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Antioxidant property of edible mushrooms collected from Ethiopia

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ABSTRACT

Two cultivated (*P. ostreatus* and *L. edodes*) and five wild (*L. sulphureus, A. campestris, T. clypeatus, T. microcarpus* and *T. letestui*) edible mushrooms were analyzed for their antioxidant activities, total phenolics, total flavonoids, phenolic profile and ergothioneine content. Results showed that *A. campestris* had the greatest antioxidant activity in all assays with lower EC_{50} (mg/ml) values of 1.4, 3.6 and 0.035 for scavenging, reducing and chelating activities, respectively. To correlate well with activities, *A. campestris* also exhibited greater total phenolics and total flavonoids content of 14.6 mg GAE/g and 1.97 mg CE/g, respectively. The maximum concentration (μg/g) of the individual phenolic compounds were 7.80 (*P. ostreatus*) for caffeic acid, 4.55 (*T. letestui*) for chlorogenic acid, 15.8 (*T. microcarpus*) for p-coumaric acid, 20.3 (*A. campestris*) for ferulic acid, 561.9 (*A. campestris*) for gallic acid, 38.7 (*A. campestris*) for p-hydroxybenzoic acid and 7.08 (*A. campestris*) for myricetin. All samples tested contained different amounts of ergothioneine ranging from 0.08 (*L. sulphureus*) to 3.78 (*P. ostreatus*) mg/g in dry weight.

1. Introduction

Mushrooms contain a variety of secondary metabolites, including various phenolic compounds, polyketides, terpenes and steroids which have been shown to act as excellent antioxidants (Ishikawa, Morimoto, & Hamasaki, 1984). The ability of phenolic compounds to act as antioxidants have been well established (Rice-Evans, Miller, & Paganga, 1996), especially that of gallic acid, catechin, caffeic acid, rutin, quercetin, ellagic acid and p-coumaric acid is well known in several models (Sun, Tang, & Powers, 2007). These polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (Rice-Evans et al., 1996).

Mushrooms were also discovered recently to be the primary source of ergothioneine (ERG), a naturally occurring thiol containing amino acid, known for its antioxidant properties (Dubost, Beelman, Peterson, & Royce, 2006). ERG is water soluble and exerts antioxidant properties through multiple mechanisms, one of which is its powerful ability to scavenge free radicals (Colognato et al., 2006). ERG is concentrated in mammalian mitochondria, suggesting a functional role in protecting it from oxidative damage due to the generation of mitochondrial superoxide (Paul & Snyder, 2010). Red beans, oat bran, and organ meats, such as liver and kidney, are dietary sources of ERG (Ey, Schömig, & Taubert, 2007). However, certain species of mushrooms are distinguished source of ERG ranging 0.1–1 mg/g (Grigat et al., 2007) and 0.4–2.0 mg/g (Dubost et al., 2006) in dry weight.

Although there are many studies on the antioxidant property of cultivated and wild edible mushrooms in other parts of the world, there is no single information available about edible mushrooms of Ethiopia. Thus in this study, for the first time, the antioxidant activities, phenolic profile and ergothioneine content occurring in the cultivated and wild edible mushrooms of Ethiopia were investigated.

2. Materials and methods

2.1. Description of sampling areas

The three mushroom sampling areas were Addis Ababa, Kaffa zone (site Bonga) and Benishangul Gumuz region (site Asosa) of Ethiopia. Addis Ababa is the capital city of Ethiopia and located 9°01 N and 038°45 E. Kaffa zone is situated in the northwestern part of the southern nations, nationalities and people region state (SNNPR) and lies within 07° 00′—7°25′N latitude and 35°55′—36°37′E longitude. Benishangul gumuz region is located in western parts of Ethiopia located between 09.17° and 12.06° north latitude and 34.10–37.04° east longitude.

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2.2. Sample collection and identification

The collection of samples was based on their abundance and availability during the rainy season of the year (May–September). The samples were either collected from the field or purchased from the indigenous people who collect edible forest resources in the region or from the local markets. Identification of the wild edible mushrooms was made by making comparisons with authentic illustrations. Moreover, confirmations of the wild mushrooms were made by mycological experts at the department of life sciences at Addis Ababa University.

2.3. Preparation of samples and storage

The mushroom samples were cleaned out of forest debris (without washing) with a plastic knife and sliced without separating the cap and the stipe of the mushrooms. Samples were dried in a drying oven in the laboratory until a constant weight. The dried samples were milled to fine powder (20 mesh) using a mill (FW 100, Yusung Industrial Ltd., China) and kept in plastic bottles until analysis.

2.4. Analysis of antioxidant activities, total phenolics and phenolic profile

2.4.1. Sample extraction

Samples were extracted based on the procedures previously outlined (Barros, Baptista, & Ferreira 2007). Briefly, ten grams of dried mushroom powder was extracted by stirring with 100 ml of methanol at 25 °C at 150 rmp for 24 h using temperature shaker incubator (ZHWY-103B) and then filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol as described above. The combined methanolic extracts were evaporated at 40 °C to dryness using rota evaporator (Stuart R3300) and re-dissolved in methanol at the concentration of 50 mg/ml and stored at 4 °C for further use.

2.4.2. Determination of free radical scavenging activity

The effect of methanolic extracts on the DPPH radical was estimated according to Kirby and Schmidt (1997). A 0.004% solution of DPPH radical solution in methanol was prepared and then 4 ml of this solution was mixed with 1 ml of various concentrations (2–14 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. Scavenging capacity was read spectrophotometrically (Perkin Elmer Lamda 950 UV/Vis/NIR) by monitoring the decrease in absorbance at 517 nm. The absorption maximum was first verified by scanning freshly prepared DPPH from 200 to 800 nm using the scan mode of the spectrophotometer. Butyl hydroxytoluene (BHT) and ascorbic acid were used as a standard and mixture without extract was used as the control. Inhibition of free radical DPPH in percent (I%) was then calculated:

$$\text{Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where *A*0 is the absorbance of the control and *A*1 is the absorbance of the sample. The extract concentration providing 50% of radicals scavenging activity (EC50) was calculated from the graph of RSA percentage against extract concentration.

2.4.3. Determination of total reducing power

Total reducing power was carried out according to the method established by Oyaizu (1986). One millilitre of the extract at different concentrations (2–12 mg/ml), phosphate buffer (0.2 M, pH 6.6, 2.5 ml) and potassium hexacyanoferrate solution (1% v/ m, 2.5 ml) were mixed in a test tube and incubated for 20 min at 50 °C. Then 2.5 ml trichloroacetic acid (10%) was added, and the mixture was centrifuged at 2000×*g* for 10 min. The upper layer (2.5 ml) was transferred into another tube and mixed with 2.5 ml deionized water and 0.5 ml ferric chloride (0.1%) and left to react for 10 min. Finally, the absorbance of the reaction mixture was measured at 700 nm. Stronger absorbance at this wavelength indicates higher reducing power of the antioxidant. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at λ = 700 nm against extract concentration. BHT was used as control.

2.4.4. Determination of chelating effects on ferrous ion

Metal chelating effects on ferrous ions was determined according to Decker and Welch (1990). Two millilitres of various concentrations (0.05–1.5 mg/ml) of the extracts in methanol was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). Total volume was adjusted to 5 ml with methanol and then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured at 562 nm. A mixture without extract was used as the control. The inhibition percentage of ferrozine Fe^{2+} complex formation was then calculated:

$$\text{Metal Chelating Effect}(\%) = \frac{A_0 - A_1}{A_0} \times 100\%$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. A lower absorbance indicates a higher ferrous ion chelating capacity and 2, 2-bipyridyl, disodium ethylenediaminetetracetate (EDTA) was used as a control. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of ferrous ion inhibition percentage against extract concentration.

2.4.5. Determination total phenolics

Concentrations of phenolic compounds in the mushroom methanolic extracts were estimated based on procedures described by Ferreira, Baptista, Vilas-Boas, and Barros (2007). One millilitre of sample (2000 μg) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used to construct the standard curve (0.5–100 μg/ml). The results were mean values ± standard error of mean and expressed as mg of gallic acid equivalents/g of extract (GAEs). Total content of phenolic in mushrooms extracts in gallic acid equivalent (GAE) was calculated by the following formula:

$$C = \frac{c \times V}{m}$$

where *C* is the total content of phenolic compounds, mg/g fresh material, in GAE; *c* the concentration of gallic acid established from the calibration curve (Absorbance = 0.0134 gallic acid μg – 0.0144, R^2 = 0.9918); *V* the volume of extract, *L*; *m* is the weight of extract, *g*.

2.4.6. Determination total flavonoids

Total flavonoid was determined by a colorimetric method as described in Xu and Chang (2007). Briefly, 0.25 ml of sample (50 mg) was mixed with 1.25 ml of deionized water and 75 μl of a 5% $NaNO_2$ solution. After 6 min, 150 μl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink colour was measured at 510 nm against the blank. For calibration curve (+)-Catechin was used with a concentration range of

10–1000 μg/ml (Absorbance = 2.9337 catechin μg +0.034, R^2 = 0.99). Results were expressed as mg (+)-catechin equivalent (CE)/g of extract.

2.4.7. Phenolic compounds profile

2.4.7.1. HPLC instrumentation and working conditions. Phenolic compound analysis was conducted with Shimadzu HPLC system (Shimadzu, Kyoto, Japan). The HPLC system consisted of a binary pumping system: pump A (LC-10AD vp) and pump B (LC-10AD), a degasser (DGU-14A), an auto-injector (SIL-10AD vp), column oven (CTO-10A vp) and UV/Vis diode array detector (SPD-M10A vp) and system controller (SCL-10A vp). Separation was achieved on RESTECK reverse phase C18 column (5 μm, 250 mm × 4.6 mm i.d) thermostatted at ambient temperature. A solvent system consisting of 0.1% acetic acid in water (solvent A) and acetonitrile (solvent B) was used with the following gradient: starting with 100% A and installing a gradient to obtain 8% B at 12 min, 10% B at 24 min, 30% B at 44 min, 90% B at 54 min, 100% B from 55 to 58 min and finally 5% B at 59 min. The system controller was stopped at the end of 64 min. The solvent flow rate was 1 ml/min.

2.4.7.2. Detection and calibration curves. Detection was carried out in a photodiode array detector (PDA), using 280 nm as the preferred wavelength. The phenolic compounds were characterised according to their UV and retention times compared with commercial standards. For the quantitative analysis of phenolic compounds, a calibration curve was obtained by injection of known concentrations (0.5–100 μg/ml) of different standards compounds: caffeic acid ($y = 322{,}940x + 79{,}128$; $R^2 = 0.9992$), chlorogenic acid ($y = 138{,}719x + 69{,}900$; $R^2 = 0.9982$), p-coumaric acid ($y = 258{,}919x - 109{,}559$; $R^2 = 0.9935$), ferulic acid ($y = 141{,}713x + 97{,}494$; $R^2 = 0.9994$); gallic acid ($y = 109{,}717x - 42{,}834$; $R^2 = 0.9976$); p-hydroxybenzoic acid ($y = 119765 \times -11{,}564$; $R^2 = 0.9899$) and myricetin, ($y = 289183x + 462227$; $R^2 = 0.9964$). The results were expressed as μg per g of dry weight (dw).

2.5. Ergothioneine (ERG)

2.5.1. Extraction of ERG

The ergothioneine content was analyzed following the method of Dubost et al. (2006). Briefly, 0.5 g dried mushroom powder, 7 ml cold ethanolic extract [10 mM dithiotreitol (DTT) in ethanol, 100 μM betaine in ethanol, 100 μM 2-Mercapto-1-methyl-imidazole (MMI) in 70% ethanol], 3 ml deionized water (DI) in a 50 ml sterile Falcon tube was blended for 10 s at medium speed in a Sorvall Omni Mixer model 17,150 115 volt 5 amp (Newtown, Connecticut, USA.) 2 ml 1% sodium dodecyl sulphate (SDS) in ethanol was added and contents blended 10 additional seconds at medium speed. Falcon tubes were centrifuged (Allegra 6R, Beckman Coulter, Fullerton, CA, USA) 20 min at 4000 rpm at 5 °C. Retained supernatant was vortexed, then two micro-centrifuge tubes with 500 μl in each was lyophilized (Savant AES 2000, Savant Instruments Inc. Holbrook, NY, USA.) These two dry samples were reconstituted in deionized water pH 7.3 and combined with a total final volume of 500 μl. These were vortexed, micro-centrifuged 1 min at 10,000 rpm (Scientific model V; VWR, Bristol, CT, USA), filtered 0.45 μm syringe filter, dispensed into clean micro-centrifuge tubes, and retained frozen until HPLC analysis.

2.5.2. ERG analysis with HPLC

Analysis was carried out using a Hewlett–Packard 1050 HPLC (Agilent, CA, USA) with a Perkin-Elmer Series 200 vacuum degasser. The separation proceeded at ambient temperature using GRACE Econosphere C-18 column was used (item number 70,066, serial number 608051125 (Alltech, Deerfield IL, USA). The column was 250 × 4.6 mm, 5 μm particle size. The degassed 0.45 μm filtered isocratic mobile phase was 50 mM dibasic sodium phosphate in water with 3% acetonitrile, 0.1% triethylamine at pH 7.3. Flow rate was 0.4 ml/min; all injections were 10 μl. Each sample was allowed to run 30 min. Detection at 254 nm was performed using a HP Series 1050 DAD. Ergothioneine in each sample was quantified by regression analysis against a standard curve of known concentration (0.01–0.5 mg/ml).

2.6. Statistical analysis

For each one of the mushroom species three parallel samples were analyzed. The results are expressed as mean values and standard error of mean (SEM). The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's Test with $\alpha < 0.05$. This analysis was carried out using SPSS version 15.0 program.

3. Results and discussion

3.1. Free radical scavenging activity

Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain how antioxidants function. Of these, total antioxidant activity, reducing power, DPPH assay, metal chelating, active oxygen species such as H_2O_2, O_2^-, and $OH^\cdot$ quenching assay are most commonly used for the evaluation of antioxidant activities of extracts (Chang, Yen, Huang, & Duh, 2002). In this study, the radical scavenging of mushroom extracts was tested using a methanolic solution of the 'stable' free radical, DPPH. A freshly prepared DPPH solution exhibits a deep purple colour with absorption maximum at 517 nm. The purple colour generally fades/disappears when an antioxidant is present in the medium (Gursoy, Sarikurkcu, Tepe, & Solak, 2010). First the concentration required to scavenge DPPH completely was optimized with pre-tests by tracking the purple to yellow colour change of DPPH with increased concentrations for each sample. This had been a crucial part of the study, since setting a much lower or higher concentration range might not indicate the difference in scavenging power between samples clearly and more so, the EC_{50} (the concentration required to scavenging 50% of the radical) might be skipped. It is after this optimization process that 2–14 mg/ml was chosen and prepared by diluting the stock solution (50 mg/ml) with methanol. This concentration optimization process was applied in similar way for reducing, chelating, total phenolics and total flavonoids assays.

Fig. 1 shows the difference in free radical scavenging activity of methanolic extracts of mushroom species evaluated. Results are expressed as the ratio percentage of sample absorbance decrease and the absorbance of DPPH solution in the absence of extract at 517 nm. Similar to other studies, the scavenging effect of the methanolic extract increased with concentration and was high for *A. campestris* with 92% inhibition at 4 mg/ml concentration. Of all the samples analyzed *L. sulphureus* had the least scavenging power with only 17% inhibition at 4 mg/ml. Overall, except *L. sulphureus (polypores),* all the wild edible mushrooms had better scavenging effect than their cultivated counter parts. The scavenging effect of the synthetic antioxidants BHT and L-ascorbic acid were higher with 95% and 96% inhibition at 4 mg/ml.

3.2. Reducing power activity

Assay of reducing power was based on the reduction of Fe^{3+}/ferricyanide complex to the ferrous form in presence of reductants (antioxidants). The yellow colour of the test solution changes to various shades of green and blue depending on the reducing power

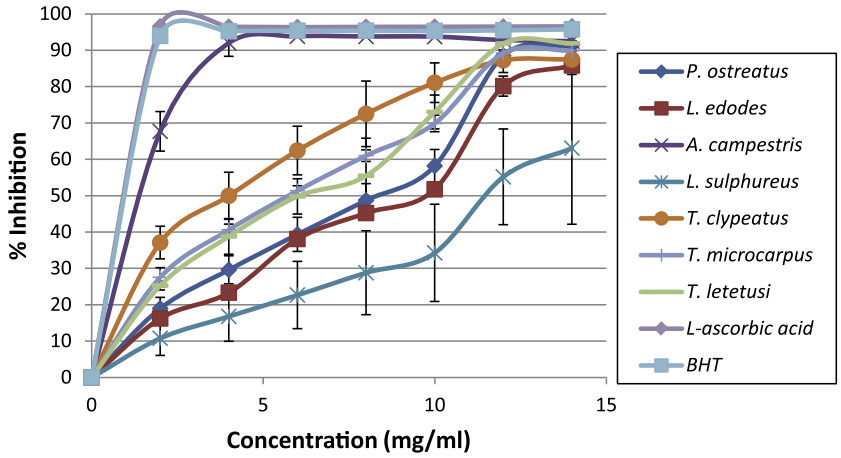

Fig. 1. Free radical scavenging of methanolic extract of edible mushrooms and controls.

of each compound (Ferreira et al., 2007). The Fe^{2+} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986).

Similar to DPPH scavenging, the appropriate reducing power concentration range was optimized with pre-tests by tracking the yellow to green colour changes. It was found that concentrations from 1–6 mg/ml were adequate enough for comparison and interpolating the EC_{50} (effective concentration at which the absorbance is 0.5). The reducing power of the wild and cultivated mushrooms is presented in Fig. 2. Since a higher absorbance at 700 nm indicates better antioxidant activity (Oyaizu, 1986), once again *A. campestris* exhibited superior reducing power with mean absorbance of 0.542 at 4 mg/ml. The lowest reducing power was shown by *L. sulphureus* with mean absorbance of 0.194 at 4 mg/ml, which is in agreement with its lower DPPH scavenging effect. Reducing power of the synthetic antioxidant BHT is much higher (mean absorbance = 2.008 at 4 mg/ml) as indicated with a straight line in the far left side Fig. 2.

3.3. Metal chelating activity

Ferrous ions, the most effective pro-oxidants, are commonly found in food systems. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted resulting in a reduction of the intensity for the red colour of the complex. Measurement of colour reduction therefore allows for an estimation of the chelating activity of the coexisting chelator (Yamaguchi, Takamura, Matoba, & Terao, 1998). In the present study, the chelating ability of the mushroom extracts towards ferrous ions was investigated by measuring the interference of the extract with the formation of ferrous and ferrozine complex. Fig. 3 shows the chelating effects of the methanolic extract of the cultivated and wild edible mushroom species. The optimized concentration range chosen to compare chelating power and to interpolate the EC_{50} (effective concentration at which 50% Fe^{2+}/ferrozine complex is inhibited) is from 0.05 to 1.5 mg/ml. Chelating effect was higher for *A. campestris* 69.7% inhibition at 0.25 mg/ml concentration to support its superior scavenging and reducing activity. On the contrary, *L. sulphureus* had the least metal chelating effect with only 27.2% at 0.25 mg/ml concentration.

3.4. Total phenolics and flavonoids content

It had been reported that the antioxidant activity of plant materials was well correlated with the content of their phenolic compounds (Velioglu, Mazza, Gao, & Oomah, 1998). Therefore, it is important to consider the effect of the total phenolic content on the antioxidant activity of mushroom extracts. Table 1 indicates the total phenolics and flavonoid content of mushrooms analyzed as milligram equivalent of gallic acid and (+) catechin per gram

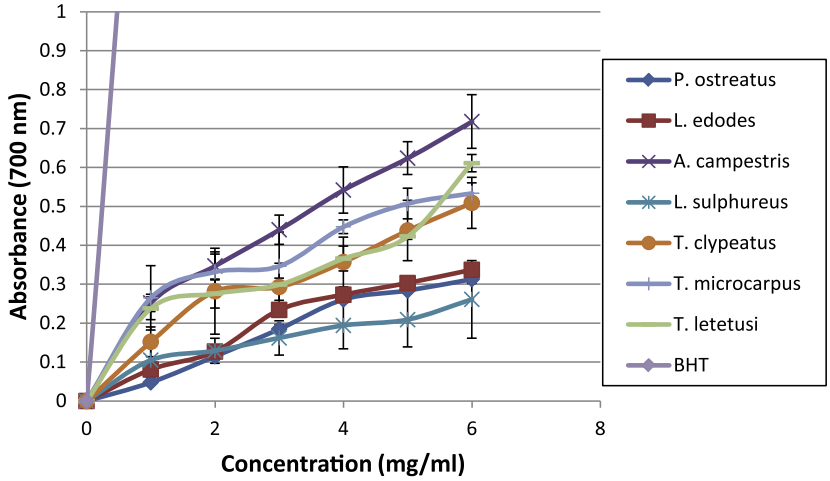

Fig. 2. Reducing power of methanolic extract of edible mushrooms and control.

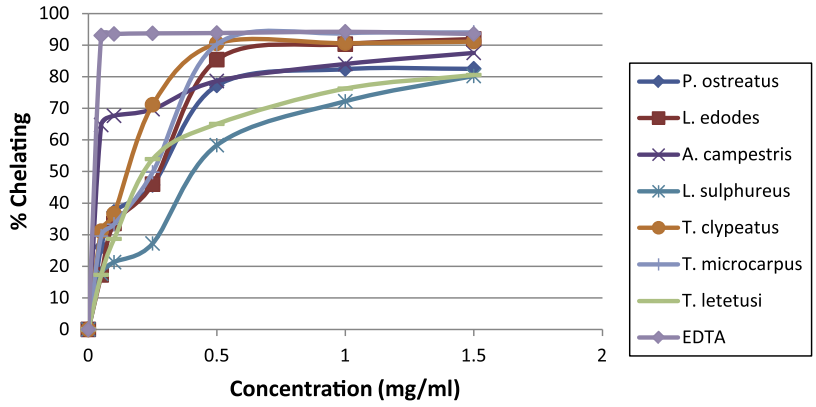

Fig. 3. Chelating effect of methanolic extract of edible mushrooms and control.

Table 1
Total phenolics, total flavonoids and effective concentration (EC_{50}) of edible mushrooms of Ethiopia.

No.	Mushroom	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH Scavenging (EC50[a])	Reducing Power (EC50[b])	Chelating Effect (EC50[c])
1	*A. campestris*	14.6 ± 0.82a	1.97 ± 0.06a	1.4	3.6	0.035
2	*T. letestui*	10.7 ± 0.32b	1.80 ± 0.07a	6.0	5.4	0.22
3	*T. clypeatus*	10.5 ± 0.59b,c	0.55 ± 0.02c	4.0	5.8	0.15
4	*T. microcarpus*	9.39 ± 0.28c	0.17 ± 0.01d	5.8	4.8	0.26
5	*P. ostreatus*	4.47 ± 0.22d	0.64 ± 0.04c	8.4	>6	0.29
6	*L. edodes*	3.45 ± 0.13d	1.50 ± 0.11b	9.8	>6	0.28
7	*L. sulphureus*	3.39 ± 0.16d	0.44 ± 0.09c	11.4	>6	0.4

Means followed by same letter in the same column are not significantly different ($p < 0.05$) according to Duncan's method.
Data are mean ± standard error of three parallel measurements ($n = 3$).
GAE, Gallic acid equivalent.
CE-Catechin equivalent.
[a] EC_{50} (mg/ml): effective concentration at which 50% of DPPH radicals are scavenged.
[b] EC_{50} (mg/ml): effective concentration at which the absorbance is 0.5.
[c] EC_{50} (mg/ml): effective concentration at which 50% Fe^{2+}/ferrozine complex are inhibited.

of extract, respectively. *A. campestris* has the highest phenolic content (14.7 mg GAE/g) among the mushroom species evaluated. This is followed by *T. letestui* and *T. clypeatus* with a value of 10.7 and 10.5 mg GAE/g, respectively, indicating the correlation of total phenolics with antioxidant activity. On the other hand, *L. sulphureus* with least antioxidant activity also had the least phenolic content (3.39 mg GAE/g). Other studies on mushrooms reported total phenolics ranging from 2.09 to 10.51 mg GAE/g extract (Gursoy et al., 2010), 0.83–42.21 mg GAE/g extract (Wong & Chye, 2009), 8.66–31.64 (Sarikurkcu, Tepe, & Yamac, 2008), 2.72–8.95 mg GAE/g (Barros et al., 2008), 4.58–58.14 mg GAE/g extract (Pereira, Barros, Martins, & Ferreira, 2012). Thus, the mushrooms evaluated in this study are somewhat comparable with the other reports.

The total flavonoid content of *A. campestris* was found to be superior with a value of 1.97 mg CE/g followed by *T. letestui* with 1.80 mg CE/g (Table 1). Other researchers reported a total flavonoid content for mushrooms ranging from 1.65 to 3.88 mg CE/g of extract (Barros et al., 2008) and 1.78–33.00 mg CE/g extract (Pereira et al., 2012). Flavonoids have been proven to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities (Cook & Samman, 1996).

3.5. Effective concentration (EC_{50})

The various antioxidant mechanisms of the mushroom species extract may be attributed to their strong reducing power by hydrogen-donating ability, a metal-chelating ability, and their effectiveness as good scavenger of free radicals, which can calculated from the EC_{50} of their assays. Table 1 also presents the EC_{50} values for scavenging, reducing power and chelating effects obtained from each mushroom methanolic extract and controls. Overall, *A. campestris* had better antioxidant properties with lower EC_{50} values of 1.4 mg/ml, 3.6 mg/ml and 0.035 mg/ml for scavenging, reducing and chelating, respectively. On the contrary, *L. sulphureus* had the least antioxidant activity with the highest EC_{50} of 11.4, >6 and 0.4 mg/ml for scavenging, reducing and cheating assays, respectively. The synthetic antioxidant (BHT, L-Ascorbic acid and EDTA), which was used as a positive control, had a superior performance with the least EC_{50} in all the assays.

3.6. Phenolic compounds profile

Individual profile of phenolic compounds can be obtained by high-performance liquid chromatography coupled to photodiode array detector (HPLC-DAD) (Palacios et al., 2011). Detection was carried out in a photodiode array detector (PDA), using 280 nm as the preferred wavelength. Phenolics occurring in edible mushrooms were identified by comparison of the absorption spectrum and the retention time with the corresponding standards. The phenolics content of the studied mushrooms in μg/g of dry weight is summarized in Table 2. Gallic acid, p-hydroxybenzoic acid and myricetin are significantly present in all the seven mushrooms. Other phenolic compounds, such as caffeic acid, chlorogenic acid, coumaric acid and ferulic acid were also detected.

The highest amount of caffeic acid was detected in *P. ostreatus* with 7.8 μg/g of dry weight. Palacios et al. (2011) reported a maximum caffeic acid concentration of 15.54 μg/g from several mushrooms. However, they did not detect any appreciable amount of caffeic acid in *P. ostreatus* unlike this study. The higher amount of

Table 2
Concentration of phenolic compounds (μg/g) in cultivated and wild edible mushrooms of Ethiopia.

No.	Mushroom	Caffeic acid	Chlorogenic acid	p-Coumaric acid	Ferulic acid	Gallic acid	p-Hydroxybenzoic acid	Myricetin
1	*P. ostreatus*	7.80 ± 1.58a	ND	ND	ND	13.0 ± 3.21c	1.27 ± 0.32c	1.67 ± 0.06c,d
2	*L. edodes*	0.79 ± 0.06b	ND	ND	0.89 ± 0.14d	61.2 ± 5.45b,c	1.58 ± 0.25c	0.13 ± 0.02d
3	*A. campestris*	ND	ND	10.9 ± 1.37b	20.3 ± 0.58a	561.9 ± 28.9a	38.7 ± 9.8a	7.08 ± 1.16a
4	*L. sulphureus*	2.53 ± 1.22b	ND	ND	ND	67.4 ± 31.2b,c	0.46 ± 0.03c	2.92 ± 0.01b,c
5	*T. clypeatus*	ND	ND	10.0 ± 0.78b	10.3 ± 1.64b,c	101.9 ± 9.76b	11.7 ± 0.92b,c	3.69 ± 0.29b
6	*T. microcarpus*	ND	ND	15.8 ± 0.22a	12.7 ± 2.96b	71.4 ± 15.1b,c	9.38 ± 0.09b,c	4.59 ± 0.29b
7	*T. letestui*	0.73 ± 0.23b	4.55 ± 3.35c	10.1 ± 1.01b	7.04 ± 0.85c	58.9 ± 3.32b,c	18.2 ± 2.71b	4.16 ± 0.80b

Means followed by same letter in the same column are not significantly different ($p < 0.05$) according to Duncan's method.
Data are mean ± standard error of three parallel measurements ($n = 3$).
ND = not detected.

caffeic acid might be due to the coffee waste substrate used for growing *P. ostreatus* mushroom in this study.

Chlorogenic acid was detected only in one of the mushrooms samples analyzed; i.e. *T. letestui* at 4.55 μg/g. Kim et al. (2008) reported a maximum of 26 μg/g chlorogenic acid, while Palacios et al. (2011) reported a maximum of 63.73 μg/g in mushrooms. As compared to other reports, the mushrooms evaluated in this study had least amount of chlorogenic acid. Highest amount of p-coumaric acid was detected in *T. microcarpus* with 15.8 μg/g. A comparable amount of coumaric acid was also observed in *A. campestris*, *T. clypeatus* and *T. letestui* with 10.9, 10.1 and 10.0 μg/g, respectively.

Ferulic acid was detected in all the samples except *P. ostreatus* and *L. sulphureus* mushrooms. Maximum and minimum concentrations of ferulic acid were 20.3 and 0.89 μg/g in *A. campestris* and *L. edodes*, respectively. Kim et al. (2008) reported a maximum of 22 μg/g in the medicinal mushroom, *Inonotus obliquus*. Ferulic acid was reported as the dominant phenolic compound in rice (Kim et al., 2008). Gallic acid was detected in much greater concentrations in all the mushrooms evaluated ranging from 13.0 (*P. ostreatus*) to 561.9 (*A. campestris*) μg/g. A similar observation was reported by Kim et al., 2008, indicating gallic acid as a strong antioxidant. Palacios et al. (2011) reported gallic acid in mushrooms ranges from 94.90 to 290.34 μg/g dry weight.

P-hydroxybenzoic acid was detected in all the mushrooms ranging from 0.46 (*L. sulphureus*) to 38.7 μg/g (*A. campestris*). Palacios et al. (2011) reported a concentration ranging from 4.69 to 24.07 μg/g for p-hydroxybenzoic acid in mushrooms. Kim et al. (2008) reported a maximum concentration of 263 μg/g in the medicinal mushroom, *Inonotus obliquus*. Recently Reis, Martins, Barros, and Ferreira (2012) reported only a maximum of 1.57 μg/g in the cultivated mushroom, *L. edodes*. Myricetin was detected in all the mushrooms samples ranging from 0.13 (*L. edodes*) to 7.08 (*A. campestris*) in μg/g. Kim et al. 2008 reported a maximum of 26 μg/g of myricetin in the cultivated mushroom, *Agaricus blazei*. Palacios et al. (2011) reported a maximum of 35.91 μg/g. As compared to other reports, the Ethiopian mushrooms evaluated had less myricetin content.

Overall, *A. campestris* had significantly ($p < 0.05$) higher concentration of p-coumaric acid, ferulic acid, gallic acid, p-hydroxybenzoic acid and myricetin which they contribute 638.9 μg/g to the total phenolic content (Table 2). There are few studies concerning the individual profiles of phenolic compounds in edible mushrooms. Cultivated species are better known in terms of composition; however, wild mushrooms are scarcely studied (Palacios et al., 2011). To the best of our knowledge, the phenolic composition of either cultivated or wild edible mushrooms of Ethiopia have not been previously described. However, the difference in the level of some phenolic antioxidants expressed in the mushrooms in this study from other reports might be dependent very much on the location and on whether the species has been exposed to stress conditions, etc.

3.7. Ergothioneine content

All samples tested contained different amounts of ergothioneine ranging from 0.08 to 3.78 mg/g in dry weight (Table 3). This result is consistent to the fact that ergothioneine is mainly synthesised in fungi (Genghof, 1970). Other studies reported EGT concentration of 0.4–2.0 mg/g (Dubost et al., 2006), 0.21–2.6 mg/g (Dubost, Ou, & Beelman, 2007) and 0.05–2.85 mg/g (Chen, Ho, Hsieh, Wang, & Mau, 2012) in dry weight basis. Assuming a serving size of 85 g (USDA. 2003), these mushrooms would provide between 6.8 and 321.3 mg of ERG/serving (Table 3). Interestingly, *Pleurotus ostreatus* (oyster) contained considerably high amount of ergothioneine with 3.78 mg/g. To support this, Chen et al. (2012) and Dubost et al. (2007) found the ergothioneine content of oyster mushrooms to be the highest with 2.85 and 2.59 mg/g dry weight, respectively. It seems that oyster mushrooms were an abundant source of ergothioneine. Another interesting observation is that both the cultivated mushrooms (oyster and shiitake) had better EGT than all the wild edible mushrooms evaluated. According to Chen et al. (2012) ergothioneine contents could be arbitrarily grouped into four levels: first level of >2.0 mg/g dry weight, second level of 1.0–2.0 mg/g, third level of 0.2–1.0 mg/kg, and fourth level of <0.2 mg/g. Among the fruiting bodies, the ergothioneine content at the first level is *P. ostreatus* whereas those at the second level are *L. edodes* and *T. clypeatus*. *L. sulphureus* had the least EGT and it is in the fourth level. Cells lacking ergothioneine were readily susceptible to oxidative stress, and thereby resulting in increased mitochondrial DNA damage, protein oxidation and lipid per-oxidation (Paul & Snyder, 2010). Since mushrooms are an abundant source of antioxidants, including ergothioneine, it is another reason to incorporate mushrooms into the human diet (Dubost et al., 2007). Besides, ergothioneine may be a new vitamin with physiologic roles in antioxidant cytoprotection (Paul & Snyder, 2010).

Table 3
Concentration of ergothioneine in cultivated & wild edible mushrooms of Ethiopia.

No.	Mushroom	mg/g of dw	mg ERG/Serving[a]
1	*P. ostreatus*	3.78 ± 0.05a	321.3
2	*L. edodes*	1.32 ± 0.02b	112.2
3	*T. clypeatus*	1.22 ± 0.11b	103.7
4	*A. campestris*	0.99 ± 0.11c	84.15
5	*T. letestui*	0.94 ± 0.01c	79.9
6	*T. microcarpus*	0.64 ± 0.02d	54.4
7	*L. sulphureus*	0.08 ± 0.01e	6.8

Means followed by same letter in the same column are not significantly different ($p < 0.05$) according to Duncan's method.
Data are mean ± standard error of three parallel measurements ($n = 3$).
[a] Serving size from USDA National Nutrient Database for standard reference for mushrooms (85 g).

4. Conclusion

The research revealed that *A. campestris* had highest antioxidant activity in all assays with lower EC_{50} values. To correlate well with activities, *A. campestris* also exhibited greater total phenolics and total flavonoids content. Gallic acid was found to be the major contributor to the total phenolic content for all the mushrooms. In addition, all mushrooms contained various amounts of ergothioneine, with *Pleurotus ostreatus* (oyster) containing substantially higher amount of ergothioneine. Thus local people eating mushrooms might be beneficial to protect themselves against oxidative damage.

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