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Original scientific paper

High-performance liquid chromatographic separation of surface flavonoid aglycones in *Artemisia annua* L. and *Artemisia vulgaris* L.

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Abstract: External flavonoid aglycones from *Artemisia annua* L. and *Artemisia vulgaris* L. were analyzed by the HPLC method. The mobile phase was composed of *t*-butanol-methanol-20 mmol l^{-1} phosphate buffer, pH 3.22. The linear gradient elution method within 22 min was applied. The main aglycones in *A. annua* and *A. vulgaris* are methyl ethers of quercetagetin and quercetin. Quercetagetin 3,6,7-trimethyl ether in *A. annua* and quercetin 3,7,3'-trimethyl ether in *A. vulgaris* are the most abundant compounds.

Keywords: *Artemisia annua*, *Artemisia vulgaris*, HPLC, flavonoid aglycones, quercetagetin 3,6,7-trimethyl ether, quercetin 3,7,3'-trimethyl ether.

INTRODUCTION

Artemisia species have received increasing interest because of their medicinal applications, especially with regards to flavonoids.[1] They are used in gastric diseases, in malaria, as antifungal, anthelmintic, sedative and an emmenagogue agents.[2] *Artemisia* species accumulate on their surface a variety of di-, tri-, tetra- and pentamethyl ethers of flavonols which are derivatives of quercetin and quercetagetin (6-methylquercetin).[3–5] Although the flavonoid constituents of *A. annua* and *A. vulgaris* have been studied[6–10] no data have been reported for the separation of the epicuticular flavonoid aglycones from the acetone exudates by the HPLC method.

The objective of this work was to analyze the external flavonoid aglycones from *A. annua* and *A. vulgaris* by HPLC.

EXPERIMENTAL

Plant material

The aerial parts of *A. annua* and *A. vulgaris* were collected from natural habitats around Sofia during flowering. Voucher specimens – Co970 and Co647 were deposited at the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences.

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Standards and chemicals

HPLC-gradient grade methanol and other chemicals (acetone, methanol, potassium dihydrogen phosphate and *ortho*- phosphoric acid) of analytical – reagent grade were purchased from Merck (Darmstadt, Germany). The authentic standards of the studied flavonol aglycones were kindly supplied by Prof. E. Wollenweber (Darmstadt).

Sample preparation and indentification of flavonoids

Air dried (not ground) plant samples were rinsed with acetone to dissolve the exudate material. After evaporation of the solvent, the residues were chromatographed on Sephadex LH-20 eluted with methanol to separate the flavonoids from the dominant terpenoids. The individual flavonoids were identified by comparison of their retention times (t_R) with those of the standards. The identies of the HPLC peaks were unambiguously determined by co-chromatography after spiking the samples with the reference compounds.

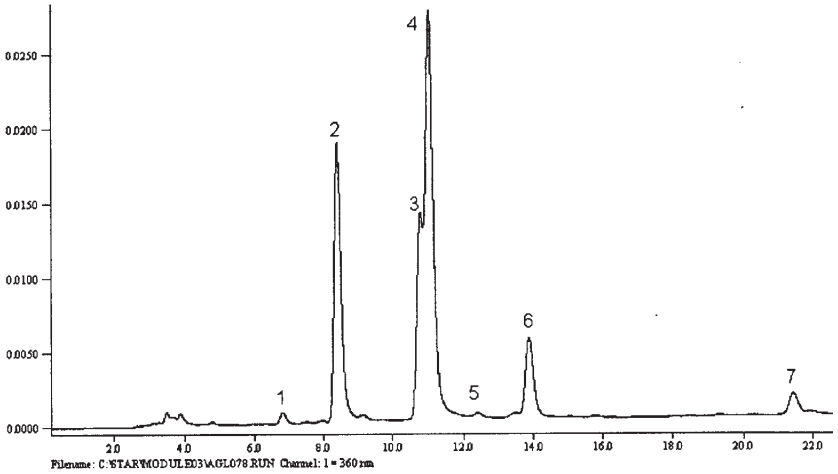

Fig. 1. HPLC Chromatogram of flavonol aglycones from *A. annua*: (1) quercetagetin 3,6-dimethyl ether, (2) quercetagetin 3,6,7-trimethyl ether, (3) quercetagetin 3,6,7,4'-tetramethyl ether, (4) artemisinic acid (5) 6-OH-kaempferol 3,6,7-trimethyl ether, (6) quercetagetin 3,6,7,3',4'-pentamethyl ether, (7) kaempferol 3,7-dimethyl ether.

Chromatographic equipment and conditions

The chromatographic analyses were performed on a Varian (USA) chromatographic system, which included a tertiary pump Model 9012, a Rheodyne injector with a 20 µl sample loop and a UV-VIS detector Model 9050. A Varian Star Chromatography workstation and computer software (version 4.5) were used to control the system and collect the data. The separation was performed using a Hypersil ODS RP18, 5 µm, 250 × 4.6 mm I.D. column (Chandon, UK) fitted with a precolumn (30 × 4.6 mm I.D., Varian, USA) dry packed with Perisorb RP-18, 30–40 µm (Merck, Germany). Both columns were maintained at room temperature. Systems (A) and (B) were comprised of the following solvent ratios – (A): *t*-butanol–methanol–20 mmol l^{-1} phosphate buffer (adjusted to pH 3.22 with *ortho*-phosphoric acid) at a volume ratio of 11:37:52 and (B): *t*-butanol–methanol–20 mmol l^{-1} phosphate buffer at a volume ratio of 15:45:40. The elution was performed employing a program from (A) – 100 % (t = 0 min) to (B) – 100 % in a period of 22 min at a flow rate 1 ml min^{-1}. The chromatograms of each sample were recorded at 254 nm and 360 nm to ensure a reliable identification of the isolated acids.

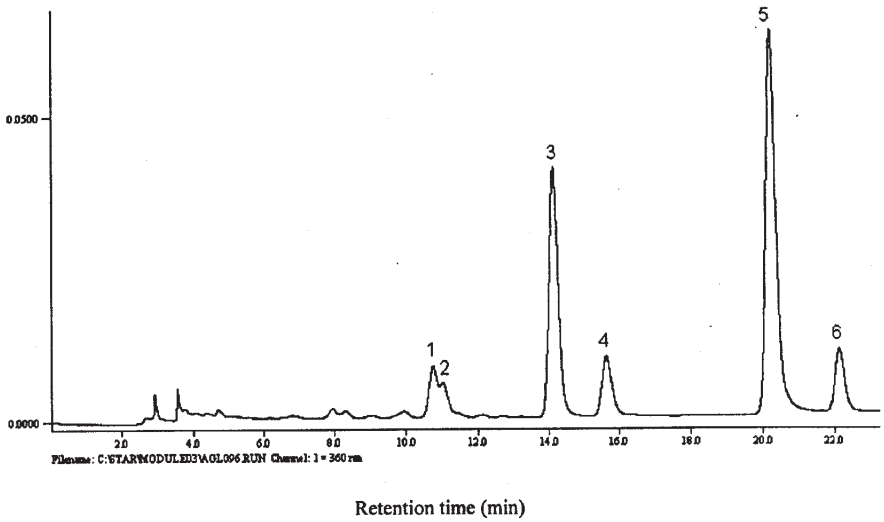

Fig. 2. HPLC Chromatogram of flavonol aglycones from *A. vulgaris*: (1) quercetagetin 3,6,7,4'-tetramethyl ether, (2) quercetin 3,3'-dimethyl ether, (3) quercetagenin 3,6,7,3',4'-pentamethyl ether, (4) quercetin 3,7-dimethyl ether, (5) quercetin 3,7,3'-trimethyl ether, (6) kaempferol 3,7-dimethyl ether.

RESULTS AND DISCUSSION

The RP-HPLC behavior of the flavonoid aglycones on the reversed phase column was tested sequentially varying the proportion of the methanol-water elution mixture. The separation was achieved by modifying the mobile phase with a small amount of *t*-butanol and using 20 mmol l^{-1} phosphate buffer adjusted to pH 3.22. The resolution parameters were improved after suitable adjustment of the linear gradient method using the ternary solvent system but both pairs of the compounds quercetagetin 3,6,7,4'-tetramethyl ether, artemisinic acid and quercetagetin 3,6,7,4'-tetramethyl ether, quercetin 3,3'-dimethyl ether were not satisfactorily separated.

The results obtained by this method revealed the presence of six flavonol aglycones of *A. annua*. The major flavonoid constituents are quercetagetin 3,6,7-trimethyl ether, 6-OH-kaempferol 3,6,7-trimethyl ether, quercetagetin 3,6,7,3',4'-pentamethyl ether, kaempferol 3,7-dimethyl ether present in traces (Fig. 1).

Derivatives of quercetin and quercetagetin are found as the main flavonoid constituents in acetone leaf exudate from *A. vulgaris*. Quercetin 3,7,3'-trimethyl ether is the most abundant compound. Quercetagetin-3,6,7,3',4'-pentamethyl ether, quercetin 3,3'-dimethyl ether, quercetagetin 3,6,7,4'-pentamethyl ether, quercetin 3,7-dimethyl ether, kaempferol 3,7-dimethyl ether were also identified from *A. vulgaris* exudate (Fig. 2).

The TLC method for separation of the flavonol aglycones has shown that the quercetin 3,3'-dimethyl ether and quercetin 3,7-dimethyl ether pair could not be well resolved.[11] The HPLC method proposed in the present work enhances the separation efficiency of this critical peak pair. The described method for sample preparation and HPLC separation of surface flavonoid aglycones is suitable for the rapid estimation of the flavonoid content in samples of plant species from different origin.

ИЗВОД

РАЗДВАЈАЊЕ ПОВРШИНСКИХ ФЛАВОНИДА АГЛИКОНА *ARTEMISIA ANNUA* L. И *ARTEMISIA VULGARIS* L. ПОМОЋУ ТЕЧНЕ ХРОМАТОГРАФИЈЕ ВИСОКЕ ПЕРФОРМАНСЕ

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Флавонид агликони из *Artemisia annua* L. и *Artemisia vulgaris* L. анализирани су методом HPLC. Мобилна фаза састојала се од *t*-бутанол-метанол-20 mmol 1^{-1} фосфатни пуфер, pH 3,22. Примењен је метод линеарног градијента елуирања током 22 мин. Главни агликони у *A. annua* и *A. vulgaris* су метил етри кверцетагетина и кверцетина. Кверцатегитин 3,6,7-триметил етар у A. annua и кверцетин 3,7,3´-триметил етар у *A. vulgaris* су најзаступљенија једињења.

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