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Original article

Identification of bioactive compounds and determination of totalphenolic and flavonoid contents in leaf extracts originated from the Algerian desert *Olea europaea* subsp. *laperrinei* and *Olea europaea* subsp. *europaea* var. *Sylvestris* and evaluation of their potential as antioxidants

Sarra Bouchoucha[1], *Habiba Boukhebti*[1], *Hani Belhadj*[2], *Abdemalek Oulmi*[1], *Adel Nadjib Chaker*[1]

[1]Department of Ecology and Vegetale Biology, Laboratory of natural resources valorization, University of Ferhat Abbas Sétif 1, Algeria; [2]Department of Applied Microbiology, Laboratory of Applied Microbiology, University of Ferhat Abbas Sétif 1, Algeria

Abstract. *Background and aim:* since ancient times, *Olea europaea* L. Notably, the olive leaf has been used in ethnopharmacology to treat fevers and malaria. Currently, this aerial part aroused the interest of researchers around the world in the fields of medicine and pharmacology due to their beneficial effects on human health, including anti-hypertensive hypoglycemic, hypocholesterol, antimicrobial properties, as well as utilized to prevent Alzheimer's disease and to provide protection from colon, breast, and ovarian cancers. Additionally,has a great antioxidant potential due to their high phenolic content.Despite the knowledge of bioactivities of olives that have been frequently reported, the majority of them were related to cultivated olives, whereas wild olives are even less recognized or unknown like our case subsp. *laperrinei*. The purpose of this work was to realize a comparative evaluation of the phytochemical profile, total phenolic, and flavonoids contents, as well as the antioxidant potential of both aqueous and methanolic extract from two subspecies such as *Olea europea* subsp.*laperrinei* from Sahara and *Olea europaea* var. *sylvestris* from north of Algeria has been done. In order to understand how environmental stress exerted on these wild species affects the capacity to synthesizesecondarymetabolites as well as antioxidant potential. *Methods:* Chromatography liquid with High-performance (HPLC) was used to identify and quantify the constituents of subspecies, the total phenolic and flavonoids content in the extracts was determined using Folin-Ciocalteu and spectrophotometric method respectively, and the antioxidant activity was analyzed in vitro using DPPH scavenging method. *Results*: HPLC analysis showed that Oleuropein is the main compound in all extracts in which the Saharan extracts showed a height level (276.157 mg/g), furthermore, all the extracts obtained showed reasonably high total phenolic and flavonoid contents and good radical scavenging activity notably those from subsp.*laperrinei* were more important. Furthermore, the highest values were obtained using methanol as solvents than water. *Conclusions:* The results also showed that wild olives have very high antioxidant potentials and it could be deduced that their leaves can under stress conditions increase the synthesis of bioactive as in the case of subsp. *laperrinei* compared to var. *sylvestris* from no stressful condition. Also, demonstrate the value of wild olive leaves as a natural antioxidant.

Key words: *Olea europaea* L, subsp. *laperrinei*, var. *sylvestris*, phytochemical profile, total phenolic, flavonoids contents, antioxidant potential

Introduction

Medicinal plants are the main source of medicines, due to the abundance of what is known as secondary metabolites (1). For this reason, people all around the world, turn to traditional medicine as an alternative to modern therapies. In which the pharmaceutical industry demonstrates that natural products continue to be a very valuable source for the production of new complex organic molecules that frequently exhibit pharmacological properties, such sources are becoming more and more important (2,3). In Algeria, many plants are traditionally used to treat many diseases, among these plants is *Olea europaea* L. (1,4,5) which is included in the *Oleaceae* family (6), it is one of the most essential fruit trees in Mediterranean regions, it is frequently employed in traditional medicine (7,8).

Olive leaves are a copious by-product generated by the olive oil industry and olive tree pruning (6). These aerial parts are currently attracting growing attention in the vast field of medicine and pharmacology (9), due to their high phenolic contents, olive leaf extract becomes one of the most effective sources of plant polyphenols with high antioxidant potential (10). Typically, the Phenolic compounds in olive leaves are numerous and of diverse nature, which the major groups of phytochemicals in *Olea europaea* L. leave extracts such as the phenolic compounds (11) and polyphenolic compounds (secoiridoids and flavonoids) (10,12).

In effect, the active constituent and frequently reported of olive leaf extract is Oleuropein a secoiridoid with great antioxidant activity in vitro (13). The health-promoting properties of this compound have been extensively studied (14), principally for their antioxidant properties and therapeutic benefits such as antimicrobial and antiproliferative activities (15,16), furthermore Flavonoids are a widely distributed group of polyphenol compounds that are recognized as antioxidants in diverse biological systems (17), it has possessed anti-inflammatory, anti-allergic, antiviral and antiproliferative activities (18,19), as well as hypoglycemic properties (20), however, the variability in qualitative and quantitative of total phenolic compounds and evidently the magnitude of the antioxidant capacity of olive leaves extracts might depend on several factors mainly rests upon the environmental conditions (21), the olive cultivars/varieties analyzed (22,23), as well as the extraction method/solvent (22-24).

Free radicals are a major contributor to the emergence of different human diseases, including cancer, Alzheimer's disease, neurological disorders, cardiac reperfusion… ect (25), in this context using synthetic antioxidants is a necessity for reducing oxidative stress but the extensive use of these additives in the food industry, exhibit genotoxic, carcinogenic effects (26,27) and hemorrhaging (28). In contrast, using natural antioxidants derived from plants does not induce side effects (28, 29) and has greater advantages over using synthetic antioxidants (30). As a result, it has become a necessity for the pharmaceutical and cosmeceuticals industry the shift towards natural products (31).

Olive leaves are classed as a source of several antioxidants (32), for the purpose of valorization of wild olive leaves as cheap and natural antioxidants we conducted this study, the current study was carried out to comparatively analyzed, the total phenolic and flavonoid content, to identify and quantify some of them by HPLC method, as well as to evaluate the antioxidant activity of both aqueous and methanolic leaves extracts of two wild subspecies of *Olea europaea* L. which belongs to two different bioclimatic levels such as *Olea europaea* subsp. *europaea* var. *sylvestris* from the northern region is characterized by a Mediterranean climate with dry summers and wet winters and *Olea europaea* subsp. *laperrinei* an endemic subspecies from the Saharan region in Algeria has a hyperarid climate with summers that are exceptionally long, and with a very low precipitation rate, In order to explain how environmental conditions affect the plant's capacity to synthesize secondary metabolites.

In light of our knowledge, only a few research have been made on the chemical profile and the antioxidant of leaves extracts issues from wild subspecies of *Olea europaea* L., furthermore no data were also provided on the antioxidant activity and the amount of total phenolic and flavonoid compounds of the Saharan endemic subspecies "*Olea europea* subsp. *laperrinei*", as well as is the first to investigate the comparative evaluation between two wild olive subspecies in Algeria.

Materials and methods

Sources and processing of plant material

The aerial parts of two wild species were collected in February 2021, were collected at two locations: *Olea europaea* subsp. *europaea* var. *sylvestris* was harvested from the northern region in Algeria "Setif, Oued El bared" (36° 37 N, 05° 40; 814 m), while *Olea europaea* subsp. *laperrinei* was collected from massifs of Hogar which are located in "central Algerian Sahara Tamanrasset (23°21N, 05°47 E; 1952m), (Figure 1) and (Figure 2).

The samples were identified at Laboratory of (L.V.R.B.N), University of Setif 1. Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Setif University, Algeria. Aerial parts of the plant material were dried at ambient temperature under obscurity.

Determination of plant extract yield

The yield of dried extracts based on a dry weight basis as obtained from 5g of the leaves was calculated from the equation (A):

$$yield\% = \frac{W1(g)}{W2(g)} * 100 \text{ .... (A) (33)}$$

W1: the weight of the extract after the solvent evaporation in grams

W2: the weight of the dry plant material in grams.

Preparation of aqueous extracts

The extraction from plants is an important step in the separation of medicinally active portions notably, bioactive constituents using selective solvents through standard procedures (31, 34), in which maceration is a technique widely used in medicinal plants research (34), However has been suggested by Vongsaka et *al*. (35) as more applicable, compared to other modern extraction methods, is known as the "Green method" (36). In our study water extract was obtained by maceration (modify (too according to Bougandoura et *al*. (37) method. 5g of dried leaves of each plant were extracted with 100ml of distilled water for 24 hours at room temperature (around 20 °C), The whole is filtered through filter paper N° 1 in order to separate the grounds from the filtrate. The aqueous extract was

Figure 1. Olive tree of *Olea europaea* subsp. *laperrinei* and *Olea europaea* subsp. *europaea* var. *sylvestris*. (a. Tree of *Olea europaea* subsp. *laperrinei*; b. Tree of *Olea europaea* subsp. *europaea* var. *sylvestris*).

Figure 2. Olive tree branch with leaves of *Olea europaea* subsp. *laperrinei* and *Olea europaea* subsp. *europaea* var. *sylvestris*. (a. leaves of *Olea europaea* subsp. *laperrinei*; b. leaves of *Olea europaea* subsp. *europaea* var. *sylvestris*)

powdered by lyophilization and stored at refrigeration (4°C) until analysis.

Preparation of methanolic extract

The areal parts of the samples were cut into very small pieces and macerated in 80 %methanol for 24 and 48 hours at laboratory temperature. The ratio of dried plant to solvent is 1:10 w/v. The extract was collected by filtration and evaporated to dryness under a vacuum (38). The dry extract was conserved at -18 °C until used.

Determination of total phenolic content

The total phenolic content of plants extracts was determined spectrophotometrically according to Foline-Ciocalteu method (39). A volume of 0.5 Ml of 50% Folin-Ciocalteu reagent was mixed in a test tube containing 0.1mL of the extract and homogenized. The resultant mixture was allowed to react for 1 min and 1.5 mL of 20% Na2CO3 was added. It was mixed thoroughly and incubated in the dark for 2 h and then the absorbance was recorded at 760 nm using a visible light spectrophotometer (Spectronic 20 genesys TM). A Gallic acid was used as a standard for the calibration curve. The total phenolic compounds content was expressed as mg equivalent of Gallic acid per gram of extract (mg GAE/gE).

Determination of total flavonoids content

Total flavonoids content was determined using a spectrophotometric method based on the formation of flavonoid complex with aluminum chloride. A volume of 1 ml of 2% $AlCl_3$ solution prepared in methanol was added to 1 ml of sample solution at room temperature. After 30 min of incubation the absorbance was measured at 430 nm using a visible light spectrophotometer.

The yellow color indicated that the extracts contained flavonoids. Quercetin was used as a standard for calibration. Total flavonoid content was calculated as mg equivalent Quercetin per gram of extract (mg EQ/GE) (40).

Evaluation of antioxidant activity in vitro

Anti-radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazil (DPPH) radical (0.004% in methanol) according to the method described by Kulsic *et al*. (41) with a minor modification. In order to prepare 0.004% (w/v) DPPH solution, 4mg of DPPH was dissolved in 100mL of methanol. Concerning the sample solutions 4mg of aqueous and methanolic extracts were dissolved in 1mL of distilled water and methanol. In parallel, serial dilutions were performed in order to prepare different concentrated solutions for each extract (20ug/mL; 40ug/mL; 60ug/mL; 80ug/mL; 100ug/mL), then one milliliter of extracts prepared at different concentrations was added to 1mL of DPPH-methanol solution. The mixtures were shaken vigorously and left standing in the laboratory conditions for 30 minutes in the dark. The optical density (DO) was measured using a Spectrophotometer at 515 nm against the blank. The blank consisted of 1mL of methanol and 1mL of DPPH solution (0.004%), and BHT (Butylated hydroxytoluene) was used as a positive control.

All determinations were performed in triplicate. The optical density was recorded and the percent of inhibition (PI) was calculated according to the mathematical formula (B):

$$PI\% = \frac{(A0 - At)}{A0} \times 100, \text{ where} \qquad (B),$$

A0: optical density of the blank (control) at starting time.

At: optical density of the sample after 30 min.

Antioxidant activity results are expressed as IC50 value (µg extract/mL) (concentration providing 50% inhibition of DPPH radicals), which was calculated graphically by interpolation from linear regression analysis.

Identification of active biomolecules by HPLC-DAD analysis

In order to detect and quantify the phenolic compounds in the different extracts, we evaluated them by Chromatographic analyses, which were achieved on an Agilent series 1260 HPLC-DAD instrument (Agilent, Waldbronn, Germany). The instrument includes a 1260 Quat pump VL quaternary pump, an online degasser, 1260 ALS auto sampler, 1260 TCC column thermostat and 1260 DAD VL diode array detector. Chromatographic separation was done in a ZORBAX Eclipse XDB-C18 column (4.6 mm × 250 mm I.D., 3.5 μm particle size). The elution conditions were as follows: mobile phase A (0.1% acetic acid in water) and mobile phase B (100% acetonitrile), flow rate of 0.5 mL/min, sample injection volume of 10 μL, and operating temperature 40°C. The running gradient was as follows: A; 0–22 min, 10%–50% B; 22–32 min, 50%–100% B; 32–40 min, 100% B; 40–44 min, 100–10% B. Re-equilibration duration lasted 6 min. DAD detector scanned from 190 to 400 nm and the samples were detected at 254, 280, and 330 nm. The injection volume was 5 µl for every sample and reference standards. Congruent retention times compared to standards were used to identify the peaks. Phenolic chemicals were quantified using HPLC by comparing peak areas with those used as internal standards. data were represented as mg/g and (mg/ml).

Statistical analysis

The average and standard deviation were used to express the results. Data were statistically analyzed, to determine if there are any significant differences between the aqueous and methanolic extracts as well as between subspecies studied in this research, which were statistically analyzed by ANOVA using the statistical package CoStat, with the criterion of P values <0.05.

Results

Plant extract yield

The percent yields of different extracts from dry plant materials were reported in (Table 1, Figure 3).

Table 1. Yield, total polyphenols and total flavonoids in aqueous and methanolic extracts of the aerial parts of *Olea europaea* subsp. *europaea* var. *sylvestris* and *Olea europea* subsp. *laperrinei*.

Plants amples	Extract type	Yield (%)	Total polyphenoles (mg GAE/g Ext)[1]	Total Flavonoids (mg QE/g Ext)[2]
Olea europaea* subsp. *europaea* var. *sylvestris	Methanolic extract	35,60 ± 3,18	18,13 ± 1.04	14.30 ± 0.47
	Aqueous extract	22,20 ± 2,24	17,57 ± 0.34	12.98 ± 0.99
Olea europea* subsp. *laperrinei	Methanolic extract	37,20 ± 6,47	26,75 ± 2 .16	22.83 ± 0.13
	Aqueous extract	25.60 ± 0,23	18,93 ± 1.17	15.24 ± 0.27

mg GAE/g Ext:mg of Galic Acid Equivalent/ g of dried Extract; mg QE/g Ext:mg of Quercetin Equivalent/g of dried Extract; each value in the table presented as mean ± standard deviation (n=3)

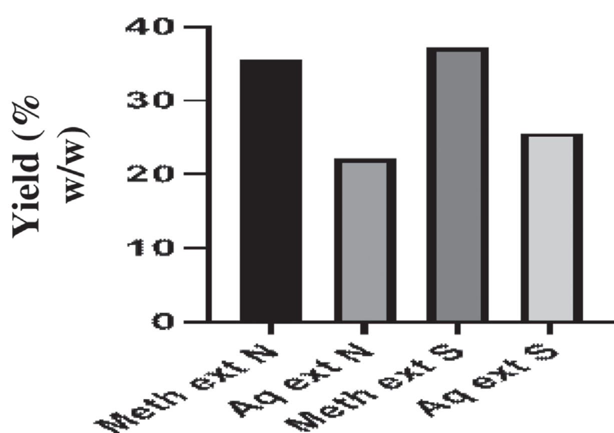

Figure 3. Yields of different extracts obtained from var. *sylvestris* and subsp. *laperrinei*. (abbreviations: AqN: aqueous extract from Northern subspecies; AqS:aqueous extract from Saharan subspecies; MethN: methanolic extract from Northern subspecies; MethS:methanolic extract from Saharan subspecies).

According to the yield values of the extracts obtained, it was observed that methanol as a solvent gives best yields than water and methanolic extract from *Olea europea* subsp. *laperrinei* gives a greater yield of (37.20 ±6.47%), followed by methanolic extract of *Olea europaea* subsp. var. *sylvestris* (35.60 ± 3.18 %). In the same way, extraction yield using water as a solvent, also is higher in the Saharan subspecies subsp. *laperrinei* (25.60 ±0.23%) than the variety from the Mediterranean region (22.20± 2.24%).

Total phenolic contents

Total phenolic contents were determined for aqueous and methanolic extracts of *Olea europaea* var. *sylvestris* as well as *Olea europea* subsp.*lapperinie*. The total quantity of phenols in the analyzed extracts are measured using the Folin–Ciocalteu, according to the gallicacid calibration curves figure 4. The values obtained for the total phenolic compounds are presented in (Table 1, Figure 5).

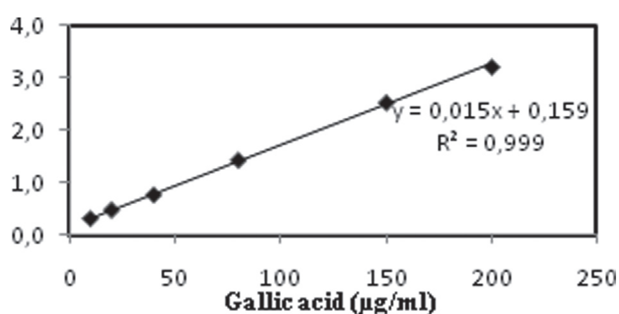

Figure 4. Gallicacid calibration curve used for polyphenols determination.

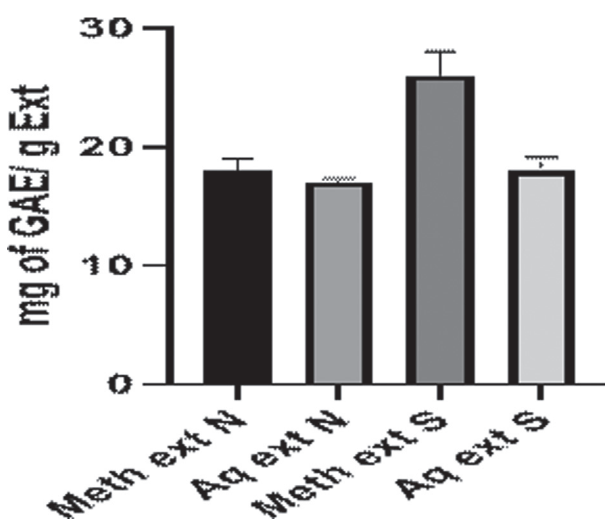

Figure 5. Total polyphenol content of different extracts samples expressed as mg of Galic Acid *Equivalent/g of extract.* (abbreviations: AqN: aqueous extract from Northern subspecies;AqS:aqueous extract from Saharan subspecies; MethN: methanolic extract from Northern subspecies; MethS: methanolic extract from Saharan subspecies).

Results suggest that the leaves extracts of both subspecies can be arich source of polyphenols, in addition, the phenolic contents varied considerably between the four leaves extracts studied, in relation to the solvent used the methanolic extracts shown to contain higher amounts of phenolic components than water extracts. As mentioned above, concerning the samples studies the greatest quantity of phenols were observed in the methanolic and aqueous extracts of the sample from the Saharan region (26,75 ± 2 .16mg GAE/g Ext), (18,93 ± 1.17 mg GAE/g) respectively, whereas the methanolic and aqueous in the sample from the Mediterranean region showed a muchlower concentration of phenols (18,13 ± 1.04 mg GAE/g), (17,57 ± 0.34 mg GAE/g) respectively.

Flavonoid concentration

The concentration of flavonoids in various extracts was determined using a spectrophotometric method based on the formation of flavonoid complex with aluminum chloride, the content of flavonoids was expressed in terms of quercetin equivalents (mg quercetin per gram of extract) (Figure 6), the summary of quantities of flavonoids in the examined extracts is reported in Table 1 and Figure 7. Overall, The concentration of flavonoids in the tested extracts ranged from 12.98 ± 0.99 to 22.83 ± 0.13 mg QE/g of extract, regarding to extracts, the methanolic extract of the two subspecies having greater contents of flavonoids compared to the aqueous extract, in fact, the extract of *Olea europaea* subsp. *laperrinei* showed the highest concentration of flavonoids in either a methanolic or aqueous (22.83 ± 0.13 mg QE/g Ext) ,(15.24 ± 0.27 mg QE/g Ext), while methanolic or aqueous extract of *Olea europaea* var. *sylvestris* showed a lowest concentrations (14.30 ± 0.47 mg QE/g Ext)(12.98 ± 0.99 mg QE/g Ext).

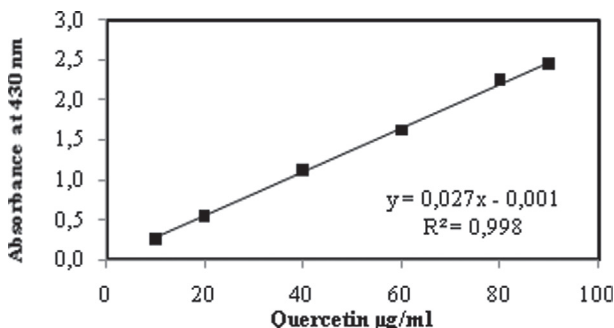

Figure 6. Quercetin calibration curve for flavonoids determination.

Antioxidant activity of plantextracts

The antioxidant activity of the methanolic and aqueous extracts of two subspecies and the positive control (BHT)against very stable free radical DPPH was evaluated using a spectrophotometric method by following the reduction of this radical which was accompanied by a color change from violet to yellow (DPPH-H), which can be measured at 515 nm. The ability of plant extracts to reduce DPPH radicals was determined by the decrease in their absorbance at 515 nm. Free radical scavenging effects results were defined as the amount of antioxidants necessary to decrease the initial DPPH radical concentration by 50% in 30 minutes (IC50), a lower IC50 value indicates higher antioxidant activity (Figure 8).

The obtained results for antioxidant activity evaluated by DPPH radical scavenging activity ranged from

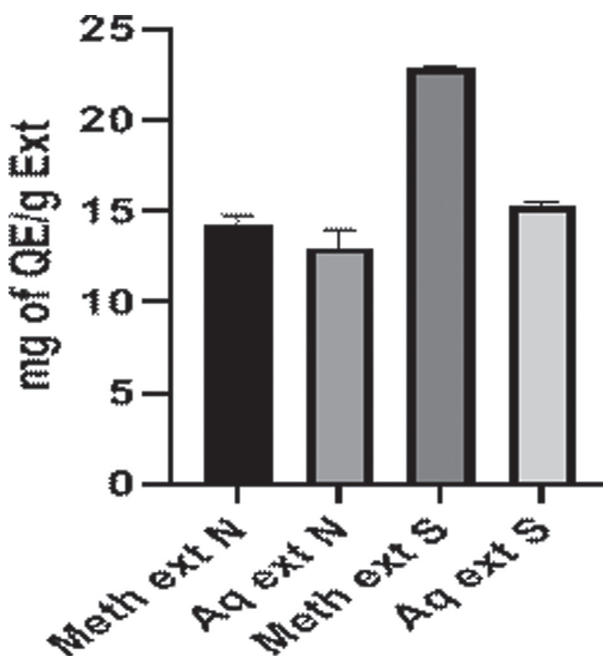

Figure 7. Total Flavonoids content of different extract samples expressed as mg of Quercetin *Equivalent/g of extract*. (abbreviations: AqN: aqueous extract from Northern subspecies; AqS:aqueous extract from Saharan subspecies; MethN: methanolic extract from Northern subspecies;MethS: methanolic extract from Saharan subspecies)

54.01 ± 0.46 to 82.33 ± 0.50 µg/mL Table 2. The highest capacity to neutralize DPPH radicals was found in the methanolic and aqueous extracts of endemic Saharan sub species *Olea europea* subsp.*laperrinei* with an IC50 value of 54.01 ± 0.46µg /ml and 66.97 ± 0.41 µg /ml respectively, these concentrations are near those exerted by positive control (BHT). In contrast, the minutest antioxidant activity was determined for extracts from *Olea europaea var. sylvestris* with values of 70.19 ± 2.09µg /ml (methanolic extract) and 82.33 ± 0.50 µg /ml (water extract) approximately two folds of that of BHT (Figure 9).

HPLC-DAD analysis of plant extracts

Identification and quantification of individual polyphenols Present in the extracts were carried out by using data from HPLC/DAD analyses, the data of phenolic compounds of each extract are listed in Tables 3 and 4.

The contents identified in all the extracts studied are numerous and diverse nature, grouped according to major molecular characteristics such as substituted phenols (hydroxytyrosol and tyrosol), phenolic acids (vanillic acid, caffeic acid, gallicacid, ferrulic acid, p-Coumaric acid, Chlorogenic acid), flavones (luteolin, Quercitin, Apigenin) and secoiridoids (Oleuropein and verbascoside) in which sixteen and seventeen phenolic compounds were identified in aqueous and methanolic extract issue from *Olea europaea* var. *sylvestris* respectively, in contrast, the aqueous and methanolic extract issue from *Olea europea* subsp. *laperrinei* showed fourteen and eleven compounds respectively.

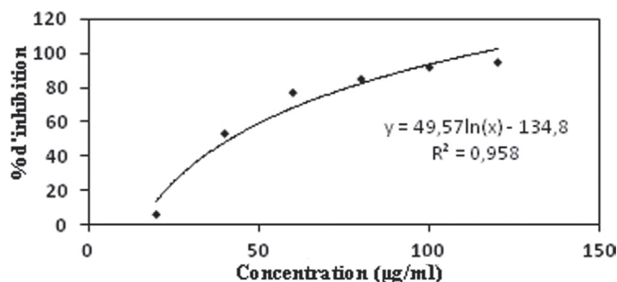

Figure 8. DPPH scavenging effect of standard antioxidant BHT.

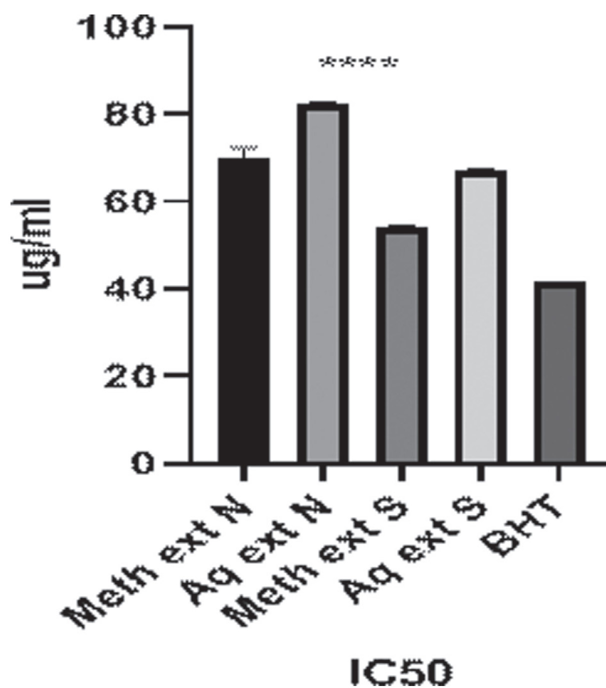

Figure 9. Comparison of antioxidant activity (IC_{50}) of different extracts with BHT.

Table 2. In vitro antioxidant activities of investigated *Olea europaea* subsp. *europaea* var. *sylvestris* and *Olea europaea* subsp. *laperrinei* leaves extracts, and positive control (BHT).

Samples	Extract type	DPPH $^{***}IC_{50}$(ug/ml) (1)
Olea europaea* subsp. *europaea* var. *sylvestris	Methanolic extract	70.19 ± 2.09
	Aqueous extract	82.33 ± 0.50
Olea europea* subsp. *laperrinei	Methanolic extract	**54.01 ± 0.46**
	Aqueous extract	66.97 ± 0.41
BHT		**41.65 ± 0.00**

(1) Eachvalueisrepresented as mean ± SD (n=3).
(2) Results were compared using ANOVA from CoStat Software p<0.001.***

Table 3. Phenolic compounds evaluated by HPLC-DAD expressed in (mg/ml)/(mg/g) of *Olea europea* subsp.*europea* var. *sylvestris* leaves extract.

Olea europaea subsp. *europaea* var. *sylvestris*								
Aqueous extract					Methanolic extract			
N°	TR	Compounds	Concentration (mg/ml)	Concentration (mg/g)	RT	Compounds	Concentration (mg/ml)	Concentration (mg/g)
1	7.072	Gallic acid	0.0024	0.165	7.076	Gallic acid	0.0014	0.121
2	10.149	**Hydroxytyrosol**	**0.0752**	**5.186**	10.151	Hydroxytyrosol	0.0222	1.93
3	11.489	**Chlorogenicacid**	**0.0508**	**3.503**	11.492	Chlorogenicacid	0.0334	2.904
4	12.857	Tyrosol	0.0467	3.220	12.855	Tyrosol	0.0157	1.365
5	13.579	Caffeicacid	0.0184	1.268	13.576	Caffeicacid	0.0113	0.982
6	14.090	Vanillicacid	0.0049	0.337	14.290	Vanillicacid	0.0062	0.539
7	15.363	**Rutin**	**0.1029**	**7.096**	15.357	**Rutin**	**0.0741**	**6.443**
8	15.742	Verbascoside	0.0272	1.875	15.983	**Verbascoside**	**0.0643**	**5.591**
9	16.387	**Luteoline7 glucoside**	**0.0716**	**4.937**	16.382	**Luteoline7 glucoside**	**0.0963**	**8.373**
10	17.061	p-Coumaric acid	0.0015	0.103	17.225	p-Coumaric acid	0.0027	0.235
11	18.057	**Apigenin-7-glucoside**	**0.0517**	**3.565**	18.050	**Apigenin-7-glucoside**	**0.0773**	**6.721**
12	18.109	Ferrulicacid	0.0229	1.579	18.238	Ferrulicacid	0.0178	1.547
13	18.841	**Oleuropein**	**0.1680**	**11.586**	18.827	**Oleuropein**	**1.6419**	**142.773**
14	21.154	Naringinin	0.0110	0.785	21.164	Naringinin	0.0298	2.591
15		Luteolin	n.d.	-	22.964	Luteolin	0.0009	0.078
16	23.335	Quercitin	0.0274	1.889	23.435	Quercitin	0.0033	0.287
17	25.804	Apigenin	0.0027	0.186	26.386	Apigenin	0.0029	0.252
∑			**0.6853**	**47.262**			**2.1015**	**182.40**

n.d.: notdetected.

Concerning var. *sylvestris* methanolic extract, Oleuropein (142.773mg/g) was the major compound followed by Luteoline7glucoside and Apigenin-7-glucoside, while Luteolin (0.078 mg/g), Gallic acid (0.121 mg/g) were the minor phenolic compounds, also Oleuropein (11.586 mg/g) represented the predominant component of the aqueous extract followed by Rutin, hydroxytyrosol and Luteoline-7-glucoside. Regarding the methanolic extract of subsp. *laperrinei*, Oleuropein (276.157 mg/g), Luteoline7glucoside (10.557 mg/g) and Rutin (8.471mg/g) were the major compounds, in the other hand the main compounds in aqueous extract as Oleuropein (28.07 mg/g), Rutin (13.40 mg/g) and Quercitin (8.41 mg/g).

The characteristic shared between the two subspecies' extracts is that oleuropein and rutin are the most abundant component of the aqueous extracts, whereas Oleuropein and Luteoline7glucoside was the predominant in the methanolic extracts.

As described in (Table 3 and 4), the number and quantity of compounds are different between extracts types and subspecies studies, which var. *sylvestri*s extracts are predominant in terms of the number of identified compounds, but subsp. *laperrinei*is dominating relative to the amount of these constituents.

Discussion

To the best of our knowledge, only a few investigations have been made about the bioactivities of leaf extracts issues from wild subspecies of *Olea europaea*

Table 4. Phenolic compounds evaluated by HPLC-DAD expressed in (mg/ml)/(mg/g) of *Olea europea* subsp. *laperrinei* leaves extract.

Olea europea subsp. *laperrinei*								
Aqueous extract					**Methanolic extract**			
N°	**RT**	**Compounds**	**Concentration (mg/ml)**	**Concentration (mg/1g)**	**RT**	**Compounds**	**Concentration (mg/ml)**	**Concentration (mg/1g)**
1	10.185	Hydroxytyrosol	**0.0525**	5.25		Hydroxytyrosol	n.d.	
2	11.496	Chlorogenicacid	0.0428	4.28	11.494	Chlorogenicacid	0.0299	4.271
3	12.830	Tyrosol	0.0235	2.35		Tyrosol	n.d.	
4	13.791	Caffeicacid	0.0102	1.02		Caffeicacid	n.d.	
5	14.051	Vanillicacid	0.0046	0.46		Vanillicacid	n.d.	
6	15.321	Rutin	**0.1340**	**13.40**	15.335	Rutin	**0.0593**	**8.471**
7	16.008	Verbascoside	0.0426	4.26	15.946	Verbascoside	0.0482	6.885
8	16.311	Luteoline7 glucoside	**0.0611**	**6.11**	16.341	Luteoline7 glucoside	**0.0739**	**10.557**
9	17.025	p-Coumaric acid	0.0183	1.83	17.324	p-Coumaric acid	0.0187	2.671
10	17.988	Apigenin-7-glucoside	0.0479	4.79	17.995	Apigenin-7-glucoside	0.0354	**5.057**
11	18.064	Ferrulicacid	0.0256	2.56	18.194	Ferrulicacid	0.0156	2.228
12	18.799	Oleuropein	**0.2807**	**28.07**	18.782	Oleuropein	**1.9331**	**276.157**
13	21.034	Naringinin	0.0090	0.90	20.683	Naringinin	0.0340	4.857
14	23. 303	Quercitin	**0.0841**	**8.41**		Quercitin	n.d.	
15		Apigenin	n.d.	-		Apigenin	n.d.	
∑			**0.8368**	**83.680**			**2.2481**	**321.153**

n.d.: not detected.

L. in addition, the present paper is the first, to investigate the antioxidant activity and quantify the total phenolic and flavonoid content of subsp. *laperrinei*, as well as, the comparative evaluation between two wild olive species in Algeria.

In the comparison between the values obtained from the two subspecies studies, different relations can be found In our study. the analysis of the yield results, total phenols, and flavonoid content in all the extracts assessed suggests that the results follow a variation with a similar dynamic,it was also noticed that the highest values were obtained using methanol as solvents than water. Numerous studies show also that extracting solvents influenced the extract yields and phenolic content, Moreover, has been reported that high yields are obtained with methanol (24). Water also plays an essential role in the extraction process of polyphenols by increasing their diffusion in the tissues of plants. The disparity in yield results is not related only to the type of solvents but could be caused by several of parameters, such as pH, temperature, extraction time, and sample composition. Additionally, the location and time of the harvest influence the yield extraction (1)**.** In our results the extract leaves issue from subsp. *laperrinei* has a greater yield than var. *sylvestris*, the values obtained were similar to those obtained byArab and Yahhiaoui (42), while other authors found less yield (1,43).

Many human diseases, such as cancer, and Alzheimer's disease, are mainly caused by free radicals. The Antioxidant compound can deactivate the free radicals by chelating metals or donating hydrogen atoms. The use of commercial antioxidants is therefore required and many of them, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are produced synthetically. However, these synthetic antioxidants are reported to be toxic. As a result, there is an

increasing interest in natural antioxidants that are present in many plants (44). In this context, the interest in the olive leaf and its chemical composition has recently been increasing. In fact, Olive leaves are considered a cheap raw material that can be used as a source of high-added-value products (45). This research was done to confirm the ability of leaf extracts issued from wild olives to synthesize secondary metabolites, to evaluate their capacity as natural antioxidants, as well as to examine how geographic differences could influence the chemical composition and bioactivity of wild olives.

In general, the results obtained in this work indicate clearly that leaf extracts obtained from the two wild olive subspecies contain high concentrations of phenols and flavonoids. According to the solvent used the Methanolic extracts of the two subspecies were shown to be containing higher phenolic component levels compared to water extracts. in relation to the two wild subspecies examined the methanolic extract of the subsp.laperrinei spread from Sahara contains a very high proportion of these phenolic compounds. These height concentrations of phenolic found in all our extracts can be explained by the type of solvent. Therefore, polar solvents were the best extraction media for phenolic compounds (46). These amounts found are in agreement with numerous research which have been done on methanolic extract of olive leaf from Tunisia, Malta, France, Serbia (47) and in Chemlali variety from west of Algeria (48) as well as Meski cultivar from Tunisia (59). In contrast to our results, other studies determined the height concentration of these phytochemicals (43, 50, 51), additionally according to the results, flavonoids represent the major fraction of polyphenol compounds; this result is in accordance with those suggested by Chu et *al*. (17).

On another side, the extracts from the Saharan region also induce an increase in the scavenging activities of free radicals more than those from the northern region related directly to their higher levels of polyphenols (22,52,53), these later can prevent the synthesis of free radicals and opposing the oxidation of macromolecules (54,55), the variability of the antioxidant activity observed between leaf extracts of the two wild olives can be explained by the structure of the phenolic compounds, notably by the number and the position of the hydroxyl groups compared to the functional carboxyl groups (1). Therefore, the antioxidant potential of the subspecies studies is height compared to many other published results in Algeria (51), France and Serbia (47), while other studies showed a high activity to our finding (43, 48), furthermore, our results are added to the other reported (32, 56, 57) to confirm that olive leaves are a source of several antioxidants.

As previously noted, the variations in the antiradical activity observed for the various extracts studied related to the bioactive content, in order to identify and quantify those chemicals and estimated their possible influence on the antioxidant potential an HPLC-DAD technique is employed, a difference in the number and quantity of compounds was detected between extracts types and subspecies studies, which var. *sylvestris* extracts are predominant in terms of the number of identified compounds while subsp. *laperrinei* is dominating relative to the amount of these constituents, consequently we can deduce that, like in the case of subsp. *laperrinei*, not only the number of identified compounds but also the quantity and structure of these phenolics can influence the bioactivity ensured by the plant (48). The characteristic shared between all extracts that is Oleuropein predominant compound, the results of other researchers studying different species of *Olea europaea* L. in various extract types were in line with that Oleuropein is the most abundant phenolic compound in water extract, methanol extract (49) and also in both methanolic and ethanolic extracts (58), therefore we can deduce that Oleuropein would be responsible for great antioxidant activities (13) observed in our subspecies, moreover our results confirm that it is a well-known antioxidant derivative (59).

The HPLC analysis showed the richness of all extracts by oleuropein, a well-known antioxidant derivative that could explain the highest antioxidant activity found (59). The health-promoting properties of this compound have been extensively studied principally for their antioxidant properties and therapeutic benefits, such as antimicrobial and antiproliferative activities (15, 16). Several studies suggest that the phenol extract with high hydroxytyrosol content obtained from olive leaves (*Olea europaea* L.) increased the oxidative stability of different food lipids (butter and lard) (60). Interestingly, some studies showed that other phenolic

compounds can be involved in the antioxidant activity of olive leaf extract for example, rutin has good effects such as antioxidant, and anti-aging, their beneficial effects are attributed to their ability to reduce oxidative stress, in high-cholesterol-diet-fed (61, 62). Besides, luteolin may have preventive benefits against the appearance of diabetes-related cardiac dysfunction by minimizing oxidative stress (63); moreover, gallic acid also possesses beneficial effects on human health and decreases oxidative stress (64).

The variability seen in the phytochemical profile and antioxidant potential between sub-species studies, as well as the predominant of the stressful one, subsp. *laperrinei*" in both types of extracts than the Mediterranean subspecies, may be explained the role play by secondary metabolites in the process of plants adapting to the ecological condition in their environments (65), in which synthesis and accumulation of those metabolites, mainly the phenolic compounds increased during stress It could support the suggestion that phenolics could play a key role in the ecophysiological adaptation of *Olea europea* L. to the specific ecological conditions (48), additionally, Edziri et *al*. (50) hypothesized that the significant differences observed in leaves methanolic extracts of four olive cultivars from Tunisia can be explained by many factors including genetic origins, geographical region, soil composition, environmental climate, altitude, rainfall and the amounts and type of phenol contents may vary according to the olive variety. Furthermore, the type of solvent used through the extraction protocol (66). These factors may directly affect the chemical profile and, as a result, their therapeutic effects, as in our study.

Conclusion

Olive leaves are widely applied in different fields due to their wide range of bioactivities. Moreover, the valorization of Algerian genetic heritage, especially wild olives and Saharan endemic subspecies which are unknown until this day like our case of subsp. *laperrinei* is becoming a necessity to demonstrate their importance, by studying their bioactive content.

From the results of this preliminary study, the optimal extraction procedure for the dried leaves of the two samples is maceration with methanol than water. It gives a high extract yield, the maximum total phenolic and flavonoid contents, the greatest antioxidant activity as well as the main active components. Moreover, it could be confirmed that not only cultivated but also wild olive leaves are considered a source of natural antioxidants due to their height amounts of total phenolic and flavonoid content and good radical scavenging activity which are detected preceding. Despite wild olive "subsp. *laperrinei*" spread in arid regions (Sahara) but containing a high amount of these phytochemicals and Greater antioxidant activity which is similar to that presented by BHT than var. *sylvestris* which collected from a habitat with more favorable conditions.

In addition, the variability demonstrated between the two subspecies explained clearly the role of habitat factors in the production of these secondary metabolites and on the other hand, the role of the secondary metabolites in the ecophysiological process of plants to adapting in stressful conditions (lower precipitation, higher temperatures). As a result, it has become a necessity for the pharmaceutical and cosmeceuticals industry the shift towards natural products.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Correspondence:
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Sara Bouchoucha, MD
Department ofecology and vegetale biology, Laboratory of natural resources valorization, Faculty of Natural and Life Sciences, University Ferhat Abbas Sétif 1
Sétif 19000, Algeria
E-mail: sarrabouchoucha41@gmail.com