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BIOLOGICAL ACTIVITIES OF THE LIGNICOLOUS FUNGUS *MERIPILUS GIGANTEUS* (PERS.: PERS.) KARST.

MAJA KARAMAN[1], SONJA KAIŠAREVIĆ[1], JELENA SOMBORSKI[1], M. KEBERT[2], and M. MATAVULJ[1]

[1]*Department of Biology and Ecology, Faculty of Science, University of Novi Sad,* 21000 Novi Sad, Serbia
[2]*Institute of Lowland Forestry and Environmental Protection,* 21000 Novi Sad, Serbia

Abstract — Crude extracts (methanolic-ME and water-WE) of the fungus *Meripilus giganteus* (Pers.: Pers.) Karst. were analyzed for their antioxidative, antibacterial, cytotoxic, neurotoxic, and hemolytic activities using tests in vitro. The highest scavenging activity was exhibited by WE on $OH^{\bullet}$ radicals, showing a 50% effective concentration (EC50) at 292.83±2.5 μg/ml and ferric-reducing ability at 14.24±2.9 mg eq of ascorbic acid per g of dried extract (mg/g). An antibacterial effect was detected mostly against Gram-positive strains of bacteria. Toxicity assays exhibited higher effects for ME, reaching EC50=403.43±5.8 μg/ml against estrogen-dependent breast cancer cell lines (MCF-7); 50% hemolytic activity at a concentration of 30±0.03 μg/ml against erythrocytes; and EC50=4.5±0.05 mg/ml for acetylcholinesterase-inhibitory activity. These results suggest possible use of this fungus as a new source of pharmaceuticals.

Key words: *Meripilus,* Antioxidative and antibacterial activity, AChE inhibition, cytotoxicity, hemolytic activity, phenolics

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INTRODUCTION

Fungal metabolites, both secondary and primary, are rich sources of new and potentially bioactive agents with diverse structures, and the screening of these metabolites leads to creation of more active (specific) medical agents (Zjawiony, 2004). Members of the diverse fungal phylum Basidiomycota, especially the order Aphyllophorales (polypores), have a long history of medicinal use and express significant biological activities, including antioxidative (Liu et al., 1997; Cui et al., 2005; Dubost et al., 2007), antimicrobial (Suay et al., 2000; Turkoglu et al., 2006; Barros et al., 2007; Kitzberger et al., 2007), cytotoxic (Ren et al., 2006), anti-inflammatory (Grace, 2006), and hepatoprotective and antidiabetic (Lee, 2005). *Ganoderma* is the most popular Asian white-rot fungus, one that has been used for medicinal purposes for centuries, particularly in China, Japan, and Korea. Its metabolites consist mainly of polysaccharides and terpenoids and possess activities against some of the major diseases of our time, including cancer (Paterson, 2006). However, many active metabolites (proteins, polysaccharides) can also exhibit some destructive effects on the (blood) cell membrane, such as hemolytic effects, indicating that such compounds cannot be used intravenously. However, some cytotoxic activities obtained against cancer lines or inhibition of enzymes (AChE) can be used in the treatment of diseases such as cancer, Alzheimer's disease, or myasthenia gravis (Mancini et al., 2004). Data on the activities analyzed in the present study could hence contribute to our knowledge about bioactive potentials of this fungus as a new source of therapeutic agents that could be used in the treatment of such diseases. Accordingly, more toxicological assays should be applied in the evaluation of fungal extract bioactivity.

The species *M. giganteus* "blackstaining polypore" (family Meripilaceae, order Polyporales, phylum Basidiomycota) originates from Europe and North America and usually appears in the summer-autumn period. It is a saprophytic, rarely weakly parasitic fungus that causes white-rot on dead trunks, logs, and roots (*Fagus*, *Quercus*), resulting in final deg-

radation of lignin through oxidative processes and enzymes (Deacon, 2006). It is edible, but data on its pharmacology are scarce. A mixture of saturated and unsaturated fatty acids and ergosterol peroxide was identified as being immunosuppressive (Narbe et al., 1991). Among fatty acids, palmitic, oleic, and linoleic acids were determined (Giovannini, 2006). In the search for novel bioactive agents, we have analyzed two kinds of extracts of the autochthonous lignicolous fungal species *M. giganteus* from the Fruška Gora low mountain chain (Northern Serbia) for their antioxidative, antimicrobial, cytotoxic, hemolytic, and neurotoxic activities *in vitro*. Further studies may generate useful research tools and ultimately yield therapeutic agents.

MATERIALS AND METHODS

Fungus material and extraction

Wild-growing fungus was collected at the Brankovac locality (Fruška Gora low mountain chain, Serbia). After classical taxonomic identification (Hermann, 1990; Courtecuisse and Duhem, 1995), it was stored in Microbiology Herbarium Laboratory (University of Novi Sad, Department of Biology and Ecology), voucher specimen No. 1001. Powdered samples (250 g) of dried mature fruiting bodies were macerated with 400 ml of 70% MeOH (or boiled distilled water for WE) on a rotary shaker (HEIDOLPH Instruments Unimax 2010, Germany) for 72 h (24 h for WE) at 120 rpm. The filtrates (organic) were rotary-evaporated at 40°C to dryness (on a Devarot evaporating unit) and freeze-dried (in a CHRIST freeze dryer, UK) at -80°C. The dry residues were redissolved in 70% MeOH (50% EtOH) and stored (at +4°C) for further use.

Chemicals and microbiological media

Trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), ethylene diamine-tetraacetic acid disodium salt dihydrate (EDTA), ascorbic acid, and 1,1-diphenyl-2-pivcrilhydrasil (DPPH) were from Sigma (St. Louis, Mo, USA). Folin-Ciocalteu (FC) reagent was purchased from Merck (Germany), while DMSO-dimethyl sulfoxide was from Sigma (St. Louis, Mo, USA). Dulbecco's modified Eagle medium (DMEM, PAA Laboratories) was used in cytotoxicity and acetylcholinesterase or AChE (Sigma, USA) in neurotoxicity assays. The following nutrient media from TORLAK (Belgrade, Serbia) were used: nutrient agar (NA), Müller-Hinton agar (MHA), and Müller-Hinton broth (MHB).

Determination of antioxidative activity

Scavenging effect on (DPPH$^{\cdot}$) radical

The antioxidant activity of fungal extracts was determined by the DPPH$^{\cdot}$ radical scavenging method (Soler-Rivas et al., 2000). This radical with violet color (515 nm) shrinks in the presence of a hydrogen donor (antioxidant), followed by a change in absorbance, which was measured spectrophotometrically after incubation (for 30 min in the dark at 25°C). The percentage (%) of radical scavenging activity (RSC) was calculated by the equation: **RSC (%) = 100 x (A_{blank}-A_{sample}/A_{blank}).** The scavenging activity of sample was expressed as the 50% effective concentration (EC_{50}).

Determination of OH$^{\cdot}$ radical content

The content of OH$^{\cdot}$ radicals generated in the Fenton reaction was determined from the reaction of 2-deoxyribose degradation. These radicals take a hydrogen atom from 2-deoxyribose, and the products formed react with the TBA reagent, which was determined spectrophotometrically at 532 nm (Cheesman et al., 1988). Each reaction was carried out in triplicate. The intensity of scavenging activity of OH$^{\cdot}$ was determined by the same equation: **RSC (%) = 100 x (A_{blank}-A_{sample}/A_{blank}).**

FRAE (ferric-reducing ability of extract)

This procedure involves the reduction of Fe^{III}-TPTZ to a blue-colored Fe^{II}-TPTZ by biological antioxidants and chemical reductants, some of which might have no antioxidant activity in a sample. The FRAE assay compares the change in absorbance at 600 nm of a sample with the change in absorbance of a known standard ($FeSO_4x7H_2O$) to determine antioxidant levels (Griffin and Bhagooli, 2004). Each reaction was repeated three times. Ascorbic acid was used to calculate the standard curve.

Determination of total phenolic content

The concentrations of phenolic compounds in the fungal extracts, expressed as gallic acid equivalents (GAEs), were measured using a slightly modified version of the method with Folin-Ciocalteu reagent (Fukumoto and Mazza, 2000). After 2 h, absorbance was read at 760 nm. Gallic acid was used to calculate the standard curve. Estimation of phenolic compounds was repeated three times. The results were expressed as mg of gallic acid equivalents (GAEs) per g of dry extract (d.w.).

Estimation of total flavonoid content

Total flavonoid content in the investigated extracts was measured using a method based on formation of a flavonoid-aluminium complex (Park et al., 1997). After incubation (for 30 min at 25°C), absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin (Q) as standard, and the results were expressed in mg of quercetin equivalents (QEs) per g of dried extract (d.e.). All measurements were carried out in triplicate (n=3).

Determination of antimicrobial activity

Test microorganisms

In vitro antimicrobial susceptibility assays were done using 13 strains from different sources (animal-^{an}, human-^{h}, and the American Type Culture Collection-^{ATCC}). The following strains were used: *Staphylococcus aureus*$^{ATCC\ 25923}$ (S.a.), *Sarcina lutea* $^{ATCC\ 9341}$ (S.l.), *Micrococcus flavus*$^{ATCC\ 40240}$ (M.f.), *Bacillus subtilis*$^{ATCC\ 6633}$ (B.s.), *Pseudomonas aeruginosa*$^{ATCC\ 9027}$, *Clostridium perfringens*^{ATCC9404}, *Salmonella enteridis*$^{ATCC\ 13076}$, *Staphylococcus aureus*^{h} (S.a.^{h}), *Pseudomonas aeruginosa*^{h} (P.a.^{h}), *Staphylococcus aureus*^{an} (S.a.^{an}), *Rhodococcus equii*^{an} (Rh.e.^{an}), *Bacillus* sp.^{an}(Bac^{an}), *Micrococcus luteus*^{an} (M.l.^{an}), and one strain isolated from soil (laboratory strain): *Pseudomonas aeruginosa*^{4U} (Table 3).

Antimicrobial activity with MIC and MBC estimation

Both agar-well and disk-diffusion assays were employed for determination of antimicrobial activity of fungal extracts (10%, w/v) according to the CLSI (2005). Bacterial suspensions (approximately 1.5 x 10^8 CFU/ml) were added (0.1 ml) to Petri dishes (90 mm) containing MHA, where agar wells (6 mm) or paper disks (9 mm) were inoculated with 50 µl of each fungal extract. After 2 h (25°C), the plates were incubated at 37°C (18-24 h). The solvent in which the extracts were prepared was used for double dilutions and for negative control. Inhibition zones (mm) were measured around the application points (24 h), including the initial diameter of the wells or disks. Each test was repeated three times and the results were analyzed for statistical significance. Reference antibiotics (ampicillin, gentamicin) were used as standards. Evaluation of MICs was done using the dilution susceptibility test in 96-well microplates (Spektar, Serbia) according to CLSI (2003). Wells in plates that were free of tested extract served as a growth control (positive). The lowest extract concentration preventing appearance of turbidity was considered as MIC. The lowest concentration of each extract that resulted in more than 99.9% reduction of the initial bacterial count, detected where turbidity was not observed, was taken as MBC.

Cytotoxicity-MTT assay

Cytotoxicity was tested on the estrogen-receptor positive (ER+) breast adenocarcinoma (MCF-7) cell line by application of the MTT assay, a colorimetric assay based on measurement of the conversion of yellow thiazolyl blue tetrazolium bromide to a purple formazan derivative by mitochondrial dehydrogenase in viable cells. Cells were seeded in 96-well plates at a density 5,000 cells/well in 0.1 ml of the MEM 10% FCS, pre-incubated 24 h to achieve attachment, and treated by the extracts' dilutions (33.3-900 µg/ml) for 24 h and 72 h. Following incubation, the medium was removed and cells were incubated for 3 h with 0.05 mg/0.1 ml/well of MTT dissolved in serum-free MEM. Formazan salts were dissolved in 0.1 ml/well of 0.04 M HCl in isopropanol, and light absorption at 540 nm was measured using a plate reader with reference wavelength of 690 nm. Cell cytotoxicity was expressed as a percentage of the corresponding control value. The 50% effect concentration (EC_{50}) values, defined as the concentration that inhibits 50% of cell growth, were

extrapolated from concentration-response curves.

Hemolytic assay

Hemolytic activity was measured by a turbidimetric method as described previously (Mancini et al., 2004). Serial extract dilutions (20 µl) were added to bovine erythrocyte suspension (100 µl) with an apparent D_{650} of 0.5±0.01. The decrease in D_{650} was recorded for 20 min at 25°C using a kinetic microplate reader (Dynex Technologies, ZDA) to define the time necessary for 50% hemolysis (t_{50}) and the maximal rate of hemolysis. The HC_{50} parameter (mg/ml) was defined as the hemolysin concentration causing 50% lysis.

Neurotoxic-AChE assay

The extent of AChE-inhibitory activity was determined by applying the colorimetric method of Ellman et al. (1961), which was modified for use in 96-well plates in microplate reader of the SPECTRAmax 340 type (Molecular Devices Corporation, Sunnydale, CA). The determination of AChE activity is dependent upon the enzyme-catalyzed hydrolysis of acetylthiocholine and the subsequent thiocholine-mediated cleavage of the DTNB chromogen to yield a yellow chromophore whose absorbance was measured at 412 nm. Negative control was applied using 70% MeOH.

RESULTS AND DISCUSSION

Extraction yield, total phenol (TP), and flavonoid (F) content

The extraction yield was higher in water extract (WE) than MeOH extract (ME), implying that most of the soluble components had higher polarity (Table

Table 1. Extraction yield and total phenolic (TP) and flavonoid content (F) in fungal extracts. a) Extracted from dried fungal sporocarp (10.00 g). Each value is the mean of three replicate determinations ± standard deviation (n=3). b) Total phenolic content is expressed as gallic acid equivalents (GAE; mg/g GAE). Each value is the mean±SD of triplicate measurements. Values within a column with different letters (A-E) differ significantly ($p<0.05$). c) Total flavonoid content is expressed as mg of quercetin equivalents (QE) per g of dried extract (de). d) Percent (%) of flavonoid content (F) in total phenol content (TP) (w/w).

Fungal species	Yield of extract[a] (g/10 g) d.w.	Extraction (w/w) %	Total phenols[b] (mg/g)	Flavonoids[c] (mg/g)	Flavonoids/ phenolics[d] %
M. giganteus ME	1.08±0.17	10.82	11.15±0.59	0.71±0.35	6.37
M. giganteus WE	1.49±0.89	14.87	10.78±0.32	0.83±0.15	7.70
Average	**1.28**	**12.85**	**10.96**	**0.77**	**7.03**

Table 2. Antioxidant activity of examined fungal extracts, expressed as EC50 or HC50. a) EC50 [µg/ml] – conc. of extracts that caused 50% of activity (neutralization of DPPH˙ and OH˙ radicals or inhibition of cell proliferation; - EC50 not detected. b) Ferric-reducing capacity of extracts (FRAP) is expressed as ascorbic acid equivalents (AAE, mg/g d.w). c) HC50 [mg/ml] - conc. of extract that caused 50% of hemolytic activity. BHA[1] - butylated hydroxyanisole (0.32 mol/dm3). BHT[2] - tert-butylated hydroxytoluene (0.25 mol/dm3). Ostreolysine[3] - protein (hemolysine) isolated from *P. ostreatus* (Sepčić et al., 2003). poly-APS[4] - synthetic poly-alkylpiridinium compound (Mancini et al., 2004) causing irreversible inhibition of AChE.

Fungal extract	Antioxidative assays			Toxicity assays			
	DPPH [a] assay (µg/ml)	OH [a] assay (µg/ml)	FRAP [b] assay (mg/g)	Cytotox.[a] MTT assay (µg/ml)		Hemol. assay [c] (mg/ml)	Neurotox. Elmann assay[d] (mg/ml)
				24 h	72 h		
M. giganteus, ME	165.75±2.4	364.83±2.6	10.59±0.9	403.43±5.8	852.94±10.6	30±0.03	4.5±0.05
M. giganteus, WE	228.03±2.5	292.83±2.5	14.24±2.9	580.00±7.6	-	-	-
BHA[1]	2.09±0.56	384.66±1.12					
BHT[2]	8.62±0.50	427.47±1.00					
Ostreolysine[3]						0.002	
poly-APS [4]							10nM

1). Contents of total phenols (TP) and flavonoids (F) were similar in both extracts (Table 1). Slightly higher values were reached for TP in ME (11.15 mg of GAE) and for F (0.83 mg of QE) in WE.

RSC and FRAE activity

The DPPH assay showed that both of extracts exhibit low ability in neutralization of the DPPH· radical when compared to standard synthetic antioxidants (BHT, BHA), although ME was more effective than WE. Conversely, in the OH assay WE was more effective and reached EC_{50} values that were even lower than those of the synthetic antioxidants. Comparing the two assays, it seems that different mechanisms (compounds) are involved in neutralization of these two radicals. The ferric-reducing capacity of WE was higher than that of ME, indicating that more polar compounds are responsible for FRAE activity. Previous studies showed a positive correlation between polyphenol content and antioxidant capacity (Ribeiro et al., 2007; Turkoglu et al., 2007). The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (Dubost et al., 2007). Furthermore, flavonoids have been proven to express antioxidative or antimicrobial activities in the lignicolous species *L. sulphureus* (Turkoglu et al., 2007). To judge from the positive correlation obtained in the present study between F content and RSC on OH· radical content and FRAP assay results (r^2=1, $p<0.05$), flavonoids seem to contribute greatly to the expressed activities. On the other hand, the negative correlation between F content (and positive correlation with TP) and RSC in the DPPH assay allows of the conclusion that some other phenolics (mechanisms) of action are responsible for the moderate RSC activity observed in this assay. Given that the crude extracts probably contained many compounds which may have acted synergistically, it is impossible to ascertain which particular compounds are responsible for the effects observed in different extracts. In view of the noticed ability of extracts to form soap-like foams in an aqueous solution, it is reasonable to expect saponins in both types of extracts (Cowan, 1999), while more proteins (lectins) and polypeptides could be found in WE and more polyphenols and terpenoids in ME. Certain other compounds, such as tannins (water-soluble polyphenols), polysaccharides, or steroids

Table 3. Antibacterial screening of analyzed fungal extracts with MICs and MBCs values (mg/ml) in two different assays. (1) Inhibition zone for agar-well method: + - < 10mm, ++ - 10-15mm, +++ - 16-20 mm, ++++ - 21-25 mm, +++++ - 26-30 mm of inhibition growth in mm. 1a Inhibition zone for disk diffusion method: + - < 15mm. ++ - 15-20 mm.+++ - 20-25 mm, ++++ - 25-30 mm, +++++ - 30-35 mm of inhibition growth in mm. (2) Source: h - human pathological strain; an - bacterium of animal origin; ATCC - American type culture collection strain; S - sensitive; I- intermediary; R – resistant. (3) Fungal extract: Mg - *M. giganteus;* ME - methanolic extract; WE - aqueous (water) extract. (4) Extract concentration (mg/ml); all extracts were diluted with the same organic solvent.

Bacterial strains[2]	***M.l.*** [an]	***S.a.*** [an]	***Rh.e.*** [a.]	***Bac*** [an]	***P.ae.*** [4U]	***S.e.*** ATCC 13076	***S.l.*** ATCC 9341	***S.a.*** ATCC 25923	***M.f.*** ATCC 40240	***B.s.*** ATCC 6633	***S.a.*** [h]	***Cl.p.*** ATCC 9404	***P. ae.*** ATCC 9027
Fungal extracts[3] (mg/ml)	**Agar-well method[1]**												
Mg ME	++	+++	++	++	-	-	++	++	+	++	++	-	-
Mg WE	-	-	-	+	-	-	-	-	-	+	-	-	-
	MIC/ MBC (mg/ml)												
Mg ME	1.12 10	0.28 4.5	2.25 ↑9.0	1.12 ↑9.0	-	-	1.12 ↑9.0	2.25 9.0	1.12 10	0.28 10	2.25 ↑9.0	-	-
Mg WE	-	-	-	-	-	-	-	0.625 ↑10.0	2.50 ↑10.0	-	-	-	-
	Disk-diffusion method[1a]												
Mg ME	++	++	+++	+++	-	-	+	++	++	++	++	+	+
Mg WE	-	-	+	+	-	-	-	+	++	+	-	+	-
Amp (µg/ml)	38 S	47 R	12 R	0 R	0 R	21 S	38 S	34 S	29 S	S	35 S	30 S	0 R
Gent (µg/ml)	26 S	20 S	22 S	22 S	27 S	22 S	38 S	20 S	25 S	23 S	19 S	27 S	23 S

(Smania, 1999), could also be responsible for the obtained activities, i.e., antimicrobial (Akiyama et al., 2001), anticancerogenic (Francis et al., 2002), or neurotoxic activities. Further investigations on the fractionation of crude extracts and identification of the bioactive components are being planned.

Antibacterial activity

The antimicrobial activities of crude extracts were tested against nine species of Gram-positive and four species of Gram-negative bacteria (Table 3). The most active extract was ME, which was able to inhibit all the Gram-positive bacteria but none of the Gram-negative ones, except for some activity against ATCC *C. perfringens* and *P. aeruginosa* in the disk-diffusion assay. The animal strains *S. aureus*^{an}, *Rh. equi,* and *Bacillus* in both applied tests were the most susceptible analyzed strains, indicating a possible application of this fungus against Gram-positive bacterial infections in animals. Since WE in the present study had a narrow antibacterial effect, the obtained properties could not be attributed to compounds like proteins or polysaccharides usually found in WE. These results are in agreement with published data for similar polypore fungi (Zjawioni, 2004; Lindequist et al., 2005) indicating sterols and lanostanoid terpenoids as the main active components. Certain phenolic compounds could also be responsible for the obtained activity (Barros et al., 2007; Elmastas et al., 2007; Turkoglu et al., 2007). Differences between the two screening methods applied were not statistically significant ($p<0.05$). Both *Meripilus* extracts showed wider inhibition zones in the disk-diffusion method, indicating that it is more appropriate for the testing of polar extracts.

Cytotoxicity

Malignant cells from (ER+) cell lines were sensitive to both extracts. This could indicate that some compounds present in the analyzed extracts have antiproliferative (antitumor) activities in human breast adenocarcinoma cells showing higher effects for ME after 24 h (EC_{50}=403.43±5.8). This activity was dose- and time-dependent. In addition, chronic activity was present after 72 h (Table 2). Although there are no data about cytotoxic activity of this species, these results are in accordance with published data for ME of some polypore fungi (Ren, 2006; Harhaji et al., 2008). Many authors (Jiang et al., 2004; Lau et al., 2004; Zjawioni, 2004; Peterson, 2006) report that terpenoids and steroids in *Ganoderma* and *Coriolus* fungi are cytotoxic compounds causing induction of apoptosis. In view of their similar habitats (wood) and white-rot degrading bioactivity, we assume that similar compounds could also be found in the analyzed fungus. Further analyses will be focused on detection of both compounds and their mechanisms of action, since cytotoxicity may be the result of different mechanisms, such as inhibition of cell division, protein synthesis, or others.

Hemolytic activity

Crude WE exhibited no hemolytic activity in the present study, while ME was able to lyze bovine erythrocytes in a concentration-dependent manner, exhibiting a linear correlation (r^2=0.96). Average t_{50} was obtained after 2.22±0.25 min at 30 mg/ml. Compared with ostreolysin from *P. ostreatus*, which exihibits HC_{50} at 1 μg/ml (Sepčić et al., 2003), it has lower activity; however, in regard to the time when 50% hemolysis occurred (t_{50}<5min), this activity is very high. The obtained activity, which caused high cell membrane lysis, could be a limiting factor in the possible application of these fungi as sources of pharmaceuticals. Further studies on the isolation of active substances are therefore needed. We assume that the maceration procedure with WE prepared from fresh material (not dry fruiting bodies) will reveal new results.

Neurotoxic activity

Concentration-dependent AchE inhibition was obtained only with ME and reached EC_{50} at 4.5 mg/ml. To our knowledge, this is the first detection of such bioactivity in this species. Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine in nervous system synapses. Several irreversible AChE inhibitors are known as warfare agents and insecticides, and some reversible inhibitors are used for treating certain neurological disorders related to AChE dysfunction, e.g., diseases such as Alzheimer's and myasthenia gravis (Mancini et al., 2004). It

is therefore possible that ME of *Meripilus* may be useful as a source of therapeutic agents for treating these diseases.

In conclusion, the presented results show that WE of the fungus *Meripilus* has very high potential RSC activity on $OH^{\cdot}$ radicals and FRAE activity, whereas ME shows remarkable hemolytic activity against erythrocytes, cytotoxic (antitumor) activity against human breast ER+ cell lines, and mild neurotoxic activity against AChE. Comparing the high hemolytic activity with the moderate cytotoxic activity, we believe that different compounds and mechanisms could be responsible for the obtained effects. Since the extracts were in the crude form, many different compounds may have produced the manifested hemolytic and cytotoxic activities. Further characterization of these extracts and isolation of active compounds may generate valuable research tools and ultimately stimulate future investigations of the species *M. giganteus*, which could be useful as a novel source of therapeutic agents if the negative side-effects such as high hemolysis are overcome.

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БИОЛОШКА АКТИВНОСТ ЛИГНИКОЛНЕ ГЉИВЕ *MERIPILUS GIGANTEUS* (PERS.: PERS.) KARST.

МАЈА КАРАМАН[1], СОЊА КАИШАРЕВИЋ[1], ЈЕЛЕНА СОМБОРСКИ[1], М. КЕБЕРТ[2] и М. МАТАВУЉ[1]

[1]*Департман за биологију и екологију, Природно-математички факултет, Универзитет у Новом Саду,* 21000 Нови Сад, Србија
[2]*Институт за низијско шумарство и заштиту животне средине,* 21000 Нови Сад, Србија

Чврсти (метанолни и водени) екстракти гљиве *Meripilus giganteus* (Pers.: Pers.) Karst. су тестовима *in vitro* анализирани на антиоксидативну, антибактеријску, цитотоксичну, неуротоксичну и хемолитичку активност. Највиша „скевенџер“ активност је добијена за водени екстракт на $OH^{\cdot}$ радикал показујући 50 % ефективну концентрацију (EC_{50}) при 292.83±2.5 μg/ml и способност редукције гвожђа на 14.24±2.9 mg еквивалента аскорбинске киселине по граму сувог екстракта mg/g. Антибактеријска активност је детектована углавном на Грам позитивне бактерије. Тестови токсичности су показали јаче ефекте у метанолном екстракту достижући EC_{50}=403.43±5.8 μg/ml на естроген зависну линију карцинома дојке (MCF-7); 50 % хемолитичку активност еритроцита при концентрацији од 30±0.03 mg/ml и 50 % инхибицију ензима ацетилхолинестеразе при концентрацији (EC_{50}=4.5±0.05 mg/ml). Ови резултати указују на могућност употребе ове гљиве као новог извора фармаколошких супстанци.