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Use of High-Performance Liquid Chromatography/Electrospray Ionization Mass Spectrometry for Structural Characterization of Bioactive Compounds in the Olive Root Bark and Wood of Chemlali Cultivar

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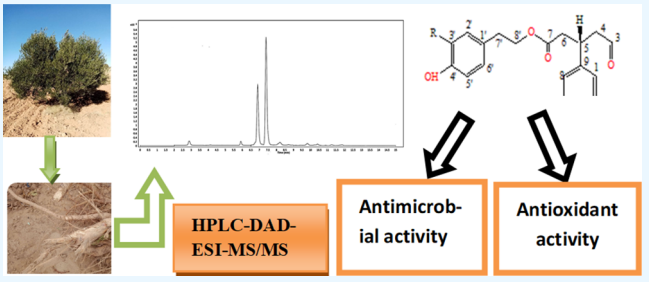
ABSTRACT: This report aims to provide complete knowledge on the polyphenol composition and biological activities of the olive tree. The extraction of the root bark and wood of *Olea europaea*. L (Chemlali cultivar) was realized by solid−liquid ethanolic extraction, whose analysis was conducted via high-performance liquid chromatography equipped with photodiode array detection and mass spectrometry (HPLC-ESI-DAD and MS/MS). Moreover, radical scavenging and antibacterial activities were determined. The results present a total of 14 phenolic compounds belonging mainly to secoiridoid and flavonoid subclasses. Oleuropein was found to be the most abundant compound at an amount of up to 7000 mg/kg followed by ligstroside and oleuropein derivatives. In addition, we found oleocanthal at a great amount (2115 mg/kg). Higher individual polyphenolic concentrations were recorded in root wood extracts compared to bark ones, except for the flavonoid group. Likewise, the total phenolic compound contents increased in the olive root wood. This trend was reflected in biological activities. In fact, root wood extracts exert more important antioxidant and antibacterial activities than bark extracts due to their high bioactive compounds.



1. INTRODUCTION

Over the last decade, the phenolic compounds as secondary metabolites deeply present in Plantae have received a great deal of attention from researchers around the world.[1−4] Such compounds have an aromatic ring holding one or more hydroxyl groups, whose arrangements vary from simple phenolic molecules to complex high-molecular mass polymers.[3,5,6] Thus, they include wide subclasses, such as phenolic alcohols, phenolic acids, flavonoids, secoiridoids, and lignans. Among these subclasses, secoiridoids and their derivatives have been gaining the increasing interest of researchers due to their biological and nutritional properties.[7−9] They are characteristic constituents of *Olea europaea* L. (Oleaceae), and they have become an essential part of the human diet.[7,10,11] Indeed, they are primarily known as potential antioxidant molecules for their ability of offering electrons to oxidant species, chelating metal ions, and improving reactive oxygen species production, along with their contribution in the pungency organoleptic attribute in olive oil.[3,12,13] The two major secoiridoids synthetized by the olive tree are oleuropein and ligstroside, conforming to esters constituted, respectively, by tyrosol or 3-hydroxy-tyrosol. Many previous studies have reported the nutraceutical properties of these compounds and their close correlation with antioxidant and antibacterial activities.[2,4,10,14−17]

Moreover, oleacein and oleocanthal are two secoiridoid dialdehyde compounds sharing very comparable structures that differ only by their aromatic moiety, which is monohydroxylated for ligstroside and dihydroxylated for oleuropein. The literature defines oleocanthal as the principal molecule in charge of the pungency of extra virgin olive oil.[3,12] Indeed, it possesses several biological properties, in particular, anti-inflammatory activity[18,19] and anti-Alzheimer's disease.[5,20,21] Likewise, oleacein has been shown to exhibit antiproliferative and antioxidant properties.[5,17,21] These compounds are widely described in olive oil and other *O. europaea* L. organs such as drupes, stems, leaves, stones, and byproducts, including pomace and olive mill wastewater.[1,6,10,14,15,22,23] The content of these compounds heavily depends on the olive biomass type, maturity stage, and extraction technology as reported by

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Table 1. Total Phenolic Contents and Antioxidant Capacities as Measured by DPPH and FRAP Assay of Olive Root Wood and Bark[a]

extracts	total phenols (mg EGA/100 g of the extract)	DPPH (μg/mL)	FRAP (mmol equiv $FeSO_4$/100 g of the extract)
root bark	311.5 ± 1.8a	6.2 ± 0.6a	46.6 ± 2.4a
root wood	480.2 ± 1.9b	4.9 ± 0.5b	63.2 ± 3.5b

[a]Each value represents the mean of three determinations (n = 3) ± standard deviation. Different letters within the same line indicate significant differences (p < 0.05) between the olive roots with different parts (bark and wood).

Gorzynik-Debicka et al.[4] However, little data about the presence of these compounds are available in the olive root, although it is the origin of water nutrients and mineral salts circulating throughout the olive tree.[14,24]

Therefore, the purpose of this paper is to highlight the potential of the olive root as a natural source of bioactive compounds. To perform the study, olive root wood and bark of cv. Chemlali were extracted by solid−liquid extraction and examined via HPLC-ESI-DAD and MS/MS. To the best of our knowledge, this is the first report to estimate qualitatively and quantitatively the phenolic fractions of olive root bark and wood separately. Moreover, radical scavenging and antibacterial activities were assessed for the first time in this work for both bark and wood extracts.

2. MATERIALS AND METHODS

2.1. Raw Materials and Reagents. *O. europaea* L. olive root cv. Chemlali (3 kg) was taken from two olive trees cultivated in the region of Sfax (Tunisia). The olive trees were about 45 years old. Olive root samples were picked in the late February of 2017. The samples were dried for 90 days at room temperature in a dark and airy room, and then, the root was scraped in a local sawmill to separate the bark from the root wood. Subsequently, all samples were ground into fine powder using a home mill before extraction.

All phenolic standards used in this work (tyrosol, caffeic, *p*-coumaric, ferulic acid, luteolin, apigenin, diosmetin) were purchased from Sigma-Aldrich (Saint-Louis-Missouri). The degree of purity of the standards was around 95% (w/w). All solvents used for HPLC-MS analysis were delivered by J.T. Baker (Phillipsburg) as used in the HPLC mobile phase. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and the Folin−Ciocalteu reagents were purchased from Sigma-Aldrich (Chemie Gmbh, Taufkirchen, Germany). Ultrapure water was used in all analyses.

2.2. Extraction Processes. Bark and wood olive root samples were extracted three times using a mixture of ethanol and water (70:30, v/v) (1 day × 2) at 37 °C as described by Abidi et al.[25] The evaporation of the extracts to dryness was then realized under vacuum at 40 °C. The extracts were filtered at the end of the experiments using grade 1 Whatman qualitative filter paper for HPLC and biological analyses.

2.3. HPLC−DAD-MS/MS Analysis. The analyses were carried out by reversed-phase liquid chromatography (RP-LC) followed by negative ion electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) detection. As for the liquid chromatography, it was realized with an Agilent (Palo Alto, California) 1200 Series LC system. It is made up of a binary pump, vacuum degasser, autosampler, and thermostated column compartment. With respect to the detection, it was conducted with an Agilent 6460 Triple Quadrupole tandem mass spectrometer, equipped with an Agilent Dual Jet Stream electrospray ionization (Dual AJS ESI) interface. In addition, the mobile phases were as follows: A: 0.1% formic acid in water and B: 0.1% formic acid in methanol.

For the absolute quantitation, the calibration models were prepared with standard solutions of pure phenols at different concentrations as reported by Ben Brahim et al.[26]

Concentrations ranged from 10 ng/mL to 5 μg/mL. Determination coefficients were in all cases above 0.999. The limit of detection (LOD) and limit of quantitation (LOQ) levels were estimated by injection of a standard at diluted concentrations. LOD values were around 0.5 ng/mL. while LOQs were around 2 ng/mL. Extraction efficiency was carried out by repetitive extractions of the same sample. Injections of additional extracts revealed extraction efficiencies between 85 and 100%.

2.4. Total Phenolic Content and Antioxidant Activity Assay. The total phenolic compounds and the radical scavenging activity of ethanolic dry olive root extracts were characterized in triplicate using a spectrophotometry method as reported by Rossi and Gulluce et al.,[27,28] modified according to Ben Brahim et al.[26,29] The FRAP assay was conducted as described by Benzie and Strain.[30]

2.5. Antibacterial Activity Assay. Our tests of antibacterial activity were conducted on both Gram-positive and Gram-negative bacteria. The used Gram-positive bacteria are *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (clinical isolate), and the Gram-negative bacteria are *Escherichia coli* (ATCC 35210) and *Pseudomonas aeruginosa* (ATCC 27853). Bacterial strains were obtained from the Department of Plant Amelioration and Agroresource Valorization (LAPVA), University of Sfax, Tunisia.

The antibacterial potentials of the *O. europaea* L. ethanol root extract (10 mg/mL) were investigated by means of the paper disc diffusion method described by Kil et al.[31] The obtained bacteria were grown in nutrient broth medium to reach a final concentration of 108 CFU/mL. Furthermore, the sterilized filter paper discs were immersed in each extract and positioned on the Mueller Hinton medium plates after streaking the test bacteria using a sterile cotton swab. Concerning the diameters of the produced inhibition zones, they were measured after 24 h of incubation of the plates at 37 °C. The positive and negative controls in this study were ampicillin (30 μg) and distilled H_2O, respectively. Experiments were performed in triplicate, however, at three different times.

2.6. Statistical Analysis. The results were expressed as the mean ± standard deviation of three measurements for the analytical determination. Significant differences between the values of all parameters were determined at p < 0.05 according to the one-way analysis of variance (ANOVA). Statistical analysis was performed using XLSTAT software for Windows (v.2013.2.03, Addin soft, New York).

3. RESULTS AND DISCUSSION

3.1. Phenolic Profiling of the Olive Root Bark and Wood. *3.1.1. Total Phenol Content.* The obtained total

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Table 2. Optimization of the MS/MS MRM Method for the Quantitative and Confirmatory Analysis of Phenolic Compounds in the Olive Root

analyte	retention time (min)	molecular formula	precursor ion (m/z)	product ion (m/z)	dwell time (ms)	fragmentor (V)	collision energy (V)
oleacein	6.2	$C_{17}H_{20}0_6$	319.1	59.0	60	65	10
oleocanthal	8.2	$C_{17}H_{20}0_5$	303.1	59.1	60	55	14
monoaldehydic open form of oleuropein aglycon	9.7	$C_{19}H_{24}0_8$	377.1	275.1	100	75	10
aldehydic closed form of oleuropein aglycon	7.2	$C_{19}H_{27}0_8$	377.1	275.1	100	75	10
monoaldehydic open form of ligstroside aglycon	11.0	$C_{19}H_{22}0_7$	361.1	291.1	100	70	10
aldehydic closed form of ligstroside aglycon	8.5	$C_{19}H_{22}0_7$	361.1	291.1	100	70	10
hydroxytyrosol	2.8	$C_8H_{10}0_3$	153.0	123.0	60	110	12
verbascoside	5.9	$C_{29}H_{36}0_{15}$	623.0	161.0	60	80	35
p-coumaric acid	6.3	$C_9H_80_3$	163.0	119.1	60	80	10
oleuropein	7.4	$C_{25}H_{32}0_{13}$	539.2	307.1	60	170	23
rutin	6.3	$C_{27}H_{30}0_{16}$	609.0	300.1	60	110	12
apigenin	10.5	$C_{15}H_{10}0_5$	269.0	117.1	100	170	35
luteolin	11.3	$C_{15}H_{10}0_6$	285.0	133.1	100	170	35
diosmetin	12.7	$C_{16}H_{11}0_6$	299.0	284.0	100	170	20

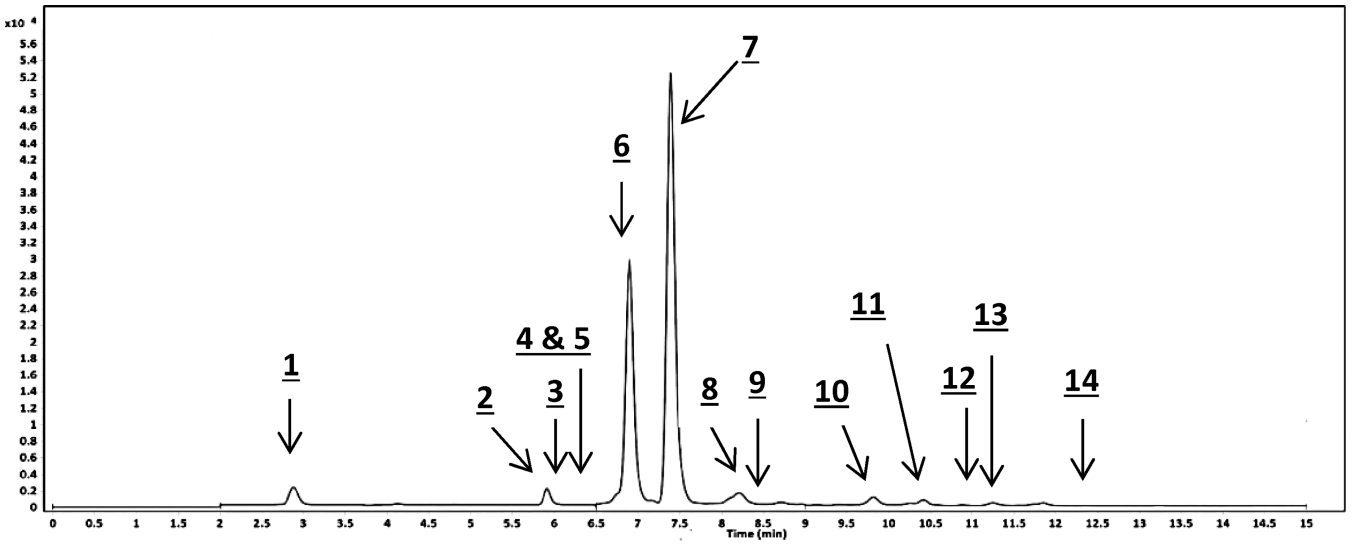

Figure 1. Base peak chromatogram (BPC) of the olive root methanol extract obtained by HPLC−DAD-ESI-MS in the negative ionization mode: **1** (hydroxytyrosol), **2** (verbascoside), **3** (oleacein), **4** (*p*-coumaric acid), **5** (rutin), **6** (aldehydic closed form (ACF) of oleuropein aglycon), **7** (oleuropein), **8** (oleocanthal), **9** (ACF of ligostroside aglycon), **10** (MOF of oleuropein aglycon), **11** (apegenin), **12** (monoaldehydic open form (MOF) of ligostrside aglycon), **13** (luteolin), and **14** (diosmetin).

phenolic content results of the tested olive root samples are depicted in Table 1. The olive root wood extract was found to hold higher total phenolic content (480.2 mg of GAE/100 g of extract) as compared to the olive root bark extract (311.5 mg of GAE/100 g of extract).

Several research studies on the determination of the total phenolic content in olive oil and biomass of different olive tree cultivars (leaves, seeds, fruit, and wood) were established.[6,32−34] In this context, Silva et al.[35] have reported that the highest level of the total phenolic content was noticed in fruits (paste and pulp extracts) and the lowest one was found in seeds. Moreover, Ben Mohamed et al.[33] have confirmed that leaves (Zalmati cultivar) have an important total phenol amount of up to 20 662 mg/kg. The variability of phenolic compounds and their diverse distribution in the plant may perhaps explicate the different levels attained for the total phenolic contents.

3.1.2. Qualitative Profiling (via) HPLC−DAD and LC-ESI-MS/MS and Their Relative MS/MS Data. The identified phenolic compounds characterized by HPLC−DAD and LC-ESI-MS/MS in the multiple-reaction monitoring (MRM) mode analysis are listed in Table 2. Each compound is associated with its retention time, molecular formula, precursor ion, product ion, dwell time, collision energy, and filtration voltage. The phenolic compounds were identified by comparing both retention times and MS spectral data between wood extract samples and authentic standard data. Figure 1 shows the base peak chromatogram (BPC) of the olive root ethanol extract in the negative ionization mode. As shown in Figure 1, 14 phenolic compounds (**1**−**14**) were identified for both root bark and wood belonging to five main classes: simple phenols (**1**), phenolic acids (**4**), flavonoids (**5**, **11**, **13**, **14**), and secoiridoids derivatives (**2**, **3**, **7**, **6**−**10**, **12**).

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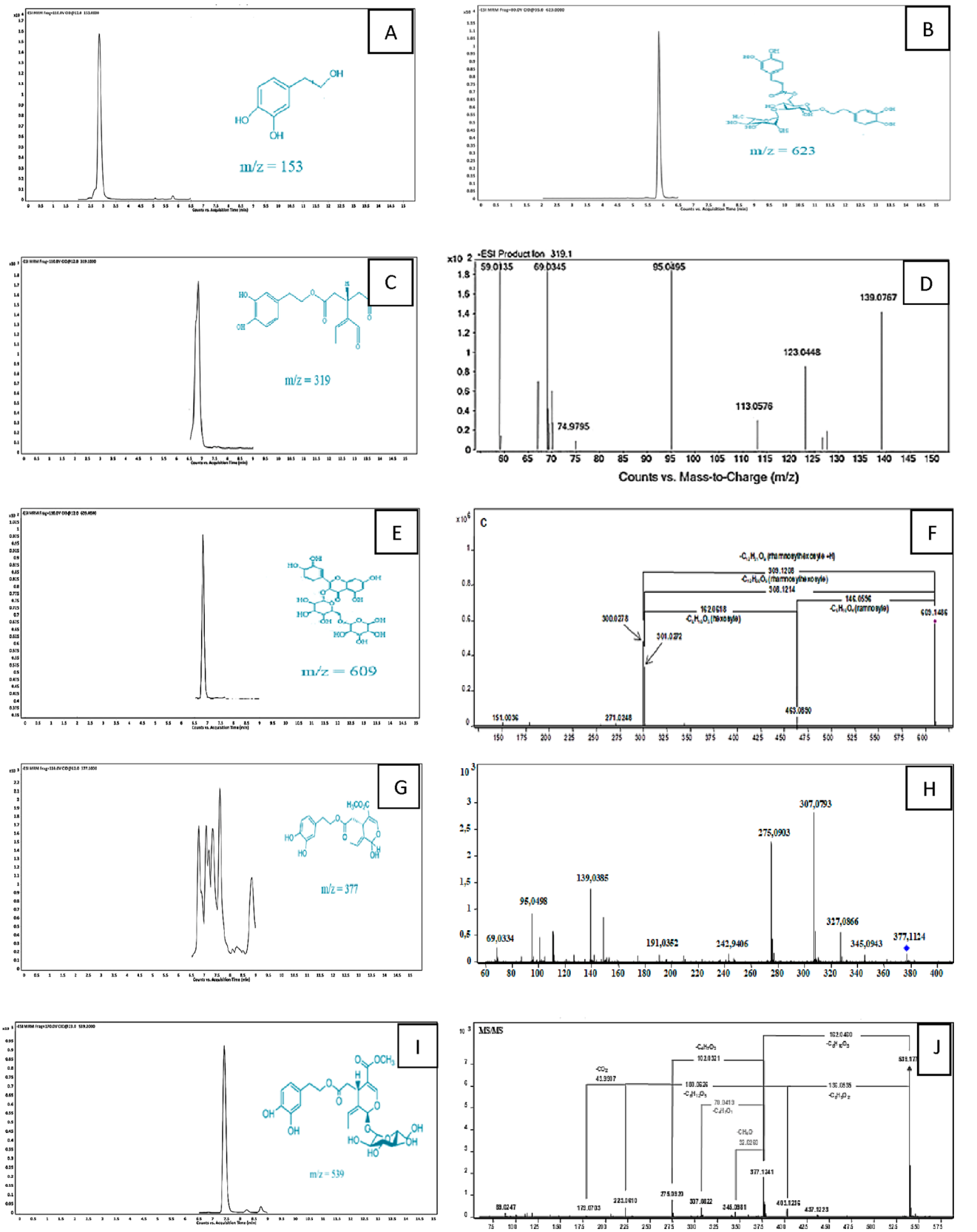

Figure 2. continued

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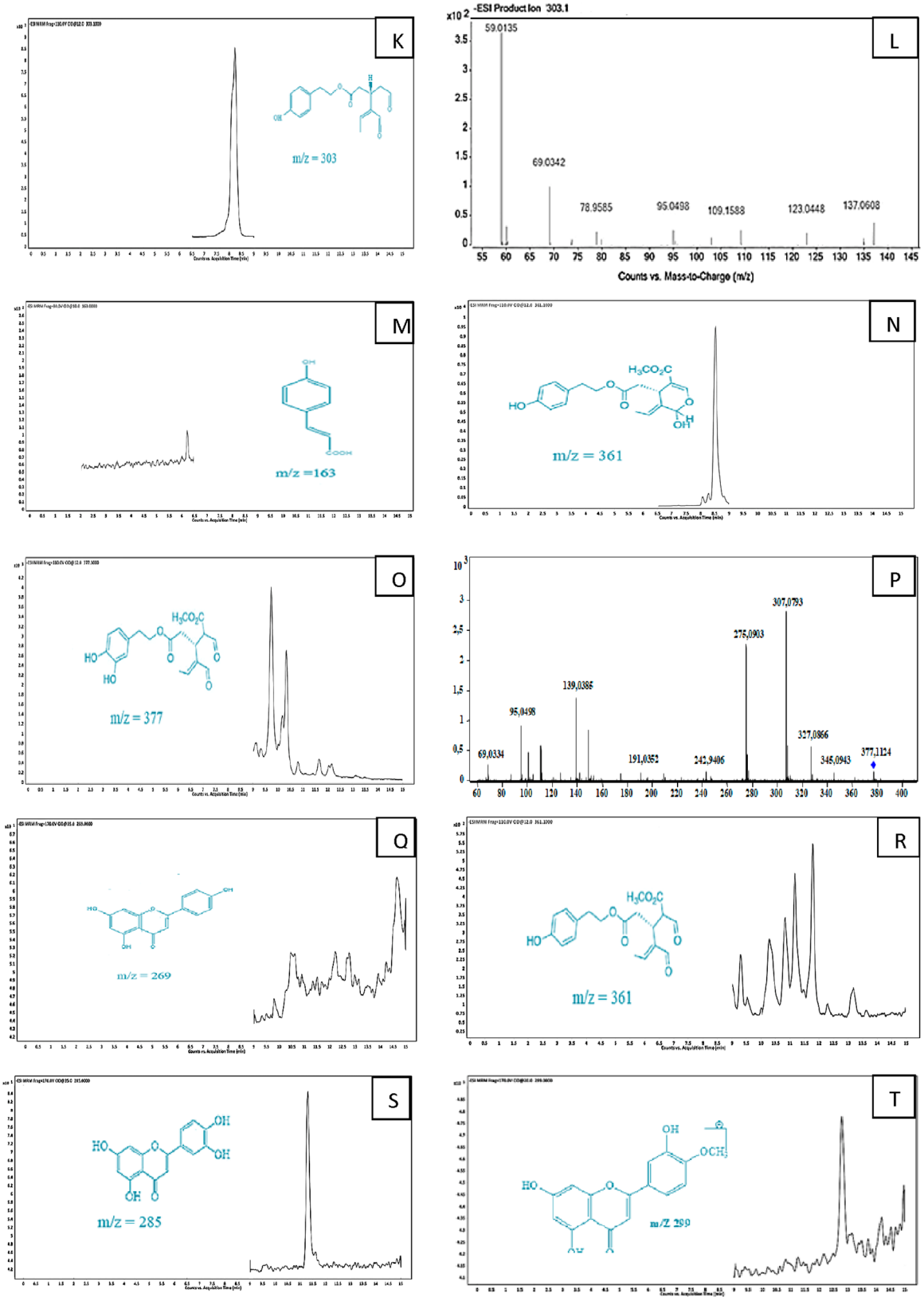

Figure 2. Extracted ion chromatogram (EIC) profiles obtained by HPLC−DAD-MS of 14 phenolic compounds identified in the Chemlali olive root bark and wood ethanol extracts and corresponding structures as A: hydroxytyrosol, B: verbascoside, C: oleacein, E: rutin, G: ACF of oleuropein aglycon, I: oleuropein, K: oleocanthal, M: *p*-coumaric acid, N: MOF of ligostrside aglycon, O: MOF of oleuropein aglycon, Q: apegenin, R: ACF of ligostroside aglycon, S: luteolin, and T: diosmetin. Negative ESI-MS/MS spectra highlighting the main fragment of detected phenolic acid in the olive root D: oleacein, F: rutin, H: ACF of oleuropein aglycon, J: oleuropein, L: oleocanthal, and P: MOF of oleuropein aglycon.

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Simple phenols were denoted with peak **1** at 2.8 min representing hydroxytyrosol (m/z 153.0). Phenolic acids were denoted with only one signal (peak **4**) at 6.3 min corresponding to *p*-coumaric acid (m/z 163.0). A total of four peaks assembled in the flavonoid group; all of them are flavones, luteolin (m/z 285.0), apigenin (m/z 269.0), diosmetin (m/z 299.0) eluted between 10.5 and 12.7 min, and rutin (m/z 609.0) at 6.3 min, which produced a fragment at m/z 300.1, corresponding to quercetin originating from the consecutive loss of a rhamnosyl group ($^{\bullet}H_{10}C_6O_4$, 146 amu) and glycosyl group ($^{\bullet}H_{10}C_6O_5$,162 amu) from their respective precursor ion (m/z 609.0). Secoiridoid derivatives were represented by eight peaks eluted within the retention time interval 5.9−11 min. Peak **2** which appears in the chromatogram at 5.9 min is verbascoside (m/z 623.0), while peak **7** corresponds to oleuropein with a precursor ion at m/z 539.2 and main fragments at m/z 403.1, m/z 377.1, m/z 371.1, m/z 327.1, m/z 307.1, m/z 275.1, m/z 223.1, m/z 179.0, m/z 149.0, m/z 165.0, and m/z 39.0 (Figure 2).

The base peak chromatogram (BPC) produced two peaks (**3** and **8**) at 6.2 and 8.2 min with precursor ion values equal to m/z 319.1 and 303.1, respectively. The extracted ion chromatograms (EIC) for $[M - H]^-$ ions of the signal at m/z 303.1 produced fragments at m/z 137.1 and m/z 119.1, which fit to tyrosol and its principal fragment. The fragment at m/z 139.1 is attributed to the dialdehydic moiety remaining after liberation of tyrosol. Likewise, the liberation of tyrosol main fragment leads to a fragment at m/z 123.0, as shown in Figure 2. However, an acetoxy ion which appeared at m/z 59.0 is associated with an ester bond. This fragmentation pattern is appropriate to the structure of the oleocanthal compound. Based on spectrophotometric data, Figure 3 displays a proposed mechanism of the fragmentation of oleocanthal.

For peak **3**, the MS1 profile generated the fragment at m/z 123.0 corresponding to the hydroxytyrosol main fragment, which is a characteristic of the oleacein structure. In addition, the signal at m/z 59.0 was also detected for this compound along with fragments at m/z 69.0 and m/z 95.0. Hence, compounds **3** and **8**, which are characterized by the same dialdehydic structure and differ only by the phenolic moiety (hydroxytyrosol or tyrosol), are well-defined as oleacein and oleocanthal, respectively. These results are consistent with those established by Sánchez de Medina et al.[21]

On the other hand, BPC chromatograms also generated two peaks, **6** (7.2 min) and **10** (9.7 min) with identical values of precursor ions (m/z 377.1). After MS/MS fragmentation of both signals, they produced fragments at m/z 275.1, related to the loss of an aldehyde group ($^{\bullet}HCO$, 29 amu) from their precursor ions. They have mass and fragmentation data very similar to those of both the aldehydic closed form (ACF) and monoaldehydic open form (MOF) of oleuropein aglycon, indicating that they are isomers. Likewise, other isomers of ligstroside aglycon (ACF and MOF) were assigned to peaks **9** and **12**, respectively (see Figure 2). Most of these compounds have been fully identified in different parts of *O. europaea* L. in several studies.[6,15,21,23,26,36,37]

Nonetheless, secoiridoid derivatives **3** and **8** have been described in rare occasions in olive trees, mainly olive oils, leaves, and fruits.[10,21,33,38] In fact, it is worth mentioning that to the best of our knowledge, this is the first time that oleacein and oleocanthal have been characterized in Tunisian olive tree root cv. Chemlali. Figure 4 explains the chemical mechanism of

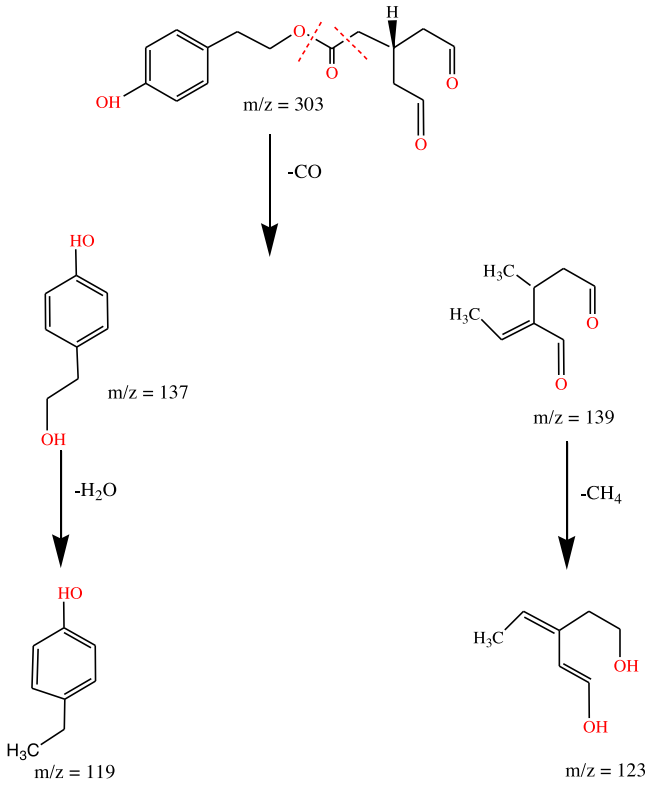

Figure 3. Proposed mechanism of oleocanthal fragmentation based on MS/MS data.

the formation of these two compounds as previousely described.[39]

3.1.3. Quantitative Profiling of Olive Root Extracts. Qualitatively, the above-mentioned results showed that secoiridoids were the main subclass phenolic compounds defined in olive root bark and wood ethanolic extracts. These findings were confirmed by the quantitative results displayed in Table 3. Indeed, the whole content of this group was in the range of 5754−18672 mg/kg.

Oleuropein is the major compound in both olive root bark and wood samples at an amount of up to 7360 mg/kg, followed by the MOF of both ligstroside and oleuropein aglycones and then oleocanthal with 989−2115 mg/kg, ACF of oleuropein aglycone, oleacein, verbascoside, and finally the ACF of ligstroside aglycone (50−60 mg/kg). The second major subclass was represented by hydroxytyrosol with a content of 50.5−359.8 mg/kg, whereas tyrosol was not detected in the olive wood extracts under study. Regardless of the root part (wood or bark), the lowest concentrations were attributed to flavonoid and phenolic acid components. In fact, rutin is the most abundant flavone with a maximum concentration of 47 mg/kg, while *p*-coumaric acid was present in traces in all root samples.

Compared to the literature, these findings are in excellent agreement with previous studies carried out on olive root extracts from Italian, Spanish, and Greek cultivars. In fact, Michel et al.[38] have detected oleuropein as the major compound in olive roots as opposed to other studied olive organs: leaves, stems, and stones. Nevertheless, other authors[14] revealed that methanolic olive root extracts provided lower amounts of oleuropein, hydroxytyrosol, and tyrosol when compared with olive stems during the maturation progress of

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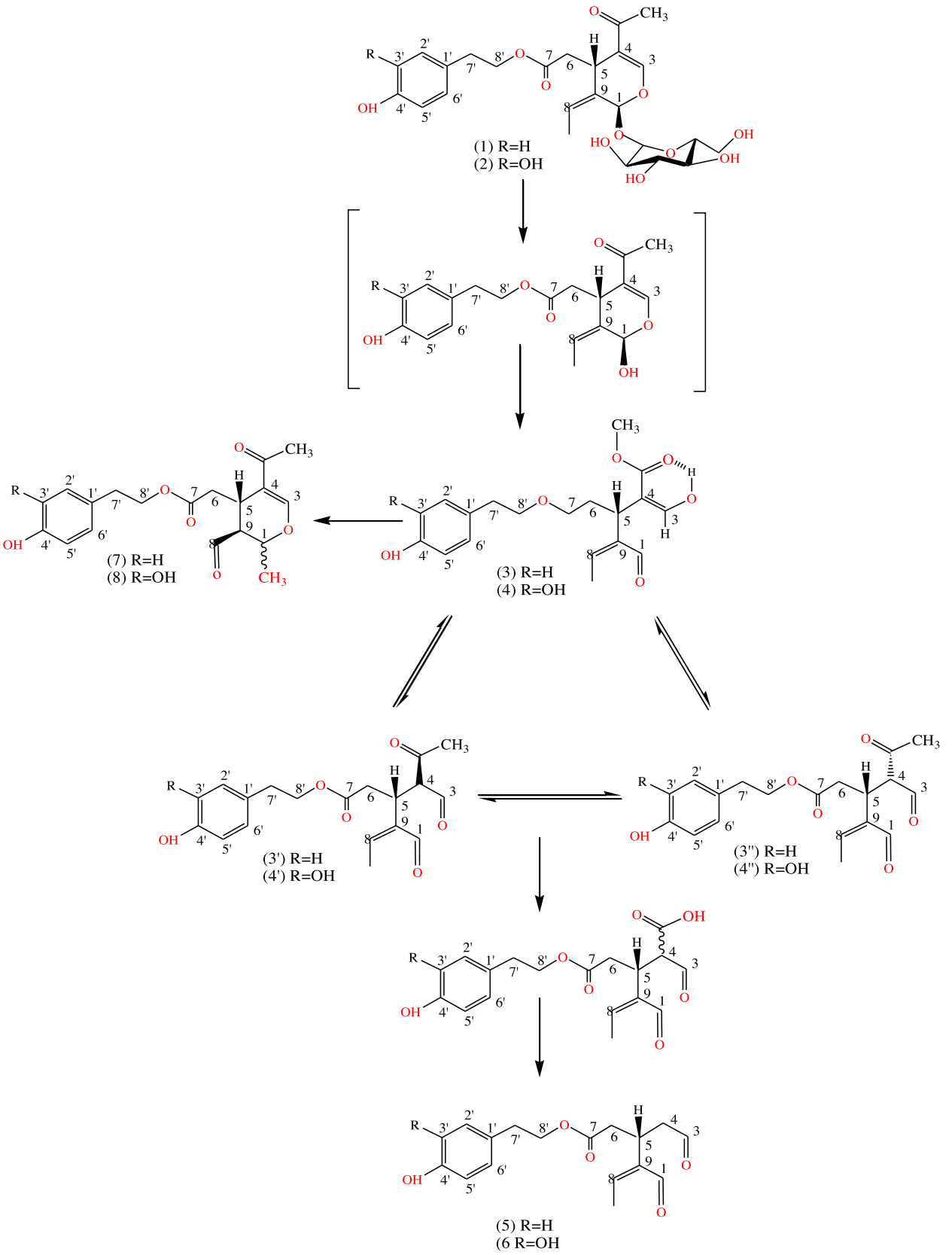

Figure 4. Chemical mechanism of formation of oleocanthal (**5**) and oleacein (**6**).

the fruit. They have explicated this phenomenon by the lower oxidative metabolism in this organ.

It is worthy to note that oleuropein detected in the extracts under study was found in a much higher concentration than that recorded in the literature.[14,38] As a matter of fact, the content of these compounds heavily depends on the olive biomass type and maturity stage. For instance, Medfai et al. have recently discovered that olive leaf byproducts are richer in

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Table 3. Phenolic Quantifications (mg/kg ± Standard Deviation) of Olive Root Bark and Wood[a]

class	phenolic compounds	concentration (mg/kg) root bark	root wood
simple phenols	hydroxytyrosol	50.5 ± 0.4a	359.8 ± 1.0b
phenolic acids	*p*-coumaric acid	0.07 ± 0.0a	0.05± 0.0a
secoiridoids and derivatives	verbascoside	51 ± 0.8a	191 ± 1.0b
	oleacein	185 ± 1.0a	890 ± 1.9b
	aldehydic closed form of oleuropein aglycon	357 ± 1.0a	1209 ± 2.0b
	oleuropein	3374 ± 2.5a	7360 ± 2.5b
	oleocanthal	989 ± 1.0a	2115 ± 1.0b
	aldehydic closed form of ligstroside aglycon	50± 0.3a	60 ± 0.9a
	monoaldehydic open form of oleuropein aglycon	654 ± 1.0a	2287 ± 1.2b
	monoaldehydic open form of ligstroside aglycon	94 ± 0.6a	4560 ± 1.5b
total		5754 ± 8.2a	18672 ± 12b
flavonoids	rutin	47 ± 0.2a	20 ± 0.1b
	apigenin	0.11 ± 0.0a	0.08 ± 0.0a
	luteolin	0.81 ± 0.0a	0.28 ± 0.0a
	diosmetin	0.57 ± 0.0a	0.19 ± 0.0a
total		48.49 ± 0.2a	20.55 ± 0.1b
total		5853.06 ± 8.8a	19052.4 ± 13.1b

[a]Each value represents the mean of three determinations (n = 3) ± standard deviation. Different letters within the same row indicate significant differences (p < 0.05) between the olive roots with different parts (bark and wood).

oleuropein, whereas olive fruits and their derived byproducts, olive pomace, contain more hydroxtyrosol, among other derivatives.[40]

Regarding the root parts (wood or bark), the wood extracts had much higher phenolic concentrations compared to the root bark for the detected phenolic compounds, especially for secoiridoid metabolites. Actually, the total concentration of this group was almost multiplied three times from 5754 mg/kg in the root bark to 18672 mg/kg in the root wood. Flavonoids seem to be an exception to this tendency. In fact, these substances reduced their contents in the root bark. For example, the rutin amount decreased nearly to the half from bark to wood (47−20 mg/kg, respectively). Perhaps, an explanation to this small content of these compounds in olive root extracts can be related to the lower need for antioxidant defense in this biomass. In addition, oleocanthal was found in important amounts in the olive root wood, 2115 mg/kg, although this component is habitually detected only in olive oil at high concentrations.[18,21]

Overall, these findings were found to confirm well those of previous researchers on the olive tree phenolic profile, yet no data pertaining to the quantitative evaluation of phenolic compounds in the olive root wood and bark exist separately.[14,38] As far as we know, this is the first report on the evolution of the concentration of these compounds in the olive root bark and wood of Chemlali cultivar.

3.2. Biological Activities of the Olive Root Bark and Wood. The biological activity datasets of the olive root bark and wood are shown in Tables 1 and 4. To the best of our knowledge, this is the first time that the antioxidant and antibacterial activities of olive root extracts were explored.

Table 4. Inhibition Effect of the Ethanolic Extracts of Olive Root Wood and Bark Parts against the Microorganisms

	inhibition zone (mm) bacteria strain (positive)		bacteria strain (negative)	
	S. aureus[a]	*B. subtilis*[a]	*P. aeruginosa*[a]	*E. coli*[a]
root wood	35 ± 0.5	34 ± 0.5	31 ± 0.5	31 ± 0.2
root bark	33 ± 0.5	33 ± 0.2	30 ± 0.3	29 ± 0.8
ampicillin	33 ± 0.2	33 ± 0.2	31 ± 0.1	30 ± 0.1

[a]S. aureus: *Staphylococcus aureus*, B. subtilis: *Bacillus subtilis*, P. aeruginosa: *Pseudomonas aeruginosa*, and E. coli: *Escherichia coli*.

3.2.1. Antioxidant Activity. The DPPH free radical scavenging activities of the olive root bark and wood extracts are summarized in Table 1. With regard to IC_{50} values (the concentration of the antioxidant providing 50% inhibition), the olive root wood extract (IC_{50} = 4.9 ± 0.5 μg/mL) was proven to have the highest radical scavenging ability, whereas the olive root bark sample (IC_{50} = 6.2 ± 0.6 μg/mL) had the lowest radical scavenging ability. These results accord well with those mentioned above for the total phenolic content. In fact, the exposition to proton radical scavengers is the cause of the considerable decline of the level of DPPH.[41] By the same token, FRAP analyses showed that olive root extracts displayed good antioxidant activities with a higher potential for the wood extract (63.2 ± 3.5 mmol equiv $FeSO_4$/100 g of the extract). It is relevant to underline the strong correlation between the antioxidant activity and polyphenol concentration and chemical structure.[6,42] Notably, flavonoids, secoiridoids, and hydrophilic phenols, such as phenolic acids and phenolic alcohols, are responsible for the high antioxidant activity in olive oil and olive byproducts.[7,16,42]

3.2.2. Antibacterial Activity. The antibacterial activities of the olive root bark and wood extracts were evaluated by the paper disc diffusion assay. The obtained results (inhibition diameter is given by (mm)) are collected in Table 4. In general, the results indicated that olive root ethanolic extracts had an antibacterial activity analogous to that of ampicillin (positive control) against all tested microorganisms. However, different responses depending on these microbes are revealed by different halo diameters. Maximal inhibition was detected for the olive root wood with inhibitory zones that are slightly higher than those of the antibiotic ranging from 31 to 35 mm. Among the four tested bacteria, *S. aureus* was the most sensitive to the olive root extract (35 ± 0.5 mm) followed by *B. subtilis*, *P. aeruginosa*, and *E. coli*. Nevertheless, no discrimination between Gram-positive and Gram-negative bacteria was detected. Both olive root bark and wood extracts exhibited an important antibacterial activity against all the studied strains.

The antibacterial activity of the plant's extracts is highly associated with their amounts of phenolic compounds. Although oleuropein is the most abundant phenolic compound in the bark and wood (up to 7360 mg/kg), it can be responsible for this strong activity, as its surfactant properties are able to affect the cell membrane permeability, as reported by Qabaha et al.[43] for olive leave samples. Other phenolic compounds such as simple phenols are thought to have a significant contribution to plant defenses against pests and pathogens.[44] Gorzynik-Debicka et al.[4] have mentioned the ability of polyphenols to participate in the immunological defense. In addition, our results are in harmony with a recent study about olive leaves.[36] In fact, the results have stated that

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ethanolic extracts have more important antibacterial activity than that of the extracts prepared using water for dried olive leaves.

Several other reports have confirmed that olive oil, fruits, and leaves exhibit antimicrobial activity against *E. coli*, *S. aureus*, *B. cereus*, and *P. aeruginosa*.[36,45−47]

4. CONCLUSIONS

To the best of our knowledge, this is the first report available to study the qualitative and quantitative phenolic profiles of olive root bark and wood separately. In addition, antioxidant and antibacterial activities have been determined for the first time in olive root samples in this paper. The obtained results emphasize that root samples contain more oleuropein than that recorded in the literature for other olive tree organs, making the Olea root a potential source of oleuropein. Moreover, other major phenolic compounds were detected in this study, namely the ACF and MOF of oleuropein aglycon and ligstroside aglycon, hydroxytyrosol, and oleocanthal. In contrast, the presence of flavonoid and simple alcohol metabolites is restricted in the extracts under study. The quantitative profiling of these samples indicates that the root bark extract of *O. europea* L. is richer in phenolic compounds than the root bark. The variations in the polyphenol content according to the root part could emanate from the low oxidative activity in the bark compared to the root wood and other olive tree parts. The results presented here suggest that olive root and especially wood extracts could be used as a natural ingredient with biological function for their antioxidant and antimicrobial properties in numerous applications in pharmaceutical, cosmetic, and food industries.

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Notes

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ABBREVIATIONS

DPPH:diphénylpicrylhydrazyle
MRM:multiple-reaction monitoring
BPC:base peak chromatogram
EIC:extracted ion chromatogram
ACF:aldehydic closed form
MOF:monoaldehydic open form
cv:cultivar

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