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K. Bouarroudj, A. Tamendjari, Romain Larbat. Quality, composition and antioxidant activity of Algerian wild olive (*Olea europaea* L. subsp. *Oleaster*) oil. *Industrial Crops and Products*, 2016, 83, pp.484-491. 10.1016/j.indcrop.2015.12.081 . hal-01351526

HAL Id: hal-01351526

<https://hal.science/hal-01351526>

Submitted on 23 Aug 2023

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1 **Quality, composition and antioxidant activity of Algerian wild olive (*Olea europaea L.***
2 ***subsp. Oleaster*) oil**

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20 **Quality, composition and antioxidant activity of Algerian wild olive (*Olea europaea L.***
21 ***subsp. Oleaster*) oil**

22
23 **Abstract**

24 Wild olive trees, also called oleasters, are largely distributed all around the Mediterranean
25 basin. The objective of this study was to evaluate the quality, the composition and the
26 antioxidant activity of four Algerian oleaster oils in comparison to an extra virgin olive oil
27 (EVOO) reference. Three of the four oleaster oils exhibited a composition in accordance to
28 the extra virgin oil category. The total phenol, *ortho*-diphenol and tocopherol contents were
29 equal to higher in the oleaster oils, than in the EVOO reference. Moreover, all the oleaster oils
30 exhibited higher antioxidant properties than the EVOO reference. Interestingly, the phenolic
31 composition of the oleaster oils differed greatly between samples. Two compounds usually
32 not described in olive oil were identified, namely eriodictyol and naringenin. Principal
33 component analysis (PCA) applied to tocopherol and phenolic compounds showed clear
34 variability between oil samples. Taken together, these results highlight the high potential of
35 oleaster oil as a phytochemical resource, a possible alternative food and a genetic resource to
36 improve the olive oil quality.

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39 **Keywords:** *virgin olive oil; wild olive; composition; phenolic compounds; antioxidant*
40 *activity.*

41 **1. Introduction**

42 The olive tree and oleaster are two subspecies *europaea* of *Olea europaea* which are naturally
43 distributed all around the Mediterranean Basin. They form a complex set of cultivated (*Olea*
44 *europaea* subsp. *europaea* var. *europaea*) and wild forms (*Olea europaea* subsp. *europaea*
45 var *sylvestris*) (Breton et al., 2006). The oleasters reproduce sexually by wind pollination and
46 their seeds are mainly dispersed by birds (Baltoni et al., 2006).

47 Oleasters differ from the cultivated olive trees by the presence of spinescent juvenile shoots,
48 smaller fruits characterized by a less fleshy mesocarp and a higher stone/mesocarp ratio,
49 relatively low oil content, a longer juvenile stage, and a higher ability to survive in harsh
50 environments (Terral and Arnold-Simard, 1996). The oleaster resources aroused a
51 considerable interest to develop and select new olive cultivars with superior oil quality
52 (Baccouri et al., 2010).

53 Several studies reported benefic effect of olive oil from cultivars on the human health, but
54 little is known about oleaster oil (Hannachi et al., 2013). Olive oil is the main source of lipid
55 in the Mediterranean diet (Tanjour, 2014). It is considered as the most useful edible oil in the
56 world due to its good characteristics such as its fine aroma and pleasant taste, and its high
57 resistance to oxidation. It was also reported that the olive oil has no adverse effect on the
58 human body, due to the absence of cholesterol (Hannachi et al., 2013).

59 Recent studies have reported that oleaster oil presents higher contents of antioxidant and oleic
60 acid than oil from cultivars (Dabbou et al., 2011). Belarbi et al. (2011) reported that the
61 consumption of oleaster oil has improved the plasma lipid profile of healthy volunteers.
62 Oleaster oil is also reported as an antidote to some poisons and is a good hair lubricant
63 (Bammi and Douira, 2002).

64 Algeria has important olive resources that are not valorized. *Olea europea* species occupies as
65 much as 2.3 % of the total cultivated surface of Algeria. Algerian oleiculture is divided into 3

66 zones: West, center (Kabylie) and East (Abdul Hussain and Abdul Hussain, 2004). Vast
67 uncultivated areas are occupied by oleaster in Bejaia (East center).

68 The present study was conducted to compare the oil composition and the antioxidant activity
69 of four oleaster olive oils from the province of Bejaia with a commercial extra virgin olive.
70 For this purpose we investigated several oil quality indices together with the composition in
71 fatty acids, tocopherols and phenolic compounds and the antioxidant activities of the four
72 oleaster oils.

73 **2. Materials and methods**

74 **2.1 Plant material**

75 Four Algerian oleaster populations originating from Bejaia (Tifra, Adekar, Sidi-Aich and
76 Tazmalt) were harvested between mid-December 2011 and early January 2012. Olives from
77 the Chemlal variety were purchased from the oil mill and were used as control.

78 The olive harvest has been done by hand from adult oleaster trees which were selected,
79 regarding their fruit load and size and the shape of their leaves. This feature allows
80 differentiating oleaster from cultivated olive tree.

81 **2.2. Oil extraction**

82 The oil extraction was performed at the Experimental Farm of the Technical Institute of Fruit-
83 bearing Arboriculture and the Vine (TIFAV), using oleodoseur (Levi-Dilon-Lerogsame)
84 equipped with a centrifuge divider (3000 rpm). Four and a half to five kilograms of olives
85 were crushed with a hammer mill and slowly mixed for 30 min in two steps, 15 min without
86 addition of water, and 15 min after addition of warm water at 30 ± 1 °C (50 mL for 920 g of
87 olive paste). The resulting paste was centrifuged to extract the oil. The oil was decanted,
88 transferred into dark glass bottles, and stored in the dark at 4°C until analysis. The extra virgin
89 olive oil (EVOO) reference was extracted from Chemlal olives with the same process than
90 oleaster oil.

91 **2.3. Quality indices**

92 The free acidity, the peroxide value and the UV spectrophotometric indices (measured at 232
93 and 270 nm) were determined according to the European Union standard methods
94 (Commission Regulation (EEC) no. 2568/91).

95 **2.4. Fatty acid composition**

96 The analytical method for the determination of fatty acid composition was described in
97 regulation to the European Union standard methods (Commission Regulation (EC) No
98 796/2002 of 6 May 2002). Fatty acids were converted to fatty acid methyl esters (FAME) by
99 shaking a solution of 0.5 g of oil in 5 mL of hexane to which were added 0.5 mL of a
100 methanolic potassium hydroxide solution (2 N). The mixture was stirred for 30 sec and then
101 centrifuged at 3000 rpm for 5 min. A hundred microliters of the supernatant were removed
102 and mixed with 1 mL of hexane.

103 One microliter of the methyl esters mixture was analyzed on a Gas Chromatograph
104 (Chrompack C 9002) with a capillary column (30 m × 0.32 mm; the film thickness 0.25 μm)
105 operated at 200°C. The temperature of the injector and the flame ionization detector were
106 maintained at 250°C. The carrier gas was nitrogen.

107 **2.5. Tocopherol analysis**

108 Tocopherols were extracted using a method adapted from Bele et al. (2013). Hence, 50 mg of
109 oil were diluted in 1 mL of hexane. After a 10 min centrifugation at 5000 rpm, the samples
110 were filtered on a 2 μm filter. β-γ and δ-tocopherols were determined from this solution,
111 whereas α-tocopherol was determined on a solution diluted 20 times in MeOH / Hexane (80 /
112 20, v / v). Ten microliters of sample were analyzed on HPLC-MS using the analytic method
113 extensively described in Nonviho et al. (2015).

114 **2.6. Extraction of the phenolic fraction**

115 Extraction was carried out according to the method described by Olivier et al. (2004). Ten
116 grams of oil were dissolved in 10 mL of MeOH / H₂O (80 / 20, v / v). The mixture was
117 vortexed and centrifuged at 3800 rpm for 15 min. The polar fraction was transferred in a 50
118 mL volumetric flask. The extraction was repeated three times and the volume was made up to
119 50 mL with MeOH / H₂O (80 / 20, v / v).

120 **2.7. Total phenol content**

121 Total phenol content was determined using the Folin-Ciocalteu method. In a 20 mL
122 volumetric flask, a volume of 0.5 mL of Folin-Ciocalteu reagent was added to 2 mL of the
123 phenolic extract. After 3 min, 4 mL of a sodium carbonate solution (10 %) was added, and the
124 total volume was adjusted with distilled water to 20 mL. After 90 min of incubation in the
125 dark, the solution was centrifuged, and the absorbance was read at 765 nm. The total phenol
126 content was expressed in mg equivalent of gallic acid per kilogram of oil (mg GAE / kg;
127 Favati et al., 1994)

128 **2.8. Colorimetric determination of *ortho*-diphenols**

129 Four milliliters of phenolic extract were dissolved in 1 mL of solution of sodium molybdate
130 dihydrate (5 %) in ethanol/water (1 : 1). The mixture was shaken vigorously. After 15 min,
131 the absorbance was measured at 370 nm and the results expressed using a caffeic acid
132 calibration curve (Mateos et al., 2001).

133 **2.9. Chromatographic analysis of phenolic fraction**

134 Compounds contained in the phenolic fraction were separated on a U-HPLC system
135 (Prominence, Shimadzu, Japan) composed by a binary solvent delivery pump connected to a
136 diode array detector and a MS spectrophotometer. One microliter of phenolic extract was
137 separated on a C18 Kinetex (100 mm × 2.1 mm) column (Phenomenex, USA) by using a
138 gradient elution from 10 to 70 % MeOH for 9 min, then 100 % MeOH for 2 min with a flow
139 rate of 500 μL min⁻¹. The column was equilibrated to 10 % MeOH for 2 min prior to the next

140 run. Compound identification was realized by comparing the retention time, the absorbance
141 maxima and the MS spectra to authentic standards whenever available. When no standard was
142 available, an identification was proposed based on the comparison of the MS spectra to
143 metabolomics databases (ReSpect: <http://spectra.psc.riken.jp>, Mass Bank:
144 <http://www.massbank.jp>) and to the available literature (Cardoso et al., 2005; Torre-Carbot et
145 al., 2005; Toth et al., 2015). Compound quantification was based on six-point standard curves
146 measured at 280 nm for tyrosol (0.54 – 18 µg / mL) and oleuropein (1.5 – 50 µg / mL), at 300
147 nm for *p*-coumaric acid (0.16 – 16 µg / mL) and at 350 nm for luteolin (0.28 – 28 µg / mL),
148 apigenin (0.27 – 27 µg / mL), naringenin (0.27 – 27 µg / mL) and eriodictyol (0.28 – 28 µg /
149 mL). All the analytical curves were linear ($r^2 = 0.99$). Phenolics for which no standard was
150 available, were expressed relative to tyrosol (hydroxy-tyrosol), oleuropein (DHPEA-EDA,
151 hydroxy-oleuropein aglycon, methyl oleuropein aglycon, oleuropein aglycon, Ligstroside
152 aglycon isomers) and luteolin (methoxy-luteolin). Limit of detection (LOD) and limit of
153 quantification (LOQ) for tyrosol were respectively 0.1 µg / mL and 0.3 µg / mL. LOD and
154 LOQ for oleuropein were respectively 0.5 µg / mL and 1.5 µg / mL. LOD and LOQ for the
155 other standards were respectively 0.05 µg / mL and 0.15 µg / mL.

156 **2.10. Bitter index K_{225}**

157 Bitter index was carried out according the method described by Inarejos-Garcia et al. (2009).
158 One gram of oil was dissolved in 5 mL of hexane, and then was extracted with 5 mL of
159 MeOH / H₂O (60 / 40, v / v). The mixture was vortexed and centrifuged at 3500 rpm for 10
160 min. After the removal of the hexane layer, the polar fraction was transferred in a 10 mL
161 volumetric flask and the volume was made up to 10 mL with MeOH / H₂O (60 / 40, v / v). An
162 aliquot of this solution (1.25 mL) was diluted to 5 mL with the same solvent (MeOH / H₂O 60
163 / 40, v / v). The absorbance was recorded at 225 nm. Bitter index K_{225} was calculated from the
164 equation: $K_{225} = A_{225} / C$, where $C = \text{g oil} / 100 \text{ mL}$, $A = \text{absorbance at } 225 \text{ nm}$.

165 **2.11. Antioxidant activities**

166 **Radical Scavenging Activity Using DPPH**

167 The radical-scavenging activity was determined using the method described by Amro et al.
168 (2002). The result of radical scavenging activity was expressed as mg gallic acid equivalent
169 per kilogram of oil (GAE / kg)

170 **Radical scavenging activity against the radical ABTS⁺**

171 The radical scavenging activity against the radical ABTS⁺, was determined according to the
172 method of Re et al. (1999). ABTS⁺ was generated by the oxidation of ABTS with potassium
173 persulfate. Prior to assay, the ABTS⁺ stock solution was diluted with ethanol to an absorbance
174 of 0.700 ± 0.020 at 734 nm. Then 990 μ L of a diluted ABTS⁺ solution was mixed with 10 μ L
175 of the test sample and the absorbance was measured at 734 nm after 30 min. The radical
176 scavenging activity was expressed as mmol Trolox / kg of oil.

177 **β -carotene bleaching assay**

178 This antioxidant assay was evaluated according to the method described by Gorinstein et al.
179 (2003) with slight modification. The method involves the oxidative degradation of β -carotene
180 in the presence of linoleic acid. An emulsion was prepared by mixing together 0.2 mg of β -
181 carotene in 0.2 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween 40. After
182 evaporation of chloroform, 40 mL of H₂O₂ were added to the emulsion. Four milliliters of the
183 emulsion was mixed with 0.2 mL of oil extracts. The tubes were placed at 50 °C in a water
184 bath. A blank emulsion was prepared as previously mentioned without β -carotene. A control
185 was prepared replacing the extract with methanol. The absorbance at 470 nm was measured
186 from time 0 ($t = 0$) and each 15 min until the color of β -carotene disappeared in the control
187 tubes ($t = 180$ min). The antioxidant activity (AA) of the extracts was evaluated in terms of
188 bleaching of the β -carotene using the following formula, $AA = 100 [1 - (A_0 - A_t) / (A^{\circ}_0 - A^{\circ}_t)]$,

189 where A_0 and A°_0 are the absorbance values measured at zero time of the incubation for test
190 sample and control, respectively, and A_t and A°_t are the absorbance measured in the test
191 sample and control respectively, after incubation for 180 min.

192 **2.12. Statistical analysis**

193 All the results are reported as the mean values ($n = 3$) and were subjected to analysis of
194 variance using the Statistica 5.0 package (StatSoft'97 edition) using the least significant
195 difference (Newman-keuls) test. Significance was defined at $p < 0.05$.

196 Principal component analysis (PCA) was applied to oil samples according to their values of
197 tocopherol and phenolic compounds. The analysis was carried out using XLSTAT 2009.1.02.

198 **3. Results and Discussion**

199 **Quality Indices**

200 All the oleaster oils, exhibited quality indices within the ranges established for “extra virgin”
201 olive oil (EVOO) category (Table 1). Free acidity is considered an important quality indice,
202 which has been used exclusively as a traditional criterion for classifying olive oil. The free
203 acidity of the oleasters oils did not exceed the upper limit of 0.8 % established by the IOOC
204 (IOOC, 2003) norm, and corresponding to the EVOO class. The four oleasters oils exhibited
205 significant differences with regard to their free acidity (from 0.20 to 0.37 %), moreover they
206 all had lower value than the EVOO reference oil.

207 The four oleasters presented lower free acidity values than oils from Turkish varieties (from
208 0.5 to 1.17 %) previously studied (Tanilgan et al., 2007). On the contrary, they compared with
209 free acidity values found in Tunisian oleasters oil (from 0.17 to 0.34 %) (Dabbou et al., 2011).

210 The peroxide value and UV characteristics are two main analytical parameters that evaluated
211 the oxidation state of the oil. As showed in Table 1, all the oleaster oils presented peroxide
212 values within the range of the Extra Virgin Olive (EVOO) category. The peroxide indice of

213 these oleasters oil were lower than those found in some Tunisians oleaster oils (Dabbou et al.,
214 2011).

215 UV spectrophotometric characteristics were expressed by measuring the specific extinction
216 coefficients at 232 and 270 nm corresponding to the maximum absorbance of hydroperoxides
217 and secondary products of oxidation. All the oleaster oils studied presented values of K_{232} and
218 K_{270} (from 1.69 to 1.79, and from 0.048 to 0.17 respectively) within the range of EVOO
219 category (Table 1).

220 Oleasters 1 and 2 exhibited similar K_{232} and K_{270} than the EVOO reference. By contrast,
221 oleasters 3 and 4 had significantly smaller K_{232} and higher K_{270} values than the EVOO
222 reference. The coefficients of specific extinction of our oleasters oils were similar to those of
223 Tunisia oleasters, (K_{232} from 1.61 to 2.36 and K_{270} from 0.12 to 0.17; Dabbou et al., 2011).

224 **Fatty acid composition**

225 The four oleaster oils exhibited a fatty acid composition qualitatively comparable to the
226 commercial EVOO reference, with palmitic, oleic and linoleic acids as the major fatty acids,
227 while palmitoleic, stearic, linolenic and arachidic acids were present in smaller amounts
228 (Table 2).

229 The analyzed oleaster oils showed fatty acid composition (Table 2) in compliance with
230 established limits by IOOC (2003), except for oleaster 4. However, the fatty acid composition
231 was specific for each oil sample. The oil of oleaster 4 distinguished from the other oleaster
232 oils by a low proportion of palmitic and palmitoleic acids (9.16 and 0.36 respectively; Table
233 2) and the presence of arachidonic (C20 : 0) and gadoleic acids (C20 : 1). The percentage of
234 saturated fatty acids (SFA) of the studied oleaster oils was also calculated and they show a
235 variation between samples and their geographical origin, and also between oleasters and
236 commercial EVOO. Moreover, the oil of oleaster 4 exhibited a ratio of unsaturated on saturated
237 fatty acids (UFA / SFA) comparable to the one of the commercial EVOO and much higher

238 than the other oleaster oils (Table 2). Within the unsaturated fatty acids, the proportion of
239 oleic (C18 : 1) and linoleic acid (C18 : 2) distinguishes oleasters 2 and 4, that exhibited a
240 higher C18 : 1 / C18 : 2 ratio than oleasters 1 and 3 (Table 2).

241 Linolenic acid ranged from 0.65 % for oleaster 2 to 1.2 % for oleaster 4 which exceeds 1 %
242 limit established by the IOOC (2003). An excess of the linolenic acid proportion has been
243 previously reported for Moroccan varieties (El Antari et al., 2003a). Also Anwar et al. (2013)
244 noted a minor discrepancy in olive oil from wild Pakistan olives with respect to the standard
245 olive oil composition (linoleic acid slight exceeding 1%). The oil of oleaster 1 is characterized
246 by the highest percentage of palmitic acid (15.4 %), compared to other oleasters.

247 The stearic acid, reported as a marker of varietal characterization by El Antari et al. (2003a),
248 distinguished the oil of oleasters 2 and 4 (3.68 and 3.38 % respectively) from the other oils
249 (oleaster and EVOO reference).

250 **Tocopherols**

251 The total tocopherol content varied from 87 to 182 mg / kg (Table 3). The oils from
252 commercial EVOO and the oleaster 2 exhibited the lowest concentrations, whereas oleaster 1,
253 3 and 4 had significantly higher concentrations (Table 3). Tocopherols play a key role in
254 preserving oil from rancidity during storage. Moreover, α -tocopherol, classically termed
255 vitamin E, constitutes a beneficial molecule for human health (Huang and Sumpio, 2008).
256 According to Baldioli et al. (1996), the total tocopherol content in good quality oils is
257 generally higher than 100 mg / kg. Regarding this indicator, all the oleaster oil tested, with the
258 exception of oleaster 2, could be classified as good quality oils. Regarding the proportion of
259 the different tocopherol isomers, α -tocopherol was the major one, ranging from 90 %
260 (commercial EVOO) to 98 % (oleaster 3) of the total content (Table 3). The δ -isomer was the
261 less represented with a proportion below 1 % in all five oil. The isomers β and γ , which
262 couldn't be distinguished since they were eluted at the same time, represented a part ranging
21

263 from 1.6 % (oleaster 3) to 10 % (commercial EVOO). These proportions are in accordance
264 with those classically found in virgin olive oil.

265 **Total phenol content**

266 The amount of phenolic compounds in EVOO is an important factor when evaluating its
267 quality, given that the natural phenols improve its resistance to oxidation, and to a certain
268 extent, are responsible for its sharp bitter taste (Gutierrez and Arnaud, 2001). The results of
269 the colorimetric determination of total phenol content in oil extracts ranged between 135 mg
270 GAE / kg (commercial EVOO) and 202 mg GAE / kg (oleaster 3). As seen in Figure 1-A
271 significant difference were observed ($p < 0.05$) between the samples. In particular, all the
272 oleaster oils had significantly higher amount of total phenol content than commercial EVOO.

273 Total phenol content of the oleaster oil samples in this study were higher than those recorded
274 in Pakistani oleaster oils (from 23.6 to 92.4 mg GAE / kg; Gulfraz et al., 2009).

275 The obtained values of *ortho*-diphenols (Figure 1-B) varied between 25 mg CAE / kg
276 (commercial EVOO reference) and 81 mg CAE / kg (oleaster 3).

277 The *ortho*-diphenol assay led to the same conclusions as the total phenol content, in the fact
278 that all the oleaster oils displayed significantly higher amounts of *ortho*-diphenols than the
279 reference EVOO.

280 **Phenolic composition**

281 A list of the main phenolic compounds found in the different olive oil, together with their
282 concentrations is given in the Table 4. The phenolic compounds could be classified into four
283 classes, namely the free phenolic alcohols (hydroxy-tyrosol, tyrosol), free phenolic acids (p-
284 coumaric acid), secoiridoid derivatives (DHPEA-EDA, ligostroside and oleuropein
285 derivatives) and flavonoids (eriodictyol, luteolin, naringenin, apigenin, methoxy-luteolin).
286 Whereas almost all these compounds could be found in all the oil samples, the concentration
287 of each compound varied significantly between them, leading to specific phenolic

288 composition for all the five oils studied (see Supplemental Figure 1). Indeed, free phenolic
289 alcohol concentrations ranged from 5.8 mg / kg in oleaster 3 to 115 mg / kg in oleaster 2.
290 Phenolic acids represent a minor part (around 1 mg / kg) of the phenolics present in all the
291 five oils. The total concentration of secoiridoid derivatives ranged from 124 mg / kg (oleaster
292 2) to 326 mg / kg (oleaster 3). The distribution of individual secoiridoid is, however, oil-
293 specific. Indeed, the high concentration of secoiridoids observed in oil of oleasters 3 and 1 is
294 mainly explained by a high concentration of a ligstroside aglycon derivative and hydroxy-
295 oleuropein aglycon, whereas their concentration in other secoiridoids is low compared to
296 other oil samples (Table 4). Total flavonoids represented between 1.3 mg / kg (oleaster 2) and
297 17 mg / kg (oleaster 1), with luteolin being the major flavonoid.

298 The proportion of each phenolic class to the total phenolic concentration highlighted that
299 secoiridoids are the main phenolics in all five oils with proportions ranging from 51 %
300 (oleaster 2) to 96 % (oleaster 3) of the total phenolics. This is in accordance with previous
301 studies on Italian olive cultivars (Cioffi et al., 2010). Free phenolic alcohols were the second
302 most represented class of phenolics in oleaster 2 (47.5 %), 4 (15.8 and commercial EVOO
303 (25.2 %), whereas they were only the third class in oleaster 1 and 3.

304 The flavonoids accounted for 0.5 to 5.2 % of the total phenolic concentration, which
305 correspond to the range currently observed in olive oils (Murkovic et al., 2004).

306 The five analyzed olive oils recorded luteolin and apigenin rates ranging from 0.76 to 1.88 mg
307 / kg, and 0.07 to 1.07 mg / kg respectively.

308 In addition to luteolin and apigenin, which are common flavonoids of olive oil, small
309 concentrations of eriodictyol in all the samples, and naringenin, in oleaster 1 and 3 were
310 detected. Since these compounds were identified recently in olive barks and leaves (Toth et
311 al., 2015), to our knowledge, this is the first time that they are reported in olive or oleaster oil.

312 They were also quantified in citrus fruits such as grapefruit, lemon and orange (Robards et al.,
313 1999).

314 Naringenin and naringin are responsible for the bitterness of some fruit juice, mainly orange
315 and grapefruit (Ribeiro and Ribeiro, 2008), and eriodictyol has the ability to reduce bitterness
316 of some molecules, such as caffeine, quinine, amarogentine, paracetameol, denatonium
317 benzoate and salicin (Ley, 2008).

318 Interestingly, the comparison of the relative part of each phenolic class highlighted the
319 similarity between the phenolic composition of oleaster 1 and 3, and between oleaster 4 and
320 the reference olive oil. However the phenolic composition in the oil of oleaster 2 is clearly
321 different with an equivalent distribution of free phenolic alcohol and secoiridoids.

322 The influence of variety on phenol compounds in olive oil was well documented in
323 bibliography. The concentration and composition of phenolic compounds in olive oil are
324 strongly affected by agronomical, biochemical and technological factors such variety (Tura et
325 al., 2007; Baccouri et al., 2008), maturation degree (Baccouri et al.2008), the content of
326 phenolic glycosides initially present in olive and the activity of various endogenous enzymes
327 acting on these glycosides (Servile et al., 2004, Romero-Segura et al., 2012). The amount of
328 endogenous enzymes in olives (beta-glucosidases, L-phenyalanine ammonia lyase,
329 peroxydases and polyphenoloxidase) depends principally on the variety and maturation
330 degree of olives (Salvador et al., 2001). Oleaster is known for its resistance to difficult
331 conditions like hydric stress which could explain the appreciable content of phenols. The
332 work of Baccouri et al., (2008) on olive oil from selected some wild olives concluded that
333 genetic factors influence olive oil quality and mainly phenolic composition.

334 **Bitter index K_{225}**

335 The values of K_{225} ranged between 1.86 and 4.88 (Figure 1-C), commercial EVOO exhibiting
336 the lowest value and oleaster 3 the highest.

337 The values of bitterness intensity displayed a high correlation with the total phenol contents (r
338 = 0.98). The bitterness is seen as a positive attribute to tolerable intensities in virgin olive oil
339 (Inarejos-Garcia et al., 2009). The accumulation of phenols with bitter characters in the olive
340 is a defense mechanism against phytopathogen (Amiot et al., 1989). Since the wild olive tree
341 is subjected to strong abiotic and biotic constraints (Nardi et al., 2010), we can postulate that
342 it may, at least partially, explain the high bitterness intensities recorded for our oleaster oils.

343 **Radical scavenging activity (DPPH test)**

344 The results of the radical-scavenging activity of the methanol extracts expressed as percent
345 inhibition of DPPH radical (Table 5), and mg GAE / kg show that the methanolic extracts
346 have different abilities to scavenge DPPH radical ($p < 0.05$). The extracts of oleaster 3 and 2
347 had the best activities with 207 and 202.61 mg GAE / kg respectively, and inhibited 85.29%
348 and 83.48% of the DPPH radical respectively, On the other hand, the extract of the
349 commercial EVOO had the lowest activity (107.84 mg GAE / kg) with an inhibition of 58.71
350 %. These results are higher than some Turkish olive oil varieties analyzed by Kiralan and
351 Bayrak (2013).

352 There was a linear correlations ($p < 0.05$) between the anti-radical power of the methanolic
353 extracts and the total phenol content ($r = 0.85$) and *ortho*-diphenol content ($r = 0.80$).

354 **Antiradical activity against ABTS radical**

355 The methanolic extracts exhibited distinct capabilities to neutralize the ABTS radical that
356 were highly correlated ($p < 0.05$) to the total phenol content ($r = 0.97$) and *ortho*-diphenol
357 content ($r = 0.92$).

358 Indeed, the methanolic extract of oleaster 3 exhibited the best anti-radical activity against
359 ABTS radical (1.806 mmol Trolox, Table 5) with inhibition of 75 %, (Table 5), follow by the
360 extracts of oleasters 2, 1, 4 and the commercial EVOO (Table 5).

361 Our results were in agreement with those obtained by Kesen et al. (2013) in Turkish with
362 Halhali variety, but lower than the Spanish varieties obtained by Gorinsetin et al. (2003).

363 **Antioxidant assay (AA) using β -Carotene**

364 All the methanolic extracts had the ability to inhibit the degradation of β -carotene with an
365 inhibition ranging from 53 % (commercial EVOO) to 65 % (oleaster 3) (Table 5).

366 Positive correlations ($p < 0.05$) were noted between the total polyphenol content and *ortho*-
367 diphenols with the percentage inhibition of degradation of β -carotene with respective
368 correlation coefficients of 0.79 and 0.76.

369 Gorinstein et al. (2003), have reported a positive correlation ($r = 0.99$) by applying this
370 method on methanol extracts of some Spanish olive oils.

371 **Principal component analysis**

372 Principal component analysis was applied to evaluate the variability between oil samples
373 regarding their tocopherol and phenolic compound composition and concentration. As shown
374 in Figure 2 (A-B), results of PCA indicate that two factors account for 77.20 % of the total
375 variance (F1: 51.44 %, F2: 26.26 %). The first factor was correlated positively with α -
376 tocopherol, total tocopherols, ligstroside aglycone isomer 1, eriodictyol, naringenin, and
377 negatively with methyl-oleuropein aglycon and tyrosol. The second axe was correlated
378 positively with $(\beta + \gamma)$ -tocopherol, δ -tocopherol, DHPEA-EDA, oleuropein aglycon,
379 ligstroside aglycon isomer 2, and negatively with *p*-coumaric acid.

380 The samples are opposed according their rates in α - tocopherol (oleaster 2), $(\beta + \gamma)$ -tocopherol
381 and δ -tocopherol (oleaster 1 and 3).

382 Concerning phenolic compounds, the three groups were opposed according to their
383 concentrations in *p*-coumaric acid, tyrosol, methyl oleuropein aglycon (in oleaster 2),
384 hydroxy-oleuropein-aglycon, methoxy-luteolin, apigenin, luteolin, naringenin, eriodictyol,

385 ligstroside aglycon isomer 1 (in oleaster 1 and 3). Oleaster 4 has a composition close to the
386 EVOO, they are structured in the same group.

387 The individualisation of samples analyzed according to the subjects was the result of high
388 intra-population variability in relation to the parameters analyzed. A clear variability (tree
389 structured groups) was shown not only between oleasters but also between EVOO and
390 oleasters. Oils from wild olive (oleaster) and cultivated olive could be differentiated by the
391 levels of polar component (phenolic compounds) and tocopherol isomers.

392 **Conclusion**

393 In conclusion, our investigation allowed to extensively characterizing the composition and
394 antioxidant properties of four different oleaster oils. Our results confirmed that there is a great
395 variation between oleaster oils in all of the parameters measured. The fatty acid composition
396 of oleaster 4 showed a similar pattern to the EVOO reference regarding notably the balance
397 between unsaturated and saturated fatty acid content, whereas the other oleaster oils contained
398 a higher proportion of saturated fatty acids. Regarding the total content of antioxidant
399 molecules (total phenol, *ortho*-diphenol and total tocopherol), all the oleaster oils (except
400 oleaster 2 for tocopherol) had a higher content than the EVOO reference. The total phenol,
401 and *ortho*-diphenol contents were highly correlated to the antioxidant properties of the oil,
402 implying that the oleaster oils exhibited higher antioxidant properties than the EVOO
403 reference. Interestingly, the phenolic composition of the oleaster oils was highly divergent
404 regarding notably the secoiridoid, flavonoid and phenolic alcohol contents. Taken together,
405 these results highlight the high potential of oleaster oil as a phytochemical resource, a possible
406 alternative food and a genetic resource to improve the olive oil quality. Additional studies are
407 necessary to corroborate these results and to better characterize the Algerian patrimony of
408 wild olives.

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410 **Acknowledgements**

411 This work has been done as part of a farm pilot of the ITAFV. We thank Algerian Ministry of
412 Higher Education and Scientific Research for financially supporting this Program. We thank
413 Mr. F. BEKDOUCHE Researcher professor at the University of Bejaia for his valuable
414 assistance. Part of this work was carried out at the Université de Lorraine-Institut National de
415 la Recherche Agronomique Agronomie Environnement Nancy-Colmar, France.

416

417 **Table list**

418 Table 1: Quality indices of oleaster oils compared to the commercial EVOO (Extra Virgin
419 Olive Oil).

420 Table 2: Fatty acid composition of studied olive oils samples (% m / m
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426 **Figure list**

427 Figure 1: Total phenol, *ortho*- diphenol and bitter index K_{225} in oil samples. Values are means
428 \pm standard deviations (n = 3). ^{a,b,c,d} Different letters indicate significantly different values (p <
429 0.05).

430

431 Figure 2: Principal component analysis (PCA) of the tocopherols and phenolic compounds of
432 olive oil samples. A: Vector Distribution of Tocopherols and Phenolic Compounds. B:
433 Distribution of oil samples with the analyzed compounds.

434

435 Supplemental Figure 1: U-HPLC chromatograms of phenolics from the oleaster and olive oils.
436 The profiles were recorded at 280 nm. (A) oleaster 1, (B) oleaster 2, (C) oleaster 3, (D)
437 oleaster 4 and (E) EVOO reference. (1) Hydroxy-tyrosol; (2) Tyrosol; (3) p-coumaric acid;
438 (4) Eriodictyol; (5) Luteolin; (6) DHPEA-EDA; (7) Ligstroside aglycon isomer 1; (8) 10-
439 Hydroxy oleuropein aglycon; (9) Naringenin; (10) Apigenin; (11) Methoxy-luteolin; (12)
440 Oleuropein aglycon; (13) Methyl oleuropein aglycon; (14) Ligstroside aglycon isomer 2.

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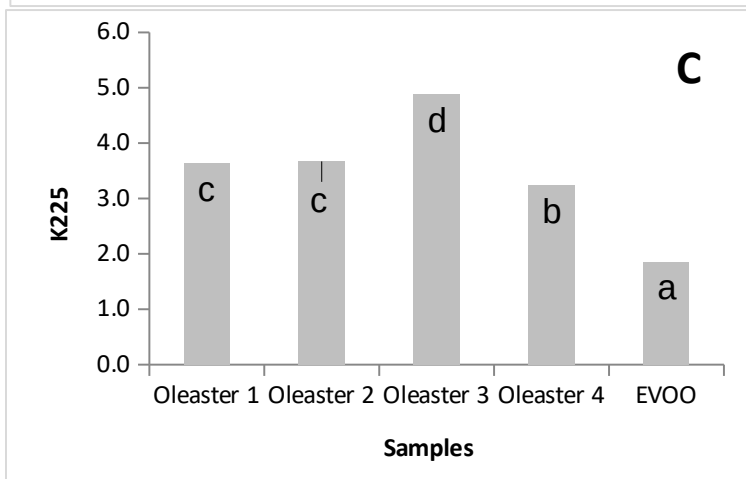
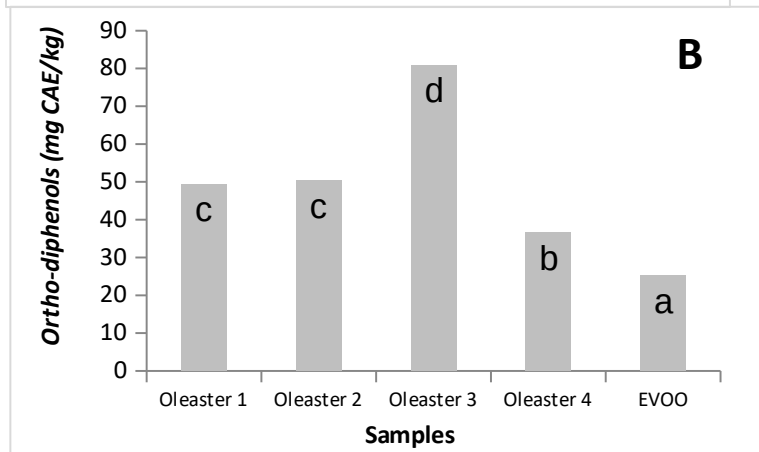
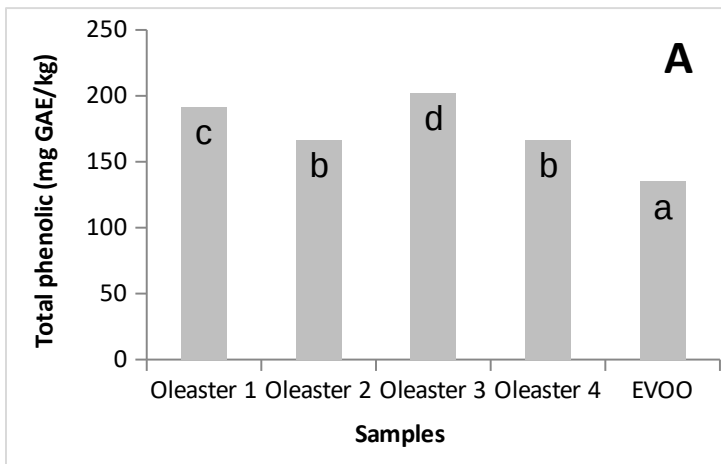


Figure 1: Total phenol (A), *ortho*-diphenol (B) and bitter index K_{225} (C) in oil samples. Values are means \pm standard deviations ($n = 3$). ^{a-d} Different letters indicate significantly different values ($p < 0.05$).

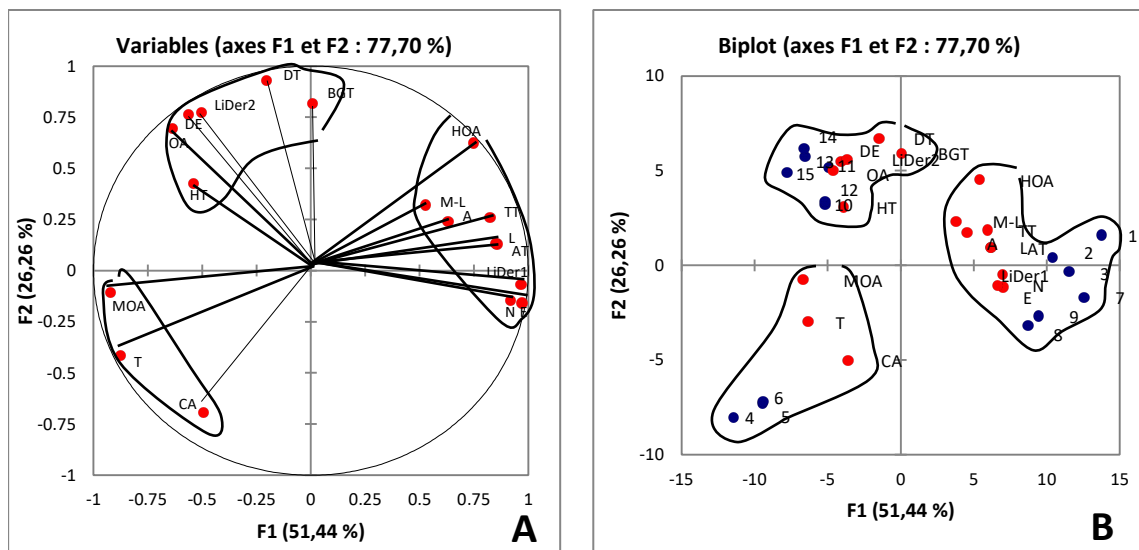


Figure 2: **Principal component analysis (PCA) of the tocopherols and phenolic compounds of olive oil samples.** **A:** Vector Distribution of Tocopherols and Phenolic Compounds. **B:** Distribution of oil samples with the analyzed compounds.

AT: α - tocopherol; **BGT :** ($\beta + \gamma$) tocopherol; **DT:** δ - tocopherol ; **TT:** Total tocopherols ; **HT:** Hydroxy-tyrosol ; **T:** Tyrosol; **CA:** *p*-coumaric acid; **DE :** DHPEA-EDA (dialdehydic form of oleuropein aglycon); **LiDer1:** Ligstroside aglycon isomer1; **HOA:** Hydroxy-oleuropein-aglycon ; **OA :** Oleuropein aglycon ; **E :** Eriodictyol ; **L:** Luteolin ; **N:** Naringenin ; **A :** Apigenin ; **M-L :** Methoxy-luteolin ; **MOA :** Methyl oleuropein aglycon; **LiDer 2:** Ligstroside aglycon isomer 2. Number **1-2-3** (oleaster 1) ; **4-5-6** (oleaster 2) ; **7-8-9** (oleaster 3); **10-11-12** (oleaster 4); **13-14-15** (EVOO).

Table 1: Quality indices of oleaster oils compared to the commercial EVOO (Extra Virgin Olive Oil)

Quality index	Oleaster 1	Oleaster 2	Oleaster 3	Oleaster 4	Commercial EVOO	(EVOO) I.O.C. 2003
Free fatty acids [% oleic acid]	0.25 ± 0.03 ^b	0.31 ± 0.03 ^c	0.20 ± 0.03 ^a	0.37 ± 0.03 ^d	0.42 ± 0.03 ^c	≤ 0,8
Peroxide index [meq O ₂ kg ⁻¹]	6.75 ± 0.25 ^c	5.5 ± 0.29 ^b	3.75 ± 0.75 ^a	7.25 ± 0.25 ^c	7.25 ± 0.25 ^c	≤ 20
K ₂₃₂	1.76 ± 0.01 ^b	1.78 ± 0.01 ^{bc}	1.69 ± 0.01 ^a	1.71 ± 0.01 ^a	1.79 ± 0.02 ^c	≤ 2.5
K ₂₇₀	0.06 ± 0.01 ^a	0.05 ± 0.01 ^a	0.18 ± 0.01 ^b	0.16 ± 0.02 ^b	0.07 ± 0.01 ^a	≤ 0.20

Values are means ± standard deviations (n = 3).

^{a, b, c, d} Different letters in the same row indicate significantly different values (p < 0.05).

Table 2: Fatty acid composition of studied oils samples (% m/m methylic ester).

Fatty acids		Oleaster 1	Oleaster 2	Oleaster 3	Oleaster 4	Commercial EVOO	(EVOO) I.O.C.2003
Palmitic acid	C 16 : 0	15.40	12.98	13.60	9.16	11.13	7.5-20
Palmitoleic acid	C 16 : 1	1.71	1.12	1.02	0.36	1.65	0.3-3.5
Stearic acid	C 18 : 0	2.15	3.68	2.98	3.38	2.62	0.5-5.0
Oleic acid	C 18 : 1	67.98	72.17	64.69	75.40	76.14	55-83
Linoleic acid	C 18 : 2	12.01	8.89	17.02	8.93	10.19	3.5-21
Linolenic acid	C 18 : 3	0.73	0.65	0.66	1.20	0.68	≤ 1.0
Arachidic acid	C 20 : 0	ND	0.48	ND	0.68	ND	≤ 0.6
Gadoleic acid	C 20 : 1	ND	ND	ND	0.45	ND	≤ 0.4
Unsaturate Fatty Acid (UFA)	UFA	82.43	82.83	83.39	86.34	88.66	
Saturate Fatty Acid (SFA)	SFA	17.55	17.14	16.58	13.22	13.75	
UFA/ SFA	UFA/SFA	4.70	4.83	5.03	6.53	6.45	
Oleic acid/ Linoleic acid	C 18 : 1 / C 18 : 2	5.66	8.12	3.80	8.44	7.47	

ND: Not detected.

Table 4: Concentrations of individual phenols (mg / kg of oil) in oil samples determined by U-HPLC.

	Oleaster 1	Oleaster 2	Oleaster 3	Oleaster 4	Commercial EVOO
Hydroxy-tyrosol	1.9 ± 0.6 ^b	10 ± 1 ^c	0.68 ± 0.24 ^a	2.2 ± 0.3 ^b	36 ± 1 ^d
Tyrosol	10 ± 1 ^b	105 ± 2 ^d	5.1 ± 1.7 ^a	47 ± 1 ^c	46 ± 1 ^c
Total Phenol alcohol	12 ± 2 ^b	115 ± 2 ^c	5.8 ± 1.9 ^a	49 ± 1 ^c	82 ± 1 ^d
Phenol acid (<i>p-coumaric acid</i>)	0.66 ± 0.10 ^c	1.5 ± 0.3 ^d	0.34 ± 0.06 ^a	0.64 ± 0.04 ^{bc}	0.39 ± 0.02 ^a
DHPEA-EDA	4.0 ± 0.6 ^a	5.5 ± 0.3a ^b	5.7 ± 1.6 ^b	12 ± 1 ^c	13 ± 1 ^c
Ligstroside aglycone isomer 1	177 ± 28 ^c	10 ± 1 ^a	225 ± 36 ^d	53 ± 6 ^b	30 ± 4a ^b
10-Hydroxy oleuropein aglycone	81 ± 12 ^d	16 ± 2 ^a	69 ± 12b ^d	61 ± 10 ^b	59 ± 9 ^b
Methy-oleuropein aglycone	0	56 ± 14 ^b	0	49 ± 3 ^b	30 ± 1 ^a
Oleuropein aglycone	24 ± 3 ^a	37 ± 5 ^b	27 ± 5 ^a	59 ± 2.12 ^c	90 ± 5 ^d
Ligstroside aglycone isomer 2	0	0	0	24 ± 2 ^b	17 ± 1.59 ^a
Total secoiridoid	286 ± 44 ^c	124 ± 19 ^a	326 ± 55 ^d	260 ± 14b ^c	240 ± 13 ^b
Eriodictyol	0.62 ± 0.09 ^b	0.04 ± 0.01 ^a	0.71 ± 0.12 ^b	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a
Luteolin	13 ± 3 ^c	0.97 ± 0.17 ^a	5.6 ± 1.0 ^b	2.2 ± 0.1 ^a	3.2 ± 0.2 ^{ab}
Naringenin	1.2 ± 0.2 ^a	0	1.4 ± 0.7 ^a	0	0
Apigenin	1.1 ± 0.1 ^c	0.07 ± 0.01 ^a	0.17 ± 0.01 ^c	0.11 ± 0.01 ^b	0.35 ± 0.01 ^d
Methoxy-luteolin	0.94 ± 0.03 ^c	0.24 ± 0.01 ^b	0.51 ± 0.04 ^c	0.12 ± 0.01 ^a	0.81 ± 0.01 ^d
Total Flavonoids	17 ± 3 ^c	1.3 ± 0.2 ^a	8.5 ± 1.8 ^d	2.5 ± 0.1 ^b	4.4 ± 0.2 ^c
Total phenol	316 ± 44 ^b	242 ± 19 ^a	341 ± 58 ^d	312 ± 14 ^b	327 ± 12 ^c

Values are means ± standard deviations (n = 3).

^{a,b,c,d} Different letters in the same row indicate significantly different values (p < 0.05).

Table 3: Total and individual tocopherol concentrations in oil samples (mg / kg of oil)

Samples	α -tocopherol	β and γ - tocopherols	δ -tocopherol	Total tocopherol
Oleaster 1	157 \pm 4 ^b	13.0 \pm 0.4 ^b	0.46 \pm 0.2 ^b	165 \pm 11 ^b
Oleaster 2	85 \pm 4 ^a	2.1 \pm 0.4 ^a	0.13 \pm 0.02 ^a	87 \pm 4 ^a
Oleaster 3	179 \pm 6 ^c	2.9 \pm 1.1 ^a	0.16 \pm 0.06 ^a	182 \pm 7 ^c
Oleaster 4	146 \pm 5 ^b	16.8 \pm 3.2 ^c	0.67 \pm 0.05 ^c	164 \pm 7 ^{bc}
Commercial EVOO	96 \pm 8 ^a	11.0 \pm 2.7 ^b	0.71 \pm 0.09 ^c	107 \pm 9 ^a

Values are means \pm standard deviations (n = 3).

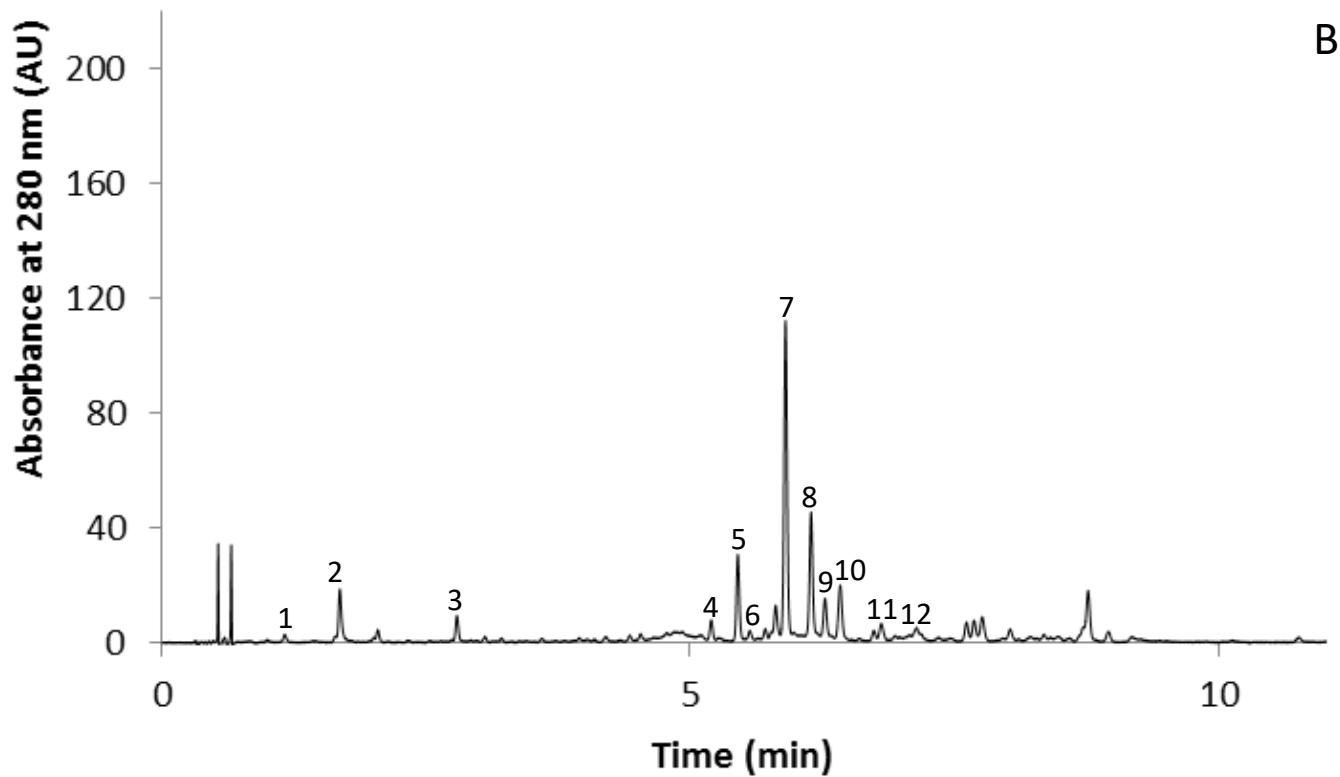
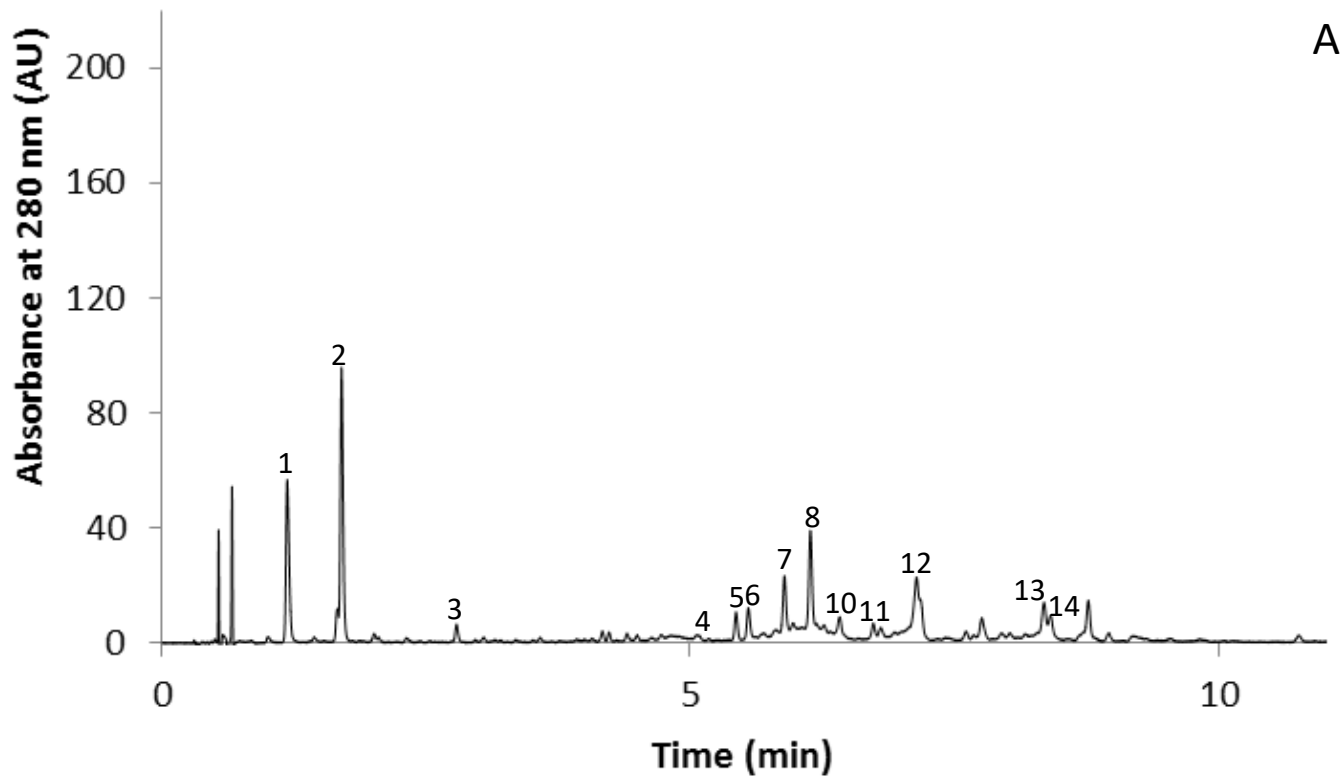
^{a,b,c,d} Different letters in the same row indicate significantly different values (p < 0.05).

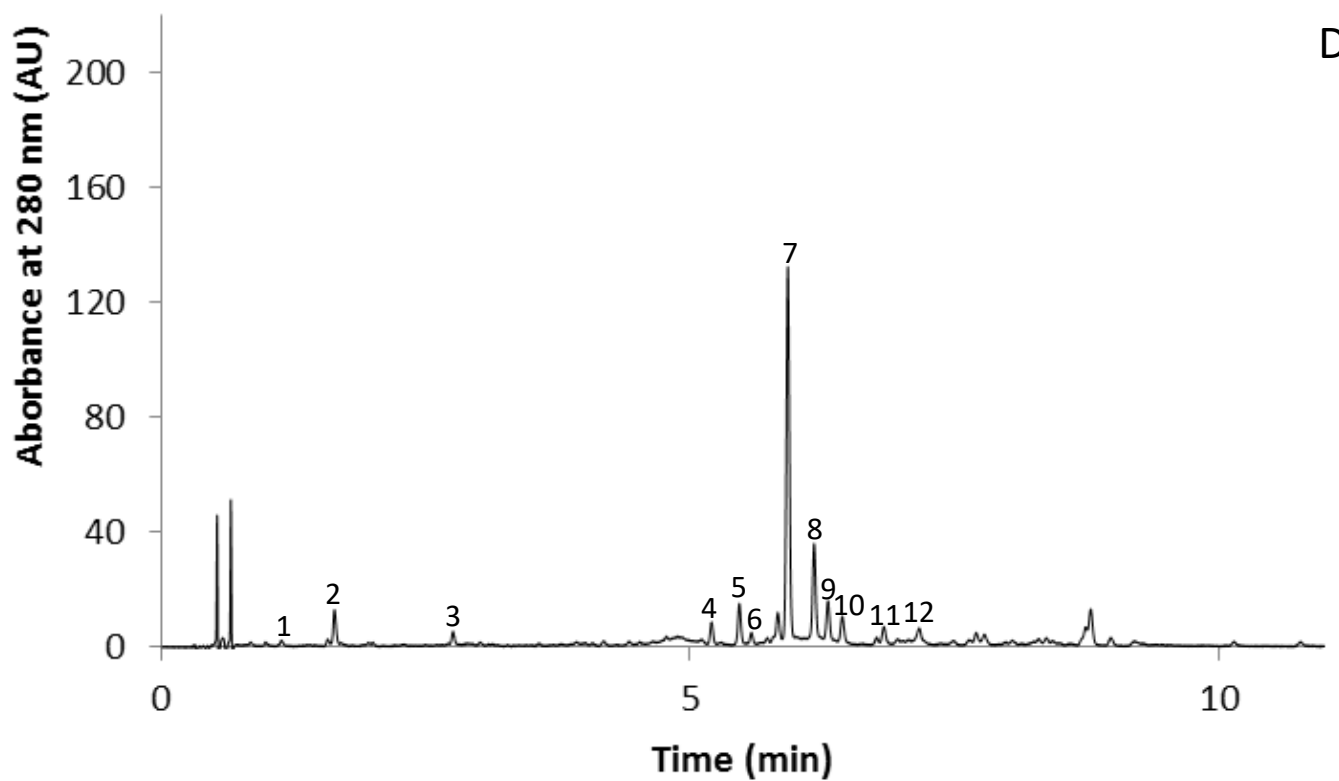
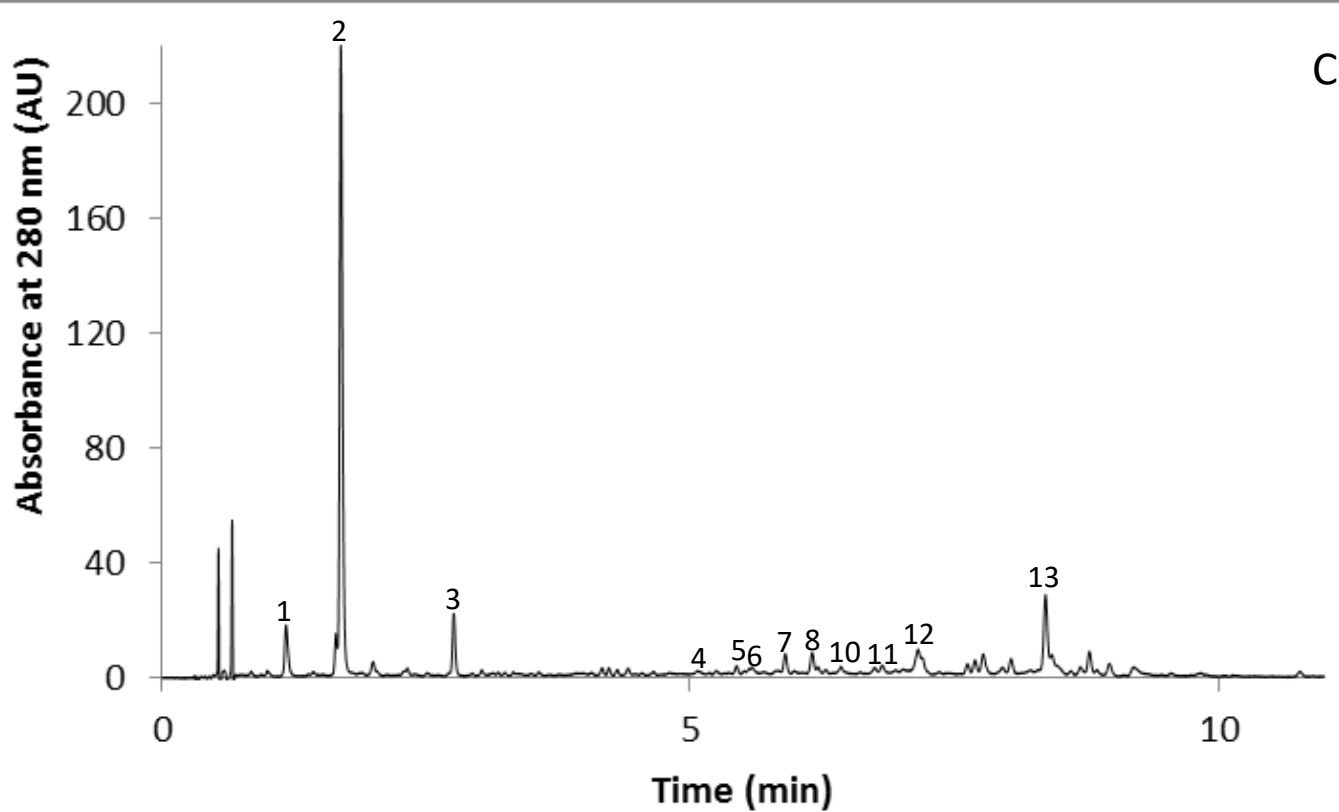
Table 5: Antioxidant activities of oil samples as determined by three different assays.

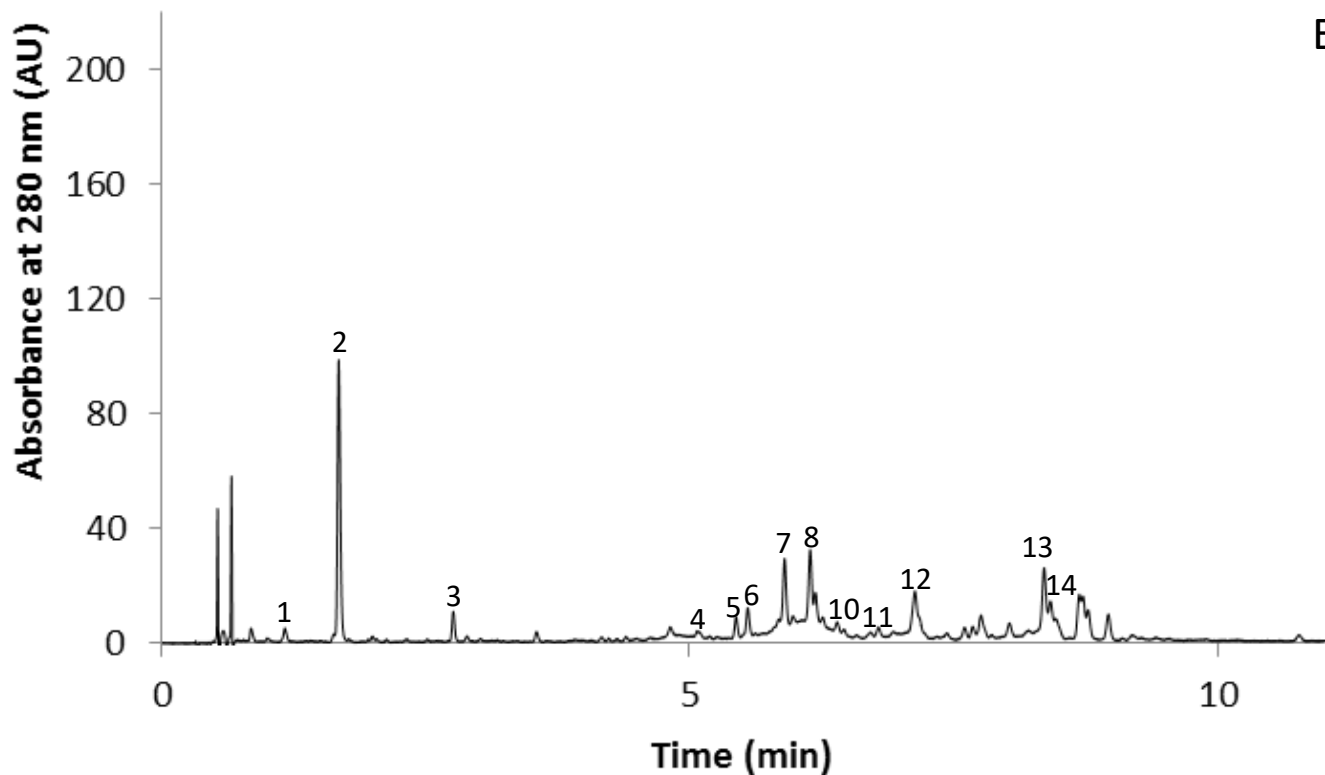
Samples	DPPH (%)	DPPH(mg GAE / kg)	ABTS (%)	ABTS mmol Trolox / kg	β -carotene (%)
Oleaster 1	81,33 \pm 0.44 ^c	197,41 \pm 1.06 ^c	42,09 \pm 1.48 ^b	1,014 \pm 0.036 ^b	64,26 \pm 0.69 ^c
Oleaster 2	83,48 \pm 0.30 ^d	202,61 \pm 0.72 ^d	51,66 \pm 2.43 ^c	1,244 \pm 0.058 ^c	56,70 \pm 2.06 ^{ab}
Oleaster 3	85,29 \pm 0.14 ^e	207,00 \pm 0.35 ^e	75,00 \pm 1.75 ^d	1,806 \pm 0.042 ^d	65,41 \pm 2.24 ^c
Oleaster 4	75,95 \pm 0.44 ^b	184,35 \pm 1.06 ^b	33,57 \pm 0.59 ^a	0,808 \pm 0.014 ^a	58,99 \pm 3.12 ^b
Commercial EVOO	58,71 \pm 0.29 ^a	142,51 \pm 0.69 ^a	30,78 \pm 1.01 ^a	0,741 \pm 0.024 ^a	53,04 \pm 1.10 ^a

Values are means \pm standard deviations (n = 3).

^{a,b,c,d} Different letters in the same row indicate significantly different values (p < 0.05).







Supplemental figure 1: U-HPLC chromatograms of phenolics from the oleaster and olive oils. The profiles were recorded at 280 nm. (A) oleaster 1, (B) oleaster 2, (C) oleaster 3, (D) oleaster 4 and (E) EVOO reference. (1) Hydroxy-tyrosol; (2) Tyrosol; (3) p-coumaric acid; (4) Eriodictyol; (5) Luteolin; (6) DHPEA-EDA; (7) Ligstroside aglycon isomer 1; (8) 10-Hydroxy oleuropein aglycon; (9) Naringenin; (10) Apigenin; (11) Methoxy-luteolin; (12) Oleuropein aglycon; (13) Methyl oleuropein aglycon; (14) Ligstroside aglycon isomer 2