Indian Journal of Natural Products and Resources
Vol. 10(4), December 2019, pp 266-271

Antioxidant, oxidant potentials and element content of edible wild mushroom *Helvella leucopus*

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Received 24 April 2019; Revised 19 December 2019

This study aims to determine the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), DPPH free radical scavenging activity and element levels of the mushroom *Helvella leucopus* Pers. Ethanol, methanol and dichloromethane extracts of the mushroom samples were obtained using a Soxhlet device. TAS, TOS and OSI values were determined using Rel Assay kits. Cr, Cu, Mn, Fe, Ni, Cd, Pb, and Zn contents were determined using an atomic absorption spectrophotometer. Consequently, this study has demonstrated that *H. leucopus* has antioxidant potential. As a result of the studies, TAS value of the mushroom was found as 2.181±0.069 mmol/L, TOS value was 14.389±0.170 μmol/L and OSI value was 0.661±0.022. DPPH free radical activity was determined as EtOH extract 49.80±0.71, MeOH extract 44.98±2.18 and DCM extract 23.68±1.37 in 1 mg/mL extract concentration of mushroom. In addition, it was found that the mushroom contains high levels of Pb and Mn. In conclusion, it was found that *H. leucopus* could be used as a natural agent in pharmacological designs due to its antioxidant activity.

Keywords: Antioxidant, Edible mushroom, Heavy metals, *Helvella leucopus*, Medicinal mushroom.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61K 36/06, A61P 39/00, A61P 39/06

Introductıon

Antioxidants reduce the negative effects of free radicals in living organisms. There are two major antioxidant groups, i.e. enzymatic and non-enzymatic antioxidants, in the cells of living organisms[1]. Enzymatic antioxidants constitute the primary and secondary defence systems. The primary defence system consists of important enzymes that inhibit the formation of and neutralize reactive oxygen species (ROS)[2]. The secondary defence system consists of enzymes that do not directly neutralize free radicals but support the primary defence system[3]. The non-enzymatic antioxidant group contains several subgroups such as vitamins (A, E, C), enzyme cofactors (Q10), minerals (zinc and selenium), peptides (glutathione), phenolic acids and nitrogen compounds (uric acid)[4]. Enzymatic and non-enzymatic antioxidants have considerably important roles in preserving the delicate balance between them and ROS molecules[3]. Oxidative stress occurs as a result of the imbalance between ROS molecules and antioxidants, which may lead to severe health problems such as cancer, cardiovascular and neurodegenerative diseases and premature ageing[3]. The antioxidants are important members of the defence system that prevent the formation of oxidative stress or reduce the effects[5]. Antioxidant supplements can be consumed within the diet when antioxidant compounds produced by living organisms fail to suffice[4].

There are many natural sources with supplementary antioxidant properties that can be consumed within the diet. It is thought that there are 53000 to 110000 macrofungi species in nature[5]. However, despite the abundance of macrofungi species, nearly 2000 species are considered safe for human consumption. In addition, it was reported that approximately 700 fungus species have therapeutic properties[6]. Mushrooms are important sources of nutrition as they are easy to digest, have a unique taste and aroma in addition to their high protein content. Mushrooms are consumed within the diet but they also draw attention due to their important antioxidant properties. There are many fungus species with high antioxidant activity due to the secondary metabolites produced by the mushrooms[7].

Some previous studies of antioxidant activities conducted on different mushrooms by various

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researchers are shown in Table 1. As shown in Table 1, many different wild mushrooms have antioxidant potentials. Within this scope, this study aims to determine the total antioxidant capacity, total oxidant capacity, oxidative stress index, DPPH free radical scavenging activity and Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn contents of the wild edible mushroom *Helvella leucopus* Pers. As a result of this study, it will be possible to determine the natural pharmacological agent potential of *H. leucopus* and the benefits of consuming this mushroom.

Table 1 — Some wild mushrooms with antioxidant properties

Mushroom species	Country	Extraction	References
Agaricus arvensis	Mongolia	Fruit bodies extracts (Ethanol)	8
A. bisporus	Iran, Romania, China	Fruit bodies extracts (methanol, ethanol, aqueous)	9-11
A. blazei	Romania, Brazil	Fruit bodies extracts (Aqueous)	11,12
A. devoniensis, A. gennadii	Iran	Fruit bodies extracts (methanol)	9
A. sylvaticus	Brazil, Mongolia	Fruit bodies extracts (Ether, ethanol, aqueous)	8,13
Aleurodiscus vitellinus, Cortinarius magellanicus, Cyttaria hariotii,Fistulina antarctica, F. endoxantha,Grifola gargal,Hydropus dusenii, Lepista nuda, Ramaria patagonica	Argentina	Fruit bodies extracts (Methanol)	14
Armilaria mellea, Lycoperdon saccatum	Croatia	Fruit bodies extracts (Ethanol)	15
Astraeus hygrometricus, Calvatia gigantea, Morchella esculenta	Pakistan	Fruit bodies extracts (Methanol)	16
Auricularia auricular-judae	Tanzania, Thailand	Fruit bodies extracts (Ethanol, water, diethylether)	17,18
Boletus aestivalis,Leccinum carpini	Serbia	Fruit bodies extracts (Acetone, methanol)	19
B. edulis	Serbia, Poland	Fruit bodies extracts (Acetone, methanol)	19,20
Cantharellus cibarius	India, Turkey	Fruit bodies extracts (Methanol)	21,22
Fomitopsis pinicola	Turkey	Fruit bodies extracts (Ethanol)	23
Lactarius deliciosus	Portugal	Fruit bodies extracts (Ethanol, methanol)	24,25
L. salmonicolor	Greece	Fruit bodies extracts (Ethanol, dichloromethane)	26
Lentinus edodes	Thailand, Poland	Fruit bodies extracts (Ethanol, methanol, water, diethylether)	18, 21
L. sajor-caju, L. squarrosulus,Panus conchatus, Polyporus tenuiculus	Tanzania	Fruit bodies extracts (Ethanol)	17
Lycoperdon perlatum	India, Serbia	Fruit bodies extracts (Methanol, ethanol, water, hexane)	21, 27
Macrolepiota dolichaula, M. rhacodes	India	Fruit bodies extracts (Methanol)	28
M. mastoidea, Sarcodon imbricatus	Portugal	Fruit bodies extracts (Methanol)	24
M. procera	India, Portugal, Tanzania, Turkey	Fruit bodies extracts (Methanol, ethanol)	17, 22, 24, 28
Clavaria vermiculris, Marasmius oreades, Pleurotus pulmonarius, Ramaria formosa	India	Fruit bodies extracts (Methanol)	21
Pleurotus eous	Thailand, India	Fruit bodies extracts (Methanol, ethanol, water, diethylether)	18, 29
P. ostreatus	Mexico, India	Fruit bodies extracts (Aqueous, methanol)	30,31
P. eryngii	Iran, India	Fruit bodies extracts (methanol)	9, 31
P. sajor-caju	Thailand, India	Fruit bodies extracts (ethanol, water, diethylether)	18, 31
Volvariella volvacea	Thailand	Fruit bodies extracts (Ethanol, water, diethylether)	18
Xerocomus badius	Poland	Fruit bodies extracts (Methanol)	20

Materials and Methods

Laboratory works

H. leucopus samples were collected during routine site studies in Gaziantep in 2018. Mushroom samples were identified[32-34] and then dried in an incubator at 40 ^{o}C. After the drying process, mushroom samples were pulverized in a mechanical grinder. This was followed by ethanol (EtOH), methanol (MeOH) and dichloromethane (DCM) extraction for nearly 6 hours at 50 ^{o}C in a Soxhlet apparatus. EtOH, MeOH, and DCM were purchased from Merck (Darmstadt, Germany). They were stored at the Ankara University, Fungarium Laboratory of Department of Biology.

TAS, TOS and OSI assays

TAS, TOS and OSI values of mushroom extracts were determined using Rel Assay brand commercial kits (Rel Assay Diagnostics Kits, Turkey). Trolox was used as a calibrator in determining the TAS value and the results were expressed in mmol Trolox equiv./L[35]. Hydrogen peroxide (H_2O_2) was used as a calibrator in determining the TOS value and the results were expressed in μmol H_2O_2 equiv./L[36]. The OSI value was calculated from the percent values of TAS and TOS after converting their units to the same unit[36]. Analyses were carried out with 5 replicates.

$$\text{OSI (AU)} = \frac{\text{TOS, μmoL } H_2O_2 \text{ equiv./L}}{\text{TAS, mmoL Trolox equiv./L X 10}}$$

DPPH free radical scavenging activity assay

The free radical scavenging activity of the mushroom extract was determined using 1-diphenyl-2-picrylhydrazyl (DPPH). Stock solutions containing 1 mg/mL mushroom extract were obtained using DMSO (Merck/Darmstadt, Germany). About 50 μL of the solution was added to 160 μL 0.039% DPPH. Incubation was carried out for 30 minutes in a dark environment at room temperature, and an absorbance value at 517 nm was obtained. The procedures were repeated separately for each concentration[37]. In addition, ascorbic acid was used as reference antioxidants.

DPPH free radical scavenging percentages were calculated according to the following formula:

$$\text{Scavenging activity (\%)} = [(A_{Blank}-A_{Sample})/(A_{Blank})] \text{ x100}$$

Determination of element contents

In order to determine the Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn content of the mushroom, samples were dried at 80 ^{o}C until constant weighing. 0.5 g of the samples was mineralized in 9 mL HNO_3 + 1 mL H_2O_2 mixture in a microwave solubilization device (Milestone Ethos Easy). Then, the element contents of the mushrooms were determined using an atomic absorption spectrophotometer (Agilent 240FS AA).

Results and Discussion

Antioxidant activity

In biological systems, ROS can damage DNA and lead to lipid and protein oxidation in cells. The antioxidant system within living organisms can suppress these radicals or reduce their effect by preserving the balance between oxidation and antioxidants. On the other hand, excessive oxidation that can occur due to environmental and inherent effects that can disrupt the antioxidant-oxidant balance. Some chronic and degenerative diseases are seen as a result of this imbalance. In such cases, consumption of exogenous antioxidants can prevent the initiation of an oxidative chain reaction or the reproduction of oxidants, thereby reducing the damage caused by oxidative stress. It is known that food products play an important role in preventing the consequences of free radical activity within an organism. It is thought that consuming sufficient amounts of antioxidants within the diet induces immunological processes and suitably increases the defence capabilities of the cells[38].

The antioxidant potential, oxidant potential, oxidative stress index and DPPH free radical scavenging activity of an edible mushroom, i.e. *H. leucopus*, were investigated in this study. As a result of the studies, TAS, TOS and OSI values EtOH extract of *H. leucopus* were determined to be 2.181±0.069 mmol/L (Troloxequiv./L X 10), 14.389±0.170 μmol/L (H_2O_2 equiv./L) and 0.661 ±0.022, respectively. There is no previous study investigating the TAS, TOS and OSI values of *H. leucopus.* However, according to the studies conducted on natural edible mushroom species collected from different regions, TAS, TOS and OSI values of *Gyrodon lividus* were 2.077 mmol/L, 13.465 μmol/L and 0.651, respectively[39]. Moreover, TAS, TOS and OSI values were reported to be 4.325 mmol/L, 21.109 μmol/L and 0.488, respectively for *Cyclocybe cylindracea*[40]. TAS, TOS and OSI values were reported to be 1.010 mmol/L, 23.910 μmol/L and 2.367, respectively for *Auricularia auricula*[41]. TAS value is an indicator of a system that reflects the entirety of the non-enzymatic and enzymatic molecules

potentially produced and stored by mushrooms. In comparison to these studies, *H. leucopus*, i.e. the mushroom used in our study, was found to have lower antioxidant compound production potential compared to *C. cylindracea*. On the other hand, it was observed that *H. leucopus* has higher antioxidant compound production capacity compared to *G. lividus* and *A. auricula*. This difference between the TAS values of different mushrooms may stem from the synthesis and release of the secondary metabolites produced by mushrooms as a response of their defence mechanism to internal and external factors, type and amount of these secondary metabolites, differences between the levels of antioxidant vitamins and changes in enzymatic/non-enzymatic antioxidant molecule levels.

Considering the TOS value, *H. leucopus* was found to have lower TOS values compared to *C. cylindracea* and *A. auricula*, but higher TOS values compared to *G. lividus*. In addition, it was observed that *H. leucopus* exhibits lower suppression of oxidant compounds by the antioxidant compounds produced by the mushroom as compared to *C. cylindracea* and *G. lividus*, but higher suppression in comparison to *A. auricula*. The main reason why mushrooms have different TOS values is their oxidant compound production and accumulation capacity that differs due to the differences in metabolic processes as a result of collecting the mushrooms from different regions and differences between mushroom species[42]. It is recommended to consume mushrooms or any natural product with high TOS values with caution when they are collected from these regions. Moreover, considering the OSI values obtained in this current study, oxidative stress induced by oxidant molecules could be eliminated and prevented by TAS that encompasses the entire enzymatic and non-enzymatic systems and consequently, the obtained OSI values were low.

DPPH radical scavenging activity of EtOH, MeOH and DCM extracts of *H. leucopus* were also determined, and inhibition (%) values at 1 mg/mL concentration are shown in Table 2.

According to Table 1, EtOH extracts of the mushroom have higher DPPH activity. The lowest activity was observed in DCM extracts. Moreover, all extracts exhibited lower activity compared to the control, i.e. ascorbic acid. Previous studies employed MeOH extracts of *H. leucopus* and reported that DPPH activity thereof at 20 mg/mL concentration was 92.49[43]. In this current study, a 1 mg/mL extract was used and lower inhibition values were obtained. It is thought that the difference between these values stems from the concentration used and differences between the regions from where the mushrooms were collected. Consequently, it was determined that the mushroom can be used as a natural antioxidant.

Element contents

Mushrooms store different levels of elements depending on the region where they have grown and the contents of their substrate. These elements can be transferred to the human body when mushrooms are consumed. Mushrooms that contain high levels of heavy metals can be toxic and negatively affect human health[44]. In this study, the levels of Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn levels in *H. leucopus* were determined. The obtained findings are shown in Table 3 in terms of dry weight in mg/kg.

Previous studies have investigated the elemental contents in *H. leucopus*, wherein Cr content was reported as 2.30, Cu content as 31.0, Mn content as 11.0, Fe content as 242.0, Ni content as 3.0, Cd content as 6.06, Pb content as 1.0 and Zn content as 354.0 mg/kg[44]. In comparison to this study, *H. leucopus* used in our study was collected from Antalya and had higher Cr, Mn, Fe and Pb contents. On the other hand, Cu, Ni, Cd and Zn contents were lower. Such different element levels are due to the differences between regions from which the mushrooms were collected. In addition, the lowest and highest element contents determined in the element studies conducted on natural mushrooms were as follows according to the literature: Cr 10. 7-42.7, Cu 71.0-95.0, Mn 18.1-103.0, Fe 14.6-835.0, Ni 1.18-5.14, Cd 2.71-7.50, Pb 2.86-6.88 and Zn

Table 2 — DPPH scavenging activity of various extracts from *H. Leucopus* andAscorbic acid

Mushroom and control (1 mg/mL)	EtOH	MeOH	DCM
H. leucopus	49.80±0.71	44.98±2.18	23.68±1.37
Ascorbic acid	93.86±0.87	93.86±0.87	93.86±0.87

Values are presented as mean±S.D
n=6 (Experiments were made as 5 parallel)

Table 3 — Element concentration

	Element Contents (mg/kg)	Literature ranges (mg/kg)[46-48]
Cr	12.03±0.55	10.70-42.70
Cu	10.35±1.42	71.00-95.00
Mn	168.08±2.99	18.10-103.00
Fe	576.12±4.08	14.60-835.00
Ni	2.09±0.07	1.18-5.14
Cd	0.88±0.10	2.71-7.50
Pb	17.19±0.69	2.86-6.88
Zn	83.36±1.01	29.80-158.00

Values are presented as mean±S.D
n=3 (Experiments were made as 3 parallel)

29.8-158.0 mg/kg[45-47]. A comparison of the element levels obtained in this current study with the literature revealed that Cr, Fe, Ni and Zn contents were within the ranges specified in the literature. Cu and Cd levels were lower, whereas Mn and Pb contents were higher than the ranges specified in the literature. It has been reported that 714 mg/kg of lead acetate (i.e., about 450 mg/kg of lead) is the lethal oral dose[48]. Consequently, it is seen that the element levels determined in this study are normal. Furthermore, it is recommended to consume mushrooms in limited amounts to prevent the problems originating from these elements, in case of overconsumption.

Conclusion

The antioxidant and oxidant capacity and element contents of the wild edible mushroom *H. leucopus* were determined in this study. Consequently, this study has demonstrated that *H. leucopus* can be a natural source of antioxidants. Moreover, it was seen that the element levels in *H. leucopus* as found in this study were within the ranges specified in the literature in general. In addition to the edible properties of the mushroom is thought to have medicinal potential. In this context, it can be used as an antioxidant source in pharmacological designs.

References

1 Selamoglu Z, Akgul Hand Dogan H, Environmental effects on biologic activities of pollen samples obtained from different phytogeographical regions in Turkey, *Fresenius Environ Bull*, 2016, **25**(7), 2484-2489.

2 Akata I, Zengin G, Picot C M N and Mahomoodally M F, Enzyme inhibitory and antioxidant properties of six mushroom species from the Agaricaceae family, *J Bot S Afr*, 2019, **120**, 95-99.

3 Bozdogan A, Ulukanlı Z, Bozok F, Eker T, Doğan H H *et al.*, Antioxidant potential of *Lactarius deliciosus* and *Pleurotus ostreatus* from Amanos Mountains, *Adv Life Sci*, 2018, **5**(3), 113-120.

4 Akbarirad H, Ardabili A G, Kazemeini S M and Khaneghah A M, An overview on some of important sources of natural antioxidants, *Int Food Res J*, 2016, **23**(3), 928-933.

5 Mueller G M, Schmit J P, Leacock P R, Buyck B, Cifuentes J, *et al.*, Global diversity and distribution of Macrofungi, *Biodivers Conserv*, 2007, **16**(1), 37–48.

6 Lima A D L, Fortes R C, Novaes M R C Gand Percário S, Poisonous mushrooms: A review of the most common intoxications, *Nutr Hosp*, 2012, **27**(2), 402-408.

7 Chye F Y, Wong J Y and Lee J S, Nutritional quality and antioxidant activity of selected edible wild mushrooms, *Food Sci Technol Int*, 2008, **14**(4), 375-384.

8 Munkhgerel L, Erdenechimeg N, Tselmuungarav B, Amartuvshin B, Bolor T, *et al.*, Chemical composition and biological activities of the *Agaricus* mushrooms, *Mong J Chem*, 2013, **14**, 41-45.

9 Rezaeian S, Saadatmand S, Sattari T N and Mirshamsi A, Antioxidant potency of Iranian newly cultivated wild mushrooms of *Agaricus* and *Pleurotus* species, *Biomed Res*, 2015, **26**(3), 534-542.

10 Kimatu B M, Zhao L, Biao Y, Ma G, Yang W, *et al.*, Antioxidant potential of edible mushroom (*Agaricus bisporus*) protein hydrolysates and their ultrafiltration fractions, *Food Chem*, 2017, **230,** 58-67.

11 Popescu M L, Costea T, Gird C E, Fierăscu I, Balaci T D, *et al.*, Antioxidant activity of romanian *Agaricus blazei* Murrill. And *Agaricus bisporus* JE lange mushrooms, *Farmacia*, 2017, **65**(3), 329-335.

12 Oliveira O M M F, Vellosa J C R, Fernandes A S, Buffa-Filho W, Hakime-Silva R A, *et al.*, Antioxidant activity of *Agaricus blazei*, *Fitoterapia*, 2007, **78**(3), 263-264.

13 Costa J V, Novaes M R C G and Asquieri E R, Chemical and antioxidant potential of *Agaricus sylvaticus* mushroom grown in Brazil, *J Bioanal Biomed*, 2011, **3**(2), 49-54.

14 Toledo C V, Barroetaveña C, Fernandes Â, Barros L and Ferreira I, Chemical and antioxidant properties of wild edible mushrooms from native *Nothofagus* spp. forest, Argentina, *Molecules*, 2016, **21**(9), 1201.

15 Zeković Z, Vidović S and Mujić I, Selenium and Zinc content and radical scavenging capacity of edible mushrooms *Armilaria mellea* and *Lycoperdon saccatum*, *Croat J Food Sci Technol*, 2010, **2**(2), 16-21.

16 Badshah H, Ullah F, Khan M U, Mumtaz A S and Malik R N, Pharmacological activities of selected wild mushrooms in South Waziristan (FATA), Pakistan, *S Afr J Bot*, 2015, **97,** 107-110.

17 Hussein J M, Tibuhwa D D, Mshandete A M and Kivaisi A K, Antioxidant properties of seven wild edible mushrooms from Tanzania, *Afr J Food Sci*, 2015, **9**(9), 471-479.

18 Boonsong S, Klaypradit W and Wilaipun P, Antioxidant activities of extracts from five edible mushrooms using different extractants, *Agri Nat Resour*, 2016, **50**(2), 89-97.

19 Kosanić M, Ranković B and Dašić M, Mushrooms as possible antioxidant and antimicrobial agents, *Iran J Pharm Res*, 2012, **11**(4), 1095-1102.

20 Heleno S A, Ferreira R C, Antonio A L, Queiroz M J R, Barros L, *et al.*, Nutritional value, bioactive compounds and antioxidant properties of three edible mushrooms from Poland, *Food Biosci*, 2015, **11**, 48-55.

21 Ramesh C and Pattar M G, Antimicrobial properties, antioxidant activity and bioactive compounds from six wild

edible mushrooms of western ghats of Karnataka, Indian, *Pharmacogn Res*, 2010, **2**(2), 107-112.

22 Yıldız S, Yilmaz A and Can Z, *In vitro* bioactive properties of some wild mushrooms collected from Kastamonu province, *Kastamonu Uni For Fac J*, 2017, **17**(3), 523-530.

23 Sevindik M, Akgul H, Akata I, Allı H and Selamoglu Z, *Fomitopsis pinicola* in healthful dietary approach and their therapeutic potentials, *Acta Aliment*, 2017, **46**(4), 464-469.

24 Barros L, Baptista P, Correia D M, SáMorais J and Ferreira I C F R, Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms, *J Agric Food Chem*, 2007, **55**(12), 4781-4788.

25 Onbasılı D, Celik G, Katırcıoglu H and Narin I, Antimicrobial, antioxidant activities and chemical composition of *Lactarius deliciosus* (L.) collected from Kastamonu province of Turkey, *Kastamonu Univ For Fac J*, 2015, **15**(1), 98-103.

26 Zavastin D E, Miron A, Gherman S P, Boerescu C M, Breaban I G, *et al.*, Antioxidant activity, total phenolic and metals contents of *Lactarius salmonicolor* (R. Heim & Leclair), *Farmacia*, 2015, **63**(5), 755-759.

27 Novaković A R, Karaman M A, Matavulj M N, Pejin B M, Belović M M, *et al.*, An insight into *in vitro* bioactivity of wild-growing puffball species *Lycoperdon perlatum* (Pers) 1796, *Food Feed Res*, 2015, **42**(1), 51-58.

28 Kumari B and Atri N S, Nutritional and nutraceutical potential of wild edible macro lepiotoid mushrooms of North India, *Int J Pharm Pharm Sci*, 2014, **6**(2), 200-204.

29 Sudha G, Vadivukkarasii S, Shree R B I and Lakshmanan P, Antioxidant activity of various extracts from an edible mushroom *Pleurotus eous*, *Food Sci Biotechnol*, 2012, **21**(3), 661-668.

30 Mishra K K, Pal R S, Arunkumar R, Chandrashekara C, Jain S K, *et al.*, Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials, *Food Chem*, 2013, **138**(2-3), 1557-1563.

31 González-Palma I, Escalona-Buendia H B, Ponce-Alquicira E, Téllez-Téllez M, Gupta V K, *et al.*, Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages, *Front Microbiol*, 2016, **7**, 1099.

32 Abbott S P and Currah R S, The Helvellaceae: Systematic revision and occurrence in northern and northwestern North America, *Mycotaxon*, 1997, **62**, 1–125.

33 Dissing H, The Genus *Helvella* in Europe with special emphasis on the species found in Norden, *Dansk Bot Ark*, 1966, **25**(1), 1-172.

34 Asef M R, The genus *Helvella* in Iran, *Mycol Iran*, 2014, **1**(1), 13-18.

35 Erel O, A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation, *Clin Biochem*, 2004, **37**(4), 277-285.

36 Erel O, A new automated colorimetric method for measuring total oxidant status, *Clin Biochem*, 2005, **38**(12), 1103-1111.

37 Shimada K, Fujikawa K, Yahara K and Nakamura T, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J Agric Food Chem*, 1992, **40**(6), 945-948.

38 Zhang D, Yan S and Song W, Photochemically induced formation of reactive oxygen species (ROS) from effluent organic matter, *Environ Sci Technol*, 2014, **48**(21), 12645-12653.

39 Bal C, A Study on antioxidant properties of *Gyrodon lividus*, *Eurasian J For Sci*, 2018, **6**(2), 40-43.

40 Sevindik M, Akgul H, Bal C and Selamoglu Z, Phenolic contents, oxidant/ antioxidant potential and heavy metal levels in *Cyclocybe cylindracea*, *Indian J Pharm Educ Res*, 2018, **52**(3), 437-441.

41 Akgul H, Sevindik M, Coban C, Allı H and Selamoglu Z, New approaches in traditional and complementary alternative medicine practices: *Auricularia auricula* and *Trametes versicolo*, *J Tradit Med Clin Natur*, 2017, **6**(4), 239.

42 Sevindik M, Akgul H, Bal C, Altuntas D, Korkmaz AI, *et al.*, Oxidative stress and heavy metal levels of *Pholiota limonella* mushroom collected from different regions, *Curr Chem Biol*, 2018, **12**(2), 169-172.

43 Dundar A, Okumus V, Ozdemir S, Celik K S, Boga M, *et al.*, Antioxidant, antimicrobial, cytotoxic and anticholinesterase activities of seven mushroom species with their phenolic acid composition, *J Hortic*, 2015, **2**(4), 1-6.

44 Ouzouni P and Riganakos K, Nutritional value and metal content profile of Greek wild edible fungi, *Acta Aliment*, 2007, **36**(1), 99-110.

45 Svoboda L and Chrastný V, Levels of eight trace elements in edible mushrooms from a rural area, *Food Addit Contam*, 2008, **25**(1), 51-58.

46 Zhu F, Qu L, Fan W, Qiao M, Hao H, *et al.*, Assessment of heavy metals in some wild edible mushrooms collected fromYunnan Province, China, *Envıron Monit Assess,* 2011, **179**(1-4), 191-199.

47 Mallikarjuna S E, Ranjini A, Haware D J, Vijayalakshmi M R, Shashirekha M N, *et al.*, Mineral composition of four edible mushrooms, *J Chem*, 2013, **2013**, 1-5.

48 Takahashi A, Problems of hygiene maintenance for food coming into contact with rubber and plastics products, *Nippon Gomu Kyokaishi*, **48**(9), 537.