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Research article

Comparative evaluation of nutritional, biochemical and enzymatic properties of the mycelium of two *Pleurotus* species

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Abstract: Aim of the present studies focuses on nutritional, antioxidant and extracellular enzymatic activity of mycelium of *Pleurotus sajor-caju* and *Pleurotus florida*. Results shows that both of the mushroom mycelium possess multiple nutritional, antioxidant components along with good extracellular enzymatic activities. Methanolic extract of *Pleurotus sajor-caju* showed higher phenolic and flavonoid content (1.01±0.57 mg.gm^{-1} and 0.14±0.01 mg.gm^{-1}) than *Pleurotus florida* (0.45±0.05 and 0.11±0.01 mg.gm^{-1}). A high alkaloid content was exhibited in *P. sajor-caju* than *P. florida*, apart from the antioxidant components. *P. sajor-caju* showed high protein and carbohydrate content *i.e.* 10.55±1.62 mg.gm^{-1} and 32.16±7.16 gm 100gm^{-1} respectively, as compared to *P. florida* which showed less amount of protein and carbohydrate content (8.50±015 mg.gm^{-1} and 8.30±1.09 gm 100gm^{-1}). Enzymatic screening showed good activity of amylase and lipase where as Xylanase and protease activity in both the mushroom mycelium was negative. Overall studies revealed that both the mushroom mycelium are potential source of antioxidants and extracellular enzymes, especially flavonoids, amylase and lipase.
Keywords: *Pleurotus* - Mycelium - Nutritional - Antioxidants - Enzymatic.

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INTRODUCTION

Wild edible mushroom have been integrated part of the diet, especially among rural, urban dwellers and tribal people. Some of the common edible mushrooms, which are predominantly consumed in India are *Pleurotus sajor-caju, P. florida, P. platypus, P. djamor, Volvariella volvacea* and *Calocybe indica* (Pan *et al*. 2008, Ramkumar *et al*. 2010). *Pleurotus* species is known as oyster mushrooms, which are widely spread saprophytic macrofungi and distributed throughout the temperate and tropical forests of the world (Gunde-Cimerman 1999). Oyster mushrooms are now in second rank among the cultivated mushrooms in the world (Chang 1991) and are known to have potent antitumor, antimicrobial activities (Zhang *et al*. 1994, Gerasimenya *et al*. 2002). *Pleurotus* sp are rich in minerals (Ca, P, Fe, K and Na) and vitamin C, B-complex (Çağlarırmak 2007). Apart from the different nutritional and antioxidant components mushroom mycelium possess different enzymes (Nonaka *et al*. 1997, Bose *et al*. 2007, Kadimaliev *et al*. 1998). *Pleurotus* is known for the different cellulolytic and amylolytic enzymes (Sławińska & Kalbarczyk 2011, Jonathan & Adeoyo 2011). Recently *Pleurotus ostreatus* and *P. sajor-caju* is characterized for the protease activity (Choi & Shin 1998, Ravikumar *et al*. 2012). In Odisha mainly oyster mushroom *Pleurotus sajor-caju* and *Pleurotus florida* are grown commercially. Both of them are liked by local people on account of unique characteristic of aroma and taste. In the present work, this was taken into the consideration because reports suggests that fruit body of both of the species possess good nutritional and antioxidant components along with industrially important enzymes and since mycelium is the miniature of fruit body, therefore same behaviour was expected from the respective mushrooms, hence it intended to evaluate the mushrooms nutraceuticals (Nutritional and Pharmaceutical) potential.

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MATERIAL AND METHODS

Nutritional analysis

Protein estimation was done by the method given by Bradford (1976). Estimation of carbohydrates was carried out by following phenol sulphuric acid method (Dubois *et al.* 1956, Hedge & Hofreiter 1962). Reducing sugars in the mycelium was done by following dinitrosalicylic acid method (Miller 1972). Non reducing sugar was calculated by following the formula of Nazarudeen (2010).

Antioxidant analysis

One gm of fresh mycelium sample was disintegrated with 10 ml of methanol. Samples were stirred for 15 minutes for effective extraction and centrifuged at 3000 rpm for 20 minutes. Supernatants were referred as methanolic extract and kept at 4 ºC until analysis (Puttaraju *et al*. 2006). The DPPH activity was estimated in the methanolic extracts by colorimetric method (Chan *et al*. 2007). Ascorbic acid equivalent Antioxidant Capacity (AEAC) was calculated by calibrating the value of above absorbance in standard ascorbic acid curve and expressed in mg per gram of dried sample. Ferric Reducing Antioxidant Power (FRAP) assay was done by following the method of Benzie & Strain (1996) and Athavale *et al*. (2012) and. The total phenolic content in the mycelium were determined through Folin-phenol method with slight modifications (Singleton & Rossi 1965). The flavonoid content of sample was estimated by using aluminium chloride colorimetric technique and flavonoid content was expressed in terms of mg quercetin equivalents per gram of extract (Chang *et al*. 2002). The concentration of β-carotene and lycopene in mushroom mycelium extracts was estimated spectrophotometrically (Nagata & Yamashita 1992, Barros *et al*. 2007). Alkaloid content in the mushroom mycelia was quantified spectrophotometrically (Srividya & Mehrotra 2003). Tannin content was estimated in the sample by Folin denis reagent tannic acid was served as standard and expressed in $mg.gm^{-1}$ (Schanderl 1970).

Extracellular enzymatic activity

i) *Amylase activity*: All the mushroom mycelium was screened for the extracellular amylase activity for which starch agar media was used. After the appreciable amount of the growth in plates they were flooded with 1% iodine solution. Clear zone around the mycelial growth was recorded for the starch hydrolysis activity.

ii) *Cellulase activity*: The medium containing 0.5% sodium salt of carboxymethylcellulose was used for the tests. After the mycelial colonization plates were flooded with congo red solution (0.2%) and washed with 1M NaCl solution followed by the incubation period of 15 minutes.

iii) *Lipase activity*: Spirit blue agar media was used for the screening of lipase activity. After the requisite amount of growth of mushroom mycelium a clear zone or precipitate was observed for the positive organism.

iv) *L-Asparaginase activity*: For screening of L- Asparaginase activity, medium containing 1% L- asparagine was used where L-asparagine served as an active ingredient, after the mycelial growth plate was flooded with Nessler's reagent. Plates showing pink coloration after the addition were recorded as extracellular L- asparaginase producer.

v) *Protease activity*: Gelatin agar media was used for the screening of protease producing organism, for which centre inoculation was done, after the incubation of 10 days plates were flooded with the reagent containing 15% $HgCl_2$ and 20% HCl.

vi) *Xylanase activity*: Medium containing xylan was used for the screening of Xylanase activity in mushroom mycelium. After the appreciable growth in the plate it was flooded with 0.1% Congo red, incubated for 30 minutes and washed with 1M NaCl subsequently. Plate was observed for the formation of clear zone for the production of Xylanase enzyme.

RESULTS AND DISCUSSION

In the present studies moderate to high nutritional components and antioxidant activities with varying levels of phenolics, proteins and alkaloids were recorded in the two species of *Pleurotus* (Table 1). Relatively higher amount of the protein content (10.55±1.62 $mg.gm^{-1}$ and 8.50±0.15 $mg.gm^{-1}$) in both of the species was observed as compared to the other species of *Pleurotus* as reported by Jean-Phillip (2005). Carbohydrate content in the *Pleurotus sajor-caju* and *Pleurotus florida* was 32.16 and 8.30 gm $100gm^{-1}$, respectively which was less than the cultivated variety of *Pleurotus* as reported by Paz *et al*. (2012) but much more than the reports of Boda *et al*. (2012). *Pleurotus* along with many other types of edible mushrooms have been known as a potent source of

nutrients as well as natural antioxidants. Findings from this research showed that fungal mycelia studied have antioxidant capacity where FRAP and DPPH free radical scavenging activities assay showed a remarkable difference between both the species. *P. sajor-caju* showed higher DPPH scavenging activities than *P. florida i.e.*

Table 1. Nutritional components and antioxidant activities of *Pleurotus* spp.

S. No.	Parameters	*P. sajor-caju*	*P. florida*
1	Protein (mg/gm)	10.55±1.62	8.50±0.15
2	Carbohydrates (gm/100gm)	32.16±7.16	8.30±1.09
3	Red. Sugars (mg/gm)	24.37±1.04	12.91±1.51
4	Non Red. Sugars(gm/100gm)	29.72±7.09	7.00±1.02
5	DPPH scavenging (%)	45.53±0.01	9.95 ±1.14
6	AEAC(mg/gm)	0.10±1.69	0.02±0.00
7	FRAP (mg AEAC/gm)	0.79±0.10	0.06±0.01
8	Phenolics (mg/gm)	1.01±0.57	0.45± 0.05
9	Flavonoids (mg/gm)	0.14±0.01	0.11 ± 0.01
10	Beta carotene (mg/gm)	0.038±0.012	0.018±0.005
11	Lycopene (mg/gm)	0.016±0.001	0.007±0.002
12	Tannins (mg/gm)	5.18±0.64	4.48±0.86
13	Alkaloids (mg/gm)	0.49±0.01	0.47±0.08

DPPH- 2, 2-Diphenyl-1-picryl hydrazyl
AEAC- Ascorbic acid Equivalent Antioxidant Capacity.
FRAP- Ferric Reducing Antioxidant Power

(45.53±0.01%) and (9.95 ±1.14%) along with their corresponding AEAC value which was 0.10±1.69 mg.gm^{-1} and 0.02±0.00 mg.gm^{-1}, respectively. Phenolic and flavonoid content in both of the species confirms the study of Vamanu (2012) and Jeena *et al.* (2014) in *P. ostreatus.* High amount of β- carotene (0.038±0.012 mg.gm^{-1}) and lycopene (0.018±0.005 mg.gm^{-1}) content was recorded in *P. sajor-caju* where as comparatively less amount of the same was recorded in *P. florida.* Presence of β-carotene and lycopene was less as compared to the investigations of Pal *et al*. (2010). Alkaloids are responsible for different cytotoxic and antimicrobial properties (Ozcelik *et al*. 2011) Present study revealed the high amount of alkaloid was recorded in *P. sajor-caju* (0.49±0.01 mg gm^{-1}) and *P. florida* (0.47±0.08 mg.gm^{-1}). Tannins are responsible for different biological activities such as antioxidant, antimicrobial and antitumor activities (Yoshizawa *et al*. 1987, Yoshida *et al*. 1989, Yoshida *et al*. 2009). Tannin content in *P. florida* and *P. sajor-caju* ranged from 4.48–5.18 mg.gm^{-1}. Comparatively *P. sajorcaju*, exhibited better scavenging of free radicals including high levels of protein, carbohydrate, reducing sugars, phenol along with both FRAP and DPPH scavenging activities than *P. florida*. In the present studies of the enzymatic activity of these mushroom mycelium, *P. florida* showed higher amylase and lipase activity than *P. sajor-caju*, both the species were negative for the Xylanase and protease activity (Table 2).

Table 2. Extracellular enzymatic activity of *Pleurotus sajor-caju* and *P. florida*.

Sl. No.	Species	Amylase	Protease	Xylanase	L-Asparaginase	Cellulase	Lipase
1	*P. sajor-caju*	+	-	-	-	+	++
2	*P. florida*	+++	-	-	+	-	+++

Note: (++++): very good activity; (+++): good activity; (++): moderate activity; (+): poor activity; (-): No activity.

CONCLUSION

Wide range of applications from the mushroom fruit bodies and mycelium has attracted much attention in regard of nutritional components and secondary metabolites. Present analysis showed varying range of bioactive compounds along with versatile production of extracellular enzymes. The preliminary study on quantification of antioxidants and screening of the enzymes can be the future prospective for the exploration of these mushroom mycelium for development of bioactive compounds.

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