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Ultraviolet absorbance of *Sphagnum magellanicum*, *S. fallax* and *S. fuscum* extracts with seasonal and species-specific variation

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Abstract
Bryophytes, including *Sphagnum*, are common species in alpine and boreal regions especially on mires, where full sunlight exposes the plants to the damaging effects of UV radiation. *Sphagnum* species containing UV-protecting compounds might offer a biomass source for nature-based sunscreens to replace the synthetic ones. In this study, potential compounds and those linked in cell wall structures were obtained by using methanol and alkali extractions and the UV absorption of these extracts from three common *Sphagnum* moss species *Sphagnum magellanicum*, *Sphagnum fuscum* and *Sphagnum fallax* collected in spring and autumn from western Finland are described. Absorption spectrum screening (200–900 nm) and luminescent biosensor (*Escherichia coli* DPD2794) methodology were used to examine and compare the protection against UV radiation. Additionally, the antioxidant potential was evaluated using hydrogen peroxide scavenging (SCAV), oxygen radical absorbance capacity (ORAC) and ferric reducing absorbance capacity (FRAP). Total phenolic content was also determined using the Folin-Ciocalteu method. The results showed that methanol extractable compounds gave higher UV absorption with the used methods. *Sphagnum fallax* appeared to give the highest absorption in UV-B and UV-A wavelengths. In all assays except the SCAV test, the methanol extracts of *Sphagnum* samples collected in autumn indicated the highest antioxidant capacity and polyphenol content. *Sphagnum fuscum* implied the highest antioxidant capacity and phenolic content. There was low antioxidant and UV absorption provided by the alkali extracts of these three species.

Keywords *Sphagnum* moss · Ultraviolet absorbance · Methanol and alkali extracts · Antioxidant potential · GC-MS

1 Introduction

Chemical sunscreens are usually synthetic aromatic compounds conjugated with a carbonyl group. They protect from the damaging effect of UV radiation by absorbing rays of UV light and releasing the wavelengths of lower energy. However, UV absorption may activate the sunscreens, and they may consequently interact with cutaneous molecules, causing adverse skin reactions, such as dermatitis or photosensitivity reactions [1]. Chemical sunscreens have also been shown to possess adverse environmental impacts. For example, oxybenzone was found to be genotoxic towards coral planulae and is therefore banned in Hawaii [2]. Sunscreen components were also demonstrated to induce coral bleaching by promoting viral infections to hard corals and their symbiotic algae [3]. These factors indicate an urgent need for broad-spectrum anti-UV radiation compounds derived from natural sources for the use of dermatology, cosmetics, and the coating industry.

Potential biomass to be studied for this use is the bryophytes, which are the dominant plant species in many UV-rich areas, such as the alpine and polar regions, and on treeless and sparsely wooded mires. Niemi et al. [4] showed that increased UV radiation decreased the capitulum dry mass and induced a 30–40% increase in the chlorophyll and carotenoid pigments of the *Sphagnum* sp. chosen for the study even with short sampling periods. This could be an indication of *Sphagnum*'s rapid ability to adjust to adverse conditions. To promote this idea, a study by Clarke et al.

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[5] showed that another bryophyte, *Ceratodon purpureus* possessed high tolerance to UV, while it only contained low concentrations of the methanol-soluble UV-screening compounds. Therefore, they suggested that the found UV-filtering photoprotective potential is mainly originated from the cell-wall-bound compounds extracted via alkali-extraction.

In this study, samples of three common *Sphagnum* moss species adapted to different growth habitats with varying UV radiation, *S. magellanicum*, *S. fuscum,* and *S. fallax,* were collected in spring and autumn to consider seasonal variation. Three specimens of each species from both sample collections were then extracted using methanol and alkali extraction. To our knowledge, there are no studies available addressing the UV-filtering potential comparing the methanol-soluble and the alkali-soluble cell-wall-bound compounds of the chosen *Sphagnum* mosses. To detect the UV absorption ability, we used traditional absorbance screening (200–900 nm) and the physical luminescent microbial biosensor methodology described by Tienaho et al. [6]. This method embodies rapid high-throughput screening potential, cost-effectiveness and extreme sensitivity. Because the exposure to UV radiation causes a formation of reactive oxygen species (ROS) in plant and animal cells and tissues, it is well justified to link the UV-filtering potential to the antioxidant activity and polyphenol contents of the methanol and alkali extracts. This was achieved using hydrogen peroxide scavenging (SCAV, also ferrous oxidation in xylenol orange FOX) [7, 8], oxygen radical absorbance capacity (ORAC) [9, 10] and ferric reducing antioxidant power (FRAP) [11] antioxidant microplate tests. Folin-Ciocalteu reagent method [12] was used for determining total phenolic content. In addition, gas chromatography–mass spectrometry (GC–MS) was used for the characterization of compounds present within the extracts.

2 Experimental

2.1 Chemicals

Methanol (Lichlorosolv), chloroform, acetone, NaOH, Na_2CO_3, NaCOOH and Folin-Ciocalteu reagent were purchased from Merck KGaA. NaCl was from J. T. Baker and sodium dodecyl sulphate (SDS, 20%) from EuroClone. Gallic acid was from Sigma and formic acid (99%) from VWR international. Ethanol (99.5%) was from Altia, Finland.

2.2 *Sphagnum* collections

Three replicate samples of the surface layer of *Sphagnum magellanicum*, *Sphagnum fallax* and *Sphagnum fuscum* were collected from pristine mire in western Finland (Kaljakankaansuo, N61°56.18′ E23°19.87′). The long term (1980–2010) annual mean temperature of the site was ca. 3.8 °C, the annual mean precipitation ca. 620 mm and the accumulative temperature sum (> + 5 °C) ca. 1150 degree-days. The mire site type of the study site was low sedge *S. papillosum* fen with large *Sphagnum* hummocks [13]. The surface of the study sites consisted of a typical pattern of boreal mires: from wet lawns to relatively dry hummocks. From a nutritional point of view, surfaces varied from poor minerotrophy (lawns) to ombrotrophy (hummocks). Dominant *Sphagnum* moss species on the hummocks were *S. fuscum* and *S. magellanicum*, and on the lawns *S. magellanicum* and *S. fallax*. The sampling was repeated twice, on the 28th of May 2018 and on the 24th of September 2018. The summer 2018 was very sunny and dry and *Sphagnum* mosses were extremely dry before the autumn rains. Visually normal and species-specific samples of the *Sphagnum* biomass were selected for this study. The fresh *Sphagnum* biomass was frozen and stored at − 20 °C for no more than 24 h prior to extraction.

2.3 Extraction

The samples were extracted first with methanol followed by consecutive alkali extraction, which was slightly modified from the study by Clarke et al. [5].

2.3.1 Methanol extraction

Two grams of the fresh *Sphagnum* capitula of each species (approximately 3 cm) with three replicate samples were ground in a mortar. After adding 10 mL of methanol, the mixture was incubated at darkness and room temperature for one h in preweighed 15 mL polypropylene tubes (BD Falcon™, VWR International Oy, Finland). The mixture was then centrifuged at 12,000 rpm for 10 min (10,350 Sigma 2-16KL Benchtop centrifuge), the supernatant was removed to a preweighed 15 mL polypropylene tube and dried using a vacuum centrifuge with a cooling unit (Rotational-Vacuum-Concentrator RVC 2–18, Cold Trap CT 02–50 SR, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The extraction yields (dry weight) in spring were 6.4–9.1 mg (0.32–0.46%) for *S. magellanicum*, 11.6–18.7 mg (0.58–0.94%) for *S. fallax*, and 6.1–7.3 mg (0.31–0.37%) for *S. fuscum*. In autumn the extraction yields (dry weight) were 5.7–7.9 mg (0.29–0.40%) of *S. magellanicum*, 10.7–11.9 mg (0.53–0.60%) of *S. fallax*, and 5.3–6.7 mg (0.27–0.34%) of *S. fuscum*. Prior to the tests, the dry extracts were dissolved in water adjusting equal concentrations for all the samples.

2.3.2 Alkali extraction

The remaining cell debris or pellet was incubated in 1 M NaCl, twice in 0.5% (w/v) SDS and again twice using

chloroform/methanol (1:1, v/v), each for 20 min and centrifuged at 12,000 rpm for 5 min in between. The pellet was then washed with acetone before drying in a vacuum centrifuge. After drying, the pellet was incubated for 16 h in darkness with 1 M NaOH and then centrifuged for 15 min in 12,000 rpm. The volume of the supernatant was then measured and moved into a new 50 mL polypropylene tube. The extraction product was neutralized using an equal amount (v/v) of 1.5 M formic acid and centrifuged again for 5 min in 12,000 rpm. The final extraction product was then pipetted into preweighed polypropylene tubes and dried using a vacuum centrifuge. Both extracts were stored at − 20 °C until analyzed. The extraction yields (dry weight) in spring were 644.0–687.6 mg (32.2–34.4%) for *S. magellanicum*, 575.5–636.8 mg (28.8–31.8%) for *S. fallax*, and 542.4–552.9 mg (27.1–27.6%) for *S. fuscum*. In autumn the extraction yields (dry weight) were 528.6–547.5 mg (26.4–27.4%) of *S. magellanicum*, 587.5–667.0 mg (29.4–33.4%) of *S. fallax*, and 482.2–536.2 mg (24.1–26.8%) of *S. fuscum*. The dry weights of alkali extracts were higher because these included the cell wall debris of the *Sphagnum* biomass. Prior to the tests, the dry extracts were dissolved in water adjusting equal concentrations for all the samples.

2.4 UV Absorbance

2.4.1 Biosensor analysis

The DNA damage-induced stress-responsive biosensor strain *E. coli* DPD2794 originally described by Vollmer et al. [14] was used with physical screening methodology described in Tienaho et al. [6]. Because the luminescent light production can vary depending on the chosen culture, the results are expressed as induction factors ($IF = Average_{sample\ triplicates}/Average_{negative\ control\ triplicates}$). Higher luminescent light signals are given for samples with low protection from UV irradiation. In the physical test methodology, the samples are not in contact with the bacterial cells. Instead, the samples are placed directly over the cell cultures, which allows UV irradiation protection but minimizes the possibility of the sample itself inducing stress reaction in the bacterial cells. Because the material was limited and the test gives evidence of the sample's ability of protecting bacteria only from the UV-C irradiation (254 nm), biosensor analysis was conducted solely for the spring collection. Both methanol and alkali extracts were dissolved in double distilled water to achieve the concentrations of 1.66; 0.83 and 0.42 mg/mL per microplate well.

2.4.2 Spectrophotometric analysis

Absorbance screening was executed for all samples. The absorbance of 100 µL of the extract with three replicates in a translucent microplate (Sarstedt AG & Co, Germany) was measured using Thermo Scientific Varioskan Flash Reader (Thermo Fischer Scientific, Thermo Electron Co. USA) in the absorbance scan mode with 5 nm intervals for the wavelength area of 200–900 nm as described by Tienaho et al. [2]. Averages and standard deviations were calculated for the sample replicates.

2.5 Antioxidant and total phenolic content tests

2.5.1 SCAV

This test measures the hydrogen peroxide scavenging ability of the samples. In order to be active in this test the substance needs to inhibit the Fenton reaction, where hydrogen peroxide oxidizes Fe(II) into Fe(III), which yields hydroxyl radicals. The used methodology was modified from the methods described by Hazra et al. [7] and Jiang et al. [8]. In brief, four technical replicates of 30 µL were measured in 96-well format as in Välimaa et al. [15] or Tienaho et al. [16]. An aliquot of 2 mM H_2O_2 (Merck KGaA, Darmstadt Germany) was added to the reaction mixture with the sample, 111 µM xylenol orange disodium salt (Sigma Aldrich Chemie GmbH, Steinheim, Germany) and 2.56 mM ammonium iron (II) sulphate·$6H_2O$ (BDH Prolabo). The absorbance was measured at 560 nm after 30 min incubation. The scavenging ability is expressed as the inhibition percentage (%) of Fe(II) oxidation to Fe(III). Sodium pyruvate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a positive control.

2.5.2 ORAC

This test was used to measure the ability of a potential antioxidant to prevent peroxyl radicals from harming the fluorescent fluorescein molecule. The used methodology is modified from the methods described by Huang et al. [9] and Prior et al. [10]. In brief, two technical replicates of 50 µL were measured in 96-well format as in Välimaa et al. [15] or in Tienaho et al. [16]. All samples were measured with a series of five dilutions (1:1–1:320) and additional dilutions were added when necessary to adjust the sample concentrations to the 0.153 mM Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) standard curve. The reaction mixture contained the sample dilution, in 75 mM phosphate buffer pH 7.5 (Merck), 150 µL of 8.16×10^{-5} mM fluorescein (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 25 µL of 2,2′-Azobis(2-methylpropionamidine) dihydrochloride (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The results are expressed as Trolox equivalents (µmol TE/g).

2.5.3 FRAP

This test measures the reducing capacity of a potential antioxidant. To be active in this test the substance must be able to reduce Fe(III) into Fe(II). The used methodology is modified from the method described by Benzie and Strain [11]. Three technical replicates of 25 µL were measured in 96-well format as in Välimaa et al. [15]. The reaction mixture contained the sample, 20 mM $FeCl_3 \cdot 6H_2O$ (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 300 mM acetate buffer pH 3.6. The formation of ferrous-tripyridyltriazine complex in the reaction mixture is measured by absorbance at 593 nm and series of dilutions to fit the sample to the standard curve. $FeSO_4 \cdot 7H_2O$ (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used as a standard compound and L(+)-ascorbic acid (150 µM and 800 µM) (VWR Chemicals) as a control and the results are expressed as µmol/L Fe(II) equivalents.

2.5.4 Folin-Ciocalteu

This test measures the total phenolic content of the samples. Phenolic compounds and polyphenols have been found to possess antioxidant capacity. They scavenge oxygen radicals and break the radical chain sequences as well as interrupt the lipid peroxidation process. In the method, molybdotungstophosphoric heteropolyanion oxidizes phenolic compounds in alkalic solution forming a blue molybdotungstophosphate complex [12]. The absorbance was measured at 750 nm and the results were compared against gallic acid (29.4 mM, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) standard curve. The reaction mixture contained the sample, Folin-Ciocalteu reagent (Merck), and 20% Na_2CO_3 (Merck). The results are expressed as Gallic acid equivalents (mg GAE/g).

2.6 Statistical methods

The effect of *Sphagnum* species, sampling date (May and September), and their interactions on total phenol contents, and on the antioxidant action levels (SCAV, ORAC and FRAP test results) were analysed by the linear regression model. The sampling date and species were included as categorical factors with fixed effects, such that their reference levels were sampling date I (May) and Species (*S. magellanicum*). The models were fitted using the function lm [17] of the R environment version 3.6.1 [18]. The analysis was performed separately for methanol and alkali extractions.

2.7 Chemical analysis

Chemical analysis of methanol and alkali extracts was performed by using a HP 6890 GC system equipped with a mass selective detector HP 5973 and ZB-SemiVolatiles capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). Helium was used as a carrier gas, at a flow rate of 1.5 mL/min. Heneicosanoic acid (C:21) and Betulin were used as internal standards. The chromatographic conditions were as follows: initial temperature 150 °C; temperature rate 7 °C/min to 230 °C and 4 °C/min final temperature 310 °C for 20 min; injector temperature 280 °C and split ratio 1:20, MS-interface temperature was 300 °C, and ion source temperature 230 °C. Mass spectra were obtained by electron impact (EI mode) ionization energy 70 eV. The samples were silylated prior to the analysis with 0.5 mL 20% TMSI-pyridine mixture (TMSI = 1-(trimethylsilyl) imidazole).

3 Results and discussion

3.1 UV absorption

UV absorption capacity was measured with two methodologies for the samples collected in spring and only using absorbance screening for the autumn collection. The results using physical biosensor methodology are shown in Fig. 1. Because the used DNA damage-induced stress-responsive sensor emits luminescence as a reaction to the stress, the higher the IF signal, the less the sample has been able to protect the bacteria. Luria broth growth medium was used as the positive control in the method and only *S. fuscum* (at the content of 1.67 and 0.84 mg/ml) and *S. magellanicum* (at the content of 1.67 mg/ml) methanol extracts gave lower IF values indicating higher UV irradiation protection with the screening method (Fig. 1a). However, all the methanol extracts gave lower values than the negative control (water). In the case of alkali extracts, all gave higher IF values than the positive or negative control (Fig. 1b) indicating no UV irradiation protection. Interestingly, in this study, alkali extracts seem to increase the UV irradiation-induced damage in the used *Escherichia coli* biosensor even without a contact with the strain. The reason for this is unclear. It may be that the refraction index of neutralized NaOH or washing solvent residues in the extracts are smaller than the water control's, which would increase the amount of UV irradiation going through to the bacteria.

The results from traditional UV–Vis region screening are shown in Fig. 2. It seems that although *S. fuscum* methanol extract had the highest absorbance in the UV-C region (250–280 nm) *S. fallax* had higher absorbance through the whole UV-B region (280–315 nm) in autumn and for 280–290 nm in spring. The concentrations of the extracts

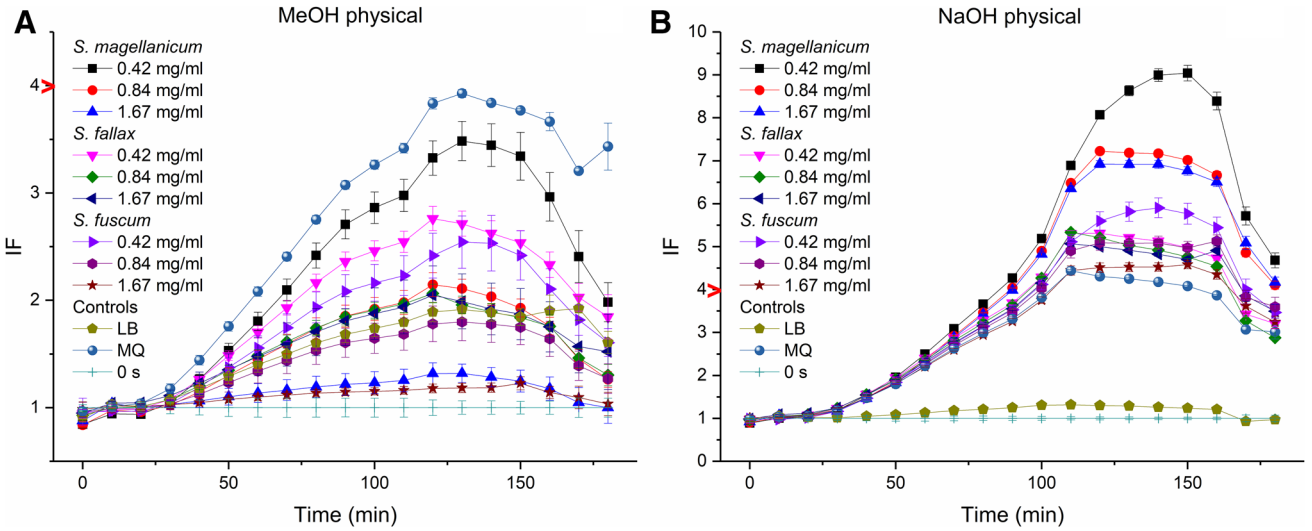

Fig. 1 The UV irradiation protection of MeOH extracts (**a**) and NaOH extracted fractions (**b**) of *Sphagnum* mosses collected in the spring (mean ± standard deviation) measured with the bioscreening method at the UV irradiation wavelength 254 nm [6]. LB = lysogeny broth growth medium, which is used as a positive control. MQ = Double-distilled water, which is used as the negative control. 0 s = shows the normal growth of the biosensor cells without UV exposure. IF = induction factor; (IF = Average(sample triplicates)/Average(negative control triplicates)). The higher the IF value of the sample at a given time point, the less the sample is protecting the bacteria from the UV irradiation

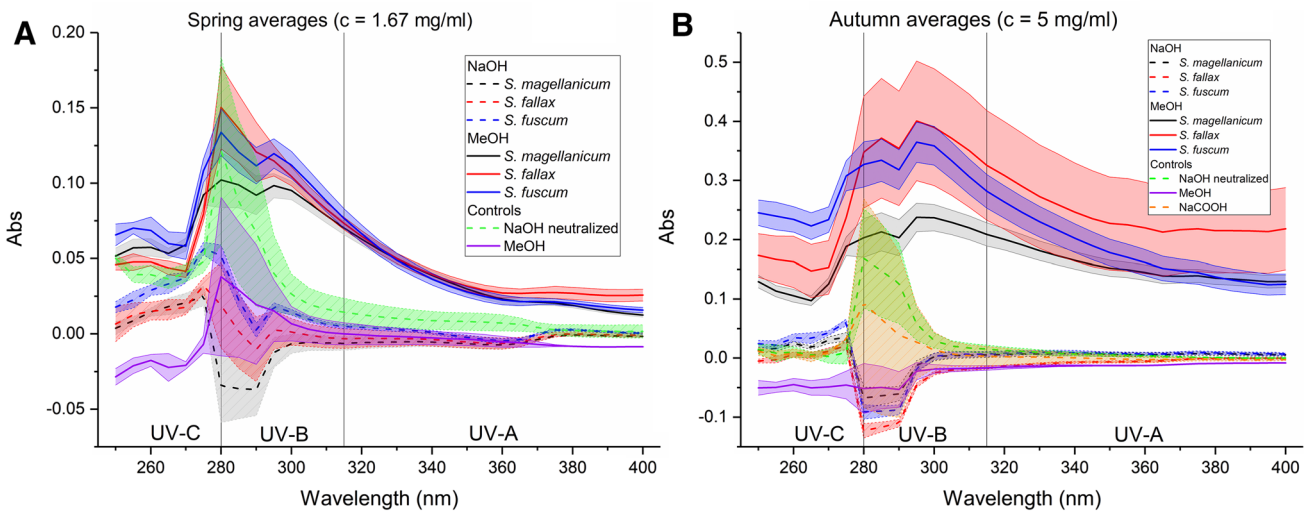

Fig. 2 UV light absorbance of MeOH and NaOH extracts of *Sphagnum* mosses in spring (**a** $c = 1.67$ mg/ml) and autumn (**b** $c = 5.00$ mg/ml) (mean ± standard deviation, $n = 3$)

differ between spring and autumn because of the altering collected *Sphagnum* biomass quantity. This difference does not impact the UV absorption profiles as can be seen from Fig. 2. *S. fallax* gives the strongest absorbance in the UV-A region as well (315–400 nm). This is particularly clear in the autumn (Fig. 2b) with slightly higher concentrations but is detectable in spring as well from 335–400 nm (Fig. 2a). Some absorbance can be detected in the UV-C region for the alkali extracts, but absorbance is negative in the UV-B region. This is unusual behavior for plant extracts. As the neutralized NaOH and the NaCOOH salt both have positive absorption in the UV-B region (Fig. 2), it could be due to some washing solvent residues in the alkali extracts causing a shift to the refraction index. Surprisingly, *S. fallax,* although adapted to the shadiest growing habitats, had the strongest UV absorbance properties. This may be due to the different growing optimum with respect to nutrition status

and water table level when compared with the two other *Sphagnum* moss species, *S. fuscum* and *S. magellanicum*. It is also possible that *S. fallax* has stronger UV absorption to adapt to various UV radiation conditions, where dark shadows caused by the trees shift suddenly to open sunshine. This sudden increase in high light is called a sunfleck and it can cause large shifts in the quality of light received by plants in the understory.

3.2 Antioxidant and phenols tests

Antioxidant and phenols test results are summarized in Fig. 3 and Supplementary Table S1. All the three antioxidant activity tests and phenol contents indicated that there was no difference between *Sphagnum* species in methanol extracts in spring. Phenol contents increased in all species in September, being on the highest level in *S. fuscum*. The phenolic content showed correlation with the antioxidant test results indicating that methanol-extractable polyphenols may contribute to the antioxidant power. The increase in the phenolic content may also contribute to the increase in the absorbance in the UV-B region (Fig. 2).

The higher antioxidant power of the methanol extracts was measured in autumn than in spring. The SCAV values differed from the other test results. The antioxidant assays used here cover the distinct antioxidant mechanisms: hydrogen atom transfer (HAT) by ORAC, single electron transfer (SET) by FRAP and the ability to chelate transition metals by SCAV. Polyphenols have been shown to absorb the UV wavelengths and to scavenge UV generated ROS and, thus, alleviate the harming effects of UV radiation [1]. Plants protect themselves from the harming physiological effects of excess solar radiation by accumulating polyphenolic compounds, such as flavonoids [19]. Additionally, plants use pigmentation to protect chlorophylls and DNA and it is usually achieved via anthocyanins, which are a form of flavonoids [20]. *Sphagnum* mosses have been shown to possess higher amounts of phenolic compounds than other

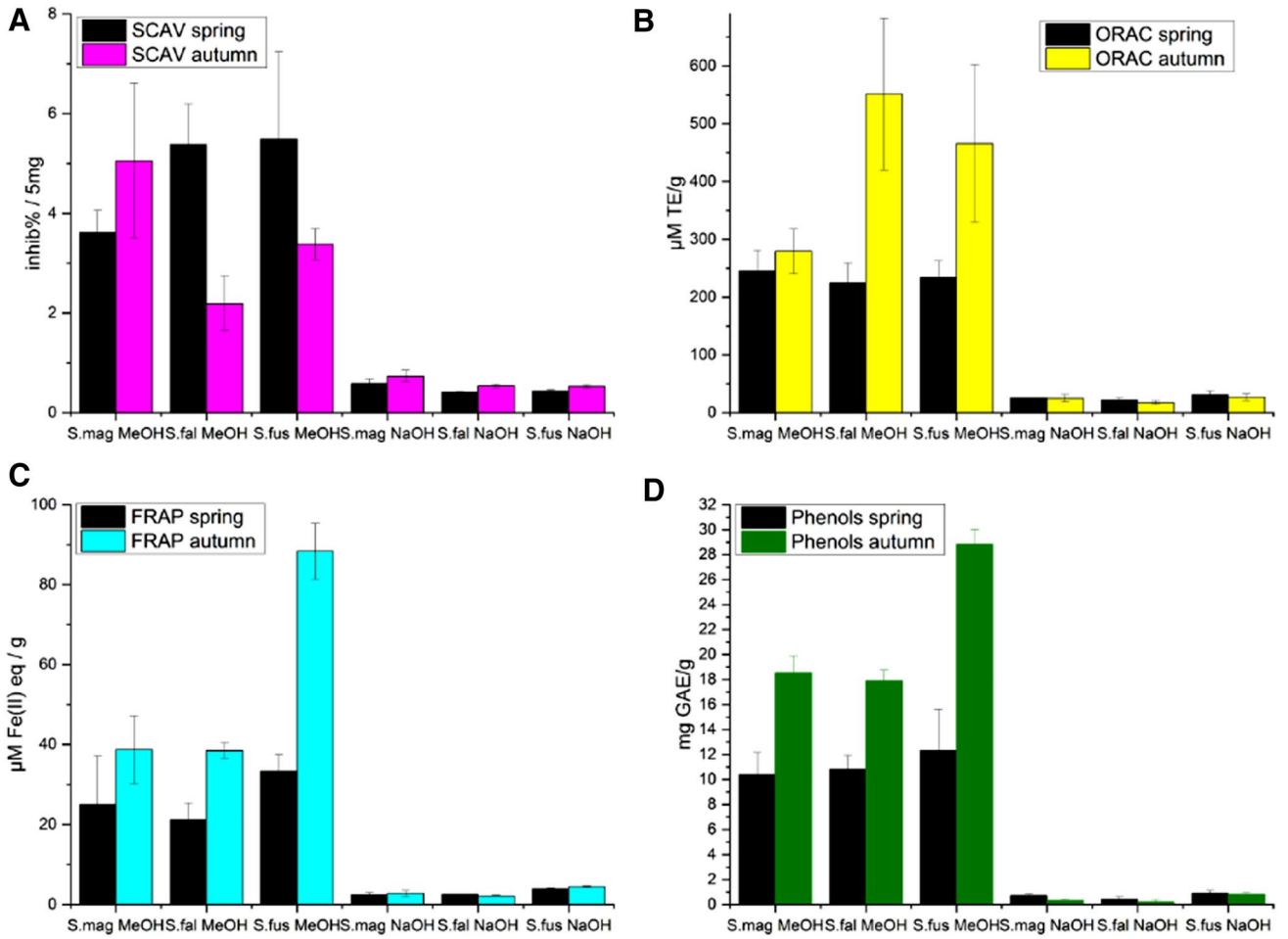

Fig. 3 H_2O_2 scavenging (SCAV) (**a**), ORAC (**b**) and FRAP (**c**) values as well as total phenolic content (**d**) of the methanol and alkali extracts of the three *Sphagnum* moss species collected in spring and autumn. The values present the averages ± standard deviations ($n = 3$) per dried extract. S.mag, *S. magellanicum*; S.fal, *S. fallax*; S.fus, *S. fuscum*

bryophytes in general although the level is still low when in comparison with other plants [21]. The comparison of different studies is challenging because of different units in the determination of the phenolic content. The highest concentration of free phenolics has been found in the capitula, which constitutes mostly living tissues with the active metabolic processes of *Sphagnum* [22, 23]. The amount of bound phenolics in *S. fallax* at 20 °C after 8 weeks was 3.4 mg/g (dry weight) and the amount of free phenolics 1.6 mg/g (dry weight) in the upper segment (0–3 cm from the capitulum) and 1.7 mg/g (dry weight) and 0.6 mg/g of dry weight in the lower segment (3–6 cm from the capitulum) of *Sphagnum*, respectively [22]. In this study, *S. fuscum* showed the highest phenolic content in the autumn and consequently the highest antioxidant power in the FRAP test. *Sphagnum fallax* autumn extract showed higher antioxidant activity in the ORAC test than the other two species *S. fuscum* and *S. magellanicum*. In the SCAV test, the highest antioxidant activity seems to account to the spring extracts whereas the SCAV activity decreased towards autumn in *S. fallax* and *S. fuscum* methanol extracts and remained stable in *S. magellanicum*. In alkali extraction, SCAV activity increased in *S. magellanicum* towards the autumn but remained stable in other species. In alkali extracts, FRAP activity was the highest in *S. fuscum* in spring. Phenolic content was probably more related to the FRAP activity due to its stronger correlation than that with ORAC. Both FRAP and ORAC activity and the phenolic content of alkali extracts showed 10–20 times lower values than those of methanol extracts. This indicates that the alkali extracted cell-wall associated compounds were not responsible for the UV absorption or the antioxidant power of the samples unlike with *Ceratodon purpureus* in an earlier study [5].

3.3 Characterization using GC–MS

Characterization results from the *Sphagnum* species can be found in Tables 1 (MeOH extracts) and 2 (NaOH extracts) and gas chromatograms can be seen on Figs. 4, 5, respectively. The amount of internal standards are the same in both figures, which emphasizes the fact that alkali extracts have smaller amounts of other substances. Most identified compounds were different carbohydrates, which is in accordance with the earlier studies [21]. However, campesterol and β-sitosterol have conjugated carbons in their ring structures, and they have been reported with UV λ_{max} at 257 nm and 251 nm in petroleum ether extracts, respectively [24]. Sitorus et al. [25] reported that β-sitosterol in ethanol has a UV λ_{max} at 268.5 nm. These compounds can therefore participate in the UV absorbance of the *Sphagnum* samples.

Table 1 GC–MS characterized compounds from the methanol extractions

MeOH		*S. fallax*		*S. magellanicum*		*S. fuscum*	
#	Compound	RT	A%	RT	A%	RT	A%
1	Fructose	6.06	25.6	6.04	10.3	6.04	21.6
2	Mannose	7.01	15.9	7.01	8.1	7.01	20.3
3	Glucose	7.14	0.6	7.15	0.6	7.14	1.3
4	Sorbitol	7.46	0.5	7.46	1.7	7.46	2.9
5	Glucose	8.15	14.8	8.15	7.5	8.15	20.2
6	Palmitic acid	9.06	1.7	9.07	1.0	9.06	1.1
7	Myo-inositol	9.53	1.3	9.53	1.1	9.53	2.7
8	Galactose	11.38	8.8	11.38	5.9	11.38	6.9
9	Glucuronic acid	13.29	0.1	13.30	0.4	13.29	0.3
10	ISTD1	15.70	11.1	15.70	10.8	15.70	13.4
11	Monopalmitin	16.41	1.5	16.41	1.4	16.41	1.4
12	Saccharose	17.08	100	17.08	100	17.07	100
13	Raffinose	18.36	0.5	18.36	0.8	18.36	1.3
14	Trisaccharose	18.67	0.4	18.67	2.7	18.66	3.8
15	Monostearin	19.41	8.6	19.41	9.1	19.41	9.3
16	Campesterol	26.30	0.5	26.30	0.2	26.69	0.4
17	β-sitosterol	27.60	0.6	27.60	0.4	27.59	0.2
18	Stachyose	28.73	11.2	28.73	11.8	28.72	10.2
19	ISTD2	30.74	17.0	30.74	16.9	30.74	20.8

RT, retention time; A%, relative peak area compared to the highest peak. Internal standards: ISTD1 = Heneicosanoic acid; ISTD2 = Betulin. The gas chromatogram of *S. fallax* is shown in Fig. 4 and other chromatograms as well as the corresponding mass spectra can be found from the Supplementary material

Table 2 GC–MS characterized compounds from the alkali extractions

NaOH		*S. fallax*		*S. magellanicum*		*S. fuscum*	
#	Compound	RT	A%	RT	A%	RT	A%
1	Arabinose	3.81	56.5	3.81	48.1	3.81	46.9
2	Arabinose	4.13	37.4	4.12	55.2	4.12	76.0
3	Fructose	5.99	17.9	5.81	22.9	5.93	42.3
4	Mannose	7.01	15.5	7.01	18.9	7.01	18.1
5	Glucose	7.13	17.2	7.13	22.4	7.13	35.1
6	Glucose	8.15	20.8	8.15	17.1	8.15	19.7
7	Palmitic acid	9.06	100	9.06	100	9.06	100
8	Stearic acid	11.58	56.0	11.58	58.2	11.58	56.3
9	ISTD1	15.70	1525	15.70	1723	15.70	1791
10	Saccharose	17.07	7.2	17.06	6.6	17.06	10.4
11	ISTD2	30.75	2656	30.75	2989	30.75	3296

The gas chromatogram of *S. fallax* is shown in Fig. 5 and other chromatograms, as well as the corresponding mass spectra, can be found from the Supplementary material

RT, retention time; A%, relative peak area compared to the highest peak; ISTD1, Heneicosanoic acid; ISTD2, Betulin

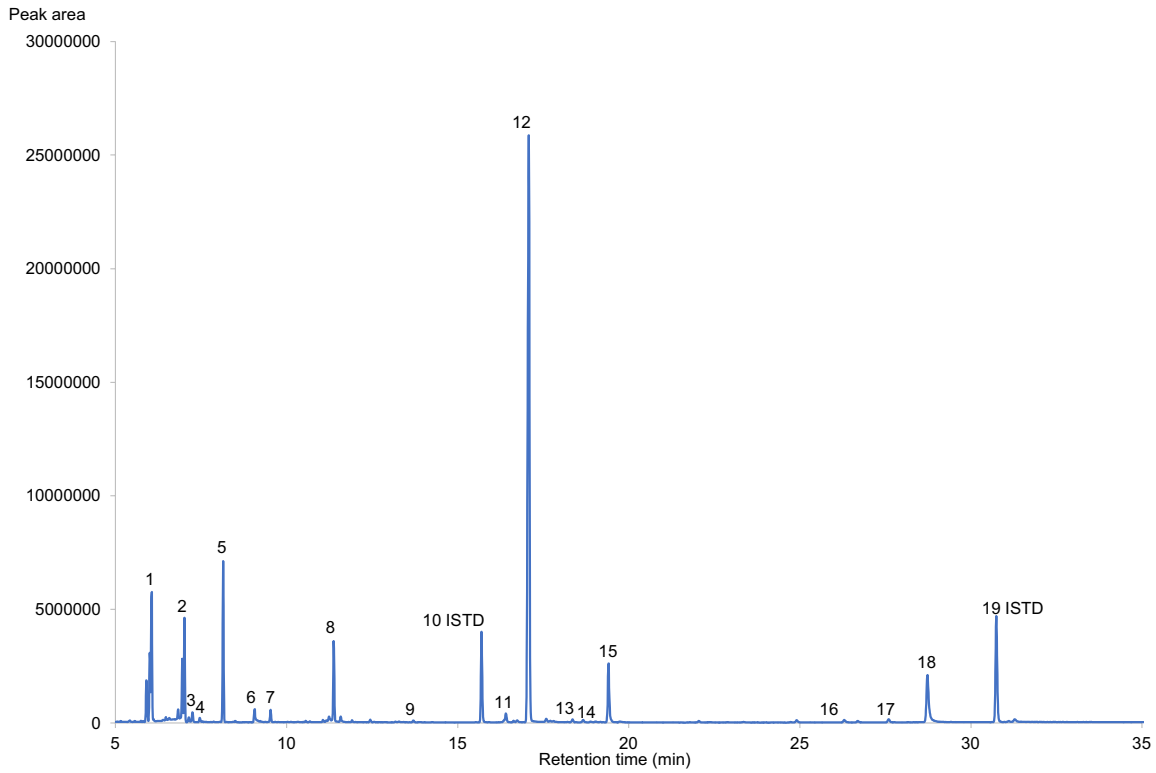

Fig. 4 The gas chromatogram of *S. fallax* methanol extract. All the chromatograms can be found from the supplementary material and they were similar for all species

Additionally, some unidentified compounds were observed (see Figs. 4 and 5 and Supplementary material). However, despite being low for all bryophytes, the phenolic composition has been reported and *Sphagnum* moss contains catechols, phenolcarboxylic acids and flavonols [26]. Additionally, lignin-like phenolics have been reported from *Sphagnum*, while structural analysis has not been able to verify their existence in moss [21]. Sphagnum acid (*p*-hydroxy-β-carboxymethyl-cinnamic acid) is considered the most abundant phenolic compound in *Sphagnum* and it

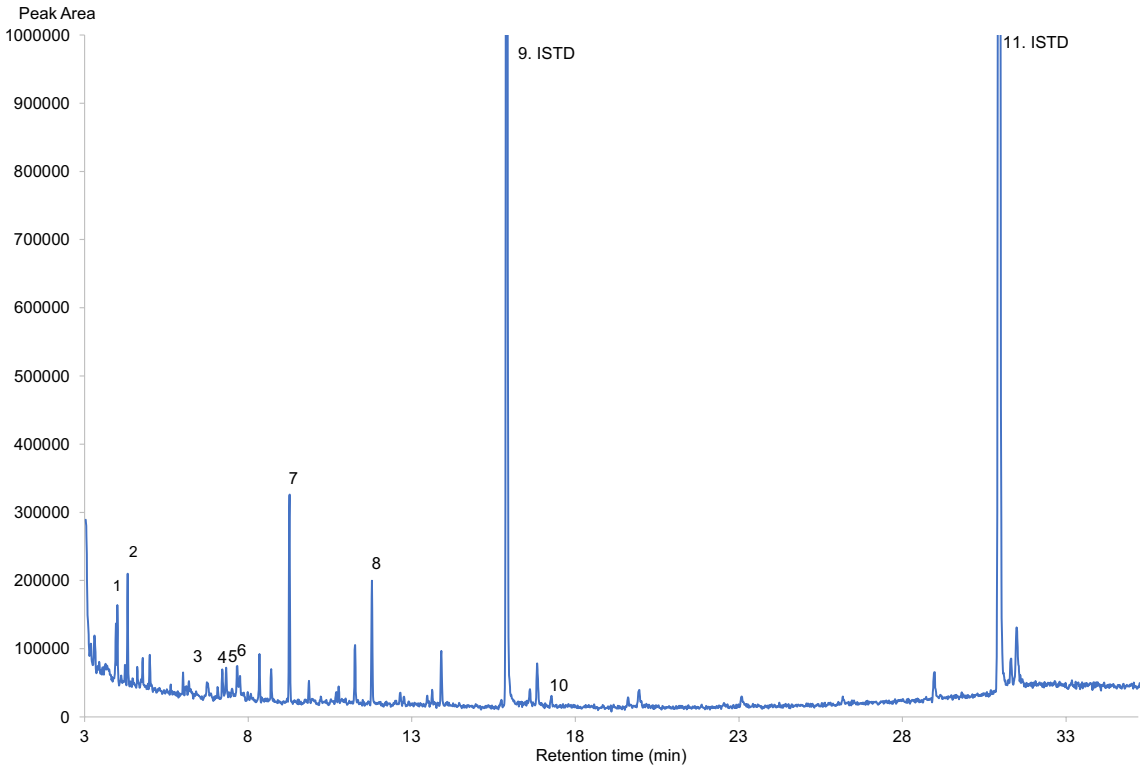

Fig. 5 The gas chromatogram of *S. fallax* alkali extract. All the chromatograms can be found from the supplementary material and they were similar for all species

is a decomposition product of lignin-like phenolics [21]. The metabolic profile of *Sphagnum fallax* has been described by Fudyma et al. [27] and a total of 655 metabolites out of which 329 were completely novel were identified using liquid chromatography–mass spectrometry LC–MS from lyophilized and Folch extracted biomass. The approach used in this study was qualitative analysis whereas more detailed analyses could be executed in further studies using LC–MS techniques.

4 Conclusions

In this study, UV absorption capacity and antioxidant power of *Sphagnum magellanicum*, *Sphagnum fuscum* and *Sphagnum fallax* both in spring and autumn were evaluated using methanol and alkali extraction. We found that methanol extractable compounds showed higher UV absorbance and antioxidant power whereas alkali extractable compounds gave significantly lower values in all tests. This must result from the extracts containing less UV absorbent, phenolic and ROS scavenging components than the methanol extracts. Interesting opportunities for future research arise from a possibility to acidify the alkali extracts down to pH 1 as has been previously examined for pine, liverworths and moss samples [28–30]. Acidification shows the importance for the extraction of compounds containing hydroxyl groups, which are often responsible for the antioxidant properties. By acidifying the alkali extracts, the antioxidant and UV absorption properties of the fraction could therefore improve. While *S. fuscum* methanol extract gave the highest absorption in the UV-C region, *S. fallax* indicated the highest absorption capacity in both UV-B and UV-A regions. UV-absorption was quite similar in spring and autumn. The highest phenolic content and the highest antioxidant power in the FRAP test were found in *S. fuscum* in autumn. The highest ORAC activity was shown in *S. fallax* and *S. fuscum* in autumn. In the SCAV test, the highest antioxidant activity accounts to the spring extracts and difference between *S. fallax* and *S. fuscum* is not significant. Only *S. magellanicum* gave higher activity in autumn. The metabolic profile of plants naturally varies during the year and metabolic changes related to the end of the growing season may affect the differences found in the activities between seasons. The GC–MS characterization verified the earlier finding that the majority of *Sphagnum* extractables are carbohydrates. However, the phytosterols characterized from the methanol extracts could participate in the UV absorbance of the samples. Earlier research has shown that bryophytes, in general, have low amounts of phenolic compounds and most of these

are catechols, phenolcarboxylic acids, flavonols, lignin-like phenolics or their decomposition products [21, 26]. LC–MS could identify further potentially active components and therefore be considered as an appropriate analysis equipment for future studies. This study indicates that the methanol extractable rather than the NaOH extractable cell-wall associated compounds are contributing to the UV absorption properties in the investigated *Sphagnum* species.

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Availability of data and material The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Electronic Supplementary Information (ESI) available: Baseline relative phenol content and antioxidant properties and estimated relative contrasts can be found in Supplementary file 1. Supplementary material also contains the gas chromatograms for all *Sphagnum* species (Supplementary file 2) and the mass spectra for the identified compounds (Supplementary file 3). See DOI: https://doi.org/10.1039/x0xx00000x.

Compliance with ethical standards

Conflicts of interest The authors have no relevant financial or non-financial interests to disclose.

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