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ORIGINAL PAPER

Antioxidant activity of polar lichens from King George Island (Antarctica)

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Abstract Antioxidant agents prevent reactive oxygen species, which can cause degenerative diseases. Natural antioxidants are preferred over many synthetic antioxidants, which can be toxic, for therapeutic applications. Five lichen species were collected from King George Island, Antarctica. Antioxidant activities as assessed by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical and $ABTS^{\bullet+}$ [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] radical scavenging capacities were determined and compared with those of commercial standards BHA (butylated hydroxyanisole) and trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid]. The results indicated that two lichens exhibited comparatively high antioxidant activities with the remaining three exhibiting less activity. The antioxidant activity was concentration-dependent. When compared, the antioxidant activity of crude extracts from polar lichens to previously published data for tropical and temperate lichen species, we concluded that lichens of Antarctic origin may be the potent sources of strong antioxidant agents. Such species should be explored as novel sources of effective antioxidant metabolites.

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Introduction

Oxidation reaction produces free radicals that promote damaging reactions for many cellular components. Reactive oxygen species (ROS) and oxygen radicals damage cells thereby enhancing a number of degenerative diseases such as premature aging, inflammation, deoxygenation of ischemic tissues, atherosclerosis and cancer (Halliwell and Gutteridge 1990). Free radicals attack unsaturated fatty acids of cell membranes resulting in lipid peroxidation, decrease in membrane fluidity and damage to membrane proteins (Dean and Davies 1993). Oxidative stress is associated with many environmental factors and aging, but some oxidative pathologies can be treated with antioxidant agents (Totour 1990). Antioxidant agents terminate oxidative chain reactions by removing free radical intermediates, thereby preventing oxidation of cellular components. Several reports of strong synthetic antioxidant propoerties have been published in past years (Funasaka et al. 1999; Shimizu et al. 2001). Because of the high carcinogenicity of synthetic antioxidants (Grice 1986; Wichi 1988), developing effective natural antioxidants may have significant implications for human health.

Lichens are non-flowering living things that are composed of an alga (phycobiont) and a fungus (mycobiont), living symbiotically. Because of a lack of an easy method for industrial scale lichen culture, and difficulties in collecting wild samples, for research, research into lichen metabolites has advanced slowly. In the past decade some investigations into lichen culture, production, biochemical analysis and metabolites have been described (Crittenden and Porter 1991; Yamamoto et al. 1998; Behera et al. 2006). Several bioactive secondary metabolites, especially antimicrobial and antioxidant compounds have been observed in lichens from the wild, and in cultured species

of tropical origin (Boustie and Grube 2005; Behera et al. 2007).

Various lichen metabolites from several chemical class types including: diterpene, usnic acid, triterpene, dibenzofuran, depside, pulvinic acids and xanthones have been described. These exhibit a range of biological activities including: cytotoxic, fungicidal, antimicrobial, herbicidal, anti-inflammatory activities, as reviewed by Dayan and Romagni (2001). A new UV-B absorbing mycosporine was isolated from the lichenized ascomycete *Collema cristatum*, collected from Israel (Torres et al. 2004). Two cytotoxic naphthopyrones, namely euplectin and coneupletin, were obtained from the Australian species *Flavoparmelia euplacta* (Ernst-Russell et al. 2000). Anti-inflammatory phenolic compounds longissiminone A and longissiminone B were isolated from *Usnea longissima* from Pakistan (Choudhary et al. 2005). Papadopoulou et al. (2007) reported ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate)] radical scavenging activities for nine secondary metabolites from the lichen *Hypotrachyna revoluta*, collected from Southern Greece.

There are very few reports (Ansaldo et al. 2000) of antioxidant activities for secondary metabolites for Antarctic and Arctic organisms. In this paper we have collected five lichen species from Antarctica and tested their antioxidant activities in comparison with commercially available antioxidant standards: DPPH (1,1-diphenyl-2-picrylhydrazyl) and a vitamin E analog, trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid].

Materials and methods

Lichen specimens and collection

Five lichen species: L3 (*Stereocaulon alpinum* Laurer) (Stereocaulaceae), L5 (*Ramalina terebrata* Hook and Taylor) (Ramalinaceae), L6 (*Caloplaca* sp.) (Teloschistaceae), L8 (*Lecanora* sp.) (Lecanoraceae) and L17 (*Caloplaca regalis* (Vain.)Zahlbr.) (Teloschistaceae), were collected from the Korean Antarctic Research Station site on King George Island (61°50′–62°15′S and 57°30′–59°01′W), Antarctica. All the species were identified by Dr. Soon Gyu Hong by comparing morphological characteristics with those previously published (Vstedal and Lewis Smith 2001). L6 and L8 were tentatively identified on the basis of morphological characteristics. The identification of L3, L5 and L17 was confirmed by comparing partial sequences of the ITS (internal transcribed spacer) region. GenBank accession number of the ITS regions for L3, L5 and L17 are EU161238, EU161239 and EU161240, respectively. Voucher specimens were deposited in the Polar Lichen Herbarium, Korea Polar Research Institute, KOPRI, Incheon, South Korea.

Lichen extraction

Various dry weights of specimens (Table 1) were separately extracted in methanol-water (90:10 v/v) at room temperature (RT). The solvent was evaporated in vacuum at 45°C and samples were then lyophilized. Crude extracts were stored at −20°C until further use.

Chemicals and reagents

Antioxidant assay kit (Product code CS0790), Butylated hydroxyanisole (BHA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. All other reagents and solvents used during this experiment were analytical grade.

Antioxidant assay

DPPH free-radical scavenging capacity

The free-radical scavenging capacity of the lichen extracts was estimated by a previously described method (Blois 1958). One mL of DPPH solution (0.1 mM of DPPH in methanol) was mixed with 3 ml of various concentrations of the lichen extract. The mixture was incubated at RT for 30 min and the absorbance was measured at 517 nm in a UV-Visible spectrophotometer (SCINCO- AMERICA) to measure the content of DPPH free radical. Reaction mixtures without the lichen extract were used as a negative control and various concentrations of BHA were used as positive controls. Experiments were conducted in triplicate.

$ABTS^{\bullet+}$ scavenging capacity

The $ABTS^{\bullet+}$ scavenging capacity of the lichen extracts was determined by comparison with a commercially available,

Table 1 Crude extract yield from five Antarctic lichens and IC_{50} values for DPPH free radical and $ABTS^{\bullet+}$ scavenging

Test samples			IC_{50} (μg ml^{-1})[a]	
Name	Lichen dry weight (g)	Crude extract yield (g)	DPPH	$ABTS^{+}$
L3	10.3	2.02	409.3 ± 6.1	370.8 ± 38.6
L5	120.3	13.6	302.4 ± 31.7	347.2 ± 22.5
L6	14.2	2.56	241.7 ± 17.6	134.8 ± 3.7
L8	22.1	3.21	242.2 ± 24.3	94.7 ± 1
L17	127.4	20.65	251.7 ± 18.9	279.6 ± 3.14
Trolox	–	–	–	46.35 ± 0.1
BHA	–	–	3.5 ± 0.2	–

[a] (Mean ± SD, $n = 3$); BHA, butylated hydroxyanisole; L3, *Stereocaulon alpinum* Laurer; L5, *Ramalina terebrata* Hook and Taylor; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis* (Vain.) Zahlbr

water soluble vitamin E analog, trolox (6-hydroxy-2,5,7, 8-tetramethylchroman-2-carboxilic acid) as a standard (Rice-Evans and Miller 1994). A standard curve for $ABTS^{\bullet+}$ scavenging capacity was obtained using 0–100 μg ml^{-1} of trolox. The water soluble methanol extract of each lichen was applied to a free radical (cation) generation system where chromogen cations of ABTS (2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) were produced by oxidizing ABTS a with ferryl myoglobin radical. The ferryl myoglobin radical came from the reaction between metmyoglobin and hydrogen peroxide. The ABTS radical was green and could be read spectrophotometrically at 405 nm. The antioxidant activity of trolox suppressed the production of the ABTS radical in a concentration-dependent manner, and the color intensity decreased proportionally. After comparison with the trolox standard, the $ABTS^{\bullet+}$ scavenging capacity of crude lichen extracts was expressed in trolox-equivalent terms.

Results and discussion

We used two electron transfer (ET) based antioxidant assays (DPPH free radical and $ABTS^{+}$ scavenging capacities) to evaluate the antioxidant activities of five lichen species from King George Island. The ET-based assays measured the capacity of an antioxidant to reduce an oxidant that, when reduced, changed color reproducibly (Huang et al. 2005). The degree of color change was correlated with antioxidant potential in lichen extracts.

The DPPH free radical scavenging capacity of the methanol extracts from all five lichen species are presented (Fig. 1). All the tested extracts, and a commercial standard sample (BHA) (figure not shown), exhibited DPPH free radical scavenging capacity in a concentration-dependent manner. However, the rate of scavenging capacity was variable for each extract. The IC_{50} (the 50% inhibition) concentration (Table 1) of each lichen extract was calculated after regression analyses of the observed data. On the basis of the calculated IC_{50} concentration, the DPPH free radical scavenging capacities of lichen extracts were found in following order: L6 ≥ L8 > L17 > L5 > L3. BHA is a strong antioxidant agent and, in the present experiment, the IC_{50} of this compound was observed to be 3.5 ± 0.2 μg ml^{-1}.

Concentration-dependent $ABTS^{\bullet+}$ scavenging capacities of water-soluble extracts from five Antarctic lichen species were observed (Fig. 2). However, the rate of $ABTS^{\bullet+}$ scavenging capacity varried between extracts. The $ABTS^{\bullet+}$ scavenging IC_{50} for each extract was calculated (Table 1). The data showed that the trend of $ABTS^{\bullet+}$ scavenging capacity for the lichen extracts examined was as follows: L8 > L6 > L17 > L5 > L3. The commercial standard, trolox showed an IC_{50} of 46.35 ± 0.1 μg ml^{-1}.

Two ET-based assays were applied to evaluate the antioxidant activities of lichen extracts. However, there was a difference in the solvent system used for different reaction mixtures. In the DPPH free radical assay, the reaction was carried out in methanol, while the $ABTS^{\bullet+}$ scavenging assay was under aqueous conditions. The antioxidant capacities shown in DPPH scavenging assays were due to the presence of methanol soluble antioxidant active agents in the lichen extracts, whereas the antioxidant capacity shown in the $ABTS^{\bullet+}$ assay was due the presence of water-soluble antioxidant agents. Comparisons of the IC_{50}s of each assay revealed that water soluble components of the lichen extracts appeared to be more active than methanol soluble components (except for L5 and L17). In the case of

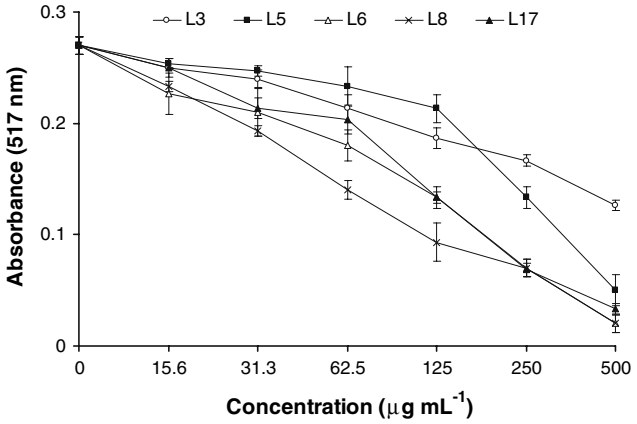

Fig. 1 DPPH free radical scavenging capacities of lichen extracts are shown here. Species are as follows: L3, *Stereocaulon alpinum* Laurer; L5, *Ramalina terebrata* Hook and Taylor; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis* (Vain.) Zahlbr

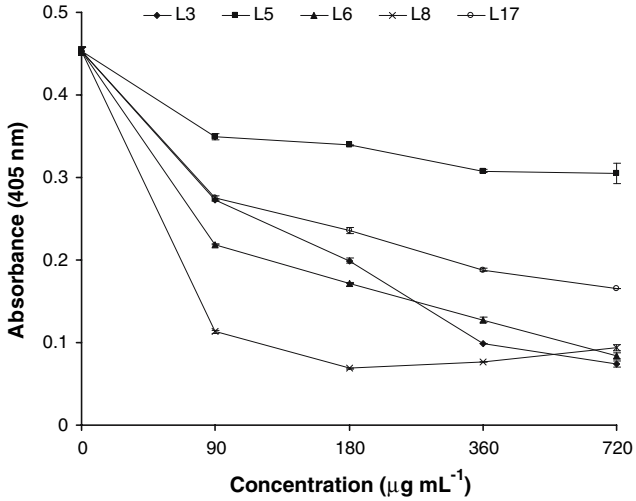

Fig. 2 $ABTS^{\bullet+}$ scavenging capacities of lichen extracts are shown here. Species are as follows: L3, *Stereocaulon alpinum* Laurer; L5, *Ramalina terebrata* Hook and Taylor; L6, *Caloplaca* sp.; L8, *Lecanora* sp.; and L17, *Caloplaca regalis* (Vain.) Zahlbr

L5 and L17, the methanol soluble extract was comparable to the aqueous extract.

The activities of putative antioxidants 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). Lichens were observed to produce several phenolic compounds as secondary metabolites (Nash 1996). Because of the ability of phenolic compounds to scavenge free radicals and reactive oxygen species (such as singlet oxygen, superoxide free radicals and hydroxyl radicals) phenolic compounds have been described as effective antioxidants (Hall and Cuppett 1997). Therefore, it is likely that effective antioxidants are present in lichen extracts. Several lichen species of tropical and temperate origin (Behera et al. 2006) have shown antioxidant activities in higher dose (mg ml^{-1}) level. We found that polar lichens exhibit antioxidant activities at a relatively low-dose level (μg ml^{-1}). Therefore, it can be concluded that polar lichens are a potentially impressive source of strong antioxidant agents when compared to tropical lichens. Therefore, these Antarctic lichen species should be explored further, especially through laboratory culture of the lichen species for mass production. Furthermore, the isolation and characterization of the antioxidant secondary metabolites from the specimens should be explored for therapeutic development.

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