



Analysis of phenolic constituents, antiradical and antimicrobial activity of edible mushrooms growing wild in Poland



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Protocatechuic acid (PubChem CID: 72)

4-OH-benzoic acid (PubChem CID: 135)

Vanillic acid (PubChem CID: 8468)

Caffeic acid (PubChem CID: 689043)

p-Coumaric acid (PubChem CID: 637542)

Ferulic acid (PubChem CID: 445858)

Salicylic acid (PubChem CID: 338)

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ABSTRACT

Mushrooms have been part of human diet for centuries. They possess great potential for both nutrition and therapeutic use. Amongst bioactive constituents occurring in mushrooms, phenolic compounds focus much attention due to their antioxidant activity.

The aim of the present study was to determine the chemical composition of phenolic compounds in wild growing edible mushrooms traditionally eaten in Poland. For this purpose, LC-ESI-MS/MS and spectrophotometric tests were used. Our findings revealed the presence of benzoic acid (protocatechuic, 4-OH-benzoic, vanillic, salicylic) and cinnamic acid derivatives (caffeic, *p*-coumaric, ferulic) in mushroom extracts. The total phenolic content in different species ranged between 1.64 and 13.53 mg GAE/g of extract. Moreover, antiradical and antimicrobial potential of 19 Polish wild growing edible mushrooms was investigated. As a result, differentiated antiradical activity was disclosed (IC₅₀ from 12.39 to 108.38 mg/mg DPPH[•]). Moderate antimicrobial properties were found, as well.

To the best of our knowledge, this is the first study conducted among such a large number of wild edible mushroom species. The results obtained indicate that mushrooms might be used directly in diet and promote health as an easily accessible source of natural antioxidants and antimicrobial agents.

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1. Introduction

Mushrooms have been part of human diet for centuries, mainly because of the variety of flavors and tastes they can provide. They are very popular in Asian countries, especially in China where the rate of mushroom consumption reaches even 20–24 kg per capita annually (Wang et al., 2014). Despite rising amounts of mushrooms consumed in recent years, they still seem to be an untapped source of food in Europe. Mushrooms possess great potential for both nutrition and therapeutic use. They supply digestible proteins, carbohydrates, fiber, certain vitamins and minerals. They are also

valuable foods due to low-calorie and low-fat content (Khatua, Paul, & Acharya, 2013; Wang et al., 2014). Recently, researchers have been increasingly interested in the chemical composition of mushrooms. A vast body of evidence indicates that edible and medicinal mushrooms contain many biologically active compounds disclosing anti-inflammatory, antitumor, antibacterial, antiviral and antioxidant activity. Moreover, their antiallergic, anti-atherogenic, hypoglycemic and hematological properties have been demonstrated (Palacios et al., 2011). Amongst bioactive constituents occurring in mushrooms, phenolic compounds focus much attention due to their antioxidant activity. Since oxidative stress resulting from imbalance between the level of free radicals and the body's defense system is considered a serious cause of many diseases, including cancer and other civilization diseases, it is essential to search for natural sources of antioxidants. Numerous

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epidemiological studies indicate that polyphenol-rich food and beverages of plant origin consumed in human everyday diet reduce the risk of cardiovascular diseases, stroke and certain types of cancer (Nowak, Olech, & Nowacka, 2013). These properties are commonly correlated with the antioxidant activity of phenolic compounds, which makes them useful for decreasing oxidative damage in the human body (Gan, Nurul Amira, & Asmah, 2013). The content of phenolic compounds in special mushroom phenolic acids has not been investigated as precisely as in the case of plants. Therefore, thorough studies of the chemical composition of mushrooms are highly desirable to identify and quantify bioactive compounds.

This work focuses on wild growing edible mushrooms from Poland. We decided to select species quite popular all over the country, i.e. *Armillaria mellea*, *Xerocomus badius*, *Macrolepiota procera* approved as edible by the Ministry of Health of the Republic of Poland according to the Regulation of the Minister of Health of 11 May 2011 (Journal of Laws of the Republic of Poland of 2011, No 115, item 672). Moreover, we collected some mushrooms not listed in this regulation but considered as edible according to Gumińska and Wojewoda (1985, pp. 1–506). Although the species are commonly eaten in some regions due to the local tradition, their chemical composition and biological activity remain unknown.

The aim of our work was to determine the chemical composition of 19 Polish wild growing edible mushrooms in terms of phenolic compounds. Moreover, antioxidant activity and antimicrobial potential against Gram-positive and Gram-negative bacteria were investigated. To the best of our knowledge, this is the first study conducted among such a large number of edible wild mushroom species growing in Poland.

2. Materials and methods

2.1. Materials

The fruiting bodies of wild growing edible mushrooms: *Armillaria mellea* (Vahl: Fr.) P. Kumm. ss. lato., *Calvatia excipuliformis* (Scop.: Pers.) Perdeck, *Clavulina cinerea* (Bull.: Fr.) J. Schröt., *Clitocybe gibba* (Pers.: Fr.) P. Kumm., *Coprinus micaceus* (Bull.: Fr.) Fr., *Craterellus cornucopiodes* (L.: Fr.) Pers., *Laccaria amethystea* (Bull.) Murrill, *Laccaria laccata* (Scop.: Fr.) Berk. & Broome, *Lactarius rufus* (Scop.: Fr.) Fr., *Laetiporus sulphureus* (Bull.: Fr.) Murrill, *Leccinum scabrum* (Bull.: Fr.) Gray, *Lycoperdon perlatum* Pers.: Pers., *Macrolepiota procera* (Scop.: Fr.) Singer, *Marasmius oreades* (Bolt.: Fr.) Fr., *Pholiota mutabilis* (Scop.: Fr.) P. Kumm., *Psilocybe capnoides* (Fr.: Fr.) Noordel., *Rozites caperatus* ('*caperata*') (Pers.: Fr.) P. Karst., *Sparassis crispa* (Wulf.: Fr.), *Xerocomus badius* (Fr.: Fr.) Kühner ex Gilbert, were collected in the Forests of Włodawa (Lublin Voivodeship, Poland) between 2012 and 2013. Mushrooms specimens were authenticated by Dr Zofia Fliśńska from the Department of Botany and Mycology, Maria Curie-Skłodowska University, Lublin, Poland and deposited at the Department of Pharmaceutical Botany, Medical University of Lublin, Poland (voucher specimens: No. MSH-005, No. MSH-008, No. MSH-010, No. MSH-011, No. MSH-014, No. MSH-016, No. MSH-030, No. MSH-031, No. MSH-034, No. MSH-075, No. MSH-036, No. MSH-042, No. MSH-045, No. MSH-046, No. MSH-029, No. MSH-025, No. MSH-054, No. MSH-076, No. MSH-071, respectively). Mushrooms were immediately lyophilized in the Free Zone 1 apparatus (Labconco, Kansas City, KS, USA) and kept in a freezer until further analysis.

2.2. Chemicals

Standards of gallic, protocatechuic, gentisic, 4-OH-benzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, salicylic, veratric, synapic, 3-OH-cinnamic, rosmarinic acids, Trolox, 2,2-diphenyl-1-

picrylhydrazyl radical (DPPH[•]), and ascorbic acid were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). Ethanol, methanol and Folin–Ciocalteu reagent were from POCH (Gliwice, Poland). All the chemicals were of analytical grade. LC grade methanol (MeOH) was purchased from J.T. Baker (Phillipsburg, USA). LC grade water was prepared using a Millipore Direct-Q3 purification system (Bedford, MA, USA).

2.3. Samples preparation

Multistep and overall extraction with pure ethanol (788.42 g/l) was used to prepare samples. Freeze-dried and milled mushrooms (5 g) were macerated twice with ethanol (50 ml) at room temperature for 24 h. The suspensions were filtered through filter paper. The residues were extracted twice by ultrasonically assisted extraction with ethanol (50 ml) for 30 min at room temperature. The combined extracts were evaporated to dryness under vacuum. Dry extracts were weighted and stored in a freezer at –70 °C. Samples were re-dissolved in the appropriate solvents for each determination.

2.4. Total phenolic content (TPC)

The total phenolic content was assayed by the modified Folin–Ciocalteu method (Olech & Nowak, 2012). All colorimetric measurements were conducted on 96-well transparent microplates (Nunc, Nunc, Roskilde, Denmark) using an Elisa Reader Infinite Pro 200F (Tecan Group Ltd., Männedorf, Switzerland). Briefly, 20 µl of the examined extract was added to 20 µl of diluted Folin–Ciocalteu reagent (with water 1:4, v/v) followed by addition of 160 µl of sodium carbonate (75 g/l). The absorbance was measured at 680 nm after 20 min using an Elisa reader with the solution containing water instead of the Folin–Ciocalteu reagent as a blank. The results were expressed as mg of gallic acid per g of extract.

2.5. LC-ESI-MS/MS conditions of analysis of phenolic acids

Phenolic acids contents were determined by reversed-phase high-performance liquid chromatography and electrospray ionization mass spectrometry (LC-ESI-MS/MS). For this purpose an Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, a degasser, an autosampler and a column oven connected to a 3200 QTRAP Mass spectrometer (AB Sciex, USA) was used. Chromatographic separations were carried out at 25 °C on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8-µm particle size; Agilent Technologies, USA) with a mobile phase consisting of water containing 0.1% HCOOH (solvent A) and methanol containing 0.1% HCOOH (solvent B), using 3 µl injections. The flow rate was 500 µl/min and the gradient was as follows: 0–0.8 min – 5% B; 2–3 min – 20% B; 5–7.5 min – 100% B; 8.5–11 min – 5% B.

The QTRAP-MS system was equipped with electrospray ionization source (ESI) operated in the negative-ion mode. ESI worked under the following conditions: capillary temperature 600 °C, curtain gas at 0.17 MPa, nebulizer gas at 0.41 MPa, negative ionization mode source voltage –4500 V. Nitrogen was used as curtain and collision gas. For each compound the optimum conditions of Multiple Reaction Mode (MRM) were determined in the infusion mode. The data was acquired and processed using Analyst 1.5 software (AB Sciex, USA). The analytes were identified by comparing the retention times and *m/z* values obtained by MS and MS2 with the mass spectra from corresponding standards tested under the same conditions. The calibration curves obtained in the MRM mode were used for quantification of all analytes. The identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the

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