



A study on Mycochemical analysis of some edible Basidiomycetes mushroom fungi

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Abstract

Fungi have been occupying a prominent position in the biological world because of their variety, economic and environmental importance. Mushrooms have been a part of fungal diversity for around 300 million years. The Basidiomycetes or agarics known by Greeks and Romans were famous since ancient times as reported in *Materia medica*. Recently there have been considerable interests in the fungi belonging to Basidiomycota due to the number of biologically active compounds isolated from the mushroom fungi. In the present study, ten edible species of mushroom fungi belonging to four genera *Hypsizygus*, *Oudemansiella*, *Schizophyllum* and *Pleurotus* were selected. All the selected mushrooms were cultured in the laboratory in SD broth and screened for the presence of bioactive compounds. After 21 days of growth, hot water extract of dried mycelia was obtained. The analysis of cell free culture filtrate and mycelia extract confirmed the presence of phytochemicals viz., total phenolics, condensed tannins, total flavanoids, proteins, carbohydrates, lipid crude fibre, and lignolytic enzymes. All the biologically active substances were quantified using standard biochemical procedures and the results obtained confirmed the presence of all the bioactive compounds. The results obtained showed the highest content of bioactive compounds in *Hypsizygus ulmarius* and *Pleurotus citrinopileatus* than that of the selected mushroom fungi for the present study indicating their potential applications. Furthermore, the presence of all the bioactive compounds in the selected mushroom fungi confirmed the potentiality of the mushrooms as antimicrobial, antioxidant, antidiabetic and hypocholesteremic agent.

Keywords: Edible Mushroom Fungi, total phenolics, total flavanoids, condensed tannins, proteins, lignolytic enzymes.

Introduction

Mushrooms are the distinctive fruiting bodies of Ascomycetes or Basidiomycetes fungi (called as macro fungi) that can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand (Chang and Miles, 1992). The study of fungal biodiversity has been carried out worldwide (Crous, 2006) and 1.5 million species have been reported so far and about 50% of them have been characterized (Hawksworth, 2001). Studies on macro fungi have been an area of importance for the scientists in particular and the people in general,

because of their role in human welfare, in food industry, in medicines, and biodegradation (Ozturk et al., 2003). Mushrooms are popular and valuable functional food, low in calories and high in minerals, essential amino acids, vitamins and fibers. Among 300 known genera of mushrooms and related fleshy Basidiomycetes, only a few species of these fungi are cultivated commercially. The most common cultivated species are the button mushroom, *Agaricus bisporus* which was widely cultivated in Europe, the Shiitake mushroom (*Lentinus edodes*) grown for

centuries in China and other oriental countries and the oyster mushroom (*Pleurotus ostreatus*) (Chakravarty, 2011). The availability of the edible mushrooms during a specific period of time in its natural habitat limit the use of it and effective utilization of mushrooms can be made possible with the cultivation of mushrooms under in vitro conditions. The fruiting bodies are characterized by seasonal occurrence; while the in vitro cultures can be prepared in reproducible conditions. The same compounds as in the fruiting bodies are produced in in vitro cultures (Muszynska et al., 2017). This can be achieved under special conditions like controlled physical parameters like pH, temperature and moisture, as well as the optimal chemical composition of the natural compost like carbon, nitrogen and mineral (Petre et al., 2010)

Polysaccharides from natural products possess wide-ranging beneficial therapeutic effects and health-promoting properties. Fungal polysaccharides, derived from *G. frondosa*, *L. edodes*, oyster mushroom, as well as *Ganoderma*, *Flammulina*, *Cordyceps*, *Coriolus*, and *Pleurotus*, and so forth, are demonstrated to have multiple bioactivities (Wang et al., 2017; Zhang et al., 2017), including immune modulating, anticancer, antimicrobial, anti-oxidant, hypocholesterolemic, and hypoglycemic effects mushrooms are rich in protein and high in essential amino acids. Fruiting bodies and mycelia of mushrooms have a wide range of biologically active proteins. Lectins, antifungal proteins, ubiquitin-like and ribonucleases proteins and peptides, fungal immunomodulatory proteins, laccases and other enzymes are the proteins found in edible mushrooms Mushrooms have a variety of secondary metabolites with antioxidant activities such as phenolic compounds. Total polyphenols (TP) are the major naturally occurring antioxidant components present in many edible mushrooms contributing directly to antioxidative action. Mushrooms seem to be a potential natural source of dietary flavonoids having a great range of compounds in significant concentrations showing healthy properties (Jinting et al., 2017).

Mushrooms are good source of several important enzymes involved in the detoxification process and destruction of superoxide free radicals as well as secondary metabolites that act as thrombin inhibitors (Karmali, 2007). Mycelia of mushrooms have been reported to contain number of extra cellular enzymes that have been shown to participate in several clinical conditions such as tumor and cancer invasion and cardiovascular disorders. The mushroom enzymes viz., laccase, peroxidases and protease are thought to prevent oxidative stress as well as to inhibit cell growth in several diseases. This enzyme system has been found in several mushrooms (Karmali, 2007). Laccases are enzymes produced by plants, bacteria, insects and fungal species such as white-rot fungi that oxidize phenolic compounds and can be used for various biotechnological and environmental applications (Tellez et al., 2008).

A high proportion of polyunsaturated fatty acids have been reported to be present in the edible mushrooms (PeDnueault et al., 2006). Polyunsaturated fatty acids present in the edible mushrooms have been a source that may contribute to the reduction of serum cholesterol (Barros et al., 2007, Guillam et al., 2010). Ergosterol is the major sterol present in the edible mushrooms which shows antioxidant activity (Guillam et al, 2010). In this above context, the present study was attempted to quantify the bioactive substances viz., carbohydrates, proteins, lipids, crude fiber, total phenolics, total flavanoids, condensed tannins and lignolytic enzymes.

Materials and Methods

The pure cultures of mushroom fungal strains used in the present study, the source from where they were obtained are listed in Table 1. Pure cultures of fungi were stored at 15°C in a refrigerator. Sub-culturing was performed every month to ensure that organisms remained viable. The mycelial agar plugs (7mm) from stock cultures were aseptically transferred every month to fresh Potato Dextrose Agar medium and then incubated at room temperature ($28 \pm 2^\circ\text{C}$) until confluent growth was achieved.

Table 1

S.No	Mushroom fungi	Common Name	Source	Strain
1	<i>Pleurotus pulmonarius</i> (Fr) Quelet	Oyster Mushroom	TNAU	M1
2	<i>Pleurotus djamor</i> (Fr.)	Oyster Mushroom	TNAU	M2
3	<i>Pleurotus citrinopileatus</i> (Fr) Singer	Oyster Mushroom	TNAU	M3
4	<i>Pleurotus eryngii</i> Singer	Oyster Mushroom	TNAU	M5
5	<i>Pleurotus florida</i> (Mont.) Singer	Oyster Mushroom	TNAU	M6
6	<i>Pleurotus flabellatus</i> (Berk and Br) Sacc	Oyster Mushroom	TNAU	M7
7	<i>Pleurotus cystidiosus</i>	Oyster Mushroom	UM	Wild
8	<i>Oudemansiella radicata</i>	Rooted Collybia	UM	Wild
9	<i>Hypsizygus ulmarius</i> (Bull.:Fr) Redhead	Oyster Mushroom	UM	Wild
10	<i>Schizophyllum commune</i> Fries	Split Gill	UM	Wild

TNAU - Tamil Nadu Agricultural University, Coimbatore, India

UM - University of Madras, Guindy campus, Chennai, India

Growth and extraction

Biomass Production in Broth Culture

Erlenmeyer flasks containing 50 ml of potato dextrose broth were plugged with cotton wool and sterilized at 121.1 °C for 15 minutes. Once the medium was cooled to room temperature, mycelial agar plugs (8mm dm) taken from the growing edge of a 7 days-old culture of the different mushroom fungi were transferred individually to the culture media. The flasks were incubated as static cultures for a period of 28 days at room temperature (28± 2 ° C) for harvesting the mycelial biomass at an interval of 7 days.

Mycelial extracts

The mycelial biomass was separated from the culture medium by vacuum filtration through Whatman No:-1 filter paper. The mycelial biomass was then rinsed with sterile water, blot dried, weighed and then used for further studies. The extraction was carried as described by Yim *et al.*, 2009 and Turkmen *et al.*, 2006) with slight modifications. One gram of mycelia powder of each mushroom species was extracted with 10 ml of distilled water. The mixture was kept in a shaker at an ambient temperature of 28±2 ° C for 3 hours and in cold (15 ° C) for 10 minutes. The filtrate was filtered using Whatman No.1 filter paper. The culture free filtrates and the aqueous mycelial extracts were used for studying anti-microbial activity and the biological activities of the different mushroom fungi.

Total carbohydrates (Dubois *et al.*, 1956)

The total carbohydrate content was estimated by phenol-sulphuric acid method proposed by Dubois *et al.* (1956¹⁸). Mycelial powder (100mg) was dissolved in 5ml of 0.5N HCl and kept in a boiling water bath for 3 hours. The solution was then neutralized with sodium hydroxide and the volume was made up to 100ml with distilled water. Aliquots of the solution (0.1ml) were used for the estimation of total carbohydrates. D Glucose (Stock: 1mg/ml) was used as the standard. Various concentrations of standard and test solutions were taken in different test tubes and the volume was made up to 1ml with distilled water. 1ml of 5% phenol was added to all the tubes followed by 5ml of 96% sulphuric acid. The mixtures were shaken well and incubated for 10 minutes. The tubes were incubated at room temperature 28 ± 2°C for 20 minutes and the absorbance was read at 490nm. The total carbohydrate in the samples was expressed in percentage.

Total protein (Bradford, 1976)

Extraction of protein

One gram of dried mushroom powder was ground with 5 ml phosphate buffer (pH 7), to this, a pinch of acid washed sand was added to facilitate the grinding. The extract was centrifuged at 10,000 g for 15 minutes and the clear supernatant was collected in a screw cap tube and the volume was made up to 10 ml with same buffer. One ml of the protein extract was precipitated

by adding 2 ml of 10% ice cold TCA for 1 hour and centrifuged at 10,000 g for 10 minutes. The pellet was collected and redissolved in 2 ml of 0.1 N NaOH.

Estimation of protein

Protein content was estimated by Bradford dye binding method (Palanivelu, 2004).

Crude fiber (Maynard, 1970)

Two gram of ground material was defatted with petroleum ether. Then 2 g of dried material was boiled with 200 ml of concentrated H₂SO₄ for 3 minutes with bumping chips. The suspension was filtered through linen cloth and washed with water until it became acid free. Then, the residue was subjected to alkali digestion by boiling with 200 ml of sodium hydroxide solution for 30 minutes, filtered again with linen cloth and washed with 25 ml of boiling 1.25% H₂SO₄, three times with 50 ml of water and 25 ml of alcohol. The residue was removed and transferred to ashing dish (pre-weighed dish W1). The residue was dried for 2 h at 130±2°C. The dish was cooled in a dessicator and weighed (W2). It was ignited for 30 min at 600±15°C and cooled in a dessicator and reweighed (W3). The crude fiber content was determined using the following formula.

% of crude fibre =

$$\frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

Lipid

2g of powdered sample was extracted with 30 ml of petroleum ether by using Soxhlet extractor for 4 h. The extract was evaporated to dryness in a weighed flask using a vacuum evaporator. The weighed flask was dried in the oven at 80°C for 2 h, allowed to cool and reweighed. The difference between the initial and final weights was regarded as the lipid content of the sample (Parent, 1977).

Quantitative analysis of phytochemicals

Total phenolics (TP)

The total phenolics in the extract was determined by the Folin-Ciocalteu method as described by Zhao and Hall, 2008 and Barros *et al.*, 2007 with slight modifications. 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu reagent. 1 ml of 7.5% of sodium

carbonate was added to the mixture after 3 minutes and the final volume was adjusted to 10 ml with distilled water. The mixture was kept in the dark for 90 minutes at room temperature. Absorbance was measured against the blank at 725nm using spectrophotometer (Systronics, India). Gallic acid was used for the calibration curve with a concentration of 50-1000µg/ml and analyzed above. Results were expressed as mg gallic acid equivalent/100g. All the experiments were carried out in triplicates.

Total flavonoids (TF)

Total flavanoids was determined by the colorimetric analysis as described by Xu and Chang (2007) and Yoo *et al.*, (2008) with slight modifications. 0.25 ml of the added.150µl of 10% AlCl₃.6H₂O was added to the mixture after 6 minutes. The mixture was then incubated at room temperature for 5 minutes. After incubation, 0.5 ml of 1M NaOH and 2.5 ml of distilled water were added to the mixture. The mixture was then vortexed briefly and the developed pink color was read at 510 nm using spectrophotometer (Systronics, India) against the blank. Catechin was used for the calibration curve with a concentration of 10-1000µg/ml and analyzed as above. Results were expressed as mg (+) - catechin equivalent /100g. All the experiments were carried out in triplicates.

Condensed tannins (CT)

Condensed tannins of the extract was analyzed by colorimetric procedure as described by Xu and Chang 2007 and Chavan *et al.*, 2001 with slight modifications. 3 ml of 4% methanolic vanillin solution was added to 50µl of sample. 1.5 ml of concentrated HCl was added to the mixture and the mixture was vortexed, allowed to stand at room temperature for 15 minutes. 5ml of 4% concentrated HCl in methanol was used as the blank. Absorbance of the sample was measured against the calibration curve and analyzed as above. Results were expressed as mg (+) – catechin equivalent/100g. All the experiments were carried out in triplicates.

Laccase activity

Laccase activity was assayed based on the oxidation of Guaiacol. The assay mixture containing 900 µl of sodium acetate buffer (50mM; pH 4.5) with 2mM Guaiacol and 100 µl of enzyme source was incubated at room temperature for 5 minutes and the absorbance of the pink colored product was measured at 440nm

using a UV – Visible spectrophotometer (Systronics, India). Laccase activity was expressed in International Units (IU/ml). One international unit is defined as the micromoles of the product formed in one minute by one ml of enzyme. Blanks were maintained with sterile water (Ramakrishna *et al.*, 2004²⁹).

$$\text{Laccase Activity} = \frac{A(nm)OD}{E_{nm} \times V \times t \times V} \text{ IU/ml}$$

$$E_{nm} = 6740 \text{ M}^{-1}\text{Cm}^{-1}$$

Lignin Peroxidases

The activity of Lignin Peroxidase (LiP) was assayed based on the oxidation of veratryl alcohol. The assay mixture contained 900µl of sodium tartarate buffer (25 mM; pH 2.5), containing 0.4 mM hydrogen peroxide, and 25mM veratryl alcohol and 100µl of enzyme source. The assay mixture was incubated at room temperature for 1 minute and the absorbance was measured at 310 nm using UV- visible spectrophotometer (Systronics, India).

Enzyme activity was expressed in International Units (IU/ml). Blanks were maintained with sterile water (Vijaya and Singaracharya, 2005). One unit LiP was defined as the amount of enzyme required to oxidize 1 µmol of veratryl alcohol in 1 min, at 30°c and pH 3.5. The reaction mixture was measured against reagent blank at 310 nm ($\epsilon_{310} = 9300$) (Oliveira *et al.*, 2016).

Manganese Peroxidases

The activity of Manganese Peroxidases (MnP) was assayed based on the oxidation of hydrogen peroxide. MnP activity was monitored using phenol red at 30°c. The assay mixture contained 25 mM lactate, 0.1mM

MnSO₄, 1mg of bovine serum albumin (Sigma) ml⁻¹, 0.1 mg of phenol red (Merck) ml⁻¹ and 0.5 ml of culture filtrate was dissolved in 20mM sodium succinate buffer (pH 4.5) to a total volume of 1ml. The reaction was started by the addition of H₂O₂ to a final concentration of 0.1mM and was stopped after 1 min with 50µl of 10% NaOH, and A₆₁₀ was measured. Control assays of phenol red oxidation in the absence of Mn²⁺ were carried out by omitting MnSO₄ from the reaction mixture. MnP activity was calculated by subtracting the value for phenol red oxidizing activity in the absence of Mn²⁺ from the value for the activity obtained in the presence of manganese. Activity was in the absence of Mn²⁺ expressed as the increase in A₆₁₀ per minute per mm. (Vares *et al.*, 1995).

Enzyme activity was expressed in International Units (IU/ml). One unit of enzyme activity was equivalent to an absorbance change of 0.01 min⁻¹. Blanks were maintained with sterile water (Mariocarlo *et al.*, 2002).

Results

Mycelial protein

Total mycelia protein was analyzed using Folin-Phenol method and the results were expressed in percent dry weight. *Hypsizyugus ulmarius* contained the highest mycelial protein (27.8%), followed by *Pleurotus djamor* (26.0%), *Pleurotus florida*, *Pleurotus flabellatus*, *P. cystidisous*, *Pleurotus pulmonarius* and *Pleurotus citrinopileatus*. Mycelia of *P. eryngii* *Oudemansiella radicata*, *Pleurotus pulmonarius* and *Pleurotus citrinopileatus* had comparatively lower protein content in the range of 13.2 – 20.8 % dry weight (Table 2).

Table 2: Content of total protein of the mycelial extract of selected mushroom fungi

1	<i>Pleurotus pulmonarius</i>	14.3 ± 0.28.
2	<i>Pleurotus djamor</i>	26.0 ± 0.27.
3	<i>P. cystidisous</i>	17.8 ± 0.39,
4	<i>Pleurotus citrinopileatus</i>	20.8 ± 0.30.
5	<i>Pleurotus eryngii</i>	14.6 ± 0.18.
6	<i>Pleurotus flabellatus</i>	21.4 ± 0.11.
7	<i>Pleurotus florida</i>	23.2 ± 0.24.
8	<i>Oudemansiella radicata</i>	13.2 ± 0.25.
9	<i>Hypsizyugus ulmarius</i>	27.8 ± 0.39
10	<i>Schizophyllum commune</i>	16.7±0.24

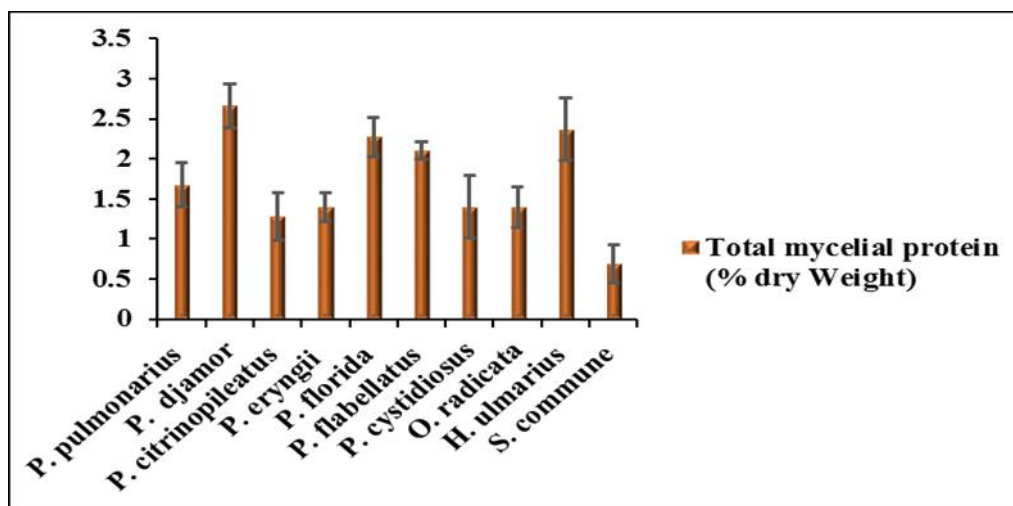


Fig 1 Content of total protein in the mycelia of selected mushroom fungi

The total carbohydrate content of the mycelia of the selected mushroom species ranged between 24.3% dry weight and 45.78% dry weight (Fig. 2). *Oudemansiella radicata* (45.78% dry weight) and *Hypsizygos ulmarius* (45.6% dry weight) had the highest mycelial carbohydrate content followed by

Pleurotus florida (44.8% dry weight), the latter having higher carbohydrate content than all the other *Pleurotus* species. *Pleurotus pulmonarius* (28.7% dry weight) and *Pleurotus flabellatus* (24.3% dry weight) had less mycelial carbohydrate content than the other mushroom species.

Table 2 Total carbohydrate content of the mycelia of selected mushroom fungi

S.No.	Organisms	Carbohydrates (% dryweight)
1.	<i>Pleurotus pulmonarius</i>	28.7 ± 2.6
2.	<i>Pleurotus djamor</i>	40.2 ± 2.0
3.	<i>Pleurotus citrinopileatus</i>	43.4 ± 1.8
4.	<i>Pleurotus eryngii</i>	42.3 ± 1.5
5.	<i>Pleurotus florida</i>	44.8 ± 2.5
6.	<i>Pleurotus flabellatus</i>	24.3 ± 2.2
7.	<i>Pleurotus cystidiosus</i>	32.6 ± 1.6
8.	<i>Oudemansiella radicata</i>	45.78 ± 1.5
9.	<i>Hypsizygos ulmarius</i>	45.6 ± 2.3
10.	<i>Schizophyllum commune</i>	36.4 ± 2.0

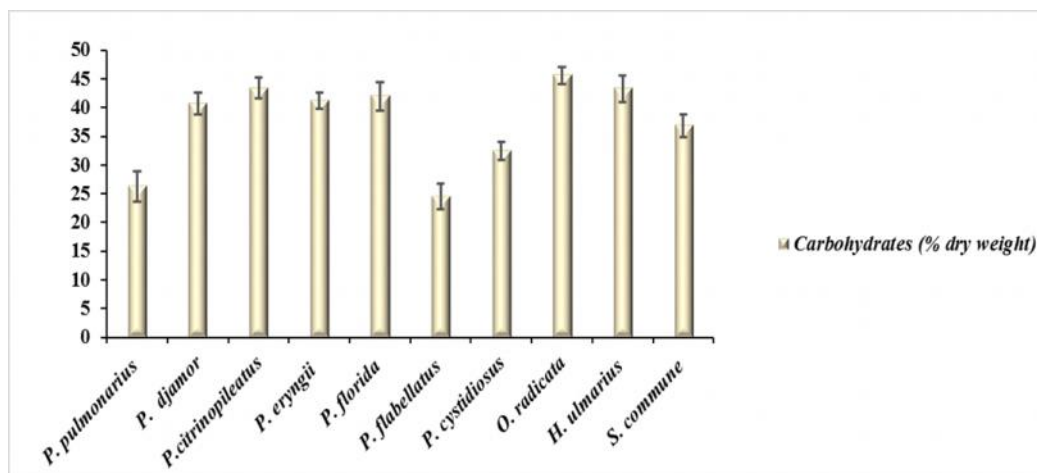


Fig. 2 Content of total carbohydrate of the mycelia of mushroom fungi

Content of crude fiber (% dry weight) in the mycelia of selected mushroom fungi

The crude fiber content of the mycelia of the selected mushroom species ranged between 13.6% dry weight and 8.1% dry weight (Fig. 3). *Hypsizygos ulmarius* (13.6% dry weight) and *Pleurotus citrinopileatus*

(13.2% dry weight) had the highest crude fiber content followed by *Pleurotus pulmonarius* (11.4 % dry weight). *Schizophyllum commune* (8.2 % dry weight) and *Pleurotus djamor* (8.1 % dry weight) had the less mycelial crude fiber content than the other mushroom species.

Table 3 Crude fiber content (% dry weight) of the mycelia of selected mushroom fungi

S.No	Organisms	Crude Fibre %
1.	<i>Pleurotus pulmonarius</i>	11.4 ± 1.95
2.	<i>Pleurotus djamor</i>	8.1 ± 1.21
3.	<i>Pleurotus citrinopileatus</i>	13.2 ± 0.75
4.	<i>Pleurotus eryngii</i>	10.3 ± 1.96
5.	<i>Pleurotus florida</i>	10.4 ± 2.06
6.	<i>Pleurotus flabellatus</i>	9.4 ± 0.80
7.	<i>Pleurotus cystidiosus</i>	10.5 ± 1.70
8.	<i>Oudemansiella radicata</i>	10 ± 1.87
9.	<i>Hypsizygos ulmarius</i>	13.6 ± 1.32
10.	<i>Schizophyllum commune</i>	8.2 ± 1.31

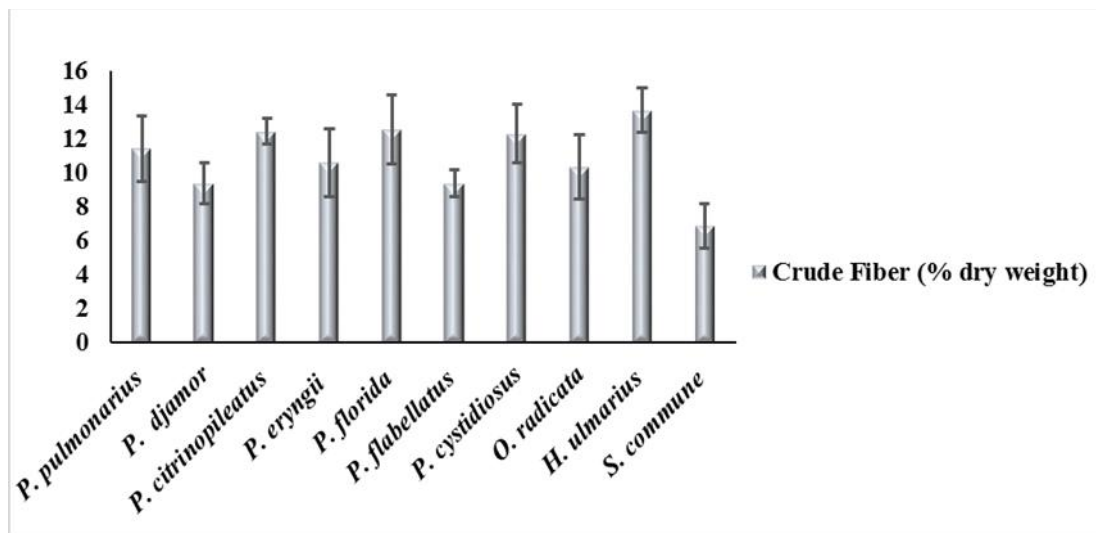


Fig 3 Crude fibre content (% dry weight) of the mycelia of selected mushroom fungi

Lipid content (% dry weight) in the mycelia of selected mushroom fungi

Figure 4 shows the lipid content of the selected mushroom species. *Pleurotus djamor* and *Pleurotus flabellatus* contained the maximum lipid content of 2.79% and 2.42% dry weight. Lipid content in the

other *Pleurotus* species ranged between 2.24% and 1.16% dry weight. *Schizophyllum commune* (2.18% dry weight), *Oudemansiella radicata* (1.89 % dry weight) and *Hypsizygos ulmarius* (1.24 % dry weight) had lipid content lower than the *Pleurotus djamor*, *Pleurotus flabellatus* and *Pleurotus cystidiosus* (Table 4).

Table 4 Lipid content in the mycelia of selected mushroom fungi

S.No.	Organisms	Lipids %
1	<i>Pleurotus pulmonarius</i>	1.24 ± 0.33
2	<i>Pleurotus djamor</i>	2.79 ± 0.39
3	<i>Pleurotus citrinopileatus</i>	1.16 ± 0.31
4	<i>Pleurotus eryngii</i>	2.04 ± 0.08
5	<i>Pleurotus florida</i>	1.36 ± 0.19
6	<i>Pleurotus flabellatus</i>	2.42 ± 0.32
7	<i>Pleurotus cystidiosus</i>	2.24 ± 0.67
8	<i>Oudemansiella radicata</i>	1.89 ± 0.42
9	<i>Hypsizygus ulmarius</i>	1.24 ± 0.21
10	<i>Schizophyllum commune</i>	2.18 ± 0.29

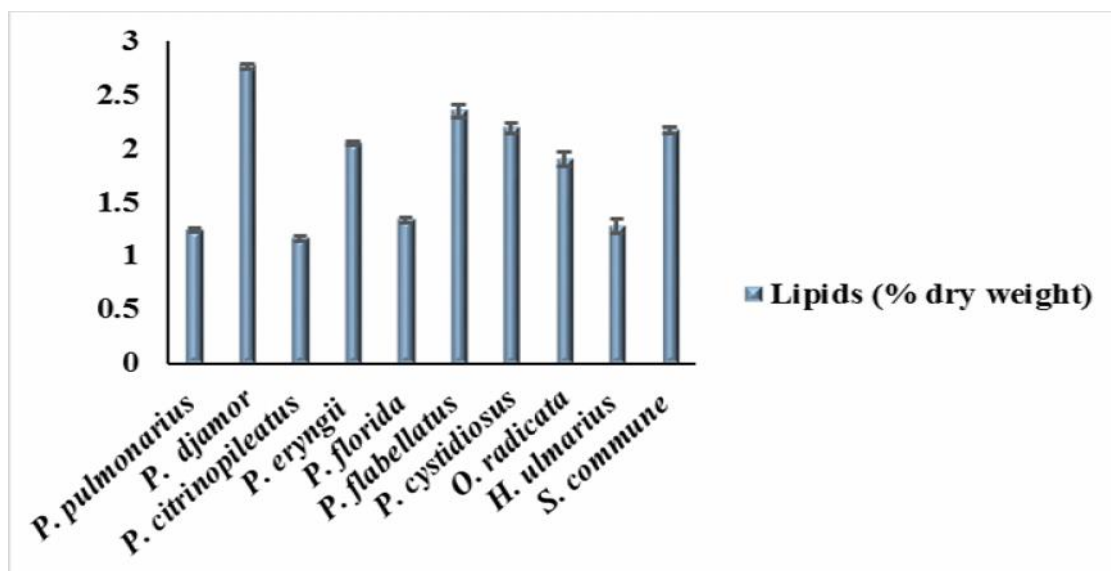


Fig 4 Content of lipid (% dry weight) of the mycelia of selected mushroom fungi

Quantitative enzyme activity of the mycelia of the selected mushroom fungi

Figure 5 shows quantitative enzyme activities of three important lignolytic enzymes Laccase, Manganese peroxidase (MnP), and Lignin peroxidase (LiP). Laccase activities of the *Pleurotus* species ranged between 1.2×10^{-4} IU/ml and 5.7×10^{-4} IU/ml. *Hypsizygus ulmarius* showed the laccase activity within the range (2.7×10^{-4} IU/ml), while *Oudemansiella radicata* showed the highest 6.5×10^{-4}

IU/ml laccase activity. *Schizophyllum commune* mycelial activity was the lowest (0.6×10^{-4} IU/ml).

Manganese peroxidase activity of the selected mushroom fungi ranged between 1.78×10^{-4} IU/ml and 6.1×10^{-4} IU/ml. Manganese peroxidase activities of the seven *Pleurotus* species ranged between 1.78×10^{-4} IU/ml (*Pleurotus flabellatus*) and 4.53×10^{-4} IU/ml (*Pleurotus djamor*). *Hypsizygus ulmarius* mycelial MnP activity was the highest of all the selected mushroom species (6.11×10^{-4} IU/ml).

Lignin peroxidase activities were lower than that of laccase and manganese peroxidase activities except for *Pleurotus florida* (4.64 x IU/ml) and *Schizophyllum commune* (1.64 x IU/ml).

The total phenolics content of the scelected mushroom fungi ranged between 32.0 and 48.2 mg/g dry weight (Fig. 6). *Hypsizygos ulmarius* and *Pleurotus*

citrinopileatus had the highest total phenolics content 48.2 and 45.1 mg/g dry weight respectively and the latter having higher total phenolics content than the other *Pleurotus* species. *Pleurotus pulmonarius* (32.6 mg/g dry weight) and *Pleurotus eryngii* (32.0 mg/g dry weight) had the lesser total phenolics content than the other mushroom fungi.

Table 5 Quantitative enzyme activity of the mycelia extract of the selected mushroom fungi

S.No.	Organisms	Laccase Activity (10x ⁻⁴ IU/ml)	Manganese peroxidase (10x ⁻⁴ IU/ml)	Lignin Peroxidase (10x ⁻⁴ IU/ml)
1	<i>Pleurotus pulmonarius</i>	2.2 ± 0.36	2.43 ± 0.86	0.76 ± 0.25
2	<i>Pleurotus djamor</i>	4.1 ± 0.71	4.53 ± 0.43	2.16 ± 0.23
3	<i>Pleurotus citrinopileatus</i>	3.2 ± 0.31	2.36 ± 0.94	1.22 ± 0.19
4	<i>Pleurotus eryngii</i>	1.6 ± 0.37	2.32 ± 0.40	1.36 ± 0.34
5	<i>Pleurotus florida</i>	5.7 ± 1.05	2.16 ± 0.49	4.64 ± 0.38
6	<i>Pleurotus flabellatus</i>	1.6 ± 0.33	1.78 ± 0.27	0.84 ± 0.16
7	<i>Pleurotus cystidiosus</i>	1.2 ± 0.31	2.48 ± 0.88	0.67 ± 0.17
8	<i>Oudemansiella radicata</i>	6.5 ± 0.26	3.16 ± 0.41	1.29 ± 2.0
9	<i>Hypsizygos ulmarius</i>	2.7 ± 0.57	6.11 ± 0.86	4.31 ± 0.43
10	<i>Schizophyllum commune</i>	0.6 ± 0.26	2.76 ± 0.67	1.64 ± 0.20

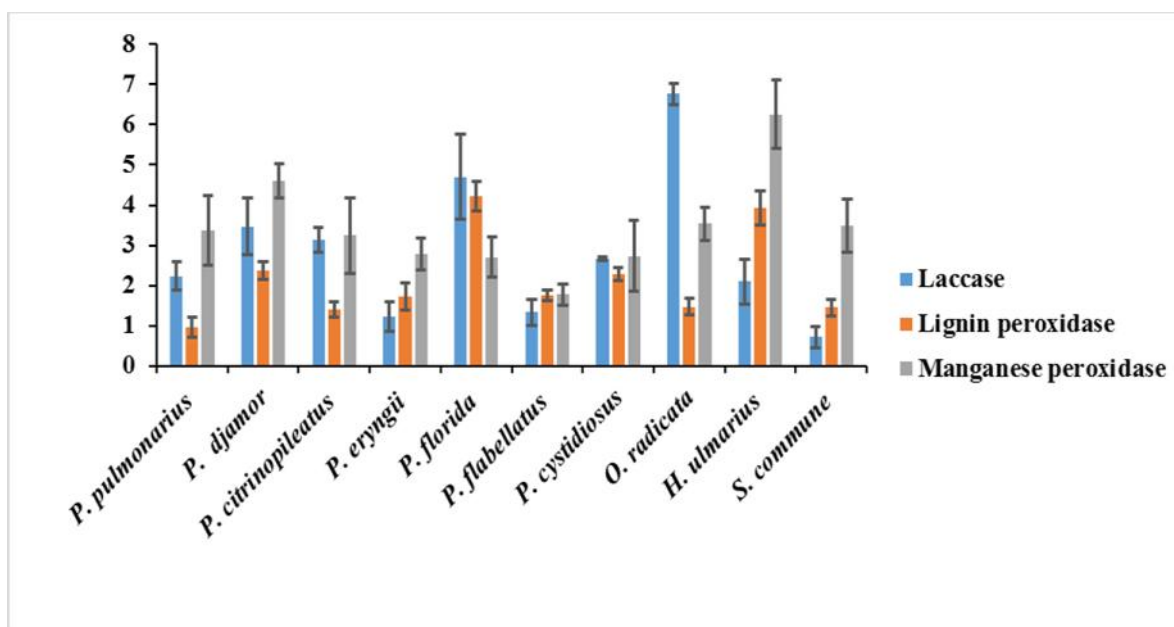


Fig. 5 Quantitative enzyme activity of the mycelia of the selected mushroom fungi

Condensed Tannins

The condensed tannins content in the mycelia of the selected mushroom fungi ranged between 1.03 and 1.30 mg per gm of dry weight of mycelia. *Pleurotus djamor* and *Hypsizyguis ulmarius* had the highest condensed tannins content 1.30 and 1.18 mg/g dry

weight respectively, followed by *Pleurotus citrinopileatus*, *P.florida*, *P.flabellatus*, *Oudemansiella radicata*, *Schizophyllum commune* (Table 7). *Pleurotus pulmonarius* (1.08 mg/g dry weight) and *P.cystidiosus* (1.03 mg/g dry weight) had the lesser condensed tannins content than the other mushroom fungi.

Table 6 Total phenolics content of mycelia of selected mushroom fungi

S.No.	Organisms	Total phenolic (mg/g dry weight)
1.	<i>Pleurotus pulmonarius</i>	32.6 ± 2.15
2.	<i>Pleurotus djamor</i>	42.6 ± 2.65
3.	<i>Pleurotus citrinopileatus</i>	45.1 ± 1.90
4.	<i>Pleurotus eryngii</i>	32 ± 2.20
5.	<i>Pleurotus florida</i>	38.4 ± 1.90
6.	<i>Pleurotus flabellatus</i>	40.8 ± 1.90
7.	<i>Pleurotus cystidiosus</i>	41.3 ± 2.05
8.	<i>Oudemansiella radicata</i>	43.4 ± 2.30
9.	<i>Hypsizyguis ulmarius</i>	48.2 ± 1.85
10.	<i>Schizophyllum commune</i>	43.6 ± 1.80

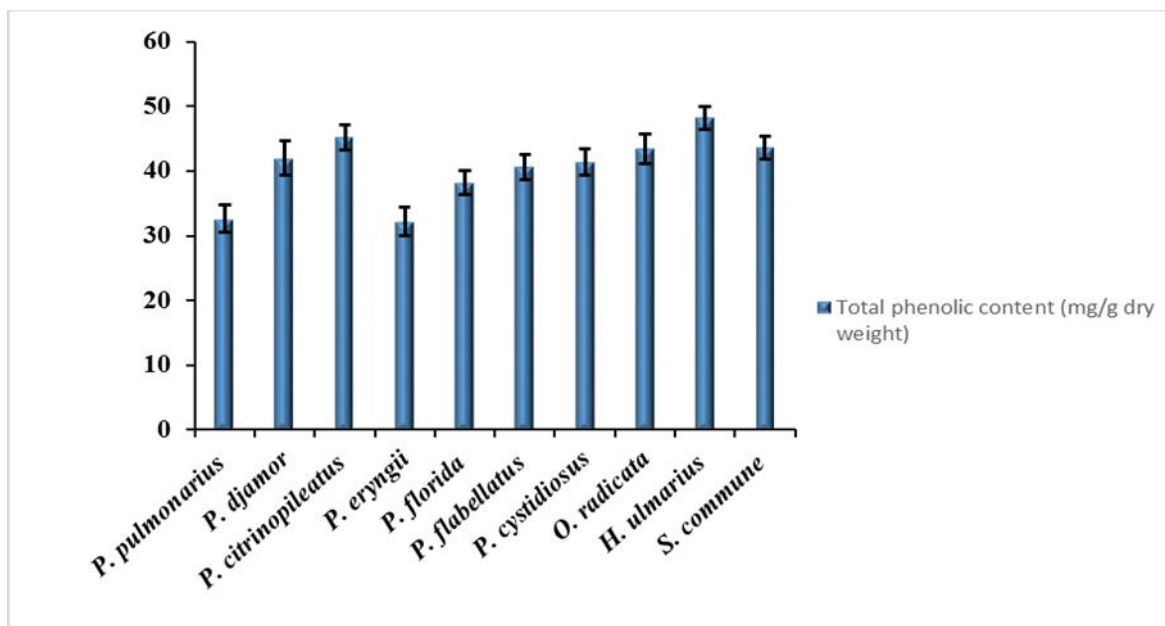


Fig. 6 Total phenolics content of the mycelia of the selected mushroom fungi

Table 7. Condensed Tannins of mycelia of selected mushroom fungi (mg/g dry weight)

S.No.	Organisms	Condensed Tannins mg/g dry weight
1	<i>Pleurotus pulmonarius</i>	1.08 ± 0.66
2	<i>Pleurotus djamor</i>	1.30 ± 0.75
3	<i>Pleurotus citrinopileatus</i>	1.15 ± 0.74
4	<i>Pleurotus eryngii</i>	1.10 ± 0.25
5	<i>Pleurotus florida</i>	1.14 ± 0.55
6	<i>Pleurotus flabellatus</i>	1.12 ± 0.38
7	<i>Pleurotus. Cystidiosus</i>	1.03 ± 0.83
8	<i>Oudemansiella radicata</i>	1.12 ± 0.75
9	<i>Hypsizygus ulmarius</i>	1.18 ± 0.13
10	<i>Schizophyllum commune</i>	1.12 ± 0.44

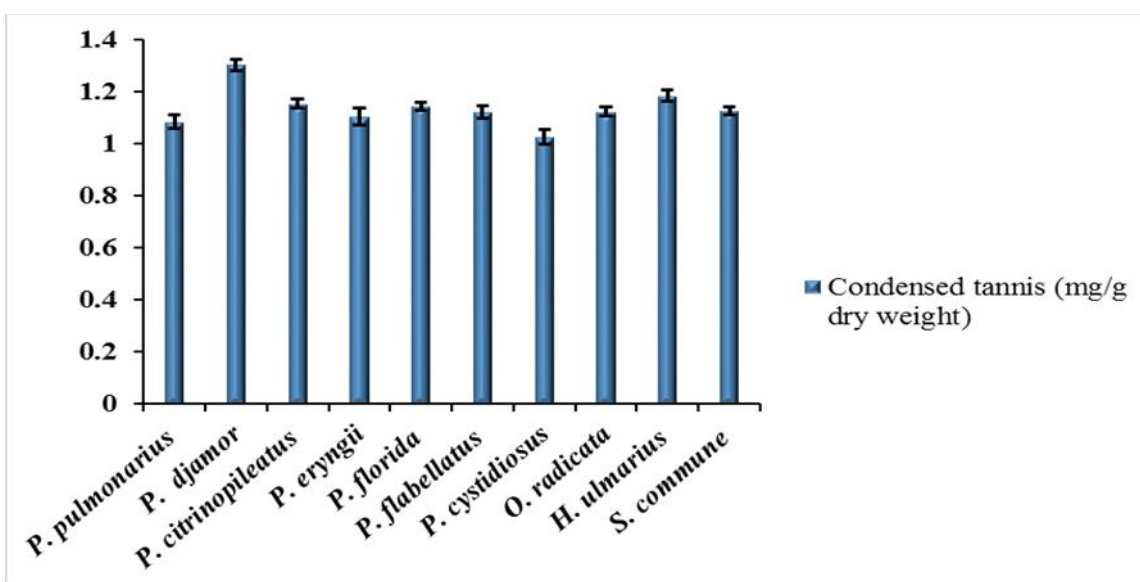


Fig. 7 Condensed tannins of the mycelia of mushroom fungi

The total flavanoids content in the mycelia of the selected mushroom fungi ranged between 0.91% and 1.5% dry weight (Fig.8). Flavanoids content was highest in the mycelia of *Hypsizygus ulmarius* (1.5 % dry weight) followed by *Schizophyllum commune* (1.4%). Except *Pleurotus pulmonarius*, (1.42% dry

weight) which had mycelial flavanoid content equivalent to that of *Schizophyllum commune*, all the other *Pleurotus* species and *Oudemansiella radicata* had comparatively lower mycelial flavanoid content than these three mushroom species (Table 8).

Table 8 Total flavanoids of mycelia of selected mushroom fungi

S.No	Mushroom	Total flavanoids (%dry weight)
1	<i>Pleurotus pulmonarius</i>	1.42 ±0.03
2	<i>Pleurotus djamor</i>	1.01±0.01
3	<i>Pleurotus citrinopileatus</i>	1.31±0.03
4	<i>Pleurotus eryngii</i>	1.01±0.02
5	<i>Pleurotus florida</i>	0.91±0.04
6	<i>Pleurotus flabellatus</i>	1.21±0.01
7	<i>Pleurotus cystidiosus</i>	1.12±0.03
8	<i>Oudemansiella radicata</i>	1.33±0.02
9	<i>Hypsizygus ulmarius</i>	1.5 ±0.01
10	<i>Schizophyllum commune</i>	1.4±0.02

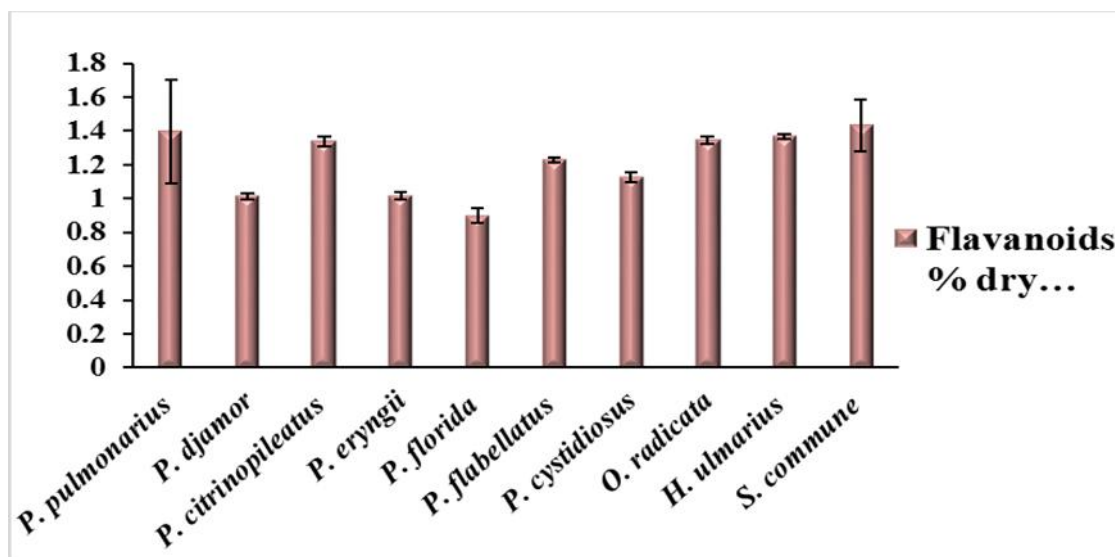


Fig. 8 Total flavanoids of mycelia of selected mushroom fungi

Discussion

Total mycelia protein content in the selected mushroom ranged between 27.8% and 13% (dry weight). *Hypsizyguis ulmarius* showed significantly higher mycelial protein content (27.8%/ dry weight) followed by *P. djamor* (26%/ dry weight). Lower mycelia protein content was observed in *Oudemansiella radicata* and *Pleurotus pulmonarius*, 13.2% and 14.3% respectively among the selected mushroom fungi.

Mushrooms are considered to be a source of nutraceuticals, strengthening the human immune system. Nutraceuticals are obtained mainly from the edible mushrooms (Rahi and Malik, 2016). Content of proteins in the edible mushrooms are in the range of 19- 35%. The results of present study are on par with results of Rashad *et al.*, 2009, citing the total mycelia protein in the range 10.5- 30.4% of dry weight. The total carbohydrate content by dry weight of the mycelia of the selected mushroom fungi ranged between 45.6% and 24.3%. The highest mycelia total carbohydrate content (45.78 %) was observed in *Oudemansiella radicata*. The apparently low total carbohydrate content of *P. flabellatus* (24.3 %) may be correlated with its high protein content as reported by Mau and Nchu, (2011³⁷). Total crude fiber was quantified from the selected mushroom fungi and the results were expressed in percentage mycelia dry weight. Mycelial crude fiber in the mushroom fungi were in the range of 8.1% - 13.6% dry weight. *Hypsizyguis ulmarius* and *Pleurotus citrinopileatus* were observed to contain the highest content of crude

fiber among the screened mushrooms (13.6 % and 13.2 % dry weight) respectively. Low crude fiber content was observed in *Pleurotus djamor* (8.1% dry weight).

Cell wall components of mushrooms are composed of non-digestible carbohydrates, resistant to human enzymes and considered as a source of dietary fiber (Cheung, 2013³⁸). Mycelial lipid content in the selected mushroom fungi was within the range of 1.24% -2.79% dry weight as reported by Ogwok *et al.* (2017³⁹) (0.24% - 5.23% dry weight). The highest mycelia lipid content was observed in *Pleurotus djamor* (2.79% dry weight) and the lowest content was observed in *Pleurotus citrinopileatus* (1.16% dry weight).

Lipid content in the mushrooms is generally low and the proportion of lipid per 100 g is 1.75% - 15.5 % (Hong *et al.*, 1988). Some edible mushrooms are reported to contain 27.4% polyunsaturated fatty acids (Barros *et al.*, 2007). Mushrooms are reported to be rich in polyunsaturated fatty acids (Ergonul *et al.*, 2013). Low lipid content in the mycelia suggests its potential to be used as an efficient food additive. Present study investigated the quantities of some biotechnologically important enzymes *viz.*, laccases, lignin peroxidases and manganese peroxidases. Laccase is a lignin degrading extracellular oxidoreductase enzyme. Mycelial laccase activity was found to be higher in *Oudemansiella radicata* (6.5 IU/ ml) among the selected mushroom fungi. The activity of laccase in the mycelia extract was in the range of 0.6 - 6.5 IU/ml. Lowest mycelia laccase activity was observed in *Schizophyllum commune* (0.6 IU/ ml).

Laccases was studied in *P.osteratus* by Palmeiri *et al.* (2000) and has applications in the production of biofuels, biocatalysts, used in food additives and in bioremediation (Mate and Alcalde, 2017). Laccase was characterized by Wang and Ng, (2006) as a 34 kDa protein in *Pleurotus eryngii*.

Mycelial Lignin peroxidase (LiP) activity in the selected mushroom fungi was in the range of 0.67 - 4.64 IU/ ml. The LiP activity was found to be higher in *P.florida* (4.64 IU/ ml) and the least LiP activity was observed in *Pleurotus cystidisous* (0.67 IU/ ml). Similar reports were observed by Xu *et al.* (2013) in *Pleurotus sp* and in *Griofola frondosa*. LiP found its applications in biopulping, bioleaching, and bioremediation and food industry. Application of Lip in the food industry was studied by Joung *et al.* (2014).

Manganese peroxidase (Mn P) activity was observed to be higher in *H. ulmarius* (6.11 IU/ ml). Quantity of mycelial MnP activity in the mushroom fungi ranged between 1.78 - 6.11 IU/ml. The least quantity of mycelia MnP activity was observed in *Pleurotus flabellatus* (1.78 IU/ml). The results were on par with observations made by Xu *et al.* (2013) in *Pleurotus sp* and *Grifola sp*

Another important observation made during the study was that all the screened mushroom mycelia contained significant quantities of all the per oxidase enzymes. The results of the study were supported by the studies of laccases, MnP and LiP in *P.osteratus* by Iwalokun *et al.* (2007⁵⁰), in *Trametes versicolor* (Ferriereira *et al.*, 2010) and in *S. commune* by Ajith and Janarthan, (2003) and Papadaki *et al.* (2019). There have been several reports emphasizing the key role of phenols as scavengers of free radicals (Moller *et al.*, 1999). Phenolic compounds in the mushrooms have been reported to involve in the stabilization of lipid oxidation, antioxidant activities and inhibitory activities on mutagenesis, carcinogenesis when taken as 1.0 g/ day as a part of regular diet (Adebayo *et al.*, 2012) .

Hypsizygyus ulmarius showed the highest quantity of total phenolics (48.2 mg/g) among the screened mushroom fungi followed by *Pleurotus citrinopileatus* (45.1mg/g). Total phenolics content in the selected mushroom fungi was in the range of 32 - 48.2 mg/g. The least total phenolics content was observed in *Pleurotus eryngii* (32 mg/g). The results corroborated with the study of Alispahic *et al.* (2015) on oyster

mushrooms (43.88 mg/g). Another report by Abugri and McElhenney (2013) on *Agaricus sp*, *Ganoderma sp*, *Schizophyllum commune* and *Pleurotus sp* also showed similar the observations.

Extensive reviews (Adebayo *et al.*, 2012) on the correlation of total phenolics content with the bioactivities of the mushrooms as antimicrobial agents, antioxidant agents reveal the importance of the investigation of total phenolic compound as a bioactive compound. Condensed tannin content in the mycelia of the selected mushroom fungi ranged between 1.03 and 1.30 mg /g dry weight. The highest condensed tannin content was found in *Pleurotus djamor* (1.30 mg /g dry weight) and the least content of condensed tannin was observed in *Pleurotus. Cystidisous* (1.03 mg /g dry weight). Phenolic compounds in the mushrooms like flavonoids, tannins and phenolic acids are reported to be natural antioxidants having the ability to quench free oxygen radicals (Barros *et al.*, 2006). Water extract of mycelia of mushrooms exhibited higher TP, TF and CT (Yim *et al.*, 2009). Significant quantities of CT in the analyzed mushroom fungi reveal their role as a promising bioactive compound. Total flavanoid (TF) content in the mycelia of selected mushroom fungi was found to be higher in *Hypsizygyus ulmarius* (15.216 mg/g dry weight). TF content in the mycelia of the selected mushroom fungi was in the range of 9.13 - 15.216 mg/g dry weight. The least total flavonoid content was observed in *P. florida* (9.13 mg/g dry weight). Total flavanoid content of *P. osteratus* and *P. eryngii* were 9.64 and 7.91 mg/g as reported by Gasecka *et al.* (2015). Total flavonoids (TF) of mushrooms are reported to exhibit antitumoral, antiallergic anti-inflammatory activities (Adebayo *et al.*, 2012). Study carried out by Abugri and McElhenney (2013) on *Agaricus sp*, *Ganoderma sp*, *Pleurotus sp* supported the results of present investigation.

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