BIOACTIVITY OF EDIBLE MUSHROOMS

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Abstract: Here we evaluated antioxidant and antimicrobial activity of the acetone and methanol extracts of the mushrooms *Amanita rubescens* and *Russula cyanoxantha*. Antioxidant activity was evaluated by free radical scavenging, reducing power activity and determination of phenolic content. As a result of the study acetone extracts from *Russula cyanoxantha* was more powerful antioxidant activities (IC_{50} = 86.279 μg/ml). Moreover, the tested extracts had effective reducing power. Total content of phenol in extracts was determined spectrophotometrically. The antimicrobial activity was estimated by determination of the minimal inhibitory concentration by using microdilution plate method. Generally, the tested mushroom extracts had relatively strong antimicrobial activity against the tested microorganisms.

Key words: Mushroom extracts; Antioxidant activity; Antimicrobial activity.

Introduction

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. At normal physiological concentrations ROS are required for cellular activities, however, at higher concentrations, ROS can cause extensive damage to cells and tissues, during infections and various degenerative disorders (Gulcin et al., 2004).

Antioxidants, both synthetic or natural, can be effective to help the human body in reducing oxidative damage by ROS. However, at the present time, suspected that synthetic antioxidants have toxic and carcinogenic effects (Kosanić et al., 2011). Therefore, the development and utilization of more effective antioxidants of natural origins are desired. In order to find new natural sources of antioxidants, our attention was focused on mushrooms.

Mushrooms possess high contents of qualitative protein, crude fibre, minerals and vitamins. Apart from their nutritional potentials, mushrooms are also sources of physiologically beneficial bioactive substances that promote good health. They produce a wide range of secondary metabolites with high therapeutic value. Health promoting properties, e.g. antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects, have been reported for some species of mushrooms. Both fruiting bodies and the mycelium contain compounds with wide ranging antioxidant and antimicrobial activities (Barros et al., 2007). Because of that, the aim of this study is to examine *in vitro* antioxidant and antimicrobial activity of the acetone and methanol extract of the mushrooms *A. rubescens* and *R. cyanoxantha.*

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Material and methods

Mushroom samples

Fungal samples of *A. rubescens* (Pers. ex Fr) Gray., and *R. cyanoxantha* (Schaeff.) Fr., were collected from Kopaonik, Serbia, in June of 2014. The demonstration samples are preserved in facilities of the Department of Biology and Ecology of Kragujevac, Faculty of Science. Determination of mushrooms was done using standard methods.

Extraction

Fresh fungal material was milled by an electrical mill. Finely ground mushrooms (50 g) were extracted using acetone and methanol for 24 h. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at -18°C until used in the tests. The extracts were dissolved in 5% dimethyl sulphoxide (DMSO).

Antioxidant activity

Antioxidant activity was evaluated by free radical scavenging, reducing power and determination of total phenolic compounds. The free radical scavenging activity of lichen extracts was measured by1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the Dorman et al's method (2004). The Oyaizu method (1986) was used to determine the reducing power. The amount of total phenols was determined as a pyrocatechol equivalent using Folin-Ciocalteu reagent according to the Slinkard and Singleton's method (1997).

Antimicrobial activity

The following bacteria were used as test organisms in this study: *Staphilococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212). All the bacteria used were obtained from the American Type Culture Collection (ATCC). The fungi used as test organisms were: *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (DBFS 310), *Candida albicans* (IPH 1316), *Paecilomyces variotii* (ATCC 22319), *Penicillium purpurescens* (DBFS 418). They were from the from the American Type Culture Collection (ATCC) and the mycological collection maintained by the Mycological Laboratory within the Department of Biology of Kragujevac, University's Faculty of Science (DBFS). Bacterial cultures were maintained on Müller-Hinton agar substrates (Torlak, Belgrade). Fungal cultures were maintained on potato dextrose (PD) agar and Sabourad dextrose (SD) agar (Torlak, Belgrade). All cultures were stored at 4°C and subcultured every 15 days.

Bacterial inoculi were obtained from bacterial cultures incubated for 24 h at 37°C on Müller-Hinton agar substrate and brought up by dilution according to the 0.5 McFarland standard to approximately 10^8 CFU/ml. Suspensions of fungal spores were prepared from fresh mature (3- to 7-day-old) cultures that grew at 30°C on a PD agar

substrate. Spores were rinsed with sterile distilled water, used to determine turbidity spectrophotometriacally at 530 nm, and then further diluted to approximately 10^6 CFU/ml according to the procedure recommended by NCCLS (1998).

The MIC was determined by the broth microdilution method using 96-well micro-titer plates (Sarker et al., 2007). A series of dilutions with concentrations ranging from 40 to 0.156 mg/ml for extracts were used in experiment against every microorganism tested. The starting solutions of extracts were obtained by measuring off a certain quantity of extract and dissolving it in DMSO. Two-fold dilutions of extracts were prepared in Müller-Hinton broth for bacterial cultures and SD broth for fungal cultures. The MIC was determined by establishing visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration.

Statistical analyses

Statistical analyses were performed with the SPSS software packages. All values are expressed as mean $\pm$ SD of three parallel measurements.

Results and discussion

The scavenging DPPH radicals of the studied extracts are shown in Table 1. The inhibition concentration at 50 % inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. The IC_{50} values of acetone extracts were 86.279 µg/ml for *Russula cyanoxantha* and 114.21 µg/ml for *Amanita rubescens*. IC_{50} for the methanol extracts were 185.70 µg/ml for *Amanita rubescens,* and 262.08 µg/ml for *Russula cyanoxantha*. The results of the reducing power assay of tested extracts are summarized in Table 2. Extracts of *Amanita rubescens* showed highest reducing power than *Russula cyanoxantha*. Total phenolic constituents of tested extracts are presented in Table 3. The amount of total phenolic compounds was determined as the pyrocatechol equivalent using an equation obtained from a standard pyrocatechol graph (Absorbance = 0.0021 x total phenols [µg PE/mg of dry extracts] - 0.0092). Results of the study showed that the phenolic compounds ranged from 4.55 to 5.23 µg PE/mg.

The antimicrobal activity of the tested mushrooms extracts against the tested microorganisms was shown in Table 4. The MIC for both extracts related to the tested bacteria and fungi were 1.25 - 10 mg/ml. Generally, the acetone extracts exerted stronger antimicrobial activity than methanol extracts. The maximum antimicrobial activity was found in the acetone extract of the mushrooms *Lactarius piperatus* against *Enterococcus foecalis* (MIC = 1.25 mg/ml).

Free radical scavenging action is one of the numerous mechanisms for antioxidation (Sini and Devi, 2004). Antiradical activity was studied by screening its possibility to bleach the stable DPPH radical. This method is based on the formation of non-radical form DPPH-H in the presence of alcoholic DPPH solution and hydrogen donating antioxidant (AH) by the reaction DPPH + AH → DPPH-H + A (Anandjiwala et al., 2008). The reducing power of a component may indicate their potential antioxidant activity. The reducing features are mainly related with the presence of reductones. Gordan et al. (1990) found that the

antioxidant effect of reductones is based on the destruction of the free radical chain by donating a hydrogen atom. Phenolic components are potential antioxidants (Shahidi and Wanasundara, 1992). Phenolic compounds can donate hydrogen to free radicals and this way to stop the chain reaction of lipid oxidation at the initial stage.

Table 1. IC_{50} values of acetone and methanol extracts of Amanita rubescens and Russula cyanoxantha

	IC_{50} (µg/ml)		
Samples Extracts	*Amanita rubescens*	*Russula cyanoxantha*	Ascor. acid
Acetone	114.21	86.279	4.22
Methanol	185.70	262.08	

Table 2. Reducing power of acetone and methanol extracts of Amanita rubescens and Russula cyanoxantha

		Absorbance (700 nm)		
Samples	Extracts	1000 µg/ml	500 µg/ml	250 µg/ml
A. rubescens	Acetone	0.0108	0.0023	0.0010
	Methanol	0.0247	0.0036	0.0023
R. cyanoxantha	Acetone	0.0072	0.0063	0.0047
	Methanol	0.0109	0.0039	0.0027
Ascorbic acid		0.2226	0.0957	0.0478

Table 3. Total phenolic content of acetone and methanol extracts of Amanita rubescens and Russula cyanoxantha

Samples	Extracts	Phenolics content µg of pyrocatechol equivalent
A. rubescenss	Acetone	4.86 ± 1.065
	Methanol	5.22 ± 1.208
R. cyanoxantha	Acetone	5.23± 1.223
	Methanol	4.55± 1.118

In the literature there are several data for the antioxidant activity of tested mushrooms. For example, Ribeiro et al. (2008) found antioxidant activity for *Amanita rubescens* and *Russula cyanoxantha.*

Numerous mushrooms were screened for antimicrobial activity in search of the new antimicrobial agents (Gezer et al., 2006; Ramesh et al., 2010). It found that different species of mushrooms exhibit different antimicrobial activity. These differences in antimicrobial activity of different species of mushrooms are probably a consequence of the presence of different components with antimicrobial activity.

Table 4. Minimum inhibitory concentration (MIC) of acetone and methanol extracts of Amanita rubescens and Russula cyanoxantha

Samples	*A. rubescens*		*R. cyanoxantha*		S - K	
Test organisms	A	M	A	M		
E. foecalis	2.5	5	5	5	15.62	-
E. coli	5	10	10	10	31.25	-
K. pneumoniae	2.5	5	2.5	5	1.95	-
P. aeruginosa	2.5	5	5	5	15.62	-
S. aureus	5	5	5	10	31.25	-
A. flavus	5	10	10	10	-	3.9
A. fumigatus	5	10	10	10	-	3.9
C. albicans	5	5	5	10	-	1.95
P. purpurescens	10	10	10	10	-	3.9
P. verrucosum	5	5	10	5	-	3.9

Values given as mg/ml for extract and as μg/ml for antibiotics.
Antibiotics: K – ketoconazole, S – streptomycin

In our experiments, the examined mushroom in the same concentrations showed a stronger antibacterial than antifungal activity. These results could be expected due to the fact that numerous tests proved that bacteria are more sensitive to the antibiotic compared to fungi (Hugo and Russell, 1983). The reason for different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall (Yang and Anderson, 2001). The cell wall of the gram-positive bacteria consists of peptidoglucans (mureins) and teichoic acids, while the cell wall of the gram-negative bacteria consists of lipopolysaccharides and lipopoliproteins, whereas, the cell wall of fungi consists of polysaccharides such as hitchin and glucan.

Conclusion

In conclusion, it can be stated that tested mushroom extracts have a strong antioxidant and antimicrobial activity *in vitro*.

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