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ANTIOXIDANT AND ANTIMICROBIAL POTENTIALS OF CHAMPIGNON MUSHROOM

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ABSTRACT: Fruiting bodies of some wild and cultivatable mushrooms contain medicinal compounds which are being used in traditional medicines and cosmetics. Champignon mushroom (*Agaricus bisporus*) is the most widely cultivated species of edible mushroom worldwide. This paper focuses on antioxidant and antimicrobial importance of *A. bisporus*. Water-soluble polysaccharide-enriched fraction was isolated from the dry carpophores of *Agaricus bisporus*. Antioxidant activities were investigated using *in vitro* assay systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and chelating ability on ferrous ions. Antimicrobial activity was tested against Gram positive and Gram negative bacteria *in vitro* by disk diffusion method in order to determine the zones of inhibition. At concentrations of 0.1-10 mg/ml, the scavenging abilities of *A. bisporus* ranged between 12.3-75.5 %. The radical scavenging ability of the positive controls -tocopherol and ascorbic acid, at the concentrations of 0.1-20 mg/ml, were between 79.9-80.8 and 80.6-91.1 %, respectively. Polysaccharide extract from *A. bisporus* showed steadily increasing chelating ability as concentrations increased to 88.2 % at 20 mg/ml. The chelating ability of the citric acid was between 7.2-10.7 %, at the concentrations of 0.1-20 mg/ml. The study of antimicrobial potential of polysaccharide extract showed more potent activity against Gram-positive *Enterococcus faecalis* ATCC 49532 (26.7 ± 0.2 mm), *Bacillus cereus* 10876 (27.5 ± 0.4 mm), *Geobacillus stearothermophylus* ATCC 7953 (22.8 ± 0.3 mm) than Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 35032 (10.4 ± 0.6 mm), *Proteus hauseri* ATCC 13315 (12.1 ± 0.1 mm) *Escherichia coli* (0157:H7) 35150 (12.7 ± 0.4 mm) with exception of *Klebsiella pneumoniae* ATCC 27736 (22.3 ± 0.2 mm).
Key words: *Agaricus bisporus, antioxidant, antimicrobial, polysaccharide extract*

INTRODUCTION

Agaricus bisporus (J. Lge) Imbach also called champignon, white mushroom, common mushroom or button mushroom is one of the most well-known, most cultivated and most used edible mushrooms. Human beings have been consuming champignons since Ancient times. Ancient Egyptians believed that the *Agaricus bisporus* held the key to immortality, while Ancient Romans revered the mushroom as one of the foods of the gods. During the 1600s, the French began to cultivate *Agaricus bisporus*, using dark underground tunnels beneath Paris that are still used for mushroom growing today (Spencer, 1985). *Agaricus bisporus* is now cultivated in at least 70 countries around the world (Cappelli, 1984). Global production in the early 1990s was reported to be more than 1.5 billion kg, worth more than US$ 2 billion (Chang, 1993).

In addition to its own unique flavor, eating this mushroom may provide important health and nutrition benefits when made a regular part of the diet. *Agaricus bisporus* is a source of excellent nutrition, providing a range of vitamins, minerals, carbohydrates, protein and phytochemicals that are important for human health. It provide the minerals selenium, copper, potassium, iron and zinc, as well as a range of vitamins including thiamin, riboflavin, pantothenic acid, niacin and vitamins C and D (Chang, 1993; Spencer, 1985).

In addition to the nutritional benefits of this mushroom, it may have useful medicinal properties that support health and well-being. *Agaricus bisporus* significantly stimulated

immune activity, specifically cytokines and enzymes that are responsible for inflammation (Kozarski et al., 2011; Ren et al., 2008; Wu et al., 2007). *Agaricus bisporus* may prevent breast cancer through an aromatase-inhibiting action that reduces enzymes that increase estrogen levels and drive breast cancer growth, making the mushroom both hormone-balancing and chemo-preventative (Chen et al., 2006; Grube et al., 2001).
Recently, there has been growing interest for polysaccharides of mushrooms, since there has been reported a great influence of these components on human health. Polysaccharides are potentially useful biologically active ingredients for pharmaceutical use, such as for immune regulation, for anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities (Klaus et al., 2009; Kozarski et al., 2009; Yang et al., 2005; Yoon et al., 2003; Lee et al., 2002). The mushroom-derived polysaccharides lentinan, schizophyllan, and krestin have been accepted as immunoceuticals in Japan, Korea and China (Zheng et al., 2005). The activity of polysaccharides is determined by their conformation, composition and size (Bohn and BeMiller, 1995).
In the present study, we have determined the antioxidant activity of polysaccharide enriched water soluble extract from *Agaricus bisporus* using assays pertaining to different ways of antioxidant action. Antimicrobial potential of polysaccharide extract was observed on several microorganisms of medicinal importance.

MATERIAL AND METHODS

A. bisporus Horst U1 fruiting bodies were obtained from the Mushroom Experimental station (Horst, The Netherlands). Crude polysaccharide extracts were prepared by hot water extraction as described before (Klaus et al., 2011). Polysaccharides were semi-purified by precipitation in 65% ethanol and repeated washing to remove the excess mannitol and ethanol-soluble phenolic compounds. The preciptitate was dried at 42 °C, in vacuum and stored for further use. The yield of extract was 3.7 ± 0.2 g/100g express on a dry weight basis of fruiting body.

Antioxidant activity assays

Antioxidant activities were investigated using *in vitro* assay systems: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capability and chelating ability on ferrous ions.

Scavenging capability for DPPH radicals

The assay was done according to the modified method of Ekanayake et al, (2005). In the first series the 2 ml of extract dissolved in dimethyl sulfoxide (DMSO) was mixed with 1 ml freshly prepared DMSO solution of 0.2 mM DPPH. In the second series, extract (2 ml) was mixed with 1 ml DMSO solution. Both series were placed in the dark at room temperature for 1 hour. The radical-scavenging activity was calculated as a percentage of DPPH discoloration using the equation: $[1-(A_i-A_j)/A_c]$ x100, where A_i was the absorbance of 2 ml extract mixed with 1 ml DPPH solution, A_j was the absorbance of 2 ml extract mixed with 1 ml DMSO solution and A_c was the absorbance of blank-2 ml of DMSO mixed with 1 ml of DPPH solution. Ascorbic acid, butylated hydroxytoluene (BHT) and α-tocopherol dissolved in DMSO were used as the positive control. The EC_{50} value (mg extract/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis**.** EC_{50} was calculated concerning concentration of 5 mg/ml at which extract reached maximum in DPPH radical scavenging activity.

Chelating ability on ferrous ions

Polysaccharide powder (0.1 to 20 mg/ml, 1 ml) in Milli-Q water was mixed with 3.7 ml of methanol and tested for Fe^{2+} chelating ability according to the method of Dinis et al., (1994). The Fe^{2+} was monitored by measuring the formation of ferrous iron-ferrozine complex at 562 nm. The lower the absorbance of the reaction mixture, the higher the Fe^{2+}-chelating ability. The EC_{50} value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50%. Citric acid and ethylenediaminetetraacetic acid (EDTA) were used for comparison.

Antimicrobial activity

Microorganisms
Antimicrobial activities of the crude polysaccharide extract of *A. bisporus* on Gram-positive *Enterococcus faecalis* ATCC 49532, *Bacillus cereus* 10876, *Geobacillus stearothermophylus* ATCC 7953 and Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 35032, *Proteus hauseri* ATCC 13315, *Escherichia coli* (0157:H7) 35150 and *Klebsiella pneumoniae* ATCC 27736 were investigated in this study. The bacteria were obtained from the culture collection of the Department for Industrial Microbiology, University of Belgrade - Faculty of Agriculture. All bacterial strains were stored at +4^{o}C on appropriate agar slants, subcultured every two weeks and checked for purity.

Screening of antimicrobial activity of extract by disc-diffusion test
The disk-diffusion method was applied for the evaluation of the antibacterial activities of the polysaccharide extract. The bacterial cells were washed from the surface of agar and suspended in sterile saline to a concentration of 1.0x10^{5} CFU/ml. Appropriate agar in Petri dish was seeded with 100 µl of bacterial suspension. The crude polysaccharide extract was dissolved in DMSO to a final concentration of 30 mg/ml and filter-sterilized through a 0.22 µm membrane filter. On the surface of the agar, the 6 mm filter discs were placed (three discs per agar plate). Ten microliters of the tested crude polysaccharide extract were added to the disc. Reference discs used for control contained penicillin and tetracycline. The plates with bacterial cultures were incubated overnight at 37^{o}C and the diameter of the resulting zone of inhibition was measured and compared with those of reference discs. Inhibitory activity of DMSO was also tested.

RESULTS AND DISCUSSION

The results of scavenging ability and chelating ability on ferrous ions of polysaccharide extract from of *A. bisporus* are shown on figure 1 and 2. As it can be seen, at concentrations of 0.1-10 mg/ml, the scavenging abilities of *A. bisporus* polisaccharide enriched extract on DPPH radicals were between 12.3-75.5% (Figure 1). At 0.1-20 mg/ml, the radical scavenging ability of the positive controls BHT, ascorbic acid and α-tocopherol were between 1.1-69.1, 80.6-91.1 and 79.9-80.8 %, respectively. The radical scavenging ability at of the extracts and positive controls, at 5 mg/ml decreased in the following order: ascorbic acid > α-tocopherol ≈ *A. bisporus* > BHT. EC_{50} values of the DPPH scavenging activity of the polysaccharides from champignon mushroom was 2.0 ± 0.18 mg/ml.

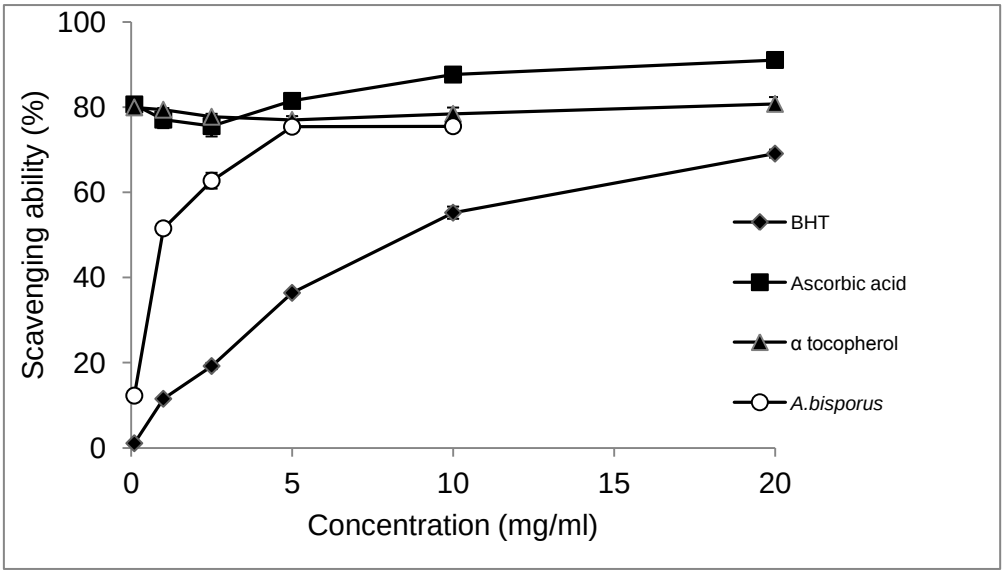

Figure 1. Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals of polysaccharide- enriched extract from of *A. bisporus*. Each value is expressed as mean ± standard deviation (*n* = 3).

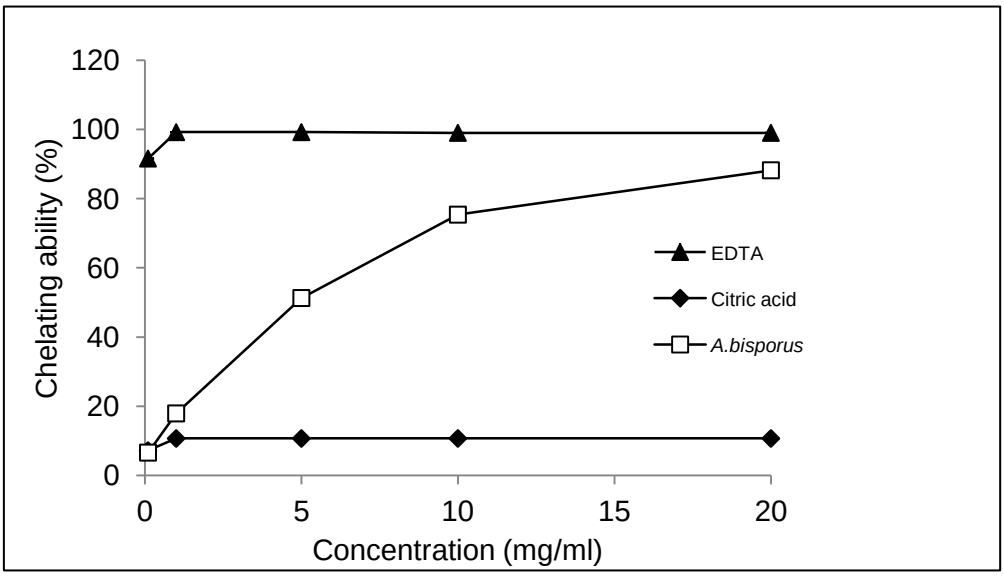

Figure 2. Chelating ability on ferrous ions of polysaccharide-enriched extract from of *A. bisporus*. Each value is expressed as mean ± standard deviation (*n* = 3).

Chelating effects of the polysaccharide extract from *A. bisporus* on ferrous ion increased with the increased concentrations (Figure 2). At 0.1-20 mg/ml, the chelating ability of *A. bisporus* polysaccharide extract was between 6.6-88.2%. The chelating effect of the synthetic metal chelator EDTA was between 91.6-99% at 0.1-20 mg/ml, while citric acid was not as good chelating agent for ferrous ions in this assay and its chelating ability was 10.7% at 20 mg/ml. EC_{50} values of the chelating ability of ferrous ions for *A.bisporus* extract was 7.80 ± 0.21 mg/ml.

The main mechanism of ferrous ion chelating activity is the ability of chelators to deactivate and/or chelate Fe^{2+} which can promote the Fenton reaction and hydroperoxide decomposition. Iron toxicity (high organ storage of iron) and/or high blood levels of iron are associated with an increased risk of free radical damage and cancer. The capability of iron to generate free radicals from peroxides by Fenton reactions has been implicated in

cardiovascular disease. Free radicals formed as a result of high iron can attack low-density lipoproteins (LDL) and subsequently lead to fatty plaque buildup, damage to the walls of arteries, as well as to heart muscle tissue. Chelation therapy may possibly reduce iron-related free radical damage and increase overall survival in cardiovascular disease (Halliwell and Gutteridge, 1990).
Factors affecting and/or attributing to chelating effects of polysaccharide-enriched extract from *A. bisporus* need to be further studied.

Antimicrobial activity of extract
As summarized in Table 1. tested Gram positive bacteria seemed to be more sensitive than Gram negative bacterial strains to the examined polysaccharide extract of *A. bisporus*. The most susceptible bacterium was *Enterococcus faecalis* ATCC 49532 (26.7 ± 0.2). Regarding the fact that all examined bacteria are serious pathogens, their sensitivity to the mushroom extract is of particular interest.

Table 1. Antibacterial acivity (inhibition zone measured in mm, including 6 mm filter discs) of the polysaccharide extract of *A. bisporus*

bacterial strain	***A. bisporus***	**P**	**T**
Enterococcus faecalis ATCC 49532	26.7 ± 0.2	12.6 ± 0.5	24.3 ± 0.6
Bacillus cereus ATCC 10876	27.5 ± 0.4	-	12.4 ± 0.6
Geobacillus stearothermophylus ATCC 7953	22.8 ± 0.3	-	17.6 ± 0.5
Pseudomonas aeruginosa ATCC 35032	10.4 ± 0.6	-	-
Proteus hauseri ATCC 13315	12.1 ± 0.1	19.8 ± 0.2	24.8 ± 0.7
Escherichia coli (0157:H7) ATCC 35150	12.7 ± 0.4	11.8 ± 0.3	13.2 ± 0.6
Klebsiella pneumoniae ATCC 27736	22.3 ± 0.2	15.4 ± 0.3	16.7 ± 0.1

P, penicillin; T, tetracycline; (–) no inhibition.
Each value is expressed as mean ± standard deviation (n = 3).

CONCLUSIONS

Measurements of antioxidant and antimicrobial properties of hot water polysaccharide extract of fruiting bodies of *A. bisporus* showed moderate antioxidant and strong antimicrobial activities. The antioxidant and antimicrobial compounds of the polysaccharide extract were resistant to high temperatures, even to a period of 45 min of boiling at 120ºC during the extraction process.
Champignon mushroom polysaccharides act as natural antioxidant. As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied.
In recent decades microorganisms are becoming resistant to antibiotics due to their excessive use in medicine, but in food industry, too. On the other hand, it is known that antibiotics can have adverse effects on the health. This study has shown that commonly used mushroom *A. bisporus* could be suitable as food preservative against food spoilage microorganisms, i.e. as antimicrobial agent in the food industry. Further investigations are necessary to verify these activities *in vivo.*

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REFERENCES

1. Bohn, J. A., BeMiller, J. N. (1995). (1–›3)-β-D-Glucan as biological response modifiers: A review of structure-functional activity relationships, *Carbohydrate Polymers 28*, 3-14.

2. Cappelli, A., (1984). Fungi Europaei: Agaricus, Libreria editrice Giovanna Biella, Saronno, Italy.
3. Chang, S.T. (1993). Mushroom biology: the impact on mushroom production and mushroom products. In S. W. Chiu, J. Buswell, & S. T. Chang, Mushroom Biology and Mushroom Products, pp. 3–20. Hong Kong: The Chinese University Press.
4. Chen, S., Oh, S. R., Phung, S., Hur, G., Ye, J. J., Kwok, S. L., Shrode, G. E., Belury, M., Adams, L. S., Williams, D. (2006). Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*), *Cancer Research, 66,* 12026–12034.
5. Dinis, T. C. P., Madeira, V. M. C., Almeida, L. M., (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives in Biochemistry and Biophysics, 315*, 161–169.
6. Ekanayake, P. M., Park, G., Lee, Y. D., Kim, S. J., Jeong, S. C., Lee, J. (2005). Antioxidant potential of Eel (*Anguilla Japonica* and *Conger Myriaster*) flesh and skin, *Journal of Food Lipids,12,* 34-47.
7. Grube, B. J., Eng, E. T., Kao, Y. C., Kwon, A., Chen, S. (2001). White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation, *Journal of Nutrition, 131***,** 3288–3293.
8. Halliwell, B., Gutteridge, J. M. C. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology, 186,* 1–85.
9. Klaus, A., Kozarski, M., Niksic, M. (2009). Influence of bioactive compounds extracted from mushroom *Ganoderma lucidum* on B and T cells, *Matica Srpska Proceedings for Natural Sciences, 116*, 17-223.
10. Klaus, A., Kozarski, M., Niksic, M., Jakovljevic, D., Todorovic, N., Van Griensven, L. J. L. D. (2011). Antioxidative activities and chemical characterization of polysaccharides extracted from the basidiomycete *Schizophyllum commune*, *LWT-Food Science and Technology 44*, 2005-2011.
11. Kozarski, M., Klaus, A., Niksic, M. (2009): Influence of structural features on immunostimulating activity of glucans extracted from *Agaricus blazei* mushroom. *Matica Srpska Proceedings for Natural Sciences, 116*, 225-233.
12. Kozarski, M., Klaus, A., Niksic, M., Jakovljevic, D., Helsper, P. F. G. J., Van Griensven L. J. L. D. (2011). Antioxidative and immunomodulating activities of polysaccharide extracts of the medicinal mushrooms *Agaricus bisporus, Agaricus brasiliensis, Ganoderma lucidum* and *Phelinus linteus, Food Chemistry 129*, 1667-1675.
13. Lee, I. H., Huang, R. L., Chen, C. T., Chen, H. C., Hsu, W. C., Lu, M. K. (2002). *Antrodia camphorate* polysaccharides exhibit antihepatitis B virus effects, *FEMS Microbiology Letters*, *209*, 61-65.
14. Ren, Z., Guo, Z., Meydani, S. N., Wu, D. (2008). White button mushroom enhances maturation of bone marrow-derived dendritic cells and their antigen presenting function in mice. *Journal of Nutrition,138,* 544–550.
15. Spencer, D. M. (1985). The mushroom–its history and importance. In P. B. Flegg, D. M. Spencer, & D. A. Wood, The Biology and Technology of the Cultivated Mushroom, pp. 1–8. New York: John Wiley and Sons.
16. Wu, D., Pae, M., Ren, Z., Guo, Z., Smith, D., Meydani, S. N. (2007). Dietary supplementation with white button mushroom enhances natural killer cell activity in C57BL/6 mice, *Journal of Nutrition, 137,* 1472–1477.
17. Yang, J. H., Du, Y. M., Huang, R. H., Sun, L. P., Liu, H., Gao, X. H., John, F. K. (2005). Chemical modification and antitumour activity of Chinese lacquer polysaccharide from lac tree *Rhus vemicifera. Carbohydrate polymers*, *59*, 101-107.
18. Yoon, S. J., Yu, M. A., Pyun, Y. R., Hwang, J. K., Chu, D. C., Juneja, L. R., Paulo, A. S. M. (2003). The nontoxic mushroom *Auricularia auricular* contains a polysaccharies with anticoagulant activity mediated by antithrombin, *Thrombosis Research*, *112*, 151-158.
19. Zhenga, R., Jiea, S., Hanchuanb, D., Mouchenga, W. (2005). Characterization and immunomodulating activities of polysaccharide from *Lentinus edodes. International Immunopharmacology*, *5*, 811– 820.