Hindawi
Journal of Chemistry
Volume 2018, Article ID 2573270, 9 pages
https://doi.org/10.1155/2018/2573270

Research Article

Determination of the Phenolic Profile and Antioxidant Activity of Leaves and Fruits of Spanish *Quercus coccifera*

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Received 30 May 2018; Revised 24 July 2018; Accepted 9 August 2018; Published 6 September 2018

Academic Editor: Jose A. Pereira

In this work, we report the phytochemical composition and antioxidant activity of methanol extracts of leaves and fruits (acorns) of *Quercus coccifera* (kermes oak). Forty-one compounds were characterized using high-performance liquid chromatography with electrospray multistage mass spectrometry (HPLC-ESI-MS^n) with an ion trap mass spectrometer. A high percentage of the detected compounds were gallic acid derivatives, although some saccharides and flavonoids were also present. This phytochemical pattern is typical in *Quercus* species, which are rich in gallotannins. These compounds are partially responsible for the cardioprotective effects observed in different food samples containing them. We evaluated the antioxidant activity by ABTS and DPPH assays. In both cases, high antioxidant activity was observed, being higher in acorns than in leaves. The high antioxidant potential of the extracts, which is related to the high total phenolic content, indicates the potential benefit of the use of this species as a source of bioactive compounds.

1. Introduction

Plants represent a rich source of natural compounds which are responsible for many multifunctional biological effects. In the last few years, exhaustive research is being carried out to obtain new raw materials from plants for the development of products with healthy characteristics, which help maintain or improve health and protect against chronic diseases.

The genus *Quercus* (Fagaceae) has 450 species estimated worldwide [1] and has been widely investigated for years, not only due to their extensive use in the wine and wood industries but also for animal feeding and medicinal purposes [2]. Acorns of *Quercus* species are a high-protein food source for a wide array of wildlife and are also used to fatten poultry and pigs [3, 4]. They are not only a source of important nutrients, namely carbohydrates, proteins, fatty acids, and sterols [5, 6], but also of phenolic constituents [3, 6, 7]. On the contrary, leaves from different *Quercus* species, which are commonly consumed as tisanes (aqueous extracts), also contain bioactive compounds, in particular phenolics [1, 8]. The antioxidant and biological activities of extracts of leaves [9, 10], acorns [4, 10, 11], and other different morphological parts of *Quercus* species such as twigs and cork [12] have been evaluated. *Quercus* species have been reported to have gastroprotective [13], antibacterial [14], cardioprotective [15], hepatoprotective [15], anti-inflammatory [16], and anticarcinogenic [16] effects, among other health benefits.

These biological activities are thought to be associated, at least in part, with the presence of phenolic compounds, such as flavonoids and tannins [3, 4, 11, 17]. The phenolic profiles vary significantly among *Quercus* species. For instance, high levels of gentisic and chlorogenic acids, as well as of the flavonoids naringin and rutin, have been found in *Quercus acuta*, *Quercus glauca*, *Quercus myrsinifolia*, *Quercus phylliraeoides*, and *Quercus salicina* [18]. Nevertheless, none of these compounds was detected in any other *Quercus* species

[6]. Likewise, several gallic acid derivatives have been solely found in *Quercus ilex*, *Quercus rotundifolia*, and *Quercus suber* [3]. Despite the phylogenetic variability, flavonoids, phenolic acids, and tannins are somehow ubiquitous in all *Quercus* species [6]. High levels of ellagitannins, a group of condensed tannins, have been reported in woods and barks of several *Quercus* species used in cooperages [19]. Gallotannins, another essential group of tannins, have also been reported in extracts of *Quercus* species [1, 20]. Other phenolic compounds such as flavonoids of quercetin and kaempferol have been found in *Quercus* leaves [1].

Kermes oak (*Quercus coccifera* L.) is a small evergreen shrub of fewer than 2 meters, whose fruits are acorns provided with stings. *Q. coccifera* is the prevailing species in the evergreen sclerophyllous shrublands, which are an important part of Mediterranean rangelands. Despite its low commercial value with regard to wood production, it plays a significant role in preventing soil erosion [21], and it is used for fodder production for domestic and wild animals [22]. Kermes oak acorns seem to be a highly energetic resource for small ruminants such as goats and lambs and are often compared to barley [23, 24].

Several biological effects have been reported for *Q. coccifera* such as neuroprotective [25], antibacterial [26, 27], antifungal [26], antihelmintic [28], and antioxidant [29] activity. Furthermore, previous studies have shown that *Q. coccifera* contains tocopherols and fatty acids [7], besides phenolic compounds such as tannins and flavonoids [27, 29]. In a recent article [30], the authors characterized individual phenolics in fruits and leaves of *Q. coccifera*, although of only seven compounds. Phenolic compounds have been shown to be responsible for many health benefits [6] and are very useful in the food industry since they can be used as dietary supplements or as preservatives instead of synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA), which have negative effects on human health. Therefore, the leaf and acorn extracts can represent a valuable source of natural antioxidants for different applications. The extracts can be obtained by using simple extraction procedures directly from the raw material, reducing the need for additional processing stages.

Considering the variety of phenolic compounds that have been reported in different *Quercus* species and that the published research concerning phenolics in *Q. coccifera* is scarce, our work aimed at identifying the extractable phenolic compounds present in acorns and leaves from *Q. coccifera* and their antioxidant activity. These data provide a better understanding of the composition of this *Quercus* species and can lead to further investigations regarding the valorization of its residues and the use of its biomass, within a biorefinery concept, for the production of biofuels; chemical, pharmaceutical, and care products; and bioenergy. The exploitation of leaves and acorns from *Q. coccifera* on an industrial scale could contribute to improving the sustainability of the agro-food chain by achieving new food products or as alternative sources of different highly-valued food ingredients. The upgrading of these products of the forest industry is an important challenge in the development of a sustainable economy and environmental friendly industrial processes.

2. Materials and Methods

2.1. Reagents and Solutions. All reagents and standards were of analytical grade unless stated otherwise. Activated charcoal (p.a.), catechin (≥99%), citric acid (≥99.5%), gallic acid (≥99%), procyanidin B2 (≥90%), kaempferol (≥97%), quercetin (≥95%), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS, ≥98%), potassium persulfate ($K_2S_2O_8$, >99%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 97%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) were purchased from Sigma-Aldrich (Madrid, Spain). Methanol (MeOH ≥99%), ethanol (96%), Folin–Ciocalteu's phenol reagent (FCR), and sodium carbonate (Na_2CO_3) (p.a.) were purchased from Panreac (Barcelona, Spain). LC–MS grade acetonitrile (CH_3CN) (99%) (Sigma-Aldrich) and ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA) were also used.

2.2. Sample Collection and Preparation. Plant materials were collected from different plants placed in Sierra Morena, a mountain range in the province of Jaén, Andalucía, south-central Spain. The materials were collected in October 2016, at an approximate height of 550 meters (38°08′01.97″ N, 3°58′30.03″W). For analysis, we separated plant materials into leaves and acorns (fully ripe) and analyzed all samples as the same batch. They were lyophilized to dryness (ModulyoD freeze dryer; Thermo Fisher Scientific, Madrid, Spain), ground to powder, and stored at −20°C.

For the extraction of the phenolic compounds, we carried out an ultrasound-assisted solid-liquid extraction using 5 g of sample powder and 100 mL of MeOH using a sonicator with a temperature controller (Bandelin Sonorex Digital 10P; Sigma-Aldrich, Madrid, Spain) at 35 Hz and 280 W for 60 min (room temperature). Then, we filtered the extracts, eliminated the chlorophylls by adsorption on activated charcoal, and concentrated the extracts to dryness using a rotary evaporator (Buchi Rotavapor R-114) at 40°C. The resulting extracts were stored at 4°C until analysis.

2.3. HPLC Analysis. The analysis of the phytochemical profile was carried out by using HPLC-MS^n. Dried extract (DE) of 5–10 mg was re-dissolved in 1 mL MeOH. After filtration through 0.45 μm PTFE membrane filters, 10 μL of each solution was injected in the chromatographic system.

An Agilent Series 1100 HPLC system (Agilent Series 1100, Agilent Technologies, Santa Clara, CA, USA) with a G1315B diode array detector was used. A reversed-phase Kinetex core-shell C_{18} analytical column of 100 × 2.1 mm and 2.6 μm particle size (Phenomenex, Torrance, CA, USA) and a C_{18} Security Guard Ultra cartridge (Phenomenex) of 2.1 mm i.d. placed before the analytical column were used. The mobile phase consisted of acetonitrile (CH_3CN) and water-formic acid (100 : 0.1, v/v). The following gradients were used: initial mobile phase, 10% CH_3CN; linear increase to 25% CH_3CN (0–10 min); 25% CH_3CN (10–20 min); linear increase to 50% CH_3CN (20–40 min); linear increase to 100% CH_3CN (40–42 min); and return to initial mobile

phase and stabilization time of 7 min. The mobile phase flow rate was 0.4 mL·min^{-1}.

The HPLC system was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray (ESI) interface. The scan range was set at m/z 100–1200 with a speed of 13,000 Da/s. The ESI conditions were as follows: drying gas (N_2) flow rate and temperature, 10 mL/min and 365°C; nebulizer gas (N_2) pressure, 50 psi; capillary voltage, 4500 V; and capillary exit voltage, −117.3 V. We used the auto MS^n mode (negative and positive modes) for the acquisition, with isolation width of 4.0 m/z, and fragmentation amplitude of 0.6 V (MS^n up to MS^4). The analysis of the phenolic composition was performed with HPLC-ESI-MS^n using negative ionization mode.

2.4. Total Phenolic and Flavonoid Contents and Antioxidant Capacity Assays. The total phenolic (TPC) and flavonoid (TFC) contents were obtained using the Folin–Ciocalteu and aluminum chloride methods, respectively. TPC was expressed as mg of gallic acid equivalents (GAE) per g of DE. TFC was expressed as mg of quercetin equivalents (QE) per g of DE. ABTS^{+} and DPPH radical scavenging activities were expressed as μmol Trolox equivalent (TE) per 100 g of DE. Detailed procedures have been previously reported [31].

3. Results and Discussion

Currently, there is scarce information concerning *Q. coccifera*, as most of the published articles regarding the *Quercus* genus have focused on *Q. suber* due to its extensive use in cork industry. For *Q. coccifera*, water and methanol [29] and water with mixtures of acetone, ethyl acetate, and methanol [27] have been used as extraction solvents. For *Q. suber*, different extraction solvents and temperature have been reported: water at 80°C [1], 100% methanol and 80% methanol : water [12], or methanol and hexane [10]. Hence, it is clear that there is no standard protocol to carry out the extraction of phenolics, as they have very different polarities. In this work, we have selected methanol—one of the most common extractants [6]—to carry out the extraction procedure. We performed an ultrasound-assisted solid-liquid extraction, whose main benefits are its simplicity and rapidity.

3.1. Phytochemical Profile. The initial step for the characterization of the compounds consisted in the determination of the molecular weight of each compound. In the negative ion mode MS^1 spectrum, the most intense peak corresponded to the deprotonated molecular ion $[M-H]^-$ or formate adduct $[M+HCOOH-H]^-$. The base peak chromatograms of extracts of acorns and leaves are shown in Figure 1, whereas the MS data for the detected compounds are reported in Table 1.

3.1.1. Tannins and Gallic Acid Derivatives. Most of the compounds found in the extracts of *Q. coccifera* corresponded to tannins, which can be classified in proanthocyanidins, ellagitannins, and gallotannins (galloylglucoses).

Compound **6** was a procyanidin dimer, (epi)catechin-(epi)catechin, B-type [32]. With an additional 152 Da (galloyl moiety), we characterized compound **17** as (epi) catechin-(epi)catechin monogallate.

Twelve gallotannins, monomers and oligomers of gallic acid with a hexoside moiety, were characterized in the analyzed extracts. These compounds were characterized by the neutral losses of 152 Da (galloyl moiety; typical 169⟶125 fragmentation) and 162 Da (hexoside moiety). Compound **4** was the only monogalloyl-hexoside. Compounds **5** and **7** were digalloyl-hexosides [33]. Five trigalloyl-hexosides were characterized (compounds **9**, **12**, **16**, **18**, and **19**); compound **25** was a tetragalloy-hexoside; compounds **27** and **28** were pentagalloyl-hexosides [20], and compound **33** was a hexagalloyl-hexoside [34].

Compounds **8** and **13** were tentatively characterized as isomers of hexahydroxydiphenyl-digalloyl-glucose based on their molecular weight and fragment ions at m/z 301 (loss of a digalloyl-hexoside residue) and 483 (loss of a hexahydroxydiphenyl residue) [20].

The fragmentation of compound **11**, with $[M-H]^-$ at m/z 183, was consistent with methyl gallate [35].

Compound **20**, with $[M-H]^-$ at m/z 473, suffered two consecutive losses of 152 Da (galloyl moieties) to yield gallic acid at m/z 169, fragmentation that corresponded to trigallic acid.

Compounds **22** and **29**, which suffered neutral losses of 152 and 15 Da, were characterized as galloyl methyl gallate isomers [36].

Compound **23** exhibited the deprotonated molecular ion at m/z 615. After the neutral losses of 152 Da (galloyl) and 162 Da (hexoside), it yielded a fragment ion at m/z 301, corresponding to quercetin. Hence, we identified this compound as quercetin hexoside-gallate.

Compound **26**, $[M-H]^-$ at m/z 441, suffered the neutral loss of a galloyl moiety to yield (epi)catechin at m/z 289, so we characterized it as (epi) catechin-*O*-gallate.

Similar to the reported results, other authors found abundance of galloyl derivatives in different *Quercus* species [1, 20]. For instance, mono-, di-, tri-, tetra-, and pentagalloyl glucosides have been found in *Q. ilex*, *Q. suber*, and *Q. rotundifolia* [6].

3.1.2. Flavonoids. Compound **10** was identified as catechin by comparison with an analytical standard. This compound has already been reported in several *Quercus* species, such as *Q. acuta*, *Q. salicina*, and *Quercus resinosa*, among others [6].

Three kaempferol derivatives were present in the extracts. The aglycone at m/z 285 was identified by comparison with an analytical standard. Compound **38** suffered the neutral loss of 308 Da (rutinoside), whereas compound **43** displayed the neutral loss of 146 (rhamnoside) and 308 Da. The exact nature of compound **44** could not be completely elucidated.

Compound **41** and **42** were characterized as quercetin-rhamnoside-hexoside-rhamnoside isomers based on the neutral losses of rhamnoside (146 Da) and hexoside (162 Da) moieties, and the aglycone quercetin observed at m/z 301 (comparison with an analytical standard).

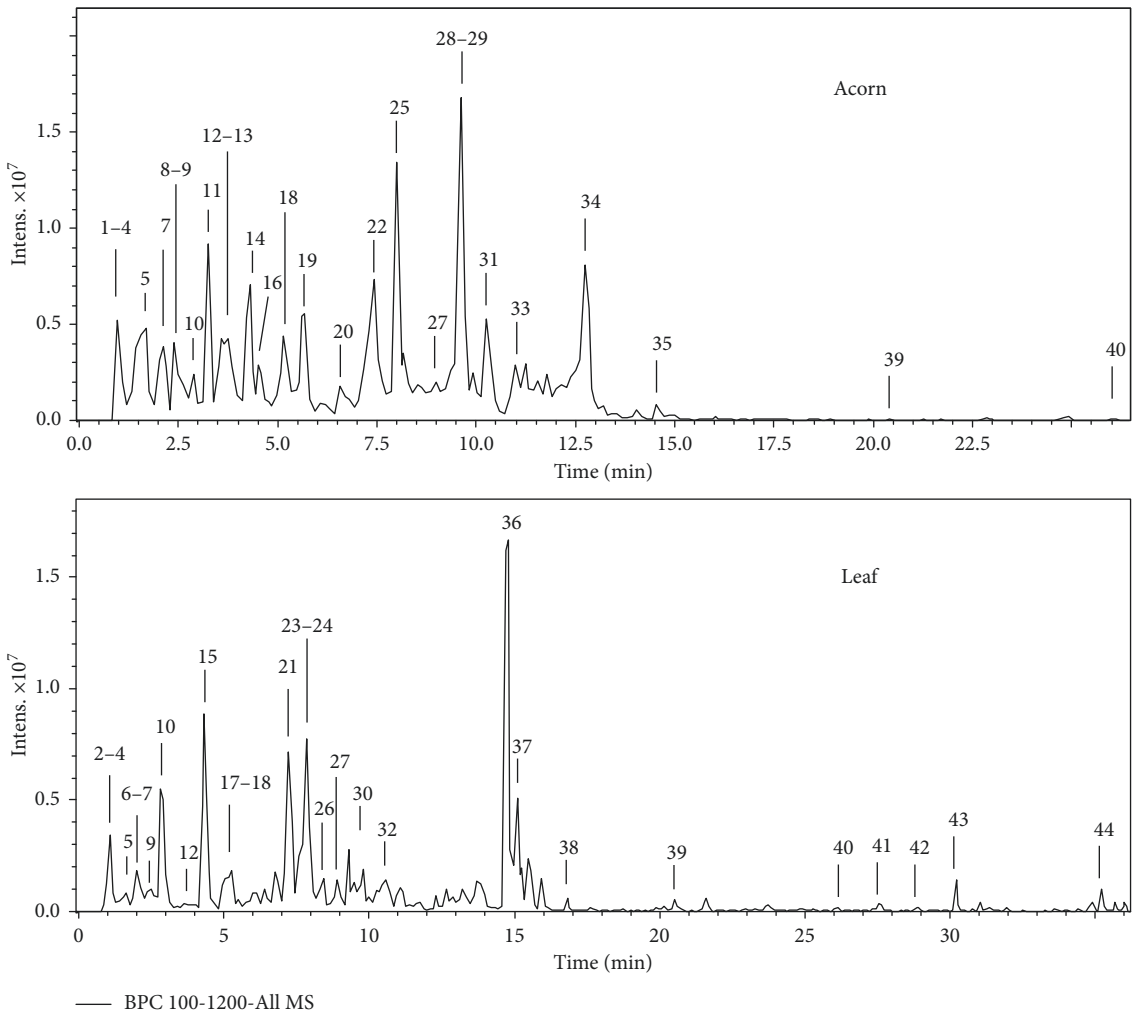

Figure 1: HPLC-ESI/MS^n base peak chromatograms (BPCs) of the methanolic extracts of acorns and leaves of *Q. coccifera*.

The presence of kaempferol derivatives and quercetin derivatives has previously been reported in the leaves of different *Quercus* species [1], although mono- and di-glycosides were detected, not tri-glycosides, which are here reported for the first time to the best of our knowledge.

3.1.3. Other Compounds. Compound **1** was characterized as a quinic acid derivative due to the 191⟶127 fragmentation.

Compound **2** was identified as a disaccharide, whereas compounds **24** and **32** were tentatively characterized as saccharide derivatives due to the MS^n fragment ions at *m*/*z* 179, 161, 143, 119, and 113 [37].

Compound **3** was identified as citric acid by comparison with an analytical standard.

Compound **14**, with a deprotonated molecular ion at *m*/*z* 387, displayed fragment ions at *m*/*z* 207 and 163, characteristic of the lignan medioresinol [38].

Compound **15** was characterized as roseoside (formate adduct) due to the deprotonated molecular ion at *m*/*z* 385 and fragment ions at *m*/*z* 223 and 153 [31].

We characterized compounds **39** and **40** as oxo-dihydroxy-octadecenoic and trihydroxy-octadecenoic acids, respectively, after comparison with bibliographic data [39, 40].

3.2. (Semi)quantification of Phenolics. We performed the semiquantitative analysis of the main compounds found in leaves and acorns. Acorn extracts were rich in gallic acid derivatives. Hence, the quantification of these compounds was carried out using gallic acid to construct the analytical graph, using the UV chromatograph at 275 nm. For leaf extracts, we used the UV signal of analytical standards of catechin (280 nm), gallic acid (275 nm), quercetin (350 nm), and kaempferol (350 nm) to construct the calibration graphs. The results are summarized in Table 2. These results are expressed in mg/g DE. In addition, humidity percentages were also calculated (62 ± 2% and 58 ± 3% for acorns and leaves, resp.), so that the amounts of phenolics in fresh samples could be calculated too.

To the best of our knowledge, the quantification of individual phenolics has not been reported for *Q. coccifera*. However, the results can be compared with data from other *Quercus* species, always keeping in mind that different solvents and extraction procedures have been used.

Table 1: Characterization of the compounds found in the methanolic extracts of acorns and leaves of *Q. coccifera*.

No.	t_R (min)	$[M-H]^- m/z$	m/z (% base peak)	Assigned identification	Acorns	Leaves
1	1.1	533	MS^2 [533]: 191 (100) MS^3 [533⟶191]: 127(100)	Quinic acid derivative	✓	
2	1.1	341	MS^2 [341]: 179 (100), 161 (20), 143 (25), 119 (11), 113 (6) MS^3 [341⟶179]: 161 (26), 149 (100)	Disaccharide	✓	✓
3	1.2	191	MS^2 [191]: 171 (27), 111 (100)	Citric acid	✓	✓
4	1.3	331	MS^2 [331]: 169 (100), 271 (43), 211 (40) MS^3 [331⟶169]: 125 (100)	Galloyl-hexoside	✓	✓
5	1.6	483	MS^2 [483]: 331 (100), 313 (2), 169 (8) MS^3 [483⟶331]: 271 (26), 211 (19), 193 (18), 169 (100), 125 (18)	Digalloyl-hexoside	✓	✓
6	2.1	577	MS^2 [577]: 451 (33), 425 (100), 407 (66), 289 (26), 287 (13)	Procyanidin dimer		✓
7	2.2	483	MS^2 [483]: 331 (27.8), 313 (24.8), 271 (100), 211 (15.8) MS^3 [483⟶271]: 211 (100), 169 (20)	Digalloyl-hexoside	✓	✓
8	2.5	785	MS^2 [785]: 785 (100), 633 (19), 483 (19), 301 (53) MS^3 [785⟶301]: 257 (32), 229 (100)	Hexahydroxydiphenyl-digalloyl-glucose	✓	
9	2.6	635	MS^2 [635]: 483 (30), 465 (100), 313 (9) MS^3 [635⟶465]: 421 (45), 313 (47), 169 (100) MS^4 [635⟶465⟶169]: 125 (100)	Trigalloyl-hexoside	✓	
10	2.9	289	MS^2 [289]: 245 (100), 205 (34), 203 (29), 179 (11)	Catechin	✓	✓
11	3.3	183	MS^2 [183]: 168 (100) MS^3 [183⟶168]: 124 (100)	Methyl gallate	✓	
12	3.6	635	MS^2 [635]: 483 (100), 465 (6), 331 (25) MS^3 [635⟶483]: 331 (100), 313 (3), 169 (3) MS^4 [635⟶483⟶331]: 271 (48), 211 (20), 169 (100), 125 (10)	Trigalloyl-hexoside	✓	✓
13	3.9	785	MS^2 [785]: 785 (100), 633 (13), 483 (51), 301 (77) MS^3 [785⟶301]: 257 (26.5) MS^3 [785⟶483]: 313 (59), 295 (30), 193 (40), 169 (100)	Hexahydroxydiphenyl-digalloyl-glucose	✓	
14	4.3	387	MS^2 [387]: 207 (100), 163 (67) MS^3 [387⟶207]: 163 (100)	Medioresinol	✓	
15	4.4	431	MS^2 [431]: 385 (100), 161 (9) MS^3 [431⟶385]: 223 (48), 205 (68), 161 (44), 153 (100)	Roseoside (formate adduct)		✓
16	4.6	635	MS^2 [635]: 483 (4), 465 (100), 313 (3) MS^3 [635⟶465]: 313 (100), 295 (21), MS^4 [635⟶465⟶313]: 295 (25), 169 (100)	Trigalloyl-hexoside	✓	
17	5.1	729	MS^2 [729]: 577 (100), 425 (38), 407 (45) MS^3 [729⟶577]: 451 (63), 425 (42), 407 (100), 289 (31), 287 (17)	(Epi)catechin-(epi)catechin monogallate (B-type)		✓
18	5.2	635	MS^2 [635]: 483 (100), 465 (80) MS^3 [635⟶483]: 331 (33), 313 (10), 271 (100), 211 (13), 169 (20) MS^4 [635⟶483⟶271]: 211 (100), 169 (26), 125 (3)	Trigalloyl-hexoside	✓	✓
19	5.7	635	MS^2 [635]: 483 (100) MS^3 [635⟶483]: 331 (10), 313 (15), 271 (100), 211 (38) MS^4 [635⟶483⟶271]: 211 (100), 169 (26), 125 (15)	Trigalloyl-hexoside	✓	
20	6.5	473	MS^2 [473]: 321 (100), 169 (14) MS^3 [473⟶321]: 169 (100), 125 (8) MS^4 [473⟶321⟶169]: 125 (100)	Trigallic acid	✓	

Table 1: Continued.

No.	t_R (min)	$[M-H]^- m/z$	m/z (% base peak)	Assigned identification	Acorns	Leaves
21	7.3	345	MS^2 [345]: 183 (100) MS^3 [345⟶183]: 165 (100), 139 (85), 121 (78)	Unknown		✓
22	7.4	335	MS^2 [335]: 183 (100) MS^3 [335⟶183]: 183 (100), 168 (21), 124 (32)	Galloyl methyl gallate	✓	
23	7.7	615	MS^2 [615]: 463 (100), 301 (36) MS^3 [615⟶463]: 301 (100), 179 (11) MS^4 [615⟶463⟶301]: 255 (100), 179 (62), 151 (62)	Quercetin hexoside-gallate		✓
24	7.9	377	MS^2 [377]: 331 (100), 179 (14) MS^3 [377⟶331]: 179 (100), 161 (34), 143 (34), 119 (19), 113 (20)	Saccharide derivative		✓
25	8.0	787	MS^2 [787]: 635 (100), 617 (65), 483 (15), 465 (8) MS^3 [787⟶635]: 483 (100), 313 (5) MS^3 [787⟶617]: 573 (49), 465 (69), 331 (100)	Tetragalloyl-hexoside	✓	
26	8.5	441	MS^2 [441]: 289 (100), 271 (10), 169 (23) MS^3 [441⟶289]: 245 (100), 203 (64), 179 (34)	(epi)catechin-*O*-gallate		✓
27	8.9	937	MS^2 [939]: 769 (100) MS^3 [939⟶769]: 617 (100)	Pentagalloyl-hexoside	✓	✓
28	9.6	939	MS^2 [939]: 787 (100), 769 (97), 635 (20), 617 (9) MS^3 [939⟶787]: 635 (56), 617 (100), 465 (19), 447 (6) MS^4 [939⟶787⟶617]: 447 (100)	Pentagalloyl-hexoside	✓	
29	9.6	335	MS^2 [335]: 183 (100) MS^3 [335⟶183]: 183 (100), 168 (21), 124 (32)	Galloyl methyl gallate	✓	
30	9.9	599	MS^2 [599]: 313 (100) MS^3 [599⟶313]: 169 (100) MS^4 [599⟶313⟶169]: 125 (100)	Gallic acid derivative		✓
31	10.3	939	MS^2 [939]: 787 (100), 769 (3) MS^3 [939⟶787]: 635 (100), 617 (95)	Pentagalloyl-hexoside	✓	
32	10.5	417	MS^2 [417]: 371 (100) MS^3 [417⟶371]: 161 (100), 179 (15), 113 (18)	Saccharide derivative		✓
33	11.0	1091	MS^2 [1091]: 939 (100), 787 (5) MS^3 [1091⟶939]: 787 (24), 769 (100) MS^4 [1091⟶939⟶769]: 617 (44), 447 (100)	Hexagalloyl-hexoside	✓	
34	12.8	487	MS^2 [487]: 335 (100), 183 (11) MS^3 [487⟶335]: 183 (100) MS^4 [487⟶335⟶183]: 168 (29), 124 (100)	Digalloyl methyl gallate	✓	
35	14.5	639	MS^2 [639]: 487 (100), 335 (48) MS^3 [639⟶487]: 335 (100), 183 (12) MS^4 [639⟶487⟶335]: 183 (100)	Trigalloyl methyl gallate	✓	
36	14.8	507	MS^2 [507]: 461 (100), 293 (45) MS^3 [507⟶461]: 293 (100), 149 (17) MS^4 [507⟶461⟶293]: 125 (100)	Unknown		✓
37	15.1	493	MS^2 [493]: 447 (100) MS^3 [493⟶447]: 191 (18), 165 (18), 149 (100), 131 (32) MS^4 [493⟶447⟶149]: 131 (100), 113 (18)	Unknown		✓
38	16.8	593	MS^2 [593]: 285 (100) MS^3 [593⟶285]: 257 (100), 151 (18)	Kaempferol-*O*-rutinoside		✓
39	20.5	327	MS^2 [327]: 291 (54), 229 (100), 211 (48), 209 (40), 171 (99)	Oxo-dihydroxy-octadecenoic acid	✓	✓
40	26.1	329	MS^2 [329]: 311 (36), 293 (28), 229 (98), 211 (100), 171 (25)	Trihydroxy-octadecenoic acid	✓	✓
41	27.5	755	MS^2 [755]: 609 (100), 301 (87) MS^3 [755⟶609]: 463 (87), 301 (100) MS^4 [755⟶609⟶301]: 179 (100)	Quercetin-rhamnoside-rhamnoside-hexoside		✓

Table 1: Continued.

No.	t_R (min)	$[M-H]^- m/z$	m/z (% base peak)	Assigned identification	Acorns	Leaves
42	28.9	755	MS^2 [755]: 609 (100), 301 (67) MS^3 [755⟶609]: 463 (84), 301 (100) MS^4 [755⟶609⟶301]: 179 (100)	Quercetin-rhamnoside-rhamnoside-hexoside		✓
43	30.2	739	MS^2 [739]: 593 (37), 453 (63), 285 (100) MS^3 [739⟶285]: 255 (100), 151 (55)	Kaempferol-rhamnose-hexose-rhamnose		✓
44	35.1	781	MS^2 [781]: 635 (25), 495 (40), 285 (100) MS^3 [781⟶285]: 151 (100)	Kaempferol derivative		✓

Table 2: Quantification of compounds in extracts of *Q. coccifera*. Results are expressed in mg/g DE.

No.	Assigned identification	Acorns	Leaves
Gallic acid derivatives			
5	Digalloyl-hexoside	2.6 ± 0.1	
7	Digalloyl-hexoside	2.1 ± 0.1	
8+9	Galloyl derivatives	8.4 ± 0.6	
11	Methyl gallate	21 ± 1	
12+13	Galloyl derivatives	5.7 ± 0.3	
16	Trigalloyl-hexoside	1.9 ± 0.1	
18	Trigalloyl-hexoside	2.4 ± 0.2	
19	Trigalloyl-hexoside	3.9 ± 0.2	
20	Trigallic acid	1.3 ± 0.1	
22	Galloyl methyl gallate	22 ± 1	
25	Tetragalloyl-hexoside	26 ± 1	
27	Pentagalloyl-hexoside		57 ± 2
28+29	Galloyl derivatives	52 ± 2	
30	Gallic acid derivative		1.7 ± 0.1
31	Pentagalloyl-hexoside	22 ± 0.2	
34	Digalloyl methyl gallate	3.5 ± 0.2	
Total		**175 ± 3**	**59 ± 2**
Flavonoids			
10	Catechin		16.4 ± 0.8
23	Quercetin hexoside-gallate		1.4 ± 0.1
26	(epi)catechin-*O*-gallate		1.06 ± 0.05
38	Kaempferol-*O*-rutinoside		0.10 ± 0.04
41	Quercetin-rhamnoside-rhamnoside-hexoside		0.08 ± 0.01
42	Quercetin-rhamnoside-rhamnoside-hexoside		0.05 ± 0.01
43	Kaempferol-rhamnose-hexose-rhamnose		0.16 ± 0.01
Total			**19 ± 2**

García-Villaba et al. [1] analyzed leaves of seven *Quercus* species, obtaining 1–5 mg/g DE of flavonoids and 2.5–285 mg/g DE of hydrolyzable tannins. In this work, we observed higher amounts of flavonoids in leaves, and the amounts of gallic acid derivatives are within the concentrations found by the mentioned authors.

3.3. Total Phenolic Content and Antioxidant Assays. We determined the total phenolic content (TPC) and the antioxidant activity ($ABTS^{\cdot+}$ and DPPH) of the extracts using the procedures previously reported [31]. The results are depicted in Figure 2. It can be observed that very high values of TPC were observed in both acorns and leaves, although acorns had higher TPC values than leaves. High TPC values

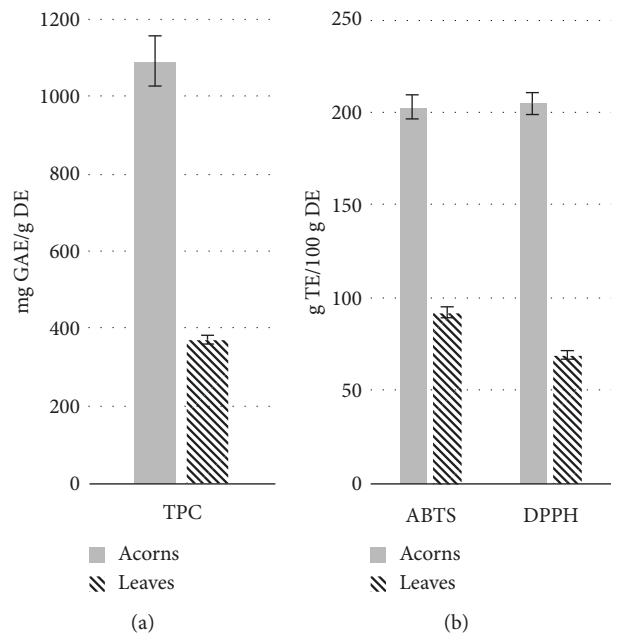

Figure 2: TPC, ABTS, and DPPH results for acorns and leaves of methanol extracts of *Q. coccifera*. GAE: gallic acid equivalents; TE: Trolox equivalents; DE: dried extract.

(similar to the ones observed here in leaves) have been previously reported in *Q. suber* [12]. In a similar way to TPC, the values obtained for the $ABTS^{\cdot+}$ and DPPH antioxidant assays were also higher in acorns compared to leaves.

4. Conclusions

We have carried out the characterization of the phenolic profile of methanolic extracts of leaves and acorns of *Q. coccifera* by using HPLC-ESI-MS^n. A total of forty-one compounds were identified or tentatively characterized. Although some flavonoids were identified, mainly kaempferol and quercetin derivatives, most of the compounds were condensed tannins and gallic acid derivatives. The extracts were particularly rich in gallotannins, which is in line with the reports in other *Quercus* species. The phytochemical profiles of acorns and leaves were similar, although (epi) catechin dimers were only detected in leaves, and hexahydroxydiphenyl-digalloyl-glucose isomers were only present in acorns. The main compounds were quantified by using HPLC with UV detection. Both acorns and leaves had

high antioxidant potential, which was in agreement with the TPC values observed, particularly in acorns. This study provides additional information concerning the phytochemical profile of this plant, which can be a valuable source of phytochemicals for the food or pharmacological industries. However, it is important to mention that these results are representative of the studied area, but samples were collected only in one year. Hence, more results are required considering that they may vary within different years or collection places.

Data Availability

The HPLC-MS data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

Technical and human support provided by CICT of Universidad de Jaén (UJA, MINECO, Junta de Andalucía, FEDER) is gratefully acknowledged. This research was supported by funding from the University of Jaén (UJA2014/10_FT/01).

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