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Short communication

SHORT COMMUNICATION

Flavonoids from flowers of *Cephalaria pastricensis* and their antiradical activity*

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Abstract: Two flavonoid glycosides **1** and **2** having the luteolin structure were isolated from flowers of the endemic plant species *Cephalaria pastricensis*. They were identified by ¹H and ¹³C NMR, as well as UV/Vis spectroscopy. The structures of **1** and **2** were also confirmed by the spectral data of aglycones and TLC of the sugars obtained after acid hydrolysis. Flavones **1** and **2** showed significant antiradical activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

Keywords: *Cephalaria pastricensis*, luteolin 7-O-glucoside, luteolin 7-O-arabino(1-6)glucoside, antiradical activity, DPPH assay.

INTRODUCTION

The genus *Cephalaria* (family Dipsacaceae) comprises about 30 species, which are widespread, mainly in the Mediterranean region and the Middle East. *Cephalaria pastricensis* Doerfl. et Hay is an endemic species that can be found on the high mountains of North Albania, Montenegro and the Serbian–Bosnian border.¹ No previous phytochemical work on this species has been reported. Some isolation studies were carried out on several *Cephalaria* species, which have been used in folk medicine for their hypothermal, alleviative, relaxant and anti-infective activities.²

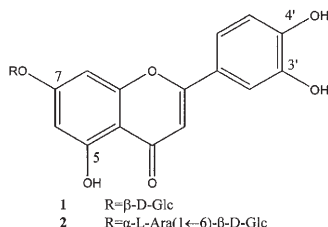
Hitherto, quite a few biologically active secondary metabolites have been isolated from the species of this genus: triterpenoid saponins, iridoid glycosides, and flavonoids.^{3–5}

Flavonoids comprise a large group of secondary plant metabolites. Presently more than 5000 individual compounds are known. Their function in plants also in-

* Dedicated to Professor Živorad Čeković on the occasion of his 70th birthday.

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volves the absorption of UV light and radical scavenging. There is an increasing interest in antioxidants such as flavonoids, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of food. Numerous papers exist on their inhibition of lipid peroxidation. Due to their structural variety, the flavonoids also offer themselves for detailed structure-activity relationship (SAR) studies.⁶ The antioxidant effect of flavonoids can reside both in their radical-scavenging activity or in their metal-chelating properties, of which the former may dominate.

In this paper, the isolation and structural elucidation of two flavonoids, luteolin 7-glucoside (**1**) and luteolin 7-arabino(1-6)glucoside (**2**) are reported. Their antiradical properties were measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test.

EXPERIMENTAL

General

¹H (200 MHz) and ¹³C (50 MHz) NMR spectra were recorded on a Varian Gemini 2000 spectrometer in DMSO-d₆. UV spectra and optical density were measured on a Cintra 40 UV-visible spectrometer. Silica gel (0.063–0.200 mm) and Sephadex LH-20 were used for column chromatography (CC). Silica gel F-254 was used for analytical thin layer chromatography (TLC).

Plant material

The flowers of *Cephalaria pastricensis* were collected in July 2000 at Veliki Stolac, mount Zvijezda, Serbian–Bosnian border. A voucher specimen, with the accession number CP28072003, is deposited in the Herbarium of the Faculty of Biology, University of Belgrade – Herbarium Code BEOU.

Extraction and isolation

The ground, air-dried flowers (150 g) were extracted with methanol–water (9:1, 3×400 ml) at room temperature. The solvent was evaporated under reduced pressure and 6.5 g of extract was obtained. This was fractionated on Sephadex LH-20 using methanol as the eluent. The fractions containing flavonoids (analysed by TLC) were combined to yield 3.5 g after evaporation of the solvent.

The crude mixture of flavonoids was subjected to silica gel column chromatography. The elution was commenced with chloroform–methanol–water (20:6.5:0.5) and the polarity was gradually increased (20:10:1, A; 20:11:2, B; 20:18:6, C). Fractions (55×10 ml) were collected and analyzed by TLC. Compounds **1** (15 mg) and **2** (20 mg) were precipitated as yellow crystals from the fractions eluted with B and C, respectively.

Acid hydrolysis of flavonoids

Solutions of compound **1** (5 mg) and **2** (5 mg) in 5 ml 6% HCl were heated for 5 h. The reaction mixture was extracted with EtOAc. The EtOAc fraction (aglycone) and the aqueous fraction (sugars) were concentrated for identification. The sugars were identified by TLC (acetonitrile–water 85:15) by comparison with authentic samples.

DPPH assay

The DPPH assay was performed according to the modified method of Kirbi and Schmidt.⁷ The analytical samples for DPPH measurements (in triplicate) were prepared by mixing methanolic solutions of compounds **1** and **2** (200 μ l), at 7 different concentrations, with 1000 μ l of 500 μ M DPPH (in MeOH) and 800 μ l of 100 mM Tris buffer (pH 7.4). The samples were shaken vigorously and kept in the dark for 30 min. The absorbance of the samples was measured at 517 nm. The DPPH control (containing no sample) was prepared using the same procedure.

RESULTS AND DISCUSSION

The structural assignments of **1** and **2** were based on the identity of their spectral data to those published in the literature.⁸ The constitution of the aromatic parts was also confirmed by comparison of the spectra of the aglycones, obtained by acid hydrolysis, to those of luteolin. The sugar anomeric configurations were confirmed by their $J_{H,H}$ -coupling constants.

While luteolin 7-glucoside is widespread in plant species,^{9–11} and also in some *Cephalaria* species,¹² isolation of luteolin 7-arabino(1–6)glucoside has been reported in only one paper to date.⁸

The DPPH radical scavenging method is a standard procedure applied to the evaluation of anti-radical activity.¹³ A parameter introduced recently for the interpretation of the results from the DPPH method is the “efficient concentration” or EC_{50} value. This is defined as the concentration of the substrate that causes 50 % loss of the DPPH activity. The EC_{50} values were calculated by the linear regression method of plots of the percent of antiradical activity against the concentration of the tested compounds.¹⁴

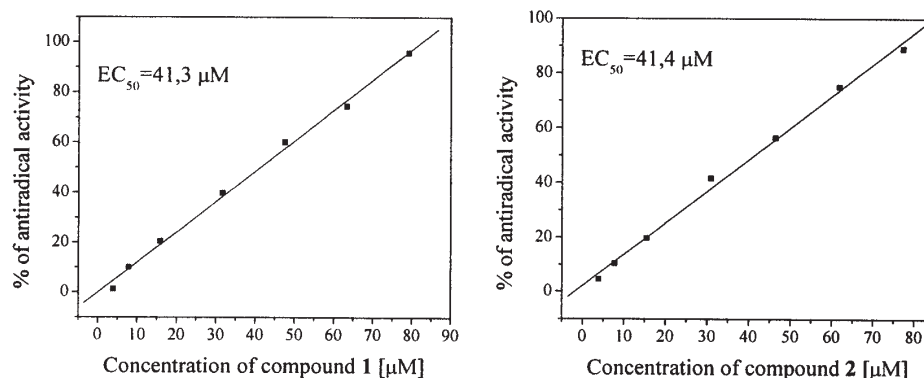


Fig. 1. Anti-radical activity of the flavonoids from *Cephalaria pastricensis*.

As shown in Fig. 1, the isolated flavonoids demonstrated almost the same significant inhibitory activity. The similar activity of **1** and **2** is to be expected, since they exhibit the same oxygenation pattern. This also fits to reported structure–activity relationship studies.¹⁵

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ИЗВОД

ФЛАВОНОИДИ ИЗ ЦВЕТОВА *Cephalaria pastricensis* И ЊИХОВА
АНТИРАДИКАЛСКА АКТИВНОСТДЕЈАН ГОЂЕВАЦ¹, ВЛАТКА ВАЈС¹, НЕБОЈША МЕНКОВИЋ², ВЕЛЕ ТЕШЕВИЋ³, ПЕЂА
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Из цветова *Cephalaria pastricensis* изолована су два хетерозидна флавоноида са лутеолинским агликоном (**1** и **2**). Њихова структура је одређена применом ¹H и ¹³C-NMR и UV спектроскопије. Структуре флавона **1** и **2** су потврђене и спектрима агликона, односно танкослојном хроматографијом ослобођених шећера након киселе хидролизе. Једињења **1** и **2** су показала значајну антирадикалску активност у 1,1-дифенил-2 пикрилхидразил (DPPH) тесту.

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