In-vitro antioxidant activity and nutritional value of four wild oyster mushroom collected from North-Eastern Part of Uttar Pradesh

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Abstract
Four species of wild edible oyster mushroom viz., Pleurotus cystidiosus OK. Mill., P. flabellatus (Berk & Br.) Sacc., P. florida (Mont.) Singer and P. ostreatus (Jacq: Fries) were selected to evaluate their antioxidant property and bioactive compounds (β-carotene, lycopene, ascorbic acid and phenolic content). The antioxidant property of all tested mushroom extracts gave a positive result with free radical scavenging potentials. Phenolic content ranged from 37.70–39.01 mg/g. P. ostreatus showed highest antioxidant activity in all tested protocols (DPPH: 0.1884mg/ml, β-carotene bleaching assay: 0.362mg/ml, H2O2 Scavenging assay: 0.820mg/ml) in comparison to other three species tested viz., P. cystidiosus, P. flabellatus and P. florida. All mushroom tested exhibited significant antioxidant properties which therefore can be promoted as natural antioxidant preference in food and can also be used as nutraceuticals.

Key words – bioactive compounds – biochemical – DPPH – phenol – Pleurotus

Introduction
Oxidation is essential for every living organism for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid arthritis and atherosclerosis as well as in degenerative processes associated with aging (Turkoglu et al. 2006). Organisms are protected against free radical damage by enzyme such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherols and glutathione (Mau et al. 2002). Mushrooms belonging to different taxa occupying diverse ecological niches have shown various degrees of antioxidant potentials. It contains various bioactive compounds such as phenolic compounds, polyketides, terpenes and steroids which are recognized as excellent antioxidants (Orsine et al. 2012). Antioxidant activity of mushroom shows strong inhibition of lipid peroxidation at high concentration of the extracts in most cases (Cheung & Peter 2005). Antioxidant activity of mushrooms includes scavenging of free radicals through hydrogen holding capacity and oxidation by peroxy radicals (Chang et al. 2007). Oyster mushrooms are preferred by many people for its taste, texture and unique aroma (Arбаyah & Umi 2013), belong to class Basidiomycetes and family Agariaceae. It is an edible mushroom and grows on logs and tree stumps in shelf like pattern (Johnsy & Okon 2013) in tropical and subtropical areas and also cultivated easily (Chirinang & Intarapichet 2009). Oyster mushrooms are good source of non-starchy carbohydrates, dietary fibre
and good quantity of proteins which include most of amino acids, minerals and vitamins (Ahmed et al. 2013). Fruiting body of *Pleurotus* possesses higher concentration of antioxidants than other commercial mushrooms (Patel et al. 2012, Roy & Prasad 2013). This property is due to the presence of pleuran (glucan) a polysaccharide isolated from *P. ostreatus* (Atri et al. 2012).

The aim of this study was to evaluate the antioxidant activity and nutritive value of four wild oyster mushroom collected from Gorakhpur forest regions.

**Materials & Methods**

**Sample collection, identification & processing**

Samples of four oyster mushrooms viz., *Pleurotus cystidiosus*, *P. flabellatus*, *P. florida*, *P. ostreatus* were collected from forest regions of Gorakhpur during the months of July to August 2011-2013. Collected samples were identified on the basis of their macro and microscopic characterization following Kong (2004) and Atri et al. (2012). Samples were cleaned and dried in shadow at room temperature for 14 days. It was then ground to make fine powder with the help of grinder and stored for further study.

**Evaluation of antioxidant property**

Following methods were used to evaluate the antioxidant activity of oyster mushroom.

**DPPH (2, 2'- diphenyl-1-picrylhydazyl) radical scavenging bioassay**

During the bioassay, a stock solution of mushroom was prepared by taking 10g fresh sample with 100 ml of 70% ethanol following Barros et al. (2008) and the prepared extract was filtered through Whatmann No. 1 filter paper. Different concentrations (1, 2, 3, 4 & 5 mg/ml) of 1 ml of prepared stock solution mushroom were mixed with 1ml methanolic solution of 0.2 mM DPPH radicals separately. The mixture was shaken vigorously and left to stand for 60 min in the dark (until stable absorption values were obtained). The reduction of the DPPH radical was determined by measuring the absorption at 517 nm by spectrophotometer (U-2900 UV/VIS, Model No. 2J1-004, Japan). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

\[
RSA(\%) = \left( \frac{A_0 - A_{s}}{A_0} \right) \times 100
\]

where \(A_0\) is the absorbance of control and \(A_s\) is the absorbance of the tested sample. The extract concentration providing 50% of radicals scavenging activity (EC\(_{50}\)) was calculated from the graph of RSA percentage against extract concentration.

**Inhibition of β-carotene bleaching**

The antioxidant activity of fresh mushroom extract was evaluated following Barros et al. (2008) using β-carotene linoleate model system. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 ml). Two milliliters of this solution were taken into a 100-ml flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 ml) were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of different concentrations (1, 2, 3, 4, & 5 mg/ml) of the mushroom extracts. The tubes were shaken and incubated at 50 °C in a water bath. As soon as the emulsion was added to each tube, the zero-time absorbance was measured at 470 nm using a spectrophotometer (U-2900 UV/VIS, Model No. 2J1-004, Japan). Absorbance readings were then recorded at 20-min intervals until the control sample had changed colour. A blank, devoid of β-carotene, was prepared for background subtraction. Lipid peroxidation (LPO) inhibition was calculated using the following equation:

\[
\text{LPO inhibition} = \frac{\text{β-carotene content after 2 h of assay}}{\text{initial β-carotene content}} \times 100
\]

The extract concentration providing 50% antioxidant activity (EC\(_{50}\)) was calculated from the graph of antioxidant activity percentage against extract concentration.
Hydrogen peroxide (H₂O₂) radical scavenging activity

For estimation of H₂O₂ scavenging activity, method of Vamanu (2012) was used. In this process H₂O₂ solution (40µM) was prepared in phosphate buffer (50mM, pH 7.4). Extract of different concentration (1, 2, 3, 4, 5 mg/ml) were mixed with H₂O₂ solution (0.6 ml) and absorbance of sample were observed at 230 nm after 10 min against blank solution (phosphate buffer without H₂O₂).

Bioactive compounds

Following methods were used to evaluate the bioactive compounds of oyster mushroom.

β-carotene and Lycopene

β-carotene and lycopene were determined by the method described by Loganathan et al. (2009). About 100 mg of dried ethanolic extract was vigorously shaken for 1 min with acetone and hexane (4:6 v/v) mixture making final volume of 10 ml and filtered through Whatmann No. 1 filter paper. The absorbance of the filtrate was measured at λ=453, 505 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations:

Lycopene (mg/100ml) = 0.0458 A663 + 0.372 A505 - 0.0806 A453

β-carotene (mg/100ml) = 0.216 A663 - 0.304 A505 + 0.452 A453

The results were expressed as μg of carotenoid/g of extract.

Ascorbic acid

The vitamin C content was determined titrmetrically using 2, 6 Dichloropheno Indophenol method following Barros et al. (2008). 10 g of grounded sample was mixed with 100 ml of 5% metaphosphoric acid solution and shaken for 30 min. The mixture was then filtered through Whtaman no 1 filter paper. 10 ml of test sample was pipetted from the extract in 250 ml conical flask and titrated against 0.025% of 2.6 Dichlorophenol Indophenol reagents. The amount of vitamin C in each extract was calculated from the equation:

\[
\text{Per cent of ascorbic acid} = \frac{\text{tinkle x Dye factor x volume made}}{\text{Aliquot of extract x weight}} \times 100
\]

Phenolic content

Phenolic compounds in the ethanol extracts were determined using Folin–Ciocalteu method (Loganathan et al. 2010). One ml of the extract was added to 10.0ml distilled water and 2.0 ml of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min and then 2.0 ml of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Gallic acid was used as a standard for the calibration curve. The phenol compound was calibrated using the linear equation based on the calibration curve. The contents of the phenolic compound were expressed as mg Gallic acid equivalent/g dry weight.

Biochemical assay

Moisture content was determined following Oyetayo et al. (2007). Protein content of macrofungal samples were determined by the method of Lowry et al. (1951), carbohydrate was estimated by Anthrone method of Thimmaiah (1999). Crude fibre content was evaluated following Alam et al. (2008) while lipid was estimated by the method of Folch et al. (1957). Ash of the sample was estimated by the method of Pearson (1976).

Statistical Analysis

Experimental values are given as mean ± standard deviation (SD). Statistical significance was determined by one way variance analysis (ANOVA). Difference at p<0.05 were considered to be significant.
Results

Characteristics of oyster mushroom collected

Table 1 represents the morphological characterization of collected oyster mushrooms. Their spore prints and substrate were also recorded.

| Mushrooms      | Locality | Host          | Fruiting body                                                                 | Collection Date | Voucher no. |
|----------------|----------|---------------|-------------------------------------------------------------------------------|-----------------|-------------|
| *P. cystidiosus* OK. Mill. | Gagaha   | *Ficus benghalensis* | Pileus 4-6 cm, smooth, grayish brown, convex, then flattened, margin smooth, narrowing at point of attachment. Stipe 1-2 cm grayish brown, smooth, eccentric, very short, wooly, stout, ring absent. Gills decurrent, at first white, becoming tinged, pallid, ochraceous, narrow, close. Spores 8-10 X 4-5.5 µm, ellipsoid, hyaline, smooth, non-amyloid. Flesh white, firm and full. Spore print creamish. | 21/7/13 | DDUNPL246 |
| *P. flabellatus* (Berk & Br.) Sacc. | Piprauli | Decaying wood | Pileus 3-7 cm, creamish white, surface smooth, thin, margin wavy, curved, narrowing at point of attachment with stipe. Stipe 0.5-1 cm, creamish white, smooth, eccentric, very short, stout, ring absent. Gills decurrent, creamish, narrow, close. Spores 7.5-8 X 2.5-4 µm, ellipsoid, smooth, hyaline, non-amyloid. Flesh white, firm, full. Spore print white. | 24/6/13 | DDUNPL248 |
| *P. florida* (Mont.) Singer | Khajni | *Ficus religiosa* | Pileus 5-8 cm, pale yellow, smooth, thick, leathery, margin wavy, flattened, narrowing at point of attachment with stipe. Stipe 1.5-2 cm long, pale yellow, smooth, leathery, firm, thick, full, stout, eccentric. Gills decurrent, pale yellow, narrow close. Spores 6.25- 8.5 X 3-4 µm, ellipsoid, smooth, hyaline, non-amyloid. Spore print whitish. Flesh yellowish, firm. | 25/8/12 | DDUNPL249 |
| *P. ostreatus* (Jacq: Fries) | Piprauli | *Mangifera indica* | Pileus 3-10 cm, convex, kidney shaped to fan shaped, greasy smooth, pale brown in colour, margin inrolled. Stipe rudimentary, eccentric, thick, stuffed, whitish. Gills close, parallel, running down the stem. Spores 7-8 X 2.5-3 µm, smooth, cylindrical. Spore print whitish. | 8/7/12 | DDUNPL250 |
Nutritional property of collected oyster mushrooms

Table 2 represents nutrients value viz., protein, carbohydrate, lipid, moisture, ash and fibre of tested oyster macrofungi. In present study, higher protein content was observed in *P. ostreatus* (43.70±1.77%) while lowest in *P. flabellatus* (40.27±0.36%). Carbohydrate ranged between 33.12±1.66-42.69±1.75%. Highest carbohydrate was recorded in *P. flabellatus* (42.69±1.75%).
Table 2 Biochemical assay of collected oyster mushrooms (%)

| Macrofungi    | Protein (%) | Carbohydrate (%) | Lipid (%) | Moisture (%) | Ash (%) | Fibre (%) |
|---------------|-------------|------------------|-----------|--------------|---------|-----------|
| P. cystidiosus| 41.11±1.69  | 40.49±0.67       | 0.42±0.05 | 85.18±0.93   | 6.65±0.78| 17.55±1.65|
| P. flabellatus| 40.27±0.36  | 42.69±1.75       | 0.65±0.08 | 84.90±1.12   | 5.72±0.79| 13.57±0.55|
| P. florida    | 42.50±0.29  | 33.12±1.66       | 0.59±0.03 | 83.70±1.75   | 6.44±1.12| 11.42±0.91|
| P. ostreatus  | 43.70±1.77  | 35.00±0.75       | 0.57±0.11 | 80.29±1.26   | 7.69±0.72| 18.08±1.23|

Values are mean of three replicates ± standard error followed by P. cystidiosus (40.49±0.67%), P. ostreatus (35.00±0.75%) and P. florida (33.12±1.66%). Lipid content varies from 0.42±0.05-0.65±0.08%, lowest in P. cystidiosus and highest in P. flabellatus. The highest moisture content was observed in P. cystidiosus (85.18±0.93%) and lowest in P. ostreatus (80.29±1.26%). Ash content of tested mushrooms was recorded between 5.72±0.79-7.69±0.72%. Highest ash content was observed in P. ostreatus (7.69±0.72%) while lowest in P. flabellatus (5.72±0.79%). Fibre content of oyster mushrooms tested ranged between 11.42±0.91-18.08±1.23%. Highest fibre was observed in P. ostreatus (18.08±1.23%) while lowest in P. florida (11.42±0.91%).

Discussion

All collected oyster mushrooms differ in their spore size and morphology (Table 1). They were found in saprotrophic association with different trees except P. flabellatus which was growing on decaying wood. This shows that oyster mushrooms are host dependent and grow on specific host (Alam et al. 2009, Musieba et al. 2011, Dhancholia 2013).

Free radical scavenging is a mechanism which inhibits lipid oxidation and is used to estimate antioxidant activity (Umamaheshwari & Chatterjee 2008). In present study, the radical scavenging activity of mushroom extracts were tested against the DPPH activity, β-carotene-linoleic acid assay and hydrogen peroxide reducing power activity.

DPPH is a stable free radical with good absorption at 517 nm, it is used to study radical scavenging activity of extracts. When antioxidant donate proton to these radicals then absorption of samples decreases. Radical scavenging activity is measured by decrease in absorption of samples (Srivastava et al. 2006). All tested extract at different concentrations in present study showed good radical scavenging activity. Earlier studies with Pleurotus species extracts showed EC₅₀ value for DPPH scavenging activity 11.56 mg/ml for P. ostreatus (Chirinang & Intarapichet 2009). The variation may be attributed to difference in the concentration in the antioxidant compounds because of the solvent used for extraction. Sample with more phenolic content exhibited more antioxidant activity (Rajasekaran & Kalaimagal 2011). In β-carotene-linoleic assay free radicals of linoleic acid...
adds up with radicals of carotenoids and results in its high antioxidant property. Different antioxidants of mushrooms hinder with the β-carotene bleaching. They neutralise the linoleate free radicals and other radicals of the system (Adebayo et al. 2014). Hydrogen peroxide is a common metabolite generated in the body during superoxide reduction. In excess H₂O₂ is cytotoxic as it is easily converted to harmful hydroxyl radical (Kuppusamy et al. 2009). It has been found that antioxidant property of mushrooms is generally due to the presence of polyphenols in them. H₂O₂ Scavenging activity (Fig. 3) was also found to present in good quantity which correlates with the finding of Vamanu (2012) for extract of A. bisporus (J. E. Lange) Imbach.

In present study β-carotene and lycopene was also found but in small concentration. Both act as a powerful antioxidant. β-carotene is a member of the carotenoid family, which are highly pigmented (red, orange, yellow), fat-soluble compounds naturally present in many fruits, grains, oils, and vegetables. Among the naturally occurring carotenoids that can be converted to vitamin A in the human body, so-called ‘provitamin A carotenoids’, β-carotene is the most abundant and most efficient one found in foods. Lycopene contain 11 conjugated and 2 non-conjugated double bond which results in its high singlet oxygen quenching ability (Pal et al. 2010). They both help to reduce the risk of cardiovascular diseases and prostate cancer. Both are found in very small concentration in tested mushroom samples.

Phenols are important component of plants and mushrooms. It has been found to be the most important component imparting antioxidant quality of mushrooms. They contribute directly to antioxidant effect of system as they have capacity to eliminate free radicals due to presence of hydroxyl group. They are important for plants, vegetables and mushrooms (Ferreira et al. 2007). The phenolic concentration of samples analysed in present study were same than those reported by Chirinang & Intarapichet (2009) for P. ostreatus and P. sajor caju.

Nutrition is one of the main concerns of all of the societies of the world. Food supply should be both an economic and ecological subject. Health and nutrition involve balanced and sufficient functional food components (Çağlarirmak 2011). Nutritional value of mushroom depends largely on the chemical composition of the compost on which they are growing (Goyal et al. 2006). Mushrooms are considered to be healthy food because of their high and qualitatively good protein content, low fat and cholesterol content, minerals and vitamins. Protein is an important constituent of dry matter of mushrooms of high quality. The protein contents of mushrooms are affected by a number of factors, namely the type of mushrooms, the stage of development, the part sampled, level of nitrogen available and the location (Longvah & Deosthale 1998). The carbohydrate content of mushroom represents the bulk of fruiting bodies accounting for 50 to 65% on dry weight basis. Free sugars amounts to about 11% (Thatoi & Singdevsachan 2014). Adejumo et al. (2015) reported 33.57 and 37.64% carbohydrate in the fruiting bodies of P. ostreatus and P. pulmonarius respectively. Alam et al. (2008) determine the carbohydrate content of P. florida, P. ostreatus, P. sajor caju and found it as 42.83, 37.8 and 39.82 g/100g respectively. Dundar et al. (2008) studied several species of oyster mushroom and reported that carbohydrate of P. eryngii, P. ostreatus and P. sajor-caju were 39.85, 37.87 and 37.72 g/100g respectively. Similar results were also recorded in present work for carbohydrate concentration in different oyster mushroom evaluated.

In mushrooms (wild and cultivated both), the fat content is very low (4-6%) as compared to proteins and carbohydrates (Thatoi & Singdevsachan 2014). Mushrooms contain all the main classes of the lipid compounds including free fatty acids, mono-, di- and triglycerides, sterols, sterol esters and phospholipids. Mushrooms are rich in linolenic acid which is an essential fatty acid (Nile & Park 2014) but are more dominated by unsaturated fatty acids (Longvah & Deosthale 1998). Due to the low fat and oil content, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid-induced disorders (Manimozhi & Kaviyarasan 2013). In present work lipid content varies from 0.42±0.05-0.65±0.08% showing the similarities with the other previous findings. Alam et al. (2008) determine the lipid content of four mushroom viz. P. florida, P. ostreatus and P. sajor caju, and found it to be 0.54, 0.68, 0.57 g/100g. Khan et al. (2013) reported lipid content of Pleurotus (flabellatus) djamor (R-22) cultivated on sawdust of different wood and mention that lipid content ranged from 0.11-0.80%.
Gbolagade et al. (2006) stated that young fruit bodies of *Pleurotus florida* contains 0.9% of lipid while mature fruit bodies contain 1.2% of lipid. Lipid content of mushroom depends upon the type of substratum on which they are growing and time of harvesting.

Mushrooms generally have high moisture content which accounts for their short shelf life as they deteriorate easily after harvest if preservative measures are not employed (Adedayo & Rachel 2011). Fresh mushroom contains about 90% moisture and 10% dry matter and dry mushroom contains 90% dry matter and 10% moisture (Johnsy & Davidson 2011). Goyal et al. (2006) reported that *P. sajor caju* contains 89.58% of moisture. Alam et al. 2008 estimated the moisture content of different species of *Pleurotus* and reported that *P. ostreatus* contains highest moisture (86.0%) followed by *P. sajor caju* (87.0%) and *P. florida* (87.5%). In present work, the moisture content of different oyster mushroom was found to be in range of 80.29±1.26% to 85.18±0.93% which completely shows the similarity with former works done.

The main constituents in the mushroom ash are K and P (totaly 60%) (Colak et al. 2009). In present work done ash content was found to be the range of 5.72±0.79-7.69±0.72% correlating the present findings with another works completely. Adejumo et al. (2015) determined the ash content of two oyster mushrooms viz., *P. ostreatus* (5.65%) and *P. pulmonarius* (7.95%). Dundar et al. (2008) estimated the ash content of 3 species of *Pleurotus* viz., *P. eryngii*, *P. ostreatus* and *P. sajor caju* and found it to be 4.89, 4.78 and 5.84% respectively, but in another work carried out by Goyal et al. (2006) for *P. sajor caju* ash content was observed to be 7.46%.

Mushroom contains good quality fibre. It helps in lowering the cholesterol. Fresh mushrooms contain both soluble and insoluble fibre. The soluble fibre is mainly β-glucans and chitosans, which are components of the cell walls (Kakon et al. 2012). Fibre content of oyster mushrooms tested ranged between 11.42±0.91-18.08±1.23%. Highest fibre content was found in *P. ostreatus* which was same as given by Jonathan et al. (2012). Oyetayo et al. (2007) analyzed the nutritional property of different parts (pileus and stipe) of cultivated and wild strains of *Pleurotus sajor caju* and observed that wild sample contains higher amount of fibre than the cultivated strain. Stalk of both strains viz. cultivated and wild sp., contains higher fibre (16.24% and 26.14% respectively). Goyal et al. (2006) in another experiment stated that *P. sajor caju* contains 12.13% fibre.

**Conclusion**

Mushrooms are rich reservoir of nutrients and also possess various medicinal prospects. Mushrooms either wild or cultivated are rich sources of protein and fibres while are low in lipid and calorie. Hence they can be recommended as good diet for peoples having various health problems. Mushroom also contain high phenol content and shows good antioxidant property. Various species of *Pleurotus* possess high bioactive compounds which imparts for its good antioxidant property. *Pleurotus* are also rich in nutrients and hence it can be included in diet to obtain nutrients and also to protect the human body against various types of free radicals generated during the process of oxidation which in turn protect the body from various diseases.

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