Evaluation of Antioxidant Activity of Some Common Mosses

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The antioxidant activity of ethanol extracts of *Atrichum undulatum*, *Polytrichum formosum* (Polytrichaceae), *Pleurozium schreberi* (Entodontaceae) and *Thuidium tamariscinum* (Thuidiaceae) was evaluated by an electrochemical method (cyclic voltammetry) and standard photometric methods: Fe(III) to Fe(II) reducing power, nitric oxide scavenging (NO) assay and simulation of Fenton-type reaction by nonsite-specific (NSSOH) and site-specific (SSOH) hydroxyl radical-mediated 2-deoxy-D-ribose degradation inhibition. The total content of phenols was determined by the Folin-Ciocalteu reagent. All tested species showed antioxidant effects lower than the positive control, caffeic acid. The extracts of *A. undulatum* and *P. formosum* contained the highest content of phenols and were the most effective in Fe(III) to Fe(II) reducing power, cyclic voltammetry and SSOH assay. By contrast, only the extract of *Pl. schreberi* showed activity in the NSSOH assay. *A. undulatum* and *T. tamariscinum* extracts were the most active in the NO assay. The results suggest that the extracts of *A. undulatum* and *P. formosum* possess stronger antioxidant activity than those of *Pl. scheberi* and *T. tamariscinum*, but they affect the Fenton-type reaction mainly by iron chelation.

Key words: Cyclic Voltammetry, Fenton Reaction, Free Radicals

Introduction

Antioxidant and free radical scavenging activities are in the focus of attention of pharmacists and nutrition scientists. Free radicals are supposed to play a key role in the pathogenesis of many diseases (Castro and Freeman, 2001). Oxidation processes may also decrease the stability of drugs and foods. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been recognized as fundamental components of stress signal cascades (Haddad, 2002), both abiotic and biotic (Wojtaszek, 1997; Gechev *et al.*, 2006). This has expanded the interest on further fields, such as physiology and biochemistry.

Mosses occupy a special position among higher plants because the haploid gametophyte dominates their live cycle. Some species have been studied for their tolerances to drought and water stress (flooding) (Robinson *et al.*, 2000; Wasley *et al.*, 2006) or high nitrogen concentrations (Koranda *et al.*, 2007). Mosses are common in the vegetation of all continents. They are still marginalized in traditional medicines. Gosuite Indians of Utah used mosses of the genera *Bryum*, *Mnium* and *Philonotis* to alleviate the pain of burns (Flowers, 1957). In China, *Polytrichum* sp. were anticipated as antipyretic and diuretic drug already in the eleventh century (Wu, 1982). Today, the effects of mosses on microbes are studied intensively (Basile *et al.*, 1999; Opelt *et al.*, 2007; Singh *et al.*, 2007), but little knowledge exists about other activities, especially about secondary metabolites with antioxidant properties (Chobot *et al.*, 2006). Antibiotic polyphenols were identified in *Atrichum, Dicranum, Mnium, Polytrichum* and *Sphagnum* sp. (McCleary and Walkington, 1966). Among the flavonoids, apigenin, luteolin, kaempferol and orobol glycosides and their dimers are usually found in mosses (Zinsmeister and Mues, 1980; Basile *et al.*, 1999). Glycosides of three- and tetraoxygenated coumarins were reported from *Atrichum undulatum* and *Polytrichum formosum* (Jung *et al.*, 1994). Volatile constituents of mosses also attracted attention (Saritas *et al.*, 2001). Lipids

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Moss species	Reported classes of constituents	References
A. undulatum	Three- and tetraoxygenated coumarin glucosides	Jung *et al.*, 1994
P. formosum	Three- and tetraoxygenated coumarin glucosides	Jung *et al.*, 1994
Pl. schreberi	Apigenin, apigenin-7-rhamnoglucoside; acetylenic acids and lipids	Vandekerkhove, 1980; Dembitsky *et al.*, 1993
T. tamariscinum	Triterpenes and lipids	Marsili and Morelli, 1970

Table I. The main classes of constituents reported in the studied species.

and fatty acids were analyzed in the various genera of families, *e.g.* Dicranacae, Ditrichaceae and Entodontaceae (Ichikawa *et al.*, 1983; Dembitsky *et al.*, 1993; Wasley *et al.*, 2006).

The present study was aimed to evaluate the antioxidant activity of ethanol extracts of four commonly occuring mosses: *Atrichum undulatum* (Hedw.) Pal. de B. (Polytrichaceae), *Pleurozium schreberi* (Brid.) Mitt. (Entodontaceae), *Polytrichum formosum* Hedw. (Polytrichaceae), and *Thuidium tamariscinum* (Hedw.) B. S. G. (Thuidiaceae). *A. undulatum* and *P. formosum* contain a series of polyhydroxylated daphnin coumarins and *Pl. schreberi* flavonoid glycosides; none of these classes of secondary metabolites have been reported from *T. tamariscinum* (Table I). The evaluation of the antioxidant effects was carried out by cyclic voltammetry, which has been utilized in several studies to evaluate flavonoids (Firuzi *et al.*, 2005; Simić *et al.*, 2007; Yakovleva *et al.*, 2007), phenolic acids (Simić *et al.*, 2007; Yakovleva *et al.*, 2007), blood plasma (Chevion *et al.*, 1997) and plant extracts (Cosio *et al.*, 2006). Further, several commonly used photometric methods, iron(III) to iron(II) reducing power (Dorman *et al.*, 2003; Koşar *et al.*, 2005), nitric oxide (NO) scavenging (Marcocci *et al.*, 1994), nonsite-specific (NSSOH) and site-specific (SSOH) hydroxyl radical-mediated 2-deoxy-D-ribose degradation inhibition (Dorman *et al.*, 2004), were applied. Caffeic acid was used as a positive control because its redox properties have been well documented (Giacomelli *et al.*, 2002; Hotta *et al.*, 2002a; Kallel Trabelsi *et al.*, 2004; Sroka, 2005; Yakovleva *et al.*, 2007). The total content of phenols in the extracts was determined by the Folin-Ciocalteau reagent (Singleton and Rossi, 1965; Dorman *et al.*, 2003).

Materials and Methods

Chemicals

Naphthylethylenediamine dihydrochloride and sulphanilamide were obtained from Merck AG (Darmstadt, Germany). All other chemicals and organic solvents used were of analytical grade and purchased from Sigma-Aldrich, Inc (St. Louis, USA). Ultrapure water (18.2 MΩ cm) was prepared using a Millipore Milli-Q 185 plus system (Millipore Corp., Billerica, USA).

Plant material and extraction

The moss gametophytes were collected in the south and east regions of The Czech Republic in spring 2005. The samples were identified by Dr. V. Chobot and voucher specimens have been deposited at the Department of Chemical Ecology and Ecosystem Research, Faculty of Life Sciences, University of Vienna, Austria.

The air-dried gametophytes (15 g) were ground and extracted by maceration with 2×200 mL of absolute ethanol for 2×12 h. The extracts were filtered and concentrated under reduced pressure to dryness. The yields of extracts are reported in Table II.

Table II. Yields and total phenolic content (TPC) of extracts of the tested mosses.

Moss species	Yield of extract (% w/w)	TPC (%)[a]
A. undulatum	6.5	8.722 ± 0.803
P. formosum	3.9	4.387 ± 0.806
Pl. schreberi	5.6	2.023 ± 0.011
T. tamariscinum	4.8	3.210 ± 0.773

[a] Values are presented with ±95% confidence limits (% of gallic acid equivalents in the dried extract).

Total content of phenols

Folin-Ciocalteau reagent was prepared and diluted according to the Pharmacopoeia (Europäisches Arzneibuch, 2002). The extract was dissolved in 50% aqueous *n*-propanol. The concentration was 0.5 mg/mL; 0.2 mL of this solution was mixed with 0.6 mL of Folin-Ciocalteau reagent and after 15 min with 0.6 mL of 10% aqueous Na_2CO_3.

After 30 min of incubation at ambient room temperature, the absorbance was measured at 760 nm and compared to a calibration curve of gallic acid (Singleton and Rossi, 1965; Dorman *et al.*, 2003). The controls contained all of the reagents except for the extract. The total phenols were determined as gallic acid equivalents and are presented as means of triplicate analyses.

Iron(III) to iron(II) reducing power (IRP)

The ability of the extracts to reduce iron(III) was assessed by the method described by Dorman *et al.* (2003). An 1-mL aliquot of each extract dissolved in water was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of an 1% aqueous potassium hexacyanoferrate, $K_3[Fe(CN)_6]$, solution. After 30 min incubation at 50 °C, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged for 10 min. A 2.5-mL aliquot of the upper layer was mixed with 2.5 mL of water and 0.5 mL of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm. The mean absorbance values were plotted against concentration, and a linear regression analysis was carried out. The data are presented as ascorbic acid equivalents in mg per g of extract.

Cyclic voltammetry (CV)

Voltammograms were recorded at room temperature in a three-electrode system μAutolab PGSTAT (EcoChemie, Inc., Utrecht, The Netherlands). The working electrode was a glassy carbon electrode of 3 mm diameter, Ag/AgCl (saturated KCl) was used as the reference electrode and a platinum wire as the counter electrode. The glassy carbon electrode was polished and the platinum electrode was cleaned by flame before every measurement. The CV scan rate was 50 mV s^{-1}. The scan potential was from −300 to + 1200 mV. The extracts were dispersed in 0.1 M phosphate buffer, pH 7.4. The concentration was 1 mg/mL. Every experiment was repeated at least twice. The antioxidant activity was calculated as the sum of anodic oxidation peak areas of a cyclic voltammogram, which was compared to a caffeic acid calibration curve. The results are presented as caffeic acid equivalents.

Nitric oxide (NO) scavenging activity

The nitroprusside assay was arranged according to Marcocci *et al.* (1994) with minor modifications.

The extracts were dissolved (2 mg/mL) in a mixture of *n*-propanol and 0.2 M phosphate buffer, pH 7.4 (1:1 v/v), and serially diluted. 0.9 mL of the extract solution was mixed with 0.1 mL of sodium nitroprusside solution (27.8 mg/mL) in 0.2 M phosphate buffer, pH 7.4. After 1 h of incubation at 25 °C, 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride) was added and the absorbance was read at 546 nm. The negative controls had the same composition without extract. The positive control was caffeic acid. The EC_{50} values for each moss extract and caffeic acid were obtained by statistical analysis of the linear part of the sigmoid curves of absorbance against the concentration of the moss extract (Table III).

Nonsite-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation (NSSOH)

The ability of the extracts to inhibit nonsite-specific hydroxyl radical-mediated peroxidation was determined essentially as described by Dorman *et al.* (2004). The reaction mixture contained 500 μL of extract dissolved in KH_2PO_4/KOH buffer (50 mM, pH 7.4), 100 μL of 28 mM 2-deoxy-D-ribose in KH_2PO_4/KOH buffer, 200 μL of premixed 100 μM $FeCl_3$ and 104 mM ethylenediaminetetraacetic acid (EDTA) (1:1 v/v) solution, 100 μL of 1.0 mM H_2O_2 and 100 μL of 1.0 mM aqueous ascorbic acid. Tubes were vortexed and incubated at 37 °C for 60 min. Thereafter, 50 μL of 2.0% butylated hydroxytoluene were added to each tube followed by 1 mL of 2.8% trichloroacetic acid and 1 mL of 1.0% 2-thiobarbituric acid. The samples were vortexed and heated in a water bath at 100 °C for 20 min. The reaction was stopped by 5 min cooling in an ice water bath. 2 mL of *n*-butanol were added to each tube, and the mixture was vigorously vortexed. After centrifugation, the extent of oxidation was estimated from the absorbance of the organic layer at 532 nm. EC_{50} values were estimated using a linear regression of the linear part of the curve.

Site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation (SSOH)

The ability of the extracts to inhibit site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation was assayed as described by Dorman *et al.* (2004), except that EDTA was replaced by KH_2PO_4/KOH buffer (50 mM, pH 7.4).

Results and Discussion

All tested species demonstrated antioxidant and free radical scavenging activities, which were lower in comparison to caffeic acid, a cinnamic acid derivative, which served as positive control (Table III). Of all studied species, those of the family Polytrichaceae showed the highest total phenolic content and the strongest iron(III) to iron(II) reducing power (IRP). These data are also supported by the cyclic voltammograms of the extracts showing the process of anodic oxidation. The voltammograms of *A. undulatum* and *P. formosum* display three well visible peaks of oxidation (Fig. 1a). The first anodic peak is quasi-reversible. The voltammetric curves of *Pl. schreberi* and *T. tamariscinum* exhibit less pronounced irreversible anodic oxidation peaks (Fig. 1b). Total areas (expressed as caffeic acid equivalents) of the anodic peaks were largest in the voltammograms of the Polytrichaceae. By far, *A. undulatum* showed the largest values obtained of all extracts (Table III).

A. undulatum and *P. formosum* were also the most active in the nitric oxide and site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation (SSOH, a simulation of the Fenton reaction without the presence of the chelator EDTA). The nonsite-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation inhibition activity (NSSOH, a simulation of the Fenton reaction in the presence of the chelator EDTA) was too low for EC_{50} estimations. In these assays, the Entodontaceae yielded much weaker results compared to the Polytrichaceae. *Pl. schreberi* was the only active species in the NSSOH test, but the least active in the SSOH test. It contains mainly apigenin de-

rivatives while hydoxycoumarins (daphnin derivatives) are reported constituents of *A. undulatum* and *P. formosum* (Table I). The observed effects suggest that the possible dominant mechanism of scavenging of *A. undulatum* and *P. formosum* coumarins might comprise not only the direct interaction with ROS but also iron ions chelating. As a consequence, iron(II) ions then become less available for Fenton-type reactions and subsequent hydroxyl radical production (Miller *et al.*, 1990; Dorman *et al.*, 2004). Similar properties were showed also by other moss species, *e.g. Ceratodon purpureus* (Ditrichaceae) and *Dicranum polysetum* (Dicranaceae) (Chobot *et al.*, 2006). The iron(II) ions directly participate in Fenton-type reactions of reducing iron(III) to iron(II). Thus they may not only contribute to the antioxidant capacity but also cause pro-oxidative effects via facilitation of the hydroxyl radical production in two steps:

1) Iron(III) is an acceptor of the electron and phenol is a donor:

$$Fe(III) + phenolic\ compound \rightarrow Fe(II)$$
$$+ phenoxy^\bullet\ radical.$$
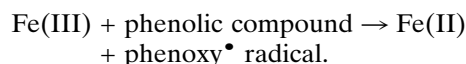
2) Hydrogen peroxide is an acceptor of the electron and iron(II) is a donor (Miller *et al.*, 1990; Sroka, 2005; see also Fig. 2):

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^\bullet OH + OH^-.$$
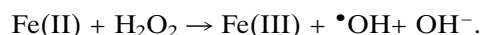
This phenomenon might explain the activity of *A. undulatum* and *P. formosum* extracts, the species with the strongest activity in the IRP and SSOH assays, but with low activity in the NSSOH assay.

Table III. Iron(III) to iron(II) reducing power, cyclic voltammetry, and 50% effective concentrations (EC_{50}) of antioxidant and free radicals scavenging activities of ethanol extracts of the mosses in comparison with caffeic acid.

Moss species	IRP [mg/g][a]	CV [mg/g][a]	NO [mg/mL][b]	NSSOH [mg/mL][c]	SSOH [mg/mL][d]
A. undulatum	79.330 ± 11.911	14.811 ± 5.059	0.088 ± 0.006	> 2.000	0.032 ± 0.005
P. formosum	48.859 ± 12.086	7.553 ± 1.688	0.029 ± 0.001	> 2.000	0.065 ± 0.006
Pl. schreberi	2.061 ± 0.352	6.808 ± 1.576	0.728 ± 0.158	0.845 ± 1.316	0.439 ± 0.203
T. tamariscinum	5.158 ± 0.889	6.286 ± 0.397	0.086 ± 0.003	> 2.000	0.174 ± 0.273
Caffeic acid	−	−	0.008 ± 0.001	0.033 ± 0.001	0.008 ± 0.001

[a] IRP, iron(III) to iron(II) reducing power (in mg of ascorbic acid equivalents per g of the dried extract); CV, cyclic voltammetry (in mg of caffeic acid equivalents per g of the dried extract).
[b] NO, nitric oxide (EC_{50} in mg/mL).
[c] NSSOH, nonsite-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation (EC_{50} in mg/mL).
[d] SSOH, site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation (EC_{50} in mg/mL); EC_{50} values were calculated from at least three replicates and are reported with 95% confidence limits.
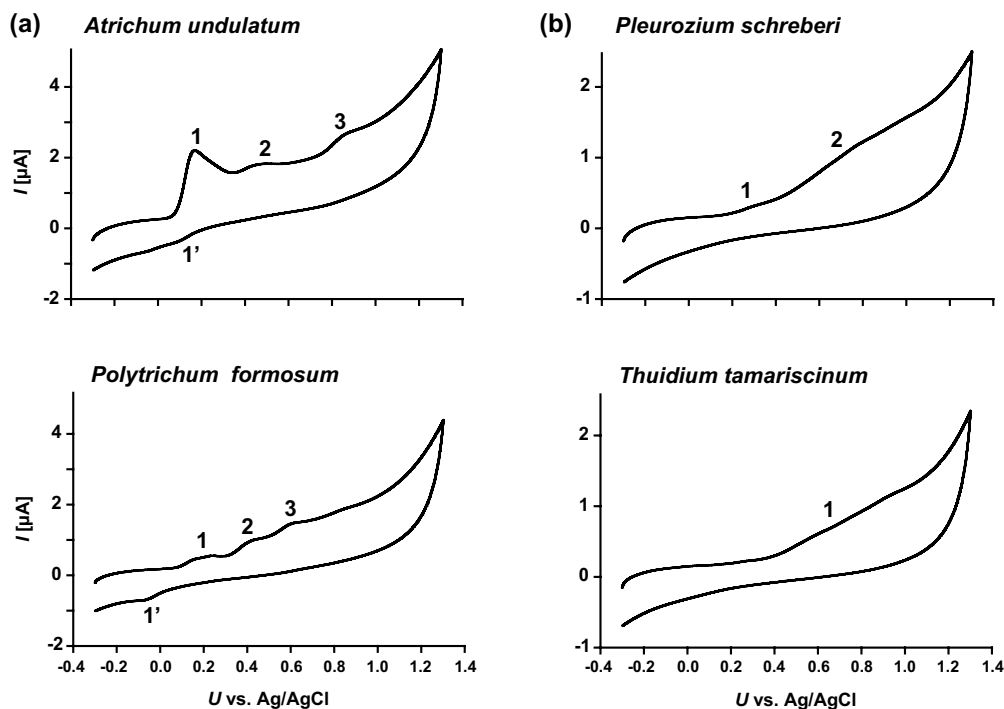
Fig. 1. (a) Cyclic voltammograms of ethanol extracts of *A. undulatum* and *P. formosum*. The curves show three peaks of oxidation (1–3) and one peak of reduction (1′), which is better visible in the *P. formosum* voltammogam. The lower reduction response is caused by polymerization reactions on the surface of the electrode. (b) Cyclic voltammograms of ethanol extracts of *Pl. schreberi* and *T. tamariscinum*. The curves show two oxidation peaks on the *Pl. schreberi* voltammogram (1, 2) and one oxidation peak on the *T. tamariscinum* voltammogram (1). The peaks are in both cases very flat and badly visible. The adsorption of extract constituents and their polymerization reactions on the electrode surface cause the absence of the back reduction.
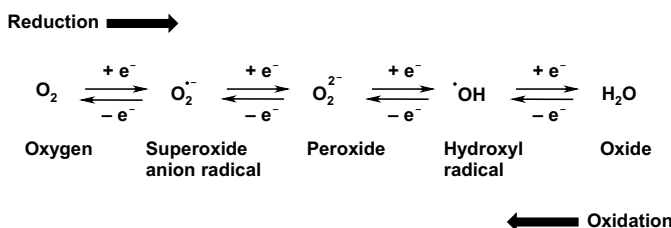

Fig. 2. Reactive oxygen species and their different oxidation stages.

Pl. schreberi showed exactly the opposite effects. The scavenging and antioxidant properties of substance mixtures containing flavonoids are crucially influenced by the equilibrium in the reaction mixture, such as the ratio of concentration of the scavenger and the ROS or RNS. The concentration of iron(II) versus iron(III) reflects the possibility of production of highly cytotoxic hydroxyl radicals. The scavenging effect is influenced also by redox reactivity and its reversibility. Thus, a flavonoid

can cause also a prooxidative effect after *e.g.* two electron transfers to oxygen when a quinone and hydrogen peroxide arise (Miura *et al.*, 1998) (Figs. 2 and 3). Their reversible redox equilibrium can be changed by oligomerization with an intermediate phenoxy radical (Hotta *et al.*, 2002b).

T. tamariscinum scored in the IRP and CV determinations between *P. formosum* and *Pl. schreberi*. It showed similar behaviour in the assays like *A. undulatum* and *P. formosum* but its activity was
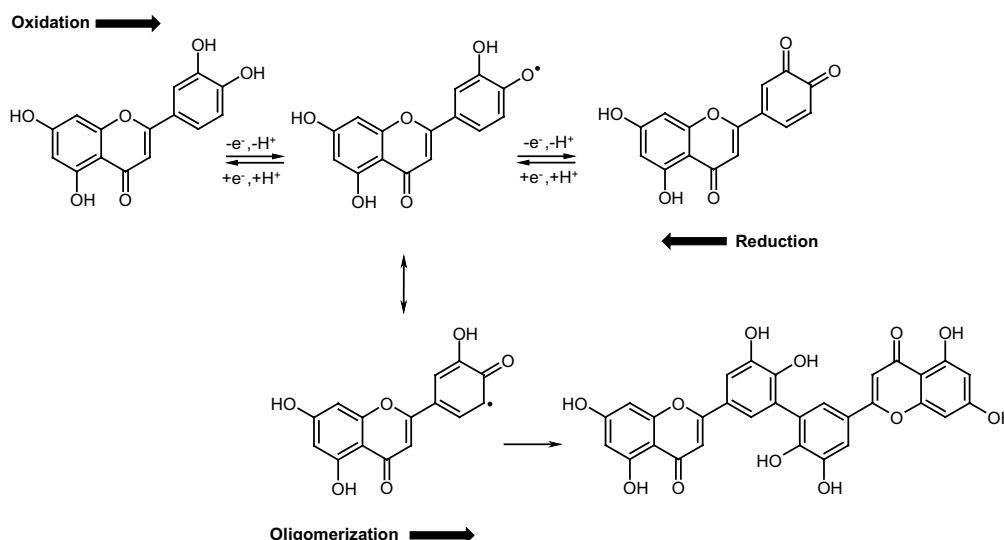
Fig. 3. Redox and oligomerization reactions of luteolin, one of the common flavonoid derivatives occurring in mosses.

weaker. *T. tamariscinum* was the second most active species in the nitric oxide scavenging assay, *i. e.* it showed good protection against nitric oxide oxidation and nitrite formation or its possible back reduction (Marcocci *et al.*, 1994; Gamal-Eldeen *et al.*, 2007). It remains object of future studies if any of the reported secondary metabolites (Table I) contribute to this effect.

The mixture of compounds present in the cell of a moss fundamentally complicates the predictability of redox reactions because we have to expect both synergistic and antagonistic effects. Their occurrence depends on the experimental milieu (Miura *et al.*, 1998; Simić *et al.*, 2007). Nevertheless, the methanolic extracts of the investigated mosses showed apparent antioxidant activity. The various experimental conditions modulated the effects, which partially correlated with known patterns of secondary metabolites in their tissues. More extensive studies using a combination of various experimental approaches as demonstrated here will certainly contribute to improved insights into the complex interactions of secondary metabolites with redox equilibrium and stress in the cell. The simple organisation of their tissues – moss cells lack a central vacuole, which is usually present in higher plants – reduces the extracted metabolites to mainly those, which we can assume to be active in the cytosol. This, on one hand, might offer attractive models to understand the functionality of these compounds in maintaining stress homeostasis. On the other hand, mixtures of secondary metabolites obtained from moss sources might provide pharmacy with evolutionary optimized model structures with high potential to be developed in medicinal applications.

Basile A., Giordano S., López-Sáez J.-A., and Cobianchi R.-C. (1999), Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry **52**, 1479–1482.

Castro L. and Freeman B.-A. (2001), Reactive oxygen species in human health and disease. Nutrition **17**, 163–165.

Chevion S., Berry E.-M., Kitrossky N., and Kohen R. (1997), Evaluation of plasma low molecular weight antioxidant capacity by cyclic voltammetry. Free Radical Biol. Med. **22**, 411–421.

Chobot V., Kubicová L., Nabbout S., Jahodář L., and Vytlačilová J. (2006), Antioxidant and free radical scavenging activities of five moss species. Fitoterapia **77**, 598–600.

Cosio M.-S., Buratti S., Mannino S., and Benedetti S. (2006), Use of an electrochemical method to evalu-

ate the antioxidant activity of herb extracts from the Labiatae family. Food Chem. **97**, 725–731.

Dembitsky V.-M., Rezanka T., Brychek I.-A., and Afonia O.-M. (1993), Acetylenic acids and lipid composition of some mosses from Russia. Phytochemistry **33**, 1021–1027.

Dorman H.-J.-D., Peltoketo A., Hiltunen R., and Tikkanen M.-J. (2003), Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem. **83**, 255–262.

Dorman H.-J.-D., Bachmayer O., Kosar M., and Hiltunen R. (2004), Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J. Agric. Food Chem. **52**, 762–770.

Europäisches Arzneibuch (2002), Grundwerk, 4th ed., Vol. 1, Verlag Österreich GmbH, Vienna, p. 1088.

Firuzi O., Lacanna A., Petrucci R., Marrosub G., and Saso L. (2005), Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. Biochim. Biophys. Acta **1721**, 174–184.

Flowers S. (1957), Ethnobryology of the Gosuite Indians of Utah. Bryologist **60**, 11–14.

Gamal-Eldeen A.-M., Djemgou P.-C., Tchundem M., Ngadjui B.-T., Tane P., and Toshifumi H. (2007), Anticancer and immunostimulatory activity of chromones and other constituents from *Cassia persiana*. Z. Naturforsch. **62c**, 331–338.

Gechev T.-S., Van Breusegem F., Stone J.-M., Denev I., and Laloi C. (2006), Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. BioEssays **28**, 1091–1101.

Giacomelli C., Ckless K., Galato D., Miranda F.-S., and Spinelli A. (2002), Electrochemistry of caffeic acid aqueous solutions with pH 2.0 to 8.5. J. Braz. Chem. Soc. **13**, 332–338.

Haddad J.-J. (2002), Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. Cell. Signalling **14**, 879–897.

Hotta H., Ueda M., Nagano S., Tsujino Y., Koyama J., and Osakai T. (2002a), Mechanistic study of the oxidation of caffeic acid by digital simulation of cyclic voltammograms. Anal. Biochem. **303**, 66–72.

Hotta H., Nagano S., Ueda M., Tsujino Y., Koyama J., and Osakai T. (2002b), Higher radical scavenging activities of polyphenolic antioxidants can be ascribed to chemical reactions following their oxidation. Biochim. Biophys. Acta **1572**, 123–132.

Ichikawa T., Namikawa M., Yamadas K., Sakai K., and Kondo K. (1983), Novel cyclopentenonyl fatty acids from mosses *Dicranum scoparium* and *Dicranum japonicum*. Tetrahedron Lett. **24**, 3337–3340.

Jung M., Zinsmeister H.-D., and Geiger H. (1994), New three- and tetraoxygenated coumarin glucosides from the mosses *Atrichum undulatum* and *Polytrichum formosum*. Z. Naturforsch. **49c**, 697–702.

Kallel Trabelsi N., Belhadj Tahar N., and Abdelhedi R. (2004), Electrochemical behaviour of caffeic acid. Electrochim. Acta **49**, 1647–1654.

Koranda M., Kerschbaum S., Wanek W., Zechmeister H., and Richter A. (2007), Physiological responses of bryophytes *Thuidium tamariscinum* and *Holocomium*

splendens to increased nitrogen deposition. Ann. Bot. **99**, 161–169.

Koşar M., Dorman H.-J.-D., and Hiltunen R. (2005), Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem. **91**, 525–533.

Marcocci L., Magire J.-J., Droy-Lefaix M.-T., and Packer L. (1994), The nitric oxide-scavenging properties of *Ginkgo biloba* extract FGb 761. Biochem. Biophys. Res. Commun. **201**, 748–755.

Marsili A. and Morelli I. (1970), Triterpenes from *Thuidium tamariscifolium*. Phytochemistry **9**, 651–653.

McCleary J.-A. and Walkington D.-L. (1966), Mosses and antibiosis. Rev. Bryol. Lichénol. **34**, 309–314.

Miller D.-M., Buettner G.-R., and Aust S.-D. (1990), Transition metals as catalysts of "autoxidation" reactions. Free Radical Biol. Med. **8**, 95–108.

Miura Y.-H., Tomita I., Watanabe T., Hirayama T., and Fukui S. (1998), Active oxygens generation by flavonoids. Biol. Pharm. Bull. **21**, 93–96.

Opelt K., Chobot V., Hadacek F., Schönmann S., Eberl L., and Berg G. (2007), Investigations of the structure and function of bacterial communities associated with *Sphagnum* mosses. Environ. Microbiol. **9**, 2795–2809.

Robinson S. A., Wasley J., Popp M., and Lovelock C.-E. (2000), Desiccation tolerance of three moss species from continental Antarctica. Aust. J. Plant Physiol. **27**, 379–388.

Saritas Y., Sonwa M.-M., Iznaguen H., König W.-A., Muhle H., and Mues R. (2001), Volatile constituents in mosses (Musci). Phytochemistry **57**, 443–457.

Simić A., Manojlović D., Šegan D., and Todorović M. (2007), Electrochemical behaviour and antioxidant and prooxidant activity of natural phenolics. Molecules **12**, 2327–2340.

Singh M., Rawat A.-K.-S., and Govindarajan R. (2007), Antimicrobial activity of some Indian mosses. Fitoterapia **78**, 156–158.

Singleton V.-L. and Rossi J.-A. (1965), Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. **16**, 144–158.

Sroka Z. (2005), Antioxidative and antiradical properties of plant phenolics. Z. Naturforsch. **60c**, 833–843.

Vandekerkhove O. (1980), Über die Verbreitung von Flavonoiden bei pleurokarpen Laubmoosen II. Apigenin und Apigenin-7-rhamnoglukosid bei *Pleurozium schreberi* (Willd.). Mitt. Z. Pflanzenphysiol. **100**, 369–372.

Wasley J., Robinson S.-A., Lovelock C.-E., and Popp M. (2006), Some like wet – biological characteristics underpinning tolerance of extreme water stress events in Antarctic bryophytes. Funct. Plant Biol. **33**, 443–455.

Wojtaszek P. (1997), Oxidative burst: an early plant response to pathogen infection. Biochem. J. **322**, 681–692.

Wu P.-C. (1982), Some uses of mosses in China. Bryol. Times **13**, 5.

Yakovleva K.-E., Kurzeev S.-S., Stepanova E.-V., Fedorova T.-V., Kuznetsov B.-A., and Koroleva O.-V. (2007), Characterization of plant phenolic compounds by cyclic voltammetry. Appl. Biochem. Microbiol. **43**, 661–668.

Zinsmeister H.-D. and Mues R. (1980), The flavonoid chemistry of bryophytes. Rev. Latinoam. Quim. **11**, 23–29.