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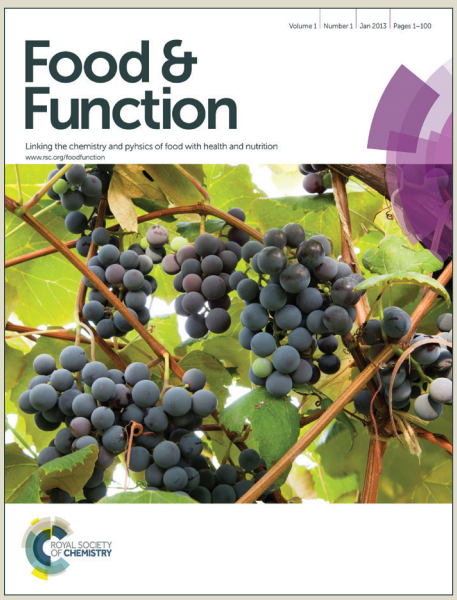

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Infusions of artichoke and milk thistle represent a good source of phenolic acids and flavonoids

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Abstract

Cynara scolymus L. (artichoke) and *Silybum marianum* (L.) Gaertn (milk thistle) are two herbs well-known for their efficiency in the prevention/treatment of liver injuries, among other chronic diseases. Therefore, the aim of this work was to characterize specific bioactive components, phenolic compounds, in hydromethanolic extracts but also in infusions (the most common used preparations) obtained from the whole plant of milk thistle and artichoke The phenolic profiles were accessed using HPLC-DAD-MS/ESI. Infusions of both species presented higher phenolic contents than the hydromethanolic extracts. Milk thistle presented a similar phenolic composition between the two preparations, revealing only differences in the quantities obtained. Nevertheless, artichoke revealed a slightly different profile considering infusion and hydromethanolic extract. Apigenin-7-*O*-glucuronide was the major flavonoid found in milk thistle, while luteolin-7-*O*-glucuronide was the most abundant in artichoke. Therefore, infusions of both artichoke and milk thistle represent a good source of bioactive compounds, especially phenolic acids and flavonoids.

Keywords: Artichoke; Milk thistle; Phenolic compounds; Infusions, Hydromethanolic extracts; HPLC-DAD-MS/ESI.

Introduction

The growing incidence of degenerative diseases, such as cancer and cardiovascular disease, has triggered an increasing number of epidemiological studies pointing natural antioxidants present in fruit and vegetables.[1-3] Indeed, some studies reported that societies whose diets are rich in these foods have a low incidence of chronic diseases, which suggest that an improved diet could reduce this kind of illnesses. [4,5]

Vegetables and derived products, such as infusions, have been considered significantly important in the prevention of cancer, diabetes, cardiovascular, inflammatory, allergic, bacterial and viral diseases,[6,7] and those health-promoting properties can be related to their extremely diverse phytochemicals, particularly phenolic compounds that provide preventive and defensive mechanisms to avoid chronic diseases.[8] These secondary metabolites of plants are well-known for their dual role: as protective agents against oxidative damages, mainly due to their redox capacity that allow them to adsorb and neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxides; and as substrates for oxidative browning reactions through enzymatic and chemical mechanisms.[9,10] Besides their strong antioxidant activity, phenolic compounds also revealed the capacity to inhibit the growth of tumor cell lines such as mammary, epidemoid, and hepatocellular carcinoma, among others, in a large number of studies.[11]

Artichoke (*Cynara scolymus* L.) and milk thistle (*Silybum marianum* (L.) Gaertn) are two medicinal plants, in which phenolic composition of hydroalcoholic extracts are documented in different studies[12-17] due to their implication in these herbs major medicinal properties, including antioxidant, chemopreventive, hepatoprotective and antiviral. [18,19]

In previous works, artichoke hydroalcoholic extracts proved to be a good source of flavonoids such as luteolin and apigenin glycosides, and mono-/di-caffeoylquinic acids

and derivatives, the main responsible for its therapeutic effects.[16,20] On the other hand, the medicinal properties of milk thistle are attributed to a polyphenolic mixture known as silymarin (present in the seeds), which contains several flavonolignans that are diastereomeric and/or constitutional isomers of each other including silybin A, silybin B, isosylibin A, isosylibin B, silychristin, isosilychristin, and silydianin.[14,17,19,21-27] Artichoke and milk thistle can be directly consumed in diet or taken as infusions, among other available formulations,[18,28] allowing the dietary polyphenolic compounds to be absorbed though the gastrointestinal tract, and reach the liver, where they are mainly metabolized.[6] Thus, in the present work, the aim was to obtain the phenolic profile of the most common parts used to prepare the infusions of these plants instead of its isolated parts that are already well-reported as rich sources of these bioactive compounds; for that purpose, the phenolic profile of hydromethanolic extracts and infusions prepared from the whole plant, were assessed and compared.

Experimental

Samples

Cynara scolymus L. (artichoke) and *Silybum marianum* (L.) Gaertn (milk thistle) were obtained from an herbalist shop in Bragança (Portugal), as dry material (mainly flowering steams, capitula and involucral bracts in both cases and leaves as well in *Silybum* sample). The botanical identification was confirmed by Ana Maria Carvalho, responsible of the medicinal plant collection of the Herbarium of the Escola Superior Agrária (BRESA), of the Polytechnic Institute of Bragança (Trás-os-Montes, Portugal), where voucher specimens were deposited (artichoke- code 9611; milk thistle- code 9612).

All the samples were reduced to powder and submitted to different preparations: i) Hydromethanolic extraction: each sample (1 g) was extracted by stirring with 25 mL of methanol:water (80:20 *v/v*, 25 ºC at 150 rpm) for 1 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with an additional 25 mL of methanol:water (80:20 *v/v*) for another hour. The combined extracts were evaporated at 40 ºC rotary evaporator (Büchi R-210, Flawil, Switzerland), frozen and lyophilized; ii) Infusion preparation: each sample (1 g) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure, afterwards the obtained infusion was frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA).

Standards and reagents

HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany). Formic acid was purchased from Prolabo (VWR International, Fontenay-sous-Bois, France). Phenolic standards were from Extrasynthèse (Genay, France). Water was treated in Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

Phenolic compounds extraction and analysis

The previously described hydromethanolic extracts and infusions were dissolved in water:methanol (80:20, *v/v*) and water, respectively (final concentration 20 mg/mL) and analysed using a Hewlett-Packard 1100 chromatograph (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, US) with a quaternary pump and a diode array detector (DAD) coupled to an HP Chem Station (rev. A.05.04) data-processing station. A Waters Spherisorb S3 ODS-2 C_{18}, 3 μm (4.6 mm × 150 mm) column thermostatted at 35 °C

was used. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% for 5 min, 15% B to 20% B over 5 min, 20-25% B over 10 min, 25-35% B over 10 min, 35-50% for 10 min, and re-equilibration of the column, using a flow rate of 0.5 mL/min. Double online detection was carried out in the DAD using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (MS) connected to HPLC system via the DAD cell outlet.

MS detection was performed in an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an ESI source and a triple quadrupole-ion trap mass analyzer that was controlled by the Analyst 5.1 software. Zero grade air served as the nebulizer gas (30 psi) and turbo gas for solvent drying (400 ºC, 40 psi). Nitrogen served as the curtain (20 psi) and collision gas (medium). The quadrupols were set at unit resolution. The ion spray voltage was set at -4500V in the negative mode. The MS detector was programmed for recording in two consecutive modes: Enhanced MS (EMS) and enhanced product ion (EPI) analysis. EMS was employed to show full scan spectra, so as to obtain an overview of all of the ions in sample. Settings used were: declustering potential (DP) -450 V, entrance potential (EP) -6 V, collision energy (CE) -10V. EPI mode was performed in order to obtain the fragmentation pattern of the parent ion(s) in the previous scan using the following parameters: DP -50 V, EP -6 V, CE -25V, and collision energy spread (CES) 0 V. Spectra were recorded in negative ion mode between *m*/*z* 100 and 1500.

The phenolic compounds were identified by comparing their retention time, UV-vis and mass spectra with those obtained from standard compounds, when available. Otherwise, peaks were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal: apigenin-7-*O*-

glucoside (y=159.62x+7.5025; R^2=0.999); caffeic acid (y=611.9x-4.5733; R^2=0.999); chlorogenic acid (y=313.03x-58.2; R^2=0.999); *p*-coumaric (y=884.6x+184.49; R^2=0.999); ferulic acid (y=505.97x-64.578); kaempferol-3-*O*-glucoside (y=288.55x-4.0503; R^2=1); kaempferol-3-*O*-rutinoside (y=239.16x-10.587; R^2=1); luteolin-7-*O*-glucoside (y=80.829x-21.291; R^2=0.999); protocatechuic acid (y=291.1x-6.4558; R^2=0.999); quercetin-3-*O*-glucoside (y=363.45x+117.86; R^2=0.999), quercetin-3-*O*-rutinoside (y=281.98x-0.3459; R^2=1). For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of other compound from the same phenolic group. The results were expressed in mg per g of lyophilized infusion or extract.

Statistical analysis

For each species, three samples were used and all the analyses were carried out in triplicate. The results were expressed as mean values and standard deviation (SD) and further analysed using one-way analysis of variance (ANOVA) followed by Tukey´s HSD Test with α= 0.05. This treatment was carried out using SPSS v.20.0 program.

Results and Discussion

Tables 1 and 2 present the data obtained from HPLC-DAD-MS analysis (retention time, λ_{max} in the visible region, mass spectral data) used for the identification and quantification of phenolic compounds in *S. marianum* (milk thistle) and *C. scolymus* (artichoke), respectively. As an example, the HPLC phenolic profiles of their infusions, recorded at 370 nm, can be observed in **Figure 1**.

Phenolic acids

Protocatechuic acid (compound **3**), 5-*O*-caffeolylquinic acid (compound **6**), quinic acid (compound **7**), caffeic acid (compound **8**), and *p*-coumaric acid (compound **15**) were positively identified according to their retention time, mass and UV-vis characteristics by comparison with commercial standards.

Hydroxycinnamic acid derivatives were detected in both samples, being mostly quinic acid derivatives, whose identities were assigned based on their MS spectra and fragmentation patterns. The assignments of the different caffeoylquinic, feruloylquinic and *p*-coumaroylquinic acid isomers were made using the recommended IUPAC numbering system, as also the hierarchical keys previously developed by Clifford et al.[29,30]

Compound **1** ($[M-H]^-$ at *m/z* 353) was identified as 3-*O*-caffeoylquinic acid, yielding the base peak at *m/z* 191 ($[quinic\ acid-H]^-$) and the ion at *m/z* 179 ($[caffeic\ acid-H]^-$) with an intensity >63% base peak, characteristic of 3-acylchlorogenic acids as reported by Clifford et al.[29,30] Monocaffeoylquinic acids have been largely reported by many authors in different parts of artichoke, such as heads and leaves,[13,15,31-38], hearts,[16] wastes such as bracts, receptacles and steams from the fruit,[39] juices and pomace,[15,32] and in dietary supplements.[15,37,40]

Compound **22** present in milk thistle and artichoke was identified as 3,5-*O*-dicaffeoylquinic acid based on its fragmentation pattern similar to the one reported by Clifford et al.[30] MS^2 base peak was at *m/z* 191, but also presented a very high relative abundance at *m/z* 353, produced by the loss of one of the caffeoyl moieties $[M-H-caffeoyl]^-$, whose subsequent fragmentation yielded the same fragments as 5-*O*-caffeoylquinic acid at *m/z* 191, 179 and 135. Compound **10** (artichoke) was identified as 1,3-*O*-dicaffeoylquinic acid (cynarin) according to its MS^2 fragmentation and elution

characteristics, being the most hydrophilic dicaffeoylquinic acid.[41] Dicaffeoylquinic acids have been extensively reported in hydroalcoholic extracts obtained from different parts of artichoke, as mentioned above.[13,15,16,31-36,38-40]

Four signals in artichoke (compounds **4**, **9**, **11** and **13**) showed a pseudomolecular ion ($[M-H]^-$) at *m/z* 337 (**Tables 1** and **2**). These compounds were assigned as the 3-acyl, 4-acyl and 5-acyl isomers of *p*-coumaroylquinic acid based on their HPLC retention and MS^2 fragmentation characteristics, as previously reported by Clifford et al.[29,42] Thus, compound **4** (artichoke) was tentatively identified as 3-*p*-coumaroylquinic acid, yielding the base peak at *m/z* 163 ($[coumaric\ acid-H]^-$). Fragmentation of compound **9** with a majority MS^2 product ion at *m/z* 173 was coherent with 4-*p*-coumaroylquinic acid, whereas compound **13** (artichoke and milk thistle), yielding the base peak at *m/z* 191, was identified as *trans* 5-*p*-coumaroylquinic acid. This latter compound was also found in the analyzed milk thistle extracts. Compound **11** (artichoke) with a UV spectrum and MS^2 fragmentation pattern identical to that of compound **13** was tentatively assigned as the *cis* isomer of 5-*p*-coumaroylquinic acid. This tentative assignment was supported by the observation that hydroxycinnamoyl *cis* derivatives are expected to elute before the corresponding *trans* ones, as previously observed before and after UV irradiation (366 nm, 24 h) of hydroxycinnamic derivatives in our laboratory.[43] Furthermore, in milk thistle compound **19** with 162+162 mu (glucosyl moieties) higher than compound **13** was tentatively identified as 5-*p*-coumarolyquinic acid dihexoside (**Table 1**). As far as we are aware, but for 3-*p*-coumaroylquinic acid identified in artichoke heart by Abu-Reidah et al.,[16] any of these *p*-coumaroylquinic acid derivatives has been previously reported neither in artichoke nor in milk thistle.

Compound **14** in milk thistle was identified as 5-*O*-feruloylquinic acid taking into account its pseudomolecular ion ($[M-H]^-$ at *m/z* 367) and MS^2 fragmentation similar to

that of 5-*O*-caffeoylquinic acid. This compound was previously identified in artichoke samples,[16,37] but, as far as we know, it has not been reported in milk thistle.

Compounds **5** present in milk thistle and artichoke showed a pseudomolecular ion $[M-H]^-$ at *m/z* 341, releasing an MS^2 fragment at *m/z* 179 ($[caffeic\ acid-H]^-$) from the loss of a hexosyl moiety (-162 mu) was tentatively assigned as caffeic acid hexoside. That compound was also identified in hydroalcoholic extracts of artichoke hearts by Abu-Reidah et al.[16]

Finally, compound **2** (artichoke) with the same UV and mass characteristics as compound **3** (protocatechuic acid, i.e. 3,4-dihydroxybenzoic acid) was just tentatively assigned as a dihydroxybenzoic acid. Protocatechuic acid was previously reported in hydroalcoholic extracts of artichoke wastes (bracts, receptacles and steams from the fruit) by Sánchez-Rabaneda et al.[39]

Flavonoids

Compounds **16**, **21** and **29** in artichoke and compound **20** in both samples, were identified as luteolin derivatives according to their UV and mass spectra characteristics (**Tables 1** and **2**). Compounds **21** and **29** were positively identified as luteolin-7-*O*-glucoside (cynaroside) and luteolin, respectively by comparison with commercial standards, being also largely identified in artichoke hearts,[16] leaves and heads,[13,15,31,32,34-37] juices and pomace,[15,32] and dietary supplements.[15,37,40]

Compound **20** presented a pseudomolecular ion $[M-H]^-$ at *m/z* 461 releasing a fragment ion at *m/z* 285 ($[M-176]^-$, loss of a glucuronyl moiety), although the position of the glycosyl moiety could not be established it was assigned to luteolin 7-*O*-glucuronide, owing to the identification of that compound in leaves, heads, hearts, juices, pomaces and dietary supplements of artichoke, mostly obtained after hydroalcoholic

extraction.[13,15,16,32,33,35-37,39,40] Compound **16** presented a pseudomolecular ion $[M-H]^-$ at *m/z* 623, yielding fragment ions at *m/z* 461 (-162 mu; loss of a hexosyl residue) and 285 (-176 mu; loss of a glucuronyl residue), which allowed its assignment as luteolin-*O*-hexoside-*O*-glucuronide. Two compounds with similar characteristics were reported by Abu-Reidah et al.[16,] in artichoke hearts, also without assigning the position of substitution of the glycosyl residues.

In accordance with their UV and mass spectra characteristics, different apigenin derivatives were also detected in the analysed samples. Compounds **24**, **27** (artichoke) and **26** (artichoke and milk thistle) showed pseudomolecular and fragment ions coherent with deoxyhexosyl-hexoside, hexoside and glucuronide derivatives of apigenin, respectively. The presence of apigenin-7-*O*-rutinoside, apigenin-7-*O*-glucuronide and apigenin-7-*O*-glucoside in different parts of artichoke was consistently reported by the previously mentioned authors, so that those identities could also be tentatively assumed for the compounds detected herein. Furthermore, the identity of apigenin-7-*O*-glucoside (compound **27**) was here confirmed by comparison with a commercial standard.

The pseudomolecular ion of compound **18** in artichoke ($[M-H]^-$ at *m/z* 607) released a fragment ion at *m/z* 269 ($[M-162-176]^-$; apigenin) allowed its tentative identification as an apigenin-*O*-hexoside-*O*-glucuronide. A compound with similar characteristics was identified as apigenin-4-*O*-hexoside-7-*O*-glucuronide by Abu-Reidah et al.[16] in artichoke hearts. Another apigenin derivative (compound **23**), was detected in the sample of milk thistle, whose mass characteristics ($[M-H]^-$ at *m/z* 591 releasing a fragment ion at *m/z* 269 ($[M-146-176]^-$) from the loss of deoxyhexosyl and glucuronyl moieties) pointed to an apigenin-*O*-deoxyhexosyl-glucuronide. To our knowledge, this compound was not previously described in milk thistle samples.

The following compounds were only present in artichoke. Compound **17** ([M-H]$^-$ at *m/z* 477) presented UV spectra with λ_{max} around 350 nm and an MS^2 product ion at *m/z* 301, comparison with a standard obtained in our laboratory[44] allowed its identification as quercetin 3-*O*-glucuronide. Compound **12** ([M-H]$^-$ at *m/z* 639) released fragment ions at *m/z* 477 and 315, from the consecutive losses of 162 mu (two hexosyl moieties). The ion at *m/z* 315 can be attributed to a methylquercetin, whilst the high abundance of the ion at *m/z* 477 indicated that each hexosyl group was located on different position of the aglycone. Therefore, the compound was tentatively assigned as methylquercetin-*O*-hexoside-*O*-hexoside. Compound **25** ([M-H]$^-$ at *m/z* 623) released fragment ions at *m/z* 315 and 300 (further loss of a methyl group) also suggesting a methylquercetin. In this case, the loss of 308 mu (146+162 mu) to yield the aglycone suggested the existence of deoxyhexose and hexose as glycosylating substituents, probably constituting a disaccharide owing to their joint loss. Although there was not further indication about the type of sugar, it might be a rutinose, taking into account the previous identification of quercetin-3-*O*-rutinoside in hydroalcoholic extracts of artichoke samples by Sánchez-Rabaneda et al.[39] and Abu-Reidah et al.[16] Thus, the compound was tentatively assigned as methylquercetin *O*-rutinoside. Finally, compound **28** with a pseudomolecular ion [M-H]$^-$ at *m/z* 431 yielding a product ion at *m/z* 285 (-146 mu, loss of a dexoyhexosyl moiety) could be associated to a kaempferol-*O*-deoxyhexoside. As far as we know, none of these latter four compounds has not been described in artichoke.

In both species, infusions presented higher phenolic contents than their hydromethanolic extracts. Milk thistle preparations presented the same composition revealing only differences in the quantities obtained. Nevertheless, artichoke revealed a different profile between infusions and hydromethanolic extracts. These differences can be mainly due to the heat treatment to which infusions were subjected. Apigenin-7-*O*-

glucuronide was the major flavonoid found in milk thistle (**Table 1**), while luteolin-7-*O*-glucuronide was the most abundant in artichoke (**Table 2**).

In literature, milk thistle phenolic composition is characterized by seven flavonolignans (silymarin).[12,14,17,19,21,23-27,31] These compounds are known to be normally present in seeds of milk thistle.[17,19] Therefore, it can be concluded that the sample studied by us did not contain seeds, but only the other parts of the plant, even though the label mentioned to the whole plant material.

Despite the many articles reporting phenolic composition of artichoke hydroalcoholic extracts,[13,15,16,31-40] the present work characterizes the phenolic composition in infusions. Moreover, infusion is the most common form to consume this plant and, to our knowledge, this is also the first report presenting results for the whole plant material and not seeds. Literature reports mainly the existence of caffeoylquinic acid, and luteolin and apigenin derivatives, as also observed in the present study. Nevertheless, the studied sample of artichoke also presented other compounds.

Conclusions

Overall, both artichoke and milk thistle represent a good source of bioactive compounds, especially phenolic acids and flavonoids, that are higher enhanced in the infusion preparations. This study also demonstrates the reason for the traditional and current uses of these plants in different formulations (dry material, pills and syrups), by deepening the knowledge of the main responsible bioactive compounds. Moreover, these plants can be used not only as excellent sources of antioxidants but also as potential natural remedies, that can easily be included in diet, thereby preventing and healing chronic diseases.

Competing interests

The authors declare no competing financial interest.

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Figure 1. Phenolic profile of the infusion of artichoke (A) and milk thistle (B) recorded at 370 nm. The profile was obtained using a Spherisorb S3 ODS-2 C_{18} column thermostatted at 35 °C, using a flow rate of 0.5 mL/min and with gradient elution, (a) 0.1% formic acid in water and (b) acetonitrile.

Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in hydromethanolic extract and infusion of artichoke (mean ± SD).

Compound	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*) (% base peak)	Tentative identification	Identification type	Quantification (mg/g)	
							Hydromethanolic	Infusion
1	5.18	326	353	191(100),179(63),135(25)	3-*O*-Caffeoylquinic acid	13,15,16,31-40	0.10 ± 0.00	nd
2	5.58	262sh294	153	109(100)	Dihydroxybenzoic acid	DAD/MS	nd	0.85 ± 0.02
3	6.18	262sh296	153	109(100)	Protocatechuic acid	Standard/DAD/MS	0.25 ± 0.01	0.23 ± 0.01
4	6.84	310	337	191(13),173(6),163(96),155(6),119(33)	3-*p*-Coumaroylquinic acid	16, 29,42	0.07 ± 0.00	nd
5	7.37	328	341	179(100),135(89)	Caffeic acid hexoside	16	0.13 ± 0.00	0.07 ± 0.00
6	7.92	326	353	191(100),179(2),161(2),135(3)	5-*O*-Caffeoylquinic acid	Standard/DAD/MS	0.49 ± 0.01	nd
7	8.44	286/333	191	175(100),148(33),103(6)	Quinic acid	Standard/DAD/MS	0.21 ± 0.01	0.08 ± 0.00
8	10.66	324	179	135(100)	Caffeic acid	Standard/DAD/MS	nd	0.51 ± 0.01
9	10.67	306	337	191(5),173(100),163(18),155(5),119(10)	4-*p*-Coumaroylquinic acid	29,42, Standard/DAD/MS	0.12 ± 0.00	nd
10	11.21	324	515	353(95),191(100),179(65),135(40)	1,3-Dicaffeoylquinic acid	13,15,16,31-40	0.37 ± 0.02	0.90 ± 0.02
11	12.95	312	337	191(100),173(6),163(10),119(4)	*cis* 5-*p*-Coumaroylquinic acid	29,42, Standard/DAD/MS	0.33 ± 0.02	nd
12	13.19	356	639	477(80),315(51)	Methylquercetin-*O*-hexoside-*O*-hexoside	DAD/MS	nd	0.14 ± 0.01
13	13.90	306	337	191(100),173(3),163(4),119(2)	*trans* 5-*p*-Coumaroylquinic acid	29,42, Standard/DAD/MS	0.03 ± 0.00	nd
15	16.81	310	163	119(100)	*p*-Coumaric acid	Standard/DAD/MS	nd	0.40 ± 0.00
16	17.02	350	623	461(7),285(100)	Luteolin-*O*-hexoside-*O*-glucuronide	16, DAD/MS	0.26 ± 0.01	0.46 ± 0.01
17	19.26	350	477	301(100)	Quercetin-3-*O*-glucuronide	Standard/DAD/MS	0.06 ± 0.00	0.09 ± 0.00
18	20.26	340	607	269(100)	Apigenin-4-*O*-hexoside-7-*O*-glucuronide	16, DAD/MS	0.12 ± 0.00	0.31 ± 0.02

24	24.01	338	577	269(100)	Apigenin-7-*O*-rutinoside	13,15,16,32,33,35-37,39,40	0.09 ± 0.02	0.16 ± 0.02
25	24.38	352	623	315(16),300(56)	Methylquercetin-*O*-rutinoside	DAD/MS	0.08 ± 0.00	0.07 ± 0.00
26	25.51	336	445	269(100)	Apigenin-7-*O*-glucuronide	13,15,16,32,33,35-37,39,40	0.20 ± 0.00	1.24 ± 0.12
27	25.67	338	431	269(100)	Apigenin-7-*O*-glucoside	Standard/DAD/MS	0.21 ± 0.01	0.68 ± 0.02
28	29.30	340	431	285(100)	Kaempferol-*O*-deoxyhexosyl	DAD/MS	0.04 ± 0.01	0.06 ± 0.00
29	34.51	346	285	175(8),151(8),133(5)	Luteolin	Standard/DAD/MS	nd	0.14 ± 0.01
					Total phenolic acids		2.12 ± 0.04[b]	3.40 ± 0.06[a]
					Total flavonoids		2.25 ± 0.01[b]	11.89 ± 0.39[a]
					Total phenolic compounds		4.37 ± 0.05[b]	15.29 ± 0.33[a]

nd-not detected; In each row different letters mean significant differences (p<0.05).

Table 2. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, identification and quantification of phenolic compounds in hydromethanolic extract and infusion of milk thistle (mean ± SD).

Compound	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*) (% base peak)	Tentative identification	Identification type	Quantification (mg/g)	
							Hydromethanolic	Infusion
3	6.19	262sh296	153	109(100)	Protocatechuic acid	Standard/DAD/MS	0.44 ± 0.01	0.08 ± 0.01
5	7.44	328	341	179(100),135(22)	Caffeic acid hexoside	DAD/MS	0.12 ± 0.00	0.05 ± 0.01
6	8.11	326	353	191(100),179(4),173(7),135(5)	5-*O*-Caffeolyquinic acid	Standard/DAD/MS	0.56 ± 0.02	0.15 ± 0.02
13	13.19	312	337	191(100),173(7),163(9),119(5)	5-*p*-Coumarolyquinic acid	29,42, Standard/DAD/MS	0.12 ± 0.00	0.03 ± 0.01
14	15.02	328	367	193(43),191(100),173(11),134(2)	5-*O*-Feruloylquinic acid	DAD/MS	0.05 ± 0.01	0.05 ± 0.01
15	17.10	306	163	119(100)	*p*-Coumaric acid	Standard/DAD/MS	0.11 ± 0.01	0.11 ± 0.01
19	20.27	322	661	499(100),337(11),179(11),173(87),163(14),119(8)	5-*p*-Coumarolyquinic acid dihexoside	DAD/MS	0.11 ± 0.00	0.38 ± 0.03
20	20.77	350	461	285(100)	Luteolin-7-*O*-glucuronide	DAD/MS	0.58 ± 0.01	1.17 ± 0.09
22	23.03	330	515	353(71),191(100),179(6),173(6),135(6)	3,5-*O*-Dicaffeolyquinic acid	30, Standard/DAD/MS	0.13 ± 0.01	0.09 ± 0.02
23	23.95	336	591	269(100)	Apigenin-*O*-deoxyhexosyl-glucuronide	DAD/MS	0.10 ± 0.01	0.36 ± 0.02
26	25.48	338	445	269(100)	Apigenin-7-*O*-glucuronide	DAD/MS	1.26 ± 0.01	3.14 ± 0.12
					Total phenolic acids		1.65 ± 0.04^a	0.91 ± 0.09^b
					Total flavonoids		1.94 ± 0.01^b	4.66 ± 0.18^a
					Total phenolic compounds		3.56 ± 0.05^b	5.57 ± 0.27^a

In each row different letters mean significant differences ($p<0.05$).

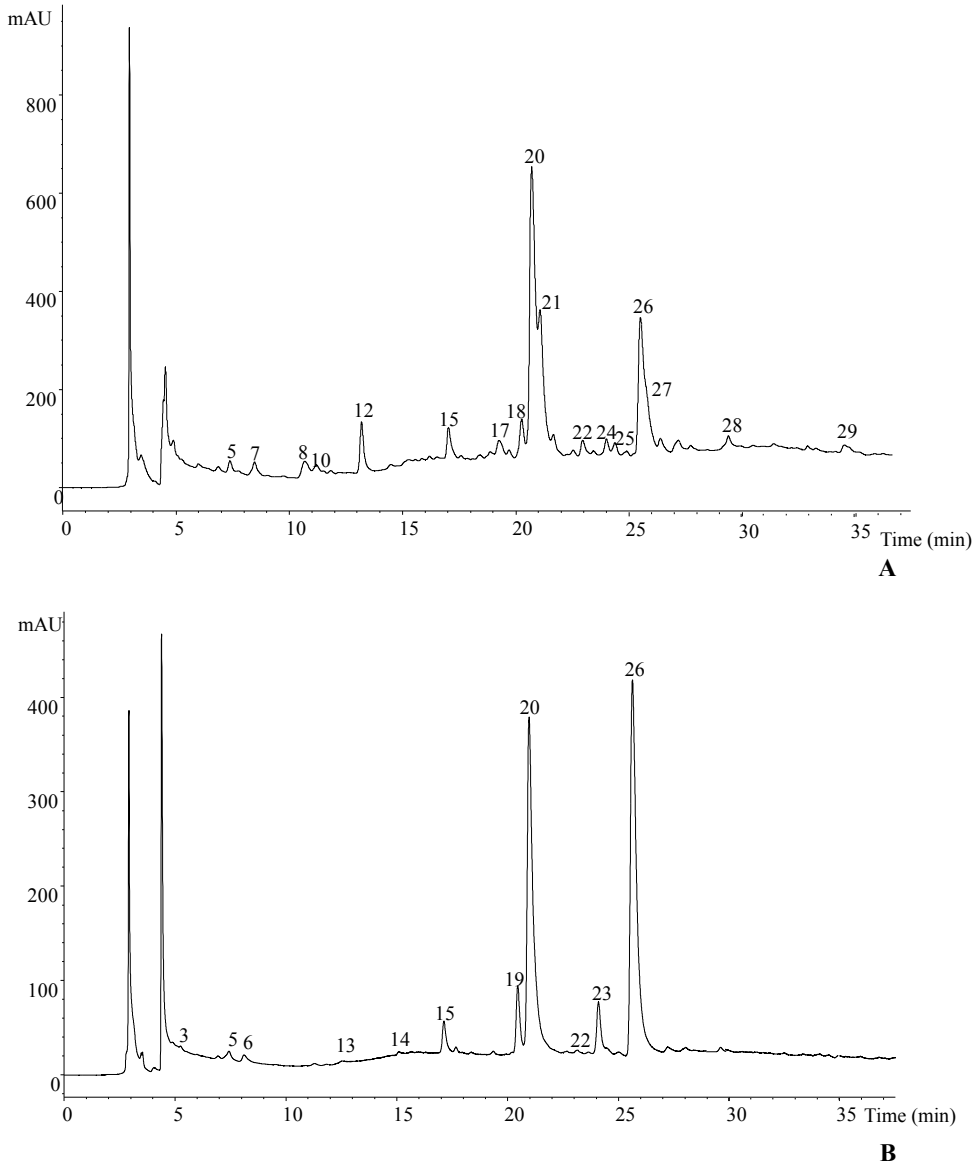

Figure 1. Phenolic profile of the infusion of artichoke (A) and milk thistle (B) recorded at 370 nm. The profile was obtained using a Spherisorb S3 ODS-2 C_{18} column thermostatted at 35 °C, using a flow rate of 0.5 mL/min and with gradient elution, (a) 0.1% formic acid in water and (b) acetonitrile.