

ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF MUSHROOMS

M. KOSANIC^{1*}, B. RANKOVIC¹ and M. DASIC¹

¹University of Kragujevac, Department of Biology, Faculty of Science, 34000 Kragujevac, Serbia

Abstract

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The aim of the study is to examine *in vitro* antioxidant and antimicrobial activity of the acetone and methanol extracts of the mushrooms *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*. Antioxidant activity was evaluated by four different methods: free radical scavenging, reducing power, determination of total phenolic compounds and determination of total flavonoid content. As a result of the study acetone extracts from *Russula cyanoxantha* was more powerful antioxidant activities than other examined mushroom extracts ($IC_{50} = 86.279 \mu\text{g/ml}$). Moreover, the tested extracts had effective reducing power. Total content of phenol and flavonoid in extracts were determined as pyrocatechol equivalent, and as rutin equivalent, respectively. The antimicrobial activity was estimated by determination of the minimal inhibitory concentration by using microdilution plate method against five species of bacteria and five species of fungi. Generally, the tested mushroom extracts had relatively strong antimicrobial activity against the tested microorganisms. The present study shows that tested mushroom species demonstrated a strong antioxidant and antimicrobial activity. It suggests that mushroom may be used as good sources of natural antioxidants and for pharmaceutical purposes in treating of various diseases.

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, such as cardiovascular disease, aging, and neurodegenerative diseases like Alzheimer's disease, mutations and cancer (Gulcin et al., 2004; Sachindra et al., 2010).

Antioxidants, both synthetic or natural, can be effective to help the human body in reducing oxidative damage by ROS (Kosanić et al., 2011). However, at the present time, suspected that synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) have toxic and carcinogenic effects (Zhang et al., 2009). Therefore, the development and utilization of more effective antioxidants of natural origins are desired. In recent years, the antioxidant properties of numerous plants, lichens and mushrooms have been widely reported. 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 wideranging antioxidant and antimicrobial activities (Oyetayo et al., 2009; Mau et al., 2004; Barros et al., 2007; Ferreira 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 *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*.

Materials and Methods

Mushroom samples

Fungal samples of *Amanita rubescens* (Pers. ex Fr) Gray., *Cantharellus cibarius* Fr., *Lactarius piperatus* (L.) Pers., and *Russula cyanoxantha* (Schaeff.) Fr., were collected from Ko-

*Corresponding author: marijanakosanic@yahoo.com

paonik, Serbia, in June of 2010. 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

Scavenging DPPH radicals

The free radical scavenging activity of mushrooms extracts was measured by using 1,1-diphenyl-picryl-hydrazil (DPPH). The method used was almost the same as the one used by other authors (Ibanez et al., 2003; Dorman et al., 2004), but was modified in details. 2 ml of methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1 ml of extract were placed in cuvettes. The mixture was shaken vigorously and left to stay at room temperature for 30 min. After that, the absorbance was measured at 517 nm in spectrophotometer ("Jenway" UK). Ascorbic acid, butylated hydroxyanisole (BHA) and α -tocopherol were used as positive control. The DPPH radical concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1)/A_0) \times 100,$$

where A_0 is the absorbance of the negative control and A_1 is the absorbance of reaction mixture or standards.

The inhibition concentration at 50 % inhibition (IC_{50}) was the parameter used to compare the radical scavenging activity. A lower IC_{50} meant better radical scavenging activity.

Reducing power

The reducing power of extracts was determined by the method of Oyaizu (1986). 1 ml of extracts were mixed with 2.5 ml of phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated at 50°C for 20 min. After that, trichloroacetic acid (10%, 2.5 ml) was added to the mixture and centrifuged. Finally, the upper layer was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% ferric chloride ($FeCl_3$). The absorbance of the solution was measured at 700 nm in spectrophotometer. Higher absorbance of the reaction mixture indicated that the reducing power is increased. Ascorbic acid, BHA and α -tocopherol were used as positive control.

Determination of total phenolic compounds

Total soluble phenolic compounds in the mushrooms extracts were determined with Folin-Ciocalteu reagent according to the method of Slinkard and Slingleton (1997) using pyrocatechol as a standard phenolic compound. Briefly, 1ml of the extract (1 mg/ml) in a volumetric flask diluted with distilled water (46 ml). One milliliter of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min 3 ml of 2% sodium carbonate (Na_2CO_3) was added and then was left to stay for 2h with intermittent shaking. The absorbance was measured at 760 nm in spectrophotometer. The total concentration of phenolic compounds in the extract was determined as microgram of pyrocatechol equivalent (PE) per milligram of dry extracts by using an equation that was obtained from standard pyrocatechol graph as

$$\text{Absorbance} = 0.0021 \times \text{total phenols} [\mu\text{g PE/mg of dry extracts}] - 0.0092 \quad (R^2 = 0.9934)$$

Total flavonoid content

The total flavonoid content was determined by using the Dowd method (Meda et al., 2005). Two ml of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (1 mg/ml). The mixture was incubated at room temperature for 10 min, and the absorbance was measured at 415 nm in spectrophotometer against blank samples. The total flavonoid content was determined as microgram of rutin equivalent (RE) per milligram of dry extracts by using an equation that was obtained from standard rutin graph as

$$\text{Absorbance} = 0.0144 \times \text{total flavonoid} [\mu\text{g RE/mg of dry extracts}] + 0.0556 \quad (R^2 = 0.9992)$$

Antimicrobial activity

Microorganisms and media

The following bacteria were used as test organisms in this study: *Staphylococcus 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). Their identification was confirmed at the Microbiological Laboratory of Kragujevac, University of Kragujevac, Department of Biology. 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.

The sensitivity of microorganisms to acetone and methanol extracts of the examined species of mushrooms was tested by determining the minimal inhibitory concentration (MIC).

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 10⁸ 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 spectrophotometrically at 530 nm, and then further diluted to approximately 10⁶ CFU/ml according to the procedure recommended by NCCLS (1998).

Minimal inhibitory concentration

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. As a positive control of growth inhibition, streptomycin was used in case of bacteria, ketoconazole in case of fungi. A DMSO solution was used as a negative control. All experiments were performed in triplicate.

Statistical analyses

Statistical analyses were performed with the SPSS software packages. To determine the statistical significance of antioxidant activity, student's t-test was used. All values are expressed as mean \pm SD of three parallel measurements.

Results

Antioxidant activity

The scavenging DPPH radicals of the studied extracts are shown in Table 1. The inhibition concentration at 50 % inhibition (IC₅₀) was the parameter used to compare the radical scavenging activity. A lower IC₅₀ meant better radical scavenging activity. Acetone and methanol extracts of the tested mushrooms showed a good scavenging activity on DPPH radical. There was statistically significant difference between extracts and control (P < 0.05). The IC₅₀ values of all extracts ranged from 86.279 – 262.08 µg/ml. Acetone extract from *Russula cyanoxantha* showed largest DPPH radical scavenging activities (IC₅₀ = 86.272 µg/ml) than those from other samples and nearly as α -tocopherol. The scavenging activity was also good for the acetone extracts from *Lactarius piperatus* (IC₅₀ = 99.197 µg/ml). Methanol extracts from tested mushrooms showed weaker DPPH radical scavenging activities than acetone. IC₅₀ for the methanol extracts were 185.70 µg/ml for *Amanita rubescens*, 192.57 µg/ml for *Cantharellus cibarius*, 172.80 µg/ml for *Lactarius piperatus* and 262.08 µg/ml for *Russula cyanoxantha*.

The results of the reducing power assay of tested extracts are summarized in Table 2. High absorbance indicates high reducing power. Measured values of absorbance varied from 0.0010 to 0.0538. The reducing power of extracts increased concentration dependently. Among the tested extracts, acetone extracts of *Lactarius piperatus* showed highest reducing power, followed by methanol extracts from *Cantharellus cibarius*. Other extracts showed weaker reducing power.

Total phenolic and flavonoid 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. Results of the study showed that the phenolic compound of the tested extracts varied from 4.55 to 5.32 µg PE/mg. Highest phenolic compounds was identified in methanol extract of *Lactarius piperatus* at a 5.32 µg PE/mg, followed by acetone extract of *Russula cyanoxantha* with 5.23 µg PE/mg.

The amount of total flavonoid compounds was determined as the rutin equivalent using an equation obtained from a standard

Table 1

IC₅₀ values of acetone and methanol extracts of *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*

Samples Extracts	IC ₅₀ (µg/ml)				Ascor. acid	BHA	α -tocoph.
	<i>Amanita rubescens</i>	<i>Cantharellus cibarius</i>	<i>Lactarius piperatus</i>	<i>Russula cyanoxantha</i>			
Acetone	114.21	158.40	99.197	86.279	4.22	6.42	62.43
Methanol	185.70	192.57	172.80	262.08			

rutin graph. As shown in Table 3, good flavonoid content was found in the methanol extract of *Lactarius piperatus* (2.81 µg RE/mg). Other lichen extracts showed lower flavonoid content.

Antimicrobial activity

The antimicrobial activity of the tested mushrooms extracts against the tested microorganisms was shown in Table 4.

Table 2

Reducing power of acetone and methanol extracts of *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*

Samples	Extracts	Absorbance (700 nm)		
		1000 µg/ml	500 µg/ml	250 µg/ml
<i>A. rubescens</i>	Acetone	0.0108	0.0023	0.0010
	Methanol	0.0247	0.0036	0.0023
<i>C. cibarius</i>	Acetone	0.0068	0.0038	0.0031
	Methanol	0.0436	0.0070	0.0050
<i>L. piperatus</i>	Acetone	0.0538	0.0224	0.0145
	Methanol	0.0139	0.0021	0.0037
<i>R. cyanoxantha</i>	Acetone	0.0072	0.0063	0.0047
	Methanol	0.0109	0.0039	0.0027
Ascorbic acid		0.2226	0.0957	0.0478
BHA		0.3465	0.1681	0.1651
α-tocopherol		0.2887	0.1651	0.0808

Table 3

Total phenolics and flavonoid content of acetone and methanol extracts of *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*

Samples	Extracts	Phenolics content µg of pyrocatechol equivalent		Flavonoid content µg of rutin equivalent	
<i>A. rubescens</i>	Acetone	4.86 ± 1.065		1.48 ± 1.099	
	Methanol	5.22 ± 1.208		1.65 ± 1.105	
<i>C. cibarius</i>	Acetone	4.82 ± 1.211		1.46 ± 1.195	
	Methanol	4.70 ± 1.318		1.49 ± 1.128	
<i>L. piperatus</i>	Acetone	4.93 ± 1.341		1.53 ± 1.213	
	Methanol	5.32 ± 1.208		2.81 ± 1.106	
<i>R. cyanoxantha</i>	Acetone	5.23 ± 1.223		1.55 ± 1.312	
	Methanol	4.55 ± 1.118		1.44 ± 1.254	

Table 4

Minimum inhibitory concentration (MIC) of acetone and methanol extracts of *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha*

Samples	<i>A. rubescens</i>		<i>C. cibarius</i>		<i>L. piperatus</i>		<i>R. cyanoxantha</i>		S	-	K
	A	M	A	M	A	M	A	M			
<i>E. faecalis</i>	2.5	5	5	5	1.25	2.5	5	5	15.62	-	
<i>E. coli</i>	5	10	10	10	2.5	5	10	10	31.25	-	
<i>K. pneumoniae</i>	2.5	5	5	5	2.5	2.5	2.5	5	1.95	-	
<i>P. aeruginosa</i>	2.5	5	5	5	2.5	2.5	5	5	15.62	-	
<i>S. aureus</i>	5	5	5	10	2.5	2.5	5	10	31.25	-	
<i>A. flavus</i>	5	10	10	10	5	5	10	10	-		3.9
<i>A. fumigatus</i>	5	10	10	10	5	5	10	10	-		3.9
<i>C. albicans</i>	5	5	5	10	2.5	5	5	10	-		1.95
<i>P. purpurescens</i>	10	10	10	10	5	5	10	10	-		3.9
<i>P. verrucosum</i>	5	5	5	5	2.5	5	10	5	-		3.9

^a Minimum inhibitory concentration (MIC); values given as mg/ml for extract and as µg/ml for antibiotics. Values are the mean of three replicate Antibiotics: K – ketoconazole, S – streptomycin

The acetone and methanol extracts of the tested mushrooms showed relatively strong antimicrobial activity. 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 faecalis* (MIC = 1.25 mg/ml). The measured MIC values for *Lactarius piperatus* against bacteria were 1.25 - 5 mg/ml for the acetone and 2.5 - 5 mg/ml for the methanol extract. Acetone extract of this mushroom inhibited the tested fungi in concentrations 2.5 mg/ml and 5 mg/ml, while the methanol extracts inhibited all fungi in concentrations 5 mg/ml.

The acetone and methanol extract of *Amanita rubescens*, *Cantharellus cibarius* and *Russula cyanoxantha* had approximately equal antimicrobial activity. They inhibited the tested bacteria and fungi in concentrations 2.5 mg/ml, 5 mg/ml and 10 mg/ml.

The antimicrobial activities were compared to streptomycin (standard antibiotic) and ketoconazole (standard antimicrobial). The results showed that streptomycin and ketoconazole had stronger activity than tested extracts as shown in Table 4. In a negative control, 5% DMSO had no inhibitory effect on the tested organisms.

Discussion

DPPH radical scavenging, reducing power, determination of total phenolic compounds and determination of total flavonoid content of the acetone and methanol extracts of the mushrooms *Amanita rubescens*, *Cantharellus cibarius*, *Lactarius piperatus* and *Russula cyanoxantha* were examined in this study.

Free radical scavenging action is one of the numerous mechanisms for antioxidation (Sini and Devi, 2004). Antiradical activity of lichen extracts 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 $\text{DPPH} + \text{AH} \rightarrow \text{DPPH-H} + \text{A}$ (Koleva et al., 2002; 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.

The reduction of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is measured by the strength of the green-blue color of solution, which absorbs at 700 nm. The result presented here indicates

that the marked ferric reducing power activity of extract to be due to presence of polyphenols which may act in a similar way as reductones react with free radicals to turn them into more stable products and abort free radical chain reactions (Sasikumar et al., 2010).

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. This ability of phenolic compounds to scavenge radicals comes due to the presence of their phenolic hydroxyl groups (Sawa et al., 1999). Flavonoids are widely group of natural compounds and the most important natural phenolics. These compounds have a large number of biological and chemical activities including radical scavenging properties (Ghafar et al., 2010).

The tested mushrooms extracts have a strong antioxidant activity against various oxidative systems *in vitro*. The intensity of antioxidant activity depended on the tested mushroom species and the solvent, which was used for extraction. The differences in the antioxidant activity of various solvents may be result of different capabilities to extract bioactive substances (Behera et al., 2005).

In the literature, there are several data for the antioxidant activity of tested mushrooms. For example, Ozen et al. (2011) found antioxidant activity for *Lactarius piperatus*. Similar results were reported for *Amanita rubescens* and *Russula cyanoxantha* (Ribeiro et al., 2008). Ramesh et al. (2010) find an antioxidant activity for *Cantharellus cibarius*. Other researchers (Ferreira et al., 2007; Choi et al., 2006; Alvarez Parrilla et al., 2007; Murci et al., 2002) also studied antioxidant activity of some other mushrooms.

Numerous mushrooms were screened for antimicrobial activity in search of the new antimicrobial agents (Ramesh et al., 2010; Gezer et al., 2006; Turkoglu et al., 2007; Mercan et al., 2006). 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.

In our experiments, the tested mushroom extracts show a relatively strong antimicrobial activity. The intensity of the antimicrobial effect depended on the species of mushroom, its concentration and the tested organism. 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 peptidoglycans (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 chitin and glucan (Jean Van, 2001; Farkas, 2003).

Conclusion

In conclusion, it can be stated that tested mushroom extracts have a strong antioxidant and antimicrobial activity *in vitro*. Based on these results, mushrooms appear to be good natural sources of antioxidants and could be of significance in human therapy, animal and plant diseases. Further studies should be done on the isolation and characterization of new compounds from mushrooms, which are responsible for antioxidant and antimicrobial activity.

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References

- Alvarez, Parrilla E., G. A. González Aguilar, L. A. de la Rosa and N. R. Martínez, 2007. Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico. *Ciencia e Tecnología de Alimentos*, **5**: 329-334.
- Anandjiwala, S., M. S. Bagul, M. Parabia and M. Rajani, 2008. Evaluation of free radical scavenging activity of an ayurvedic formulation, Panchvalkala. *Indian Journal of Pharmaceutical Sciences*, **70**: 31-35.
- Barros, L., R. C. Calhella, J. A. Vaz, I. C. F. R. Ferreira, P. Baptista and L. M. Estevinho, 2007. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, **225**: 151-156.
- Behera, B. C., N. Verma, A. Sonone and U. Makhija, 2005. Antioxidant and antibacterial activities of lichen *Usnea ghattensis* *in vitro*. *Biotechnology Letters*, **27**: 991-995.
- Choi, Y., S. M. Lee, J. Chun, H. B. Lee and J. Lee, 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shitake (*Lentinus edodes*) mushroom. *Food Chemistry*, **99**: 381-387.
- Dorman, H. J., O. Bachmayer, M. Kosar and R. Hiltunen, 2004. Antioxidant properties of aqueous extracts from selected Lamiales species grown in Turkey. *Journal of Agricultural and Food Chemistry*, **52**: 762-770.
- Farkas, V., 2003. Structure and biosynthesis of fungal cell walls: Methodological approaches. *Folia Microbiologica*, **48**: 469-478.
- Ferreira, I. C. F. R., P. Baptista, M. Vilas-Boas and L. Barros, 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chemistry*, **100**: 1511-1516.
- Gezer, K., M. E. Duru, I. Kivrak, A. Turkoglu, N. Mercan, H. Turkoglu and S. Gulcan, 2006. Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *African Journal of Biotechnology*, **5**: 1924-1928.
- Ghafar, M. F. A., P. K. Nagendra, K. K. Weng and A. Ismail, 2010. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from Citrus species. *African Journal of Biotechnology*, **9**: 326-330.
- Gordan, M. H., 1990. Food antioxidants. Elsevier, London, New York, 18 pp.
- Gulcin, I., O. I. Kurfrevioglu, M. Oktay and M. E. Buyukokuroglu, 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *Journal of Ethnopharmacology*, **90**: 205-215.
- Hugo, W. B. and A. D. Russell, 1983. Pharmaceutical microbiology, 3rd edition, (Blackwell Scientific Publications). 470 pp.
- Ibanez, E., A. Kubatova, F. J. Senorans, S. Caverro, G. Reglero and S. B. Hawthorne, 2003. Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry*, **51**: 375-382.
- Jean Van, H., 2001. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology*, **11**: 25-36.
- Koleva, I. I., T. A. van Beek, J. P. Linsen, A. de Groot and L. N. Evstatieva, 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis*, **13**: 8-17.
- Kosanić, M., B. Ranković and J. Vukojević, 2011. Antioxidant properties of some lichen species. *Journal of Food Science and Technology*, **48**: 584-590.
- Mau, J. L., C. N. Chang, S. J. Huang and C. C. Chen, 2004. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Chemistry*, **87**: 111-118.
- Meda, A., C. E. Lamien, M. Romito, J. Millogo and O. G. Nacoulma, 2005. Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. *Food Chemistry*, **91**: 571-577.
- Mercan, N., M. E. Duru, A. Türkoğlu, K. Gezer, İ. Kivrak and H. Türkoğlu, 2006. Antioxidant And Antimicrobial Properties of Ethanollic Extract from *Lepista nuda* (Bull.) Cooke. *Annals of Microbiology*, **56**: 339-344.
- Murci, M. A., M. Martínez-Tomé, A. M. Jiménez, A. M. Vera, M. Honrubia and P. Parras, 2002. Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *Journal of Food Protection*, **65**: 1614-1622.
- NCCLS (National Committee for Clinical Laboratory Standards). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-forming Filamentous Fungi: Proposed Standard M38-P. NCCLS, Wayne, PA, USA. 1998.
- Oyaizu, M., 1986. Studies on products of browning reaction prepared from glucoseamine. *Japanese Journal of Nutrition*, **44**: 307-314.

- Oyetayo, V. O., C. H. Dong and Y. J. Yao, 2009. Antioxidant and Antimicrobial Properties of Aqueous Extract from *Dictyophora indusiata*. *The Open Mycology Journal*, **3**: 20-26.
- Ramesh, C. and G. P. Manohar, 2010. Antimicrobial Properties, Antioxidant Activity and Bioactive Compounds from Six Wild Edible Mushrooms of Western Ghats of Karnataka, India. *Pharmacognosy Research*, **2**: 107-112.
- Ribeiro, B., R. Lopes, P. B. Andrade, R. M. Seabra, R. F. Goncalves, P. Baptista, I. Quelhas and P. Valentao, 2008. Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. *Food Chemistry*, **110**: 47-56.
- Sachindra, N. M., M. K. W. A. Airanthi, M. Hosokawa and K. Miyashita, 2010. Radical scavenging and singlet oxygen quenching activity of extracts from Indian seaweeds. *Journal of Food Science and Technology*, **47**: 94-99.
- Sarker, S. D., L. Nahar and Y. Kumarasamy, 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.
- Sasikumar, J. M., G. M. Mathew and P. D. D. Teepica, 2010. Comparative studies on antioxidant activity of methanol extract and flavonoid fraction of *Nyctanthes arbortristis* leaves. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **9**: 227-233.
- Sawa, T., M. Nakao, T. Akaike, K. Ono and H. Maeda, 1999. Alkylperoxyl radical scavenging activity of various flavonoids and other phenolic compounds: Implications for the anti-tumor promoter effect of vegetables. *Journal of Agricultural and Food Chemistry*, **47**: 397-492.
- Shahidi, F. and P. K. J. P. D., 1992. Wanasundara. Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, **32**: 67-103.
- Sini, H. and K. S. Devi, 2004. Antioxidant activities of chloroform extract of *Solanum trilobatum*. *Pharmaceutical Biology*, **42**: 462-466.
- Slinkard, K. and V. L. Singleton, 1997. Total phenolic analyses: automation and comparison with manual method. *American Journal Enology and Viticulture*, **28**: 49-55.
- Turkoglu, A., M. E. Duru and M. Mercan, 2007. Antioxidant and antimicrobial activity of *Russula delica* fr: an edible wild mushroom. *Eurasian Journal of Analytical Chemistry*, **2**: 54-66.
- Yang, Y. and E. J. Anderson, 1999. Antimicrobial activity of a porcine myeloperoxidase against plant pathogenic bacteria and fungi. *Journal of Applied Microbiology*, **86**: 211-220.
- Zhang, W. M., B. L. Lin Han and H. D. Zhang, 2009. Antioxidant activities of extracts from Areca (*Areca catectu* L.) flower, husk and seed. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **8**: 740-748.
- Ozen, T., C. Darcan, O. Actop and I. Turkekul, 2011. Screening of Antioxidant, Antimicrobial Activities and Chemical Contents of Edible Mushrooms Wildly Grown in the Black Sea Region of Turkey. *Combinatorial Chemistry & High Throughput Screening*, **14**: 72-84.

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