

Polyphenolic profile characterization of *Agrimonia eupatoria* L. by HPLC with different detection devices

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ABSTRACT: Liquid chromatography coupled to diode array and electrospray ionization mass spectrometry detection was used to establish the polyphenolic profile of an ethyl acetate fraction from *Agrimonia eupatoria* L. aqueous-alcoholic extract. Additionally, an HPLC technique with post-column derivatization by *p*-dimethylaminocinnamaldehyde was employed for the selective detection and quantification of flavan-3-ols. Important information was obtained by combining the data of these two HPLC techniques. Flavan-3-ols (catechin and procyanidins B1, B2, B3, B6, B7, C1, C2 and epicatechin–epicatechin–catechin), quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-(6''-*O*-*p*-coumaroyl)-glucoside, apigenin 6-*C*-glucoside and various phenolic acids were identified. Antioxidant activity of the *Agrimonia eupatoria* L. fraction containing these compounds was assessed through the 1,1-diphenyl-2-picrylhydrazyl, trolox equivalent antioxidant capacity and thiobarbituric acid reactive substances methods. Significant activity was observed for this fraction, where compounds with recognized anti-inflammatory properties such as procyanidins, kaempferol 3-*O*-(6''-*O*-*p*-coumaroyl)-glucoside and quercetin glycosides were identified for the first time. These results are predictive of the beneficial effects of this fraction, or some of its compounds, in human health, as possible anti-inflammatory drug. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: HPLC; diode array detection; ESI-MS/MS; post-column derivatization; *Agrimonia eupatoria* L.; polyphenols

INTRODUCTION

The aerial parts of agrimony (*Agrimonia eupatoria* L., Rosaceae) are used as infusions, decoctions or tinctures (aqueous-alcoholic extracts) in traditional medicine, for their anti-inflammatory, astringent and diuretic properties (Garnier *et al.*, 1961; Newall *et al.*, 1996). Nonetheless, only a small number of scientific investigations have been carried out concerning these activities and the constituents involved. Aromatic acids, triterpenes and tannins have been cited. The presence of kaempferide, kaempferol and their derivatives, such as kaempferol 3-glucoside, kaempferol 3-rhamnoside and kaempferol 3-rutinoside, have also been reported (Bilia *et al.*, 1993). However, to our knowledge, very

little scientific information has been published on the flavonoids, particularly on flavan-3-ols and its oligomers.

Flavonoids belong to the extensive group of polyphenols and several biological activities, such as anti-inflammatory, anti-ulcer, anti-cancer, antiviral, antibacterial, antispasmodic, neuroprotectant, anti-atherosclerotic and anti-thrombotic, have been attributed to these compounds (Middleton *et al.*, 2000). Oxidative stress and antioxidant defence imbalance have been associated with inflammatory, carcinogenic and coronary diseases. The recognized antioxidant potential of flavonoids could therefore be responsible for their beneficial actions (Pietta, 2000). An inverse relation between flavan-3-ols (monomeric and oligomeric) intake and ischemic heart disease and the incidence of cancers has also been found (Arts *et al.*, 2001a,b). Moreover, anti-inflammatory properties, which are sometimes correlated with antioxidant activities, have also been verified (Mackenzie *et al.*, 2004; Hosseini *et al.*, 2001).

In the online polyphenolic characterization and quantification of complex biological matrices, HPLC/diode array detection (DAD), in conjunction with mass spectrometry detection and post-column detection by derivatization with *p*-dimethylaminocinnamaldehyde

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Abbreviations used: ABTS, [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]; DAD, diode array detection; DMACA, *p*-dimethylaminocinnamaldehyde; DPPH, (1,1-diphenyl-2-picrylhydrazyl); TBARS, thiobarbituric acid reactive substances; TEAC, trolox equivalent antioxidant capacity.

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(DMACA), plays an important role when oligomeric flavan-3-ols (proanthocyanidins) are present. These compounds have identical UV spectra. Furthermore, their low concentration in the extracts and their interference with other phenolic compounds make proanthocyanidins difficult to analyse by HPLC/DAD. HPLC/DAD/ESI-MS/MS and HPLC/DMACA were usefully applied to study proanthocyanidins from unripened sweet almond fruits and grape seed (de Pascual-Teresa *et al.*, 1998a,b). In this work, the purpose was to use those two techniques as valuable tools to establish the polyphenolic profile of one medicinal plant, *Agrimonia eupatoria* L., and to verify its correlation with antioxidant activity, in support of the traditional use of this medicinal plant as an anti-inflammatory.

EXPERIMENTAL

Samples

The aerial parts of *Agrimonia eupatoria* L. were collected, dried and provided by Segredo da Planta, Portugal. Dr J. Paiva, of the Botany Department, identified the plant and a voucher specimen has been deposited at the Department of Pharmacognosy of the Faculty of Pharmacy, University of Coimbra (Portugal).

Standards

Different procyanidins (B1, B2, B3, B6, B7, C1, C2 and EEC) were isolated from either grape seeds or unripe fruits of almond (*Prunus amygdalus*) as previously described (de Pascual-Teresa *et al.*, 1998a). Protocatechuic, *p*-coumaric and ascorbic acids, and catechin were purchased from Sigma. Apigenin 6-*C*-glucoside (isovitexin), quercetin 3-*O*-glucoside (isoquercitrin), quercetin 3-*O*-galactoside (hyperoside), kaempferol 3-*O*-glucoside and kaempferol 3-*O*-(6''-*O*-*p*-coumaroyl)-glucoside (tiliroside) were purchased from Extrasynthese.

Extracts

The extracts (infusion and tincture) were prepared according to the usages in traditional medicine. The tincture (the most active extract) was prepared by adding 30 mL of 45% aqueous ethanol to 2 g of the pulverized agrimony and leaving to stand for 13 days in the dark. Subsequently, the aqueous-alcoholic extract was filtered and concentrated under vacuum to a volume of 10 mL. An aliquot of this extract was washed with *n*-hexane to eliminate the fat-soluble compounds and fractionated by repeated extraction with ethyl acetate. The organic phases, containing the polyphenols, were collected, dehydrated with anhydrous sodium sulfate and concentrated with water, in a rotavapor at 30°C, until all the ethyl acetate had been removed. The aqueous-alcoholic extract and the respective ethyl acetate fraction were frozen with liquid N₂ and freeze-dried.

Phytochemical characterization

High-performance liquid chromatography (HPLC), using double online detection with a diode array spectrophotometer and a mass spectrometer (MS), was used to characterize the phenolic profile in the ethyl acetate fraction of the *Agrimonia eupatoria* L. aqueous-alcoholic extract. HPLC using detection after post-column derivatization with *p*-dimethylaminocinnamaldehyde (HPLC/DMACA) was performed for further flavan-3-ols analysis.

HPLC/DAD/MS. The analyses were performed on an HP 1100 liquid chromatography system interfaced with a Finnigan LCQ mass spectrometer equipped with an API-ES ionization chamber.

Separation was carried out in a 3 μm Waters Spherisorb ODS2 column (150 × 4.6 mm) at 25°C. The mobile phase consisted of 2.5% aqueous acetic acid (solvent A), a mixture of acetonitrile–2.5% aqueous acetic acid, 10:90 (solvent B) and acetonitrile (solvent C). The gradient profile used was 0–5 min, 0–100% B; 5–30 min, 0–15% C in B; 30–35 min, 15–50% C in B; 35–40 min, 50% C in B isocratically. The flow rate was 0.5 mL/min. The first detection was made in the diode array spectrophotometer using 280 and 360 nm as preferred wavelengths, followed by a second detection in the mass spectrometer. The capillary temperature and voltage were 225°C and 45 V, respectively. Mass spectra were obtained in positive ion mode and the MS was programmed to perform a series of two scans: a full mass and an MS-MS of the most abundant ion.

HPLC/DMACA post-column derivatization detection. A Waters 600E HPLC system coupled to an auto sampler Waters 717 plus was used with the conditions previously described (de Pascual-Teresa *et al.*, 1998b). First, detection at 280 nm was carried out in a diode array detector (Hewlett-Packard HP 1040 M series II). Thereafter, the reagent (1% DMACA in 1.5 M sulphuric acid in methanol) was added and the product of the reaction was measured at 640 nm in a UV-vis detector (Shimadzu SPD-10AV). Data treatment was performed on an HP data treatment station.

The flavan-3-ols were quantified by comparison of the areas of the peaks recorded at 640 nm with a calibration curve obtained with a standard of catechin.

Antioxidant activity

Antioxidant activity of the extracts was monitored using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The DPPH, trolox equivalent antioxidant capacity (TEAC) and thiobarbituric acid reactive substances (TBARS) methods were used to evaluate the antioxidant activity of the aqueous-alcoholic extract and the ethyl acetate fraction. All the assays were performed at least in triplicate.

Antiradical activity assays. Antiradical activity was assessed by the DPPH method (Blois, 1958). Briefly, different concentrations of samples (100 μL) were added to 500 μL of DPPH (500 μM) in ethanol. After 30 min, the absorbance was measured at 517 nm in a Hitachi U-2000 spectrophotometer. Scavenger activities were expressed in EC₅₀ (micrograms of freeze-dried samples required to scavenge 50% of the DPPH

radical). The ascorbic acid and the catechin EC_{50} were determined and used as reference.

Cation radical ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] scavenging activity was measured by TEAC assay (Miller *et al.*, 1993). The samples (8.4 μ L) were mixed with 30 μ L of ABTS radical (5 mM). The absorbance was monitored at 734 nm for 6 min using a Hewlett Packard 8453 spectrophotometer. The results were compared with Trolox C (water-soluble analogue of vitamin E) and expressed as TEAC per μ g freeze-dried samples.

Lipid peroxidation assay. This method measures the thiobarbituric acid reactive substances that result from the lipid peroxidation (Plumb *et al.*, 1998). Various concentrations of the sample (4 μ L) were added to an ascorbate/iron-induced peroxidation solution of phosphatidylcholine in absolute ethanol (final concentration 1.25 mg/mL). The mixture was added to 800 μ L of TBA [trichloroacetic acid 20% (w/v), thiobarbituric acid 0.4% (w/v), hydrochloric acid 0.25 M] and the lipid peroxidation was evaluated at 535 nm using a UV-vis Perkin-Elmer spectrophotometer model Lambda 3B. The results were expressed in IP_{50} (μ g freeze-dried samples required to inhibit 50% of the induced lipid peroxidation).

Total phenols

A modified version of the Folin-Ciocalteu method (Wang *et al.*, 1997) was used. Absorbance was measured at 700 and 735 nm in a Hitachi U-2000 spectrophotometer and the results expressed as grams of gallic acid equivalents by 100 g freeze-dried samples. All samples were analysed in triplicate.

Total flavonols

Reagent [$AlCl_3 \cdot 6H_2O$ 2% in methanol (w/v), 2 mL] was added to the samples in ethanol (2 mL). The absorbance was measured

after 10 min, at 430 nm (Lamaison and Carnat, 1990) in a Hitachi U-2000 spectrophotometer, and the results were expressed in grams rutin equivalents by 100 g of freeze-dried samples. All samples were analysed in triplicate.

RESULTS

Phytochemical characterization

Figure 1 shows the HPLC profile recorded at 280 nm of the ethyl acetate fraction. Absorption spectra obtained with the diode array detector allowed most peaks to be identified as polyphenols, i.e. flavan-3-ols, flavonols (kaempferol and quercetin derivatives), flavones and phenolic acids. Table 1 summarizes the retention time and the UV and mass spectra characteristics obtained in the HPLC/DAD/ESI-MS/MS analysis, and, on this basis, the identification of the polyphenols present in the fraction. To our knowledge, no information has yet been published about flavan-3-ols in *Agrimonia eupatoria* L.

Flavan-3-ols. In order to characterize the flavan-3-ols present in the *Agrimonia eupatoria* L. ethyl acetate fraction, HPLC-MS chromatograms for the molecular ions $[M+H]^+$ at m/z 291, 579, 867 and 1155 were recorded. Thus, the occurrence of one (epi)catechin monomer and various procyanidin dimers (five), trimers (six) and tetramers (four), as the more representative flavan-3-ols, was detected.

A characteristic fragmentation pattern was observed in the MS^2 spectra of the flavan-3-ols, showing signals corresponding to the *retro* Diels Alder fission (RDA;

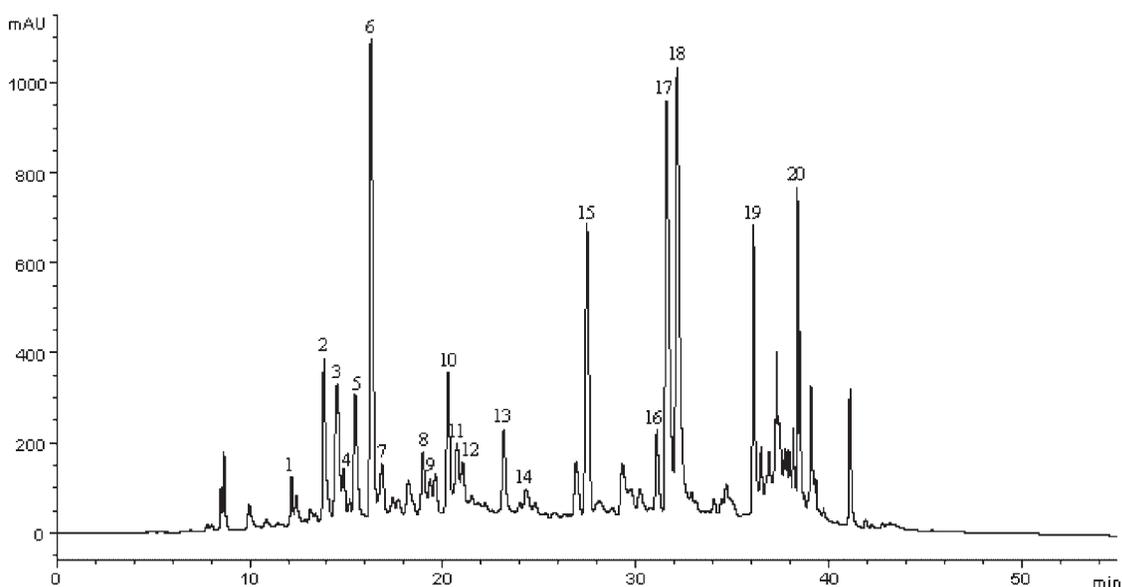


Figure 1. HPLC polyphenolic profile of an ethyl acetate fraction of *Agrimonia eupatoria* L., recorded at 280 nm. The identification of the peaks is shown in Table 1.

Table 1. UV-vis and MS spectra, and identification of the compounds of *Agrimonia eupatoria* L. ethyl acetate fraction

Compounds	Rt (min)	HPLC-DAD (nm)	HPLC-ESIMS	
			Molecular ion [M+H] ⁺ (<i>m/z</i>)	[M+H] ⁺ fragments [<i>m/z</i> (relative abundance,%)]
1 Protocatechuic acid	12.17	260, 294	—	—
2 Procyanidin dimer B1	13.85	280	579	427 (99), 409 (100), 291 (27), 287 (37)
3 Procyanidin dimer B3	14.54	280	579	453 (13), 427 (99), 409 (100), 291 (12), 287 (17)
4 Procyanidin trimer	14.87	280, 330sh	867	697 (17), 577 (100), 409 (15), 287 (20)
5 Procyanidin trimer C2	15.48	280	867	715 (90), 697 (76), 577 (57), 427 (52), 409 (100), 287 (20)
6 Catechin	16.27	280	291	273 (8), 165 (86), 139 (100), 123 (81)
7 Procyanidin trimer EEC	16.86	—	867	715 (19), 697 (42), 577 (31), 571 (100), 427 (16), 409 (53)
8 Procyanidin dimer B2	18.99	292sh, 310	579	561 (12), 453 (26), 435 (50), 427 (25), 409 (100), 291 (13), 287 (17)
9 Procyanidin dimer B6	19.35	280, 340	579	561 (8), 453 (67), 427 (41), 435 (88), 409 (100), 287 (40)
10 Procyanidin trimer	20.28	298sh, 324	867	715 (28), 714 (100), 696 (70), 579 (29), 533 (90), 427 (23), 409 (28), 287 (21)
11 Procyanidin tetramer	20.75	288, 310	1155	—
12 Procyanidin trimer	21.04	280	867	715 (9), 697 (37), 577 (100), 517 (47), 287 (12)
13 Procyanidin dimer B7	23.16	280	579	435 (28), 427 (65), 409 (100), 291 (17), 287 (27)
14 Procyanidin trimer C1	24.32	278	867	714 (53), 697 (29), 589 (68), 438 (100), 427 (7), 409 (80)
15 <i>p</i> -Coumaric acid	27.48	298sh, 310	—	—
16 Apigenin 6- <i>C</i> -glucoside	31.10	270, 338	433	415 (59), 397 (40), 379 (36), 367 (100), 337 (68), 313 (33), 283 (22), 271 (2), 343 (2)
17 Quercetin 3- <i>O</i> -galactoside	31.60	256, 266sh, 294sh, 354	465	303 (100), 285 (15), 257 (44), 229 (11)
18 Quercetin 3- <i>O</i> -glucoside	32.13	256, 264sh, 298sh, 354	465	303 (100), 447 (49), 429 (28), 399 (66), 376 (26), 369 (42), 345 (21)
19 Kaempferol 3- <i>O</i> -glucoside	36.11	265, 294sh, 348	449	431 (69), 413 (41), 395 (27), 383 (72), 353 (84), 329 (79), 299 (22), 287 (100)
20 Kaempferol 3- <i>O</i> -(6''- <i>O</i> - <i>p</i> -coumaroyl)-glucoside	38.50	268, 314, 348sh	595	576 (39), 565 (84), 489 (59), 473 (89), 459 (57), 447 (39), 443 (100), 433 (43)

−152 m.u.), loss of water (−18 m.u.), RDA + water loss (−170 m.u.), loss of ring A (−126 m.u.), cleavage of the upper heterocyclic ring (C-ring; −246 m.u.) and/or cleavage of the interflavanic bond following the quinone–methide mechanism (−288 m.u.) (Fig. 2).

Spectral and chromatographic (retention time) characteristics of the detected flavan-3-ols are included in Table 1. Compound identification was based on their mass spectra (molecular ion and fragmentation pattern), chromatographic behaviour (elution order) and comparison with procyanidin standards previously isolated in the laboratory. The degree of polymerization does not significantly influence the elution, although the lower subunit and the interflavanic linkage are critical factors. Compounds with an epicatechin unit in the lower position or possessing (4→6) interflavanic linkage(s) usually elute later than the corresponding catechin derivatives and (4→8)-linked procyanidins, respectively (Table 1).

Some UV results show interference between different phenolic compounds. Characteristic mass spectra of flavan-3-ols were obtained, although the UV spectra match with different compounds, mainly phenolic acids or their derivatives. Since the ionization of these compounds in these ESI conditions is poor, the co-elution observed does not constitute a problem for the flavan-3-ols identification.

With a molecular ion at *m/z* 291, compound **6** was identified as the monomer catechin. The elution and mass and UV characteristics were the same as those of the catechin standard. Dimeric procyanidins, compounds **2**, **3**, **8**, **9** and **13**, with molecular ions at *m/z* 579 were identified as type B procyanidins: epicatechin-(4β→8)-catechin (B1), catechin-(4β→8)-catechin (B3), epicatechin-(4β→8)-epicatechin (B2), catechin-(4β→6)-catechin (B6) and epicatechin-(4β→6)-catechin (B7), respectively. Identification of procyanidins B1, B2 and B3 was confirmed by comparison with standards. The B6 and B7 identification was supported in their elution characteristics. The fragmentation profile of these dimers shows an unusual ion at *m/z* 287, which might be the result of an oxidation reaction that produces another quinone structure (Rohr *et al.*, 2000). Trimers and tetramers were also found in *Agrimonia eupatoria* L. ethyl acetate fraction, although the absence of standards and the lack of information relating to the order of elution only allowed the identity of compounds **5**, **7** and **14** to be suggested as the procyanidin trimers catechin-(4→8)-catechin-(4→8)-catechin (C2), epicatechin-(4→8)-epicatechin-(4→8)-catechin (EEC) and epicatechin-(4→8)-epicatechin-(4→8)-epicatechin (C1), respectively.

To confirm the identification and to quantify the flavan-3-ols, a post-column derivatization HPLC/

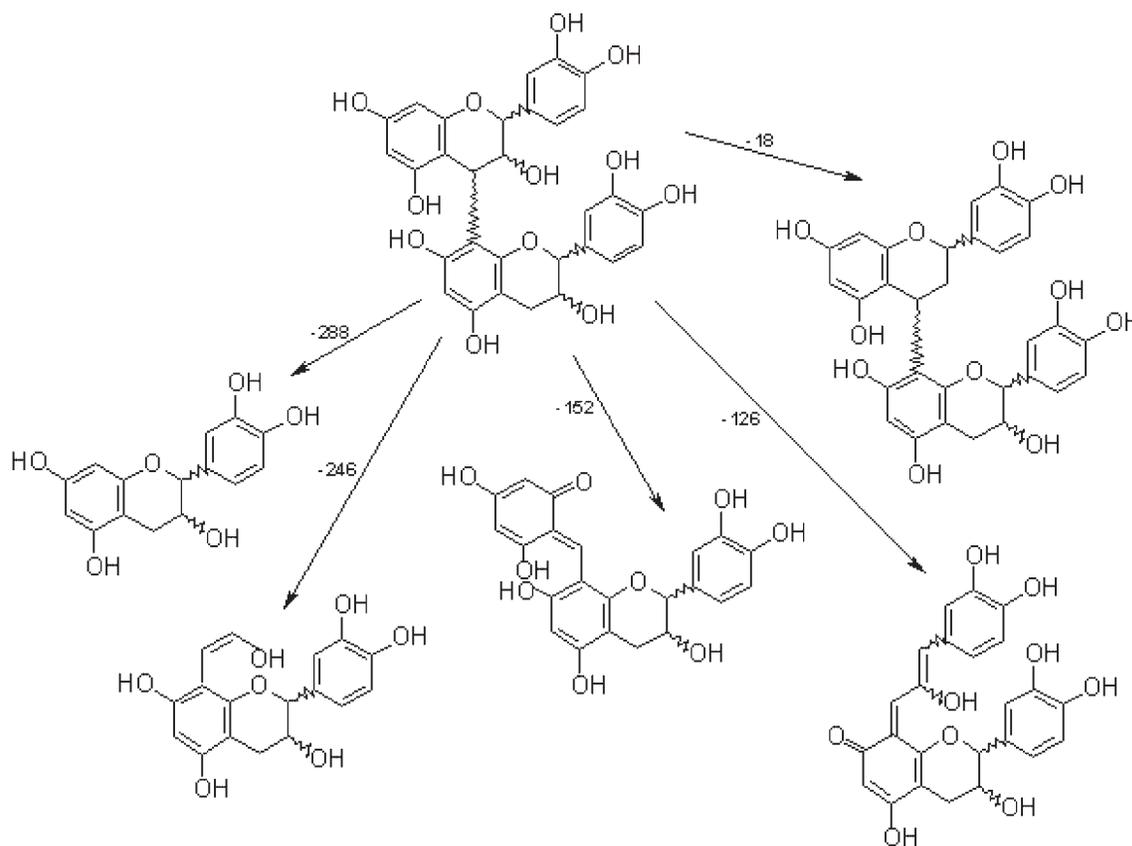


Figure 2. Characteristic fragments of procyanidins in the positive ionization mode.

DMACA method was used. The coloured complex, resulting from the reaction between the DMACA and the flavan-3-ols, shows a distinctive maximum at 640 nm, thus overcoming co-elution with other compounds and allowing selective detection and rigorous quantification of the flavan-3-ols.

For identification, the chromatographic behaviour and the ratio between the areas of the peaks recorded at 640 and 280 nm (A_{640}/A_{280}) were considered according to Treutter *et al.* (1994). Individual quantification of compounds was made from the areas of their peaks recorded at 640 nm by comparison with a calibration curve prepared with catechin. Total flavanols, calculated as the sum of the concentrations of the individual flavan-3-ols, represented 18.96% of the freeze-dried ethyl acetate fraction, of which 6.34% corresponded to the monomer (catechin), 5.81% to dimers (B1, 1.86%; B3, 1.38%; B6, 0.35%; and B7, 1.63%) and 1.07% to the trimer EEC.

Flavones and flavonols. One flavone and four flavonols were identified in the *Agrimonia eupatoria* L. ethyl acetate fraction. Preliminary analysis of the compound **16** UV spectrum suggested the presence of an apigenin derivative. The mass spectrum of this compound showed a molecular ion at m/z 433 nm, releasing

a wide range of MS^2 fragments, one of them at m/z 271, related to apigenin, and others at m/z 283, 313 and 343, which correspond to the losses of 150, 120 and 90 mass units. These fragments are due to the cross-ring cleavages that occurred in the sugar unit and are characteristic of the flavone C-hexoses (Bakhtiar *et al.*, 1994; Sanchez-Rabaneda *et al.*, 2003). This assumption was corroborated by the losses of one, two and three water moieties (fragments at m/z 415, 397 and 379) that are more intense than the ions resulting from the sugar cleavage (Grayer *et al.*, 2000). According to these features and by its retention time, compared with a commercial standard, compound **16** was proposed to be the apigenin 6-*C*-glucoside (isovitexin).

Compounds **17** and **18** were identified as quercetin derivatives according to their UV spectra and the existence in their MS^2 spectra of a fragment at m/z 303, corresponding to quercetin aglycon. Retention characteristics and comparison with standards allowed compounds **17** and **18** to be identified as quercetin 3-*O*-galactose (hyperoside) and quercetin 3-*O*-glucoside (isoquercitrin), respectively.

According to their UV spectra, compounds **19** and **20** were associated with kaempferol derivatives. The mass spectra of compound **19** showed a molecular ion at m/z 449 and a fragment at m/z 287, indicating kaempferol

Table 2. Antioxidant activity and polyphenolic content of the *Agrimonia eupatoria* L. aqueous-alcoholic extract and ethyl acetate fraction

	DPPH (EC ₅₀)	TEAC	TBARS (IP ₅₀)	Total phenols	Total flavonols
	μg			(g % ± S.D.)	
Tincture ^a	18.120	0.196	15.078	15.780 ± 2.931	1.853 ± 0.000
EtOAc ^a	9.800	0.608	2.122	27.637 ± 2.060	2.353 ± 0.000

^a Results expressed in freeze-dried samples.

linked to a hexose moiety. Comparison with a standard confirmed the compound identity as kaempferol 3-*O*-glucoside. The molecular ion of compound **20** was 146 m.u. greater than that of compound **19**. In its UV spectrum, an additional absorption maximum was observed at 314 nm, which is consistent with a *p*-coumaroyl moiety. Its later elution with regard to peak 19 is also coherent with the presence of such an acyl substituent. Based on these characteristics and comparison with a commercial standard, compound **20** was identified as kaempferol 3-*O*-(6''-*O*-*p*-coumaroyl)-glucoside (tiliroside).

Antioxidant activity

Table 2 show the scavenger activities (DPPH and TEAC assays), the lipid peroxidation inhibition (TBARS assay) and the phenolic content (total phenols and flavonols) obtained for the *Agrimonia eupatoria* L. aqueous-alcoholic extract and its respective ethyl acetate fraction. The relevant content of phenols and significant antioxidant activity was observed for the aqueous-alcoholic extract. Its DPPH radical scavenge activity was quite similar to that obtained for ascorbic acid (EC₅₀ = 17 μg) and much higher than that achieved for catechin (EC₅₀ = 140 μg). Both antioxidant activity and phenolic content were appreciably increased in the ethyl acetate fraction, suggesting a straight relation between them.

DISCUSSION AND CONCLUSION

Evidence of a relevant antioxidant activity for the ethyl acetate fraction of *Agrimonia eupatoria* L. aqueous-alcoholic extract (about double that of ascorbic acid) was found by the DPPH method (Table 2). Several polyphenols were identified in this fraction (Table 1), which probably contribute to or are responsible for that activity. Indeed, significant antioxidant activity has been ascribed to quercetin, kaempferol and flavone glycosides and to flavan-3-ols (Rice-Evans *et al.*, 1996). In the *Agrimonia eupatoria* L. ethyl acetate fraction, the flavan-3-ols are present at a high percentage

(18.96%). Catechin is the main individual compound (6.34%) among them, although catechin-type oligomers (i.e. procyanidins) are the most representative flavan-3-ols in the fraction. The difference between the antioxidant activity of a catechin standard and that of the fraction (EC₅₀ = 140 and 9.8 μg, respectively, by the DPPH method) was considerable, indicating the involvement of other compounds in the fraction antioxidant activity besides catechin. Proanthocyanidins, namely procyanidins, have relevant antioxidant properties and other important activities: anti-inflammatory effects, inhibition of platelet aggregation, metal complexation and protein interactions have also been attributed to them (Li *et al.*, 2001; Santos-Buelga and Scalbert, 2000). It is believed that some of these properties, namely anti-inflammatory, are associated with the antioxidant activities of these compounds.

Valuable antioxidant and anti-inflammatory activities have been referred to kaempferol 3-*O*-(6''-*O*-*p*-coumaroyl)-glucoside, quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside (Sala *et al.*, 2003; Melzig *et al.*, 2001), which are also important constituents in the *Agrimonia eupatoria* L. ethyl acetate fraction. Moreover, hepatoprotective properties were also attributed to tiliroside, as well as to kaempferol 3-*O*-glucoside (Matsuda *et al.*, 2002), which is also present in the studied fraction.

Studies *in vivo* suggest that the catechin and smaller oligomeric procyanidins can be absorbed (Cos *et al.*, 2004). The intestinal absorption of quercetin glycosides, namely quercetin 3-*O*-glucoside, has also been reported (Crespy *et al.*, 2001).

In conclusion, compounds that had not been previously reported in *Agrimonia eupatoria* L., but with recognized biological activities and bioavailability, were detected in this medicinal plant by the combination of two HPLC techniques, one of which shows high specificity for flavanols. This study provided very important information for targeting the potentially active compounds of *Agrimonia eupatoria* L. The compounds identified can contribute to explaining the beneficial effects of this plant on human health. The high antioxidant activity registered is also predictive that such beneficial biological properties could be related, at least

in part, to its reported anti-inflammatory activity in traditional medicine.

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