BIOACTIVITY OF EDIBLE MUSHROOM *AGARICUS CAMPESTRIS*

Marijana Kosanić[1], Branislav Ranković[1], Tatjana Stanojković[2]

Abstract: Here we determined antioxidant, antimicrobial and anticancer properties of edible mushroom *Agaricus campestris.* Antioxidant activity was evaluated by free radical scavenging, superoxide anion scavenging and reducing power. *A. campestris* extract had moderate free radical scavenging activity (IC_{50}=179.65 µg/mL) and potent superoxide anion scavenging potential (IC_{50}=35.204 µg/mL). Measured values of absorbance for reducing power varied from 0.3701 to 0.141. Further, the antimicrobial potential was determined by a microdilution method on 12 microorganisms. Extract of *A. campestris* showed relatively strong antimicrobial activity with minimum inhibitory concentration values ranging from 0.08 to 7.5 mg/mL. Finally, the cytotoxic activity was tested using MTT method on the human epithelial carcinoma Hela cells, human lung carcinoma A549 cells and human colon carcinoma LS174 cells. *A. campestris* extract expressed cytotoxic activity with IC_{50} values ranging from 9.54 to 16.87 µg/mL.

Key words: anticancer activity, antimicrobial activity, antioxidant activity, mushrooms.

Introduction

In many parts of the world, wild mushrooms are regularly collected and used directly as a main source of food or as a condiment. Today mushrooms are valuable not only because of the appealing texture and unique taste, but because of their important chemical and nutritional characteristics (Kalac, 2012).

Besides the important nutritional values, mushrooms also have a valuable health benefits. Their consumption has consistently been shown to have beneficial effects on human health. One of the noticeable features of many mushrooms is their ability to function as immunomodulators and thus to improve the resistance to disease. While much attention in recent years has focused on various immunological and anti-cancer properties of certain mushrooms, they also offer other potentially important health benefits, including antioxidants, anti-hypertensive and cholesterol-lowering properties, liver protection, as well as anti-inflammatory, anti-diabetic, anti-viral and anti-microbial properties (Mishra et al., 2015; Pandimeena et al., 2015; Kosanić et al., 2017). These properties have attracted the interest of many pharmaceutical companies, which are viewing the medicinal mushroom as a rich source of innovative biomedical molecules.

[1]Department of Biology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, Kragujevac, Serbia (marijanakosanic@yahoo.com);
[2]Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000 Belgrade, Serbia

Material and methods

Fungal materials

Fungal sample of *A. campestris* L.:Fr., was collected from Šumarice, Kragujevac, Serbia, in September of 2016. The demonstration samples are preserved in facilities of the Department of Biology and Ecology of Kragujevac, Faculty of Science. The determination of mushrooms was done using standard keys (Uzelac, 2009).

Finely dry ground thalli of the examined mushrooms (100 g) were extracted using methanol (500 mL) in a Soxhlet extractor. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at -18^0C until they were used in the tests. The extracts were dissolved in 5% dimethyl sulphoxide (DMSO) for the experiments (Kosanić et al., 2017). DMSO was dissolved in sterile distilled water to the desired concentration

Antioxidant activity

Antioxidant activity was evaluated by free radical scavenging, superoxide anion radical scavenging and reducing power. The free radical scavenging activity of lichen extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the Kosanić et al. method (2017). Oyaizu method (1986) was used to determine the reducing power. The superoxide anion radical scavenging activity was detected according to the Nishimiki et al. method (1972). The amount of total phenols in the lichen extracts was determined as pyrocatechol equivalent using Folin-Ciocalteu reagent according to Slinkard and Singleton's method (1997).

Antimicrobial activity

The sensitivity to extracts of the investigated species of microorganisms was tested by determining the minimal inhibitory concentration (MIC) by the broth microdilution method with using 96-well micro-titer plates (Sarker et al., 2007).

Cytotoxic activity

Human epithelial carcinoma Hela cells, human lung carcinoma A549 cells and human colon carcinoma LS174 cells were obtained from American Type Culture Collection (Manassas, VA, USA). All cancer cell lines were cultured as a monolayer in the RPMI 1640 nutrient medium, with 10% (inactivated at 56ºC) FBS, 3 mM of L-glutamine, and antibiotics, at 37ºC in humidified air atmosphere with 5% CO_2.

Stock solutions (50 mg ml^{-1}) of the extracts, made in DMSO, were dissolved in a corresponding medium to the required working concentrations. The final concentrations applied to the cells were 200, 100, 50, 25 and 12.5 μg ml^{-1}. In the control wells only the nutrient medium was added. The effect on cancer cell survival was determined 72 h after the addition of the extract, by the MTT test (Mosmann, 1983).

Results and discussion

The scavenging DPPH radicals, superoxide anion radical scavenging, reducing power and phenolics content of the studied extract are represented in Table 1 and Table 2. The IC_{50} values were 179.65 and 35.204 μg ml^{-1} for DPPH radicals and superoxide anion radicals scavenging activity, respectively. Measured values of absorbance for reducing power varied from 0.3701 to 0.141. The total phenolic compounds was determined as the pyrocatechol equivalent using an equation obtained from a standard pyrocatechol graph (y = 0.0057x - 0.1646, R_2 = 0.9934).

Table 1. DPPH radical scavenging activity, superoxide anion scavenging activity and phenolics content of extract of Agaricus campestris

Mushroom species	DPPH radical scavenging IC_{50} (μg ml^{-1})	Superoxide anion scavenging IC_{50} (μg ml^{-1})	Phenolics content (μg PE mg^{-1} of extract)
Agaricus campestris	179.65 ± 1.11	35.204 ± 3.08	50.57 ± 1.024
Ascorbic acid	6.42 ± 0.18	115.61 ± 1.16	

Table 2. Reducing power of extract of Agaricus campestris

	Absorbanca (700 nm)		
Mushroom species	1000 μg ml^{-1}	500 μg ml^{-1}	250 μg ml^{-1}
Agaricus campestris	0.3701 ± .031	0.195 ± .025	0.141 ± .008
Ascorbic acid	3.862 ± .992	2.113 ± .032	1.654 ± .021

Extract from *A. campestris* acted on all the microorganisms tested. The MIC fluctuated in a range of 0.08–0.16 mg ml^{-1} for bacteria and 0.4–7.5 mg ml^{-1} for fungi (Table 3).

Table 3. Minimum inhibitory concentration (MIC) of extract Agaricus campestris

Mushroom species	*A. campestris* (mg ml^{-1})	Streptomycin (mg ml^{-1})	Ketoconazole (mg ml^{-1})
Bacillus cereus	0.08	0.02	-
Bacillus subtilis	0.08	0.02	-
Eschrichia coli	0.16	0.06	-
Proteus mirabilis	0.16	0.03	-
Staphylococcus aureus	0.16	0.03	-
Trichophyton mentagrophytes	0.81	-	0.16
Geotrichum candidum	1.62	-	0.08
Paecilomyces variotii	0.81	-	0.16
Fusarium solani	1.62	-	0.16
Candida albicans	0.4	-	0.04
Aspergillus flavus	1.62	-	0.31
Penicillium italicum	7.5	-	0.16

The data obtained for anticancer effect of *A. campestris* extract are shown in Table 4. The IC_{50} against Hela, A549 and LS174 cell lines was 9.54, 16.87 and 12.84 μg ml^{-1} respectively.

Table 4. Growth inhibitory effect of Agaricus campestris on Hela, A549, and LS174 cell survival

Cell lines	Hela	A549	LS174
Mushroom species		IC_{50} (μg ml^{-1})	
Agaricus campestris	9.54 ± 0.63	16.87 ± 1.57	12.84 ± 2.23
Cis-DDP	0.86 ± 0.33	4.91 ± 0.42	3.18 ± 0.29

The extract tested had strong antioxidant activity against various oxidative systems in vitro. In this study, we used DPPH test just to verify that extracts exhibit antioxidant activity, and in future complex research we would use some other tests to explain the mechanisms of antioxidant activity**.** Several researchers explored antioxidant activity of *A. campestris* (Kosanić et al., 2017; Woldegiorgis et al., 2014) by using different extraction solvents. In this study, the antioxidant activity of selected mushroom was confirmed by acetone extracts. Depending on the polarity, different extraction solvents can extract different metabolites which contribute to the great antioxidant potential, which means that between antioxidative components in the extracts there is a synergistic effect, which leads to prominent antioxidant activity of mushrooms.

Similar to our results, numerous researchers found relatively strong antimicrobial activity for *A. campestris* (Giri et al., 2012; Kosanić et al., 2017). The probable mechanisms of antimicrobial action of tested mushroom are inhibition of cell wall synthesis, protein synthesis, or nucleid acid synthesis, like antibiotics, but less effective. In our experiments, the examined mushroom in the same concentrations showed a stronger antibacterial than antifungal activity. This different sensitivity between the fungi and bacteria is probably due to the different permeability of their cell walls. The cell wall of the gram-positive bacteria consists of mureins and teichoic acids, the gram-negative bacteria consist of lipopolysaccharides and lipopoliproteins, while the cell wall of fungi consists of hitchin and glucan (Kosanić et al., 2017).

Numerous findings suggest that some mushrooms in combination with commercial anti-cancer drugs work in synergy as an effective tool for treating drug-resistant cancers. Mushrooms also are known to complement chemotherapy and radiation therapy by countering the side-effects of cancer, such as nausea, bone marrow suppression, anemia, and lowered resistance (Patel and Goyal, 2012). The evidences from various researchers across the globe, regarding anti-tumor application of mushroom extracts unarguably make it a fast-track research area worth mass attention.

Results of our study promise that the *A. campestris* extract can be very effective therapy for malignant cancer cells. *A. campestris* was previously tested on cytotoxic activity by several researchers. For instance, Kosanić et al. (2017) shown that methanol extract from *A. campestris* has anticancer activity. Also, Li et al. (2005) found antitumor activities of different solvent extracts of *A. campestris.* The mechanism of action of the tested extracts is yet to be tested. For further research is necessary to isolate active anticancer compounds from this extract.

Conclusion

The present investigation can be concluded that the tested mushroom appear to be good natural antioxidant, antimicrobial and anticancer agent. The identification of active antioxidant, antimicrobial and anticancer compounds of this mushroom species can lead to their potential commercial usage in food production, medicine, and the cosmetic industry.

Acknowledgment

This work was financed in part by the Ministry of Science, Technology, and Development of the Republic of Serbia (projects no. 173032 and 175011).

References

Giri, S., Biswas G., Pradhan P., Mandal S.C., Acharya K. (2012). Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. International Journal of PharmTech Research. 4: 1554-1560.

Kalac P. (2012). Chemical composition and nutritional value of European species of wild growing mushrooms. In *Mushrooms: types, properties and nutrition*, S. Andres and N. Baumann. (ed.), 130-151. Nova Science Publishers.

Kosanić M., Ranković B., Rančić A., Stanojković T. (2017). Evaluation of metal contents and bioactivity of two edible mushrooms *Agaricus campestris* and *Boletus edulis.* Emirates Journal of Food and Agriculture. doi:10.9755/ejfa.2016-06-656

Li S., Chen G., Bi Y. (2005). Studies on antioxidative and Antitumor Activities for Two Wild Edible Fungi. Edible Fungi of China. 3: 58-63.

Mishra K.K., Pal R.S., Bhatt J.C. (2015). Comparison of antioxidant properties in cap and stipe of *Lentinula edodes* – a medicinal mushroom. Emirates Journal of Food and Agriculture. 27: 562-569.

Mosmann T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65:55-63.

Nishimiki M., Rao N.A., Yagi K. (1972). The occurrence of super-oxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications. 46:849-853.

Oyaizu M. (1986). Studies on products of browning reaction prepared from glucoseamine. Japanes Journal of Nutrition. 44:307-314.

Pandimeena M., Prabu M., Sumathy R., Kumuthakalavalli R. (2015). Evaluation of Phytochemicals and in vitro Anti-Inflammatory, Anti Diabetic Activity Of The White Oyster Mushroom, *Pleurotus florida*. International Research Journal of Pharmaceutical and Applied Sciences. 5:16-21.

Patel S., Goyal A. (2012). Recent developments in mushrooms as anti-cancer therapeutics: a review. 3 Biotech. 2: 1-15.

Sarker S.D., Nahar L., Kumarasamy Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods. 42:321-324.

Slinkard K., Slingleton V.L. (1997). Total phenolic analyses: automation and comparison with manual method. American Journal of Enology and Viticulture. 28: 49-55.

Uzelac B. (2009). Gljive Srbije i Zapadnog Balkana. Beograd: BGV Logik.

Woldegiorgis A.Z., Abate D., Haki G.D., Ziegler G.R. (2014). Antioxidant property of edible mushrooms collected from Ethiopia. Food Chemistry. 157: 30-36.