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COMPARATIVE ANALYSIS OF POLYPHENOLIC PROFILES AND ANTIOXIDANT ACTIVITY OF *Agaricus bisporus* AND *Agaricus campestris*

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Abstract

In the context of a worldwide growing population there is a need for renewable biological resources for food and animal feed, safe and healthy, as well as materials, energy and other products. Thus, an important and sustainable use of renewable resources has become one of the major goals of bio-economic strategy at the national and European levels. Quality, food safety and human health are some of the major concerns of Romania. Due to considerable genetic resources and remarkable biological quality, mushrooms are currently considered functional foods with important nutritional and therapeutic qualities. Nationally, 90% of the mushroom production is covered by Agaricus bisporus species (champignon), being consumed mainly in urban areas. At the same time in rural areas is harvested from spontaneous mycoflora and consumed, mainly Agaricus campestris species. These two species are related, belonging to the same Agaricaeae family. Nutritional and therapeutic important properties of these species are given by their bioactive metabolites including polysaccharides, proteins, dietary fibres, polyphenolic compounds and other bio-molecules. In this paper is presented comparative qualitative (HPTLC) and quantitative (spectrophotometric) chemical composition in polyphenolic compounds and the scavenger potential of free radical DPPH of the two species. The comparative analysis is made for alcoholic and hydroalcoholic extracts of the two species. The results shows the differences and similarities in terms of composition in polyphenolic compounds, dependence between chemical composition and antioxidant potential and also the importance of the solvent used to obtain the extracts. Thus, this study contributes to understanding the importance of the valorisation of indigenous natural resources potential, respectively of the species Agaricus campestris.

Key words*: polyphenolic profiles, Agaricus campestris, Agaricus bisporus, antioxidant activity.*

INTRODUCTION

At national level, the bio-economy, benefits of the huge potential of Romanian agriculture and wild flora. Capitalization of this potential, in the context of an increasingly active local food industries and rising standards, achievements of applied research in the field and in the food and pharmaceutical industries, is a major economic target. The need for renewable biological resources for food and animal feed, safe and healthy, as well as materials, energy and other products, is increasing in the same time with the growth of population number. In Romania, the interest for quality, food safety and human health become one of the major concern at national level. Due to considerable genetic resources and remarkable biological quality, mushrooms are currently considered functional foods with important nutritional and therapeutic qualities (Perera and Li, 2011, Mizuno et al., 1995).

Rich in high-quality protein, containing a high level of dietary fiber and a high proportion of unsaturated fatty acids, rich in various vitamins and minerals, and having an acceptably low level of nucleic acids, mushrooms are suitability for daily use as a vegetable (Chang, 1999).

As therapeutic agents, mushrooms are useful in preventing diseases as hypertension, diabetes, hypercholesterolemia and cancer (Bobek and Galbavy, 1999; Bobek et al., 1995). There are also studies that have shown antioxidant, antitumor, antiviral, antithrombotic and immunomodulating properties (Mau et al., 2002; Subrata et al., 2012). With the increasing awareness of the population to food quality, nutritional and medicinal properties of them,

the mushroom demand will have a significant growth both nationally and internationally levels. Nationally, 90% of the mushroom production is covered by *Agaricus bisporus* species (champignon) (http://biotehnol. eu / mycoind/ abstract.html), being consumed mainly in urban areas. At the same time, in rural areas is harvested from spontaneous mycoflora and consumed, mainly, *Agaricus campestris* species. These two species are related, belonging to the same *Agaricaceae* family.

Nutritional and therapeutic important properties of these species are given by their bioactive metabolites including polysaccharides, proteins, dietary fibers, polyphenolic compounds and other bio-molecules (Popescu, 2006; Parvu, 1997).

In this paper is presented comparative qualitative (HPTLC- high performance thin layer chromatography) and quantitative (spectrophotometric) chemical composition in polyphenolic compounds and the scavenger potential of free radical DPPH of the two species. The comparative analysis is made for alcoholic and hydroalcoholic extracts obtained from both mushrooms. The results shows the differences and similarities in terms of composition in polyphenolic compounds, dependence between chemical composition and antioxidant potential and also the importance of the solvent used to obtain the extracts. Thus, this study contributes to understanding the importance of the valorisation of indigenous natural resources potential, respectively of the *Agaricus campestris* mushroom.

MATERIALS AND METHODS

Raw material - *Agaricus bisporus* (cultivated) sample was obtained from SC Camimar SRL (Arges County) - local producer of champignon mushroom. *Agaricus campestris* (wild) sample was obtained from University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnologies - mushroom collection.

Sample preparation: the samples was prepared by extraction with methanol or ethanol 50%(v/v) - vegetal material/ solvent rate - 1/20m/v for 2h at boiling temperature of the solvent. The solutions was filtered and frozen until analysis

Total phenol content - Total phenol content was determined with Folin - Ciocalteu method (European Pharmacopoeia 6,0). Brifly, 1 ml of extract was transferred to a 25 ml volumetric flask, 10 ml of water and 1 ml of Folin-Ciocalteu reagent was added. The volume was made to 25 ml 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 760 nm with a UV/VIS spectrophotometer (Helios λ, Thermo Electron Corporation). Distilled water was used as a blank. Total phenol content was determined from the extrapolation of the calibration curve (*$y=1,474x-0,438$, $R_2 = 0.992$)*, which was obtained for gallic acid (Sigma Chemical Co., St. Louis, USA) The results was expressed as miligrams of gallic acid equivalents (GAE) per gram of dried raw material.

(HP)TLC Analysis for phenols:

The densitometric analysis (HPTLC) was made according to TLC Atlas - Plant Drug Analyses (Wagner H Balt S. 1997). The characteristic fingerprint profile for phenolic compounds was determined. 3-3.5µl of the samples and 1-3µl of references substances (10^{-3} M rutin, hyperoside, chlorogenic acid, caffeic acid- Sigma-Aldrich) were loaded as 10 mm band length in the 20 x 10 Silica gel 60F254 TLC plate using Hamilton- Bonaduz, Schweiz syringe and CAMAG LINOMAT 5 instrument. Ethyl acetate-acetic acid-formic acid-water 100:11:11:27 (v/v/v/v) was the mobile phase. After development, plates were dried and derivatized in NP-PEG (Natural Product - Polyethylene glycol) reagent. The fingerprints were evaluated at 366nm in fluorescence mode with a WinCats and VideoScan software.

*Free radical scavenging assay- w*as evaluated using the Sanchez-Moreno et al. (1998) assay. The extracts concentration were 1%, 0.1%, 0.01%, 0,.01% in methanol. 50µl aliquots of the extract were mixed with 2950µl of the DPPH methanolic solution (0.025g/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. IC50 (the half maximal inhibitory concentration) was calculated from the graph of RSA percentage against extract concentration and expressed as mg/ml.

RESULTS AND DISCUSSIONS

Total phenol content

Agaricus campestris and *Agaricus bisporus* are both species of *Agariaceae* family.
An important study report that antioxidant activity of natural vegetal extracts are well correlated with their content in phenolic compounds (Velioglu et al., 1998).
Agaricus campestris - commonly known as field or meadow mushroom is white, wild and edible. Is can be seen from spring to autumn on manure or in well fertilized places on meadows, pastures and gardens. (Parvu, 1997).
Agaricus bisporus - also known as button mushroom is white or brown, cultivated and edible. At the present is one of the most cultivated mushrooms in the world.
Both species are reported regarding the content phenolic compounds (Woldegiorgis, et al 2014; Jun et al, 2013).
Therefore, is important to investigated the antioxidant activity of phenolic compounds of mushrooms methanolic and hydroalcoholic extracts. Table 1 shows the total phenol content of the four extracts expressed as gallic (GAE) acid equivalents per g of raw material.

Table 1. Total phenol content of mushroom extracts

No	Extract	mg (GAE)/g
1	Agaricus campestris (methanolic)	4.96
2	Agaricus bisporus (methanolic)	4.63
3	Agaricus campestris (hydroalcoholic)	12.19
4	Agaricus bisporus (hydroalcoholic)	5.2

In the present study the total phenol content in the methanolic extracts is similar in *Agaricus campestris* (4.96 mg (GAE)/g) and *Agaricus bisporus* extract (4.63 mg (GAE)/g). For *Agaricus bisporus* methanolic extracts the total phenol content obtained in other studies are 3.4 mg GAE/g dw (Palacios et al, 2011) and 4.5 mg GAE/g dw, from Spain (Ramirez-Anguiano et al., 2007), as for ethanolic extracts - 6.18 mg GAE/g dw, from China (Liu et al, 2013) and 8.0 mg GAE/g dw, from the United States (Dubost et al., 2007). For *Agaricus campestris* the total phenol content obtained by us 12.19 mg(GAE)/g for hydroalcoholic extract is similar to other results – 10.2 mg/g (Schaffer et al., 2004) and 14.6 mg GAE/g dw (Woldegiorgis et al., 2014).

(HP)TLC Analysis for phenols

Figure 1 shows the (HP)TLC phenolic profiles of the extracts: tracks - T2, T3 *Agaricus campestris* hydroacoholic extract (duplicate sample), track T4 - *Agaricus campestris* methanolic extract, tracks T5, T6 - *Agaricus bisporus* hydroalcoholic extract (duplicate sample) track T7 - *Agaricus bisporus* methanolic extract, tracks T1, T8 - references substances (rutin, chlorogenic acid, hyperoside and caffeic acid - duplicate sample).
The present HPTLC study have revealed that there are no differences between the methanolic and hidroalcoholic extracts (for both species).
For *Agaricus campestris* HPLC-DAD study have shown the presence of p-coumaric, acid ferulic acid, gallic acid p-hydroxybenzoic acid and myricetin (Woldegiorgis et al., 2014). In our study the phenolic profile (HP)TLC of *Agaricus campestris* have 7 major phenolic spots, that are phenolic acid derivate (caffeic acid Rf~0.97 and gallic acid Rf~0.92), according to Wagner and Bladt, (1996) and based on the relationship spot color - Rf.
For *Agaricus bisporus* caffeic acid (Rf~0.97) and gallic acid (Rf~0.92) are detected.

Free radical scavenging assay

When DPPH (2,2 - diphenyl -1-picrylhydrazil) radical accepts an electron from other compounds became very stable and the color of intensive violet disappears (becoming yellow). This action is considered radical scavenging properties (Brighente et al., 2007, Sharma et al., 2009, Ionita, 2005; Huang et al., 2005). Free radicals are chemical species associated with unpaired electron. They are highly reactive species and can be formed when oxygen interacts with certain molecules. Free radicals cause cell damage leading to various helth problems such as cancer, atherosclerosis,

malaria, and rheumatoid arthritis and neurodegenerative diseases. Antioxidant are capable to trap free radicals preventing oxidative damage (Shiv Kumar, 2011; Pham-Huy et al, 2008).

Table 2 presents IC50 value obtained for concentration between 1-0.001% of the extracts (results are presented as mean ± SD (n = 3).

The results indicated that all the extracts have antioxidant activity in a concentration-dependent manner. Hydroalcoholic extracts show the best DPPH scavenger activity for *Agaricus campestris* (IC50 – 0.13 mg/ml) and *Agaricus bisporus* (IC50 – 0.86 mg/ml).

Table 2. IC50 value of the extracts.

No	Extract	IC50 (mg/ml)	R^2
1	Agaricus campestris (methanolic)	1.48	0.997
2	Agaricus bisporus (methanolic)	2.00	0.997
3	Agaricus campestris (hydroalcoholic)	0.13	0.980
4	Agaricus bisporus (hydroalcoholic)	0.86	0.994

Our results are similar to the DPPH scavenger activity obtained in other study that are comprised between 0.18 µg/ml and 9.61 mg/ml for *Agaricus bisporus,* depending on the extraction solvent (Elmastas et al., 2007; Barros et al., 2008).

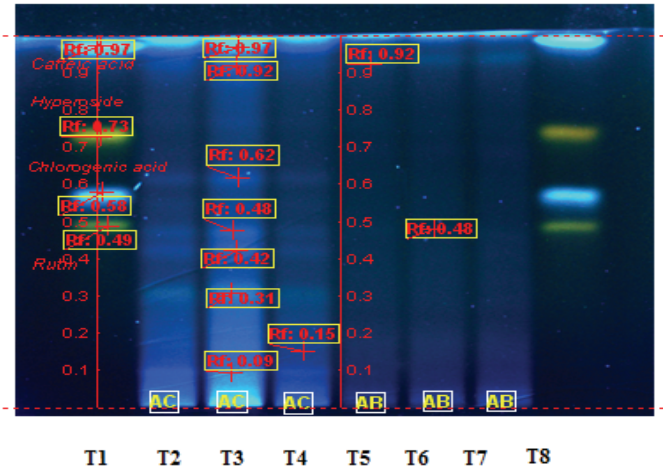

Figure 1. Phenolic profiles of the extracts of *Agaricus campestris* (AC) and *Agaricus bisporus* (AB) comparative with references substances

CONCLUSIONS

Given the fact that mushrooms are considered functional food, having both nutritional and therapeutic properties is important to take into consideration the wild species. Nutritional properties of meadow mushroom are known for many years.

Our results shows that the both species proved to have antioxidant properties, namely radical scavenging activity. The solvent used influences directly the content and bioactivity of the extracts.

For both species, hydroalcoholic extracts have shown both a higher content in total phenolic compounds and a stronger antioxidant activity than methanolic extracts.

Presenting the lowest IC50 value in DPPH assay, *Agaricus campestris* hydroalcoholic extract was the most efficient, having the antioxidant activity correlated with the total phenol content.

Agaricus bisporus hydroalcoholic extract have a lower antioxidant activity comparative with *Agaricus campestris* hydroalcoholic extract, but not inconsiderable.

In the same time, the chromatographic profile shows 7 major compounds for meadow mushroom, comparative with champignon mushroom that have three phenolic derivates, but there was no difference between the extracts (regarding to the extraction solvent).

As mushrooms are important natural resources, these extracts represent considerable source of

compounds that can be used in preparation of bioproducts with therapeutic value. Such extracts can be useful in preventing disease caused by free radical.

Our results shows that *Agaricus campestris* can be taken into consideration for the introduction in controlled culture, which can be a first step in superior valorification of this wild species.

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REFERENCES

Barros, L., Falcão, S., Baptista, P., Freire, C., Vilas-Boas, M., Ferreira, I.C.F.R., 2008. Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chem. 111, 61–66.

Brighente I.M.C., Dias M., Verdi L.G. and. Pizzolatti M.G, 2007. Antioxidant Activity and Total Phenolic Content of Some Brazilian Species Pharmaceutical Biology 45, 156 .

Bobek P, Galbavy S. 1999. Hypocholesterolemic and Antiatherogenic Effect of Oyster Mushroom (Pleurotus ostreatus) in Rabbit. Nahrung; 43: 339-342;

Bobek P, Ozdyn L, Kuniak L, 1995. The Effect of Oyster (Pleurotus ostreatus) Ethanolic Extracts and Extraction Residues on Cholesterol Levels in Serum Lipoproteins and Liver of Rat ; Nahrung 39: 98-99)

Chang S.T., 1999, Global impact of edible and medicinal mushrooms on human welfare in the 21st century nongreen revolution, Int. J. Med. Mushrooms, 1, 1-7

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

Elmastas, M., Isildaka, O., Turkekulb, I., Temura, N., 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J. Food Compos. Anal. 20, 337–345;

European Pharmacopoeia 6,0

Huang DJ, Ou BX, Prior R L. 2005 The chemistry behind antioxidant capacity assays. J Agric Food Chem;53:1841-1856.

Ionita P., 2005, Is DPPH Stable Free Radical a Good Scavenger for Oxygen Active Species? Chem. Pap. 59 (1) 11-16

Kumar Shiv 2011, Free Radicals and Antioxidants: Human and Food System Advances in Applied Science Research, 2 (1): 129-135;

Liu Jun, Jia Liang, Kan Juan, Jin Chang-Hai. 2013 In vitro and in vivo antioxidant activity of ethanolic extract of white button mushroom (Agaricus bisporus) Food and Chemical Toxicology 51 310–316).

Mau LL, Lim HC, Chen CC. 2002. Antioxidant Properties of Several Medicinal Mushrooms, J. Agric. Food Chem 50: 6072 6077;

Mizuno Takashi, Sakai Tadamoto, Chihara Goro 1995 (published online 2009) Health foods and medicinal usages of mushrooms Food Reviews International, Volume 11, Issue 1, Special Issue: Mushrooms: The versatile Fungus-Food and Medicinal Properties

Palacios, I., Lozano, M., 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 Chem. 128, 674–678.

Pathirage Kamal Perera, Yunman Li -2011, Mushrooms as a functional food mediator in Preventing and ameliorating diabetes Functional Foods in Health and Disease; 4:161-171, review

Parvu C. 1997– Universul plantelor Mica enciclopedie – Ed. Enciclopedica , Bucuresti).

Pham-Huy, L. A., He, H., & Pham-Huy, C. 2008. Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science : IJBS*, *4*(2), 89–96).

Popescu Maria Lidia - 2006, Ciuperci. Potential terapeutic si toxicologic. Editura Tehnoplast. Company S.R.L., Bucuresti,ISBN 973-8932-08-4, ISBN 978-973-8932-08-1;

Ramirez-Anguiano, A.C., Santoyo, S., Reglero, G., Soler-Rivas, C., 2007. Radical scavenging activities, endogenous oxidative enzymes and total phenols in edible mushrooms commonly consumed in Europe. J. Sci. Food Agric. 87, 2272– 2278.

Sebastian Schaffer, Gunter P. Eckert, Walter E. Müller, Rafael Llorach Diego Riverab, Simona Grande, Claudio Galli, and Francesco Visioli. 2004. Hypochlorous Acid Scavenging Properties of Local Mediterranean Plant Foods Lipids, Vol. 39, no. 12

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.

Sharma Om P. Tej. K Bhat, 2009, DPPH antioxidant assay revisited Food Chemistry, Volime 113, Issue 4, 15 April 2009, Pages 1202–1205

Subrata Giri, Gunjan Biswas, Prakash Pradhan Subhash C. Mandal, Krishnendu Acharya1 2012. Antimicrobial Activities Of Basidiocarps Of Wild Edible Mushrooms Of West Bengal, India International Journal of PharmTech Research Vol.4, No.4, pp 1554-1560, ISSN : 0974-4304;

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables grain. Journal of Agricultural and Food Chemistry, 46, 413–417.

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

Woldegiorgis Ashagrie Z., Abate Dawit, Haki Gulelat D., Ziegler Gregory R., 2014. Antioxidant property of edible mushrooms collected from Ethiopia, Food Chemistry 157, 30–36;

http://biotehnol. eu / mycoind/ abstract.html