DE GRUYTER OPEN

Material Science and Applied Chemistry doi: 10.1515/msac-2015-0011

2015/32

Solid Phase Extraction of Bryophyte Lipids

Laura Klavina[1], Jorens Kviesis[2],
[1–2]*University of Latvia*

***Abstract* — A simple, fast and reproducible analytical method for fractionation of freely available bryophyte lipids was developed. Lipid fractionation was achieved by solid phase extraction using porous silica and silica derivatized with -NH_2 groups and the compounds were quantified as their trimethylsilyl derivatives by gas chromatography-mass spectrometry. The suggested fractionation method allows to identify several groups of substances prospective in respect of search of new biologically active compounds in bryophytes as well as in respect of understanding of their metabolism.**

***Keywords* — Bryophytes, GC-MS, SPE, fractionation, lipids, sterols.**

I. Introduction

Bryophytes are the second largest taxonomic group in the plant kingdom (25000 species, including mosses, liverworts and hornworts) and during the last decades have been an object of intensive research, considering the presence of a large number of unique substances amongst their secondary metabolites [1], [2]. Bryophytes are considered to be an importance source of potentially biologically active compounds and already now their ability to suppress growth of fungi and antimicrobial activity has been demonstrated [3]. At the same time composition and metabolism of bryophytes is of interest from perspective of evolutionary biology and stress ecology, considering many unique properties of these plants, such as high drought resistance and others. Considering biological activity of bryophytes, studies of their secondary metabolites are of especial importance; number of studies on bryophyte secondary metabolites is rapidly growing. Recent publications sum up the knowledge on chemistry of bryophytes and highlight findings of several hundreds of new compounds with many interesting kinds of activities [2] and huge prospects to develop new applications in biomedicine. Still, major part of recent studies of biologically active compounds present in bryophytes have concentrated on relatively low-polar substances (lipids) found in oil-bodies of liverworts, but the most abundant group of bryophytes — leaf mosses are not studied as much. However, leaf mosses are the most widespread group of bryophytes in the Northern Hemisphere, are dominant in bogs and wetlands, occupying large areas (in Latvia ~ 10 % of the territory). Also, a high number of lipids, sterols, waxes, terpenoids and other substances have been found in mosses [4]; and their analysis, evaluation of factors affecting lipid composition and their changes could be of importance. Further reason to study moss lipids is related to their application as biomarkers [5] of processes characterizing climate change and paleoclimate reconstruction. The n-alkane distribution can be used as a sensitive indicator of chemotaxonomy of bryophyte species in bog environment and raised bog peats (Baas [5], [6]. Alkane composition and their ratios can be used as the hydrological regime indicator and higher C_{23}/C_{25} *n*-alkane values for long chain *n*-alkanes are found in sites with elevated groundwater levels and, thus, increased wetness [7], [8]. Lipid composition also provides information about contribution of different bryophytes to the development of peat, especially *Sphagnum* species [8], [9].

The large number of compounds appearing in bryophyte extracts (especially, if changes in their abundance are an object of the studies) requires fractionation approaches to study bryophyte secondary metabolite composition. The use of solid phase extraction on polymeric sorbents with a differing polarity and eluent systems (widely used for analysis of lipid classes of higher plants and animal fats and oils) can be considered to be a versatile method [10]. Solid phase extraction with polar adsorbents is often used to separate lipids and low-polarity compounds into groups of compounds, depending on their interaction with solid phase [11], [12].

The aim of this paper is to study the composition of bryophyte lipids and possibilities of their fractionation with solid phase extraction (SPE).

II. Materials and Methods

Materials. All solvents were analytical grade (*Sigma-Aldrich*). Internal standards (palmitic acid methyl ester, progesterone) were obtained from *Sigma-Aldrich* and were used as solutions in chloroform in the indicated concentrations. Pre-packed SPE cartridges SPE-NH_2 (500 mg, 3 ml) were obtained from *Chromabond*. Silica (60 Å) was obtained from *Sigma-Aldrich*. Silica (60 Å) was packed in 2.5×5 cm glass column. The vacuum manifold was from *Alltech*. For derivatization (*Fluka analytical*) N,O-bis(trimethylsilyl) trifluoroacetamide + trimethylchlorosilane were used.

Plant material. Bryophyte samples were collected in the summer of 2013 and 2014 in Cenas Bog and in the surrounding mixed forests (*Polytrichum commune*, *Dicranum polysetum* — common moss species for wet forests), Latvia. After collection the plants were dry cleaned of contamination of other bryophytes, needles, soil, peat, etc. The samples were stored at −20 °C. Voucher specimen was deposited in Laboratory of Environmental Quality, University of Latvia, Riga, Latvia.

Lipid extraction. Bryophyte samples (*Polytrichum commune* (PC), *Dicranum polysetum* (DP), were dried at +40 °C in an oven until mass was constant. Dry samples were ground in a mill, 1 g of bryophyte sample was weighed into 100 mL bottles with a screw cap and 40 mL of extrahent (Table I) was added. Afterwards samples were treated with of ultrasound (100 W) in an ultrasound bath (*Cole Parmer*) for 40 min, temperature was regulated with regular addition of

cold water to keep it constant at +40 °C. Then the bottles were shaken in a shaker for 24 h at 140 rpm. Extracts were filtered and stored until analysis at 4 °C for up to 1month. Extraction was done in triplicate and the yield (Table I) was estimated after removal of solvent at reduced pressure.

TABLE I

YIELD OF BRYOPHYTE LIPID EXTRACTS (MG/G)

Bryophyte species	C_6H_{12}	CH_2Cl_2	$CHCl_3$	$C_2H_5OOCCH_3$	$(C_2H_5)_2O$
Polytrichum commune	7.2	6.8	10.8	8.8	8.0
Dicranum polysetum	4.0	11.7	12.4	9.2	12.0

Analysis of lipid extracts by gas chromatography-mass spectrometry. GC-MS instrumentation consisted of a *Clarus 680* chromatograph and a *Clarus SQ 8 C* mass spectrometer. The separations were performed on an *Elite-5ms* (5 % phenyl-, 95 % methyl polysiloxane) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium (99.9999 %) was used as carrier gas at initial flow 2.0 mL/min for 2 min and following this held constant at 1.0 mL with split flow 10.0 mL/min. Column temperature was maintained at 75 °C for 2 min, then programmed from 75 °C to 130 °C at 20 °C 1/min and from 130 °C to 310 °C at 4 °C 1/min and finally held at 310 °C for 12 min, total run time 59.25 min. The 1.0 µl sample was injected into a split mode injector (4/1) with an auto sampler. The mass spectrometer was operated in the electron impact mode at 70 eV ionization energy and scanned from 42 Da to 650 Da with a cycle time of 0.5 s. Multiplier was operated at 1700 V. The column injector and transfer line temperatures were set at 290 °C and 250 °C, respectively, and the ion source temperature was 230 °C. Retention time (Rt, min) and MS fragmentation patterns of the known compounds were compared with literature and data base NIST. All peaks were quantified by peak area.

SPE fractionation of lipid mix/bryophyte lipid extracts. The scheme used to separate lipid classes is shown in Table II. The SPE columns were placed on a vacuum manifold and conditioned by washing 3 times with a total volume of 9 mL of hexane. The vacuum was adjusted to generate a flow rate ~0.5 ml/min. Five replicate samples of bryophyte lipid extract were analysed. The procedures for the fractionation of lipids were as follows: the dried lipid extract was taken up in a minimal volume of (< 0.5 mL) of hexane-chloroform (1:1) and loaded into the cartridge column. The column was eluted with eluents (each eluent 10 ml) in a sequence, as indicated in Table II. Eluates were dried under mild stream of nitrogen. First fraction after dissolving in chloroform was directly submitted for analysis with GC-MS, but the remaining two after dissolving in acetonitrile and derivatization with BSTFA. Repeatability was evaluated by measuring the variability of five consecutive analyses of lipid mixture and bryophyte lipid extract.

TABLE II

ELUTION SCHEME FOR SEPARATION OF LIPID CLASSES USING BONDED PHASE SILICA AND AMINOPROPYLSILICA SOLID PHASE EXTRACTION COLUMNS

Sorbent	Eluent	Groups of substances
Silica	Hexane	Alkanes, sterols, fatty alcohols
	Hexane/chloroform 5:1	Esters, ketones, aromatic substances
	Chloroform	Sterols
Amino-propylsilica	Hexane	Alkanes, sterols, fatty alcohols
	Hexane/chloroform 5:1	Esters, ketones
	Chloroform	Fatty acids, sterols
	Diethylether/acetic acid 98:2	Fatty acids

III. RESULTS AND DISCUSSION

In the study well characterized bryophyte species, *Polytrichum commune* and *Dicranum polysetum* [13], common in forest and bog vegetation in Northern Europe, were used. As bryophytes represent much simpler organisms than vascular plants, studies of their composition are of interest in respect to better understanding of evolution of living organisms, as well as to consideration of possibilities to obtain new prospective ubstances for application in medicine — bioprospecting. Bryophytes contain many substances of low polarity, generically described as lipids and represented by high number of structurally different compounds with relatively low polarity, such as alkanes, fatty acids, polar lipids (glyco-and phospholipids), and neutral lipids, including triacylglycerols, fatty alcohols, carotenoids, sterols, steryl esters, hydrocarbons and terpenoids.

Bryophyte lipids can be considered to be one of the functionally most important groups of substances with potentially high indicator value in respect to bryophyte physiology studies and, considering their application potential, several hundreds of new substances from bryophytes have been isolated (first isolated from *Marchantiophyta*).

Lipids may be traditionally fractionated as neutral lipids (triglycerides), phospholipids, glycolipids, etc. ([4]. Fatty acids and sterols of bound lipids can be analysed using transesterification. However, from a perspective of search of biologically active compounds and study of bryophyte metabolism, the most interesting are freely bound substances, as biological activity of glycolipids and others usually is lower than that of freely available substances.

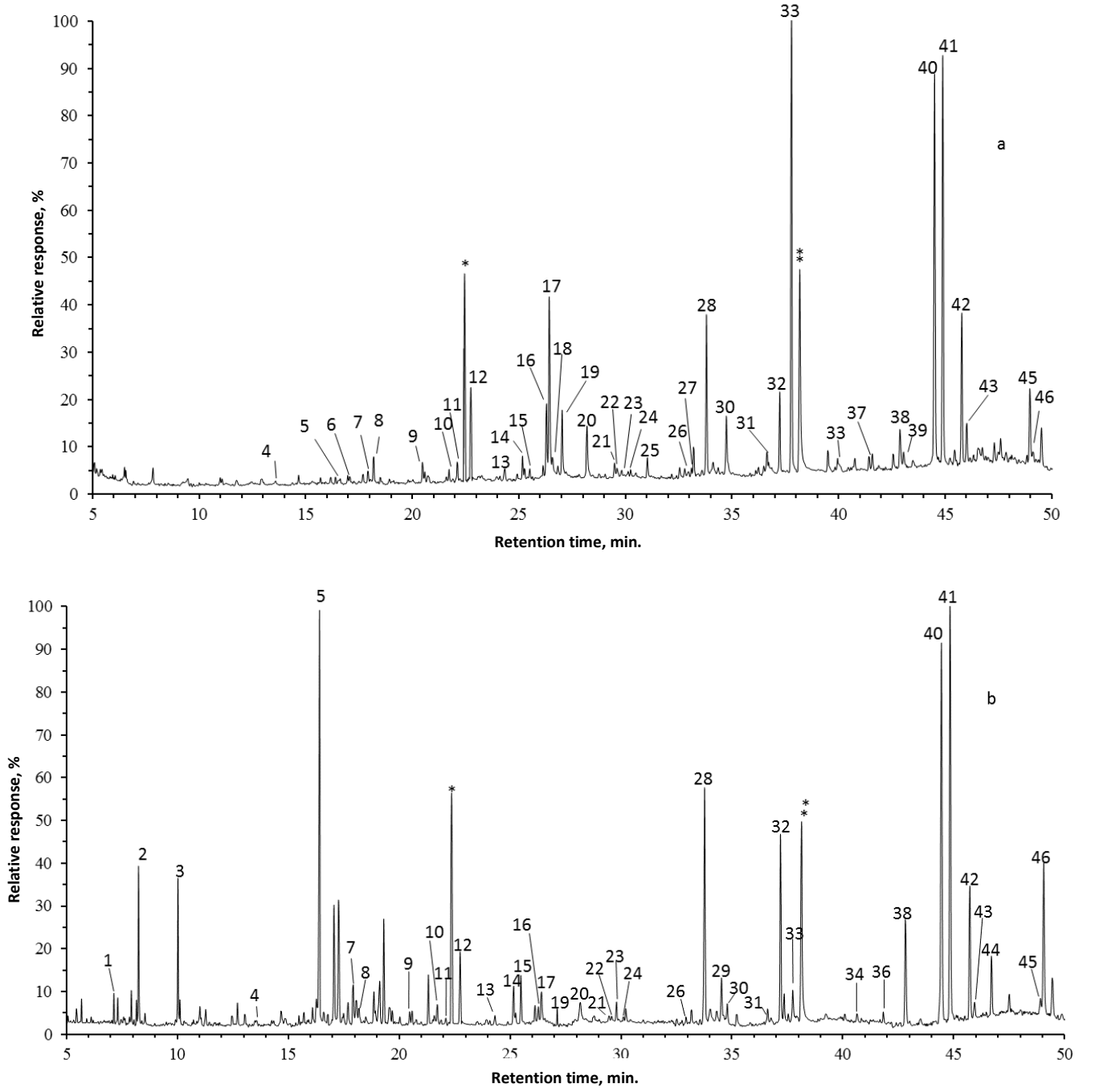

Fig. 1. Gas chromatogram with mass-spectrometric detection of total lipid extracts of a) *P. commune* (PC)*;* b) *D. polysetum* (DP). IS- internal standard (* — methylheptadecanoic acid; ** — progesterone); numbers refer to compounds listed in Table I. Acid and alcohol groups were derivatised prior to GC-MS analysis.

TABLE III

CONCENTRATIONS (μG/G DRY WEIGHT) OF LIPIDS IN MOSS *P. COMMUNE* AND *D. POLYSETUM* EXTRACTS. PEAK NUMBERS AS IN FIG. 1

Peak nr.	Retention time, min	Compounds	*D. polysetum* Concentration, μg/g	*P. commune* Concentration, μg/g
4	13.59	Dodecanoic acid,	5.3 ± 0.2	3.8 ± 0.5
5	16.42	2-Methyl fructofuranoside	1.4 ± 0.1	3.2 ± 0.2
6	16.97	Azelaic acid	1.1 ± 0.4	4.9 ± 0.4
7	17.93	Neophytadiene	19.8 ± 0.8	6.3 ± 0.3
8	18.2	Tetradecanoic acid	10.6 ± 0.5	11.5 ± 0.8
9	20.47	Pentadecanoic acid	6.8 ± 0.7	9.5 ± 0.5
10	21.73	Eicosane	4.3 ± 0.1	6.5 ± 0.3
11	22.1	7,10,13-Hexadecatrienoic acid	3.1 ± 0.1	9.6 ± 0.7
12	22.75	Hexadecanoic acid	4.1 ± 0.1	3.2 ± 0.2
13	24.38	9-Heptadecenoic acid	4.1 ± 0.1	6.4 ± 0.3
14	25.17	1-Octadecanol	21.8 ± 1.2	9.6 ± 0.6
15	25.52	Phytol	19.2 ± 0.9	5.5 ± 0.3
16	26.31	9,12-Octadecadienoic acid	6.5 ± 0.2	31.3 ± 7.4
17	26.42	9-Octadecenoic acid	16.5 ± 0.5	74 ± 5
18	26.57	11-Octadecenoic acid	1.1 ± 0.1	11.4 ± 0.6
19	27.03	Octadecanoic acid	18.9 ± 1.1	26.6 ± 1.8
20	28.2	Pimaric acid	19.5 ± 1.6	28.5 ± 1.8
21	29.48	5,8,11,14-Eicosatetraenoic acid	3.1 ± 0.2	8.0 ± 0.3
22	29.61	5,8,11,14,17-Eicosapentaenoic acid	3.6 ± 0.2	5.0 ± 0.3
24	30.13	Tetradecanoic acid, 2,3-dihydroxypropyl ester	2.5 ± 0.2	4.1 ± 0.9
25	31.03	Eicosanoic acid	2.3 ± 0.1	8.4 ± 0.2
27	33.1	1-Docosanol	0	29.1 ± 0.5
28	33.8	Hexadecanoic acid, 2,3-dihydroxypropyl ester	110.5 ± 3.5	58 ± 3
29	34.53	Sucrose	9.4 ± 0.4	1.1 ± 0.1
30	34.73	Docosanoic acid	11.4 ± 0.5	29.4 ± 1.2
32	37.19	Octadecanoic acid, 2,3-dihydroxypropyl ester	78.3 ± 1.6	31 ± 1
33	37.8	Squalene	19.5 ± 0.5	248 ± 32
34	39.96	1-Hexacosanol	1.1 ± 0.1	8.4 ± 0.5
35	40.63	γ-Tocopherol	9.3 ± 0.3	1.1 ± 0.1
36	41.42	Hexacosanoic acid	1.1 ± 0.1	6.7 ± 0.3
37	41.82	Decanyl tetradecanoate	10.3 ± 0.3	5.2 ± 0.2
38	42.82	α-Tocopherol	121 ± 4	56 ± 8
39	43.05	Octacosanol	3.0 ± 0.2	10.3 ± 0.4
40	44.45	Campesterol	434 ± 23	454 ± 21
41	44.83	Stigmasterol	445 ± 25	409 ± 25
42	45.73	β-Sitosterol	139 ± 3	154 ± 4
43	46.02	Isofucosterol	1.1 ± 0.1	45 ± 3
44	46.69	Cycloartenol	73.0 ± 1.6	2.3 ± 0.4
45	48.97	Lupa-13(18),22-dien-3-ol	32.7 ± 1.5	79 ± 3
46	49.06	Phytylhexadecanoate	199 ± 15	7.3 ± 0.3

For extraction of lipids from bryophytes different solvents have been used, usually chloroform, however the extraction conditions and solvent selection has not been much studied. A brief comparison (Table I) of different solvents demonstrates that chloroform and diethyl ether could be considered the most preferable. Treatment with ultrasound can be applied as an significant tool to increase extraction yield. Studied leaf moss species (*Polytrichum commune*, *Dicranum polysetum*) contained < 12 mg/g of lipids. Exhaustive extraction (repeated treatment with fresh solvent) might yield up to 21−60 mg/g [4] depending on the moss species (however such yield requires intensive and repeated treatment).

A chromatogram of total lipid extracts of the studied bryophytes (Fig. 1, numbers refer to compounds listed in Table III) demonstrated presence of a significant number (total number identified substances is 46) of different groups of low-polarity compounds. Hydrocarbons occurred in relatively low concentrations (100 ± 290 μg/g dry weight; Table IV). The distributions of alkanes were dominated by C_{21+} odd-carbon numbered homologues. These results were in agreement with literature data about other moss species. These data can help to differentiate lower plants (n-alkanes with carbon numbers C_{21}–C_{25}) from higher plants (alkane distribution is dominated by C_{29}, C_{31}, C_{33}) in peat and soil studies [5], [6].

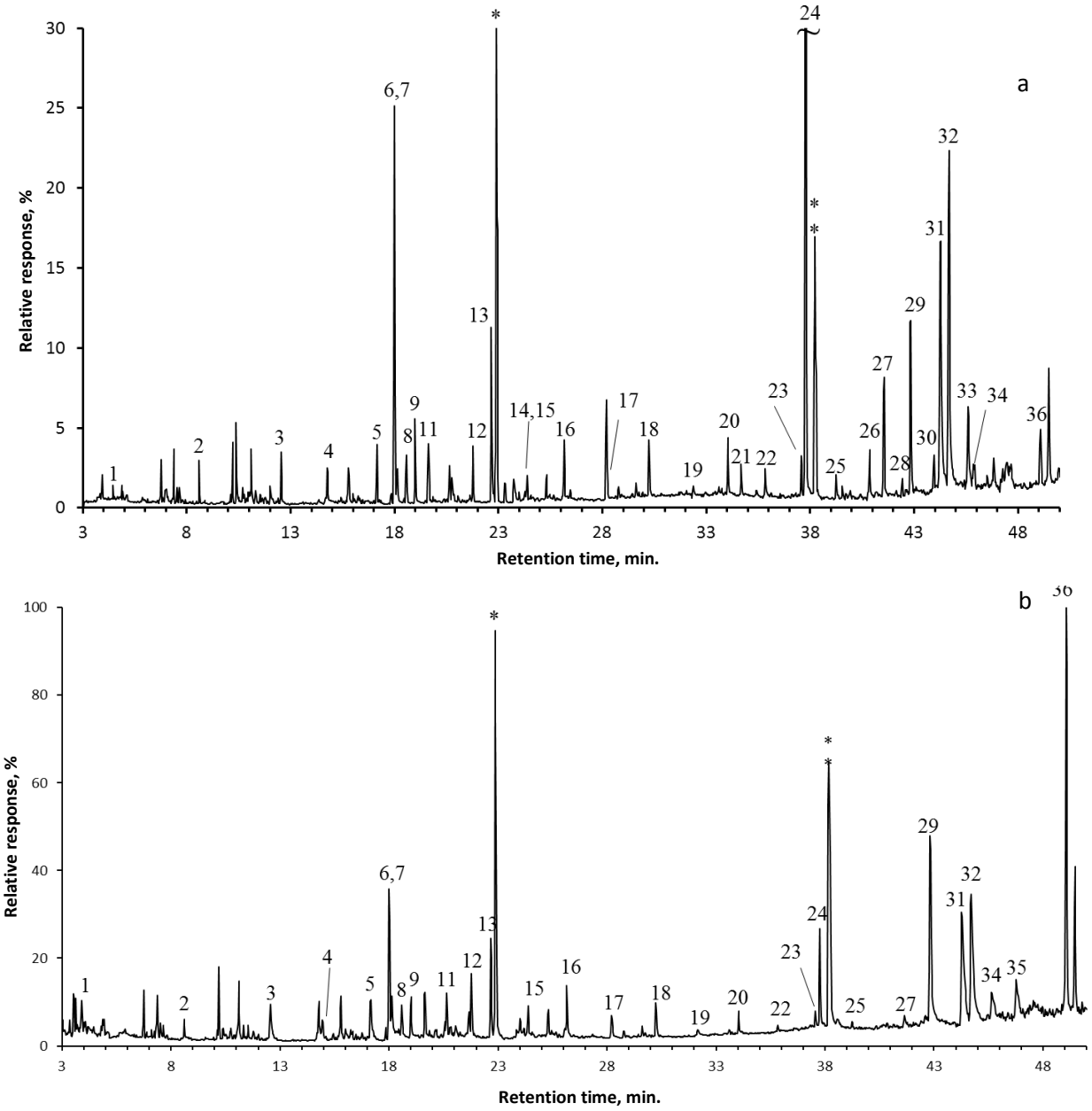

Fig. 2. Gas chromatogram with mass-spectrometric detection of the fraction of a lipid extract of *P. commune* eluted with hexane from the a) silica SPE; b) SPE-NH_2. IS- internal standard (* — methylheptadecanoic acid; ** — progesterone); numbers refer to compounds listed in Table IV.

The differences between studied moss samples (*Polytrichum commune, Dicranum polysetum*) are remarkable; this is reflected by the number of the identified and quantified substances. Considering this, only *Polytrichum commune* was selected for further studies and fractionation.

However alkane concentrations in total lipid extracts were low and thus isolation of alkane fraction from total lipid chromatogram was one of the aims of the present study. Sterols were the most abundant lipids in the extracts of studied bryophytes and their concentrations varied from 450 μg/g to 1600 μg/g dry weight (Table I). For the studied samples the dominance of the C_{29} sterols in respect to C_{28}. sterols was common and 24-methyl-cholest-5-en-3b-ol, 24-ethylcholesta-5,22-dien-3b-ol, 24-ethylcholest-5-en-3b-ol, 24-ethylcholest-5-en-3b-ol, 24-methylcholest-5-en-3b-ol, 24-methylcholesta-5,22-dien-3b-ol, and 24-methylcholesta-5,7,22-trien-3b-ol could be found in the highest concentrations.

Sterols are an important component of bryophyte secondary metabolites and also their fractionation might help to identify new application options of bryophyte extracts as well as physiological processes going on in them.

The concentrations of terpenoids were much more variable than those of sterols, ranging from 20 μg/g to 3500 μg/g dry weight (Table III). Amongst terpenoids ursolic acid, oleanoic acid, amyrin and lupeol dominated. The concentrations of fatty acids also differed much amongst the studied bryophytes

and ranged from 200 μg/g to 1050 μg/g dry weight (Table III). $C_{16} \pm C_{18}$ fatty acids (and especially unsaturated compounds) could be considered to be a bacterial contribution. Only higher fatty acids (C_{24} or C_{26} members) are from bryophytes [14]. Total concentrations of fatty alcohols were much lower than concentrations of fatty acids and varied from 80 μg/g to 340 μg/g dry weight (Table III). Fatty alcohols were dominated by even-carbon numbered compounds in the range C_{22}−C_{30}. A significant group of bryophyte lipids were waxes and they were comprised of $C_{16:0}$ fatty acids esterified with $C_{22:0}$, $C_{24:0}$ and $C_{26:0}$ fatty alcohols. Lipid extracts of bryophytes also contained α-tocopherol as well as C_{25} and C_{27} methyl ketones. In the studied extracts several carbohydrates also were found, for example, 2-methyl fructofuranoside and sucrose.

TABLE IV

CONCENTRATIONS (μG/G DRY WEIGHT) OF LIPIDS IN MOSS *P. COMMUNE* IN THE FRACTION ELUTED WITH HEXANE FROM THE SILICA SPE AND SPE-NH_2. PEAK NUMBERS AS IN FIG. 2

Peak nr.	Compounds	Silica SPE conc., μg/g	SPE-NH_2 conc., μg/g
1	Decane	2.3	0.0
2	Tetradecane	3.2	1.1
3	Hexadecane	4.9	1.5
4	Heptadecane	4.7	0.9
5	Octadecane	6.2	2.1
6	Neophytadiene	38.9	5.4
7	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	3.0	0.8
8	Phyta-1,3(Z)-diene	5.7	1.3
9	Phyta-1,3(E)-diene	9.9	1.8
10	Nonadecane	0.3	0.1
12	Eicosane	6.8	2.1
13	Octadeca-3,6,9,12-tetraene	17.1	5.7
14	Phytol	1.2	—
15	Heneicosane	2.7	1.3
16	Docosane	6.0	2.6
17	Tricosane	13.7	1.2
18	Tetracosane	7.0	1.5
19	Pentacosane	0.5	0.3
20	Hexacosane	6.8	0.8
21	Tetracosanal	4.0	—
22	Heptacosane	4.5	0.2
23	Octacosane	5.0	0.5
24	Squalene	232.3	3.4
25	Nonacosane	4.2	0.4
26	Triacontane	5.6	0.4
27	Octacosanal	17.8	0.9
28	Hentriacontane	1.5	—
29	α-Tocopherol	27.9	9.2
30	Dotriacontane	3.9	-
31	Campesterol	53.3	9.4
32	Stigmasterol	55.1	9.6
33	γ-Sitosterol	15.2	—
34	Fucosterol	2.5	2.3
35	Cycloartenol	—	3.5
36	Phytylhexadecanoate	9.5	19.6

Silica modified with aminopropyl groups has been suggested for separation of individual lipid classes [14] and later this approach has been extended for lipids from different sources.

In the present study bryophyte lipids obtained by chloroform extraction from moss *Polytrichum commune* were fractionated on aminopropyl SPE in comparison with silica SPE. Considering the interest in specific groups of substances found in bryophytes, the elution system was selected based on polarity of eluents and fractions, as a separate class of identifying acids (Table II). The fraction eluted with hexane contained relatively most low-polar compounds. Remarkable was the presence of a high number of hydrocarbons, starting from C_{10} and up to C_{30} compounds. Amongst other substances identified was phytol, phytadiene, as well as some aldehydes, α-tocopherol, as well as campesterol, stigmasterol, γ-sitosterol and cycloartenol. Significant differences between fractions obtained from silica SPE and SPE-NH_2 were found; silica SPE could be recommended as the most appropriate for fractionation, supporting the identification hydrocarbons as biomarkers of importance in biogeochemical studies [5], [6]. Elution with eluents with an increased polarity — hexane/chloroform (5:1) yielded (Fig. 3, Table V) relatively few compounds, including several dicarboxylic acids and their esters, phytol, squalene, α-tocopherol, cycloartenol and others. Also, for this eluent system the use of silica SPE columns is preferable.

TABLE V

CONCENTRATIONS (μG/G DRY WEIGHT) OF LIPIDS IN MOSS *P. COMMUNE* IN THE FRACTION ELUTED WITH HEXANE/CHLOROFORM (5:1) FROM THE SILICA SPE AND SPE-NH_2. PEAK NUMBERS AS IN FIG. 3

Peak nr.	Compounds	Silica SPE conc., μg/g	SPE-NH_2 conc., μg/g
1	Succinic acid	—	—
2	Hexanedioic acid	—	—
3	Malic acid	—	—
4	2-Methyl Fructofuranoside	—	—
5	Hexadecanoic acid methyl ester	1.3	0.1
6	Octadeca 3,6,9,12 tetraene	0.3	—
7	9,12-Octadecadienoic acid, methyl ester	0.3	—
8	Linolenic acid, methyl ester	0.6	—
9	Phytol	0.8	0.8
10	Tetracosanal	1.2	—
11	Squalene	5.4	0.2
12	Octacosanal	2.8	—
13	Decanyl tetradecanoate	0.9	0.5
14	α-Tocopherol	18.9	7.8
15	Triacontanal	0.7	—
16	Cycloartenol	—	—
17	Phytylhexadecanoate	1.9	—

Still both in respect to number of substances identified in chromatogram (Fig. 3), both in respect to quantified substance concentrations. The eluent system hexane/chloroform did not provide the intended fractionation aims.

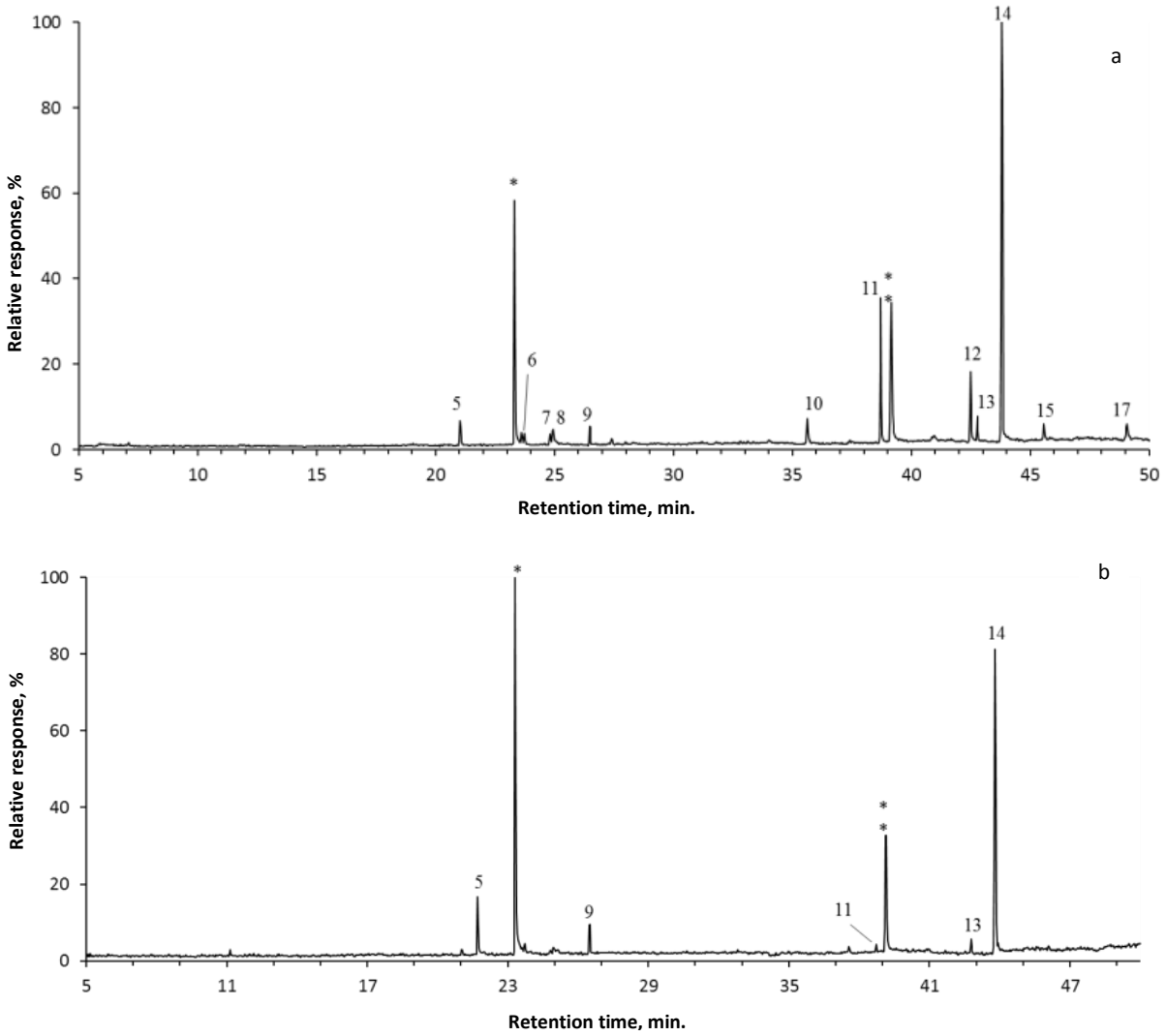

Fig. 3. Gas chromatogram with mass-spectrometric detection of the fraction of a lipid extract of *P. commune* eluted with hexane/chloroform (5:1) from the a) silica SPE; b) SPE-NH_2. IS- internal standard (* — methylheptadecanoic acid; ** — progesterone); numbers refer to compounds listed in Table V.

Elution with eluents with an increasing polarity supported the possibility to get analytical characterisation of lipid ingredients, characterising differing groups of bryophyte metabolites. In this respect possibility to quantify substances (like tocoferol, squalene, phytol and others) are of interest. However, further eluent systems could be suggested to achieve more efficient separation. However, several of these substances can be found in all fractions, thus the aim of their isolation in one fraction was not achieved using the suggested system. If eluent polarity change principle is used to achieve acceptable separation efficiency, silica (more polar column) has evident preferences on comparison with aminopropyl bonded phase columns SPE-NH_2.

Elution of both silica SPE and SPE-NH_2 with chloroform (Fig. 4, Table VI) yielded a relatively high number of substances with evidently high polarity. Amongst chloroform fraction many substances with high biological activity were found, for example, neophytadiene, phyta-1,3(Z)-diene, phyta-1,3(E)-diene, phytol, several fatty acids (saturated and unsaturated), campesterol, stigmasterol, β-sitosterol, fucosterol. Still, significant differences between the studied eluent systems could be observed, both in number and quantity of identified substances.

Elution with chloroform ensured the removal of an absolute majority of extracted substances from silica SPE and further eluent on this SPE was not applied.

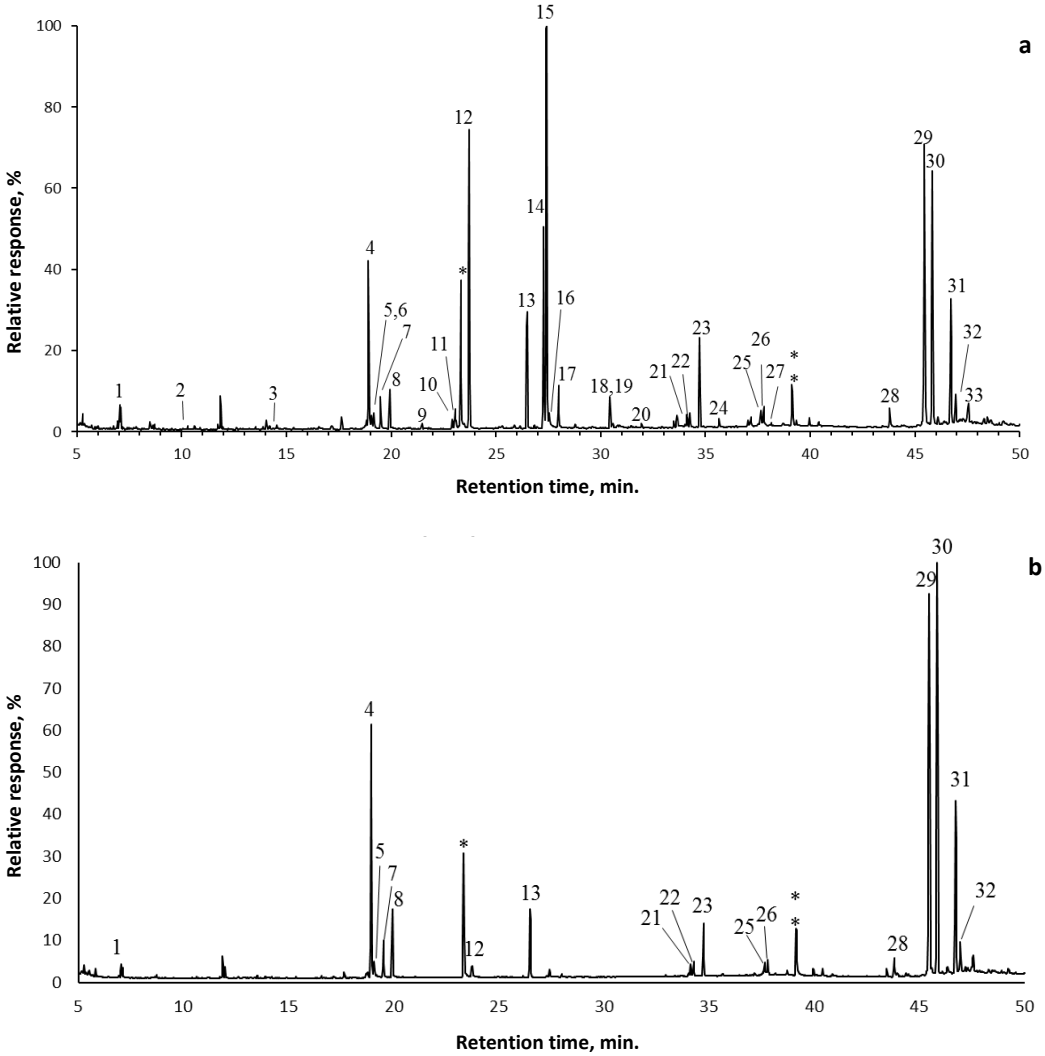

Fig. 4. Gas chromatogram with mass-spectrometric detection of the fraction of a lipid extract of *P. commune* eluted with chloroform from the a) silica SPE; b) SPE-NH_2. IS- internal standard (* — methylheptadecanoic acid; ** — progesterone); numbers refer to compounds listed in Table VI.

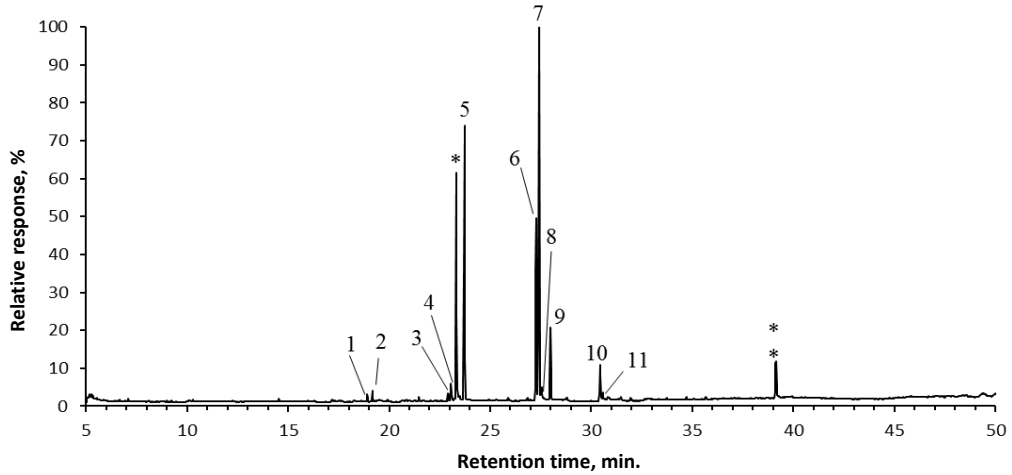

Fig. 5. Gas chromatogram with mass-spectrometric detection of the fraction of a lipid extract of *P. commune* eluted with diethyl ether/acetic acid (98:2) from the SPE-NH_2. IS- internal standard (* — methylheptadecanoic acid; ** — progesterone); numbers refer to compounds listed in Table VII.

TABLE VI

CONCENTRATIONS (μG/G DRY WEIGHT) OF LIPIDS IN MOSS *P. COMMUNE* IN THE FRACTION ELUTED WITH CHLOROFORM FROM THE SILICA SPE AND SPE-NH_2. PEAK NUMBERS AS IN FIG. 4

Peak nr.	Compounds	Silica SPE con., μg/g	SPE-NH_2 con., μg/g
1	Glycerol	13.2	12.6
2	Decanoic acid,	0.9	—
3	Dodecanoic acid	1.4	—
4	Neophytadiene	62.8	165.7
5	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	3.1	6.9
6	Tetradecanoic acid,	4.6	—
7	Phyta-1,3(Z)-diene	10.9	21.8
8	Phyta-1,3(E)-diene	15.3	41.9
9	n-Pentadecanoic acid	2.1	—
10	7,10-Hexadecadienoic acid	3.5	—
11	7-Hexadecenoic acid	8.8	—-
12	Hexadecanoic acid	109.2	8.4
13	Phytol	52.6	49.4
14	9,12-Octadecadienoic acid (Z,Z)	81.3	—
15	9-Octadecenoic acid	190.6	—
16	11-Octadecenoic acid	3.2	—
17	Octadecanoic acid	15.7	—
18	Arachidonic acid	12.0	—
19	cis-5,8,11,14,17-Eicosapentaenoic acid	1.6	—
20	Eicosanoic acid	1.9	—
21	7,10-Hexadecadienoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	5.1	7.0
22	7,10,13-Hexadecatrienoic acid, 2,3-dihydroxypropyl ester	4.9	8.2
23	Hexadecanoic acid, 2,3-dihydroxypropyl ester	34.4	32.7
24	Docosanoic acid	3.6	—
25	9,12-Octadecadienoic acid, 2,3-dihydroxypropyl ester	4.6	7.1
26	6,9,12-Octadecatrienoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	7.6	10.3
27	Octadecanoic acid, 2,3-dihydroxypropyl ester	1.7	—
28	α-Tocopherol	8.5	16.0
29	Campesterol	391.9	405.9
30	Stigmasterol	276.0	383.0
31	β-Sitosterol	151.1	163.4
32	Fucosterol	31.6	26.2
33	Lupa-13(18),22-dien-3-ol	25.8	—

TABLE VII

CONCENTRATIONS (μG/G DRY WEIGHT) OF LIPIDS IN MOSS *P. COMMUNE* IN THE FRACTION ELUTED WITH DIETHYLETHER/ACETIC ACID (98:2) FROM THE SPE-NH_2. PEAK NUMBERS AS IN FIG. 4

Peak nr.	Compounds	Con., μg/g
1	Neophytadiene	2.5
2	Tetradecanoic acid,	3.3
3	7,10-Hexadecadienoic acid	2.9
4	7,10,13-hexadecatrienoate	6.5
5	Hexadecanoic acid,	90.5
6	9,12-Octadecadienoic acid (Z,Z)	65.8
7	9-Octadecenoic acid	160.6
8	11-Octadecenoic acid	3.1
9	Octadecanoic acid	24.3
10	cis-5,8,11,14-Eicosatetraenoic acid	13.2
11	cis-5,8,11,14,17-Eicosapentaenoic acid	1.9

On the aminopropylsilica SPE column acids were most strongly sorbed (Fig. 5, Table VII) and they might be eluted as the final fraction using diethyl ether/acetic acid (98:2) as suggested by Giacometti and others (2002). Thus functional fractionation aim could be achieved. Amongst identified substances in moss *Polytrichum commune* long chain unsaturated fatty acids were identified.

V. CONCLUSION

1. Studied leaf moss species (*Polytrichum commune*, *Dicranum polysetum*) contained < 12 mg/g of lipids. Total lipid extracts of the studied bryophytes had a significant number (total number identified substances is 46) of different groups of low-polarity compounds.
2. Solid phase extraction fractionation on silica and aminopropyl bonded silica with an eluent system with increasing eluent polarity allowed to identify many substances with high biological activity or possible significance to bioindication purposes and studies of biogeochemical processes. Significance of the SPE fractionation was in their ability to identify substances, not visible in total chromatograms.

ACKNOWLEDGEMENT

The study was financially supported by ERAF project 2DP/2.1.1.1.0/14/APIA/VIAA/016 „Development of environmentally friendly surface cleaning agent and research of its potential applications". Authors acknowledge contribution of Polina Naidjonoka at elaboration of the SPE procedures.

REFERENCES

1. **Asakawa, Y.** Biologically active compounds from bryophytes. *Pure Applied Chemistry*, 2007, vol 4, N 79, pp. 557−580. http://dx.doi.org/10.1351/pac200779040557
2. *Chemical constituents of bryophytes: bio- and chemical diversity, biological activity, and chemosystematics (Progress in the chemistry of organic natural products)*, Maksimova, V., Klavina, L., Bikovens, O., Zicmanis, A., Purmalis, O. Structural characterization and chemical classification of some bryophytes found in Latvia. *Chem. Biodiver.* 2013, 10(7), 1284–1294. Y. Asakawa, A. Ludwiczuk, F. Nagashima, Springer, Vien, 2013, 605 p.
3. **Mellegård, H., Stalheim, T., Hormazabal, V., Granum, P. E., Hardy S. P.** Antibacterial activity of sphagnum acid and other phenolic compounds found in *Sphagnum papillosum* against food-borne bacteria. *Letters of Applied Microbiology*, 2009, N 49, pp. 85−90. http://dx.doi.org/10.1111/j.1472-765X.2009.02622.x
4. **Dembitsky, V.M.** Lipids of bryophytes. *Progress in Lipid Research*, 1993, vol 3, N 32, pp 281−356. http://dx.doi.org/10.1016/0163-7827(93)90010-T
5. **Baas, M., Pancost, R., van Geel, B., Damste, J.S.S.** A comparative study of lipids in *Sphagnum* species. *Organic Geochemistry*, 2000, N 31, pp 535−541. http://dx.doi.org/10.1016/S0146-6380(00)00037-1
6. **Ronkainen, T., McClymont, E.L., Väliranta, M., Tuittila, E.S.** The *n*-alkane and sterol composition of living fen plants as a potential tool for palaeoecological studies. *Organic Geochemistry*, 2013, N 59, pp. 1−9. http://dx.doi.org/10.1016/j.orggeochem.2013.03.005
7. **Zhou, W., Xie, S., Meyers, P.A., Zheng, Y.** Reconstruction of late glacial and Holocene climate evolution in Southern China from geolipids and pollen in the Dingnan peat sequence. *Organic Geochemistry*, 2005, N 36, pp. 1272−1284. http://dx.doi.org/10.1016/j.orggeochem.2005.04.005
8. **Huang, X., Xue, J., Zhang J., Quin, Y., Meyers, P.A., Wang, H.** Effect of different wetness conditions on *Sphagnum* lipid composition in

the Erxianyan peatland, central China. *Organic Geochemistry*, 2012, N 44, pp 1−7. http://dx.doi.org/10.1016/j.orggeochem.2011.12.005

9. **Vonk, J.E., Gustafson, Ö.** Calibrating n-alkane *Sphagnum* proxies in sub-Arctic Scandinavia. *Organic Geochemistry*, 2009, N 40, pp. 1085−1090. http://dx.doi.org/10.1016/j.orggeochem.2009.07.002
10. **Ruiz-Gutierez, V., Perez-Camino, M.C.** Update on solid-phase extraction for the analysis of lipid classes and related compounds. *Journal of Chromatography A*, 2000, N 885, pp. 321−341. http://dx.doi.org/10.1016/S0021-9673(00)00181-3
11. **Pernet, F., Pelletier, C.J., Milley,J.** Comparison of three solid-phase extraction methods for fatty acid analysis of lipid fractions in tissues of marine bivalves. *Journal of Chromatography A*, 2006, N 1137, pp 127−137. http://dx.doi.org/10.1016/j.chroma.2006.10.059
12. **Mills, C.T., Goldhaber, M.B.** On silica-based solid phase extraction techniques for isolating microbial membrane phospholipids: ensuring quantitative recovery of phosphatidylcholine-derived fatty acids. *Soil Biology & Biochemistry*, 2010, N 42, pp. 1179−1182. http://dx.doi.org/10.1016/j.soilbio.2010.03.023
13. **Maksimova, V., Klavina, L., Bikovens, O., Zicmanis, A., Purmalis, O.** Structural characterization and chemical classification of some bryophytes found in Latvia. *Chem. Biodiver.* 2013, vol 7, N 10, pp. 1284–1294. http://dx.doi.org/10.1002/cbdv.201300014
14. **Kaluzny, M.A., Duncan, L.A., Merrit, M.Y., Epps, D.E.** Rapid separation of lipid classes in high yield and purity using bonded phase columns. *Journal of Lipid Research*, 1985, N 26, pp. 135−140
15. **Giacometti, J., Milosevic, A., Milin, C.** Gas chromatographic determination of fatty acids contained in different lipid classes after their separation by solid-phase extraction. *Journal of Chromatography A*, 2002, N 976, pp. 47−54. http://dx.doi.org/10.1016/S0021-9673(02)01159-7

Laura Kļaviņa is a PhD student of Environmental Sciences at the University of Latvia. Her scientific interests are linked to plant chemistry, pharmacognosy and plant reaction to environmental stress. She has participated in experiments on invetigation of bryophyte chemical composition, bryophyte secondary metabolites and their separation and identification.
Address: Raina blvd. 19, LV-1586, Riga, Latvia
Phone: +371 28348067
E-mail: laura.klavina@lu.lv

Jorens Kviesis holds *Dr. chem.* degree since 2007. His interests are related to studies of biologically active substances in vegetation and development and new applications of chromatographic techniques, firstly GC-MS.
Address: Raina blvd. 19, LV-1586, Riga, Latvia
Phone: +371 26321034
E-mail: cations@inbox.lv

Laura Kļaviņa, Jorens Kviesis. Briofītu lipīdu cietfāzes ekstrakcijas frakcionēšana.
Pētījuma ietvaros izstrādāta vienkārša, ātra un viegli atkārtojama analītiska metode briofītu lipīdu frakcionēšanai. Lipīdu frakcijas frakcionēšanai izmantoja cietfāzes ekstrakciju ar silikagēlu un ar NH_2 grupām modificētu silikagēlu, iegūtās vielu grupas kvantificēja kā to trimetilsilil atvasinājumus, izmantojot gāzes hromatogrāfiju-masas spektrometriju. Frakcionēšanu veica, izmantojot eluentu polaritātes gradienta principu (eluenti: heksāns, heksāns/hloroforms 5:1, hloroforms), kā pēdējo eluentu ar NH_2 grupām modificēta silikagēla gadījumā izmantojot skābes (etiķskābes) šķīdumu. Izpētes objekti bija divi Latvijā plaši izplatīti sūnaugi (*Polytrichum commune, Dicranum polysetum*), kuru sastāvā identificēja vairāk nekā 45 dažādus savienojumus, daudzi no kuriem ir ar augstu bioloģisko aktivitāti. Sūnaugu sastāvā identificēja sterolus, tokoferolus, taukskābes un citi savienojumus. Izmantotā frakcionēšanas shēma pavēra iespējas identificēt daudzus savienojumus, kuri kopējā ekstrakta hromatogrammā nebija uzrādīti, piemēram, alkānu rindas savienojumi, vairāki steroli. Līdz ar to sūnaugu lipīdu cietfāzes frakcionēšanas metode ļāva noteikt vielu klātbūtni, kuras atrodas mikrodaudzumos, bet kuras varēja ļaut izsekot brīofītu pārvērtībām bioģeoķīmiskos procesos. Ieteiktā frakcionēšanas metode ļāva arī identificēt vairākas vielu grupas, kuras varētu būt perspektīvas kā jaunas bioloģiski aktīvas vielas, kā arī palīdzēja labāk izprast briofītu metabolismu, piemēram starpsugu atšķirības un sastāva sezonālās mainības raksturu.