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STUDIES ON ANTIOXIDANT, ANTIHYPERGLYCEMIC AND ANTIMICROBIAL EFFECTS OF EDIBLE MUSHROOMS *BOLETUS EDULIS* AND *CANTHARELLUS CIBARIUS*

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Abstract: The study evaluated the antioxidant, antihyperglycemic and antimicrobial effects of both ethanolic and hydromethanolic extracts of the fruiting bodies of wild edible mushrooms *Boletus edulis* (penny bun) and *Cantharellus cibarius* (golden chanterelle) sampled in Poiana Stampei (Suceava county, Romania). The total phenolic contents of extracts were also determined. *Boletus edulis* hydromethanolic extract showed the highest total phenolic content (72.78±0.29 mg/g). This extract was also the most active as scavenger of DPPH and ABTS radicals (EC_{50}=151.44±0.85 and 65.4±0.4 µg/mL, respectively) and reducing agent (EC_{50}=46.77±0.34 µg/mL). *Cantharellus cibarius* ethanolic extract showed high ferrous ion chelating (EC_{50}=82.9±0.6 µg/mL), 15-lipoxygenase (EC_{50}=236.7±1.5 µg/mL) and α-glucosidase (EC_{50}=9.77±0.06 µg/mL) inhibitory activities. For both mushrooms, the ethanolic extracts were more active against *Staphylococcus aureus* ATCC 25923 than the hydromethanolic ones. The antioxidant and antihyperglycemic effects revealed in this study support further investigations for a possible valorization of both mushrooms in the dietary supplement and pharmaceutical industries.

Keywords: *Boletus edulis, Cantharellus cibarius,* ferrous ion chelation, free radical scavenging, α-glucosidase, 15-lipoxygenase, reducing power.

Introduction

Edible mushrooms are consumed for their nutritional and functional properties in fresh or dried form [CHEUNG 2013; VALVERDE & al. 2015]. Bioactive compounds such as polysaccharides, proteins, triterpenoids, phenols and flavonoids have been isolated from edible mushroom species [LIU & al. 2016]. Moreover, numerous studies have reported that some edible mushrooms have antioxidant, antitumor, antiallergic, anti-inflammatory, anticholesterolemic, antiviral, antibacterial and immunomodulatory effects [CHANG & WASSER, 2012]. In oriental medicine, many edible mushrooms are widely used to prevent chronic diseases [SARIKURKCU & al. 2008]. Edible mushrooms are also known for their low glycemic index and high mannitol and dietary fibers content that recommend them for diabetic patients diet [CHANG & WASSER, 2012]. Evaluation of chemical composition and pharmacological activities of edible mushrooms is still an active research area.

Boletus edulis Bull. (*Boletaceae*, penny bun) is a widespread mushroom that grows in deciduous and coniferous forests in Europe, North America and Asia. Due to its nutritional value and unique taste, it is considered a culinary delicacy and a functional food [TSAI & al.

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2007; WANG & al. 2014]. Fruiting bodies of *Boletus edulis* are an important source of carbohydrates (mannose, rhamnose, glycans), lectins (boledulin A, B, C), organic acids (malic, oxalic, quinic, ketoglutaric acids), aminoacids (glutamine, alanine, serine, proline) and microelements (Co, Cu, Fe, Ni) [FAURE & al. 2014]. Polysaccharides isolated from *Boletus edulis* are responsible for many biological activities such as antitumor, anti-inflammatory and antioxidant effects. Oral administration of a water-soluble polysaccharide purified from *Boletus edulis* proved to have antitumor effect on renal cell carcinoma in mice [WANG & al. 2014]. Ethyl acetate fractions rich in boledulin A, B and C showed moderate cytotoxic activity against human myeloid leukemia HL-60, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, colon cancer SW480 and lung cancer A-549 cells [FENG & al. 2011]. Hot water extract of *Boletus edulis* (5-320 μg/mL) showed immunomodulatory activity due to a stimulatory effect on splenic lymphocytes proliferation [WANG & al. 2013]. *Boletus edulis* extracts exhibited antioxidant and antiviral effects. Strong free radical scavenging activity was reported for methanolic and hot-water extracts of *Boletus edulis* fruiting bodies [SARIKURKCU & al. 2008; TSAI & al. 2007]. Methanolic and water extracts rich in polyphenols and α,β-glycans exhibited antiviral activity on type-1 *Herpes simplex* virus (HSV-1), while hot-water extract rich in lectins showed antiviral properties against type-1 human immunodeficiency virus (HIV-1) [SANTOYO & al. 2012; ZHENG & al. 2007].

Cantharellus cibarius Fr. (*Cantharellaceae*, golden cantherelle) is the most common wild edible mushroom in European coniferous forests and hardwood forests that can be harvested from early spring to fall [DREWNOWSKA & FALANDYSZ, 2015; HONG & al. 2012]. *Cantharellus cibarius* mushrooms are rich in ergocalciferol but also carotenoids; the latter are responsible for the yellow-to gold pigmentation of the fruiting bodies [FALANDYSZ & al. 2012; DREWNOWSKA & FALANDYSZ, 2015]. Other constituents such as polysaccharides, lectins, phenolic acids, lipids, sterols and indolic compounds have been recently isolated from *Cantharellus cibarius* extracts. Similar to *Boletus edulis*, *Cantharellus cibarius* is of great interest due to its antitumor, anti-inflammatory and immunomodulatory effects but also for its antimicrobial and antigenotoxic potential [VALENTAO & al. 2005; DREWNOWSKA & FALANDYSZ, 2015]. The immunomodulatory effect is apparently related to acetylenic acid derivatives as these compounds, isolated from the methanolic extract of *Cantharellus cibarius*, were able to enhance gene expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) [HONG & al. 2012]. At the same time, a polysaccharide-rich fraction of *Cantharellus cibarius* stimulated the proliferation of mouse splenocytes [HAN & al. 2013]. Regarding possible benefits of *Cantharellus cibarius* in Alzheimer's disease, a slight inhibition of acetylcholinesterase was reported for the methanolic extracts rich in polyphenols [ORHAN & USTUN, 2011]. This mushroom could also have a beneficial role in other chronic diseases as it contains phytochemicals with anti-inflammatory properties. MORO & al. (2012) investigated the anti-inflammatory mechanism of *Cantharellus cibarius* methanolic extracts and concluded that these extracts could reduce the expression of inducible nitric oxide synthase (iNOS*),* interleukins IL-1β and IL-6 in lipopolysaccharide-stimulated macrophages.

Further investigation is necessary to broaden the therapeutic applications of *Boletus edulis* and *Cantharellus cibarius* in pharmaceutical and functional food industries. The purpose of our study was to evaluate the polyphenolic content of edible mushrooms *Boletus edulis* and *Cantharellus cibarius* sampled in Suceava county, Romania. Our further objective

was to assess their antioxidant, antihyperglycemic and antimicrobial effects. In this respect, ethanolic and hydromethanolic extracts were prepared and investigated.

Materials and methods

Mushroom material

Fruiting bodies of *Boletus edulis* (Bull.) and *Cantharellus cibarius* Fr. were collected in September 2011 in Poiana Stampei, Suceava county, Romania. The mushroom material was cleaned and stored at -18 °C. For further analysis, the samples were defrosted and air dried in shade. Voucher specimens are deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania.

Ethanolic extracts preparation

Dried and powdered mushroom samples (50 g) were extracted twice with 500 mL of 96% ethanol at room temperature for 3 h under continuous stirring. The combined ethanolic extracts were evaporated at 40 °C under reduced pressure resulting in the final ethanolic extracts.

Hydromethanolic extracts preparation

After ethanolic extraction, the mushroom residue was further extracted with methanol:water mixture (1:1, v/v) using the same procedure. The extracts were evaporated at 40 °C under reduced pressure resulting in the final hydromethanolic extracts.

Total phenolic content

The total phenolic contents of both extracts were evaluated by Folin-Ciocalteu assay [WANGENSTEEN & al. 2004].

DPPH radical scavenging effect

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was assessed according to a previously described method [LIU & al. 2012; WANGENSTEEN & al. 2004] with slight modifications. Briefly, DPPH radical scavenging activity was determined after 60 min reaction time in darkness at room temperature.

ABTS radical cation scavenging effect

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid] radical cation scavenging activity was determined using the method of RE & al. (1999).

Reducing power

Reducing power was evaluated according to the method described by ZHANG & al. (2011).

Ferrous ion chelating effect

Ferrous ion chelation assay was performed according to VENDITTI & al. (2010).

15-Lipoxygenase inhibition

The ability to inhibit the peroxidation of polyunsaturated fatty acids was investigated using 15-lipoxygenase inhibition assay as previously described [BITO & al. 2014; WANGENSTEEN & al. 2004].

α-Glucosidase inhibition

The capacity to inhibit α-glucosidase was performed as described by LIU & al. (2012) with slight modifications. α-Glucosidase from *Saccharomyces cerevisiae* was dissolved in phosphate buffer (67 mM, pH 6.8 at 37 °C) to a concentration of 0.86 IU/mL. An aliquot of 0.05 mL of each extract was mixed with 0.5 mL phosphate buffer, 0.02 mL glutathione (3 mM) and 0.02 mL α-glucosidase. After 5 min incubation at 37 °C, a volume

of 0.05 mL *p*-nitrophenyl α-D-glucopyranoside (10 mM) was added followed by 15 min incubation at 37 °C. The reaction was stopped with 2.36 mL sodium carbonate (0.1 M). The absorbance was determined at 400 nm.

Antibacterial and antifungal effects

Antibacterial activity was evaluated against Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 278523), while the antifungal activity was tested against *Candida albicans* ATCC 10231, *Candida glabrata* ATCC MYA 2950 and *Candida parapsilosis* ATCC 22019. The strains belonged to the Culture Collection of the Microbiology Department, Grigore T. Popa University of Medicine and Pharmacy, Iasi, Romania.

Antibacterial and antifungal effects were evaluated by agar diffusion assay [WAYNE, 2015].

Results and discussion

Extraction

The solvent used for extraction has a great influence on the phenolic content and consequently on the biological effects of vegetal extracts. In our study, the hydromethanolic extracts showed higher yields than the ethanolic ones (Tab. 1).

Total phenolic content

The highest phenolic contents were detected in the hydromethanolic extracts (Tab. 1). Regarding *Boletus edulis* extracts, the values found in our study were higher than those reported by TSAI & al. (2007) for *Boletus edulis* samples collected in Taiwan (5.73±0.05 and 5.81±0.10 mg/g for the ethanolic and hot water extracts, respectively). Lower phenolic contents were also reported for the methanolic extract of *Boletus edulis* from Portugal (5.03±0.11 mg/g) [BARROS & al. 2008]. *Cantharellus cibarius* extracts showed higher phenolic contents than those reported for the methanolic extract of *Cantharellus cibarius* from India (3.20±0.05 mg/g) [RAMESH & PATTAR, 2010] and Portugal (1.75±0.5 mg/g) [BARROS & al. 2009]. These different values reported in literature might be due to the harvest moment, substrate on which mushrooms grew, storing conditions and duration [HELENO & al. 2010]. Our results indicate that the total phenolic content depends on the mushroom species and solvent used for extraction.

Tab. 1. Extraction yields and total phenolic contents of *Boletus edulis* and *Cantharellus cibarius* extracts

Mushroom	Extract	Abbreviations	Yields (%)	Total phenolic content (mg/g)
Boletus edulis	ethanolic	Be-E	14.69	35.83±0.92
	hydromethanolic	Be-HM	24.31	72.78±0.29
Cantharellus cibarius	ethanolic	Cc-E	9.50	11.27±0.32
	hydromethanolic	Cc-HM	31.69	11.53±0.03

DPPH radical scavenging effect

For all tested concentrations, the hydromethanolic extracts showed higher scavenging activity against DPPH radical than the ethanolic extracts. As it can be concluded from the low effective concentrations 50% (EC_{50}) (μg/mL) (Tab. 2), *Boletus edulis* extracts exhibited stronger DPPH radical scavenging effects than *Cantharellus cibarius* extracts. In the same assay, an EC_{50} of 2.33±0.06 μg/mL was found for quercetin [ZAVASTIN & al.

2015]. In contrast to our study, TSAI & al. (2007) reported lower scavenging activity for the ethanolic and hot water extracts from commercial samples of *Boletus edulis* from Taiwan (EC_{50}=1.75±0.02 and 15.78±0.10 mg/mL, respectively). Moreover, other researchers reported higher EC_{50} values for the methanolic extracts of *Boletus edulis* from Portugal (EC_{50}=1.54±0.03 mg/mL) [FERNANDES & al. 2013] and Poland (EC_{50}=1.80±0.01 mg/mL) [HELENO & al. 2015].

With respect to *Cantharellus cibarius*, KOSANIC & al. (2013) found lower EC_{50} values for the methanolic and acetonic extracts of *Cantharellus cibarius* from Serbia (EC_{50}=192.57 and 158.4 µg/mL, respectively).

ABTS radical cation scavenging effect

In this assay, hydromethanolic extracts displayed higher ABTS radical cation scavenging effects than the ethanolic ones (Tab. 2). *Boletus edulis* hydromethanolic extract was the most active; at 250 µg/mL, it almost completely scavenged ABTS radical cation (90.62±0.15% scavenging activity). In the same assay, quercetin showed an EC_{50} value of 1±0 µg/mL [ZAVASTIN & al. 2015].

Reducing power

The highest reducing power was determined for *Boletus edulis* hydromethanolic extract. However, both *Boletus edulis* extracts were more active than the extracts of *Cantharellus cibarius* (Tab. 2). The reducing effects of the tested extracts were lower than that found for quercetin in our previous studies (EC_{50} =2.98±0.12 µg/mL) [ZAVASTIN & al. 2015]. In contrast to our study, other researchers reported a lower reducing power for the methanolic extracts of *Boletus edulis* from Portugal (EC_{50}=0.71±0.01 mg/mL) [FERNANDES & al. 2013] and Poland (EC_{50}=0.63±0.02 mg/mL) [HELENO & al. 2015]. However, the ethanolic extract of *Cantharellus cibarius* from Turkey had a similar reducing capacity (0.315±0.10 at 500 µg/mL) as the one found in our study (0.34±0.00 at 533.34 µg/mL) [ORHAN & USTUN, 2011].

Ferrous ion chelating effect

In this assay, the ethanolic extracts were more active than the hydromethanolic ones. The strongest ferrous ion chelating effect was exerted by *Cantharellus cibarius* ethanolic extract (Tab. 2). At 576 µg/mL, this extract chelated ferrous ions by 91.47±0.44%. At the same concentration, *Boletus edulis* ethanolic extract showed 55.32±0.26% chelating activity. In the same assay, an EC_{50} value of 6.34±0.06 µg/mL was determined for ethylenediaminetetraacetic acid (EDTA), a very potent metal chelator [ZAVASTIN & al. 2015]. KHALILI & al. (2015) also reported the capacity of the methanolic and ethyl acetate extracts of *Cantharellus cibarius* to chelate plasmatic ferrous ions in iron overloaded mice. In our study, *Boletus edulis* ethanolic extract showed a weaker capacity of chelating ferrous ions than the methanolic extract of *Boletus edulis* from Turkey (90.2±0.85% chelating activity at 500 µg/mL). Extracts with strong chelating capacity might be able to chelate the excess of pro-oxidant ferrous ions in the human body [SARIKURKCU & al. 2008].

15-Lipoxygenase inhibition

In contrast to the hydromethanolic extracts, the ethanolic ones exhibited a stronger inhibition of 15-lipoxygenase (Tab. 2). It is worth noting that at 833.34 µg/mL, both *Boletus edulis* and *Cantharellus cibarius* ethanolic extracts almost completely inhibited 15-lipoxygenase (100% and 97.53% inhibition, respectively). At the same concentration, the percentages of 15-lipoxygenase inhibition showed by the hydromethanolic extracts were very low (20.14±0.23% for *Boletus edulis* extract and 1.02±0.18% for *Cantharellus cibarius* extract). With respect to the EC_{50} values, all extracts were less effective than quercetin; the

latter was found to inhibit 15-lipoxygenase with an EC_{50} value of 19.5±0.7 µg/mL [ZAVASTIN & al. 2015]. The inhibition of 15-lipoxygenase could not be associated with the phenolic content; other compounds seem to be responsible for this effect. 15-Lipoxygenase inhibitors are able to restrain lipid peroxidation in the human body [BITO & al. 2014].

Antihyperglycemic activity

α-Glucosidase is an important enzyme involved in the hydrolysis of starch and disaccharides to glucose units. α-Glucosidase inhibitors slow the absorption of carbohydrates thus being able to control postprandial hyperglycemia [KUMAR & al. 2013]. The ethanolic extracts proved to be strong α-glucosidase inhibitors (Tab. 2). Their activity was significantly higher than that of acarbose (anti-diabetic drug) that inhibits α-glucosidase. In the same assay, acarbose inhibited the enzyme with an EC_{50} of 70.7±0.3 µg/mL, as determined previously [ZAVASTIN & al. 2015]. *Boletus edulis* ethanolic extract had a remarkable α-glucosidase inhibitory activity (84.27±0.19% at 83.34 µg/mL). Further studies should be done to identify the constituents responsible for this activity. Other researchers stated that polysaccharides from mushrooms are involved in the antihyperglicemic effect as they elevate insulin level in plasma, increase hepatic glycogen and reduce carbohydrates decomposition by restraining α-glucosidase [WANG & al. 2016].

Tab. 2. Antioxidant and antihyperglycemic effects of *Boletus edulis* and *Cantharellus cibarius* extracts

Type of activity	EC_{50} values (µg/mL)			
	Be-E	Be-HM	Cc-E	Cc-HM
DPPH radical scavenging effect	411.63±0.25	151.44±0.85	>833.34	730.37±3.05
ABTS radical cation scavenging effect	124.77±2.80	65.4±0.4	387.1±6.0	179.57±1.65
Reducing power	98.54±0.55	46.77±0.34	872.99±6.69	241.92±1.20
Ferrous ion chelating effect	449.13±5.15	7954.5±45.3	82.9±0.6	3752.57±35.65
15-Lipoxygenase inhibition	348.27±1.55	-	236.7±1.5	-
Antihyperglycemic activity	13.2±0.00	-	9.77±0.06	131.3±2.2

Antibacterial and antifungal effects

In the last decades, antibiotic resistance among microbial strains has dramatically increased. Since the current treatment often failed to overcome multidrug-resistance, researchers have investigated the antimicrobial activity of natural products [NOWACKA & al. 2014]. In our study, all extracts acted selectively against Gram-positive bacteria (*Sarcina lutea* ATCC 9341 and *Staphylococcus aureus* ATCC 25923). According to the inhibition zone diameter (IZD), the most sensitive bacteria was *Sarcina lutea* ATCC 9341 (Tab. 3). The ethanolic extracts showed the highest antimicrobial activity. According to IZD, *Boletus edulis* ethanolic extract had a slightly lower antibacterial activity against *Sarcina lutea* ATCC than chloramphenicol (20 *vs*. 25 mm). These results are in agreement with other data reporting antibacterial activity against *Staphylococcus aureus* ESA 7 (strain isolated from pus) for the methanolic extracts of *Boletus edulis* and *Cantharellus cibarius* showing minimum inhibitory concentrations (MIC) of 5 and 50 µg/mL, respectively [BARROS & al. 2008]. NOWACKA & al. (2015) found that the extracts of wild growing mushrooms from Poland were more active against Gram-positive bacteria than Gram-negative bacteria. Not only the activities of individual compounds in mushroom extracts, but also the interactions between them might be responsible for these differences in the antibacterial potencies [KOSANIC & al. 2016].

Our study also revealed that all extracts were inactive against Gram-negative bacteria although other studies found that *Cantharellus cibarius* ethanolic extract was active against *Escherichia coli* ATCC 25922 (MIC=15 µg/mL) and *Pseudomonas aeruginosa* ATCC 27853 (MIC=13 µg/mL) [RAMESH & PATTAR, 2010]. *Boletus edulis* ethanolic extract was the only extract with antifungal activity against *Candida parapsilosis* ATCC 22019 but its efficacy was lower than that of nystatin. In contrast to our results, KOSANIC & al. (2013) reported that the methanolic and acetonic extracts of *Cantharellus cibarius* were active against *Candida albicans* IPH 1316 (MIC=10 and 5 mg/mL, respectively).

Differences in the microbial cell wall structure might also explain, in part, the different antimicrobial effects of investigated mushroom extracts. Gram-positive bacteria cell wall is composed of several layers of peptidoglycans, Gram-negative bacteria cell wall consists of one peptidoglycan layer and an outer membrane containing phospholipids and lipopolysaccharides, whereas the fungal cell wall contains chitin and other polysaccharides [KOSANIC & al. 2016; SILHAVY & al. 2010].

Tab. 3. Antibacterial and antifungal activity of *Boletus edulis* and *Cantharellus cibarius* extracts

Extract/ Positive control	**Diameter of inhibition zone (mm)**						
	***S. aureus* ATCC 25923**	***S. lutea* ATCC 9341**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 278523**	***C. albicans* ATCC 10231**	***C. glabrata* ATCC MYA 2950**	***C. parapsilosis* ATCC 22019**
Be-E	14	20	0	0	0	0	0
Cc-E	14	17	0	0	0	0	0
Be-HM	11	13	0	0	0	0	10
Cc-HM	11	12	0	0	0	0	0
Ampicillin (25 µg/disc)	23	30	17	0	n.d.	n.d.	n.d.
Chloram phenicol (30 µg/disc)	20	25	22	0	n.d.	n.d.	n.d.
Nystatin (100 µg/disc)	n.d.	n.d.	n.d.	0	20	20	21
	n.d. – not determined						

Conclusions

As far as we know, this is the first report that underlines the antioxidant, antihyperglycemic and antimicrobial effects of *Boletus edulis* and *Cantharellus cibarius* mushrooms from Suceava county, Romania. The present study demonstrates that these mushrooms are valuable sources for the development of antioxidant and antihyperglycemic dietary supplements. *Boletus edulis* hydromethanolic extract showed a remarkable free radical scavenging activity which is related to its high content in phenolic compounds, while *Cantharellus cibarius* ethanolic extract proved to be an important 15-lipoxygenase inhibitor and ferrous ion chelator. Furthermore, *Boletus edulis* and *Cantharellus cibarius* ethanolic extracts were effective α-glucosidase inhibitors. The components of both ethanolic extracts should be further investigated for antidiabetic activity. The ethanolic extracts showed a moderate antimicrobial activity against Gram-positive bacteria and therefore a possible synergism of these extracts with conventional antibiotics should be further evaluated. Taking into consideration the results of the present study, we can conclude that *Boletus edulis* and *Cantharellus cibarius* can bring important positive effects on the human health as functional foods.

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