

Wild mushrooms of Odisha: prospective candidates of antioxidant sources

Abstract

Wild medicinal mushrooms, mostly woody mushrooms comprised *Lenzites betulina*, *Lentinus polychrous*, *Trametes versicolor*, *Pycnoporus cinnabarinus*, *Pycnoporus sp*, *Inonotus radiatus* and *Microporus xanthopus* were analyzed for antioxidant properties. Of the seven wild mushrooms, *Inonotus radiatus* had highest content of flavonoid content (13.50 ± 1.33 mg/gm), FRAP (1.47 ± 0.09 mg AEAC/gm) and total phenolics content (3.5 ± 0.052 mg/gm). *Pycnoporus* and *P. cinnabarinus* showed considerably good amount of carotenoids. *M. xanthopus* showed higher amount of ascorbic acid (5.70 ± 0.01 mg/gm) than other species studied. Results in this study revealed that the composition of all the woody mushrooms are below the phytotoxicity level and ongoing research in this regard can lead for the formulation of drugs in the future.

Keywords: mushroom, antioxidant, ergosterol, flavonoids

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Abbreviations: GAE, gallic acid equivalent; FRAP, ferric reducing antioxidant power; RSA, radical scavenging assay

Introduction

Antioxidants are compounds that inhibit or slows down the oxidation of other molecules by preventing oxidizing chain reactions. Antioxidants can scavenge free radicals and inhibit lipid peroxidation.¹⁻³ Antioxidant supplementations from fruits and vegetables have been shown to increase level of plasma antioxidant capacities. Similar kind of research has also shown that the intake of plant products lowers the incidences of cancer.² Several naturally occurring substances have been known to have antioxidant properties. Flavonoids and other phenolic compounds are among the major components which are present in them which contribute to such sort of activity.⁴ Recent studies have highlighted the bioactive compounds obtained from fungi,³ particularly antioxidants⁵ which significantly delay or prevent the oxidation of biomolecules in the human body. Mushrooms have been known for functional foods as well as source of physiologically beneficial therapeutic agent, especially antioxidants, β -glucans, polysaccharides and other bioactive substances which are responsible for anticancer, immunostimulating, analgesic and neuroprotective activities. Medicinal mushrooms contain various classes of secondary metabolites with potent antioxidant activity.^{6,7}

Odisha possess varied range of flora which synergistically or individually supports growth of many macrofungi of edible and/or non edible nature. Although various reports are available regarding antioxidant profiling of edible mushrooms from India but studies related to wild non edible mushrooms are very few. Present studies focus mainly on the antioxidant characterization of some wild non edible mushrooms, for which no records are available from Odisha (India).

Materials and methods

Collection

Mushrooms species of *L. betulina*, *T. versicolor*, *L. polychrous*, *P. cinnabarinus*, *Pycnoporus sp*, *I. radiatus*, *M. xanthocarpus* were collected from tropical moist deciduous and semi ever green forest

of Similipal of Mayurbhanj district in India and stored in dried form. Macroscopic and microscopic examination of pileus, stipe, veil, ring, volva, lamellae and gills properties of mushrooms were made to identify species following procedure of Largent et al.⁸ All the assays were performed using the entire mushroom fruiting body including stipe. A fine dried mushroom powder (100mesh) was prepared for each species, stored in the room temperature at 28°C and used for further biochemical analysis.

Total phenolic content

Total phenolic content in the wild mushrooms were estimated through folin phenol method as described by Singleton et al.,⁹ 1gm of the powdered sample of each of dried mushroom sample was extracted with 10ml of absolute methanol by grinded in mortar pestle for effective extraction and centrifuged at 2000g for 15minutes. Supernatant collected and stored at 4°C for further analysis. A sample of 100 μ l was made up to 1ml with distill water and 1ml of folin ciocalteu reagent and 2ml of 10% sodium carbonate solution was added to the extract. The total phenolic content in different extract were measured and expressed as gallic acid equivalent (GAE) in gram per 100gram of sample. Gallic acid was used to draw the standard curve using 10–80 μ g (0–0.75mg/ml) of Gallic acid. The optical density was measured at 765nm using Analytic Jena spectrophotometer.

Ascorbic acid content

The ascorbic acid content in the wild mushrooms was determined by volumetric method.¹⁰ The dye solution was prepared by dissolving 42mg of sodium carbonate into a small volume of distill water and 52mg of 2,6 Dichlorophenolindophenol. The final volume was made up to 200ml. Sample (0.5–5g) was extracted in 4% oxalic acid and made up to a known volume (100ml) and centrifuged for 15minutes. 5ml supernatant from the extract was carefully taken and added with 10ml of 4% oxalic and titrated against the dye (V_2 in ml). The initial and final volume of dye consumed while the appearance of pinkish color for each sample was noted down. The amount of ascorbic acid in mg/100g sample is calculated by using formula; $0.5 \text{mg} / V_1 \text{ml} \times V_2 / 5 \times 100 / \text{weight of the sample} \times 100$, when V_1 is the standard ascorbic acid consumed against dye.

Flavonoids

The flavonoid content of dried sample was estimated by using aluminum chloride colorimetric technique in terms of quercetin equivalents per gram of extract.¹¹ 0.1ml of methanol extract of samples were diluted with 1.5ml of methanol and incubated for 5min at room temperature. 0.1ml of $AlCl_3$ was added and again incubated at room temperature for 5min. The reaction mixture was mixed with 0.1ml of 1M Potassium acetate and total volume was made up to 5ml with distilled water. The mixture was incubated for 30minutes at room temperature and optical density was measured at 415nm.

Beta carotene and Lycopene

The concentration of β -carotene and lycopene in mushroom extracts was estimated spectrophotometrically following.^{12,13} Methanolic extract was evaporated to dryness at 40°C and about 100mg of methanolic extract was prepared. The dried methanolic extract i.e., 100mg was shaken vigorously with 10ml of acetone and hexane (4:6) for one minute continuously and filtered through whatman filter paper. The absorbance was measured at 453, 505, and 663nm. The contents of β -carotene and Lycopene were estimated by following formula:
Lycopene: $-0.0458 \times A_{663nm} + 0.372 \times A_{505nm} - 0.0806 \times A_{453nm}$.

β -carotene: $0.216 \times A_{663nm} - 0.304 \times A_{505nm} + 0.452 \times A_{453nm}$.

Carotenoid

The carotenoid was estimated in 500mg of dried mushroom powder treated with 10ml of 80% acetone and centrifuged at 3000 rpm for 10minutes at 4°C. This procedure was repeated until the residue became colorless. The residue was made to 10ml with 80% acetone and measured for absorbance at 480, 645 and 663nm separately. The quantity of carotenoid was calculated by using following formula and values were expressed in mg/gm by using formula:¹⁴

$$\text{Carotenoid} = A_{480} + (0.114 \times A_{663} - 0.638 \times A_{645}).$$

Where A=Absorbance.

Tannins

1gm of mushroom powdered sample was boiled in distilled water for 30minutes and filtered through whatman filter paper. 0.1ml of the sample extract prepared by above process was treated with 0.5ml of Folin Denis reagent. 1ml of saturated sodium carbonate and 1ml of distilled water was added to the reaction mixture and shaken well, and the optical density measured at 760nm. Tannic acid was served as a standard and tannin content estimated was expressed in mg/gm.¹⁵

Alkaloids

1mg of dried powdered sample was extracted with 100ml of 10% glacial acetic acid in alcohol. It was filtered and concentrated to 25% of its original volume. A 5ml amount of the extract solution was adjusted to 2–2.5 pH by adding HCl. 2ml of Dragendroff's reagent was added to it, and the precipitate was separated through centrifugation and was further washed with alcohol. The residue was treated with 2ml sodium sulfide solution. The brownish black precipitate formed was again centrifuged. Completion of precipitation was checked by adding 2drops of sodium sulfide. The residue was then dissolved in 2ml concentrated nitric acid which was diluted to 10ml with distilled water. 1ml of the solution was added with 5ml of urea solution and the absorbance was measured at 435nm.¹⁶

Ergosterol

Sample preparation was done by preparing 1g dry weight of the fruiting body with 10ml of mixture of chloroform and methanol (2:1 V/V). Extraction was carried out for 24hrs. Homogenate was filtered and the filtrate was transferred to a separation funnel, shaken well and with 1/5 volume of aqueous $NaCl_2$ (0.9%). The layers were allowed to separate and the lower chloroform phase was collected and used as the test sample. 1ml of sample was evaporated to dryness. 6ml glacial acetic acid was added to this, immediately followed by mixing 4ml Ferric chloride reagent. The contents were mixed thoroughly, cooled and the color developed was read at 550nm against blank. The sterol content was estimated from the standard curve plotted using Ergosterol 10–25ug/ml.¹⁷

Antioxidant assay

Free radical scavenging activity

The DPPH activity was estimated in the methanolic extracts by a colorimetric method.¹⁸ 1ml of methanolic extract was added with 2ml of DPPH solution (1:2) and incubated for 30 minutes in dark after vigorous mixing. Absorbance was measured at 517 nm and scavenging activity of each extract on DPPH radical was calculated.

Reducing power ability

Each mushroom extract (0.5–4mg/ml) in methanol (2.5ml) was mixed with 2.5ml of 200mM sodium phosphate buffer (pH–6.6) and 2.5ml of 1% potassium ferricyanide and the mixture was incubated at 50°C for 20minutes. After 2.5ml of 10% trichloroacetic acid was added, the mixture was centrifuged at 2000 rpm for 10 minutes. The upper layer (5ml) was mixed with 5ml of deionized water and 1ml of 0.1% ferric chloride and the absorbance was taken at 700nm (Analytic jena) spectrophotometer. Ec 50 value was calculated in mg/ml at 0.5 optical density against reagent blank.

Ferric Reducing Antioxidant Power (FRAP)

100 μ l of the methanolic extract was mixed with 3ml of FRAP reagent and incubated in the room temperature in dark for 10minutes and finally absorbance was read at 593nm. FRAP value was expressed in terms of mg AEAC/gm of sample.^{19,20}

Results and discussion

Table 1 represents the antioxidant components of mushroom sp. studied in this research. The total phenol exhibited varied with concentrations ranging from 3.50 \pm 0.05 to 0.20 \pm 0.01mg GAE/g of dry extract. Highest phenolic content was estimated in *I. radiatus* (3.50 \pm 0.05mg/gm) followed by *Pycnoporus* sp. (0.90 \pm 0.045 mg/gm) and *Pycnoporus cinnabarinus* (0.60 \pm 0.004mg/gm). We observed low phenolic content in *L. polychrous*, *T. versicolor* and *L. betulina*.

Carotenoid content in the mushroom is relevant to intensity of its color, colorful mushroom showed high amount of the carotenoid content than the colorless ones in our study (Table 2). The highest carotenoid was found in the *Pycnoporus* sp. (23.93 \pm 0.87mg/gm) followed by *Pycnoporus cinnabarinus* (20.84 \pm 1.56mg/gm) and *I. radiatus* (4.61 \pm 1.23mg/gm) where *L. polychrous* had the lowest concentration (1.31 \pm 0.1mg/gm).

β - carotene is precursor for the synthesis of Vitamin A which acts as powerful antioxidants as well. In general, β - carotene and lycopene

are found in rudimentary concentration in mushrooms.^{21,22} The content of β -carotene differed considerably between the mushroom species studied, ranging from 0.030mg/gm to 0.693mg/gm, highest β -carotene content was found on *P. cinnabarinus* (Table 2). Relatively higher lycopene was observed in *Pycnoporus* sp. i.e. 0.067mg/gm of the dry weight where as lowest lycopene concentration was recorded in *Lenzites betulina* and *I. radiatus* (0.008 mg/gm).

The ascorbic acid content of *M. xanthopus* (5.7 ± 0.010 mg/gm) was more than *P. cinnabarinus* and *T. versicolor* 5.0 ± 0.044 mg/gm and 3.9 ± 0.042 mg/gm, respectively. Most of the known functions of alkaloids are related to protection or self defence against microbes. Estimation of the alkaloids in the analyzed sample revealed that highest amount of alkaloid in *I. radiatus* (14.70 ± 2.29) followed by *P. cinnabarinus* and *Pycnoporus* sp when *L. betulina* and *L. polychrous* (1.45mg/gm and 0.88mg/gm) contained very small proportion of alkaloid. Ergosterol content acts as a precursor for the formation of Vitamin D and responsible for bone density.²³ Ergosterol content was maximum in *Pycnoporus* sp followed by *L. polychrous* and *M. xanthopus*, respectively. Ergosterol content in *I. radiatus* was lowest (0.023mg/gm) in our study.

Reducing power of methanolic extracts from wild edible mushrooms increased steadily with increase in concentration (Figure 1). At 4mg/ml, reducing powers were in order of: *I. radiatus* (1.214) > *Pycnoporus* sp. (0.5) > *M. xanthopus* (0.272) > *P. cinnabarinus* (0.263) > *L. polychrous* (0.186) > *L. betulina* (0.173) > *T. versicolor* (0.166). Except *I. radiatus* and *Pycnoporus* sp., all other mushrooms showed very negligible reducing power ability. The reducing power ability of wild edible mushrooms might be due to their hydrogen donating ability.

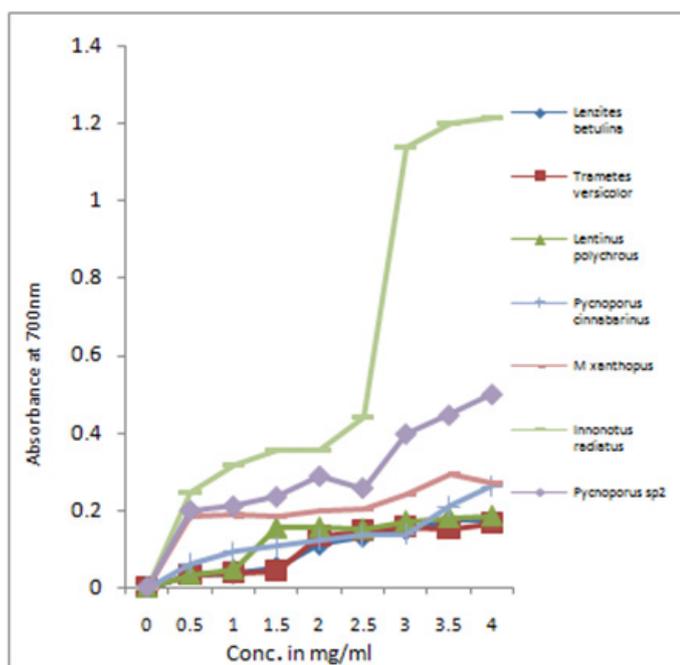


Figure 1 Reducing power ability of wild medicinal mushrooms.

Concentration dependent scavenging activity was observed in all samples, three mushrooms showing very high radical scavenging assay (RSA). Methanol extract of *Pycnoporus* sp (84.93%), *P. cinnabarinus* (73.72%) and *L. polychrous* (71.09%) can be grouped as high, *I. radiatus* (57.10%), exhibited moderate whereas species

L. betulina (36.71%) and *T. versicolor* (48.37%) showed very little radical scavenging assay. *Pycnoporus* sp. represents the lowest IC₅₀ value having and highest DPPH activity (Table 1) (Figure 2).

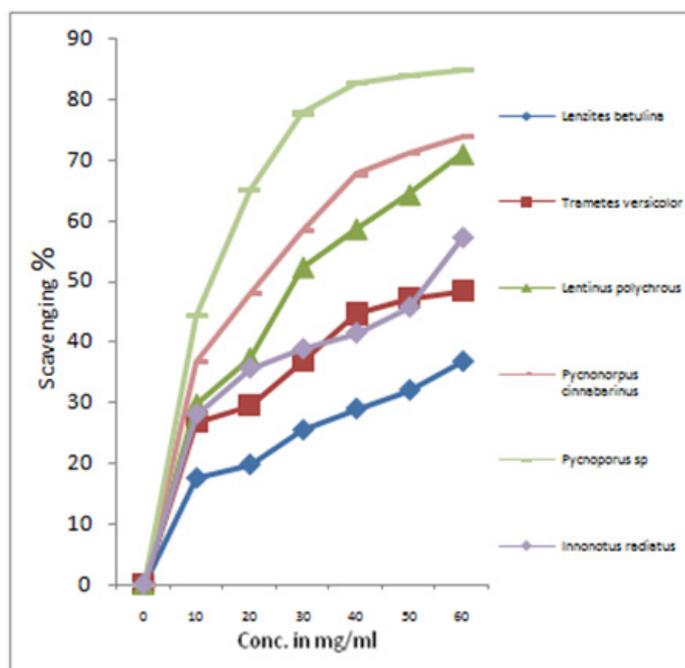


Figure 2 Radical scavenging activity of wild medicinal mushrooms.

Table 1 Representing antioxidant assay of seven different wild medicinal mushrooms

Species	DPPH in %	IC ₅₀ mg/ml	FRAP (mg AEAC/gm)
<i>Lenzites betulina</i>	36.70	>100	0.24±0.02
<i>Trametes versicolor</i>	48.37	70	0.43±0.01
<i>Lentinus polychrous</i>	71.09	28	0.80±0.08
<i>Pycnoporus cinnabarinus</i>	73.72	22	0.12±0.02
<i>Pycnoporus</i> sp.	84.93	13	0.25±0.01
<i>Inonotus radiatus</i>	57.10	54	1.47±0.09
<i>Microporous xanthopus</i>	nd	nd	0.04±0.01

AEAC, ascorbic acid equivalent antioxidant capacity Where \pm represents average and standard deviation of three replicates

FRAP value ranged from 1.47 to 0.04mg AEAC/gm. A high value of FRAP was seen for *I. radiatus* which also contained maximum total phenolic content and ascorbic acid indicating a strong correlation of these antioxidant component contributing to the bioactivity. It suggested the antioxidant components in these wild mushrooms are capable of reducing oxidants and free radicals. Within the

studied mushrooms such as *L. polychrous* and *T. versicolor* showed comparatively good FRAP value with those reported from peaches, which ranged from 0.84–1.2mg AEAC/gm²⁴ (Table 1).

Highest total flavonoid was observed in *I. radiatus* (13.50±1.33mg/gm) followed by *Pycnoporus* sp. and *P. cinnabarinus* and lowest in *L. betulina* (0.23±0.15mg/gm) (Table 2).

Table 2 Representing antioxidant components of wild mushrooms of Odisha in this study

Species	Tannins (mg/gm)	Flavonoids (mg/gm)	β-Carotene (mg/gm)	Lycopene (mg/gm)	Total Phenolic (mg/g)	Ascorbic acid (mg/g)	Ergosterol (mg/gm)	Alkaloids (mg/gm)	Carotenoids (mg/gm)
<i>L. betulina</i>	0.94±0.16	0.23±0.15	0.030±0.000	0.008±0.000	0.30±0.010	2.8±0.037	0.04±0.021	1.45±0.05	1.17±0.27
<i>T. versicolor</i>	0.41±0.08	0.86±0.16	0.159±0.069	0.038±0.003	0.20±0.010	3.9±0.042	0.033±0.010	3.80±0.38	1.30±0.05
<i>L. polychrous</i>	0.47±0.02	0.80±0.01	0.193±0.003	0.023±0.011	0.30±0.007	2.9±0.049	0.046±0.005	0.88±0.17	1.15±0.19
<i>P. cinnabarinus</i>	1.33±0.44	5.60±0.65	0.693±0.002	0.060±0.002	0.60±0.004	5.0±0.044	0.033±0.010	5.90±4.60	20.84±1.56
<i>Pycnoporus</i> Sp	2.40±0.51	11.66±0.90	0.458±0.004	0.067±0.001	0.90±0.045	2.1±0.029	0.066±0.013	4.51±0.13	23.93±0.87
<i>I. radiatus</i>	1.42±0.19	13.50±1.33	0.101±0.005	0.008±0.006	3.50±0.052	3.4±0.022	0.023±0.008	14.70±2.29	4.61±1.23
<i>M. xanthocarpus</i>	1.81±0.42	0.53±0.20	0.059±0.001	0.009±0.001	0.30±0.016	5.7±0.010	0.036±0.005	2.10±1.09	1.07±0.14

Where ± represents average and standard deviation of three replicates

The mushrooms we analyzed especially *Pycnoporus* sp. and *Pycnoporus cinnabarinus* contains good amount of carotenoid besides useful phytochemicals such as phenolics, ascorbic acid and other components related to the antioxidant properties.

The methanol extract of *Pycnoporus cinnabarinus*, *Pycnoporus* sp. and *Lentinus polychrous* of eastern India in particular showed the most promising scavenging activity both in term of DPPH scavenging and FRAP assay values. Phenolic compounds at high concentrations may inhibit cell proliferation and simultaneous exposure to hydrogen peroxide. Phenolics have been also shown to lead to the amplification of proliferation inhibition.²⁵ Our results revealed that total phenolic component and ascorbic acid content of *I. radiatus* are comparatively appreciable of all our studied mushrooms, 3.50mg/g and 3.40mg/gm respectively, which were similar to findings of Anguiano et al.,²⁶ and even the concentration of phenolics is more than the edible variety *Pleurotus djamor* as reported by Saha et al.²⁷

Generally fungi do not possess flavonoid content they have been reported in some macrofungi like *Lactarius piperatus*.²⁸ Bioactive compounds as flavonoid content was found to be near about similar 13.50mg/gm (*I. radiatus*) significant compared to other edible varieties like *Ramaria botrytis* (16.56mg/gm) or non edible species as *Hypholoma fasciculare* (5.09mg/g) as reported by Barros et al.²⁹ Even the concentration of flavonoids was found to be quite high as compared to *Agaricus bisporus* as reported by Rao et al.³⁰ Tannic acid is an example of plant tannins and is polyphenolic– polyhydroxy class of compound. Chemical nature of tannins form complexes with proteins, enzymes and other macromolecules and is generally referred to as an anti–nutrient as well as an antimicrobial agent.³¹ Tannic acid content in the mushrooms we analyzed were quite low as compared to the edible varieties as reported by Puttaraju et al.,³² ranging from 2.40±0.51mg/gm to 0.41±0.08mg/gm.

Results depicted in present study revealed the potential of wild mushrooms of Odisha as a good source of bioactive metabolites specifically when compared with the previous analysis done by Ferreira et al.,³³ in addition to that, to best of our knowledge present studies is the first report from the Odisha state of India. Phytochemicals

present in the mushrooms after analysis shows that they can be a good alternative source if compared with the plant sources for the health benefits. Present data can be well compared with the results of plant sources as reported by Edeoga et al.,³⁴ which shows that mushroom is the source of medicinally important agents which is no less than many medicinal and edible plant sources.³⁵ Further studies are targeted towards extraction and purification of these compounds and to evaluate to reach the final conclusion.

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Conflict of interest

The author declares no conflict of interest.

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