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COMPARATIVE ELEMENTAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE FRUITING BODIES OF *Pleurotus djamor* CULTIVATED ON SAWDUST AND RICE STRAW-BASED FORMULATIONS

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ABSTRACT

Our team initiated a study to determine and compare the elemental composition as well as anti-oxidant activities of the fruiting bodies of *P. djamor* grown separately on two substrate-formulations, namely: sawdust and rice straw. Elemental analysis of the air dried fruiting bodies was done using Thermo EDX System (Noran System 6, Ultra Dry, 10mm2 SDD crystal, 129eV resolution, NORVAR window, LN2-Free Type Detector) installed in Hitachi SU1510 Scanning Electron Microscope. The anti-oxidant activity was evaluated based on the DPPH free radical scavenging activity. Nine elements (magnesium, nitrogen, sodium, potassium, chlorine, sulfur, phosporus, silicon and oxygen) were detected in the fruiting bodies of *P. djamor* grown on sawdust-based formulation while six elements (magnesium, nitrogen, potassium, sulfur, phosporus and oxygen) were present in fruiting bodies harvested on rice straw – based

formulation. The radical scavenging activity increased with an increase in the concentration of the extract. Moreover, fruiting bodies grown on rice straw based substrate exhibited higher scavenging activity (93.82% at 1000 mg/L extract) than those grown in sawdust based substrate (60.67% at 1000 mg/L extract). In conclusion, the elemental composition and antioxidant activity of *P. djamor* are substrate dependent.

Keywords: antioxidant, mineral composition, mushroom, *Pleurotus djamor*

INTRODUCTION

Mushroom production is a lucrative agri-business undertaking particularly in regions where substrates are abundant. Mushroom growers oftentimes use locally available substrates which are derived from agro-industrial residues. These substrates include cotton waste, coffee hull, banana leaves, rice straw, sawdust and other lignocellulosic residues [1, 2, 3, 4]. Among these substrates, rice straw and sawdust are the most widely utilized by the growers in producing different species of wood degrading mushrooms such as *Pleurotus*, *Ganoderma* and *Auricularia*. In recent years, mushrooms are regarded as nutraceuticals which contain important nutrients and exhibit different functionalities such as anti-hypertensive, anti-microbial and anti-oxidant [5, 6, 7, 8, 9]. Damage of cell due to reactive oxygen species (ROS) has been associated in several lifestyle diseases such as diabetes, hypertension and cancer. In recent years, antioxidants which protect the body from this damage have significantly gained important role in human health [10]. *Pleurotus djamor* is one of the fancy species of commercially cultivated mushrooms due to its distinctly pinkish fruiting bodies that usually grow in cluster. Local mushroom growers usually cultivate this mushroom either on sawdust-based or rice straw-based formulation depending on the availability and ubiquity of these substrates for cultivation. Recently, *P. djamor* was reported to possess ergosterol, triacylglycerols, and fatty acid methyl esters which are believed to exhibit anti-tumor, anti-microbial and anti-oxidant activities [11]. In this paper, we are reporting the elemental composition as well as the anti-oxidant activity of the crude extract of the fruiting bodies of *P. djamor* grown separately in rice straw and sawdust – based formulations respectively.

MATERIALS AND METHODS

Cultivation of mushrooms

Following the standard protocol for cultivation [12], *P. djamor* was separately grown on sawdust-based (i.e. 78 parts sawdust, 20 parts rice bran and 2 parts

agricultural lime, v/v) and rice straw – based mixtures (i.e. 7 parts composted rice straw and 3 parts sawdust, v/v).

Preparation of samples

Marketable size of the fruiting bodies of *P. djamor* (Figure 1) were harvested and air-dried at 18ºC.

Elemental analysis

Elemental analysis of the air - dried fruiting bodies of *P. djamor* was done using Thermo EDX System (Noran System 6, Ultra Dry, 10mm2 SDD crystal, 129eV resolution, NORVAR window, LN2-Free Type Detector) installed in Hitachi SU1510 Scanning Electron Microscope.

Anti-oxidant assay

The DPPH free radical scavenging method of Chan et al [13] was adopted with minor modifications as follows: the sample crude extract and the standard (L-ascorbic acid and butylated hydroxylanisole) were diluted to various concentrations using methanol at 1, 10, 100, 300 and 1000 ppm. From each dilution, 1.5 ml was added into the test tube containing 2.5 ml DPPH solution in methanol. The mixtures were incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance of the resulting solution was measured using visible spectrophotometer at 517nm. The DPPH free radical scavenging activity was expressed following the equation: DPPH free radical scavenging activity (%) = (1- Abs sample/Abs control) x 100 where Abs sample was the absorbance of the sample or standard while Abs control was the absorbance of the control (i.e. without a sample extract or anti-oxidant compound).

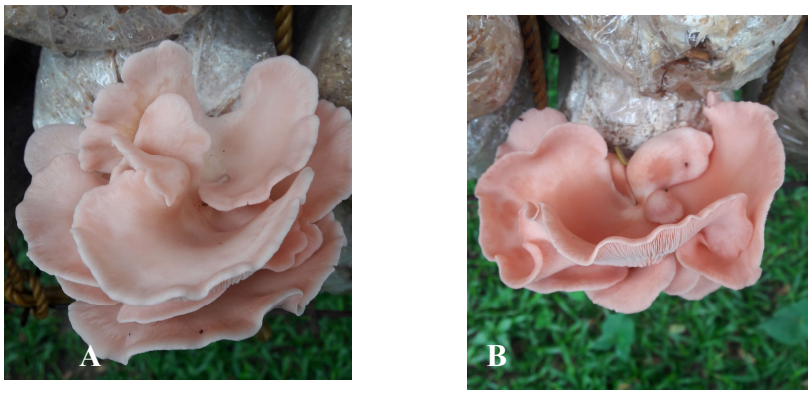

Figure 1: Cluster of fruiting bodies of *P. djamor* grown on rice straw - (A) and sawdust - (B) based substrate formulations

RESULTS AND DISCUSSION

Elemental composition of *P. djamor*

The amount and kinds of element present in mushroom may vary depending on the species and substrate. In this study, the elemental composition of *P. djamor* cultivated on two substrate formulations was determined and the results are presented in Table 1. Nine elements (magnesium, nitrogen, sodium, potassium, chlorine, sulfur, phosporus, silicon and oxygen) were detected in the fruiting bodies of *P. djamor* grown on sawdust-based formulation while six elements (magnesium, nitrogen, potassium, sulfur, phosporus and oxygen) were found on fruiting bodies cultured on rice straw – based formulation. Among the elements analyzed chlorine, silicon and sodium were not detected in rice straw grown fruiting bodies. Potassium and oxygen were the most abundant elements in fruiting bodies from both substrates. With regards to the distribution of elements in four different regions within the cluster of fruiting bodies, oxygen and potassium were found in all the regions of mushrooms cultured on both substrates. However, phosphorous was evenly distributed in sawdust grown fruiting bodies. Magnesium and nitrogen were mostly concentrated in the central part of the cluster of fruiting bodies grown in sawdust-based formulation. On the other hand, magnesium was detected in both right and left regions in a cluster of the fruiting bodies grown in rice straw-based formulation. These results suggest that type of substrate influence the type of elements that may be present as well as their distribution within the cluster of fruiting bodies.

The difference in the elemental composition of the fruiting bodies of *P. djamor* grown on two substrate formulations indicates that the elemental composition varies according to the substrates where the mushroom is cultivated. This confirms previous report that the mineral content of mushroom not only depend on mushroom species but also on the substrates used [14, 15]. Moreover, Mallikarjuna et al. [16] revealed that the mineral composition reflects the growth conditions of the mushrooms and reported eleven elements namely: potassium, phosphorous, calcium, sodium, magnesium, iron, zinc, copper manganese and selenium in *Lentinula edodes, Lentinus cladopus, Pleurotus florida and Pleurotus djamor* collected from the forest. Kalmis et al. [17] reported that the mineral composition of mushroom varies according to the growth stages. They reported that the zinc content of mycelial and young fruiting bodies was found to be lower than the content of the mature fruiting bodies.

Table 1: Distribution of elements within the cluster of the fruiting bodies of *P. djamor* grown in rice straw and sawdust – based formulations

Substrate formulation	Element	Region within the cluster of the fruiting bodies			
		Left	Center	Right	Bottom
Rice straw-based	O	67.05	70.63	78.79	74.87
	Si	ND	ND	ND	ND
	P	7.65	5.59	5.44	ND
	S	3.08	2.89	1.9	ND
	Cl	ND	ND	ND	ND
	K	16.64	20.88	12.2	25.13
	Na	ND	ND	ND	ND
	N	4.66	ND	ND	ND
	Mg	0.92	ND	1.67	ND
Sawdust-based	O	60.85	58.06	64.21	64.55
	Si	1.58	7.01	1.53	ND
	P	5.76	5.3	7	5.1
	S	3.74	2.39	1.96	ND
	Cl	3.18	ND	2.58	ND
	K	24.88	18.84	21.42	30.35
	Na	ND	ND	1.3	ND
	N	ND	4.23	ND	ND
	Mg	ND	4.17	ND	ND

Anti-oxidant assay

The oxidative properties of oxygen perform a significant role in various biological activities which include the utilization of nutrients, generation of ATP thru electron transport and the removal of xenobiotics. However, oxygen though vital in the different life processes can also instigate cellular damage. When oxygen is transformed into more reactive forms such as superoxide radical (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) can damage important enzymes, DNA and structural proteins. It can also provoke uncontrolled chain reactions such as lipid peroxidation or autooxidation reactions [18, 19]. Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of specific compounds or extracts in a short time [20]. The comparative ability of the fruiting bodies of *P. djamor* grown separately on sawdust and rice straw - based formulations to scavenge super oxide, hydroxyl, nitric oxide, 2,2′-diphenyl-1-picrylhydrazyl (DPPH) when compared with ascorbic acid and butylated

hydroxylanisole is shown in Table 2. The scavenging activity of the extracts of mushrooms increased with an increased concentration. This significant finding contradicts with the previous report of Ajith and Janardhanan [21] who reported that the methanolic extract of *Phellinus rimosus* at 0.005% had higher antioxidant activity compared to 0.01 and 0.1% concentrations respectively. The type of cultivation media also influences the antioxidant activity of mushroom. *P. djamor* grown on rice straw-based formulation had higher antioxidant activity compared with *P. djamor* which was grown on sawdust-based formulation. This important observation suggests that the type of growing media plays an important role in the expression of the functionalities of mushrooms [22].

Table 2. Antioxidant activity of the crude extract of *P. djamor* at different concentrations*

Concentration (mg/L)	% Scavenging Activity (Mean ± SD)			
	L-ascorbic acid, (as standard)	butylated hydroxylanisole, (as standard)	*P. djamor* grown on sawdust-based formulation	*P. djamor* grown on rice straw-based formulation
1	13.34 ± 1.33	15.23 ± 0.06	1.28 ± 0.93	1.31 ± 0.48
10	97.84 ± 0.32	85.60 ± 3.02	2.44 ± 0.62	3.73 ± 0.51
100	97.77 ± 0.12	96.93 ± 0.85	13.88 ± 1.26	23.72 ± 1.05
300	98.27 ± 0.06	97.56 ± 0.11	31.47 ± 3.41	53.88 ± 6.41
1000	98.44 ± 0.06	97.46 ± 0.38	60.37 ± 3.82	93.82 ± 1.11
EC_{50} (mg/L)	2.57	3.12	651.80	233.30

***based on %DPPH free radical scavenging activity**

The EC_{50} values of ascorbic acid, butylated hydroxyl anisole, crude extract of *P. djamor* grown on sawdust and rice straw -based formulations are also presented in Table 2. The EC_{50} is inversely proportional to the DPPH free radical scavenging activity of the sample extracts [23]. Thus, lower value suggests higher antioxidant activity. The ascorbic acid and butylated hydroxylanisole being pure compounds have relatively higher antioxidant activity compared with the mushroom crude extracts. Crude extract of *P. djamor* grown on rice straw - based formulation recorded higher antioxidant activity than the crude extract of *P. djamor* grown on sawdust-based formulation. This result implies that the antioxidant activity of the *P. djamor* may vary according to the substrate where the mushroom is grown. This could possibly be explained by the nutrient composition of the substrate.

CONCLUSION

In conclusion, the elemental composition and antioxidant activity of *P. djamor* is greatly influenced by the type of substrate.

REFERENCES

[1] Sanchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms.

Applied Microbiology and Biotechnology, 2010, 85(5),1321-1337.

[2] Tisdale TE, Miyasaka SC, Hemmes DE. Cultivation of the oyster mushroom (*Pleurotus ostreatus*) on wood substrates in Hawaii. World Journal of Microbiology and Biotechnology, 2006, 22:201-206.

[3] Belewu MA, Belewu KY. Cultivation of mushroom (*Volvariella volvacea)* on banana leaves. African Journal of Biotechnology, 2005, 4 (12), 1401-1403.

[4] Philippoussis A, Zervakis G, Diamantopoulou P. Bioconversion of agricultural lignocellulosic wastes through the cultivation of the edible mushrooms *Agrocybe aegerita*, *Volvariella volvacea* and *Pleurotus* spp. World Journal of Microbiology & Biotechnology, 2001, 17, 191-200.

[5] Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RMV, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. Journal of Applied Microbiology, 2013. 115, 346–357.

[6] Lau CC, Abdullah N, Shuib AS, Aminudin N. Proteomic analysis of antihypertensive proteins in edible mushrooms. Journal of Agriculture and Food Chemistry, 2012, 60 (50):, 12341–12348.

[7] Ozen T, Darcan C, Aktop O, Turkekul I. Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea Region of Turkey. Combinatorial Chemistry & High Throughput Screening, 2011, 14 (2),72 – 84.

[8] Öztürk M, Duru ME, Kivrak S, Mercan-Doğan, N, Türkoglu A, Özler MA. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: A comparative study on the three most edible mushrooms. Food and Chemical Toxicology, 2011, 49(6), 1353-1360.

[9] Zanabaatar B, Kim MK, Seo GS, Lee YW, Lee JS. Screening and physiological functionality of *Hypsizygus marmoreus* (white cultivar) fruiting body. The Korean Journal of Mycology, 2011, 39(3), 185–188.

[10] Nitha B, Strayo De, Adhikari SK, Devasagayam TPA, Janardhanan

KK. Evaluation of free radical scavenging activity of morel mushroom, *Morchella esculenta* mycelia: A potential source of therapeutically useful antioxidants. Pharmaceutical Biology, 2010, 48(4), 453-460.

[11] Ragasa CY, Tan MCS, Ting J, Reyes RG, Brkljaca R, Urban S. Chemical constituents of *Pleurotus djamor*. Der Pharma Chemica, 2016, 8(2), 343-346.

[12] Reyes RG, Eguchi F, Kalaw SP, Kikukawa T. Mushroom Growing in the Tropics: A Practical Guide. Science City of Munoz, Central Luzon State University Press, 2009, 89.

[13] Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. Food Chemistry, 2007, 104, 1586-1593.

[14] Okoi AI, Iboh CI. The effects of different substrates on sporophore yield, mineral and nutrient composition of *Pleurotus tuberregium* fries singer in Calabar, Nigeria. International Journal of Agricultural Science Research, 2015, 4(6), 126-131.

[15] Hoa HT, Wang CL, Wang CH. The effects of different substrates on the growth, yield and nutritional composition of two Oyster mushrooms *(Pleurotus ostreatus* and *Pleurotus cystidiosus*. Mycobiology, 2015, 43 (4), 423-434.

[16] Mallikarjuna SE, Ranjini A, Haware DJ, Vijayalaksmi MR, Shashirekha MN, Rajarathnam. Mineral composition of four edible mushrooms. Journal of Chemistry, 2013, 1-5.

[17] Kalmis E, Yildiz H, Ergonul B, Kalyoncu F, Solak MH. Chemical composition and nutritional value of a wild edible ectomycorrhizal mushroom, *Trocholoma anatolicum*. Turkey Journal of Biology. 2011, 35, 627-633.

[18] Halliwell B, Cross CE. Oxygen-derived species: Their relation to human disease and environmental stress. Environmental Health Perspective, 1994, 102, 5-12.

[19] Hemnani T, Parihar MS. Reactive oxygen species and oxidative oxygen DNA damage. Indian Journal of Physiology and Pharmacology, 1998, 42, 440-452.

[20] Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry, 2003, 81, 249-255.

[21] Ajith TA, Janardhanan KK. Indian medicinal mushrooms as source of antioxidants and antitumor agents. Journal of Clinical Biochemistry and Nutrition, 2007, 40, 157-162.

[22] Kumakura K, Kumakura H, Ogura M, Eguchi F. Pharmacological effects of *Ganoderma lucidum* collected from ume (Japanese apricot) trees. Journal of Wood Science, 2008, 54, 502–508.

[23] Tudor V, Manole CG, Teodorescu R, Asanica A, Barbulescu ID. Analysis of some phenolic compounds and free radical scavenging activity of strawberry fruits during storage period. Agriculture and Agricultural Science Procedia, 2015, 6,157-164.