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ANTIOXIDANT, ANTIFUNGAL AND ANTICANCER ACTIVITIES OF Se-ENRICHED *PLEUROTUS* SPP. MYCELIUM EXTRACTS

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Abstract - The goal of this study was the evaluation of antifungal, antioxidant and anticancer potentials of *Pleurotus eryngii*, *P. ostreatus* and *P. pulmonarius* mycelial extracts, and the influence of mycelium enrichment with selenium on these activities. Both Se-amended and non-amended extracts showed the same or similar minimal inhibitory concentration for 14 studied micromycetes, while a fungicidal effect was not noted, contrary to ketoconazole, which had inhibitory and fungicidal effects at very low concentrations. Se-non-amended extracts exhibited antioxidant activity, especially at higher concentrations. Selenium enrichment influenced activity, its effects decreasing in *P. eryngii* and *P. pulmonarius*, while in *P. ostreatus* no effect was noted. The $DPPH^{\cdot}$ radical scavenging capacity of the extracts was in direct correlation with their phenol and flavonoid contents. Cytotoxic activity against both HeLa and LS174 cell lines was very low compared with *cis*-DDP. These features suggest that mycelium should be an object of intensive studies.

Key words: Biological activities; *Pleurotus eryngii*; *P. ostreatus*; *P. pulmonarius*; selenium

INTRODUCTION

The main characteristics of the end of the 20th and the beginning of the 21st century are population explosions and the desire to strengthen the economy that have led to the intense development of agriculture and industry, a consequence of which is environmental pollution. Contemporary life style weakens an organism's ability to defend itself from microorganisms and free radicals, which are abundantly present in the environment. Currently, with the rise in awareness about the negative effects of commercial antibiotics and antimycotics, antioxidative drugs and chemotherapy, special attention is given to natural remedies originating from various plant species. The medicinal properties of numerous mushroom species, known since ancient times, represent a base for the study of their antimicrobial, antioxidant and anticancer potentials. Species of the genus *Pleurotus* have significant nutritional and medicinal values (Gunde-Cimerman, 1999; Stamets, 2000) and therefore some of them are commercially cultivated. Antihypercholesterolemic, anticancer, antiviral, antimicrobial and immunomodulating activities are the most important medicinal effects of these species (Gunde-Cimerman, 1999). In addition to a significant content of microelements, *Pleurotus* species also have the ability to absorb some of them from substrate. Stajić et al. (2006) showed that the mycelia of 10 *Pleurotus* species with 41 strains of different origin can assimilate selenium (Se) and can therefore present an excellent dietary source of Se. Se has important biological functions such as enhancement of immune functioning (Combs and Gray,

1998; Rayman, 2000), participation in the biosynthesis of thyroid hormone (Clement, 1998), energy metabolism and gene expression (Falandysz, 2008), and protection of cell membranes from oxidative stress (Costa-Silva et al., 2011). Its antioxidant effect is based on the reduction of hydrogen peroxide, lipid and phospholipid hydroperoxide by glutathione peroxidase, in whose active site Se enters and basically prevents the occurrence of cancer, cardiovascular diseases, diabetes, atherosclerosis, cataract, as well as neurodegenerative disorders. Combs and Gray (1998) demonstrated that Se could inhibit tumor and cancer growth by either improvement of the immune system or perturbation of tumor cell metabolism. Currently, consumption of Se supplements is on the increase, but little is known about the risk of resistance occurrence in some consumers. Therefore, priority is given nowadays to the use of natural Se products based on plants and mushrooms. Numerous studies of *P. ostreatus* extracts and compounds demonstrated that Se-containing polysaccharides can inhibit the growth of lung, breast and ovarian cancer cells, and that Se-containing proteins enhance antioxidant activity (Zhao et al., 2004).

The above-mentioned data influenced the formulation of the aim to carry out a comparative analysis of the antifungal, antioxidant and anticancer potentials of Se-non-amended and Se-amended mycelial extracts of three *Pleurotus* species.

MATERIALS AND METHODS

Organisms and cultivation conditions

The cultures of *Pleurotus eryngii* HAI 507, *P. ostreatus* HAI 592, and *P. pulmonarius* HAI 573, originating from the Institute of Evolution, University of Haifa (Israel), were maintained on malt agar (MA) medium in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade.

The inoculum was processed in several steps as follows: (i) inoculation of 100 mL of synthetic medium (glucose, 10 g L^{-1}; NH_4NO_3, 2 g L^{-1}; K_2HPO_4, 1 g L^{-1}; $NaH_2PO_4 \times H_2O$, 0.4 g L^{-1}; $MgSO_4 \times 7H_2O$, 0.5 g L^{-1}; yeast extract, 2 g L^{-1}; pH 6.5) with 25 mycelial discs (Ø 0.5 cm, from 7-day-old culture from MA) in 250 mL Erlenmeyer flasks; (ii) incubation at room temperature (22±2°C) on a rotary shaker (100 rpm) for 7 days; (iii) washing of obtained biomass (3 times) with sterile distilled water (dH_2O); (iv) homogenization of biomass with 100 mL of sterile dH_2O in a laboratory blender. The homogenized inoculum (30 mL) was used for the inoculation of 400 mL modified synthetic medium (with 65 g L^{-1} glucose and 2 g L^{-1}peptone, previously determined as the optimal carbon and nitrogen sources and concentrations for biomass production) enriched with sodium selenite (Na_2SeO_3) at an initial Se concentration of 1.3 mg L^{-1}. Medium without Se was used as the control.

Submerged cultivation was carried out in 1 L flasks at room temperature on a rotary shaker for 21 days. The obtained biomass was filtered, washed 3 times with dH_2O at 30°C in order to remove the remaining Se from the cell wall, and dried at 50°C to a constant weight.

Preparation of fungal extracts

Dry Se-amended and non-amended mycelia (3.0 g) were extracted by stirring with 90 mL of 96% ethanol at 30°C for 72 h. The obtained extracts were centrifuged (20°C, 3 000 rpm, 10 min) and supernatants were filtered through Whatman No. 4 filter paper, concentrated under reduced pressure in a rotary evaporator (BÜCHI R-114, Switzerland) at 40°C to dryness, and redissolved in 96% ethanol (for the testing of antioxidant activity) or in 5% dimethyl sulfoxide (DMSO) (for the analysis of antifungal and anticancer activity) to a concentration of 32.0 mg mL^{-1}.

Antifungal activity

The tested micromycetes (Table 1) were maintained on MA at 4°C in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade. The tested micromycetes were cultivated on Sabouraud dextrose agar (SDA) at 25±2°C for 21 days. Spore suspensions were prepared by washing the agar surface with sterile 0.9% saline containing 0.1% Tween

80 (v/v). Turbidity was determined spectrophotometrically at 530 nm and spore number was adjusted to 10^6 CFU mL^{-1} (NCCLS, 1998).

DMSO extracts of Se-amended and non-amended mycelia were sterilized by filtration through Whatman No. 4 filter paper and a 0.2 μm membrane filter. The antifungal potential of the tested extracts was studied by microdilution using a 96 well microtiter plate (Sarker et al., 2007). Series of double extract dilutions (from 32.0 mg mL^{-1} to 0.5 mg mL^{-1}) were analyzed. Each well was comprised of Sabouraud dextrose broth (SDB), spore suspension, resazurine and extract of defined concentration. The mixture without extract was used as the negative control, while the positive control contained the commercial antimycotic, ketoconazole, instead of extract. Tested ketoconazole concentrations ranged from 0.031 mg mL^{-1} to 0.002 mg mL^{-1} (series of double dilutions). The effect of 5% DMSO on spore germination was also analyzed by its addition to the mixture instead of SDB. Microtiter plates were incubated at 25±2°C for 72 h. The lowest extract concentration without visible mycelium growth was defined as the minimal inhibitory concentration (MIC) (Andrews, 2001). Minimal fungicidal concentration (MFC) was determined as the lowest extract concentration with no mycelial growth after reinoculation of 2.0 μL of the mixture on SDA. The experiments were repeated three times.

Antioxidant activity

DPPH assay

Antioxidant activity was assessed by measuring the bleaching of the purple-colored methanol solution of stable 1,1-diphenyl-2-picryl-hydrazil radical ($DPPH^{\cdot}$) (Blois, 1958); 1.8 mL of 4% methanol solution of $DPPH^{\cdot}$ and 200 μL of extract of defined concentration (series of double dilutions from 32 mg mL^{-1} to 0.5 mg mL^{-1}) were mixed and shaken. After 30 min of incubation in the dark, the absorbance was measured at 517 nm against methanol as blank using a CECIL spectrophotometer (CE2501, U.K.). The negative control contained all the reaction reagents except the extract. Scavenging effects were calculated using the equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_{sample})/A_0] \times 100,$$

where A_0 is the absorbance of the negative control and A_{sample} is the absorbance of the reaction mixture.

The EC_{50} value (mg extract/mL) is the effective concentration at which the $DPPH^{\cdot}$ radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. The commercial antioxidant, butylated hydroxyanisole (BHA), was used as a positive control.

Determination of the total phenolic content

Total soluble phenolic compounds in the ethanolic extracts of Se-amended and non-amended mycelia were estimated with Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965), using gallic acid as a standard. 1.0 mL of 10% Folin-Ciocalteu reagent and 200 μL of the extract were reacted in the dark for 6 min before the addition of 800 μL of 7.5% Na_2CO_3. The reaction mixture was vortexed vigorously and incubated on a rotary shaker (100 rpm) in the dark at room temperature for 2 h. The absorbance was measured at 760 nm. The total concentration of phenolic compounds in the tested extracts was determined as μg of gallic acid equivalents (GAE) per mg of dry extract, using an equation that was obtained from a standard gallic acid graph as:

$$\text{Absorbance} = 0.012 \times \text{total phenols (μg of gallic acid)} - 0.029 \ (R^2 = 0.999)$$

Determination of total flavonoid content

The total flavonoid content was determined according to Park et al. (1997). One mL of the extract was diluted with 4.3 mL of mixture containing 4.1 mL of 80% ethanol, 100 μL of 10% aluminum nitrate and 100 μL of 1 M aqueous potassium acetate. The reaction mixture was incubated at room temperature for

40 min, and absorbance was measured spectrophotometrically at 415 nm. A mixture with ethanol instead of the extract served as the blank. The amount of total flavonoids was expressed as μg of quercetin equivalents (QE) per mg of dry extract, using an equation that was obtained from the standard quercetin hydrate graph as:

Absorbance = 0.011 x total flavonoid (μg quercetin hydrate) + 0.080 ($R^2 = 1.0$)

Cytotoxic activity

Cell lines

Human cervix adenocarcinoma HeLa and human colon carcinoma LS174 cell lines, obtained from the American Type Culture Collection (ATCC) were used. Both cancer cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 100 g L^{-1} heat-inactivated (56°C) fetal bovine serum (FBS), 3 mM L-glutamine, 100 mg mL^{-1} streptomycin, 100 IU mL^{-1} penicillin, and 25 mM 4-(2 hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and adjusted to pH 7.2 with bicarbonate solution. Cells were grown in a humidified atmosphere of 95% air/5% CO_2 (v/v) at 37 °C.

Treatment of cell lines

Stock solutions (100 mg mL^{-1}) of extracts, made in 50.0 g L^{-1} DMSO, were dissolved in enriched RPMI 1640 medium to the required working concentrations. HeLa cells (2 000 cells per well) and LS174 cells (7 000 cells per well) were seeded into 96-well microtiter plates. 24 h later, after cell adherence, five different doubly diluted concentrations of the extracts were added to the wells. The final concentrations applied to target cells were 200, 100, 50, 25 and 12.5 μg mL^{-1}, except in the control wells where only the nutrient medium was added to the cells. The cultures were incubated for 72 h.

Determination of cell survival (MTT test)

The effect of extracts on cancer cell survival was determined by microculture tetrazolium test (MTT test), according to Mosmann (1983) with modification by Ohno and Abe (1991). 20 μL of MTT solution [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in phosphate-buffering saline] of 5 mg mL^{-1} concentration, was added to each well. Samples were incubated for 4 h at 37°C in a humidified atmosphere of 95% air/5% CO_2 (v/v). Then, 100 μL of 10% sodium dodecyl sulfate was added to the extract to dissolve the insoluble product formazan resulting from the conversion of the MTT dye by viable cells. The number of viable cells in each well was proportional to the intensity of the light absorbance (A), which was read in an enzyme-linked immunosorbent assay (ELISA) plate reader at 570 nm after 24 h. The inhibition rate was calculated according to the formula:

Cell growth inhibition rate (%) = $(A_{control} - A_{sample})/A_{control}$ x 100

Cis-diamminedichloroplatinum (*cis*-DDP), a commercial cytostatic, was used as a positive control. All experiments were done in triplicate.

Statistical analysis

The results were expressed as the mean ± standard error of data obtained from three parallel measurements. One-way analysis of variance (ANOVA) was performed using STATISTICA software, version 5.0 (StatSoft, Inc) to test any significant differences. P-values less than 0.01 were considered statistically significant.

RESULTS

Antifungal activity

The antifungal activity of ethanol extracts of Se-amended and non-amended mycelia was tested against 14 micromycetes, including saprobes as well as plant, animal, and human pathogens. The presence of Se in the mycelia of the studied *Pleurotus* species had almost no effect on the antifungal activity of the extracts (Table 1). Thus, in *P. ostreatus* identical

Table 1. Antifungal activity (MIC and MFC) of ethanol extracts of Se-non amended and Se-amended mycelia of *Pleurotus* spp. and commercial antimycotic

Tested organisms	*Pleurotus ostreatus*				*Pleurous eryngii*				*Pleurotus pulmonarius*				Ketokonazole	
	MIC (mg mL^{-1})		MIC (mg mL^{-1})		MIC (mg mL^{-1})		MIC (mg mL^{-1})		MIC (mg mL^{-1})		MIC (mg mL^{-1})		MIC (mg mL^{-1})	MIC (mg mL^{-1})
	Control	1.3 mg of Se	Control	1.3 mg of Se	Control	1.3 mg of Se	Control	1.3 mg of Se	Control	1.3 mg of Se	Control	1.3 mg of Se		
Acremonium strictum W.Gams	8.0	8.0	-	-	8.0	8.0	-	-	4.0	4.0	-	-	0.0078	0.0156
Aspergillus flavus Link	8.0	8.0	-	-	8.0	16.0	-	-	4.0	4.0	-	-	0.0078	0.0078
Aspergillus fumigatus Fresen.	8.0	8.0	-	-	8.0	8.0	-	-	4.0	4.0	-	-	0.0078	0.0156
Aspergillus niger Teigh.	32.0	32.0	-	-	8.0	8.0	-	-	16.0	16.0	-	-	0.0156	0.031
Aspergillus terreus Trom	32.0	32.0	-	-	8.0	8.0	-	-	16.0	16.0	-	-	0.0156	0.031
Candida albicans (C.P.Robin) Berkhout	16.0	16.0	-	-	8.0	8.0	-	-	8.0	8.0	-	-	0.0078	0.0156
Candida krusei (Castell.) Berkhout	8.0	8.0	-	-	8.0	8.0	-	-	4.0	4.0	-	-	0.0078	0.0156
Candida parapsilosis (Ashford) Langeron & Talice	16.0	16.0	-	-	8.0	8.0	-	-	8.0	8.0	-	-	0.0078	0.0156
Cladosporium sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.0019	0.0039
Fusarium verticilloides (Sacc.) Nirenberg	8.0	8.0	-	-	8.0	16.0	-	-	8.0	4.0	-	-	0.0156	0.0156
Microsporum gypseum (E. Bodin) Guiart & Grigoraki	-	-	-	-	-	-	-	-	-	-	-	-	0.0019	0.0039
Penicillium funiculosum Thom	8.0	8.0	-	-	8.0	8.0	-	-	4.0	4.0	-	-	0.0039	0.0078
Trichoderma viride Pers.	4.0	4.0	-	-	4.0	8.0	-	-	8.0	8.0	-	-	0.0039	0.0078
Trichophyton mentagrophytes (C.P.Robin) Sabour.	-	-	-	-	-	-	-	-	-	-	-	-	0.0019	0.0039

Table 2. Total phenol and flavonoid content in Se-non amended and Se-amended mycelium extracts of *Pleurotus* spp.

Tested species	Total phenol content (μg GAE/mg dried extract)		Total flavonoid content (μg QE/mg dried extract)	
	Se-non amended extract	Se-amended extact	Se-non amended extract	Se-amended extact
Pleurotus eryngii	21.9 ± 3.4[a]	24.2 ± 5.1	2.8 ± 0.7	2.9 ± 0.0
Pleurotus ostreatus	9.5 ± 1.2	8.9 ± 0.8	-	-
Pleurotus pulmonarius	14.1 ± 1.2	17.9 ± 0.3	2.3 ± 0.0	5.4 ± 0.2

[a]Each value is expressed as mean ± standard deviation (n = 3)

Table 3. Cytotoxic activity of Se-non amended and Se-amended mycelium extracts of *Pleurotus* spp. and commercial cytostatic against HeLa and LS 174 cell lines.

Tested species	IC_{50} (μg mL^{-1})			
	HeLa		LS 174	
	Se-non amended extract	Se-amended extact	Se-non amended extract	Se-amended extact
Pleurotus eryngii	251.3 ± 3.6[a]	335.5 ± 1.8	196.1 ± 2.8	325.9 ± 3.5
Pleurotus ostreatus	396.2 ± 1.5	> 400.0	381.4 ± 1.2	> 400.0
Pleurotus pulmonarius	> 400.0	333.2 ± 2.5	> 400.0	286.8 ± 1.8
Cis-DDP	0.62 ± 0.11		2.57 ± 0.27	

[a]Each value is expressed as mean ± standard deviation (n = 3)

MIC values for all studied micromycetes were noted both for Se-non-amended and Se-amended extracts. However, 2-fold MIC of the Se-amended extracts of *P. eryngii*, compared with non-amended ones, was observed for *Aspergillus flavus* and *Fusarium verticillioides* (16 and 8 mg mL^{-1}, respectively), as well as *Trichoderma viride* (8 and 4 mg mL^{-1}, respectively). Contrary to *P. eryngii*, in *P. pulmonarius* Se-enrichment of mycelium only strongly inhibited the growth of *F. verticillioides* (4 and 8 mg mL^{-1}, respectively). Comparing the efficiency of the tested extracts, *P. pulmonarius* was the most effective with a MIC of 4 mg mL^{-1} for five micromycete species, while extracts of two other species at the same concentration only inhibited the growth of *T. viride* (Table 1). None of the tested extracts and concentrations inhibited the growth of *Cladosporium* sp., *Microsporum gypseum*, and *Trichophyton mentagrophytes*, and they even showed a stimulatory effect. After reinoculation of the tested micromycete species on SDA, none of the tested extracts displayed a fungicidal effect.

Sensitivity of the tested species to the commercial antimycotic, ketoconazole, was higher. Thus, the lowest tested concentration of 0.002 mg mL^{-1} was the MIC for *Cladosporium* sp., *M. gypseum* and *T. mentagrophytes*, while *A. niger*, *A. terreus*, and *F. verticillioides* were the most resistant to the fungicide and had a MIC of 0.015 mg mL^{-1}. The MFC was 2-fold higher than the MIC for all tested species except for *A. flavus* and *F. verticillioides*, where the same concentrations (0.008 mg mL^{-1} and 0.015 mg mL^{-1}, respectively) had both fungistatic and fungicidal effects (Table 1).

No difference was noted in the mycelium growth between the negative control and 5% DMSO.

Antioxidant activity

Ethanol extracts of both Se-amended and non-amended mycelia of the studied *Pleurotus* species showed antioxidant potential that was dependent on concentration. At higher concentrations the extracts

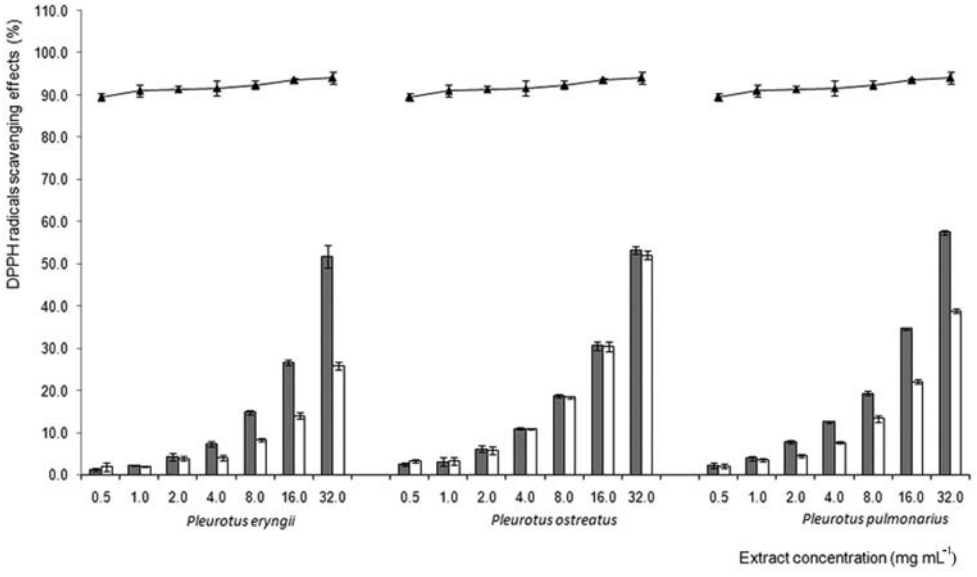

Fig. 1. DPPH radical scavenging capacity of mycelium ethanol extracts of tested *Pleurotus* species and commercial antioxidant. Se non-amended (■); Se-amended (□); BHA (–▲–). (Data represent mean value of activities of three different samples. Variations are given as standard errors).

were more effective in $DPPH^{\cdot}$ radical scavenging (Fig. 1). In the tested species, the extracts of the Se-enriched mycelia had mainly lower DPPH scavenging activity than Se-non-amended ones. These differences in the activities were significant (P <0.01) in *P. eryngii* and *P. pulmonarius*, especially at higher concentrations (≥4 mg mL^{-1}), while in *P. ostreatus* there was no significant difference in the activity between Se-amended and non-amended extracts. Se-non-amended mycelium extract exhibited a progressive increase of the activity with concentration. Thus, in *P. eryngii* the activity values ranged between 7.3% and 51.8%, and in *P. pulmonarius* between 12.6% and 57.5% (at concentrations of 4 mg mL^{-1} and 32 mg mL^{-1}, respectively), which were significantly lower than those in Se presence. However, in *P. ostreatus* the differences were minor (about 1%) at all tested concentrations (Fig. 1). These results were confirmed by the EC_{50} values. In *P. eryngii* and *P. pulmonarius* these values were 30.9±1.4 mg mL^{-1} and 27.1±0.4 mg mL^{-1}, respectively, in Se-non-amended mycelium extract, and 63.7±1.6 mg mL^{-1} and 41.2±0.2 mg mL^{-1}, respectively, in Se-amended extracts. On the contrary, in *P. ostreatus* the differences were minimum, 28.8±1.5 mg mL^{-1} and 29.2±1.5 mg mL^{-1}, respectively. The commercial antioxidant BHA was more efficient compared to the mycelium extracts, especially at lower concentrations. Noted values of BHA antioxidant activity ranged from 89.5% to 94% (Fig. 1).

The total phenolic contents in Se-non-amended and Se-amended mycelium extracts of the tested *Pleurotus* species were similar (Table 2). The phenolic content in the non-amended extracts of *P. ostreatus* was higher than in the amended ones, unlike *P. eryngii* and *P. pulmonarius* where the Se-amended extracts were slightly richer. Comparing the DPPH radical scavenging activity and phenolic proportions in the extracts, a direct correlation was noted in all of the studied species (R^2 values were from 0.937 to 0.995 in Se-non-amended mycelium extracts and from 0.977 to 0.999 in Se-amended ones).

Analysis of the flavonoid proportion showed that *P. pulmonarius* was the best producer especially during cultivation in a Se-enriched medium where pro-

duction was more than two-fold higher than in non-amended medium (5.4 and 2.3 μg QE/mg of dried extract). The difference between flavonoid amount in Se-amended and in non-amended extracts of *P. eryngii* was insignificant (2.9 and 2.8 μg QE/mg of dried extract, respectively), while *P. ostreatus* was characterized by the absence of their production (Table 2). Comparing DPPH radical scavenging activity and flavonoid proportion in the extracts, a direct correlation was noted in all studied species (R^2 values were from 0.958 to 0.990 in Se-non-amended mycelium extracts and from 0.928 to 0.987 in Se-amended ones).

Cytotoxic activity

The tested extracts showed low cytotoxic activity against both HeLa and LS174 cell lines compared with *cis*-DDP (Table 3). IC_{50} values of non-amended mycelium extracts against HeLa cells were in the range of 251.3 μg mL^{-1} to >400.0 μg mL^{-1}, and against LS174 cells between 196.1 μg mL^{-1} and >400.0 μg mL^{-1}, which was several hundred-fold higher than the values obtained for *cis*-DDP (0.62 μg mL^{-1} and 2.57 μg mL^{-1}, respectively). Se-amended mycelium extracts were weaker cytotoxic agents than the control, especially for the HeLa cell line where IC_{50} values were from 333.2 μg mL^{-1} to >400.0 μg mL^{-1}.

DISCUSSION

According to the obtained results, significant antioxidant capacity is one more characteristic that should be added to the list of medicinal properties of *Pleurotus* spp. However, unlike this feature, the species possesses almost no antifungal and anticancer activities. Although the mycelium of the species has significant ability of Se absorption and accumulation (Stajić et al., 2006; Milovanović et al., 2013), and Se, as a constituent of the active center of glutathione peroxidase, can exhibit a significant role in the protection from oxidative stress and inhibition of tumor growth in both initiation and post-initiation events (Combs and Gray, 1998), the presence of this trace element in the mycelial extract did not influence either the antioxidant or the anticancer potential.

Pleurotus species represent a deposit of numerous biologically active compounds which alone or in combination with others have significant antioxidant capacity and could be considered as potential natural antioxidants. The capacity of the fruiting bodies of *Pleurotus* species, as well as compound holders of the activity were the objects of numerous studies (Lin, 1999; Jose and Janardhanan, 2000; Jayakumar et al., 2006, 2007, 2008; Lee et al., 2007; Tsai et al., 2009; Liu et al., 2010; Xia et al., 2011). Thus, the ethanol extracts of *P. citrinopileatus* fruiting bodies and mycelia showed high scavenging of DPPH (94.9% at the concentration of 5 mg mL^{-1} and 92.8% at 20 mg mL^{-1}, respectively) (Lee et al., 2007), as did the ethanol extracts of *P. ostreatus* and *P. ferulae* fruiting bodies whose EC_{50} values for DPPH radical scavenging were less than 14 mg mL^{-1} (Tsai et al., 2009), and the water extracts of *P. sajor-caju* fruiting bodies whose EC_{50} values were in the range from 9.0 to 10.4 (Finimundy et al., 2013). Similar results were obtained by Jose and Janardhanan (2000) and Jayakumar et al. (2007) for *P. ostreatus* and *P. florida* fruiting body extracts, which showed excellent reducing power on ferric ions, and potent scavenging of hydroxyl radicals and inhibition of lipid peroxidation activities, respectively. Contrary to the presented data, the antioxidant potential of mycelium ethanol extracts of the selected *Pleurotus* species was approximately two times weaker, but still significant. Considering that mycelium could be produced faster and cheaper than fruiting bodies and that it has a high absorption ability of trace elements (Stajić et al., 2006; Milovanović et al., 2013), it could be good food supplement. These features suggest that mycelia should be studied further.

Numerous compounds with antioxidant activity have been isolated from fruiting bodies and characterized (Jayakumar et al., 2006; Lee et al., 2007; Liu et al., 2010; Xia et al., 2011). Phenolics are major and excellent antioxidants and have an important potential in protection against several degenerative diseases, including brain dysfunction, cancer and cardiovascular diseases (Finimundy et al., 2013). However, their proportion in the mushroom extracts is different from species to species, even from strain

to strain (Lee et al., 2007). Thus, the phenol content in the methanol extract of *P. cystidiosus* fruiting body was 10.2 mg GAE/mL of extract (Yang et al., 2002), in cold and hot water extracts of *P. sajor-caju* it was between 34.8 and 36.0 % mg of the catechin equivalent (Finimundy et al., 2013), in the ethanol extracts of *P. florida* and *P. ostreatus* it was 6.7 and 7.1 mg GAE/g, respectively (Tsai et al., 2009), and in the extract of the *P. ostreatus* strain studied by Jayakumer et al. (2009) it was 54.9 mg GAE/g. However, compared to these values obtained for fruiting body extracts, phenol amount was negligible in the mycelial extracts of the species tested in this study. Flavonoids, a class of phenol compounds, are also represented in the fruiting body extracts of *Pleurotus* species, and could be holders of antioxidant activity. Jayakumar et al. (2009) noted high concentrations of the widely distributed flavonoids, rutin and chryzin (31.2 mg/100 g and 40 mg/100 g, respectively) in *P. ostreatus* and a linear correlation between the amount and the antioxidant potential. According to Lee et al. (2007), the high antioxidant capacity of phenol compounds is based on the presence of hydroxyl groups, proton donors, which react with free radicals converting them to stable molecules. However, Jayakumar et al. (2008) have gone further and suggest that the mushroom extracts express antioxidant effects at the level of the antioxidant defense system genes.

The fruiting body extracts of *Pleurotus* species have already been shown to possess significant cytotoxic effects against various tumor/cancer cells both *in vitro* and *in vivo* (Jayakumar et al. 2006; Li et al. 2008; Tong et al. 2009; Finimundy et al. 2013). Thus, Jayakumar et al. (2006) noted *in vivo* inhibition of solid tumor growth by treatment with extracts of *P. florida* fruiting bodies. Finimundy et al. (2013) demonstrated the high activity of *P sajor-caju* extracts against Hep-2 and HeLa cell lines, whose IC_{50} values were between 0.23% and 1.17%, and 0.31% and 1.21%, respectively, depending on the temperature preparation. The high anticancer cell potential of the mushroom extracts has been described (Li et al., 2008; Tong et al., 2009; Finimundy et al., 2013). Lectins isolated from *P. ostreatus* and *P. citrinopileatus* fruiting bodies reduced tumor size by 88.5% and 80%, respectively (Li et al., 2008), while polysaccharides from *P. ostreatus* fruiting bodies inhibited HeLa cell growth from 23.1% to 63.3%, depending on the dose (Tong et al., 2009).

Unlike the antioxidant and anticancer capacities which are significant for numerous *Pleurotus* species, their antifungal activity is insignificant (Tim Cushnie and Lamb, 2005; Li et al., 2008). Li et al. (2008) reported the absence of any effect of *P. citrinopileatus* extracts. The antifungal activity in some mushroom extracts are due to flavonoids which inhibit the germination of micromycete spores, as was demonstrated in *Candida* spp., *Aspergillus tamarii*, *A. flavus*, *Cladosporium sphaerospermum*, *Penicillium digitatum* and *P. italicum* (Tim Cushnie and Lamb, 2005).

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