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BIOMEDICAL POTENTIAL OF SELECTED MUSHROOM SPECIES

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Abstract: The aim of this study was to determine neuroprotective, antioxidant, antimicrobial and cytotoxic potential of acetone extracts of *Coprinus comatus* and *Coprinopsis picacea* mushrooms. The neuroprotetive activity was tested against acetylcholinesterase enzyme using the Ellman method. Antioxidant activity was evaluated by free radical scavenging ability, superoxide anion radical scavenging activity and reducing power. The total phenol content was examined using Folin-Ciocalteu reagent. The antimicrobial potential was determined by a microdilution method against 12 microorganisms. The cytotoxic activity was tested using MTT method on the Hela, A549 and LS174 cells. Our results indicate that *C. comatus* expressed a stronger neuroprotective effect (the percentage of inhibition of acetylcholinesterase was within the range 19.66-51.73%) than *C. picacea*. In antioxidant effect *C. comatus* had more potent free radical scavenging activity (IC_{50} = 276.69 μg/mL) and superoxide anion radical scavenging activity (IC_{50} = 39.40 μg/mL), while reducing power was relatively similar for both species. The total amount of phenols for *C. comatus* and *C. picacea* was 50.57 and 50.20 μg PE/mg of dry extracts, respectively. In antimicrobial activity, *C. picacea* showed a better effect with MIC values from 0.1 to 7.5 mg/mL. Finally, *C. picacea* expressed stronger cytotoxicity toward A549 and LS174 cells, while *C. comatus* was more active against Hela cell.

Keywords: Acetone extract; bioactivity; mushrooms.

1. INTRODUCTION

Every day there is a growing number of new data on the activity of natural products and their application to human health. In nature there is great chemical diversity that can be utilized, for example, as bioactive components that are extracted from mushrooms [1,2].

Medicinal mushrooms are especially interesting for possession of a large number and richness on bioactive compounds such as lectins, polysaccharides, triterpenoids, phenolics, flavonoids, tocopherols, ascorbic acid, carotenoids, ergothioneine, glutathione and selenium [3]. In the constant searching for new therapeutic alternatives, investigated were many different mushroom species and found were some important activities such as antiviral, antimicrobial, anticancer, antihyperglycemic, cardioprotective, as well as antiparasitic, anti-inflammatory and antibiotic effects [4,5].

Coprinoid mushrooms are some of the most medically important mushrooms. Extracts from several members of the genus *Coprinus* are known in many parts of the world to their bioactivity [6]. Thus, the objective of the present study is to examine biomedicinal properties of extracts of edible mushroom *Coprinus comatus* and non-edible mushroom *Coprinopsis picacea* in order to compare their neuroprotective, antioxidant, antimicrobial and anticancer potential *in vitro.*

2. MATERIALS AND METHODS

2.1. Collection of mushroom samples and preparation of the extract

Fungal samples of *C. comatus* (O.F.Mull.: Fr.) *Pers.*, and *C. picacea* (Bull.) Redhead, Vilgalys & Moncalvo, were collected from Šumarice, Kragujevac, Serbia, in October of 2018. 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 literature [7].

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Finely dry ground thalli of the examined mushrooms were extracted using acetone 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.

2.2. The evaluation of acetylcholinesterase inhibition

The effect of tested mushrooms on the acetylcholinesterase (AChE) inhibition rate was measured spectrophotometrically, using 96-well microtiter plates, according to the method of Ellman et al. [8].

2.3. Antioxidative activity

Antioxidant activity of tested samples was evaluated by free radical scavenging, superoxide anion radical scavenging and reducing power assays. The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to the Kosanić et al's method [9]. The superoxide anion radical scavenging activity was studied according to the method of Nishikimi et al. [10]. The Oyaizu method [11] was used to determine the reducing power. In all antioxidant assays, ascorbic acid was used as positive control. In addition, the estimation of total phenolic content in mushroom extracts was done in accordance with the method of Slinkard and Singleton [12], using pyrocatechol as a standard phenolic compound.

2.4. Antimicrobial activity

The antimicrobial activity of selected mushrooms was tested on five bacteria: *Bacillus cereus* (ATCC 11778), *B. subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (ATCC 12453); and ten fungi: *Aspergillus flavus* (ATCC 9170), *A. niger* (ATCC 16888), *Candida albicans* (ATCC 10231), *Mucor mucedo* (ATCC 20094), *Trichoderma viride* (ATCC 13233), *Cladosporium cladosporioides* (ATCC 11275), *Fusarium oxysporum* (ATCC 62506), *Alternaria alternata* (ATCC 11680), *Penicillium expansum* (ATCC 20466) and *P. chrysogenum* (ATCC 10106). All cultures are provided from the American Type Culture Collection (ATCC). Minimum inhibitory concentrations (MIC) were evaluated by the 96-well microtiter assay using resazurin as the indicator of cell growth [9].

2.5. Cytotoxic activity

Human epithelial carcinoma Hela cells, human lung carcinoma A549 cells, human colon carcinoma LS174 cells and normal MRC5 human embryonic lung fibroblast 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% heat-inactivated (56ºC) fetal bovine serum, (Sigma Chemical Co. St Louis, MO, USA) supplemented with 3 mmol L-1L-glutamine, 100 mg/mL streptomycin, 100 IU/mL penicillin. Cells were grown in a humidified atmosphere of 95% air and 5% CO_2 at 37ºC. The effect on cancer cell survival was determined 72 h after the addition of extract, by the MTT test [13].

2.6. Statistical analysis

Statistical analyses were performed using Microsoft Excel and SPSS software packages. Data are expressed as means ± standard deviations (mean ± SD) of three parallel measurements.

3. RESULTS

The result of inhibition of AChE activity is summarized in Table 1. As shown in the table, inhibition of AChE activity was concentration dependent. The percentage inhibitions of AChE activity for *C. comatus* and *C. picacea* extracts was within the range 19.66-51.73% and 14.21-39.54%, respectively.

Table 1. Acetylcholinesterase inhibition of acetone extracts of mushrooms Coprinus comatus and Coprinopsis picacea

Tested mushroom	Acetylcholinesterase (AChE) inhibition (%)			
	1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml
C. comatus	51.73 ± 0.94	39.47 ± 0.89	29.68 ± 0.79	19.66 ± 0.71
C. picacea	39.54 ± 0.85	29.25 ± 0.78	20.15 ± 0.62	14.21 ± 0.43
Galanthamine	92.23 ± 0.56	87.08 ± 0.51	74.31 ± 0.44	68.12 ± 0.32

Radical scavenging activity of mushrooms extracts are presented in Table 2. It is evident that extract of *C. comatus* has higher DPPH radical scavenging activity (IC_{50} = 276.69 μg/mL) and superoxide anion radical scavenging activity (IC_{50} = 39.40 μg/mL) than extract of *C. picacea*. These results can be considered as a moderate antioxidant capacity, in comparison with the ascorbic acid standard.

The reducing power of mushrooms extracts was shown in Table 3. Reducing power of extracts increased as the concentration increased. Among the mushroom extracts, the higher reducing power activity was obtained from *C. comatus* (absorbance values varied from 0.056 to 0.311) than *C. picacea* (absorbance values varied from 0.045 to 0.205). Ascorbic acid as positive controls showed quite higher activities than extracts.

Table 2. DPPH radical scavenging activity and superoxide anion scavenging activity of extracts of Coprinus comatus and Coprinopsis picacea

Mushroom species	DPPH radical scavenging IC_{50} (μg/ml)	Superoxide anion scavenging IC_{50} (μg/ml)
Coprinus comatus	276.69 ± 2.35	39.40 ± 1.01
Coprinopsis picacea	455.48 ± 1.28	235.13 ± 2.02
Ascorbic acid	6.42 ± 0.18	115.61 ± 1.16

Table 3. Reducing power of extracts of Coprinus comatus and Coprinopsis picacea

Mushroom species	Absorbanca (700 nm)			
	1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml
C. comatus	0.311 ± .031	0.113 ± .025	0.078 ± .008	0.056 ± .004
C. picacea	0.205 ± .043	0.106 ± .030	0.066 ± .012	0.045 ± .009
Ascorbic acid	2.113 ± .032	1.654 ± .021	0.095 ± .008	0.048 ± .008

Total phenolic constituents of tested extracts are given in Table 4. The total phenolics was determined as the pyrocatechol equivalent using an equation obtained from a standard pyrocatechol graph ($y = 0.0057x - 0.1646$, $R_2 = 0.9934$). The data clearly show that the extracts had the very similar phenolic content in *C. comatus* (5.57 μg PE/mg of extract) and *C. picacea* (5.20 μg PE/mg of extract).

The antimicrobial activity of the both extracts is depicted in Table 5. The results indicated that the mushrooms extracts showed a very important activity against the tested bacteria, as well as the very good to the fungi. Especially, the extract of mushroom *C. picacea* (MIC values ranging from 0.1 to 7.5 mg/mL) has several times stronger activity of the extract of *C. comatus*, which indicates notable antimicrobial potential of this mushroom. The antimicrobial activity was compared with the standard antibiotics, streptomycin (for bacteria) and ketoconazole (for fungi). The results showed that standards had stronger activity than tested samples.

Table 4. Total phenolics content of extracts of Coprinus comatus and Coprinopsis picacea

Mushroom species	Phenolics content (μg PE/mg of extract)
Coprinus comatus	50.57 ± 1.024
Coprinopsis picacea	50.20 ± 1.125

PE - pyrocatechol equivalents

Table 5. Minimum inhibitory concentration of Coprinus comatus and Coprinopsis picacea

	C. comatus	*C. picacea*	S	K
Bacillus cereus	0.81	0.1	0.016	-
Bacillus subtilis	0.81	0.2	0.016	-
Eschrichia coli	0.81	0.2	0.062	-
Proteus mirabilis	1.625	0.4	0.062	-
Staphylococcus aureus	1.625	0.2	0.031	-
Aspergillus flavus	15	3.25	-	0.312
Candida albicans	7.5	2.25	-	0.039
Geotrichum candidum	15	7.5	-	0.078
Paecilomyces variotii	15	7.5	-	0.156
Penicillium italicum	15	15	-	0.156
Fusarium solani	3.25	3.25	-	0.156
Trichophyton mentagrophytes	1.625	7.5	-	0.156

Values given as mg/ml

Investigated *C. comatus* and *C. picacea* extracts were used for evaluation of their potential cytotoxic activity against three human cancer cell lines and normal human MRC5 cells. Obtained data shown in Table 6, indicate that both extracts have moderate cytotoxic activity against investigated malignant cells. Though, according to the LS174 cells, extract of *C. picacea* show good activity (IC_{50} 35.76 ± 2.49 μg/mL) contrary, to *C. comatus*, cytotoxic activity was not detected in the range of up to 200 μg/mL. Also, none of the investigated extract does not show significant cytotoxicity against normal MRC5 cell line.

Table 6. Growth inhibitory effects of extracts of Coprinus comatus and Coprinopsis picacea on Hela, A549, LS174 and MRC5 cell survival

Cell lines	Hela	A549	LS174	MRC5
Mushroom species	IC_{50} (μg/ml)			
C. comatus	135.22 ± 4.28	180.23 ± 2.47	> 200	> 200
C. picacea	154.03 ± 4.26	158.08 ± 4.55	35.76 ± 2.49	> 200
Cis-DDP	0.86 ± 0.33	4.91 ± 0.42	3.18 ± 0.29	13.21 ± 0.37

4. DISCUSSION

In spite of the fact that the etiology of neurodegenerative disorders, primarily Alzheimer's disease has not been fully explained, it is known that at the base of these diseases is a decreased level of acetylcholine i.e dopamine. Enzyme AChE hydrolyses the neurotransmitter acetylcholine, thereby it is stopping the synaptic transmission. Therefore, AChE inhibitors are considered to be the most effective agents in the treatment of these disorders, by reason of reducing the activity of this enzyme, they help to restore the level of acetylcholine in cholinergic synapses. Since synthetic inhibitors of AChE are expensive and have different side effects more attention is paid to finding natural alternative sources.

When it comes to the inhibition of AChE activities of mushrooms, previous researches showed that extracts of few mushroom species can contain compounds which inhibit AChE activity [14]. In comparison with AChE activity inhibition rate, which was obtained by other mushrooms, tested mushrooms especially *C. comatus* can be considered as a good neuroprotective agent. Similar to our research, the polysaccharide extracts from the *C. comatus* and *Coprinellus truncorum* were screened in liquid for their AChE activity by Pejin et al. [15]. Both extracts were found to display inhibition of the aforementioned enzyme reaching similar IC_{50} values of 0.62 ± 0.07 and 0.61 ± 0.03 mg/mL, respectively.

Natural antioxidants have been proved to be effective protectors of body from the adverse effects of free radicals caused oxidative stress. Mushrooms are found to be rich source of these antioxidants. In this study, we used DPPH test, as well as superoxide anion radical scavenging and reducing power activity just to verify antioxidant activity of tested mushroom extracts. The results presented here indicate that the acetone extract from examined mushrooms showed relatively strong DPPH radical scavenging and superoxide anion radical scavenging activity, while reducing power was less pronounced.

In the literature there are several data for the antioxidant activity of *C. comatus* [16-18]. They determined antioxidant activity for this species, but for other extraction solvents used. In this study, the antioxidant activity of selected mushroom was confirmed by acetone extract. Different extraction solvents, according to their polarity, may extract various compounds which can participate in great antioxidant activity. This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of mushroom extract (containing the antioxidant active components).

Extracts of edible mushrooms are also extensively used in traditional medicine to treat various microbial infections. Our obtained results for antimicrobial activity for *C. comatus* edible mushroom are in accordance with the previous data obtained for this mushroom [19-21]. 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. The intensity of the antimicrobial effect of *C. comatus* depended on the used concentration of extract and the tested microorganisms. The extract of *C. picacea* has several times stronger activity of the extract of *C. comatus*, which indicates notable antimicrobial potential of this mushroom. Generally, slightly higher activities of mushroom against gram-positive than gram-negative strains were observed. These results are comparable with previous results regarding the antimicrobial activity of mushrooms, where it was found that mushrooms were more active against gram-positive bacteria [22,9]. 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 antibiotics compared with fungi. [9,23].

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 [2]. The evidence from various researchers across the globe, regarding anti-tumor application of mushroom extracts unarguably make it a fast-track research area worth mass attention.

There were several available informations about anti-cancer potential of *C. comatus.* For example, Asatiani et al. [24] showed that the IC_{50} value of the ethyl acetate extract obtained from *C. comatus* on MCF7 cell viability was only 32 μg/mL. They also found that the effect of ethyl acetate extract of this mushroom was comparable to the effect of curcumin, a known NF-κB pathway inhibitor, as well as that this extract inhibited the activity of IKK complex, at close to 90% as compared to the control of the untreated sample. These results promise that the *C. comatus* extract can be an effective therapy for malignant estrogen-independent breast cancer. Zaidman et al. [25] studied the selective inhibition of prostate cancer LNCaP cells by ethanol and ethyl acetate extracts of this mushroom. It was observed that this extract inhibits dihydrotestosterone-induced LNCaP cell viability and causes a G1 phase arrest. These findings suggested the therapeutic mechanism of the extracts as androgen receptor or non-androgen receptor mediated. And some other researchers have been shown anticancer potential for *C. comatus* [26,1].

In literature no data for neuroprotective, antioxidant, antimicrobial and anticancer potential for *C. picacea*, probably because it is an inedible mushroom. In this study, we wanted to show that in addition to edible even inedible mushrooms can be used as a natural antineurodegenerative, antioxidant, antimicrobial and anticancer agents. From inedible mushroom can be created diverse preparations which in various forms can be used for different biomedicinal purposes.

In the present investigation it can be concluded that the tested mushrooms appear to be good natural neuroprotective, antioxidant, antimicrobial and anticancer agents. However, more detailed studies are needed to be conducted, such as identification of the bioactive compounds and study of mechanisms of actions, in order to use these mushrooms as a possible natural drug for treating various diseases.

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БИОМЕДИЦИНСКИ ПОТЕНЦИЈАЛ ОДАБРАНИХ ВРСТА ГЉИВА

Сажетак: Циљ истраживања је био да се одреди неуропротективна, антиоксидативна, антимикробна и цитотоксична активност ацетонских екстраката гљива *Coprinus comatus* и *Coprinopsis picacea*. Неуропротективна активност процењена је одређивањем степена инхибиције ензима ацетилхолинестеразе Елмановом методом. У оквиру антиоксидативне активности испитиван је утицај екстраката на DPPH и супероксид анјон радикале, као и њихов редукциони капацитет. Садржај фенола у екстрактима измерен је коришћењем Folin-Ciocalteu реагенса. Антимикробна активност испитивана је одређивањем минималне инхибиторне концентрације (МИЦ) микродилуционом методом у односу на 12 врста

микроорганизама. Цитотоксичност је испитивана MTT методом у односу на Hela, A549 и LS174 ћелијске линије. Екстракт врсте *C. comatus* испољио је јачу неуропротективну активност (ниво инхибиције ацетилхолинестеразе кретао се у опсегу 19,66 – 51,73%) у односу на врсту *C. picacea*. Екстракт гљиве *C. comatus* је такође показао јачи инхибиторни утицај на DPPH радикале (IC_{50} = 276,69 μg/mL) и супероксид анјон радикале (IC_{50} = 39,40 μg/mL), док је редукциони капацитет био сличан код обе врсте. Садржај фенола износио је 50,57 μg PE/mg сувог екстракта за *C. comatus* и 50.20 μg PE/mg сувог екстракта за *C. picacea*. Што се тиче антимикробне активности, екстракт врсте *C. picacea* је испољио јачи ефекат (добијене МИЦ вредности варирале су у опсегу 0,1 – 7,5 mg/mL). Екстракт врсте *C. picacea* је показао јачу цитотоксичност према A549 и LS174 ћелијама, док је естракт гљиве *C. comatus* испољио јачи ефекат према Hela ћелији.

Кључне речи: Ацетонски екстракт, биоактивност, гљиве.

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