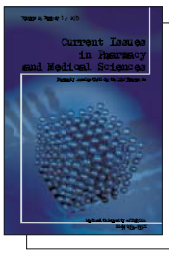

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Determination of coumarins from aerial part of two *Artemisia* species

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ABSTRACT

The methanolic and methanolic-water extracts were received from a blooming aerial part (herb) of two species: *Artemisia gmelini*i Webb ex Stechmann and *Artemisia umbelliformis* Lam. Two methods were used for extracting the plant material: Accelerated Solvent Extraction (ASE) and Soxhlet Extraction. The analysis of samples was performed by the HPLC-DAD method. All obtained extracts were subjected to the process of the solid phase extraction. Samples for HPLC-DAD analysis were purified using C-18 (Octadecyl, 500 mg) Baker Bond (J.T. Baker, USA) SPE-micro-columns. The quantitative indications of individual constituents (coumarins) were converted into the content of scopoletin.

Keywords: Accelerated Solvent Extraction (ASE), Asteraceae, *Artemisia gmelinii* Webb ex Stechmann, *Artemisia umbelliformis* Lam., coumarins, HPLC-DAD analysis method.

INTRODUCTION

The genus *Artemisia* (Asteraceae) consists of about 500 species, appearing worldwide [1]. A large number of species from *Artemisia* genus have been used for centuries in folk medicine (tradicional herbal medicines) due to their anti-viral, anti-tumor, anti-pyretric, anti-malarial, anti-hemorrhagic, anti-coagulanr, antifungial, anti-coagulant, anti-spasmodic, antioxidant, hepatoprotective, anti-ulcerogenic, choleretic and interferon-inclucing properties [4,6,7,9,11,12,15].

The *Artemisia* genus has recently emerged as a source of naturally occurring therapeutic agent for diabetes and diabetic complication [2-5]. *Artemisia* plants are important medicinal material in traditional Asian medicines (Chinese-, Japanis- or Koreas medicine) [8,16]. Terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes constitute major classes of phytoconstituents of the genus [1].

This paper reports the isolation and chromatographic HPLC-DAD quantitative analysis of coumarins in the methanolic and methanolic-water (v/v) extracts from the aerial part of two *Artemisia* species. The plant material was extracted with Accelerated Solvent Extraction (ASE). In available literature, the application of this method for the isolation of plant constituents, also of coumarins [7,13,14] has been reported.

MATERIAL AND METHODS

Plant Materials. The blooming herbs (the aerial part) of two *Artemisia* species were collected from the Botanical Garden of Dept. of Pharmacognosy with Plant Medicinal Plant Unit in September 2011 and 2012 and then air-dried in the shade..

The seeds for cultivations came from two botanic gardens: Botanischer Garten Karl-Franzes-Universität Graz, Institut für Pflanzenwissenschaften/ 2009 – *Artemisia gmelinii* Webb ex Stechmann and Giardino Botanico Alpino "Chanousia" c/o Museo Regionale Scienze Naturali Aosta Italy/ 2009-2010 – *Artemisia umbelliformis* Lam. The raw material was air dried and then crumbled in an electric mill. The proper fraction was obtained by sieving according to the Polish Pharmacopoeia IX [10].

Soxhlet Extraction (SE). Plant materials (75g of *A. gmellini* Webb & Stechmann and 85g of *A.umbelliformis* Lam.) were placed in a 500 ml Soxhlet apparatus. The extractions were carried out using in turn: petroleum ether, chloroform (for purifying from low-polar compounds and chlorophyll), methanol (100%) and the mixture of metha-

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nol–water (1:1v/v). The extracts obtained with each solvent were collected separately and evaporated to 100 mL.

Accelerated Solvent Extraction (ASE). Plant materials (1g) were placed in the stainless steel extraction cell (11 mL) of a Dionex (Sunnyvale, CA, USA) ASE 100 Accelerated Solvent Extractor. Glass fibres filters were placed at the outlet of the extraction cell, to prevent blocking up of the frit. Plant materials were extracted with methanol (100%) and methanol- water (9:1, 8:2, 7:3, 6:4, 1:1, 3:7 v/v) mixtures.

Extractions were performed in the following conditions: pressure – 100 bar (10 MPa), temperature – 70°C, set time – 300 s, flush volume – 60%, purge time – 100 s and static cycle -3. The obtained extracts were concentrated under reduced pressure and transferred (after dissolving in small portions of solvents) to graduated flask (10 mL).

Sample purification. All obtained extracts were subjected to the process of the Solid Phase Extraction. Samples for HPLC-DAD analysis were purified using C-18 (Octadecyl, 500 mg) Baker Bond (J.T. Baker, USA) SPE-micro-columns. The microcolumn with the c-18 batch was rinsed out with 5 mL of the methanol – 80% (for receiving dry abundance) and 3 mL of the methanol – 50% (the batch is supposed to remain humid – to the line of the batch). A portion of 2 mL of extract was sampled and passed through the batch for getting dry abundance. Next the batch was rinsed out with 4 ml of 40% methanol and 4 mL of 80% methanol. The obtained extracts were taken to test tubes of about 15 mL capacity and were supplemented with the methanol (80%). After the analysis the batch was rinsed out with 5 mL of the methanol (100%), 1 mL of the dichloromethane and 3 mL of the methanol (100%).

HPLC-DAD Analysis. The samples were analysed using the Agilent 1100 liquid chromatograph with diode–array detector (DAD). Compounds were separated on XDB-C8 column (150 x 4.6 mm I.D., dp = 5µm) and mobile phase comprising acetonitrile (B) – water + acetic acid (1%) (A) in gradient mode was used. Gradient conditions were: 7 min – 10% B in A, 10 min – 14% B in A, 15 min – 22% B in A, 25 min – 30% B in A, 35min – 35% B in A, 40 min – 55% B in A, 50 min – 60% B in A. The sample injection volume was 20 µL. Elution was carried out at temperature 25°C with a flow rate of 1 mL min-1.

Identification was performed by comparing retention times and by comparision of spectra of components and standards in UV (λ= 254, 280, 325 nm).

Each extract examined was injected in triplicate during a day. Quantitative determination was performed in maximum of absorbtion for each of examined substances. The quantitative indications of individual constituents (coumarins) were converted into the content of scopoletin (λ= 325 nm).

RESULTS AND DISCUSSION

The identification of coumarins was performed based on tR values and UV spectra. The purity of the peaks in the sample was ascertained by comparison of absorption spectra with those obtained from the standards. Good linearity ($R^2 > 0.9991$) of calibration curves in concentration range 0.05–1.5 mg was achieved for scopoletin. Each examined extract was injected in triplicate during a day. The method precision for obtained results (expressed in RSD) was within the range: 0.13–4.56%. The linear regression equation for scopoletin was: y = 1072.2x–933.27 (Fig. 1).

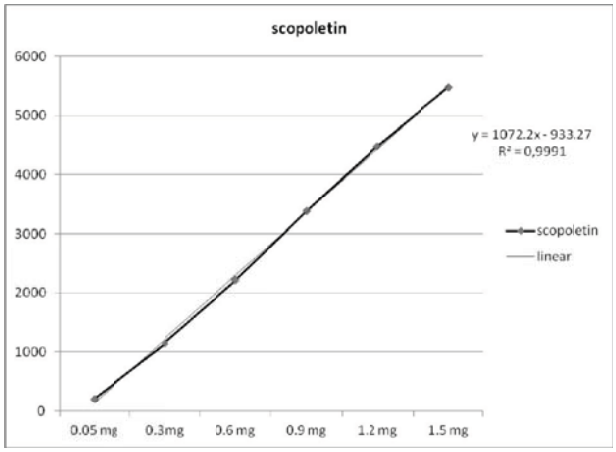

Fig. 1. Calibration curve for determination of scopoletin

Typical for the Asteraceae family coumarins – scopoletin and 6,7 dimethoxy-coumarin were identified from aerial parts of two investigated *Artemisia* sp. 6,7 dimethoxy-coumarin first time in two examined species, but only after Soxhlet Extraction (SE) with mixtures methanol-water (1:1 v/v).

Table 1. The results of quantification of 3 coumarins from investigated extracts from *Artemisia gmelinii* Webb ex Stechmann (mg/g dry wt.)

Extracts	Scopoletin	Unknown coumarin (A)	6,7 dimethoxy--coumarin
SE 100%	0.489 SD=0.03 RSD=0.60	–	–
SE 1:1 v/v	–	–	0.091 SD=0.003 RSD=0.13
ASE 100%	–	0.24 SD=0.016 RSD=2.45	–
ASE 9:1 v/v	–	0.06 SD=0.007 RSD=0.24	–
ASE 8:2 v/v	–	0.06 SD=0.048 RSD=1.09	–
ASE 7:3 v/v	–	0.05 SD=0.0022 RSD=1.0	–
ASE 6:4 v/v	–	0.07 SD=0.017 RSD=0.39	v
ASE 1:1 v/v	0.082 SD=0.002 RSD=0.59	0.48 SD=0.002 RSD=1.55	–
ASE 3:7 v/v	0.126 SD=0.004 RSD=0.36	0.20 SD=0.002 RSD=1.27	–

Scopoletin was identified in extracts obtained with SE method (Soxhlet Extraction) with the use of 100% methanol – the highest content, and with ASE method, with the use of methanol-water mixture (1:1, 3:7 v/v) (Tab. 1, 2).

Table 2. The results of quantification of 3 coumarins from investigated extracts from *Artemisia umbelliformis* Lam. (mg/g dry wt.)

Extracts	Scopoletin	Unknown coumarin (A)	6,7 dimethoxy--coumarin
SE 100%	1.457 SD=0.09 RSD=0.5	–	–
SE 1:1 v/v	–	–	0.062 SD=0.01 RSD=1.78
ASE 100%	–	0.17 SD=0.01 RSD=1.04	–
ASE 9:1 v/v	–	0.14 SD=0.02 RSD=0.66	–
ASE 8:2 v/v	–	0.15 SD=0.02 RSD=0.59	–
ASE 7:3 v/v	–	0.11 SD=0.088 RSD=4.56	–
ASE 6:4 v/v	–	0.044 SD=0.002 RSD=0.51	–
ASE 1:1 v/v	0.084 SD=0.002 RSD=0.21	0.39 SD=0.01 RSD=0.14	–
ASE 3:7 v/v	0.128 SD=0.03 RSD=0.60	0.21 SD=0.07 RSD=0.23	–

The unknown compound (named A) was identified exclusively in two examined species after extraction with ASE method (all concentrations of the extractant). The highest content was obtained applying a mixture of methanol-water (1:1 v/v) (Tab. 1, 2).

CONCLUSIONS

Chromatographic analysis of 9 extracts from the investigated plant materials showed the presence of 3 coumarins: scopoletin, 6,7 dimethoxy-coumarin, and unknown compound (A) with tR 4.85 and UV spectra similar to similar to UV spectra of remaining coumarins. The chosen chromatograms obtained from investigated extracts (with ASE method) are present in Fig. 2, 3. Scopoletin is a predominant constituent of complex of coumarins. Unknown compound (A), they identified exclusively in extracts received with ASE method. The structure of the examined compound needs further investigation, using spectroscopic methods. 6,7 dimethoxy – coumarin was identified only in Soxhlet extraction with mixtures of methanol–water 1:1 v/v. The role of coumarins in chemotaxonomic studies of *Artemisia* species could be discussed as well.

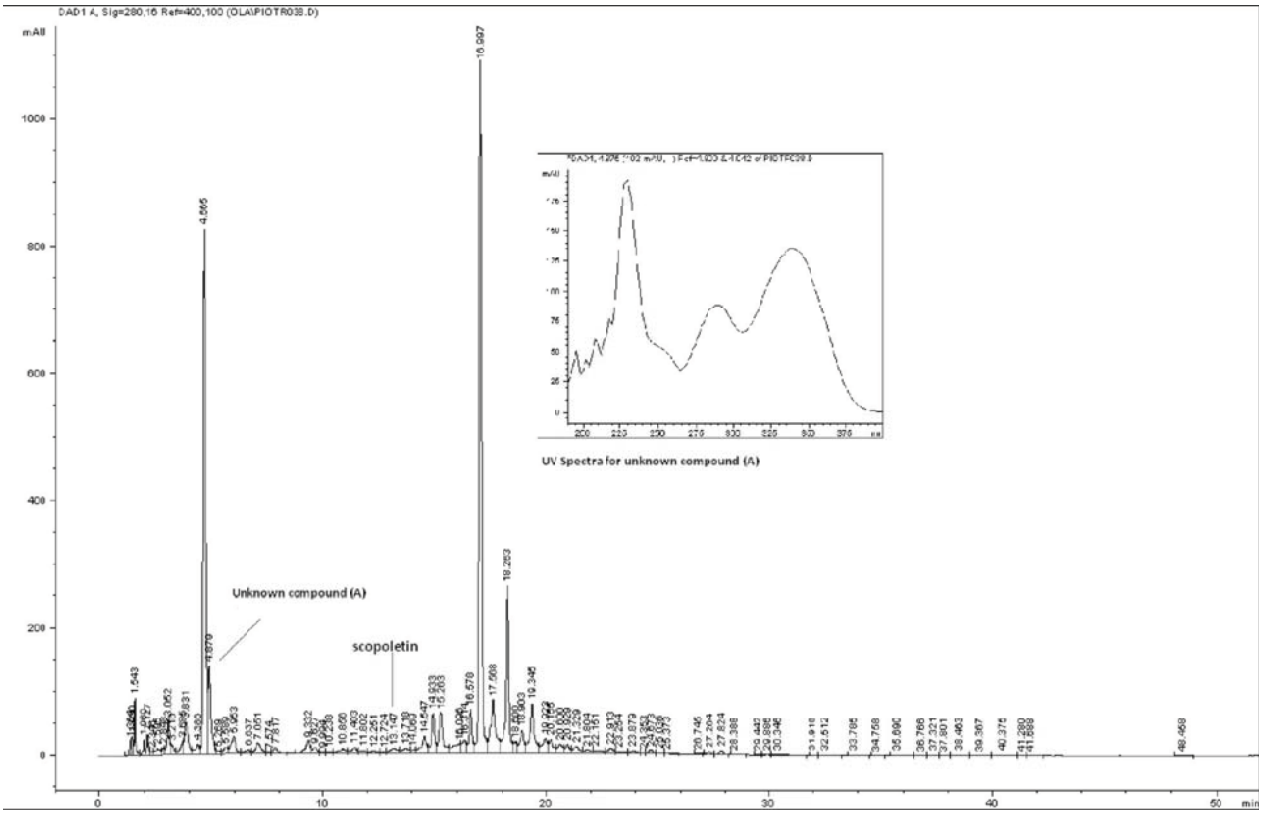

Fig. 2. HPLC chromatogram and UV spectra for examinated fractions – ASE method with methanol-water 1:1 v/v from *A. gmelini* Webb ex Stechmann

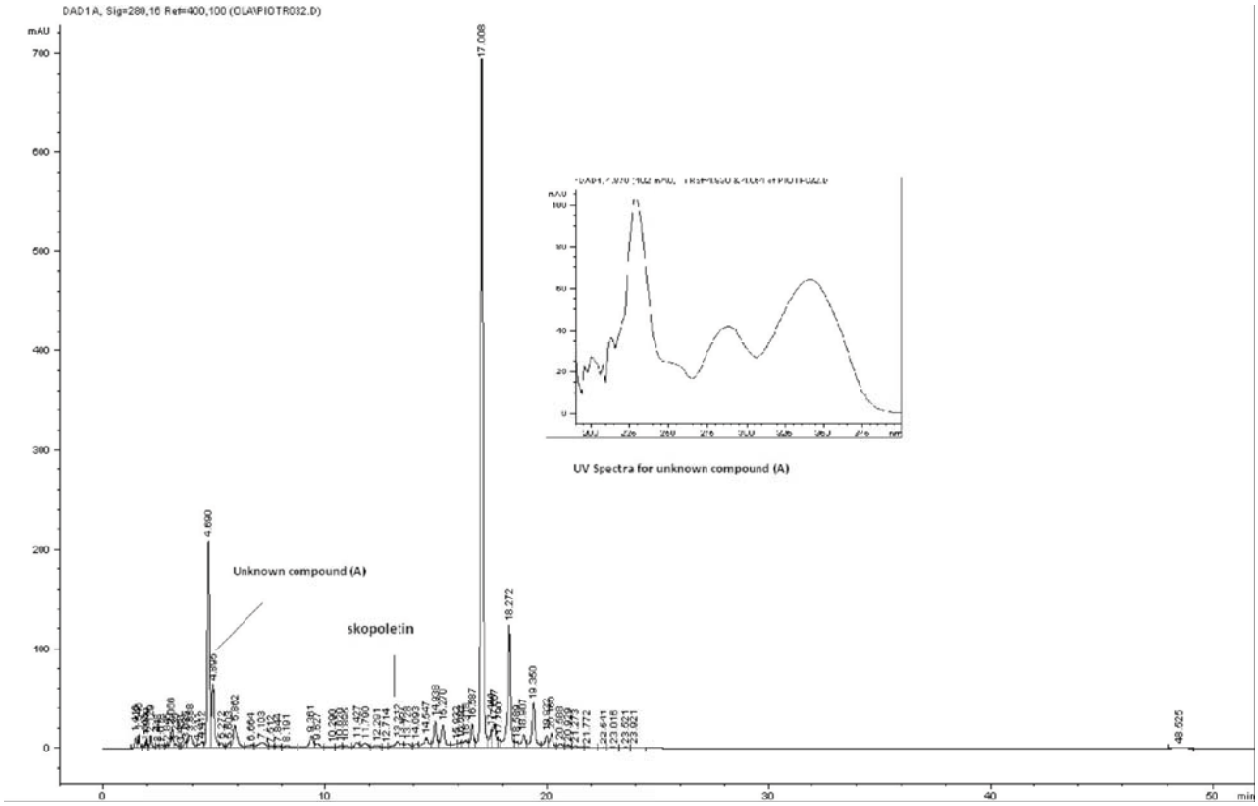

Fig. 3. HPLC chromatogram and UV spectra for examinated fractions – ASE method with methanol-water 1:1 v/v from *A. umbelliformis* Lam

ACKNOWLEDGEMENTS

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