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Indústria, Ciência, Formação e Inovação



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Phenolic profile of different *Cichorium spinosum* L. ecotypes

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ABSTRACT

The flora of the Mediterranean basin includes many wild edible horticultural species and most represent an important food and medicinal source for the rural communities. In the present study, the profile of phenolic compounds of various *Cichorium spinosum* L. ecotypes was evaluated. For this purpose, six ecotypes of *C. spinosum* purchased by retail supermarkets, collected *in situ*, and cultivated at the farm of the University of Thessaly were examined. Significant differences were observed among the various studied ecotypes in their phenolic compounds' content and profile, especially between wild and cultivated ecotypes. Wild ecotypes had lower content of total phenolic compounds than cultivated ones, whereas commercial products were very rich in flavonoids, especially in kaempferol-3-*O*-glucuronide, isorhamnetin-3-*O*-glucuronide and apigenin-*O*-glucuronide, in comparison to the other ecotypes (wild and cultivated). Commercial cultivation of *C. spinosum* should be encouraged since it ensures higher content of phenolic compounds. Thus, this species has a great potential since it could be used as a new alternative in the food industry.

1. INTRODUCTION

Several studies demonstrated the importance of wild edible species in the traditional Mediterranean diet as well as their pharmaceutical and medicinal properties. Leafy vegetables are an important source of phytonutrients that are essential for the human body and can be included in many vegetable salad dishes in order to enrich dietary sources of health promoting compounds [1].

Phenolic compounds are secondary metabolites that can be found in many plants and have a great importance in various physiological and morphological features, such as defense mechanisms, cell wall structure, interaction with phytohormones, proteins and enzymes, scavenging of free radicals and signaling for gene expression [2].

Cichorium spinosum L., also known as “stamnagathi” in Greek language, belongs to the Asteraceae family. It is a native plant in the Mediterranean basin and can be found in the Balearic Islands, Cyprus, Greece, Italy and Spain. It usually grows in coastal areas or plateaus of the mainland and constitutes a very common ingredient of the so-called Mediterranean diet [3].

Therefore, the aim of the present study was the identification of specific phenolic compounds of *C. spinosum* ecotypes from the eastern Mediterranean; a comparison between experimentally cultivated and/or commercial products with wild plants collected *in situ* was carried out, in order to identify the best conditions to increase polyphenols content.

2. MATERIALS AND METHODS

2.1. Sampling material

Samples of six ecotypes of *Cichorium spinosum* L. were evaluated, namely, commercial products obtained from retail supermarkets in Greece (samples 2 and 3); wild ecotypes collected *in situ* in the area of Velanidia, Greece (samples 4 and 5); and plants grown at the farm of the University of Thessaly (samples 1 and 6).

Hydromethanolic extracts were prepared by stirring the dried plant material (1 g) with 30 mL methanol/water (80:20, v/v, at 25 °C) for 1 h and subsequently filtering. The residue was then re-extracted with an additional portion of 30 mL of the hydromethanolic mixture. The combined extracts were evaporated under reduced pressure until complete removal of methanol, and the aqueous phase was frozen and lyophilized. The extracts were redissolved in methanol/water (80:20, v/v) at a final concentration of 5 mg/mL.

2.2. Phenolic compounds' analysis

The phenolic compounds' analysis was carried out by liquid chromatography coupled to a diode array detector and an electrospray ionization multistage mass spectrometry operating in negative mode [4]. Phenolic compounds were identified by comparison of commercial standards when available or tentatively identified using reported data from literature. Calibration curves were obtained by using commercial standards and were based on the UV-Vis signal. When no commercial standard was available, a similar compound from the same phenolic group was used as a standard. Results were expressed as g per kg of dry extract.

2.3. Statistical analysis

For all the analyses, three samples were analyzed for each treatment and all of the assays were carried out in triplicate (n=9). The results were expressed as mean values and standard deviations (SD). Statistical analysis of data was applied using SPSS v. 22.0 program (IBM Corp., Armonk) through a one-way analysis of variance (ANOVA) while, for means where a statistical difference was detected, means comparisons were carried out using Tukey's HSD test ($p = 0.05$).

3. RESULTS AND DISCUSSION

The phenolic compounds' profile of *C. spinosum* hydromethanolic extracts are presented in Table 1. These samples were characterized by the presence of 17 phenolic compounds, from which eight were classified as phenolic acids and nine as flavonoid glycoside derivatives. Phenolic acids were the most abundant phenolic compounds for all the studied ecotypes,

accounting for 63.4-70.7% of the total phenolic content for commercially cultivated ecotypes, while for wild and experimentally cultivated ecotypes the corresponding amount was approximately 70% and 90%, respectively (Table 2). The wild ecotypes (samples 4 and 5) had lower amounts of chicoric acid isomers compared to cultivated ecotypes and especially sample 3 (conventional commercial ecotype), which had the highest amount. Caftaric, 5-*O*-caffeoylquinic, fertaric, and 3,5-*O*-dicafeoylquinic acids were detected in higher amounts in sample 6, whereas the lowest amounts were detected in wild ecotype (sample 4).

Nevertheless, commercial samples 2 and 3 were very rich in flavonoids, especially kaempferol-*O*-glucuronide and quercetin-3-*O*-glucuronide, followed by kaempferol-3-*O*-glucuronide and isorhamnetin-3-*O*-glucuronide (Table 2), compared to the other ecotypes (wild and cultivated). Concerning growing conditions and cultivation practices, significant differences were detected between wild and cultivated plants, with wild ecotypes having significantly lower amounts of phenolic acids and flavonoids and consequently of total phenolic compounds, compared to cultivated plants.

Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{\max}), mass spectral data and tentative identification of phenolic compounds in *C. spinosum* leaves.

Compound	Rt (min)	λ_{\max} (nm)	Molecular ion	MS ² (m/z)	Tentative identification
1	5.1	328	311	179(85), 149(54), 135(100)	Caftaric acid
2	8.2	328	353	191(100), 179(71), 135(43)	5- <i>O</i> -Caffeoylquinic acid
3	14.8	330	473	313(68), 293(83), 219(13), 179(93), 149(100), 135(42)	<i>cis</i> -Chicoric acid
4	14.9	330	473	313(68), 293(83), 219(13), 179(93), 149(100), 135(42)	<i>trans</i> -Chicoric acid
5	19.5	312	457	295(100), 277(68), 219(26), 179(24), 163(58), 149(8)	Coutaric acid hexoside
6	19.9	358	477	301(100)	Quercetin-3- <i>O</i> -glucuronide
7	20.3	350	461	285(100)	Kaempferol- <i>O</i> -glucuronide
8	21.2	310	295	219(27), 179(5), 163(22), 149(5), 135(11)	Coutaric acid
9	21.2	330	325	193(100), 134(98)	Fertaric acid
10	22.3	356	505	463(10), 301(100)	Quercetin-7- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside
11	22.6	328	515	353(), 191(98), 179(77), 161(4), 135(22)	3,5- <i>O</i> -Dicafeoylquinic acid
12	23.5	348	593	285(100)	Kaempferol-3- <i>O</i> -rutinoside
13	24.4	348	461	285(100)	Kaempferol-3- <i>O</i> -glucuronide
14	25.2	336	445	269(100)	Apigenin- <i>O</i> -glucuronide
15	25.5	358	491	315(100)	Isorhamnetin-3- <i>O</i> -glucuronide
16	26.9	338	489	285(100)	Kaempferol-3- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside
17	28.0	358	519	315(100)	Isorhamnetin-3- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside

Table 2. Phenolic compound quantification (g/kg dry extract) in the studied *C. spinosum* ecotypes (mean±standard deviation).

Compound	Phenolic compound	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	Caftaric acid	0.84 ± 0.03	0.49±0.01	1.50±0.01	0.30±0.02	0.84±0.03	2.39±0.02
2	5- <i>O</i> -Caffeoylquinic acid	3.32 ± 0.03	2.40±0.02	5.29±0.05	1.75±0.01	1.77±0.03	9.46±0.03
3	<i>cis</i> -Chicoric acid	16.85 ± 0.08	13.31±0.05	17.4±0.2	4.47±0.01	7.06±0.03	16.0±0.2
4	<i>trans</i> -Chicoric acid	nd	13.45±0.06	16.2±0.5	0.082±0.001	0.082±0.001	15.1±0.1
5	Coutaric acid hexoside	0.097 ± 0.003	0.71±0.02	1.04±0.04	0.037±0.001	0.16±0.01	0.57±0.02
6	Quercetin-3- <i>O</i> -glucuronide	0.951 ± 0.001	5.57±0.03	5.57±0.01	1.070±0.001	1.47±0.02	1.04±0.01
7	Kaempferol- <i>O</i> -glucuronide	nd	6.18±0.02	3.80±0.01	0.64±0.01	0.75±0.01	0.53±0.01
8	Coutaric acid	tr	nd	nd	nd	nd	nd
9	Fertaric acid	0.46 ± 0.03	2.45±0.05	3.27±0.05	0.36±0.01	1.01±0.01	6.45±0.04
10	Quercetin-7- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside	0.050 ± 0.005	0.56±0.02	0.876±0.01	tr	0.204±0.002	0.53±0.03
11	3,5- <i>O</i> -Dicafeoylquinic acid	nd	1.37±0.03	1.71±0.01	0.79±0.01	1.99±0.01	2.40±0.02
12	Kaempferol-3- <i>O</i> -rutinoside	0.064 ± 0.002	0.094±0.002	0.070±0.003	tr	0.019±0.004	tr
13	Kaempferol-3- <i>O</i> -glucuronide	0.758 ± 0.005	3.13±0.02	3.35±0.05	0.90±0.01	1.02±0.01	0.62±0.01
14	Apigenin-7- <i>O</i> -glucuronide	nd	1.26±0.08	1.31±0.01	0.24±0.01	0.16±0.01	0.66±0.02
15	Isorhamnetin-3- <i>O</i> -glucuronide	nd	2.194±0.09	2.63±0.04	0.237±0.004	0.26±0.01	0.11±0.01
16	Kaempferol-3- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside	0.12 ± 0.01	0.54±0.04	0.84±0.01	0.12±0.01	0.38±0.01	0.26±0.01
17	Isorhamnetin-3- <i>O</i> -(6''- <i>O</i> -acetyl)glucoside	nd	0.17±0.03	0.13±0.02	0.032±0.002	0.140±0.002	0.925±0.004
	Total phenolic acids	21.6 ± 0.3d	34.2±0.2c	46±1b	7.79±0.01e	12.9±0.1e	52.3±0.3a
	Total flavonoids	1.94 ± 0.01e	19.7±0.1a	18.6±0.2b	3.24±0.04d	4.40±0.02c	4.7±0.1c
	Total phenolic compounds	23.5 ± 0.3d	53.9±0.1c	65±1a	11.04±0.05f	17.3±0.1e	57.0±0.1b

tr- traces; nd- not detected. In each row, different letters mean significant statistical differences between samples ($p < 0.05$).

4. CONCLUSION

Cichorium spinosum L. phenolic compounds consist mainly of chicoric acid and 5-*O*-caffeoylquinic acid, while significant differences were observed among the studied ecotypes, as well as between the growing conditions (wild and cultivated); no significant differences were observed between the studied conventional and organic commercial products. Wild ecotypes had lower content of total phenolic compounds than cultivated ones, whereas commercial products were richer in flavonoids glycoside, in comparison to the other ecotypes.

Commercial cultivation of *C. spinosum* (either conventional or organic) should be encouraged since it ensures higher content of phenolic compounds and minimizes the danger of genetic erosion from irrational harvesting of wild plants.

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