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Bioactive compounds content and antimicrobial activities of wild edible Asteraceae species of the Mediterranean flora under commercial cultivation conditions

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A B S T R A C T

Nine wild edible species belonging to Astreaceae family, native to the Mediterranean basin were tested for their chemical composition (phenolic compounds, tocopherols, and organic acids) and antimicrobial activities over two growing periods, apart from *Scolymus hispanicus* and *Hedypnois cretica* which were tested for only one growing period. Flavonoids were the most abundant phenolic compounds in all the species, except for the case of *Taraxacum* species where significant amounts of chicoric acid were detected, while phenolic compounds content increased in the 2nd growing period by 4.6–397.4% for the tested species. α- and β-tocopherols were the main tocopherols, apart from *Taraxacum* sp. where significant amounts of γ-and δ-tocopherols (18.32 and 16.31 μg/100 g fresh weight) were detected, while total tocopherols content either increased (*Reicardia picroides*, *Picris echioides*, *Urospermum picroides*, and *Taraxacum officinale*) or decreased (*Hymenonema graecum*, *Sonchus oleraceus*, *Taraxacum* sp.) in the 2nd growing period. Oxalic acid was the most abundant organic acid, with the highest content (972 mg/100 g fresh weight) being observed in *H. graecum* (L.) DC. in the 1st growing period. Moreover, with the exception of *H. graecum* and *S. olearaceus,* total organic acids content increased in the 2nd growing period. Significant antimicrobial activities were observed against *Bacillus cereus*, *Salmonella typhimurium* and *Penicillium ochrochloron* for all the studied species. In conclusion, the studied species showed great potential for commercial cultivation, while plant extracts could find use in the food industry as alternative food preservatives.

1. Introduction

Mediterranean basin is abundant with native species which have been traditionally used by the locals for medicinal and therapeutic purposes throughout centuries, while Greece in particular is thriving with numerous ecotypes of such species due to terrain morphology (Psaroudaki, Nikoloudakis, Skaracis, & Katsiotis, 2015; Vardavas, Majchrzak, Wagner, Elmadfa, & Kafatos, 2006). Several studies report the significance of these species in human diet, since they have been considered as famine food during harsh time periods of history (Leonti, Nebel, Rivera, & Heinrich, 2006; Łuczaj & Dolina, 2015; Pieroni & Quave, 2014). Consumption of native species is integral part of the so-called Mediterranean diet and has been associated with several beneficial health effects against the maladies that afflict the modern world (Fragopoulou et al., 2012; Guarrera & Savo, 2013; Pinela, Carvalho, & Ferreira, 2017; Trichopoulou et al., 2000). Apart from essential nutrients and microelements that enrich and diversify human diet, native species contain high amounts of bioactive compounds that contribute to

Abbreviations: DMSO, dimethyl sulfoxide; fw, fresh weight; HPLC, High performance liquid chromatography; LC-DAD–ESI/MSn, liquid chromatography coupled diode array detection and electrospray ion-trap tandem mass spectroscopy; nd, not detected; n/a, not available; N-P-K, nitrogen-phosphorus-potassium; MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration; Rt, retention times; SD, standard deviation; TE, trace elements; UHPLC, Ultra high performance liquid chromatography; UFLC, Ultra fast liquid chromatography; UV, ultraviolet

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healthy diets and to maintain or improve physical condition (Sánchez-Mata et al., 2012).

Asteraceae family includes numerous wild edible species that are native in the Mediterranean basin, most of which are consumed for their fresh and tender leaves as salad vegetables (Petropoulos, Ntatsi, Levizou, Barros, & Ferreira, 2016) or as vegetable mixtures (Guarrera & Savo, 2016). Chemical composition of native species has gained great research interest during the last decades, due to increased demands from the food industry and consumers for healthy and functional foods, rich in bioactive compounds (Albuquerque et al., 2018; Backes et al., 2018; Giacometti et al., 2018). Many of the reported studies refer to polyphenols, sesquiterpenes, and organic and fatty acids composition which have been associated with positive effects against cardiovascular diseases, cancer, oxidative stress and other diseases (Morales et al., 2012, 2014; Simopoulos, 2004). According to Antonia et al. (2011) and Schütz, Kammerer, Carle, and Schieber (2005), the main phenolic compounds detected in wild *Sonchus oleraceus*, *S. asper* and *Taraxacum officinale* were mainly cichoric acid and cichoric acid derivatives, while verbascoside and isoverbascoside glucosides and chlorogenic acid derivatives were detected in lower amounts. Moreover, Huo and Qin (2008) reported luteolin, apigenin and quercetin as the main flavonoids detected in *S. oleraceus*, while Dias et al. (2014) identified α-tocopherol as the main vitamer of vitamin E in the vegetative parts of *Taraxacum* species (*Taraxacum* sect. *Ruderalia*). Organic acids are also essential bioactive molecules in native species since they are involved in many biosynthetic pathways, while its composition is also important, especially oxalic acid content which has been associated with antinutritional properties (Guil, Rodríguez-García, & Torija, 1997).

Although most of these species are traditionally hand-picked in the wild by the locals, the increasing demand for such food products has created a market niche for commercial exploitation of native species in order to fulfill consumer demands for product availability throughout the year, as well as to lessen the risk for genetic erosion due to irrational gathering (Petropoulos et al., 2018). Therefore, recently there are reports for cultivation practices of native species and how these practices may affect its chemical composition and bioactive compounds content (Chatzigianni et al., 2017; Petropoulos et al., 2017; Petropoulos, Karkanis, Martins, & Ferreira, 2018), as well as reports for potential commercial cultivation of such species (Martinez et al., 2015).

Moreover, apart from raw food consumption the use of wild and under-utilized native species extends to other purposes, including uses in traditional and folk medicine due to their bioactive compounds content and antioxidant potential (Leonti et al., 2006; Sánchez-Mata et al., 2012). Many ethnopharmacological studies confirm the therapeutic effects of wild edible herbs extracts against diabetes, as well as its immunomodulatory, anti-inflammatory and antioxidant properties (Guarrera & Savo, 2013; Li et al., 2017). Antibacterial properties have been also reported for herb extracts from species such as *Sonchus* sp. (Xia, Yu, Zhu, & Zou, 2011) and *Taraxacum officinale* (Ghaima, Hashim, & Ali, 2013; Kenny et al., 2014), while several herbal extracts have shown important antifungal activities as alternative post-harvest preservatives in the food industry (Antonia et al., 2011; Antonia, Sergio, Ippolito, & Di, 2016; Domínguez et al., 2018; Mousavi, Hashemi, & Limbo, 2018; Pateiro et al., 2018).

The studied species were selected since they have been traditionally used for human consumption and there is a customary use in specific regions of the Mediterranean basin. However, irrational gathering has rendered native species vulnerable to genetic erosion, therefore commercial cultivation could be a useful means towards the conservation of valuable genetic material as well as the creation of a market niche for these products. Considering the importance and the benefits of these species on human diet and their beneficial health effects, the aim of the present study was to report the bioactive compounds content of selected native species of the Asteraceae family, as well as to evaluate their antifungal and antibacterial activities which is rarely explored. Although there are available reports in the literature regarding the chemical composition for several non-cultivated native species, the present study refers to bioactive compounds content of such species under commercial cultivation conditions in order to further evaluate the potential of introducing them as alternative crops.

2. Materials and methods

2.1. Plant material and sampling

Seeds of selected native species of the Asteraceae family were collected in situ from the broader region of western Crete during spring of 2014 in order to obtain genetic material for species propagation. The selected species were the following: *Hedypnois cretica* (L.) Schmidt, *Hymenonema graecum* (L.) DC., *Picris echioides* (L.) Holub, *Reichardia picroides*, *Scolymus hispanicus*, *Sonchus oleraceus*, *Urospermum picroides, Taraxacum officinale* and *Taraxacum* sp. Collected seeds were put in plastic seed trays containing peat (Traysubstrat; Klasmann-Deilmann Gmbt) on October 17th, 2015 (1st growing period; all the species except of *S. hispanicus* and *H. cretica* where no adequate number of seedlings was obtained) and January 17th, 2016 (2nd growing period). Young seedlings were transplanted to 2 L pots containing peat (Base Substrate 1; Klassman-Deilmann KTS2,1.0 L) and perlite (1.0 L) between December 5th and 13th, 2015 (1st growing period) and March 7th, 2016 (2nd growing period) and when they reached the stage of 3–4 true leaves. After transplantation, pots were transferred in the unheated plastic experimental greenhouse of the University of Thessaly, Greece. During cultivation, plants were irrigated at regular intervals with nutrient solution containing 150 mg L^{-1} of nitrogen, phosphorus and potassium (N-P-K; water soluble fertilizer 20–20-20 + trace elements (TE)). Harvest took place when plants from each species reached the edible stage by cutting the rosette of leaves and cleaning them with distilled water. Then batch samples of leaves from each species were put in air sealed plastic food bags, stored at deep freezing conditions (−80 °C), and lyophilized prior to further analyses.

Seed collections for all the studied species are deposited in the Laboratory of Vegetable Production, University of Thessaly, Greece.

2.2. Chemical composition analysis

2.2.1. Phenolic composition

To obtain the extracts, a maceration was performed using a 30 g/L solid/liquid ration with methanol/water (80:20 *v*/v, at 25 °C at 150 rpm) during 60 min, and afterwards filtered (Whatman paper No. 4). The residue was re-extracted with the same solid/liquid ratio and at the same temperature (25 °C). The combined extracts were evaporated under reduced pressure at 35 °C (rotary evaporator Büchi R-210, Flawil, Switzerland) until complete removal of methanol, and afterwards the aqueous phase was frozen and lyophilized (−49 °C for 48 h, FeeZone 4.5, Labconco, Kansas City, MO, USA).

The hydromethanolic extracts (the lyophilized aqueous phase obtained from the evaporation of extracts) were re-dissolved in methanol/water (80:20 *v*/v) to a final concentration of 10 mg mL^{-1} for phenolic compound identification and quantification, which was performed in a LC-DAD–ESI/MSn analyses (Dionex Ultimate 3000 UHPLC instrument, Thermo Scientific, San Jose, CA, USA) equipped with a diode-array detector and coupled to a mass detector, following a procedure previously reported by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). The chromatogram was recorded at several wavelengths, characteristic of different classes of polyphenols (280, 330 and 370 nm). For quantitative analysis, a calibration curve for each available phenolic standard (5–100 μg mL^{-1}) was constructed based on the UV signal, namely chlorogenic acid, caffeic acid, *p*-coumaric acid, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-rutinoside (Extrasynthesis, Genay, France). For the identified phenolic compounds for which a commercial standard was not available, the quantification was

Table 1
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification, and quantification of phenolic compounds (mg 100 g^{-1} fw) in *Reichardia picroides* and *Hymenonema graecum* in relation to growing period (1st and 2nd).

Peaks	*Reichardia picroides*	Growing period		*t*-Students test *p*-value
	Compounds	1st	2nd	
2	*trans* 5-*O*-Caffeoylquinic acid	0.43 ± 0.03	0.64 ± 0.04	< 0.05
7	Caffeic acid	tr	tr	
9	Kaempferol-*O*-glucuronyl-*O*-hexoside	1.48 ± 0.01	1.84 ± 0.02	< 0.05
20	Luteolin-7-*O*-rutinoside	4.28 ± 0.01	3.56 ± 0.09	< 0.05
24	Luteolin-7-*O*-glucoside	3.5 ± 0.2	3.98 ± 0.02	< 0.05
30	Apigenin-7-*O*-rutinoside	1.44 ± 0.01	1.89 ± 0.01	< 0.05
31	Apigenin-*O*-glucuronide	1.54 ± 0.05	1.73 ± 0.03	< 0.05
33	Apigenin-7-*O*-glucoside	1.43 ± 0.07	1.99 ± 0.06	< 0.05
34	Luteolin-*O*-malonylhexoside	2.3 ± 0.1	2.28 ± 0.03	0.49
35	Kaempferol-*O*-glucuronide isomer 2	6.26 ± 0.05	5.5 ± 0.3	< 0.05
37	Kaempferol-glucuronide isomer 3	2.01 ± 0.08	2.2 ± 0.1	0.06
38	Apigenin-*O*-acetylhexoside	1.61 ± 0.05	1.96 ± 0.06	< 0.05
	Total phenolics acids	0.43 ± 0.03	0.64 ± 0.04	< 0.05
	Total flavonoids	25.8 ± 0.6	26.9 ± 0.2	< 0.05
	Total phenolics compounds	26.3 ± 0.6	27.5 ± 0.2	< 0.05
	Hymenonema graecum	Growing period		*t*-Students test *p*-value
Peaks	Compounds	1st	2nd	
6	*cis* 5-*p*-Coumaroylquinic acid	0.93 ± 0.01	0.95 ± 0.02	0.08
8	*trans* 5-*p*-Coumaroylquinic acid	0.560 ± 0.002	0.54 ± 0.02	0.07
9	Kaempferol-*O*-glucuronyl-*O*-hexoside	2.28 ± 0.05	2.29 ± 0.01	< 0.05
11	Luteolin-*O*-glucuronyl-*O*-rutinoside	6.4 ± 0.1	7.8 ± 0.1	0.68
12	Luteolin-*O*-glucuronyl-*O*-hexoside	1.50 ± 0.02	1.64 ± 0.01	< 0.05
15	Luteolin-*O*-hexoside	1.60 ± 0.03	1.80 ± 0.01	< 0.05
16	Apigenin-*O*-glucuronyl-hexoside	1.46 ± 0.03	1.60 ± 0.01	< 0.05
20	Luteolin-7-*O*-rutinoside	2.44 ± 0.03	2.73 ± 0.08	< 0.05
21	Luteolin-*O*-deoxyhexosyl-*O*-hexoside	1.55 ± 0.03	2.30 ± 0.08	< 0.05
24	Luteolin-7-*O*-glucoside	2.1 ± 0.1	3.1 ± 0.1	< 0.05
25	Luteolin-*O*-malonylglucuronyl-hexoside	1.62 ± 0.05	2.29 ± 0.07	< 0.05
26	Luteolin-*O*-malonylhexoside	1.66 ± 0.04	1.49 ± 0.01	< 0.05
	Total phenolics acids	1.49 ± 0.01	1.49 ± 0.01	0.07
	Total flavonoids	22.7 ± 0.2	28.2 ± 0.2	< 0.05
	Total phenolics compounds	24.2 ± 0.2	29.7 ± 0.2	< 0.05

For peak information refer to Table S1.tr – traces (below limit of quantification (LOQ) of the standard compound).

performed through the calibration curve of the most similar available standard. The results were expressed as mg per 100 g of fresh weight.

2.2.2. Tocopherols analysis

Tocopherols were determined following a procedure previously described by Barros et al., 2013; Barros, Pereira, and Ferreira (2013), using the internal standard methodology and using a Knauer Smartline system 1000 (HPLC, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, USA). Tocopherols were identified by comparing their retention times with authentic standard compounds and quantification was conducted by comparison with dose–response curves constructed from authentic standards, using the IS (tocol) method. The results were expressed as μg per 100 g of fresh weight.

2.2.3. Organic acids analysis

Organic acids were determined by ultra-fast liquid chromatography (UFLC) (Shimadzu 20A series UFLC, Shimadzu Corporation, Kyoto, Japan) coupled to a diode-array detector (DAD) operating in the conditions described by Barros, Pereira, and Ferreira (2013). The organic acids found were identified and quantified by comparison of the area of their peaks recorded at 215 nm or 245 nm (for ascorbic acid) with calibration curves obtained from commercial standards of each compound. The results were expressed as mg per 100 g of fresh weight.

2.3. Antimicrobial assays

The following Gram (+) bacteria: *Bacillus cereus* (food isolate), *Staphylococcus aureus* (ATCC 6538) and *Listeria monocytogenes* (NCTC 7973), Gram (−) bacteria: *Escherichia coli* (ATCC 35210), *Enterobacter cloacae* (human isolate), *Salmonella typhimurium* (ATCC 13311), and fungi: *Aspergillus fumigatus* (ATCC 1022), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839) and *Penicillium verrucosum* var. *cyclopium* (food isolate) were used in this studied. The antimicrobial assay was carried out by the microdilution method as previously described by Petropoulos, Karkanis, et al. (2018). The concentrations that completely inhibited bacterial growth (minimum inhibitory concentration: MICs) were determined by a colorimetric microbial viability assay, and MBC and MFC (minimum bactericidal concentration and minimum fungicidal concentration, respectively) were also calculated. Streptomycin, aqmpicillin, ketoconazole and bifonazole (Sigma-Aldrich, St. Louis, MO, USA) were used as positive controls and 5% dimethyl sulfoxide (DMSO) was used as a negative control.

2.4. Statistical analysis

For each composition parameter and antimicrobial assays, three samples were analyzed for each treatment, whereas all the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (SD), and analyzed using a Student's *t*-test, with $\alpha = 0.05$ (SPSS v. 23.0 program, IBM Corp., Armonk, NY, USA).

3. Results and discussion

The phenolic compounds were identified based on their chromatographic, UV–vis and mass spectra characteristics and 38 individual compounds were detected in total: seven phenolic acids and derivatives, 16 flavonol glycoside derivatives and 15 flavone glycoside derivatives (Supplementary Material; Table S1). Compounds 1, 2, and 4–8

Table 2
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification, and quantification of phenolic compounds (mg 100 g^{-1} fw) in *Sonchus oleraceus* and *Picris echioides* in relation to growing period (1st and 2nd).

Peaks	Compounds	Growing period		*t*-Students test *p*-value
		1st	2nd	
Sonchus oleraceus				
2	*trans* 5-*O*-caffeoylquinic acid	2.81 ± 0.04	1.37 ± 0.02	< 0.05
4	*cis* Chicoric acid	2.28 ± 0.05	2.03 ± 0.06	< 0.05
5	*trans* Chicoric acid	1.61 ± 0.04	1.98 ± 0.09	< 0.05
9	Kaempferol-*O*-glucuronyl-*O*-hexoside	2.84 ± 0.03	2.99 ± 0.05	< 0.05
13	Luteolin-*O*-dihexoside	2.8 ± 0.1	3.53 ± 0.05	< 0.05
19	Luteolin-*O*-glucuronide	79.9 ± 0.5	143.8 ± 0.1	< 0.05
24	Luteolin-7-*O*-glucoside	12.8 ± 0.3	23.7 ± 0.3	< 0.05
26	Quercetin-*O*-malonylhexoside	3.50 ± 0.02	3.42 ± 0.04	< 0.05
31	Apigenin-*O*-glucoronide	30.2 ± 0.6	44 ± 1	< 0.05
34	Luteolin-*O*-malonylhexoside	6.32 ± 0.08	8.29 ± 0.04	< 0.05
	Total phenolics acids	6.70 ± 0.06	5.4 ± 0.1	< 0.05
	Total flavonoids	138.2 ± 0.5	230 ± 2	< 0.05
	Total phenolics compounds	144.9 ± 0.5	235 ± 2	< 0.05
Picris echioides				
2	*trans* 5-*O*-Caffeoylquinic acid	0.17 ± 0.01	0.67 ± 0.01	< 0.05
3	Quercetin-*O*-hexosyl-*O*-pentoside isomer 1	1.75 ± 0.01	4.47 ± 0.06	< 0.05
6	Chicoric acid	0.42 ± 0.01	1.61 ± 0.02	< 0.05
10	Quercetin-*O*-hexosyl-*O*-pentoside isomer 2	2.16 ± 0.02	3.27 ± 0.04	< 0.05
14	Kaempferol-*O*-dihexoside	1.49 ± 0.01	1.49 ± 0.03	0.96
19	Luteolin-*O*-glucuronide	10.0 ± 0.1	23.1 ± 0.2	< 0.05
24	Luteolin-7-*O*-glucoside	3.65 ± 0.01	13.4 ± 0.3	< 0.05
29	Kaempferol-*O*-glucuronide isomer 1	1.39 ± 0.01	1.64 ± 0.04	< 0.05
31	Apigenin-*O*-glucuronide	1.68 ± 0.02	1.48 ± 0.05	< 0.05
33	Apigenin-7-*O*-glucoside	1.39 ± 0.06	1.51 ± 0.07	< 0.05
	Total phenolics acids	0.586 ± 0.004	2.28 ± 0.03	< 0.05
	Total flavonoids	23.47 ± 0.03	50.37 ± 0.6	< 0.05
	Total phenolics compounds	24.06 ± 0.03	52.65 ± 0.7	< 0.05

For peak information refer to Table S1.

Table 3
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification, and and quantification of phenolic compounds (mg 100 g^{-1} fw) in *Scolymus hispanicus* and *Hedypnois cretica* in the 2nd growing period.

Peaks	Compounds	Growing period
		2nd
Scolymus hispanicus		
2	*trans* 5-*O*-caffeoylquinic acid	0.097 ± 0.003
18	Quercetin-3-*O*-glucuronide	1.46 ± 0.01
19	Luteolin-*O*-glucuronide	11.41 ± 0.24
27	Kaempferol-3-*O*-rutinoside	1.35 ± 0.01
29	Kaempferol-*O*-glucuronide isomer 1	4.67 ± 0.01
31	Apigenin-*O*-glucuronide	3.1 ± 0.1
32	Isorhamnetin-*O*-glucuronide	1.81 ± 0.05
	Total phenolics acids	0.097 ± 0.003
	Total flavonoids	23.8 ± 0.1
	Total phenolics compounds	23.88 ± 0.09
Hedypnois cretica		
6	*cis* 5-*p*-Coumaroylquinic acid	0.51 ± 0.01
10	Quercetin-*O*-hexosyl-*O*-pentoside isomer 2	1.75 ± 0.02
17	Kaempferol-*O*-hexosyl-*O*-pentoside	1.76 ± 0.01
20	Luteolin-7-*O*-rutinoside	3.07 ± 0.01
24	Luteolin-7-*O*-glucoside	1.90 ± 0.01
29	Kaempferol-*O*-glucuronide isomer 1	1.79 ± 0.01
	Total phenolics acids	0.51 ± 0.01
	Total flavonoids	10.27 ± 0.01
	Total phenolics compounds	10.78 ± 0.01

For peak information refer to Table S1.

consisted of phenolic acids, more precisely hydroxycinnamic acid derivatives, while compounds 2 (5-*O*-caffeoylquinic acid) and 7 (caffeic acid) were positively identified according to their retention, mass and UV–vis characteristics by comparison with commercial standards. Compound 1 ([M–H]$^-$ at *m/z* 353) presented the same pseudomolecular ion and fragmentation pattern as compound 2, being identified as *cis* 5-*O*-caffeoylquinic acid. Compounds 6 and 8 ([M–H]$^-$ at *m/z* 353) presented a base peak at *m/z* 191, accompanied by a weak fragment at *m/z* 163 ([*p*-coumaric acid-H]$^-$), being identified as *cis* and *trans* 5-*p*-coumaroylquinic acid. Compounds 4 and 5 ([M–H]$^-$ at *m/z* 473) presented a fragmentation pattern that allowed assigning both peaks as *cis* and *trans* chicoric acids (dicaffeoyltartaric acid).

The remaining compounds corresponded to flavonoids, namely flavonols (quercetin and kaempferol glycoside derivatives) and flavones (apigenin and luteolin glycoside derivatives). Luteolin glycoside derivatives (compounds 11–13, 15, 19, 20, 21, 24, 25, and 34) were the main detected flavones, while kaempferol glycoside derivatives (compounds 9, 14, 17, 27–29, 35, 36, and 37) were the main flavonols. Luteolin (λ_{max} around 255, 265, 346 nm, and MS^2 fragment at *m/z* 285) and kaempferol (λ_{max} around 270, 342 nm, and MS^2 fragment at *m/z* 285) presented the same product ion at *m/z* 285 ([luteolin-H]$^-$ and ([kaempferol-H]$^-$), being their UV spectra the only method to distinguish these two aglycones. Compounds 18 (quercetin-3-*O*-glucuronide), 20 (luteolin-7-*O*-rutinoside), 22 (quercetin-3-*O*-glucoside), 24 (luteolin-7-*O*-glucoside), 27 (kaempferol-3-*O*-rutinoside), 28 (kaempferol-3-*O*-glucoside), 30 (apigenin-7-*O*-rutinoside), and 33 (apigenin-7-*O*-glucoside) were positively identified after comparison with commercial standards. Compounds 9 ([M–H]$^-$ at *m/z* 623), 12 ([M–H]$^-$ at *m/z* 623), and 16 ([M–H]$^-$ at *m/z* 607) showed a similar fragmentation pattern with the alternative losses of a glucuronyl (−176 u) and hexosyl (−162 u) residues, indicating the location of each residue in different positions of the aglycone; however, no information about the identity of the sugar moieties and the location on the aglycone could be obtained, so the compounds were tentatively identified as kaempferol-*O*-glucuronyl-*O*-hexoside, luteolin-*O*-glucuronyl-*O*-hexoside, and apigenin-*O*-glucuronyl-*O*-hexoside, respectively. Similarly, peak 11 ([M–H]$^-$ at *m/z* 769) presented a MS^2 fragmentation with the alternative loss of glucuronyl (*m/z* at 593; −176 u) and deoxyhexosylhexoside (*m/z* at 285; −308 u) residues. Nevertheless, the positive identification of different rutinosides, including luteolin-7-*O*-rutinoside, may suggest the tentative identification of this compound as luteolin-*O*-glucuronyl-*O*-rutinoside. Compounds 3 and 10 ([M–H]$^-$ at *m/z* 595), and 17 ([M–H]$^-$ at *m/z* 579), revealed the alternative losses of hexosyl (−162 u) and pentosyl (−132 u) moieties; taking into account the above findings, these compounds were identified as quercetin-*O*-hexosyl-*O*-pentoside isomers 1 and 2, and kaempferol-*O*-hexosyl-*O*-pentoside, respectively.

The mass characteristics and UV spectra of compounds 13 and 14 ([M − H]$^-$ at *m/z* 609) showed that they correspond to luteolin and kaempferol derivatives bearing two hexosyl residues, while the observation of only one MS^2 fragment suggested that the two sugars were linked together, thus being tentatively identified as luteolin-*O*-dihexoside and kaempferol-*O*-dihexoside, respectively. Compounds 15 ([M − H]$^-$ at *m/z* 447) and 23 ([M − H]$^-$ at *m/z* 463) presented the loss of a hexosyl moiety (−162 u), and showed the same pseudomolecular ion as compounds 24 (luteolin-7-*O*-glucoside) and 22 (quercetin-3-*O*-glucoside), thus presenting a different retention time in comparison with the commercial standard. Therefore they could be assumed as a luteolin-*O*-hexoside and quercetin-*O*-hexoside,

Table 4
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification, and quantification of phenolic compounds (mg 100 g^{-1} fw) in *Urospermum picroides*, *Taraxacum* sp. and *T. officinale* in relation to growing period (1st and 2nd).

Peaks	Compounds	Growing period		*t*-Students test *p*-value
		1st	2nd	
Urospermum picroides				
1	*cis* 5-*O*-caffeoylquinic acid	0.68 ± 0.02	1.80 ± 0.02	< 0.05
2	*trans* 5-*O*-caffeoylquinic acid	0.53 ± 0.01	2.06 ± 0.04	< 0.05
6	*cis* 5-*p*-coumaroylquinic acid	0.50 ± 0.01	0.57 ± 0.01	< 0.05
22	Quercetin-3-*O*-glucoside	2.41 ± 0.06	3.47 ± 0.09	< 0.05
23	Quercetin-*O*-hexoside	1.7 ± 0.1	2.04 ± 0.04	< 0.01
26	Quercetin-*O*-malonylhexoside	4.12 ± 0.01	4.22 ± 0.03	< 0.05
28	Kaempferol-3-*O*-glucoside	1.48 ± 0.01	1.55 ± 0.01	< 0.05
36	Kaempferol-*O*-malonylhexoside	1.40 ± 0.01	1.46 ± 0.01	< 0.05
	Total phenolics acids	1.71 ± 0.03	4.43 ± 0.08	< 0.05
	Total flavonoids	11.1 ± 0.2	12.74 ± 0.2	< 0.05
	Total phenolics compounds	12.8 ± 0.2	17.18 ± 0.3	< 0.05
Taraxacum sp.				
5	*trans* Chicoric acid	0.67 ± 0.01	14.9 ± 0.3	< 0.05
20	Luteolin-7-*O*-rutinoside	3.25 ± 0.06	4.51 ± 0.01	< 0.05
	Total phenolics acids	0.67 ± 0.01	14.9 ± 0.3	< 0.05
	Total flavonoids	3.25 ± 0.06	4.51 ± 0.01	< 0.05
	Total phenolics compounds	3.92 ± 0.07	19.5 ± 0.4	< 0.05
Taraxacum officinale				
5	*trans* Chicoric acid	1.65 ± 0.02	2.79 ± 0.05	< 0.05
20	Luteolin-7-*O*-rutinoside	2.49 ± 0.1	2.88 ± 0.04	< 0.05
	Total phenolics acids	1.65 ± 0.02	2.79 ± 0.05	< 0.05
	Total flavonoids	2.49 ± 0.01	2.88 ± 0.04	< 0.05
	Total phenolics compounds	4.14 ± 0.01	5.68 ± 0.02	< 0.05

For peak information refer to Table S1.

respectively. Similarly, compound 21 ([M − H]$^-$ at *m/z* 593) was identified as luteolin-*O*-deoxyhexosyl-hexoside. Peaks 19 ([M − H]$^-$ at *m/z* 461), 31 ([M − H]$^-$ at *m/z* 445), 29, 35, and 37 ([M − H]$^-$ at *m/z* 461) and 32 ([M − H]$^-$ at *m/z* 491) were identified as glucuronyl derivatives (−176 u) of luteolin, apigenin, kaempferol and isorhamnetin, respectively.

Compounds 25 ([M − H]$^-$ at *m/z* 709), 26 ([M − H]$^-$ at *m/z* 549), 34 ([M − H]$^-$ at *m/z* 533) and 36 ([M − H]$^-$ at *m/z* 533) possessed molecular weights 86 u higher than peaks 12, 22, 24, and 28, indicating the existence of an additional malonyl residue, thus being tentatively identified as luteolin-*O*-malonylglucuronyl-hexoside, quercetin-*O*-malonylhexoside, luteolin-*O*-malonylhexoside, and kaempferol-*O*-malonylhexoside. Likewise, peak 38 ([M − H]$^-$ at *m/z* 473) presented a molecular weight 42 u higher than peak 33, revealing the existence of an acetyl moiety, thus being tentatively identified as apigenin-*O*-acetylhexoside.

The phenolic composition of *Reichardia picroides* and *Hymenonema graecum* is presented in Table 1. For *R. picroides* twelve phenolic compounds were identified with kaempferol-3-*O*-glucuronide being the most abundant compound, followed by luteolin-7-*O*-rutinoside and luteolin-7-*O*-glucoside. Luteolin derivatives in methanolic extracts of *R. picroides* have been previously reported by Recio et al. (1992) who also detected chlorogenic and isochlorogenic acid. In the case of *H. graecum*, twelve individual phenolic compounds were identified with luteolin-*O*-glucuronyl-*O*-hexoside being the most abundant molecule, followed by other luteolin derivatives which were detected in lower amounts. Moreover, phenolic compounds content was higher in the second growing period for both species due to a slight increase of both phenolic acids and flavonoids.

Table 2 presents the phenolic compounds profile of *Sonchus oleraceus* and *Picris echioides*. Ten individual phenolic compounds were detected in *S. oleraceus* aerial parts extracts, with luteolin and apigenin derivatives being the most abundant ones, accounting for 91.1% and 95.0% of total phenolic compounds in the 1st and 2nd growing period, respectively. Similarly, ten individual phenolic compounds were also identified in *P. echioides*, with luteolin and apigenin derivatives being the most abundant ones, accounting for 91.1% and 95.0% of total phenolic compounds in the 1st and 2nd growing period, respectively. However, a difference in the overall composition was observed between these two species. Moreover, a significant increase in phenolic compounds content was observed in the 2nd growing period for both species. According to Giner et al. (1993), luteolin and apigenin derivatives were also detected in *S. oleraceus* after using a Soxhlet extraction with CH_2Cl_2 and MeOH, while the authors reported that luteolin-7-*O*-glucoside is wide-present among the various *Sonchus* species. However, a different composition of phenolic compounds was detected in that study which could be due to different extraction method comparing to the method implemented in our study (non-thermal stirring hydromethanolic extraction). Similarly, Kenny et al. (2014) reported that luteolin derivatives were the main flavone glucosides detected in ethanolic extracts of *S. asper* aerial parts, while they also identified chlorogenic acid and quinic acid in significant amounts. In contrast, Morales et al. (2014) have reported that a lower portion of total phenols consists of flavonoids, which could be due to different growing conditions and cultivation practices (wild versus cultivated species), as well as to different geographical origin (Schaffer, Schmitt-Schillig, Müller, & Eckert, 2005) and extraction method (Dias et al., 2014), comparing to our study.

The phenolic composition of *Scolymus hispanicus* and *Hedypnois cretica* is presented in Table 3. For both species only data from the 2nd growing period are available due to insufficient number of plants in the 1st growing period. The most abundant compounds were luteolin-*O*-glucuronide and luteolin-7-*O*-rutinoside for *S. hispanicus* and *H. cretica*, respectively, while phenolic compounds consisted mostly of flavonoids in both species. Table 4 shows the phenolic compounds profile of *Urospermum picroides*, *Taraxacum* sp., and *T. officinale*. Quercetin-*O*-malonylhexoside was the most abundant phenolic compound in the case of *U. picroides*, while for both *Taraxacum* species the main compounds were luteolin-7-*O*-rutinoside and chicoric acid, especially in the case of *Taraxacum* sp. and the 2nd growing period where the highest amount of chicoric acid was observed. The results for *Taraxacum* species are in agreement with the study of Dias et al. (2014) who also reported chicoric acid as the main phenolic compound in vegetative parts of *Taraxacum* sect. Ruderalia, as well as with the study of Chen, Inbaraj, and Chen (2012) who evaluated phenolic compounds composition of *T. formosanum* Kitam. The overall phenolic compounds content increased in the 2nd growing period, as it is already reported for the rest of the studied species, however in the case of *Taraxacum* species this was due to the increase of phenolic acids content instead of an increase in flavonoids content.

Considering that 2nd growing period and harvest of plants took place under higher temperatures and light intensity levels (mid-late spring comparing to winter-early spring for the 2nd and 1st growing period, respectively) it could be assumed that plants were subjected to stress conditions under the 2nd growing period that could induce the biosynthesis of phenolic compounds (Fallovo, Schreiner, Schwarz, Colla, & Krumbein, 2011), while Pérez-López, Miranda-Apodaca, Muñoz-Rueda, and Mena-Petite (2015) have also reported that high light intensity may increase total phenols content in lettuce leaves. Similarly, according to Petropoulos, Karkanis, et al. (2018) not only growing season but also harvesting stage has a significant effect on total phenolic content and phenolic compounds composition, while Becker, Klaering, Schreiner, Kroh, and Krumbein (2014) reported that plant age

Table 5
Composition in tocopherols (μg 100 g^{-1} fw) and organic acids (mg 100 g^{-1} fw) of the studied wild species (mean ± SD).

Species	Growing period	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total Tocopherols
Reichardia picroides	1st	2.54 ± 0.08	nd	nd	nd	2.54 ± 0.08
	2nd	3.51 ± 0.07	1.4 ± 0.2	nd	nd	4.9 ± 0.2
	t-Students test *p*-value	< 0.05	n/a	n/a	n/a	
Hymenonema graecum	1st	2.78 ± 0.08	2.10 ± 0.08	nd	nd	4.88 ± 0.01
	2nd	1.05 ± 0.06	2.14 ± 0.06	nd	nd	3.2 ± 0.1
	t-Students test *p*-value	< 0.05	0.39	n/a	n/a	< 0.05
Sonchus oleraceus	1st	2.2 ± 0.2	1.8 ± 0.2	nd	nd	3.93 ± 0.05
	2nd	2.29 ± 0.05	1.55 ± 0.01	nd	nd	3.84 ± 0.05
	t-Students test *p*-value	0.15	0.08	n/a	n/a	< 0.05
Scolymus hispanicus*	2nd	0.68 ± 0.02	0.85 ± 0.07	nd	nd	1.53 ± 0.09
Hedypnois cretica*	2nd	2.33 ± 0.07	nd	nd	nd	2.33 ± 0.06
Picris echioides	1st	1.26 ± 0.03	nd	nd	nd	1.27 ± 0.02
	2nd	2.64 ± 0.06	nd	nd	nd	2.65 ± 0.06
	t-Students test *p*-value	< 0.05	n/a	n/a	n/a	< 0.05
Urospermum picroides	1st	1.54 ± 0.09	nd	nd	nd	1.54 ± 0.08
	2nd	1.58 ± 0.08	nd	nd	nd	1.58 ± 0.08
	t-Students test *p*-value	0.46	n/a	n/a	n/a	0.46
Taraxacum sp.	1st	9.6 ± 0.6	5.2 ± 0.3	18.3 ± 0.2	16.3 ± 0.6	49.4 ± 0.5
	2nd	4.02 ± 0.01	2.9 ± 0.2	nd	nd	6.9 ± 0.2
	t-Students test *p*-value	< 0.05	< 0.05	n/a	n/a	< 0.05
Taraxacum officinale	1st	2.05 ± 0.08	2.8 ± 0.1	nd	nd	4.9 ± 0.2
	2nd	2.13 ± 0.09	1.57 ± 0.09	nd	nd	3.7 ± 0.2
	t-Students test *p*-value	0.21	< 0.05	n/a	n/a	< 0.05
Species	Growing period	Oxalic acid	Malic acid	Shikimic acid	Total organic acids	
Reichardia picroides	1st	625 ± 6	91.7 ± 0.8	2.22 ± 0.02	719 ± 3	
	2nd	937 ± 4	73 ± 2	144.0 ± 0.2	1154 ± 2	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
Hymenonema graecum	1st	972 ± 2	110 ± 4	244 ± 1	1326 ± 2	
	2nd	877 ± 3	61 ± 3	166 ± 4	1103 ± 1	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
Sonchus oleraceus	1st	777 ± 3	415 ± 2	167 ± 3	1359.7 ± 0.4	
	2nd	644 ± 1	444 ± 2	146 ± 6	1233 ± 2	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
*Scolymus hispanicus**	2nd	564 ± 2	18 ± 1	34.4 ± 0.1	617 ± 1	
*Hedypnois cretica**	2nd	631 ± 5	299 ± 2	128 ± 2	1059 ± 2	
Picris echioides	1st	562.7 ± 0.7	105 ± 2	87.1 ± 0.5	755 ± 1	
	2nd	638 ± 6	216 ± 3	149 ± 2	1003 ± 3	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
Urospermum picroides	1st	574 ± 1	248 ± 3	75 ± 2	897 ± 1	
	2nd	660 ± 4	383 ± 4	89 ± 3	1132 ± 1	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
Taraxacum sp.	1st	370 ± 4	133 ± 1	59 ± 3	562 ± 1	
	2nd	541 ± 2	136.3 ± 0.1	86 ± 3	763 ± 1	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	
Taraxacum officinale	1st	386.8 ± 0.3	220 ± 4.0	53.2 ± 0.2	660 ± 2	
	2nd	462 ± 1	175 ± 5	72 ± 2	708 ± 2	
	t-Students test *p*-value	< 0.05	< 0.05	< 0.05	< 0.05	

nd: not detected (below limit of quantification: LOQ); n/a: non available data *Calibration curves: oxalic acid (y = 9E + 06× + 459,731, R^2 = 0.990; LOD: 12.6 μg mL^{-1}, LOQ: 41.8 μg mL^{-1}), malic acid (y = 912,441 x + 92,665; R^2 = 0.999; LOD: 35.8 μg mL^{-1}, LOQ: 119.2 μg mL^{-1}), and shikimic acid (y = 7E + 07 x + 175,156; R^2 = 0.999; LOD: 10.2 μg mL^{-1}, LOQ: 56.5 μg mL^{-1}).

and ontogeny are essential for phenolic compounds composition. Therefore, differences in growing conditions between the growing periods of the present study could induce the biosynthetic pathways of phenolic compounds and result in a higher content in the 2nd growing period comparing to the 1st one.

Phenolic compounds composition showed a great diversity among the studied species with 38 different compounds being detected (Supplementary Material; Table S1). Among these compounds, only 12 were common in two or more species, whereas the rest of the compounds were unique for the studied species (Supplementary Material; Table S2). In addition, *S. oleraceus* had the highest content in total phenolic compounds among the studied species due to the highest content in flavonoids, and luteolin-*O*-glucuronide in particular. The abundance of *S. oleraceus* in phenolic compounds has been reported by Xia et al. (2011) who also suggested that the species had the highest content in total flavonoids among six *Sonchus* species. The prevalent phenolic compounds in the species of our study were mostly flavonoids derivatives (luteolin, apigenin, kaempferol, and quercetin glycoside derivatives), whereas only in the case of *T. officinale* phenolic acids comprised a significant portion of total phenolic compounds, especially in the 2nd growing period where the amount of phenolic acids was equal to that of total flavonoids (Table 4).

Tocopherols content is presented in Table 5. The main tocopherol was α-tocopherol which was detected in all the studied species for both growing periods, while β-tocopherol was detected only in *H. graecum* and *S. oleraceus* in both growing periods and in *R. picroides* and *S. hispanicus* in the 2nd growing period. Moreover, *Taraxacum* sp. was the only species where all tocopherol isoforms were detected in the 1st growing period. Similarly, Morales et al. (2014) reported that α-tocopherol was the main tocopherol in *S. oleraceus*, while α- and β-tocopherols were detected in similar amounts in *S. hispanicus*. However, in contrast with our study they also detected γ- and δ-tocopherols in *S. oleraceus* plants, which could be due to different growing conditions comparing to our study. In addition and in contrast with the above mentioned study, Conforti et al. (2011) did not detect tocopherols in leaves samples of *S. oleraceus* collected in Italy, which further highlights

Table 6
Antimicrobial activity of the studied wild species (MIC and MBC mg mL^{-1}).

Species	Growing period	Antibacterial activity						
			Bacillus cereus	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Enterobacter cloacae*	*Salmonella typhimurium*
Reichardia picroides	1st	MIC	0.15	0.30	0.30	0.15	0.30	0.30
		MBC	0.30	0.60	0.60	0.30	0.60	0.60
	2nd	MIC	0.15	0.30	0.30	0.30	0.30	0.60
		MBC	0.30	0.60	0.60	0.60	0.60	0.90
Hymenonema graecum	1st	MIC	0.20	0.60	0.60	0.60	0.45	0.30
		MBC	0.30	0.90	1.20	0.90	0.90	0.60
	2nd	MIC	0.20	0.60	0.60	0.60	0.60	0.30
		MBC	0.30	1.20	0.90	1.20	1.20	0.60
Sonchus oleraceus	1st	MIC	0.20	0.45	0.45	0.45	0.60	0.45
		MBC	0.30	0.60	0.60	0.60	0.90	0.90
	2nd	MIC	0.15	0.30	0.60	0.30	0.30	0.30
		MBC	0.30	0.60	0.90	0.60	0.60	0.60
Scolymus hispanicus	2nd	MIC	0.10	0.30	0.20	0.10	0.15	0.15
		MBC	0.15	0.60	0.60	0.15	0.30	0.30
Hedypnois cretica	2nd	MIC	0.15	0.60	0.45	0.20	0.30	0.30
		MBC	0.30	0.90	0.60	0.30	0.60	0.60
Picris echioides	1st	MIC	0.075	0.45	0.60	0.45	0.30	0.60
		MBC	0.15	0.60	0.90	0.60	0.60	0.90
	2nd	MIC	0.15	0.30	0.30	0.15	0.20	0.20
		MBC	0.30	0.60	0.60	0.30	0.30	0.30
Urospermum picroides	1st	MIC	0.15	0.90	0.90	0.90	0.45	0.45
		MBC	0.30	1.20	1.20	1.20	0.90	0.90
	2nd	MIC	0.15	0.90	0.30	0.45	0.60	0.30
		MBC	0.30	1.20	0.60	0.90	0.90	0.90
Taraxacum sp.	1st	MIC	0.075	0.60	0.45	0.20	0.20	0.20
		MBC	0.15	0.90	0.60	0.30	0.30	0.60
	2nd	MIC	0.075	0.30	0.45	0.90	0.20	0.30
		MBC	0.15	0.60	0.60	1.20	0.30	0.60
Taraxacum officinale	1st	MIC	0.037	0.30	0.30	0.15	0.15	0.15
		MBC	0.075	0.60	0.60	0.30	0.30	0.30
	2nd	MIC	0.20	0.90	0.90	0.30	0.30	0.60
		MBC	0.30	1.20	1.20	0.45	0.45	0.90
Streptomycin		MIC	0.10	0.04	0.20	0.20	0.20	0.20
		MBC	0.20	0.10	0.30	0.30	0.30	0.30
Ampicillin		MIC	0.25	0.25	0.40	0.40	0.25	0.75
		MBC	0.40	0.45	0.50	0.50	0.50	1.20
Species	Growing period	Antifungal activity						
			Aspergillus fumigatus	*Aspergillus ochraceus*	*Aspergillus niger*	*Penicillium funiculosum*	*Penicillium ochrochloron*	*Penicillium cyclopium*
Reichardia picroides	1st	MIC	0.60	0.075	0.60	0.30	0.60	–
		MFC	1.20	0.15	1.20	0.60	1.20	–
	2nd	MIC	0.30	0.037	0.30	0.30	0.60	–
		MFC	0.60	0.075	0.60	0.60	1.20	–
Hymenonema graecum	1st	MIC	–	0.15	–	–	–	–
		MFC	–	0.30	–	–	–	–
	2nd	MIC	–	0.075	–	0.60	0.60	–
		MFC	–	0.15	–	1.20	1.20	–
Sonchus oleraceus	1st	MIC	0.45	0.10	0.60	0.45	–	–
		MFC	0.90	0.15	1.20	0.90	–	–
	2nd	MIC	0.90	0.037	0.90	0.60	0.90	–
		MFC	1.20	0.075	1.20	1.20	1.20	–
Scolymus hispanicus	2nd	MIC	0.45	0.10	0.60	0.45	0.30	0.60
		MFC	0.90	0.30	0.90	0.90	0.90	1.20
Hedypnois cretica	2nd	MIC	0.90	0.15	0.45	0.30	0.20	0.30
		MFC	1.20	0.30	0.90	0.60	0.60	0.60
Picris echioides	1st	MIC	0.15	0.075	0.30	0.45	0.30	0.45
		MFC	0.30	0.15	0.60	0.60	0.60	0.60
	2nd	MIC	0.20	0.037	0.30	0.15	0.15	0.15
		MFC	0.60	0.075	0.60	0.30	0.30	0.30
Urospermum picroides	1st	MIC	0.60	0.20	0.60	0.45	0.60	0.60
		MFC	0.90	0.30	1.20	0.60	1.20	1.20
	2nd	MIC	0.15	0.075	–	0.20	0.20	0.45
		MFC	0.30	0.15	–	0.30	0.30	0.90
Taraxacum sp.	1st	MIC	0.30	0.10	0.60	0.15	0.30	0.30
		MFC	0.60	0.15	0.90	0.30	0.60	0.60
	2nd	MIC	0.15	0.037	0.30	0.60	0.20	0.30
		MFC	0.30	0.075	0.60	1.20	0.30	0.90

(*continued on next page*)

Table 6 (*continued*)

Species	Growing period		Antibacterial activity					
			Bacillus cereus	*Staphylococcus aureus*	*Listeria monocytogenes*	*Escherichia coli*	*Enterobacter cloacae*	*Salmonella typhimurium*
Taraxacum officinale	1st	MIC	0.45	0.20	–	0.45	–	–
		MFC	0.60	0.30	–	0.60	–	–
	2nd	MIC	0.45	0.20	–	0.60	0.60	–
		MFC	0.90	0.30	–	0.90	1.20	–
Ketoconazole		MIC	0.25	0.20	0.20	0.20	2.50	0.20
		MFC	0.50	0.50	0.50	0.50	3.50	0.30
Bifonazole		MIC	0.15	0.10	0.15	0.20	0.20	0.10
		MFC	0.20	0.20	0.20	0.25	0.25	0.20

– No activity.

that microclimate conditions and genetic material are essential for chemical composition of wild edible species. Regarding *Taraxacum* species, α-, β- and γ-tocopherol were also detected in vegetative parts of *Taraxacum* sect. *Ruderalia* by Dias et al. (2014). However, δ-tocopherol was not detected in that study, which is in contrast with our results, as well as with the results reported by Morales et al. (2014) for *Taraxacum obovatum* and Šircelj, Mikulic-Petkovsek, Veberič, Hudina, and Slatnar (2018), who did not detect any tocopherol isoforms in *T. officinale* edible parts.

Organic acids content is presented in Table 5, with oxalic acid being the most abundant organic acid in all the studied species and growing periods, followed by malic and shikimic acid. According to the review study of Pinela et al. (2017), wild edible greens show a great diversity in organic acids content and oxalic acid in particular, while the same authors indicated that *S. oleraceus* presents a value higher than 2.5 regarding the ratio of oxalic acid/Ca and could be considered as potentially toxic if consumed in high amounts. Moreover, in most cases oxalic acid and shikimic acid, as well as total organic acids increased in the second growing period of our study, except for the case of *H. graecum* and *S. oleraceus* where the opposite trend was observed. Similarly, malic acid increased in the second growing period except for the cases of *R. picroides*, *H. graecum* and *T. officinale*. Oxalic acid is considered an antinutrient and results in adverse health effects when consumed in great amounts (5 g is the minimum lethal dose for an adult) (Morales et al., 2014). However, considering that most of the wild edible species are usually consumed in low amounts as side dishes or in mixed vegetable dishes, there is no risk of high ingestion of oxalic acid even in the case of species that contain high amounts of this organic acid (e.g. *H. graecum* and *R. picroides* which contain up to 972 and 937 mg 100 g^{-1} fresh weight, respectively).

The antimicrobial activity of plant extracts is presented in Table 6. Bacteriostatic activity ranged from 0.037 to 0.90 mg mL^{-1}, whereas positive controls of ampicillin and streptomycin presented MIC values ranging from 0.04 to 0.75 mg mL^{-1}. In addition, bactericidal activity varied between the species and growing periods with MBC values ranging between 0.15 mg mL^{-1} and 1.20 mg mL^{-1}, whereas positive controls of ampicillin and streptomycin presented values between 0.10 mg mL^{-1} and 1.20 mg mL^{-1}. *S. hispanicus*, *P. echioides*, *Taraxacum* sp. and *T. officinale* showed higher bacteriostatic and bactericidal potency against *Bacillus aureus* than both positive controls, while the rest of the studied species were more potent only comparing to ampicillin. Similarly, *S. hispanicus*, *P. echioides* and *T. officinale* showed significant and higher than positive controls bactericidal and bacteriostatic activity against *Salmonella tymphimurium*, while the rest of the species were more effective than ampicillin. Antibacterial activities have been already reported for *Taraxacum* sp. (Ghaima et al., 2013; Kenny et al., 2014; Wang, 2014), *Sonchus* spp. (Xia et al., 2011) and *Scolymus hispanicus* (Marmouzi et al., 2017) against various food borne pathogens, such as *Salmonella enterica*, *S. typhi*, *Staphylococcus aureus*, *B. cereus* and *E. coli*. Regarding antifungal activities, all the studied species showed higher efficiency against *Penicillium ochrochloron* than ketoconazole, whereas only *P. echiodes*, *U. picroides*, and *Taraxacum* sp. (samples from the 2nd growing period) had higher or similar fungistatic efficiency to bifonazole against *P. funiculosum* and *P. ochrochloron*. Similarly, the same species were more effective than ketoconazole against *Aspergillus fumigatus*, while they also presented similar fungistatic efficiency to bifonazole. Finally, the studied species presented significant efficacy against *A. ochraceus*, where in most cases MIC and MFC values of plant extracts were lower than those of positive controls (bifonazole and ketoconazole), especially for *R. picroides*, *S. oleraceus*, *P. echioides* and *Taraxacum* sp. which presented the lowest values. Antonia et al. (2011) have also suggested significant antifungal properties of *S. oleraceus* extracts against *Aspergillus niger* and *Penicillium italicum*, whereas to the best of our knowledge there are no available reports in the literature for the rest of the species.

The antimicrobial properties of these species could be attributed to their high content in phenolic compounds which have been associated with the control of food borne pathogens (Kenny et al., 2014).

4. Conclusion

The investigation of wild edible species belonging to Asteraceae family showed a great diversity in terms of chemical composition of the edible parts, while to the best of our knowledge phytochemical composition for some of the studied species is presented for the first time. All the studied species contained mostly flavonoids, whereas only in the case of *Taraxacum officinale* flavonoids and phenolic acids content was detected in similar amounts. *In* addition, the highest content of phenolic compounds was detected in *Sonchus oleraceus*, while the highest content of total tocopherols was observed in *Taraxacum* sp. plants during the 1st growing period, mostly due to the presence of high amounts of γ- and δ-tocopherols. In terms of organic acids, oxalic acid was the most abundant acid for all the studied species, while its content was the lowest in *Taraxacum* species, regardless of the growing period. Moreover, cultivation of these species for commercial purposes shows a great potential for using them as rich dietary sources of bioactive compounds such as phenolic compounds and tocopherols, although special attention should be given to oxalic acid content which is considered as an antinutritional factor. Therefore, these species could be considered as functional ingredients of healthy diets contributing to wellbeing of consumers, while at the same time all-year availability could be ensured and risk for genetic erosion could be minimized. Moreover, with commercial cultivation practices and the scheduling of growing period, chemical composition could be improved and a further increase of the nutritional value of the studied species could be achieved. Finally, antimicrobial properties of the plant extracts showed promising results and could be incorporated in the food processing industry as alternative food preservatives in processed or minimally processed food products.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.10.069.

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