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Original Article

BIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF WILD EDIBLE MUSHROOMS USED FOR FOOD BY TRIBAL OF EASTERN INDIA

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ABSTRACT

Objective: The main objective of this research was to analyze some selected indigenous wild edible mushrooms in Eastern India for their novel antioxidant components and their properties specifically used by primitive tribal groups of Eastern India.

Methods: The antioxidant components were analyzed by standardized spectrophotometric methods. The antioxidant properties were analyzed by DPPH Free radical scavenging & Reducing power ability assay.

Results: The TPC (phenolic content) in the studied edible mushroom varied from 4.55 mg/g (*Russula nigricans*) to 0.9 mg/g GAE (*Lentinus tuberigium)*. Measured in term of antioxidants *Termitomyces* group ranked higher than *Russula* and *Volvariella* sp. The scavenging effect of studied mushrooms on 1,1 DPPH varied from 61% to as high as 94%. On the other hand, reducing power (RPA) in methanolic extracts were in the order of *T. clypeatus* (4.21) *T. heimi* (2.20) ~*R. breviceps* (1.73) ~ *Termitomyces eurrhizus* (1.11) ~ *T. rufum* (1.07). Antioxidant potential inedible wild mushrooms are found to be on account of combinations of biochemicals, rather than any such significant individual components as TPC, AA, or alkaloid.

Conclusion: This is for the first time wild edibles such as *Termitomyces clypeatus, Termitomyces eurrhizus, Termitomyces heimii, Russula brevipes, Tuber rufum, Russula nigricans, Volvariella volvaceae, Lentinus fusipes, Lentinus tuberigium* and *R. lepida* from eastern India were observed, collected and subjected to nutritional and biochemical analysis. Of significance is the identification of *Tuber rufum* and *Volvariella volvaceae* growing wild as edible mushrooms which have not been profiled in the Indian context. The analysed mushroom especially *Lentinus fusipes* and *Lentinus tuberigium* was found valuable in terms of iron and calcium, besides having useful phytochemicals such as phenolics, ascorbic acid, carotenoids.

Keywords: Deciduous forests, Orissa, Wild food, Phenolics, Ergosterol, Termitomyces

INTRODUCTION

Oxidative stress induced by reactive oxygen species (ROS) that are generated by metabolic activity as well as lifestyle factors is responsible for the advancement of several chronic diseases. Many epidemiological studies suggest that consumption of polyphenol-rich food is associated with reduced risk of cardiovascular diseases, strokes and cancer in which the phenolics are linked to antioxidant properties [8, 17]. Availability of antioxidants that can mitigate damaging effects of ROS. Therefore, continue to be the focus of nutrition and health research [15]. A multitude of antioxidants has been isolated from different plant materials such as oilseed, vegetables, fruits, herbs [29]. Many of the indigenous herbs and wild edibles used in India and China reportedly contains medicinal and antioxidant properties. Mushrooms such as *Ganoderma lucidum, Ganoderma tsugae* and *Coriolus versicolor* have been widely acclaimed to control a host of diseases, for which it is commercially grown in various countries. But a number of wild mushrooms consumed in tropical countries are yet to be investigated for such values. Mushrooms have also added advantage as an ideal diet, due to high protein content (19-35%) in comparison to traditional food, i.e., wheat (13.3%) and milk (25.2 %) besides having low-fat content and better vitamin C and B level [28, 2, 6].

Mushrooms are now considered as an important source of food and income both in the developing and developed countries as well [39]. Edible mushrooms, in particular, attract attention as functional food and source of natural phytotherapeutic agent, bereft of side effects [32]. Mushrooms have anti-diabetic, cardiovascular and immune-modulating effect in addition to preventing the risk of cancer and controlling blood sugar level [18] with substantive antioxidant activity recorded in both wild and cultivated species [26]. The phenolics, organic acids, and alkaloid contents in mushroom contribute to the antioxidant and free radical scavenging properties of mushrooms on account of their ability to capture metals, inhibit lipo oxydase and scavenge free radicals [34, 1]. The wild edible ones-those consumed by local and tribal communities, in particular, are of interest which could impact the health and nutrition, as because it contains significant protein, flavonoid, β-carotene, lycopene, etc usually not available in a poor man's diet. However, only a few selected species have been worked out to estimate antioxidant components such as phenols, flavonoid, carotene, etc including its antioxidant properties [34, 30] and a number of such valuable wild species are awaiting evaluation.

Biochemical evaluation of mushrooms from Indian Himalayan and wet forest region has been conducted [21]. However, characterization of macrofungal species of dry and deciduous forest region in India is rare. The eastern Indian region has a high tribal concentration and a higher proportion of poverty and hunger compared to the rest of India. Evaluation of food consumed by tribals is, therefore, essential to address malnutrition and for determining the need for dietary supplements from natural sources. Search for low cost and natural ingredients which can provide essential macro and micronutrients could be useful in this context for ensuring access to quality food. The objective of this study was, therefore, to profile the biochemical composition and antioxidant properties often commonly consumed wild mushrooms in tribal dominated areas of Odisha, India.

The eastern Indian state of Orissa harbors mainly *Shorea robusta* along with mixed deciduous forests, with limited patches of moist deciduous and semi-evergreen vegetation. It has a subtropical monsoon and humid climate that receive 1100-1300 mm of average annual rainfall. The diversity of mushroom flora in forests and agroforests of the region is yet to be comprehensively investigated [23], notwithstanding the fact that in tribal dominated districts of Sundargarh, Koraput, Kandhamal, Mayurbhanj, and Keonjhar mushrooms are consumed in many villages during June-September. Selected weekly markets, tribals also sell mushrooms in limited quantity. No attempt has been made so far to estimate the actual

volume of mushrooms harvested from forests or potential yield from wild. On account of the limited quantity coming for sale, the price of mushrooms often exceeds even costliest vegetable in the local market of eastern India. While *Russula* sp. costs Rs. 20-50/-per kg. on average, species as *Tuber rufum* can fetch as high as Rs.130/kg. Unlike other regions, mushrooms are not dried or stored, only fresh mushrooms from the wild are consumed within a day or two. Mushrooms from the border districts also get exported from Orissa to nearby big cities of adjoining states such as Kolkata (West Bengal), Raipur (Chattisgarh), and Tatanagar (Jharkhand).

Ten most commonly consumed mushrooms by tribals and rural people for food were identified (*Termitomyces clypeatus, Termitomyces eurrhizus, Termitomyces heimi, Russula brevipes, Tuber rufum, Russula nigricans, Volvariella volvaceae, Lentinus fusipes, and Lentinus tuberigium, Russula lepida*) by the authors and chemical assays on the antioxidant activity of these wild specimens were conducted. The antioxidant activity of the species was evaluated through reducing power assay and radical scavenging activity on 2,2-DPPH radicals. Bioactive compounds such as phenols, flavonoids, carotenoid, ascorbic acid, and ergosterols available in the fruiting body (cap and stipe) were also determined.

MATERIALS AND METHODS

Sample

Healthy, fresh and clean mushrooms of *Termitomyces clypeatus, Russula brevipes, Termitomyces eurrhizus, Volvariella volvaceae, Tuber rufum, Termitomyces heimi, Lentinus fusipes, Russula nigricans, R. lepida* and *Lentinus tuberigum* were collected from moist deciduous and semi-evergreen forests through field survey across 44 forest blocks of Odisha state during July-September 2011-12. Macroscopic and microscopic examination of pileus, Stipe, veil, ring, volva, lamellae and gills, etc. was done. Further, samples were sent to Agarkar Research Institute, Pune for confirmation of the identity of species. The identified species i.e. *Termitomyces clypeatus* (RPRC/KRPD25), *Termitomyces eurrhizus* (RPRC/MIS58), *Termitomyces heimii* (RPRC/GB28), *Russula brevipes* (RPRC/MIS07), *Tuber rufum* (RPRC/KP33), *Russula nigricans* (RPRC/MIS08), *Volvariella volvaceae* (RPRC/SIM61), *Lentinus fusipes* (RPRC/BG05), *Lentinus tuberigium* (RPRC/GB21) and *R. lepida* (RPRC/BG10) were stored in the herbarium collection centre of Regional Plant Resource Centre, Bhubaneswar. All of the assays were performed by using the whole mushroom fruiting body including stipe. The specimens were cleaned, and subsequently, air dried in the oven at 50 °C for about 2 h. The dried mushrooms were ground to fine powder and stored in an airtight plastic bag in room temperature for further analysis.

Extract preparation

1 gm of dried mushroom powder sample (100 meshes) was mixed with 10 ml of methanol. Samples were stirred for 15 min for effective extraction and centrifuged at 3000 rpm for 20 min. Supernatants were referred to as methanolic extract and kept at 4 °C until analysis [41]. Mushrooms samples were analysed in the microbiology laboratory of Regional Plant Resource Centre, Bhubaneswar, Orissa, India.

Determination of total phenolic content

The total phenolic content in the wild edible mushroom was determined through Folin-phenol method with some modification [35]. The powdered dried mushroom sample (1 gm) was extracted with 10 ml of absolute methanol. Samples were ground in mortar pestle for effective extraction and centrifuged at 2000g for 15 min. A sample of 100 µl was made up to 1 ml with distill water and 1 ml of Folin and Ciocalteau's reagent and thereafter 2 ml of 10% sodium carbonate solution was added to the extract. The total phenolic content in the different extract was measured by the methods of Folin-phenol and expressed as gallic acid equivalent (GAE) in gram per 100 grams of the sample [37]. Gallic acid was used to draw the standard curve using 10-80 µg of gallic acid. The optical density was measured at 765 nm using Analytic Jena spectrophotometer.

Determination of ascorbic acid content

The ascorbic acid content in the edible wild mushrooms was determined by volumetric method [16]. The dye solution was prepared by dissolving 42 mg of sodium carbonate in a small amount of distill water to which 52 mg of 2,6 di-chlorophenol indophenols was added to make the volume up to 200 ml. The powdered sample (0.5-5g) was extracted in 4% oxalic acid and made up to a known volume (100 ml) and centrifuged at 2000g for 15 min. 5 ml supernatant from the extract was added with 10 ml of 4 % oxalic and titrated against the dye (V2 in ml). The initial and final volume of the dye consumed while the appearance of pinkish colour for each sample was noted down. The amount of ascorbic acid in the mg/100g sample were calculated by using formula; 0.5 mg/V_1 ml × V_2/5 ×100/weight of sample ×100 when V_1 is the standard ascorbic acid consumed against dye.

Estimation of flavonoids

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

Estimation of Beta carotene and lycopene

The concentration of β carotene and lycopene in mushroom extracts was estimated spectrophotometrically [25]. Methanolic extract was evaporated to dryness at 40 °C and about 100 mg of methanolic extract was prepared. The dried methanolic extract, i.e., 100 mg was shaken vigorously with 10 ml of acetone and hexane (4:6) for one minute continuously and filtered through Whatman filter paper. The absorbance was measured at 453, 505, and 663 nm. The contents of β-carotene and Lycopene were estimated by:

$$\text{LYCOPENE} = 0.0458 \times \text{A.663nm} + 0.372 \times \text{A.505nm} - 0.0806 \times \text{A.453nm}$$

$$\text{BETA CAROTENE} = 0.216 \times \text{A.663nm} - 0.304 \times \text{A.505nm} + 0.452 \times \text{A.453nm}.$$

Determination of carotenoid content

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

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

$$\text{where A} = \text{Absorbance.}$$

Estimation of tannins

5 gm of mushroom powdered sample was boiled in distilled water for 30 min and filtered through Whatman filter paper [28]. 0.1 ml of the sample extract prepared by above process was treated with 0.5 ml of Folin denis reagent. 1 ml of saturated sodium carbonate and 1 ml of distilled water was added to the reaction mixture and shaken well, and the optical density measured at 760 nm**.** Tannic acid was served as a standard and tannin content estimated was expressed in mg/gm.

Estimation of alkaloids

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

water. 1 ml of the solution was added with 5 ml of urea solution, and the absorbance was measured at 435 nm [33].

Estimation of ergosterol

Sample preparation was done by preparing 1g dry weight of the fruiting body with 10 ml of a mixture of chloroform and methanol (2:1 V/V). The extraction was carried out for 24 h. The homogenate was filtered, and the filtrate was transferred to a separation funnel, shaken well and mixed 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. 1 ml of sample was evaporated to dryness. 6 ml glacial acetic acid was added to this, immediately followed by mixing 4 ml Ferric chloride reagent. The contents were mixed thoroughly, cooled and the colour developed was read at 550 nm against blank. The sterol content was estimated from the standard curve plotted using Ergosterol 10-25ug/ml [31].

Radical scavenging activity assay

The DPPH activity was estimated in the methanolic extracts by a colorimetric method [10]. 1 ml of methanolic extract was added with 2 ml of diphenyl-1-picrylhydrazyl (DPPH) solution (1:2) and incubated for 30 min in dark after vigorous mixing. The methanolic extract was centrifuged, and the supernatant was taken in six different concentrations (0.1 ml-0.6 ml) and treated with DPPH reagent. The optical density was measured at 517 nm and used in the formula to calculate the scavenging percentage and the IC50 value was analyzed from the graph.

Scavenging activity (%) = (1-Absorbance of sample/Absorbance of control) ×100

Ascorbic acid equivalent Antioxidant Capacity (AEAC) was calculated by calibrating the value of above absorbance in a standard ascorbic acid curve and expressed in mg per gram of dried sample.

Reducing power assay

Each mushroom extract (0.5-4 mg/ml) in methanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH-6.6) and 2.5 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After 2.5 ml of 10% trichloroacetic acid had been added, the mixture was centrifuged at 2000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride, and the absorbance was taken at 700 nm (Analytika jena) spectrophotometer. EC 50 value was calculated in mg/ml at 0.5 optical density against reagent blank [26].

Ferric antioxidant reducing power (FRAP)

Methanolic extract was mixed with 0.95 ml of ferric-TPTZ reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-striazine), in 40 mM HCL and 20 mM FeCl3 in the ratio 10:1:1) and absorbance was measured at 593 nm. The calibration curve was obtained with an ascorbic acid stock solution in the concentration of 0.1 mg/ml. Series dilutions of the stock solution were made by pipetting out 10 µl, 20 µl, 30 µl……100 µl and final volume was made up to 100 µl. To the resultant diluted sample of 100 µl, 3 ml of FRAP reagent was added and incubated in the room temperature in dark for 10 min and finally absorbance was read at 593 nm. FRAP value was expressed in terms of mg AEAC/gm of the sample [5].

RESULTS

Biochemical components

Phenolics are naturally occurring antioxidant components found in a methanolic extract from edible wild mushrooms. The total phenolics content had wide variation across studied species. It varied from 0.90±0.01 to 4.55±0.37 mg GAE/g of dry extract. The nine wild edible mushroom species could be categorized into low, moderate and high phenolic species on the basis of the total phenolic component. *R nigricans* and *Russula brevipes* ranked high phenolic species whereas *Lentinus tuberigum*, *Tuber rufum* and *T eurrhizus* can be categorized as low phenolic *and Lentinus fusipes*, *Termitomyces heimii* and *Termitomyces clypeatus* are moderate phenolic mushrooms. The high content of total phenols in *R. nigricans* (4.55±0.37) and *R. brevipes* (3.20±0.04) extracts compared to other studied species account for their reducing power and radical scavenging effect on DPPH radicals (table 1).

Polyphenolic compounds, ascorbic acids and flavonoids are considered as antioxidants in many fruits, vegetables and mushrooms [12, 21]. It had been reported that the antioxidant activity of plant materials is strongly correlated to their phenolic compounds [20] especially BHT (butylated hydroxytoluene) and Gallate, which are known antioxidants. While phenol was the major antioxidant components found in the mushroom extracts, ascorbic acid was found in smaller amounts (0.10-0.069 g/100g). Some similar findings showed a similar trend for *Leucopaxillus, Sarcodon and Agaricus* sp. [7]. Amongst the edibles we studied total phenolic component was highest for *R. nigricans* but ascorbic acid content was maximum for *T heimii*. Although total phenolics was in relatively moderate scale in case of *T. heimi* (2.44 mg/g) the free radical scavenging activity was observed to be highest amongst all edible specimens evaluated in term of DPPH assay value (93.3%). This could be on account of higher ascorbic acid content (0.75g/100g) in this edible species. The result while supports findings of other studies reveal that the phenolic level has not only distinct interspecific variation [41] but also is dependent upon solvent (water/ethanol/methanol) in which the mushroom was extracted. Further, as expected, the level of total phenol content in mushrooms is of much lower level than higher plants having antioxidant properties [33].

Table 1: Antioxidant components of wild edible mushrooms

Species	Carotenoids (mg/g)	Flavonoids (mg/g)	β-carotene (mg/g)	Lycopene (mg/g)	Alkaloids (mg/g)	Tannins (mg/g)	Ergosterol (mg/g)	Total Phenolics (mg of GAE/g)	Ascorbic Acid (g/100g)
Termitomyces clypeatus	1.31±0.10	0.80±0.10	0.208±0.02	0.0762±0.05	0.61±0.06	15.26±0.46	14.2±1.45	2.80±0.01	0.54±0.05
Termitomyces eurrhizus	3.90±0.74	1.18±0.09	0.015±±0.07	0.012±0.06	0.30±0.06	3.36±0.20	5.51±0.31	1.30±0.01	0.32±0.08
Termitomyces heimii	2.89±0.12	0.55±0.16	0.009±0.00	0.035±0.01	0.35±0.10	6.63±0.23	10.73±0.36	2.44±0.01	0.75±0.04
Russula brevipes	1.58±0.09	2.33±0.37	0.037±0.02	0.003±0.02	0.50±0.03	6.81±0.29	9.71±1.07	3.22±0.04	0.42±0.04
Russula nigricans	1.40±0.01	1.09±0.23	0.002±0.01	0.022±0.01	0.58±0.04	7.28±0.27	6.68±0.05	4.55±0.37	0.29±0.05
Tuber rufum	5.42±0.58	1.03±0.19	0.014±0.02	0.005±0.01	0.30±0.09	2.95±0.36	17.9±0.24	1.10±0.02	0.69±0.06
Volvariella volvaceae	5.55±0.41	1.25±0.15	0.053±0.02	0.016±0.00	0.70±0.03	13.36±0.58	12.76±2.83	2.90±0.01	0.10±0.00
Lentinus fusipes	1.81±0.20	2.08±0.37	0.017±0.02	0.004±0.00	0.53±0.04	9.16±0.27	3.95±0.86	3.00±0.04	0.66±0.07
Lentinus tuberigium	2.10±0.32	1.83±0.13	0.025±0.09	0.011±0.00	1.05±0.06	4.83±0.19	18.06±0.55	0.90±0.01	0.40±0.09
Russula lepida	*5.75±0.3*	1.88±0.13	0.056±0.004	0.029±0.002	3.85±0.58	8.33±0.10	0.50±0.10	3.20±0.50	0.58±0.04

Where±represents average and standard deviation of three replicates. *Russula lepida* contained highest carotenoids as compared to other wild edible mushrooms. Total phenolics content of *R. nigricans* was high i.e. (4.22 mg/g). Ergosterol content in *T. rufum* & *L. tuberigium* were comparatively mushed higher than that of other wild mushrooms analyzed.

Table 2: Result of antioxidant assay of wild edible mushrooms

Species	DPPH Scavenging (%)	EC50 (mg/ml)	Reducing Power EC50 (mg/ml)	FRAP (mgAEAC/g)
Termitomyces clypeatus	93.45	8.0	0.30	1.67±0.06
Termitomyces eurrhizus	94.24	15.0	1.50	0.34±0.04
Termitomyces heimi	93.37	10.0	0.80	0.59±0.04
Russula brevipes	93.19	16.0	1.50	0.39±0.03
Russula nigricans	66.70	28.0	2.00	0.53±0.04
Tuber rufum	77.97	43.0	4.00	1.00±0.01
Volvariella volvaceae	65.01	7.00	0.70	1.57±0.10
Lentinus fusipes	90.59	18.0	2.00	0.14±0.01
Lentinus tuberigium	92.94	3.0	0.80	0.36±0.03
Russula lepida	84.38	10	5.0	0.18±0.01

±Standard deviation, n = 3 (replications), AEAC= Ascorbic Acid Equivalent Antioxidant Activity.

Table 3: Correlation between biochemical and antioxidant activities

	Phenol	AA	Beta carotene	Flavonoid	Alkaloid	Tannin
DPPH	-0.42	0.50*	0.20	0.19	-0.04	-0.19
Red. power	0.035	0.35	0.57	0.25	0.64*	0.37
FRAP	-0.08	-0.25	0.65*	-0.56	-0.29	0.64*

Pearson correlation coefficient (*P<0.05)

The total *flavonoid* content of the wild edible species was estimated by a colorimetric technique in terms of 4.4 mg of quercetin equivalent per gram extract of dried mushroom. The maximum total flavonoid was observed in *Russula brevipes* (2.33±0.37 mg/gm) followed by *Lentinus fusipes* and *R lepida*. The lowest amount was recorded for *T. heimii* (0.55±0.16 mg/gm). The level of flavonoid content in edibles of Orissa was observed to be better compared to the other common edible variety, i.e. *Agaricus bisporous* (button mushroom) as reported [40]. But the concentration of flavonoid in the studied species are of much smaller dimension reported for Portugese specimens as *Ramaria botrytis* (16.56 mg/g) or non-edible species as *Hypholoma fasciculare* (5.09 mg/g) [7]. Flavonoids are responsible for radical scavenging activity in plants and show antioxidant action on human health and fitness [14]. The presence of this important phytochemical underscore values of mushrooms in the tribal diet of the region.

Carotenoids content in the mushroom usually corresponds with the colour of the stripe or cap. Colorful mushroom is likely to show a high amount of carotenoid than the colourless ones. In our study highest carotenoid was found for *R lepida* (5.75 mg/gm±0.4) followed by *V. volvaceae* and *T. rufum* The least was in *T. clypeatus* 1.31±0.1 mg/gm (table 1). β-carotene is a precursor to Vitamin A which acts as a powerful antioxidant. In general, the β-carotene and lycopene are found in rudimentary concentration in mushrooms. The content of β-carotene differed considerably amongst the studied edible mushroom species, ranging from 0.002 mg/gm to 0.208 mg/gm. A similar result is also reported for Portuguese mushrooms [7]. In Orissa specimens, highest β-carotene content was found on *T. clypeatus*. Relatively higher lycopene was also observed in *T. clypeatus* i.e. 0.208 mg/gm of the dry weight whereas lowest found in *R nigricans* (0.002 mg/gm). The comparatively higher content of β-carotene and *lycopene* in the *T. clypeatus* accounted for the bright colour of mushroom.

Ergosterols in mushrooms are a precursor for Vitamin D synthesis. *Lentinus tuberigium* showed maximum ergosterol followed by *Tuber rufum* and *T. clypeatus respectively.* Ergosterol content in *R. lepida* was found to be lowest. On the other hand, the ascorbic acid content of *T. heimii* (0.75±0.04 g/100g) was more than *T. rufum, L. fusipes* and *V. volvaceae* (0.10 g/100g).

Secondary metabolites including alkaloid provide medicinal attributes to plants including mushrooms. Estimation of *alkaloids* revealed the highest amount of this active compound is available in *R lepida* (3.85±0.58) followed by *L. tubergium, where* as *T. eurrhizus* and *Tuber rufum* (0.30 mg/gm) contained a very small proportion of alkaloid. Compared to the results of alkaloid reported in the *Boletus edulis* [30]our results shows the relatively high amount of alkaloids in the *Lentinus tubergium* (1.05 mg/gm), *Volvariella volvaceae* (0.70 mg/gm) and *T. clypeatus* (0.61 mg/gm). Similarly, wide variation in tannic acid content was also noticed in the studied samples i.e. 15.26 mg/gm to 2.95 mg/gm. the highest tannin was found in *T. clypeatus* whereas lowest in *Tuber rufum*.

Radical scavenging activity

Free radical scavenging activity is considered an established phenomenon in inhibiting radicals generated from the oxidants. Unlike laboratory generated free radicals such as the hydroxyl radical and superoxide anions, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition [15]. The methanol extract of fruit bodies was subjected to screening for possible antioxidant activity by the DPPH free radical scavenging method [6]. Results are expressed as the ratio of % of sample absorbance decrease, and the absorbance of extract at 517 nm. Free radical scavenging values of extracts as a percentage are shown in Table-2. Scavenging effects of mushroom methanolic extracts on DPPH radicals increased with increase in the concentration (fig.1). Four mushrooms showed very high RSA. The methanol extract of *T clypeatus* (93.45%), *T. eurrhizus* (94.24%), *T heimii* (93.37%), *R brevipes* (93.19) can be grouped as high, since their scavenging ability was as good as the standard Ascorbic acid (Fig.2). *R lepida* (84.38 %), *Lentinus fusipes* (90.59%), exhibited moderate action whereas *R nigricans* (66.70%) and *V volvaceae (*65.01) showed very little RSA. The lower EC 50 value also supports the stronger antioxidant ability of *T clypeatus* and *L. tubergium*.

Reducing power

Reducing the power of methanolic extracts from edible wild mushrooms increased steadily with the increase in the concentration (fig. 3). The presence of reducers causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. At 4 mg/ml, reducing powers was in order *Termitomyces clypeatus* (4.212)>*Volvariella volvaceae* (2.27)>*Termitomyces heimii* (2.201)>*Russula brevipes* (1.734)> *Russula nigricans* (1.152)>*Termitomyces eurrhizus (*1.119)>*L fusipes* (1.075)>*T. rufum* (0.503)>*R lepida (0.174)>L. tuberigium* (0.263). Thus, *R lepida* and *L. tuberigium* showed a lower reducing power than *Termitomyces sp*. The reducing power ability of edible wild mushrooms might be due to their hydrogen donating ability also reflected in their EC 50 value (table 2). The result indicated wild mushrooms such as *T. clypeatus* and *V. Volvaceae* are likely to contain a higher amount of reductone, which could react with radicals to stabilize and terminate radical chain reactions.

FRAP value ranged from 1.67 to 0.14 mg/gm. A high value of FRAP was seen for *V. volvacea* which also contained maximum TPC and

ascorbic acid indicating a strong correlation of these antioxidant component contributing to the bioactivity. The result suggested that the antioxidant components in edible wild mushrooms are capable of reducing oxidants and scavenging free radicals. Interestingly, edible mushrooms such as *V. volvaceae, T. rufum and T. clypteaus* showed higher FRAP value than fruit as Peaches, which ranged from 0.84-1.2 mg AEAC/g [13].

Free radical scavenging is one of the mechanisms commonly used to estimate antioxidant activity. Many of our samples showed significant antioxidant activity compared to commonly consumed mushroom as *Agaricus bisporus* (67.86%). The methanol extract of *Tuber rufum,* and *Russula nigricans* of eastern India, in particular, showed promising scavenging activity both in term of DPPH scavenging and FRAP assay.

Antioxidant activities of methanolic extracts of several commercial and medicinal mushrooms have been reported [24]. And as observed in our study, wild mushrooms compare very well with commercial varieties [42, 43]. The phyto-theraputic ability of macrofungus is on account of the free radical scavenging enzymes and antioxidant substance, resulting from not any single but combinations bioactive compound such as phenolics, flavonoids, carotenoids etc.

The relationship between the antioxidant properties of mushrooms with biochemical constituents was statistically evaluated. The significant coefficient value obtained from the correlation analysis indicated no specific pattern, rather different constituents independently and in combination seem to influence RSA, FRAP assay, etc (table 3). Ascorbic acid seems to be more potent than total phenolic content in defining DPPH antioxidant behavior whereas alkaloid and tannin or β carotene had a stronger positive association to explain the ferric antioxidant, reducing power. The result points to the fact that differential biochemical parameter both in term of its variety and concentration is linked to phyto-therapeutic and nutritional potential of different mushrooms.

DISCUSSION

Reports from Black sea region of Turkey represent phenolics content of dry weight *Russula nigricans* to be 4.66 mg of GAE/gm. The *Russula nigricans* analyzed from Orissa contains 4.55 mg of GAE/g. The scavenging capacity of *R. nigricans* from Black sea region of Turkey was reported to be 78.16%, and our sample showed 66.70 % scavenging capacity [19]. Research studies show that flavonoid content of popular edible mushrooms like *Pleurotus florida* is which is comparatively less than the analyzed *Russula brevipes* (2.33 mg/g) and *Lentinus fusipes* (2.08 mg/g) [21]. Therefore edible wild mushrooms can be stated as a good potential holder of antioxidant components in comparison with the traditionally used edible mushrooms.

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CONCLUSION

Wild mushrooms are becoming more and more important in our diet for their nutritional and constituents [12, 20]. Simultaneously, their demand by the urban population is also increasing due to good amount of proteins and trace minerals [20]. Especially mushroom is sought after as an alternative to meat and fish for health conscious urban dwellers. The study informs the result of biochemical assay in respect of 10 wild mushrooms used for food by the tribals in the eastern state of India. This is for the first time wild edibles such as *Termitomyces clypeatus, Termitomyces eurrhizus, Termitomyces heimii, Russula brevipes, Tuber rufum, Russula nigricans, Volvariella volvaceae, Lentinus fusipes, Lentinus tuberigium* and *R lepida* from eastern India were observed, collected and subjected to nutritional and biochemical analysis. Of significance is the identification of *Tuber rufum* and *Volvariella volvaceae* growing wild as edible mushrooms which have not been profiled in the Indian context. The analyzed mushroom especially *Lentinus fusipes* and *Lentinus tuberigium* was found valuable in terms of iron and calcium, besides having useful phytochemicals such as phenolics, ascorbic acid, carotenoids. The antioxidant properties of commonly consumed wild mushrooms emphasizes the necessity for detailed biochemical and analytical profiling on amino acid content, and proximate for other wild varieties which are also used for treating disease in some localities of the region.

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CONFLICT OF INTERESTS

Declare none

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