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External flavonoid aglycones from *Veronica chamaedrys* L. *(Scrophulariaceae)*

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External flavone aglycones in the overground parts of *Veronica chamaedrys* L. (*Scrophulariaceae*) have been analysed by isocratic reversed-phase high performance liquid chromatography. Luteolin, apigenin, luteolin-3'-methyl ether and scuterallein-6,4'-dimethyl ether have been identified. The latter compound is a new flavonoid identified in *Veronica* genus.

Keywords: *Veronica chamaedrys* (*Scrophulariaceae*), flavone aglycones, scuterallein-6,4'-dimethyl ether, HPLC

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External flavonoid aglycones (mainly methylated derivatives) are accumulated in several medicinal species, on their leaf and stem surfaces (1). Many studies have indicated the increasing importance of flavonoid aglycones as biologically active natural products (2–4). This emphasizes the importance of further studies on their distribution in medicinal plants and natural cures.

Some *Veronica* species are used for their diaphoretic, astringent, anti-inflammatory, cytotoxic, radical scavenging and anti-ulcer activities (5–7). *Veronica chamaedrys* L. is a perennial herb, growing wild and abundantly in north hemisphere (8).

Although high performance liquid chromatography (HPLC) has been applied to the analyses of flavone aglycones (9) no method has been reported for separation of this compounds in *Veronica* species. The aim of this work was to analyse the external flavonoid aglycones in *Veronica chamaedrys* using HPLC method.

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EXPERIMENTAL

Plant material

The overground part of *Veronica chamaedrys* was collected in the region of Black Sea during April, 2000. Voucher specimen of the plant sample (SOM – Co468) was deposited in the Herbarium of the Institute of Botany, Sofia, Bulgaria.

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 (Germany). The bidistilled water was used. The authentic standards of the studied flavone aglycones were kindly supplied by Prof. E. Wollenweber (Institute of Botany, Darmstadt, Germany).

Sample preparation

Air dried (not ground) plant sample was rinsed with acetone for several minutes to dissolve the surface flavonoids. The acetone exudate was concentrated in rotary vacuum evaporator and the dried extract was chromatographed on Sephadex LH-20 (Farmacia, Sweden). The flavonoids were eluted with methanol and separated from the predominant terpenoids.

Chromatographic equipment and conditions

The chromatographic analyses were performed on Varian (USA) chromatographic system, which includes tertiary pump Model 9012, Rheodyne injector with 20-μL sample loop and UV-Vis detector Model 9050. Varian Star Chromatography workstation and computer software (version 4.5) were used for controlling the system and collecting the data. The separation was performed using Hypersil ODS RP18, 5 μm, 250 × 4.6 mm I.D. column (Chandon, UK) fitted with precolumn (30 × 4.6 mm I.D., Varian, USA) dry packed with Perisorb RP-18, 30–40 μm (Merck, Germany), the both maintained at room temperature. The mobile phase comprised *t*-butanol, methanol and 20 mmol L^{-1} potassium dihydrogen phosphate buffer (adjusted to pH 3.22 by *ortho* phosphoric acid) at a volume 11:37:52, filtered through a 0.45-μm filter (Millipore, Ireland) and degassed before use in ultrasonic bath. The flow rate was 1 mL min^{-1}. The chromatograms were recorded at 360 nm selected on the specific UV absorption of the assayed compounds.

RESULTS AND DISCUSSION

The RP-HPLC behavior of the aglycones on the reversed-phase column was tested sequentially varying the proportion in aqueous-organic elution mixture. The separation was improved by modifying the mobile phase with small amount of *t*-butanol. The flavone aglycones were separated isocratically and identified by comparison of their reten-

tion times with those of the standards. The identity of HPLC peaks was definitely assessed by co-chromatography after spiking the samples with reference compounds.

The results obtained by this method revealed the presence of four flavone aglycones: luteolin (t_R 8.38 min), luteolin-3'-methyl ether (chrisoeryol) (t_R 11.26 min), apigenin (t_R 15.11 min) and scutellarein-6,4'-dimethyl ether (pectolinaringenin) (t_R 24.59 min) (Fig. 1). The current results confirm our earlier data from TLC analysis (10). Main structures of the flavones found in this study are presented in Fig. 2.

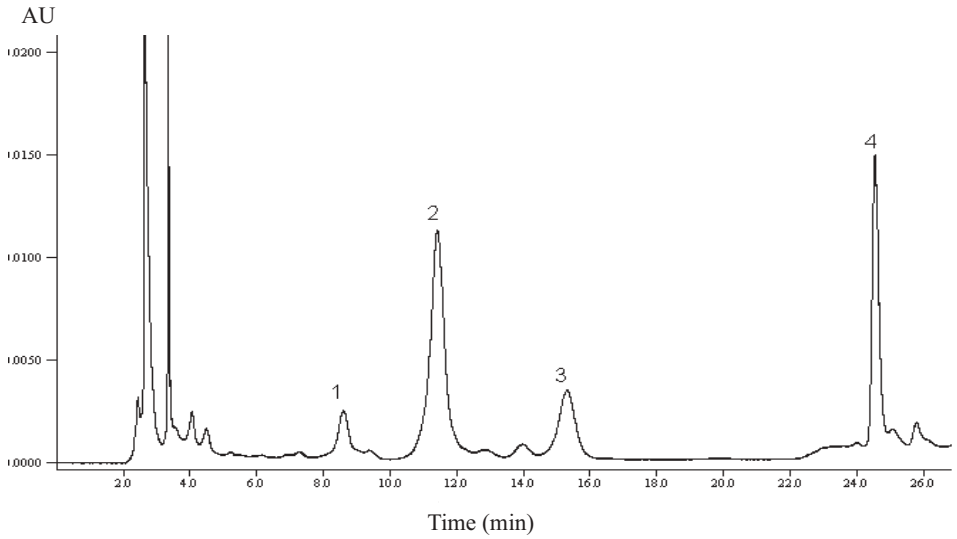

Fig. 1. HPLC chromatogram of flavone aglycones: (1) luteolin, (2) luteolin-3'-methyl ether, (3) apigenin, (4) scutellarein-6,4'-dimethyl ether.

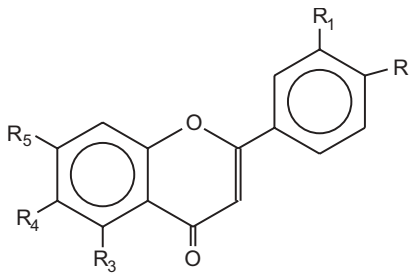

Fig. 2. Basic structures of flavone aglycones detected in *V. chamaedrys*.

apigenin – $R_1 = R_4 = H$, $R_2 = R_3 = R_5 = OH$
luteolin – $R_1 = R_2 = R_3 = R_5 = OH$, $R_4 = H$
scutellarein-6,4'-dimethyl ether – $R_1 = H$, $R_2 = R_4 = OCH_3$, $R_3 = R_5 = OH$
luteolin-3'-methyl ether – $R_1 = OCH_3$, $R_2 = R_3 = R_5 = OH$, $R_4 = H$

There is a considerable amount of evidence for pharmacological effects of flavonoids (11). According to these data pectolinaringenin reduced the serum cholesterol, triglycerides and lipoproteins. Chrysoeriol exhibited antimicrobial activity, while apigenin and luteolin show anti-inflamatory and antifungal activities.

External accumulation of epicuticular, lipophilic flavonoids is an advantage because they could thus be isolated much easier than water soluble vacuolar flavonoids. Therefore simple and fast techniques for preparation of sample and analysis are suitable for investigation of external flavonoid aglycones in medicinal plants.

CONCLUSIONS

Four epicuticular flavone aglycones (luteolin, apigenin, luteolin-3-methyl ether, scutellarein-6,4'-dimethyl ether) have been identified from crude acetone exudate of *Veronica chamaedrys*. External accumulation of this lipophilic flavonoids is an advantage because they could be isolated much easier than water soluble vacuolar flavonoids. Therefore simple and fast techniques for preparation of sample and analysis are suitable for investigation of external flavonoid aglycones in medicinal plants. Biological effects of acetone exudate from *V. chamaedrys* are a goal of our further investigation.

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S A Ž E T A K

Flavonoidni aglikoni iz biljke *Veronica chamaedrys* L. (*Scrophulariaceae*)

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Flavonoidni aglikoni iz nadneznih dijelova biljke *Veronica chamaedrys* L. analizirani su izokratičnom reverzno-faznom tekućinskom kromatografijom visoke učinkovitosti. Identificirani su sljedeći spojevi: luteolin, apigenin, luteolin-3'-metil eter i skuteralein-6,4'-dimetil eter. Posljednji spoj je novi flavonoid identificiran u rodu *Veronica*.

Ključne riječi: Veronica chamaedrys (*Scrophulariaceae*), flavonoidni aglikoni, skuteralein-6,4'-dimetil eter, HPLC

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