic acid, tocopherol, b-carotene, flavonoids, and phenol compounds (Xu *et al*., 2009). A phenol extract of high hydroxytyrosol content obtained from olive leaves (*Olea europaea* L.) increased the oxidative stability of different food lipids (butter, lard and cod liver oil) (De

Table 3: Polyphenosl, flavonoids contents and DPPH and ABTS scavenging activities of oleaster and two olive cultivar stone extracts

	Polyphenols mg GAE/100 g DW	Flavonoids mg RE/100 g DW	DPPH mM TEAC	ABTS mM TEAC
OlGab1	178.77 ± 1.08	12.05 ± 0.07	6.22 ± 0.31	0.60 ± 0.001
OlGab2	123.52 ± 0.71	11.46 ± 0.70	8.15 ± 0.21	0.31 ± 0.001
OlGab3	178.00 ± 1.53	12.05 ± 0.17	6.22 ± 2.04	0.58 ± 0.18
OlGab4	123.20 ± 1.26	11.47 ± 1.69	8.37 ± 0.22	0.31 ± 0.14
OlGab5	124.60 ± 0.71	12.37 ± 0.47	6.12 ± 0.76	0.48 ± 0.25
OlGab6	178.76 ± 0.34	12.06 ± 1.33	6.22 ± 1.73	0.58 ± 0.33
Mean oleasters	151.14 ± 0.94	11.91 ± 0.74	6.88 ± 0.88	0.48 ± 0.15
Chemlali	212.19 ± 6.56	13.14 ± 2.15	14.11 ± 0.15	0.62 ± 0.001
Zarrazi	761.83 ± 1.97	22.33 ± 0.08	10.18 ± 3.28	0.60 ± 0.001
Mean cultivars	487.01 ± 4.27	17.73 ± 1.11	12.14 ± 1.72	0.61 ± 0.001
F	13968.98*	21.45*	6.72*	1.21
P	0.0001	0.0001	0.008	0.40

GAE: Gallic acid equivalents Each value is the mean ± Standard deviation. F: test F.
*significant difference at $p < 0.05$. DW: dry weight

Leonardis *et al*., 2008), showing the increasing interest in the use of natural antioxidants. The results in the present study showed that oleaster stone extracts presented antioxidant activity reflecting their richness on natural antioxidants. Therefore, the oleaster olive trees seemed to be valorised not only by its fatty acid profile but also by its richness on natural antioxidants. It was previously anticipated that the chemical components and bioactivity of the African wild olive might differ from that of the European olive leaves due to geographic differences (Hutchings *et al*., 1996). Relatively little is known about phyto-chemicals of oleaster stone; this work offers some indication of the chemical potentialities of oleasters stone as new source of natural components which would be tested to some pharmaceutical assay.

CONCLUSION

In this study, oil extracted from oleaster stones displayed good fatty acids profile as cultivars. Therefore, they constitute important oil resources which would be used in cosmetic and/or pharmaceutical products. The oleaster stones were richer in soluble proteins which the globulins as the major fraction followed by the albumin, the prolamin and the glutelin fractions. The oleaster stone seem to be valuable because its richness in polyphenols and flovonoids content and characterizing by antioxidant activities. All these parameters suggested qualifying the oleaster stone as a valuable oil and natural source of antioxidants.

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