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Identification of phenolic compounds by liquid chromatography-mass spectrometry in seventeen species of wild mushrooms in Central Mexico and determination of their antioxidant activity and bioactive compounds

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ABSTRACT

Wild mushrooms are important for the diet of some communities in Mexico. However, limited information exists on their chemical composition, contribution to the diet, and health effects. We characterized seventeen wild mushroom species growing in the state of Queretaro in Central Mexico. Most species analyzed were edible, but also included nonedible, medicinal, poisonous and toxic specimens. Whole mushrooms (caps and stipes) were characterized for water content, color, and total content of phenolic compounds, flavonoids and anthocyanins. *In vitro* antioxidant capacity was measured by FRAP and DPPH assays. Phenolic compounds were identified and quantified by HPLC-mass spectrometry. All species analyzed were found to possess antioxidant activity *in vitro* and a wide range of phenolic and organic compounds were identified. Our results add to the limited information available on the composition and potential nutritional and health value of wild mushrooms. Further analyses of their bioactivities are warranted.

1. Introduction

Mushrooms have been extensively used for food and medicinal purposes all around the world. In Mexico, mushrooms represent an important component of the diet for many communities, especially for some ethnic groups. It has been estimated that about 185,000 species of mushrooms exist in Mexico (Guzmán, 1996). In spite of this diversity, little is known about their taxonomical description, and more importantly, about their chemical composition, contribution to the diet, and health effects.

The nutritional significance of mushrooms has been long recognized due to their high fiber, vitamin, mineral and protein, and low fat contents (Alispahic et al., 2015; Mattila, Sounpa, & Piironen, 2000). Several studies have shown that in addition to providing these important nutrients, mushrooms are rich in bioactive compounds with antioxidant properties, attributed mostly to phenolic compounds (Barros, Ferreira, Queirós, Ferreira, & Baptista, 2007; Cheung & Cheung, 2005; Kim et al., 2008; Mau, Lin, & Chen, 2002; Puttaraju, Venkateshaiah, Dharmesh, & Urs, 2016; Yang, Lin, & Mau, 2002). Antioxidant containing-foods have gained great interest in the last few years since they can protect against the effects of excessive free radicals in the body, thus preventing oxidative damage linked to the development of chronic diseases such as cancer, cardiovascular disease, and other degenerative disorders associated with aging (Barja, 2004; Shah & Channon, 2004; Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006).

Several studies have investigated the chemical and antioxidant content of mushrooms in different parts of the world (Barros et al., 2007; Chen, Xia, Zhou, & Qiu, 2010; Elmastas, Isildak, Turkekul, & Temur, 2007; Kim et al., 2008; Mattila et al., 2001; Nakajima, Sato, & Konishi, 2007; Puttaraju et al., 2016). However, to the best of our knowledge, only one study has analyzed the total phenolic and antioxidant contents of five species of mushrooms in the north of Mexico (Alvarez-Parrilla, de la Rosa, Martinez, & Gonzalez Aguilar, 2007). Therefore, we characterized seventeen species of wild mushrooms from the State of Queretaro in Central Mexico for their antioxidant and phytonutrient content. From these 17 species, ten were edible mushrooms. Analyses included antioxidant capacity, total content of phenolics, flavonoids, and anthocyanins, and identification and quantification of phenolic compounds using liquid chromatography coupled to mass spectrometry (HPLC-MS).

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2. Materials and methods

2.1. Chemicals

Reagents were obtained from Sigma-Aldrich (St. Louis, Mo., U.S. A.) unless otherwise stated. Phenolic compounds standards used included cinnamic, gallic, protocatechuic, catechin, chlorogenic, sinapic, quercetin, *p*-hydroxybenzoic, *p*-coumaric, caffeic, kaempferol, ferulic, myricetin, vanillic, epicatechin, isoramnethin, and *o*-coumaric, were HPLC (high-performance liquid chromatography) grade. HPLC-grade methanol, acetonitrile, and acetone were purchased from J.T. Baker (Baker Mallinckrodt, Mexico). All other solvents were ACS grade. HPLC-grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA, USA).

2.2. Samples and sample preparation

Fresh fruiting bodies of seventeen species of mushrooms were collected under live oak (*Quercus pyrenaica* Willd.) and pine (*Pinus* sp.) trees in Laguna de Servin, Queretaro, Mexico (20°16′ latitude, 100°16′longitude, 2760 m over sea level), during the rainy season. The morphological identification of the wild mushrooms was made according to macroscopic characteristics and following available classification guides (Garcia Jimenez, Pedraza Kamino, Silva Barrón, Andrade Melchor, & Castillo Tovar, 1998). Identification of samples were as follows: 1. *Lactarius indigo* (edible), 2. *Amanita flavoconia* (non-edible), 3. *Russula emetica* (non-edible), 4. *Strobilomyces floccopus* (non-edible), 5. *Hygrophorus sordidus* (edible), 6. *Amanita pantherina* (toxic), 7. *Boletus edulis* (edible), 8. *Agaricus arvensis* (edible), 9. *Amanita virosa* (poisonous), 10. *Boletus frostii* (edible), 11. *Ramaria flava* (edible), 12. *Lycoperdon perlatum* (edible in immature stage), 13. *Ganoderma lucidum* (medicinal), 14. *Cortinarius alboviolaceus* (non edible), 15. *Sarcodon imbricatus* (non edible), 16. *Boletus luridus* (edible), and 17. *Hypomyces lactiflorum* (edible). Only mature mushrooms (based on size and color development) were collected, except for sample 12 which was collected in its immature stage at which it is edible. Up to eight specimens of each mushroom species were collected based on their sizes, placed in plastic bags on ice, and taken immediately to the Phytochemistry and Nutrition Laboratory of the Autonomous University of Queretaro (about 2 h road distance). Color was measured with a Minolta spectrophotometer (Minolta, Co. Ltd., Osaka, Japan), which was calibrated with the white pattern during each sampling time. Color was determined on four points of the pileus. L^*, C^*, and h° values were recorded. Samples were cut in small pieces and immediately frozen with liquid nitrogen and kept at −80 °C until freeze drying. Moisture content was determined by freeze drying. Freeze-dried samples were kept in the dark at room temperature during the analysis period. All mushrooms from the same species were homogenized for analysis to minimize variability among individuals, and analyses were done in triplicate.

2.3. Antioxidant capacity

Antioxidant capacity (AOC) was measured in the lipophilic (LPE) and hydrophilic extracts (HPE) which were obtained as reported (Wu et al., 2004; Yahia, Gutierrez-Orozco, & Arvizu-de León, 2011), with some modifications. Samples of 0.2 g of freeze-dried powder were homogenized in 10 mL of hexane/dichloromethane (1:1, v/v) using an Ultra Turrax model T25 Basic homogenizer (IKA Works, Willmington, NC, USA). The homogenate was sonicated for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co., Danbury, CT, USA) and then centrifuged at 15,000*g* for 10 min at 4 °C. The supernatant was collected, and the sediment was subjected to an additional extraction using the same procedure. Both supernatants were mixed and roto-evaporated at 40 °C. The dried extract was re-suspended in 10 mL of HPLC-grade acetone, filtered through a 0.45 μm nylon membrane, and designated as LPE. The residue, after the second extraction process, was homogenized in 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v), sonicated, and centrifuged using the same conditions mentioned above. The supernatant was collected, and the sediment was subjected to extraction again. Both supernatants were mixed and designated as hydrophilic extract (HPE).

2.3.1. DPPH assay

DPPH (2,2′-diphenyl-1-picrylhydrazyl) assay was performed as previously reported (Kim, Lee, Lee, & Lee, 2002; Yahia et al., 2011), with some modifications, using a microplate reader. Aliquots of 280 μL of 100 μM DPPH/methanol solution per well were placed in a 96-well plate, and 20 μL of extracts, diluted to different concentrations, and were added to each well to complete 300 μL. Aliquots of 300 μL of methanol were used as a blank. The plates were incubated for 30 min in the dark, and readings were taken at 490 nm in a MRX microplate reader (Dynex Technology, Chantilly, VA, USA). A calibration curve was constructed using Trolox as standard. Results were expressed as Trolox equivalents (TE) in μmol/100 g dry weight (dw) (Rice-Evans & Miller, 1994).

2.3.2. FRAP assay

For FRAP (ferric ion reducing antioxidant power) assay (Benzie & Strain, 1996; Yahia et al., 2011), aliquots of 280 μL of FRAP reagent were placed in 96-well plates, and 20 μL of extracts were added. The plates were incubated for 30 min in the dark and read at 630 nm in a MRX microplate reader (Dynex Technology). Calibration curves were prepared using Trolox as a standard, and results were expressed as Trolox equivalents (TE) in μmol/100 g dw. FRAP reagent was prepared by mixing 50 mL of 300 mM acetate buffer (pH 3.6), 5 mL of 10 mM 2, 4, 6-tripyridyl-2-triazine (TPTZ) in 40 mM HCl, and 5 mL of 20 mM $FeCl_3$.

2.4. Determination of bioactive compounds

Mushroom phenolic extracts were prepared as described before (Wolfe, Xianzhong, & Liu, 2003; Yahia et al., 2011), with some modifications. A total of 0.2 g of freeze-dried powder samples were homogenized in 10 mL of 80% acetone (2% formic acid) using an Ultra Turrax model T25 basic homogenizer (IKA Works) at room temperature. The homogenate was subjected to sonication for 5 min in a Bransonic 2510 sonicator (Bransonic Ultrasonic Co.) and centrifugation at 19,000*g* for 15 min at 2 °C in a Hermle Z323 K centrifuge (Labortechnik, Wehingen, Germany). The supernatant was collected, and an additional extraction was done in the sediment following the same procedure. Both supernatants were mixed and evaporated at 40 °C using a rotary evaporator (Buchi R-205, Labortechnik, Switzerland). The concentrate was diluted with 25 mL of methanol and completed to 50 mL with HPLC-grade water. These extracts were used to analyze total content of soluble phenols, flavonoids and anthocyanins.

2.4.1. Total soluble phenols (TSP)

Aliquots of 30 μL of sample per well were placed in 96-well plates, and 150 μL of Folin-Ciocalteu reagent (dilution 1:10) and 120 μL of 7.5% Na_2CO_3 were added. The plates were incubated for 2 h in the dark, and absorbance was measured at 630 nm using a Dynex MRX microplate reader (Dynex Technol.). Gallic acid was used to prepare a calibration curve. Results were expressed as milligrams of gallic acid equivalents (GAE)/100 g dw.