J Food Sci Technol (May 2020) 57(5):1866–1876
https://doi.org/10.1007/s13197-019-04221-7

ORIGINAL ARTICLE

Determination of antioxidant activities and chemical composition of sequential fractions of five edible mushrooms from Turkey

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Revised: 4 December 2019 / Accepted: 11 December 2019 / Published online: 17 December 2019

Abstract *Tricholoma scalpturatum*, *Tricholoma populinum*, *Neolentinus cyathiformis*, *Chlorophyllum agaricoides*, and *Lycoperdon utriforme* have been traditionally utilized as food in Turkey for a long time. The present study focused on determining antioxidant activities, total phenolic groups contents (flavonols, hydroxycinnamic acids, proanthocyanidins, and anthocyanins), phenolic compounds and fatty acids of sequential extracts (*n*-hexane, ethyl acetate, chloroform, acetone, ethanol, and pure water) obtained from five wild edible macrofungi species. Ethanol and acetone (or ethyl acetate) were found as the most efficient solvents in terms of antioxidant activities and extraction efficiency. Antioxidant studies showed that *L. utriforme*, *C. agaricoides*, and *T. populinum* exhibited the highest radical scavenging and reducing activities and contained the highest phenolic contents. Chromatographic studies revealed that phenolic acids (protocatechuic, gallic, and chlorogenic acids) and fatty acids (oleic, linoleic, and palmitic acids) were the major contributors of the antioxidant activities of the extracts. The results obtained suggest the utilization of these macrofungi species as significant sources of natural antioxidants.

Keywords Antioxidant · Phenolic compound · Fatty acid · Extraction · Mushroom

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Introduction

Since prehistoric times, mushrooms have been used as significant food sources because of their nutritive, flavour and biologically active ingredients. For instance, *Lentinus edodes* (Shiitake) and *Inonotus obliquus* (Chaga) have been used for centuries in China, Japan, Korea, and Eastern Russia for food and medicinal purposes (Wasser 2002). Besides their traditional medicinal value, mushrooms have been among the basic biological materials for increasing the quality of life because of their nutritional value and biologically active secondary metabolites (Lindequist et al. 2005). Mushrooms contain various physiologically active chemical components including phenolic acids, terpenes, alkaloids, lactones, protein polysaccharide compounds (beta-glucan, lentinan, ganoderan etc.) and enzymes (laccase, glucose oxidase, and peroxidase) (Ajith and Janardhanan 2007; Islam et al. 2016; Sanchez 2017; Bach et al. 2017).

Scientific studies revealed that edible mushrooms exhibited significant in vitro antioxidant, antimicrobial, antiallergic, antiviral, hypoglycaemic, antifungal, antiatherogenic, antidiabetic, antihypertensive, anticholesterol, cardiovascular, hepatoprotective, cytotoxic and enzyme inhibitory activities (Barros et al. 2007; Ferreira et al. 2009; Vaz et al. 2010; Yim et al. 2010; Kozarski et al. 2015; Dundar et al. 2015; Ng and Rosman 2019) and in vivo anticancer (Li et al. 2014a, b), antiobesity (Handayani et al. 2014), antidiabetic (Li et al. 2014a, b), and antihypertensive (Kang et al. 2013) according to their chemical composition.

Antioxidant compounds and/or rich extracts have been used in food industry for a long time. Since many of the synthetic antioxidant additives used in the food industry can lead to toxicity, cancer and neurological degeneration,

the demand of natural antioxidant sources with minimum or less toxicity has been increased (Stone et al. 2003). Phenolic compounds are potent antioxidants, which are able to suppress the formation of free radicals and autoxidation. Natural source-based extracts are generally used for their flavouring characteristics due to their strong H-donating activity, which makes them effective antioxidants. This antioxidant activity is most often associated to the presence of phenolic acids (gallic, protocatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol, carnosic acid, rosmanol, and rosmadial), flavonoids (quercetin, catechin, naringenin, and kaempferol), and volatile oils (eugenol, carvacrol, thymol, and menthol). Some natural pigments (anthocyanin and anthocyanidin) can chelate metals and donate H to oxygen radicals thus slowing oxidation (Brewer 2011). Mushrooms are potential candidates of such antioxidant sources due to the presence of bioactive compounds including polysaccharides, vitamins and minerals, polyphenols and carotenoids (Cheung et al. 2003; Barros et al. 2007; Yim et al. 2010).

Several wild grown macrofungi species are being consumed and sold in Turkey due to the biological diversity and rich flora (Gençcelep et al. 2009). During our regular ethnomycological surveys conducted between 2012 and 2015 (Sezgin et al. 2015), we detected *Tricholoma scalpturatum, Neolentinus cyathiformis, Chlorophyllum agaricoides, Tricholoma populinum,* and *Lycoperdon utriforme* as the most utilized macrofungi species in Eastern Anatolia Region of Turkey. However, there is no or limited data regards to antioxidant capacities and chemical composition of these wild edible mushroom species. Our research group (Sezgin et al. 2018) reported only mineral composition of these species previously. On the other hand, scientific studies mainly focused on one-way antioxidant determination mechanism (principally single electron transfer) and limited extraction methods based on single solvent, which hinder the revealing of the chemical nature and comprehensive antioxidant activities of such natural sources. Therefore, within this study, sequential extracts based on increasing polarity principle using *n*-hexane, chloroform, ethyl acetate, acetone, ethanol, and pure water, respectively in order to obtain both of lipophilic and hydrophilic compounds of five commonly consumed mushroom samples (*Tricholoma scalpturatum, Neolentinus cyathiformis, Chlorophyllum agaricoides, Tricholoma populinum, Lycoperdon utriforme*) were researched. Antioxidant abilities of the 30 extracts were evaluated through hydrogen atom transfer (ORAC method) and single electron transfer (FCR and FRAP methods) mechanisms. Bioactive composition of the extracts was analyzed through reagent based on spectrophotometric (flavonols, hydroxycinnamic acids, proanthocyanidins, and anthocyanins) and chromatographic (HPLC–MS/MS and GC–MS) methods.

Materials and methods

Mushroom materials

Fruiting bodies of mushroom samples (*Tricholoma scalpturatum* (Fr.) Quél. (GPS coordinates 38°34′09.81″N, 43°16′53.23″E), *Tricholoma populinum* J.E. Lange (GPS coordinates 37°17′24.35″N, 44°35′47.56″E), *Neolentinus cyathiformis* (Schaeff.) Della Maggiora & Trassinelli (GPS coordinates 38°17′31.69″N, 43°05′25.12″E), *Chlorophyllum agaricoides* (Czern.) Vellinga (GPS coordinates 37°23′59.2″N, 44°29′49.02″E) and *Lycoperdon utriforme* Bull. (GPS coordinates 37°23′59.92″N, 44°29′49.02″E)) (Fig. 1) were harvested from Eastern Anatolia Region of Turkey, on 5–19 May 2016.

Mushroom materials were isolated in clean polythene bags including dried ice and transferred to the laboratory within a maximum of 3 h after harvest. The identities of mushroom materials were confirmed by Yusuf Uzun, Ph.D. at Mycology Research Fungarium, Science Faculty, Van Yuzuncu Yil University, Turkey and a voucher specimen was stored at the university's fungarium (Fungarium codes: 7484, Acar 481, 7485, Acar 636 and 7486 respectively). The mushroom materials were properly cleaned from dust and contaminants in a manner to minimize the loss of chemical components and left at room temperature in the dark until dry. The dried mushroom materials were subsequently ground for a fine powder and stored at − 20 °C until analyzed.

Chemicals

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich, Inc. (St Louis, MO, USA) and were of analytical or HPLC grade.

Preparation of sequential extracts

The ground mushroom materials were extracted sequentially using a range of solvents with increasing degrees of polarity; *n*-hexane, chloroform, ethyl acetate, acetone, ethanol and pure water respectively. Briefly, the ground mushroom samples were mixed with a 10-fold volume of *n*-hexane (g/ml) shaken for 2 h at room temperature (22 °C) using magnetic stirrer and centrifuged for 20 min at 15,320×*g* (10,000 rpm) at 4 °C (Sorvall RC-5B; DuPont, Wilmington, DE, USA; rotor Beckman JA14 (137 mm) serial no. 02U8152, USA), and the supernatant was collected. The same extraction procedures were applied to the pellet using 10-fold of chloroform, ethyl acetate, acetone (80%), ethanol (80%) and pure water (g/ml) respectively, with the supernatants collected. The obtained fractions from different solvents were evaporated

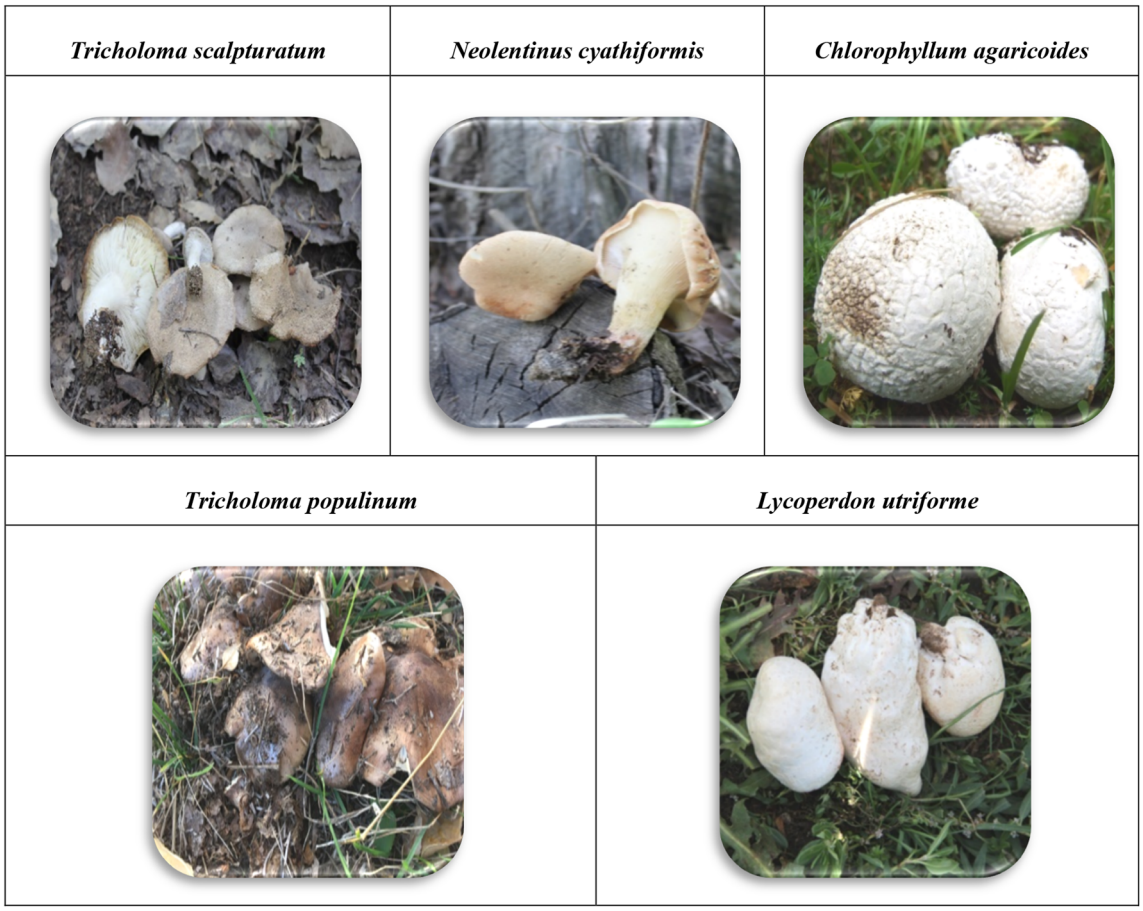

Fig. 1 Edible mushrooms materials

individually under reduced pressure at 37 °C using a rotary evaporator (Rotavapor R-205; Buchi, Switzerland). The derived concentrated fractions were freeze-dried under a vacuum at − 51 °C to obtain fine lyophilized powders.

Antioxidant activity

Antioxidant capacities of the extracts were evaluated via ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacities (ORAC) assays. Total reducing capacity was determined using the FRAP assay as described previously (Dalar et al. 2012) and the reducing capacity of the extract was expressed as μM of iron (Fe^{2+}) per gram of dry weight of lyophilized extract (μM Fe^{2+}/g DW) based on an iron sulphate standard (Fe_2SO_4) curve against a blank control. Oxygen radical scavenging capacity was determined using the ORAC assay according to Dalar et al. (2012) and antioxidant capacity of the sample was expressed as μM of Trolox equivalent per gram of dry weight of lyophilized extract (μM T Eq./g DW) based on a Trolox standard curve. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

Total phenolic content

Total phenolic contents of the extracts were determined by Folin–Ciocalteu method as described previously (Dalar et al. 2012) and the results were expressed as mg gallic acid equivalents per gram of dry weight of the lyophilized extract (mg GAE/g DW), based on Gallic acid standard curve and against a blank control. Standardized Reishi-Shiitake-Maitake extract was used as positive control. The analyses were conducted in triplicate.

Determination of total phenolic groups contents

Total flavonoid, hydroxycinnamic acids, anthocyanin and proanthocyanidin contents of the extracts were determined as described previously (Dalar et al. 2012). The total proanthocyanidin content was determined using DMACA–HCL (4-dimetilamino cinnamaldehyde–hydrochloric acid) protocol the absorbance was measured at 640 nm. The results were expressed as mg of total proanthocyanidin content (Catechin equivalent, CE) per gram of dry weight of the extract (mg CE/g extract), based on Catechin standard curve. The total 4-hydroxycinnamic acid content was

determined using the Glories' method. For 4-hydroxycinnamic acids, the absorbance was measured at 320 nm. The results were expressed as mg of total 4-hydroxycinnamic acids (Caffeic acid equivalent, CAE) per gram of dry weight of the extract (mg CAE/g extract), based on Caffeic acid standard curve against a blank control. For quantification of anthocyanins the absorbance was measured at 520 nm and the results were expressed as mg of total anthocyanin content (Cyanidin 3-glucoside Equivalent, C3-GE) per gram of dry weight of the extract (mg C3GE/g extract), based on Cyanidin 3-glucoside standard curve against a blank control. The total flavonols content was measured at 510 nm. The results were expressed as μg of total flavonols content (Rutin equivalent, RE) per gram of dry weight of the extract (μg RE/g extract), based on Rutin standard curve against a blank control. The analyses were conducted in triplicate.

HPLC–MS/MS analysis

The identification of phenolic compounds was performed on a Quantum triple stage quadrupole (TSQ) mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an auto sampler as previously described (Dalar and Konczak 2013). The quantification of phenolic compounds was performed on HPLC system consisting of two LC-10ADVP pumps, SPD-M10ADVP diode array detector, CTO-1-ADVP column oven, DGU-12A degasser, SIL-10ADVP auto injector, and SCL-10A system controller (Shimadzu Corporation, Kyoto, Japan) and equipped with an Atlantis column (dC18, 4.6 mm i.d × 100 mm length, 5 μm particle size, Waters Associates, Chippendale, NSW, Australia) as described previously (Dalar and Konczak 2013).

GC–MS analysis

Volatile compounds and fatty acids present in extracts were analyzed by gas chromatography mass spectrometry (GC/MS) using a head space solid phase micro extraction and identified by the fragment ions and relative retention indices of their peaks with those of the MS library standards as described previously (Uzun et al. 2017). Volatile compounds prepared by HS-SPME procedure were analyzed on Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) equipped with a flame ionization detector (FID), at the injector temperature of 260 °C in injection mode, splitless. The compounds were identified using the National Institute of Standards and Technology (NIST) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices. Results were expressed as FID response area in relative percentages.

Data analysis

The mean values were calculated based on at least three determinations (n = 3). One-way ANOVA followed by the Bonferroni post hoc test was performed to assess differences between the samples at the level of $p < 0.05$ through GraphPad Prism 5 (GraphPad Software, CA, USA).

Results

Extraction yields

The extraction yields were shown in the Table 1. The highest yields were obtained from water-based extracts. The yields of apolar solvents (hexane, chloroform, and ethyl acetate) were found at very low levels compared to the polar solvents (ethanol and water). These findings indicate the presence of high amounts of hydrophilic compounds in the extracts.

Antioxidant activity

Determination of antioxidant activities of fungal extracts were carried out using complementary methods represent to different mechanisms and were presented in Table 1. The ORAC assay was performed in order to measure the hydrogen atom transfer (HAT) and FRAP was applied for measuring single electron transfer (SET) abilities of the extracts in acidic and alkaline conditions, respectively.

Hexane fractions exhibited very low levels of ORAC activities (18.1–55.6 μmol Trolox eq./g extract). However, there are relatively high increases of ORAC values in chloroform fractions (principally in *L. utriforme, C. agaricoides, and T. populinum*) which were also higher than that of the positive control (Reishi Shitake Maitake Mushroom Extract). The ORAC values of ethyl acetate fractions were showed the pattern of the *L. utriforme* > *T. scalpturatum* > *T. populinum* ≥ *N. Cyathiformis* > *C. agaricoides* (Table 1). The highest ORAC values were determined in acetone, ethanol and water-based fractions (Table 1). *T. populinum* and *L. utriforme* were found as the most oxygen radicals scavenging capable sources among all mushroom samples. Similar to ORAC values hexane fraction showed the weakest antioxidant activities (Table 1). Acetone and ethyl acetate were the most effective solvents in terms of extraction of the antioxidant compounds capable to reduce free radicals (Table 1). However, ethanol was detected as the most proper solvent in terms of antioxidant compounds which were able to

Table 1 Extraction yields, antioxidant activities, total phenolic, flavonol and hydroxycinnamic acid content levels

	n-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Water
Extraction yields (%)						
Tricholoma scalpturatum	0.24 ± 0.0b	0.36 ± 0.0c	0.06 ± 0.0d	0.09 ± 0.0c	6.93 ± 0.42c	20.93 ± 1.64a
Neolentinus cyathiformis	0.27 ± 00a	0.32 ± 0.0d	0.09 ± 0.0c	0.02 ± 0.0d	9.47 ± 0.54a	17.27 ± 1.87b
Chlorophyllum agaricoides	0.27 ± 0.0a	0.16 ± 0.0e	0.15 ± 0.0b	0.14 ± 0.0b	9.33 ± 1.12ab	9.73 ± 1.29d
Tricholoma populinum	0.17 ± 0.0c	0.53 ± 0.1a	0.18 ± 0.0a	0.17 ± 0.0a	8.40 ± 0.74b	15.53 ± 1.41c
Lycoperdon utriforme	0.05 ± 0.0d	0.36 ± 0.0b	0.14 ± 0.0b	0.13 ± 0.0b	5.70 ± 0.56d	10.50 ± 0.92d
Oxygen radical absorbance capacity (ORAC) levels (µmol Trolox Eq./g extract)						
Tricholoma scalpturatum	18.1 ± 0.7d	32.3 ± 0.4d	183.3 ± 3.3b	410.6 ± 10.5d	385.2 ± 6.8d	175.2 ± 1.9e
Neolentinus cyathiformis	18.4 ± 0.3d	25.4 ± 0.5e	142.9 ± 4.4c	425.3 ± 17.9d	709.4 ± 3.2c	332.6 ± 2.3b
Chlorophyllum agaricoides	24.2 ± 0.4c	241.9 ± 3.5b	75.4 ± 5.8d	776.3 ± 2.5b	1117.2 ± 3.7a	309.2 ± 2.0c
Tricholoma populinum	29.7 ± 0.5b	202.8 ± 3.3c	151.9 ± 9.5c	1267.2 ± 12.9a	363.4 ± 4.2e	191.7 ± 4.2d
Lycoperdon utriforme	55.6 ± 2.0a	406.0 ± 1.6a	427.9 ± 8.2a	533.7 ± 3.4c	975.6 ± 2.4b	536.4 ± 3.6a
Positive control (Reishi Shitake Maitake mushroom extract) 218.9 ± 7.3						
Ferric reducing antioxidant power (FRAP) levels (µmol Fe + 2/g extract)						
Tricholoma scalpturatum	8.2 ± 0.2e	111.8 ± 6.3ab	166.6 ± 11.3c	30.6 ± 1.4d	142.5 ± 3.4d	34.2 ± 2.0c
Neolentinus cyathiformis	13.1 ± 1.2d	102.2 ± 8.9b	233.4 ± 11.0b	30.0 ± 2.3d	202.7 ± 4.8c	68.5 ± 3.0b
Chlorophyllum agaricoides	17.7 ± 0.1c	119.7 ± 3.1a	242.4 ± 11.2b	43.8 ± 2.0c	533.8 ± 6.1a	30.0 ± 2.3c
Tricholoma populinum	31.8 ± 0.2a	108.8 ± 2.0ab	484.9 ± 18.2a	50.3 ± 2.4b	45.0 ± 3.1e	14.3 ± 1.2d
Lycoperdon utriforme	20.2 ± 0.4b	101.6 ± 5.2b	228.6 ± 12.9b	132.9 ± 3.4a	365.8 ± 3.0b	213.5 ± 1.4a
Positive control (Reishi Shitake Maitake mushroom extract) 73.5 ± 3.8						
Total phenolic content analysed by Folin–Ciocalteu method (mg Gallic acid Eq./g extract)						
Tricholoma scalpturatum	12.1 ± 0.5bc	12.3 ± 0.5b	48.6 ± 2.6bc	66.1 ± 2.4b	16.7 ± 1.4d	13.2 ± 0.2c
Neolentinus cyathiformis	11.3 ± 0.1c	12.3 ± 0.6b	51.1 ± 3.7b	80.7 ± 8.2b	27.4 ± 1.4c	16.2 ± 0.1b
Chlorophyllum agaricoides	13.4 ± 1.0b	13.3 ± 1.0a	35.5 ± 2.6c	27.3 ± 1.7d	46.5 ± 1.4a	16.3 ± 0.3b
Tricholoma populinum	13.5 ± 0.8b	11.2 ± 0.6b	69.3 ± 7.6a	195.2 ± 7.9a	14.8 ± 0.6d	12.0 ± 0.1d
Lycoperdon utriforme	22.8 ± 0.6a	12.2 ± 1.2ab	55.4 ± 1.0ab	45.8 ± 1.6c	34.9 ± 1.1b	28.1 ± 1.3a
Positive Control (Reishi Shitake Maitake mushroom extract) 6.2 ± 0.4						
Flavonols (µg Rutin Eq./g extract)						
Tricholoma scalpturatum	3.9 ± 0.1b	12.9 ± 04a	3.2 ± 0.1c	0.5 ± 0.1d	8.2 ± 0.1d	7.0 ± 0.5d
Neolentinus cyathiformis	4.0 ± 0.1b	7.9 ± 0.0c	2.7 ± 0.3 cd	1.8 ± 0.1b	21.1 ± 0.1a	14.4 ± 0.2b
Chlorophyllum agaricoides	5.3 ± 0.0a	12.7 ± 0.2a	2.4 ± 0.2d	1.9 ± 0.1b	8.0 ± 0.1d	10.9 ± 0.1c
Tricholoma populinum	3.1 ± 0.2c	11.0 ± 0.1b	7.9 ± 0.2a	5.8 ± 0.2a	14.1 ± 0.2c	4.8 ± 0.3e
Lycoperdon utriforme	2.9 ± 0.2c	12.7 ± 0.2a	4.7 ± 0.2b	0.8 ± 0.1c	17.3 ± 0.2b	22.0 ± 0.1a

Table 1 continued

	n-Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Water
Hydroxycinnamic acids (mg Caffeic acid Eq./g extract)						
Tricholoma scalpturatum	14.6 ± 0.1b	23.2 ± 0.9d	9.9 ± 1.1b	1.3 ± 0.0d	28.5 ± 1.5d	20.8 ± 0.5d
Neolentinus cyathiformis	12.3 ± 0.7c	34.7 ± 1.2c	8.0 ± 0.3b	10.6 ± 1.0b	73.7 ± 1.5a	49.6 ± 1.0b
Chlorophyllum agaricoides	18.5 ± 1.7a	54.2 ± 2.5a	9.0 ± 0.7b	7.6 ± 1.0c	26.8 ± 0.4d	38.3 ± 1.0c
Tricholoma populinum	9.5 ± 0.7d	46.3 ± 1.1b	26.0 ± 2.2a	12.9 ± 1.4a	48.0 ± 0.5c	17.3 ± 0.6e
Lycoperdon utriforme	4.4 ± 0.3e	48.7 ± 3.6ab	11.4 ± 1.5b	1.8 ± 0.2d	55.2 ± 0.4b	77.7 ± 1.1a

Means with different letters in the same column were significantly different at the level ($p < 0.05$); n = 3

reduce free radicals in acidic conditions via FRAP method. Among all samples tested, *L. utriforme* extracts were relatively exhibited the highest antioxidant capacities (Table 1). Antioxidant result obtained within this study were comparable to seven mushroom species including *Pleurotus ostreatus*, *Boletus edulis*, *Tricholoma populinum*, *Helvella queletii*, *Armillaria tabescens*, *Psathyrella candolleana*, and *Helvella leucopus* collected from Turkey (Dundar et al. 2015).

Chemical composition

Total phenolic content

Total phenolic contents of the extracts were analyzed by using Folin–Ciocalteu method and were presented in Table 1. The highest amounts of the total phenolics were found in the extracts of *Lycoperdon utriforme* and *Tricholoma populinum*. The reducing abilities of the extracts in alkaline medium, which measure the total phenolic content, showed that polar solvents (12–195 mg Gallic acid Eq. per gram extract) had higher amounts of phenolic concentration compare to those of the apolar solvents (11–69 mg Gallic acid Eq. per gram extract). These levels of total phenolics were higher than total phenolic concentration of selected mushrooms from Malaysia (Ng and Rosman 2019) and India (Ao and Deb 2019).

Total phenolics groups analysis

Total phenolics groups contents were presented in Table 1. The extracts were found as rich sources of hydroxycinnamic acids, followed by very low levels of flavonol compounds. The levels of proanthocyanidins were found at trace levels, while no any anthocyanins were determined in the extracts (Table 1). Chloroform extracts had the highest levels of flavonols, followed by ethanol and water extracts. Similar pattern was detected for hydroxycinnamic acids contents of the extracts with the superiority of the ethanol or water extracts. The lowest levels of total phenolics groups were found in hexane fractions (Table 1).

HPLC–MS/MS Analysis

Among all extracts evaluated *L. utriforme* extracts showed the relatively higher antioxidant capacities and contained higher amounts of phenolic contents. Therefore, detailed chromatographic analyses were conducted in *L. utriforme* extracts HPLC–MS/MS studies showed that the extracts were principally contain three major compounds (Table 2 and Fig. 2). The maximal absorption was detected at 280 nm, followed by 326 nm. The dominant compounds of the extracts were phenolics acids. The results showed that

polar solvents were more efficient in terms of extraction capability of such compounds. Ethanol extract contained the highest levels, while hexane had the lowest levels of major compounds (Table 2). The neutral mode analysis revealed the non-presence of any polymeric and glycoside/or glucuronide compounds and suggest the aglycons as the major compounds of the extracts. This finding was also confirmed through acid hydrolysis studies, which did not show any differences in liquid chromatography before and after hydrolysis. Based on further MS/MS data (molecular weight, fragment ions and fragmentation pattern) and liquid chromatography studies (co-chromatogram, spectral characteristics and comparison to authentic standards), three major compounds were tentatively identified in the extracts (Table 2 and Fig. 2).

Compound 1 had the highest absorption at 280 nm. A charged molecular ion of 153 m/z at negative mode was detected. According to MS/MS data, comparison of spectral characteristic of the compound and authentic standard and co-chromatogram findings, this compound was tentatively identified as protocatechuic acid. This compound was detected in all extracts with the highest amount in water extract. Second major compound was detected in all extracts except *n*-hexane fraction. This compound had a maximum absorbance at 280 nm and showed typical spectral characteristic of gallic acid. The comparison of the compound with authentic standard and co-chromatogram studies suggested the identical of gallic acid compound. In order to confirm HPLC–DAD results, MS/MS studies were conducted. The SRM mode revealed the presence of 125 m/z fragment ion, which had a charged ion of 169 m/z. The neutral loss mode revealed the non-presence of glycoside and/or glucuronide compounds. According to MS/MS and spectral findings, compound 2 was tentatively characterized as gallic acid. The third compound was only detected at ethyl acetate and ethanol extracts. It was tentatively identified as chlorogenic acid according to spectral data, co-chromatogram studies and MS/MS data, which showed the presence of 191 m/z fragment ion at 353 m/z charged ion at negative mode. No any finding was detected at neutral loss mode.

GC–MS Analysis

The GC–MS analysis of the extracts showed that fatty acids were the dominant compounds of lipophilic composition (Table 3 and Fig. 3). According to molecular weight, fragment ions, and comparison of the compounds with authentic standards three major fatty acids and an alkyl benzene compound were identified. Palmitic acid was the major fatty acid compound of the extracts, followed by stearic, linoleic acids and 2,4-ditertbutylphenol compounds. Oleic acid was found as trace levels in the extracts.

Discussion

Mushrooms have been commonly used as significant food source because of their specific taste, low calorie and sodium contents, high content of vitamins, minerals, phenolics antioxidative compounds. Therefore, it is important to recognize edible mushrooms and elucidate their disease preventive or therapeutic potentials and physiological active ingredients. Extraction of physiologically active chemical compounds from natural sources, including fungi, depends on many factors such as method of extraction, solvent, temperature, pH, ratio, purification process etc. Bioactive substances in natural sources consist of a variety of chemical contents roughly lipophilic and hydrophilic such as terpenes, phenolics, alkaloids, peptides, polysaccharides that have different solubility, chemical structure and properties. The use of various solvents having different polarity in extraction processes will significantly contribute to the comprehensive extraction of chemical compounds

Table 2 Phenolic composition of *Lycoperdon utriforme* sequential extracts

Phenolic compound (mg/g extract)	Protocatechuic acid	Gallic acid	Chlorogenic acid
MS/MS			
$[M + 1]^+/[M - 1]^-$	−/153	−/169	−/353
Fragments (m/z) (±)	−/−	−/125	−/191
Sequential extractions			
n-Hexane	6.3 ± 0.1c	ND	ND
Chloroform	7.1 ± 0.1b	38.9 ± 1.2a	T
Ethyl acetate	6.0 ± 0.1c	8.9 ± 0.4b	11.7 ± 0.2b
Acetone	7.1 ± 0.1b	7.1 ± 0.3c	T
Ethanol	5.2 ± 0.1d	5.4 ± 0.3e	26.1 ± 0.4a
Water	7.8 ± 0.0a	6.0 ± 0.1d	T

Means with different letters in the same column were significantly different at the level ($p < 0.05$); n = 3

ND not detected, *T* trace

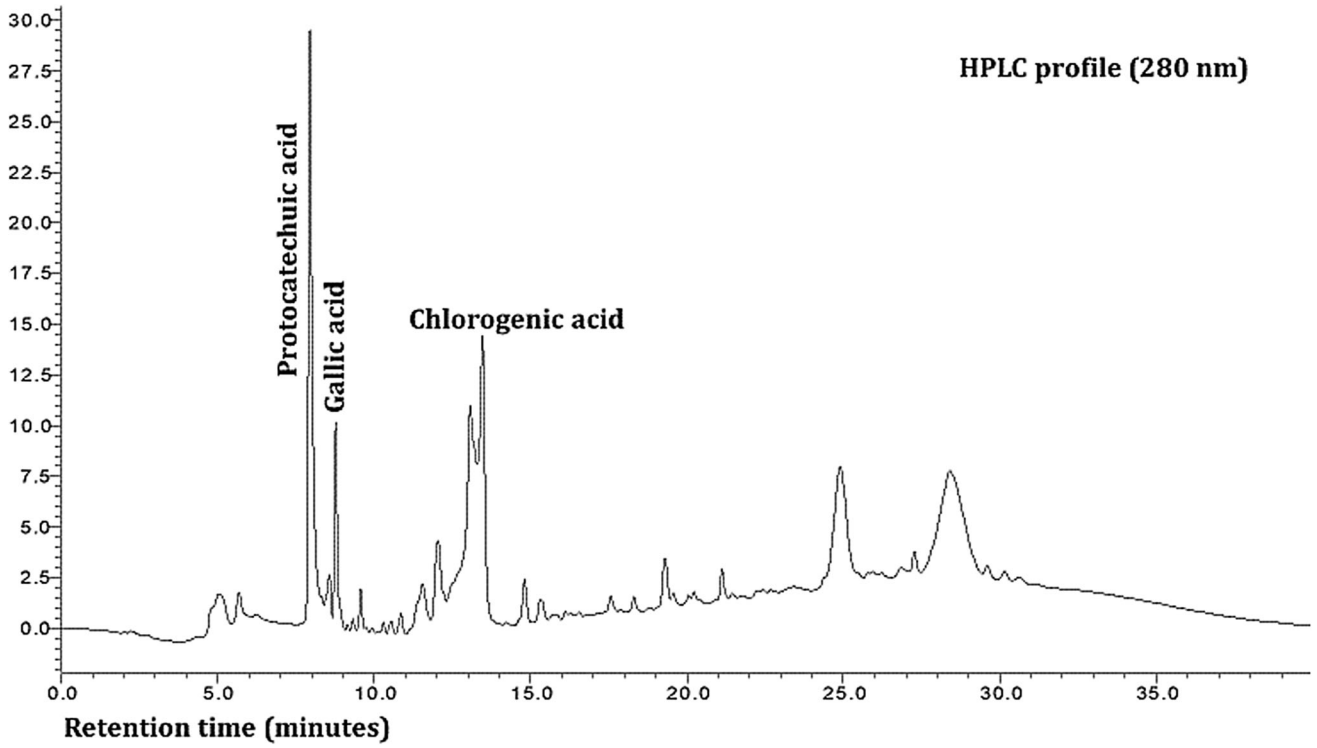

Fig. 2 Representative HPLC profile of *Lycoperdon utriforme* sequential extracts

from the matrix. For example, some compounds such as polar phenolics, vitamin C, and reducing agents that exhibit pronounced antioxidant activities could be successively extracted through polar solvents, whereas some compounds including terpenes and less polar phenolics can be extracted through apolar solvents (Smolskaitė et al. 2015). The extraction yields findings showed that polar solvents were more efficient than apolar solvents, which indicates the dominancy of the hydrophilic compounds in the extracts, and this argument was in accordance to HPLC–MS/MS and GC–MS findings.

Ao and Deb (2019) evaluated the total phenolic contents of the single solvent based extracts obtained from ten wild edible mushroom species naturally grown in India and reported very low levels (a range of 7.3 to 17.4 of mg Gallic acid equivalent per 100 g) compare to our findings and therefore it can be suggested the use of multiple solvents that have different polarities for the efficient extraction of biologically active compounds from mushroom materials. When the data were analyzed, it was observed that antioxidant activity positively correlated with the increase in solvent polarity. Antioxidant activities showed that the *n*-hexane extracts had the lowest and ethanol and acetone (or ethyl acetate) extracts had the highest antioxidant activity which can be explained by the presence of hydrophilic compounds as major compounds of mushroom samples since apolar solvents particularly hexane is not able to extract phenolics from mushroom matrix (Smolskaitė et al. 2015).

Phenolic acids were detected as key phenolic components of the extracts and these findings were in agreement data which were reported that phenolic acids including gallic, protocatechuic and chlorogenic acids were the major phenolic compounds of mushroom samples (Li et al. 2011; Leal et al. 2013; Kalogeropoulos et al. 2013; Islam et al. 2016; Sanchez 2017). Dundar et al. (2015) analyzed methanol-based extracts of seven mushroom species from Turkey and reported high antioxidant activities and the presence of phenolic acids such as protocatechuic, caffeic, syringic, coumaric acids. Barros et al. (2009) investigated sixteen Portuguese wild mushrooms for their chemical composition and reported the presence of phenolic acids as the major components. The authors also reported the highest total phenolic contents in *Lycoperdon* species (*L. molle* and *L. perlatum*) which is conformed to our findings. According to our findings no any UV spectra or mass data was found during chromatographic studies that could be linked to the presence of flavonoid compounds and therefore it was determined that these mushroom materials didn't contain flavonoid compounds with the exception of very low levels (0.8 to 22 µg/g extract, Table 1) of flavonols which can be derived through the interferences or sample contaminants or

Table 3 Fatty acid composition of *Lycoperdon utriforme* sequential extracts

	Palmitic acid	2,4-Ditert-butylphenol	Stearic acid	Oleic acid	Linoleic acid
Fragment ions	55, 74, 87, 97, 129, 143, 185, 213, 227, 239, 270	57, 74, 91, 163, 191, 206	55, 57, 73, 87, 99, 113, 129, 141, 143, 157, 185, 199, 241, 255, 284	55, 69, 83, 97, 110, 123, 137, 180, 222, 235, 264, 296	67, 81, 95, 150, 220, 263, 294
Contribution (%)					
n-Hexane	43.98	7.61	29.51	T	15.47
Chloroform	41.12	8.13	28.54	T	12.17
Ethyl acetate	42.18	9.12	28.14	T	12.78
Acetone	41.78	10.21	31.12	T	10.12
Ethanol	40.54	12.15	30.19	T	8.65
Water	40.97	13.89	33.94	T	2.06

T trace; contribution $\leq$ 2%

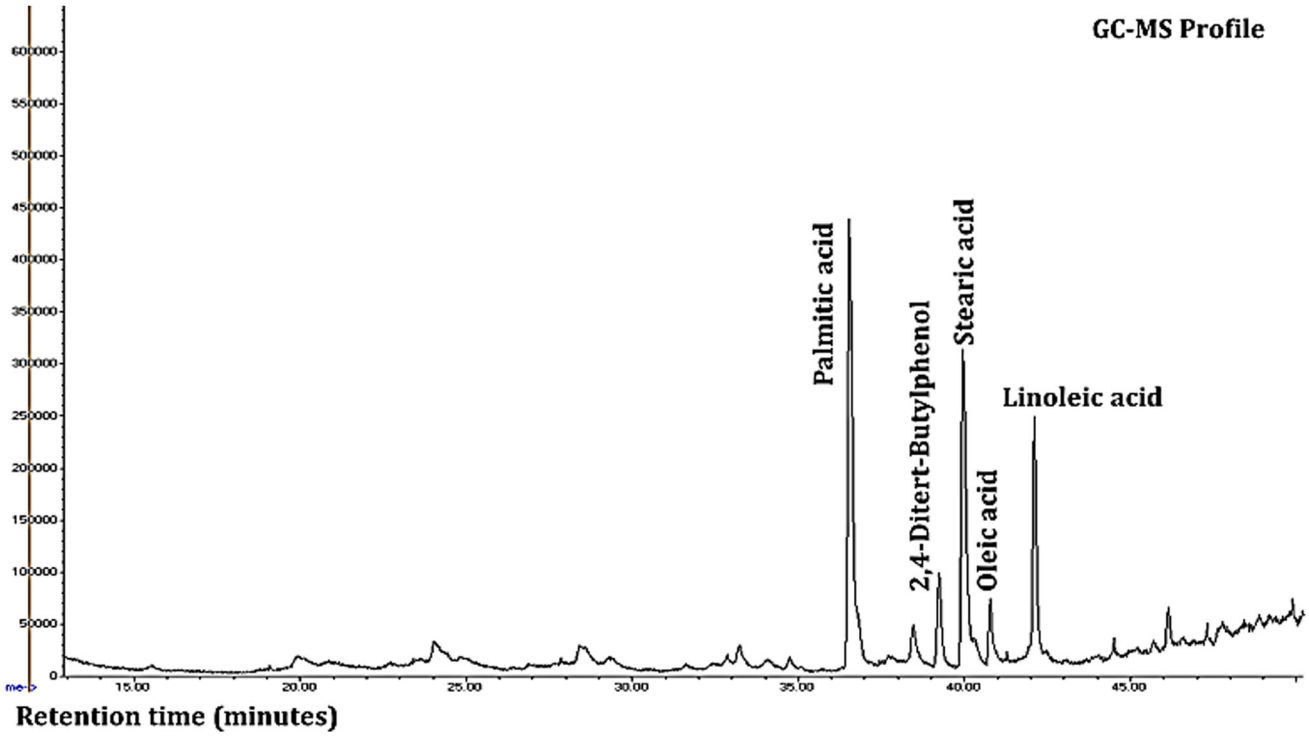

Fig. 3 Representative GC–MS profile of *Lycoperdon utriforme* sequential extracts

other pitfalls within the utilized colorimetric method. This argument can be explained by the non-presence of the key enzymes such as chalcone synthase or chalcone isomerase, which involved in the flavonoid biosynthetic pathway in the mushrooms and their inability of absorbing such compounds from onion wastes and media supplemented with specific flavonoids (Gil-Ramírez et al. 2016).

It was reported the superior antioxidant activities of protocatechuic acid than Trolox, a commercial antioxidant agent, and suggested its activity through chelating metal transition ions and scavenging free radicals (Kakkar and Bais 2014). Protocatechuic acid was also reported for its potential protective properties in vivo (Wang et al. 2009). Fatty acids identified in the extract were composed of biologically active linoleic, oleic and palmitic acids, which are common fatty acids, found in mushroom species (Barros et al. 2007; Sanchez 2017).

Conclusion

Within this study in vitro antioxidant activities and chemical profiles of sequential extractions obtained from five wild-edible mushroom samples including *Tricholoma scalpturatum*, *Tricholoma populinum*, *Neolentinus cyathiformis*, *Chlorophyllum agaricoides*, and *Lycoperdon utriforme* from Turkey were analyzed. Sequential extraction method showed that polar solvents were more effective in terms of antioxidant capacities. The extracts were found as rich sources of phytochemical compounds such as phenolics acids (gallic, protocatechuic and chlorogenic) and fatty acids (linoleic, oleic and palmitic acids). Findings obtained from this study revealed that *Lycoperdon utriforme* extracts might be useful in human diet because of pronounced antioxidant potential and biologically active ingredients.

Acknowledgements None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices or with commercial providers of medical services. The authors are grateful to the Van Yuzuncu Yil University Scientific Research Projects Council for providing financial assistance of the research (Project Number: 2015-SBE-YL268). This study represents Sema Sezgin MSc thesis.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests.

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