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Mycochemical Screening, Mineral Analysis and Antioxidant Efficacy of Some Commercially Cultivated and Pharmaceutically Important *Pleurotus* Species

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Abstract

The present study aims to evaluate the presence of secondary metabolites, mineral contents, and antioxidant potential of *Pleurotus floridanus, P. ostreatus and P. cystidiosus* that are the potentially edible and pharmaceutically efficient mushroom species. Mycochemical screening revealed the presence of important secondary metabolites i.e. saponins, terpenoids, flavonoids, tannins, cardiac glycosides, and phenolic compounds. The mineral analysis was carried out to determine the presence of essential and non-essential nutrients in the *Pleurotus* species. Potassium was found to be maximum among all the nutrients i.e. 45 mg/g, 57mg/g and 62 mg/g in *P. cystidiosus P. ostreatus* and *P. floridanus* respectively. Nickel and cobalt were absent in all three species. Antioxidant potential of the ethanol extracts of these valuable mushrooms was appraised by using assays i.e. ABTS (2, 2'- Azino-Bis-3-Ethylbenzothiazoline-6-Suphonic Acid), FRAP (Ferric Reducing Antioxidant Power Assay) and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), Flavonoids (TFC) and Phenolic (TPC) were also determined. Maximum % inhibition on ABTS (92.5 ± 0.288), DPPH (56.45 ± 0.22) and Fe3+ (0.541 ± 0.006) radical was shown by *P. floridanus* followed by *P. cystidiosus and P. ostreatus*. *P. floridanus* also contains the highest phenolic and flavonoids contents. This study identified that all three species exhibit important mycochemical compounds, antioxidant potential and essential minerals that showed their potential to use them as the natural medicine and also can utilized for the production of pharmaceutical drugs in future.

Keywords: *Pleurotus floridanus; P. ostreatus; P. cystidiosus;* Mycochemical; Mineral analysis; Antioxidant potential

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Introduction

Mushrooms edibility always allures man to use them as the food [1]. Around 7000 of the 16,000 recognized mushroom species, exhibit different ranges of edibility, above 3000 species are primary edible mushrooms, and 700 are considered as safe therapeutic mushrooms [2,3]. Mushrooms may exhibit a number of elements that have Antigenotoxicity, Antioxidation, Anti-hypertensive, Anti-nociceptive, Immuno-stimulation and stress diminishing attribute [4-7].

Pleurotus genus is placed in Tricholomataceae family of the Phylum Basidiomycota [8,9]. Worldwide *Pleurotus* (Fr.) P. Kumm spp. have been used due to of their nutritional, therapeutic, and other beneficial effects. *Pleurotus* spp. possesses antithrombotic, antimicrobial and hypoglycaemic properties, prevent inflammation, stimulate immune system and also possess numerous other activities [10]. *Pleurotus* species are abundant source of minerals (P, Ca, K, Fe and Na), proteins, and vitamin C, B-complex (riboflavin, folic acid, thiamine and niacin) [11].

Pleurotus species have been appreciated as superfood due to their ability to use them both as a food and medicine [12]. Traditionally, *Pleurotus* species extracts have been described to be used in treating some diseases [13-15]. The aim of this research is to assess and compare the presence of mycochemical

constituents, minerals concentration, and antioxidant potential of three globally recognized cultivated *Pleurotus* species that may be used as the natural medicine in future.

Materials and Methods

Sampling and experimental design

Basidiomata of the *P. floridanus and P. ostreatus* were collected from Himalayan moist temperate forests (Khanaspur-Ayubia, KP) in Pakistan while Basidiomata of the *P. cystidiosus* was collected from Girls Hostel Area, University of the Punjab, Lahore, Punjab, Pakistan and all the field characteristics were noted. Samples were placed in small card boxes and then brought to the lab for further proceeding. They were dried under fan heater, and then these dried samples were put in new and clean plastic zipper bags for preservation and further analysis. Specimens were identified by and accordingly to the literature already reported [16-18]. The experiments were carried out in Fungal Biology and Systematics Research lab, Department of Botany, University of the Punjab, Lahore. Specimens were submitted in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Extracts preparation

To obtain the crude extracts, 5 g of each mushroom specimen were subjected to the maceration technique. Firstly the fruiting bodies of mushrooms were sun dried and then converted into fine powdered form by the use of grinder. To extract the active constituent of the mushrooms, 50 milliliter of each solvent for the 5g of the specimens were used, firstly the non-polar solvents i.e. Petroleum ether and Chloroform were used, then gradual transfer to the polar solvents i.e. Ethanol and Distilled water. After 8 days these were filtered through Whatman No. 1 filter paper and then filtrate was subjected to further analysis i.e., mycochemical composition and antioxidant potential [19].

Mycochemical analysis

The dried *Pleurotus* species extracts were subjected to qualitative mycochemical screening by using ordinary protocol. These include the tests for the Anthraquinones (Borntrager's test), Reducing sugars (Fehling Test), Saponins (Frothing test), Tannins, Terpenoids (salkowaski's test), Flavanoids, Alkaloids and Cardiac glycosides (Keller-Killiani test) [20].

Mineral analysis

Mineral analysis was pursued by using standard procedures described by Horwitz et al. Powder samples were subjected to digestion by wet digestion method to check the mineral contents present in the samples. Minerals i.e., Potassium (K), Zinc (Zn), Calcium (Ca), Nickle (Ni), Copper (Cu) and Cobalt (Co) were analyzed through wet digestion method. Standard solutions of 5, 10, 15 20 and 25 ppm were prepared from the stock solution of the five required metals except potassium. For potassium standard solution of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm were prepared from the prepared stock solution (1000 mL) of potassium and they were analyzed in Atomic Absorption (AA) Spectrophotometer (Perkin-Elmer Analyst-100) and in Flame Photometer (JANWAY PFP 7).

Determination of antioxidant potential

The antioxidant activity of ethanolic extracts of three *Pleurotus* species was assessed by using assays i.e. DPPH, ABTS and FRAP, TPC, and TFC.

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) Free Radicle Scavenging Assay: DPPH free radicle scavenging activity of the extracts of *Pleurotus* species was carried out by using the method of Blois (1958) To find out the radical scavenging potential of the mushrooms extracts, 0.1 mL of extract and 3.9 mL of DPPH (0.06 mM) were added in each test tube and the absorbance was noted at 517nm for each species.

2, 2'- Azino-Bis-3-Ethylbenzothiazoline-6-Suphonic Acid ABTS+ Radical Cation Assay: To assess the ABTS scavenging potential, mixture of potassium persulphate (2.45 mM) and ABTS (7 mM) was prepared with same ratio. The mixture was incubated for 24 hours and then diluted it with ethanol. After it 0.1 mL of sample was mixed 0.9 mL of prepared solution and absorbance was measured at 734 nm [21].

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Ferric Reducing Antioxidant Power Assay (FRAP) Assay: Firstly the FRAP reagent was prepared by using acetate buffer (300 mmol/liter), 2.5 mL TPTZ (10 mmol/liter in 40 mmol/liter HCl) and 2.5 mL $FeCl_3.6H_2O$ (20 mmol/liter). Then 0.3 mL of FRAP reagent was added into 0.01 mL of ethanol extracts along with 0.03 mL of distilled water. Ferrous sulphate ($FeSO_4.7H_2O$) was used as standard and absorbance was taken as 593 nm against blank [23].

Total Phenolic Content (TPC): Total phenolic contents in the ethanol extracts of mushrooms samples were evaluated by using Folin-Ciocalteu reagent while gallic acid was used as the standard 0.2 mL of extract was added in 0.8 mL of freshly prepared FC reagent. After 5 minutes shaking 2.0 mL of (7.5%) Na_2CO_3 and 7 mL distilled water were added. The reaction mixtures were kept in dark for 2 hours and absorbance was noted at 765nm against blank.

Total Flavonoids Content (TFC): To determine the TFC of the *P. floridanus, P. cystidiosus* and *P. ostreatus*, their ethanol extracts (1.0 ml) were added in double distilled water. Immediately 0.3 mL of $NaNO_2$ (5%) was added and then 0.3 mL of $AlCl_3$ (10%) was added after 5 minutes. 2 mL of NaOH (1M) was added in flask after 1 minute. The solution was diluted with double distilled H_2O (2.4 mL) at 0 time and then mixed thoroughly. Absorbance was noted at 510 nm and catechin was used as the standard.

Statistical Analysis

All the experiments were conducted in triplicates and data is

expressed as mean value ± S.E. Analysis of Variance (ANOVA) with Duncan's multiple range tests ($p<0.05$) by using co-stat software (Version 3.03) was employed to analyze the significant value of analysis.

Results and Discussion

Mycochemical screening

Mycochemical screening of *P. floridanus, P. ostreatus* and *P. cystidiosus* crude extracts disclose the presence of important secondary metabolites i.e., flavonoids, terpenoids, saponins, tannins and cardiac glycosides in all types of used mushrooms extracts but with different intensity while anthraquinones was absent in all the extracts used for mycochemical screening except in the ethanol extract of *P. cystidiosus* **(Table 1).** Presence of active secondary metabolites in the *Pleurotus* species showed their potential to use them as the natural therapeutic drugs. These findings were similar with the study of Obodai et al., and Rahimah et al., [23-25].

Mineral analysis

The mineral analysis was carried out to find the presence and concentration of macro and trace elements in the nutritionally and medicinally important oyster species. *Pleurotus* species were enriched with the macronutrients i.e., Potassium and calcium while nickel and cobalt that are trace elements were not detected **(Table 2).** Enrichment of essential elements and absence of toxic metal showed their efficacy to use them as the superfood. These results were in agreement with the findings of Mallikarjuna et al., and Ahmed et al., [2,21].

Analysis of antioxidant potential

The ethanol extracts of *Pleurotus* species were used to evaluate their antioxidant efficacy. The ethanolic extracts of mushroom samples has demonstrated the great capability to scavenge free radicles such as DPPH, ABTS, high reducing power, total phenolic content and total flavonoid contents. BHA and BHT that are the synthetic antioxidants were used as the standards and the % anti-radical activity of *Pleurotus* species was compared with standard antioxidants. *P. floridanus* extract showed the maximum scavenging activity on DPPH, ABTS and Fe^{3+}. Percent inhibition of *P. floridanus* on DPPH was 56.45 ± 0.22 (mg/ml) while the 50.67 ± 0.31 and 52.24 ± 0.123 (mg/ml) scavenging efficacy revealed by the BHA and BHT that were used as the standard antioxidants. *P. floridanus*, *P. cystidiosus* and *P. ostreatus* antiradical value on DPPH is given **(Table 3)** [20-28].

Similarly, the % inhibition activity of oyster species on ABTS was find out **(Table 3)** and compared with the standard antioxidants BHA and BHT that had percentage inhibition of 92.33 ± 0.22% and 92.15 ± 0.144% respectively. The *Pleurotus* species also showed the reducing power of Fe^{+3}. The total phenolic and flavonoids contents were also noticed. The TPC was determined as gallic acid equivalents while the total flavonoids contents was reported as catechin equivalent to grams. The antioxidant potential of the crude ethanolic extract of *P. floridanus*, *P. cystidiosus* and *P. ostreatus* was quantified and results are given in **Table 3.** These findings were in agreement with Jeena et al., 2016 and Bozdogan et al., [19-28].

Table 1 Mycochemical screening of crude extracts of *P. floridanus, P. ostreatus and P. cystidiosus.*

Constituents	Mycochemical test	Extracts of *Pleurotus* species											
		Petroleum ether			Chloroform			Ethanol			Distilled water		
		PF	PC	PO	PF	PC	PO	PF	PC	PO	PF	PC	PO
Reducing Sugars	Fehling's test	++	+	+	+	+	++	+++	++	++	+	-	-
Anthraquinones	Borntrager's test	-	-	-	-	-	-	-	++	-	-	-	-
Terpenoids	Salkowski test	+	-	++	+	++	-	+++	+++	++	+	-	+
Flavonoids	Sulfonic test	++	+	+	++	+++	+	+++	++	+	-	-	+
Saponins	Fronthing test	++	++	++	+	++	-	+	++	+++	++	+	++
Tannins	$FeCl_3$ test	++	+	+++	+	+	+	+++	+++	+	++	+	+
Cardiac Glycosides	Keller-Killiani test	+	+	++	+	++	++	++	+	+	+	++	+

*Mycochemical detection key: - = Absent, + = Present in small amount (concentration), ++ = Moderately present, +++ = Present in large numbers
PC: *Pleurotus cystidiosus*; PF: *Pleurotus floridanus*; PO: *Pleurotus ostreatus*

Table 2 Minerals concentration present in *the three Pleurotus species.*

Mushrooms	Essential and Trace minerals (mg/g)					
	Ca	Co	Cu	K	Zn	Ni
P. floridanus	1.332	ND	0.524	62	0.21	ND
P. ostreatus	0.28	ND	0.233	57	0.627	ND
P. cystidiosus	1.34	ND	0.155	45	0.44	ND

ND indicates not detected; Calcium (Ca), Cobalt (Co), Copper (Cu), Potassium (K), Zinc (Zn), Nickel (Ni)

Table 3 Antioxidant potential of ethanol extracts of *P. floridanus, P. cystidiosus, and P. ostreatus.*

Mushrooms	Total Phenolics contents (mg GAE/g)	Total Flavonoids contents (mg CE/g)	FRAP Assay (nmol/L of $FeSO_4$)	DPPH Scavenging activity (mg/ml)	ABTS Assay (mg/ml)
P. floridanus	0.24 ± 0.007c	0.28 ± 0.009c	0.541 ± 0.006d	56.45 ± 0.22b	92.5 ± 0.288a
P. cystidiosus	0.112 ± 0.001 ab	0.25 ± 0.002c	0.523 ±0.004c	54.166 ± 0.023c	91.069± 0.007c
P. ostreatus	0.23 ± 0.008c	0.25 ± 0.0029c	0.29 ± 0.007c	54.944 ± 0.014d	90.738± 0.022h
LSD	0.018	0.017	0.021	0.045	0.019

*The results reported were run in triplicates and stated as Mean± Standard error. *In each column different letters mean significant (p <0.05) differences between mean (p<0.05) according to Duncan's new multiple range test while ± indicates standard error.

Conclusion

From these results it could be concluded that *P. floridanus* possess the highest antioxidant potential and essential mineral concentration among all the three *Pleurotus* species but overall all the three *Pleurotus* species viz., *P. floridanus, P. cystidiosus* and *P. ostreatus* exhibit the significant anti-oxidant potential and important macro-nutrients which showed their efficient potential to utilize them for the production of pharmaceutical drugs in future and to use them as the superfood. This work was supported by grants from the Science and Technology Innovation Committee of Shenzhen Municipality (Grant No. JCYJ20180228162607111, JCYJ20190809104601662), the Health and Family Planning Commission of Shenzhen Municipality Research Project (Grant No. SZBC2018018), China Scholarship Council (CSC, 201908440124).

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