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Research Article

Characteristics, Phytochemical Analysis and Biological Activities of Extracts from Tunisian Chetoui *Olea europaea* Variety

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This study selected 10 extracts from Tunisian chetoui *O. europaea* variety for their total phenolics, flavonoids, and phytochemical analyses as well as for their antioxidant and antimicrobial activities determination. The *in vitro* antioxidant property was investigated using DPPH, ferric reducing antioxidant capacity (FRAP), oxygen reducing antioxidant capacity (ORAC), and β-carotene-linoleic acid bleaching assays while antimicrobial activity was evaluated using macrodilutions method. For all organs of chetoui *O. europaea* variety, the investigated activities were found to be higher in the polar extracts (ethyl acetate, methanol, and methanol/water). These activities were correlated with the presence of phenolic compounds. Phytochemical analyses revealed that the crude extracts contain triterpenoids, quinones, and flavonoids. High performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) confirmed the presence of phenolic compounds in the studied extracts.

1. Introduction

Globally, research into biologically active natural products from plants has attracted many natural products chemists. In recent years, increasing attention has been paid to the exploration of naturally occurring antioxidants and antimicrobials because of the growing consumer demand for food products free from synthetic chemical additives [1]. In this context, the Mediterranean region has attracted special interest because of its remarkable diversity and it constitutes a reservoir for the production of medicinal plants. *O. europaea* (Oleaceae) has an important social and economic significance in the Mediterranean basin which occupies 98% of the world's cultivated olive trees. Olive is a long lived ever green tree that adapts to many soil types and environmental conditions [2–4].

The beneficial properties of *O. europaea* seem to be due to its richness in antioxidant constituents. This could be related to the phenolic compounds, which are considered to be responsible for conferring specific organoleptic and antioxidant proprieties [5, 6]. Olive contains important secondary metabolites, such as secoiridoid derivatives (oleacein, ligstroside, verbascoside, and oleuropein) and phenyl alcohols (hydroxytyrosol and tyrosol). These compounds have been shown to possess antioxidant activity based on their hydroxyl group donation to free radicals [7].

Antioxidants are substances that delay or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen species, activating a battery of detoxifying proteins or preventing the generation of reactive oxygen species [8]. As recommended by certain research groups [9], the use of more than one assay to determine the antioxidant potential of food extracts or single compounds is necessary, since different methods can yield widely diverging results. Hence various methods, based on different mechanisms, should be used.

In the present study, we investigated phytochemical characteristics and antimicrobial and antioxidant activities

of extracts from different organs of Tunisian chetoui *O. europaea* variety. For antioxidant activity determination, we used assays based on the main reaction mechanisms of antioxidants: FRAP (activity to reduce metal ions), DPPH (activity to scavenge free radicals), ORAC (oxygen reducing antioxidant capacity), and β-carotene-linoleic acid bleaching assays.

2. Materials and Methods

2.1. Experiment Material. The different organs of chetoui *O. europaea* variety were collected from Sfax (Tunisia). The plant identification was carried out by the olive institute of Sfax. The compounds, 2,2-Diphényl-1-picrylhydrazyl (DPPH), linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween 40), potassium phosphate, β-carotene, BHT (butylated hydroxytoluene), α-tocopherol, gallic acid, potassium ferricyanide, ferric chloride, and Folin-Ciocalteu phenol reagent were purchased from Biochemika Fluka. Visible spectra measurements were done using Jenway 6320D spectrophotometer.

2.2. Extraction. Leaves (272 g), stems (660 g), and seeds (993 g) of chetoui *O. europaea* variety were extracted by maceration with hexane, ethyl acetate, and methanol three times at room temperature according to the extraction diagram presented in (Figure 1). Following filtration of the suspension the crude extracts were concentrated under vacuum at 40°C.

2.3. HPTLC Analysis. A Camag (Muttenz, Switzerland) HPTLC system equipped with an automatic TLC sampler (Linomat 4) and a horizontal developing chamber were used for the phytochemical analyses. Sample (20 μL at 10 mg/mL) and standard (10 μL at 1 mg/mL) solutions were laid on using an automated TLC sampler in 7 mm bands, at 10 mm from the bottom, 5 mm from the sides, and with 3.5 mm space between the two bands. The HPTLC plate and solvent system were chosen according to phytochemicals studied. After development, the plate was removed and dried and spots were visualized under visible and UV (254 and 366 nm) light. The plates were then sprayed with a mixture of sulfuric acid and vanillin [10].

2.4. HPLC Analysis. Analytical HPLC was carried out using a Thermo Finnigan HPLC system coupled with a Spectral System UV6000LP PDA detector. A two solvent gradient method was used: A. H_2O and B. Acetonitril (ACN). The applied conditions were 0 min, 95% A – 5% B,: 5–10 min, 85% A – 15% B,: 15–35 min, 75% A – 25% B,: 68 min, 20% A – 80% B, 68 min, 20% A – 80% B, 68 min, 20% A – 80% B, 68 min, 20% A – 80% B. The flow rate was set at 1 mL/min. Available standard solution was prepared in 50% H_2O/ACN (v/v) and run under the same conditions as the samples. The analysis was maintained at room temperature and the injection volume was 10 μL. The detection was done at 280 nm and the column used was Analytical Discovery HS C18 (25 cm × 4.6 mm i.d., 4.6 μm).

2.5. Phytochemical Analysis. The preliminary phytochemical screening was performed according to the Harborne methods [11]. Plant extracts hexane, ethyl acetate, methanol, and methanol water were subjected to chemical tests for the presence of sterols, triterpenoids, carotenoids, tropolone, quinones, alkaloids, and flavonoids. A sample of each extract or fraction obtained is dissolved in a minimum amount of solvent adequate for a solution E. This will be added to various reagents according to the protocols experimental T1, T2, T3, T4, T5, and T6.

T1 (Reaction of Liebermann). An aliquot of solution E (1 mL) is mixed with acetic anhydride (0.2 mL) and 4 drops of H_2SO_4 (cc). Violet changing to blue-green indicates the presence of sterols and (or) triterpenoids.

T2 (Reaction of Carr and Price). An aliquot of solution E (1 mL) is mixed with 4 drops of $SbCl_3$ 20% in $CHCl_3$. Coloring fleeting blue to blue violet could be a sign of the presence of carotenoids and (or) triterpenoids.

T3 (Reaction of Wiustater). An aliquot of solution E (1 mL) is mixed with MeOH (0.2 mL), 1 drop of $FeCl_3$ (0.005 M), water (0.6 mL) and $CHCl_3$ (0.4 mL). Red coloration of the chloroform layer indicates the presence of a tropolone nucleus.

T4 (Reaction of Borntraeger). An aliquot of solution E (2 mL) is mixed with 2 mL of NaOH (0.1 M). If the aqueous phase is colored of red to violet, we can deduce the presence of free quinones.

T5 (Test of Flavonoids). An aliquot of solution E (1 mL) is mixed with EtOH (1 mL), H_2O (1 ml), 2 drops of HCl (cc) and Mg chips. Orange to red violet coloration and blue indicate the presence of flavonoids.

T6 (Mayer Reaction). An aliquot of solution E (1 mL) is mixed with 0.5 mL of HCl (0.1 N) and 5 drops of Mayer reagent. Formation of a white precipitate indicates the presence of alkaloids.

Mayer Reagent. Two solutions A and B are prepared as follows; Solution A: $HgCl_2$ (13.5 g) is dissolved in water (20 mL); solution B: KI (49.8 g) is dissolved in water (20 mL). These two solutions are mixed and diluted with water to one liter [12, 13].

2.6. Determination of Total Phenolics. The total phenolic content was determined using the Folin-Ciocalteu method, described by Heimler et al. with some modifications [14]. To 125 μL of the suitably diluted sample extract, 0.5 mL of deionised water and 125 μL of the Folin-Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 mL of a 7% aqueous Na_2CO_3 solution was added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/per g of extract) through the calibration curve of gallic acid.

2.7. Determination of Total Flavonoids. The total flavonoid content in extracts was determined according to Akrout et al., using a method based on the formation of a flavonoid-aluminium complex, having the maximum absorbance at 430 nm [15]. Quercetin was used to make the calibration

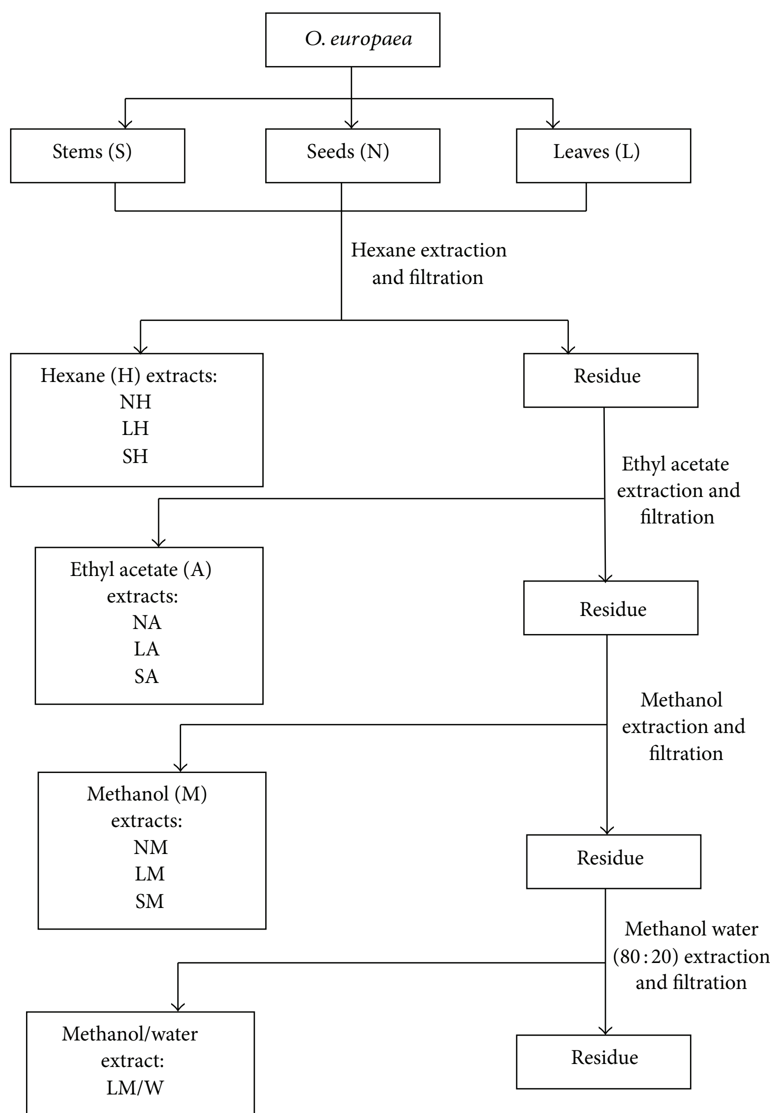

Figure 1: General scheme of extraction protocol.

curve. About 1 mL of diluted sample was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Shimadzu Jenway 6320D spectrophotometer and the total flavonoid content was expressed as quercetin equivalent (QE mg quercetin/per g of extract).

2.8. Antioxidant Capacity Estimation

2.8.1. Ferric-Reducing Antioxidant Power Assay. The reducing power of extracts was determined according to the method of Oyaizu [16]. Briefly, extracts (1 mg) were dissolved in 1 mL of distilled water and mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v). The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 mL of a 10% (w/v) trichloroacetic acid was added to the mixtures. Following that, samples were centrifuged for 10 min. Aliquots of 2.5 mL of the upper layer were combined with 2.5 mL of water and 0.5 mL of the 0.1% (w/v) solution of ferric chloride. Absorbance of the reaction mixture was read spectrophotometrically at 700 nm.

2.8.2. Total Antioxidant Capacity. The total antioxidant capacity was evaluated by the method of Prieto et al. [17]. Suitable working standards (0.24, 1.0, 5.0, and 10 mg/mL) were prepared by dissolving the extracts in distilled water. Aliquots (0.30 mL) were mixed with 3 mL of the reagent solution (600 mM sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with aluminium foil and incubated at 95°C for 90 min. The tubes were cooled to room temperature and absorbance was measured at 695 nm against a blank. Vitamin E was used as a standard. Total antioxidant capacity was expressed as equivalents of vitamin E.

2.8.3. DPPH Radical Scavenging Assay. The antioxidant activity was measured using the DPPH method with a minor modification [18]. Briefly, 2 mL of 0.1 Mm DPPH methanolic solution was added to a 1 mL of either methanolic solution of extract (sample) or methanol (control). The mixtures were vortexed for 1 min and then left to stand at room temperature in the dark. After 30 min absorbance was read at 517 nm. Radical-scavenging activity (RSA) for DPPH free radical was calculated using the following equation:

$$1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}} \times 100, \quad (1)$$

where the A_{Control} is the absorbance of the methanol control and the A_{Sample} is the absorbance of the extract. Synthetic antioxidant, BHT and Vitamin E, was used as positive control.

Bleached DPPH solution, prepared by adding 2 mL of 0.1 Mm DPPH solution to 1 mL of BHA solution, was used as blank. DPPH radical-scavenging activity was calculated as the concentration that scavenges 50% of DPPH free radical and thus has RSA = 50% (IC_{50}) [18–20].

2.8.4. β Carotene Bleaching Assay. An aliquot (1 mL) of a β carotene solution in chloroform (0.2 mg/mL) was pipetted into a flask containing purified linoleic acid (20 mg) and Tween 40 (200 mg). After removing chloroform by evaporation, oxygenated distilled water (50 mL) was added to the flask under vigorous stirring, and 5 mL aliquots of the emulsion formed were pipetted into test tubes containing ethanolic antioxidant solution (0.2 mL). The test and control (ethanol) tubes were stoppered and placed in a water bath at 50°C.

Absorbance readings at 470 nm were taken at regular intervals until the carotene was decolorized. Vitamin E was used as a reference compound. Tests were carried out in triplicate [18].

2.9. Antimicrobial Activity Evaluation. The extracts were tested against *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442), *Staphylococcus aureus* (ATCC 6538) *Micrococcus luteus* (ATCC 9341) *Enterococcus faecalis* (ATCC 19434) *Candida albicans* (ATCC 5314) *Agrobacterium tumefaciens* (ATCC 33970) *Listeria monocytogenes* (ATCC 7644) *and Salmonella enterica* (ATCC 13314). All strains were cultured in liquid LB (1% Bactotryptone, 0.5% Yeast extract, 0.5% NaCl). The estimation of the antimicrobial effect against microbial strains was performed by the method of microdilution in ELISA plates. The procedure is the following. A DMSO stock solution of the tested compound was prepared at a concentration of (250 μg/mL, 125 μg/mL, 62.5 μg/mL, and 31.25 μg/mL). A series of 8 wells of ELISA plate was used for the test. 160 μL of LB liquid (Lysogeny broth) was introduced in the first well and each of the remaining 7 wells was filled with 100 μL of the same liquid. 40 mL of the test compound was added into the first well. 1/2 dilutions were obtained by transferring 100 mL of the first well to the second well and this operation was repeated from the second well and subsequently to the last well. Then, 100 μL of the inoculum of the tested strain were added into each well. ELISA plates for a negative control (noninoculated wells) and for a positive control (inoculated wells without the active compound) were kept at 37°C for 20 hours. The inhibition of proliferative activity was determined with a plate reader at 600 nm [21].

3. Results and Discussion

3.1. HPTLC. HPTLC, combined with chemical detection, is an effective technique for the phytochemical screening of plant extracts. Detection of different phenol is attempted using different stationary and mobile phases and using specific derivation system. Presence and/or absence of specific molecules were identified by matching the colors of spots after development and after derivatisation. Nuzheinide and oleuropein were used as references. The HPTLC fingerprints (data not shown) exhibit a good separation which allows a qualitative analysis of the studied extarct. Ethyl acetate, methanol, and methanol/water extracts were rich in phenolic compounds. Indeed, they showed fluorescent spots with different colors in the corresponding plates (white, blue, pink, brown, yellow-orange, and yellow-green color). Oleuropein and nuzheinide were the more abundant phenolic compounds detected in the studied extracts due to their intense spots compared with the other ones. Hexane extracts did not present any fluorescent spots indicating the absence of phenolic compounds. In a previous study it was reported that hexane is a good solvent only for lipids of low polarity and apolar compounds [22]. On the other hand, it was established that ethyl acetate exhibits a higher extraction power in respect to other solvents, such as methyl isobutyl ketone, methyl ethyl ketone, and diethyl ether, even though it is somewhat selective towards low (about 180 Da) and medium (about 13 kDa) molecular masses of phenolic compounds from *O. europaea*. Methanol is usually used for the extraction of phenolic and flavonoid contents from plants [23]. In our study methanol appears ideal for the extract of a high amount of phenolic compounds from organs of chetoui *O. europaea* variety. This finding is in agreement with that of Karimi who observed the highest phenolics content in the methanolic extract [7].

3.2. HPLC. A reversed phase high performance liquid chromatographic technique was used to identify and quantify the major phenolic compounds in the crude extracts of organs from chetoui *O. europaea* variety. The identification was based on comparison of the chromatographic retention times and UV absorbance spectra of compounds in *O. europaea* extracts with those of standards analysed in the same condition (Table 1) including hydroxytyrosol, tyrosol, oleuropein, ligstroside, nuzheinide, quercetin, and luteolin. The HPLC profiles (Figure 2) showed several peaks corresponding to different biophenols, among which seven compounds were identified: phenyls alcohols (hydroxytyrosol and tyrosol), secoiridoids (oleuropein, ligstroside, and nuzheinide), and flavonoids (quercetin and luteolin). Oleuropein was the abundant constituent in the leaves extract; however nuzheinide was the major compound in the nuts extract. Tyrosol and hydroxytyrosol which result from the hydrolysis of ligstroside and oleuropein, respectively, were also detected in very low concentration in comparison with the other products. Flavonoids (quercetin and luteolin) were also found in extracts from chetoui *O. europaea* variety

Table 1: Identification of the principal compounds in chetoui *O. europaea* variety extracts with their retention times and UV spectrum.

Standards	Retention times (min)	λmax (nm)
Hydroxytyrosol	8.7	221, 275
Tyrosol	10.5	220, 270
Nuzheinide	19	270, 290
Oleuropein	24.05	224, 274
Ligstroside	28.6	235, 270
Quercetin	30	370, 255
Luteolin	30.6	250, 350

especially in the nuts (NA) and the leaves (LA) ethyl acetate extracts. Quantification of biophenols in the *O. europaea* extracts was performed using standard curves, in the concentration range 100 to 1000 ppm, according to the method reported by Tsimidou. The calculated concentrations were reported in (Table 2). Several factors are known to affect the quantitative phenolic profiles of *O. europaea* organs [24]. Among these factors the degree of ripeness, the geographical origin, the irrigation treatment, and the nature of the cultivar are certainly those having a pronounced influence on the phenolic composition [23].

Oleuropein is the more abundant phenolic compound in leaves of chetoui *O. europaea* variety (887.80 mg/g in methanol/water extract) and stems (230.34 mg/g in methanol extract) followed by hydroxytyrosol (251.43 mg/g in methanol leaves extract). However, nuzheinide is the major compound in Chetoui *O. europaea* variety nuts (340.06 mg/g in ethyl acetate extract and 563.89 mg/g in methanol extract). The other compounds were found in very low concentrations by comparison with the two main products [23–25]. This result is in full agreement with that of Bouaziz et al. who reported levels of oleuropein and hydroxytyrosol equal to 832 mg/g and 230 mg/g, respectively, in leaves [23]. On the other hand, oleuropein level in methanol leaves extract from chetoui variety of *O. europaea* obtained in our study (557.6 mg/g) is higher than that reported by Jemai and coresearchers (432 mg/g in leaves extracts) related to chemlali variety of *O. europaea* [26]. This finding can be explained by the difference in the used *O. europaea* varieties.

3.3. Phytochemical Analysis. Phytochemical screening (Table 3) revealed that the different chetoui *O. europaea* variety extracts contained triterpenoids, quinones and flavonoids. Alcaloids and troplones were not present in the tested extracts. The phytochemicals tested would reveal some medicinal and biological activities of the studied natural substances. Steroids and triterpenoids displayed analgesic and anti-inflammatory properties [27, 28]. Quinones have been reported to possess antimicrobial activity [29]. The presence of biologically important phytochemicals in chetoui *O. europaea* variety extracts, as established in this study, contributes to their medicinal value and therefore point to potential sources for useful drugs.

3.4. Determination of Total Phenolics and Flavonoids in Extracts. The total phenols content was determined using the Folin-Ciocalteu reagent and the results were expressed in milligram of gallic acid equivalents per gram of extract. The flavonoids content was estimated by the method of Akrout and the results were expressed in milligram of quercetin equivalents per gram of extract [15]. The obtained values for phenolic and flavonoid contents are summarized in (Table 4). Among the different chetoui extracts the methanol-water fraction showed the highest amount of phenolic and flavonoid compounds. This amount decreases with the polarity of used solvent in the following order methanol, ethyl acetate and hexane. In previous studies it was reported that solvents, such as methanol and ethanol, in combination with water have usually been used for the extraction of phenolic and flavonoid contents from plants [20]. Furthermore, the polarity of solvent is also one of interest in the processing of phenolics and flavonoids extraction. Usually, the more polar solvents are considered to be suitable for the extraction of phenolic and flavonoid contents.

3.5. Antioxidant Activity of the Extracts

3.5.1. Ferric-Reducing Antioxidant Power Assay. Determination of ferric reducing antioxidant power is a simple direct test for measuring antioxidant capacity. This assay is often used to evaluate the ability of a natural antioxidant to donate electron to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power of test specimen. The presence of reductants (antioxidant) in the tested samples would result in Fe^{3+} reduction or reduction of the ferricyanide complex to the ferrous ion (Fe^{2+}) [30]. The values of the reducing power (absorbance at 700 nm) at different concentrations are shown in (Figure 3). All the extracts exhibited reducing power that increased with concentration. The reducing powers for the different extracts related to the same organs were in the following order: methanol/water > methanol > ethyl acetate > hexane. In this way, it was established that phenolic compounds contribute directly to the antioxidant activity of plant extracts. Allouche and coworkers have explained that many aromatic rings and hydroxyl groups present in polar extracts are important for their free radical scavenging activity [18]. Linear correlation coefficients (r^2) for all the extracts were between (0.96 and 0.99) (data not shown). The most active extracts were obtained from leaves of chetoui *O. europaea* variety: LA, LM, and LM/W which were more active than Vitamin E used as a reference.

This result can be related to phenolic compounds which were identified in the leaves extracts. Those compounds have a good ability to donate electrons to reactive free radicals, converting them into more stable products and terminating the free radical chain reaction [31].

3.5.2. Total Antioxidant Capacity. The antioxidant capacity of extracts was measured spectrophotometrically through phosphomolybdenum method, which was based on the reduction of Mo^{6+} to Mo^{5+} by the sample analyte and the subsequent

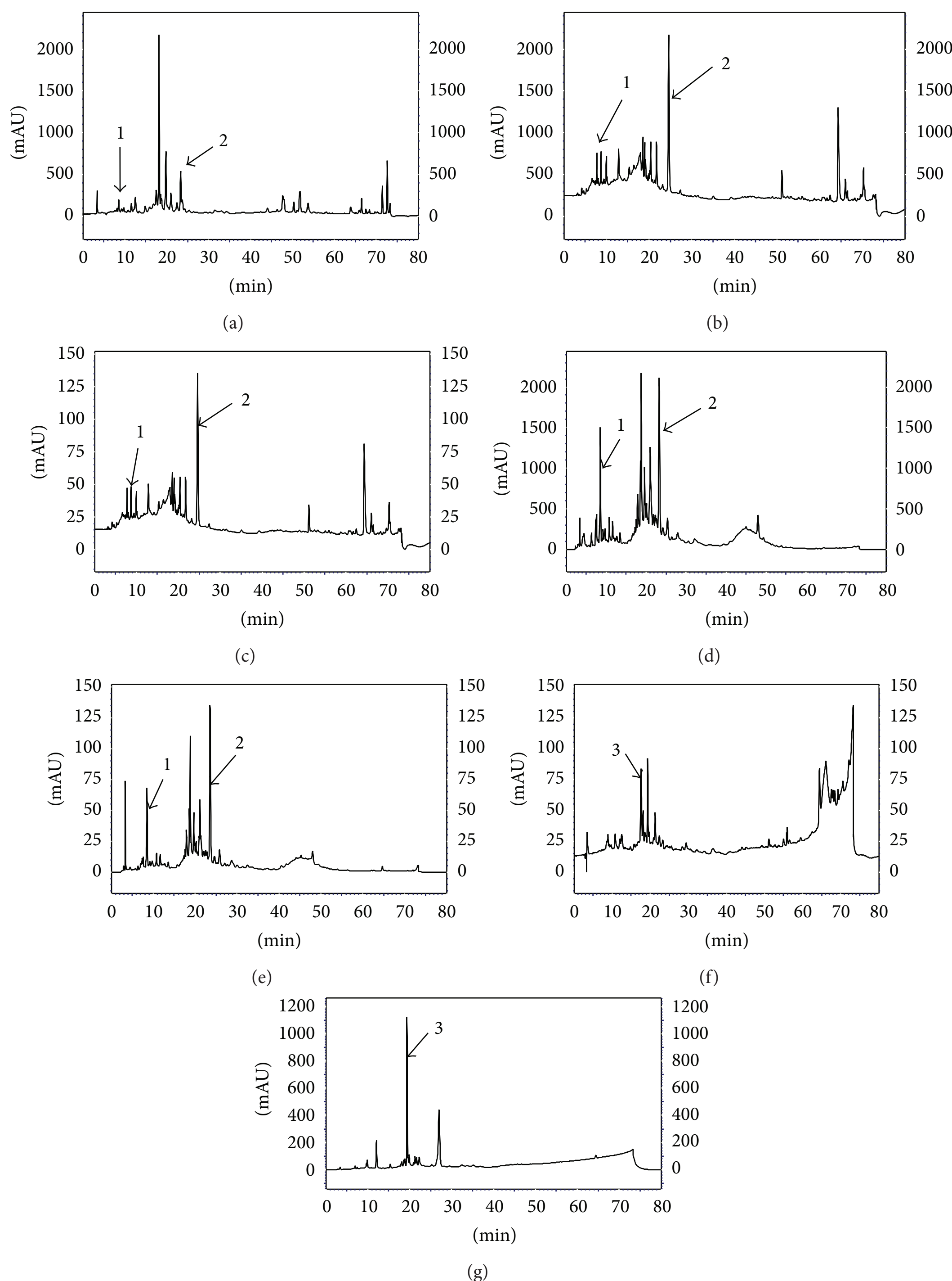

Figure 2: Representative HPLC chromatograms of chetoui *O. europaea* variety extracts. (a) Stems ethyl acetate extract. (b) Stems methanol extract. (c) Leaves ethyl acetate extract. (d) Leaves methanol extract. (e) Leaves methanol water extract. (f) Nuts ethyl acetate extract. (g) Nuts methanol extract. 1: Hydroxytyrosol, 2: Oleuropein, 3: Nuzheinide.

formation of green phosphate/Mo^{5+} compound with a maximum absorption at 695 nm [32]. High absorbance values indicate that the sample possesses significant antioxidant activity. The antioxidant capacity of the different extracts related to the same organs was found to decrease in this order: methanol/water > methanol > ethyl acetate > hexane (Table 5). The stem methanol extract is endowed with the highest activity. This result is in full agreement with that obtained from the Ferric-reducing antioxidant power assay.

3.5.3. DPPH Radical Scavenging Assay. Being a stable free radical, $DPPH^{\bullet}$ is frequently used to determine radical-scavenging activity of natural compounds. In its radical form, DPPH absorbs at 517 nm, but upon reduction with

Table 2: Quantification of the principal compounds in chetoui *O. europaea* variety extracts.

Standards	Amount (mg/g of extracts)						
	SA	SM	LA	LM	LM/W	NA	NM
Hydroxytyrosol	15.31	36.12	168.34	251.43	199.67	2.29	3.45
Tyrosol	0.53	7.92	4.58	1.87	2.50	1.23	3.25
Nuzheinide	0.15	1.13	8.50	—	3.67	340.06	563.89
Oleuropein	159.86	230.34	315.43	557.60	887.80	10.69	13.7
Ligstroside	0.33	0.80	19.12	0.87	0.31	3.85	2.67
Quercetin	0.17	0.08	—	0.15	0.32	0.12	1.65
Luteolin	1.89	0.13	7.13	0.54	6.16	0.66	—

SA: stems ethyl acetate extract, SM: stems methanol extract, LA: leaves ethyl acetate extract, LM: leaves methanol extract, LM/W: leaves methanol water extract, NA: nuts ethyl acetate extract, and NM: nuts methanol extract.

Table 3: Phytochemical analysis of chetoui *O. europaea* variety extracts.

Phytochemicals analysis	Plant extracts								
	Stems H	Stems A	Stems M	Leaves H	Leaves A	Leaves M	Nuts H	Nuts A	Nuts M
Triterpenoids	+	+	−	+	+	+	−	+	+
Quinones	−	+	+	−	+	+	−	−	−
Flavonoids	−	−	+	−	+	−	−	−	−
Alkaloids	−	−	−	−	−	−	−	−	−
Tropolones	−	−	−	−	−	−	−	−	−

+: presence and −: absence.
H: hexane, A: ethyl Acetate, and M: methanol.

Table 4: Total phenolic and flavonoid contents in the different extracts from chetoui *O. europaea* variety.

Extracts	Total phenolics (mg gallic acid/per g of extract)	Total flavonoids (mg quercetin/per g of extract)
Hexane (nuts)	5.41 ± 0.11	4.40 ± 0.24
Ethyl acetate (nuts)	57.13 ± 0.52	18.20 ± 1.34
Methanol (nuts)	83.20 ± 0.46	20.23 ± 0.35
Hexane (stems)	6.30 ± 0.54	4.22 ± 1.68
Ethyl acetate (stems)	65.10 ± 0.80	26.27 ± 3.41
Methanol (stems)	90.01 ± 0.62	30.17 ± 1.92
Hexane (leaves)	15.87 ± 0.56	1.62 ± 2.56
Ethyl acetate (leaves)	86.64 ± 1.42	43.29 ± 1.51
Methanol (leaves)	105.21 ± 1.83	22.70 ± 0.41
Methanol/water (leaves)	185.54 ± 0.98	66.90 ± 4.61

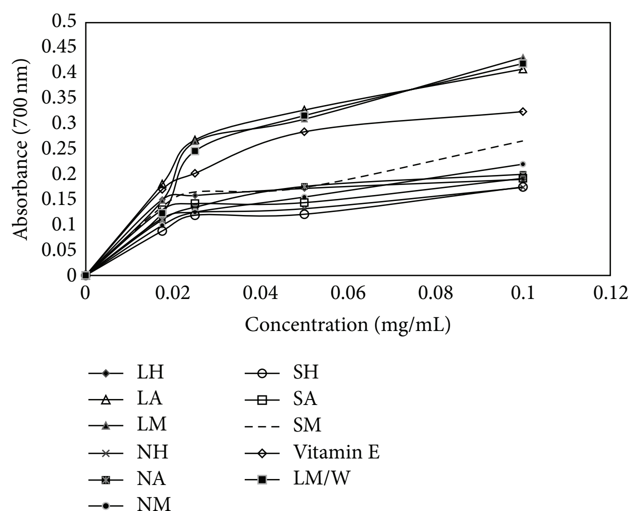

Figure 3: Reducing power of extracts from *O. europaea* organs.

an antioxidant, its absorption decreases due to the formation of its nonradical form, DPPH-H. Thus, the radical-scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored as a decrease in absorbance of DPPH solution. The concentration of antioxidant needed to decrease the initial $DPPH^{\bullet}$ concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity [3]. (Table 6) shows free radical-scavenging activity of extracts from chetoui *O. europaea* variety, Vitamin E and BHT. The order of scavenging activity of extracts is NA < NM < SA < LA < SM < LM < LM/W. The most active extracts were from leaves: LM and LM/W which were more active than Vitamin E and BHT used as a reference. This activity would be due to the high content of phenolic compounds in methanol and methanol/water extracts from leaves of chetoui *O. europaea* variety especially oleuropein and hydroxytyrosol. The chemical activities of polyphenols in terms of their reducing properties as hydrogen or electron-donating agents redirect their potential for action as free-radical scavengers (antioxidants). Polyphenols possess ideal chemical structure for free radical-scavenging activities and most of them have been shown to be more effective antioxidants *in vitro* than

Table 5: Total antioxidant capacity of different extracts from chetoui *O. europaea* variety.

Extracts	mg of antioxidant/g of extract (expressed as equivalents of vitamin E)
Hexane (nuts)	6.30 ± 0.23
Ethyl acetate (nuts)	9.28 ± 1.10
Methanol (nuts)	13.04 ± 2.40
Hexane (stems)	13.27 ± 0.13
Ethyl acetate (stems)	43.40 ± 1.08
Methanol (stems)	76.97 ± 1.21
Hexane (leaves)	10.02 ± 1.64
Ethyl acetate (leaves)	38.69 ± 0.34
Methanol (leaves)	43.81 ± 0.45
Methanol/water (leaves)	73.94 ± 1.98

Table 6: IC_{50} of different extracts from chetoui *O. europaea* variety.

Extracts	IC_{50} (mg/mL)
Ethyl acetate (nuts)	0.6
Methanol (nuts)	0.5
Ethyl acetate (stems)	0.05
Methanol (stems)	0.043
Ethyl acetate (leaves)	0.045
Methanol (leaves)	0.02
Methanol/water (leaves)	0.014
Vitamin E	0.027
BHT	0.03

vitamin E [33]. Allouche and coworkers have demonstrated that hydroxytyrosol is endowed with a powerful antioxidant activity which is due to the ortho diphenol function. Consequently, the high antioxidant activities of oleuropein can be explained by the presence of hydroxytyrosol unit in its structure [18].

3.5.4. β Carotene Bleaching Assay. In this method, β-carotene was added to the model system as a monitor for linoleate oxidation. Antioxidant activity is measured by the ability of a compound to minimize the loss of β-carotene during the oxidation of linoleic acid in an emulsified aqueous system. Each extracts was added to linoleic acid oxidation system at 200 ppm. An experiment, using Vitamin E at the same concentration, was conducted along with the other experiments in order to compare the antioxidative activity of the extracts with synthetic antioxidants currently used in the industry. The presence of samples with antioxidant activity can hinder the extent of β-carotene bleaching by neutralizing the linoleate-free radical formed in the system. The reduction in absorbance of β-carotene-linoleate emulsion in presence of the extracts is shown in Figure 4.

Accordingly, the absorbance decreased rapidly in samples without antioxidant whereas, in the presence of the tested extracts, they retained their color and thus their absorbance, for a longer time. The decreasing rate of absorbance for an

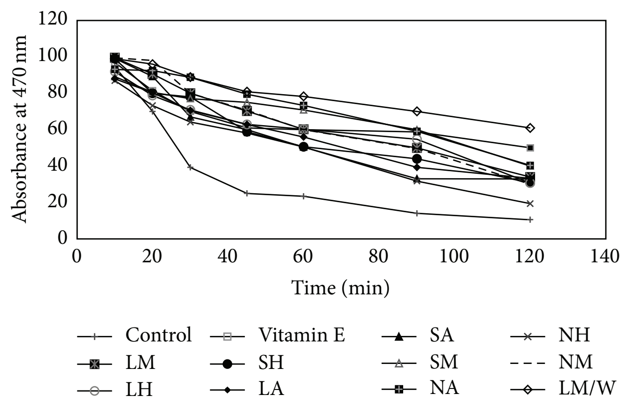

Figure 4: β carotene bleaching assay of chetoui *O. europaea* variety nuts, stem and leaves extracts.

emulsion sample with the added of hexane and ethyl acetate extracts was significantly lower than the sample with the addition of other extracts. The methanol and methanol water extracts are endowed with the highest activity. This activity may be attributed to the content of phenolic components in the extracts of chetoui *O. europaea* variety.

The ability of extracts to inhibit lipid peroxidation which was evaluated by β carotene bleaching test, showed that the peroxidation of lipids was effectively inhibited by chetoui *O. europaea* variety extracts.

3.6. Antimicrobial Activity. The results for antimicrobial tests of extracts from chetoui *O. europaea* variety are listed in Table 7. The extracts (LA and LM) were active against all assayed bacteria. The ethyl acetate nuts extract was the most active against *Candida albicans* justified by lowest MBC (62.5 μg/mL) and MIC (31.25 μg/mL) values followed by the ethyl acetate stem extract (MBC: 125 μg/mL and MIC: 31.25 μg/mL). All of the extracts have a high activity against *Micrococcus luteus*, *Enterococcus faecalis*, *Candida albicans*, *Agrobacterium tumefaciens*, and *Escherichia coli*. The antimicrobial action of phenolics is well known and is related to their ability to denaturize proteins. They act by causing the leakage of cytoplasmic constituents such as proteins or minerals and testifying their ability to cross the cells wall. Polyphenols are also known to bind to the peptidoglycan leading to the breaking of the bacterial cell-wall integrity [34, 35].

4. Conclusion

In conclusion, we found that the antioxidant activity varied between extracts from organs of chetoui *O. europaea* variety but the methanolic extracts obtained from stems, leaves and seeds exhibited the highest antioxidant activities, as determined by scavenging effect on the DPPH free radical, reducing power, antioxidant capacity, and β carotene linoleic acid model system.

High Performance Liquid Chromatography (HPLC) and High performance Thin Layer Chromatography (HPTLC)

Table 7: The MIC and MBC values of extracts from chetoui *O. europaea* variety.

Bacteria species	NH		NA		NM		SH		SA		SM		LH		LA		LM		LM/W	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
Micrococcus luteus	2	3	3	2	2	2	3	3	2	2	2	3	2	3	2	3	2	3	2	3
Enterococcus faecalis	1	2	1	2	2	1	1	2	2	2	1	2	2	3	1	2	2	3	1	2
Candida albicans	2	3	3	4	2	2	3	3	2	4	2	3	2	3	2	3	2	3	2	3
Agrobacterium tumefaciens	2	3	2	3	2	2	2	3	2	3	1	2	2	3	2	3	2	3	2	3
Pseudomonas aeruginosa	2	3	2	3	1	2	2	3	1	3	×	×	2	3	2	3	1	2	1	2
Listeria monocytogenes	×	1	×	1	×	×	×	1	×	1	1	2	1	2	2	3	1	2	1	2
Staphylococcus aureus	2	3	2	3	2	2	2	3	2	3	×	×	2	3	2	3	1	2	2	3
Salmonella enterica	×	1	×	1	×	×	×	1	×	1	1	2	×	1	1	2	1	2	×	1
Escherichia coli	1	2	1	2	1	1	1	2	1	2	1	2	1	2	1	2	1	2	1	2

1: 250 μg/mL, 2: 125 μg/mL, 3: 62.5 μg/mL, 4: 31.25 μg/mL, ×: no activity.

confirmed the presence of oleuropein in the stems and the leaves while nuzheinide is present in the nuts. Both compounds present a high antioxidant activity.

Furthermore, the antimicrobial activity showed that all the extracts from organs of chetoui *O. europaea* variety have a high activity against *Micrococcus luteus*, *Enterococcus faecalis*, *Candida albicans*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa,* and *Escherichia coli*. Extracts from Chetoui *O. europaea* variety would be used as natural antioxidant and antimicrobial agents in the agro food industries.

Conflict of Interests

The authors of this paper report no conflict of interests and have no financial and personal relationships with other people or organizations that could influence their work.

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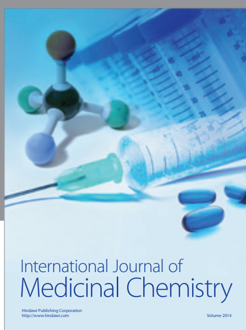
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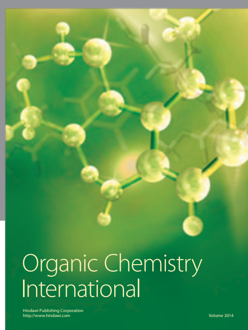
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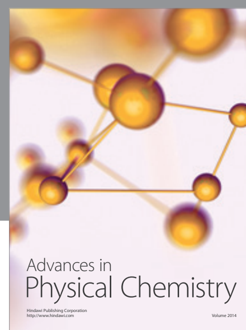
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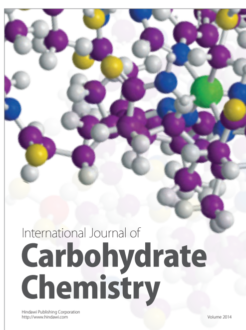
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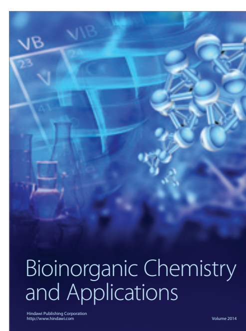
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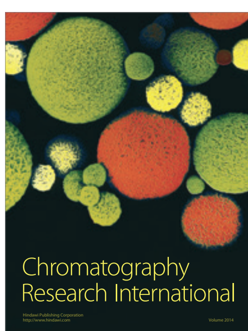
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