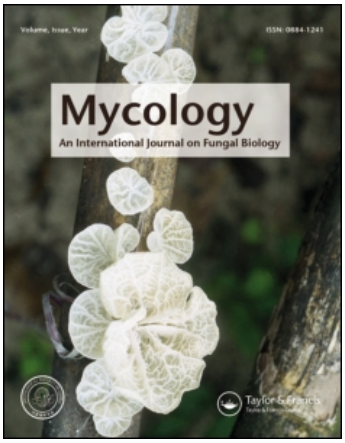

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Antioxidant activity of the mycelium of 21 wild mushroom species

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In this study, the antioxidant activity of mycelia from 21 wild mushrooms – *Agaricus bresadolanus, Auricularia auricula-judae, Chroogomphus rutilus, Fomes fomentarius*, *Ganoderma lucidum, Gloeophyllum trabeum, Gymnopus dryophilus, Infundibulicybe geotropa, Inocybe flocculosa* var. *crocifolia*, *Inocybe catalaunica*, *Lentinula edodes, Lentinus sajor-caju, Lycoperdon excipuliforme, Macrolepiota excoriata, Morchella esculenta* var. *rigida*, *Morchella intermedia*, *Omphalotus olearius*, *Pleurotus djamor, Postia stiptica, Rhizopogon roseolus* and *Stropharia inuncta* – were investigated. Antioxidant properties of ethanol, chloroform and water extracts of these 21 mycelia were studied by two methods: free radical scavenging (DPPH) and the scavenging activity of 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation ($ABTS^{\cdot+}$). Among the 21 mushroom extracts, *Omphalotus olearius* displayed the most potent antioxidant activity. The study has shown that these wild macrofungi have potential as natural antioxidants.

Keywords: antioxidant activity; mycelium; Turkey; wild mushroom

Introduction

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, uncontrolled production of oxygen-derived free radicals results in the onset of many diseases, such as cancer, rheumatoid arthritis and atherosclerosis, as well as in degenerative processes associated with aging (Halliwell and Gutteridge 2003). Almost all organisms are well protected against free radical damage by enzymes, such as superoxide dismutase and catalase, or compounds, such as ascorbic acid, tocopherols and glutathione (Niki et al. 1994). When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur, resulting in diseases and accelerated ageing. However, antioxidant supplements or antioxidant-containing foods may help the human body to reduce oxidative damage (Mau et al. 2001; Gülçin et al. 2002).

Many species of fruits, vegetables, herbs, cereals, sprouts and seeds have been investigated for antioxidant activity over the past decade (Assimopoulou et al. 2004; Elmastas et al. 2005). Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress; the latter being considered a cause of ageing and degenerative diseases (Cazzi et al. 1997). Like plants, mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Turkoglu et al. 2007). Mushrooms are appreciated, not only for texture and flavour but also for their chemical and nutritional properties. Wild mushrooms are traditionally used in many Asian countries in both food and medicine (Sanmee et al. 2003; Isildak et al. 2004). Mushrooms have also been reported as therapeutic foods that are useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. These functional characteristics are mainly due to their chemical composition (Manzi et al. 2001). Wild mushrooms are becoming more and more important in our diet for their nutritional and pharmacological properties (Elmastas et al. 2007). Like the fruiting bodies, mycelia are used as food and food-flavouring materials and also in the formulation of nutraceuticals and functional foods.

Although there are many studies on cultivated and wild mushrooms in the northern hemisphere, there is little information available about antioxidant properties of wild mushrooms collected from different parts of Anatolia. Our objective was to evaluate the antioxidant activities of ethanol, chloroform and water extracts of mycelium of 21 wild mushrooms by free radical scavenging and $ABTS^{\cdot+}$ decolorisation methods.

Material and methods

Mushrooms and growth of mycelia

Mycelia obtained from 21 wild mushroom species (*Agaricus bresadolanus*, *Auricularia auricula-judae*, *Chroogomphus rutilus*, *Fomes fomentarius*, *Ganoderma lucidum*,

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Gloeophyllum trabeum, *Gymnopus dryophilus*, *Infundibulicybe geotropa*, *Inocybe flocculosa* var. *crocifolia*, *Inocybe catalaunica*, *Lentinula edodes*, *Lentinus sajor-caju*, *Lycoperdon excipuliforme*, *Macrolepiota excoriate*, *Morchella esculenta* var. *rigida*, *Morchella intermedia*, *Omphalotus olearius*, *Pleurotus djamor*, *Postia stiptica*, *Rhizopogon roseolus* and *Stropharia inuncta*) collected from different parts of Anatolia were grown at 25°C in submerged liquid cultures. The families and culture collection numbers of these species are given in Table 1. The liquid Hagem medium (pH 6.5) contained malt extract (4.0 g/l), yeast extract (1.0 g/l), glucose (5.0 g/l), KH_2PO_4 (0.5 g/l), $MgSO_4{\cdot}7H_20$ (0.5 g/l), NH_4Cl (0.5 g/l), $FeCl_3$ (1% aqueous solution: 0.5 ml), thiamine (1 mg/ml aqueous solution), distilled water (1 l) (Kalmış and Kalyoncu 2008).

Erlenmeyer flasks of Hagem medium were inoculated with agar plugs (Potato Dextrose Agar – 6 mm diameter) covered with mycelium (Hatvani 2001). After 30 days incubation in the dark, the liquid medium was filtered and the mycelium separated from the liquid.

DPPH radical scavenging activity

The hydrogen atom or electron donation abilities of the mycelial extracts were measured from bleaching of the purple-coloured methanol solution of 1,1-diphenly-2-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Gezer et al. 2006). First, 1000 µl of a 1 mg/ml concentration of the extracts in ethanol were added to 4 ml of a 0.004% methanol solution of DPPH. After a 30-min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition (I) of free radical by DPPH in percent (I (%)) was calculated as follows:

$$I(\%) = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. α-tocopherol (TOC) was used for comparison.

Scavenging activity of $ABTS^+$ radical cation

The scavenging activity of the extracts was estimated using the $ABTS^{\cdot+}$ decolourisation method (Re et al. 1999; Arumagam et al. 2006). ABTS with potassium per sulphate generates blue/green $ABTS^{\cdot+}$. The radical formed shows a maximum absorbance at 734 nm. The antioxidants cause discoloration by transferring a hydrogen atom to the radical cation. In this experiment, 5 ml of 7 mM ABTS and 88 µl of 140 mM $K_2S_2O_8$ were mixed and allowed to complete radical generation for 12–16 h in the dark at room temperature. The stock solution was diluted with ethanol and PBS (pH 7.4) to give an absorbance of 0.75 at 734 nm. Then, 1 ml of the extract was added to 1 ml of diluted stock solution and the absorbance measured at 734 nm, 5 min after the initial mixing, using ethanol as the blank. All determinations were performed in triplicate. The total antioxidant activity (TAA) percentage was calculated by the equation given below:

$$\text{TAA}\ \% = (A_c - A_s/A_c) \times 100$$

Table 1. Families and culture collection numbers of macrofungus species.

No.	Species	Families	Mushroom culture collection number
1	*Omphalotus olearius* (DC.) Singer	Marasmiaceae	MCC-03
2	*Gloeophyllum trabeum* (Pers.) Murrill	Gloeophyllaceae	MCC-05
3	*Inocybe flocculosa* var. *crocifolia* (Berk.) Sacc.	Inocybaceae	MCC-06
4	*Gymnopus dryophilus* (Bull.) Murrill	Marasmiaceae	MCC-09
5	*Infundibulicybe geotropa* (Bull.) Harmaja	Tricholomataceae	MCC-10
6	*Lycoperdon excipuliforme* (Scop.) Pers.	Agaricaceae	MCC-12
7	*Postia stiptica* (Pers.) Jülich	Fomitopsidaceae	MCC-13
8	*Macrolepiota excoriata* (Schaeff.) Wasser	Agaricaceae	MCC-14
9	*Pleurotus djamor* (Rumph. ex Fr.) Boedijn	Pleurotaceae	MCC-15
10	*Inocybe catalaunica* Singer	Inocybaceae	MCC-17
11	*Rhizopogon roseolus* (Corda.) Th. Fr.	Rhizopogonaceae	MCC-18
12	*Fomes fomentarius* (L.) J. Kickx f.	Polyporaceae	MCC-19
13	*Chroogomphus rutilus* (Schaeff.) O.K. Mill.	Gomphidiaceae	MCC-21
14	*Morchella esculenta* var. *rigida* (Krombh.) I.R. Hall	Morchellaceae	MCC-24
15	*Agaricus bresadolanus* Bohus	Agaricaceae	MCC-28
16	*Lentinus sajor-caju* (Fr.) Fr.	Polyporaceae	MCC-29
17	*Morchella intermedia* Boud.	Morchellaceae	MCC-30
18	*Auricularia auricula-judae* (Bull.) Quel.	Auriculariaceae	MCC-47
19	*Ganoderma lucidum* (Curtis) P. Karst.	Ganodermataceae	MCC-52
20	*Lentinula edodes* (Berk.) Pegler	Marasmiaceae	MCC-55
21	*Stropharia inuncta* (Fr.) Quel.	Strophariaceae	MCC-59

A_c: absorbance of stock solution, A_s: absorbance of the extract

Statistical analysis

The data presented are the averages of the results of five replicates with a standard error of less than 5%.

Results and discussion

Extraction yield

Generally, the yields from water extracts were significantly higher than those of ethanol and chloroform extracts (Table 2). The discrepancy in the yields from the water, ethanol and chloroform extracts may be due to the fact that water extracts contained a certain amount of soluble polysaccharides which could be precipitated by ethanol and chloroform (Lee et al. 2007).

Free-radical scavenging activity

The chloroform, ethanol and water extracts of mycelia were subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method. The model of scavenging the stable DPPH radical is widely used to evaluate antioxidant activities over a relatively short time compared to other methods. DPPH is a stable free radical with a characteristic absorption at 517 nm and, as antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging values of mycelia extracts as percentage are shown in Table 3.

Mycelial extracts in ethanol exhibited varying scavenging capacities. Ethanol extracts of *Omphalotus olearius* showed the strongest radical scavenging effect (60.25%) at 1 mg/ml. This activity was followed by water extracts of *Chroogomphus rutilus* (40.84%) and *Rhizopogon roseolus* (35.38%), respectively (Table 3). The lowest scavenging activity was exhibited by *Inocybe catalaunica* (2.42%). However, the scavenging effect for α-tocopherol (TOC) was 91.6% at 0.5 mg/ml, i.e. 1 mg of *Omphalotus olearius* ethanol extract has an equivalent inhibition value of 6.57 µg α-tocopherol. The equivalent TOC inhibition values for all mushroom extracts are shown in Table 3. The scavenging effect of TOC is higher than all mushroom extracts, which has been reported previously (Mau et al. 2004; Elmastas et al. 2007).

Huang (2000) found that methanolic extracts of mycelia of *Antrodia camphorata* and *Agaricus blazei* scavenged 97.1 and 98.8% of DPPH radicals at 5 mg/ml, respectively. At 10 mg/ml, the methanolic extracts of *Agrocybe cylindracea* and *Ganoderma tsugae* mycelia scavenged 91.4 and 95.6% of DPPH radicals, respectively (Tsai 2002). According to Mau et al. (2004) the scavenging effects of *Termitomyces albuminosus*, *Grifola frondosa* and *Morchella esculenta* mycelia at 10 mg/ml were 78.8, 79.4 and 94.1%, respectively. Lee et al. (2008) reported that an ethanolic extract of *Hypsizigus marmoreus* mycelium had a scavenging ability of 75.5% at 5 mg/ml.

The results revealed that ethanolic extracts of the mushrooms were free radical scavengers, acting possibly as primary antioxidant. Ethanolic extracts of wild mushrooms may react with free radicals, which are major initiators in the autoxidation of fat, thereby terminating the chain reaction (Gordon 1990; Frankel 1991).

Table 2. Yields from chloroform, ethanol and water mycelial extracts (%).

No.	Species	Chloroform	Ethanol	Water
1	*Omphalotus olearius*	0.66	7.06	11.58
2	*Gloeophyllum trabeum*	18.60	7.73	15.88
3	*Inocybe flocculosa* var. *crocifolia*	5.64	7.60	19.05
4	*Gymnopus dryophilus*	21.84	15.08	20.90
5	*Infundibulicybe geotropa*	8.39	19.01	19.89
6	*Lycoperdon excipuliforme*	2.82	14.01	24.95
7	*Postia stiptica*	1.82	32.20	71.45
8	*Macrolepiota excoriata*	4.13	6.25	10.15
9	*Pleurotus djamor*	1.26	2.16	13.99
10	*Inocybe catalaunica*	11.83	7.73	17.30
11	*Rhizopogon roseolus*	2.30	6.72	9.41
12	*Fomes fomentarius*	4.29	5.79	13.52
13	*Chroogomphus rutilus*	0.34	5.40	47.21
14	*Morchella esculenta* var. *rigida*	7.97	6.39	5.02
15	*Agaricus bresadolanus*	2.52	4.30	13.47
16	*Lentinus sajor-caju*	3.34	8.40	8.24
17	*Morchella intermedia*	21.47	21.09	27.25
18	*Auricularia auricula-judae*	0.54	21.47	32.90
19	*Ganoderma lucidum*	1.97	4.94	4.26
20	*Lentinula edodes*	13.44	26.94	22.03
21	*Stropharia inuncta*	3.29	6.15	12.36

Table 3. Antioxidant activity of chloroform, ethanol and water mycelial extracts.

Species	Chloroform			Ethanol			Water		
	A	B	C	A	B	C	A	B	C
Omphalotus olearius	47.11	5.55	55.53	60.25	6.57	88.01	22.97	3.68	48.38
Gloeophyllum trabeum	18.27	3.45	–	21.63	3.61	29.12	6.41	2.32	27.99
Inocybe flocculosa var. *crocifolia*	–	–	–	–	–	–	3.45	2.06	–
Gymnopus dryophilus	7.41	2.47	20.58	15.77	3.12	46.43	35.10	4.62	59.11
Infundibulicybe geotropa	5.30	2.31	14.77	12.50	2.86	37.20	12.93	2.90	50.39
Lycoperdon excipuliforme	4.60	2.33	15.32	6.80	2.56	15.93	5.75	2.40	19.63
Postia stiptica	1.82	3.13	–	14.66	3.03	36.14	8.17	3.07	4.97
Macrolepiota excoriata	–	–	–	–	–	15.20	3.40	2.16	–
Pleurotus djamor	3.92	2.20	21.68	–	–	–	9.06	2.60	81.71
Inocybe catalaunica	–	–	–	–	–	20.42	2.42	2.08	76.50
Rhizopogon roseolus	10.75	2.73	20.05	32.22	4.40	83.13	35.38	4.64	71.76
Fomes fomentarius	–	–	–	5.97	2.36	5.22	31.10	4.31	77.19
Chroogomphus rutilus	2.25	2.07	21.10	14.28	3.00	61.47	40.84	5.06	35.88
Morchella esculenta var. *rigida*	16.54	3.21	15.56	27.41	3.72	87.07	15.34	3.09	81.26
Agaricus bresadolanus	12.28	2.85	–	18.89	3.36	64.11	24.70	3.81	75.98
Lentinus sajor-caju	–	–	–	13.65	3.03	9.73	6.40	2.39	68.97
Morchella intermedia	21.15	3.54	27.41	25.20	3.68	24.69	18.45	3.17	61.40
Auricularia auricula-judae	4.14	2.21	–	9.95	2.67	44.06	35.10	4.62	59.10
Ganoderma lucidum	6.83	2.42	12.13	10.75	2.73	22.28	21.51	3.56	70.71
Lentinula edodes	13.44	2.94	38.52	6.20	1.90	43.00	13.66	2.95	28.23
Stropharia inuncta	5.95	2.47	–	8.84	2.32	–	7.63	2.51	26.70

Note: A: % DPPH values; B: alfa-tocopherol equivalent values (µg/ml); C: % ABTS inhibition values; – : no activity

ABTS$^+$ radical cation activity

As can be seen from the Table 3, at a 1 mg/ml concentration, the ethanol extract of *Omphalotus olearius* exhibited the highest radical scavenging activity (88.01%) when reacted with ABTS* radicals. This activity was closely matched by ethanol extracts of *Morchella esculenta* var. *rigida* and *Rhizopogon roseolus* at 87.07 and 83.13%, respectively. *Inocybe flocculosa* var. *crocifolia* showed no antioxidant activity using ABTS* radical cation activity method.

Gursoy et al. (2009) found that a methanolic extract of the fruiting bodies of *Morchella conica* scavenged 78.66% of ABTS* radicals at a 40 µg/ml concentration. At 0.14 mg/ml, methanol extracts of the fruiting bodies of *Boletus edulis* and *Amanita cesarea* scavenged 85.8 and 92.0% of ABTS* radicals, respectively (Ramirez-Anguiano et al. 2007). According to Bruijn et al. (2009), the scavenging effects of an ethanol extract of *Grifolia gargal* was 94.5%.

Conclusions

Antioxidant properties of mushrooms are usually related to low-molecular weight compounds, in particular to the phenolic fractions. Therefore, a wide range of these potentially beneficial phenolic compounds could be natural substrates for oxidative enzymes, such as peroxidases or polyphenol oxidases, which are present in high levels in mushrooms (Gursoy et al. 2009).

On the basis of the results, it is suggested that extracts of the mushroom species evaluated here could be of use as an easily accessible source of natural antioxidants. However, at present, the active components in the extracts, responsible for the observed antioxidant activity, are unknown. Further work is necessary on the isolation and purification of the active components from crude extracts of mushrooms to ascertain their mode of action. To the best for our knowledge, this is the first report of the antioxidant activity of these Turkish mushroom species.

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