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Research Article

Physicochemical, Antioxidative and Cytotoxic Properties of *Entoloma lividoalbum*

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ABSTRACT

Recent investigations are establishing *Entoloma lividoalbum* as an important medicinal mushroom which is found in the hilly regions of West Bengal, India, that makes it extremely important to authenticate the crude mushroom samples before processing for drugs in the modern times, providing quality and health assurance for both the developers and thousands of unaware consumers. The present study attempts to standardize *Entoloma lividoalbum* based on physico-chemical and chromatographic features and to partially screen its antioxidant activity and cytotoxicity on human alveolar adenocarcinomic cell line A549. The physico-chemical parameters included loss on drying, ash value, fluorescence analysis which were determined by standard methods. Chromatographic parameters entailed the HPLC profile of methanolic extract of *E. lividoalbum*, the chromatographic peaks were noted as signature parameters for identification of the mushroom. Preliminary mycochemical analysis of the methanol extract indicated presence of flavonoids and phenols. Quantitatively the major bioactive components in the extract were present in the following order: phenol> flavonoid> β-carotene~ lycopene in the methanolic extract. In terms of partially screening the antioxidant potential of the extract, DPPH radical scavenging activity (EC_{50} value 0.978 mg/ml) and total antioxidant capacity (70.96 µg AAE/mg of extract) were determined. The distinctive features established in this work are steps in identification, standardization and quality control of the medicinal mushroom.

Keywords: Antioxidant property, cytotoxicity, HPLC, Physico-chemical evaluation.

INTRODUCTION

Mushrooms have been used in oriental therapies since the Neolithic age. The traditional knowledge was validated by contemporary research, which has proved mushrooms to possess antioxidative[1,2], antidiabetic[3], antimicrobial[4,5], antiparasitic[6,7], antineoplastic[8,9], antiulcer[10] and hepatoprotective[11] effects. This has irrevocably established the fact that mushrooms make up a vast source of new powerful pharmaceutical products. Compositional analyses of medicinal mushrooms revealed that they are an unlimited source of bioactive polysaccharides, polysaccharide-protein complexes and high molecular weight and low molecular weight compounds[12]. These bioactive components have been reported to be effective anticancers[13], and also as strong immunomodulators[14-16].

Entoloma lividoalbum is a wild edible mushroom commonly found in the hilly regions of West Bengal, India. Very recent research has reported potential antioxidative effect of the ethanolic extract of this mushroom[17]. Purified β- glucans from *E. lividoalbum* have been shown to have antioxidant[18] and immunomodulatory activities[19], as well as protective effect on human lymphocytes[20].

In view of the diverse medicinal prospects of *E. lividoalbum*, the current study has been undertaken to evaluate the detailed pharmacognostic profile of the powdered fruitbodies of the mushroom, which will be useful to pharmaceutical industries for the authentication of their commercial samples. In the process, we have also shown that *E. lividoalbum* has potential cytotoxic effect against human alveolar adenocarcinomic cell line A549.

MATERIALS AND METHODS

Mushroom sample

The mushroom *Entoloma lividoalbum* was purchased from the local markets of Darjeeling, West Bengal, India. Identity of this mushroom was authenticated using standard literature. A specimen was also kept in the herbarium following the process described by Prakash et al., (2015)[21]. Basidiocarps were dried at 40°C for 24 hours and pulverized using an electric blender and sieved through 160 mesh and stored in an air-tight container.

Physicochemical, and organoleptic characterization of the powdered basidiocarps

Different characters like color, odor, taste, nature of powdered samples were evaluated. Physico-chemical constants such as loss on drying, total ash[22], water holding capacity, swelling capacity[23] and methanol soluble extractive value were also evaluated.

Fluorescence analysis

A small quantity of dried sieved mushroom powder was placed on a grease free clean microscopic slide and 1-2

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drop of freshly prepared reagent was added to it. Then the slide was placed inside the UV viewing chamber and viewed in visible light, short (254 nm) and long (365 nm) UV light. The color observed by application of Hager's, Mayer's, Dragendroff's, iodine solution, 1N nitric acid, phloroglucinol, Barfoed's, sodium nitroprusside, ninhydrin, ferric chloride, 1N NaOH, acetic acid, 1N HCl, methanol and 1N NaOH in methanol in different radiations were recorded.

Quantitative estimation of some important phytochemicals from methanol soluble extract

10 grams of the dried and powdered basidiocarps were extracted with methanol or 48 h with regular agitation. The extract was then dried in a rotary evaporator and stored for further experiments. Total phenol, flavonoid, β-carotene and lycopene contents were estimated using standard published procedures[24-26]. Phenols were expressed as µg of gallic acid equivalents per mg of dry extract, while flavonoid content was expressed as µg of quercetin equivalents per mg of dry extract. Ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye[27].

HPLC of methanol soluble extract

The methanolic extract was filtered through 0.2 µm filter and 20 µl filtrate was loaded in the HPLC system (Agilent, USA). Separation was achieved on an Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) using a flow rate of 0.8 ml/min at 25℃. The mobile phase consisted of eluent A (acetonitrile) and eluent B (aqueous phosphoric acid solution, 0.1% v/v). A gradient program was used for elution: 0-2 min, 5% A; 2-5 min, 15% A; 5-10 min, 40% A; 10-15 min, 60% A; 15-18 min, 90% A. The absorbance of sample solution was measured at 280 nm[28].

Antioxidant assay

Total antioxidant capacity and DPPH radicals scavenging activity of methanolic extract of *E. lividoalbum* was analysed using previously published methods[29]. In brief, different concentrations of the methanolic extract was added to a 0.004% solution of DPPH and incubated in the dark for 30 min. Absorbance was then measured at 517 nm. In case of Total antioxidant capacity assay, tubes containing extract (1 mg/ml) and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95˚C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

Cell culture and Cytotoxic effect of E. lividoalbum

Human alveolar adenocarcinomic cell line A549 was purchased from the cell line repository at NCCS, Pune and maintained in Ham's F-12 medium, with 10% Foetal Bovine Serum in a humidified CO_2-Incubator at 5% CO_2 level and 37° C. confluent cells were trypsinized with 0.25% trypsin-EDTA and seeded in a 96-well microtiter plate (10^4 cells per well) and incubated under the same conditions for 24 hours. The methanolic extract was dried in a rotary evaporator and redissolved in DMSO to obtain a concentration of 500 mg/ml. This extract was further diluted for experiments. At all circumstances, the percentage of DMSO was not greater than 1%. The cells seeded in the 96-well plate were treated with different concentrations of the extract. After incubating the plate for 24 hours, cytotoxicity was measured with iMark™ Microplate Absorbance Reader (BIO-RAD, USA) employing WST-I reagent (TaKaRa) according to manufacturer protocol.

For the partial determination of the nature of cytotoxicity, change in nuclear morphology was studied by staining the nuclei of the treated cells with DAPI. To that effect, the cells were washed with PBS, fixed with 4% paraformaldehyde and incubated for 10 mins with 300nM DAPI solution. The cells were visualized under Floid Cell Imaging Station (Life Technologies, USA).

RESULT AND DISCUSSION

Organoleptic and physicochemical characterization

Organoleptic study was conducted with the sieved powder. The powder was brown in color, with biscuit like odor, it was salty to taste and a little pungent and of sandy texture. Physico-chemical parameters of the powder were evaluated as they are important for identifying adulterants and improper handling of drugs. The percentage of water and volatile matter in crude drug was determined by loss on drying test. Result showed presence of low of moisture content, revealing a value of 9.375%. Determination of total ash is important in the evaluation of purity of drug because it confirms the presence or absence of foreign inorganic matter. The total ash was found to be 6.3461% w/w. The hydration properties of the powdered mushroom, including the solubility, swelling capacity are important traits for evaluating physiological and functional characteristics. Water holding capacity and swelling capacity of the powder were 4.5 ml/g and 1.8974 g per gram o powder respectively.

Fluorescence analysis

Fluorescence is useful for authenticating samples and recognizing adulterants and thus is an important pharmacognostic character. The drug was allowed to react with different reagents which resulted in fluorescence under UV light. As a result fluorescence analysis displayed an array of colors that could be employed for identification of probable classes of compounds in the mushroom[30]. In the present study, the powdered drug was treated with 16 different chemical reagents which showed respective colour, characteristic to the reagent when viewed under UV light (365 nm and 254 nm) and was compared with the color observed under ordinary light (Table 1). In recent times powdered basidiocarps of different medicinal mushrooms were subjected to fluorescence analysis[31-34] and it has been found to be an useful tool for authenticating the purity of the material.

Phytochemical screening

The methanol soluble extract was prepared to determine the presence of some important bio-organic groups which confirms the medicinal properties of this mushroom. The extractive value of the methanol soluble fraction was 17.68%. Table 3 summarizes the total phenol, flavonoids,

Table 1: Fluorescence analysis of dry powder *Entoloma lividoalbum.*

Sl No.	Reagent	Visible	UV	
			Long (365nm)	Short (254nm)
1	Powder as such	Brown	Black	Light Green
2	Hager's	Yellowish Brown	Black	Greenish
3	Mayer's	Yellow	Black	Light green
4	Dragendroff's	Yellow	Black	Green
5	Iodine solution	Yellowish Brown	Black	Green
6	1(N) HNO_3	Brown	Black	Light green
7	50% HNO_3	Brown	Black	Light green
8	FLORO	Yellowish Brown	Black	Greenish
9	Barfoed reagent	Green	Black	Green
10	Sodium nitroprusside	Yellowish brown	Black	Green
11	Ninhydrin	Brown	Black	Black
12	$FeCl_3$	Yellow	Black	Yellowish Green
13	1(N)NaOH	Yellowish Brown	Black	Light Green
14	Acetic acid	Yellow	Black	Greenish
15	1(N)HCl	Brown	Black	Greenish
16	Methanol	Brown	Black	Light green
17	1(N) NaOH in methanol	Black	Black	Light green
18	Ethanol	Brown	Black	Green
19	Chloral hydrate solution	Dark Brown	Black	Brown

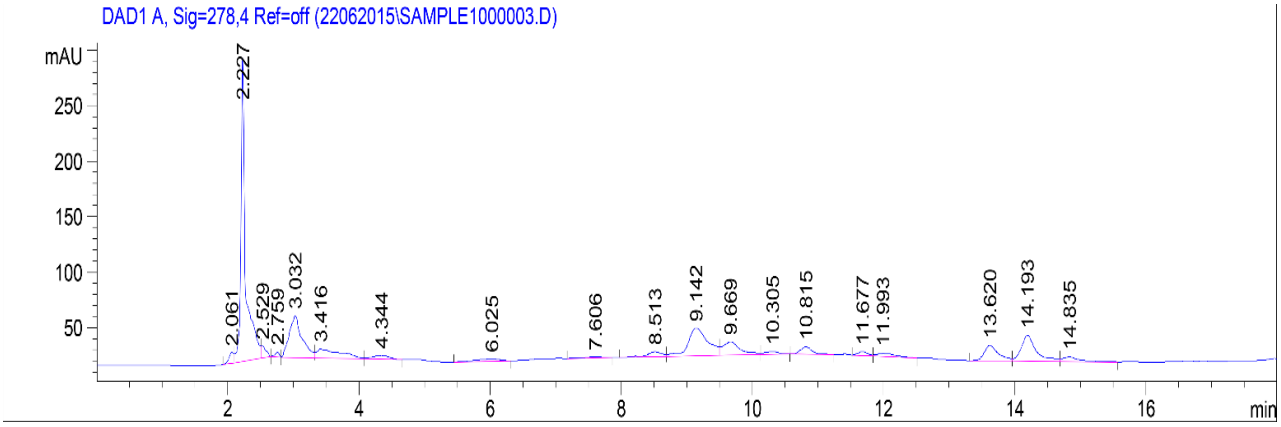

Figure 1: Enlarged HPLC chromatogram of methanolic extract from *E. lividoalbum.*

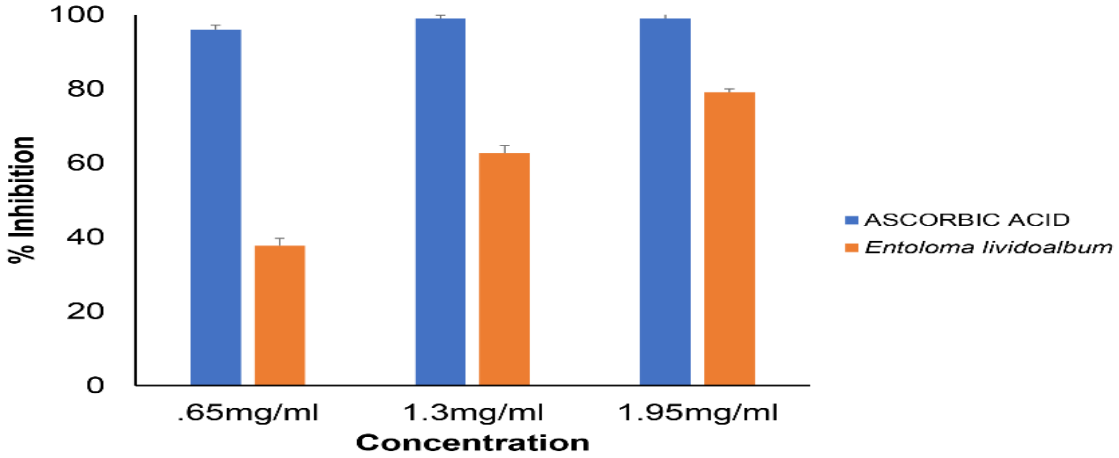

Figure 2: DPPH radical scavenging activity of the methanolic extract of *E. livioalbum* compared with standard. The values are the means of three separate experiments each in triplicate.

beta-carotene and lycopene content in the Methanolic extract of *E. lividoalbum*. Data shows that total phenols and flavonoids were the major antioxidant components, whereas, ascorbic acid, β- carotene and lycopene were found in vestigial amounts.

Chromatographic characterization

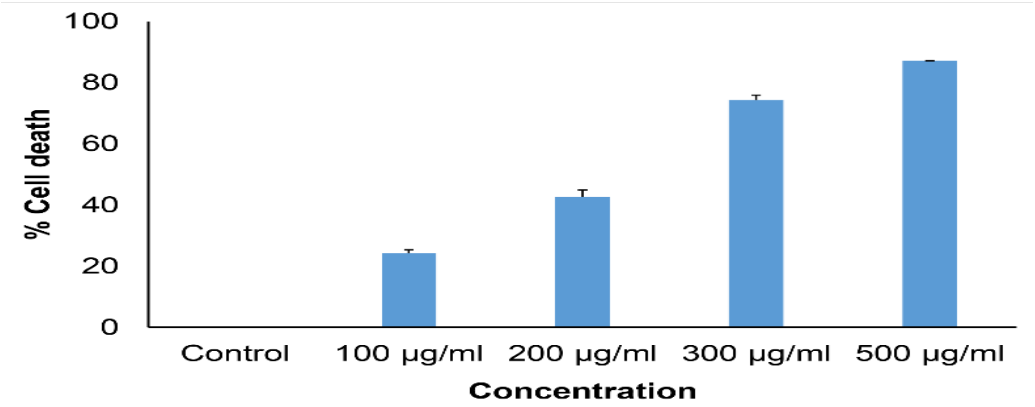

Figure 3: Cytotoxicity assay for methanolic extract of *E. lividoalbum* against A549 cell line.

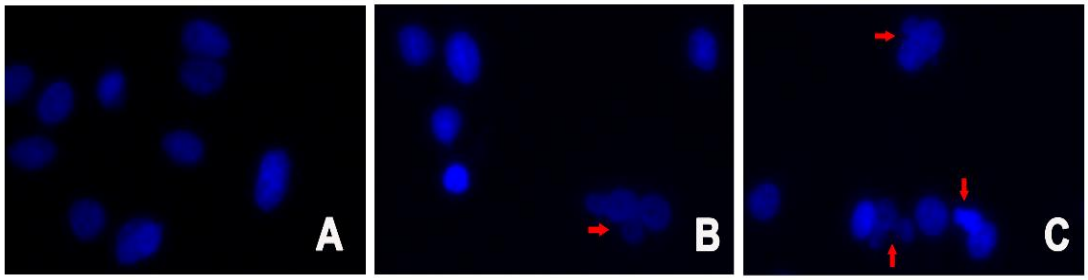

Figure 4: Changes in nuclear morphology by DAPI staining. A: Control nuclei, B: 300 µg/ml, C: 500 µg/ml.

Table 2: Bioactive phytochemicals in the methanol extract of *E. lividoalbum*.

Flavonoids (µg/mg)	Total phenols	β- carotene		Lycopene
11.538846 ±0.064102	17.692± 0.1538	1.9769 1.708	±	1.7137± 1.058533

HPLC is an efficient and valuable technology for the separation and determination of constituents. Figure 2 represents HPLC chromatogram of the extract consisting of 16 peaks excluding the solvent peaks. The retention times of each peak have been marked in the figure. The peaks provide a specific fingerprint towards authentication of the crude samples[35].

Antioxidant assay

DPPH is one of the most stable free radicals and thus used widely to assess the antioxidant property of various food samples. The purple DPPH solution takes up electrons from reducing agents and loses its colour, giving us a measure of the free radicle scavenging activity of the tested sample. The colour change is measured spectrophotometrically at 517nm. Our extract exhibited radical scavenging activity at the range of 37.73%, 62.65% and 79.15% at 0.65, 1.3 and 1.95 mg/ml concentrations respectively. EC_{50} value was found to be at 0.978 mg/ml.

Phosphomolybdenum method is a good method for evaluation of total antioxidant capacity. The method is based on reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of green phosphate /Mo (V) complex at acidic pH. Total antioxidant capacity of all five fractions was investigated and compared against ascorbic acid. The extract presented 70.96 ± 5.2846 µg AAE/ mg of extract total antioxidant activity.

Cytotoxic effect

The methanolic extract of *E. lividoalbum* showed significant cytotoxicity against the lung cancer cell line A549 (Fig 3). At a concentration as low as 100 µg/ml the extract showed 24% cell death, which went up with increasing concentration of the drug. At the maximum concentration tested (500 µg/ml), cell death increased to 87%. The LD_{50} was determined to be 226 µg/ml.

Analysis of the changes in nuclei of the treated cells is a useful indicator for cellular apoptosis. Apoptosis entails an organized nuclear breakdown which can be easily studied by nuclear staining. Nuclei of the extract treated cells appeared smaller, indicating nuclear shrinkage. With an increase in concentration, a notable degree of nuclear fragmentation was observed (Fig 4), with characteristic blebbing which may have been triggered by extract-induced apoptosis.

CONCLUSION

The study deals with the identification of salient physico-chemical characters along with chromatographic fingerprint of an emerging medicinal mushroom *E. lividoalbum*, which may become of utmost importance to authenticate the crude mushroom samples before processing for drugs in the modern times, providing quality and health assurance for the thousands of unaware consumers. Furthermore, this mushroom also showed potential antioxidant activity as well as cytotoxicity towards human lung cancer cell line, possibly by inducing apoptosis which needs to be studied further.

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REFERENCES

1. Khatua S, Paul S, Acharya K. Mushroom as the potential source of new generation of antioxidant: a review. Research Journal of Pharmacy and Technology 2013; 6(5):496-505.
2. Kozarski M, Klaus A, Jakovljevic D, Todorovic N, Vunduk J, Petrović P, Niksic M, Vrvic MM, Griensven LV. Antioxidants of Edible Mushrooms. Molecules 2015; 20:19489-19525.
3. Biswas G, Acharya K. Hypoglycemic activity of ethanolic extract of *Astraeus hygrometricus* (Pers.) Morg. in alloxan-induced diabetic mice. International Journal of Pharmacy and Pharmaceutical Sciences 2013; 5(1):391-394.
4. Giri S, Biswas G, Pradhan P, Mandal SC, Acharya K. Antimicrobial activities of basidiocarps of wild edible mushrooms of West Bengal, India. International Journal of PharmTech Research 2012; 4(4): 1554-1560.
5. Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Medica 2012; 78(16):1707-18.
6. Mallick S, Dutta A, Dey S, Ghosh J, Mukherjee D, Sultana SS, Mandal S, Paloi S, Khatua S, Acharya K, Pal C. Selective inhibition of *Leishmania donovani* by active extracts of wild mushrooms used by the tribal population of India: An in vitro exploration for new leads against parasitic protozoans. Experimental Parasitology 2014; 138:9-17.
7. Mallick S, Dey S, Mandal S, Dutta A, Mukherjee D, Biswas G, Chatterjee S, Mallick S, Lai TK, Acharya K, Pal C. A novel triterpene from *Astraeus hygrometricus* induces reactive oxygen species leading to death in *Leishmania donovani*. Future Microbiology 2015; 10(5):763-789.
8. Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Chemopreventive effect of *Tricholoma giganteum* against benzo[a]pyrene-induced forestomach cancer in Swiss albino mice. International Journal of Pharmaceutical Sciences Review and Research 2014; 26(2):189-196.
9. Chatterjee S, Chatterjee A, Chandra S, Khatua S, Saha GK, Acharya K, *Tricholoma giganteum* ameliorates benzo[a]pyrene – induced lung cancer in mice. International Journal of Current Pharmaceutical Review and Research 2016; 7(5):283-290.
10. Chatterjee A, Khatua S, Chatterjee S, Paloi S, Mukherjee S, Mukherjee A, Acharya K, Bandyopadhyay SK. Polysaccharide-rich fraction of Termitomyces eurhizus accelerate healing of indomethacin induced gastric ulcer in mice. Glycoconjugate Journal 2013; 30:759-768.
11. Chatterjee A, Acharya K. Include mushroom in daily diet—A strategy for better hepatic health. Food Reviews International 2016; 32(1):68-97.
12. Wasser SP. Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology 2011; 89:1323-1332.
13. Chatterjee S, Biswas G, Basu SK, Acharya K. Antineoplastic effect of mushrooms: A review. Australian Journal of Crop Science 2011; 5(7):904-911.
14. Samanta S, Nandi AK, Sen IK, Maity P, Pattanayak M, Devi KSP, Khatua S, Maiti TK, Acharya K, Islam SS. Studies on antioxidative and immunostimulating fucogalactan of the edible mushroom *Macrolepiota dolichaula*. Carbohydrate Research 2015; 413:22-29.
15. Nandi AK, Sen IK, Samanta S, Maity K, Devi KSP, Mukherjee S, Maiti TK, Acharya K, Islam SS. Glucan from hot aqueous extract of an ectomycorrhizal edible mushroom, *Russula albonigra* (Krombh.) Fr.: Structural characterization and study of immunoenhancing properties. Carbohydrate Research 2012; 363:43-50.
16. Nandi AK, Samanta S, Maity S, Sen IK, Khatua S, Devi KSP, Acharya K, Maiti TK, Islam SS. Antioxidant and immunostimulant β-glucan from edible mushroom *Russula albonigra* (Krombh.) Fr. Carbohydrate Polymers 2014; 99:774-782.
17. Dasgupta A, Rai M, Acharya K. Phytochemical analysis and in vitro antioxidant activity of a wild edible mushroom *Entoloma lividoalbum*. Asian Journal of Pharmaceutical and Clinical Research 2015; 8:3-6.
18. Maity P, Samanta S, Nandi AK, Sen IK, Paloi S, Acharya K, Islam SS. Structure elucidation and antioxidant properties of a soluble β-d-glucan from mushroom *Entoloma lividoalbum*. International Journal of Biological Macromolecules 2014; 63:140-149.
19. Maity P, Sen IK, Maji PK, Paloi S, Devi KSP, Acharya K, Maiti TK, Islam SS. Structural, immunological, and antioxidant studies of β-glucan from edible mushroom *Entoloma lividoalbum*. Carbohydrate Polymers 2015; 123:350-358.
20. Maity P, Nandi AK, Sen IK, Pattanayak M, Chattopadhyay S, Dash SK, Roy S, Acharya K, Islam SS. Heteroglycan of an edible mushroom *Entoloma lividoalbum*: Structural characterization and study of its protective role for human lymphocytes. Carbohydrate Polymers 2014; 114:157-165.
21. Pradhan P, Dutta AK, Acharya K. A low cost long term preservation of macromycetes for fungarium. Protocol Exchange 2015. doi:10.1038/protex.2015.026.
22. Tripathi AK, Tripathy S, Verma NK. A study on pharmacognostic and chemomicroscopic features of *Streblus asper* L. Der Pharmacia Lettre 2014; 6:30-36.
23. Phat C, Li H, Lee D-U, Moon BK, Yoo Y-B, Lee C. Characterization of *Hericium erinaceum* powders prepared by conventional roll milling and jet milling. Journal of Food Engineering 2015; 145:19-24.

24. Adebayo EA, Oloke JK, Ayandele AA, Adegunlola CO. Phytochemical, antioxidant and antimicrobial assay of mushroom metabolite from *Pleurotus pulmonarius*-LAU 09 Journal of Microbiology and Biotechnology Research 2012; 2(2):366–374.
25. Nagata M, Yamashita I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaishi 1992; 39:925–928.
26. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal Enology and Viticulture 1965; 16:144–158.
27. Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithra DJ, Vijay KHT, Prashith KTR. Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits. Chemical Science Transactions 2012; 1(2):303-310.
28. Khatua S, Dutta AK, Acharya K. Prospecting *Russula senecis* : a delicacy among the tribes of West Bengal. PeerJ 2015; 3:e810. doi:10.7717/peerj.810.
29. Dasgupta A, Rai M, Acharya K. Chemical Composition and Antioxidant Activity of a Wild Edible Mushroom *Pleurotus flabellatus*. International Journal of PharmTech Research 2013; 5(4):1655-1663.
30. Bhattacharya S, Zaman MK. Pharmacognostical evaluation of *Zanthoxylum nitidum* bark. International Journal of PharmTech Research 2009; 1(2):292-298.
31. Acharya K, Mukherjee S, Mitra P. Pharmacognostic standardisation of *Lentinula edodes*: A widely explored medicinal mushroom. International Journal of Pharmacognosy and Phytochemical Research 2015; 7(4):866-872.
32. Acharya K, Ghosh S, Dutta AK. Pharmacognostic standardization based on physicochemical and molecular parameters of a medicinal mushroom *Schizophyllum commune*. Oriental Pharmacy and Experimental Medicine 2016; 16(4):259-266.
33. Acharya K, Ghosh S, Khatua S, Mitra P. Pharmacognostic standardization and antioxidant capacity of an edible mushroom *Laetiporus sulphurous*. Journal fur Verbraucherschutz und Lebensmittelsicherheit 2016; 11(1):33-42.
34. Acharya K, Ghosh S, Ghosal S. Pharmacognostic standardization of a widely explored medicinal mushroom, *Pleurotus ostreatus*. Der Pharmacia Lettre 2016; 8(6):66-72.
35. Acharya K, Bera M, Tarafder E, Dasgupta A. Pharmacognostic standardization of *Ganoderma lucidum*: A commercially explored medicinal mushroom. Der Pharmacia Lettre 2015; 7(7):175-181.