

Research Article

Chemical characterization and antimicrobial potential of two extra virgin olive oils of *Olea europaea* var. *sylvestris* and *Olea europaea* subsp. *laperrinei*

Habiba Boukhebt^{1*}, Sarra Bouchoucha¹, Loubna Amor², Yacine Mouhamadi¹

¹Department Plant Biology and Ecology, Faculty of Natural and Life Sciences, Laboratory of Natural Resources Valorization, Ferhat Abbas Sétif 1 University, Sétif 19000, Algeria

²Department Plant Biology and Ecology, Faculty of Natural and Life Sciences, Ferhat Abbas Sétif 1 University, Sétif 19000, Algeria

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*Corresponding author: Habiba Boukhebt, E-mail: habibaboukhebt@gmail.com

Abstract

The purpose of this research was to investigate a comparative evaluation of the chemical profiles of extra virgin olive oils (EVOOs) obtained from two wild olives such as *Olea europaea* subsp. *laperrinei* from the Sahara and *Olea europaea* var. *sylvestris* from north of Algeria, as well as to assess their antibacterial activities. Gas Chromatography-Mass Spectrometry analysis (GC-MS) was used to identify the constituents of the two EVOOs, and the disc diffusion method was used to evaluate their antibacterial activity against seven bacterial strains. GC-MS analysis revealed 22 components from *O. europaea* var. *sylvestris*, with squalene (27.87%), oleic acid (17.27%), palmitic acid (14.31%) and linoleic acid (6.87%) as major constituents, while 14 components were identified in *O. europaea* subsp. *laperrinei* oil, with palmitic acid (27.98%) is the main compound followed by Linoleic acid (25.99%), oleic acid (17.43%) and squalene (13.43%). Furthermore, unsaturated fatty acids are the predominant fraction in the two EVOOs. The results indicate that the effect of the two EVOOs on positive bacteria is greater than on negative bacteria. In addition, the EVOO from *O. europaea* subsp. *laperrinei* exhibited the highest range of inhibition with all bacterial strains tested, in which *S. aureus* (MRSA), *P. aeruginosa*, *S. aureus* and *B. subtilis* are the sensitive strains, however, *K. pneumoniae* and *E. faecalis* are extremely resistant to the two EVOOs. These results demonstrate the nutritional value and the medicinal properties of EVOOs.

Keywords: Wild olives, *Olea europaea* var. *sylvestris*, *Olea europaea* subsp. *laperrinei*, Extra virgin olive oils (EVOOs), Chemical profiles

Introduction

The olive tree, *Olea europaea* L., considered a crucial fruit tree, which is native to the Mediterranean countries (Šarolić *et al.*, 2013), such as Palestine, Syria, Spain, Italy, Greece, France, Turkey, Algeria and Morocco (Martins *et al.*, 2022), and it has been used historically for important purposes related to nutrition and medicine (Salih *et al.*, 2019). Additionally, a low incidence of diseases and maintenance of health have been linked to frequent use of the two main olive tree products: olive oil and olive fruits. These products include considerable amounts of compounds with health advantages (Hashmi *et al.*, 2015). Furthermore, it has been widely used as a major ingredient in face and body creams, lotions, soaps, and shampoos (Cicerale *et al.*, 2009). This tree represents an extremely significant floristic heritage entity with nutritional agro-economic, environmental, and medicinal importance in Algeria (Arab *et al.*, 2013; Addab *et al.*, 2020).

Olive oil is distinguished by its oxidative stability which allows for extended shelf life with high sensory quality (Hannachi *et al.*, 2013). In addition, it is regarded as one of the most essential components of the “Mediterranean diet”. As part of a balanced diet, and is favored for its flavor and high nutritional content; also, it is the most beneficial edible oil in the world (Espínola *et al.*, 2021). Moreover, regular consumption of olive oil is inversely linked to cardiovascular disease, inflammatory illnesses, and certain cancer types, including breast and pancreatic cancer (Hannachi *et al.*, 2013). In this context, among the olive oils, the extra virgin olive oil (EVOO) is considered

a key component of the Mediterranean diet and serves as its primary source of fat (Servili *et al.*, 2014; Fuentes *et al.*, 2018), which is a pure olive juice, extracted directly from olives. It is regarded as an excellent olive oil quality, with low acidity, and higher sensory features since it has a perfect flavor and aroma (Román *et al.*, 2019; Seçmeler & Galanakis, 2019). In general, EVOO has a median of zero faults and a strong fruity note (Mariotti, 2014).

The chemical profile of EVOOs has been previously studied. It includes several significant chemical components that contribute to their nutritional and biological properties (Jimenez-Lopez *et al.*, 2020). It was found that the chemical composition differed depending on several parameters, such as olive variety, pedoclimatic conditions (Lombardo *et al.*, 2018), storage conditions, timing of harvest and extraction (Jimenez-Lopez *et al.*, 2020). Regarding the chemical composition, EVOOs are mainly formed by triglycerides and other minor compounds. Among the glyceride fraction, it has a high content of monounsaturated fatty acids, such as oleic acid, and some polyunsaturated fatty acids, such as linoleic acid. Concerning the saturated fatty acids composed basically by palmitic and stearic acids. In addition, it must be highlighted the minor compounds, such as phenolic compounds including hydroxytyrosol and tyrosol, as well as hydrocarbons mainly composed by squalene (Jimenez-Lopez *et al.*, 2020). This later can only be detected in this sort of oil, though, because it may be lost during refinement (Lombardo *et al.*, 2018).

All of these compounds are attributed to their nutritional value, as well as to their biological properties

(Šarolić *et al.*, 2013). Antibiotic-resistant human pathogenic microorganisms are a worldwide problem (Hussain *et al.*, 2014), and can be prevented by employing novel plant-based molecules or plant-lead synthetic antimicrobial chemicals (Shinwari *et al.*, 2012; Hussain *et al.*, 2014). The literature revealed that olive oils are greatly effective in inhibiting the activity of both gram-negative and gram-positive bacteria (Medina *et al.*, 2007; Hussain *et al.*, 2014; Boukhebt *et al.*, 2020).

Based on our objectives on the study of wild forms of olive trees, two forms has been inventoried in Algeria, and has been fixed in the present paper, one of which is commonly referred to Oleaster "*Olea europaea* L. var. *sylvestris* [Mill.] Lehr", mainly in the northern region (Breton & Bervillé, 2012), and the other is known as ālew or āliwan among the Tuareg Berbers, it is an endemic subspecies was detected in the central Sahara which is "*Olea europaea* L. subsp. *laperrinei* [Batt.& Trab.] Cif." (Chaker, 2013), and represents a fundamental element of the ecosystem of the Hoggar mountains (Green, 2002; Besnard & Baali-Cherif, 2009). To the best of our knowledge, only one report has been made in Algeria on the chemical profile of Oleaster oil. Despite Oleaster yields high-quality oil, especially for pharmacological applications (Bouarroudj *et al.*, 2016), little attention has been attracted to the production of this oil in Algeria, because it is more expensive (Quézel & Santa, 1962), and has a lower oil yield than cultivated one (Bouarroudj *et al.*, 2016). Furthermore, the present research is the first to investigate the chemical composition of Saharan endemic subspecies olive oil, as well as the antimicrobial potential of the extra virgin olive oils from these two wild species in Algeria. In this respect, the present study aims to valorize their extra virgin oils through their chemical characterization, and the evaluation of their antibacterial activity (Bouarroudj *et al.*, 2016).

Materials and methods

Plant material

The fruits of two wild subspecies were collected during the beginning of November, concerning the subspecies that were collected in the central Sahara Hoggar "Tamanrasset" (23° 21 N, 05°47 E; 1952 m) in the case of *O. europaea* subsp. *laperrinei*, and in the beginning of December for the other subspecies which were sampled from the region of the mountainous massifs of the high plateau of Setif (36° 37 N, 05° 40 E; 814 m) in the case of *O. europaea* var. *sylvestris*.

The samples were identified at the Laboratory of L.V.R.B.N, University of Setif 1, using the identification keys; Quézel & Santa and Ozenda keys (Quézel & Santa, 1962; Ozenda, 1991), and online herbariums (such as ENSA (National Superior School of Agronomy; Algeria) and MPU (Herbarium of North African Vascular Plants of Montpellier; France). Furthermore according to online databases (Tela Botanica and APD «African Plant Database"), the two wild olives were confirmed by Pr. Tarek Hamel (University of Annaba). Reference specimens have been deposited at the herbarium of the Valorization of Natural Biological

Resources Laboratory L.V.R.B.N of the Faculty of Nature and Life Sciences, Ferhat Abbas University. The specimens are registered under the numbers 23/OEL 27 and 23/OES 28.

Extra virgin olive oils isolation

The two extra virgin olive oils were extracted using a traditional method based on cold pressing. The extra virgin olive oils obtained were stored in sealed brown vials at 4 °C until use (Boukhebt *et al.*, 2020).

Chemical analysis of the two extra virgin olive oils

The two extra virgin olive oils isolated were analyzed using Gas Chromatography on a Hewlett Packard G 6890 apparatus, equipped with an aglient mass spectrophotometer with a direct capillary interface and fused silica capillary column Agilent 19091S- 433, HP-5MS 5% Phenyl Methyl Siloxane (30 m x 250 µm x 0.25 µm film thickness) in order to separate and identify the extra virgin oils constituents (all fractions were identified. Not only the fatty acid fraction). Helium was used as a carrier gas (1 mL/min) pulsed splitless mode and the injector size was 10.0 µL/min.). The used system was equipped with an Agilent mass spectrophotometer. The injected volume was 10.0 µL. The mass spectral detector was operated in an electron impact mode with an ionizing energy of 70 eV scanning from *m/z* 35.0 to 550.0. The ion source temperature was 230 °C and the quadruple temperature was 150 °C. The GC temperature program was started at 190 °C for 15 min, and then elevated to 280 °C at a rate for 4 °C/min. The injector and the detector temperatures are set at 250 °C and 280 °C respectively. The compounds were identified by comparing their mass spectra to those of authentic standards, analyzed under the same experimental conditions.

In vitro assessment of the antibacterial activity of the two EVOOs

The disk diffusion method was used to evaluate the antibacterial activity of *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei* extra virgin olive oils. Seven bacterial strains referenced were used to assess the antibacterial activity of the essential oils from the two subspecies. Three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* ATCC 700603), and Four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 43300 and *Enterococcus faecalis* ATCC 51299). All bacterial strains were obtained from Setif University's applied microbiology lab and the hospital-university center. An optical density ranging from 0.08-01 at 625 nm was obtained by preparing the bacterial inoculums from an overnight broth culture in physiological saline (0.8% of NaCl).

Before inoculation, MH agar, also known as Muller-Hinton agar, was put into Petri dishes, solidified, and surface-dried, sterile discs (6 mm) with diluted essential oils (1:10, 1:4, and 1:2 v/v of Hexen), and mother solution

(10 μ L) were placed on the inoculated agar by test bacteria, Hexen was used as a negative control. The antibiotic, Gentamicin, was used as a positive control. Then Petri dishes were incubated at 37 °C for 18 to 24 h aerobically. After the incubation period, the inhibition zones' diameters (mm) were measured around the discs. Means were calculated following the execution of each test in triplicate. The sensitivity to extra virgin olive oils was evaluated according to the diameters (mm) of the inhibition zones (Hussain *et al.*, 2014).

Statistical analysis

The analysis of variance was carried out using "Costat 6.400,1998" Software and the comparison was done at 95% (P=0.05) to determine if there are any significant differences between the results of the antibacterial activity of the two extra virgin wild olive oils.

Results

Chemical composition of the two extra virgin olive oils (EVOOs)

Based on the chromatographic analysis results of the two extra virgin olive oils (EVOOs), 22 components from *O. europaea* var. *sylvestris* oil were detected; these components represent an average of 98.61% of the total oil, while 14 components were identified in *O. europaea* Subsp. *laperrinei* oil making up an overall percentage of 98.61%. Table 1 summarizes the identified compounds and their relative abundances according to their order of appearance.

Chemical profile of *O. europaea* var. *sylvestris* EVOO

The predominant component of *O. europaea* var. *sylvestris* EVOO, was squalene, which had a rate of 27.87%,

followed by oleic acid (17.27%), palmitic Acid (14.31%), linoleic acid (6.87%). Other low concentration compounds with low amounts were detected including apiole (2.81%), tyrosol (2.45%) and stearic acid (1.19%) (Table 1).

Chemical profile of *O. europaea* Subsp. *laperrinei* EVOO

The chemical composition of EVOO obtained from *O. europaea* subsp. *laperrinei*, the most prevalent component was palmitic acid, which accounted for 27.98% of the total oil. Linoleic acid (25.99%), oleic acid (17.43%), squalene (13.43%), β -Sitosterol (5.66%), were the next most dominant components. The remaining constituents, such as palmitoleic acid (2.61%), stearic acid (1.77%), lauric acid (0.24%) and myristic acid (0.23%) were detected in trace amounts (Table 1).

Different fractions of the two EVOOs

The different fractions of the two EVOOs extracted from *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei* are listed in Table 2.

The saponifiable fraction is the predominant fraction of the identified compounds of *O. europaea* subsp. *laperrinei* EVOO contains a total of 76.36%, and within this fraction the unsaturated fatty acids (UFAs) represent a larger proportion (46.03%) than saturated fatty acids (SFAs) (30.33%). In contrast, the unsaponifiable fraction had the lowest amount of compounds (19.54%), with hydrocarbons and sterols being the most prevalent classes with a rate of 13.51% and 5.66% respectively. On the other hand, EVOO from *O. europaea* var. *sylvestris*, showed that the unsaponifiable fraction represented the main fraction (52.03%) with hydrocarbons as a dominant class (30.21%), while the saponifiable fraction had lower amounts with a rate of 39.64%, in which the unsaturated

Table 1: Chemical composition of extra virgin olive oils extracted from *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei*

<i>O. europaea</i> var. <i>sylvestris</i>					<i>O. europaea</i> subsp. <i>laperrinei</i>			
S. No.	TR	Compounds	Chemical Formula	%	TR	Compounds	Chemical Formula	%
1	5.981	Tyrosol	C ₈ H ₁₀ O ₂	2.45	6.616	Dillapiol	C ₁₂ H ₁₄ O ₄	0.18
2	6.228	Allyltetramethoxybenzene	C ₁₃ H ₁₈ O ₄	0.49	7.022	8-Heptadecene	C ₁₇ H ₃₄	0.08
3	6.275	Spathulenol	C ₁₅ H ₂₄ O	0.55	9.716	Myristic acid	C ₁₄ :0	0.23
4	6.610	Apiole	C ₁₂ H ₁₄ O ₄	2.81	12.198	n-Pentadecanoic acid	C ₁₅ :0	0.11
5	8.416	Hydroxytyrosol	C ₈ H ₁₀ O ₃	1.19	14.551	Glucose	C ₆ H ₁₂ O ₆	0.19
6	9.116	D-Ribofuranose	C ₅ H ₁₀ O ₅	0.76	14.998	Palmitoleic acid	C ₁₆ :1	2.61
7	9.328	D-Fructose	C ₆ H ₁₂ O ₆	2.15	15.8	Palmitic Acid	C ₁₆ :0	27.98
8	9.669	Sorbopyranose	C ₆ H ₁₂ O ₆	1.42	23.76	Linoleic acid	C ₁₈ :2	25.99
9	10.19	Glucofuranose	C ₆ H ₁₂ O ₆	1.54	24.4	Oleic acid	C ₁₈ :1	17.43
10	10.61	Nd	-	1.1	25.15	Stearic acid	C ₁₈ :0	1.77
11	11.45	α -D Mannopyranose	C ₆ H ₁₂ O ₆	2.88	35.49	Lauric acid	C ₁₂ :0	0.24
12	11.55	d-Xylose	C ₅ H ₁₀ O ₅	1.71	35.491	nd		2.66
13	11.76	Glucopyranose	C ₆ H ₁₂ O ₆	1.01	38.52	β -Sitosterol	C ₂₉ H ₅₀ O	5.66
14	14.52	Glucose	C ₆ H ₁₂ O ₆	3.35	39.596	Squalene	C ₃₀ H ₅₀	13.43
15	15.5	Palmitic Acid	C ₁₆ :0	14.31				
16	18.104	Nd	-	5.84				
17	23.76	Linoleic acid	C ₁₈ :2	6.87				
18	24.09	Oleic acid	C ₁₈ :1	17.27				
19	25.009	Stearic acid	C ₁₈ :0	1.19				
20	26.78	Tetracosane	C ₂₄ H ₅₀	1.06				
21	32.444	Pentacosane	C ₂₅ H ₅₂	0.79				
22	39.520	Squalene	C ₃₀ H ₅₀	27.87				
Σ				98.61%				98.56%

fatty acids had a higher level (31.58%) than the saturated fatty acids (8.06%).

According to these findings *O. europaea* subsp. *laperrinei* contained higher amounts of UFAs and SFAs compared to *O. europaea* var. *sylvestris*, however, the latter poses a high level of other minor compounds, such as hydrocarbons, phenolic compounds, and volatile compounds with the exception of sterols which are absent.

Evaluation of the antibacterial activity of the two EVOOs

Using disc diffusion assays, the antibacterial activity of the two EVOOs was assessed in vitro by measuring the mean growth inhibition zone diameters (mm) of the seven bacterial strains at various EVOO doses. The antibiotic gentamicin was used as a positive control in this investigation (Table 3). The results indicate that the effect of the two EVOOs on positive bacteria is greater than that on negative bacteria. In addition, the EVOO from *O. europaea* subsp. *laperrinei* exhibited the highest range of inhibition among all bacterial strains tested compared to other EVOOs. *S. aureus* (MRSA), *P. aeruginosa*, *S. aureus* and *B. subtilis* are the sensitive strains to the two EVOO. In which *O. europaea* var. *sylvestris* shows a smaller inhibition diameter range than other one with inhibition diameter varying between (15.33±0.57; 14.66±1.55; 14±1.00; 12.66±0.57) respectively. Furthermore *E. coli* exhibited a moderate sensitivity to the *O. europaea* subsp. *laperrinei* EVOO and no effect was observed with the other EVOO. However, *K. pneumoniae* and *E. faecalis* was extremely resistant to the two EVOO. The greatest activity for all bacterial strains tested was observed at pure doses of both EVOOs compared the other dilutions.

The statistical analysis revealed that there was a highly significant (P<0.001) in the interaction between the two

EVOOs, bacterial strains, and the doses (Table 4). Also the statistical study revealed significant variation in bacterial sensitivity. According to their extreme sensitivity to the two EVOOs investigated and their higher mean diameter of inhibition (12.06 mm), followed by, the other strains with a higher sensitivity such as *S. aureus* (MRSA) which classified in the category “a”, and *S. aureus* strain categorized in group “b”, then *P. aeruginosa* and *B. subtilis* were classified in the same category “c”. *E. coli*, *K. pneumoniae*, and *E. faecalis*, were categorized in ranges “d”, “e” and “f” respectively, which are the resistant strains to both *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei* EVOO (Table 5). The statistical analysis of the effect of EVOO dilutions and antibiotic on the inhibitory zone of bacterial tested revealed that category “a” contains the antibiotic Gentamicin which has the most powerful antibacterial activity, followed by class “b” of pure EVOO with moderate activity, the sensitivity of the bacteria decreases with increasing in dilution, therefore 1/2, 1/4 and 1/10 dilutions had minimal antimicrobial activity (Table 6). Concerning antibacterial activity potential, for all evaluated bacterial strains, EVOO extracted from the Saharan population has a greater potential of growth bacterial inhibition than the EVOO extracted from the Northern population (Table 7).

Discussion

Limited knowledge of the chemical composition and antibacterial potential of wild olive oils either in Algeria or in the worldwide been found, only some studies have been conducted on *O. europaea* var. *sylvestris*, but no research has been done on the endemic subspecies *O. europaea* subsp. *laperrinei*.

Our findings indicated that the two wild olives that were extracted had different colors, a green dark colored oil from *O. europaea* var. *sylvestris* and a yellowish oil from

Table 2: Different fractions of the two EVOOs extracted from *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei*

Fractions	Saponifiable fraction			Unsaponifiable fraction			
	Saturated fatty acids	Unsaturated fatty acids	Hydrocarbons	Phenolic compounds	Sterols	Volatile compounds	Other minor compounds
<i>Olea europaea</i> var. <i>sylvestris</i>	8.06%	31.58%	30.21%	3.64%	0%	3.36%	14.82%
Σ		39.64%				52.03%	
<i>Olea europaea</i> subsp. <i>Laperrinei</i>	30.33%	46.03%	13.51%	0%	5.66%	0.18%	0.19%
Σ		76.36%				19.54%	

Table 3: Inhibition diameter zone (mm) of the two EVOOs from *O. europaea* var. *sylvestris* and *O. europaea* subsp. *laperrinei* oils

Concentration (W/W)	<i>O. europaea</i> var. <i>sylvestris</i>				<i>O. europaea</i> subsp. <i>laperrinei</i>				Gentamicin
	Pure	1/2	1/4	1/10	Pure	1/2	1/4	1/10	
<i>S. aureus</i> ATCC 43300 (MRSA)	13.33±1.15	10.66±0.57	9.33±0.57	0±0.0	15.33±0.57	11.66±0.57	12.33±0.58	0±0.0	24±1.00
<i>S. aureus</i> ATCC 25923	13.33±0.57	10±0.00	0±0.0	0±0.0	14±1.00	11.66±1.53	11.33±1.16	10.66±0.57	22±1.00
<i>B. subtilis</i> ATCC 6633	11±1.00	9±1.00	0±0.0	0±0.0	12.66±0.57	11.33±1.52	0±0.0	0±0.0	30±1.00
<i>E. coli</i> ATCC 25922	7.66±0.57	0±0.0	0±0.0	0±0.0	9.77±0.57	0±0.0	0±0.0	0±0.0	22±1.00
<i>K. pneumoniae</i> ATCC 700603	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	25±1.00
<i>P. aeruginosa</i> ATCC 27853	13±1.00	11.33±0.57	0±0.0	0±0.0	14.66±1.55	11.66±0.57	0±0.0	0±0.0	27±1.00
<i>E. faecalis</i> ATCC 51299	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	21±1.00

Table 4: Main effects and interactions of the two EVOOs

Sources	Df	MS	F	P
Main Effects				
Oils	61.88571429	61.885714	141.26087	0.0000***
Dosses	14982.02857	3745.5071	8549.5272	0.0000***
Bacteria	1960	326.66667	745.65217	0.0000***
Interaction				
Dosses* oils	4 25.59047619	6.397619	14.603261	0.0000***
Bacteria* oils	6 128.9142857	21.485714	49.043478	0.0000***
Bacteria* dosses	24 1904.571429	79.357143	181.1413	0.0000***
Bacteria* dosses* oils	24 193.9428571	8.0809524	18.445652	0.0000***

*Significant at P<0.05; **Significant at P<0.01; *** Significant at P<0.001

Table 5: Sensitivity of the two EVOOs on all bacterial strains tested

Rank	Bacteria	Mean (mm)	n
1	<i>S. aureus</i> ATCC 43300 (MRSA)	12.06 ^a	30
2	<i>S. aureus</i> ATCC 25923	11.47 ^b	30
3	<i>P. aeruginosa</i>	10.47 ^c	30
4	<i>B. subtilis</i>	10.4 ^c	30
5	<i>E. coli</i> ATCC	6.13 ^d	30
6	<i>K. pneumonia</i>	5 ^e	30
7	<i>E. faecalis</i>	4.2 ^f	30

LSD0.05=0.33. Letters in the super script denotes significant differences

Table 6: EVOOs dilutions and antibiotic effects on the inhibitory zone of tested bacteria

Rank	Doses	Mean (mm)	n
1	Gentamicin	24.43 ^a	42
2	Pure	8.90 ^b	42
3	½	6.21 ^c	42
4	¼	2.36 ^d	42
5	1/10	0.76 ^e	42

Table 7: Effectiveness of two EVOOs from several sampling locations

Rank	Station	Mean	n
1	EVOO of <i>O. europaea</i> var. <i>sylvestris</i>	9.08 ^a	105
2	EVOO of <i>O. europaea</i> subsp. <i>laperriniae</i>	7.99 ^b	105

LSD0.05=0.18. Letters in the super script denotes significant differences

the other Jimenez-Lopez *et al.* (2020) explained these color differences of the EVOOs by the number of pigments, as increasing the number of pigments elevates the greenish-yellow color and vice versa.

As previously mentioned, unsaturated fatty acids, of which oleic acid is the predominant FA of *O. europaea* var. *sylvestris* EVOO, accounted for 17.27%, and was followed in order of dominance by palmitic and linoleic acids. Since our research includes the percentage of all oil fractions, the results mentioned below have higher fatty acid concentrations because their analysis only evaluated the composition of fatty acids. Our findings were similar to those obtained from *O. europaea* var. *sylvestris* oil from different locations in the Mediterranean basin, in that oleic

acid was the predominant constituent, but not in terms of their respective amounts, Such as in Baccouri *et al.* (2008), who detected that oleic acid was the main FA in VOO from some selected wild olives in Tunisia, with an average of 48.4% to 71.1%. Moreover, Hannachi *et al.* (2013) were found that the main FA of nine Tunisian wild olives was oleic acid and their contents varied from 47.03% to 71.55%. As well as Bouarroudj *et al.* (2016) obtained that the oleic acid was the major FA in Four Algerian oleaster populations originating from Bejaia, with values ranging from 64.7% to 76.1%. Additionally, the oleic acid is the main source FA (67.79%) in both ssp. *maroccana* and var. *sylvestris* oils, followed by palmitic acid (18.66%) and linoleic acid (8.20%) (Elgadi *et al.*, 2021). Similar results were obtained from another investigation, in Northeastern Portugal, with the Oleic acid (68.9-70.6%) being the most abundant FA, subsequent to palmitic acid, (ranging from 14.2% to 15.2%), and linoleic acid (varying from 7.9% to 9.9%) (Rodrigues *et al.*, 2020), as well as in the Spanish wild olive, the oleic acid is the major FA (76.41 % to 78.07%) followed by palmitic acid (13.47 % to 13.69 %) (Espínola *et al.*, 2021)

Numerous researches have indicated an association between the beneficial effects of olive oil and oleic acid (Perez-Martinez *et al.*, 2011; Kabaran, 2018). In addition, phenolic compounds, primarily hydroxytyrosol and tyrosol, have a multitude of beneficial effects, such as anti-oxidant activity, antimicrobial, and anti-inflammatory properties, as well as regulating a variety of signaling molecules involved in inflammation, cell adhesion, growth, apoptosis, and aging, and considered as cardio-protective and neuro-protective agents (Flori *et al.*, 2019).

Additionally, unsaturated fatty acids (UFAs) made up the majority of the chemical composition of *O. europaea* subsp. *Laperriniae* EVOO. However, the quantity and variety of FAs in this subspecies' EVOO differ from this of *O. europaea* var. *sylvestris*, which produces only three FAs (oleic acid, palmitic acid, and linoleic acid). In contrast, many FAs, such as palmitic acid, linoleic acid, oleic acid, palmitoleic acid, stearic acid and lauric acid, were detected in *O. europaea* subsp. *laperriniae* EVOO, the latter showed higher amounts of fatty acids in common with the other. This subspecies differs from the other, where oleic acid was one of the dominant FAs but not the dominating one, in this case palmitic acid is the major fatty acid followed by linoleic acid, then oleic acid. Our results were similar to the results of numerous investigations that were done on the chemical composition of the other subspecies of *O. europaea*, which revealed that oleic acid, palmitic acid, and linoleic acid were the major FAs, but did not agree with those later on in terms of the amounts of each one, such as, the oil of *O. europaea* subsp. *cuspidata* from Kenya, oleic acid was found to be the highest at 44.3%, followed by linoleic acid (33.3%) and palmitic acid (12.1%) (Elgadi *et al.*, 2021), also found that oleic acid (63.22 to 67.79%) was the main fatty acid, followed by linoleic acid (8.20 to 19.81%) and palmitic acid (10.65 to 19.02%) in *O. europaea* subsp. *maroccana* oil. Our oil contains higher concentrations of linoleic acid, which has been shown in numerous studies to have many health advantages, such as Ramsden *et al.* (2012), who reported

that vegetable and seed oils containing linoleic acid are the most frequent sources of polyunsaturated fats in the human diet, also noted that linoleic acid is an omega-6 fatty acid which represents a principal component of human tissues. Linoleic acid applied topically is thought to be beneficial in treating melasma patients (Lee *et al.*, 2009). It has also been linked to a lower risk of hypercholesterolemia (Ramsden *et al.*, 2021) and atherosclerosis (Das, 2021). Additionally reduces headache pain when used in conjunction with omega-3 fatty acid supplements (Mercola & D'Adamo, 2023). It has also been demonstrated that omega-6 linoleic acid has a hypolipidemic effect when paired with palmitic acid (Ahmad & Beg, 2016).

The two EVOOs investigated generated a height amount of UFAs than SFAs while *O. europaea* subsp. *laperrinei* EVOO, has a more important amount of UFAs which represents an average of 46.03% of UFA, than *O. europaea* var. *sylvestris* (31.58%). Owing to these highest concentrations it can be regarded as a source of UFAs, and as such, offers a variety of therapeutic benefits (Amiot, 2014), including protection against cardiovascular disorders (Kabaran, 2018). Numerous studies have shown that a diet high in unsaturated fat raises HDL cholesterol and lowers TG levels, making it useful for treating hypercholesterolemia (Schwingshackl & Hoffmann, 2016).

These variability in the chemical profiles of EVOOs between the two wild olives can be attributed to a variety of factors, including the pedo-climatic conditions and olive variety (Lombardo *et al.*, 2018), Other factors that may have affected the composition but were taken into account in our study include storage conditions, timing of harvest and extraction (Jimenez-Lopez *et al.*, 2020).

Overall, the results of this study clearly show that the two EVOOs have effective antibacterial activity, with the greatest activity observed against Gram-positive bacteria as compared to Gram-negative bacteria. Several authors, including Medina *et al.* (2007) and Boukhebt *et al.* (2020), have confirmed these findings. According to Janakat *et al.* (2015), the resistance of the Gram-negative to olive oil related to their outer cell (wall) membrane which offered their protection, which Gram-positive bacteria lack.

As previously stated, *S. aureus* (MRSA), *P. aeruginosa*, *S. aureus* and *B. subtilis* were the sensitive strains, However *K. pneumoniae* and *E. faecalis* were the resistant strains to the two EVOO investigated, the antimicrobial activity of olive oil were also analyzed by Hussain *et al.* (2014), where they found a similar result that *S. aureus* and *P. aeruginosa* showed a height antibacterial activity, while *E. faecalis* as resistant bacteria, while showed an important antibacterial activity of *K. pneumonia* which was resistant in our results. Furthermore, Boukhebt *et al.* (2020) noticed that olive oil has a remarkable antibacterial activity toward *B. subtilis*, *S. aureus* and *E. coli* as well as *K. pneumonia* and *P. aeruginosa* were the resistant bacteria.

According to our finding, the EVOO extracted from *O. europaea* subsp. *laperrinei* has the highest potential of

inhibition of all bacterial tested, this is mainly due to their abundance of fatty acids which was greater in this oil for 76.36%, than the other, where the majority of them are absent. Many previous studies reported FAs as antibacterial agents that cause partial solubilization and membrane fission in order to disrupt bacterial cell membranes (Yoon *et al.*, 2018). According to Desbois and Smith (2010), FAs affect oxidative phosphorylation and the electron transport chain. In addition to disrupting the creation of cellular energy, and caused the limitation of enzyme activity, hinders nutrition uptake, produces harmful peroxidation and auto-oxidation of the breakdown products, or directly lyses bacterium cells. Many other authors confirmed the antibacterial properties of FAs (Yang *et al.*, 2017; Ivanova *et al.*, 2017; Kumar *et al.*, 2020; Casillas-Vargas *et al.*, 2021). Notably palmitic acid, linoleic acid, oleic acids, lauric acid (Huang *et al.*, 2010; Yoon *et al.*, 2018). β -Sitosterol is another compound that appeared in *O. europaea* subsp. *laperrinei* EVOO and absent in the other, and it has been shown in the literature to have strong antibacterial potential (Alawode *et al.*, 2021).

Conclusion

Olive oil is widely applied in different fields due to their nutritional value, and their wide range of health benefits. Moreover, the valorization of Algerian genetic heritage, especially wild olives and Saharan endemic subspecies that are unknown until this day like our case of subsp. *laperrinei* is becoming a necessity to demonstrate their importance, by studying their olive oil contents and their biological activities, which were tested for the first time in this research. Clear distinction was recorded on the chemical composition particularly in terms of the number of detected constituents and the amount of the main components and the antimicrobial activity between the two extra virgin olive oils examined. In fact, *O. europaea* var. *sylvestris* revealed the identification of 22 components with squalene, oleic acid and palmitic acid as major constituents, while 14 components were identified in *O. europaea* subsp. *laperrinei* oil, with palmitic acid, Linoleic acid and oleic acid. In addition, the endemic olive oil showed the highest amounts of both unsaturated and saturated fatty acids. In addition the two EVOOs had a high potential of inhibition on positive bacteria than negative bacteria, and the EVOO from *O. europaea* subsp. *laperrinei* exhibited the highest range of inhibition on all bacterial strains tested. Moreover, these findings highlight the extra virgin olive oils from wild olive trees' nutritional worth and therapeutic qualities.

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Authors' contribution

Collecting plant samples SB; the chromatographic analysis and treatment of results were performed by HB, SB, MY, OL; writing of the original draft by SB, review and editing by HB,

MY, OL. All authors revised and agreed the manuscript before submission.

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