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Original Article

Mycelia from *Pleurotus* sp. (oyster mushroom): a new wave of antimicrobials, anticancer and antioxidant bio-ingredients

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Abstract

Introduction: There has been an upsurge of interest in mushrooms, such as *Pleurotus* species, as an important source of bioactive compounds. Mycelia-submerged culture represents a promising approach to search new safe and healthy myco-products with standardized quality in addition to mushroom fruiting bodies.

Methods: The study examined the in vitro antimicrobial, antitumor and antioxidant activities of a hot-water extract from *Pleurotus* sp. mycelium. The antimicrobial activity was screened through the activation of the microbial autolytic system of four bacteria and four yeast strains. The anti-proliferative effects on NB4 human leukemia cells were measured by flow-cytometry analyses. The antioxidant activity was investigated by the scavenging of DPPH and ABTS radicals, the reducing power and the inhibition of lipid peroxidation.

Results: The extract activated the microbial autolytic system of eight strains: 7 autolyzing strains with intensity values (Is) ranging from 2.7% in Candida sp. to 36.1% in *Saccharomyces cerevisiae*. Thus, the microbial autolytic system of the strains tested (including Gram-positive and gram-negative bacteria as well as yeasts) could be activated in vitro by mycelial extract. *Pleurotus* extract reduced the viability of NB4 leukemia cells, particularly at the concentration of 200 µg/mL to 82% compared to control cells, and induced apoptosis demonstrated by an increase in annexin V-FITC+ cells (25% at 200 µg/mL). At 10 mg/mL, the extract showed the most potent scavenging effects for DPPH and ABTS radicals (96% and 55%, respectively) and the inhibition of lipid peroxidation (52%). The mushroom extract at 5 mg/mL manifested reducing power of 1.105. Although carbohydrates (76.8%, w/w) appear to be the most important bioactive compounds, secondary metabolites, like phenolics, would also con- tribute to the antioxidant, antimicrobial and anti-proliferative activities.

Conclusion: The hot-water extract obtained from *Pleurotus* mycelium, in light of its in vitro antimicrobial, antitumor and antioxidant effects could be considered a good candidate for developing nutraceuticals and for designing innovative myco-therapeutics and phytocosmetics applications.

Keywords: Anticancer, Antimicrobials, Antioxidant, Nutraceuticals, *Pleurotus*, Phytocosmetics, Phototherapy

Introduction

The use of mushrooms to improve health represents an important cultural heritage as they have been used since time immemorial as a source of highly tasty/nutritional foods and medicinal preparations by the greatest early civilizations.[1-4] Mushrooms are now attracting more attention as a functional food and as a source of drugs and nutraceuticals.[5-7]

Mushrooms are thought to exert approximately 130 pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial, and antiviral activities.[8]

Modern scientific data has documented that mushrooms represent an unlimited source of bioactive molecules as an example of molecular diversity with recognized potential in drug discovery and development.[9] The bioactive molecules

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comprise high molecular weight compounds, mainly polysaccharides, and low molecular weight secondary metabolites.[10] Polysaccharides (especially β-glucans) are the best known and most potent mushroom derived substances, with antitumor and immunomodulatory effects, thus acting as biological response modifiers (BRMs).[11,12] In addition, the vast structural diversity of mycochemicals (phenolic compounds, terpenes, lactones, steroids, alkaloids, among others) provides unique opportunities for discovering new drugs that target and modulate biochemical signal transduction pathways.[13-15]

Of the known mushroom species, approximately 700 are considered to be safe with medicinal properties.[3] Pharmacological effects have been demonstrated for many traditionally used mushrooms, including species from genera *Ganoderma*, *Lentinus (Lentinula)*, *Agaricus*, *Auricularia*, *Flammulina, Grifola, Hericium*, *Pleurotus*, *Trametes (Coriolus)*, *Schizophyllum, Lactarius*, *Phellinus*, *Cordyceps*, *Inonotus*, *Inocybe*, *Tremella*, and *Russula*.[2,14,16,17] In this wonderful world, *Ganoderma*, mushroom of immortality, has been considered the king of medicinal mushrooms, followed by *Lentinula* and others, including *Pleurotus*.[7]

On the other hand, with the increasing concern of public health and consumers' awareness, there has been a metamorphosis in the cosmetic industry with natural products being more in demand than their synthetic counterparts.[18] Natural ingredients could be an attractive candidate in cosmetic formulation due to: (*a*) antimicrobial activities could protect against the growth of microorganisms in cosmetics and skin and thus, minimize or replace the use of synthetic preservatives in cosmetics, and (*b*) antioxidant properties could protect the cells from aging caused by the oxidative damage of free radicals and enhance skin nutrition.[19] In this context, mushrooms and their products are now finding their way into cosmetics and purported as highly active ingredients in these products.[20] Nutritive, anti-inflammatory, regenerative and antioxidant properties of several mushrooms makes their usage prospective in manufacturing of cosmetic products. There are numerous potential medicinal products from mushrooms that could be used in cosmeceuticals (products applied topically, such as creams, lotions, and ointments) or nutricosmetics (products that are ingested orally). The mushrooms presently used are traditionally known to produce medicinal compounds and thus were the first to be incorporated in cosmetic applications.[21]

Species of the genus *Pleurotus* (Fries) Kummer (Basidiomycota, Agaricales) (Figure 1), like many edible and medicinal mushrooms, are a good source of antitumor, immunostimulating, antimicrobial and antioxidant substances besides a wide array of biotechnological and environmental applications.[7,22,23] Usually regarded as oyster mushrooms, these edible basidiomycetes are among the most popular worldwide, as much as they achieved the third position in the production of edible mushrooms, behind the species of the genus *Agaricus* and *Lentinula*.[24] More recently, extensive research has been done on isolation and characterization of new functional compounds, besides the in-depth study of their medicinal properties.[7,25,28]

Figure 1. Production of *Pleurotus* sp. (oyster mushroom) in the research-development plant of the Center for Studies on Industrial Biotechnology (CEBI) (a), and *Pleurotus* fruiting bodies (strain CCEBI-3024) after harvesting.

Both fruiting bodies and mycelia of *Pleurotus* spp. have been studied in search of biological effector molecules.[29,30] However, between 80% and 85% of all medicinal mushroom products are derived from fruiting bodies; only 15% of all products are based on extracts from mycelia.[31] For this reason, mushroom mycelia may also constitute a good source of healthy compounds, which may be useful in the formulation of nutraceutical, medicinal and cosmetic products.

In previous studies, we reported the immunomodulating activity of a hot-water mycelial extract from *Pleurotus ostreatus* against the immunosuppression caused by cyclophosphamide or whole-body irradiation in mice.[32,33] To our knowledge, this is the first report linking some bioactivities of *Pleurotus*' mycelia with their potential applications in phyto- (myco-) cosmetics. Better insight into the different roles of multiple active compounds and the mechanisms underlying their biological action will accelerate commercial production of novel pharmaceuticals and cosmetic products based on water-extracts from mycelia of *Pleurotus* sp.

Materials and Methods

Pleurotus sp. Strain and Preparation of Hot-Water Mycelial Extract

Pleurotus sp. strain (CCEBI-3024) is deposited at the Culture Collection of the Center of Studies for Industrial Biotechnology (CEBI, Cuba). The strain was maintained on slants with solid medium of potato dextrose agar (PDA) incubated at 5°C.

Mycelium was inoculated in Erlenmeyer flasks, which contained YPG medium (yeast-peptone-glucose). The flasks were incubated at 27°C with continuous stirring at 100 rpm for 15 days. After the submerged fermentation was carried out, *Pleurotus* sp. mycelia were collected by centrifugation at 4000 g and washed twice with distilled water. Isolated mycelia, suspended in 200 g (wet weight)/L of distilled water, were extracted with boiling water for 10 hours and the final extracts were collected

by centrifugation and filtration.[26] Figure 2 shows a simple production platform for mushroom extract using submerged mycelium fermentation.

The extracts (F-I) at a yield of 5.5 g/L culture (dried weight) were stored at -20°C and freeze dried. They are composed of 76.8% of carbohydrate, 15% of protein, 0.11% of nucleic acids, and 5% of minerals. The presence of different secondary metabolites such as phenolic compounds, i.e. flavonoids and tannins, and alkaloids has also been demonstrated in *Pleurotus* mycelia-derived hot water extract.[34]

Determination of Antimicrobial Activity

The antimicrobial activity of *Pleurotus* mycelial extract was determined through its ability to activate the autolytic enzyme system of the microbial cell.[35] Four bacterial strains (*Bacillus cereus* ATCC 11776, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25953 and *Pseudomonas aeruginosa* ATCC 27853) as well as 4 yeast strains (*Candida* sp. CCEBI 2034, *Candida intermedia* CCEBI 2034, *Saccharomyces cerevisiae* CCEBI 2010 and

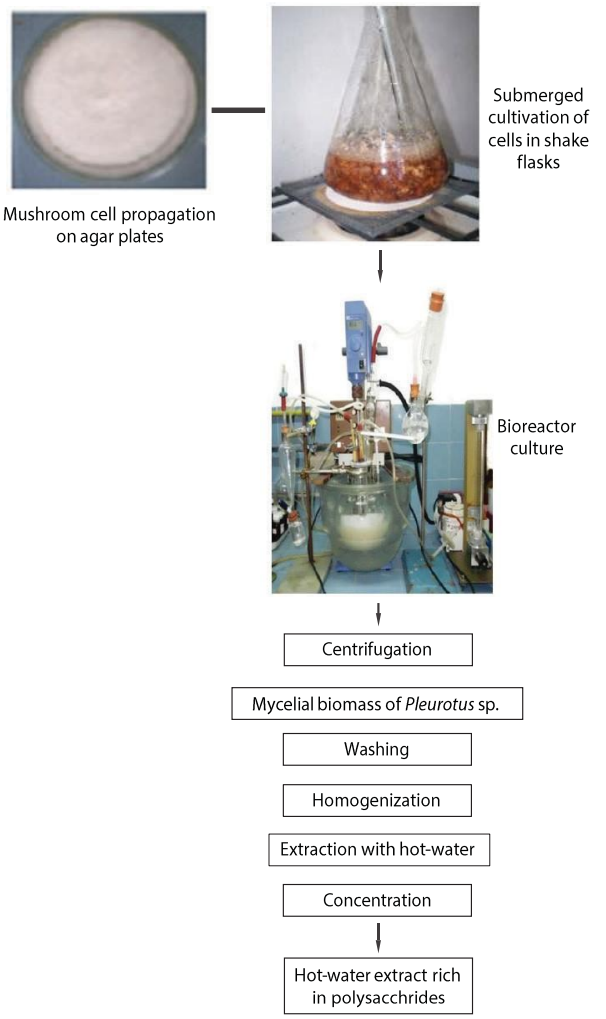

Figure 2. Process for Mushroom Extract Production by *Pleurotus* sp. Using Submerged Mycelium Fermentation.

Saccharomyces fermentii CCEBI 2008), were used as test microorganisms. Suspensions of microbial cells were prepared in distilled water to an absorbance at 520 nm of approximately 1.0 (Genesys 10 uv, Thermospectronic Rochester, NY, USA) and were mixed with equal quantities of the *Pleurotus* extract at 2.4 mg/mL. This concentration was chosen taking into account the lack of antimicrobial effects found with lower double-diluted concentrations of *Pleurotus* extract ranging from 0.5 to 2 048 µg/mL, using the agar-well diffusion technique and the resazurine reduction test (data not shown).

Controls were distilled water only. Absorbance at 520 nm of this mixture was measured at zero time (D0) and after their exposure for 60 min at 37°C (D60). The antimicrobial activity, expressed as the intensity of autolysis (I) in both the study and control samples, was calculated using the formula:

$$I = \frac{D_0 - D_{60}}{D_0} \times 100\%$$

For naturally non-autolyzing strains $Ic>0$, whereas for naturally non-autolyzing strains $Ic=0$.

Apoptosis Induction in Human Acute Promyelocytic Leukemia (NB4) Cells

The cells were maintained in RPMI medium (Gibco-Life Technologies) with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 0.02 mg/mL gentamycin. Cells were cultured at 37°C in a humidified air atmosphere with 5% CO2. Apoptosis induction was evaluated by the Annexin V-FITC assay measuring the Phosphatidylserine exposure on the outer side of membrane. For this assay, 5×10^5 treated cells were collected and centrifuged at 1500 rpm for 3 minutes. Then, they were incubated for 15 minutes in the dark at room temperature in 500 µL PBS containing 10 µL of binding reagent and 1.25 µL Annexin V-FITC (Annexin V-FITC Apoptosis Detection Kit, Calbiochem, EMD Millipore, USA). Subsequently, the cells were centrifuged at 2300 rpm for 5 minutes and resuspended in 500 µL binding buffer 1× diluted in PBS. After that, 10 µL PI was added and the samples were analyzed in a FACScan cytometer (Becton Dickinson, San José, CA, USA). Data were analyzed using WinMDI 2.8 software. All Annexin V+/PI– cells were scored as early apoptotic, while double-stained Annexin V+/PI+ cells were considered as late apoptotic.

Assays for In Vitro Antioxidant Activity

DPPH assay. Radical scavenging ability (RSA) of mushroom extract against 1,1-diphenyl-2-picryl-hydrazyl (DPPH, Sigma-Aldrich) was measured according Cheung et al.[36] using spectrophotometry. The extract (1 mL) at 10 mg/mL was mixed with 0.5 mL of 0.1 mM DPPH ethanolic solution. Then, the mixture was shaken vigorously and incubated at 25°C for 1 hour in the dark. The absorbance of the sample was measured at 520 nm (VIS-

723G spectrophotometer, Beijing Rayleigh Analytical Instrument Corporation) and the scavenging ability against DPPH radicals was calculated as a percentage of DPPH discoloration using the equation:

$$\% \text{ RSA} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

where A_S is the absorbance in the solution when the sample extract has been added, and A_{DPPH} is the absorbance of DPPH solution. L-ascorbic acid was used as a standard.

ABTS assay. Scavenging effect on 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, Sigma-Aldrich) radicals was measured according to Choi et al.[37] ABTS radicals were generated by mixing 7 mM of the ABTS stock solution and 2.45 mM of potassium persulfate (Sigma-Aldrich) in distilled water, and storing this mixture overnight at room temperature in the dark. The mixture (10 mL) was diluted with 840 mL of distilled water. *Pleurotus* extract (50 µL) at 10 mg/mL was added to 3 mL of ABTS solution and after 90 minutes, the absorbance was measured at 414 nm (VIS-723G spectrophotometer, Beijing Rayleigh Analytical Instrument Corporation). The scavenging ability against ABTS radicals was calculated using the equation:

$$\% \text{ RSA} = [(A_{ABTS} - A_S)/A_{ABTS}] \times 100$$

where A_S is the absorbance in the solution when the sample extract has been added, and A_{ABTS} is the absorbance of ABTS solution. L-ascorbic acid was used as a standard.

Reducing power. The reducing power was determined according to the method of Lee et al.[38] The mushroom extract (2.5 mL) at 5 mg/mL was mixed with 2.5 mL of 10 g/L potassium ferricyanide (Sigma-Aldrich) and the mixture was incubated at 50°C for 20 minutes. Then 2.5 mL of 100 g/L trichloroacetic acid (Merck) was added, and the mixture was centrifuged at 2 000 g for 10 minutes. A sample of the supernatant (5 mL) was mixed with 5 mL of distilled water and 1 mL of 1 g/L FeCl3 (Merck), and the absorbance was measured at 700 nm (VIS-723G spectrophotometer, Beijing Rayleigh Analytical Instrument Corporation). Butylated hydroxyltoluene (BHT) was used as a standard.

Determination of inhibitory activity on lipid peroxidation. A reaction mixture containing 8 mL of a suspension of 20% sheep erythrocytes, 7.52 mL of physiological saline, 80 µL of 0.5 mol/L $FeCl_3$, 0.4 mL of ascorbic acid (0.5 mM) and 1 mL of mycelial extract (10 mg/mL) was incubated at 37°C in a water bath for 120 minutes. The lipid peroxide formed was estimated by measuring thiobarbituric acid reacting substances (TBARS) with some modifications.[39] For this, 2 mL of the incubation mixture was treated with 1 mL of trichloroacetic acid at 10% and the samples were shaken in a vortex for 1 minute and centrifuged at 6000 g for 15 minutes. Then, 2 mL of the supernatant was transferred to test tubes with 2.5 mL of 0.335% thiobarbituric acid (TBA) and the reaction tube was kept in a water bath at 100°C for 1 hour. After cooling, the absorbance was measured at 532 nm. The percentage of inhibition of lipid peroxidation (LPO) was determined by comparing the results of the test compounds (treated with mushroom extract) with those of control (not treated with the mushroom extract). The % of lipid peroxide-scavenging ability of the extract was calculated by the formula described in DPPH and ABTS radicals scavenging effect.

Statistical Analysis

Experimental results were mean ± SD of three measurements. The values $P < 0.05$ were regarded as significant. Statistical analysis of the data was performed by the Mann-Whitney test or the Kruskal-Wallis rank test followed by the Student-Newman-Keuls test to determine the significance of differences between treatments. The software Statgraphics Plus version 5.1 (Statistical Graphics Corporation, 1994-2001) was used in all the analysis.

Results and Discussion

The wide spectrum of natural products with biological activity produced by Basidiomycetes includes antimicrobial agents, as well as immunomodulatory, antioxidant and antiproliferative activities, and some have already been clinically applied.[40] To broaden their use in food, cosmetic and pharmaceutical industries, a comprehensive study of the effects exerted as BRMs is still needed. Thus, in order to expand the properties of a hot water mycelial extract from *Pleurotus* sp. as a natural ingredient, we screened for several activities in this study, and found that it showed: (*i*) antimicrobial activity as judged by its ability to activate the microbial autolytic system; (*ii*) antiproliferative activity of NB4 leukemia cells and ability to induce apoptosis; and (*iii*) in vitro antioxidant activity in view of the degree of LPO inhibition and the free RSA. All of these activities suggested it to be a good candidate for developing innovative myco-cosmetic and other healthcare biotech-based products.

Antimicrobial Activity of *Pleurotus* sp. Hot-Water Mycelial Extract

Antimicrobial ingredients are materials that protect against the growth of microorganisms in cosmetics and skin, including bacteria, viruses and fungi. They can also kill organisms that may be present in ingredients that may be used to make cosmetics and thus, play an important role in making sure that cosmetics are free of microorganisms during storage and after they are opened.[41]

Clinical microbiologists have 2 reasons to be interested in the topic of antimicrobial natural extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. Second, the public is becoming increasingly aware of problems with the overprescription and misuse of

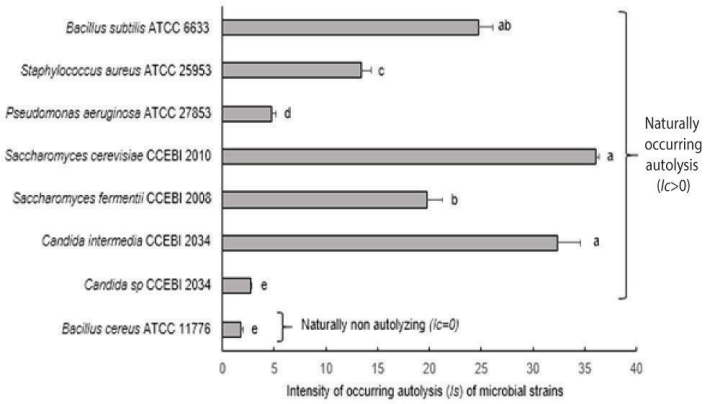

Figure 3. Intensity of autolysis (Is) of microbial strains achieved when using a hot-water mycelial extract from *Pleurotus* sp.
Results are showed as means ± standard deviation of three replicates; means with different letters indicate statistically significant differences according to the Kruskal-Wallis rank test followed by the Student Newman-Keuls test (P 0.05)

traditional antibiotics.[42]

The antimicrobial activity was screened through the ability of mushroom extract to activate the microbial autolytic system of eight strains (4 bacteria and 4 yeast strains) (Figure 3). Autolysis of seven naturally autolyzing strains (Ic>0) was activated with intensity values (I_S) ranging from 2.7% in *Candida* sp. to 36.1% in *S. cerevisiae*. Among the tested bacterial autolyzing strains, the highest *IS* was showed in the gram-positive *B. subtilis*. On the other hand, autolysis was of 1.8% in one naturally non-autolyzing strain (*B. cereus*), for which Ic=0. Although the control of autolysin activity still remains a mystery, strategies for lysing microbes by direct activation of their autolytic system are among the possible approaches for antimicrobial drug development.[35] In our study, the microbial autolytic enzyme system of the strains tested (including gram-positive and gram-negative bacteria as well as yeasts) could be activated in vitro by *Pleurotus* mycelial extract.

Many secondary metabolites isolated from edible mushrooms have shown potentialities as antimicrobial agents. In this context, *Pleurotus* spp. extracts have been explored to combat simple and multiple drug resistant microbial strains.[43] Antimicrobial activity of oyster mushroom depends upon the nature of the solvent.[12] Although in some cases ethanol extracts contain more bioactive substances and potent effects, the results for *Pleurotus* spp. are contradictory. Our results compare favorably with those reported for an ethanolic extract of lyophilized mycelium of *P. ostreatus* PQMZ91109 when cultured in presence of the same nitrogen source (yeast extract and peptone).[44]

The presence of secondary metabolites like phenolic compounds (38 ± 5 mg/g) such as flavonoids and tannins, and alkaloids was demonstrated in the *Pleurotus* hot-water mycelial extract tested here.[34] The phenolic constituents of *Pleurotus* spp. may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lysis, and the inhibition of protein synthesis, proteolytic enzymes and microbial adhesion.[45] These bioactive substances would also contribute to the antimicrobial activity of the extract.

Antiproliferative Activity of NB4 Leukemia Cells and Apoptosis Induction

Since research has tended to focus on the dietary value of species of the genus *Pleurotus*, there is relatively little information pertaining to their anticancer mechanisms, particularly in mycelia derived products.[46] In this study, the treatment with *Pleurotus* mycelial extract reduced viability of NB4 human leukemia cells, particularly at the concentration of 200 µg/mL, to 82% with respect to control untreated cells (P<0.05) (data not shown). The extract also induced apoptosis of NB4 cells, which was demonstrated by an increase in the number of annexin V-FITC positive cells, mostly at the dose of 200 µg/mL (25%, P<0.05) (Figure 4).

Apoptosis is the physiological controlled process by which cells actively commit a gene-mediated cell death program essential for cell homeostasis. It is also the key

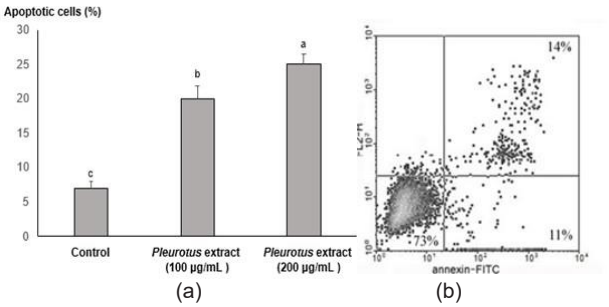

Figure 4. Induction of apoptosis in human leukemia NB4 cells treated with the *Pleurotus* sp. mycelial extract for 24 h at concentrations of 100 and 200 µg/mL (a), and a flowcytometry profile of cell cycle of treated NB4 cells with 200 µg/mL (b). The cell populations shown in the right bottom quadrant (PI–/Annexin V+) represents early apoptotic cells, whereas the right upper quadrant (PI+/Annexin V+) represents late apoptotic cells.
The values in (a) represent the mean of three different experiments. Different letters indicate significant differences among the groups according to the Kruskal-Wallis rank test followed by the Student-Newman-Keuls test (P<0.05).

mechanism for acting the chemotherapeutic agents.[47] The apoptosis percentage values obtained in this work were lower compared to those reported for *P. ostreatus* mycelia-derived proteoglycans (5 mg/kg) in the in vivo Sarcoma-180-bearing mouse model (45.36%-75.36%).[48] However, the apoptosis ratios achieved in our study were higher than those referenced by Lee et al[49] on Molt 4 human leukemia cells treated with 1 mg/mL of the polysaccharopeptide (PSP) isolated from the mycelia of *Trametes* (=*Coriolus*) *versicolor* (5.92 ± 0.7%). Taking into account that the evasion of apoptosis is a hallmark of human cancers (e.g., hematological malignancies),[47] products capable of inducing apoptosis deserve special interest.

In this respect, some mushrooms and their extracts are either presently investigated to be used as promising anticancer products for their antiproliferative effects on certain skin cancers. *L. edodes* has been shown to reduce cell proliferation and induce apoptosis in CH72 mouse skin carcinoma cells via an induction of a transient G1 arrest.[50] Similarly, reduction of cell proliferation of B-16 melanoma cells by arrest in the G_0/G_1 phase of the cell cycle, followed by both apoptotic and secondary necrotic cell death has been demonstrated for a methanol extract of *C. versicolor*.[51]

In Vitro Antioxidant Activities

Until now, research has tended to focus on the dietary significance of edible mushrooms; however, there is relatively little information relating to the antioxidant activity and the possible use of such mushrooms to neutralize oxidative stress.[52]

The antioxidant potential of a compound could be attributed to its various characteristics, the most important of these being the ability to scavenge and reduce free radicals, to chelate transition metal ions, to inhibit LPO, among others.[53] DPPH free radical scavenging activity assay is one of the most common methods for the determination of antioxidant capacity. As the data presented in Table 1 show, the DPPH radical scavenging activity of an aqueous extract obtained from *Pleurotus* mycelium was of 96%, similar to the values achieved with ascorbic acid used as control. Scavenging effects of extracts from several specialty and commercial mushrooms on DPPH radicals augmented with increased extract concentrations. Thus, DPPH radical scavenging activity varied from 9% (*P. nebrodensis*) to 57% (*P. cystidiosus*). Moreover, DPPH scavenging action for *P. citrinopileatus* and some other fungi significantly improved with the gradual elevation of sample concentration from 0.5 to 9.0 mg/mL.[54]

With the ABTS radical scavenging test, we can measure the activity of hydrophilic and lipophilic compounds; therefore, it is useful in the simultaneous study of several natural ingredients.[55] In this study, the ABTS radical scavenging activity of an aqueous extract obtained from *Pleurotus* mycelium at 10 mg/mL was of 55%, lower than those of 80% reported for a fruiting bodies extract (Table 1).[56] On the other hand, hydro-alcoholic extracts of *Grifola gargal* showed an ABTS radical scavenging ability of 90.9-93.3 ascorbic acid equivalents (milligram of ascorbic acid per liter of sample).[57] This method has been also used in the evaluation of the antioxidant activity of neutral polysaccharides from *Auricularia auricula*r and their homologues sulfated in the concentrations of 0.2-10 mg/mL, without significant differences.[58]

Moreover, it has been discussed that the diphenylpropane structure of flavonoids and the aromatic ring structure of phenolics, such as aromatic oxy phenol acids, might contribute to the free RSA of these compounds.[59]

The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity and the efficacy of certain antioxidants is known to be associated with their reducing power.[60] In the present study, the reducing power of a 5 mg/mL concentration of the mushroom extract was found to be 1.107, which was relatively more pronounced than that of BHT (0.7) ($P<0.05$) (Table 1). The reducing power of medicinal mushrooms might be due to their hydrogen-donating ability.[52] Possibly, medicinal mushrooms contain high amounts of reductones, which could react with radicals to stabilize and terminate radical chain reactions. The reducing power of the ethanolic extract of *P. ostreatus* fruiting bodies was found to steadily increase in direct proportion to the increasing concentration of the extract. The reducing power of a 10 mg/mL concentration of the mushroom extract was found to be 1.367, which was relatively higher than that of BHT (1.192).[61] Further ethanolic extract from the fruiting bodies of the mushroom *Pleurotus citrinopileatus* has been reported to exhibit the reducing power of 1.05 at 10 mg/mL.[38]

LPO, a process induced by free radicals, leads to oxidative deterioration of polyunsaturated lipids and inactivates cellular components and therein plays a key role in oxidative stress in biological systems.[62] Hence, the inhibitory activity of the mushroom extract on LPO was evaluated. Both the mushroom extract and the ascorbic acid standard inhibited LPO (Table 1). At a concentration of 10 mg/mL, the mushroom extract effected 52% inhibition of LPO activity and the ascorbic acid standard effected 92%. At least until we know, there

Table 1. In Vitro Antioxidant Activity of a Hot-Water Mycelial Extract of *Pleurotus* sp.

Sample	Scavenging of DPPH Radicals (%)	Scavenging of ABTS Radicals (%)	Reducing Power ($A_{700\,n}$)	Inhibition of Lipid Peroxidation (%)
Pleurotus extract	96 0.4 (10 mg/mL)	55 0.8 (10 mg/mL)	1.105 0.022* (5 mg/mL)	52 2.4 (10 mg/mL)
Control	96.3 0.6 (ascorbic acid)	98 0.2* (ascorbic acid)	0.700 0.018 (BHT)	92 1.4* (ascorbic acid)

Results are shown as means ± standard deviation of three replicates; means with an (*) differ when compared with the Mann-Whitney test.

Table 2. Mycochemical Profile and Activities - Verified in Our Laboratory or in Agreement With Other Mushroom Studies - of Mycelial Extract of *Pleurotus ostreatus*

Carbohydrates/Glycosides	Molisch	++	Immunomodulatory, Antitumor, Antimicrobial, Antioxidant
Reducing Sugar	Fehling	+	
	Benedict	+	
Quinones	Borntrager	-	
Phenols and tannins	$FeCl_3$	+	Antioxidant, anti-inflammatory, antimicrobial
Amino acids	Ninhidrine	++	Structural units of proteins (immunomodulatory, antioxidant, antimicrobial)
Flavonoids	Concentrated H_2SO_4	+	Antioxidant, antimicrobial, anti-inflammatory
	Rosenheim	+	

(-) none, (+) present, (++) mild, (+++) marked.

is no data available for comparison of our results obtained with *Pleurotus* mycelial extract on LPO in the in vitro erythrocyte membrane model estimated by TBARS. Erythrocytes are excellent subjects for studies of biological effects of free radicals, since they are structurally simple, are continuously exposed to high oxygen tensions, the membrane lipids are composed partly of polyunsaturated fatty acid side chains which are vulnerable to peroxidation, and they have antioxidant enzyme systems.[63]

An ethanolic extract of *P. ostreatus* at a concentration of 10 mg/mL effected 56.20% inhibition of LPO activity of rat liver homogenate, and the ascorbic acid standard effected 67.15%. The concentration of the extract and ascorbic acid needed for 50% inhibition of LPO was 8 and 6 mg/mL, respectively. Moreover, the pronounced antioxidant activity of *P. ostreatus* mushroom, manifested as inhibition of LPO, scavenging of hydroxyl and superoxide radicals, reducing power and chelating activity on ferrous ions, has been related with its high phenolic content.[52] Therefore, the in vitro antioxidant properties exhibited by the tested *Pleurotus* mushroom preparation may be due to the presence of these antioxidant mycochemicals inherent in it. In addition, the antioxidant activities (chelating ability of ferrous ion, inhibition of LPO and reducing power) found in polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum, Ganoderma lucidum, Lentinus edodes* and *Trametes versicolor*[64] does not discard the possible contribution of β-1,3-1,6-glucans polysaccharides to the antioxidant effect. Actually, mushroom extracts that are rich in antioxidant components will have high potential to be developed into cosmetic formulations. For example, schizophyllan derived from *Schizophyllum commune* contains β-1,6-branched-β-1,3-glucan, decreases inflammation, irritation and other damage due to UV and toxic environment exposure of the skin.[21]

There are numerous potential mycocompounds from mushrooms that could be used in cosmeceuticals or nutricosmetics. The main families of mycochemicals previously reported in the hot-water extract from *Pleurotus* mycelia tested here are listed in Table 2, with their biological activities experimentally verified in our laboratory or in agreement with other mushroom studies.

Species presently used, or patented to be used, in cosmeceuticals and nutricosmetics include *Agaricus subrufescens* (=*A. blazei, A. brasiliensis*), *Choiromyces maeandriformis*, *Cordyceps sinensis*, *Ganoderma lucidum*, *Grifola frondosa*, *Hypsizygus ulmarius*, *Inonotus obliquus*, *Lentinula edodes*, *Polyporus* spp., *Trametes versicolor*, *Tremella fuciformis*, *Schizophyllum commune* and many other lesser used taxa.[65] However, there are numerous other mushroom species that are untested, undescribed or not yet cultivatable and that have huge potential for use in the cosmetic industry. To our knowledge, there is only one cosmetic product formulated with *Pleurotus ostreatus* (fruiting bodies) and *Ganoderma lucidum*: Hankook Sansim Firming Cream (Tan Ryuk SANG, Korea) which makes skin tight and vitalized.[21,66] Therefore, it is not surprising that intrinsic biological properties demonstrated in vitro in our study with *Pleurotus* mycelial extract can be transferred in vivo after mushroom use as food, nutraceutical or cosmeceutical/nutricosmetic.

Conclusion

It may be concluded that in view of the above results, the mycelial hot-water extract from *Pleurotus* sp. could be used as a natural ingredient with antimicrobial, anticancer and antioxidant properties, thus representing a potential alternative to some commercially available synthetic products, which may have a large number of side effects. The various experiments conducted revealed it to be a very good source of mycochemicals that could play–owing to their synergistic action–a very important role in drug development and as a health supplement. With interdisciplinary studies, this mushroom extract can find its way into cosmetics for its prospective usage in cosmeceuticals and nutricosmetics. Further research is also required for isolation and identification of active biomolecules and principles present in these extracts, so that they could be exploited at the industrial scale.

Competing interests

The authors declare no conflict of interest.

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