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STUDIES ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *PLEUROTUS OSTREATUS* PSI101109 MYCELIUM

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Abstract

The antioxidant and antimicrobial potential of the ethanolic extract from *Pleurotus ostreatus* PSI101109 mycelium was determined based on inorganic and organic nitrogen sources in a culture medium. The presence of ammonium sulphate and corn extract resulted in a greater accumulation of bioactive compounds. The results were confirmed by the EC_{50} and minimum inhibitory concentration (MIC). The extracts of *P. ostreatus* PSI101109 mycelium selectively inhibited the strains used, both of which were of the genus *Candida*, at an MIC value of 12.5 mg ml^{-1}. The most resistant strains were found to be *Escherichia coli* and *Listeria innocua*. The capacity of the antioxidant was evaluated by the DPPH scavenging activity, β-carotene-linoleic acid, the reducing power, the scavenging effect on superoxide radical, the scavenging effect on hydroxyl radical and the scavenging activity on nitric oxide. Compounds with antioxidant effects were present in the extracts. Lycopene was an exception because it was found to be lacking in values when culturation was performed in the presence of peptone and yeast extract, but had a maximum value on ammonium sulphate.

Introduction

Therapeutic applications and use of fungi have long been a tradition in the history of Asian countries. The empirical knowledge from these countries has been scientifically demonstrated in the recent decades due to the increasing interest shown toward this form of therapy in Europe and the USA (Smith *et al.*, 2002). The increased interest in exploiting the properties of mushrooms for medicinal purposes reveals the importance of natural sources of biologically active substances (Hobbs, 2000). Recently, fermentation in solid layer with *Pleurotus* sp., was used to convert wastes into animal feed or for production of enzymes (Murcia *et al.*, 2002). Fermentation in liquid medium can, on the other hand, lead to the production and obtention of a more consistent and reproducible biomass and may prove useful in the production of mycelium with high nutritional value and healing properties (Tang *et al.*, 2007).

Pleurotus ostreatus is a fungus found in the mountain forests of Romania (Petre *et al.*, 2010). It is used as food, but not in quantities as high as *Agaricus bisporus* species. In terms of nutrition, it has a low fat content while it is rich in dietary fibres, proteins, vitamins and minerals (Chirinang & Intarapichet, 2009). In addition to food consumption, biologically effective extracts can be obtained from the oyster mushroom's mycelium. The most powerful and direct action in combating some common health problems is the inhibiting of free radicals (Barros *et al.*, 2007). These extracts exhibit antioxidant effects similar to those of some synthetic compounds, such as butylated hydroxyltoluene (BHT), vitamin E, or ascorbic acid (Jayakumar *et al.*, 2009).

The presence of some compounds, such as ascorbic acid, α-tocopherol, β-carotene and phenolic compounds is an indication that mushrooms are equipped with antioxidant properties (Murcia *et al*., 2002). Of these, flavonoids and phenolic acids contribute largely to the antioxidant effect. The carotenoid compounds and the ascorbic acid are, generally, available in the *P. ostreatus* mycelium extracts with a level of about 3 mg 100 g^{-1} and 25 mg 100 g^{-1} of mycelium, respectively (Jayakumar *et al.*, 2009). The quantities differ from one strain to another and are directly influenced by the culture medium used for producing the mycelium (Petre *et al.*, 2010).

The carbon source – and especially the nitrogen sources – has a direct influence on the quantum of biologically active substances in the extracts (Barros *et al.*, 2007). Thus, various processes for submerged cultivation of mycelium were developed in order to quickly yield substances possessing biological characteristics. Other important compounds that can be determined are the amino acids like cysteine methionine (Mattila *et al*., 2002) and lovastatin, which have the effect of reducing blood cholesterol and blood pressure (Jayakumar *et al.*, 2009). There have been researches carried out, which also show its anti-inflammatory and anti-cancer properties (Thekkuttuparambil & Kainoor, 2007).

Because the effect of the extract depends on the mycelium source, the purpose of these researches was to find the best nitrogen source wherewith to obtain mycelium that is endowed with antioxidant and antimicrobial capabilities. Antioxidant activities were demonstrated by β-carotenelinoleic acid, reducing power and scavenging effects on radicals. The contents of phenolics, flavonoids, ascorbic acid, β-carotene and lycopene components were also determined.

Materials and Methods

Chemicals: All chemicals and reagents were purchased from Merck (Sigma, Aldrich GmbH, Sternheim, Germany). All other unlabelled chemicals and reagents were of analytical grade.

Culture and storage requirement: The mushroom, *P. ostreatus* PSI101109, was isolated from the stem of a poplar (Băneasa forest, Romania). It was authenticated by Dr. D. Pelinescu, Faculty of Biology, University of Bucharest, Romania (Gezer *et al.*, 2006). The mycelia were maintained on potato dextrose agar (PDA) at 4°C (Atlaf *et al.*, 2010). The microorganisms were subcultured at regular intervals (45 days) to retain viability (Vamanu, 2012).

Media preparation and fermentation condition: The fungi were initially grown on a PDA medium for 10 days at 25°C. The inoculum was obtained by growing mycelium in

a LabTech rotary shaker. The cultivation parameters applied were 150 rpm, for 5 days and at 25°C, in 500 ml Erlenmeyer flasks containing 250 ml of the following synthetic medium (per liter): 6.0 g glucose, 100.0 g malt extract, 20.0 g yeast extract, 1.0 g KH_2PO_4 and 0.5g $MgSO_4 \times 7H_2O$. The medium was adjusted to pH 5.5 with 0.2 M NaOH (Altaf *et al.*, 2010). The submerged fermentation was carried out in Erlenmeyer flasks of 1000 ml, containing 700 ml of liquid medium (KH_2PO_4 0.2%, $CaSO_4$ 0.5%, $MgSO_4$ 0.05%, Na_2HPO_4 0.01% in 5% extract solution of corn flour), and the procedure was performed using four different nitrogen sources: corn extract (dry matter 40%); peptone; yeast extract; and ammonium sulphate. The nitrogen sources were added as 10 g l^{-1}. The inoculated flasks were shaken and maintained on a rotary shaker (LabTech, Korea) at 150 rpm and at 25°C. After 7 days of growth, the mycelium was recovered from the liquid medium by centrifugation at 4000 × g for 15 min. Thereafter, the obtained mycelia were washed thrice with distilled water and were submitted to freeze-drying in an Alpha 1-2 LD freeze-dryer in the absence of a cryoprotector agent (Barros *et al.*, 2007; Naraian *et al.*, 2009; Vamanu, 2012; Khandakar *et al*., 2008).

Preparation of mushroom extract: The extract was obtained by ethanol extraction of freeze-dried mushroom mycelia. Mycelia were submitted to ethanol extraction through stirring at 150 rpm for 24 h, at 20°C, while the ratio was 1 g freeze-dried biomass for 10 ml solvent. The broth was centrifuged at 3000 × g for 15 minutes and the supernatant was filtered using Whatman No. 1 filter paper. The ethanol extract was freeze-dried. The freeze-dried extract was then re-dissolved in 80% ethanol (v/v) to yield solutions containing 1.0, 2.5, 5.0, 10.0, 15.0 and 20.0 mg of extract per ml (Barros *et al.*, 2007; Vamanu, 2012).

Antimicrobial activity: *In vitro* antimicrobial susceptibility tests were performed using a panel of microorganisms from the laboratory collection of the Faculty of Biotechnology, Bucharest, Romania, using Gram positive bacteria (*Listeria innocua* CMGB 218, *Bacillus cereus* CMGB 215, *Staphylococcus aureus* ATCC 6588), Gram negative bacteria (*Escherichia coli* CBAB 2, *Pseudomonas aeruginosa ATCC* 15442), and yeasts (*Candida albicans* ATCC 20231, *Candida sp*. ICCF15). The yeasts and bacteria were maintained in 20% glycerol, kept at –80°C (Jagadish *et al.*, 2009; Vamanu, 2012; Ajaib *et al*., 2011).

Determination of minimum inhibitory concentration (MIC): The standard agar dilution protocol with doubling dilution was used. The MICs of the extract for each test microorganism were regarded as the agar plate with the lowest concentrations without growth (Oboh *et al.*, 2007; Vamanu, 2012).

1,1-Diphenyl-2-picrylhydrazyl radical scavenging activity of mushroom freeze-dried extracts: The DPPH assay was performed as described by Hussain *et al*., 2011. Ascorbic acid was used for comparison. The extract concentration providing 50% of free radical scavenging activity (EC_{50}) was calculated from the graph of radical scavenging activity percentage against extract concentration (Rop *et al*., 2011; Vamanu, 2012; Win *et al*., 2011).

Antioxidant activity by β-carotene-linoleic acid: The antioxidant activity was also assessed by measuring the inhibition of the conjugated diene hydroperoxides arising from the linoleic acid oxidation as described by Hussain *et al*., 2011.

Reducing power: Reducing power was determined by the method of Kim *et al*., 2009.

Determination of antioxidant component: The total phenolic and flavonoid content of ethanolic extract were determined using Folin-Ciocalteu reagent and the aluminium chloride colorimetric method, respectively (Fu *et al*., 2010; Al-Juhaimi & Ghafoor, 2011).

Determination of β-Carotene and lycopene were determined by the method described by Barros *et al.*, 2008.

Superoxide radical scavenging activity of freeze-dried extracts: This was assayed as described by Lobo *et al*., 2010. Ascorbic acid was used for comparison. EC_{50} value (milligram extract ml^{-1}) was the effective concentration at which hydroxyl radicals were scavenged by 50% (Xiao *et al*., 2005; Vamanu, 2012).

Hydroxyl radical scavenging of freeze-dried extracts: This was assayed as described by Philips *et al*., 2010. Ascorbic acid was used for comparison. EC_{50} value (milligram extract ml^{-1}) was the effective concentration at which hydroxyl radicals were scavenged by 50% (Xiao *et al*., 2005; Vamanu, 2012).

Nitric oxide scavenging of freeze-dried extracts: Nitric oxide scavenging activity was measured spectrophotometrically (Kumar *et al*., 2008a). Ascorbic acid was used for comparison. EC_{50} value (milligram extract ml^{-1}) was the effective concentration at which hydroxyl radicals were scavenged by 50% (Xiao *et al*., 2005; Vamanu, 2012).

Statistical analysis: All the parameters for antimicrobial and antioxidant activity were assessed in triplicate, and the results were expressed as mean ±SD values of three observations. The mean values and standard deviation were calculated with the EXCEL program of Microsoft Office 2007.

Results and Discussion

1. Effect of nitrogen source on mycelia growth: It is confirmed that the use of corn extract as a nitrogen source may be recommended for obtaining a mycelium with nutraceutical activity. It was suggested that certain essential amino acids for growth could also be synthesised in the presence of inorganic nitrogen sources. The development of PSI101109 was achieved in an uneven manner, the developed colonies having a different diameter. Colony sizes were small, with an average diameter of 0.5 cm. It was observed that in the presence of corn extract, the hyphae color was darker – toward brown – while in the other cases the color was beige. The difference between the presence of ammonium sulphate and the most productive source of organic nitrogen was almost 55%. The use of ammonium sulphate resulted in the best value of 15.42 g l^{-1}. For the three organic nitrogen sources, the order was: yeast extract> corn extract> peptone (Fig. 1).

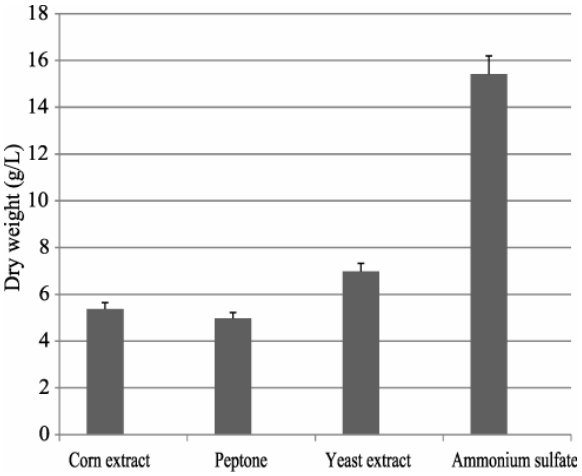

Fig. 1. Effect of nitrogen source on mycelia growth of the *Pleurotus ostreatus* PSI101109.
Values are expressed as means ± SD (n = 3).

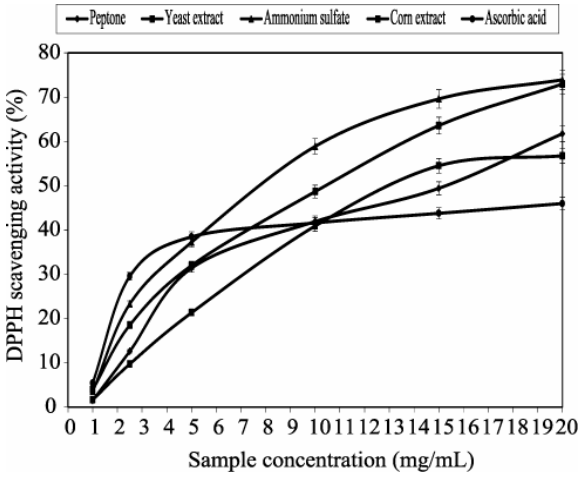

Fig. 2 Scavenging activity of extracts from the *Pleurotus ostreatus* PSI101109 mycelium against 1,1-diphenyl-2-picrylhydrazyl.
Values are expressed as means ± SD (n = 3)

Although peptone and corn extract are considered to be efficient nitrogen sources for fungal biomass production, in the case of PSI101109 mycelium, the resultant amount depended on the strain used (Petre *et al.*, 2010). The results obtained for other strains, such as *Leucopaxillus giganteus*, *Ganoderma lucidum*, *Cordyceps militaris* and *Pleurotus citrinopileatus* showed similar behaviors (Barros *et al.*, 2007; Vamanu, 2012).

2. Scavenging effect on DPPH: Using the DPPH free radicals is a common practice in order to assess the scavenging activity of antioxidant extracts, because it is a fast and reliable method to detect the hydrogen-donating ability of the different alcoholic extracts at low concentration (Mustaffa *et al.*, 2010). The decrease in absorbance is due to the reaction between the antioxidant components of the extract and the stable radical. The scavenging effect of PSI101109 extracts on DPPH radicals increased with the increase in sample concentration. The maximum values were obtained for mycelium grown in the presence of ammonium sulphate and also corn extract, with a very small difference for a concentration of 20 mg ml^{-1} (Fig. 2). The obtained order, according to the nitrogen source was: ammonium sulphate> corn extract> peptone> yeast extract. Thus, the EC_{50} for ammonium sulphate was 7.6 mg ml^{-1}. Additionally, ascorbic acid revealed a much higher EC_{50} value.

In the case of *Ramaria flava*, similar percentages of DPPH scavenging activity have been reported for the ethanolic extract. The values of DPPH scavenging activity are similar in the case of inorganic nitrogen source and of corn extract, with those of the alcoholic extracts of *Lentinus lepideus* fruiting bodies (Yoon *et al.*, 2011). The performance of ethanol extracts of PSI101109, regardless of organic or inorganic nitrogen source used was higher than the standard ascorbic acid, which is in agreement with the previous studies, but only half when compared to the synthetic antioxidant compounds like BHT and BHA (Jagadish *et al.*, 2009; Vamanu, 2012).

3. Antioxidant activity on β-carotene-linoleic acid: β-carotene–linoleic acid assay is used to assess the ability of the extracts in protecting β-carotene, and the data are expressed as antioxidant activity (Zargar *et al.*, 2011). This method is based on the determination of the coupled oxidation of carotene and linoleic acid (Kumar *et al*., 2008a,b). The most effective nitrogen source was the inorganic ammonium sulphate, followed by a very small difference of about 3% by the corn extract (Fig. 3).

For organic sources, the order was: corn extract> peptone> yeast extract. EC_{50} is about 8 mg ml^{-1} for ammonium sulphate, but corn extract shows an increase of 50%, namely, about 12 mg ml^{-1}. In contrast, for BHT, the value is 0.2 mg ml^{-1}. The inhibition ratio corresponds to previous studies on antioxidant activities for *Leucopaxillus giganteus* and *Agaricus arvensis* which were of 61.4 and 46.7%, respectively, for concentrations below 10 mg ml^{-1}. Higher values presented the alcoholic and aqueous extracts of *Lentinus edodes* fruiting bodies which have exceeded 95% for a concentration of 20 mg ml^{-1} (Yoon *et al.*, 2011; Vamanu, 2012).

4. Reducing power assay: Determination of reduction capacity is an indicator of the antioxidant potential of ethanolic extract of *P. ostreatus* PSI101109 mycelium (Fig. 4). The reaction mixture, yellow in color, exhibits a color change to blue, depending on the reducing power of each sample (Ferreira *et al.*, 2007). The reduction capacity is dependent on the extract concentration in the analysed sample. In the case of the extracts obtained from the PSI101109 strain, a very good reducing power was obtained, up to a maximum of 1.1, for ammonium sulphate. For the organic nitrogen sources, the order was as follows: corn extract> peptone> yeast extract. The difference was 21.8% less compared with the inorganic sources of nitrogen, and 23.61% compared to ascorbic acid, at a concentration of 20 mg ml^{-1}. In comparison with other similar strains of *P. ostreatus* at 1 mg ml^{-1} extract concentration, the reducing power values were higher, except the yeast extract (Kim *et al.*, 2009).

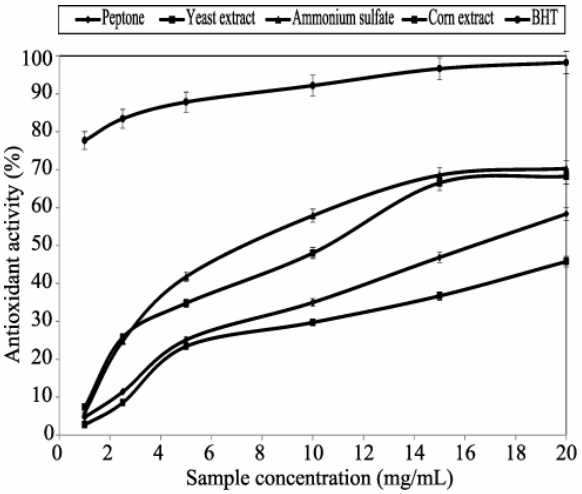

Fig. 3 Antioxidant activity against β-carotene-linoleic acid of ethanolic extracts from *Pleurotus ostreatus* PSI101109.
Values are expressed as means ± SD (n = 3)

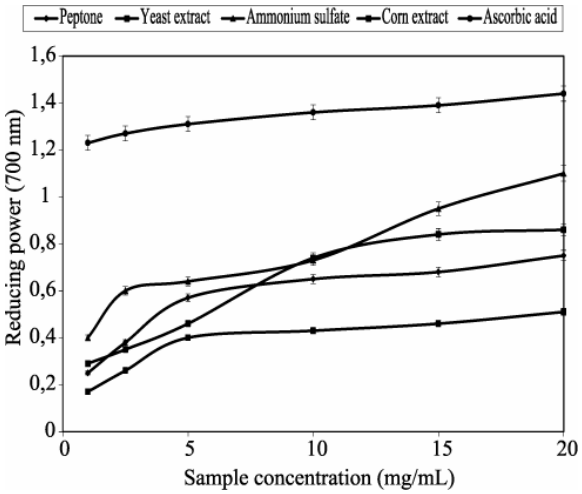

Fig. 4. Reducing power of ethanolic extracts from the mycelium of *Pleurotus ostreatus* PSI101109.
Values are expressed as means ± SD (n = 3)

5. Antioxidant components: The content in compounds with antioxidant effect shows that these types of lyophilised extracts can exert a protection against diseases, such as cancer and cardiovascular dysfunctions. Following the studies carried out, it was demonstrated that the quantitative distribution of bioactive compounds depends on the source of nitrogen, with a significant role of the inorganic source. Also, corn extract represents an important new source of organic nitrogen, it being a cheap alternative to standard organic nitrogen sources.

The extracts of mushrooms contain, in general, a significant amount of phenolic compounds, the most common being gallic acid. The amount of phenolic compounds is therefore directly proportional to their antioxidant capacity (Nuhu *et al.*, 2011). The total phenolic content recorded a maximum of 22.65 ± 0.06 mg 100 g^{-1} extract gallic acid equivalent. The lowest amount, 57.39%, was obtained for yeast extract. The determined values (Table 1) are comparable to previous studies related to total phenolic content in edible mushrooms (Kim *et al.*, 2008).

Table 1. EC_{50} values for radical scavenging effect and bioactive compounds obtained from different mycelium extracts of *Pleurotus ostreatus* PSI101109.

	EC_{50} (mg/mL)			
	Peptone	**Yeast extract**	**Ammonium sulfate**	**Corn extract**
Hydroxyl scavenging activity	8.8 ± 0.14	14.82 ± 0.11	7.4 ± 0.1	10.05 ± 0.07
Superoxide radical scavenging activity	2.53 ± 0.05	2.69 ± 0.12	1.14 ± 0.03	2.11 ± 0.11
Nitric oxide scavenging activity	3.01 ± 0.22	4.57 ± 0.23	1.58 ± 0.14	2.71 ± 0.26
Bioactive compounds				
Ascorbic acid (mg/100 g)	16 ± 0.06	13 ± 0.03	22 ± 0.07	23 ± 0.08
Total free phenolics (mg gallic acid/100 g)	22.65 ± 0.06	8.04 ± 0.16	12 ± 0.12	11.01 ± 0.15
Flavonoids (mg quercetin/100 g)	258.45 ± 0.9	161.1 ± 0.17	319.35 ± 0.01	96.6 ± 0.07
Lycopene (mg/100 g)	-	-	0.18 ± 0.1	0.12 ± 0.6
β-carotene (mg/100 g)	-	-	0.21 ± 0.08	0.3 ± 0.11

Values are mean ±SD of 3 separate determinations, each in triplicate.

The quantum of flavonoids present was expressed in quercitin equivalent with a maximum of 319.35 mg 100 g^{-1} lyophilised extract. For organic sources of nitrogen, the peak was 258.45 mg 100 g^{-1} lyophilised extract for peptone, followed by corn extract and then the yeast. The flavonoid content of the lyophilised extract from PSI101109 mycelium was higher than that of alcoholic extracts from the mycelium of some *Pleurotus florida* and *Pleurotus ostreaus* strains (Imran *et al.*, 2011).

Only alcoholic extracts obtained from mycelium grown in the presence of ammonium sulphate and corn extract contained carotenoid compounds. The ammonium sulphate determines the presence of a lycopene quantity that was 33.33% higher than the extract of the mycelium grown in the presence of corn extract (Vamanu, 2012). Conversely, β-carotene is present in an amount 30% higher in the extract of the mycelium grown in medium with corn extract. The content of freeze-dried extracts is consistent with the one determined for other strains

belonging to species *Boletus edulis*, *A. bisporus* and *Macrolepiota procera*, but is less than *Suillus variegatus*, *Suillus bovinus* and *Tricholoma equestre* strains (Robaszkiewicz *et al.*, 2010).

The amount of ascorbic acid ranged from 13 to 23 mg 100 g^{-1} lyophilised extract. For ascorbic acid, the amount is lower than for other strains such as, *A. bisporus*, *Calocybe gambosa* or *Cantharellus cibarius*. By contrast, in extracts from strains like *Boletus edulis* or *Marasmius oreades*, this bioactive compound could not be found (Barros *et al.*, 2008).

6. Scavenging effect on hydroxyl radical: The hydroxyl radical is the most reactive of the reactive oxygen species (Vamanu, 2012). It can cause damage to tissues and death at the cellular level. Because of these effects, using some protectors against the hydroxyl radical action represents a necessity (Luo *et al.*, 2011). The obtained results show that the efficiency of the extracts depends on their concentration according to the source of nitrogen (Table 1). The order, depending on the EC_{50} value was as follows: ammonium sulphate> peptone> corn extract> yeast extract. As in previous studies, the efficiency of using ethanol to obtain extracts with important biological effects was demonstrated (Krishnendu *et al.*, 2011). Therefore, the extracts obtained from lyophilised mycelium of PSI101109 can be considered as good scavengers of hydroxyl radicals.

7. Scavenging effect on superoxide radical: The superoxide anion radical is not very reactive, but its generation favours the production of hydrogen peroxide and of hydroxyl radicals. At the cellular level, the damages are determined by its derivatives (Sarikurkcu *et al.*, 2010). Table 1 shows the inhibition capacity of lyophilised mycelium extracts from PSI101109, in the form of EC_{50}. According to obtained data, the most effective extract was that obtained from mycelium grown in the presence of an inorganic nitrogen source. For the three organic sources, the order was as follows: corn extract> peptone> yeast extract. The value of the scavenging effect on superoxide radicals is relatively similar to values obtained for the mushrooms, *Clitocybe geotropa* and *Leucoagaricus pudicus*, at a concentration of 10 mg ml^{-1}. The value is 50% lower than the inhibition achieved for *Amanita caesarea* at the same concentration (Sarikurkcu *et al.*, 2010; Vamanu, 2012).

8. Scavenging activity on nitric oxide: Nitric oxide radical is associated with the development of inflammatory disorders (Wagay, 2011). Nitric oxide radicals generated from sodium nitroprusside were found to be inhibited by the PSI101109 mycelium extract. Ethanolic extracts showed inhibition effects on nitric oxide, with a maximum for the extract of mycelium grown in the presence of inorganic nitrogen source ($p<0.05$). For the organic sources of nitrogen, the obtained order, according to the value of EC_{50}, was: corn extract> peptone> yeast extract. Thus, the concentration of the extract of mycelium which achieved a 50% inhibition was approximately 1.58 mg ml^{-1} for the ammonium sulphate. As regards the organic sources of nitrogen, the sample concentration was with 41.7, 47.5 and 65.4%, respectively, higher in the order presented above. The data are comparable with those obtained from *Morchella esculenta* methanolic extract and are generally 20% lower than ascorbic acid (Ningappa *et al.*, 2007; Vamanu, 2012).

9. Effect of nitrogen source on antimicrobial activity: The ethanol extracts of the PSI101109 mycelium were screened against some human pathogenic bacteria and two fungal ones to check antibacterial and antifungal activities, with the results being presented in Table 2. The two yeast strains were inhibited by the extract of mycelium grown in the presence of corn extract and ammonium sulphate, resulting in a MIC value of 12.5 mg ml^{-1}. *E. coli* CBAB 2 and *L. innocua* CMGB 218 were found to be resistant to all extracts. Yeast extract resulted in the highest MIC value of 25 mg ml^{-1}, regardless of the strain used. In general, the inorganic nitrogen source, ammonium sulphate, and the corn extract led to obtaining mycelia whose extracts had the most pronounced antimicrobial effect (Vamanu, 2012). The obtained results are similar to those presented by Barros *et al.*, (2007), on *Leucopaxillus giganteus* mycelium, whose extract showed a similar MIC for the same microbial species tested. The same is true for the comparison with ethanolic and methanolic extracts from *Ganoderma lucidum* (Quereshi *et al.*, 2010).

Table 2. Antimicrobial activity of mycelia extracts obtained by using four different nitrogen sources.

Nitrogen source	MIC (mg/ml)						
	Escherichia coli **CBAB 2**	***Bacillus cereus*** **CMGB 215**	***Listeria innocua*** **CMGB 218**	***Candida*** **sp. ICCF15**	***Candida albicans*** **ATCC 20231**	***Pseudomonas aeruginosa ATCC*** **15442**	***Staphylococcus aureus*** **ATCC 6588**
Corn extract	25	12.5	25	12.5	12.5	12.5	12.5
Ammonium sulfate	25	12.5	25	12.5	12.5	12.5	12.5
Yeast extract	25	25	25	25	25	25	25
Peptone	25	25	25	25	25	12.5	12.5

Values are mean 3 separate determinations, each in triplicate

Conclusions

Using such extracts from the lyophilised mycelium of the PSI101109 mushroom may represent a significant component in order to find alternatives to the classical medication. The extracts of the tested mycelium demonstrated their capacity to inhibit the development of some microorganism strains which can facilitate the evolvement of infectious diseases in humans. The obtained results can have a positive effect on human health and may represent an important component in the manufacture of products used in ethno-medicine.

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References

Ajaib, M., Khan, Z.U.D., Khan N., Abbasi, M.A., Shahwar, D., M. Wahab and M.F. Saddiqui. 2011. Antibacterial and antioxidant activities of an ethnobotanically important plant *Sauromatum venosum* (ait.) Schott. of district Kotli, Azad jammu & Kashmir. *Pak. J. Bot*., 43: 579-585.

Al-Juhaimi, F. and K. Ghafoor. 2011. Total phenols and antioxidant activities of leaf and stem extracts From coriander, mint and parsley grown in saudi arabia. *Pak. J. Bot*., 43: 2235-2237.

Altaf, S.A., D.M. Umar and M.S. Muhammad. 2010. Production of xylanase enzyme by *Pleurotus eryngii* and *Flamulina velutipes* grown on different carbon sources under submerged fermentation. *World Appl. Sci. J*., 8: 47-49.

Barros, L., P. Baptista, L.M. Estevinho and I.C.F.R. Ferreira. 2007. Bioactive properties of the medicinal mushroom *Leucopaxillus giganteus* mycelium obtained in the presence of different nitrogen sources, *Food Chem.,* 105: 179-186.

Barros, L., T. Cruz, P. Baptista, L.M. Estevinho and I.C.F.R. Ferreira. 2008. Wild and commercial mushrooms as source of nutrients and nutraceuticals, *Food Chem. Toxicol*., 46: 2742-2747.

Chirinang, P. and K.O. Intarapichet. 2009. Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju, Science Asia,* 35: 326-331.

Ferreira, I.C.F.R., P. Baptista, M. Vilas-Boas and L. Barros. 2007. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem.*, 100: 1511-1516.

Fu, L., B.T. Xu, X.R. Xu, X.S. Qin, R.Y. Gan and H.B. Li. 2010. Antioxidant capacities and total phenolic contents of 56 wild fruits from south China. *Molecules,* 15: 8602-8617.

Gezer, K., M.E. Duru, I. Kivrak, A. Turkoglu, N. Mercan, H. Turkoglu and S. Gulcan. 2006. Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *Afr. J. Biotechnol*., 5: 1924-1928.

Hobbs, C.R. 2000. Medicinal value of *Lentinus edodes* (erk.) Sing. (Agaricomycetideae). A literature review. *Int. J. Med. Mush.*, 2: 287-302.

Hussain, A.I., F. Anwar, T. Iqbal and I.A. Bhatti. 2011. Antioxidant attributes of four *Lamiaceae* essential oils. *Pak. J. Bot*., 43: 1315-1321.

Imran, M., M.M.M. Raja, M.A. Basith and A. Asarudeen. 2011. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *Int. Food Res. J.,* 18: 579-582.

Jagadish, L.K., V.V. Krishnan, R. Shenbhagaraman and V. Kaviyarasan. 2009. Comparitive study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.E. Lange) Imbach before and after boiling. *Afr. J. Biotechnol*., 8: 654-661.

Jayakumar, T., P.A. Thomas and P. Geraldine. 2009. *In-vitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*, Innov. *Food Sci. Emerg. Tech.,* 10: 228-234.

Khandakar, J., S. Yesmin and M. Moonmoon. 2008. Mycelial growth of *Pleurotus citrinopileatus* on different environmental condition. *Bangladesh J. Mushroom*., 2: 55-62.

Kim, Y.J., K.S. Kang and T. Yokozawa. 2008. The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. *Food Chem. Toxicol*., 46: 2466-71.

Kim, Y.J., K.S. Kang and T.Yokozawa. 2008. The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. *Food Chem. Toxicol*., 46: 2466-71.

Kim, J.H., S.J. Kim, H.R. Park, J.I. Choi, Y.C. Ju, K.C. Nam, S.J. Kim and S.C. Lee. 2009. The different antioxidant and anticancer activities depending on the color of oyster mushrooms. *J. Med. Plants Res*., 3: 1016-1020.

Krishnendu, A., S. Giri and G. Biswas. 2011. Comparative study of antioxidant activity and nitric oxide synthase activation property of different extracts from *Rhododendron arboreum* flower. *Int. J. Pharm. Tech. Res.,* 3: 757-762.

Kumar, H.V., C.R. Gnanendra, N Naik and D.C. Gowda. 2008. *In Vitro* antioxidant activity of dibenz[*b,f*]azepine and its analogues. *E-J. Chem*., 5: 1123-1132.

Kumar, S., D.K. Manjusha, K. Saroha, N. Singh and B. Vashishta. 2008. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharm.,* 58: 215-220.

Lobo, V.C., A. Phatak and N. Chandra. 2010. Antioxidant and free radical scavenging activity of *Hygrophila schulli (Buch.-Ham.) Almeida and Almeida.* seeds. *Biores.,* 1: 72-78.

Luo, A., F. Yijun and A. Luo. 2011. *In vitro* free radicals scavenging activities of polysaccharide from *Polygonum Multiflorum Thunb*. *J. Med. Plants Res*., 5: 966-972.

Mattila, P., P.S. Vaananen, K. Kongo, H. Aro and T. Jalava. 2002. Basic composition and amino acid contents of mushrooms cultivated in Finland. *J. Agr. Food Chem.,* 50: 6419-6422.

Murcia, A.M., M. Tome, M. Jimenez, A.M. Vera, A.M. Honrubia and P. Parras. 2002. Antioxidant activity of edible fungi (truffles and mushrooms): Losses during industrial processing. *J Food Prot.,* 65: 1614-1622.

Mustaffa, F., J. Indurkar, S. Ismail, M.N. Mordi, S. Ramanathan and S.M. Mansor. 2010. Antioxidant capacity and toxicity screening of *Cinnomomum iners* standardized leaves methanolic extract. *Int. J. Pharmacol.,* 6: 888-895.

Naraian, R., K.A. Naveen and S.K. Garg. 2009. Improved submerged fermentation of corn cob with mechanically broken oil seed cakes and decolorisation of textile dyes by enzyme extract of *Pleurotus florida* PF05. *Res. Environ. Life Sci.,* 2: 83-90.

Ningappa, M.B., R. Dinesha and L. Srinivas. 2007. Antioxidant and free radical scavenging activities of polyphenolenriched curry leaf extract (*Murraya koenigii* L.). *Food Chem.,* 10: 101-117.

Nuhu, A., K.N. Yoon, K.R. Lee, H.Y. Kim, P.G. Shin, J.C. Cheong, Y.B. Yoo, M.J. Shim, M.W. Lee and T.S. Lee. 2011. Assessment of antioxidant and phenolic compound concentrations as well as xanthine oxidase and tyrosinase inhibitory properties of different extracts of *Pleurotus citrinopileatus* fruiting bodies. *Mycobiology,* 39: 12-19.

Oboh, I.E., J.O. Akerele and O. Obasuyi. 2007. Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta* burm.f. (malvaceae). *Trop. J. Pharm. Res.,* 6: 809-813.

Philips, A., S. Philip, V. Arul, B. Padmakeerthiga, V. Renju, S. Santha and S. Sethupathy. 2010. Free radical scavenging activity of leaf extracts of indigofera aspalathoides – An *In vitro* analysis. *J. Pharm. Sci. & Res.,* 2: 322-328.

Petre, M., A. Teodorescu, E. Ţuluca, C. Bejan and A. Andronescu. 2010. Biotechnology of mushroom pellets producing by controlled submerged fermentation. Rom. *Biotechnol. Lett.,* 15s: 50-55.

Quereshi, S., A.K. Pandey and S.S. Sandhu. 2010. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts*, People's J. Sci. Res.,* 9: 9-13.

Robaszkiewicz A., G. Bartosz, M. Ławrynowicz and M. Soszy´nski. 2010. The role of polyphenols, *β*-Carotene, and lycopene in the antioxidative action of the extracts of dried, edible mushrooms. *J. Nutr. Metab.,* ID 173274.

Rop, O., V. Reznicek, J. Mlcek, T. Jurikova, J. Balik, J. Sochor and D. Kramarova. 2011. Antioxidant and radical oxygen species scavenging activities of 12 cultivars of blue honeysuckle fruit. *Hort. Sci*. (Prague), 38: 63-70.

Sarikurkcu, C., B. Tepe, D.K. Semiz and M.H. Solak. 2010. Evaluation of metal concentration and antioxidant activity of three edible mushrooms from Mugla, Turkey. *Food Chem. Toxicol.,* 48: 1230-1233.

Smith, J.E., N.J. Rowan and R. Sullivan. 2002. Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities, *Biotech. Lett.,* 24: 1839-1845.

Tang, Y.J., L.W. Zhu, H.M. Li and D.S. Li. 2007. Submerged cultivation of mushrooms, *Food Technol. Biotechnol.,* 45: 221- 229.

Thekkuttuparambil, A.A. and K.J. Kainoor. 2007. Indian medicinal mushroom as a source of antioxidant and antitumor agents. *J. Clin. Biochem. Nutr.,* 40: 157-162.

Vamanu, E. 2012. *In vitro* antimicrobial and antioxidant activities of ethanolic extract of lyophilized mycelium of *Pleurotus ostreatus* PQMZ91109. *Molecules*, 17: 3653-3671.

Win, M.M., A. Abdul-Hamid, B.S, Baharin, F. Anwar, M.C. Sabu and M.S. Pak-Dek. 2011. Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. *Pak. J. Bot*., 43: 1635-1642.

Xiao, L., H. Takada, K. Maeda, M. Haramoto and N. Miwa. 2005. Antioxidant effects of water-soluble fullerene derivatives against ultraviolet ray or peroxylipid through their action of scavenging the reactive oxygen species in human skin keratinocytes. *Biomed. Pharmacother*., 59: 351-358.

Yoon, K.N., N. Alam, L.K. Rim, P.G. Shin, J.C. Cheong, Y.B. Yoo and T.S. Lee. 2011a. Antioxidant and antityrosinase activities of various extracts from the fruiting bodies of *Lentinus lepideus. Molecules,* 16: 2334-2347.

Zargar, M., A.H. Azizah, A.M. Roheeyati, A.B. Fatimah, F. Jahanshiri and M.S. Pak-Dek. 2011. Bioactive compounds and antioxidant activity of different extracts from *Vitex negundo* leaf. *J. Med. Plants Res*., 5: 2525-2532.

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