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Phenolic Components and Antioxidant Activity of Wood Extracts from 10 Main Spanish Olive Cultivars

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ABSTRACT: The chemical composition and radical-scavenging activity of wood samples from 10 main Spanish olive cultivars were studied. The wood samples were collected during the pruning works from trees growing under the same agronomical and environmental conditions. The 10 ethyl acetate extracts were submitted to HPLC-DAD/ESI-MS analysis to determine the phenolic constituents. Seventeen compounds were identified (10 secoiridoids, 3 lignans, 2 phenol alcohols, 1 iridoid, and 1 flavonoid) by comparison with authentic samples. Significant quantitative and qualitative differences were found among olive cultivars. The lignan (+)-1-hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside was the major compound in all olive cultivars, except in cultivars 'Farga' and 'Picual'. The multivariate analysis of all data revealed three sets of cultivars with similar compositions. Cultivars 'Gordal sevillana' and 'Picual' had the most distinct chemical profiles. With regard to the radical-scavenging activity, cultivar 'Picual', with oleuropein as the major phenolic, showed the highest activity (91.4 versus 18.6−32.7%).

KEYWORDS: *Olea europaea, olive tree wood, agricultural byproducts, Spanish olive cultivars, 'Arbequina', 'Cornicabra', 'Empeltre', 'Farga', 'Gordal sevillana', 'Hojiblanca', 'Lechín de Sevilla', 'Manzanilla de Sevilla', 'Picual', 'Verdial de Badajoz', radical-scavenging activity, simple phenols, lignans, flavonoids, iridoids, secoiridoids, multivariate analysis*

INTRODUCTION

The search for (new) natural antioxidants is an expanding research area in the cosmetic, pharmaceutical, and natural food supplements markets.[1−3] Considerable research is being conducted to find novel sources of potentially safe natural antioxidants in materials of vegetable origin,[4] including agroindustrial byproducts.[5]

Olive (*Olea europaea* L., Oleaceae) is one of the main agricultural tree crops worldwide, cultivated since ancient times to produce oil and table olives. Its cultivation covers about 10 million hectares (ha) and is predominantly concentrated in the Mediterranean basin. Spain, Italy, and Greece, in that order, are the world's principal olive oil producers.[6] Virgin olive oil, which is obtained from olive fruit by mechanical means (without refining), shows very interesting nutritional and sensorial properties, and it is one of the pillars of the so-called Mediterranean diet. Its fatty acid composition and its natural antioxidants provide important health benefits.[7] Olives are known to be rich in antioxidants.[8] They are the source of the antioxidants found in virgin olive oil. However, the whole olive tree is an extraordinary source of natural antioxidants and other valuable compounds.[9,10] Phenolic compounds have been found in olive leaves, stems, bark, wood, stones, and roots.[11−15] Considerable efforts have been directed toward their recovery from byproducts and residues (e.g., waste waters, olive pomace, leaves, or pits) generated during olive oil and table olive industrial production.[16−18]

Large amounts of biomass from olive tree pruning are generated periodically in all olive oil-producing countries. In Spain alone, >8 million tonnes per year is produced from this biomass, which consists mainly of wood with leaves and stems.[19] Olive tree wood is currently used for home heating in rural areas. Thus, this abundant and renewable biomass is being underutilized. Several attempts to find more profitable uses for this resource are known: conversion in activated carbon,[20] as feedstock for the production of lignin,[21] cellulose pulp,[22] or bioethanol,[23] and as a fuel source in power plants for energy production.[24] Several years ago, our group assessed for the first time the radical-scavenging activity of olive tree wood extracts and concluded this byproduct could be a promising source of natural antioxidants.[25] Subsequently, we initiated a program for the identification of the components with high antioxidant activity. This resulted in the isolation and structural characterization of 25 compounds.[14,26−29] This activity-guided isolation of antioxidants was conducted by the use of the online HPLC-DAD-DPPH/ABTS assay,[30] which has proven to be a very useful tool for the rapid identification of antioxidants in plant extracts, foods, and beverages.[31−33] The major components of the olive wood extracts were oleuropein and ligustroside (secoiridoids). Indeed, the group of secoiridoids, with 12 compounds isolated, is the most representative of olive wood. However, the most active antioxidant was the simple phenol, hydroxytyrosol. As a result of this research and subsequent optimization, a procedure to obtain D-mannitol and elenolic acid from olive tree wood, along with extracts enriched in the antioxidants hydroxytyrosol and oleuropein, has been patented.[34] Additional research on the composition and applications of fractions obtained from olive tree wood has recently been reviewed.[19]

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The olive wood samples used in our past studies were collected from trees belonging to cultivar Picual. This olive cultivar is the most abundant in Spain (860,000 ha) and is extensively cultivated in the province of Jaén and partially in Córdoba and Granada provinces, southern Spain.[35] There are approximately 260 olive cultivars in Spain, of which 24 are the dominant cultivar (i.e., "main cultivar") in at least one region. In this paper, we report on the phenolic composition of olive wood samples from 10 Spanish olive cultivars, growing under the same environmental and agronomical conditions. Herein, we report on differences and similarities among olive cultivars. The chosen cultivars, in order of cultivated areas in Spain, were 'Cornicabra' (cv. Cor), 'Hojiblanca' (cv. Ho), 'Lechín de Sevilla' (cv. LS), 'Manzanilla de Sevilla' (cv. MS), 'Empeltre' (cv. Em), 'Arbequina' (cv. Ar), 'Farga' (cv. Far), 'Gordal sevillana' (cv. Gs), and 'Verdial de Badajoz' (cv. VB), along with 'Picual' (cv. Pi) for comparative purposes. Fruits from cv. MS and cv. Gs are used for the preparation of table olives, whereas fruits of the rest of the cultivars are utilized for olive oil production, with the exception of cv. Ho fruits, which are used for both purposes. These 10 olive cultivars are "main cultivars" and are representative of olives cultivated throughout Spain. No study encompassing the phenolics of these 10 cultivars has been reported.

MATERIALS AND METHODS

Chemicals. The solvents used for olive wood extraction (dichloromethane and ethyl acetate) were glass-distilled prior to use. Methanol used for radical-scavenging activity assays and HPLC analyses was of HPLC grade. Two reference compounds were obtained from commercial sources: oleoside 11-methyl ester (**3**) (PhytoLab GmbH & Co., Vestenbergsgreuth, Germany) and eriodictyol (**9**) (Extrasynthèse, Genay, France). Other identified compounds in this work were previously isolated in the authors' laboratory.[14,27,28] 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) (95%, Sigma-Aldrich Chemie, Steinheim, Germany) was used for radical-scavenging assays.

Plant Material. The olive tree (*O. europaea* L.) wood samples used in this work were collected on March 28, 2014 (during an ordinary day of pruning work), in the "garden of olive cultivars" of the Centro IFAPA "Venta del Llano", Mengíbar (Jaén province), Spain. This experimental farm, located at 280 m above sea level (37° 56′ 26″ N; 3° 47′ 6″ O), consists of a collection of one-trunk olive trees (30 years old), growing at 7 × 7 m distance, under standard growing practices and without artificial irrigation. The farm comprises one or two individual trees per each olive cultivar (around 180 different olive cultivars from Spain and the rest of the world). Thus, a single tree per olive cultivar was chosen to take pruning material. The olive cultivars selected were 'Arbequina', 'Cornicabra', 'Empeltre', 'Farga', 'Gordal sevillana', 'Hojiblanca', 'Lechín de Sevilla', 'Manzanilla de Sevilla', 'Picual', and 'Verdial de Badajoz' (Table 1). Because only one or two old olive branches were cut per tree during the pruning work, it was necessary to take a single piece of wood (approximately 3−4 cm diameter and 50 cm length) from each olive cultivar as not all cultivars had two branches pruned from the south side. In all cases, one old branch was utilized having been pruned from the south-facing side of the tree to minimize microhabitat variation. Thus, all single wood pieces collected for this work were taken from the south side of the trees in order to make the selected pruning material as homogeneous as possible for comparative purposes. Every wood sample was stored for 3 months in a dry and dark place at room temperature with passive ventilation, prior to extraction. Just before the start of the extraction process, the wood piece was shaved in a local sawmill (wood shavings: length, 3−5 cm; thickness, 0.1−0.3 mm).

Extraction. Wood shavings (40 g) were extracted successively with dichloromethane (DCM) and ethyl acetate (EtOAc) (350 mL of each) for 2 h at reflux under nitrogen atmosphere, following a protocol previously optimized by the authors.[29] The solvents were evaporated under reduced pressure to give the corresponding DCM and EtOAc extracts (i.e., dry extracts). DCM dry extracts were discarded, whereas all EtOAc dry extracts were stored under argon in sealed vials at −20 °C until analysis. All of the extractions were carried out in duplicate. Extraction yields are reported in Table 2.

Table 1. Selection of Spanish Olive Cultivars (*Olea europaea* L.) Included in This Work

olive cv. name	abbrev[a]	surface[b] (×1.000 ha)	crop land (provinces)[b]	fruit destination[b]
Picual	Pi	860	Jaén, Córdoba, Sevilla	olive oil
Cornicabra	Cor	269	Ciudad Real, Toledo	olive oil
Hojiblanca	Ho	217	Córdoba, Málaga, Sevilla	olive oil, table olives
Lechín de Sevilla	LS	105	Sevilla, Cádiz	olive oil
Manzanilla de Sevilla	MS	85	Sevilla, Badajoz	table olives
Empeltre	Em	72	Zaragoza, Teruel, Baleares	olive oil
Arbequina	Ar	71	Lérida, Tarragona	olive oil
Farga	Far	45	Castellón, Tarragona	olive oil
Gordal sevillana	Gs	30	Sevilla	table olives
Verdial de Badajoz	VB	29	Badajoz, Cáceres	olive oil

[a]These abbreviations for olive cultivar names are used throughout the text. [b]Information included in this column has been taken from ref 35.

Table 2. Extraction Yields and Radical-Scavenging Percentages of Olive Wood Samples from 10 Spanish Olive Cultivars (*Olea europaea* L.)

olive cultivar[a]	DCM[b]	EtOAc[c]	RSP[d]
Pi	0.7	1.6	91.4 a
Cor	0.4	0.5	32.7 ab
Far	0.4	0.4	31.3 bc
LS	0.5	0.5	28.4 bcd
MS	0.4	0.5	26.4 cde
Ar	0.6	0.4	24.0 de
Gs	0.5	0.5	21.6 e
Em	0.5	0.3	21.0 e
Ho	0.5	0.3	21.0 e
VB	0.4	0.5	18.6 e

[a]Abbreviated olive cultivar names. See Table 1 for meaning. [b]Yields for dichloromethane extracts. Values are means of two replicates. [c]Yields for ethyl acetate extracts. Values are means of two replicates. [d]RSP (radical-scavenging percentage) values for the ethyl acetate extracts are expressed as DPPH$^{\bullet}$ scavenging (%). Values are means of three replicates, and the RSD is <1%. RSP data values followed by common letters are not significantly different (F = 230.0; P < 0.05).

HPLC-DAD Analysis. High-performance liquid chromatography (HPLC) analyses were performed by an analytical RP-HPLC (Spherisorb ODS-2 column, 250 mm × 3 mm i.d., 5 μm, Waters Chromatography Division, Milford, MA, USA) on a Waters 600E instrument (Waters Chromatography Division) equipped with a diode array detector, with a scan range of 190−800 nm (Waters CapLC 2996 photodiode array detector, Waters Chromatography Division) and operating at 30 °C. Samples for injection were prepared by dissolving each EtOAc dry extract in MeOH at a concentration of 10 mg/mL (injection volume, 5 μL). HPLC separations were achieved following a method previously used by the authors to analyze olive wood extracts:[14] H_2O/CH_3COOH, 99.8:0.2, v/v (solvent A) and CH_3OH/

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Table 3. Chromatographic and Spectral Characteristics of the Phenolic Compounds Studied in 10 Spanish Olive Cultivars (*Olea europaea* L.)

compound	t_R, min	λ_{max}, nm	$[M - H]^-$	fragment ions
hydroxytyrosol (**1**)[a]	5.1	226, 280	152.8	123.1
tyrosol (**2**)[a]	6.6	224, 278	136.8	119.0
oleoside 11-methyl ester (**3**)[b]	10.0	239	402.7	332.7, 222.6
aldehydic form of oleuropein aglycone (**4**)[c]	10.5	226, 284, 346	–[d]	176.8
(−)-olivil 4-*O*-*β*-D-glucopyranoside (**5**)[c]	13.0	230, 279	537.1	375.2
(−)-olivil (**6**)[c]	19.4	230, 282	375.2	179.7
2″-hydroxyoleuropein (**7**)[e]	20.4	226, 279	555.1	539.0, 393.1
7-deoxyloganic acid (**8**)[a]	23.5	232	359.2	197.6
eriodictyol (**9**)[b]	28.3	288	286.7	150.6
(+)-1-hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside (**10**)[c]	29.7	230, 280	535.1	355.1
oleuropein (**11**)[a]	34.1	230, 284	539.0	376.9
ligustroside (**12**)[a]	39.9	230, 279	522.8	361.2
jaspolyoside (**13**)[e]	44.6	233, 284	925.2	539.1
jaspolyoside isomer (**14**)[f]	49.2	233, 284	925.2	539.1
jaspolyanoside (**15**)[e]	49.6	228, 277	909.2	523.1
isojaspolyoside A (**16**)[e]	49.7	233, 283	925.2	539.1
jaspolyanoside isomer (**17**)[f]	50.0	228, 277	909.2	523.1

[a]An authentic sample of this compound was isolated by the authors from cv. Pi.[27] [b]This compound was identified by comparison with a commercial standard. [c]An authentic sample of this compound was isolated by the authors from cv. Pi.[14] [d]The $[M - H]^-$ ion for this compound was not observed. [e]An authentic sample of this compound was isolated by the authors from cv. Pi.[28] [f]This compound was tentatively identified on the basis of its UV−vis and MS spectra and MS fragmentation pattern.

CH_3COOH, 99.8:0.2, v/v (solvent B) at a flow rate of 0.7 mL/min: linear gradient from 20 to 70% B for 55 min and another 10 min to return to the initial conditions. Quantification of identified compounds in each EtOAc extract was carried out by the external-standard method, using HPLC peak areas at 230 nm (**5**−**7**, **10**−**17**), 239 nm (**3**, **8**), 280 nm (**1**, **2**), 288 nm (**9**), or 346 nm (**4**). The concentration of each compound was measured by comparing it with calibrations made with the pure compound analyzed under the same conditions. Two of them are commercial reference standards (**3** and **9**), and the remainder of the compounds were isolated and characterized previously by us.[14,27,28] Calibration curves were obtained for each standard in the following concentration ranges: hydroxytyrosol (**1**) in the range of 1.0−50.0 μg/mL; oleoside 11-methyl ester (**3**) in the range of 1.0−100.0 μg/mL; aldehydic form of oleuropein aglycone (**4**) in the range of 48.7−325.0 μg/mL; (−)-olivil 4-*O*-*β*-D-glucopyranoside (**5**) in the range of 47.5−316.6 μg/mL; (−)-olivil (**6**) in the range of 51.9−692.5 μg/mL; eriodictyol (**9**) in the range of 29.2−729.0 μg/mL; (+)-1-hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside (**10**) in the range of 100.0−1000.0 μg/mL; oleuropein (**11**) in the range of 120.0−1000.0 μg/mL. Concentration values for compounds **1** and **2** were calculated by external calibration against hydroxytyrosol (**1**). Concentration values for compounds **3** and **8** were calculated by external calibration against oleoside 11-methyl ester (**3**). Concentration values for compounds **4**−**6**, **9**, and **10** were calculated by external calibration against the corresponding pure compounds. Concentration values for compounds **7** and **11**−**17** were calculated by external calibration against oleuropein (**11**). The results were expressed as milligrams of compound per gram of dry weight EtOAc extract.

Because the number of wood samples studied for each olive cultivar was limited (see discussion above), the concentration data were analyzed on the basis of two extraction replicates per cultivar for ANOVA and SNK multiple-range tests of significance.

HPLC-DAD/ESI-MS Analysis. Analyses were performed using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector and an Esquire 6000 ion mass spectrometer (MS, Bruker Daltonics, Bremen, Germany) fitted with an electrospray ionization (ESI) interface, operating in negative mode. Chromatographic separation was achieved using an analytical reverse-phase column (Spherisorb ODS-2, 150 mm × 3 mm i.d., 5 μm, Waters Chromatography Division) using the same solvents and conditions as described above. Ionization and mass spectrometric conditions were optimized by infusing a solution of pure compounds. The nebulizer pressure was 40 psi; dry gas flow, 9 L/min; dry gas temperature, 350 °C; and capillary voltage, 4 kV. Analyses were performed using scans from m/z 50 to 1200.

DPPH Radical-Scavenging Activity Assay. The radical-scavenging activity of EtOAc dry extracts was determined spectrophotometrically with the stable DPPH radical.[36] Methanolic solutions (2.4 mL) of DPPH$^\bullet$ (~7×10^{-5} M) with an absorbance at 515 nm of 0.80 ± 0.03 AU were mixed with methanolic solutions (1.2 mL) of EtOAc dry extracts at a concentration of 50 μg/mL. The resulting mixtures were shaken and kept in the dark for 15 min at room temperature. The decrease of absorbance was measured at 515 nm. Results are expressed as radical-scavenging percentage (RSP) and were calculated by using the formula[36]

$$\mathrm{RSP} = \left[\frac{A_B - A_A}{A_B}\right] \times 100$$

where A_B is the absorbance of the blank (t = 0 min) and A_A is the absorbance of tested sample solution (t = 15 min). The experiments were carried out in triplicate.

Statistical and Multivariate Analyses. Data were collected from two independent extractions from each olive cultivar. Thus, each compound (as mg/g DM) was compared among the cultivars (two replicates per cultivar) by one-way ANOVA SNK (Student−Newman−Keuls multiple-range test).[37] Gower or Manhattan metric similarities[38,39] were computed among all cultivars using character weighting of the square root ($F - 1$) (where F is the F ratio of variance among cultivars/variance within cultivars for each compound, taken from ANOVA). Using the square root of ($F - 1$) for character weighting factors places more importance on characters that vary more among the cultivars, thus improving their separation (see discussion in ref 39). Principal coordinate ordination (PCO) factored the association matrix using the formulations of Gower[40] and Veldman.[41]

RESULTS AND DISCUSSION

The olive cultivars included in this work were selected from the group of 24 main Spanish olive cultivars.[35] Table 1 presents information on the 10 olive cultivars, their cultivated areas in Spain, the Spanish provinces where they are mainly cultivated, and the use of their fruits. Each of the 10 olive varieties studied

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are the dominant cultivar at least in one district. Thus, they represent all of the olive-growing areas of Spain, from the southern half to northeastern Spain. All of the olive wood samples were collected on the same day and location, so any differences in the phenolic compositions of the samples should be due to genetics rather than different environmental and/or agronomical conditions. The olive wood samples used in the work came from the pruning materials collected at an experimental farm, Jaén province, southern Spain.

Extraction of Olive Wood Samples and Radical-Scavenging Activity. Olive wood shavings were sequentially extracted with dichloromethane and ethyl acetate under reflux conditions. Dichloromethane extractions (0.4−0.7% yield) removed nonpolar components, which are known to have negligible antioxidant activities.[29] Hence, the dichloromethane extracts were discarded. Because it is known that ethyl acetate is an excellent solvent for the extraction of antioxidants,[29] the resulting ethyl acetate extracts (0.3−1.6% yield) were submitted to the DPPH assay. The DPPH assay is based on the ability of the antioxidants to scavenge the model free radical, DPPH$^{\bullet}$, and has been widely used due to its simplicity and worldwide acceptance for comparative purposes.[36] Table 2 presents the extraction yields from wood samples and the antioxidant activities of the ethyl acetate extracts expressed in terms of radical-scavenging percentage (RSP). The ethyl acetate yields for all olive cultivars were <1%, with the exception of cv. Pi (1.6%). ANOVA of antioxidant activities revealed a highly significant difference (F = 230.0, $P = 0.2 \times 10^{-5}$) between the RSP value of cv. Pi and the other cultivars (Table 2). The RSP value for cv. Pi was 91.4%, whereas the RSP of the other olive cultivars ranged from 18.6 to 32.7% (Table 2).

Identification and Quantitative Analysis of Phenolic Compounds. The 10 ethyl acetate extracts were submitted to HPLC-DAD and HPLC-DAD/ESI-MS analyses. The identifications of components were carried out by comparison of retention times and mass and UV spectra with those of authentic samples. Table 3 shows the phenolic compounds studied in order of elution from a C_{18} HPLC column, along with their chromatographic and spectral characteristics. Figure 1 shows the HPLC chromatograms (recorded at 230 nm) of cultivars Ar, Gs, and Pi, which typify the different component profiles of the selection of olive cultivars. Compounds **1**, **2**, **4**−**8**, **10**−**13**, **15**, and **16** have been previously isolated and fully characterized by our laboratory from ethyl acetate extracts of cv. Pi.[14,27,28] Compounds **3** and **9** were identified as oleoside 11-methyl ester and eriodictyol, respectively, by comparison with the corresponding commercial standards (Figure 2). On the other hand, compounds **14** and **17** have mass and UV spectra very similar to those of jaspolyoside (**13**) and jaspolyanoside (**15**), respectively, indicating they are isomers. The 17 compounds belong to five groups of phenolic compounds: (I) simple phenols (**1**, **2**); (II) lignans (**5**, **6**, **10**); (III) flavonoids (**9**); (IV) iridoids (**8**); and (V) secoiridoids (**3**, **4**, **7**, **11**−**17**). All of these compounds have previously been isolated or detected in different parts of the olive tree, although some of them (**5**−**9**, **13**, **15**, and **16**) have been described in *O. europaea* on rare occasions.[14,27,28,42,43] In contrast, it is the first time that compounds **3** and **9** have been described in olive tree wood. Several studies on the biological activities and potential applications of some of these compounds, especially focused on hydroxytyrosol or oleuropein, have been conducted.[44−46]

After identification of the HPLC peaks, the concentration of each compound in all ethyl acetate extracts was calculated by the external standard method, correlating the peak area for each component in a given HPLC chromatogram with the calibration curves obtained using our own standards (**1**, **2**, **4**−**8**, **10**−**13**, **15**, **16**) and those commercial ones (**3**, **9**) (Table 4). Significant differences, both quantitative and qualitative, were found among olive cultivars (Table 4). Compounds **1**, **2**, **4**−**7**, **9**, **10**, and **12** were present in all olive cultivars, whereas compounds **3**, **8**, and **13** were present in only five, three, and four cultivars, respectively (Table 4). In contrast, compounds **11** and **14**−**17** were absent in three cultivars (Table 4). Compound **10** was the major phenolic compound in all cultivars, except in cv. Far and cv. Pi, where it was the second most abundant component. Compound **9** was the second highest concentration component in four cultivars (Cor, Gs, MS, and VB) and the third highest in cv. Ho (Table 4). Compound **6** was also quite abundant, being the third highest concentration component in three cultivars (Em, Gs, and LS), the highest in cv. Far, and the second highest in cv. Ar and cv. Ho (Table 4). Other prominent constituents are compounds **4**, **7**, **12**, and **11**, the latter being the major component in cv. Pi. In

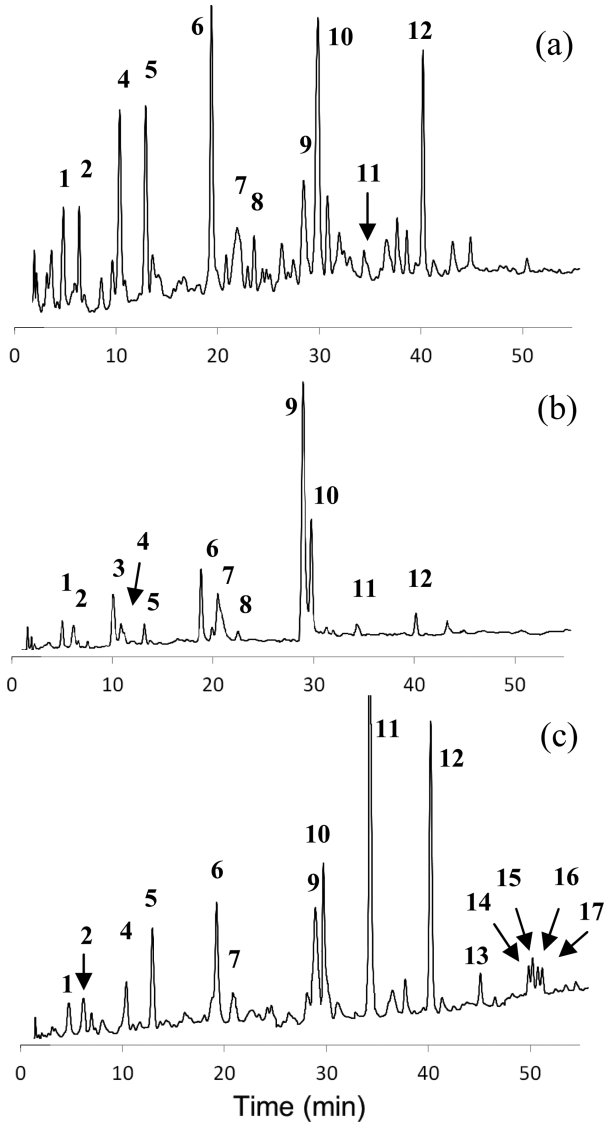

Figure 1. HPLC profiles of ethyl acetate extracts from olive tree wood at 230 nm: (a) cv. 'Arbequina'; (b) cv. 'Gordal sevillana'; (c) cv. 'Picual'. See Table 3 for compound names.

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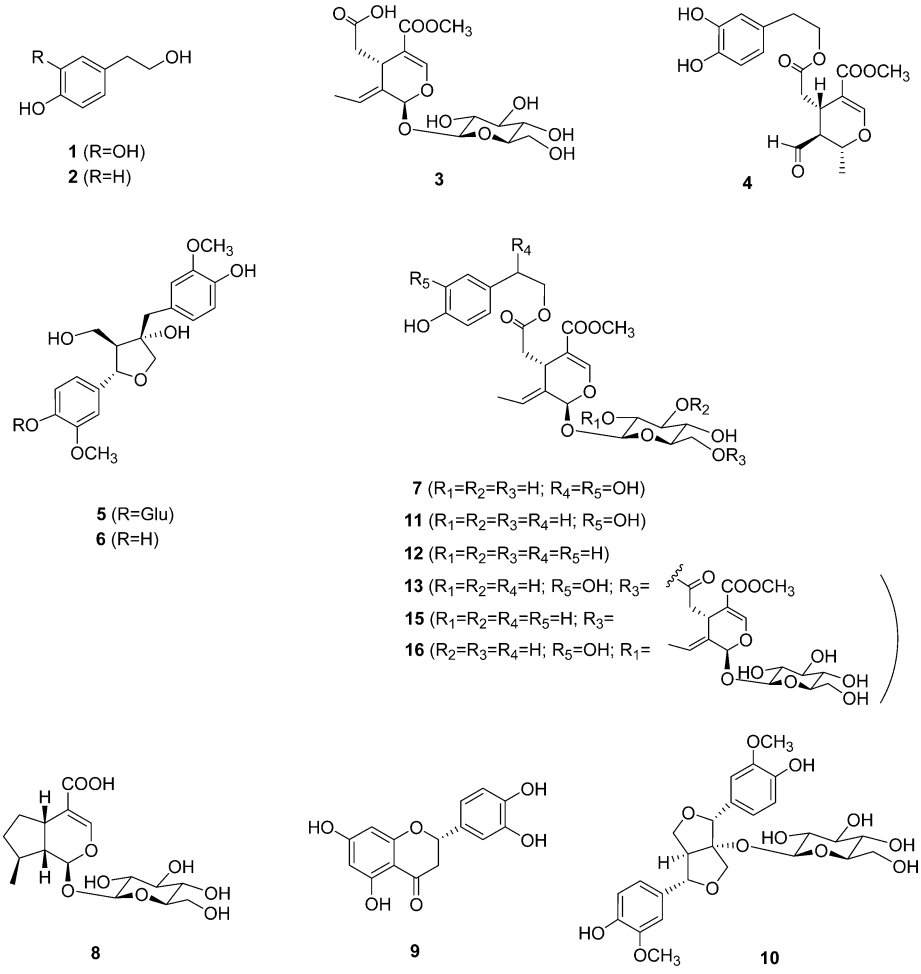

Figure 2. Compounds identified in this work from olive wood EtOAc extracts of 10 Spanish olive cultivars (*Olea europaea* L.).

Table 4. Composition of Olive Wood Ethyl Acetate Extracts from 10 Spanish Olive Cultivars (*Olea europaea* L.)

	olive cultivars[a]											
compd[b]	Ar	Cor	Em	Far	Gs	Ho	LS	MS	Pi	VB	*F* ratio[c]	*P*[d]
1	0.34 c	0.29 c	0.62 c	0.63 c	2.96 a	0.17 c	1.23 b	0.23 c	0.34 c	0.21 c	38.2	0.3×10^{-4}
2	1.46 a	0.64 bc	1.24 ab	1.10 ab	0.65 bc	1.25 ab	0.81 b	0.21 c	0.92 ab	0.62 bc	6.51	0.4×10^{-2}
3	– c	0.24 c	– c	– c	3.76 a	0.21 c	– c	0.73 b	– c	0.61 b	239.7	0.2×10^{-5}
4	17.71 c	18.68 b	20.29 b	26.81 a	26.74 a	15.37 c	25.74 a	14.91 c	17.92 bc	21.03 b	28.4	0.5×10^{-4}
5	13.33 cd	6.41 g	5.30 g	14.64 bc	15.98 b	11.37 e	9.26 f	8.38 f	19.20 a	11.98 de	66.6	0.1×10^{-4}
6	18.52 e	12.02 f	13.93 f	51.81 a	42.82 b	25.38 c	22.63 cd	18.13 e	20.70 de	20.69 de	192.7	0.2×10^{-5}
7	14.50 e	22.03 c	11.92 f	22.50 c	29.61 a	15.47 e	17.72 d	18.49 d	15.57 e	26.19 b	145.7	0.3×10^{-5}
8	1.62 b	– d	– d	1.88 a	0.62 c	– d	– d	– d	– d	– d	146.4	0.3×10^{-5}
9	5.67 e	23.19 c	3.00 f	13.94 d	71.03 a	21.62 c	15.92 d	22.20 c	7.42 e	27.61 b	779.8	0.8×10^{-6}
10	32.63 e	50.03 b	21.02 f	36.68 d	90.21 a	43.10 c	89.39 a	37.90 d	38.99 d	49.73 b	448.4	0.1×10^{-5}
11	13.11 b	12.92 b	– c	– c	11.88 b	– c	12.38 b	13.01 b	42.30 a	12.29 b	310.8	0.2×10^{-5}
12	16.68 c	20.40 b	13.28 d	16.52 c	15.97 c	17.53 c	16.52 c	17.09 c	35.00 a	21.45 b	94.1	0.6×10^{-5}
13	– c	– c	– c	– c	– c	11.22 b	11.20 b	– c	12.31 a	10.91 b	839.5	0.7×10^{-6}
14	– d	12.24 ab	– d	10.98 c	– d	13.40 a	12.13 ab	11.91 ab	13.11 a	12.09 ab	244.0	0.2×10^{-5}
15	– b	13.03 a	– b	12.13 a	– b	12.43 a	12.54 a	11.92 a	13.01 a	12.43 a	127.0	0.4×10^{-5}
16	– b	12.48 a	– b	11.41 a	– b	12.22 a	12.27 a	12.02 a	12.90 a	11.63 a	232.6	0.2×10^{-5}
17	– c	11.92 ab	– c	11.61 ab	– c	11.24 b	12.41 ab	12.13 ab	13.30 a	11.70 ab	183.1	0.3×10^{-5}

[a]See Table 1 for complete olive cultivar names. Concentration values are expressed as milligrams of compound per gram of dry weight EtOAc extract. Data values in the same row followed by common letters are not significantly different ($P < 0.05$). [b]See Table 3 for complete compound names. [c]*F* ratio = variance among cultivars/variance within cultivars, from ANOVA. [d]*P* = probability of significant difference among the cultivars. Sixteen compounds differ among cultivars at very highly significantly difference ($P < 0.001$) levels; compound **2** is highly significantly different among cultivars ($P < 0.01$).

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contrast, compound **2**, with a concentration that ranged from 0.21 to 1.46 mg/g, was a very minor component (Table 4) as were compounds **8** (0−1.88 mg/g) and **1** (0.17−2.96 mg/g). With respect to the phenolics found in our past analyses on olive wood samples from cv. Pi[14,27,28] and those identified in the Picual wood sample analyzed in this work, some minor differences are found; the flavonoid **9** has been detected now for the first time, and the group of lignans (**5**, **6**, **10**) has increased its presence, representing now ca. 30% (in weight) of the reported components. However, oleuropein (**11**) still is the major component, and the group of secoiridoids (**4**, **7**, **11**−**17**) remains the most representative (ca. 70% in weight).

Multivariate Analysis. To investigate the pattern of the chemical relationships among the cultivars, PCO was performed. A similarity matrix was produced using the average values for each of the 17 compounds. Factoring the similarity matrix resulted in five eigenroots that appeared to asymptote after the fifth root. These eigenroots accounted for 43.91, 16.51, 13.68, 8.06, and 4.87% (87.03% total) of the variance among the 10 cultivars. Ordination of the cultivars onto the first three principal coordinates (Figure 3) reveals three major

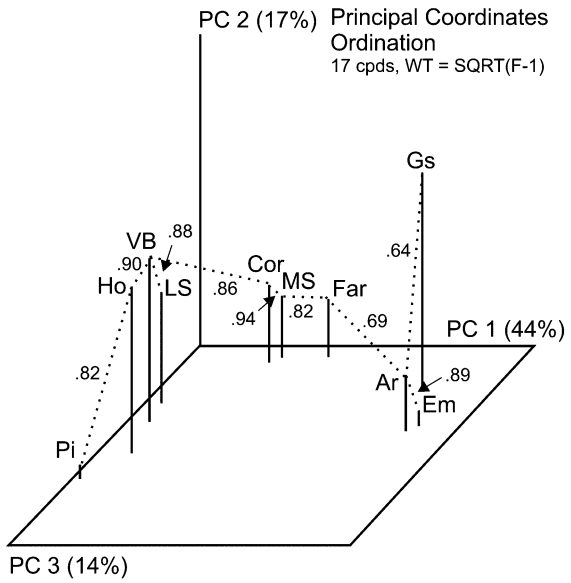

Figure 3. Principal coordinates ordination (PCO) of 10 olive cultivars based on 17 compounds with matches weighted by square root of (F − 1), where F is the F ratio from ANOVA (the dotted line is the minimum spanning network; the numbers next to the dotted line are the similarity between cultivars).

groups (cv. Ar-Em, Cor-MS, and Ho-VB-LS). Cultivar Gs has the most distinct chemical profile (being only 0.64 similar to cv. Ar), followed by cv. Pi (0.82 to cv. Ho) and cv. Far (0.82 to cv. MS) (Figure 3).

An alternative view of the chemical relationships among the cultivars is the minimum spanning network (Figure 4). Three sets of cultivars are very similar in their phenolics chemistry (cv. Cor-MS, Ho-VB, and Ar-Em) (Figure 4; Table 4). Cultivar Gs has the most distinct chemical profile (Figure 4; Table 4) with cv. Pi and Far being somewhat similar to cv. Ho and MS, respectively (Figure 4). It should be noted that the PCO and minimum spanning network reflect similarity in the cultivar's phenolics. This may or may not indicate their genetic relationships. Additional research utilizing DNA sequencing

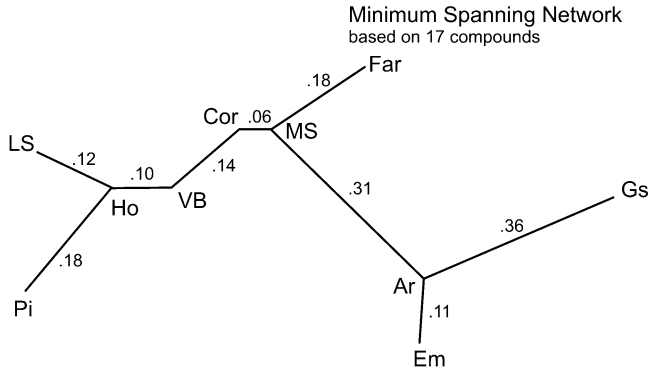

Figure 4. Minimum spanning network for 10 olive cultivars based on 17 compounds (the numbers next to the lines are distances (1 − similarity) between cultivars).

would be needed to more fully explore the overall genetic relationships among these cultivars.

In conclusion, 17 compounds were identified and quantified after analysis of the ethyl acetate extracts of 10 Spanish olive cultivars by HPLC-DAD and HPLC-DAD/ESI-MS. The lignan (+)-1-hydroxypinoresinol 1-*O*-*β*-D-glucopyranoside (**10**) (21.02−90.21 mg/g) and the flavonoid eriodictyol (**9**) (3.00−71.03 mg/g), together with compounds **6** (12.02−51.81 mg/g), **4** (14.91−26.81 mg/g), or **7** (11.92−29.61 mg/g), were prominent components in most cultivars. Multivariate analysis revealed significant quantitative and qualitative differences among the cultivars. The principal coordinates ordination and minimum spanning network established the chemical relationships among cultivars. Three sets of cultivars were found to be similar in their phenolic composition: (a) 'Cornicabra' and 'Manzanilla de Sevilla', (b) 'Hojiblanca' and 'Verdial de Badajoz', and (c) 'Arbequina' and 'Empeltre'. In contrast, cultivars 'Gordal sevillana', 'Picual', and 'Farga' had the most distinct chemical profiles. Despite having only two replicate extractions per cultivar, highly significant differences were found in ANOVA and SNK multiple-range comparisons of the chemical components among cultivars for this important biomass. Considering the abundance of this renewable agricultural byproduct and the influence of the cultivar genotype on the chemical composition, new opportunities for the industry are developing to recover diverse value-added products and explore potential applications from olive tree waste byproduct wood.

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Notes

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