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HPLC–DAD of phenolics in bryophytes *Lunularia cruciata*, *Brachytheciastrum velutinum* and *Kindbergia praelonga*

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Abstract: The chemistry of bryophytes is not well known. The available data indicate interesting chemical constitutions of some bryophyte species, *i.e.*, active and new compounds are to be found within bryophytes, especially liverworts. In this study, one liverwort and two moss species were studied: *Lunularia cruciata* (L.) Dumort, *Brachytheciastrum velutinum* (Hedw) Ignatov & Huttunen and *Kindbergia praelonga* (Hedw) Ochyra. The phenolic compositions of these bryophyte species have not hitherto been reported. Their methanolic extracts were analyzed by reversed-phase HPLC, coupled to a diode-array detector (DAD). Luteolin-7-*O*-glucoside and quercetin were found in the *L. cruciata* extract. The extract obtained from *B. velutinum* contained four phenolic acids (4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, caffeic and ellagic acids) and three flavonoids (apigenin-7-*O*-glucoside, luteolin and apigenin). The *K. praelonga* extract was characterized by the presence of several phenolic acids and their derivatives (4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, caffeic, *p*-coumaric, ferulic and ellagic acids, and caffeic and *p*-coumaric acid derivatives) and three flavonoids (apigenin-7-*O*-glucoside, luteolin, apigenin and an unidentified flavanone).

Keywords: bryophytes; phenolics; *Lunularia cruciata*; *Brachytheciastrum velutinum*; *Kindbergia praelonga*.

INTRODUCTION

Bryophytes (mosses, liverworts and hornworts) with approximately 15,000–25,000 species[1] are, after flowering plants, worldwide the most diverse plant group. They are to be found in all ecosystems, from desert to alpine, except marine, and the bryophyte biomass productivities can vary in each ecosystem, from

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negligible to the most significant producers. However, the ecological role of bryophytes in any ecosystems is significant.

The chemistry of bryophytes is poorly known and the results on are very scattered.[2–4] The reason for this is the difficulty in identification and small amount of the same species available for analyses, usually by sophisticated methods. Liverworts are very interesting for chemical analysis due to their oil bodies containing many scientifically new compounds.

However, worldwide bryophytes are known to be used in ethno-botany and are applied to cure diseases, threat to plants and animals, or in the household.[5–7] Therefore, bryophytes are indicated as a source of chemically new and unknown compounds.[3,4,8–10] Studies of the chemical constituents of bryophytes were recently performed but are still inadequate and neglected.[2,11–18] These data help in the systematics of barely morphologically classified bryophytes.[19] Also, some scattered data on the biological activities of bryophyte extracts and/or chemical constituents are available for not very many bryophyte taxa.[20–23]

Generally, based on the species studied to date, bryophytes are known to possess extremely high amounts of terpenoids, phenolics (flavonoids and bibenzyl derivatives), glycosides, fatty acids and also some rare aromatic compounds. Bryophytes are considered as a "remarkable reservoir" of new, natural products or secondary compounds, many of which have shown interesting biological activity. These activities of bryophytes include antimicrobial, antifungal, cytotoxic, antitumor, vasopressin (VP) antagonist, cardiotonic, allergy causing, irritancy and tumour effecting, insect anti-feedant, insecticidal, molluscicidal, pesticidal, plant growth regulatory, superoxide anion radical release inhibition and 5-lipoxygenase, calmodulin, hyaluronidase and cyclooxygenase inhibition features. Some latest results also predict a beneficial influence of bryophytes in AIDS therapy (some bibenzyls of liverworts).[24–36]

The liverwort *Lunularia cruciata*, a Mediterranean Atlantic species, expresses antimicrobial and, to a less extent, antifungal activities.[37–39] The plant--growth-regulator lunularic acid was isolated for the first time from this species.[40] The chemical constituents of *L. cruciata* are unknown.[4,41]

The palearctic mosses *Brachytheciastrum velutinum* and *Kindbergia praelonga* have hitherto not been chemically screened; nor are their bioactive effects known.[4]

EXPERIMENTAL

Samples

Fresh material was collected in July 2003 in the Oporto City Park (Portugal). A voucher of each Bryophyte sample is deposited in the Bryophyte Collection of Belgrade University (BEOU).

The material was cleaned and dried to constant weight at room temperature.

Extraction of phenolics

5 g dry mass of each bryophyte sample was used for the extraction of the phenolics. The material was previously ground in an electric mill to a rough powder. The extraction consisted of two consecutive steps employing 175 and 125 mL methanol, respectively, on a magnetic stirrer for 10 min. These two extracts were combined and the solvent removed under reduced pressure at 30 °C To this residue, 20 mL of 2.0 M HCl were added and the obtained solution was passed through a C18 Bond Elut cartridge, preconditioned with methanol and 2.0 M HCl. The retained phenolics were eluted with methanol. This solution was taken to dryness under reduced pressure (30 °C), dissolved in methanol and 20 μL were analyzed by HPLC–DAD.

HPLC–DAD analysis of the phenolics

The extracts were analyzed on an analytical HPLC instrument (Gilson), using a Spherisorb ODS2 column (25.0 cm×0.46 cm; 5 μm particle size Waters, Milford, MA, USA) with a C18 ODS guard column. The mobile phase consisted of solvent A (water–formic acid (19:1)) and solvent B (methanol) (Table I).

The flow rate was 0.9 mL/min and the injection volume 20 μL. Detection was performed using a Gilson diode array detector. The phenolic compounds in each sample were identified by comparing their retention times and UV–Vis spectra in the 200–600 nm range with individual standards. The chromatograms were registered at 280, 320 and 350 nm.

TABLE I. Gradient flow

Time, min	Solvent A content, %	Solvent B content, %
0.00	95	5
3.00	85	15
13.00	75	25
25.00	70	30
35.00	65	35
39.00	60	40
42.00	55	45
44.00	50	50
47.00	45	55
50.00	30	70
56.00	25	75
60.00	0	100
62.00	5	95

RESULTS AND DISCUSSION

The chromatogram of the methanol extract of *Lunularia cruciata* is presented in Fig. 1. Based on a comparison of the retention time (R_t) and UV–Vis spectra with standard substances, the presence of the flavonoid heteroside luteolin-7-*O*-glucoside and the flavonoid aglycone quercetin was confirmed. The presence of these two compounds is for the first time reported in *L. cruciata.*

The chromatogram of the methanol extract of *Brachytheciastrum velutinum* is presented in Fig. 2. The following substances were evidenced as constituents of this species: phenolic acids, *i.e.*, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, caffeic and ellagic acid, flavonoids, *i.e.*, heteroside apigenin-7-*O*-glucoside, and flavonoid aglycones, *i.e.*, luteolin and apigenin.

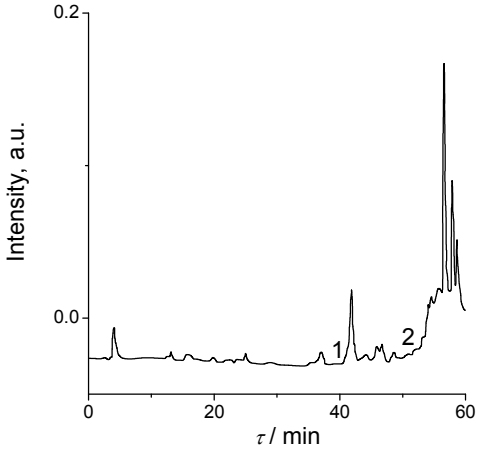

Fig. 1. Chromatograms of the methanol extract of liverwort *L. cruciata*: luteolin-7-*O*--glucoside (1) and quercetin (2).

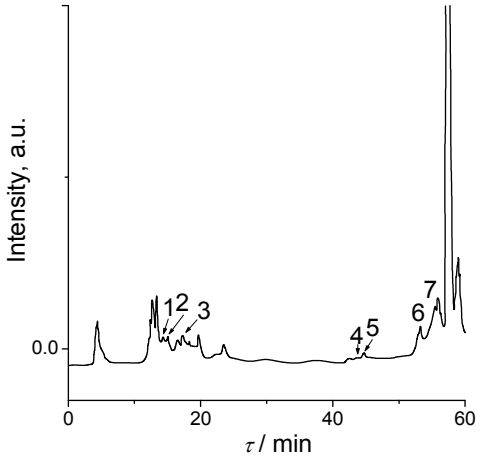

Fig. 2. Chromatogram of the moss methanol extract of *B. velutinum*: 4-*O*-caffeoylquinic acid (1), 5-*O*-caffeoylquinic acid (2), caffeic acid (3) and ellagic acid (5), apigenin-7-*O*-glucoside (4), luteolin (6) and apigenine (7).

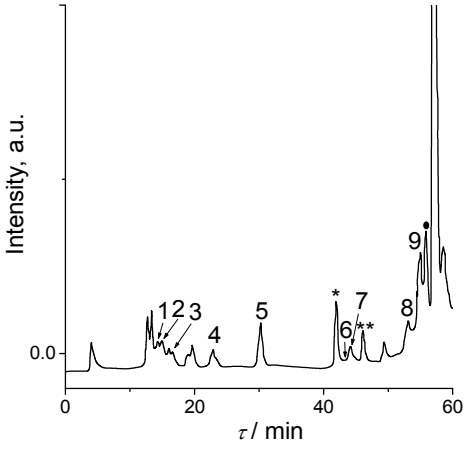

Fig. 3. Chromatogram of the methanol extract of the moss *K. praelonga*: 4-*O*-caffeoylquinic acid (1), 5-*O*-caffeoylquinic acid (2), caffeic acid (3), *p*-coumaric acid (4), ferulic acid (5), ellagic acid (7), caffeic acid derivative (*), *p*-coumaric acid derivative (**), apigenin-7-*O*--glucoside (6), luteolin (8), apigenin (9) and unidentified flavanone (●).

In the methanol extract of the moss *Kindbergia praelonga*, 4-*O*-caffeoylquinic, 5-*O*-caffeoylquinic, caffeic, *p*-coumaric, ferulic and ellagic acid, caffeic acid derivative, *p*-coumaric acid derivative, flavonoid heteroside apigenin-7-*O*-glucoside, aglycones luteolin and apigenin, as well as one unidentified flavanone were evidenced, as shown in Fig. 3.

The chemical contents of *B. velutinum* and *K. praelonga* have not been screened previously.

Luteolin is present in many vascular plants, especially from the family Resedaceae, *Genista tinctoria* (Fabaceae) and *Petroselinum crispum* (Apiaceae).[42] However, the heteroside form of luteolin-7-*O*-glucoside is not common and this compound was not previously known from *L. cruciata*. This form is known from some *Mentha* plants.[43] The yellowish pigment quercetin is widespread in many plants but was not detected previously in *L. cruciata*. Quercetin was found to be the most biologically active of the flavonoids and many medicinal plants owe much of their activity to their high quercetin content.[44]

Artichoke (*Cynara scolymus*) is known to have rich content of 4-*O*-caffeoylquinic and 5-*O*-caffeoylquinic acids.[45] Previously they were not evidenced from mosses among the other phenolic acids.[4]

Caffeic acid is already known from some mosses.[46] Apigenine is a pale yellow pigment present in many plants from the families Apiaceae and Asteraceae with an antitumor effect. Apigenin and its derivates are known to be present in mosses and to have biological effects.[47] In mosses, *p*-coumaric and ferulic acids are known to be present in moss spores. They are precursors of lignin, which is not common in moss gametophytes, but both *p*-coumaric and ferulic are present in moss gametophytes where lignin was not detected.[48]

Although phenolic compounds are known to be present in bryophytes, this knowledge is mainly based on liverworts not mosses and their presence; diversity and distribution within different species remain for further studies.[49–51]

Thus, the paper presents one first approach to the identification of phenolics in the bryophytes *L. cruciata*, *B. velutinum* and *K. praelonga*, until now unknown.

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ИЗВОД

HPLC–DAD ФЕНОЛА КОД БРИОФИТА *Lunularia cruciata*, *Brachytheciastrum velutinum* И *Kindbergia praelonga*

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Хемијски састав бриофита је слабо познат. Досадашњи подаци указују на интересантне хемијске састојке бриофита, биолошки активна и нова једињења, нарочито код јетрењача. У овом раду изучаване су једна јетрењача *Lunularia cruciata* (L.) Dumort и две маховине *Brachytheciastrum velutinum* (Hedw) Ignatov & Huttunen и *Kindbergia praelonga* (Hedw) Ochyra. Фенолни састав ових врста бриофита од раније није познат. Њихови метанолни екстракти су анализирани путем HPLC типа реверсне фазе, повезаног са DAD детектором. U екстракту *L. cruciata* пронађени су лутеолин-7-*O*-глукозид и кверцетин. Екстракт добијен од *B. velutinum* показао је присуство четири фенолне киселине (4-*O*-кафеоилхина, 5-*O*-кафеоилхина, кофеинска и елагинска киселина) и три флавоноида (флавоноидни агликони лутеолин и апиге-

нин, и његов хетерозид апигенин-7-*O*-глукозид). Екстракт од *K. praelonga* је окарактерисан присуством неколико фенолних киселина и њихових деривата (4-*O*-кафеоилхина, 5-*O*-кафеоилхина, кофеинска, *н*-кумаринска, ферула и елагинска киселина, деривати кофеинске и *н*-кумаринске киселине) и следећих флавоноида: апигенина, апигенин-7-*O*-глукозида, лутеолина и једног неидентификованог флаванона.

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