Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

HPTLC IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF *Pleurotus ostreatus* AND *Lentinus edodes* EXTRACTS

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Abstract

Because of the special flavour and of the therapeutic properties, Pleurotus ostreatus and Lentinus edodes are among the most cultivated and consumed mushrooms species. This work aimed to evaluate the antioxidant properties and free radical scavenger activities of Pleurotus ostreatus and Lentinus edodes methanol extracts by phosphomolibdenum and DPPH- assays respectively, total phenolic content (Folin – Ciocalteu) and identification of bioactive compounds by HPTLC (high-performance thin layer chromatography). The obtained fingerprints of extracts, in tree systems (for phenols, coumarins and triterpenoid saponins) have shown the presence of ferulic acid as main compounds in Pleurotus ostreatus extract and ergosterol (provitamin D2) in both of them. Total phenol content was 2.03 g/100g for Lentinus edodes extract and 1.24 g/100g for Pleurotus ostreatus extract. Radical scavenger activity and total antioxidant capacity, was higher for Lentinus edodes extract (90.14%, 203.05 mg AA) in comparison with the results obtained for Pleurotus ostreatus extract (85.32%, 168.34mg AA). The mushrooms examined in this work could represent important and accessible sources of natural antioxidants for food, food supplement and cosmetic industry.

Key words*: mushrooms, Pleurotus ostreatus, Lentinus edodes, HPTLC, antioxidant.*

INTRODUCTION

The use of natural remedies is one of the most exciting areas of interest that is supported by a long history of traditional use and of scientific research results. Mushrooms are part of natural ecosystems having an important role in the health maintaining process. Also, for centuries, mushrooms are used in food industry and in traditional medicine. Edible and/or medicinal mushrooms are either collected from nature or cultivated. Mushrooms cultivation is important for nutritional security, for recycling solutions of agro-waste and also for providing quality controlled products. The nutritional and chemical composition of mushrooms is responsible for their therapeutic properties. *Pleurotus ostreatus* - oyster mushroom (*Pleurotaceae*) was cultivated for the first time during the Word War I, as a subsistence measure (Eger et al., 1976). Now is the third most cultivated mushroom around the world, having a high nutritional value because of the content in essential amino-acids as alanine, arginine, glutamine and glutamic acid, carbohydrates–specially glucans, water, proteins, vitamins B,C,D,K and minerals Ca, P, Fe, K, Mn, Cu, Zn, Mg and Se, ascorbic and folic acids (Fernandes et al., 2015; Fontes Vieira et al., 2013; Xia et al., 2011). The mushroom also, contain essentials unsaturated fatty acids palmitic, stearic, oleic and linoleic acids (Hadar and Cohen-Arzi 1986) and phenolic compounds as ferulic, p-coumaric, galic, gentisic and homogentisic acids (Palacios et al., 2011) and ergothioneine (Dubost et al. 2007; Bhattacharya et al., 2014). Correlated with the chemical composition, *Pleurotus ostreatus* extracts have antioxidant, immunomodulatory, anti-inflammatory properties and lowering cholesterol and triglycerides levels activity (Fengguo et al. 2011; Fontes Vieira et al, 2013; Facchini et al. 2014; Sanjana et al. 2013; Tong et al. 2009).
Lentinus edodes (Pleurotaceae) has excellent nutritional values. The Japanese name of this

mushroom is Shiitake derived from the association with Shii tree (*Castanopsis cuspidate* Schottky) and – take the Japanese word for mushroom. Fructification body contains 88-92% water, proteins, lipids, carbohydrates, vitamins and minerals. Dry mushroom has higher nutritional values as of most of the vegetable consumed every day. Dry mushroom is rich in carbohydrates and protein, containing 58-60% carbohydrates, 20-30% protein (which are digested at a rate of 80-87%) 9-10% fibre, 3-4% fat and 4-5% ash (Rahman and Choudhury, 2012).

Shiitake is a considerable source of vitamins, particularly provitamin D_2 (ergosterol) - 325mg%, which under ultraviolet light (UV) and heat turn into calciferol (vitamin D_2), containing also B vitamins, thiamine (B_1), riboflavin (B_2), niacin (B_{12}) and pantothenic acid (Hobbs 1995; Przbylowicz et al., 1990; 199, Mizuno 1995, 11, 7–21; Hobbs 2000; Wasser and Weis, 1997). Minerals identified in dry mycelium are Fe, Mn, Ca, Mg, Cd, Cu, P, Zn, Ge, Br and Sr (Casaril et al., 2011). Shiitake contains water-soluble ((1-4)-,(1-6)-α-D-glucans) and antitumor polysaccharides (lentinan) and fatty acids (linoleic, palmitic, oleic, stearic, tetradecenoic and myristic acids). Being one of the most studied mushrooms, with a large utilization in traditional medicine shiitake has proved pharmacological effects as: immunomodulatory, anticarcinogenic, antitumor, cardiovascular, hepatoprotective, antiviral, antibacterial and antiparasitic (Wasser 2005).

This paper aims to present the HPTLC profiles of bioactive compounds – phenolic – of the methanolic extracts obtained from the two mushroom species *Pleurotus ostreatus* and *Lentinus edodes* cultivated in Romania, as well as total polyphenolic content and antioxidant activity by DPPH and phosphomolibdenum assays.

MATERIALS AND METHODS

Raw material – Pleurotus ostreatus (fruiting body) sample and *Lentinus edodes* sample (fruiting body) were obtained from local mushroom producers. Voucher specimens are deposited in INCDCF-ICCF Plant Material Storing Room.

Sample preparation: the samples were prepared by extraction with methanol - vegetal material/ solvent rate -1/10m/v for 1h at boiling temperature of the solvent. The solutions were filtered and frozen until analysis.

HPTLC Analysis - The analysis was made according to TLC Atlas - Plant Drug Analyses (Wagner and Bladt, 1996) for the determination of characteristic fingerprint for chemical compounds. 3-7µl of the samples and 1-5µl of references substances (10^{-3}M ferulic acid T1, chlorogenic acid –T2 and 1mg/ml ergosterol T3-Sigma-Aldrich) were loaded as 10mm band length in the 20 x 10 Silica gel 60F254 TLC plate using Hamilton- Bonaduz, Schweiz syringe and CAMAG LINOMAT 5 instrument. Polyphenolic compounds: the mobile phases (A) consisted in 100:11:11:27 (v/v/v/v) ethyl acetate-acetic acid-formic acid-water and (B) consisted in 1:1 (v/v) toluene-diethyl ether. The TLC twin chamber was pre-saturated with mobile phase for 30 min at ~20°C. The plate was developed in the mobile phase up to 90mm. After development, plates were dried and derivatized in for A system in Natural Product followed by PEG4000 reagent and for B in KOH 10%. The fingerprints were evaluated at UV with a WinCats and VideoScan software. Triterpenoid saponins according to American Herbal Pharmacopoeia®, Reishi Mushroom, 2006: 3-12µl of the samples were loaded as 10 mm on band length in the 20 x 10 Silica gel 60F254 TLC. The mobile phase (C) consisted in 9:1 (v/v) dichloromethane: methanol. The plate was developed in the mobile phase up to 70mm. The plate was dried and derivatized in vanillin–sulphuric acid reagent. The fingerprint was evaluated in visible light.

Total phenol content - Total phenol content (TPC) determined according to Folin – Ciocalteu method (Ph Eur.6). Briefly, 1ml of the extract was transferred to a 25ml volumetric flask, 10ml of water and 1ml of Folin Ciocalteu reagent was added. The volume was made to 25ml with 5% sodium carbonate (w/v). The blend was left at room temperature for 30 minutes. Then the absorbance of the samples was read at 760nm with a UV/VIS spectrophotometer (Helios λ, Thermo Electron Corporation). Distilled water was used a as blank solution.

*Free radical scavenging assay- w*as evaluated using the Sanchez-Moreno et al. (1998) assay. The extracts concentration were 0.1% in methanol. 50µl aliquots of the extract were mixed with 2950µl of the DPPH methanolic solution (0.0025g/l). The radical scavenging activity of the extracts against 2,2-diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) was determined by measuring UV absorbance at 517nm. A blank solution was prepared containing the same amount of methanol and DPPH and measured after standing at room temperature 30 minutes. The radical scavenging activity (RSA) was calculated using the following formula:

% inhibition = {(AB – AA)/AB} x 100.

Where AB is the absorption of blank sample and AA is the absorption of tested extract solution.

Total antioxidant capacity (TAC) assay

Was assessed by phosphomolybdenum method, according to Prieto et al. 1999. To 0.3ml ethanolic solution of the sample (concentration 0.1mg/ml) was added 2.7ml of reagent solution (0.6M sulphuric acid, 28mM sodium molybdate, and 4mM ammonium phosphate). The mixtures were incubated at 95^0C for 90 minutes. After cooling the samples to room temperature, their extinction was measured at 695nm with UV-VIS spectrophotometer. Ethanol was used as negative control. The antioxidant capacity was expressed as ascorbic acid equivalent (AA) to 1mg of active substance. The calibration curve is linear for ascorbic acid in the range of 0.001 to 1mg/ml, n= 6, r^2=0.999.

RESULTS AND DISCUSSIONS

The HPTLC fingerprint profile is utilized as identification method of a species. This is a technique that can be use either in research or in production processes.

In figure 1, the polyphenolic fingerprint of *Pleurotus ostreatus* extract (Figure 1- Track 1) is characterized by two major blue fluorescent zones, one of them – ferulic acid (Rf~0.9) in system A. C system is characteristic for coumarins according to Wagner and Bladt**,** 1996. In the extract were identified ferulic acid (Rf~0.55) and coumarins aglycones as tree bright blue prominent fluorescent spots at Rf~0.1, 0.6 and 0.8.

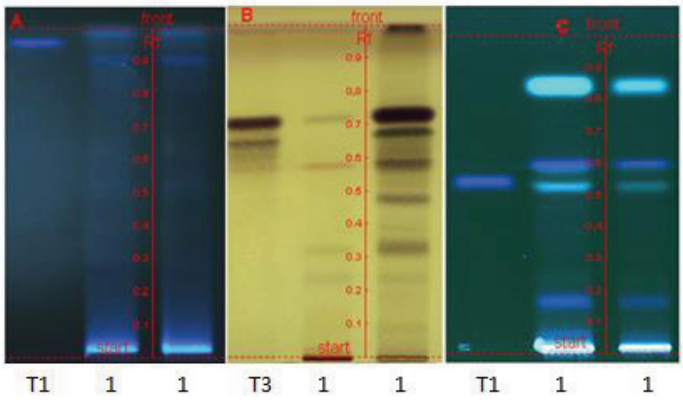

Figure 1. HPTLC fingerprint profile of *Pleurotus ostreatus* mushroom
1 - *Pleurotus ostreatus* extract; T1- ferulic acid (ref.); T3 - ergosterol (ref.)
System A - 100:11:11:27 (v/v/v/v) ethyl acetate-acetic acid-formic acid-water;
B - 1:1 (v/v) toluene-diethyl ether;
C - 9:1 (v/v) dichloromethane: methanol.

B system is used (American Herbal Pharmacopoeia®, Reishi Mushroom, 2006) for identification of triterpenoid saponins in *Ganoderma lucidum* mushroom. *Pleurotus ostreatus* fingerprint is characterized by the presence of ergosterol (T3) and of four major purple - brown spots between Rf ~0.3-0.9.

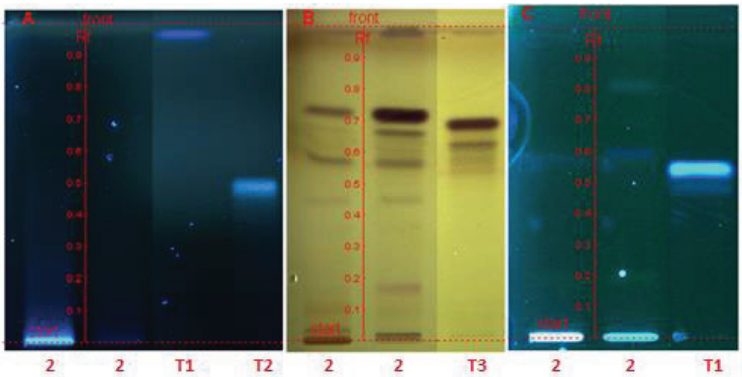

Figure 2. HPTLC fingerprint profile of *Lentinus edodes* mushroom

1 – *Lentinus edodes* extract; T1- ferulic acid (ref.); T2 –chlorogenic acid ; T3 - ergosterol (ref.)

System A - 100:11:11:27 (v/v/v/v) ethyl acetate-acetic acid-formic acid-water;

B - 1:1 (v/v) toluene-diethyl ether;

C - 9:1 (v/v) dichloromethane: methanol.

In figure 2, in A system for *Lentinus edodes* extract there are not specific spots/zones for polyphenolic compounds. In C system, in UV-365 nm, two prominent blue fluorescent zones are found in the Rf ranges 0.6 and 0.8, specific to simple coumarins and one yellow-green zone (non-substituted coumarin) (Rf~0.2) with KOH reagent.

In C system ergosterol was identified at Rf~ 0.7. Also, there are tree purple-brown zones at Rf ranges 0.2-0.9.

Total phenol content was determined from the extrapolation of the calibration curve (y=0.0525x-0.020, R2 = 0.992), which was obtained for gallic acid reference substance (Sigma Chemical Co., St. Louis, USA). The results were expressed as grams of gallic acid equivalents (GAE) per 100g extract. The results obtained for methanolic extract mushrooms was 2.03 g/100g for *Lentinus edodes* extract and 1.24 g/100g for *Pleurotus ostreatus* extract. Redox properties of phenolic compounds allow them to act as antioxidants agents (Soobrattee et al., 2005). Determination of free radical scavenging activities by DPPH assay is routinely used for plant extracts (Aksoy Lacine, et al., 2013).

DPPH is a stable free radical, that accepts an electron or hydrogen radical to become a stable molecule.

For evaluation of total antioxidant capacity was used phosphormolybdenum method that is based on the reduction of Mo (VI) to Mo (V). This reduction is based on the antioxidant compound and the formation of green phosphate /Mo (V) complex at acidic pH.

Table 1 presents the antioxidant activity of the extracts obtained by the two methods DPPH and TAC.

Table 1 Antioxidant activity

No	Extract	DPPH radical scavenging activity (%)	TAC (mg ascorbic acid equivalents)
1	*Pleurotus ostreatus*	85.32 ±0.35	168.34 ±0.84
2	*Lentinus edodes*	90.14±1.21	203.05±2.35

The results obtained in our research showed that *Lentinus edodes* methanolic extract have a higher content in TPC, comparative with *Pleurotus ostreatus* methanolic extract. Also, radical scavenging activity and TAC are in a dose- dependent manner with the total phenol content. Research results showed that poly-phenols are responsible for the antioxidant activity of fruits, vegetables and mushrooms (Ferreira et al., 2007). Also, ergosterol com-pound identified in both extract was found to have antioxidant activity correlated with the content, in a study conducted by Shao S et al, 2010.

CONCLUSIONS

Mushrooms are important sources of antioxidant compounds that are capable of reducing the effects of free radicals. Our results show that *Lentinus edodes and Pleurotus*

ostreatus mushroom species could play a protective role in diseases related to oxidative stress and also can be important sources of phenolic acids and ergosterol.

ACKNOWLEDGEMENTS

This work was made with the support of the MEN – UEFISCDI through the "Partnerships in priority areas - PN II" research program, project no. 174/2014 and of the ANCSI through the "Nucleu Program", project no. PN 16 27 01 02

REFERENCES

American Herbal Pharmacopoeia® • Reishi Mushroom • April 2006.

Bhattacharya M., Srivastav P.P., Mishra H.N, 2014. Optimization of process variables for supercritical fluid extraction of ergothioneine and polyphenols from *Pleurotus ostreatus* and correlation to free-radical scavenging activity Aaccepted manuscript J. of Supercritical Fluids.

Casaril, Kérley Braga Pereira Bento, Kasuya, Maria Catarina Megumi, & Vanetti, Maria Cristina Dantas.,2011. Antimicrobial activity and mineral composition of shiitake mushrooms cultivated on agricultural waste.*Brazilian Archives of Biology and Technology*,*54*(5), 991-1002.

Dubost N. J., Ou B., Beelman R. B., 2007. Quantification of polyphenols and ergothioneine in cultivated mushrooms and correlation to total antioxidant capacity Food Chemistry 105, 727–735.

Eger, G., Eden, G. & Wissig, E., 1976 . Theoret. Appl. Genetics 47: 155. doi:10.1007/BF00278373).

European Pharmacopoeia 6,0

Facchini J. M., Endi P. A., Aguilerac C., Gerna R. M. M., Lange Silveira M. L., Wisbeck E., Furlana S. A., 2014. Antitumor activity of *Pleurotus ostreatus* polysaccharide fractions on Ehrlich tumor and Sarcoma 180 - International Journal of Biological Macromolecules 68, 72–77.

Fernandes A., Barros L., Martins A., Herbert P.,. Ferreira I. C.F.R, 2015. Nutritional characterisation of *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm.produced using paper scraps as substrate Food Chemistry 169, 396–400.

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

Fontes Vieira P. A., Gontijo D. C., Vieira B. C., Fontes E. A.F., Soares de Assunção L., Leite J. P. V., Goreti de A. Oliveira M., M. Kasuya M. C., 2013. Antioxidant activities, total phenolics and metal contents in *Pleurotus ostreatus* mushrooms enriched with iron, zinc or lithium - LWT - Food Science and Technology 54, 421e425.

Hadar Y., Cohen-Arzi E., 1986. Chemical Composition of the Edible Mushroom *Pleurotus ostreatus* Produced by Fermentation Applied and environmental microbiology, p. 1352-1354.

Hobbs, Ch. 2000., Medicinal value of *Lentinus edodes* (Berk.),. Sing. A literature review. Int. J. Med. Mushr, 2, 287–302;

Hobbs, Ch., 1995. Medicinal Mushrooms: An Exploration of Tradition, Healing, and Culture, 2nd Ed.; Botanica Press, Inc.: Santa Cruz, CA, USA.

Lacine A., Erdi K., Yasin A., Zeyneb A., Kargıoğlu M., 2013. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic *Thermopsis turcica* Saudi Journal of Biological Sciences Volume 20, Issue 3, Pages 235–239 .

Mizuno, T., 1995. Shiitake, *Lentinus edodes*: functional properties for medicinal and food purposes. Food Rev. Int., 11, 7–21.

Palacios, M. Lozano, Moro C., D'Arrigo M., Rostagno M.A., Martínez J.A., García-Lafuente A., Guillamón E., Villares A., 2011. Antioxidant properties of phenolic compounds occurring in edible mushrooms Food Chemistry 128, 674–678.

Prieto P., Pineda M., Aquilar M., 1999. Spectrophotometric qantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. /Analytical Biochemistry;269(2):337-41.

Przbylowicz, P.; Donoghue, J., 1990. Shiitake Grower's Handbook: The Art and Science of Mushroom Cultivation; Hunt Publ. Co.: Dubugue, Kendall; 199.

Rahman T., Choudhury MBK,2012 Shiitake Mushroom: A Tool of Medicine, Bangladesh J Med Biochem; 5(1): 24-32.

Sanchez-Moreno, C. Larrauri, J.A., Saura-Calixto, F., 1998. A procedure to measure the antiradical efficiency of polyphenols. Journal of Agricultural and Food Chemistry 76, 270-276.

Sanjana K. P. D., Bibhas R., Pradip P., Banalata S., Syed S. I., Tapas K. M., 2013. Characterization and lectin microarray of an immunomodulatory heteroglucan from *Pleurotus ostreatus* mycelia Carbohydrate Polymers 94, 857– 865.

Shao S, Hernandez M, Kramer JK, Rinker DL, Tsao R. 2010, Ergosterol profiles, fatty acid composition, and antioxidant activities of button mushrooms as affected by tissue part and developmental stage. J Agric Food Chem. 24;58(22):11616-25.

Soobrattee M.A., Neergheen V.S., Luximon-Ramma A., O.I. Aruoma, O.T. Bahorun., 2005. Phenolics as potential antioxidant therapeutic agents:

mechanism and actions Mutat. Res. Fundam. Mol., 579, pp. 200–213.

Tong H., Fengguo X., Kai F., Guangren S., Xiaoxv G., Liwei S., Rui J., Dan T., Xin S., 2009. Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus* Bioresource Technology 100, 1682–1686.

Wagner H, Bladt S, 1996. Plant Drug Analysis, Second Edition, Springer.

Wasser, S.P.; Weis, A.L., 1997. Medicinal Mushrooms. *Lentinus edodes* (Berk.) Singer; Nevo, E., Ed.; Peledfus Publ. House: Haifa, Israel; 6pp.

Wasser S.P., 2005. Shiitake (*Lentinus edodes*) Encyclopedia of Dietary Supplements DOI: 10.1081/E-EDS-120024880 Copyright by Marcel Dekker. All rights reserved.

Xia F., Fan J., Zhu M., Tong H., 2011. Antioxidant effects of a water-soluble proteoglycan isolated from the fruiting bodies of *Pleurotus ostreatus*. Journal of the Taiwan Institute of Chemical Engineers 42, 402–407.