Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

HPLC-DAD-MS STUDY OF POLYPHENOLS FROM *Artemisia absinthium*, *A. annua*, and *A. vulgaris*

B. Ivanescu,[1*] L. Vlase,[2] A. Corciova,[3] and M. I. Lazar[3]

UDC 547.587.52

The species studied is widespread in Romanian spontaneous flora. Due to the poor data available on the native plants, we have initiated a comparative phytochemical study of three *Artemisia* species [1–3]. We continue our work by analyzing some polyphenols from the species before and after acid hydrolysis. The best approach for qualitative identification of flavonoids was considered to be HPLC-MS because it has been successfully used to identify all kinds of flavonoids in different plant samples, including *Artemisia* species [4, 5].

HPLC-DAD-MS analysis is based on a HPLC-DAD method already published in the literature [6–9]. The initial method was modified in order to be compatible with mass spectrometry detection; the most important change was the replacement of the potassium phosphate from the mobile phase with acetic acid. Thus, the mobile phase contains only volatile compounds, and we can introduce the eluent from the chromatographic column directly into the mass spectrometer.

These polyphenolic compounds contain in their molecule at least one phenolic function, so they can be easily transformed into negative ions $[M - H]^-$ and be analyzed by negative ionization. The spectrometer isolates the ions of interest and then fragmentizes them, recording the MS spectra. We recorded the mass spectra of the analyzed polyphenolic compounds as standards in the analytical method. We present the MS analysis mode and the specific ions from the mass spectra that we can use to identify every compound in Table 1.

Eighteen polyphenolic compounds have been studied in the aerial parts of *Artemisia absinthium* L., *A. annua* L. and *A. vulgaris* L. The substances were one hydroxybenzoic acid, six cinnamic acid derivatives, four quercetin glycosides, and seven aglycones of flavonol and flavone type. The results are summarized in Table 2; the polyphenolic compounds are shown in order of their retention time. Quantification of constituents was performed using UV detection at 330 nm for phenol carboxylic acids and 370 nm for flavonoids.

The results indicate the presence of caffeic and chlorogenic acid in all samples. *p*-Coumaric acid was found in all the herbal drugs except *Artemisia absinthium,* in which it exist only in ester form. Ferulic acid was present in all the analyzed samples, except *Artemisia vulgaris,* before hydrolysis. Caftaric acid and sinapic acid are absent; gentisic acid is present only as an ester in *Artemisia annua* and *A. vulgaris*.

In the case of flavonoids, rutoside and isoquercitrin were present in all three herbal drugs; hyperoside was found only in *Artemisia absinthium*, and quercitrin only in *A. vulgaris*. Fisetin was identified in *A. absinthium* and *A. annua*, after hydrolysis. Quercetin is present as the free aglycon in *A. absinthium* and *A. annua*. Patuletin was found in *A. annua* before and after hydrolysis and in *A. absinthium* as glycoside. All analyzed samples contain the flavonic aglycon luteolin. Kaempferol was found as the free aglycon in *A. absinthium* and *A. annua*, and in glycoside form in *A. vulgaris*. Myricetin could not be detected in any of the samples. *A. absinthium* and *A. annua* both have apigenin as the free aglycon.

Apparatus and Chromatographic Conditions. Analysis was performed on an Agilent 1100 HPLC Series system equipped with a G1322A degasser, a G1311A quaternary gradient pump, and a G1313A autosampler. For the separation, a Zorbax SB-C18 reversed-phase analytical column was employed (100 × 3.0 mm i.d., 5 μm particle), fitted with precolumn Zorbax SB-C18, both operated at 48°C.

1) Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy, Iasi, Romania, e-mail: biancaivanescu@yahoo.com; 2) Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, University Iuliu Hatieganu, Cluj, Romania; 3) Department of Drugs Analysis, Faculty of Pharmacy, University of Medicine and Pharmacy, Iasi, Romania. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 394–396, May–June, 2010. Original article submitted December 9, 2008.

TABLE 1. Retention Time of Polyphenolic Compounds (min), MS Analysis Mode, and the Specific Ions for Identification from the Mass Spectra of Polyphenolic Compounds

Compound	Retention time	MS analysis mode	Specific ions for identification *m/z* ion [M – H] > *m/z* ions of spectra
Gentisic acid	2.15	MRM	153 > 108.7
Caffeic acid	5.6	MRM	179.4 > 134.7
Chlorogenic acid	5.6	MRM	353.5 > 178.7, 190.7
p-Coumaric acid	8.7	MRM	163 > 118.7
Ferulic acid	12.2	MRM	193.2 > 133.7, 148.7, 177.6
Hyperoside	18.6	AUTO MS	463 > 254.9, 270.9, 300.7
Isoquercitrin	19.6	AUTO MS	463 > 254.9, 270.9, 300.7, 342.8
Rutoside	20.2	AUTO MS	609.1 > 254.9, 270.9, 300.7, 342.8
Fisetin	22.6	AUTO MS	285 > 134.6, 162.6, 240.7, 256.7
Quercitrin	23	AUTO MS	447.1 > 178.8, 300.7
Quercetin	26.8	AUTO MS	301 > 150.6, 178.6, 272.7
Patuletin	28.7	AUTO MS	331 > 315.7
Luteolin	29.1	AUTO MS	285 > 150.6, 174.6, 198.6, 240.7
Kaempferol	31.6	AUTO MS	285 > 150.6, 256.7
Apigenin	33.1	AUTO MS	269.2 > 148.6, 150.6, 224.7, 226.7

MRM monitoring the compounds′ specific ion; it is used when compound ionization is difficult, the corresponding ions have low intensity, and the spectrometer might confused them with the "background noise"; AUTO MS – the apparatus scans all the compounds′ masses from the mobile phase, and it automatically selects the main ions present and automatically records the mass spectra. We can use it when the compounds ionization is very good or when we make a screening to identify the unknown compounds.

TABLE 2. The Content in Polyphenolic Compounds of Aerial Parts from *Artemisia absinthium*, *A. annua,* and *A. vulgaris* (mg/100 g dry herb)

Polyphenols	*Artemisia absinthium*		*Artemisia annua*		*Artemisia vulgaris*	
	NH	H	NH	H	NH	H
Gentisic acid	–	–	–	*	–	*
Caffeic acid	*	*	*	*	*	*
Chlorogenic acid	*	*	*	*	*	*
p-Coumaric acid	–	12.6	*	5.616	11.152	42.136
Ferulic acid	0.608	2.432	7.488	59.056	–	7.28
Hyperoside	*	–	–	–	–	–
Isoquercitrin	*	–	42.088	–	*	–
Rutoside	*	–	*	–	*	–
Fisetin	–	0.792	–	*	–	–
Quercitrin	–	–	–	–	20.152	–
Quercetin	*	*	2.456	3.336	–	8.84
Patuletin	–	0.616	0.872	8.248	–	–
Luteolin	*	*	2.424	6.848	*	*
Kaempferol	2.456	–	4.584	–	–	1.664
Apigenin	*	–	1.144	–	–	–

NH: nonhydrolyzed sample; H: hydrolyzed sample.

*Only MS qualitative determination was done; UV signal < LoQ (limit of quantification) or interferences/peak overlapping from other compounds does not allow the quantitative determination of these substances.

Not found: caftaric acid, sinapic acid, myricetin.

The mobile phase was a binary gradient prepared from methanol and 0.1% acetic acid. The elution started with a linear gradient, beginning with 5% methanol and ending at 42% methanol, for 35 min; isocratic elution followed for the next 3 min with 42% methanol. The system was subsequently returned to the initial condition and equilibrium for 7 min before the next injection. The flow rate was 1 mL/min and the injection volume was 5 μL. All solvents were filtered through 0.5 μm filters (Sartorius) and degassed through ultrasonication.

UV detection was performed at 330 nm in the first 17 min and then at 370 nm for the next 21 min using a G1315A diode array detector system.

Mass spectrometric analyses were performed on an Agilent Ion Trap 1100 VL mass spectrometer fitted with an electrospray ionization (ESI) interface. The instrument was operated in the negative ion mode. Operating conditions were optimized in order to achieve maximum sensitivity values: gas temperature 350°C at a flow rate of 12 L/min, nebulizer pressure 60 psi, and capillary voltage 3000 V.

The chromatographic data were processed using ChemStation software from Agilent, USA.

Identification and Quantitative Determination of Polyphenols. Retention times were determined with a standard deviation ranging from 0.04 to 0.19 min. The detection limits were calculated as the minimal concentration producing a reproducible peak with a signal-to-noise ratio greater than 3. Quantitative determinations were performed using an external standard method. Calibration curves in the 0.5–50 μg/mL range with good linearity ($R2 > 0.999$) for a five-point plot were used to determine the concentration of polyphenols in plant samples. Accuracy was checked by spiking samples with a solution containing each phenolic compound in a concentration of 10 mg/mL. The full identification of compounds was performed by comparing the retention time and UV and mass spectra with those of standards in the same chromatographic conditions.

Plant Material. The aerial parts of *Artemisia absinthium*, *A. annua,* and *A. vulgaris* were collected at the flowering stage (July–September, 2007) from the countryside around Iasi, Romania. Plants were identified and a voucher specimen of each was deposited at the Herbarium of the Pharmaceutical Botany Department, Faculty of Pharmacy, University of Medicine and Pharmacy, Iasi.

Sample Preparation. Samples of 10 g pulverized plant material were extracted at room temperature by maceration with 100 mL methanol for 24 h. In order to study the flavonoid aglycons that can be obtain by hydrolysis, 0.5 mL of each extract was treated with 0.5 mL of 2 M hydrochloric acid in a centrifuge tube. In parallel, 0.5 mL of each extract was diluted with 0.5 mL water in a centrifuge tube. All the mixtures were heated 30 min at 90°C on a water bath and then centrifuged at 10000 rpm. The supernatant was diluted with water 1:4 (v/v) and centrifuged again at 8000 rpm. The resulting supernatant was filtered through a 0.45 μm filter before injection into an HPLC instrument.

Chemicals. Methanol of HPLC analytical grade and acetic acid and hydrochloric acid of analytical grade were purchased from Merck (Germany). Standards: caffeic acid, chlorogenic acid, *p*-coumaric acid, kaempferol, apigenin, rutoside, quercetin, quercitrin, isoquercitrin, fisetin, hyperoside, and myricetin from Sigma (Germany), ferulic acid, gentisic acid, sinapic acid, patuletin, and luteolin from Roth (Germany), and caftaric acid from Dalton (USA). Methanolic stock solutions (100 g/mL) of the above standards were prepared and stored at 4°C, protected from daylight. Before being used as working solutions, they were appropriately diluted with double distilled water.

REFERENCES

1. B. Ivanescu, A. Corciova, A. Ionescu, and M. I. Lazar, 11th Panhellenic Pharmaceutical Congress, 2003, Athens, P035.
2. B. Ivanescu, D. Lazar, M. I. Lazar, and A. Ionescu, *"Ovidius" University Annals of Medical Science–Pharmacy*, **2**, 107 (2004).
3. B. Ivanescu, A. Poiata, C. Tuchilus, and M. I. Lazar, *Drugs and Pain*, ed. "Gr.T.Popa" Iasi, 2007, 297 pp.
4. J-P. Lai, Y. H. Lim, J. Su, H-M. Shen, C. N. Ong, *J. Chromatogr. B.*, **848**, 215 (2007).
5. A. R. Bilia, P. Melillo de Malgalhaes, M. C. Bergonzi, and F. F. Vincieri, *Phytomedicine*, **13**, 487 (2006).
6. C. I .Peev, L. Vlase, D. S. Antal, C. A. Dehelean, Z. Szabadai, *Chem. Nat. Comp*., **43** (3), 259 (2007).
7. C. S. Fodorea, L. Vlase, S. E. Leucuta, and M. Tamas, *Chem. Nat. Comp*., **41** (4), 400 (2005).
8. S. Leucuta, L. Vlase, S. Gocan, L. Radu, and C. Fodorea, *J. Liquid Chromatogr. Relat. Technol.*, **28**, 3109 (2005).
9. S. Suciu, L. Vlase, C. S. Fodorea, M. Tamas, and S. E. Leucuta, *Clujul Medical*, **LXXVII** (4), 777 (2004).