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Short communication

FLAVONOIDS OF *VERBASCUM SCARDICOLUM* AND *MELAMPYRUM SCARDICUM*

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Aerial parts of two *endemic* plant species, *Verbascum scardicolum* and *Melampyrum scardicum*, were examined for the presence of twenty-one flavonoid and two phenolic acids by RP HPLC. The components were detected by comparison with standards. From the inflorescences of *V. scardicolum* luteolin 7-*O*-glucoside was isolated and purified by chromatographic methods. In the leaves' extract of *V. scardicolum,* caffeic acid was detected. The *M. scardicum* extract showed the presence of luteolin, luteolin 7-*O*-glucoside and apigenin 7-*O*-glucoside. The results were compared with literature data for the other species of *Verbascum* and *Melampyrum*.

Key words: flavonoids; *Verbascum; Melampyrum*; reverse-phase high performance liquid chromatography

INTRODUCTION

It is generally known that the most of the species of the *Verbascum* group (*Scrophulariaceae* family) are quite abundant in the European flora. Due to their biological activity, some species have been traditionally used for medical purposes. The biennial herb *Verbascum scardicolum* Bornm., however, is described as endemic form found only in certain areas of Šar Mt., Central Balkan Peninsula [1]. Unlike the most of the species *of Scrophulariaceae* family, no literature data could be found about its chemical components.

Another species of the *Scrophulariaceae* family, *Melampyrum scardicum* Wettst., is known to be a Balkan endemic species [2]. Up to now, nothing has been reported on its chemical composition.

Due to many advantages, such as the structural specificity with respect to the origin and simple detection, flavonoids can serve as useful taxonomic markers [3]. This is of special importance in cases where the discernment of close plant groups as done by other means (for example, morphologically or cytologically) is insufficient. Furthermore, the knowledge of the flavonoid composition makes it possible to examine the eventual relationship among chosen plant groups on a chemotaxonomical basis. Therefore, numerous methods for separation and identification of various naturally occuring flavonoids based on high-performance liquid chromatography (HPLC) have been reported lately [4].

The present paper reports on the preliminary results of the chemical investigations on *V. scardicolum* and *M. scardicum* species. Reverse-phase HPLC technique was used to screen these species for the presence of certain flavonoids and phenolic acids and eventually relate them to the other species of their group.

EXPERIMENTAL

Aerial parts (blossomed) of *V. scardicolum* and *M. scardicum* were collected near Tri Vodi region of Šar Mt., Macedonia (July, 1996). The samples were identified by Dr Ljubčo Melovski.

Voucher specimens are deposited in the herbarium of the Institute of Biology.

Fresh leaves (20 g) and blossoms (15 g) of *V. scardicolum* were treated separately. Since the separation of the blossoms from the green parts of *M. scardicum* was difficult, whole aerial parts (approximately 30 g) were further treated in this case. The ground material was extracted twice with 200 ml portions of 96 % (v) ethanol overnight at ambient temperature. The extract was decanted and the plant material was refluxed for three hours with three successive 100 ml 96 % (v) ethanol portions by gently heating the mixture. The ethanol extracts were then collected and concentrated under reduced pressure until yellow-brownish (from *V. scardicolum* blossoms) and dark green (leaves of *V. scardicolum* and aerial parts of *M. scardicum*) oily residues were obtained. The remaining residues were partitioned between water (20 ml) and chloroform (20 ml).

The water layer was analyzed with a HPLC system (Varian), equipped with a ternary pump (model 9012), diode array UV detector (model 9065) and C_{18} column (250 nm in length, 46 mm in diameter, with particle size of 5 μm). The components of the standard mixture, as well as of the extracts, were successfully separated using binary mixtures of acetonitrile (ACN) and 0.1 % (v) HCOOH in water. The following method was used: 0–10 minute 10 % (v) of ACN, 10–30 minute 30 % (v) of ACN, 30–50 minute 40 % (v) of ACN. The flow was kept at 0.02 $ml \cdot s^{-1}$. The absorption at 254 nm was accounted for in comparison of the retention times (denoted t_r hereafter). The standard mixture contained nineteen flavonoid aglycons (acacetin, apigenin, chrysoeriol, chrysin, cirsilineol, cirsimaritine, 5,4-dihydroxy-6,7,8,3'-tetramethoxyflavone, diosmin, eriodictyol, galangin, genkwanin, kaempferol, luteolin, myricetin, naringenin, quercetin, rutin, thymonyn, xantomicrol), the 7-*O*-glucosides of luteolin and apigenin, as well as two phenolic acids, rosmarinic and caffeic. The retention times and the spectral data obtained with these standards were stored in a data library and later on compared with those obtained from the samples. Besides the retention times and the UV spectral data, the identity of the components was additionally confirmed by the standard addition method. The choice of the standards was set, including the most abundant *Scrophulariaceae*, but at the same time aglycons and glycosides that were available to us.

The water layer of *V. scardicolum* inflorescences was further concentrated under reduced pressure (0.93 g) and chromatographed on a silica gel (0.2–0.5 mm, 35–70 mesh) column by gradient elution with $CHCl_3$/CH_3OH mixtures, increasing the methanol content. Fractions of 5 ml were collected. The fractions containing luteolin 7-*O*-glucoside were concentrated under reduced pressure. The glucoside was further purified by TLC, using silica gel plates and a mixture of 78.4 % CH_3OH, 19.6 % $CHCl_3$ and 2.0 % CH_3COOH (v/v/v). The identity of the glucoside was confirmed by comparison with the standard. Iodine vapors were used to detect the components.

RESULTS AND DISCUSSION

The relevant part of the chromatogram of the standard mixture is shown in Fig. 1d. As can be noticed, using the present elution method, a good separation of the investigated compounds is achieved. Although the separation between apigenin 7-*O*-glucoside and caffeic acid (corresponding to the peaks 4 and 5, respectively, in Fig. 1d) might seem unsatisfactory, the same were clearly distinct by their UV spectral data. This separation method, therefore, can be tested in rapid identification of flavonoids in similar plant extracts. It can be applied, for instance, to chemotaxonomic purposes [5].

Parts of the sample chromatograms are given in Fig.1a–c. It can be concluded from there that, as expected, the two extracts from *V. scardicolum* (Figs. 1a and 1b) differ by their overall composition. The presence of common components, however, although in rather different concentrations, can be also supposed. The chromatogram of the *M. scardicum* extract (Fig. 1c), on the other hand, is complicated by the large number of components (again in accordance with the intuitive expectations) with close retention times. Previous separation of the components by some other method in this case is recommended for further study.

According to the retention time (7.3 minutes) and the UV spectral data ($\lambda_{max,1}$ = 254 nm, $\lambda_{max,2}$ =348 nm), in the blossom extract of *V. scardicolum* (Fig. 1a) luteolin 7-*O*-glucoside (the sysematic name of the corresponding aglycon is: 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-

ran-4-one.) was detected. Its isolation by CC and TLC was as described in the experimental. The spectral UV data of the glucoside in chloroform solution corresponded to those in the literature [6]. In the leaf extract chromatogram (Fig. 1b) a very small peak (with retention time of about 13.2 minutes, as shown by the inbox figure) of caffeic acid (the systematic name: 3-(3,4-dihydroxyphenyl)-2-propenoic acid.) appeared (λ_{max} = 329 nm). The water layer of the *M. scardicum* extract (as it can be seen from Fig. 1c) showed the presence of luteolin (at t_r = 22.4 min, $\lambda_{max,1}$ = 253 nm, $\lambda_{max,2}$ = 348 nm), luteolin 7-*O*-glucoside (at t_r = 7.3 min, $\lambda_{max,1}$ = 254 nm, $\lambda_{max,2}$ = 347 nm) and apigenin 7-*O*-glucoside [the systematic name of the corresponding aglycon is: 5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one] (at t_r = 11.6 minutes, $\lambda_{max,1}$ =265 nm, $\lambda_{max,2}$ = 336 nm). Besides the detected components, the spectral feature characteristic for the flavonoids followed several other peaks in each chromatogram. The characterization of these components, however, is in progress and will be published in a subsequent paper.

According to the literature data, besides in *V. scardicolum* (this work), caffeic acid was also found in *V. phlomoides* [7, 8] and in *V. thapsiforme* [8]. Luteolin 7-*O*-glucoside was previously isolated from *V. lychnitis* [9], *V. phlomoides* [10] and *V. thapsiforme* [11], but contrary to them, *V. scardicolum* did not contain detectable amounts of apigenin and its 7-*O*-glucoside. These facts might lead towards the possible relation of *V. scardicolum* to *V. phlomoides* and *V. thapsiforme* and should be considered when making chemotaxonomical conclusions within the *Verbascum* group.

From the few data available about the flavonoid components of *Melampyrum*, luteolin 7-*O*-glucoside (besides detected in *M. scardicum* by this work) was isolated from *M. elatius* (together with the corresponding 7-*O*-arabinoside) [12].

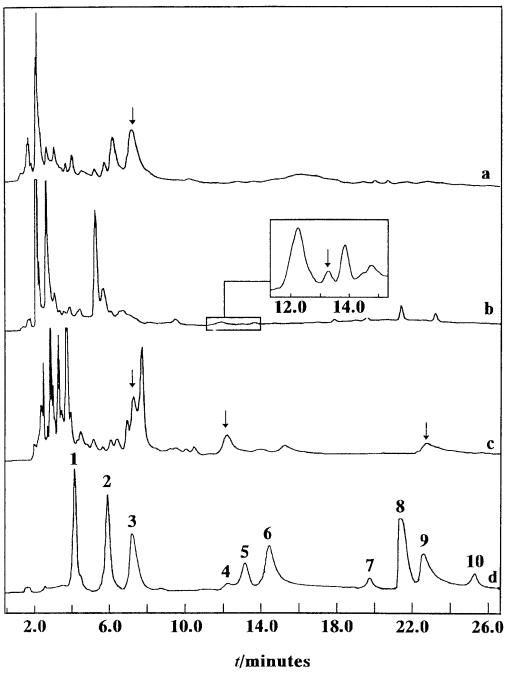

Fig. 1. Part of the chromatograms (at 254 nm) of the extracts of *V. scardicolum* blossoms (a), *V. scardicolum* leaves (b), *M. scardicum* (c) and the standard mixture (d)
1 – rosmarinic acid, 2 – rutin, 3 – luteolin 7-*O*-glucoside, 4 – apigenin 7-*O*-glucoside, 5 – caffeic acid, 6 – myricetin, 7 – eriodictyol, 8 – quercetin, 9 – luteolin, 10 – naringenin

In the course of further research, detection and isolation of the components that are specific for these endemic species will be of interest. In this sense, a development of the method as well as modifications in the extraction and purification will be needed.

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Р е з и м е

ФЛАВОНОИДИ КАЈ *VERBASCUM SCARDICOLUM* И *MELAMPYRUM SCARDICUM*

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Клучни зборови: флавоноиди; *Verbascum; Melampyrum*; реверзно-фазна високоефикасна течна хроматографија

Надземни делови од два ендемични растителни вида, *Verbascum scardicolum* и *Melampyrum scardicum*, се испитани за присуството на дваесет и еден флавоноид и две фенолни киселини со реверзно-фазна високоефикасна течна хроматографија. Компонентите се детектирани со споредба со стандарди. Со хроматографски методи од цветови на *V. scardicolum* изолиран и пречистен е лутеолин 7-*O*-глукозид. Во екстрактот од листови на *V. scardicolum* е детектирана кофеинска киселина. Во екстрактот од *M. scardicum* е утврдено присуство на лутеолин, лутеолин 7-*O*-глукозид и апигенин 7-*O*-глукозид. Резултатите се споредени со литературни податоци за други видови од родовите *Verbascum* и *Melampyrum*.