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RESEARCH / REVIEW ARTICLE

Chemical constituents and antioxidant activity of the Arctic mushroom *Lycoperdon molle* Pers

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Abstract

The biochemical adaptations of fungi to the harsh conditions of the Arctic may mean that these organisms have properties useful to people. Using samples of the puffball mushroom *Lycoperdon molle* Pers. (Basidiomycota, Fungi) collected at Ny-Ålesund, Svalbard, we examined the *in vitro* antioxidant potential of this species by investigating its free-radical scavenging (FRS) activity, inhibition of lipid peroxidation (ILP) and Trolox equivalent antioxidant capacity (TEAC). The FRS activity of the samples in various organic solvents, including methanol, ethanol, acetone and dimethyl sulfoxide (DMSO), were found to be in the range of 44.00–89.60%, while ILP activities ranged from 32.00 to 54.41%. The methanol extract showed the highest levels of FRS (89.60%) and ILP (54.41%) compared to standard antioxidants butylated hydroxyanisol and butylated hydroxytoluene (BHT). The TEAC value was also found to be higher compared to the standard water soluble vitamin E analogue Trolox (3.9 mM). Antimicrobial screening of *Lycoperdon molle* extracts was negative to the tested microorganisms. Using electrospray ionization mass spectrometry (ESI-MS), we determined that the samples contained compounds such as phosphoethanolamine, monomethyl arsenic acid, phosphatidyl glycerol, phosphoionositol, phosphoserine and lysophosphatidyl choline. We found that *Lycoperdon molle* showed strong antioxidant abilities compared to the standards, suggesting that this and perhaps other Arctic mushrooms could be valuable sources of natural antioxidants for the pharmaceutical industry. To our knowledge, this is the first report of the antioxidant activity in any Arctic mushroom.

Keywords:
Lycoperdon; fungi; antioxidative potential; HPLC; ESI-MS; Arctic.

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Around 2.3% of the world fungal biota exists in the Arctic. The investigation of the macromycete flora of Svalbard began with the studies of Sommerfelt (1933) and a comprehensive account on Svalbard Basidiomycota fungi has been published (Gulden & Torkelsen 1996; Aarnæs 2002). The macromycete *Lycoperdon molle* Pers. (Lycoperdaceae, Agaricales, Agaricomycetidae, Agaricomycetes, Basidiomycota, Fungi) is an edible puffball found in the region. The micromycetes flora of Svalbard has also been documented (Lind 1928).

Studies of polar mosses (Rozema et al. 2001; Huttunen et al. 2005) have shown that ultraviolet-B radiation and low temperatures induce the production of some specific metabolites that protect these organisms from environmental stresses. Similarly, biochemical adaptations allow macromycete species to survive extreme environmental factors that include low temperatures, intermittent freezing and relatively high exposure to ultraviolet rays (Jang et al. 2000; Feller & Gerday 2003; Hoshino et al. 2003). These adaptations may provide compounds, such as cold tolerant enzymes and antimicrobial compounds (Fiedurek et al. 2003; Frisvad 2008), with biotechnological potential (Leary 2008). There is therefore a need to screen Arctic mushrooms for biotechnological prospecting.

In recent years, mushrooms have earned attention on account of their pharmacological characteristics and their high-protein/low-fat nutritional value (Barros, Baptista et al. 2007). The fatty acid composition of mushrooms may

also have beneficial effects on blood lipid profiles. Substitution of saturated fatty acids with monounsaturated fatty acids leads to increased high-density lipoprotein cholesterol and decreased low-density lipoprotein cholesterol, triacylglycerol, lipid oxidation and low-density lipoprotein susceptibility to oxidation (Kanu et al. 2007). There are few reports of antioxidant activities in polar organisms, e.g., Antarctic fish (Ansaldo et al. 2000), lichens (Paudel et al. 2007) and moss (Bhattarai et al. 2008). To our knowledge, there are no published reports of antioxidant activities in Arctic mushrooms.

Human physiological systems produce reactive oxygen species (ROS) in the form of free radicals such as superoxide, hydroxyl radicals and other different non-free radicals (Halliwel & Gutteridge 1999; Yildirim et al. 2000; Gulcin et al. 2002). ROS react with biomolecules such as proteins, lipids, lipoproteins and DNA and cause oxidative stress. They are thus directly responsible for creating a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity (Kourounakis et al. 1999; Gulcin et al. 2002). To overcome these problems, living organisms possess a number of endogenous protective mechanisms against the oxidative stress caused by ROS. To overcome the physiological burden of free-radical generation in the body, a supply of exogenous antioxidative compounds is required. Antioxidants are compounds that scavenge free radicals and help in maintaining the human physiological system (Halliwel & Gutteridge 1999). They are used as food additives to provide protection from oxidative degradation of food and oils (Senevirathne et al. 2006). The most extensively used synthetic antioxidants are propylgallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone. These are, however, suspected of causing liver damage and carcinogenesis (Grice 1986; Wichi 1988; Hettiarachchy et al. 1996).

Natural antioxidants can protect against ROS without any side effects by retarding the progress of many age-associated diseases (Pryor 1991; Kinsella et al. 1993; Lai et al. 2001; Gulcin et al. 2003). Mushrooms produce a variety of compounds (Almendros et al. 1987; Sun & Xie 2004; Barros et al. 2008) that are known to have antioxidant (Mau et al. 2002; Barros, Ferreira et al. 2007) and antimicrobial properties (Puttaraju et al. 2006; Barros, Calhelha et al. 2007; Coolak et al. 2009; Ramesh & Pattar 2010).

In this study, we investigated the chemical composition, antimicrobial activities and antioxidant potential of the Arctic wild mushroom *Lycoperdon molle*, with the aim of beginning to fill a knowledge gap regarding the properties of Arctic fungi that may be beneficial to people.

Materials and methods

Collection and preparation of the specimens

The natural fruit bodies of *Lycoperdon molle* were collected at different localities in Ny-Ålesund (78°54.605'N – 12°05.365'E), on the west coast of Spitsbergen Island in the Arctic archipelago of Svalbard. The samples were placed in sterile Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA) and a sample collector (Himedia) and stored at–20°C until studied. Specimens were identified using the standard literature (Moser 1983; Courtecuisse & Duhem 1995) and representative voucher specimens were deposited at the herbarium of the National Centre for Antarctic and Ocean Research in Goa and the National Fungal Culture Collection of India (NFCCI-WDCM 932), in Pune.

The fruit bodies (3 g) were extracted in 20 ml of methanol, ethanol, acetone and DMSO using a soxhlet extractor. The extract was dried overnight at 37°C and weighed. The dried extracts were again dissolved in the different solvents and used for the determination of antioxidant activities.

Chemicals and reagents

Folin-ciocalteu reagent, coomassie brilliant blue G-250, peroxidase, linoleic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and hydrogen peroxide were procured from Himedia (Mumbai, India), while 2-,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate (ABTS), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), (BHA), (BHT) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were procured from Sigma-Aldrich (St. Louis, MO, USA). All other routine chemicals used were of analytical reagent grade.

High-performance liquid chromatography of the extract

The fruit bodies were extracted in methanol using a soxhlet extractor. High-performance liquid chromatography **(**HPLC) analysis was carried out on a e2695 HPLC system (Waters, Milford, MA, USA) using a C18 column (Waters) and a 2998 photodiode detector (Waters), with a solvent system of methanol–water–phosphoric acid (80:20:0.9, v/v/v). The detection wavelength was 254 nm and the injection volume was 10 µl, with a flow rate of 1 ml/min. Retention times of 3.003 min, 3.758 min, 5.294 min and 6.002 min were recorded.

Mass spectrometric and nuclear magnetic resonance analyses of the compounds

Electrospray ionization mass spectrometry (ESI-MS) analysis was performed on a Qstar XL quadrapole time-of-flight mass spectrometer (Applied Biosystems, Rotkreuz Zug, Switzerland) equipped with analyst software. The compounds isolated from the *Lycoperdon molle* samples were dissolved in methanol and directly analysed by

ESI-MS. The samples were introduced at a constant flow rate into the electrospray source using an integrated syringe pump. The mass/charge (m/z) range was from 100–2000 in positive and negative mode. The declustering potential and the collision energy were optimized for the tandem mass spectra (MS/MS) experiments so as to cause fragmentation of the selected molecular ion species, as evident by the appearance of fragment ions and decrease in the intensity of the molecular ion. ^{1}H, ^{13}C nuclear magnetic resonance (NMR) spectra were recorded, in CD_3OD, on an Avance-300 spectrometer (Bruker, Switzerland) with tetramethylsilane as the internal standard. Silica gel, 60–120 mesh (Merck, Mumbai, India) was used for column chromatography, while pre-coated Kieselgel 60 F254 plates (Merck) were used for thin layer chromatography.

Free-radical scavenging activity

The free-radical scavenging (FRS) activity of the extracts was measured following DPPH using the method of Blois (1958), with minor modifications. Briefly, 0.1 mM solution of DPPH was prepared in ethanol. Two ml of this solution was added to 100 µl of fruit bodies extract and was allowed to stand at 25°C for 2 h, and the absorbance was read at 517 nm against blank samples using a Specord 205 UV VIS spectrophotometer (Analytik Jena, Jena, Germany). The percentage of inhibition was calculated against a negative control (only DPPH solution). Lower absorbance of the reaction mixture indicated higher FRS activity, as shown in this equation:

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

where $A_{C(0)}$ is the absorbance of the control at t=0 h and $A_{A(t)}$ is the absorbance of mushroom extract at 2 h.

Inhibition lipid peroxidation assay

The inhibition of lipid peroxidation (ILP) activity of the extracts was determined following the method of Liegeois et al. (2000). Thirty µl of 16 mM linoleic acid dispersion was added to a UV cuvette containing 2.81 ml of 0.05M phosphate buffer with a pH of 7.4 which had been brought to 40°C. The oxidation reaction was initiated at 37°C in a waterbath by adding 150 µl of 40 mM AAPH solution. Oxidation was carried out in aliquots (20 µl) of extract (0.3 mg/ml). The rate of peroxidation at 37°C was monitored by recording the increase in absorbance at 234 nm, caused by conjugated diene hydroperoxides. ILP in per cent was calculated using the following equation:

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

where A_0 is the absorbance of the blank sample without extract and A_1 is the absorbance in the presence of the extract.

Trolox equivalent antioxidant capacity assay

The Trolox equivalent antioxidant capacity (TEAC) of the mushroom extracts was measured using the assay described by Miller et al. (1995), with minor modifications. $ABTS^{\cdot+}$ radicals were generated by the interaction of $ABTS^{\cdot+}$ (100 µM), H_2O_2 (50 µM) and peroxidase (4.4 unit/ml) in the assay system. To measure the antioxidant capacity of the mushroom fruit bodies, 200 µl of mushroom extract (0.3 mg/ ml) was mixed with an equal volume of $ABTS^{\cdot+}$, H_2O_2, peroxidase and deionized water. Absorbance was measured at 734 nm for 10 min. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. TEAC value is expressed as mM of Trolox solution having the antioxidant equivalent to a 0.1% (w/v) extract solution. Higher TEAC values suggest stronger antioxidant ability.

Total polyphenolic assay

To determine the correlation between the antioxidant activities and the phytochemicals present in the fruit bodies extracts, polysaccharide, protein and total polyphenolic content present in the fruit bodies extracts were estimated.

Total soluble phenolics in the fruit bodies extracts were determined with Folin-Ciocalteu reagent, according to the method of Slinkard & Singleton (1977), using pyrocatechol as the standard. Briefly, 0.1 ml of extract solution and 1 ml of Folin-Ciocalteu reagent were added and shaken well. After 5 min, 3 ml of Na_2CO_3 (2%) was added, and the mixture was allowed to stand for 1 h with intermittent shaking. The absorbance was measured at 760 nm. Total polyphenol was determined as µg pyrocatechol equivalent using the equation given below (obtained from standard pyrocatechol curve):

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

Polysaccharide assay

The polysaccharide content in the fruit bodies extracts was determined using the method described by Dubois et al. (1956). One ml of extract solution was added to 25 µl of 80% phenol and one ml concentrated sulfuric acid (H_2SO_4). The mixture was shaken and allowed to stand at 30°C for 30 min. The absorbance was measured using a UV VIS spectrophotometer at 490 nm. Polysaccharide content was estimated by a standard curve, using known amounts of standard polysaccharide solution.

Protein assay

The protein content of the fruit bodies extracts was determined using the method described by Bradford (1976). The protein content was estimated using a standard curve for a known amount of bovine serum albumin.

Antimicrobial activity

The cut-well agar method (Takahiro et al. 1991) was followed for antimicrobial activity against multidrug resistant pathogens. The pathogens *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were obtained from the Microbial Type Culture Collection (Institute of Microbial Technology, Chandigarh, India). These were cultured aerobically in brain heart infusion (BHI) broth at 37°C in a Biomulti incubator (NK Systems, Tokyo, Japan). A 100 µL freshly grown pathogens were spread on BHI agar plate and wells were made with a well borer (depth 3 mm, diameter 4 mm). The wells were filled with 200 µL of the mushroom extract in methanol, ethanol, acetone and DMSO and kept at 37°C. The extracts were screened for the different pathogens, and the inhibitory zones were observed. A control was used for comparison.

Results

Free-radical scavenging activity

The extracts showed DPPH FRS activity ranging from 44 to 89.60% (Fig. 1). The methanol extract showed the highest activity at 89.60%. The FRS activity of the methanol extract was found to be higher than shown by the BHA extract (56%) but less that of the BHT extract (69%). The extracts with ethanol, acetone and DMSO showed moderate FRS activity (87.27, 57.86 and 44%, respectively; Fig. 1). In general, the antioxidant activity of fruit bodies is solvent specific, and the extraction preference for fruit bodies is probably more in methanol and ethanol than in other solvents, which would account for the variation in FRS activity.

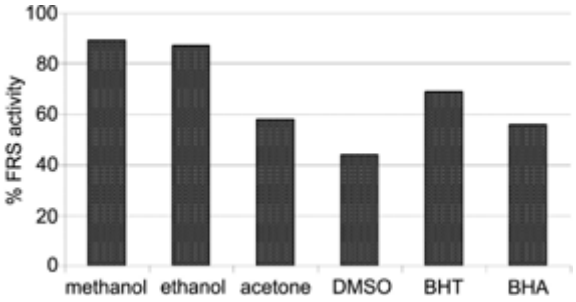


Fig. 1 Free-radical scavenging (FRS) activity of the Arctic mushroom *Lycoperdon molle* in various solvents: methanol, ethanol, acetone, dimethyl sulfoxide (DMSO), butylated hydroxytoluene (BHT); and butylated hydroxyanisol (BHA).

Inhibition of lipid peroxidation

ILP values of the different solvent extracts are presented in Fig. 2. Almost all the solvent extracts showed greater percentages of ILP than the standard antioxidants, BHA (38%) and BHT (47%). The methanol extract showed the highest activity at 54.41%, followed by ethanol at 45.00%, acetone at 39.00% and DMSO at 32.00%, in that order.

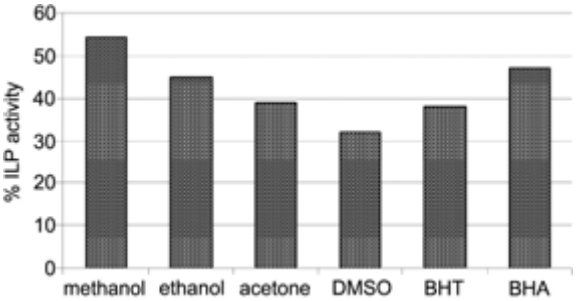


Fig. 2 Inhibition of lipid peroxidation (ILP) activities of the Arctic mushroom *Lycoperdon molle* in various solvents:

methanol, ethanol, acetone, dimethyl sulfoxide (DMSO), butylated hydroxytoluene (BHT); and butylated hydroxyanisol (BHA).

Trolox equivalent antioxidant capacity

The results of the TEAC assays of the various solvent extracts are presented in Table 1. All the extracts showed good activity. The acetone and ethanol extracts showed higher antioxidant capacity (7.4 mM–7.0 mM, respectively) compared to other solvents. TEAC values of our mushroom extracts were found to be higher than the activity shown with standard Trolox (3.9 mM).

Table 1 Antioxidant activity, polysaccharide, protein and total polyphenol content of *Lycoperdon molle* extracts in various solvents. Data presented are the average of three consecutive readings of the extract in each assay system.

		Antioxidant activities					
Solvent extracts	Extract dry wt. g	FRS[1] (%)	ILP[2] (%)	TEAC[3] (mM)	Polysaccharide content (mg/ml)	Protein content (µg/ml)	Total polyphenol content (mg/ml)
Acetone	0.06	57.87	39.00	7.4	14.92	24.0	0.031
Ethanol	0.08	87.27	45.00	7.0	14.85	8.04	0.090
Methanol	0.64	89.60	54.41	–	14.68	8.63	0.304
DMSO[4]	0.11	44.00	32.00	2.4	4.00	8.0	0.325

[1]Free-radical scavenging activity.

[2]Inhibition of lipid peroxidation.

[3]Trolox equivalent antioxidant capacity.

[4]Dimethyl sulfoxide.

Protein, polysaccharide and total phenol content of the fruit bodies

The polysaccharide, protein and total phenol contents present in the mushroom extracts were estimated and are presented in Table 1. Regardless of the solvent used, the protein content obtained in different solvents ranged from 8.00 µg to 24.00 µg/g dry wt. The polyphenol content ranged from 0.031 mg to 0.325 mg/g dry wt. High polysaccharide dry content was found in all the solvent extracts, ranging from 4.00 mg to 14.92 mg/g wt. The acetone extract showed the highest polysaccharide content: 14.92mg/g dry wt.

Antioxidant activities in relation to phytochemical content

Like higher plants, fruit bodies of *Lycoperdon molle* also produce primary and secondary compounds such as proteins, polysaccharides and polyphenols that have been reported for various biological activities (Barros et al. 2008). Accordingly, we determined protein, polysaccharide and polyphenol content present in the *Lycoperdon molle* (Table 1) and correlated these with the antioxidant activities. A significant correlation (R^2=0.968, p<0.01) was found between polysaccharides content and antioxidant activities. We deduce from this study that the very high content of polysaccharide in *Lycoperdon molle* is mainly responsible for the antioxidative activity that is essential for their survival in the Arctic. Our results are also in agreement with those of Liu et al. (1997), who reported the ability of polysaccharide to scavenge free radicals.

Purification and ESI-MS analysis of purified fractions A and B

The crude methanolic extract of *Lycoperdon molle* exhibited six distinguishable spots on thin layer chromatography (TLC) in the solvent system butanol:acetic acid:water (60:15:25, BAW) and then detected with methanolic ninhydrin. These spots were separated using the preparative TLC technique to give six fractions. TLC of the concentrated fractions was carried out and only fraction 2, labelled fraction A, which showed a single spot (R_f=0.7) weighing 5 mg. NMR and ESI-MS were carried out on fraction A. NMR was done in CD_3OD. Fraction 3 showed two spots but the amount was very small. Preparative TLC of fraction 3 gave one fraction, labelled fraction B, which showed only one spot, which weighed (10.8 mg, R_f=0.512). NMR and ESI-MS analyses were carried out on fraction B. The identification of the compounds from fractions A and B using ESI-MS analysis is explained in detail below. The remaining four fractions weighed less than 1 mg so a detailed spectral analysis could not be undertaken on them.

ESI-MS (negative ion mode) of fraction A

In the negative ion mode, the ESI-MS profile (Fig. 3) of one of the chromatographed fractions—fraction A—showed peaks in the mass range of m/z 200–350 that were consistent with assignment as carboxylate ions. These peaks were m/z 227, 253, 255, 283, 305, 325 and 339, which are attributable to C14:0 (myristic), C16:1 (methyl palmitoleate), C 16:0 (palmitic), C18:0 (stearic), C20:3 (methyl eicosatrienoate), C21:0 (heneicosanoic) and C22:0 (hehenic) carboxylate ions, respectively. These include ions formed from phospholipids as well as free fatty acids

present in the organism. Palmitic acid, at m/z 255, was the most prominent ion, indicating it to be one of the major components. A peak at m/z 140.8 was assigned to the presence of phosphoethanolamine. It could also have been due to the presence of monomethyl arsenic acid, since it is known that arsenic molecular species are recorded from *Lycoperdon molle*. An additional signal at m/z 135.9 could have been due to trimethyl arsine oxides. The signals at m/z 171 and 199 corresponded to a phosphatidylglycerol moiety and the peak at 184.99 was due to phosphoserine unit. The signal at m/z 240 corresponded to phosphoionositol after the elimination of a water molecule.

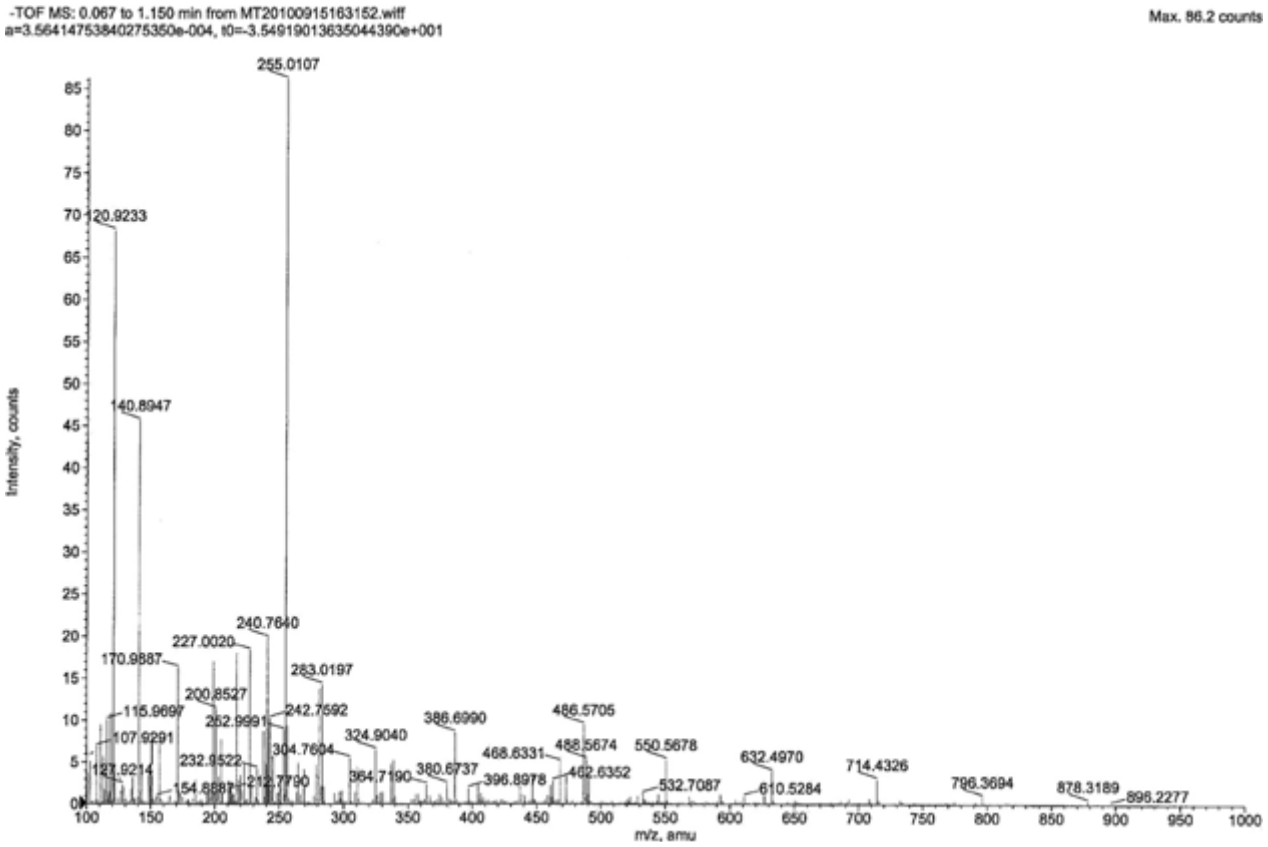
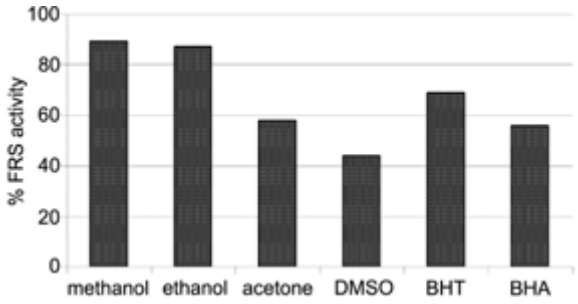


Fig. 3 Electrospray ionization mass spectrometry profile of fraction A in the negative mode.



The mass range at m/z 500–1000 included peaks at 610.5 and 632.4 due to phosphatidic acids. It also included signals at 714.4 and 796.6, representing phosphatidylglycerols. Less intense signals at m/z 878 and 896 were consistent with the expected presence of digalactosyldiacylglycerol, but in the present sample a sugar moiety did not seem to be present as corresponding signals were not observed in the δ3.0–4.5 region of ^{1}HNMR.

ESI-MS (positive ion mode) of fractions A and B

The ESI-MS profile (Fig. 4) in the positive ion mode of fraction A showed signals in the range of m/z 200–500, which probably represented fragments of phospholipids. A molecular species with m/z 540.7 was identified as lysophosphatidylcholine on the basis of fragmentation observed in its electrospray ionization—tandem mass spectrum (ESI–MS/MS; Fig. 5) and as shown in the diagram of its molecular structure.

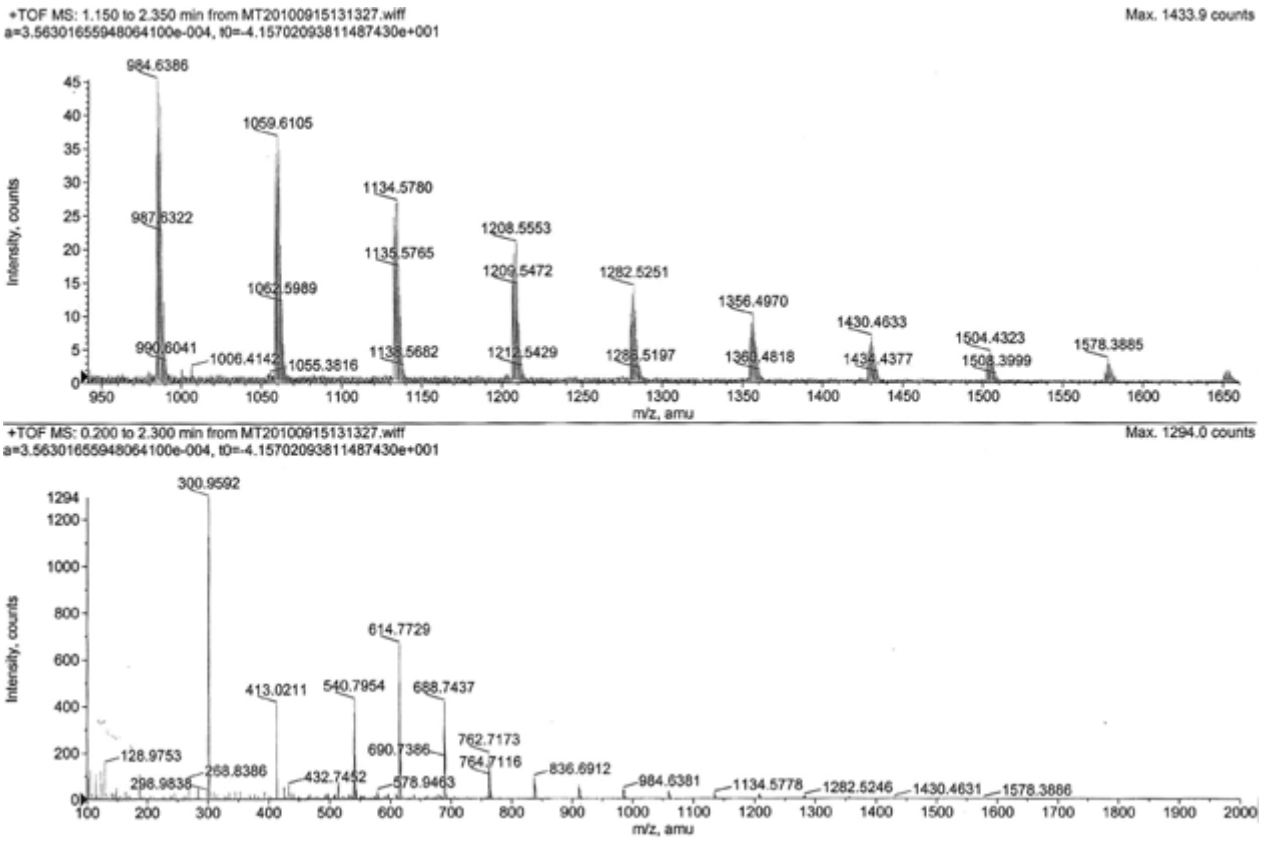


Fig. 4 Electrospray ionization mass spectrometry profile of fraction A in the positive mode.

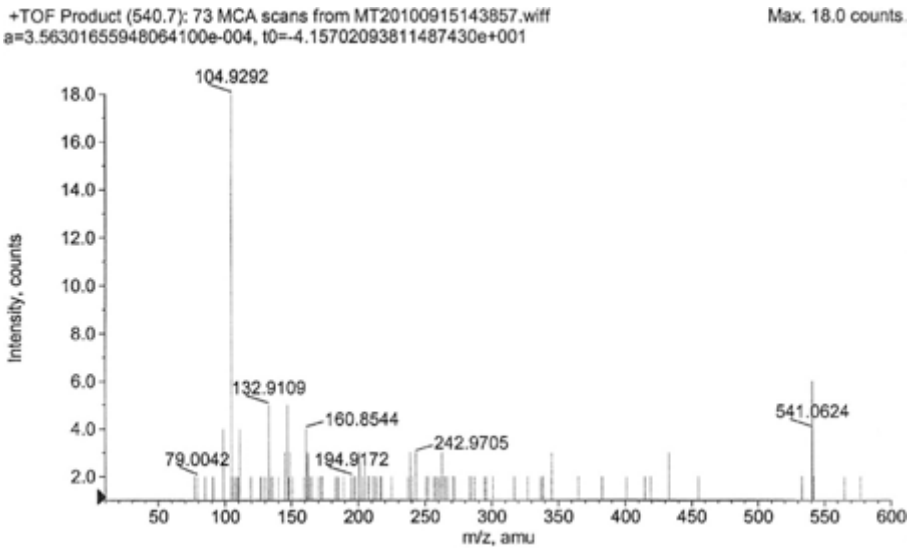
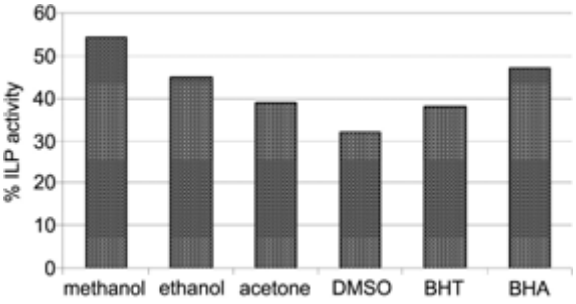
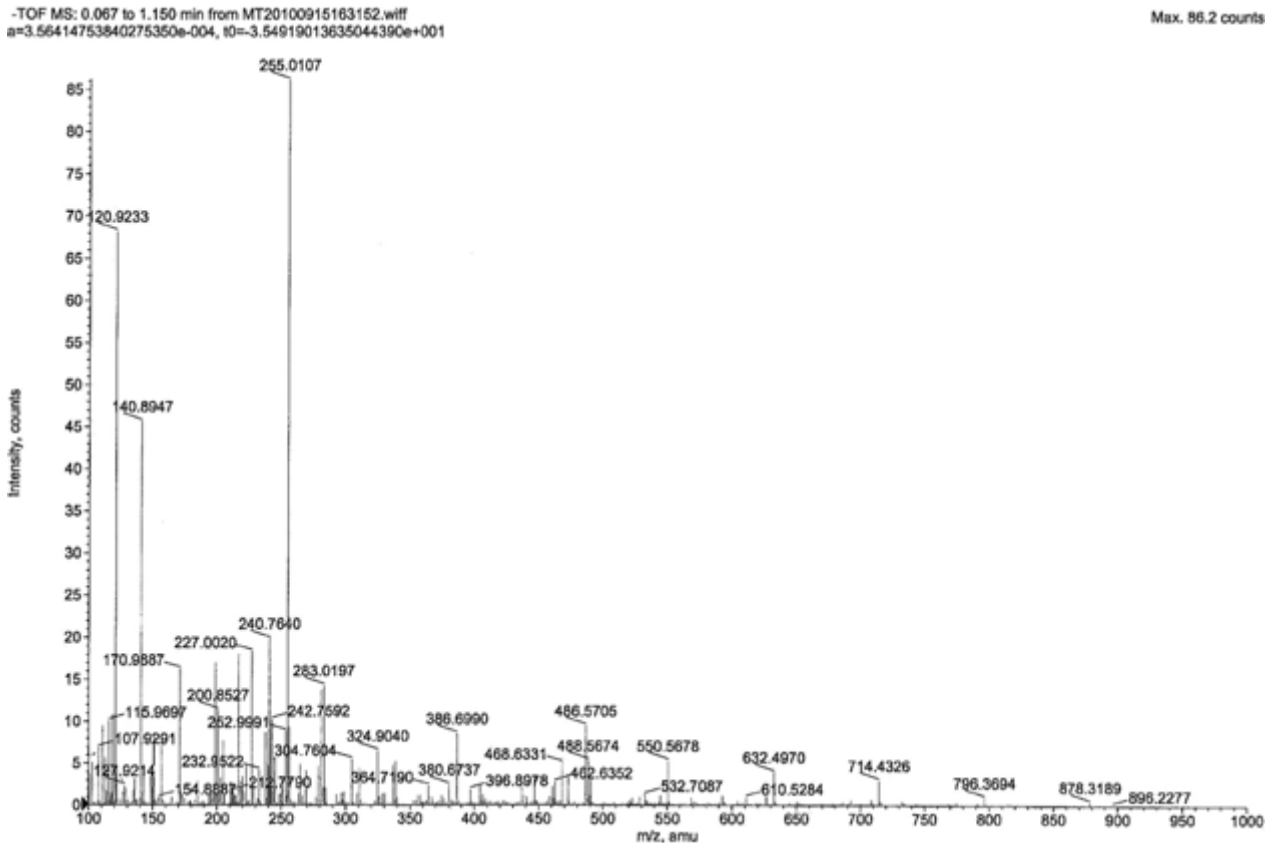


Fig. 5 Electrospray ionization mass spectrometry/tandem mass spectra of m/z 540.7.



An interesting observation here is that beyond molecular species with [M+H]+ at 540.7 the signals at m/z 614.77, 688.74, 762.71, 836.69, 910, 984, 1058, 1132, 1206, 1280, 1354, 1428, 1502, 1576 are observed. A close look at these values indicates that all these molecular species differ by 74 amu, which probably resulted from the oxidation of the double bonds to the corresponding vicinal diols, as shown below:



This indicates that addition of these units to the lysophosphatidylcholine (541) will lead to the structure of the remaining molecular species present in fraction A. The presence of these groups is reinforced by the presence of NMR signals in the region of δ3.5–3.8 for protons and 73 ppm for carbons of hydroxymethines. In addition, methylene groups were observed at δ1.2 and 30 ppm in ^{1}H and ^{13}C NMR spectra.

The ESI-MS analysis of fraction B showed similar pattern as A in its mass profile.

Antimicrobial screening

Antimicrobial screening of *Lycoperdon molle* extracts against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* revealed no activity. Similar results were reported by Barros, Calhelha et al. (2007).

Discussion

Antioxidants play a vital role in scavenging harmful free radicals generated during any physiological stress. Presumed antioxidant activities have been attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continuous hydrogen abstraction and radical scavenging (Diplock 1997; Yilidirim et al. 2001). Both FRS and ILP assays reveal parts of the antioxidant profile of the tested material, giving a clear picture of any substances that have antioxidative potential in the samples. In the FRS assay, the DPPH free radicals generated by the action of alcohol are scavenged by the mushroom extract while in the ILP test, mushroom extract prevents oxidation of lenoleic acid by the oxidizing agent 2,2′-azobis (2-aminopropane) dihydrochloride.

Mushrooms produce a number of secondary metabolites, many of which are phenolic and polysaccharide compounds (Barros, Ferreira et al. 2007). Since significant correlations were found between the polysaccharide content and the FRS, ILP and TEAC activity levels in the *Lycoperdon molle* samples, it is presumed that the antioxidant activity exhibited by the extracts was probably due to the presence of high polysaccharide content (Table 1). These results corroborate the findings of Liu et al. (1997), who reported that the polysaccharides have the ability to scavenge free radicals. ESI-MS analysis of the *Lycoperdon molle* extract showed the presence of compounds such as phosphoethanolamine, monomethyl arsenic acid, phosphatidylglycerol, phosphoionositol, phosphoserine, and lysophosphatidylcholine. In the Arctic, ROS-induced peroxidation and de-esterification of glycerolipids, caused by dehydration and rehydration cycles, are responsible for cell membrane disruptions. Compounds such as polyols, act as osmoprotectants, and quenches the ROS, thereby protecting the intimate cellular structures of the organism against the potentially deleterious effects of dehydration (Jennings et al. 1998). We believe that the chemical constituents we identified in the *Lycoperdon molle* extracts contribute to the survival of this mushroom in the Arctic's extreme environmental conditions.

In the present study, *Lycoperdon molle* showed strong antioxidant abilities compared to the standards. To the best of our knowledge, this is the first report of the antioxidant activity in any Arctic mushroom. On the basis of our results, it can be concluded that the extracts of mushrooms collected in the Arctic region could be a valuable source of natural antioxidants for the pharmaceutical industry. Arctic mushroom species should be explored further and possibly cultivated in the laboratory for mass production.

In addition, antioxidant secondary metabolites from Arctic mushrooms should be explored for therapeutic purposes and to aid our understanding of the biology of these species

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