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# Study of nutritional and antibacterial potential of some wild edible mushrooms from Gurguripal Ecoforest, West Bengal, India

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Mushrooms are important natural resources concerning human health, nutrition and disease prevention. The present study was conducted for the exploitation of wild edible mushrooms of Gurguripal Ecoforest. The analysis of nutrients in *Termitomyces heimii*, *Astraeus hygrometricus*, *Leucopaxilus* sp., *Amanita vaginata*, *Agaricus campestris*, *Russula delica*, *Schizophyllum commune*, *Pleurotus ostreatus* and *Cantharellus* sp. on dry wt basis showed that these mushrooms were rich in proteins (20.4-38.3%) and carbohydrates (33.2-48.4%), while lipid contents were relatively low (0.8-6.2%). The fibre and ash contents ranged 2.0-18.6% and 2.3-14.9%, respectively. Antibacterial properties of mushroom extracts were studied against some human pathogens like *Escherichia coli* MTCC118, *Shigella flexneri* MTCC7061, *Staphylococcus aureus* MTCC96, *Streptococcus faecalis* MTCC5383, *Salmonella typhi* MTCC734, *Klebsiella pneumoniae* MTCC109, *Enterobacter aerogenes* MTCC111, *Vibrio cholerae* MTCC3906, *Pseudomonas aeruginosa* MTCC741 and *Bacillus subtilis* MTCC441. Acetone extracts of *P. ostreatus* and *T. heimii* showed noticeable antibacterial potentialities against *S. aureus* and *K. pneumoniae*, respectively. Biosynthesis of nanoparticles using mushroom extracts was performed to increase the effectiveness of antibacterial potentials. Silver nanoparticles were synthesized using extracts of *Volvariella volvacea* and applied against *E. coli* and *S. flexneri*, which showed inhibition zones of 15 and 18 mm respectively. The silver nanoparticles were also characterized through UV-Visible spectroscopy and FTIR analysis.

**Keywords:** Antibacterial properties, silver nanoparticle, wild mushroom

## Introduction

Mushrooms are the macroscopic fruit bodies of higher fungi. They can be epigeous or hypogeous, large enough to be seen with the naked eyes and can be picked by hand[1]. Wild edible mushrooms have been collected and consumed by people for thousands of years. Due to the high content of vitamin, protein and minerals, fibres and trace elements, and no or low calories and cholesterol[2,3], they are considered as " poor man's protein". During the early days of civilization, nutraceutical potentials of wild mushrooms were evident. In the present century, wild mushrooms are increasingly popular in our diet for their nutritional and pharmacological potentials in order to promote health status.

Mushrooms need antibacterial and antifungal compounds to survive in their natural environment. It is, therefore, not surprising that antimicrobial compounds with more or less strong activities could be isolated from many mushrooms and that they could be of benefit for human health[4]. The secondary metabolites of these mushrooms are chemically diverse and possess a wide spectrum of biological activities, which are explored in traditional medicines[5]. *Astreaus hygrometricus* and *Pleurotus ostreatus* reported with immune enhancing activity[6]. Antioxidant and antimicrobial activities are well proved in *Cantharellus cibarius*[7]. Species of *Agaricus* showed efficient antioxidant, antimicrobial and also antiproliferative activity[8]. Despite the huge diversity of antibacterial compounds, bacterial resistance to first choice antibiotics has been drastically increasing. Natural resources have been exploited in the last few years and among them mushrooms could be an alternative source of new antimicrobials.

Nanotechnology is mainly concerned with the synthesis of nanoparticles of variable size (10-1000 nm), shape, chemical composition and their potential use for human benefits. In the past decade there has been a tremendous amount of research interest in nanomaterials with respect to its production, properties and applications[9,10]. Biosynthesis of silver nanoparticles showed strong antibacterial activity against a few pathogenic bacteria[11].

The medicinal use of mushrooms has a very long tradition in the Asian countries in general and India in particular, with rich biodiversity of mushrooms has been exploited for their use as food, medicine,

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minerals, drugs[12]. As per all the available literature, there are no reports about the nutritional compositions and bioactive potentials of wild mushrooms of Gurguripal Ecoforest. Concerning this fact, the aim of the present investigation was to evaluate nutritional values and antibacterial potentials of wild mushrooms occurring in Gurguripal Ecoforest, West Bengal, India.

## Materials and Methods

**Collection and Identification of Mushrooms**

The mushroom samples were collected from Gurguripal Ecoforest during rainy season between June to October 2016 from different habitats like damp pits, decaying woods, forest litters and termite nests. The specimens were carefully uprooted to avoid damaging the tissue portions of mushrooms. The collected mushroom specimens were analysed vividly by macroscopic and microscopic characteristics and the identification were done by comparing with descriptions given in authentic identification manuals and standard literatures. Mushroom samples were preserved in 4% formaldehyde solution and the major amount of specimen was dried for further analysis.

**Moisture Content**

About 20 g of fresh mushroom was weighed into a weighed moisture box and dried in an oven at ~100-105ºC for 1 h 30 min and cooled in a desiccator. The moisture content was calculated as per the equation by Raghuramulu *et al*[13].

**Determination of Total Protein**

About 5 g of dried and grinded mushroom powder was taken with 50 mL of 0.1 N NaOH and boiled for 3 min. The solution was cooled at room temperature and centrifuged at 1000× g by a tabletop centrifuge. The supernatant was collected and total protein content was measured according to the method of Lowry *et al*[14].

**Determination of Total Lipid**

About 5 g of dried and grinded mushroom powder was suspended in 50 mL of chloroform: methanol (2:1 v/v) mixture, then mixed thoroughly and let stand for 3 d. The solution was filtered and centrifuged at 1000× g. The upper layer of methanol was removed by Pasteur pipette and chloroform was evaporated by heating. The remaining substance was the crude lipid and its amount was calculated by the method of Floch *et al*[15].

**Determination of Crude Fibre and Total Ash**

The amount of crude fibre and total ash of dried mushroom were determined using the method described by Raghuramulu *et al*[13].

**Determination of Carbohydrate**

The content of the available carbohydrate was determined by the following equation[13]:

$$\text{Carbohydrate (g/100 g) sample} = \frac{100 - (\text{moisture} + \text{protein} + \text{ash} + \text{crude fibre})\ \text{g}}{100\ \text{g}}$$

**Preparation of Mushroom Extracts**

Dried and powdered mushroom samples were mixed with extracting solvents like water (W), acetone (A), chloroform(C) and methanol (M) in a ratio 1:5 (w/v). Samples were then kept in shaking condition at 130 rpm for 24 h at 37°C. The extracts were filtered by using Whatman no. 4 filter paper and stored at 4°C until further use.

**Antibacterial Assay of Mushroom Extracts**

The antibacterial properties of methanol, chloroform, acetone and aqueous extracts of the selected mushroom species were studied by agar well diffusion method against some pathogenic bacteria like *Escherichia coli* MTCC118, *Shigella flexneri* MTCC7061, *Staphylococcus aureus* MTCC96, *Streptococcus faecalis* MTCC5383, *Salmonella typhi* MTCC734, *Klebsiella pneumoniae* MTCC109, *Enterobacter aerogenes* MTCC111, *Vibrio cholerae* MTCC3906, *Pseudomonas aeruginosa* MTCC741 and *Bacillus subtilis* MTCC441. Antibacterial spectrum of extracts was determined in terms of zone of inhibition.

**Synthesis and Characterization of Silver Nanoparticles**

About 5 g of fresh *Volvariella volvacea* was boiled with double distilled water for 10 min and then filtered through Whatman No. 1 filter paper. For the biosynthesis of silver nanoparticles the filtrate was added to 100 mL of 0.001M $AgNO_3$ (prepared in deionized water) and incubated at 40ºC with 150 rpm for 24 h. The gradual change in colour after 12 h was observed and the formation of nanoparticles were characterized by UV-Visible spectroscopy under the range of 200-600 nm using a Hitachi UV-Vis spectrophotometer.

***Fourier Transform Infrared Spectroscopy (FTIR) Study***

The residual solution after reaction was centrifuged at 10,000 rpm for 15 min and the resulting suspension was washed with deionized water to get pure nanoparticles free of proteins/enzymes. After that the nanoparticles were analyzed through FTIR and the spectra were recorded from 400 to 4000 $cm^{-1}$, with a resolution of 4 $cm^{-1}$.

## Results and Discussions

A total of 22 mushroom species (Fig. 1) were collected and identified during the study. The nutritional composition of 10 prime wild edible mushrooms of Gurguripal Ecoforest is shown in Fig. 2. The moisture content of the studied mushroom ranged 76.2-88.4% on dry wt basis, while the protein content of mushrooms ranged 20.4-38.3% on dry wt basis. Particularly the species like *P. ostreatus*, *V. volvacea*, *A. campestris* and *T. heimii* showed high (33.2-38.3%)

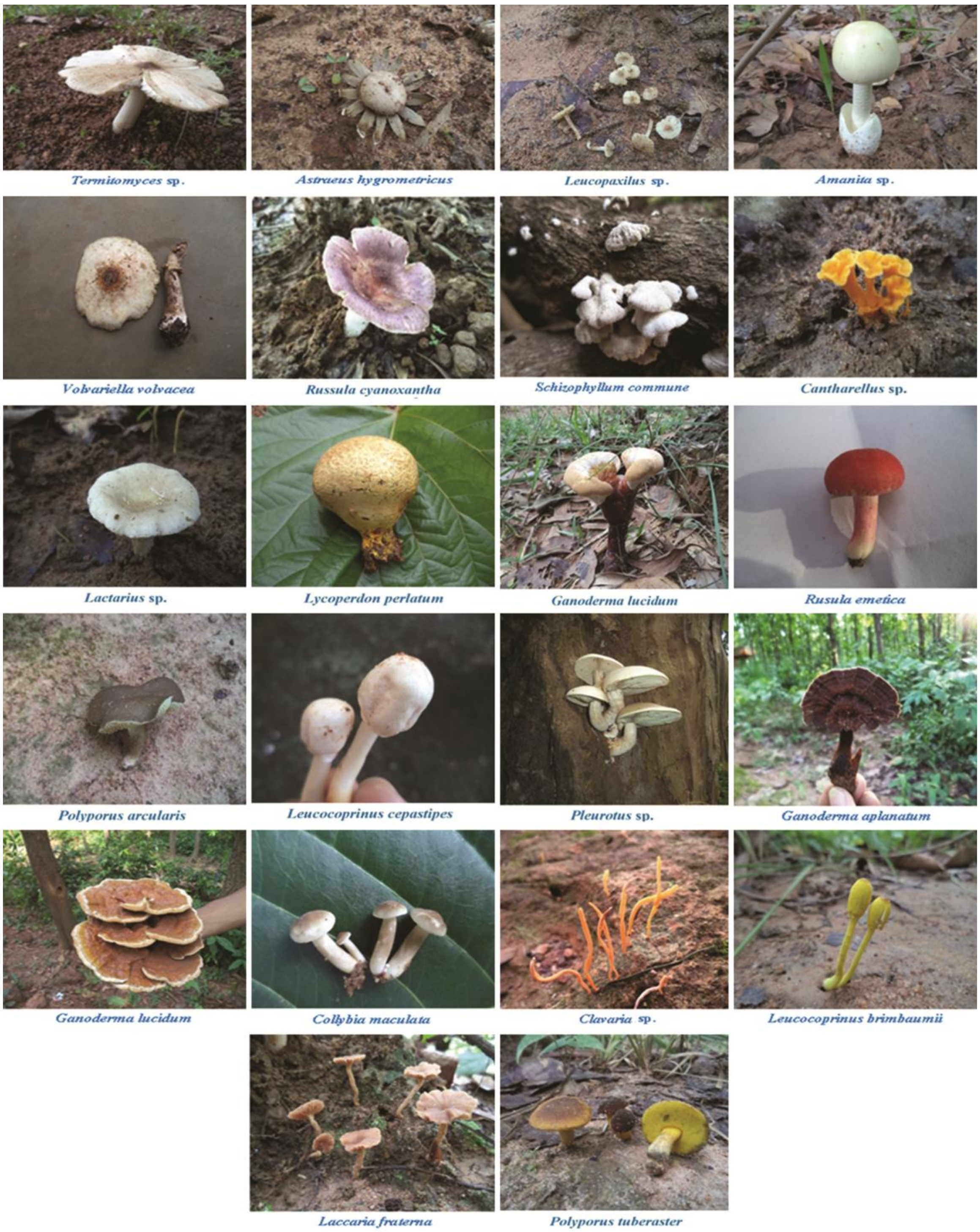


Fig. 1 — Some wild edible mushrooms from Gurguripal Ecoforest.

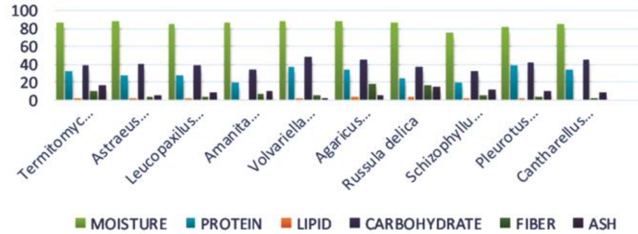


Fig. 2 — Nutrient profile of wild edible mushrooms from Gurguripal Ecoforest.

protein content. However, it is known that the protein contents of mushrooms are affected by a number of factors, namely, type of mushrooms, stage of development, part sampled, level of nitrogen available and the location[16]. The results pertaining to lipid (fat) content in mushrooms studied showed very low amount of lipid ranging 0.8-6.2%. These high protein and low fat characteristics makes the mushrooms an ideal snack material. The carbohydrate content of mushrooms ranged from 33.2-48.4%, where *V. volvacea*, *A. campestris*, *C. cibarius* and *P. ostreatus* showed high (42.6-48.4%) content of carbohydrate. The edible mushrooms rich in high sugar contents are regarded as biologically and medicinally active and can be used as nutraceuticals[17]. Among the studied-mushroom species, *A. campestris*, *R. delica* and *T. heimii* showed high (10.8-18.6%) fibre content, which is significant and desirable as fibre plays an important role in digestion. These mushrooms were also found rich in mineral contents. Analysis of total ash ranged 2.3 to 16.7% on dry wt basis. The nutritional value of wild mushrooms differs according to the species but this difference also depends on the mushroom substratum, atmospheric conditions, age and part of the fructification. Earlier Pushpa and Purushothama[18] have found protein, carbohydrate, lipid/fats, ash and fibre contents in mushrooms as 26.25, 34.88, 5.38, 17.92 and15.42%, respectively. These results show similarity with our results, where protein, carbohydrate, lipid, total ash and fibre contents were recorded as of 24.2, 36.7, 6.2, 14.9 and 16.4%, respectively. Wild edible mushrooms are known to have high nutritional value and are important sources of food supplement for tribal communities that live in consonance in nature and heavily dependent on forest resources to solve malnutrition problem[19]. Thus, the present study clearly shows that wild edible mushrooms are substantially rich in nutrients with relatively high protein, which can enrich human diet especially in villages of our country.

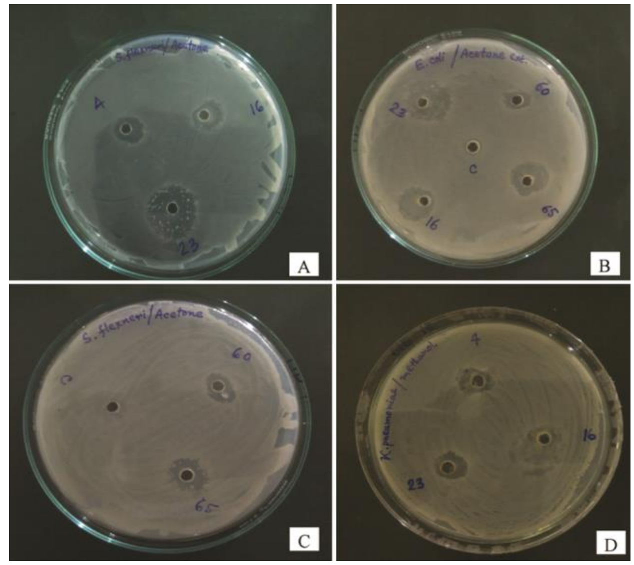


Fig. 3 — Antibacterial activity of mushroom extracts against human pathogens. [Pathogens: A & C, *S. flexneri*; B, *E. coli*; D, *K. pneumonia*. Mushroom extracts: 4, *A. hygrometricus*; 16, *P. ostreatus*; 23, *V. volvacea*; 60, *R. delica*; 65, *T. heimii*.]

The increasing resistance of bacterial pathogens to antibiotics has drawn the attention of researchers to natural alternative treatments of bacterial infections as potential sources of novel antimicrobial agents. Acetone extracts of *P. ostreatus* and *T. heimii* showed noticeable antibacterial potentialities against the pathogen *S. aureus* and *K. pneumoniae*, respectively. Methanol extract of *V. volvacea* showed maximum inhibitory zone in case of *E. coli* (Fig. 3). These results reveal that mushrooms could play an important role for the future development of next generation antibacterial drugs.

The mushroom extract was responsible for the reduction of silver ions and absorbed on the surface of silver nanoparticles accounting for their stabilization[20]. In the preparation of 7 nm silver nanoparticles, the reduction reaction was carried out at pH 10. At this pH value, it is expected that phenol groups are ionized, so that the reduction reaction occurs very fast. The mushroom extract reduced the silver ions and the protein secreted by the mushroom became coated on the nanoparticles (core-shell), which increased their stability. The reduction of silver nitrate to silver nanoparticles was indicated by the colour change from pale yellow to reddish brown. The colour arises due to excitation of surface plasmon resonance (SPR) in the metal nanoparticles. The absorption spectrum of the silver nanoparticles is presented in Fig. 4. The result of FTIR study of the nanoparticles is depicted in Fig. 5

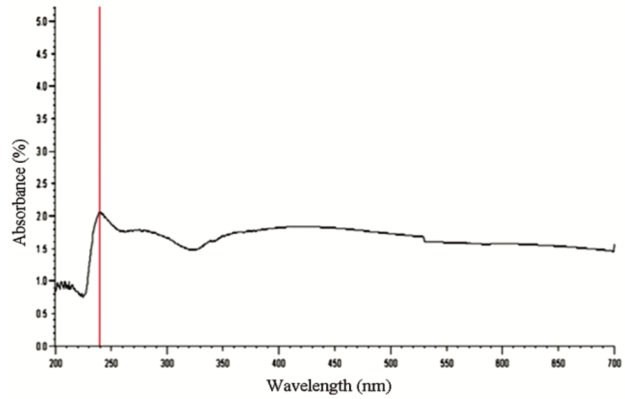


Fig. 4 — UV-Vis spectrum analysis of silver nanoparticles during synthesis using *V. volvacea*.

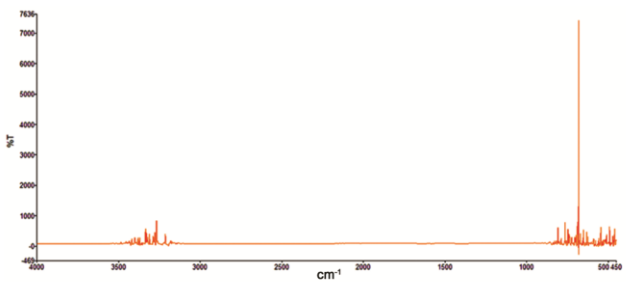


Fig. 5 — FT-IR study of silver nanoparticles synthesized using *V. volvacea*.

and reveals that the absorption bands were around at 3400 $cm^{-1}$ and 690 $cm^{-1}$, which are characteristics of carbohydrate rings and halogen compounds, respectively[21]. FTIR measurement is one of the alternatives to identify the possible biomolecules of the nanoparticles synthesized using mushroom extract[22].

In the present work, silver nanoparticles (AgNPs) were synthesized using paddy straw mushroom, *V. volvacea*, which exhibited increased antibacterial activity and showed 15 and 18 mm clear zone against *E. coli* and *S. flexneri*, respectively.

## Conclusion

The wild edible mushrooms of Gurguripal Ecoforest were found to be rich in nutrients as well as exhibited significant antibacterial potentials. So, these wild mushrooms could play a vital role by not only developing lower cost and better quality drugs but also improving the food status and create employment especially in villages of our country. The present study also suggests the need to intensify research in identifying and screening of wild mushrooms having nutraceutical and medicinal properties in order to commercialize their production and marketing, which in turn will boost the food and pharmacological industry.

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## References

1 Chang S T & Miles P G, Mushroom biology—A new discipline, *Mycologist*, 6 (1992) 64-65.
2 Agrahar-Murugkar D & Subbulakshmi G, Nutritional value of edible wild mushrooms collected from the Khasi hills Meghalaya, *Food Chem*, 89 (2005) 599-603.
3 Wani B A, Bodha R H & Wani A H, Nutritional and medicinal importance of mushrooms, *J Med Plants Res*, 4 (2010) 2598-2604.
4 Lindequist U, Teuscher E, Narbe G, Neue Wirkstoffe aus Basidiomyceten, *Z Phytother*, 11 (1990)139-149 (in German).
5 Rai M, Tidke G & Wasser S P, Therapeutic potential of mushrooms, *Nat Prod Rad*, 4 (2005) 246-257.
6 Maity K K, Patra S, Dey B, Bhunia S K, Mandal S *et al*, A heteropolysaccharide from aqueous extract of an edible mushroom, *Pleurotus ostreatus* cultivar: Structural and biological studies, *Carbohydr Res*, 346 (2011) 366-372.
7 Ramesh C & Pattar M G, Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of Western Ghats of Karnataka, India, *Pharmacogn Res*, 2 (2010) 107-112.
8 Jagadish L K & Krishnan V V, Shenbhagaraman R & Kaviyarasam V, Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J.E. Lange) Imbach before and after boiling, *Afr J Biotechnol*, 8 (2009) 654-661.
9 Narayanan K M & Sakthivel N, Biological synthesis of metal nanoparticles by microbes, *Adv Colloid Interface Sci*, 156 (2010) 1-13.
10 Huang C C, Yu C C, Chen S Y, Yao Y D & Lai J Y, Magnetization reversal of Co/Pd multilayers on nanoporous template, *Nanoscale Res Lett*,7(2012)41
11 Muthukrishnan L & Nanda A, Geno-toxic study of silver bio-nanoparticles toward Gram-positive and Gram-negative clinical isolates, *J Pharm Res*, 6 (2013) 725-729.
12 Ajith T A & Janardhanan K K, Indian medicinal mushrooms as a source of antioxidant and antitumor agents, *J Clin Biochem Nutr*, 40 (2007) 157-162.
13 Raghuramulu N, Madhavan N K & Kalyanasundaram S, *A manual of laboratory techniques* (National Institute of Nutrition, Hyderabad, India) 2003, 56-58.
14 Lowry O H, Rosebrough N J, Farr A L & Randall R J, Protein measurement with the Folin phenol reagent, *J Biol Chem*, 193 (1951) 265-275.
15 Folch J, Lees M & Sloane-Stanely G H, A simple method for the isolation and purification of total lipids from animal tissues, *J Biol Chem*, 226 (1957) 497-509.
16 Flegg P B & Maw G, Mushrooms and their possible contribution to world protein needs, *Mushroom J*, 48 (1977) 395-403.
17 Wasser S P & Weis A L, Medicinal properties of substances occurring in higher Basidiomycetes

mushrooms: Current perspective (review), *Int J Med Mushrooms*, 1 (1999) 47-50.

18 Pushpa H & Purushothama K B, Biodiversity of mushrooms in and around Bangalore (Karnataka), India, *Am-Euras J Agric Environ Sci*, 12 (2012) 750-759.

19 Panda M K & Tayung K N, Documentation and ethnomedicinal knowledge on wild edible mushrooms among ethnic tribes of northern Odhisa, India, *Asian J Pharm Clin Res*, 8 (2015) 139-143.

20 Wang H, Ng T B & Ooi V E C, Lectins from mushroom―A review, *Mycol Res*, 102 (1998) 897-906.

21 Peng Y, Zhang L, Zeng F & Xu Y, Structure and activity of extracellular polysaccharides from mycelium, *Carbohydr Polym*, 54 (2003) 297–303.

22 Philip D, Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract, *Spectrochim Acta (A) Mol Biomol Spectrosc*, 73 (2009) 374-381.