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A study on bryophyte chemical composition–search for new applications

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Abstract. Bryophytes are the taxonomic group in the plant kingdom represented with about 25'000 species. They contain a high number of biologically active compounds; however their use as a food source is negligible. The aim of this paper is to evaluate bryophyte chemical composition and new possible applications. In order to evaluate bryophyte potential usage as a raw food material, bryophyte basic chemical content and the secondary metabolite profile was determined. To obtain best results bryophyte secondary metabolite extraction options has been studied. Couple of extraction methods were used (conventional, ultrasound, microwave, supercritical CO_2 extraction etc.) and different solvents (ethanol, water etc.). A total concentration of polyphenols and substances determining free radical scavenging activity has been determined. The extracts obtained from bryophytes have remarkable antioxidant activity, the extent of which depends on the extraction conditions and bryophyte species. Comparison of five extraction methods and several solvents indicates that microwave assisted extraction and supercritical CO_2 extraction is the most promising approach to obtain highest yields of extractives.

Key words: bryophytes; extraction, polyphenols, antiradical activity, optimization, food.

INTRODUCTION

Bryophytes are the second largest group in the plant kingdom with about 25'000 bryophyte species (Asakawa et al., 2013) and they can be found in any kind of ecosystems (Glime, 2007). In comparison with higher plants use of bryophytes for human consumption is negligible due to their low caloric value (Forman, 1968) and poor organoleptic properties. Traditionally, use of bryophytes as a food source is limited for famine periods, however in Northern regions of Europe and America bryophytes are used as an ingredient of bread or soup. In circumpolar regions bryophytes are a common animal feed (Glime, 2007).

Recent research demonstrates a presence of a large number of biologically active substances in the composition of bryophytes. Bryophytes due to the presence of high number of biologically active compounds in their composition are commonly used in ethnopharmacology and as medical plants for treatment of wounds and burns (Singh et al. 2006; Cheng et al., 2012; Fu et al., 2012; Asakawa et al., 2013). More specifically bryophytes demonstrate antibacterial, antifungal, antiviral activities, antioxidant, antiplatelet, antithrombin, insecticidal, neuroprotective activities, as well as cytoxicity in respect to cancer cells (Spjut et al., 1986; Cheng et al., 2012).

Bryophytes are mainly composed of hemicelluloses and pectin (30 to 60% respectively, cellulose content from 15 to 25%). Bryophytes also contain 5 to 10% proteins, 5 to 10% lipids and phenolic compounds (Orlov et al., 2005). The content of lignin has been found to be insignificant (Klavina, 2015), however controversies in this respect exist (Painter et al., 2003). Notably, the chemical composition of bryophytes differs depending on species, growth environment, and season (Goffinet & Shaw 2008; Glime 2007; Xie et al., 2009).

Growth of human population and a need to develop healthy diets requires looking for new, alternative food as well as for sources of important food ingredients, for example antioxidants. Further, caloric value of food considering abundance of high calority food nowadays, is by far not the main direction of activities in search for alternative food plants (Haines&Renwick 2009). In this respect bryophytes are highly relevant and prospective object of studies. Nowadays bryophyte availability for practical applications does not depend on their field sampling possibilities, but they can be grown using biotechnological approaches (in reactors) as axenical cultures, but also in large scales (ton quantities – *Sphagnum* farming) (Beike et al., 2012; Pouliot et al., 2014). To advance the studies of alternative food sources, it is important to study bryophyte and their extract composition.

The aim of this paper is to evaluate potential of bryophyte use in food and extraction possibilities of their secondary metabolites with a special emphasis on polyphenolic compounds as a source of natural antioxidants.

MATERIALS AND METHODS

Materials

In this study 16 bryophyte species common for Northern Europe (from moist coniferous forests, moist deciduous forests and bogs in Latvia) were selected. Bryophyte living parts were collected, identified (Strazdiņa et al., 2011), and cleaned from biotic contamination, washed with distilled water and air dried. Top parts (2–5 cm) of bryophytes were used and before analysis samples were stored at -20°C. Each bryophyte species specimen voucher is stored in Department of Environmental Science, University of Latvia.

Conventional extraction of bryophytes

Sample of *Rhytidiadelphus triquetrus* was dried at +40°C in an oven until constant mass. Dry sample was grinded in a mill and 0.3 g of bryophyte sample was weighed into 100 mL bottles with screw cap and 50 ml of solvent was added. Solvents such as ethanol (96, 80, 60, 40, and 20%) diluted with demineralised (*Millipore*) water and water. The bottles were shaken in a shaker for 24 h at 140 rpm. All extracts were filtered and stored until analysis at 4°C up to 1 month.

Ultrasound-assisted extraction of bryophytes

Solvents such as ethanol (96, 80, 60, 40, and 20%) diluted with demineralised (*Millipore*) water and water were used. Samples afterwards were treated with 20 and 40 min of ultrasound (100W) in ultrasound bath (*Cole Parmer*), temperature was regulated with regular adding of cold water to keep constant temperature of +40°C. The bottles then were shaken in a shaker for 24 h at 140 rpm.

Extraction of bryophytes by microwave treatment

Dry samples were grinded in a mill and 0.3 g of bryophyte sample were weighted into Teflon extraction tubes and 50 ml of solvent (96%, 80%, 60%, 40%, 20% ethanol) were added and sealed using *Milestone Twister*. Extraction was performed using *Milestone Ethos One* microwave oven in 120°C and 150°C with power of 1500 W. Extraction took 40 min: 10 min to reach chosen temperature, 20 minutes for a steady extraction at a set temperature and 10 minutes for oven to cool down.

Extraction of bryophytes with supercritical CO_2

Dry sample was grinded in a mill and 15 g of bryophyte sample was weighted in metallic column. Column was inserted in preheated (+102°C) oven, after CO_2 flow of 10 ml per minute was set. Extraction was done using *Separex* CO_2 supercritical extractor. After first trials it was concluded that coupled extraction was required for best results, therefore 96 % ethanol flow (5 ml min^{-1}) also was set. Extraction experiments were conducted under 20 MPa pressure for 30 minutes and 60 minutes.

Soxhlet extraction of bryophytes

Dry bryophyte sample (20 g) was weighted in fabric bag. Fabric bag was sealed and inserted in extraction tube. Extraction was done using Soxhlet extractor and as a solvent 96% ethanol was used. Extraction process was done in 80°C for 8 h and 24 h time period.

Estimation of dry weight

Prepared extracts were kept in room temperature for an hour and total amount of extract measured using pipette; afterwards 5 ml of each extracts were measured in previously prepared and weighted weight glasses. Extrahent was evaporated on a stove (70°C) until dry. After weight glasses have cooled down it was placed in exicator for 24 h. After that weight glasses were weighted in triplicate. Dry weight of extracts was expressed as mg 100 g^{-1} dry moss weight.

^{13}C-NMR spectroscopy

Solid-state ^{13}C–NMR spectroscopy was carried out using the technique of cross-polarization with magic angle spinning (CP/MAS). The spectra were recorded on a *Bruker Avance wide-bore* 600 MHz solid state NMR spectrometer equipped with a 4 mm MAS double-resonance probe. 2 ms contact time and 2 s repetition time were used. The sample magic angle spinning was 10 kHz, and chemical shifts were referenced to adamantane at 38.48 ppm.

Total polyphenol concentration determination in bryophyte extracts

Before all analysis, bryophyte extracts were kept at room temperature for ~1 hour. 1 ml of bryophyte extract was added to a test tube and 5 ml of 10% Folin-Ciocalteu reagent (*Aldrich*) was added, after 5 minutes 4 ml of 7.5% sodium carbonate (*Aldrich*) was added. The test tube was shaken thoroughly and kept in a dark place at room temperature for 2 hours. Absorption was then measured using a quartz cuvette (d = 1 cm) on a spectrophotometer (*Hach-Lange DR 2800*) at 725nm wavelength Narwal et al. 2011. Results were calculated using a standard curve (gallic acid concentration

5–150 mg l^{-1}), which was expressed as gallic acid 100 g^{-1} (GE 100 g^{-1}) dry matter (Singleton et al., 1999). Three parallel measurements were carried out.

Radical scavenging activity determination in bryophyte extracts using DPPH

In a test tube 0.3 ml of bryophyte extract was added and was mixed with 3.6 ml of 4% solution in 96% ethanol 2,2-diphenyl-1-picrylhydrazyl (DPPH) (*Aldrich*). Mixture was incubated for 20 minutes in a dark place in room temperature. Absorption was measured using a quartz cuvette (d = 1 cm) with a spectrophotometer at 517 nm wavelength. Three parallel measurements were carried out. Results were calculated using a standard curve (gallic acid concentration 1–5 mg l^{-1}), which is expressed as gallic acid 100 g^{-1} (GE 100 g^{-1}).

RESULTS AND DISSCUSION

The selection of the bryophyte species was done considering their abundance in the Northern Europe, sampling and cultivation possibilities, use in traditional medicine and food, and possible presence in their composition of biologically active substances. The studied bryophytes showed a relatively low variability in their elemental composition and the ranges of basic elements in bryophyte species were: C 41–44%; O 49–52%; H 5.5–6%; N 0.4–2%; S 0% (Maksimova et al., 2013). Metal content and basic chemical characteristics of studied bryophyte species were given in previous study (Maksimova et al., 2013). To study basic chemical composition, the cross-polarization magic angle spinning ^{13}C nuclear magnetic resonance spectra (CPMAS ^{13}C NMR) of solid samples of the studied bryophyte species was used (Fig. 1). This method supports quantitative estimation of major structural elements of organic matter, including biota samples (Nierop et al., 2001). Chemical shifts in spectra were compared with literature data (Karlström et al., 1995) and they were divided into chemical shift regions, depending on different functionalities of chemical compounds.

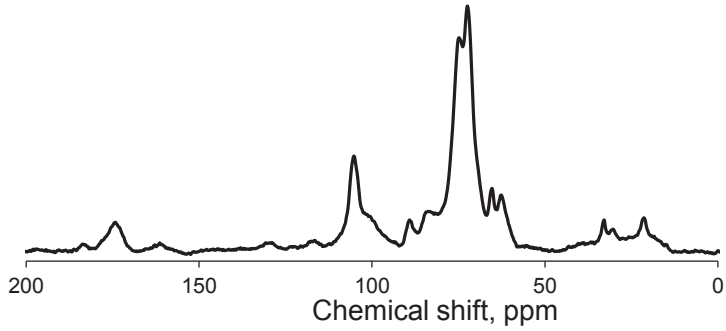

Figure 1. ^{13}C NMR spectra of moss *Rhytadiadelphus triquetrus*.

The chemical shift region from 0 to 50 ppm corresponds to aliphatic carbon and amount of aliphatic structures on average has been ~ 10%. The chemical shift region 50 to 92 ppm corresponds mainly to the ring carbon of carbohydrates (Karlström et al., 1995). The chemical shift region from 92 to 112 ppm corresponds to the axial carbon of carbohydrates (Karlström et al., 1995; Nierop et al., 2001). CPMAS ^{13}C NMR spectra

confirm that carbohydrates were the main constituents of bryophytes as it was stated already before (Maksimova et al., 2013). The chemical shift region from 112 to 136 ppm corresponds to the aromatic carbon (Karlström et al., 1995). The chemical shift region from 136 to 159 ppm corresponds to the phenolic and N–substituted aromatic carbon. Amount of aromatic compounds, including lignin and its derivatives was low in studied bryophytes. The chemical shift region from 159 to 190 ppm corresponds to the carbon in fatty acids, including free carboxylic acids, their esters, and also amides. The chemical shift region from 190 to 212 ppm corresponds to the carbonyl group carbon (Silverstein et al., 2005). Fatty acids and alcohols were present in bryophytes in minimal amounts. Composition of bryophytes partly explains low caloric value of bryophytes (3,800–5,000 cal g^{-1} dry weight, Forman 1968, Rastorfer 1976), however accessibility of structural carbohydrates for animal consumption could be another reason (Klavina 2015) to this. So the composition of secondary metabolites thus could become a major direction of research of bryophyte composition and their application possibilities.

Optimization of extraction was performed using moss – *Rhytidiadelphus triquetrus* as representative sample of mosses common for mixed wood forest ecosystems. As criteria for the evaluation of extraction efficiency, the following parameters were used: yield of extracts (dry residue), total polyphenolic content, radical scavenging activity, carbohydrate content analysis. Amongst criteria of extraction efficiency major stress was put on the yield of extracted substances and the antioxidant activities of the extracts, considering recent interest just in this kind of activity of natural compounds (Cheynier et al., 2013) and for this purpose DPPH radical scavenging activity analysis was used. Considering interest in studies of bryophyte biologically active compounds and more broadly in the composition of bryophyte secondary metabolites, for the extraction low-cost, low-toxicity, volatile solvents and their mixtures were selected with ability to extract substances with possibly wider range of properties (water, ethanol, CO_2). Five different extraction methods ensuring possibly highest extraction yield, prospective for obtaining of preparative amounts of extracts of were used: a) conventional extraction (shaking at room temperature); b) Soxhlet extraction; c) ultrasound-assisted extraction; d) extraction using treatment with microwaves; e) extraction with supercritical CO_2. To study impact of the extraction procedures, extraction conditions (time and temperature) were changed to compare efficiency of each selected method.

The most efficient extraction method (Table 1) proved to be microwave extraction at 150°C both judging by the total polyphenol content and the radical scavenging activity. Conventional and Soxhlet extraction provided high yields, but in comparison with intensive extraction methods, required much more time. Conventional extraction consumed also much more solvent than the other studied methods. Soxhlet and extraction showed good results when extracts were tested in respect to radical scavenging activity; however total polyphenol levels were lower than, for example, in case of ultrasound assisted extraction. This implies that not only polyphenolic compounds in bryophytes were responsible for radical scavenging activity. Supercritical CO_2 extraction provided good results in total polyphenol content, but the overall yields and radical scavenging activity was relatively low.

Table 1. Extraction efficiency of different extraction methods of biologically active compounds from *Rhytadiadelphus triquetrus* (solvent 60% ethanol*). Data are average from three replicates ± SE

Extraction method	Time of extraction, h dry weight	Total polyphenol content, GE 100 g^{-1} dry weight	Radical scavenging activity, GE 100 g^{-1} dry weight	Extraction yield, mg 100 g^{-1} dry weight
Soxhlet*	165.5 ± 8.3	116.4 ± 5.8	205.2 ± 10.3	165.5 ± 8.3
	239.6 ± 11.9	142.6 ± 7.1	231.5 ± 11.6	239.6 ± 11.9
Microwave	111.2 ± 5.6	167.8 ± 8.4	195.6 ± 9.8	111.2 ± 5.6
	486.9 ± 24.4	172.9 ± 8.6	150.2 ± 7.5	486.9 ± 24.4
Ultrasound	243.7 ± 12.2	54.1 ± 2.7	195.5 ± 9.7	243.7 ± 12.2
	254.6 ± 12.7	63.3 ± 3.2	150.6 ± 7.5	254.6 ± 12.7
Supercritical* CO_2	230.4 ± 11.5	162.4 ± 8.1	125.1 ± 6.3	230.4 ± 11.5
	274.9 ± 13.5	143.5 ± 7.2	124.8 ± 6.2	274.9 ± 13.5
Conventional	150.7 ± 7.6	25.8 ± 1.3	99.5 ± 4.9	150.7 ± 7.6
	194.3 ± 9.7	36.9 ± 1.8	97.2 ± 4.9	194.3 ± 9.7

* Extraction done using 96% ethanol.

For a better understanding of the extraction efficiency using ultrasound the effect of treatment time, as well as composition of extrahents, in respect to application potential, ethanol: water mixture ratio was compared (Fig. 2). Ultrasound-assisted extraction helped to improve extraction yield due to mechanical stress which cavitation induces, following cellular breakdown and release of secondary metabolites.

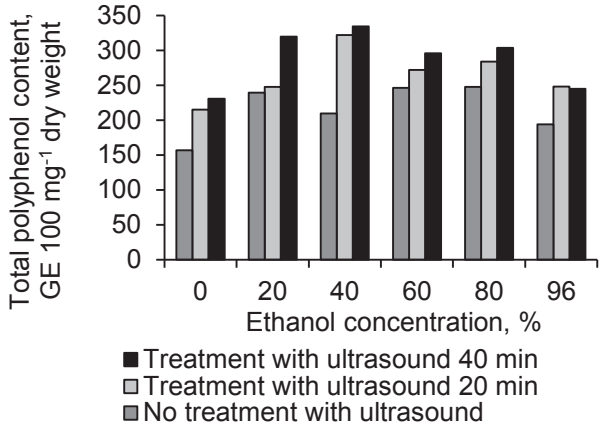

Figure 2. Effect of ethanol: water ratio, extraction and treatment with ultrasound time on total polyphenol content in extracts from bryophyte *Rhytadiadelphus triquetrus*.

The increase of the treatment time with the ultrasound helped to significantly increase yield of polyphenolics (Fig. 2). Difference of extraction efficiency with 40 min ultrasound treatment and without was approximately 20–40% in some cases, but the difference between the treatments for 20 or 40 min was less than 10%. It may be concluded that it was effective to use ultrasound to increase extraction efficiency but the sonification duration if it exceeds 20 min do not have major impact. Highest yield of polyphenolics in case of *Rhytadiadelphus triquetrus* ensured use of 20–60% ethanol.

Detected differences in the extraction yield of total polyphenolics indicated differences in the bryophyte composition. For screening of bryophyte secondary metabolite composition 60% ethanol could be suggested and were further used in this study.

As a next step in polyphenolic, radical scavenging and total mass of extracted substances extraction optimization process various types of solvents were used ensuring secondary metabolite and especially phenolic compound isolation from bryophytes (Table 2). Solvents used were water and ethanol. Selection of solvents was based considering economic reasons and toxicity of solvents. Microwave extraction was concluded to be the most effective extraction technique; nevertheless solvent optimization was done using sample treatment with ultrasound with subsequent shaking for 24 h.

Table 2. Extraction efficiency of *Rhytadiadelphus triquetrus* using different solvents and solvent mixtures (extraction conditions–ultrasound treatment for 20 min with following shaking for 24 h and repeated 20 min ultrasound treatment). Data are from three replicates ± SE

Extrahent		Total polyphenol content, GE 100 g^{-1} dry weight	Radical scavenging activity, GE 100 g^{-1} dry weight	Extraction yield, mg 100 g^{-1} dry weight
Water		230.0 ± 11.5	10.5 ± 0.5	36.8 ± 1.8
Ethanol	96%	254.0 ± 12.7	11.5 ± 0.6	652.5 ± 32.6
	80%	304.0 ± 15.2	50.4 ± 2.5	667.5 ± 33.4
	60%	296.0 ± 14.8	24.0 ± 1.2	195.0 ± 9.8
	40%	334.0 ± 16.7	7.9 ± 0.4	187.5 ± 9.4
	20%	320.0 ± 16.0	6.3 ± 0.3	96.6 ± 4.8

Higher polyphenolic concentrations were found using ethanol. Antioxidant capacity (measured with DPPH method) was also higher using ethanol, but the differences were not as significant as in case of total polyphenolics content and the total dry extract mass. The optimal yields of polyphenolics, radical scavenging substances and total dry extract mass could be obtained from mosses using aqueous ethanol in concentration range from 60% till 80%.

As mentioned in Table 1 and 2 the best results were obtained using microwave assisted extraction with ethanol as the solvent. For better understanding the controlling factors of extraction efficiency, another experiment using microwave assisted extraction was carried out in order to see how ethanol concentrations affecting extraction efficiency (Fig. 3).

Higher polyphenol content was reached when microwave treatment was used at 150°C in comparison with 120°C. Microwave assisted extraction in comparison with ultrasound assisted extraction showed similarities when it comes to differences between different optimal solvent concentrations. Optimal ethanol concentration for *Rhytadiadelphus triquetrus* at 120°C temperature was 60%. Meanwhile optimal ethanol concentrations when used ultrasound assisted extraction was 40%. The extraction conditions elaborated for *Rhytadiadelphus triquetrus* were efficiently applied for extraction of biologically active secondary metabolites from bryophytes common in Northern Europe (Table 3).

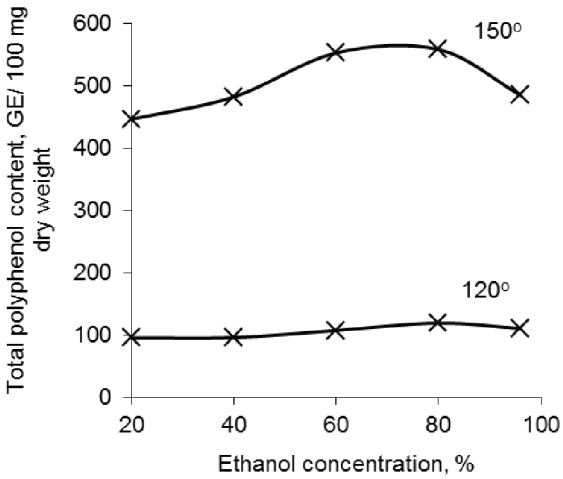

Figure 3. Extraction optimization from *Rhytadiadelphus triquetrus* using microwave assisted extraction with aqueous ethanol in concentration from 20 to 96%.

The studied bryophytes contained significant amounts of secondary metabolites and using elaborated extraction approach they could be isolated (Table 3). The yields of extracts altered for different species and evidently depended on the factors specific for each species individually (remarkable are differences of extraction yield for *Polytrichum juniperum* and *Polytrichum commune* as well as differences in the extraction yields from *Sphagnum rubellum* in comparison with other *Sphagnum* species).

Table 3. Bryophyte extracts obtained with optimal extraction method (microwave extraction) with solvent 60% ethanol. Data are from three replicates ± SE

Bryophyte	Total polyphenol content, GE 100 g^{-1}, dry weight	Radical scavenging activity, GE 100 g^{-1}, dry weight	Extraction yield, mg 100 g^{-1}, dry weight
Aulocomnium palustre	267.5 ± 13.1	39.0 ± 1.9	114.2 ± 5.7
Climacium dendroides	301.0 ± 14.7	14.9 ± 0.7	155.6 ± 7.8
Dicranum scoparium	477.3 ± 23.2	11.9 ± 0.5	194.6 ± 10.1
Hylocomnium splendens	298.4 ± 14.6	59.3 ± 3.1	102.6 ± 5.1
Pleurozium shreberi	275.6 ± 13.4	15.6 ± 0.8	145.2 ± 7.3
Polytrichum commune	800.7 ± 39.1	12.6 ± 0.6	154.8 ± 7.7
Polytrichum juniperum	416.2 ± 20.2	24.1 ± 1.2	88.5 ± 4.4
Ptilium crista-castrensis	237.9 ± 11.7	19.6 ± 1.0	145.5 ± 7.3
Rhytadiadelphus squarosum	399.2 ± 19.5	16.4 ± 0.8	133.5 ± 6.7
Rhytadiadelphus triquetrus	379.1 ± 18.5	11.3 ± 0.5	144.8 ± 7.2
Sphagnum girgensonii	783.2 ± 38.1	17.6 ± 0.9	142.5 ± 7.1
Sphagnum magelanicum	370.6 ± 18.1	18.2 ± 0.9	140.6 ± 7.0
Sphagnum rubellum	345.7 ± 16.7	16.3 ± 0.8	65.3 ± 3.3
Sphagnum squarosum	280.1 ± 13.7	14.9 ± 0.7	149.3 ± 7.4

Optimized extraction conditions allowed obtaining extracts with relatively high content of polyphenolics and high radical scavenging activity, also much dependent on the studied species. In comparison with much widely studied higher vegetation species

(Häkkinen et al., 1999; Kolesnikov and Gins. 2001), especially, berries and plants used as a source of polyphenolics and antioxidants in food, the values of total polyphenol content and radical scavenging activity in studied bryophytes was significantly lower, however, the composition of bryophyte secondary metabolites could be considered as prospective to continue studies of their composition.

CONCLUSION

Bryophytes as abundant plant group, especially in Northern regions are interesting group of plants for studies of alternative food sources, despite low caloric value of them. Elemental composition analysis and ^{13}C nuclear magnetic resonance spectra indicate that carbohydrates are a major structural component of bryophytes. Bryophytes contain numerous secondary metabolites. This study indicates that the extracts obtained from bryophytes have remarkable antioxidant activity, the extent of which depends on the extraction conditions. The principal factors that contribute to the efficiency of extraction are the type of solvent, temperature, ratio solvent: bryophyte mass, etc. Some of these parameters have been evaluated in this work on the extraction of polyphenolics and antioxidants.

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REFERENCES

Asakawa, Y., Ludwiczuk, A. & Nagashima, F. 2013. *Chemical constituents of bryophytes: bio- and chemical diversity, biological activity, and chemosystematics (Progress in the chemistry of organic natural products)*. Springer, Vien, 796 pp.

Beike, A.K., Decker, E.L., Frank, W., Lang, D., Vervleit–Scheebaum, M., Zimmer, A.D. & Reski, R. 2010, Applied Bryology- Bryotechnology. *Tropical Bryology* **31**, 22–32.

Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., Martens, S. 2013. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry*, **72**, 1–20.

Cheng, X., Xiao, Y., Wang, X., Wang, P., Li, H., Yan, H. & Liu Q. 2012. Anti-tumor and pro-apoptic activity of ethanolic extract and its various fractions from *Polytrichum commune* L. Ex Hedw in L1210 cells. *Journal of Ethnopharmacology* **143**, 49–56.

Forman, R.T.T. 1968. Caloric values of bryophytes. *Bryologist*, **71**, 344–347.

Fu, P., Lin, S., Shan, L., Lu, M., Shen, Y.H., Tang, J., Liu, R.H., Zhang, X., Zhu, R.L. & Zhang, W.D. 2012. Constituents of the moss *Polytrichum commune*. *Journal of Natural Products* **72**, 1335–1337.

Glime, J.M. 2007. *Bryophyte Ecology*. Physiological Ecology. E-book. Michigan Technological University, International Association of Bryologists. Vol. 1. Accessed on 2.03.2012 at <http://www.bryoecol.mtu.edu/>

Goffinet, B. & Shaw, A.J. 2008. *Bryophyte biology*. Cambridge University Press: Cambridge, 565 p.

Haines, W.P. & Renwick, J.A.A. 2009. Bryophytes as food: comparative consumption and utilization of mosses by a generalist insect herbivore. *Entomologia experimentalis et apllicata*, **133**, 296–306.

Häkkinen, S.H., Kärenlampi, S.O., Heinonen, M., Mykkänen, H.M., Törrönen, A.R. 1999. Content of the flavonols quercetin, myrucetin, and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry*, **47**, 2274–2279.

Karlström, H., Nilsson, M., Norden, B. 1995. Using multivariate methods on solid-state ^{13}C NMR data of complex material. *Analytica Chimica Acta*. **315**, 1–14.

Klavina, L. 2015, *Polysaccharides from Lower Plants: Bryophytes.* In: Ramawat, Kishan Gopal and Mérillon, Jean-Michel (eds) *Polysaccharides*. Springer International Publishing, Switzerland.

Kolesnikov M.P. & Gins V.K. 2001. Phenolic substances in medical plants. *Applied Biochemistry and Microbiology,* **37**(4), 392–399.

Maksimova, V., Klavina, L., Bikovens, O., Zicmanis, A., Purmalis, O. 2013. Structural characterization and chemical classification of some bryophytes found in Latvia. *Chemistry & Biodiversity,* **10**(7), 1284–1294.

Narwal, S.S., Szajdak, L., Sampietro, D.A. 2011. *Research methods in plant sciences Vol. 1. Soil allelochemicals.*, Studium press LTD, USA, 440 pp.

Nierop, K.G.J., van Lagen, B., Buurman, P. 2001. Composition of plant tissues and soil organic matter in the first stages of a vegetation succession. *Geoderma*, **100**, 1–24.

Orlov, D.S., Sadovnikova, L.K. 2005. Soil organic matter and protective functions of humic substances in the biosphere 37-52, in Use of Humic substances to remediate Polluted Environments: From theory to practice. Edited by Irina V. Perminova, Kirk Hattfield and Norbert Hertkorn, NATO Science Series IV Earth and Environmental Science Vol. 52.

Painter, T.J. 2003. Concerning the wound healing properties of *Sphagnum* holocellulose: the Mailard reaction in pharmacology. *Journal of Ethnopharmacology*, **88**, 145–148.

Pouliot, R., Hugron, S., Rochefort, L. 2014. *Sphagnum* farming: a long term study on producing peat moss biomass sustainably. *Ecological Engineering*, **74**, 135–147.

Rastorfer, J.R. 1976. Caloric values of three Alaskan- Arctic mosses. *Bryologist*, **79**, 76–78.

Silverstein, R.M., Webster, F.X., Kiemle, D.J. 2005. *Spectrometric identification of organic compounds.* 7th ed. Wiley, Hoboken, 512 pp.

Singh, M., Govindarajan, R., Nath, V., Rawat, A.K.S., Mehrotra, S. 2006. Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *Journal of Ethnopharmacology,* **107**, 67–72.

Singleton, V.L., Orthorfer, R. & Lamuel- Raventos, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteu reagent. *Methods of Enzymology,* **299**, 152–178.

Spjut, R.W., Suffness, M., Cragg, G.M., Norris, D.H. 1986. Mosses, liverworts and hornworts screened for antitumor agents. *Economic Botany,* **40**(3), 310–338.

Strazdiņa, L., Liepiņa, L., Mežaka, A., Madžule, L. 2011. *Sūnu ceļvedis dabas pētniekiem*. LU Akadēmiskais apgāds, Rīga, 50 pp. (in Latvian)

Xie, C.F., Lou, H.X. 2009. Secondary metabolites in bryophytes: an ecological aspect. *Chemistry & Biodiversity*, **6**, 303–312.