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An insight into antidiabetic properties of six medicinal and edible mushrooms: Inhibition of α -amylase and α -glucosidase linked to type-2 diabetes

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ABSTRACT

As a continuation of our search for biologically active mushroom species the present study investigates *in vitro* antidiabetic properties of six edible and medicinal mushroom species: *Agaricus blazei* Murrill, *Coprinus comatus* (O.F.Müll.) Pers., *Cordyceps militaris* (L.) Fr., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Morchella conica* Pers. and *Phellinus linteus* Berk. & M.A. Curtis. *In vitro* assays on α -amylase and α -glucosidase enzyme inhibition were performed with methanolic extracts of the selected mushrooms. Furthermore, we calculated the necessary daily intake of mushroom extracts and dry mushroom powders based on the equivalent doses of therapeutic drug acarbose given to diabetic patients per day. Our comparative study on enzyme inhibition showed that the most promising potential is ascribed to *I. obliquus* extract, while no inhibition of α -amylase was recorded with *M. conica* and *C. militaris* methanolic extract at the tested concentration. This comparative study is the first highlighting *in vitro* antidiabetic potential by inhibition of α -amylase and α -glucosidase with methanolic extracts; which makes the investigated species more promising for the diabetes type-2 treatment by an additional and different mechanism of action.

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1. Introduction

Mushrooms are important dietary components in some cultures, consumed in daily diet as supplementary food since olden times (Soković et al., 2016). Mushroom extracts are increasingly consumed because of their health beneficial effects, including the enhancement of immune function and antitumor activity (Popović et al., 2013). It is confirmed that mushroom extracts contain diverse compounds such as alkaloids, fibers, lectins, protein, polyphenols and polysaccharides, which are contributing to their biological activities (Popović et al., 2013). Mushrooms are thought to exert many pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial and other activities (Lindequist, 2013; Paterson and Lima, 2014). Mushrooms as functional food and dietary supplements can help in the intervention of sub-health states and may prevent the full-blown consequences of life-threatening diseases. An equilibrated diet linked with the mushroom consumption has an advantage of the nutritional/medicinal features of mushrooms (Soković et al., 2016) and their pharmacological

applications. The term "mushroom" is used here for a medicinal/edible fruiting body of higher fungi.

Diabetes mellitus is a chronic disorder of metabolism followed by abnormal rise in plasma glucose levels, as a consequence of unequilibrated insulin production and/or insensitivity to the effect of this hormone in signal transduction of cellular receptors. These metabolic changes are accompanied by modifications in carbohydrate, lipid and protein metabolism. Most of the diabetes type 2 complications in patients are due to hyperglycemia as their main cause (Ortiz et al., 2007; Shobana et al., 2009). One of the effective strategies for diabetes type-2 management is the inhibition of complex polysaccharide hydrolysis by pancreatic α -amylase and absorption limitation of glucose by inhibiting intestinal α -glucosidase enzyme. Acarbose, miglitol, voglibose are commercial drugs used for α -glucosidase inhibition in the control of the diabetic disease (Saito et al., 1998). Chronic α -amylase and α -glucosidase inhibitions may be useful in the management of type-2 diabetes and obesity (Melo et al., 1999; Koike, 2005). The drugs currently used as reversible inhibitors of α -amylase and α -glucosidase for the treatment of diabetic patients exhibit side effects such as abdominal distension, meteorism, bloating, flatulence and possibly diarrhea (Fujisawa et al., 2005).

To continue our investigations (Stojković et al., 2013; Reis et al., 2014; Stojković et al., 2014; Bizarro et al., 2015; Glamoclija et al.,

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2015; Vieira et al., 2016) on biological activities of mushroom species, we designed this study to explore the antidiabetic properties of six edible and medicinal mushrooms: *Agaricus blazei* Murrill, *Coprinus comatus* (O.F.Müll.) Pers., *Cordyceps militaris* (L.) Fr., *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Morchella conica* Pers. and *Phellinus linteus* Berk. & M.A. Curtis. *In vitro* assays on α -amylase and α -glucosidase enzyme inhibition were performed with methanolic extracts of the selected mushrooms.

2. Material and methods

2.1. Mushroom samples

Six edible and medicinal mushroom species were used in this study: *A. blazei* Murrill (commercial sample), *C. comatus* (O.F.Müll.) Pers. (collected - wild growing in Serbia), *C. militaris* (L.) Fr. (commercial sample), *I. obliquus* (Ach. ex Pers.) Pilát (commercial sample), *M. conica* Pers. (collected - wild growing in Serbia) and *P. linteus* Berk. & M.A. Curtis (commercial sample). The origin of samples, identification and preparation of dried powder were described previously (Stojković et al., 2013; Reis et al., 2014; Stojković et al., 2014; Bizarro et al., 2015; Glamočlija et al., 2015; Vieira et al., 2016).

2.2. Extraction procedures

Mushroom samples (~5 g) were extracted by stirring with 150 mL of methanol (–20 °C at 150 rpm) for 24 h and subsequently filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland) to dryness.

2.3. α -Amylase and α -glucosidase inhibition assays

Mushroom extracts at different concentrations dissolved to 500 μ L and 500 μ L of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Porcine pancreatic α -amylase (Sigma Aldrich, Germany) (0.5 mg/mL) were incubated at 25 °C for 10 min. Then, 500 μ L of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures were incubated at 25 °C for 10 min and stopped with 1.0 mL of dinitrosalicylic acid color reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 mL of distilled water, and absorbance measured at 540 nm using the spectrophotometer (Agilent 8453, Agilent Technologies, Waldbronn, Germany).

Mushroom extracts at different concentrations dissolved to 50 μ L and 100 μ L of α -glucosidase (Sigma Aldrich, Germany) solution (1.0 U/mL) in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 °C for 10 min. Then, 50 μ L of 5 mM p-nitrophenyl- α -D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures

were incubated at 25 °C for 5 min. Then, 2 mL of Na₂CO₃ was added to terminate the reaction before reading the absorbance at 405 nm in the spectrophotometer (Agilent 8453, Agilent Technologies, Waldbronn, Germany).

The α -amylase and α -glucosidase inhibitory activities were expressed as percentage of inhibition (IC₅₀). Acarbose was used as a positive control (Worthington, 1993; Apostolidis et al., 2007).

3. Results and discussion

The results of α -amylase and α -glucosidase inhibition linked to diabetes type-2 therapy are summarized in Table 1. *C. comatus* methanolic extract had the most prominent inhibitory activity on α -amylase with IC₅₀ 714.45 μ g/mL, while *C. militaris* and *M. conica* did not have inhibitory potential on this enzyme. All of the investigated mushroom species inhibited α -glucosidase with *I. obliquus* as the most potent inhibitor among the studied species (IC₅₀ 220.31 μ g/mL).

Table 1 also summarizes calculations of the daily intake of mushroom methanolic extracts and dry mushroom powders necessary to achieve the same effect as commercial therapeutic drug acarbose on the daily basis. Acarbose is given to diabetic patients in an initial dose of 3 \times 25 mg per day. Calculations were done on the basis of α -glucosidase inhibition by acarbose. The lowest daily intake of mushroom powder was predicted for *I. obliquus* with the dose of 3 \times 1.148 g/day, while the highest was predicted for *P. linteus* 3 \times 2.215 g/day (Table 1).

Kim et al. (2005) demonstrated that β -glucans and their enzymatically hydrolyzed oligosaccharides from *Agaricus brasiliensis* (syn. *A. blazei*) show the activities of antihypercholesterolemic, antihypertriglyceridemic, antiarteriosclerotic and antihyperglycemic, indicating antidiabetic activity in diabetic rats. The data suggested that both β -glucans and oligosaccharides might enhance insulin secretion from pancreatic islets as well as proliferation of islets in diabetic or normal rats (Kim et al., 2005). The results of our study showed that *A. blazei* could also have an inhibitory influence on key enzymes involved in the degradation of complex sugars (Table 1), thus delaying the process of glucose uptake. Our previous study (Stojković et al., 2014) on chemical composition of *A. blazei* revealed that this species possessed oxalic, malic and fumaric organic acids. The total content of organic acids was 1.87 g per 100 g dw. The phenolic acids found in the studied mushroom were p-coumaric acid (0.28 mg per 100 g dw) and cinnamic acid (0.12 mg per 100 g dw), (Stojković et al., 2014). Different compounds present in methanolic extracts and their different combinations are probably linked to *A. blazei* enzyme inhibitory potential.

Recent studies described *in vivo* antidiabetic activity of different extracts and fractions of *C. comatus* (Yamaç et al., 2009; Ding et al., 2010; Zhou et al., 2015). According to our knowledge, no previous records were published on α -amylase and α -glucosidase enzyme inhibition with *C. comatus* methanolic extract. Methanolic extract of *C. comatus* studied here had inhibitory potential on both enzymes (Table 1). Regarding *C. comatus*, our previous study (Stojković et al., 2013) revealed organic acid profile, with five different compounds namely oxalic,

Table 1
Inhibitory potential of mushroom species on α -amylase and α -glucosidase linked to type-2 diabetes treatment.

Mushroom species	Yield of extracts (%)	α -Amylase inhibition; IC ₅₀ (μ g/mL)	α -Glucosidase inhibition; IC ₅₀ (μ g/mL)	mg of extracts per day equivalent to acarbose 3 \times 25 mg tablets given to a diabetic patient daily ^a	g of dry mushroom powder per day equivalent to acarbose 3 \times 25 mg tablets given to a diabetic patient daily ^a
<i>Agaricus blazei</i>	14.24	1719.20 \pm 89.53	357.23 \pm 20.11	3 \times 227.65	3 \times 1.598
<i>Coprinus comatus</i>	10.57	714.45 \pm 44.44	322.74 \pm 11.21	3 \times 205.67	3 \times 1.945
<i>Cordyceps militaris</i>	13.25	na ^b	415.66 \pm 36.12	3 \times 264.88	3 \times 1.999
<i>Inonotus obliquus</i>	12.23	830.32 \pm 28.24	220.31 \pm 7.37	3 \times 140.40	3 \times 1.148
<i>Morchella conica</i>	15.41	na	521.12 \pm 16.45	3 \times 332.09	3 \times 2.155
<i>Phellinus linteus</i>	13.73	1479.46 \pm 92.86	477.33 \pm 17.55	3 \times 304.19	3 \times 2.215
Acarbose	–	87.15 \pm 2.93	39.23 \pm 5.41	–	–

^a Calculated on the basis of α -glucosidase inhibition.

^b na – not active at the tested concentrations.

quinic, malic, citric and fumaric acids. Concerning phenolic acids, the studied sample revealed the presence of dominant *p*-hydroxybenzoic acid (0.09 mg/100 g) and presence of *p*-coumaric acid, as also the related compound cinnamic acid (Stojković et al., 2013). Phenolic compounds and organic acids are probably related to the anti-diabetic activity investigated herein.

Water and polysaccharidic extracts of *C. militaris* previously showed antidiabetic properties in diabetes induced rats (Liu et al., 2016). Our study showed that methanolic extract of *C. militaris* did not inhibit the activity of α -amylase (Table 1), while it had inhibitory potential on α -glucosidase. A recent study showed that water extract of *C. militaris* had inhibitory activities on α -glucosidase with values of 45.77% with 0.1 mg/mL, 54.76% with 0.25 mg/mL, 58.74% with 0.5 mg/mL, and 60.5% with 1.0 mg/mL (Kim et al., 2017). The organic acid we found previously in higher amounts in *C. militaris* was citric acid 7.97 g/100 g (Bizarro et al., 2015). Regarding phenolic acids, *p*-hydroxybenzoic acid was the only compound found in the studied species (0.02 mg/100 g dw). However, cinnamic acid was also found (0.11 mg/100 g dw), (Bizarro et al., 2015). The link between chemical composition of methanolic extract of *C. militaris* and the enzyme inhibitory activity certainly exists.

Water extracts and polysaccharidic fractions of *I. obliquus* are recorded to possess *in vivo* antidiabetic properties (Qin et al., 2015). Extract of *I. obliquus* tested in our study possessed inhibitory potential of both enzymes investigated (Table 1). A previous study by Ying et al. (2014) revealed terpenoids from this species as inhibitors of glucosidase. In our previous study (Glamočlija et al., 2015) oxalic acid was the only organic acid detected in the extract of *I. obliquus* (72 mg/g extract). The phenolic acids found were gallic, protocatechuic and *p*-hydroxybenzoic acids, as also the related compound cinnamic acid (Glamočlija et al., 2015). Relation between organic, phenolic acids, their combination in the extract and antidiabetic activity explored herein certainly exists.

According to our knowledge, no previous studies were published on *M. conica* antidiabetic activity. Our results indicate that methanolic extract of this species did not inhibit α -amylase, while α -glucosidase was inhibited (Table 1). Regarding *M. conica* organic acid profile, previously (Vieira et al., 2016) we have quantified three different organic acids: oxalic, quinic, and fumaric acids. The phenolic compounds gallic and *p*-hydroxybenzoic acids were only present in the wild growing sample, as well as cinnamic acid.

A polysaccharide isolated from *P. linteus* has previously shown hypoglycemic properties in alloxan-induced diabetic mice (Zhao et al., 2014). *P. linteus* methanolic extract exhibited enzyme inhibitory potential in this study (Table 1). The hydrophilic compounds recorded in *P. linteus* earlier (Reis et al., 2014) were only organic acids — oxalic acid was the only detected molecule (0.295 g/100 g dw).

The investigated mushroom species possessed antidiabetic properties regarding the inhibition of the enzymes investigated. *C. militaris* and *M. conica* failed to inhibit the activity of α -amylase, while inhibiting the activity of α -glucosidase. *I. obliquus* methanolic extract was the most potent inhibitor of α -glucosidase, while *C. comatus* has the highest potential in inhibition of α -amylase. The lowest antidiabetically effective daily intake of mushroom powder was predicted for *I. obliquus* with the dose of 3×1.148 g/day, while the highest was predicted for *P. linteus* 3×2.215 g/day. The activity of the extracts investigated herein could be linked to their chemical compositions previously published by our research groups. Organic acids, phenolic acids, fatty acids and their specific combination in each species contributed to unique *in vitro* antidiabetic activity of investigated mushrooms.

Although majority of previous studies showed *in vivo* antidiabetic potential of water and polysaccharidic mushroom extracts by different experimental approaches, our study is the first highlighting *in vitro* antidiabetic potential by inhibition of α -amylase and α -glucosidase with methanolic extracts; which makes the investigated species more promising for the diabetes type-2 treatment by another additional and different mechanism of action.

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