

Phytochemical profiles and antioxidant potential of four Arctic vascular plants from Svalbard

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Abstract: Environmental stress in the Arctic region leads to damage in plant membranes as a result of oxidation processes. To withstand these stress conditions, plants are expected to produce antioxidants that differ from phenolics. Here we investigated the chemical composition and antioxidative activities of four Arctic flowering plant species (*Dryas octopetala*, *Carex rupestris*, *Silene uralensis*, and *Deschampsia alpina*.) through *in vitro* measurements of the Free Radical Scavenging activities (FRS), Inhibition of Lipid Peroxidation (ILP) and Trolox Equivalent Antioxidant Capacities (TEAC). *D. octopetala* exhibited the highest ILP (76.45%) and FRS (86.58%) activities. The TEAC values were higher than those of the Trolox vitamin E standard in all four species. Overall, the antioxidative activity was highest in *D. octopetala*, followed by *C. rupestris*, *S. uralensis*, and *D. alpina*. Electrospray ionization tandem mass spectrometric (ESI-MS/MS) analysis of methanolic extracts of these plants revealed the presence of organoselenides, linear alkylbenzenesulfonates (LAS) and oligosaccharides, some of which are reported as antioxidants in the literature. Hence, it is likely that the antioxidant activities exhibited by these plants are not only related to the production of phenolics. This is the first report of the antioxidant potential of four Arctic flowering plants and the presence of selenides in *D. octopetala* and *S. uralensis*, and the production of LAS in *C. rupestris*. Our findings suggest that these plants can be used as nutraceutical sources of selenium and as biomarkers for environmental pollution.

Key words Antioxidants, Angiosperm, Ny-Ålesund, Selenium, Svalbard.

Introduction

Free radicals are strong oxidants that potentially lead to membrane impairment, modification in proteins and enzymes, and DNA damage (Brawn and Fridovich 1981; Smirnov 1993). As a result, they are considered to play an important role in aging and several degenerative diseases, such as heart disease, cataracts, cognitive dysfunction, and cancer (Halliwell and Gutteridge 1998). Research on antioxidants, and particularly the search for the presence of natural compounds with low cytotoxicity in plants, has become an important research field in biomedicine.

The high-Arctic flora is dominated by lichens, mosses, liverworts and algae, while vascular plants are less abundant (Matveyeva and Chernov 2000). The vascular flora of Svalbard has been described by Rønning (1996), and an annotated list of 173 species can be found in Elven and Elvebakk (1996). Although this flora is comparatively well known, there is a lack of research of its biotechnological potential. Despite extreme environmental factors including low temperature, freezing events, desiccation, a high intensity of ultraviolet radiation and a prolonged period of winter darkness, these plant species are widely distributed in Arctic terrestrial ecosystems. Different Arctic regions experience variable climate regimes, but all regions experience extremes in solar radiation both during summer and winter. Variations in the frequency of cloud cover can cause significant variability in the amount of solar radiation reaching the surface at locations situated at a comparable latitude.

Extreme conditions of desiccation and irradiation lead to an increase in the formation of reactive oxygen species (ROS), which can potentially damage cellular components via oxidation or the activation of programmed cell death (Raff 1998; Hengartner 2000). To deal with the damaging effect of different types of ROS, plants possess antioxidant defence systems by the production of enzymatic (superoxide dismutase, catalases and peroxidases) and non-enzymatic low-molecular mass antioxidants (glutathione, ascorbate phenolic compounds and lignans) (Foyer et al. 1994; Pietta 2000). Antioxidants are therefore expected to play an important role in the survival of plants in environments which are characterised by high desiccation and irradiation stress, such as those in the polar regions.

Antioxidants which act as free radical scavengers help in maintaining physiological systems (Halliwell and Gutteridge 1998) and also provide protection against the oxidative degradation of food and oils (Senevirathne et al. 2006). The most extensively used synthetic antioxidants are propylgallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-Butylhydroquinone (TBHQ). However, these synthetic compounds potentially cause liver damage and carcinogenesis in humans (Grice 1986, 1988; Hettiarachchy et al. 1996). Hence, strong restrictions have been placed on their application, and there is a trend to substitute them with naturally occurring antioxidants.

Although studies have revealed the presence of natural sources of antioxidants in Antarctic lichens (Paudel et al. 2008), mosses (Bhattarai et al. 2008), fish (Ansaldò 2000), as well as in Arctic macroalgae (Aguilera et al. 2002; Dummermuth et al. 2003), lichens (Singh et al. 2011), and mushrooms (Singh et al. 2012), there have been no systematic studies on antioxidant activity in Arctic vascular plants.

Here we evaluate the *in vitro* antioxidant potential of four plant species, namely *Carex rupestris* All. (Cyperaceae), *Dryas octopetala* L. (Rosaceae), *Deschampsia alpina* (L.) Roem. Sch. (Poaceae) and *Silene uralensis* (Rupr.) Bocq (Caryophyllaceae), by (i) measuring their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, (ii) inhibiting the linoleic acid peroxidation (ILP) and (iii) assay of the Trolox Equivalent Antioxidant Capacity (TEAC). To identify the type of molecules responsible for the observed antioxidant properties, extracts of plant species were also subjected to electrospray ionization tandem mass spectrometric analyses (ESI-MS/MS). The structures of these molecules were identified based on the observed fragmentation and comparison with published values (Sakairi and Kambara 1993; Andreu and Picó 2004; Cappiello et al. 2007). There are no published reports of the chemistry of these plants except for the presence of phytoecdysteroids in *Silene* sp., with 20-hydroxyecdysone being most abundant (Meng et al. 2001), and of volatile components including ethylbenzene and benzaldehyde from the flower scent of this genus (Jurgens et al. 2002).

Materials and methods

Collection and processing of plant specimens

Four native plant species, namely *Carex rupestris* (location: 78°55'.569"N 11°54'.033"E), *Deschampsia alpina* (location: 78°55'.569"N 11°54'.003"E), *Dryas octopetala* (location: 78°55'.569"N 11°54'.033"E), and *Silene uralensis* (location: 78°54'.962"N 11°48'.734"E), were collected in the vicinity of Ny-Ålesund, Svalbard, and identified on the basis of their morphological characteristics (Rønning 1996). The samples were stored at -20°C. Whole plants (including root, stem, leaves and flowers) of the studied species were collected from one single habitat. Four plant specimens of each species were extracted in four different solvents (methanol, ethanol, acetone and DMSO). A subsample of each species was preserved as a dried herbarium specimen and deposited in the Polar Herbarium of the NCAOR, Goa, India.

Identification of plant substances by High-Performance Liquid Chromatography (HPLC)

Samples were extracted at the refluxing temperature of methanol in a Soxhlet extractor. Extracts were filtered and the organic solvent was removed by rotary evaporation at 40 °C in vacuum to yield a residue of methanolic extract. High-performance liquid chromatography (HPLC, Alliance Waters e2695 HPLC system, USA) of the extracts was performed using a C18 column, a PDA detector (2998), a solvent system of methanol-water-phosphoric acid (79:20:1, v/v/v). Detection occurred at a wavelength of 254 nm. The

injection volume was 10 μ l, and the flow rate 1 ml/min. (Feige et al. 1993). Major components detected by HPLC were purified by thin layer chromatography (TLC), using precoated Kieselgel 60 F₂₅₄ plates (Merck).

Mass spectrometry of the purified fractions

Electrospray ionization mass spectrometry (ESI-MS) was performed on a quadrupole-time of flight mass spectrometer (Model: Qstar XL MS/MS, Applied Biosystems, Switzerland). The isolated compounds were dissolved in methanol and directly infused into the ESI-source at a constant flow rate using a syringe pump. The mass spectra were acquired by scanning over the 100-2000 m/z range. The declustering potential and the collision energy were optimized for MS/MS experiments in order to cause fragmentation of the selected molecular ion species, as evidenced by the appearance of fragmented ions and a decrease in the intensity of the original molecular ion.

Measurement of antioxidant activities

Fresh plant material (1g of each species) was extracted in 20 ml of each of the four different solvents viz. ethanol, methanol, dimethylsulfoxide (DMSO) and acetone:water (9:1) using a Soxhlet apparatus. The extract was dried overnight at 37°C and weighed. The dried extracts were again dissolved in the respective solvents and used for the determination of antioxidant activities.

Measurement of free radical scavenging (FRS) activities

The scavenging potential of plant extracts were tested using the method of Blois (1958) with minor modifications (i.e., the mixture was allowed to stand for 2 hrs before reading the absorbance). The degree of decolouration of the solution is a measure of the scavenging efficiency of the added substances. 2 ml of 0.1 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol was added to 100 μ l of plant extract (0.3 mg/ml), and allowed to stand at 25°C for 2 hrs. The absorbance was read at 517 nm against blank samples with a UV-Vis spectrophotometer (Analytikjena Specord 205, Germany). Percentage inhibition was calculated against the negative control (solution). Lower absorbance of the reaction mixture is an indication for higher FRS activity. The antiradical activity was calculated as the percentage of DPPH decolouration using the following equation:

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$$

with $A_{C(0)}$: the absorbance of the control at $t=0$ hrs, $A_{A(t)}$: the absorbance of the plant extract at 2 hrs.

Inhibition of Lipid peroxidation (ILP) assays

Inhibition of Lipid Peroxidation (ILP) activities of plant extracts were determined following the method of Liegeois et al. (2000). 30 μ l of linoleic acid in Tween 20 (16 mM) (Hi-Media, Mumbai, India) were added to

2.81 ml of 0.05M phosphate buffer (pH 7.4), which was equilibrated at 40°C. The oxidation reaction was initiated at 37°C in a water bath by adding 150 µl of 40 mM AAPH solution. Oxidation was carried out in aliquots (20 µl) of the extract (0.3 mg/ml). The rate of peroxidation at 37°C was monitored by recording the increase in absorbance at 234 nm, due to the formation of conjugated diene hydroperoxides. The absorbance was measured using the UV-Vis spectrophotometer described above. The inhibition ratio (percent) was calculated using the following equation:

$$\% \text{ inhibition} = ([A_0 - A_1] / A_0) \times 100$$

With A_0 : the of the blank, and A_1 : the absorbance in the presence of the extract.

Both FRS and ILP assays provide information on different elements of the antioxidant profile, but focus on different types of free radicals and hence give a more overall picture of the antioxidative potential. In the FRS assay, the DPPH free radicals generated by the action of alcohol are scavenged by the plant extract while, in ILP, the plant extract prevents oxidation of linoleic acid by the oxidizing agent AAPH. In both FRS and ILP assays, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standard controls.

Assay of the Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox Equivalent Antioxidant Capacity (TEAC) of plant extracts was measured using the method described by Miller et al. (1995) and modified by Tyrakowska et al. (1999) with metmyoglobin being replaced by microperoxidase-8 (MP8). 2-, 2'- azinobis 3-ethylbenzothiazoline-6-sulfonate radicals ($ABTS^+$) were generated by the interaction of $ABTS^+$ (100 µM), 50 µM H_2O_2 (hydrogen peroxide), and peroxidase (4.4 unit/ml). To measure the antioxidant capacity, 200 µl of plant extract (0.3 mg/ ml) was mixed with an equal volume of $ABTS^+$, H_2O_2 (Hi-Media), peroxidase and deionized water. Absorbance was measured at 734 nm for 10 min using the UV-Vis spectrophotometer described above. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. The TEAC value is expressed as mM of Trolox solution, which has an antioxidant equivalent to 0.1% (w/v) extract solution.

Estimation of the phytochemical contents

In order to determine the correlation between the measured antioxidant activities and the phytochemicals present in the plant extracts, we estimated the polysaccharide, protein and total polyphenolic content present in the plant extracts. Some of the phytochemicals were tentatively identified from the methanol extract by tandem mass spectrometry (ESI-MS/MS).

Determination of polyphenolics in the plants

Polyphenolics in the plant extracts were determined spectrophotometrically using a Folin-Ciocalteu reagent based on a colorimetric oxidation/reduction reaction (Slinkard and Singleton 1977). Pyrocatechol was used as

the standard. The extract (0.1 ml) was added to 1 ml of Folin-Ciocalteu reagent and shaken well manually for 5 minutes. After 5 min., 3 ml of Na₂CO₃ (2%) was added and the mixture was allowed to stand for 1 hr with intermittent shaking. The absorbance was measured at 760 nm using the UV-Vis spectrophotometer described above. Total polyphenol value was expressed as µg pyrocatechol equivalent using the following equation (obtained from the standard pyrocatechol curve):

$$\text{Absorbance} = 0.001 \times \text{Pyrocatechol } (\mu\text{g}) + 0.0033$$

Determination of polysaccharide contents in the plants

The polysaccharide content in plant extracts was determined as described by Dubois et al. (1956). The extract (0.1ml) was added to 25 µl of 80% phenol and 1 ml conc. H₂SO₄. The mixture was shaken and allowed to stand at 30°C for 30 min. The absorbance was measured at 490 nm by using the UV-Vis spectrophotometer described above. The polysaccharide content was estimated by a standard curve prepared using known amounts of standard sucrose solution (100µg/ml).

Determination of protein contents in the plants

The protein content in plant extracts was determined following Bradford (1976), and estimated from a standard curve prepared using known amounts of bovine serum albumin (BSA).

Results

High-Performance Liquid Chromatography of plant extracts

HPLC analysis of plant extracts revealed the presence of several detectable chemical substances. The retention time (in minutes) was recorded for each plant species: *Carex rupestris* (3.033, 3.246, 3.939, 4.675, 5.253, 8.254), *Deschampsia alpina* (3.123, 4.068, 5.337, 6.072), *Dryas octopetala* (3.607, 3.915, 4.102, 4.689) and *Silene uralensis* (3.053, 3.914, 4.272, 4.802, 5.366, 5.748).

Identification of antioxidants by electrospray ionization tandem mass spectrometry of purified fractions

Electrospray ionization tandem mass spectrometry (ESI-MS/MS) revealed the presence of several active constituents (Table1). The ESI-MS profiles for *Silene uralensis* and *Dryas octopetala* suggest the presence of organoselenium compounds. The ESI-MS profile for *Carex rupestris* was indicative of the presence of linear alkyl benzene sulfonates and their oxidized metabolites, and sulphophenyl carboxylic acid (SPC). ESI-MS was not performed for *Deschampsia alpina* due to the paucity of material.

Silene uralensis

Thin layer chromatography (TLC) of the methanolic extract of *Silene uralensis* revealed the presence of 3 compounds designated as Silene-1, Silene-2 and Silene-3, in decreasing order of polarity. The ESI-MS of Silene-1, with each signal in the spectrum representing a chemical substance present in the fraction (Online Resource 1). The fraction contains several compounds with protonated molecular masses at m/z 107, 187, 209, etc. (Table 1). The tandem mass spectrum of each of these signals produced fragments which are characteristic for each class of compounds. Thus, tandem mass spectrum (MS/MS) of the signal at m/z 107 yielded a fragment at m/z 77, which is indicative of the presence of a benzene ring in the molecule. The difference between the protonated molecular mass and the fragment equals 30 amu (atomic mass units), which is indicative of an ethyl group being attached to the benzene nucleus. Hence the compound with a protonated molecular mass of 107 amu was identified as being ethyl benzene (1).

The signal at m/z 187 was identified as being benzoyl selenol (2) based on the fragment at m/z 105, which is characteristic of benzoyl moiety. The difference between the $[M+H]^+$ 187 and the fragment at m/z 105 is 82 amu, which is the atomic mass of the selenium isotope.

The compounds present in Silene-3 (Online Resource 2) were in agreement with the isotopic pattern characteristic of compounds containing selenium (Dumont, 2005). A difference of 74/82 amu observed in the subsequent signals is an indication of the accumulation of selenium isotopes. The fragmentation observed in tandem mass spectrometry led to a tentative identification of constituents as given in Table 1. Silene-1 and Silene -2 showed a more or less similar profile with the exception of the presence of an additional signal at m/z 284 in Silene-2, which corresponds to the presence of sphingosine.

Dryas octopetala

The ESI-MS profile in the positive ion mode of the fraction in *Dryas octopetala* (Online Resource 3) revealed the potential presence of oligosaccharides although other chemical constituents were also present. Oligosaccharides were present as sodium adducts which are known to be quite resistant towards dissociation. Each ion is likely to represent a single component of the mixture rather than being fragments of other heavier ions. The ionization behaviour is typical of neutral oligosaccharides, due to their capability to form strong ligands to alkali metal ions (Tinke et al. 1993; Bahr et al. 1997; Zaia 2004). These dextrans were identified as sodium adducts of glucose [m/z 203], maltose [m/z 365], maltotetraose [m/z 689], and the potassium adduct of maltotriose [m/z 543] (Fig. 2) through comparison with Sakairi and Kambara (1993) and Cappiello et al. (2007). The fragmentation pathway observed for maltotetraose in its ESI-MS/MS profile is shown in figure 2, scheme-1. The ESI-MS profile also showed the presence of signals indicative of selenium being associated with the maltotetraose moiety. It yielded molecular species with pseudomolecular ions at m/z 837, 911, 1059, 1135, 1209, and 1283 due to the consecutive incorporation of 1-6 selenium atoms in the maltotetraose

molecule as shown in 2. The only binding location for selenium is next to hydrogen as there are no reports in the literature where selenium is attached to oxygen.

ESI-MS analysis in the negative mode of the same fraction showed the presence of hydroxyl benzoic acid as evidenced by the presence of signals at m/z 93 and m/z 137. The signals at m/z 279, 325 and 339 are an indication for the presence of linolenic acid, heneicosanoic and docosanoic acids.

Carex rupestris

The ESI-MS profile of the methanolic extract of *Carex rupestris*, taken in negative ion mode is illustrated in Online Resource 4. Anionic compounds lose a proton in solution and become negatively charged and hence detected by ESI in the negative ion mode. The deprotonated molecule was the only ion observed.

It is evident from the negative ESI-MS profile (Online Resource 4) and the ESI-MS/MS spectra (Table 1) of *Carex rupestris* that linear alkyl benzene sulfonates (LAS) and their polar biotransformation products sulfophenyl carboxylic acid (SPC) are present (Fig. 3). The diagnostic ion corresponded to m/z 297, 311, 325 and 339 for C_{10} LAS, C_{11} LAS, C_{12} LAS and C_{13} LAS, respectively. For SPC identification the diagnostic peak corresponded to m/z 313, 327, 355 and 365 for C_9 SPC, C_{10} SPC, C_{12} SPC and C_{13} SPC respectively (Andreu and Picó 2004).

The purified fraction of this plant extract also showed the presence of fatty acids in the ESI-MS profile in negative ion mode. The fatty acids identified were lauric ($[M-H]^-$,199), myristic ($[M-H]^-$,227), pentadecylic ($[M-H]^-$,241), palmitic ($[M-H]^-$,255), palmitoleic ($[M-H]^-$,253), heptadecanoic ($[M-H]^-$,269), oleic ($[M-H]^-$,281), and stearic acids ($[M-H]^-$,283).

Free radical scavenging (FRS) activities

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical is stable and has been widely used as a tool to estimate the free radical-scavenging activity of antioxidants. Antioxidants, after interaction with DPPH, either transfer electrons or hydrogen atoms to DPPH, thereby neutralizing the free radical character. The colour of the reaction mixture changed from purple to yellow, and a decrease in absorbance occurred. The DPPH-radical scavenging activity of the organic extracts is shown in Fig. 4 and was compared to butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

All plant extracts, except that of *D. alpina*, showed very strong DPPH free radical scavenging activity with the methanol extract of *Carex rupestris* exhibiting the highest activity (86.99%). Methanolic extracts are better antioxidants than the standard BHA (56%) and BHT. The acetone extract of *D. octopetala* was comparable in activity to the standard BHT. The order of activity was, *C. rupestris* > *D. octopetala* > *S. uralensis* > *D. alpina* (Fig. 4).

Inhibition of Lipid Peroxidation (ILP)

The lipid peroxidation inhibition (ILP) of the different solvent extracts is shown in Fig. 5. Almost all the studied plants showed greater percentage of ILP than the standard antioxidants BHA (38%) and BHT (47%). The ethanol extract of *Dryas octopetala* showed the highest activity (76.45%), followed by *Silene uralensis* (64.00%), *Carex rupestris* (54.2%) and *Deschampsia alpina* (53.01%). Acetone extracts of the plants showed activity between 50.26 and 69.97%. However, the activity of other solvent extracts ranged between 34.78 and 52.16%, which is higher than the activity of standard antioxidants, but lower than those of methanol and acetone extracts. The order of ILP activity of the different solvent extracts was ethanol > acetone > methanol > DMSO. The order of activity across the four plant species was *D. octopetala* > *S. uralensis* > *C. rupestris* > *D. alpina* (Fig. 5).

Trolox Equivalent Antioxidant Capacity (TEAC)

All the four plant species studied showed good Trolox Equivalent Antioxidant capacities (TEAC) (Table 2). The acetone and ethanol extracts showed higher antioxidant capacity (4.2 mM to 8.0 mM) in all the plant species compared to other solvents. The TEAC of all plant extracts was higher than the capacity shown by standard Trolox (3.9 mM), which suggests a stronger antioxidant ability.

Protein, polysaccharide and total phenol contents

The polysaccharide, protein and total phenol contents present in the plant extracts are presented in Table 2. Regardless of the solvent used, the protein contents of the different species obtained ranged from 10.52 to 53.89 $\mu\text{g/g}$ dry wt. The polyphenol content ranged from 0.003 to 0.244 mg/g dry wt. A high polysaccharide content was found in all the solvent extracts and ranged from 0.53 to 14.91 mg/g dry wt. The polysaccharide content was highest in the acetone extracts and ranged from 14.45 to 14.91 mg/g dry wt. A significant correlation ($R^2 = 0.968$, $p < 0.01$) was found between the polysaccharide content and antioxidant activities (Table 2).

Discussion and conclusion

Phenolic compounds are known to have direct antioxidant properties due to the presence of hydroxyl groups, which function as hydrogen donor (Dreosti 2000). DPPH bleaching is elicited by a great variety of compounds which possess a widely different free radical scavenging capacity (Pérez et al. 2006). Hence, the observed antioxidant activity could also be related to some other classes of compounds different than phenolics. Previous studies on polar lichens, bryophytes, algae and alpine plants (Nybakken et al. 2004a,b; Huttunen et al. 2005; Pannowitz et al. 2005; Green et al. 2007; Snell et al. 2009; Schroeter et al. 2010) revealed that overall radiation intensity, exposure to high energy short wavelength ultraviolet radiation (UV-B) and temperature fluctuations induce the production of specific metabolites which protect them against

environmental stress. Recently, antioxidant assays were conducted for the first time for Antarctic lichens (Paudel et al. 2008). The significant correlation found here between the polysaccharide content and the FRS, ILP and TEAC activities of the different plant extracts is consistent with the antioxidant activity being linked to the presence of a high polysaccharide content (Table 2). These results are also consistent with those of Liu et al. (1997), who reported that some polysaccharides have the ability to scavenge free radicals. There are also reports on antioxidant activity of glucans (Babincová et al. 1999; Tsiapali et al. 2001) and polysaccharides produced by marine biota (Xue et al. 1998). Some sulphated, acetylated and phosphorylated derivatives of carrageenan oligosaccharides are known to exhibit significant antioxidant activities as revealed by *in vitro* experiments. The antioxidant activity is in some cases higher than that of polysaccharides and oligosaccharides, and the effect was independent of molecular mass as both poly- and oligosaccharides showed similar activities (Yuan et al. 2005).

Electrospray ionization tandem mass spectrometric (ESI-MS/MS) analysis of *Dryas octopetala* revealed the presence of dextrin oligosaccharides consisting mainly of maltose units with maltotetraose being associated with 1-6 selenium atoms (Online resource 3). *D. octopetala* is also known to contain flavonoid glycosides (Servettaz et al. 1984). The chemical composition of *D. octopetala* can explain its high IPL and free radical scavenging activity. Selenium compounds similarly accumulate in *Silene uralensis*. Plano et al. (2010) evaluated organoselenium derivatives for their biological activity as superoxide generators and cytotoxic agents against PC-3, MCF-7 and CCRF-CEM cell lines. Some of the derivatives were found to be much more active as superoxide generators, as well as cytotoxic agents, than the standard diselenide propionic acid and etoposide/methylseleninic acid. *Dryas octopetala* and plants belonging to the genus *Silene* are known to be accumulators of metals. This is particularly the case in *Silene acualis*, which is known to absorb manganese (Dowdall et al. 2005). Selenites and selenates in plants accumulate primarily as methylated selenium compounds, which evolved in plants as dimethyldiselenide (DMDSe) and dimethylselenide (DMSe). Alternatively, microbial conversion of DMDSe to DMSe occurs, which is an indication that DMDSe is not a stable molecule (Spallholz 1997).

The genus *Silene* was shown to contain new and structurally varied ecdysteroid analogues (Zibareva et al. 2009). Phytoecdysteroids from *Silene guntensis* are also antioxidants (Mamadaliyeva et al. 2011). Hence, *Dryas octopetala* and *Silene uralensis* may be used not only for their antioxidant properties but also as bio-indicators of environmental pollution.

Carex rupestris contains linear alkyl benzene sulfonates (LAS) and their polar biotransformation products, i.e. sulfophenyl carboxylic acids (SPCs). LAS are also present in the shallow marine environment (Léon et al. 2002; Lara-Martin et al. 2006) and the organisms living there potentially suffer from LAS and SPCs accumulation. Scott and Jones (2000) suggested that in aerobic metabolism LAS is transformed via ω -oxidation of the alkyl terminus and subsequent α and β -oxidation (loss of one or two carbon atoms respectively) to sulfophenyl alkanolic acids (SPCs). This process generates intermediate compounds, which

differ not only in the length of the alkyl chain but also in the link between the sulfophenyl group and the alkyl chain (Álvarez-Munoz 2007). Another class of metabolites, dicarboxylated SPCs (SPdC), are also generated in the pathway of LAS biodegradations. SPdCs are formed by oxidative attack on both sides of the alkyl chain and subsequent breakdown through β -oxidation. These intermediates have been identified in sewage treatment plants and surface waters (Di Corcia et al. 1999). Hence, the concentrations of LAS in organism are reduced. LAS also provide protection against UV radiation because the surfactants act as UV screen.

In the present study, Arctic vascular plants were shown to possess strong antioxidant abilities. We therefore suggest that extracts of plants collected from the Arctic region could be a valuable source of natural antioxidants for biotechnological applications. Furthermore, selenium-accumulating plants concentrate the element in their shoots and roots. Such ability may provide a cost-effective technology to help remediate selenium-contaminated soils and waters that can pose major environmental and human health problems. The ability to transform selenium into bioactive compounds has also important implications for human nutrition and health (Kahakachchi et al. 2004).

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FIGURE LEGENDS

Figure 1: Structures of compounds identified in *Silene uralensis*: (1) Ethyl benzene, (2) Benzoyl selenol, (3) Benzaldehyde, (4) Diethyldiselenide, (5) Selenium methyl selenium propanamine, (6) Selenium cystamine, (7) Cysteine diselenide, (8) Sphingosine, (9) 2-stearoyl-1-palmitoyl 3-phosphatidyl glycerol, (10) Ethyltriselenide, (11) Benzyltriselenide, (12) Ethyltetraselenide, (13) Ethylhexaselenide, (14) Ethylheptaselenide, (15) Ethylnonaselenide, (16) Ethyldecaselenide, (17) Ethylheptaselenide

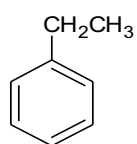
Figure 2 Structures of compounds identified in *Dryas octopetala*: (27) Maltotetraose fragmentation, (28) Sodiatered Maltotetraose, (29) Sodiatered Maltotetraose diselenol, (30) Sodiatered Maltotetraose triselenol, (31) Sodiatered Maltotetraose pentaselenol, (32) Sodiatered Maltotetraose hexaselenol, (33) Sodiatered Maltotetraose heptaselenol, (34) Sodiatered Maltotetraose octaselenol.

Figure 3 Structures of compounds identified in *Carex rupestris*: (18) 6-(p-sulfophenyl) decane, (19) 6-(p-sulfophenyl) undecane, (20) 6-(p-sulfophenyl) dodecane, (21) 6-(p-sulfophenyl) tridecane, (22) 4-(p-sulfophenyl) nonanoic acid, (23) 5-(p-sulfophenyl) decanoic acid, (24) 7-(p-sulfophenyl) dodecanoic acid, (25) 8-(p-sulfophenyl) tridecanoic acid (26) 9-(p-sulfophenyl) tetradecanoic acid.

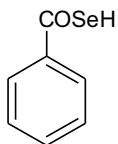
Figure 4 Free radical scavenging activity of plant species in various solvents. *C. rupestris* = *Carex rupestris*, *D. alpina* = *Deschampsia alpina*, *D. octopetala* = *Dryas octopetala*, *S. uralensis* = *Silene uralensis*, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, DMSO = dimethylsulfoxide.

Figure 5 Lipid peroxidation inhibition activity of plant species in various solvents. *C. rupestris* = *Carex rupestris*, *D. alpina* = *Deschampsia alpina*, *D. octopetala* = *Dryas octopetala*, *S. uralensis* = *Silene uralensis*, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, DMSO = dimethylsulfoxide..

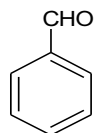
Figure-1



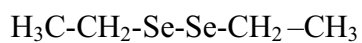
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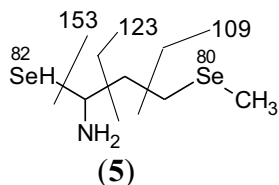
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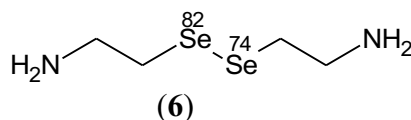
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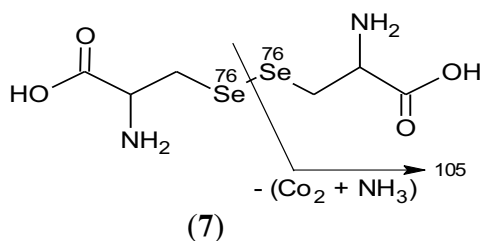
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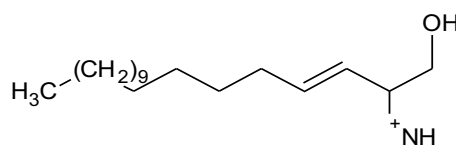
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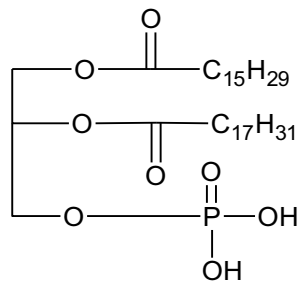
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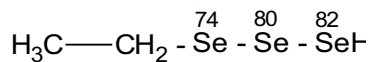
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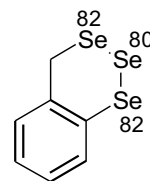
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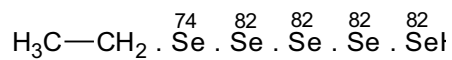
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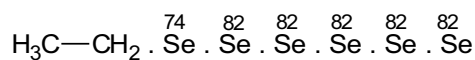
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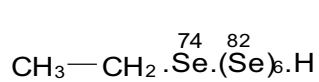
(11)-335 Benzyl triselenide



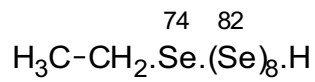
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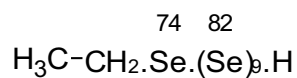
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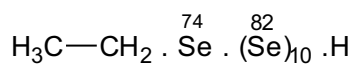
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(15)- 761

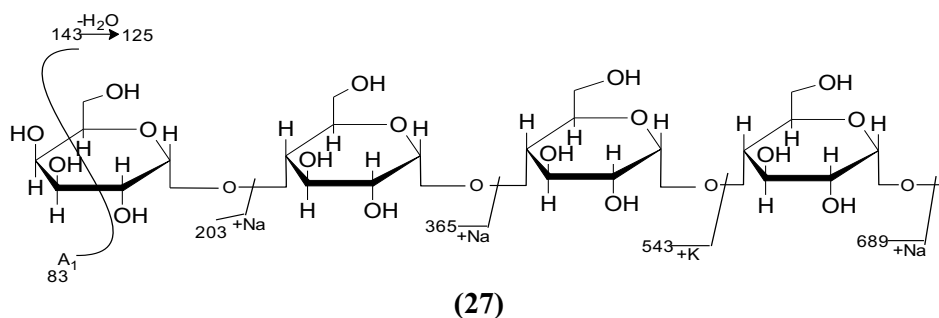


(16)- 843



(17)- 925

Fig. 1 Structures of compounds identified in *Silene uralensis*: (1) Ethyl benzene, (2) Benzoyl selenol, (3) Benzaldehyde, (4) Diethyldiselenide, (5) Selenium methyl selenium propanamine, (6) Selenium cystamine, (7) Cysteine diselenide, (8) Sphingosine, (9) 2-stearoyl-1-palmitoyl 3-phosphatidyl glycerol, (10) Ethyltriselenide, (11) Benzyltriselenide, (12) Ethyltetraselenide, (13) Ethylhexaselenide, (14) Ethylheptaselenide, (15) Ethylnonaselenide, (16) Ethyldecaselenide, (17) Ethylheptaselenide,



Scheme-1: Maltotetraose along with fragmentation pathway observed in its tandem mass spectrum

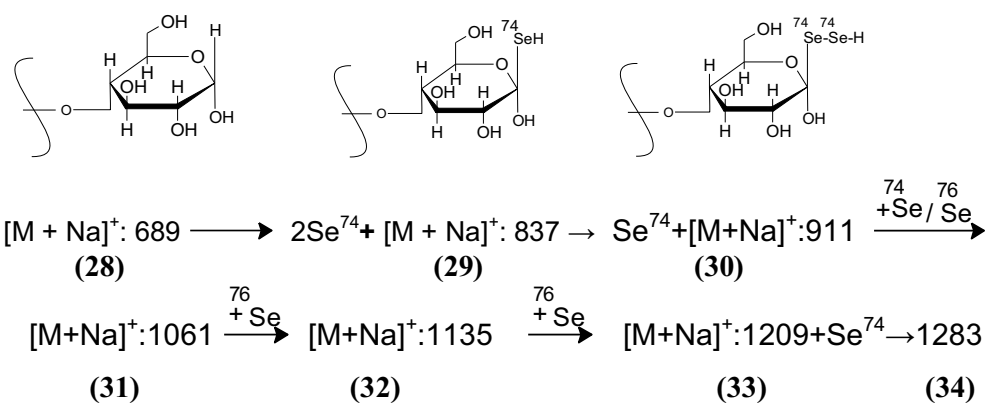
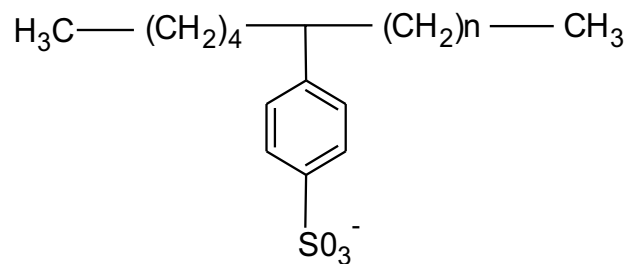
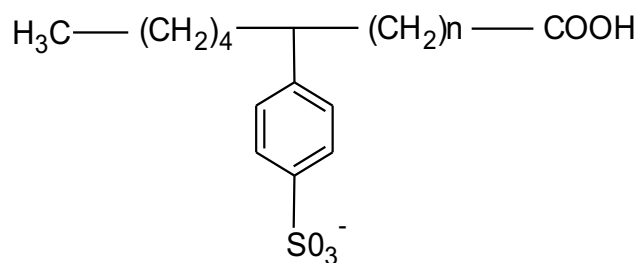


Fig. 2 Structures of compounds identified in *Dryas octopetala*: (27) Maltotetraose fragmentation, (28) Sodiatered Maltotetraose, (29) Sodiatered Maltotetraose diselenol, (30) Sodiatered Maltotetraose triselenol, (31) Sodiatered Maltotetraose pentaselenol, (32) Sodiatered Maltotetraose hexaselenol, (33) Sodiatered Maltotetraose heptaselenol, (34) Sodiatered Maltotetraose octaselenol.



LAS- n = 3 : 297; n = 4 : 311; n = 5 : 325; n = 6 : 339
(18-21)



SPCs- n = 2 : 313; n = 3 : 327; n = 5 : 355; n = 6 : 369; n = 7 : 383

(22-26)

Fig. 3 Structures of compounds identified in *Carex rupestris*: (18) 6-(p-sulfophenyl) decane, (19) 6-(p-sulfophenyl) undecane, (20) 6-(p-sulfophenyl) dodecane, (21) 6-(p-sulfophenyl) tridecane, (22) 4-(p-sulfophenyl) nonanoic acid, (23) 5-(p-sulfophenyl) decanoic acid, (24) 7-(p-sulfophenyl) dodecanoic acid, (25) 8-(p-sulfophenyl) tridecanoic acid (26) 9-(p-sulfophenyl) tetradecanoic acid.

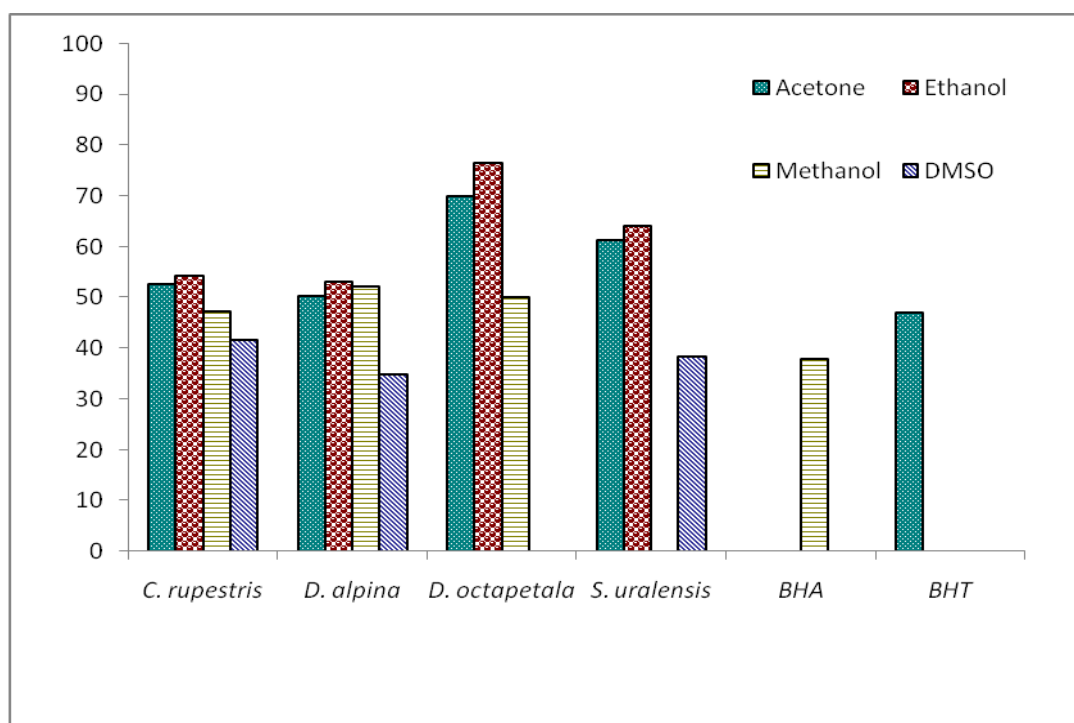


Fig. 4 Free radical scavenging activity of plant species in various solvents. (*C. rupestris* = *Carex rupestris*, *D. alpina* = *Deschampsia alpina*, *D. octopetala* = *Dryas octopetala*, *S. uralensis* = *Silene uralensis*, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, DMSO = dimethylsulfoxide).

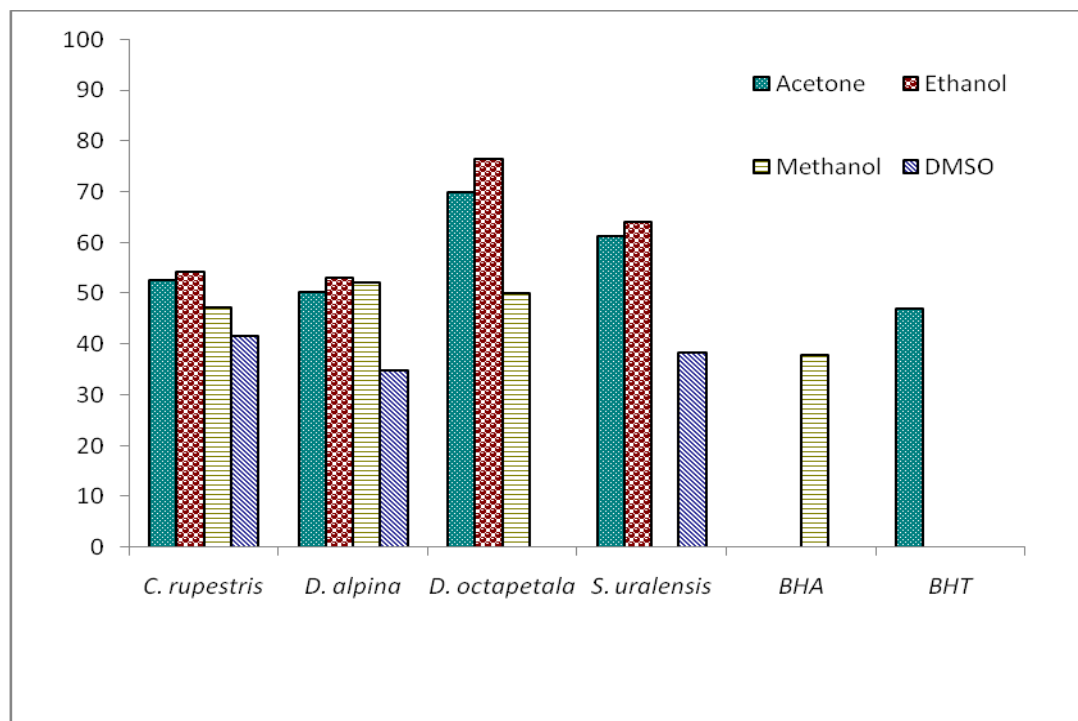


Fig. 5 Lipid peroxidation inhibition activity of plant species in various solvents. (*C. rupestris* = *Carex rupestris*, *D. alpina* = *Deschampsia alpina*, *D. octopetala* = *Dryas octopetala*, *S. uralensis* = *Silene uralensis*, BHA = butylated hydroxyanisole, BHT = butylated hydroxytoluene, DMSO = dimethylsulfoxide).

Table 1 Structural assignments and tandem mass spectral data for compounds found in Arctic vascular plants

Plant name	Structural assignment	[M-H] ⁻	[M+H] ⁺	Fragment ions (m/z) observed
<i>Dryas octapetala</i>				
Maltotetraose	27		689	689, 527, 365, 203
Maltotetraose-selenium complex (1-6 selenium atoms)	28-34		837-1283	
<i>Carex rupestris</i>				
Linear-C ₁₀₋₁₃ Alkyl Benzene Sulfonate	18-21	297-339		297, 183(base peak), 71.8
LAS C ₁₀₋₁₃				311, 183 (base peak), 170.2, 119
				325, 183 (base peak), 170, 119, 197
				339, 183 (base peak), 170, 118
Linear Alkyl-Sulfohenyl carboxylate (SPCs-C ₉₋₁₄)	22-26	313-383		383, 321, 311, 255, 113
				369, 351, 325, 307, 281, 113, 89
				327, 197, 183, 170, 119

-continued

Table 1 - continued

Plant name	Structural assignment	[M-H] ⁻	[M+H] ⁺	Fragment ions (m/z) observed
<i>Silene uraltensis</i>				
Ethyl benzene/ benzaldehyde	1/3		107	107, 77
Benzoyl selenol	2		187	187, 105
Diethyldiselenide	4		209	209, 189, 108, 106
Selenium methyl selenium propanamine	5		237	237, 153, 125, 123, 109
Selenium cystamine	6		245	245, 105
Cysteine diselenide	7		329	329, 245, 189, 171, 165, 145, 135, 105
Sphingosine	8		284	284, 85, 71, 60 (base peak), 57, 43
Phosphatidyl glycerol	9		691	691, 413, 301
Ethyltriselenide	10		269	269, 187, 105
Benzyltriselenide	11		335	335, 91
Ethyl tetraselenide	12		433	433, 269, 187, 105
Ethyl (Se) ₆	13		515	515, 433, 351, 269, 187, 105
Ethyl (Se) ₇	14		597	597, 433, 351, 269, 187, 105
Ethyl (Se) ₉	15		761	761, 597, 515, 433, 351, 269, 187
Ethyl (Se) ₁₁	17		925	925, 761, 679, 597, 515, 433, 351, 269, 187

See Figure 3, 5 & 7

Table 2 Antioxidant activity (TEAC), polysaccharide, protein and total polyphenol content of plant extracts in various solvents. Data presented are the average of three consecutive readings of the extract in each assay system

Antioxidant activity		Polysaccharide	Protein content	Total polyphenol
Solvent extracts	TEAC (mM)	content (mg/ml)	($\mu\text{g/ml}$)	Content (mg/ml)
<i>Carex rupestris</i>				
Acetone	7.3	14.45	31.85	0.014
Ethanol	6.3	0.53	53.89	0.007
Methanol	2.3	11.75	20.21	0.126
DMSO	2.3	13.85	24.00	0.079
<i>Deschampsia alpina</i>				
Acetone	7.8	14.91	29.89	0.008
Ethanol	7.8	0.466	34.10	0.003
Methanol	4.4	3.72	31.13	0.018
DMSO	-	9.62	13.47	0.105
<i>Dryas octapetala</i>				
Acetone	8.0	14.66	26.73	0.020
Ethanol	7.6	1.070	26.31	0.020
Methanol	-	14.54	25.47	0.104
DMSO	3.9	14.04	10.52	0.177
<i>Silene uralensis</i>				
Acetone	4.2	14.27	28.63	0.019
Ethanol	7.8	1.85	31.15	0.010
Methanol	-	14.89	15.57	0.244
DMSO	1.9	14.67	17.05	0.116