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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