ASSAY OF FLAVONE AGLYCONES IN HELICHRYSUM PLICATUM DC. (ASTERACEAE)

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Abstract. Flavone aglycones were examined in different parts of *H. plicatum* DC. (*Asteraceae*) from Macedonia. Plant material was collected during summer 1996, on the Golak mountain, dried and separated into flowers and steams+leaves. Flavone aglycones in the extracts were analyzed by high performance liquid chromatography (HPLC) equipped with UV diode-array detection. Identification was made according to the retention times and UV-spectra of the components compared to those of authentic samples of flavonoids. The quantity of these components varied a lot depending on the extraction procedure. Primary extraction with ethanol:water (7:3) and secondary extraction of the aqueous phase with ethyl acetate provided lower concentration of flavones compared to that obtained with methanol. Apigenin and naringenin as free aglycones and glycosides of apigenin, naringenin, kaempferol and quercetin were identified in the flowers of *H. plicatum*. Almost the same quantities of apigenin and naringenin were determined before and after hydrolysis implying that they are present in the plant as free aglycones. In the steams+leaves parts of *H. plicatum* quercetin and luteolin glycosides and luteolin as free aglycone were identified.

Key words: *Helichrysum plicatum*, *Asteraceae*, flavonoids, aglycones, HPLC

1. Introduction

Helichrysum plicatum DC. has been used in Macedonian folk medicine for a long time in a treatment for gastric or hepatic disorders, usually in combination with other plants with similar effects [1]. This plant is treated as very similar to *Helichrysum arenarium* L., the flowers of which (*Helichrysi flos*) are used for the preparation of home made remedies. Until now, *H. arenarium* was not recognized in the flora of Macedonia. Two other species that occur in this climate were identified as *H. plicatum* DC. and *H. zivojinii* Cernjavski and Soska, the latter one representing an endemic in Macedonian flora [2].

Helichrysum plicatum DC. (*Asteraceae*) is 30-40 cm tall perennial plant, with greenish lanceolate leaves and small flower-heads arranged in cymes, comprising yellow tubular florets and bearing membranaceous involucral bracts. It grows on dry grass-lands on mountains in the southern part of the Balkan Peninsula, mostly in Albania, Macedonia and Greece. It is also spread in Turkey, where few different subspecies and varieties of the taxa occur [1].

The chemical composition of *H. plicatum* has been investigated by many authors and flavonoids seem to be the most interesting constituents. Thus, in three various subspecies of *H. arenarium* (ssp. *aucheri*, ssp. *erzincanicum* and ssp. *rubicundum*) apigenin, luteolin, naringenin, kaempferol and 3,5-dihydroxy-6,7,8-trimethoxy-flavone were identified as well as seven different glycosides [3]. Almost the same flavone aglycones were identified in a few other *Helichrysum* species [4-8]. Mainly apigenin, naringenin, 3,5-dihydroxy-trimethoxy-flavone, often kaempferol and rarely luteolin and quercetin are found in the flowers of *Helichrysum* sp., whereas the stems and leaves of *Helichrysum* contain mainly quercetin and kaempferol glycosides and then naringenin and luteolin glycosides.

Helichrysum species from Macedonia has not been investigated, so the aim of this study is the assay of the flavonoid pattern of *H. plicatum* from our climate using high performance liquid chromatography (HPLC) for separation and identification of the flavone aglycones.

2. Experimental

2.1. Materials and instruments

Aerial parts of *Helichrysum plicatum* DC. collected on the Golak Mountain, Eastern Macedonia, during the flowering period of the plant, were air-dried; identified by Dr. V. Matevski, Department for Botany, Faculty of Science, Skopje, Republic of Macedonia.
Authentic samples of apigenin, luteolin, naringenin, eriodyctiol, chrysoeriol, luteolin-7-glycoside (Extrasinthese, Lyon), quercetin dihydrate (Merck, Germany) were used.
Varian HPLC system equipped with ternary pump model 9012 and UV diode array detector (UV-DAD) model 9065 was used.

2.2. Extraction procedures

Dried flowers and steams+leaves, separately, were cut into small pieces and extracted by the following three procedures: 1. with methanol; 2. with ethanol-water (7:3), then evaporated and extracted with ethyl acetate; 3. hydrolysis with conc. HCl, aglycones extracted with ethyl acetate. All extracts were evaporated and dried residues were dissolved in small volume of methanol (2 ml).

2.3. HPLC analysis

A reverse phase C18 column with dimensions 250 x 4.6 mm, with particle diameter of 5 μm was used. Plant extracts were injected in the column using manual loop valve injector (20 μl). The mobile phase flow rate was 1.2 ml/min and it consisted of the following three solvents: A- HCOOH:H_2O (0.1:99.9), B-acetonitrile and C-methanol. The method was: at the start 70 % A, 10 % B and 20 % C; then 1-5 min 65 % A, 15 % B and 20 % C; 15-35 min 60 % A, 20 % B and 20 % C; up to 45 min 55 % A, 20 % B and 20 % C; up to 50 min 40 % A, 40 % B and 20 % C. UV detection was carried out at 254, 286 and 360 nm. The retention times and UV-spectra were compared with those for authentic samples.

3. Results and discussion

In order to investigate the flavonoid pattern of *Helichrysum plicatum* from Macedonia, extracts were prepared from the flowers and stems+leaves of the plant, separately. Flavone aglycones in the extracts were analyzed by HPLC equipped with UV diode-array detection and identification was made according to the retention times and UV-spectra of the components compared to those for authentic samples of available flavonoids (luteolin-7-glycoside, eriodictyol, quercetin, luteolin, naringenin, apigenin, kaempferol and chrysoeriol). Chromatograms obtained using HPLC are presented in Fig. 1, where a. stands for a mixture of authentic samples, b. and c. for ethyl acetate extracts (glycosides previously hydrolyzed) from flowers and steams+leaves, respectively.

Extraction with methanol was found to be more effective then the one with ethanol:water (7:3) and secondary extraction of the aqueous phase with ethyl acetate. Hydrolysis was used for releasing the flavone aglycones bound in glycosides, and their subsequent identification. The results from the identification of flavone aglycones in the extracts with and without hydrolysis are presented in Table 1.

Table 1. Identification of flavone aglycones in extracts of *Helichrysum plicatum*

Extract	Apigenin	Luteolin	Naringenin	Kaempferol	Quercetin
Flowers					
- Methanol	++*	-**	++	-	-
- Ethyl acetate	+	-	+	-	-
- After hydrolysis	++	-	+++	+++	+
Steams+leaves					
- Methanol	-	+	-	-	-
- Ethyl acetate	-	+	-	-	-
- After hydrolysis	-	+	-	-	+

* relative content, ** not found

In the flowers of *H. plicatum*, apigenin and naringenin as free aglycones were identified. Almost the same quantities of apigenin and naringenin were found before and after hydrolysis, implying that they are present in the flowers mostly as free aglycones. On the other hand, apigenin, naringenin, kaempferol and quercetin were identified after hydrolysis, which means that the latter two flavonoids are present in the flowers bound with sugars in glycosides. As can be seen in Fig. 1b, the most abundant of all was kaempferol, followed by smaller content of apigenin whereas very low quantity of quercetin and naringenin was found in the flower extract.

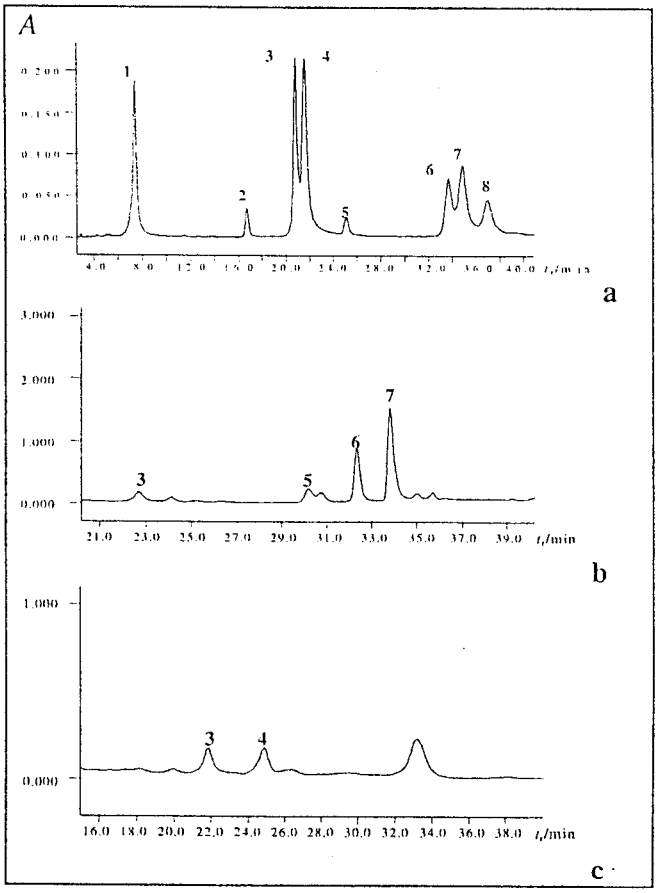

Fig. 1. HPLC chromatograms: **a**. mixture of authentic samples (1-luteolin-glycoside; 2-eriodictyol; 3-quercetin; 4-luteolin; 5-naringenin; 6-apigenin; 7-kaempferol; 8-chryseriol); **b**. ethyl acetate extract of flowers, after hydrolysis; **c**. ethyl acetate extract of steams and leaves after hydrolysis.

Comparison of the chromatograms obtained for the extracts of the stems+leaves before and after hydrolysis enabled us to identify quercetin and luteolin glycosides and luteolin as free aglycone. The identification of quercetin and luteolin, after hydrolysis, is shown in Fig. 1c.

The results obtained are in good accordance with data of flavonoids in *H. arenarium* [9, 10]. It can be concluded that apigenin and naringenin are present in the flowers of *H. plicatum* as free aglycones, whereas the most abundant flavonoids are the glycosides of kaempferol. Further examination will be carried out on isolation and identification of the glycoside forms in *H. plicatum* from Macedonia.

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