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ASSESSMENT OF BIOLOGICAL ACTIVITY OF SELECTED SPECIES MUSHROOMS OF THE ORDER *AGARICALES* AND *BOLETALES*

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ABSTRACT. The aim of this study was to examine the antineurodegenerative, antimicrobial and antioxidant potential and to determine the total phenolics and total flavonoids of acetone extracts of selected mushrooms species (*Suillus luteus, Leccinum aurantiacum, Agaricus xanthoderma* and *Tricholoma terreum*) belonging to the genera *Boletus* and *Agaricus*. The content of phenolic components in the tested mushroom species varied in the total amount from 94.95 to 147.81 μg PE/mg extract, while the content of flavonoids varied in the range from 2.43 to 23.71 μg RE/mg extract. The tested acetone extracts showed acetylcholinesterase inhibition ranging from 11.49 to 17.46%. The strongest antimicrobial activity for the tested bacteria and fungi was shown by the species *Agaricus xanthoderma*. The antibacterial effect was stronger than the antifungal. Acetone extracts of the tested mushroom species showed moderate antioxidant activity. This study shows that the tested species of mushrooms possess different biological activity and that they can be used as a good source of natural agents are beneficial for human health.

Keywords: *mushroom, biological activity, antioxidants*

INTRODUCTION

Since ancient times, mushrooms have aroused different interests in people. In the last decades, mushrooms are valuable because of their nutritional value due to the content of high-quality proteins, crude fiber, minerals and

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vitamins [1, 2]. By drying the mushrooms at 40 degrees, the protein content is stable, while other types of heat treatment significantly reduce the protein content. They contain all the essential amino acids of which lysine is the most abundant. In addition to lysine, mushrooms also contain other essential amino acids such as arginine, glutamic acid, aspartic acid and serine [3]. Of the fatty acids, unsaturated fatty acids are largely present in the fruiting bodies of mushrooms, the most common of which is linoleic, while carbohydrates represent from 50% to 60% of the mushrooms content. The most common carbohydrates are pentose (xylose, ribose) and hexose (glucose, fructose, galactose, mannose) [4].

The mineral components of the mushrooms are divided into micro and macro elements that are necessary for the normal functioning of the organism. The group of macroelements includes: K, Na, Mg, P and S, while the group of microelements includes: Cu, Co, Fe, J, Mn, Mo, Zn and Se. The content of macronutrients such as sodium, potassium, and phosphorus is constant, while the content of calcium, magnesium and sulfur varies depending on the composition of the substrate on which the mushrooms grow [5]. Mushrooms are a significant source of vitamins, especially B vitamins, thiamine (B_1) and riboflavin (B_2), pyridoxine (B_6) and niacin (B_3) and ascorbic acid (vitamin C). Mushrooms also contain aromatic ingredients that can spice up and raise the quality of other dishes with their magical vinegar and aroma [6-7].

In addition to their nutritional value, mushrooms are a valuable source of many different biologically active compounds, which is why they are becoming an increasingly popular subject of research [8-9]. Among the biologically active compounds in mushrooms are polysaccharides, terpenoids and alkaloids that exhibit antioxidant, antimicrobial, anticancer, immunomodulatory, anti-inflammatory and antineurodegenerative effects, as shown by numerous studies [10-12]. Many antibiotics are still used today, e.g. such as penicillin, streptomycin, chloramphenicol, and others, just derived from mushrooms [13-14]. A large number of compounds with antioxidant action in mushrooms are found in the fruiting body and mycelium. Antioxidants, synthetic or natural, can have a positive effect on human health by reducing oxidative damage caused by ROS [15-16]. Recently, however, it has been suspected that some of synthetic oxidants such as butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHK) and propyl gallate (PG) have toxic and carcinogenic effects [17]. In the search for natural antioxidants, we focused our research on mushrooms, which are a potential source of natural antioxidants.

Today, there is a growing interest in traditional medicine, and an increasing number of researchers are focused on finding new medicines from mushrooms. In this study, we considered the bioactive activity of mushrooms from the genera *Boletus* and *Agaricus*. From the genus *Boletus* we examined

the species *Suillus luteus* and *Leccinum aurantiacum*, and from the genus *Agaricus,* species *Agaricus xanthoderma* and *Tricholoma terreum*. Some species of mushrooms that belong to these genera are known producers of biologically active substances, which is why they are used in the production of antibiotics, organic acids, cheeses and many other products [18-19].

The aim of this study is to describe and identify mushroom samples collected in the nearness of the cities Kragujevac and Niš in the Republic of Serbia and to determine total phenolics and total flavonoids in the acetone extracts of examined mushrooms spectrophotometrically. Also, the aim of the study is to examine the biological activities of mushrooms: antibacterial activity of acetone extract of studied mushrooms species in relation to certain bacterial species, antifungal activity of acetone extracts of studied mushrooms species in relation to certain fungal species, antineurodegenerative activity by determining the degree of inhibition of acetylcholinesterase enzyme activity and to determine the antioxidant activity of extracts (determination of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, determination of superoxide anion radicals scavenging activity and total reducing power).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

It has been demonstrated that the phenol content of mushrooms extracts depended on the solvent used and its polarity and also shown that the acetone extract gave the highest polyphenol content [20]. The high efficiency of acetone to extract phenolic compounds from samples may be due to its ability to prevent the protein–polyphenol binding, which is insoluble complex, through solvent extraction [21]. It has been postulated that acetone is able to inhibit the formation of the protein–polyphenol complex during extraction, or to break down the interaction between the functional group of polyphenols ($^{-}$OH) and the carboxyl group of proteins [22].

The total content of phenolic and flavonoid compounds in mushroom extracts is shown in Figure 1. The content of phenolic components in the tested mushroom species varied in the total amount from 94.95 to 147.81 μg PE/mg extract. The highest content of measured phenol is in the species *Suillus luteus* 147.81 μg PE/mg extract. Slightly lower phenol content was observed *in Agaricus xanthoderma* 100.29 μg PE/mg extract. The lowest phenol content was measured in *Tricholoma terreum.* The content of flavonoids in the extracts of the tested mushrooms varied in the range from 2.43 to 23.71 μg RE/mg extract. The highest flavonoid content was measured in *Suillus luteus*

23.71 μg RE/mg extract, while the lowest flavonoid content was measured in *Agaricus xanthoderma* 2.43 μg RE/mg extract. The difference perhaps could be attributed to genetic factors (different species) [23]. The tested mushroom species showed similar or higher content of flavonoids and phenols than the same species collected in other localities around the world, as well as in relation to other species belonging to the same genus, which is why we expected strong bioactivity of their extracts [24-27].

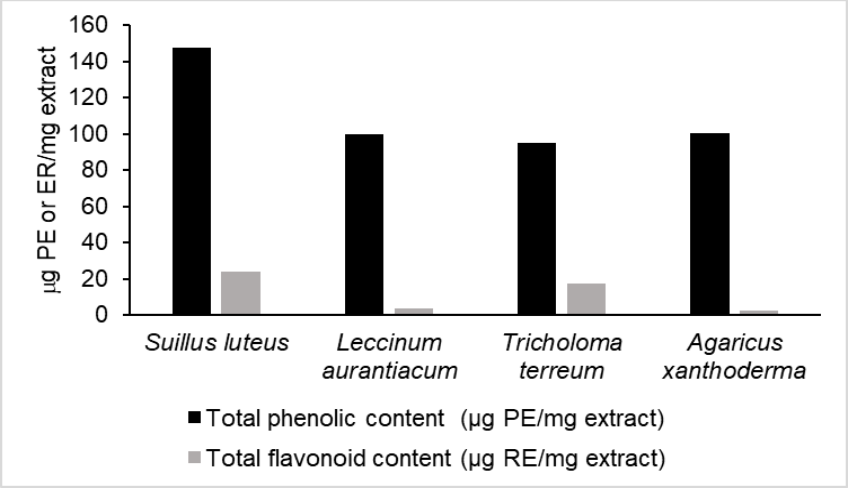

Figure 1. Total phenolic and flavonoid content of tested mushrooms extracts

Inhibition of acetylcholinesterase enzyme activity

Extracts of the studied mushrooms species showed significant potential in inhibiting acetylcholinesterase (AChE) activity compared to the synthetic galantamine inhibitor (Table 1). Acetylcholinesterase activities with these extracts ranged from 11.49 to 17.46%. *Agaricus xanthoderma* extract was the most effective and inhibited acetylcholinesterase activity by 17.46%, while *Suillus luteus* extract was the weakest and inhibited acetylcholinesterase activity by 11.49%.

Despite the fact that the etiology of neurodegenerative disorders, primarily Alzheimer's and Parkinson's disease, has not been fully explained, it is known that there is a reduced level of acetylcholine. The enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine, stopping synaptic transmission. Therefore, AChE inhibitors are considered to be the most effective agents in the treatment of these disorders, since by reducing the activity of this

enzyme, it helps to restore the level of acetylcholine in cholinergic synapses (28-29). Because synthetic AChE inhibitors are expensive and have a variety of side effects, more and more attention is being paid to finding natural alternative sources. Selected Mushroom species were tested for the first time in terms of inhibition of acetylcholinesterase activity. *Agaricus xanthoderma* extract was the most effective and inhibited acetylcholinesterase activity by 17.46%.

Table 1. Acetylcholinesterase inhibitory activity of tested mushroom extracts in different concentrations

Mushrooms extracts	**Inhibition of acetylcholinesterase (%)**			
	125 µg/ml	**250** µg/ml	**500** µg/ml	**1000** µg/ml
Suillus luteus	**11.49**	12.19	15.24	17.45
Leccinum aurantiacum	11.82	13.53	14.20	15.91
Tricholoma terreum	13.13	14.13	15.01	16.71
Agaricus xanthoderma	11.82	14.15	14.20	**17.46**

Antioxidant activity

The antioxidant activity of the tested extracts was determined through several tests: DPPH free radical scavenging activity, superoxide anion scavenging activity and total reducing power assay**.** Edible and medicinal mushroom species are a depot of numerous biologically active compounds that individually or in combination with others have significant antioxidant capacity and can be considered potential natural antioxidants [30]. Until now, many researchers have investigated the antioxidant properties of many mushroom extracts from order *Agaricales* and *Boletales* and some of them showed very good antioxidant activity [24, 31-32].

Table 2. The antioxidant activity of examined extracts of mushrooms

Mushrooms extracts	**Type of antioxidant activity**	
	DPPH IC_{50} (µg/ml)	Superoxide anion radical IC_{50} (µg/ml)
Suillus luteus	**73.41**	**24.47**
Leccinum aurantiacum	107.24	**136.08**
Tricholoma terreum	**142.34**	85.24
Agaricus xanthoderma	105.17	116.47

Table 2 showcases the results of DPPH scavenging activity for the examined extracts. The IC_{50} values of tested extracts ranged from 73.41 to 142.34 μg/ml. The concentration at which 50% of the radicals were neutralized (IC_{50}) was the parameter for comparing antiradical activity (lower IC_{50} values represent better antiradical activity). Among the examined extracts, the extract of the mushrooms *Suillus luteus* showed the largest DPPH scavenging activity (IC_{50} = 73.41 μg/ml).

The results of the superoxide anion scavenging activity (Table 2) of the tested extracts showed that the highest activity had the extract of *Suillus luteus* (IC_{50} = 24.47 μg/ml), while the extract of *Leccinum aurantiacum* showed the lowest activity (IC_{50} = 136.08 μg/ml).

The reducing power of the tested extracts was dose-dependent (Table 3). The measured values of absorbance in all species ranged from 0.04 to 0.25 (higher value of absorbance indicates stronger reduction power). The strongest reducing capacity was shown by the extract of the fungus *Suillus luteus* (0.3026 at a concentration of 1 mg/ml, 0.1875 at a concentration of 0.5 mg/ml 0.0864 at a concentration of 0.25 mg/ml, and 0.0522 at a concentration of 125 mg/ml), while the reduction capacity of *Tricholoma terreum* was least pronounced (0.0390 at 1 mg/ml, 0.0318 at 0.5 mg/ml, 0.0265 at 0.25 mg/ml, and 0.0102 at 0.25 mg/ml). The remaining tested species exhibited approximately similar reducing power.

Table 3. Reducing power of examined extracts of mushrooms

Mushrooms extracts	**Absorbance**			
	125 μg/ml	250 μg/ml	500 μg/ml	1000 μg/ml
Suillus luteus	**0.0522**	**0.0864**	**0.1875**	**0.3026**
Leccinum aurantiacum	0.0260	0.0510	0.0845	0.1488
Tricholoma terreum	**0.0102**	**0.0265**	**0.0318**	**0.0390**
Agaricus xanthoderma	0.0318	0.0386	0.0561	0.0813

The degree of strength of antioxidant activity differed in the examined species, which correlates with the amount of phenols present in the mushrooms itself. The tested extracts of mushrooms show strong antioxidant activity against different oxidative systems. The strong antioxidant activity is the result of high total phenolic content of tested extracts, because between the total phenolic content and the antioxidant activity is a positive correlation, and there are reports about it [33-35].

Antimicrobial and antifungal activity

Acetone extracts of the tested species of mushrooms showed different antimicrobial activity according to the tested species of bacteria and fungi. If we take into account the MIC values as well as the number of tested microorganisms in relation to which the antimicrobial effect was found, the highest antibacterial activity was shown by acetone extract of *Agaricus xanthoderma*. The inhibitory effect was manifested in a concentration of 0.78 mg/ml on two tested species of bacteria (*Bacillus cereus and Escherichia coli*), in a concentration of 1.56 mg/ml also on two species of tested bacteria (*Bacillus subtilis* and *Proteus mirabilis*), and in a concentration of 3.12 mg/ml per test bacterial species (*Staphylococcus aureus*). The weakest antibacterial activity was shown by *Suillus luteus*, where the MIC was 12.5 mg/ml for one species of tested bacterium (*Bacillus subtilis*), and 25 mg/ml for four species of tested bacteria (*Bacillus cereus, Staphylococcus aureus, Proteus mirabilis* and *Escherichia coli*) (Table 4).

The strongest antifungal effect was shown by the extract of the *Agaricus xantoderma* where the MIC concentration varied from 3.12 to 12.5 mg/ml. The inhibitory effect was shown at a concentration of 3.12 mg/ml per test fungus (*Candida albicans*), at a concentration of 6.25 mg/ml on three tested fungi (*Geotrichum candidum, Penicillium chrysogenum* and *Aspergillus fumigatus*), and at a concentration of 12.5 mg/ml showed an inhibitory effect on two tested fungi (*Trichophyton mentagrophytes* and *Paelicomyces variotii).* The weakest antifungal effect was shown by the extract of the fungus *Tricholoma terreum* where the MIC concentration varied from 12.5 to 25 mg/ml (Table 4).

The antibacterial effect was stronger than the antifungal. Such results could be expected given that numerous studies have shown that bacteria are more sensitive to antibiotics than fungi. The reason for the different susceptibility between fungi and bacteria could be attributed to the different permeability of the cell wall. The cell wall of gram-positive bacteria is made of peptidoglycan (murein) and teichoic acid, and the cell wall of gram-negative bacteria is made of peptidoglycan, lipopolysaccharides and lipoproteins, while the cell wall of fungi contains polysaccharides such as chitin and glucan. Selected species of mushrooms are a possible source of bioactive compounds that have antibiotic activity [36, 37]. The strongest antimicrobial effect was shown by the species *Agaricus xantxoderma*.

Table 4. Minimum inhibitory concentration (mg/ml) for mushroom extracts for tested bacterial and fungal strains

Bacterial/fungal strain	Mushrooms species				Control	
	Suillus luteus	*Leccinum aurantiacum*	*Tricholoma terreum*	*Agaricus xanthoderma*	Streptomycin	Ketoconazole
Bacillus subtilis (IPH 189)	12.5	6.25	3.12	**1.56**	0.016	/
Bacillus cereus (ATCC 11778)	**25**	25	12.5	**0.78**	0.016	/
Staphylococcus aureus (ATCC 25923)	25	12.25	12.5	**3.12**	0.031	/
Escherichia coli (ATCC 25922)	25	25	12.5	**1.56**	0.062	/
Proteus mirabilis (ATCC 12453)	25	25	6.25	**0.78**	0.062	/
Trichophyton mentagrophytes	6.25	12.5	25	**12.5**	/	0.156
Geotrichum candidum	6.25	25	25	**6.25**	/	0.078
Paecilomyces variotii	6.25	12.5	25	**12.5**	/	0.156
Fusarium solani	25	25	12.5	**25**	/	0.156
Candida albicans	3.12	3.12	12.5	**3.12**	/	0.039
Aspergillus flavus	25	25	25	**25**	/	0.312
Penicillium chrysogenum	12.5	12.5	12.5	**6.25**	/	0.312
Aspergillus fumigatus	3.12	12.5	12.5	**6.25**	/	0.156

CONCLUSIONS

To the best of our knowledge, this study presented for the first time a comparison of biological activity and total phenolic and flavonoid content in mushrooms of the orders *Agaricales* and *Boletales*. The results of biological activity tests show that the extract of *Suillus luteus* has the best antioxidant and antineurodegenerative activity, while the extract of *Agaricus xanthoderma* has the best antibacterial and antifungal activity. Based on the obtained results, it can be concluded that edible mushroom species are a rich source of various biologically active compounds that have significant antimicrobial, antioxidant and antineurodegenerative activity, in addition to their nutritional value, particular aroma and other beneficial properties.

The discovery of new bioactive metabolites of mushrooms and more detailed studies of the same will enable the discovery of new biologically active agents in the control of various types of diseases, which is of great interest to the human population.

EXPERIMENTAL SECTION

Chemicals/ Reagents

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MO, USA), Aldrich Chemical Co. (Steinheim, Germany) and Alfa Aesar (Karlsruhe, Germany).

Plant material

The studied species of mushrooms were collected in the vicinity of Kragujevac and Nis, in the period September-October 2017 (Table 5). Dry mushroom material is kept in the Department of Biology and Ecology, Faculty of Science in Kragujevac. The identification of collected mushrooms was performed using standard herbarium keys [38-40].

Table 5. Examined mushrooms species

Examined mushrooms species	Code	Vicinity	Year of sampling
Suillus luteus	DBFS92	Nis	2017
Leccinum aurantiacum	DBFS93	Nis	2017
Tricholoma terreum	DBFS94	Kragujevac	2017
Agaricus xanthoderma	DBFS97	Kragujevac	2017

Preparation of extracts

The extraction of selected mushroom specimens was performed in the Laboratory for Microbiology and Mycology, Faculty of Science in Kragujevac. The acetone extracts of the studied mushroom species were analyzed. Preparation of acetone extract: Collected mushrooms were firstly dried at room temperature. Dried, main parts (body of mushrooms) of selected mushrooms species were then pulverized and extracted with acetone for 48h at room temperature. The resulting extract was filtered through filter paper (Whatman No. 1) and then evaporated at room temperature to get the dry extract. The obtained acetone extracts were stored at -20 °C, which prevented the possibility of degradation of bioactive molecules. To check

the antibacterial, antifungal, antioxidant, and antineurodegenerative activity, solutions of different concentrations of extracts were obtained by dissolving a certain amount of dry matter in a certain volume of solvent (5% DMSO).

Determination of total flavonoid content

Total flavonoids were determined by the Dovd method (41). The same volume of extract was added to 2 ml of 2% methanolic aluminum (III) chloride solution. After one hour, the absorbance was measured at 415 nm. Based on the measured absorbances, the concentration (μg) of total flavonoids was read from the standard rutin calibration curve, and then the content of total flavonoids in the extract was expressed as rutin equivalent (RE)/mg of extract, according to:

Absorbance = 0.0296 x total flavonoids [μg (RE)/mg extract] + 0.0204,
(R^2 = 0.9992)

Determination of total phenolic content

The total phenolic content in the extracts was determined with Folin-Ciocalteu reagens by the method of Slinkard and Slingleton [42]. The reaction mixture was prepared by mixing 1 ml of extract, 46 ml of distilled water, 1 ml of Folin-Ciocalteu reagent and 3 ml of 2% sodium carbonate solution. After 2 h of incubation, absorbances were measured spectrophotometrically at 760 nm. The standard curve equation was determined by measuring the absorbance of the pyrocatechol concentration series (0.1 - 0.02 mg/ml). The content of the polyphenols was expressed in μg of pyrocatechol equivalents per milligram of dry extract (μg PE/mg dry extract), according to:

Absorbance = 0.0021 x total phenolic [μg PE/mg of extract] - 0.0092,
(R2 = 0,9934)

Inhibition of acetylcholinesterase enzyme activity

The degree of inhibition of acetylcholinesterase activity (AChE) was determined spectrophotometrically using 96-well microtiter plates. The reaction mixture (140 μl 0.1 mM sodium phosphate buffer pH 8.0, 20 μl 5,5-dithiobis-2-nitrobenzoic acid (DTNB), 20 μl extract, 20 μl AChE (5IU) was incubated for 15 min at 25ºC. The reaction was initiated by the addition of 10 μl of acetylthiocholine iodide whose hydrolysis was followed by a change in absorption at 412 nm due to the conversion of DTNB to yellow 5-thio-2-nitrobenzoate anion (TNB^-) in an AChE catalyzed reaction 6-15 min after initiation [43]. The degree of AChE inhibition was determined by the formula:

Degree of inhibition of AChE activity (%) = [(E - S) / E] × 100

where E is the activity of the enzyme without the extract and C is the activity of the enzyme with the extract. The values obtained were compared with a commercial inhibitor, galantamine.

Antioxidant activity

Determination of DPPH free radical scavenging activity

The antioxidant activity of mushroom extracts was determined by a modified (1,1-diphenyl-2-picryl-hydrazyl) DPPH assay [44]. A mixtures of methanolic solution of DPPH (concentration 0.05 mg/ml) and mushrooms extracts of different concentrations (1, 0.5, 0.25 and 0.125 mg/ml) were incubated at room temperature, in the dark, for 30 minutes. After incubation, absorbance was measured at 517 nm on a spectrophotometer ("Jenway" UK). Ascorbic acid was used as a positive control. The capacity to neutralize free radicals was calculated by the following formula:

$$\text{Neutralization capacity of DPPH radicals (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the negative control and A_1 is the absorbance of the reaction mixture or standard. IC_{50} values (concentration at which 50% of DPPH radicals were neutralized) were calculated from the obtained inhibition values.

Determination of superoxide radical scavenging activity

The effect of the extracts on the superoxide anion radical was determined by the Nishimikietal method [45]. The reaction mixture was prepared by mixing 0.1 ml of extract of different concentrations (1, 0.5, 0.25 and 0.125 mg/ml), 1 ml of nitroblue tetrazolium (NBT) solution (156 μM in 0.1 M phosphate buffer, pH=7.4), 1 ml of NADH solution (468 μM in 0.1 M phosphate buffer, pH=7.4) and 100 μl phenazine methosulphate (PMS) solution (60 μM in 0.1 M phosphate buffer, pH=7.4). After incubation at room temperature for 5 minutes, the absorbance was measured at 560 nm on a spectrophotometer. Ascorbic acid was used as a positive control. The percentage inhibition of superoxide anion radicals was calculated using the following equation:

$$\text{Inhibition of superoxide anion radical (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the negative control and A_1 is the absorbance of the reaction mixture or standard. IC_{50} values (concentration at which 50% of superoxide anion radicals were neutralized) were calculated from the obtained inhibition values.

Total reducing power assay

The total reduction capacity of the extracts was determined by the Oyaizu method (46). Mushroom extract (1ml) of different concentrations (1, 0.5, 0.25 and 0.125 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 ml, 1%). Then, the mixture was incubated for 20 min at 50°C, and then 2.5 ml of 10% trichloroacetic acid solution was added. After centrifugation, the supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% iron (III) chloride solution (0.5 ml). The absorbance was measured at 700 nm on a spectrophotometer. Ascorbic acid was used as a positive control.

Antibacterial and antifungal activities of extracts of selected mushroom species

Tests of antibacterial activity of extracts of selected mushrooms species were performed against five bacterial species: *Bacillus subtilis* (IPH 189), *B. cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), (Gram-positive bacteria); *Escherichia coli* (ATCC 25922) and *Proteus mirabilis* (ATCC 12453) (Gram-negative bacteria).

Tests of antifungal activity of extracts of selected mushrooms species were performed in relation to 5 fungal species: *Aspergillus niger* (ATCC 16888), *Candida albicans* (ATCC 10231), *Mucor mucedo* (ATCC 20094), *Penicillium italicum* (ATCC 10454) and *Trichoderma viride* (ATCC 13233). The fungi and bacteria tested come from the American Culture Sample Collection (ATCC).

The sensitivity of microorganisms to acetone extracts of the tested mushrooms species was performed by measuring the inhibition for a given concentration of the extract by determining the minimum inhibitory concentration (MIC) by the microdilution method [47].

In the experiment, 96-well microtiter plates were used to which 100 µl of broth (Müller-Hinton for bacteria and SD for fungi) was added. In the first row, 100 µl of extract of a certain initial concentration was added and a series of double dilutions in the range of 50 to 0.0475 mg/ml was made. Then, 10 µl of a solution of resazurin of prepared growth of 270 mg of resazurin in 40 ml of sterile distilled water was added to all wells. Finally, 10 µl of the appropriate inoculum was added to all wells. After inoculation, the plates were incubated for 24 h at 37°C (bacteria) and 48 h at 27°C (fungi), respectively. MIC was determined visually, by changing the color of resazurin (oxido-reduction indicator for assessing microbial growth). Resazurin is a blue non-fluorescent dye that turns pink fluorescent after reduction of resazurin to resofurin oxidoreductases. The lowest concentration at which there is no discoloration of resazurin is defined as MIC.

The antibiotic streptomycin was used as a positive control to inhibit bacterial growth, and the antifungal ketoconazole to inhibit fungal growth. A positive control was also set up, which contained a substrate with microorganisms to monitor their unhindered growth. Negative control of the solvent effect was performed in parallel.

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