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Research Article

Phenolic Profile, Antioxidant Activity, and Enzyme Inhibitory Properties of *Limonium delicatulum* (Girard) Kuntze and *Limonium quesadense* Erben

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In this work, we report the phytochemical composition and bioactive potential of methanolic and aqueous extracts of leaves from *Limonium delicatulum* (Girard) Kuntze and *Limonium quesadense* Erben. The characterization and quantitation of individual phytochemicals were performed with liquid chromatography with diode array and electrospray-tandem mass spectrometry detection. Myricetin glycosides were abundant in *L. delicatulum*, whereas *L. quesadense* was rich in gallo(epi)catechin-*O*-gallate. Total phenolics, flavonols, and flavonoids were assayed with conventional methods. Antioxidant and radical scavenging assays (phosphomolybdenum, DPPH, ABTS, CUPRAC, FRAP, and metal chelating activity), as well as enzyme inhibitory assays (acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase, glucosidase, and lipase), were performed to evaluate the potential bioactivity. The methanolic extracts of both species presented higher phenolic content and bioactivity than the aqueous extracts. Overall, *L. quesadense* extracts exhibited the most potent activity for most assays, representing a potential source of bioactive compounds for the pharmaceutical and food industries.

1. Introduction

Plants represent a rich source of many bioactive compounds, particularly polyphenols, which are well known for their high antioxidant activity and various health benefits. As a result, an increasing number of plant species are constantly used in folk medicine. In fact, several recent studies have focused on the enzyme inhibitory properties of plant extracts as an interesting approach to prevent different chronic diseases, such as inflammation, diabetes mellitus, Alzheimer, and cancer. Here, we present an investigation concerning two species of the genus *Limonium*: *L. delicatulum* (Girard) Kuntze and L. *quesadense* Erben.

Limonium Miller is a genus that belongs to the *Plumbaginaceae* family, specifically to the *Staticoideae* subfamily. There are two subgenera (*Pteroclados* and *Limonium*) with different sections—depending on the authors—and at least 10 subsections [1, 2]. There are between 350 and 470 species, mainly distributed in the western Mediterranean region [2–4]. This genus comprises perennial species and, rarely, annual herbaceous plants. *Limonium* species usually grow in arid or semiarid areas, occupying small isolated spaces over gypsic or saline soils. There are numerous local endemic species and, due to their isolation, many of them are threatened and protected species. Some species are cultivated as ornamental plants, whereas others have important medicinal properties [5, 6]. Research on species of this genus has revealed important bioactivity concerning the free radical scavenging [7, 8], antioxidant [5, 9–11], anti-inflammatory [10], antibacterial [12], antimicrobial [9], and

antiviral [13] properties. The main phenolics identified as responsible for these activities were gallic acid, epigallocatechin gallate, and myricetin and isorhamnetin flavonoids [7, 8, 10, 11, 14].

L. delicatulum is an Iberian-North African endemism [3], growing up to 100 cm. Leaves are green, usually ovate to elliptic or obovate (3.5–15 cm length × 2–5 cm width), with 4–10 lateral nerves. It blooms from February to October depending on the altitude, developing shoots of 20–90 cm with spikes of 5–25 mm and spikelets of 4-5 mm. It inhabits coastal and inland saline habitats between 0 and 800 m.a.s.l. It is not considered a threatened species [15] and its chemical composition and bioactivity have been scarcely studied. Its antioxidant activity has been reported [16, 17], as well as total phenolics, flavonoids, tannins, and antimicrobial activity [17]. However, the detailed phytochemical composition and potential enzyme inhibitory activities have not been reported so far.

L. quesadense is endemic to the province of Jaén (southeastern Iberian Peninsula, Spain) of 35–60 cm. Leaves are green-bluish to green-violetish, oblanceolate to spathulate (4–12 cm length × 1.5–3 cm width), with 4 (rarely 6) lateral nerves. It blooms from June to August, developing shoots of 20–50 cm with spikes of 7–20 mm and spikelets of 4.5-5 mm. It takes part in continental halophytic vegetation and gypsophyte scrubs in the Guadiana Menor valley between 500 and 700 m.a.s.l. It is regarded as a threatened plant under the category of "endangered" (EN) [15, 18]. To date, there are no studies concerning the phytochemical composition and bioactivity of this species.

Taking into account the lack of information concerning the two target species—as well as the reports of the bioactivity of other *Limonium* species—this research aims at providing information concerning the phenolic composition of leaves of *L. delicatulum* and *L. quesadense*, examining their antioxidant activity (radical scavenging, reducing power, and metal chelating) and enzyme inhibitory properties (against acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase, glucosidase, and lipase).

2. Materials and Methods

2.1. Sample Preparation. Leaves of *L. quesadense* and *L. delicatulum* were collected at the Native Flora Garden of the University of Jaén (Jaén, Andalusia, Spain; 37°47′18.879″N 3°46′31.583″W, 427 m a.s.l.) in September 2018. Samples are stored at the Herbarium of the University of Jaén. Photographs of both species are shown in Figure 1.

The taxonomical classification was confirmed by botanist Dr. Carlos Salazar-Mendías. Samples were washed with Milli-Q water and extracted by two different procedures:

(i) Ultrasound-assisted extraction with MeOH: leaves were lyophilized (ModulyoD-23, Thermo Savant; Waltham, MA USA) and powdered; 2.5 g of sample was extracted with 50 mL of MeOH in an ultrasonic liquid processor (Qsonica Sonicator; Newtown, CT, USA; power of 55 W and frequency of 20 kHz) at 50% power for 10 min.

(ii) Decoction: 2.5 g of sample (fresh and powdered) was extracted with 150 mL of boiling Milli-Q water for 30 minutes.

Both extracts were filtered through Whatman No. 1 filters, and the solvent was evaporated under reduced pressure in a Hei-Vap Precision Rotary Evaporator (Heidolph; Schwabach; Germany) at 40°C. Dried extracts (DE) were stored at −20°C until analysis.

2.2. HPLC Analysis. Dried extracts (5–10 mg) were redissolved in 1 mL of MeOH and filtered with 0.45 μm nylon filters, and 10 μL of the sample was injected. The HPLC system was an Agilent Series 1100 with a G1315B diode array detector. The separation of the compounds was performed with a reversed-phase Luna Omega Polar C_{18} column (150 × 3.0 mm and 5 μm particle size; Phenomenex) with a Polar C_{18} Security Guard cartridge (Phenomenex) of 4 × 3.0 mm. The HPLC system was connected to an ion trap mass spectrometer (Esquire 6000, Bruker Daltonics) with an electrospray interface. Chromatographic conditions have been previously detailed [19]. All standards required to perform phenolic quantitation were purchased from Sigma-Aldrich (Madrid, Spain); individual stock solutions were prepared in methanol (MeOH, LC-MS grade, >99.9%; Sigma). LC-MS grade acetonitrile (CH_3CN, 99%; LabScan; Dublin, Ireland) and ultrapure water (Milli-Q Water Purification System; Millipore; Milford, MA, USA) were also used.

2.3. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). TPC and TFC were determined using the Folin–Ciocalteu and $AlCl_3$ assays, respectively [20]. Results were expressed as gallic acid equivalents (mg GAEs/g extract) and rutin equivalents (mg REs/g extract) for the respective assays.

2.4. Determination of Antioxidant and Enzyme Inhibitory Effects. The metal chelating, phosphomolybdenum, FRAP, CUPRAC, ABTS, and DPPH activities of the extracts were assessed following the methods described by Uysal et al. [20]. The antioxidant activities were reported as trolox equivalents, whereas EDTA was used for metal chelating assay. The possible inhibitory effects of the extracts against cholinesterases (AChE (E.C. 3.1.1.7) from *Electrophorus electricus* (electric eel) and BChE (E.C. 3.1.1.8) from equine serum, by Ellman's method), tyrosinase (from mushroom, E.C. 1.14.18.1), α-amylase (from porcine pancreas, E.C. 3.2.1.1), α-glucosidase (E.C. 3.2.1.20), and lipase (from porcine pancreas, E.C 3.1.1.3) were evaluated using standard in vitro bioassays [21].

2.5. Data Analysis. Bioactive compounds and biological activity data were prepared for univariate and multivariate statistical analysis. Firstly, one-way ANOVA followed by post hoc test, namely, Tukey's multiple range was performed under Xlstat 2018 to investigate significant differences



(a) (b)

Figure 1: Photographs of (a) *L. delicatulum* and (b) *L. quesadense*.

($p < 0.05$) between the studied samples. The data were subjected to unsupervised multivariate analysis PCA and HCA using R software v. 3.5.1 for the discrimination between the extracts and their classification according to biological activities. Finally, the relationship between biological activities and phenolic classes based on the estimation of Pearson's correlation coefficients were conducted.

3. Results and Discussion

3.1. Phytochemical Characterization. The characterization of phytochemicals was performed by HPLC-ESI-MS^n using negative and positive ion modes. Base peak chromatograms are shown in Figure 2, whereas the characterization of compounds is detailed in Table 1.

Compound **1** was identified as the HCl adduct of a disaccharide (dihexoside) due to its fragmentation pattern [22]. Compound **2**, with deprotonated molecular ion at *m/z* 191 and base peak at *m/z* 111, was identified as citric acid.

Compound **4**, with $[M\text{-}H]^-$ at *m/z* 169 and base peak at *m/z* 125, was identified as gallic acid by comparison with an analytical standard. Compound **3**, after the loss of 80 Da (sulfate moiety), displayed fragment ions at *m/z* 331, 169, and 125, typical of galloyl hexoside. Compound **7** also presented gallic acid in its fragmentation pattern and was tentatively characterized as a derivative. Compound **9** was tentatively characterized as prodelphinidin dimer B-type gallate (2 units of gallo(epi)catechin) based on bibliographic information [23].

Compounds **14**, **16**, and **18** presented $[M\text{-}H]^-$ at *m/z* 457 and fragment ions at *m/z* 331, 305, 169, and 125, consistent with gallo(epi)catechin-*O*-gallate isomers [23]. Compound **15** was characterized as a dimer.

Compound **8** exhibited fragment ions at *m/z* 153 and 109/108, which corresponded to protocatechuic acid (comparison with an analytical standard), so it was characterized as a derivative.

Compound **11**, identified using positive ion mode, suffered the neutral loss of 162 Da, yielding cyanidin at *m/z* 287, so it was characterized as cyanidin 3-glucoside [24].

Several myricetin derivatives were characterized in the analyzed extracts. In all of them, myricetin was observed at *m/z* 317 (main fragment ions at *m/z* 179 and 151). The following neutral losses were observed in compounds **17**, **19**, **20**, **23**, **35**, and **36**: 152 Da (galloyl moiety), 146 Da (deoxyhexoside), 162 Da (hexoside), 308 Da (rutinoside). We could not elucidate the exact structure of compounds **26** and **30**, so they were characterized as myricetin derivatives.

Following the same neutral losses than myricetin, several quercetin (**21**, **22**, **24**, **31**, **38,** and **39**) and kaempferol (**28**, **29, and 37**) derivatives were characterized. Quercetin and kaempferol aglycones were detected at *m/z* 301 and 285, respectively.

Compound **27** suffered the neutral loss of 80 Da (sulfate) to yield the lignan syringaresinol at *m/z* 417, which was identified by the fragment ions at *m/z* 181, 166, and 151 [25]. Compound **34** was also characterized as a sulfate adduct of a lignan, pinoresinol [25].

Compound **32** was characterized as delphinidin due to the 303⟶257 fragmentation using positive ion mode. With an additional hexoside moiety, **25** was characterized as delphinidin-*O*-hexoside [24].

Finally, compound **33**, with deprotonated molecular ion at *m/z* 477, suffered the neutral loss of 146 Da (deoxyhexoside) to yield mearnsetin at *m/z* 331 (main fragment ion at *m/z* 316) [26].

3.2. Quantitation of Phenolic Compounds. We quantified 16 compounds in the methanolic and aqueous extracts of *L. quesadense* and *L. delicatulum*. The results are summarised in Table 2. Total individual phenolic content (TIPC) was defined as the sum of all the individual compounds that were quantified by HPLC-DAD (phenolic acids at 320 nm and flavonoids at 350 nm).

L. quesadense presented higher TIPC (54 and 32 mg/g DE for MeOH and H_2O extracts, respectively) than *L. delicatulum* (17 and 3.1 mg/g DE for MeOH and H_2O extracts, respectively). In both cases, methanol extracts presented the highest concentration of phenolics due to the highest solubility of flavonoids in MeOH compared to water. The most abundant compounds in *L. delicatulum* MeOH extract were myricetin glycosides (compounds **19**, **20**, and **23**), which have been previously reported as

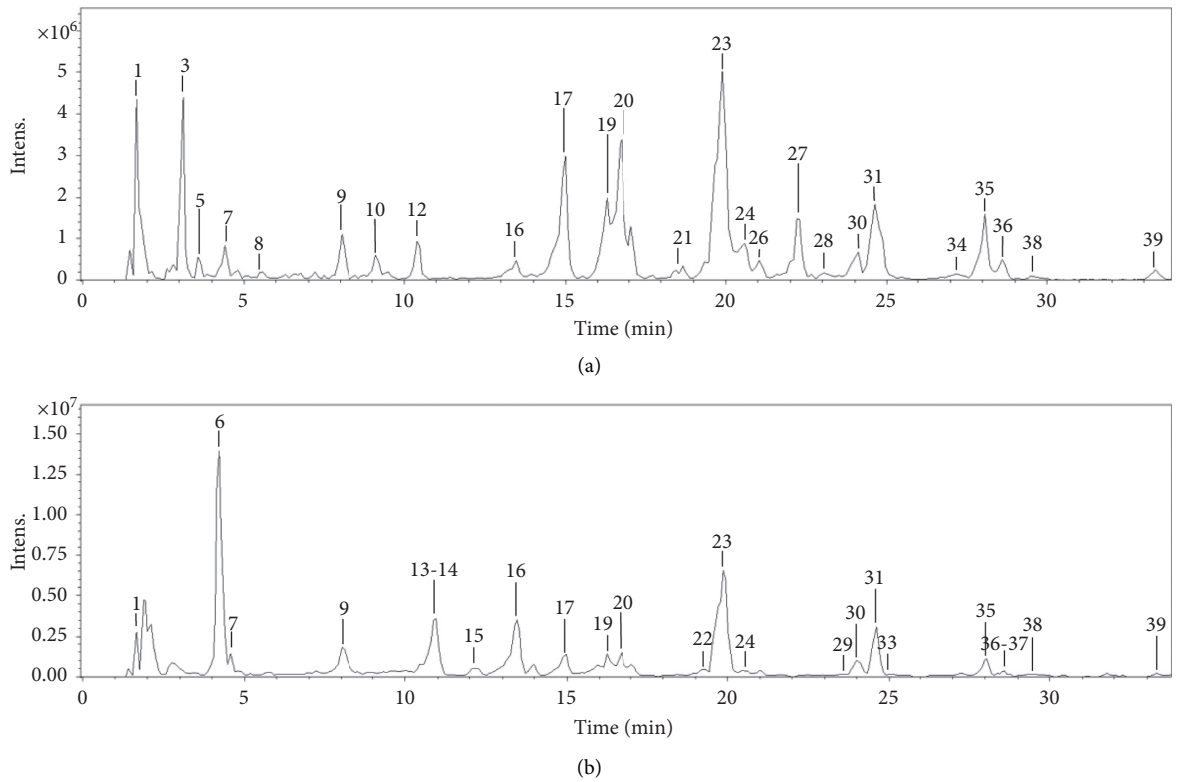


Figure 2: HPLC-ESI-MS^n base peak chromatograms of the methanolic extracts of (a) *L. delicatulum* and (b) *L. quesadense*.

bioactive compounds in *L. densiflorum* [10]. On the other hand, the most abundant compounds in *L. quesadense* extracts were **15** and **16** (gallo(epi)catechin-*O*-gallate monomer and dimer), followed by myricetin glycosides. Epigallocatechin gallate has been previously reported as the potential mainly responsible for the bioactive properties of *L. brasiliense* and *L. algarvense* [11, 14].

3.3. Antioxidant Properties. The majority of plant-based aromatic natural products are phenols, which comprise flavonoids, tannins, flavonols, flavanols, and anthocyanins, among others. In the present study, the different prepared extracts have been screened for the presence of phenolics, flavonols, and flavonoids. Indeed, all extracts showed a good level of phenolics, followed by flavonols and flavonoids (Table 3), observing higher levels in *L. quesadense* than *L. delicatulum*. In addition, MeOH extracts presented higher levels of phenolics than aqueous extracts. These results are in agreement with TIPC quantified by chromatography. The methanolic extract of *L. quesadense* possessed the highest TPC (172 ± 4 mg GAE/g·DE) and flavonol (74 ± 3 mg RE/g·DE). However, flavonoids were most abundant in the MeOH extract of *L. delicatulum* (42.1 ± 0.8 mg RE/g·DE).

Moreover, a series of antioxidant assays were conducted on the extracts of both *Limonium* species, namely, total antioxidant capacity (phosphomolybdenum), radical scavenging (ABTS and DPPH), reducing power (CUPRAC and FRAP), and metal chelating. These results are presented in Tables 3 and 4.

In terms of total antioxidant capacity, the most potent extract was the methanolic extract of *L. quesadense* (5.1 ± 0.4 mmol TE/g·DE). However, it is essential to point out that there is no statistical difference between this extract and the aqueous and methanolic extracts of *L. delicatulum*. Interestingly, the methanolic extract of *L. quesadense* displayed the highest activity in reducing power and radical scavenging assays. It showed significant activity with ABTS (510 ± 30 mg TE/g·DE) and DPPH (620 ± 10 mg TE/g·DE), CUPRAC (940 ± 10 mg TE/g·DE), and FRAP (520 ± 10 mg TE/g·DE). The most abundant flavonoid identified in the methanolic extract of *L. quesadense* was gallo(epi)catechin-*O*-gallate (26 ± 1 mg·g^{-1}·DE). Thus, it can be extrapolated that this compound, along with its dimer, might be mainly responsible for the observed antioxidant properties.

In contrast to the aforementioned antioxidant assays, the results obtained in the metal chelating assay classified the aqueous extract of *L. delicatulum* as the most effective metal chelator, with a significant activity of 28.43 ± 0.01 mg EDTAE/g·DE. Considering the quantitation analysis of phenolic compounds of all extracts (Table 2), it can be said that there exists a good correlation between the antioxidant results and the quantitation of polyphenols. As ample evidence, the total quantified phenolic content (TIPC) with HPLC reported the methanolic extract of *L. quesadense* as most rich in flavonoids (54 ± 1 mg·g^{-1} DE) which as discussed above presented the highest antioxidant property. In this sense, the observed significant antioxidant properties could be attributed mainly to the presence of compounds containing galloyl moieties, such a gallo(epi)catechin-*O*-

Table 1: Characterization of phenolics in *L. delicatulum* and *L. quesadense* extracts.

No.	t_R (min)	$[M-H]^- m/z$	m/z (% base peak)	Assigned identification	*L. delicatulum*	*L. quesadense*
1	1.7	377	MS^2 [377]: 341 (100) MS^3 [377⟶341]: 179 (100), 131 (14), 113 (18) MS^4 [377⟶341⟶179]: 161 (42), 143 (93), 119 (100)	Disaccharide (HCl adduct)	✓	✓
2	2.0	191	MS^2 [191]: 173 (25), 127 (19), 111 (100)	Citric acid		✓[b]
3	3.1	411	MS^2 [411]: 331 (7), 241 (100), 169 (14), 125 (6)	Galloyl hexoside (sulfate adduct)	✓	
4	3.3	169	MS^2 [169]: 125 (100)	Gallic acid	✓[b]	✓[b]
5	3.6	439	MS^2 [439]: 241 (100) MS^3 [439⟶241]: 223 (96), 139 (100), 165 (16)	Unknown	✓	
6	4.3	379	MS^2 [379]: 379 (100), 241 (20)	Unknown		✓
7	4.5	325	MS^2 [325]: 169 (100), 125 (9) MS^3 [325⟶169]: 125 (100)	Gallic acid derivative	✓	✓
8	5.6	365	MS^2 [365]: 321 (68), 153 (100) MS^3 [365⟶153]: 109 (100), 108 (61)	Protocatechuic acid derivative	✓	
9	8.0	761	MS^2 [761]: 609 (89), 593 (96), 575 (74), 423 (100) MS^3 [761⟶423]: 297 (50), 283 (100), 243 (28)	Prodelphinidin dimer B-type gallate (2 units (epi)GC)	✓[a]	✓
10	9.1	443	MS^2 [443]: 275 (24), 245 (100), 167 (27)	Unknown	✓	
11	9.5	449 (+)	MS^2 [449]:431 (8), 288(18), 287(100)	Cyanidin 3-glucoside	✓[a]	
12	10.4	759	MS^2 [759]: 481 (100), 423 (96), 301 (87) MS^3 [759⟶423]: 297 (84), 283 (50), 243 (100)	Unknown	✓	
13	11.0	363	MS^2 [363]: 363 (100), 241 (10)	Unknown		✓
14	11.0	457	MS^2 [457]: 331 (20), 305 (17), 193 (85), 169 (100) MS^3 [457⟶169]: 125 (100)	Gallo(epi)catechin-*O*-gallate isomer		✓[a]
15	12.3	913	MS^2 [913]: 761 (100), 423 (60) MS^3 [913⟶761]: 423 (100), 609 (90), 305 (49) MS^4 [913⟶761⟶423]: 297 (100), 253 (49), 405 (33)	Gallo(epi)catechin-*O*-gallate dimer		✓
16	13.3	457	MS^2 [457]: 331 (22), 305 (13), 169 (100) MS^3 [457⟶169]: 125 (100)	Gallo(epi)catechin-*O*-gallate isomer	✓	✓
17	15.0	631	MS^2 [631]: 479 (80), 317 (100) MS^3 [631⟶479]: 317 (100), 316 (91), 179 (9) MS^4 [631⟶479⟶317]: 271 (100), 179 (54), 151 (18)	Myricetin-galloyl-hexoside	✓	✓
18	15.4	457	MS^2 [457]: 331 (16), 305 (15), 169 (100) MS^3 [457⟶169]: 125 (100)	Gallo(epi)catechin-*O*-gallate isomer		✓[b]
19	16.2	625	MS^2 [625]: 317 (100), 316 (89) MS^3 [625⟶317]: 271 (100), 179 (48), 151 (26)	Myricetin-*O*-rutinoside	✓	✓
20	16.6	479	MS^2 [479]: 317 (79), 316 (100), 179 (5) MS^3 [479⟶317]: 271 (100), 179 (67), 151 (16)	Myricetin-*O*-hexoside	✓	✓
21	18.5	615	MS^2 [615]: 463 (100), 301 (33) MS^3 [615⟶463]: 301 (100) MS^4 [615⟶463⟶301]: 179 (75), 151 (100)	Quercetin-galloyl-hexoside	✓	✓[b]
22	19.2	609	MS^2 [609]: 301 (100) MS^3 [609⟶301]: 271 (98), 179 (76), 151 (100)	Rutin		✓
23	19.9	463	MS^2 [463]: 317 (77), 316 (100), 179 (10) MS^3 [463⟶316]: 271 (100), 179 (60), 151 (18)	Myricetin-*O*-deoxyhexoside	✓	✓
24	20.6	463	MS^2 [463]: 301 (100), 151 (5) MS^3 [463⟶301]: 255 (21), 179 (70), 151 (100)	Quercetin-*O*-hexoside	✓	✓
25	20.6	465 (+)	MS^2 [465]: 303 (100) MS^3 [465⟶303]: 257 (100), 137 (39)	Delphinidin-*O*-hexoside		✓
26	21.0	659	MS^2 [659]: 317 (85), 316 (100) MS^3 [659⟶316]: 271 (100), 179 (74), 151 (47)	Myricetin derivative	✓[a]	
27	22.2	497	MS^2 [497]: 417 (100) MS^3 [497⟶417]: 371 (14), 181 (100), 166 (50), 151 (24) MS^4 [497⟶417⟶181]: 166 (100)	Syringaresinol (sulfate adduct)	✓	
28	23.0	593	MS^2 [593]: 286 (20), 285 (100) MS^3 [595⟶285]: 257 (100), 239 (76), 213 (75), 151 (45)	Kaempferol-*O*-rutinoside	✓	
29	23.5	599	MS^2 [599]: 313 (100), 285 (94) MS^3 [599⟶313]: 169 (100)	Kaempferol-*O*-galloyl-hexoside		✓
30	24.1	549	MS^2 [549]: 505 (100) MS^3 [549⟶505]: 317 (44), 316 (100) MS^4 [549⟶505⟶316]: 271 (100), 179 (57), 151 (16)	Myricetin derivative	✓[a]	✓
31	24.6	447	MS^2 [447]: 301 (100), 179 (5) MS^3 [447⟶301]: 179 (91), 151 (100)	Quercetin-*O*-deoxyhexoside	✓	✓
32	24.7	303 (+)	MS^2 [303]: 257 (100)	Delphinidin	✓[a]	
33	24.9	477	MS^2 [477]: 331 (100) MS^3 [477⟶331]: 316 (100), 315 (57)	Mearnsetin-*O*-deoxyhexoside		✓[a]
34	27.2	437	MS^2 [437]: 357 (100), 151 (39) MS^3 [437⟶357]: 342 (48), 151 (100), 136 (40)	Pinoresinol (sulfate adduct)	✓	
35	28.0	615	MS^2 [615]: 317 (100) MS^3 [615⟶317]: 179 (100), 151 (40)	Myricetin-*O*-galloyl-deoxyhexoside	✓[a]	✓

Table 1: Continued.

No.	t_R (min)	$[M-H]^-$ m/z	m/z (% base peak)	Assigned identification	*L. delicatulum*	*L. quesadense*
36	28.6	615	MS^2 [615]: 317 (100) MS^3 [615⟶317]: 179 (100), 151 (54)	Myricetin-*O*-galloyl-deoxyhexoside	✓[a]	✓
37	28.6	431	MS^2 [431]: 286 (17), 285 (100), 284 (25), 255 (10) MS^3 [431⟶285]: 257 (82), 255 (100), 197 (39)	Kaempferol-*O*-deoxyhexoside		✓
38	29.5	533	MS^2 [533]: 489 (100) MS^3 [533⟶489]: 447 (19), 301 (100) MS^4 [533⟶489⟶301]: 271 (100), 179 (22), 151 (42)	Quercetin derivative	✓[a]	✓
39	33.4	599	MS^2 [599]: 301 (100) MS^3 [599⟶301]: 179 (81), 151 (100) MS^4 [599⟶301⟶179]: 151 (100)	Quercetin-O-galloyl-deoxyhexoside	✓	✓

[a]Only in MeOH extract; [b] only in H_2O extract.

Table 2: Contents (mg g^{-1} DE) of the main phenolic compounds present in *L. delicatulum* and *L. quesadense* extracts.

No.	Assigned identification	*L. delicatulum*		*L. quesadense*	
		MeOH	H_2O	MeOH	H_2O
Phenolic acids					
3	Galloyl hexoside	0.67 ± 0.01	1.91 ± 0.1	—	—
7	Gallic acid derivative	0.84 ± 0.02	—	—	—
Total		**1.51 ± 0.02**	**1.9 ± 0.1**		
Flavonoids					
9	Prodelphinidin dimer	0.58 ± 0.01^c	—	5.10 ± 0.01^b	6.3 ± 0.3^a
15	Gallo(epi)catechin-*O*-gallate dimer	—	—	10.0 ± 0.7	—
16	Gallo(epi)catechin-*O*-gallate	1.7 ± 0.2^c	—	26 ± 1^a	15.0 ± 0.6^b
17	Myricetin-galloyl-hexoside	1.2 ± 0.1^a	—	0.89 ± 0.07^b	0.69 ± 0.05^c
18	Gallo(epi)catechin-*O*-gallate	—	—	—	2.68 ± 0.05
19 + 20	Myricetin glycosides	4.40 ± 0.01^a	0.30 ± 0.02^d	2.22 ± 0.06^b	1.41 ± 0.08^c
23	Myricetin-*O*-deoxyhexoside	5.1 ± 0.1^b	0.64 ± 0.05^d	7.5 ± 0.4^a	4.1 ± 0.1^c
24	Quercetin-*O*-hexoside	0.54 ± 0.05^a	0.21 ± 0.02^b	—	0.21 ± 0.02^b
30	Myricetin derivative	—	—	0.50 ± 0.07	0.39 ± 0.02
31	Quercetin-*O*-deoxyhexoside	0.93 ± 0.04^b	—	1.37 ± 0.06^a	0.75 ± 0.04^c
35	Myricetin-*O*-galloyl-deoxyhexoside	0.56 ± 0.03^a	—	0.48 ± 0.01^b	0.24 ± 0.01^c
36	Myricetin-*O*-galloyl-deoxyhexoside	0.28 ± 0.01	—	—	0.25 ± 0.01
39	Quercetin-*O*-galloyl-deoxyhexoside	0.18 ± 0.01	—	—	—
Total		**15.5 ± 0.3^c**	**1.15 ± 0.05^d**	**54 ± 1^a**	**32.0 ± 0.7^b**
TIPC		**17.0 ± 0.3^c**	**3.1 ± 0.1^d**	**54 ± 1^a**	**32.0 ± 0.7^b**

Values are means ± SD of three parallel measurements. Means in the same line not sharing the same letter are significantly different ($p < 0.05$).

Table 3: Total bioactive components, total antioxidant capacity (by phosphomolybdenum assay), and radical scavenging abilities of *L. delicatulum* and *L. quesadense* extracts.

Plant species	Solvents	Total phenolic content (mg GAE/g·DE)	Total flavonol content (mg RE/g·DE)	Total flavonoid content (mg RE/g·DE)	Phosphomolybdenum (mmol TE/g·DE)	ABTS (mg TE/g DE)	DPPH (mg TE/g DE)
L. delicatulum	MeOH	151 ± 1^b	55 ± 2^b	42.1 ± 0.8^a	4.5 ± 0.6^a	360 ± 10^b	470 ± 10^b
	H_2O	31.1 ± 0.4^c	1.08 ± 0.03^d	5.80 ± 0.09^d	0.67 ± 0.04^b	53 ± 8^d	56 ± 1^d
L. quesadense	MeOH	172 ± 4^a	74 ± 3^a	30.8 ± 0.5^b	5.1 ± 0.4^a	510 ± 30^a	620 ± 10^a
	H_2O	152 ± 1^b	10.4 ± 0.2^c	12.98 ± 0.07^c	4.6 ± 0.7^a	248 ± 6^c	428 ± 8^c

Values expressed are means ± SD of three parallel measurements. GAE: gallic acid equivalent; RE: rutin equivalent; TE: trolox equivalent. Means in the same column not sharing the same letter are significantly different ($p < 0.05$).

Table 4: Reducing power and metal chelating abilities of *L. delicatulum* and *L. quesadense* extracts.

Plant species	Solvents	CUPRAC (mg TE/g·DE)	FRAP (mg TE/g·DE)	Metal chelating activity (mg EDTAE/g·DE)
L. delicatulum	MeOH	853 ± 5^b	470 ± 10^b	26.74 ± 0.01^b
	H_2O	94 ± 2^d	62.5 ± 0.6^d	28.43 ± 0.01^a
L. quesadense	MeOH	940 ± 10^a	520 ± 10^a	19.43 ± 0.01^d
	H_2O	640 ± 10^c	431 ± 5^c	22.30 ± 0.01^c

Values expressed are means ± SD of three parallel measurements. TE: trolox equivalent; EDTAE: EDTA equivalent. Means in the same column not sharing the same letter are significantly different ($p < 0.05$).

Table 5: Enzyme inhibitory properties of *L. delicatulum* and *L. quesadense* extracts.

Plant species	Solvents	AChE inhibition (mg GALAE/ g·DE)	BChE inhibition (mg GALAE/ g·DE)	Tyrosinase (mg KAE/ g·DE)	Amylase (mmol ACAE/ g·DE)	Glucosidase (mmol ACAE/ g·DE)	Lipase (mg OE/ g·DE)
L. delicatulum	MeOH	4.8 ± 0.7^{a}	3.5 ± 0.4^{a}	155.87 ± 0.01^{a}	0.95 ± 0.03^{b}	2.70 ± 0.01^{c}	27 ± 4^{b}
	H_2O	1.0 ± 0.2^{c}	na	18.87 ± 0.01^{d}	0.08 ± 0.00^{c}	2.74 ± 0.01^{a}	na
L. quesadense	MeOH	4.3 ± 0.2^{a}	2.63 ± 0.02^{b}	155.27 ± 0.01^{b}	1.00 ± 0.02^{b}	2.72 ± 0.01^{b}	65 ± 7^{a}
	H_2O	1.7 ± 0.2^{b}	0.86 ± 0.01^{c}	135.34 ± 0.01^{c}	1.5 ± 0.3^{a}	na	na

Values expressed are means ± SD of three parallel measurements. GALAE: galantamine equivalent; KAE: kojic acid equivalent; ACAE: acarbose equivalent; OE: orlistat equivalent; na: not active. Means in the same column not sharing the same letter are significantly different ($p < 0.05$).

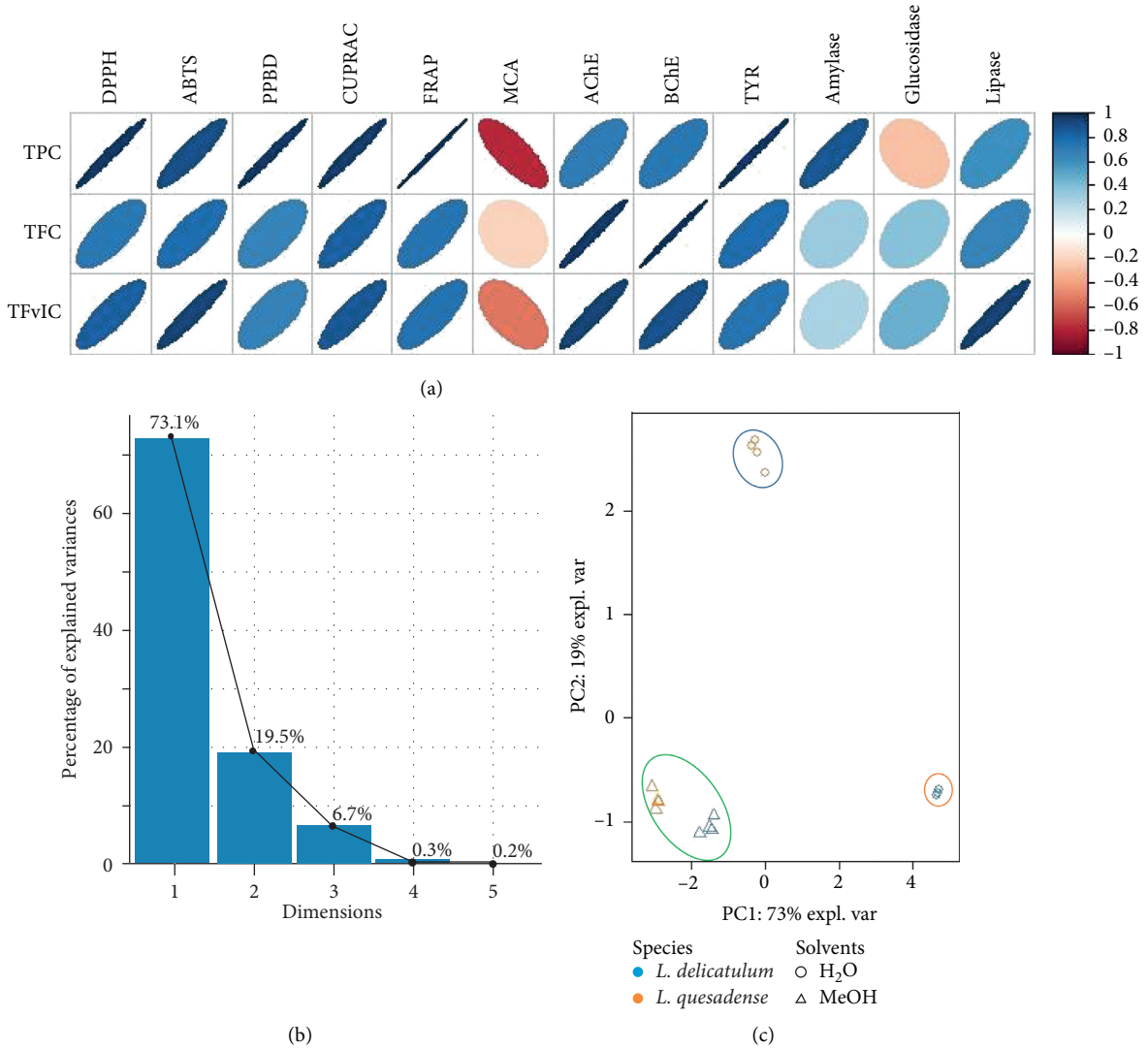
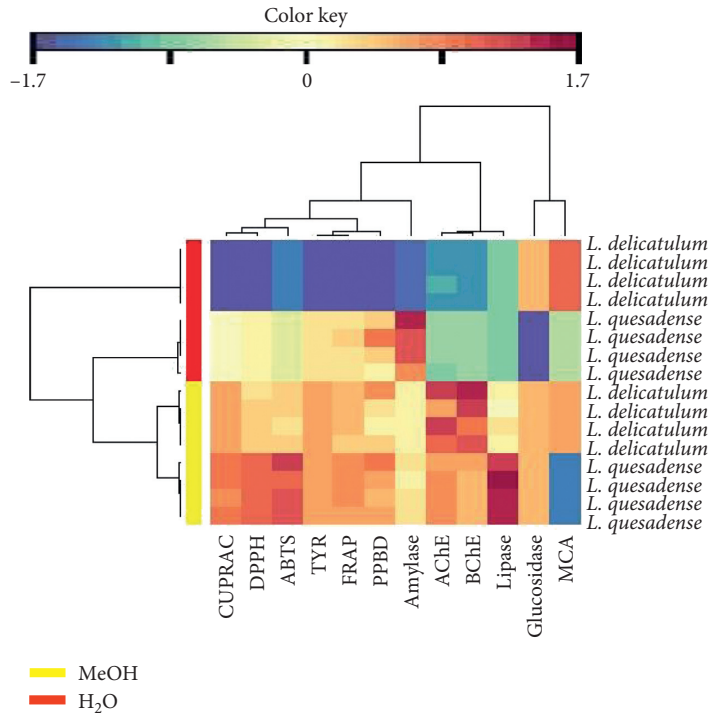


Figure 3: Continued.



(d)

Figure 3: Relationship between total bioactive compounds and biological activities and multivariate analysis of *Limonium* species. (a) Pearson's correlation heatmap; (b) screen plot of explained variance by PCA components; (c) score plot of principal components 1 and 2; (d) hierarchical cluster dendrogram of extracts. The color box indicates the standardized biological activities of each extract. The red, yellow, and blue colors indicate high, middle, and low bioactivity, respectively.

gallate dimer. Our findings were also supported by several researchers [27, 28].

3.4. Enzyme Inhibitory Effects. Enzymes are the main targets to control the constantly emerging global health issues [29]. As an example, tyrosinase is an important enzyme involved in the melanogenesis process during which the pigment, melanin, is produced [30]. However, the inhibitor of tyrosinase, kojic acid, which is used inexhaustibly by the pharmaceutical and cosmetic industries, represents various side effects [31]. Furthermore, the drug orlistat, which is the only clinically approved pharmacologic agent against pancreatic lipase, is associated with considerable side effects [32]. Thus, there is a dire need to search for new and safer enzymatic inhibitors for future pharmaceutical development. Accordingly, this present study is in line with the current trend and has screened the prepared extracts from the two *Limonium* species against α-amylase, glucosidase, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and lipase.

Tyrosinase inhibitors from the methanolic extract of *L. delicatulum* (155.87 ± 0.01 mg KAE/g·DE) and lipase inhibitors from the methanolic extract of *L. quesadense* (65 ± 7 mg OE/g·DE) seemed promising candidates. The methanolic and aqueous extracts of *L. delicatulum* and *L. quesadense* were screened for their inhibitory activities on both AChE and BChE (Table 5).

We observed that the methanolic extract of *L. delicatulum* exhibited the highest activity (4.8 ± 0.7 mg GALAE/g·DE); nevertheless, there is no statistical difference between the latter extract and the methanolic extract of *L. quesadense*. Hence, the two mentioned extracts represent the most potent cholinesterase inhibitors. Furthermore, the aqueous extract of *L. quesadense* was the most active inhibitor for α-amylase (1.5 ± 0.3 mmol ACAE/g·DE). On the other hand, in terms of glucosidase enzymatic assay, we observed the aqueous extract of *L. delicatulum* to be more potent (2.74 ± 0.01 mmol ACAE/g·DE) followed by the methanolic extract of *L. quesadense* (2.72 ± 0.01 mmol ACAE/g·DE) and the methanolic extract of *L. delicatulum* (2.70 ± 0.01 mmol ACAE/g·DE). Further data collected in this present study showed that the methanolic extract of *L. quesadense* exhibited the most effective lipase inhibitor. A substantial amount of reports showed that several plant metabolites are prospective pancreatic lipase inhibitors. Principally, it is projected that the presence of galloyl moiety of flavan-3-ols is essential for lipase inhibition [33]. Indeed, the methanolic extract of *L. quesadense* contained the highest levels of gallo(epi)catechin-*O*-gallate and its dimer, as well as myricetin-*O*-hexoside (Table 2) which might be linked to the significant lipase activity. It is noteworthy to point out that although the methanolic extract of *L. quesadense* possessed the highest bioactive components, we did not observe the most significant activity in all enzymatic assays. These results display that there may not always a correlation between polyphenol contents and enzymatic inhibition assays.

3.5. Unsupervised Multivariate Data Analysis of Biological Activities of Limonium Extracts. The analysis of *Limonium* species extracts encompassing 12 biological activities justified the employment of multivariate data analysis tools. Thus, with the help of unsupervised PCA and hierarchical clustering analysis, the biological activity data allowed for discrimination between the different extracts.

The first two principal components showed 73.1% and 19.5% of the total variance, respectively, suggesting only the two components could outline 90% information of the original data. As presented in Figure 3, the extracts were clearly classified into three clusters. Likewise, hierarchical cluster analysis (HCA) based on the concept of Euclidean similarity measure and Wards as linkage rule between the extracts confirmed the PCA results.

The MeOH extracts of two studied species were close enough. That suggested both extracts have similar properties against all evaluated biological activities. Otherwise, as opposed to MeOH extracts, the H_2O extracts were different. It allowed to better discriminate the two species. Accordingly, *L. delicatulum* and *L. quesadense* had different properties against all biological activities excepted AChE, BChE, and lipase; nevertheless, a more significant difference between the two species was obtained with MCA and amylase assays. Therefore, it can be concluded that *L. delicatulum* was most active against MCA while *L. quesadense* showed better amylase inhibitory activity.

4. Conclusions

The phenolic composition and bioactive properties of leaves of *L. delicatulum* and *L. quesadense* have been examined. *L. delicatulum* was rich in myricetin glycosides, whereas some of the most abundant compounds in *L. quesadense* were gallo(epi)catechin-*O*-gallate and its dimer. The presence of these compounds has been previously reported in other *Limonium* species and has been suggested as the main responsible for the bioactivity of *Limonium* extracts. In general, methanolic extracts presented the highest amounts of phenolics, along with the highest bioactive properties, although the most potent activities were observed in *L. quesadense* leaves. Not only the antioxidant activity was evaluated, but also the enzyme inhibitory properties against several key enzymes. The overall results indicate that leaves of *L. quesadense* may represent an interesting source of bioactive compounds. As L. *quesadense* is a threatened plant that is not currently protected by law, its cultivation on gypsic soils could be tested under the permission of the authorities by using seeds collected in the wild. It may also be an economic impulse for the population of semiarid areas in Jaén province.

Data Availability

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

Disclosure

The abstract of this manuscript has been presented in the conference SEQA 2019.

Conflicts of Interest

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

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