Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: an underexploited and highly disseminated species

SÍLVIA M.F. BESSADA[a], JOÃO C.M. BARREIRA[a,b,*], LILLIAN BARROS[b], ISABEL C.F.R. FERREIRA[b,*], M. BEATRIZ P.P. OLIVEIRA[a]

[a]*REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, nº 228, 4050-313*

[b]*CIMO-ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal*

[*]Authors to whom correspondence should be addressed (e-mail: jbarreira@ipb.pt, telephone +351 273 303909; fax: +351 273 325405; e-mail: iferreira@ipb.pt, telephone +351 273 303219; fax: +351 273 325405).

Abstract

Coleostephus myconis (L.) Rchb.f. (Asteraceae) is a species with ruderal growth and persistence in abandoned soils, being characterized for its plentiful yellow flowering between March and August. Despite its botanical relevance, *C. myconis* had never been studied neither for its antioxidant activity, nor individual phenolic compounds. Herein, the antioxidant activity of different botanical parts: stems and leaves (green parts), floral buds, flowers in anthesis and senescent flowers, was studied in selected extracts (ethanol, ethanol:water 1:1 and water) through different chemical and biochemical assays. In addition, the phenolic profiles of the hydroethanolic extracts of each botanical part were also characterized by liquid chromatography with dioade array detection and electrospray ionization tandem mass spectrometry (LC-DAD-ESI/MS^n). The antioxidant activity was significantly modulated by the extract type, with the hydroethanolic extracts showing the highest antioxidant activity, especially those obtained from the senescent flowers and floral buds. The phenolic profiles were the same for all flowering stages (with quantitative differences), but that characterized in the green parts was quite different. Floral buds gave the highest contents in phenolic compounds, mainly due to the contribution of 3,5-*O*-dicaffeoylquinic acid and myricetin-*O*-methyl-hexoside. Overall, *C. myconis* showed an interesting potential to be included in different industrial applications.

Keywords: *Coleostephus myconis*; hydro/alcoholic extracts; antioxidant activity; phenolic profile.

1. Introduction

Some researchers suggest that two-thirds of the world's plant species have medicinal value. Actually, medicinal plants used in folk medicine are being increasingly studied and used in pharmaceutical and nutraceutical fields. Furthermore, the so called phytomedicines are playing a progressively higher role in human health care system. In fact, in a society increasingly concerned with health and nutrition, these products are emerging as a strong alternative to the synthetic ones (Phillipson, 2007, Krishnaiah et al., 2011).

The Asteraceae family has a worldwide distribution (with special relevance in the Mediterranean, Eastern Europe and Asia Minor), being acknowledged about 25 000 species integrated in approximately 1000 genera. In addition to the anti-inflammatory, analgesic and antipyretic potential of some of these species, their high antioxidant power, as proven in research works with extracts (of roots, stems, bark, leaves, flowers, fruits and seeds) should be highlighted (Bessada et al., 2015; Cabral et al., 2013; Krishnaiah et al., 2011). In Portugal, there are nearly 314 Asteraceae species, having a large representation in the Portuguese flora. *Coleostephus myconis* (L.) Rchb.f. belongs to Asteraceae family and is characterized as being a species with ruderal growth and persistence in abandoned soils. *C. myconis* is available throughout all the territory (mainly in the north) and has a seasonal growth, with plentiful yellow flowering between March and August.

Some of the biological properties of plant-derived products are related to their antioxidant activity. Oxidative stress, which results from a lack of balance between reactive species (and their metabolites) and antioxidant defense, plays a pivotal role in the development of human diseases and skin aging (López-Alarcón and Denicola, 2013).

The antioxidant activity of plants is often related to its individual phenolic compounds. These compounds occur frequently conjugated with glycosides, being usually located in the cell vacuolar structures. It is generally accepted that solvent extraction is the most commonly used procedure to extract and liberate phenolic compounds (Proestos et al., 2008). However, the effectiveness of the solid-liquid extraction is significantly influenced by the type of solvent, mainly due to the varying polarity or solvents' proportions, being also swayed by the chemical composition and physical characteristics of the samples (Radojkovi et al., 2012). Accordingly, the process of extraction should be standardized for each material *vis-a-vis* with the solvent, which is the underlying reason for the different solvents tested in this work.

As far as we know, *C. myconis* species was not previoulsy studied for its antioxidant activity and individual phenolic compounds, since no related references could be found in literature. Besides the innovative character, studying *C. myconis* is also relevant for its high dissemination in the Portuguese territory (mainly in the northern region).

2. Materials and methods

2.1. Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). HPLC-grade acetonitrile was obtained from Fisher Scientific (Lisbon, Portugal). The phenolic compound standards were from Extrasynthese (Genay, France). All other chemicals and solvents were of analytical grade and purchased from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.2. Samples

C. myconis plants were collected in the northwest of Portugal (Riba de Mouro, Minho) in June (2014) and, after taxonomical identification, were further divided in i) green parts (stems and leaves); ii) floral buds; iii) flowers in anthesis (fully open and functional flowers); iv) senescent flowers. The vegetal material was then frozen, lyophilized (48 h, -78 ºC, 0.015 mbar) (Telstar Cryodos-80, Terrassa, Barcelona), reduced to powder, mixed to obtain homogenized samples and stored in plastic tubes at room temperature for subsequent use.

2.3. Preparation of extracts

For the extracts preparation, a fine dried powder (20 mesh; ~0.5 g) of each sample was stirred (150 rpm) with 50 mL of one of three different solvents: ethanol, water or ethanol:water (1:1 v/v), at 25 °C for 1 h. The residues obtained in each case were then extracted with additional 50 mL portions of each solvent under the same conditions. The combined extracts were filtered through Whatman No. 4 paper, evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), re-dissolved in the specific solvent at 10 mg/mL (stock solution), and stored (4 °C) for further evaluation of the antioxidant activity. From the 10 mg/mL solution, several sequential dilutions were made (0.08-5.00 mg/mL).

2.4. Evaluation of antioxidant activity

2.4.1. DPPH radical-scavenging activity

This methodology was performed using an ELX800 microplate reader (Bio-Tek Instruments, Inc.) (Barreira et al., 2013). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 30 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

2.4.2. Reducing power

This methodology was performed using the microplate reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). The mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm. The concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 690 nm against extract concentration (Barreira et al., 2013).

2.4.3. Inhibition of β-carotene bleaching

A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two milliliters of this solution were pipetted into a round-bottom flask. The chloroform was removed at 40 ºC under vacuum and linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into test tubes containing extracts with different concentrations (0.2 mL). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm (AnalytikJena 200 spectrophotometer, Jena, Germany). The tubes were shaken and incubated at 50 ºC (2 h) in a water bath and the absorbance was measured again. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene content after 2h of assay/initial β-carotene content) × 100. The concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration (Barreira et al., 2013).

2.4.4. Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Brain porcine tissue was homogenized in Tris-HCl buffer (20 mM, pH 7.4) 1:2 (w/v), and further centrifuged at 3000*g* for 10 min. An aliquot (0.1 mL) of the supernatant was incubated with the extracts at different concentrations (0.2 mL) in the presence of $FeSO_4$ (10 mM; 0.1 mL) and ascorbic acid (0.1 mM; 0.1 mL) at 37 °C for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28%, w/v, 0.5 mL), followed by thiobarbituric acid (TBA, 2%, w/v, 0.38 mL), and the mixture was then heated at 80 °C for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA-TBA) complex in the supernatant was measured by its absorbance at 532 nm.

The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively. The concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of TBARS formation inhibition percentage against sample concentration (Barreira et al., 2013).

2.5. Characterization of phenolic compounds by LC-DAD-ESI/MS^n

The hydroethanolic extracts obtained with i) green parts- stems and leaves, ii) floral buds, iii) flowers in anthesis and iv) senescent flowers were dissolved in 20% aqueous ethanol at 5 mg/mL and filtered through a 0.22-µm disposable LC filter disk. Chromatographic analysis were performed in a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector coupled to a electrospray ionization mass detector (LC-DAD-ESI/MS^n), a quaternary pump, an auto-sampler (kept at 5 ºC), a degasser and an automated thermostatted column compartment.

Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 µm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C.

The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B to 20% B (5 min), 20-25% B (10 min), 25-35% B (10 min), 35-50% B (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 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 negative mode, using a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source.

Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 5 kV, a source temperature of 325 °C, a capillary voltage of -20 V. The tube lens offset was kept at a voltage of -66 V. The full scan covered the mass range from *m*/*z* 100 to 1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

The phenolic compounds were identified by comparing their retention times, UV-vis and mass spectra with those obtained from standard compounds, when available. Otherwise, compounds 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. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of the most similar available standard. The results were expressed as μg/g of extract.

2.6. Statistical analysis

For each botanical part, three independent experiments were performed, and each of them was analyzed in triplicate. The results were expressed as mean values±standard deviation (SD). The statistical differences represented by letters were obtained through one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference *post hoc* test (homoscedastic distributions) or Tamhane's T2 test (heteroscedastic distributions) with $\alpha = 0.05$, coupled with Welch's statistic. All statistical tests were performed with the SPSS v.22.0 software. The normality within groups and homogeneity of variances and of variance-covariance matrices, were checked using the Kolmogorov-Smirnov with Lilliefors correction, the Levene and M-Box tests, respectively.

3. Results and discussion

3.1. Antioxidant activity of the C. myconis extracts

The solvent type had some influence on the antioxidant potential, as it was exemplified by the higher activity measured in the hydroethanolic extracts, when compared to the remaining assayed extraction solvents (**Table 1**). Even so, it was possible to infer some general conclusions regarding the antioxidant activity of each botanical part. The extracts obtained from the green parts (stems and leaves), for instance, showed lower antioxidant activity in all assays (except for the TBARS formation inhibition in the ethanolic extracts), independently of the used solvent (**Figure 1**). On the other hand, floral buds and senescent flowers gave the highest levels of antioxidant activity, with the best results obtained for the hydroethanolic extracts from senescent flowers (DPPH scavenging activity: 0.25 mg/mL; reducing power: 0.13 mg/mL; β-carotene bleaching inhibition: 0.41 mg/mL; TBARS formation inhibition: 0.08 mg/mL, in the latter with similar values to those obtained with the other flowering stages).

Since there are no similar reports on *C. myconis* antioxidant activity, the determined values cannot be directly compared to those obtained in parallel laboratorial conditions.

3.2. Characterization of phenolic compounds in the C. myconis hydroethanolic extracts

The characterization of the phenolic compounds, as performed by LC-DAD/ESI-MS^n analysis, was conducted only in the hydroethanolic extracts, owing their best results in the antioxidant activity assays. Data of the retention time, λ_{max}, pseudomolecular ion, main fragment ions in MS^2 and tentative identification of phenolic acid and flavonoid

derivatives are presented in **Table 2**. As examples, the HPLC phenolic profiles, recorded at 280 nm, of the senescent flowers (A) and green parts (B) are presented in **Figure 2**.

UV and mass spectra obtained by LC-DAD-ESI/MS^n analysis showed that the phenolic composition of both the green parts and flowers of *C. myconis* was characterized by the presence of hydroxycinnamoyl (caffeoylquinic and diccafeoylquinic acids), hydroxybenzoyl (protocatechuic acid), flavonols (myricetin and quercetin glycosides) and flavone (luteolin glycoside) derivatives.

Four hydroxycinnamic acid derivatives were detected (peaks 1, 3, 15 and 16) in the flowers (all stages) and 3 of those were also detected in the green parts (peaks 3, 15 and 16). The detected compounds showed UV spectra with λ_{max} = 314-330 nm and pseudomolecular ions $[M-H]^-$ at *m/z* 353 and 515, all producing a fragment ion with *m/z* 191, corresponding to the deprotonated quinic acid, so that they could be clearly identified as quinic acid derivatives containing one or two caffeic acid units. The assignment of the different peaks to caffeoylquinic acid isomers was made according to the numbering system recommended by IUPAC (IUPAC, 1976) and the hierarchical keys developed by Clifford et al. (2003, 2005). Peak 3 was identified as 5-*O*-caffeoylquinic acid by comparison with the commercial standard. Peak 1, with the same fragments as peak 3, presented a deprotonated quinic acid (*m/z* 191), as the base peak, and a caffeate (*m/z* 179) with a relative percentage higher than 50% (comparing to the base peak) and was identified as 3-*O*-caffeoylquinic acid, considering that these are common features for 3-acyl chlorogenic acids (Clifford et al., 2003, 2005). The predominant phenolic compound (**Table 3**) in all *C. myconis* parts (stems and leaves: 62 μg/g extract; floral bud: 271 μg/g extract; flower in anthesis: 102 μg/g extract; senescent flower: 190 μg/g extract) was detected as peak 15, which corresponds to 3,5-*O*-

dicaffeoylquinic acid, according to its pseudomolecular ion [M-H]$^-$ at *m*/*z* 515, fragmentation pattern, and relative abundances of the fragment ions as described by Clifford et al. (2003; 2005). Considering the same criteria, peak 16 was identified as 4,5-*O*-dicaffeoylquinic acid. A fifth phenolic acid was detected as peak 2, identified from its retention time and spectral characteristics by comparison with a commercial standard. These same criteria allowed identifying peaks 6, 10 and 12 as luteolin-6-*C*-glucoside, quercetin-3-*O*-rutinoside and quercetin-3-*O*-glucoside, respectively, Peaks 8, 9, 11 and 14 were also identified as quercetin derivatives, considering the common fragment at *m*/*z* de 301 [quercetin-H]$^-$. Peak 8, with a pseudomolecular ion [M-H]$^-$ at *m*/*z* 741, may correspond to a quercetin-*O*-pentosyl-rutinoside, according to the fragments at *m*/*z* 609 (-132 Da; loss of pentosyl residue) and *m*/*z* 301 (-132-308 Da; loss of pentosyl + rutinosyl residues). Compound 9, identified only in the green parts, showed the same pseudomolecular ion as peak 12 (quercetin-3-*O*-glucoside), but presented a different retention time, therefore being tentatively identified as quercetin-*O*-hexoside, despite the position and nature of the sugar could not be identified. Peak 11 ([M-H]$^-$ at *m*/*z* 477) released a fragment at *m*/*z* 301 [M-H-176]$^-$, which corresponds to the loss of a glucuronyl unit; the corresponding compound was tentatively identified as quercetin-*O*-glucuronide, despite the sugar position remained unknown. Peak 14 yielded a pseudomolecular ion [M-H]$^-$ at *m*/*z* 549, releasing a MS^2 fragment at *m*/*z* 301 [M-H-162-86]$^-$, which might correspond to the loss of a malonyl-hexoside moiety, being assigned as quercetin-*O*-malonyl-hexoside. Peaks 4, 5, 7 and 13 were assigned as glycosylated derivatives of myricetin, according to the common fragment at *m*/*z* 317 [myricetin-H]$^-$. Compound corresponding to peak 4 was hypothesized as myricetin-*O*-rutinoside, considering its pseudomolecular ion at *m*/*z* 625 and the fragment at *m*/*z* 317 ([M-H-rutinosyl]$^-$). Peak 5, in turn, should correspond to myricetin-*O*-hexoside,

considering the pseudomolecular ion at *m*/*z* 479 and the fragment at *m*/*z* 317 [M-H-hexosyl]$^-$. Although MS analysis does not allow concluding about the nature and position of the substituting sugar, these peaks could be speculated as myricetin-3-*O*-glucoside and myricetin-3-*O*-rutinoside, due to the identification of other compounds with these designations. A similar pattern was determined for compound 7, but this peak presented an additional loss of 46 Da, which remained unidentified, thus this compound was assumed as an unknown derivative of myricetin-*O*-hexoside. Finally, compound 13 was identified as myricetin-*O*-methyl-hexoside, as suggested by its pseudomolecular ion at *m*/*z* 493 and the fragments at *m*/*z* 331 [M-H-hexosyl]$^-$ and *m*/*z* 316 [M-H-hexosyl-methyl]$^-$.

In general, phenolic acids, especially caffeoylquinic acid derivatives, represent the majority of phenolic compounds in *C. myconis* samples. The floral bud showed significantly higher amounts of all phenolic compounds, except protocatechuic acid (higher in stems and leaves). On the other hand, the green parts showed the lowest contents in phenolic acids (99 μg/g extract) and flavonoids (18.4 μg/g extract).

The antioxidant activity is frequently modulated by the phenolic profile of a determined matrix (Cheung et al., 2003; Li et al., 2014). The present results are generally in agreement with this principle, since the floral buds and the senescent flowers, which showed the highest contents in phenolic acids and flavonoids contents, exhibited the most powerful antioxidant activity. On the other hand, the extracts from stems and leaves presented the weakest antioxidant activity (DPPH scavenging activity: EC_{50} = 0.51-3.9 mg/mL, reducing power: EC_{50} = 0.30-0.78 mg/mL, β-carotene bleaching inhibition: EC_{50} = 1.4-1.5 mg/mL; and TBARS inhibition: EC_{50} = 0.09-0.20 mg/mL), which is also in agreement with their lower amounts of phenolic compounds.

4. Conclusion

The antioxidant activity exhibited by each of the extracts obtained from different botanical parts of *C. myconis* showed to be modulated by the extraction solvent, with the best results being obtained for the hydroethanolic mixture. Furthermore, stems and leaves (green parts) showed the lowest values of this bioactivity indicator, whilst the floral buds and the senescent flowers presented the highest antioxidant activity. The phenolic profiles of the hydroethanolic extracts revealed high predominance of phenolic acids (mainly 3,5-*O*-dicaffeoylquinic acid). The same compounds were detected throughout the flowering stages (despite the significant quantitative differences), but the profile of the green parts was quite different (7 of the detected compounds were only detected in the stems and leaves).

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Table 1. Antioxidant activity (EC_{50}, mg/mL) of the extracts prepared from different botanical parts of *Coleostephus myconis* (L.) Rchb.f.

	DPPH scavenging effect[1]	Reducing power[1]	β-carotene bleaching inhibition[1]	TBARS formation inhibition[1]
		Ethanol		
Stems and leaves	3.9±0.1 a	0.78±0.01 a	1.4±0.1 a	0.09±0.01 b
Floral bud	0.41±0.02 c	0.15±0.01 d	0.62±0.01 c	0.11±0.01 a
Flower in anthesis	0.52±0.01 b	0.24±0.01 b	0.85±0.03 b	0.08±0.01 c
Senescent flower	0.46±0.01 bc	0.18±0.01 c	0.48±0.01 d	0.08±0.01 c
Homoscedasticity[2]	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3]	<0.001	<0.001	<0.001	<0.001
		Water		
Stems and leaves	2.1±0.1 a	0.72±0.01 a	1.5±0.1 a	0.20±0.01 a
Floral bud	0.39±0.05 c	0.20±0.01 d	0.50±0.01 c	0.08±0.01 c
Flower in anthesis	1.2±0.1 b	0.54±0.01 b	0.89±0.02 b	0.19±0.01 a
Senescent flower	0.47±0.01 c	0.35±0.01 c	0.47±0.01 d	0.11±0.01 b
Homoscedasticity[2]	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3]	<0.001	<0.001	<0.001	<0.001
		Ethanol: water 1:1, *v/v*		
Stems and leaves	0.51±0.04 a	0.30±0.01 a	1.4±0.1 a	0.09±0.01 a
Floral bud	0.34±0.01 b	0.21±0.01 b	0.49±0.01 c	0.08±0.01 b
Flower in anthesis	0.33±0.01 b	0.18±0.01 c	0.72±0.01 b	0.08±0.01 b
Senescent flower	0.25±0.01 c	0.13±0.01 d	0.41±0.01 d	0.08±0.01 b
Homoscedasticity[2]	<0.001	<0.001	<0.001	<0.001
1-way ANOVA[3]	<0.001	<0.001	<0.001	<0.001

[1]Different letters in each column and for each extract type indicate mean values with significant differences (p<0.001). These differences were classified by the Tamhane's T2 test, since the homoscedasticity criterion was not fulfilled. [2]Homoscedasticity among the botanical parts results was verified through the Levene's test. [3]Since p<0.001, the mean value of this parameters differs from the others in at least one of the botanical parts; thereby the multiple comparison could be performed.

Table 2. Retention time (Rt), wavelengths of maximum absorption (λ_{max}), mass spectral data, relative abundances of fragment ions and tentative identification of the phenolic compounds in the hydroethanolic extracts from different botanical parts of *Coleostephus myconis* (L.) Rchb.f.

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion [M-H]- (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification
1	5.1	328	353	191(100), 179(74), 173(7), 161(7), 135(37)	3-*O*-Caffeoylquinic acid
2	5.8	260,294sh	153	108(100)	Protocatechuic acid
3	7.5	328	353	191(100), 179(48), 173(49), 161(22), 135(28)	5-*O*-Caffeoylquinic acid
4	12.6	340	625	317(100)	Myricetin-*O*-rutinoside
5	13.4	336	479	317(100)	Myricetin-*O*-hexoside
6	14.7	341	447	429(17), 357(58), 327(100)	Luteolin-6-*C*-glucoside
7	14.8	357	525	479(100), 317(80)	Myricetin-*O*-hexoside (derivative)
8	15.6	350	741	609(100), 301(76)	Quercetin-*O*-pentosyl-rutinoside
9	16.9	362	463	301(100)	Quercetin-*O*-hexoside
10	17.7	352	609	301(100)	Quercetin-3-*O*-rutinoside
11	18.2	358	477	301(100)	Quercetin-*O*-glucuronide
12	19.0	354	463	301(100)	Quercetin-3-*O*-glucoside
13	20.0	353	493	331(35), 316(100)	Myricetin-*O*-methyl-hexoside
14	20.7	362	549	301(100)	Quercetin-*O*-malonyl-hexoside
15	21.3	327	515	353(92), 335(8), 191(100), 179(89), 173(14), 161(8), 135(46)	3,5-*O*-Dicaffeoylquinic acid
16	23.4	327	515	353(95), 335(5), 191(30), 179(72), 173(100), 161(4), 135(26)	4,5-*O*-Dicaffeoylquinic acid

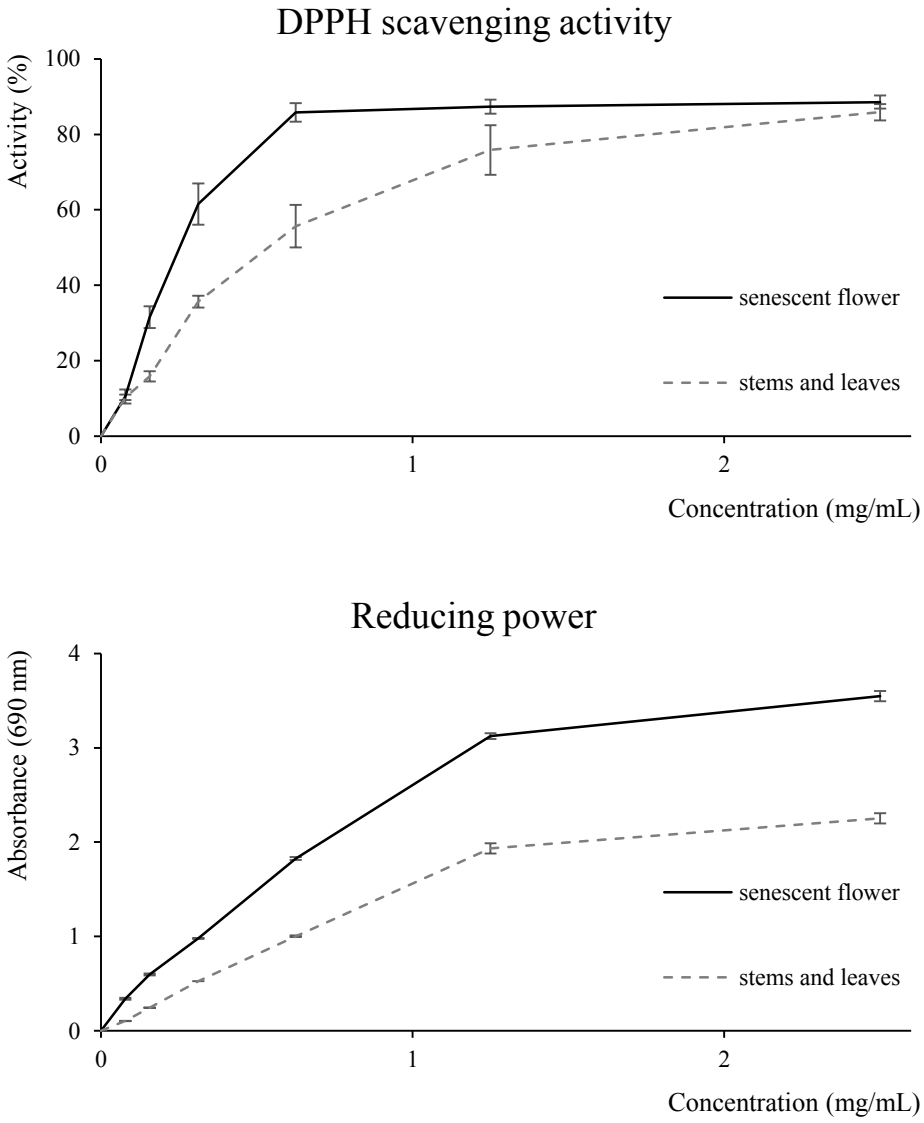
Table 3. Quantification of the phenolic compounds in the hydroethanolic extracts prepared from different botanical parts of *Coleostephus myconis* (L.) Rchb.f.

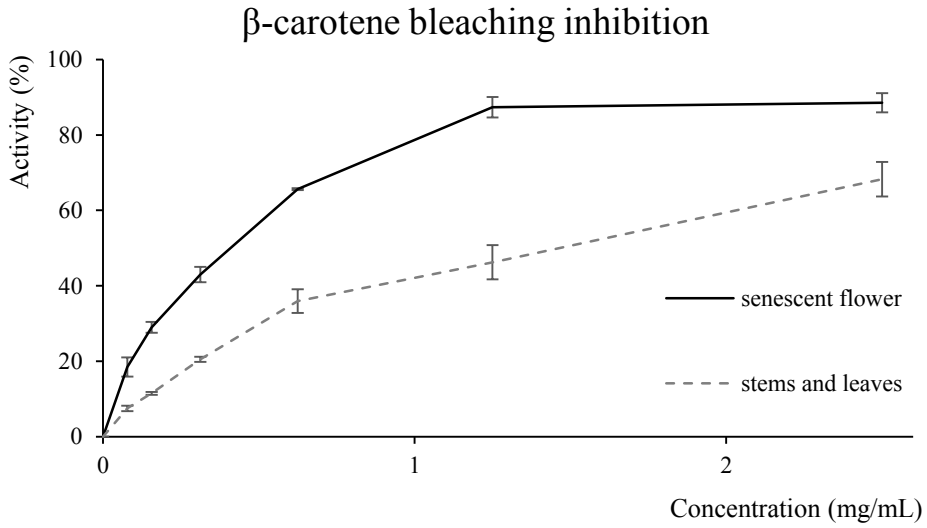
Peak	Rt (min)	Tentative identification	Quantification (μg/g extract)[1]				Homoscedasticity[2]	1-way ANOVA[3]
			Stems and leaves	Floral buds	Flowers in anthesis	Senescent flowers		
1	5.1	3-*O*-Caffeoylquinic acid	nd	7.0±0.1 a	3.0±0.1 c	3.6±0.1 b	<0.001	<0.001
2	5.8	Protocatechuic acid	2.8±0.1 a	2.4±0.1 b	1.0±0.1 c	1.0±0.1 c	<0.001	<0.001
3	7.5	5-*O*-Caffeoylquinic acid	31±1 c	79±3 a	23±1 d	48±1 b	<0.001	<0.001
4	12.6	Myricetin-*O*-rutinoside	0.80±0.03	nd	nd	nd	-	-
5	13.4	Myricetin-*O*-hexoside	1.04±0.05	nd	nd	nd	-	-
6	14.7	Luteolin-6-*C*-glucoside	1.07±0.04	nd	nd	nd	-	-
7	14.8	Myricetin-*O*-hexoside (derivative)	nd	75±1 a	24±1 b	21±1 c	<0.001	<0.001
8	15.6	Quercetin-*O*-pentosyl-rutinoside	0.52±0.02	nd	nd	nd	-	-
9	16.9	Quercetin-*O*-hexoside	4.3±0.2	nd	nd	nd	-	-
10	17.7	Quercetin-3-*O*-rutinoside	3.6±0.1	nd	nd	nd	-	-
11	18.2	Quercetin-*O*-glucuronide	nd	77±2 a	14±1 c	17±1 b	<0.001	<0.001
12	19.0	Quercetin-3-*O*-glucoside	nd	15±1 a	4.3±0.1 c	4.9±0.1 b	<0.001	<0.001
13	20.0	Myricetin-*O*-methyl-hexoside	nd	100±3 a	22.7±0.4 b	23.6±0.1 b	<0.001	<0.001
14	20.7	Quercetin-*O*-malonyl-hexoside	7.1±0.2	nd	nd	nd	-	-
15	21.3	3,5-*O*-Dicaffeoylquinic acid	62±2 d	271±12 a	102±2 c	190±3 b	<0.001	<0.001
16	23.4	4,5-*O*-Dicaffeoylquinic acid	3.1±0.1 d	16.6±0.3 a	5.9±0.2 c	10.4±0.1 b	<0.001	<0.001

	Phenolic acids	99±1 d	376±13 a	135±2 c	253±3 b	0.001	<0.001
	Flavonoids	18.4±0.3 c	268±5 a	65±1 b	67±1 b	<0.001	<0.001

[1]Different letters in each column indicate mean values with significant differences (p<0.001). These differences were classified by the Tamhane's T2 test, since the homoscedasticity criterion was not fulfilled. [2]Homoscedasticity among the botanical parts results was verified through the Levene's test. [3]Since p<0.001, the mean value of this parameters differs from the others in at least one of the botanical parts; thereby the multiple comparison could be performed.

Figure 1. Antioxidant activity of the hydroethanolic extracts from *C. myconis* stems and leaves (green parts) and senescent flowers.




β-carotene bleaching inhibition
Activity (%)
100
80
60
40
20
0
0
1
2
Concentration (mg/mL)
senescent flower
stems and leaves

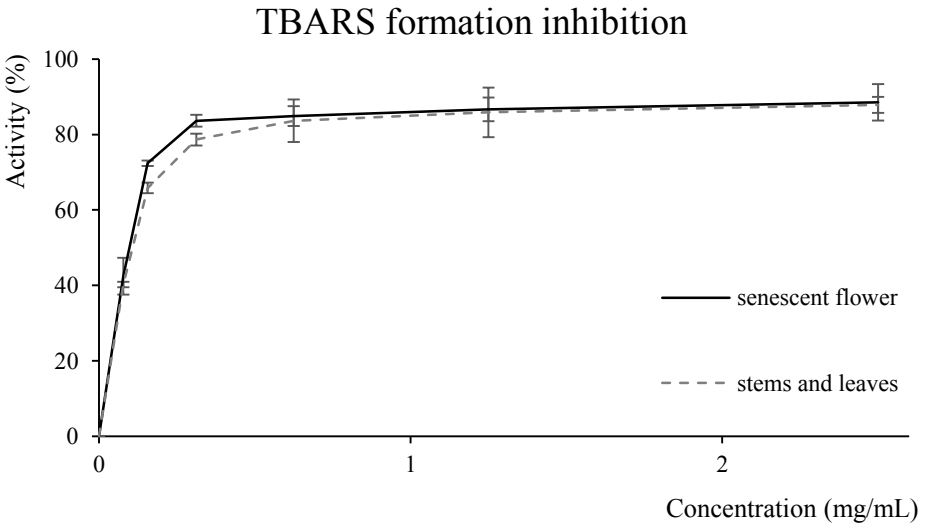
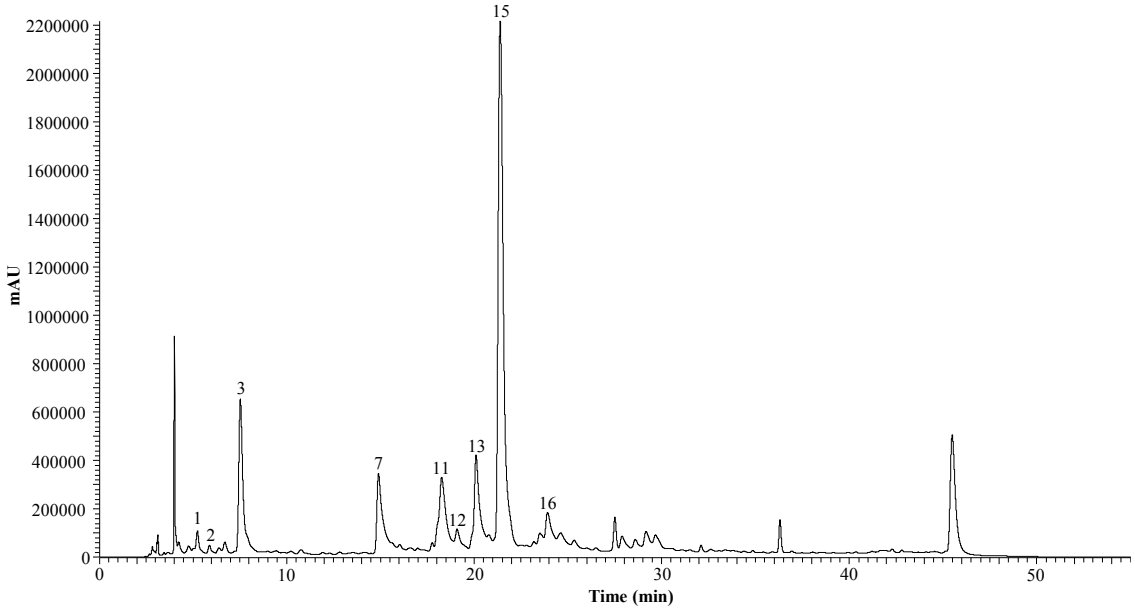
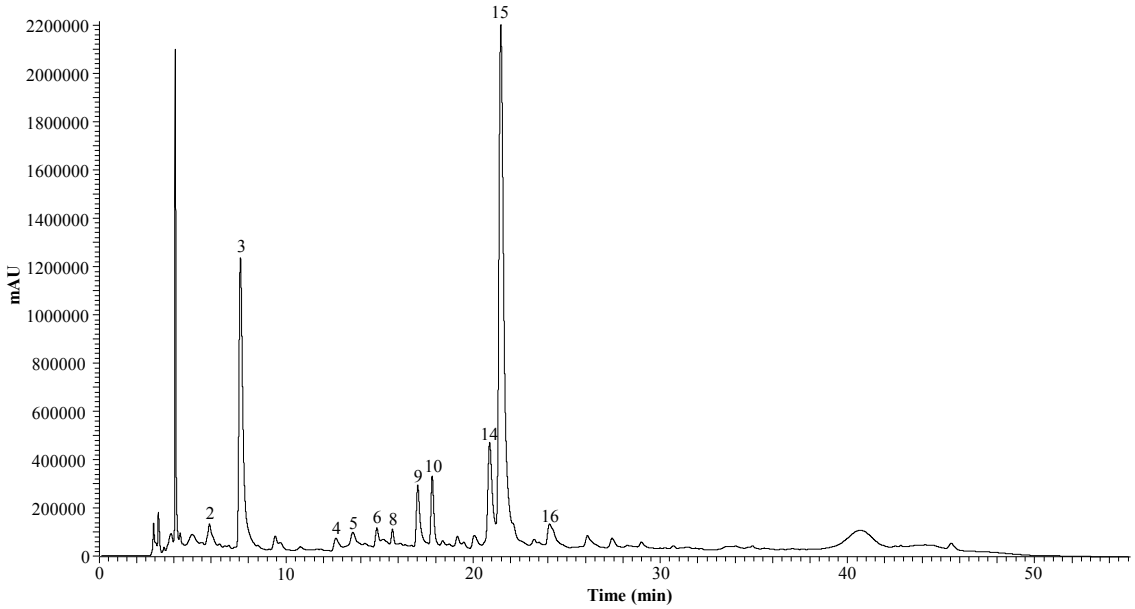

TBARS formation inhibition
Activity (%)
100
80
60
40
20
0
0
1
2
Concentration (mg/mL)
senescent flower
stems and leaves

Figure 2. HPLC chromatograms (recorded at 280 nm) of the phenolic compounds in the hydroethanolic extracts of the senescent flower (A) and green parts (steams and leaves) (B).



(A)



(B)