Chapter 4
Studies on Antioxidant Properties of Lichen Secondary Metabolites

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Abstract At the present time, it is suspected that much used synthetic antioxidants have toxic and carcinogenic effects. Consequently, there is a growing interest towards finding new antioxidants of natural resources without any undesirable effect. Numerous in vitro studies on plants, micro- and macroalgae, macromycetes and lichens strongly support the fact that their constituents with antioxidant capacity are capable of exerting protective effects against oxidative stress in biological systems. Therefore, it is of prime importance to utilise natural antioxidants for their protective effect against oxidative stress and physiological dysfunctions. In the quest for novel natural antioxidant sources, our prime interest has focused on lichens. In recent time, numerous studies point to the importance of lichens in the neutralisation of free radicals. Lichens are rich in the secondary metabolites, primarily phenols, which are well known for its antioxidant properties. Because of that, the present chapter focuses on the role of lichens and their secondary metabolites in combating danger posed by overproduced free radicals.

4.1 Free Radicals, Oxidative Stress and Antioxidants

Free radicals (reactive oxygen species, such as the hydroxyl radical, superoxide anion and hydrogen peroxide, and reactive nitrogen species, such as nitric oxide and peroxynitrite) (Fig. 4.1) play an important role in many chemical processes in the cells. At low or moderate concentrations, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are necessary for the maturation process of cellular structures and can act as weapons for the host defence system (Halliwell 1995; Squadriato and Pelor 1998; Young and Woodside 2001; Droge 2002; Sangameswaran et al. 2009; Huda-Faujan et al. 2009). Under normal conditions, the balance between the generation and diminution of ROS is controlled by the antioxidant defence system. However, under certain pathological conditions such as

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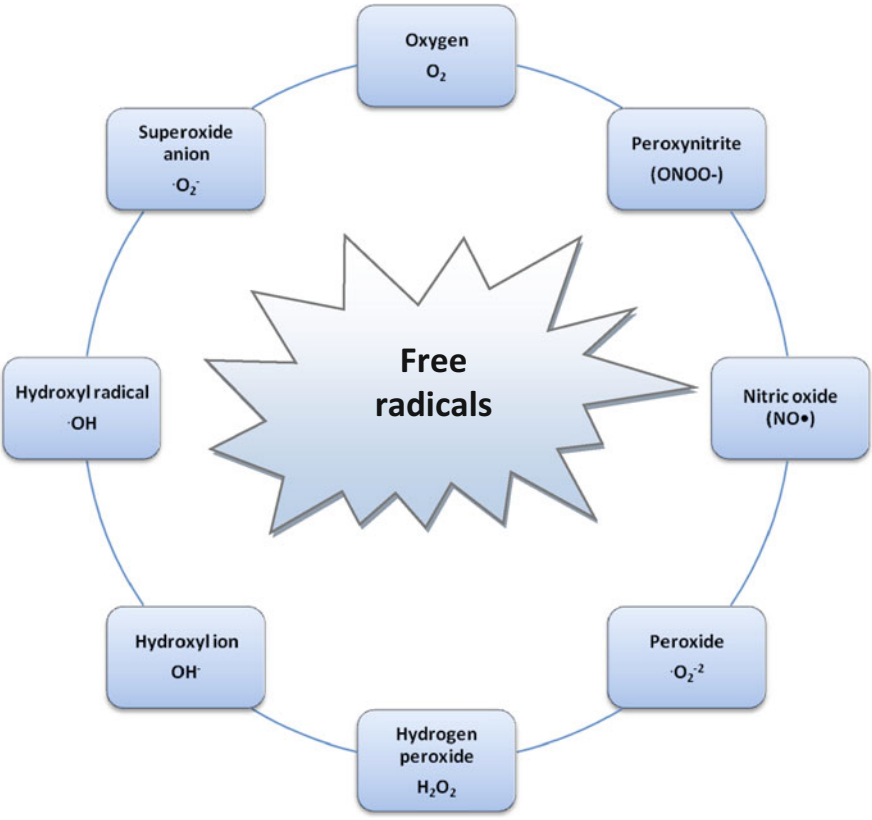

Fig. 4.1 Free radicals (reactive oxygen species and reactive nitrogen species)

drought, salinity, chilling, metal toxicity and UV-B radiation as well as pathogens, when ROS are not effectively eliminated by the antioxidant defence system, the dynamic balance between the generation and diminution of ROS is broken (Lobo et al. 2010).

When produced in excess, free radicals and oxidants generate a phenomenon called oxidative stress, a deleterious process that can seriously alter the cell membranes and other structures such as proteins, lipids, lipoproteins and deoxyribonucleic acid (Betteridge 2000). If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases such as Alzheimer's disease, atherosclerosis, emphysema, hemochromatosis, many forms of cancer (e.g. melanoma, Parkinson's disease and schizophrenia) as well as the ageing process (Fig. 4.2) (Sangameswaran et al. 2008; Sachindra et al. 2010).

Antioxidants are substances which possess the ability to protect the body from damage caused by oxidative stress (Souri et al. 2008). The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants) or externally supplied through foods (exogenous antioxidants) (Fig. 4.3).

Fig. 4.2 Diseases induced by oxidative stress

In addition, in order to protect biomolecules against the attack of ROS and/or to suppress the resultant damage, synthetic antioxidants have been used for industrial processing in recent years. The most extensively used synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) (Ramachandra et al. 2012). They are used widely in the food industry because of their effectiveness and generally being less expensive than natural antioxidants. However, since synthetic antioxidants are often carcinogenic, finding natural substitutes is of great interest (Zhang et al. 2009). Concerns regarding toxicological effects and carcinogenic potential of synthetic antioxidants have prompted the need for natural alternatives in the last few decades (Lu and Foo 2001; Aligiannis et al. 2003; Vagi et al. 2005; Es-Safi et al. 2006). Therefore, the importance of search for the exploitation of effective natural antioxidants has greatly increased in recent years (Pokorny et al. 2001; Gulcin et al. 2004; Naveena et al. 2008).

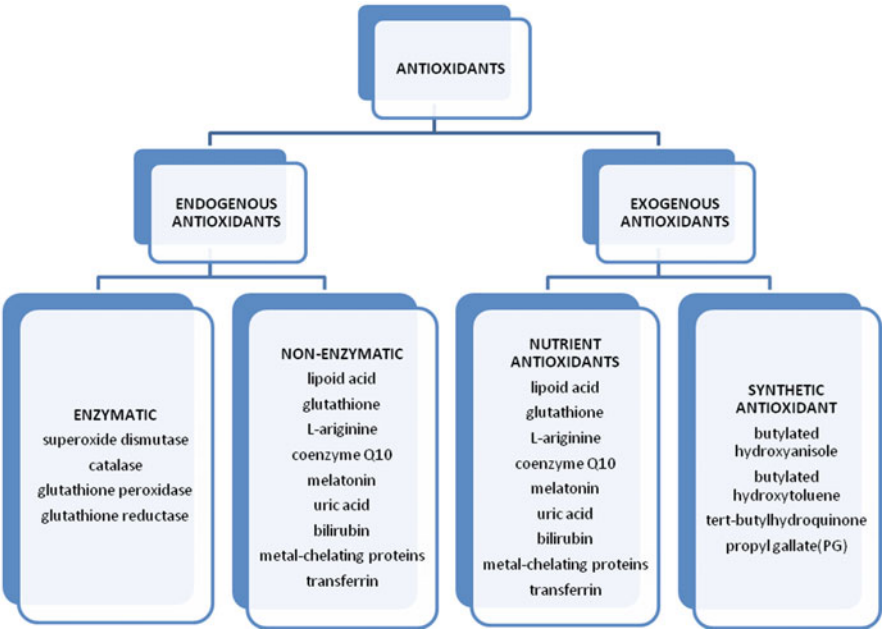

Fig. 4.3 Endogenous antioxidants and exogenous antioxidants for protecting the body from damage caused by oxidative stress

4.2 Antioxidant Properties of Lichens

Lichens are a good source of natural antioxidants. Although mainly hydrophobic, the phenolic nature of the major secondary metabolites of lichens is expected to afford antioxidant properties. Phenolics are the major secondary metabolites from lichens that play crucial function in the regulation of lichen growth and development in stressful and unfavourable climatic conditions. Lichens grow in extreme environmental conditions which in turn could cause up-regulated pathways of secondary metabolite synthesis and increased production of polyphenolics that have protective effects against oxidative stress owing to their antioxidant capacities (Kumar et al. 2014). Lichens produce many phenolic compounds including depsides, depsidones, dibenzofurans and pulvinic acid derivatives (Paudel et al. 2012).

Phenolic compounds are widespread products of secondary metabolism of lichen, and antioxidant activity is most frequently associated with their presence. Lichen phenolics are mainly depsides, depsidones and dibenzofurans, whereas vascular plant phenolics include tannins, lignins and flavonoids. Orsellinic acid is the basic unit in the biosynthesis of lichen phenolics. Lichen phenols are generally secreted by the fungal partner and deposited as crystals on the surface of the cell wall of the fungal hyphae. Lichen phenols are primarily acetate-polymalonate derived with the exception of pulvinic acid derivates which are synthesised via

the shikimic acid pathway. Lichen phenolics are composed of two monocyclic phenols joined either by an ester bond as in depsides or by both ester and ether bonds in depsidones or a furan heterocycle bond as found in dibenzofurans, such as usnic acid (Watson 2014).

Phenolic compounds contain in its structure an aromatic ring by one or more hydroxyl groups. It is believed that the antioxidant activity of the phenol is primarily a result of their ability to be the donor of hydrogen atoms and eliminate the free radicals to form less reactive phenoxyl radicals (Sawa et al. 1999).

$$\mathrm{Ph-OH + ROO \rightarrow Ph-O^{\bullet} + ROOH}$$

$$\mathrm{Ar-OH + OH \rightarrow H2O + ArO^{\bullet}}$$

It has been shown that with the increase of the number of hydroxyl groups in the molecule, as well as the extension of the side chain of an antioxidant increases the activity of these compounds, probably due to the possibility of stabilisation free radical forms the side chain conjugation (Yanishlieva et al. 1999). Because of the large number of potential reactive centres, the phenol molecule has the ability to react with the radicals of the same time.

Phenols possess ideal structural chemistry for antioxidant activity and have been shown to be more effective in vitro than vitamins E and C on molar basis (Michalak 2006). As described by Bors et al. (1990), there are three structural features that are important determinants for the antioxidant potential of phenols:

(a) The *orto* 3′,4′-dihydroxy structure in the B ring
(b) The 2,3-double bond in conjunction with the 4-oxo group in the C ring (which allows conjunction between the A and B ring or electron delocalisation)
(c) The presence of a 3-OH group in C ring and a 5-OH group in the A ring

Among them, the 3-OH group is the most significant determinant of electron-donating activity.

Mainly, the antioxidant activity of phenolic compounds is affected by their chemical structure. The antioxidant activity of phenols also depends on the type and polarity of the extracting solvent, the isolation procedures, purity of active compounds, as well as the test system and substrate to be protected by the antioxidant (Moure et al. 2001).

When antioxidant capacities of the lichens are compared with their phenolic constituents, it could be concluded that antioxidative nature of the lichens might depend on their phenolics. There are many reports which correlate the total phenolic content of lichens and their antioxidant activity. For example, for the methanol extract of *Lobaria pulmonaria*, there was a strong correlation between antioxidant activity and total phenolic content (Odabasoglu et al. 2004). Kekuda et al. (2012) found highly positive relationship between total phenols and antioxidant activity in methanol extract of a foliose macrolichen *Everniastrum cirrhatum*.

Pavithra et al. (2013) found a high correlation between antioxidant efficacy of a macrolichen *Usnea pictoides* and its phenolic content. A positive correlation was seen between the phenolic content and total antioxidant activity of *Anaptychia ciliaris*, *Nephroma parile*, *Ochrolechia tartarea* and *Parmelia centrifuga* having correlation coefficient values (r) of 0.990 (Ranković et al. 2010a). Kosanić and Ranković (2011a) found that content of total phenolic of the extracts of *Cetraria islandica*, *Lecanora atra*, *Parmelia pertusa*, *Pseudevernia furfuracea* and *Umbilicaria cylindrica* was strongly related with DPPH radical scavenging activity ($r = 0.966$), with reducing power ($r = 0.944$) and with superoxide anion radical scavenging ($r = 0.823$). A certain correlation also was established between the antioxidant activity and the total phenol content for the *Toninia candida* methanol, chloroform and petrol ether extracts (Manojlović et al. 2012).

Many other studies have shown a direct correlation between the phenolic content and the antioxidant activity (Tilak et al. 2004; Coruh et al. 2007; Rekha et al. 2012; Poornima et al. 2012).

However, the total phenolic content and antioxidant potency did not always correlate. Odabasoglu et al. (2005) determined the total antioxidant activity, total phenolic content and the reducing power of methanol and water extracts of four lichen species, *Bryoria fuscescens*, *Dermatocarpon intestiniformis*, *Peltigera rufescens* and *Pseudevernia furfuracea*. Water and methanol extracts of *P. rufescens* showed the highest antioxidant activity. However, there was no correlation between antioxidant activity and total phenolic content of the extracts. Although the methanol extract of *P. furfuracea* had the highest total phenolic contents, it exhibited low antioxidant activity. In contrast, there was a strong correlation between reducing power and total antioxidant activity of the extracts. Stanly et al. (2011) evaluated the antioxidant activity and total phenol content of four lichen species belonging to the genus *Ramalina*, *Parmotrema*, *Bulbothrix* and *Cladia* collected from Malaysia. There was no correlation between total phenolic content and radical scavenging activity of methanol extracts of all the tested species. For example, the antioxidant properties of some isolated phenols are not so impressive. For instance, two depsidones were found only slightly more active than the commercial quercetin in a superoxide scavenging assay with IC50 # 600 lM (Lohézic-Le Dévéhat et al. 2007), and none of the orcinol or orsellinate derivatives were as active as the commercial gallic acid to reduce DPPH (Lopes et al. 2008).

These results suggest that the antioxidant activity of some tested extracts might be attributed to the presence of non-phenolic compounds. Nevertheless, it should be taken into consideration that individual phenolics may have distinct antioxidant activities; there may be antagonistic or synergistic interactions between phenolics and other compounds like carbohydrates, proteins, etc. (Odabasoglu et al. 2005).

4.3 Assays to Determine Antioxidant Capacities of Lichens

Methods to assess the antioxidant activity of lichens are based on hydrogen atom transfer (HAT) and others on electron transfer (ET).

HAT-based assays measure the capability of phenolic antioxidants to quench free radicals (generally, peroxyl radicals considered to be biologically more relevant) by H-atom donation. The HAT mechanisms of antioxidant action in which the hydrogen atom (H) of a phenol (Ar–OH) is transferred to a $ROO^{\bullet}$ radical can be summarised by the reaction

$$ROO^{\bullet} + AH/ArOH \rightarrow ROOH + A^{\bullet}/ArO^{\bullet}$$

where the aryloxy radical ($ArO^{\bullet}$) formed from the reaction of antioxidant phenol with peroxyl radical is stabilised by resonance. The AH and Ar–OH species denote the protected biomolecules and phenolic antioxidants, respectively. Effective phenolic antioxidants need to react faster than biomolecules with free radicals to protect the latter from oxidation. Since, in HAT-based antioxidant assays, both the fluorescent probe and antioxidants react with $ROO^{\bullet}$, the antioxidant activity can be determined from competition kinetics by measuring the fluorescence decay curve of the probe in the absence and presence of antioxidants, integrating the area under these curves and finding the difference between them (Huang et al. 2005; Prior et al. 2005).

HAT-based assays include oxygen radical absorbance capacity (ORAC) assay, TRAP assay using R-phycoerythrin as the fluorescent probe, crocin bleaching assay using 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) as the radical generator and β-carotene bleaching assay, although the latter bleaches not only by peroxyl radical attack but by multiple pathways (Huang et al. 2005; Prior et al. 2005).

In most **ET-based assays**, the antioxidant action is simulated with a suitable redox-potential probe, namely, the antioxidants react with a fluorescent or coloured probe (oxidising agent) instead of peroxyl radicals. Spectrophotometric ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant, which changes colour when reduced. The degree of colour change (either an increase or decrease of absorbance of the probe at a given wavelength) is correlated to the concentration of antioxidants in the sample. 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)/trolox-equivalent antioxidant capacity (TEAC) and 2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) (Brand-Williams et al. 1995; Bondet et al. 1997; Sanchez-Moreno et al. 1998) are decolourisation assays, whereas in Folin total phenols assay (Folin and Ciocalteu 1927; Singleton et al. 1999), ferric reducing antioxidant power (FRAP) (Benzie and Strain 1996; Benzie and Szeto 1999) and cupric reducing antioxidant capacity (CUPRAC) (Apak et al. 2004), there is an increase in absorbance at a prespecified wavelength as the antioxidant reacts with the chromogenic reagent (i.e. in the latter two methods, the lower valencies of iron and copper, namely, Fe(II) and Cu(I), form charge transfer complexes with the corresponding ligands, respectively).

ET-based assays generally set a fixed time for the concerned redox reaction and measure thermodynamic conversion (oxidation) during that period. Although the reducing capacity of a sample is not directly related to its radical scavenging capability, it is a very important parameter of antioxidants (Apak et al. 2013).

4.4 Antioxidant Activities of Lichen Extracts

Lichens are proven to be good source of antioxidants, and a plenty of literatures have supported the antioxidant action of these organisms. Few reports concerning the antioxidative nature of pure lichen metabolites are available in the literature; most of the publications describe the antioxidant activities of crude lichen extracts.

There was a first report in 1993 (Yamamoto et al. 1993) on antioxidation activity in lichens by the method using SOD. Thereafter, antioxidant activities of many lichen species were assessed by numerous researchers. For example, Aslan et al. (2006) investigated antioxidant activity of methanol extract of *Evernia divaricata*, *Evernia prunastri*, *Cladonia foliacea*, *Dermatocarpon miniatum* and *Neofuscella pulla* by scavenging of free radical DPPH and the inhibition of linoleic acid oxidation. They found that extracts of *Cladonia foliacea*, *Evernia divaricata*, *Evernia prunastri* and *Neofuscella pulla* did not exert any activity in both assays, whereas those of *Dermatocarpon miniatum* provided 50 % inhibition at 396.1 μg/ml concentration in the former and gave 49 % inhibition in the latter. Mitrović et al. (2011) also studied antioxidant activity of methanol extract of *Parmelia sulcata*, *Flavoparmelia caperata*, *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia foliacea* and found than they exhibited DPPH radical scavenging activity.

The antioxidant activity of ethanol extract of *Sanionia uncinata* was evaluated by Bhattarai et al. (2008a) by analysing its reducing power, superoxide scavenging activity, ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) cation scavenging activity and DPPH free radical scavenging activity. They found that tested lichen species could be an important source of natural antioxidant agents. Kumar et al. (2010b) reported significant antioxidant activity for methanol extracts of *Ramalina hossei* and *Ramalina conduplicans*; Yucel et al. (2007) for chloroform, methanol and aqueous extracts of *Cladonia rangiformis*; and Odabasoglu et al. (2005) for methanol and aqueous extracts of *Bryoria fuscescens*, *Dermatocarpon intestiniformis*, *Peltigera rufescens* and *Pseudevernia furfuracea*. Strong antioxidant effect was also found in the methanol extract of *Usnea ghattensis* (Verma et al. 2008), in methanol extract of *Parmotrema pseudotinctorum* (Kumar et al. 2010a, b), as well as in acetone, methanol and aqueous extracts of lichens *Cladonia furcata*, *Hypogymnia physodes*, *Lasallia pustulata*, *Parmelia caperata* and *Parmelia sulcata* (Kosanić et al. 2011).

Sisodia et al. (2013) evaluated antioxidant activity for different extract of *Ramalina roesleri* species and found that the DPPH radical scavenging activity of extracts ranged from 29.42 to 87.90 %. Sharma et al. (2012) examined the antioxidant activity of two common lichens, namely, *Parmotrema reticulatum* and *Usnea*

sp. from Darjeeling hills. The antioxidant assay of different concentration of ethanolic and methanolic extracts of lichens was determined with respect to five parameters, i.e. DPPH radical scavenging activity, total antioxidant activity, reducing power ability and flavonoid and phenolic content. The DPPH radical scavenging ranged from 10 to 31.5 % for methanol extracts of *Parmotrema reticulatum* and *Usnea* sp., respectively, and for reducing power measured values of absorbance varied from 0.376 to 0.514. In addition, total phenolic content of the extracts was high, and total flavonoid content was moderate.

The methanol extracts of 24 lichen species were tested for antioxidant activities in vitro. It was found that in DPPH assay, three species *Peltigera* sp., *Cladonia* sp. and *Canoparmelia* sp. showed comparable activity with commercial standard, BHA. In ABTS+ assay, extracts of *Parmotrema* sp., *Ramalina* sp., *Peltigera* sp. and *Cladonia* sp. showed stronger activity than ascorbic acid. The observed data indicated that the high altitude lichens contain stronger antioxidant constituents (Paudel et al. 2012).

In the study described by Mastan et al. (2014), the secondary metabolites of lichens *Cladonia fimbriata*, *Parmeliopsis ambigua*, *Punctelia subrudecta* and *Evernia mesomorpha* were extracted in the two solvents methanol and water. The lichen extracts showed comparable and strong antioxidant activity and exhibited higher DPPH and hydroxyl radical scavenging activity. Among the tested lichen extracts, water extract of *Evernia mesomorpha* gave the highest reducing power, although the reducing activity was lower than the standard ascorbic acid. These findings provided evidence that crude aqueous and organic solvent extracts of lichens contain antioxidant important compounds.

Gulcin et al. (2002) found that the aqueous extracts of *Cetraria islandica* had a strong inhibition on peroxidation of linoleic acid, reducing power, superoxide anion radical scavenging and free radical scavenging activities. Similar results were reported by Behera et al. (2005) for different extracts from the lichen *Usnea ghattensis* which was found their strong effect on DPPH radical, superoxide anion radical, nitric oxide and the strong inhibition of lipid peroxidation. Kekuda et al. (2009) find strong antioxidant activity for the extracts of the lichen *Parmotrema pseudotinctorum* and *Ramalina hossei*. Manojlovic et al. (2010) explored antioxidant properties of *Laurera benguelensis* by scavenging of DPPH radical and found that this lichen provided a weak radical scavenging activity.

Anaptychia ciliaris, *Nephroma parile*, *Ochrolechia tartarea* and *Parmelia centrifuga* were screened for antioxidant activity by Ranković et al. (2010a, b). They found that the methanol extract of the *Parmelia centrifuga* showed a strong antioxidant activity, in comparison to the extracts from *Anaptychia ciliaris*, *Nephroma parile* and *Ochrolechia tartarea* which were relatively weaker. *Lecanora muralis*, *Parmelia saxatilis*, *Parmeliopsis ambigua*, *Umbilicaria crustulosa* and *Umbilicaria polyphylla* were tested for DPPH radical scavenging, superoxide anion radical scavenging and reducing power (Kosanić et al. 2014a), and it has been found that of the lichens tested, *Umbilicaria polyphylla* had the largest antioxidant activities.

Antioxidant activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Usnea pictoides* lichen was determined by DPPH free radical scavenging assay and ferric reducing assay (Pavithra et al. 2013). The scavenging potential of methanol extract was higher than other extracts, and also, in ferric reducing assay, methanol extract showed stronger reducing power than other extracts.

Usnea pictoides was found to be a strong antioxidant agent by Pavithra et al. (2013). The lichen was powdered and extracted sequentially using solvents of increasing polarity, viz., petroleum ether, chloroform, ethyl acetate and methanol. Antioxidant activity of solvent extracts was determined by DPPH free radical scavenging assay and ferric reducing assay. A dose-dependent scavenging of DPPH radicals by solvent extracts was observed. The scavenging potential of methanol extract was higher than other extracts. In ferric reducing assay, methanol extract showed stronger reducing power than other extracts. Overall, extracts containing high phenolic contents exhibited stronger antioxidant activity. A positive correlation was observed between total phenolic content and the antioxidant activity of lichen extracts.

In related study, Odabasoglu et al. (2004) determined the antioxidant activities, reducing powers and total phenolic contents of methanol and water extracts of three lichen species, *Usnea longissima*, *Usnea florida* and *Lobaria pulmonaria*. Of the extracts tested, the methanol extracts of *Lobaria pulmonaria* and *Usnea longissima* showed potent antioxidant activities. The methanol extract of *Lobaria pulmonaria* also had the highest total phenolic contents (87.9 mg/g lyophilisate). For the methanol extract of this species, there was also a strong correlation between antioxidant activity and total phenolic contents. However, a similar correlation was not observed for *Usnea longissima*. Although the methanol extract of *Usnea longissima* had a lower phenolic content (38.6 mg/g lyophylisate), it exhibited potent antioxidant activity. On the other hand, there was a strong correlation between the reducing powers and the total phenolic contents of the extracts. The highest reducing power was determined for the methanol extract of *Lobaria pulmonaria*.

Ranković et al. (2010a, b) evaluated methanol extracts of the lichens *Cetraria pinastri*, *Cladonia digitata*, *Cladonia fimbriata*, *Fulgensia fulgens*, *Ochrolechia parella* and *Parmelia crinite* for their antioxidant activity. They found that the methanol extract of the *Cetraria pinastri* showed a strong antioxidant activity, whereas the extracts of the species *Fulgensia fulgens*, *Cladonia fimbriata* and *Parmelia crinite* showed the moderate one and the extract of the species *Ochrolechia parella* and *Cladonia digitata* the weak one. The methanol extract of the lichen *Cetraria pinastri* had the biggest total phenol content (32.9 mg/g of the dry extract). A certain correlation was established between the antioxidant activity and the total phenol content for the researched lichen extracts.

Acetone, methanol and aqueous extracts of the lichens *Cetraria islandica*, *Lecanora atra*, *Parmelia pertusa*, *Pseudevernia furfuracea* and *Umbilicaria cylindrica* were found to possess effective antioxidant activities (Kosanić and Ranković 2011a). Antioxidant activities of the tested extracts were studied by DPPH radical scavenging, superoxide anion radical scavenging and reducing

power. The DPPH radical scavenging activity for studied species ranged from 32.68 to 94.70 %. For reducing power, measured values of absorbance varied from 0.016 to 0.109. The superoxide anion scavenging activity for different extracts was 7.31–84.51 %. In addition, the high contents of total phenolic compounds suggest that phenols might be the major antioxidant compounds in tested extracts. The same authors also found relatively strong antioxidant activity for acetone, methanol and aqueous extracts of the lichens *Cladonia furcata*, *Hypogymnia physodes* and *Umbilicaria polyphylla* (Kosanić and Ranković 2011b).

Antioxidant effect of methanol, chloroform and petrol ether extracts from the lichen *Toninia candida* was assayed for their antioxidant activity by Manojlović et al. (2012). The lichen extracts showed comparable and strong antioxidant activity and exhibited higher DPPH and hydroxyl radical scavenging, chelating activity and inhibitory activity towards lipid peroxidation.

Stereocaulon paschale, *Parmeliopsis ambigua*, *Parmelia pertusa*, *Parmelia caperata*, *Parmelia sulcata*, *Parmelia saxatilis*, *Hypogymnia physodes*, *Cladonia furcata*, *Lecanora atra*, *L. muralis*, *Umbilicaria crustulosa*, *Umbilicaria cylindrica* and *Umbilicaria polyphylla* were studied for their antioxidant capacity by Serbian group of scientists (Ranković et al. 2011, 2014b; Kosanić et al. 2012a, b). They found that the tested lichens have effective free radical scavenging activity, reducing power and superoxide anion radical scavenging, and based on strong relationships between total phenolics contents and the antioxidant effect of tested species, it could be concluded that antioxidative nature of these lichens depends on their phenolics.

The *n*-hexane, methanol and water extracts of 14 saxicolous lichens from trans-Himalayan Ladakh region were evaluated for their antioxidant capacities by Kumar et al. (2014). The ferric reducing antioxidant power (FRAP), 2,29-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO) radical scavenging capacities and b-carotene-linoleic acid bleaching property exhibited analogous results where the lichen extracts showed high antioxidant action. The lichen extracts were also found to possess good amount of total proanthocyanidin, flavonoid and polyphenol as the main antioxidant components in extracts. The methanolic extract of *Lobothallia alphoplaca* exhibited the highest FRAP value. Methanolic extract of *Xanthoparmelia stenophylla* showed the highest ABTS radical scavenging capacity. The *n*-hexane extract of *Rhizoplaca chrysoleuca* exhibited the highest DPPH radical scavenging capacity. The highest antioxidant capacity in terms of b-carotene linoleic acid bleaching property was observed in the water extract of *Xanthoria elegans*. Similarly, *Melanelia disjuncta* water extract showed the highest NO scavenging capacity. Among *n*-hexane, methanol and water extracts of all lichens, the methanolic extract of *Xanthoparmelia mexicana* showed the highest total proanthocyanidin, flavonoid and polyphenol content.

In the study reported by Vivek et al. (2014), radical scavenging potential of three *Parmotrema* species, viz., *Parmotrema tinctorum*, *Parmotrema grayanum* and *Parmotrema praesorediosum* from Maragalale and Guliguli Shankara, Western Ghats of Karnataka, India, was determined. The powdered lichen materials were

extracted using methanol. Radical scavenging activity of lichen extracts was determined by DPPH free radical scavenging assay. Total phenolic content of lichen extracts was estimated by Folin–Ciocalteu reagent method. Scavenging of DPPH radicals by lichen extracts was concentration dependent. Among the lichen species, *Parmotrema grayanum* showed higher scavenging potential as indicated by lower IC50 value. Total phenolic content was also high in *Parmotrema grayanum*.

Antioxidant capacity of many other lichen extracts was confirmed by other researchers (Ranković et al. 2012; Kosanić et al. 2013a, b, 2014b).

4.5 Antioxidant Activities of Lichen Secondary Metabolites

Lichens have been found to contain a variety of secondary lichen substances with strong antioxidant activity. There are reports concerning the antioxidative nature of pure lichen metabolites that are available in the literature (Table 4.1).

Methanol–water (90:10 v/v) extracts of five polar lichen species, namely, *Stereocaulon alpinum*, *Ramalina terebrata*, *Caloplaca* sp., *Lecanora* sp. and *Caloplaca regalis*, from King George Island were analysed using thin layer chromatography (TLC) followed by a DPPH (2,2-diphenyl-1-picrylhydrazyl) spray technique. The experimental data showed that 33–50 % of the major constituents of the test extracts were active antioxidants (Bhattarai et al. 2008a, b).

Hidalgo et al. (1994) reported the antioxidant activity of some depsides, such as atranorin (isolated from *Placopsis* sp.) and divaricatic acid (isolated from *Protousnea malacea*), and depsidones, such as pannarin (isolated from *Psoroma pallidum*) and 1′-chloropannarin (isolated from *Erioderma chilense*). All of these secondary compounds inhibited rat brain homogenate auto-oxidation and β-carotene oxidation, and depsidones were found to be the most effective. Russo et al. (2008) found that both sphaerophorin (depside) and pannarin (depsidone) inhibited superoxide anion formation in vitro, pannarin being more efficient, confirming Hidalgo et al. (1994). Similarly, de Barros Alves et al. (2013) found high the antioxidant power of fumarprotocetraric acid produced by the lichen *Cladonia verticillaris* evaluated using the thiobarbituric acid reactive species assay in mouse lung tissue.

Thadhani et al. (2011) were assessed antioxidant activity of several classes of lichen metabolites in the in vitro superoxide radical (SOR), nitric oxide radical and 2,2-diphenyl-1-picrylhydrazil radical scavenging assays. The despsides sekikaic acid and lecanoric acid showed promising antioxidant activity in SOR assay with IC_{50} values of 82.0 ± 0.3 μmol and 91.5 ± 2.1 μmol, respectively, while the depsidone lobaric acid exhibited an IC_{50} value of 97.9 ± 1.6 μmol, all relative to the standard, propyl gallate ($IC_{50} = 106.0 \pm 1.7$ μmol). One of the most abundant mononuclear phenolic compounds, methyl-β-orcinol carboxylate, was found to be a potent NO scavenger ($IC_{50} = 84.7 \pm 0.1$ μmol), compared to the standard rutin ($IC_{50} = 86.8 \pm 1.9$ μmol).

Table 4.1 Literature sources mentioning data for lichen substances responsible for antioxidant activity of lichens

Lichen substances	References
Lecanoric acid	Jayaprakasha and Rao (2000), Lopes et al. (2008), Thadhani et al. (2011), Verma et al. (2011), Buçukoglu et al. (2013)
Atranorin	Hidalgo et al. (1994), Jayaprakasha and Rao (2000), Melo et al. (2011), Sisodia et al. (2013). Ranković et al. (2014a, b), Kosanić et al. (2014a, b)
2,4-Dihydroxy-6-propyl	Sisodia et al. (2013)
Ramalin	Paudel et al. (2008)
Barbatic acid	Verma et al. (2011)
Divaricatic acid	Hidalgo et al. (1994)
Pannarin	Hidalgo et al. (1994), Russo et al. (2008)
Zeorin	Verma et al. (2011)
Gyrophoric acid	Buçukoglu et al. (2013)
Umbilicaric acid	Buçukoglu et al. (2013)
Protocetraric acid	Manojlović et al. (2012)
Fumarprotocetraric acid	de Barros Alves et al. (2014), Kosanić et al. (2014a, b)
Stictic acid	Lohézic-Le Dévéhat et al. (2007)
Salazinic acid	Verma et al. (2012), Manojlović et al. (2012)
Usnic acid	Odabasoglu et al. (2006), Manojlović et al. (2012), Ranković et al. (2012), Verma et al. (2012), Sisodia et al. (2013), Ranković et al. (2014a, b)
Variolaric acid	Brisdelli et al. (2013)
2-Hydroxy-4-methoxy-6-propyl benzoic acid	Sisodia et al. (2013)
Evernic acid	Kosanić et al. (2013a, b)
Erythrin	Choudhary et al. (2009)
2,4-Dihydroxy-3,6-dimethyl benzoate	Sisodia et al. (2013)
Lobaric acid	Thadhani et al. (2011), Brisdelli et al. (2013)
Physodic acid	Kosanić et al. (2013a, b), Ranković et al. (2014a, b)
Protolichesterinic acid	Sisodia et al. (2013)
Norstictic acid	Ranković et al. (2012)
Cuculloquinone	Stepanenko et al. (2002)
Homosekikaic acid	Sisodia et al. (2013)
Sekikaic acid	Thadhani et al. (2011), Verma et al. (2012), Sisodia et al. (2013)
Benzoic acid	Sisodia et al. 2013
Diffractaic acid	Brisdelli et al. (2013)
Vicanicin	Brisdelli et al. (2013)
Sphaerophorin	Russo et al. (2008)
Isidiophorin	Atalay et al. (2011)
Rhizonaldehyde	Atalay et al. (2011)
Rhizonyl alcohol	Atalay et al. (2011)
Pulmonarianin	Atalay et al. (2011)

(continued)

Table 4.1 (continued)

Lichen substances	References
Hypotrachynic acid	Papadopoulou et al. (2007)
Deoxystictic acid	Papadopoulou et al. (2007)
Cryptostictinolide	Papadopoulou et al. (2007)
8′-Methylconstictic acid	Papadopoulou et al. (2007)
8′-Methylstictic acid	Papadopoulou et al. (2007)
8′-Methylmenegazziaic acid	Papadopoulou et al. (2007)
Ethylstictic acid	Papadopoulou et al. (2007)

Brisdelli et al. (2013) investigated the effects of six lichen metabolites (diffractaic acid, lobaric acid, usnic acid, vicanicin, variolaric acid, protolichesterinic acid) on reactive oxygen species (ROS) level towards three human cancer cell lines, MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma) and HCT-116 (colon carcinoma). All tested lichen compounds did not exhibit free radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The lichen metabolites did not significantly increase the intracellular ROS level and did not prevent oxidative injury induced by *t*-butyl hydroperoxide in tested cells.

Choudhary et al. (2009) studied the superoxide radical scavenging activity assay. They found that the para depsides lecanoric acid, erythrin and the meta depside sekikaic acid showed exceptionally high percentage of radical scavenging activity in the SOR assay along with the depsidone lobaric acid. The common structural feature in all of the above compounds is two aromatic rings connected by an ester linkage, an ortho to the carbonyl bearing carbon of ring A and an oxygen atom which may act as the electron acceptor from the antibonding orbitals of superoxide radical leading to molecular oxygen. The electron thus obtained could be stabilised due to extended conjugation available in such compounds. In the case of depsidone lobaric acid, the electron accepted by C-2-O could be stabilised by both aromatic rings. The SOR activity of both the depsides lecanoric acid and erythrin was lost on permethylation suggesting that when C-2-O is methylated, the molecule loses its ability to accept electrons. Importantly, the IC_{50} values of the sekikaic acid, lecanoric acid and lobaric acid were lower than the propyl gallate standard.

Atalay et al. (2011) determined the lipid peroxidation inhibition potential for nine lichen compounds in two lipid peroxidation test systems (liposome and emulsion systems). They found that isidiophorin, rhizonaldehyde, rhizonyl alcohol and pulmonarianin retarded lipid peroxidation at in both test systems. However, stictic acid and ergosterol peroxide exhibited antioxidant activity in only the liposome test system. Usnic acid and diffractaic acid were not antioxidants in either system, while stictic acid was not lipid peroxidation inhibitor in the emulsion test system. All compounds, which inhibited lipid peroxidation in both test systems, were also DPPH radical scavengers.

Odabasoglu et al. (2006) investigated gastroprotective effect of usnic acid isolated from *Usnea longissima* in the indomethacin-induced gastric ulcers in

rats. They found that the gastroprotective effect of usnic acid can be attributed to its reducing effect on the oxidative damage. Namely, all tested doses of usnic acid showed a significant increase in the levels of superoxide dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) and a reduction in the lipid peroxidation (LPO) level in tissues.

For some stictic acid derivates, Lohézic-Le Dévéhat et al. (2007) found moderate antiradical activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and very high superoxide anion scavenging activity.

Methanolic extracts and lichen acids (gyrophoric acid, lecanoric acid and umbilicaric acid) obtained from six *Umbilicaria* species were tested for their antioxidant activity (Buçukoglu et al. 2013). The antioxidant ability was measured using a free radical scavenging activity assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The methanolic extracts showed moderate DPPH radical scavenging activity. Among the lichen acids, umbilicaric acid showed the highest antioxidant activity with 68.14 % inhibition.

Jayaprakasha and Rao (2000) extracted with benzene and acetone the lichen *Parmotrema stuppeum* in order to investigate its antioxidant effect. Both the extracts were fractionated on 1 % oxalic acid impregnated silica gel column to obtain methyl orsenillate, orsellinic acid, atranorin and lecanoric acid, respectively. Antioxidant activities of benzene extract, acetone extract and isolated compounds were evaluated in a carotene–linoleate model system. The obtained results showed that the pure compounds and extracts have moderate antioxidant activity.

Four new β-orcinol metabolites, hypotrachynic acid, deoxystictic acid, cryptostictinolide and 8′-methylconstictic acid along with the metabolites 8′-methylstictic acid, 8′-methylmenegazziaic acid, stictic acid, 8′-ethylstictic acid and atranorin that have been previously described, were isolated for the first time from the tissue extracts of the lichen *Hypotrachyna revoluta* (Papadopoulou et al. 2007). The structures of the new metabolites were elucidated on the basis of extensive spectroscopic analyses. Radical scavenging activity of the metabolites isolated in adequate amounts was evaluated using luminol chemiluminescence. The evaluation of the lichen metabolites by the chemiluminescence method showed most of them to possess noteworthy antioxidant activity, with the highest levels being exhibited by compound 8′-methylmenegazziaic acid, which was only seven times less potent than the standard antioxidant Trolox® that was used for comparison reasons. The results showed that compounds 8′-methylmenegazziaic acid and atranorin that posses an additional hydroxyl group on the aromatic ring are the most active ones and the activity is reduced by half when the hydroxyl of C-3 is replaced by an aldehyde moiety. Finally, the scavenging activity of the metabolites possessing an aldehyde group on C-3 seems to be drastically reduced when the methylene of the γ-lactone ring is substituted by a hydroxy or methoxy moiety, as observed in the cases of metabolites deoxystictic acid, stictic acid and 8′-methylstictic acid.

For cuculloquinone, a bisnaphthoquinone of *Flavocetraria cucullata* was found to inactivate DPPH to an 80 % extent, while the BHT that was used as a standard antioxidant was twofold less active (Stepanenko et al. 2002).

Lopes et al. (2008) found that lecanoric acid and some depside isolated from a *Parmotrema tinctorum* were very active DPPH radical scavengers.

In various antioxidant assays, Paudel et al. (2008) used to evaluate the antioxidant capacities of the ramalin isolated from the methanol–water extract of the Antarctic lichen *Ramalina terebrata*. The experimental data showed that ramalin was five times more potent than commercial butylated hydroxyanisole (BHA) in scavenging l-diphenyl-2-picryl-hydazil (DPPH) free radicals, 27 times more potent in scavenging 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid free radicals (ABTS*+)) than the vitamin E analogue, trolox, and 2.5 times more potent than BHT in reducing Fe3+ to Fe2+ ions. Similarly, ramalin was 1.2 times more potent than ascorbic acid in scavenging superoxide radicals and 1.25 times more potent than commercial kojic acid in inhibiting tyrosinase enzyme activity, which ultimately leads to whitening of skin cells. Furthermore, ramalin was assessed to determine its antioxidant activity in vivo. One microgram per millilitre ramalin significantly reduced the released nitric oxide (NO) and 0.125 μg/ml ramalin reduced the produced hydrogen peroxide in lipopolysaccharide (LPS)-stimulated murine macrophage Raw264.7 cells. Considering all the data together, ramalin can be a strong therapeutic candidate for controlling oxidative stress in cells.

Melo et al. (2011) evaluated free radical scavenging activities and antioxidant potential of atranorin using various in vitro assays for scavenging activity against hydroxyl radicals, hydrogen peroxide, superoxide radicals and nitric oxide. Besides, the total reactive antioxidant potential and total antioxidant reactivity indexes and in vitro lipoperoxidation were also evaluated. They found that atranorin exerts differential effects towards reactive species production, enhancing hydrogen peroxide and nitric oxide production and acting as a superoxide scavenger; no activity towards hydroxyl radical production/scavenging was observed. Also, total reactive antioxidant potential and total antioxidant reactivity analysis indicated that atranorin acts as a general antioxidant, although it demonstrated to enhance peroxyl radical-induced lipoperoxidation in vitro.

Atranorin, protolichesterinic acid, usnic acid, 2-hydroxy-4-methoxy-6-propyl benzoic acid, homosekikaic acid, sekikaic acid, benzoic acid, 2,4-dihydroxy-6-propyl and 2,4-dihydroxy-3,6-dimethyl benzoate isolated from the hexane extract from *Ramalina roesleri* were assayed for antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (Sisodia et al. 2013). Maximum DPPH radical scavenging activity was exhibited by sekikaic acid followed by homosekikaic acid.

Verma et al. (2012) found high scavenging of radicals of salazinic acid, sekikaic acid and usnic acid isolated from three terrestrial natural lichen species *Ramalina celastri*, *Ramalina nervulosa* and *Ramalina pacifica*. On the contrary, Brisdelli et al. (2013) investigated the effects of six lichen metabolites (diffractaic acid, lobaric acid, usnic acid, vicanicin, variolaric acid, protolichesterinic acid) on reactive oxygen species (ROS) using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. They found that lichen compounds did not exhibit free radical scavenging activity and did not significantly increase the intracellular ROS level.

Manojlović et al. (2012) investigated antioxidant activities for protocetraric and usnic acids from *Parmelia caperata* lichen and depsidone salazinic acid from

Parmelia saxatilis species. Antioxidant activities of these isolated metabolites were evaluated by free radical scavenging, superoxide anion radical scavenging and reducing power. As a result of the study, usnic acid had stronger antioxidant activity than salazinic acid and protocetraric acid in all used tests.

In the study described by Ranković et al. (2012), lichen compounds, norstictic acid isolated from *Toninia candida* and usnic acid from *Usnea barbata*, exhibited high antioxidant potential in vitro, but it should be noted that the norstictic acid had a larger antioxidant capacity.

Evernia prunastri and *Pseudevernia furfuracea* lichens and their major metabolites evernic acid and physodic acid were screened for their antioxidant effects by Kosanić et al. (2013a) who found varying antioxidant success in free radical scavenging, superoxide anion radical scavenging and reducing power, and physodic acid was found to be the most effective.

Antioxidant activities of major lichen metabolites in *Hypogymnia physodes* lichen (physodic acids, atranorin and usnic acid) were studied by Ranković et al. (2014b). An physodic acid was found to be the most effective antioxidant in free radical and superoxide anion scavenging, as well as in reducing power assays among tested lichen metabolites.

Kosanić et al. (2014a) investigated antioxidant activities of acetone extracts of the lichens *Cladonia furcata*, *Cladonia pyxidata* and *Cladonia rangiferina* and their atranorin and fumarprotocetraric acid constituents. Antioxidant activities were evaluated by free radical scavenging, superoxide anion radical scavenging and reducing power. As a result of the study, isolated components had larger antioxidant activity than tested extracts. Atranorin had the largest free radical scavenging activity with IC_{50} values 131.48 μg/ml. Moreover, atranorin had the most effective reducing power and superoxide anion radical scavenging.

This work reveals that the lichens can be an interesting source of new antioxidative agents, with a potential use in different fields (food, cosmetics, pharmaceutical). Further work should be focused on the isolation of new pure compounds from lichens and investigation of their antioxidant activity.

References

Aligiannis N, Mitaku S, Tsitsa-Tsardis E et al (2003) Methanolic extract of *Verbascum macrurum* as a source of natural preservatives against oxidative rancidity. J Agric Food Chem 51: 7308–7312

Apak R, Güçlü K, Özyürek M et al (2004) A novel total antioxidant capacity index for dietary polyphenols, vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: the CUPRAC method. J Agric Food Chem 52:7970–7981

Apak R, Gorinstein S, Bohm V et al (2013) Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC technical report). Pure Appl Chem 85:957–998

Aslan A, Güllüce M, Sökmen M et al (2006) Antioxidant and antimicrobial properties of the lichens *Cladonia foliacea*, *Dermatocarpon miniatum*, *Evernia divaricata*, *Evernia prunastri and Neofuscella pulla*. Pharm Biol 44:247–252

Atalay F, Halici MB, Mavi AA et al (2011) Antioxidant phenolics from *Lobaria pulmonaria* (L.) Hoffm. and *Usnea longissima* Ach. Lichen species. Turk J Chem 35:647–661

Behera BC, Verma N, Sonone A et al (2005) Antimicrobial activity of Various solvent extracts of Lichen Usnea ghattensis. Agarkar Research Institute, Pune, India. Biotechnol Lett 27:991–995

Benzie IFF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Benzie IFF, Szeto YT (1999) Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. J Agric Food Chem 47:633–636

Betteridge DJ (2000) What is oxidative stress? Metabolism 49:3–8

Bhattarai HD, Paude B, Lee HS et al (2008a) Antioxidant activity of *Sanionia uncinata*, a polar mass species from King George Island, Antarctica. Phytother Res 22:1635–1639

Bhattarai HD, Paudel B, Hong SG et al (2008b) Thin layer chromatography analysis of antioxidant constituents of lichens from Antarctica. J Nat Med 62:481–484

Bondet V, Brand-Williams W, Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Food Sci Technol 30:609–615

Bors W, Heller W, Michel C et al (1990) Flavonoids as antioxidants*:* determination of radical-scavenging efficiencies. Methods Enzymol 186:343–355

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 28:25–30

Brisdelli F, Perilli M, Sellitri D et al (2013) Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an *in vitro* study. Phytother Res 27:431–437

Buçukoglu TZ, Albayrak S, Halici MG et al (2013) Antimicrobial and antioxidant activities of extracts and lichen acids obtained from some *Umbilicaria* species from Central Anatolia, Turkey. J Food Process Preserv 37:1103–1110

Choudhary MI, Ali S, Thadhani VM et al (2009) Natural novel antioxidants. Patent application number: 11/838567, publication date 02/19/2009

de Barros Alves GM, de Sousa Maia MB, de Souza FE et al (2014) Expectorant and antioxidant activities of purified fumarprotocetraric acid from Cladonia verticillaris lichen in mice. Pulm Pharmacol Ther 27:139–143

Droge W (2002) Free radicals in the physiological control of cell function (Review). Physiol Rev 82:47–95

Coruh N, Celep AGS, Ozgokce F et al (2007) Antioxidant capacities of Gundelia tournefortii L. extracts and inhibition on glutathione-S-transferase activity. Food Chem 100:1249–1253

Es-Safi N, Khlifi S, Kerhoas L et al (2006) Iridoid glucosides from the aerial parts of *Globularia alypum* L. (*Globulariaceae*). Chem Pharm Bull 54:85–88

Folin O, Ciocalteu V (1927) Tyrosine and tryptophan determination in proteins. J Biol Chem 73: 627–650

Gulcin I, Oktay M, Kufrevioglu OI et al (2002) Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol 79:325–329

Gulcin I, Kufrevioglu OI, Oktay M et al (2004) Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J Ethnopharmacol 90:205–215

Halliwell B (1996) Antioxidants in human health and disease. Annu Rev Nutr 16:33–50

Hidalgo ME, Fernández E, Quilhot W et al (1994) Antioxidant activity of depsides and depsidones. Phytochemistry 37:1585–1587

Huang D, Ou B, Prior RL (2005) The chemistry behind antioxidant capacity assays. J Agric Food Chem 53:1841–1856

Huda-Faujan N, Noriham A, Norrakiah AS et al (2009) Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr J Biotechnol 8:484–489

Jayaprakasha GK, Rao LJ (2000) Phenolic constituents from the lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. Z Naturforsch 55:1018–1022

Kekuda PTR, Vinayaka KS, Kumar PSV et al (2009) Antioxidant and antibacterial activity of lichen extracts, honey and their combination. J Pharm Res 2:1875–1878

Kekuda TRP, Raghavendra HL, Swathi D et al (2012) Antifungal and cytotoxic activity of *Everniastrum cirrhatum* (Fr.) Hale. Chiang Mai J Sci 39:76–83
Kosanić M, Ranković B (2011a) Lichens as possible sources of antioxidants. Pak J Pharm Sci 24: 165–170
Kosanić M, Ranković B (2011b) Antioxidant and antimicrobial properties of some lichens and their constituents. J Med Food 14:1624–1630
Kosanić M, Ranković B, Vukojević J (2011) Antioxidant properties of some lichen species. J Food Sci Technol 48:584–590
Kosanić M, Ranković B, Stanojković T (2012a) Antioxidant, antimicrobial, and anticancer activities of three *Parmelia* species. J Sci Food Agric 92:1909–1916
Kosanić M, Ranković B, Stanojković T (2012b) Antioxidant, antimicrobial and anticancer activity of 3 *Umbilicaria* species. J Food Sci 77:T20–T25
Kosanić M, Manojlović N, Janković S et al (2013a) *Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents. Food Chem Toxicol 53:112–118
Kosanić M, Ranković B, Stanojković T (2013b) Investigation of selected Serbian lichens for antioxidant, antimicrobial and anticancer properties. J Anim Plant Sci 23:1628–1633
Kosanić M, Ranković B, Stanojković T et al (2014a) *Cladonia* lichens and their major metabolites as possible natural antioxidant, antimicrobial and anticancer agents. LWT Food Sci Technol 59:518–525
Kosanić M, Šeklić D, Marković S et al (2014b) Evaluation of antioxidant, antimicrobial and anticancer properties of selected lichens from Serbia. Dig J Nanomater Bios 9:273–287
Kumar SVP, Kekuda TRP, Vinayaka KS et al (2010a) Studies on antibacterial, anthelmintic and antioxidant activities of a macrolichen *Parmotrema pseudotinctorum* (des. Abb.) Hale (parmeliaceae) from Bhadra wildlife sanctuary, Karnataka. Int J Pharm Tech Res 2:1207–1214
Kumar SVP, Prashith Kekuda TR, Vinayaka KS et al (2010b) Anthelmintic and antioxidant efficacy of two macrolichens of *Ramalinaceae*. Pharmacogn J 1:4
Kumar J, Dhar P, Tayade AB et al (2014) Antioxidant capacities, phenolic profile and cytotoxic effects of saxicolous lichens from trans-Himalayan cold desert of Ladakh. PLoS One 9(6): e98696. doi:10.1371/journal.pone.0098696
Lobo V, Patil A, Phatak A et al (2010) Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4:118–126
Lohézic-Le Dévéhat F, Tomasi S, Elix JA et al (2007) Stictic acid derivatives from the lichen *Usnea articulate* and their antioxidant activities. J Nat Prod 70:1218–1220
Lopes TIB, Coelho RG, Yoshida NC et al (2008) Radical scavenging activity of orsellinates. Chem Pharm Bull 56:1551–1554
Lu Y, Foo LY (2001) Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem 75:197–202
Manojlovic NT, Vasiljevic PJ, Gritsanapan W et al (2010) Phytochemical and antioxidant studies of *Laurera benguelensis* growing in Thailand. Biol Res 43:169–176
Manojlović N, Ranković B, Kosanić M et al (2012) Chemical composition of three *Parmelia* lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites. Phytomedicine 19:1166–1172
Mastan A, Sreedevi B, Kumari J et al (2014) Evaluation of the *in vitro* antioxidant and antibacterial activities of secondary metabolites produced from lichens. Asian J Pharm Clin Res 7:193–198
Melo MG, dos Santos JP, Serafini MR et al (2011) Redox properties and cytoprotective actions of atranorin, a lichen secondary metabolite. Toxicol In Vitro 25:462–468
Michalak A (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal. Pol J Environ Stud 15:523–530
Mitrović T, Stamenković S, Cvetković V et al (2011) Antioxidant, antimicrobial and antiproliferative of five lichen species. Int J Mol Sci 12:5428–5448
Moure A, Cruz JM, Franco D et al (2001) Natural antioxidants from residual sources. Food Chem 72:145–171

Naveena BM, Sen AR, Kingsly RP et al (2008) Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. Int J Food Sci Technol 43:1807–1812
Odabasoglu F, Aslan A, Cakir A et al (2004) Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res 18:938–941
Odabasoglu F, Aslan A, Cakir A et al (2005) Antioxidant activity, reducing power phenolic content of some lichen species. Fitoterapia 76:216–219
Odabasoglu F, Cakir A, Suleyman H et al (2006) Gastroprotective and antioxidant effects of usnic acid on indomethacin-induced gastric ulcer in rats. J Ethnopharmacol 103:59–65
Padmalatha Rai S (2012) Antioxidant activity of Alstonia scholarsis extracts containing flavonoids and phenolic compounds. Int J Pharm Pharm Sci 4:424–426
Papadopoulou P, Tzakou O, Vagias C et al (2007) Beta-orcinol metabolites from the lichen *Hypotrachyna revolute*. Molecules 12:997–1005
Paudel B, Bhattarai HD, Lee JS et al (2008) Antioxidant activity of polar lichens from King George Island (Antarctica). Polar Biol 31:605–608
Paudel B, Bhattarai HD, Pandey DP et al (2012) Antioxidant, antibacterial activity and brine shrimp toxicity test of some mountainous lichens from Nepal. Biol Res 45:387–391
Pavithra GM, Vinayaka KS, Rakesh KN et al (2013) Antimicrobial and antioxidant activities of a macrolichen *Usnea pictoides* G. Awasthi (*Parmeliaceae*). J App Pharm Sci 3:154–160
Pokorny J, Yanishlieva N, Gordan M (2001) Antioxidants in food: practical applications. Woodhead, Cambridge, UK
Poornima G, Kekuda PTR, Vinayaka KS (2012) Antioxidant efficacy of Olea dioica Roxb (Oleaceae) leaves. Biomedicine 32:506–510
Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 53:4290–4302
Ramachandra YL, Ashajyothi C, Padmalatha RS (2012) Antioxidant activity of *Alstonia scholaris* extracts containing flavonoids and phenolic compounds. Int J Pharm Pharma Sci 4:424–426
Ranković B, Ranković D, Kosanić M et al (2010a) Antioxidant and antimicrobial properties of the lichen *Anaptychia ciliaris*, *Nephroma parile*, *Ochrolechia tartarea* and *Parmelia centrifuga*. Cent Eur J Biol 5:649–655
Ranković B, Ranković D, Marić D (2010b) Antioxidant and antimicrobial activity of some Lichen species. Microbiology 79:809–815
Ranković B, Kosanić M, Stanojković T (2011) Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata*, *Lecanora atra* and *Lecanora muralis*. BMC Complement Altern Med. doi:10.1186/1472-6882-11-97
Ranković B, Kosanić M, Stanojković T et al (2012) Biological activities of *Toninia candida* and *Usnea barbata* together with their norstictic acid and usnic acid constituents. Int J Mol Sci 13: 14707–14722
Ranković B, Kosanić M, Stanojković T (2014a) *Stereocaulon paschale* lichen as antioxidant, antimicrobial and anticancer agent. Farmacia 62:306–317
Ranković B, Kosanić M, Manojlovic N et al (2014b) Chemical composition of *Hypogymnia physodes* lichen and biological activities of some its major metabolites. Med Chem Res 23: 408–416
Rekha C, Poornima G, Manasa M et al (2012) Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chem Sci Trans 1:303–310
Russo A, Piovano M, Lombardo L et al (2008) Lichen metabolites prevent UV light and nitric oxide-mediated plasmid DNA damage and induce apoptosis in human melanoma cells. Life Sci 83:468–474
Sachindra NM, Airanthi MKWA, Hosokawa M et al (2010) Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds. J Food Sci Technol 47:94–99
Sanchez-Moreno C, Larrauri JA, Saura-Calixto FA (1998) A procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric 76:270–276

Sangameswaran B, Balakrishnan BR, Chumbhale D et al (2009) In vitro antioxidant activity of roots of Thespesia lampas dalz and gips. Pak J Pharm Sci 22:368–372
Sawa T, Nakao M, Akaike T et al (1999) Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effect of vegetables. J Agric Food Chem 47:397–402
Sharma P, Jha AB, Dubey RS et al (2012) Reactive oxygen species, oxidative damage *and* antioxidative defense mechanism *in* plants under stressful conditions. J Bot 2012:1–26
Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299:152–178
Sisodia R, Geol M, Verma S et al (2013) Antibacterial and antioxidant activity of lichen species *Ramalina roesleri*. Nat Prod Res 27:2235–2239
Souri E, Amin G, Farsam H et al (2008) Screening of thirteen medicinal plant extracts for antioxidant activity. Iran J Pharm Res 7:149–154
Squadriato GL, Pelor WA (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. Free Radic Biol Med 25:392–403
Stanly C, Ali DMH, Keng CL et al (2011) Comparative evaluation of antioxidant activity and total phenolic content of selected lichen species from Malaysia. J Pharm Res 4:2824–2827
Stepanenko LS, Krivoshchekova OE, Skirina IF (2002) Functions of phenolic secondary metabolites in lichens from Far East Russia. Symbiosis 32:119–131
Thadhani VM, Choudhary MI, Ali S et al (2011) Antioxidant activity of some lichen metabolites. Nat Prod Res 25:1827–1837
Tilak JC, Adhikari S, Devasagayam TPA (2004) Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin. Redox Rep 9:220–227
Vagi E, Rapavi E, Hadolin M et al (2005) Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. J Agric Food Chem 53:17–21
Verma N, Behera BC, Sonone A et al (2008) Lipid peroxidation and tyrosinase inhibition by lichen symbionts grown *in vitro*. Afr J Biochem Res 2:225–231
Verma N, Behera BC, Joshi A (2012) Studies on nutritional requirement for the culture of lichen Ramalina nervulosa and Ramalina pacifica to enhance the production of antioxidant metabolites. Folia Microbiol 57:107–114
Vivek MN, Kambar Y, Manasa M et al (2014) Radical scavenging and antibacterial activity of three *Parmotrema* species from Western Ghats of Karnataka, India. J App Pharm Sci 4: 086–091
Watson RR (2014) Polyphenols in plants: isolation, purification and extract preparation. Academic, London
Yamamoto Y, Miura Y, Higuchi M et al (1993) Using lichen tissue cultures in modern biology. Bryologists 96:384–393
Yanishlieva NV, Marinova EM, Gordon HM et al (1999) Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 64:59–66
Young I, Woodside J (2001) Antioxidants in health and disease. J Clin Pathol 54:176–186
Yucel O, Odabasoglu F, Gulluce M et al (2007) Antioxidant and antimicrobial properties of a lichen species, Cladonia rangiformis growing in Turkey. Turk J Pharm Sci 4:101–109
Zhang XY, da Chen C, Xiu MH (2009) The novel oxidative stress marker thioredoxin is increased in first-episode schizophrenic patients. Schizophr Res 113:151–157