Antioxidant Components and Antioxidant Activities of Hydroglyceroalcoholic Extract from Fresh Mushroom Mycelium

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The in vitro antioxidant activity, as well as the hydroglyceroalcoholic extracts main active components from mycelia of Pleurotus ostreatus and Coprinus comatus, were determined. The mycelium submitted for extraction was obtained by submerged cultivation in a bioreactor. The hydroglyceroalcoholic extracts, in particular, those of P. ostreatus PSI101109 and PQMZ91109 mycelium had a considerable antioxidant activity, and was compared to the overall constituent of flavonoids plus phenolic compounds. According to the extracts HPLC assessment, the key phenolic acid evident from the extracts was gentisic acid. The homogentisic, gallic and protocatechuic acids were also determined. Catechin was identified as well, except for C. comatus M8102 and P. ostreatus PBS281009 mycelia extracts. Since catechol and gentisic acid levels were high, respectively, there was a correlation with the absence of carotenoid compounds in hydroglyceroalcoholic extracts (P. ostreatus PSI101109). There is a correspondence between the hydroglyceroalcoholic extracts' key antioxidant activities with the catechin and polyphenolic acids levels identified in each extract.

Keywords: antioxidant activity, gentisic acid, glycerol, mycelium, Pleurotus

The antioxidant activity and the content in polyphenols are two interdependent parameters when considering products of nature. Finding natural products that are easy to obtain, and that have a high content of these compounds is a current research trend. This results from the fact that human metabolism can produce a number of free radicals that have significant adverse effects on human health. The derived free radicals take part in cancer instigation plus cardiovascular and degenerative ailments [1]. The mushrooms and their derived products contain a large phenol amounts, inclusive of phenolic acids and flavanoids. They exert most of the antioxidant activities which were demonstrated for the fungi [2]. Besides these compounds, significant levels of ascorbic acid and carotenoid compounds have been found, which have a significant antioxidant effect [3].

The extracts of mushroom mycelium represent a current way of rapidly extracting the biologically active components of mushrooms. This mycelium can also be grown in bioreactors, obtaining amounts equal to 50 g/L biomass WCW (wet cell weight) [4]. The scavenging activity of submerged mycelium from mushroom depends on the cultured species, on the solvent used, and on the applied method of extraction [5]. The present study's goals includedassessment of antioxidant activities, lipid peroxidation inhibition, power reduction, as well as chelation on hydroglyceroalcoholic extracts' ferrous ions from fresh mycelium of four *Pleurotus ostreatus* strains, to compare with the *Coprinus comatus* strain, and to determine their active antioxidant compounds.

Experimental part

Materials and methods

Chemicals

The reagents and chemicals used were procured from a German firm, Merck and Sigma-Aldrich GmbH, in Sternheim. The rest unlabeled reagents and chemicals entailed an analytical grade.

Biologic material and culture media

The mushrooms *Pleurotus ostreatus* M2191, *P. ostreatus* PQMZ91109, *P. ostreatus* PBS281009, *P. ostreatus* PSI101109, and *Coprinus comatus* M8102, were acquired from the collection of Bucharest's Biotechnology Faculty in Romania. Verification was ensured by the Biology Faculty's Dr. D. Pelinescu, from Romania's Bucharest University.Preservation of mycelia was achieved by freezing it at –85°C in 10% glycerol. A LabTech gyratory shaker rotating at 150 revolutions per minute was engaged in the growing mushrooms and employed in preparing the inoculums. The shaker was maintained at 25°C, for 5 days and in 500 mL Erlenmeyer flasks with 250 mL of the subsequent synthetic medium (for every liter): glucose (6.0 g); malt extract (100.0 g); KH_2PO_4 (1.0 g); yeast extract (20.0 g); and $MgSO_4 \times 7\ H_2O$ (0.5 g). NaOH (0.2M) were used in regulating the medium to *p*H 5.5 [6].

Subsequently, a 500mL flask with 300mL of the media upon 10% (v/v) inoculation of the initial inoculum culture within the above-stipulated circumstances was engaged in performing the other inoculum. Inoculation of the second inoculum culture (10% v/v) with a fermentation medium of, $CaSO_4$ 0.5%, KH_2PO_4 0.2%, Na_2HPO_4 0.01%, $MgSO_4$ 0.05%, corn extract (40% of dry matter) 1% in 5% cornstarch solution, was done and later grown in a 5–l New Brunswick BioFlo 310 bioreactor. Various conditions considered during fermentations engagements included agitation speed 150 rpm, the temperature of 25^{0}C, *p*H 5.5–6, aeration rate 1 vvm, as well as working volume 4 l. A 7-day cultivation was engaged when conveying the inoculum culture to the fermentation medium until the mycelia reached growing phase [7,8]. Centrifuging of the fermented mediums was done for a period of 15 min, while distilled water was used in washing the biomass twice [9].

Extraction procedures

A fresh mushroom mycelia sample (10%) was blended in a mixture of water, ethanol and glycerin (1:1:1) for 10 days, 150 rpm and in cool temperature. The extracts were

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evaporated for all the alcohol, and the water poured. After that, the solution was incubated at 65°C with the remnant alcohol evaporated to acquire a semisolid extract. An amount of 80% ethanol was used in dissolving the extracts,resulting in solutions of different concentrations: 2–10 mg/mL [9].

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity

The content of the reaction solution entailed test samples (50 μL) plus 0.04% (w/v) DPPH solution (5 mL) in ethanol. Subsequent to a 30 min incubation period, discoloration was gauged at 517 nm. The equation used in determining DPPH radical concentration was:

$$DPPHscavengingeffect(\%) = A0 - AP/A0 \times 100,$$

with A_0 the control absorbance and A_p the sample absorbance. The ideal absorption decline generated by the test compounds was assessed against the positive controls. The objectives of Ascorbic acid were based on positive control and were for comparison purposes. A graph of *RSA percentage against concentration of extract* was used in achieving extract concentration offering free radical scavenging activity (50%) (RSA; EC_{50}) [10-12].

Reducing power

The technique delineated by Gulcin et al. [13] was engaged in determining the reducing power. For every extract (2.5 mL ethanol), a mixture of 1% potassium ferricyanide (2.5 mL) and 200 mM sodium phosphate buffer (2.5 mL and *p*H 6.6) was considered and later incubated at 50°C for a period of 20 min. Subsequent to that, there was an addition of 10% trichloroacetic acid (2.5 mL), and later a centrifuging of the mixture at 3000 g for a period of 10 min. A mixture of the outer layer (2.5 mL) with 0.1% ferric chloride (0.5 mL) and deionized water (2.5 mL) was subsequently considered. Eventually, 700 nm was the absorbance measure in relation to a blank, with a value of EC_{50} (mg extract/mL) being the effectual concentration whose absorbance was identified being 0.5. Ascorbic acid centered on the comparison purposes [12,14].

Superoxide RSA of freeze-dried extracts

The superoxide radicals' scavenging actions were analyzed in line with the techniques delineated by Chou et al. [15]. An identical volume of 120 μM PMS, freeze-dried extract, 936 μM NADH, plus 300 μM NBT was considered in a reaction mixture of 100 mM phosphate buffer (1 mL and *p*H 7.4). The mixture was incubated for 5 min at an ambient temperature and the resultant solution's absorbance gauged at 560 nm. The following technique was employed in determining superoxide radical activity:

$$scavenging\ effect\ =$$

$$= (1 - absorbance\ of\ sample/absorbance\ of control) \times 100.$$

For the comparison purposes, ascorbic acid was employed. After scavenging the hydroxyl radicals at 50%, the resultant effective concentration was EC_{50} (mg extract/mL) [16].

Hydroxyl radical scavenging activity

Just as portrayed by Varshneya et al. [17], the present study assessed the hydroxyl radical scavenging although by considering a minor modification. This analysis is derived on computing for the 2-deoxyribose (degradation product) by having it condensed courtesy of TBA (thiobarbituric acid). Creation of hydroxyl radicals was done courtesy of a Fenton reaction using Fe^{3+}-ascorbate-EDTA-H_2O_2. The components contained in the eventual volume (1 mL) of the reaction included 2.8 mM of 2-deoxy-2-ribose, 20 mM of KH_2PO_4-KOH buffer (*p*H 7.4), 1.0 mM of H_2O_2, 100 μM of $FeCl_3$, 100 μM of ascorbic acid, 100μM of EDTA, among a range of hydroglyceroalcoholic extracts concentrations. Subsequent to a 1h incubation period at a temperature of 37°C, integration of 0.5 mL of the reaction mixture was done on 2.8% TCA (1mL), and later an addition of 1 mL of 1% aqueous TBA was considered, and then the resultant mixture underwent 90°C incubation for a period of 15 min to have the color developed accordingly. Subsequent to the cooling, the absorbance was gauged at 532 nm alongside an apt blank solution. Inhibition percentage was calculated by having the test compared with blank solutions, with the effective concentration being identified at a value of EC_{50} (mg extract/mL), where scavenging of hydroxyl radicals was done at 50% [18].

Establishing antioxidant component

Establishing total phenolic content

There was an addition of 0.5mL of the extract sample to 2.5 mL of Folin-Ciocalteu reagent in a dilution ratio of 1:10, and a saturated solution of sodium carbonate (75 g/L, 2 mL on average) was added 4 min later. The reaction mixture's absorbance was identified at 760 nm after a 2h incubation period at room temperature. The reference standard engaged was Gallic acid, with the outcome provided as mg GAE (Gallic acid equivalent) of the extract [19-21].

Establishment of total flavonoids

Measuring flavonoid content was achieved by engaging the simple and consistent technique provided by Jia et al., [21] and Barros et al. [22]. Ethanolic extract (50μL) was mixed with 5% $NaNO_2$ (37 μL) and deionized water (700 μL). After incubation for 5 min at room temperature, a 10% $AlCl_3$ (75μL) was added initially then 1M NaOH (250 μL) during the subsequent 6 min. Centrifuging (5000 × g, for 15 min at room temperature) was then done, with the supernatant's absorbance realized at 515 against a blank. The standard engaged was Quercetin [23]. Establishment of flavones and polyphenol carboxylic acids was achieved courtesy of top-performance liquid chromatography as offered in an earlier study [24].

Establishing β-carotene and lycopene.

In establishing lycopene and β-carotene, a comprehensive shaking of dried ethanolic extract 100 mg was done together with an acetone-hexane mixture (10 mL) (at a ratio of 4:6) for 1min period and later filtered via Whatman No. 4 filter paper. The filtrate's absorbance was gauged at 663, 505, 453 nm. Lycopene and β-carotene content were established respective of the equations below:

$$lycopene(mg/100\,mL) =$$

$$= -0.0458 \times A663 + 0.372 \times A505 - 0.0806 \times A453$$

$$\beta - carotene(mg/100\,mL) =$$

$$= 0.216 \times A663 - 0.304 \times A505 + 0.452 \times A453.$$

Expression of the results is done in terms of mg of carotenoid/g of extract , as stated by Barros et al. [22].

Statistical breakdown

All antioxidant and antimicrobial aspects' activities were evaluated in triplicate, while the outcome was delineated as mean ± SD values of 3 outcomes. Microsoft Office 2010 EXCEL software was used in determining both the standard deviation and mean values.

Results and discussions

Extraction yield plus antioxidant compounds content ofhydroglyceroalcoholic extracts

Generally, any increase in yield of the extract is associated with an increase in the quantity of biomolecules that possess antioxidant effects. The efficiency in obtaining the hydroglyceroalcoholic extracts ranged between 3.72 and 25.22% for *P. ostreatus*, being lower at ≈12% for *P. ostreatus* M2191 compared to *C. comatus* M8102. Following the use of this type of solvent, the content of biomolecules with antioxidant effects is also expected to increase [9].

Table 1 presents the determined values of molecules with antioxidant effect. The values of the total quantities of phenols and flavonoids have been partly described previouslyin a preliminary study [9]. Thus resulted a maximum quantity for *P. ostreatus* PSI101109, of 58.8±0.56 mg/100 mL of extract, followed by *P. ostreatus* M2191 with 42.54±2.53 mg/100 mL, and finally by the strain *C. comatus* M8102, with the minimum determined value of 13±0.67 mg/100 mL. As in other previous studies, the flavonoid content is not correlated with that of the total phenolics [25]. The exception was species *C. comatus* M8102's mycelium extract that also presented the lowest content of flavonoids, compared with the tested species.

The breakdown of both the flavonoids and phenolic compounds was accomplished by top-performance liquid chromatography, within the limits of the mentioned controls. It was noted that in the hydroglyceroalcoholic mycelium extract of *P. ostreatus* PBS281009, some other compounds can be present, which have not been tested in this study. The most important phenolic acid was gentisic acid and situated entirely within the extract of the species *P. ostreatus* PQMZ91109 (18.827 ± 0.02 mg/100 mL – fig. 1, Peak 2), and PSI101109 (19.340 ± 0.01mg/100 mL – fig. 2, Peak 2). As with other conventional extracts, the gallic acid was the second component from the quantitative point of view, with a maximum of 5.591 ± 0.06 mg/100 mL in the extract of *P. ostreatus* PQMZ91109 (fig. 1, Peak 1). In the same two pecies of *P. ostreatus*, the homogentisic and protocatechuic acids have been identified. In the hydroglyceroalcoholic mycelium extract of *C. comatus* M8102, an amount of only 0.487 ± 0.0 mg/100 mL of homogentisic acid was identified. It is of significance that the presence of catechin with 12.370 ± 0.1 mg/100 mL in *P. ostreatus* PSI101109 extracts. This compound is found in quantities below 1 mg/100 mL in mycelium extracts of

Table 1

ANTIOXIDANT COMPOUNDS CONTENT OF HYDROGLYCEROALCOHOLIC EXTRACTS FROM FRESH MUSHROOM MYCELIUM

Antioxidant compounds	M2191	PBS281009	PQMZ91109	PSI101109	M8102
Total phenols (mg GAE/100 mL)	42.54±2.53	36.24±0.18	31.86±0.77	58.8±0.56	13.00±0.67
Gallic Acid (mg/100 mL)	0.025±0.0	-	5.591±0.06	3.701±0.004	0.154±0.0
***Gentisic Acid* (mg/100 mL)**	-	-	18,827±0.02	19.340±0.01	-
Homogentisic Acid (mg/100 mL)	-	-	1.776±0.01	4.737±0.0	0.487±0.0
Protocatechuic *Acid* (mg/100 mL)	-	-	0.124±0.001	2.937±0.003	-
Total flavonoids (µg quercitine/100 mL)	74.7±0.49	77.1±0.34	59.25±0.08	12.00±0.41	1.5±0.03
Catechin (mg/100 mL)	0.154±0.0	-	0.644±0.0	12.370±0.1	-
β-carotene (mg/100 mL)	0.006±0.0001	0.017±0.0003	0.024±0.0009	-	-
Lycopene (mg/100 mL)	0.001±0.0004	0.014±0.0007	0.017±0.0003	-	-
Extraction yield (%)	39.65±0.31	11.35±0.19	9.45±0.03	3.72±0.15	28.69±1.52

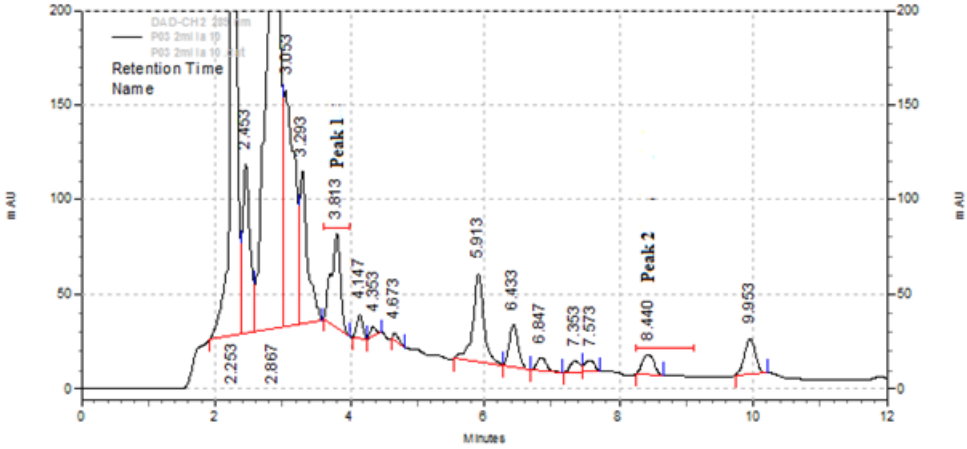

Fig. 1. The phenolic profile of the extract of hydroglyceroalcoholic extracts (*P. ostreatus* PQMZ91109) at 289 nm

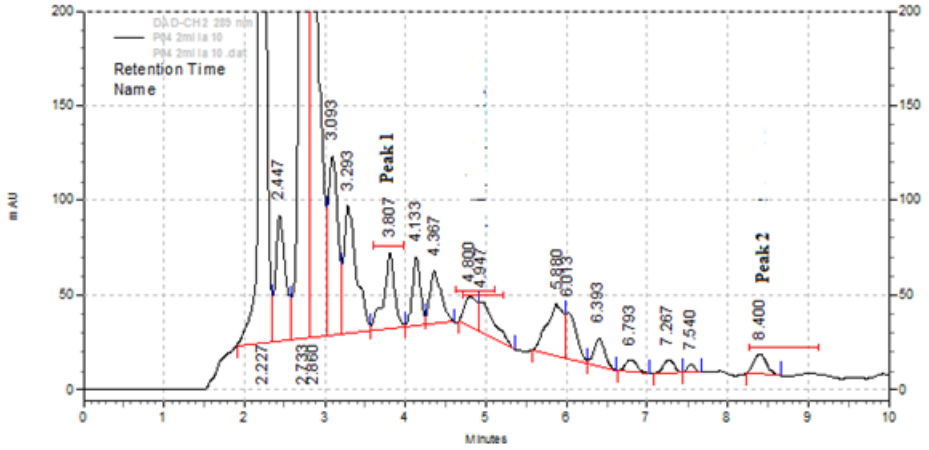

Fig. 2. The phenolic profile of the extract of hydroglyceroalcoholic extracts (*P. ostreatus* PSI101109) at 289 nm

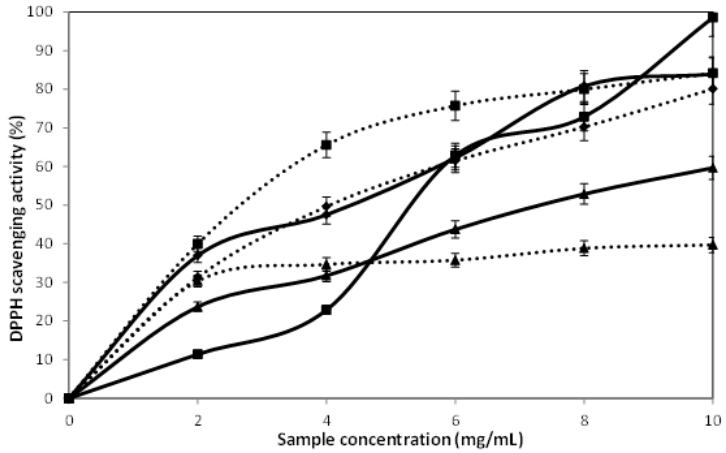

Fig. 3. DPPH radical scavenging activity of hydroglyceroalcoholic extracts

the species *P. ostreatus* PQMZ91109 and *P. ostreatus* M2191, along with only gallic acid, has been identified.

It was also noticed that a high amount of phenolic acids, namely catechin, may be correlated with the lack of carotenoid compounds in hydroglyceroalcoholic extracts (*P. ostreatus* PSI101109). In the rest of the extracts, except for the one from the mycelium of *C. comatus* M8102, β-carotene amounts ranging from 0.006 ± 0.0001– 0.024 ± 0.0009 mg/100 mL of extract have been identified, as well as lycopene between 0.0004 ± 0.001–0.017 ± 0.0003 mg/100 mL extract.

Antioxidant Activity

DPPH RSA

'DPPH' radicals are extensively utilized in the antioxidant activity's analysis because they are very stable radicals, and the method is quick and accurate. The antioxidant activity was determined as a reduction percentage of primary DPPH absorbanceby the hydroglyceroalcoholic extracts at diverse concentrations (fig. 3) [26]. The radicals' scavenging effect was augmented with an increment in the free radical inhibition's percentage [27]. The hydroglyceroalcoholic extracts' scavenging impact on DPPH radicals goes up with sample concentration. With respect to the value obtained, at 10 mg/mL, the decreasing order was the following: PSI101109 > M8102 ≈ M2191 > PQMZ91109 > PBS281009. Thus, the EC_{50} was of 5.85 mg/mL for *P. ostreatus* PSI101109 value, which is ≈ 74% more compared to that of ascorbic acid.

Thus, both *C. comatus* M8102 and *P. ostreatus* M2191 hydroglyceroalcoholicextractshowed similar values with the ethanol extract of *Leucopaxillus giganteus* mycelium grown within $(NH_4)_2HPO_4$ as source of nitrogen [28]. Instead, the hydroglyceroalcoholic extract of *C. comatus* M8102 mycelium had a similar DPPH scavenging activity with the ethanol extract of fruit bodies, with a 10 mg/mL concentration [29]. The outcome depends not only on the strain, but also on the extraction technique and on the solvent from the protocol. The sample with 10 mg/mL of the lyophilizate mycelium extract of *P. ostreatus* PQMZ91109 cultured within sources of organic nitrogen had lower values of up to 60% compared to the yeast extract [30]. On account of the outcome obtained, it was shown how such an extract contains molecules with antioxidant effect in an amount at least similar to the conventional extracts. By the values of DPPH scavenging activity, superiority of the hydroglyceroalcoholic extracts could be linked with enhanced amounts of flavonoid and phenolic compounds, determined by the use of three polar solvents [31].

Reducing power

The hydroglyceroalcoholicextracts' reducing power from fresh mycelium of *P. ostreatus* and *C. comatus* increased with their concentration and it is a direct indicator of their antioxidant capacity. Depending on the sample concentration, the test solution's yellow coloris altered different blue shades,respective of the quantity of antioxidant compounds [32].

The acquired results showed that at the maximum concentration, the hydroglyceroalcoholic extract from mycelium of *C. comatus* M8102 had the highest value of 2.12 (fig. 4). The value is ≈19% higher than the maximum that was obtained from the mycelium of *P. ostreatus* PSI101109, and was ≈ 36% higher than that of standard ascorbic acid. As regards to themycelium's extracts of the other three strains of *P. ostreatus*, the value of the reducing power was lower and ranged between 0.575 and 0.94 (at 10 mg/mL). At this concentration of the sample, the value of the reducing power in *C. comatus* M8102 was significantly higher. The ethanolic extracts of mycelium and fruit bodies had a reducing power which varied

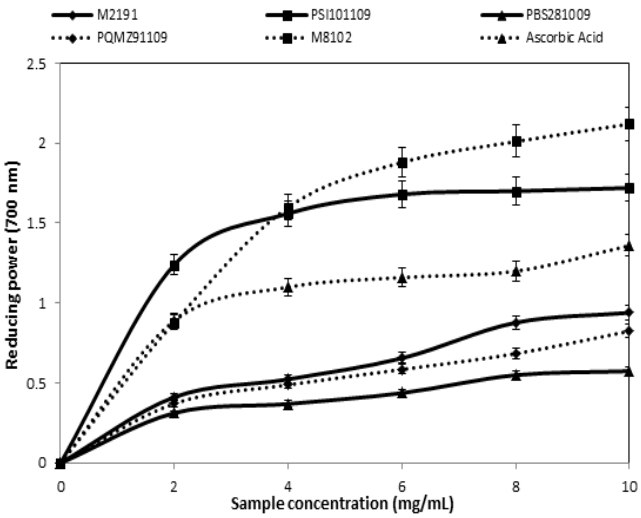

Fig. 4. Reducing power of hydroglyceroalcoholic extracts

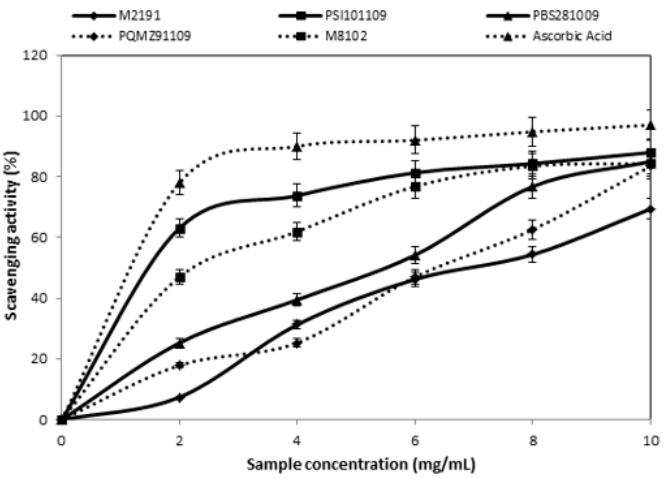

Fig. 5. Superoxide anion scavening activity of hydroglyceroalcoholoc extracts

between 0.2 and 0.6, while the aqueous ones varied between 0.5 and 0.85 [29]. For the hydroglyceroalcoholic extract from mycelium of *P. ostreatus*, the values are comparable to those acquired from the lyophilized extract of the mycelium cultured amid ammonium sulfate as nitrogen source [30]. These values of the hydroglyceroalcoholic extracts are no more than 10% higher than the power reduction of the alcoholic extract from dried mushroom powder of *Lactarius deliciosus* [32]. Thus, these extracts have shown their presence in a larger quantity of some molecules with antioxidant effect, which corresponds to previous studies regarding the association of the reduction power values with that of reductones. This direct relationship of reductones with reducing properties of the extract is effective in preventing peroxide formation, by reaction with precursors of peroxide [33].

Superoxide RSA

Cytotoxicity of this radical must be controlled because it is an antecedent of some reactive classes of oxygen (single oxygen, hydroxyl radical, and hydrogen peroxide) [34]. The superoxide radical is generated *in vivo* and initiates lipid peroxidation due to the creation of hydrogen peroxide, forming precursors which are capable of forming stronger reactive oxidative species [35]. The scavenging effects of hydroglyceroalcoholic extracts against superoxide radicals are presented in figure 5. The extract from *P. ostreatus* PSI101109 mycelium had theutmost scavengingeffect (88%) at 10 mg/mL sample absorption.The lowest effect was determined for the hydroglyceroalcoholic extract from fresh mycelium of *P. ostreatus* M2191. Otherwise, the other three strains showed, at 10 mg/mL, a value of ≈ 84% (± 1%). Compared to EC_{50} for ascorbic acid, *P. ostreatus* PSI101109 showed a value 63% higher (1.65 mg/mL). Compared with the alcoholic extract of *Armillaria mellea* mycelium, the EC_{50} values are ≈32% superior for *P. ostreatus* PSI101109, and 71% higher for *C. comatus* M8102. Generally, the values of EC_{50} index did not exceed 9.5 mg/mL for all hydroglyceroalcoholic extracts and, thus, can be deduced as having a strong scavenging effect against the superoxide radical [36].

Hydroxyl RSA

The highly reactive type of active oxygen is the hydroxyl radical, which is responsible for the cytotoxic effect in plant and animal cells. The radical is produced in different conditions of stress, such as inflammatory processes, the

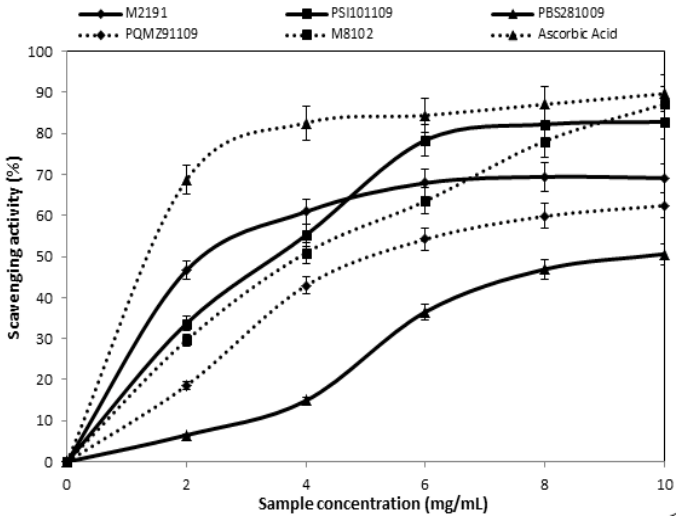

Fig. 6. Hydroxyl RSA of hydroglyceroalcoholic extracts

action of some herbicides, cell death, or when the organism is defending against various pathogens [37]. Its negative action is manifested at the level of DNA and of the cell membrane, and combating these negative effects is of great interest today. Figure 6 illustrates 'the hydroglyceroalcoholic extract's scavenging hydroxyl radical as well as that of standard ascorbic acid. The hydroglyceroalcoholic extract of *C. comatus* M8102 mycelium had the highest scavenging effect of 87.1% at 10 mg/mL. Instead, the same type of extract from *P. ostreatus* PSI101109 mycelium presented a value ≈ 5% lower, and the standard with 2.8% higher. The other strains showed values of the scavenging activities of at least 50.6% for the maximum tested concentration. The EC_{50} value of the extracts from *C. comatus* M8102 mycelium, and of *P. ostreatus* PSI101109, were within the limit of 4.2–4.6 mg/mL. The results showed that the hydroxyl scavenging activities are directly dependent on the sample concentration. This property of inhibiting the hydroxyl radical is important because it prevents, or limits, the rate of the chain reaction that causes adverse physiological effects manifested by disturbances of the normal cellular activities [35].

Metal chelating activity

It is acknowledged that metals (particularly iron) are significant in different phases of the inflammatory process. Iron can catalyze the production of free radicals, instigating the lipid peroxidation procedure and cell membrane damage [38]. Ferrous iron is capable of instigating

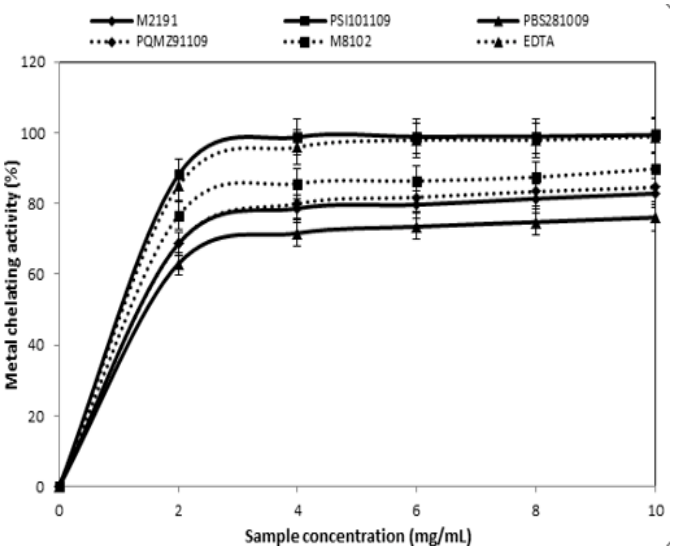

Fig. 7. Metal chelating activity of hydroglyceroalcoholic extracts

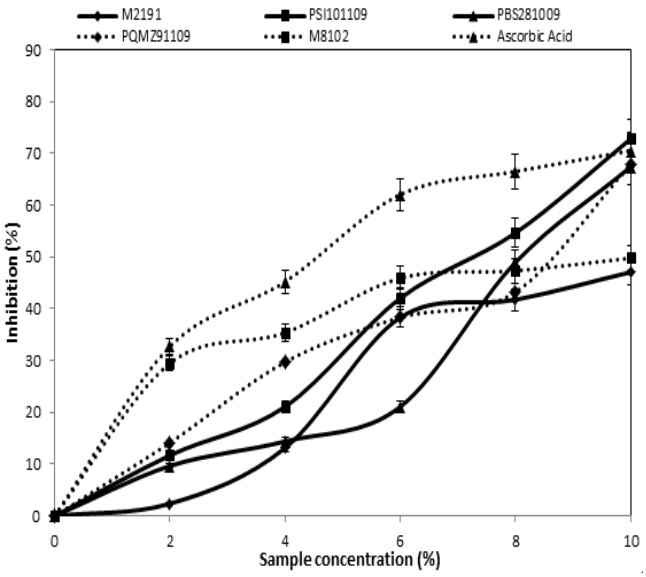

Fig. 8. Inhibition of lipid peroxidation of hydroglyceroalcoholic extracts

peroxidation procedure through a Fenton reaction [39]. Ferrozine establishes composites with ferrous iron, and amidsthydroglyceroalcoholic extracts, the interruption of the process occurs and hence resulting to fading away of the violet-red color, allowing the spectrophotometric determination of the chelating ability [40]. The values obtained for the 10 mg/mL ranged between 76.08 and 99.45% for *P. ostreatus* PSI101109 (fig. 7), being 0.5% higher than that of the used standard (EDTA). The maximum values were comparable to those obtained for water extracts from fruit bodies of *Grifola frondosa*. The obtained results are also significantly higher than the ethanolic extracts of *Agaricus bisporus* and of *Agrocybe cylindracea*, which had a maximum value of ≈ 59%. The results suggest that hydroglyceroalcoholic extracts from fresh mushrooms mycelium possess a significant chelating iron capacity, and may determine the reducing of lipid peroxidation and inhibition of free radicals [41]. Chelating agents seem to act as lesser antioxidants, hence stabilizing the oxidized iron ions. According to data obtained on ferrous ions, the chelating effects of these hydroglyceroalcoholic extracts results show that their use has beneficial effects against oxidative disorders and reduce the effects determined by the degenerative processes of aging [42].

Inhibition of Lipid Peroxidation

The testedhydroglyceroalcoholicextracts have the capacity to hold back the lipid peroxidation of polyunsaturated fatty acids present in egg yolk homogenates. The'lipid peroxidation's inhibition process is very important because it is merged with various forms of biological damage [43]. As shown in figure 8, lipid peroxidation inhibition is directly comparative to concentration increment in each extract separately. The maximum inhibition was determined for *P. ostreatus* PSI101109 extract, with an EC_{50} equal to 7.7 mg/mL. This is 25% over that of ascorbic acid. For the other extracts of *C. comatus* M8102 mycelium and of *P. ostreatus* M2191, the differences are higher, because the value of EC_{50} index exceeds 10 mg/mL. Instead, the hydroglyceroalcoholic extracts from fresh *P. ostreatus* PBS281009 and *P. ostreatus* PQMZ91109 presented a value of ≈8.3 mg/mL. At the maximum concentration of 10 mg/mL, the hydroglycero-alcoholic extracts from *C. comatus* M8102 and *P. ostreatus* M2191 showed similar values with a range of known culinary-medicinal mushrooms (*Flammulina velutipes, Hericium erinaceus, Pleurotus eryngii, Termitomyces heimii, Pleurotus sajor-caju*), which do not exceed 50% inhibition of lipid peroxidation [44]. The values that were obtained show that the oxidative changes that occur at the level of polyunsaturated fatty acids from LDL can be inhibited, or at least diminished, by these hydroglycero-alcoholic extracts. This effect was demonstrated *in vitro* by various extracts of *Lentinus edodes* and *Volvariella volvacea* and was directly correlated with the intensity of phenoliccompounds [45]. Additionally, the results of some studies performed *in vivo* on rats, have recently been published, that support the validity of the obtained results, by the EC_{50} value, which has been of maximum 8 mg/mL of extract [2,46].

The significant correlation among different methods of assessing the antioxidant activities *in vitro* and between them, and the content of phenolic compounds, reflects the high degree of complexity of these extracts. Even if the methods used belong to the group of the accepted ones for such tests,the values show that in the first place there are the cultured species and the solvent mixture that count, since there should be a correlation between the two variables in order to obtain a valuable product. The results support the idea that according to the technology for processing the substrate subjected to an extraction, the results are not automatically similar, because there are structural and physiological differences that are reacting in a species-specific manner. The results support the possibility of achieving gemo products with antioxidant activities from freshly obtained mycelium by submerged cultivation of the medicinal mushrooms.

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