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Short communication

SHORT COMMUNICATION

Preliminary analysis of fatty acid chemistry of *Kindbergia praelonga* and *Kindbergia stokesii* (Brachytheciaceae)

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Abstract: Moss species of the family Brachytheciaceae, *Kindbergia praelonga* (Hedw.) Ochyra and *Kindbergia stokesii* (Turn.) Ochyra, were preliminarily analysed for their fatty acid composition with the aim of studying the chemical relationship of these two entities. Fatty acid methyl esters were examined by GC and GC–MS in their methanol extracts. Thirteen fatty acids were identified. It is likely that the mosses are chemically distinguishable and should be treated as separate entities. However, additional chemical constituents of various moss samples, such as phenolic acids, their derivatives and flavonoids, must be also analyzed in order to support the re-examination of the relationship between these two species.

Keywords: bryophytes; mosses; *Kindbergia*; fatty acids; chemotaxonomy.

INTRODUCTION

Kindbergia praelonga (Hedw.) Ochyra (typified by *Hypnum praelongum* Hedw.) and *Kindbergia stokesii* (Turn.) Ochyra (typified by *Hypnum stokesii* Turn.) belong to the subgenus *Oxyrrhynchium*. The latter species is now usually considered as a synonym for or variety of *K. praelonga*. The genus *Kindbergia* Ochyra was recently treated as a separate genus from the *Eurhynchium* Schimp.[1,2] According to Hill *et al.*,[1] in Europe only one species is present within the genus: *K. praelonga* (Hedw.) Ochyra (syn. *Eurhynchium praelongum* (Hedw.) Schimp.). The same authors classified *Eurhynchium praelongum* var. *stokesii* (Turner) Dixon and *Eurhynchium stokesii* (Turner) Schimp. as a syno-

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nym for *K. praelonga*. These two entities were separated into the genus *Stokesiella* (Kindb.) H. Rob., which was later considered as an incorrect homonym of the algal generic name *Stokesiella* Lemmerm. Ochyra renamed the genus *Kindbergia* Ochyra. Now molecular results support this generic independence.[3]

Worldwide, eleven taxa found at various locations are known, namely *K. africana* (Herz.) Ochyra, *K. altaica* Ignatov, *K. arbuscula* (Broth.) Ochyra, *K. brittoniae* (Grout) Ochyra, *K. dumosa* Mitt., *K. kenyae* (Dix. Ex Tosco & Piovano) O'Schea *et* Ochyra, *K. oedogonium* (C. Müll.) Ochyra, *K. oregana* (Sull.) Ochyra, *K. praelonga*, *K. stokesii* and *K. squarrifolia* Broth. *ex* Iishiba.

The main reason for the repeated nomenclatural confusion and the resulting complications surrounding *Oxyrrhynchium* is that the name *E. praelongum* was widely used with two different meanings. Schimper employed it for *E. hians* (syn. *Hypnum hians*, *Oxyrrhynchium hians* (Hedw.) Loeske) including the conspecific *E. swartzii* Turn. (syn. *Hypnum swartzii* Turn.) and several closely related elements) for varietal synonymy, whereas his *Eurhynchium stokesii* corresponded to *E. praelongum*, or following the recent nomenclature *K. praelonga*. Although many bryologists knew of the problem, frequently the use of the name *E. praelongum*, instead of *E. hians*, continued.[4] Thus, *K. stokesii* was often easily overlooked.

Analyses were performed to test if chemotaxonomy can be helpful in the assignment of the two entity species, *K. praelonga* and *K. stokesii*.

EXPERIMENTAL

Both moss species, which were available as fresh material, were collected in Germany in December 2007: *Kindbergia praelonga* (Hedw.) Ochyra (BEOU4701) in Kologne and *Kindbergia stokesii* (Turn.) Ochyra (BEOU4703) in the surroundings of Bonn. Voucher specimens were deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (bryophyte collection – BEOU).

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 both mosses were ground (1 g) and extracted 3 times with 90 % MeOH for 1 h at room temperature. The extracts were evaporated to dryness and were further transesterified with 5 % H_2SO_4 in MeOH (v/v) for 4 h at 80 °C. The resulting methyl esters of the 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 libraries.

The GC analyses were performed on an Agilent 7890A GC system equipped with a 5975C MSD and an FID, 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^{-1}, while the column temperature was linearly programmed in the range of 150–240 °C at a rate of 4 °C min^{-1} and held at 240 °C for 10 min. The transfer line was maintained 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

For *K. stokesii*, 13 fatty acids were identified (Fig. 1): palmitic acid (C16:0, 25.04 %), arachidonic acid (C20:4,*n*-6, 18.29 %), linolelaidic acid (C18:2,*n*-6*t*, 14.57 %), *α*-linolenic acid (C18:3,*n*-3, 11.13 %), *cis*-5,8,11,14,17-eicosapentaenoic acid (C20:3,*n*-6, 8.94 %), elaidic acid (C18:1,*n*-9*t*, 6.32 %), behenic acid (C22:0, 3.04 %), lignoceric acid (C24:0, 2.90 %), palmitoleic acid (C16:1, 2.76 %), stearic acid (C18:0, 2.31 %), myristic acid (C14:0, 1.31 %), oleic acid (C18:1,*n*-9*c*, 1.30 %) and arachidic acid (C20:0, 1.28 %). *K. praelongum* showed less variety in these constituents: only two fatty acid constituents were found (Fig. 2), palmitic acid (C16:0, 88.58 %) and stearic acid (C18:0, 11.42 %).

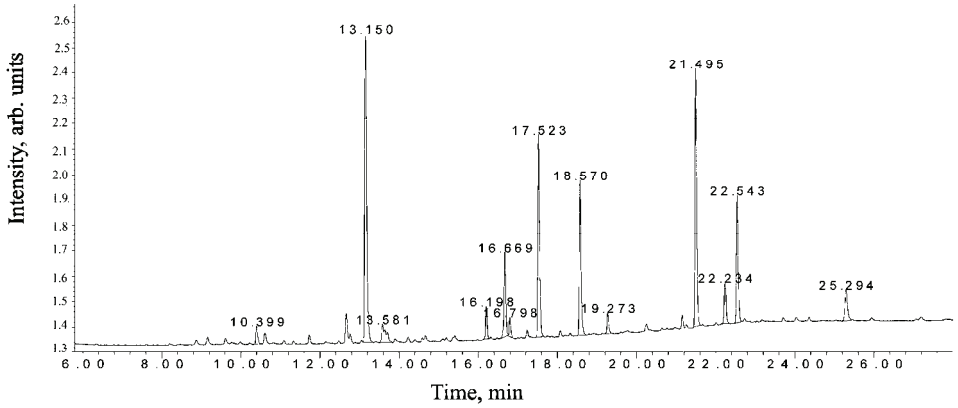

Fig. 1. Fatty acid methyl ester chromatogram for *K. stokesii*; myristic acid (*RT* 10.399 min); palmitic acid (*RT* 13.150 min); palmitoleic acid (*RT* 13.581 min); stearic acid (*RT* 16.198 min); elaidic acid (*RT* 16.669 min); oleic acid (*RT* 16.798 min); linolelaidic acid (*RT* 17.523 min); *α*-linolenic acid (*RT* 18.570 min); arachidic acid (*RT* 19.273 min); arachidonic acid (*RT* 21.495 min); behenic acid (*RT* 22.234 min); *cis*-5,8,11,14,17-eicosapentaenoic acid (*RT* 22.543 min) and lignoceric acid (*RT* 25.294 min).

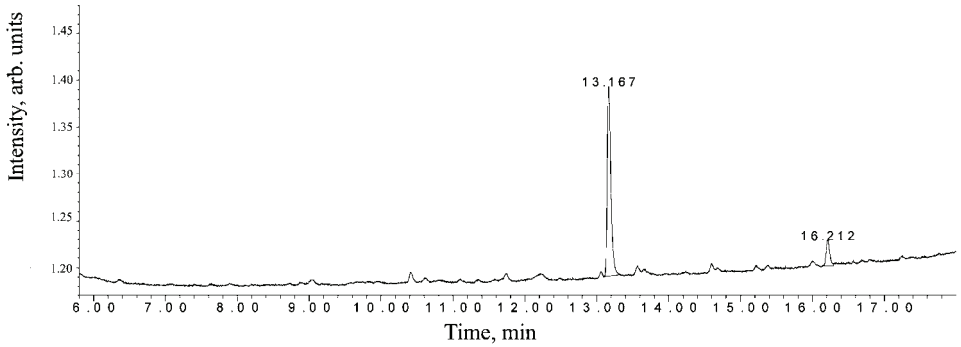

Fig. 2. Fatty acid methyl ester chromatogram for *K. praelongum*; palmitic acid (*RT* 13.167 min) and stearic acid (*RT* 16.212 min).

CONCLUSIONS

The fatty acid composition of the two related and often synonymized *Kindbergia* species strongly suggested that they are chemically distinguishable and, thus, could be treated as separate entities. This, however, has still to be confirmed by the analyses of additional chemical constituents, such as phenolic acids and their derivatives as well as flavonoids,[5] of various moss samples in order to support a re-examination of the relationship between them. For a general consideration of the quality of fatty acid profiling for chemotaxonomy, more replicates and additional species would have to be included in a follow-up study.

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И З В О Д

ПРЕЛИМИНАРНА АНАЛИЗА ХЕМИЈЕ МАСНИХ КИСЕЛИНА ВРСТА *Kindbergia praelonga* И *Kindbergia stokesii* (BRACHYTHECIACEAE)

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Прелиминарно је испитиван састав виших масних киселина две маховине из фамилије Brachytheciaceae, *Kindbergia praelonga* (Hedw.) Ochyra и *Kindbergia stokesii* (Turn.) Ochyra, са хемотаксономским циљем. Укупно је идентификовано 13 виших масних киселина GC и GC–MS анализом. На основу добијених експерименталних резултата се може закључити да се наведене биљне врсте значајно хемијски разликују и да би се могле сматрати засебним ентитетима уколико се то потврди и додатним анализама.

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REFERENCES

1. M. O. Hill, N. Bell, M. A. Bruggemann-Nannenga, M. Brugues, M. J. Cano, J. Enroth, K. I. Flatberg, J.-P. Frahm, M. T. Gallego, R. Garilleti, J. Guerra, L. Hedenäs, D. T. Holyoak, J. Hyvönen, M. S. Ignatov, F. Lara, V. Mazimpaka, J. Munoz, L. Söderström, *J. Bryol.* **28** (2006) 198
2. M. Sabovljević, R. Natcheva, G. Dihoru, E. Tsakiri, S. Dragićević, A. Erdag, B. Papp, *Phytol. Balcan.* **14** (2008) 159
3. M. S. Ignatov, S. Huttunen, *Arctoa* **11** (2002) 245
4. M. Ignatov, P. Isoviita, *Taxon* **52** (2003) 352
5. N. Jocković, P. B. Andrade, P. Valentao, M. Sabovljević, *J. Serb. Chem. Soc.* **73** (2008) 1161.