

# Fatty acid chemistry of *Atrichum undulatum* and *Hypnum andoi*

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## Abstract

The fatty acid compositions of the moss species *Atrichum undulatum* (Hedw.) P. Beauv. (Polytrichaceae) and *Hypnum andoi* A.J.E. Sm. (Hypnaceae) collected in winter time were analysed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) as a contribution to their chemistry. Eight fatty acids were identified in the chloroform/methanol extract 1:1 of *A. undulatum* (linoleic acid, 26.80%, palmitic acid, 22.17%,  $\alpha$ -linolenic acid, 20.50%, oleic acid, 18.49%, arachidonic acid, 6.21%, stearic acid, 3.34%, *cis*-5,8,11,14,17-eicosapentaenoic acid, 1.52% and behenic acid, 1.01%), while six fatty acids were found in the same type of extract of *H. andoi* (palmitic acid, 63.48%, erucic acid, 12.38%, stearic acid, 8.08%, behenic acid, 6.26%, lignoceric acid, 5.16% and arachidic acid, 4.64%). According to this study, the moss *A. undulatum* can be considered as a good source of both essential fatty acids for humans (linoleic acid and  $\alpha$ -linolenic acid) during the winter.

**Keywords:** Bryophytes, Mosses, phytochemistry, GC FID, GC–MS, linoleic acid;  $\alpha$ -linolenic acid.

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Bryophytes are the second largest group of land plants, after the flowering plants, with about 15,000 to 25,000 species and many intraspecific taxa [1]. They have settled all known ecosystems from desert to arctic with the exception of seas. In general, bryophytes consist of three very diverse groups: hornworts, liverworts, and mosses. The phytochemistry of bryophytes has been neglected for a long time because they are very small and difficult to collect in large amounts as pure samples [2]. Today these plants are known to be a reservoir of interesting and useful chemicals such as lipophilic terpenoids [3,4], acetogenins [5] and bisbibenzyl compounds [6]. Indeed, many new compounds are described from bryophytes, mainly liverworts, which possess oil-bodies rich in terpenes and terpene like substances. Mosses, another group of bryophytes, remain less chemically studied since its members do not possess oil-bodies in their cells [7]. Fatty acids are known to be present within moss species but there are many assumptions and generalisations from small number of species studied [8–10]. Our continuing investigation of these compounds from mosses [11–15] has recently led to the identification of acetylenic fatty acids [16].

The objective of this study was to identify fatty acids of the moss species *Atrichum undulatum* (Hedw.) P. Beauv. (Polytrichaceae) and *Hypnum andoi* A.J.E. Sm.

(Hypnaceae) by gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS) in corresponding chloroform/methanol extracts 1:1 as a contribution to their chemistry. To the best of our knowledge, there is no previous report on fatty acid chemistry of *H. andoi*, while only one report is available to date in the case of *A. undulatum* collected in sun-exposed habitat during summer time [9].

## EXPERIMENTAL

Both mosses were collected in Germany in December 2007: *A. undulatum* in Königsforst near Köln and *H. andoi* in surrounding of Bonn. Voucher specimens were deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection – BEOU No. 4707 and 4709, respectively).

The moss samples were carefully selected and cleaned from soil and other contaminants. The gametophyte tips were used for the extraction. Air-dried parts of *A. undulatum* and *H. andoi* species were ground (1 g) and extracted three times with the system chloroform/methanol 1:1 for 1 h at room temperature (9.76 and 7.07% extract yield, respectively). The extracts were evaporated to dryness and further transesterified with 5% sulphuric acid in methanol (v/v) for 4 h at 80 °C. The resulting methyl esters of fatty acids were analysed by comparing their GC FID chromatograms with the chromatogram of a standard mixture (Supelco 37) obtained under the same conditions, and/or by analysis of GC–MS data using NIST 5 and Wiley 7

RESEARCH NOTE

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libraries. Three analysis replicates and three biological replicates were done.

The GC analyses were performed on an Agilent 7890A GC system equipped with a 5975C MSD and an FID detector, using a DB-23 column (30 m×0.25 mm×0.25 μm). The injection volume was 1 μL and injector temperature was 220 °C with 10:1 split ratio. The carrier gas was He at a flow rate 0.9 mL/min, while the column temperature was linearly programmed in the range of 150–240 °C at a rate of 4 °C/min and held at 240 °C for 10 min. The transfer line was heated at 240 °C. The FID detector temperature was 300 °C. The EI mass spectra (70 eV) were acquired in the *m/z* range 40–500.

## RESULTS AND DISCUSSION

Eight fatty acids were identified in *A. undulatum*: linoleic acid, 26.80%, palmitic acid, 22.17%, α-linolenic acid, 20.50%, oleic acid, 18.49%, arachidonic acid, 6.21%, stearic acid, 3.34%, *cis*-5,8,11,14,17-eicosapentaenoic acid, 1.52% and behenic acid, 1.01%, while six fatty acids were found in *H. andoi*: palmitic acid, 63.48%, erucic acid, 12.38%, stearic acid, 8.08%, behenic acid, 6.26%, lignoceric acid, 5.16% and arachidonic acid, 4.64%.

The obtained results for *A. undulatum* are in good agreement with previous studies [9], since arachidonic acid and *cis*-5,8,11,14,17-eicosapentaenoic acid in monogalactosyldiacylglycerol (MGDG) fraction have been presented in small-scale as well (8 and 1%, respectively). This moss species collected in winter time can be considered as a good source for *in vitro* production of both essential fatty acids for humans, linoleic acid and α-linolenic acid [17]. A possible and the most appropriate way for the production of large amounts of *A. undulatum* that can be used for the isolation of highlighted fatty acids are corresponding *in vitro* culture. Therefore, the axenical culture of *A. undulatum* has been established. Further studies are needed to compare the content of fatty acids of *in vitro* grown and the plants from natural habitats.

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**IZVOD****HEMIJA MASNIH KISELINA *Atrichum undulatum* I *Hypnum andoi***Boris Pejin<sup>1</sup>, Ljubodrag Vujsić<sup>1</sup>, Marko Sabovljević<sup>2</sup>, Vele Tešević<sup>1</sup>, Vlatka Vajs<sup>3</sup><sup>1</sup>Univerzitet u Beogradu, Hemijski fakultet, Katedra za organsku hemiju, Beograd, Srbija<sup>2</sup>Univerzitet u Beogradu, Institut za botaniku i Botanička bašta, Biološki fakultet, Beograd, Srbija<sup>3</sup>Univerzitet u Beogradu, Institut za hemiju, tehnologiju i metalurgiju, Centar za hemiju, Beograd, Srbija

(Kratko saopštenje)

Kao doprinos izučavanju njihovog hemijskog sastava, u ovom radu GC i GC–MS analizom ispitivane su masne kiseline mahovina *Atrichum undulatum* (Hedw.) P. Beauv. (Polytrichaceae) i *Hypnum andoi* A.J.E. Sm. (Hypnaceae) sakupljene za vreme zimskog doba. U ekstraktu hloroform/metanol 1:1 vrste *A. undulatum* identifikovano je osam viših masnih kiselina (linolna kiselina, 26,80%, palmitinska kiselina, 22,17%,  $\alpha$ -linolenska kiselina, 20,50%, oleinska kiselina, 18,49%, arahidonska kiselina, 6,21%, stearinska kiselina, 3,34%, *cis*-5,8,11,14,17-eikosapentaenska kiselina, 1,52% i behenska kiselina, 1,01%), dok je u istom ekstraktu vrste *H. andoi* nađeno šest viših masnih kiselina (palmitinska kiselina, 63,48%, erukinska kiselina, 12,38%, stearinska kiselina, 8,08%, behenska kiselina, 6,26%, lignocerinska kiselina, 5,16% i arahidinska kiselina, 4,64%). Dobijeni rezultati ukazuju da u ispitivnom vremenskom periodu mahovina *A. undulatum* predstavlja dobar izvor linolne i  $\alpha$ -linolenske kiseline, esencijalnih masnih kiselina za ljude.

*Ključne reči:* Briofite • Mahovine • Fito-hemija • GC FID • GC–MS • Linolna kiselina •  $\alpha$ -Linolenska kiselina