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Pharmacognostic standardization of a widely explored medicinal mushroom, *Pleurotus ostreatus*

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ABSTRCT

From ancient times Pleurotus ostreatus is a popular edible mushroom for its test, flavor, texture and gastronomic delicacy. In addition, recent literature reports have revealed diverse therapeutic importance of this mushroom. However quality standard of this species is still unexplored. Therefore the present study aims at investigating the microscopic, physical and fluorescent characters of dry powder derived from basidiocarp with the objective to establish pharmacognostic standard of this popular oyster mushroom. Furthermore, a methanolic fraction was prepared from the macrofungus and preliminary mycochemical screening revealed presence of phenols, flavonoids, ascorbic acid, β-carotene and lycopene. For chromatographic study, HPLC was performed to achieve a phenolic fingerprint which indicated existence of 13 components. DPPH and total antioxidant assays were executed to determine the antioxidant potential of this methanolic fraction. Result demonstrated high efficiency of the mushroom as radical scavenger indicating potentiality to be used as a therapeutic drug. The aforementioned data presented in this work are valuable to assist identification of crude drugs prepared from P. ostreatus.

Keywords: Antioxidant, HPLC, Mycochemicals, Pharmacognosy, Powder microscopy

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INTRODUCTION

One of the most simple, efficient and accurate method for identification and evaluation of natural products is pharmacognostical study that includes investigation from several aspects such as macroscopic, microscopic, physicochemical as well as phytochemical screening [1, 2]. Natural drugs are easily prone to be adulterated with low quality materials that decrease therapeutic efficacy of that product [3]. Another major problem associates with natural product is its identity issue [4]. Initially standard description was consulted for identification of any particular crude drug but in recent times this area is becoming more diverse with the advancement of modern technologies. Therefore a multidimensional pharmacognostic study should be carried out to ensure identity, quality, and purity of raw material to be used as medicine and that will also help in authentication of the exact material.

Medicinal mushrooms are considered to be a part of herbal medicines as they contain several bioactive compounds that exhibit tremendous medicinal properties such as anticancer [5, 6], immunomodulation [7-11], hepatoprotective [12-14], antioxidant [15, 16], antidiabetic [17], antiparasitic [18] etc. *Pleurotus ostreatus* (Jacq.: Fr.) Kumm, popularly known as Hiratake in Japanese and sometimes referred to as oyster mushroom. Around the world it is considered as one of popular edible mushroom after the species of *Agaricus* and *Lentinus* [19]. The chemical composition of this mushroom revealed the presence of high amount of fatty acids [20, 21], vitamins specially vitamin B complex [22], minerals [23], dietary fibers [24] and very less amount of cholesterol [25] which is

appropriate for heart patients. It has already been reported that this mushroom possesses remarkable medicinal activities such as antioxidant, anticancer, antiviral, antimutagenic, hepatoprotective, immunomodulatory [26] effects. Polysaccharides extracted from this mushroom have demonstrated high antineoplastic activity [27]. Literature studies revealed that its better antioxidant and cytotoxic effect may be due to the presence of flavonoids and phenolic compounds in their fruiting bodies [28, 29]. Several glucans have been reported to posses various therapeutic activities [30, 31, 32].

To the best of our knowledge, there is no scientific report on the pharmacognostic standardization of this mushroom. Therefore, the present study has been undertaken to standardize the medicinal mushroom, *P. ostreatus*, on the basis of following pharmacognostical features.

MATERIALS AND METHODS

Mushroom materials:
Basidiocarps of *P. ostreatus* were collected during the rainy season from of West Bengal. The basidiocarps were identified by using standard literature [33, 34, 35] and authenticated by comparing the voucher specimen present in CUH herbarium. A representative specimen (AMF 573) was deposited in the same herbarium for future reference as per the method by Pradhan et al. [36]. For powder analysis basidiocarps were first dried by field drier at 40°C for overnight, then pulverized to make them crumbly using electric blender and sieved through 160 mesh. After that the finely sieved powder was stored in an airtight container for future use.

Powder-microscopic and organoleptic evaluation of powdered basidiocarp:
For microscopic evaluation, powder was taken in a grease free slide and hydrated with 10% KOH and then examined with Congo red and Melzer's reagent. Various identifying characters such as cellular structures of hyphae, basidia and spores were recorded and pictomicrography studies were undertaken using compound binocular microscope aided with camera and computer attachment (Leica DMLS). Different organoleptic characters of the powder such as colour, flavor, texture, taste were evaluated.

Fluorescence analysis:
A small quantity of sieved powder was placed on a grease free slide and treated with 16 different chemical agents and mixed properly by gentle tilting the slide. Then the slide was placed inside the UV viewer chamber and exposed to visible, short (254 nm) and long (365 nm) ultraviolet light to study their fluorescence behavior.

Preparation of methanol extract:
2 gm of dried sieved powder was soaked with 100 ml of methanol for 72 hours before extraction. Thereafter it was filtered through Whatman filter paper No. 1. The filtrate was then concentrated at 40°C using Rota vapor (R3 Buchi, Switzerland) and preserved in refrigerator at -20°C in a dark bottle until analysis. Percentage yield and organoleptic parameters of the extract were recorded.

Quantitative estimation of mycochemicals:
The methanolic extract was subjected for quantitative estimation of mycochemicals such as phenols, flavonoids, β carotene, lycopene and ascorbic acid. Total phenol content of the extract was estimated using Folin-Ciocalteu reagent and gallic acid used as standard [37]. The result was expressed as µg of gallic acid equivalent per mg of extract. Flavonoid content was estimated using aluminium nitrate and potassium acetate.The result was expressed as µg of quercetin equivalent per mg of extract. β carotene and lycopene content of the extract was determined using a standard protocol [38]. Ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye in presence of 0.6% oxalic acid [39].

High performance liquid chromatography (HPLC) of methanol extract:
The methanolic extract was filtered through 0.2 µm filter and 20 µl of the filtrate was loaded in the HPLC system (Agilent, USA). Separation was done on an Agilent Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) and flow rate was maintained at 0.8 ml/min (at 25°C) throughout the run. 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 [39].

Antioxidant assays:
In order to determine antioxidant activity, the methanolic extract of *P. ostreatus* was subjected to *in vitro* antioxidant activity by using two well-known assays such as DPPH radical scavenging assay and total antioxidant activity.

DPPH radical scavenging assay:
Radical scavenging activity of the extract was performed using purple coloured DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals according to the method of Shimada et al. [40]. 2 ml of reaction mixture consisted of 0.1 mM methanolic solution of DPPH and various concentrations of extract. The mixture was then shaken and incubated for 30 minutes in dark at room temperature. After incubation, absorbance was measured at 517 nm against blank. The scavenging ability was calculated using the following equation:

Scavenging effect (%) = {(A0 –A1)/A0} ×100

A0 was absorbance of the control and A1 was absorbance in the presence of sample. The % of inhibition was plotted against respective concentrations used and EC_{50}, the effective concentration at which DPPH radicals were scavenged by 50 %.

Total antioxidant activity:
Total antioxidant assay was carried out following methods of Prieto *et al* [41] with little variation. The reaction mixture consisted of 0.3 ml sample solution and 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium sulphate and 4 mM ammonium molybdate). Tubes were capped and incubated at 95ºC for 90 min. Samples were cooled at room temperature and absorbance was measured at 695nm against blank. Blank solution contained 3 ml of reagent solution and appropriate volume of the same solvent used for the sample and was also incubated under the same conditions. Concentrations of ascorbic acid (1 –30 µg/ml) were used to obtain a standard curve. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Statistical analysis:
The experimental results were expressed as mean ± standard deviation (SD) of three replicates and were subjected to analysis of variance using Microsoft® Office Excel (Microsoft®, USA). Significant levels tested at $p < 0.05$.

RESULTS AND DISCUSSION

Organoleptic evaluation of the powder is considered as one of the important parameter of pharmacognostical studies which is based on morphological and sensatory profile of the whole drugs. Organoleptic characters of the powder showed greyish yellow in colour, granular in texture, without any significant taste and possessed a chocolaty odour [Figure 1].

Figure 1: Fruit body of *Pleurotus ostreatus*. A. Dried basidiocarp B. Sieved powder C. Methanolic extract

As per WHO (1998), both macroscopic and microscopic description of a plant is the pioneer step to be implemented first for establishing the identity and purity of such material before any other methodological tests are taken [42]. For microscopic characterization, the dried sieved powder was macerated with 10% KOH and examined with Congo red. The following characters of the powder were observed under light microscope: Densely interwoven branched

hyphal system; 8.34- 9.84 µm wide, clamp connection present; cystidia almost absent; basidia 21.80-26.46 µm × 5-7.3 µm slenderly clavate, 4-sterigmate, a basal clamp connection present; spores cylindric to cyclindrical ellipsoid, smooth, hyaline with vacuoles, length 6.42-12.84 µm, breadth 3.68-5.14 µm [Figure 2]. Melzer's reaction showed negative result which signifies that the spores are non-amyloid in nature. Microscopic data of the dried powder of *P. ostreatus* were found to be similar with that of the standard description.

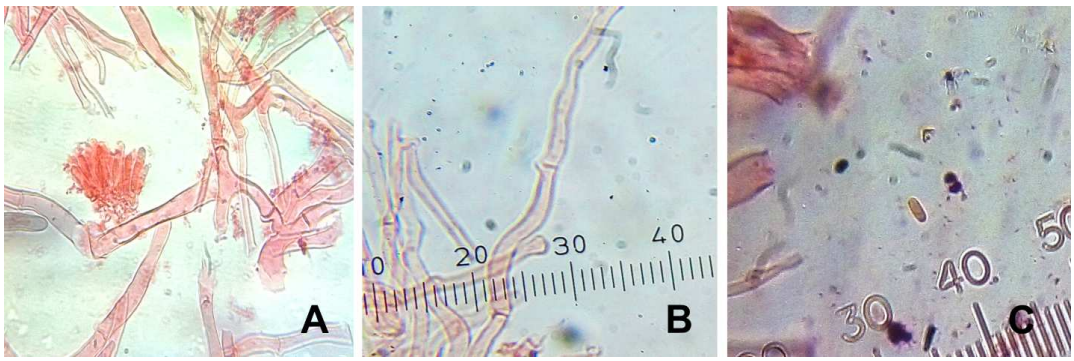

Figure 2: Microscopic characterization of dried sieved powder of *Pleurotus ostreatus*. A. Hyphal characters B. Hypha with clamp C. Spore

Fluorescence analysis of crude drugs is an important qualitative parameter of pharmacognostical evaluation. Some organic constituents do not visibly fluoresce under the daylight but show fluorescence in the ultra violet light due to the conversion of those compounds into fluorescent derivatives with the application of different chemical reagents. The powdered sample on treatment with different chemical reagents displayed an array of colours when observed under long and short wavelengths of UV light and were compared with that, observed under visible light (Table 1). These characteristic fluorescent colours could be employed as reference information for correct identification of drug.

Table 1: Fluorescence analysis of dry powder from *Pleurotus ostreatus*

Serial No.	Reagents	Visible	UV	
			Long (365 nm)	Short (254 nm)
1	Powder as such	Creamish white	Light brown	Creamish white
2	Hager's	Yellow	Reddish yellow	Green
3	Mayer's	Greenish yellow	Reddish yellow	Light green
4	Dragendroff's	Yellowish orange	Brown	Yellow
5	Iodine solution	Deep orange	Black	Deep brown
6	1(N) HNO_3	Creamish white	Light brown	Greyish yellow
7	50% HNO_3	Light yellow	Yellowish brown	Light yellow
8	Phloroglucinol	Whitish grey	Light brown	Pale cream
9	Barfoed	Sea green	Brown	Olive green
10	Sodium nitroprusside	Creamish white	Orangish brown	Greyish green
11	$FeCl_3$	Pale cream	Greenish brown	Pale cream
12	1(N) NaOH	Pale cream	Light brown	Cream
13	Acetic acid	Yellowish cream	Light brown	Yellowish cream
14	1(N)HCl	Cream	Light brown	Greyish yellow
15	Methanol	Yellow	Light brown	Yellow
16	1(N) NaOH in Methanol	Yellow	Light brown	Yellow

A methanolic extract was prepared from *P. ostreatus* and organoleptic characters of the extract were documented. The fraction was creamish white in colour, sticky in nature with extractive value of 17±2.3%. The methanolic extract was then subjected for quantitative analysis of different mycochemicals using standard protocol. Edible mushrooms are considered as a huge resource of certain potent antioxidant compounds. Quantitative analysis of methanolic extract revealed the presence of certain mycochemicals such as phenols, flavonoids, β carotene, lycopene and ascorbic acid. The results were presented in Table 2.

Table 2: Mycochemical estimation of methanolic extract of *Pleurotus ostreatus* (mean ± SD)

Mycochemical constituents	Amount
Total phenols (µg GAE/mg of extract)	3.125±0.61
Total flavonoids (µg QE/mg of extract)	0.625±0.22
β carotene (µg/mg of extract)	0.082±0.005
Lycopene (µg/mg of extract)	0.060±0.007
Ascorbic acid (µg/mg of extract)	11.1±0.202

In the present study, quantitative estimations showed that ascorbic acid, phenol and flavonoid were the major antioxidant components whereas β carotene and lycopene were found in negligible amount. The ascorbic acid content of *P. ostreatus* was found to be higher in comparison with the reported values in other mushroom such as *Ganoderma lucidum* (2.77 µg/mg of extract) [43], *Macrocybe crassa* (1.81 µg/mg of extract) [44], *Laetiporus sulphureus* (2 µg/mg of extract) [45], *Lentinula edodes* (3.96 µg/mg of extract) [46] and *Griffola frondosa* (0.37 µg/mg of extract) [47]. However, these chemical components have a great value in human health care system. Ascorbic acid, the functional form of vitamin C, is reported to interact directly with the free radicals thereby preventing the oxidative damage [48]. Similarly phenols have been reported as an active quencher of free radicals by donating hydrogen atom or electron to stabilize free radicals [49].

Chromatographic fingerprint is considered as an authentic approach for quality evaluation of herbal preparation as it represents the active chemical constituents present in the sample. For this purpose HPLC was carried out using the methanolic extract of *P. ostreatus*. UV spectrum analysis of the chromatogram at 278 nm revealed existence of 13 peaks that might be of phenolic compounds. Chromatographic profile along with the retention time of each peak was presented in Figure 3. Respective areas of each peak were documented in Table 3.

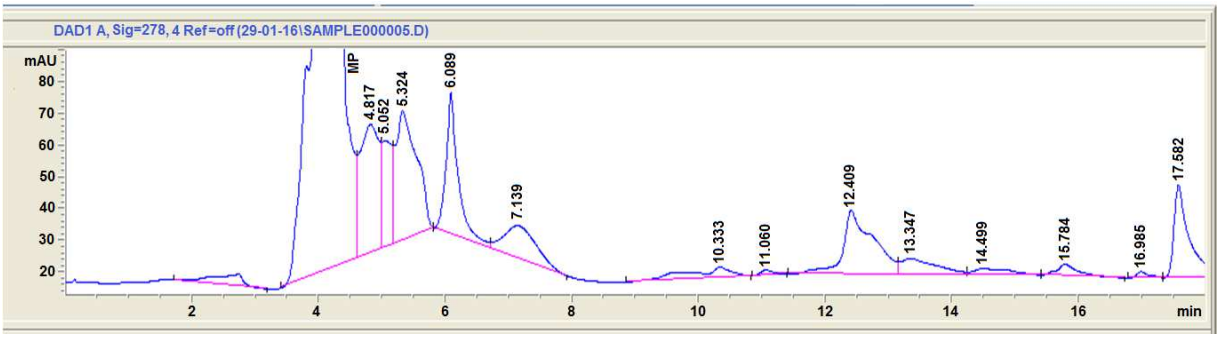

Figure 3: Enlarged HPLC chromatogram of methanol extract from *Pleurotus ostreatus*.

Table 3: HPLC profile of methanol extract of *Pleurotus ostreatus* at 278nm

Peak no.	Retention time (min)	Area (AU)
1	4.817	211.008
2	5.052	154.644
3	5.324	597.473
4	6.089	538.196
5	7.139	294.684
6	10.333	39.582
7	11.060	22.848
8	12.409	477.972
9	13.347	44.041
10	14.499	53.699
11	15.784	83.199
12	16.985	27.015
13	17.582	461.057

Antioxidant screening of methanolic extract of *P. ostreatus* showed a high effective free radical scavenging activity in the DPPH assay at the rate of 20.1%, 45.50%, and 62.96% at 0.5, 1 and 1.5 mg/ml concentrations as represented in Figure 4. Results showed that the radical scavenging activity of methanolic extract increased with increasing concentration of the extract. Methaolic extract of *P. ostreatus* performed well as indicated by its low EC_{50} value

(1.232±0.283 mg/ml) in comparison with that of *M. crassa* was 2.45 mg/ml [44]. Therefore, the scavenging activity of *P. ostreatus* could be considered more effective than *M. crassa.*

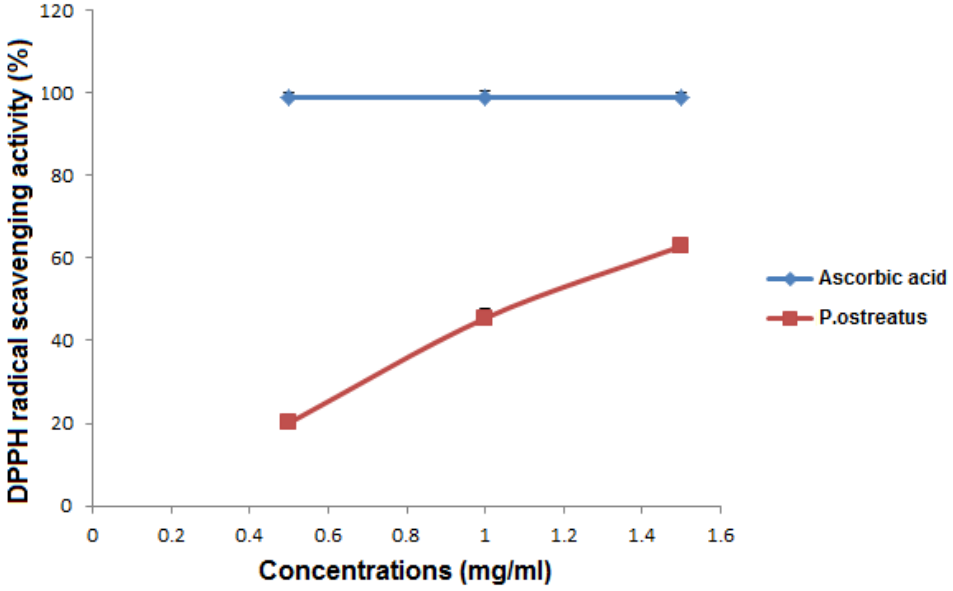

Figure 4. DPPH assay of methanol extract from dried basidiocarp of *Pleurotus ostreatus*.

The antioxidant capacity of the extract was also measured through phosphomolybdenum method which is based on the reduction of Mo (IV) to Mo (V) and formation of green phosphate / Mo (V) complex with characteristic absorption at 695 nm. The total antioxidant capacity was determined using linear regression equation expressed as the number of equivalent of ascorbic acid which was found to be 12.5±1.25 µg ascorbic acid equivalent (AAE) per mg of extract.

CONCLUSION

The present study highlights the knowledge of quality and identity of the mushroom *P. ostreatus*. It is very difficult to identify the desired material form dry powdered mushroom samples only on the basis of microscopic characters. So these microscopic features may act as a preliminary support towards the identity of the mushroom. All the additional data starting from microscopic, biochemical quantification, chromatographic fingerprinting as well as antioxidant screening might provide a pharmacognostic standard for identity of the mushroom which will be helpful in the further study and also in drug formulation. From the present study it also can be concluded that the high antioxidant activity of the methanolic extract from *P. ostreatus* as evidenced in *in vitro* assays was possibly due to its high ascorbic acid and phenol content.

REFERENCES

[1] SD Sarker. *Pharmacogn. Mag.*, **2012**, 8, 91–92.
[2] MA Sonibare; OV Olatubosun. *Phrmacogn. J.*, **2015**, 7, 107–116.
[3] S Chanda. *J. Pharmacogn. Phytochem.*, **2014**, 2, 69-73.
[4] VK Dixit; NP Yadav. *Integr. Biol.*, **2008**, 2, 195-203.
[5] G Biswas; S Chatterjee; K Acharya. *Dig. J. Nanomater. Bios.*, **2012**, 7, 185-191.
[6] S Chatterjee; G Biswas; S Chandra; GK Saha; K Acharya. *Bioproc. Biosyst. Eng.*, **2013**, 36, 101-107.
[7] S Patra; P Patra; KK Maity; S Mandal; SK Bhunia; B Dey; KSP Devi; S Khatua; K Acharya; TK Maiti; SS Islam. *Carbohydr. Res.*, **2013**, 368, 16-21.
[8] AK Nandi; IK Sen; S Samanta; K Maity; KSP Devi; S Mukherjee; TK Maiti; K Achrya; SS Islam. *Carbohydr. Res.*, **2012**, 363, 43-50.

[9] S Samanta; K Maity; AK Nandi; IK Sen; KSP Devi; S Mukherjee; TK Maiti; K Acharya; SS Islam. *Carbohydr. Res.*, **2013**, 367, 33-40.
[10] AK Nandi; S Samanta; IK Sen; KSP Devi; TK Maiti; K Acharya; SS Islam. *Carbohydr. Polym.*, **2013**, 94, 918-926.
[11] AK Nandi; S Samanta; S Maity; IK Sen; S Khatua; KSP Devi; K Acharya; TK Maiti; SS Islam. *Carbohydr. Polym.*, **2014**, 99, 774-782.
[12] S Chatterjee; R Datta; A Dey; P Pradhan; K Acharya. *Res. J. Pharm. Technol*., **2012**, 5, 1034-1038.
[13] G Biswas; S Sarkar; K Acharya. *Dig. J. Nanomater. Bios*., **2011**, 6, 637-641.
[14] S Chatterjee; A Dey; R Datta; S Dey; K Acharya. *Int. J. PharmTech Res.*, **2011**, 3, 2162-2168.
[15] K Acharya; S Chatterje; S Ghosh. *Pharmacologyonline*, **2011**, 1, 440-450.
[16] S Chatterjee; GK Saha; K Acharya. *Pharmacologyonline*, **2011**, 3, 88-97.
[17] G Biswas; K Acharya. *Int. J. Pharm. Pharm. Sci.*, **2013**, 5, 391-394.
[18] S Mallick; S Dey; S Manda; A Dutta; D Mukherjee; G Biswas; S Chatterjee; S Mallick; TK Lai; K Acharya; C Pal. *Future Microbiol*., **2015**, 10, 763-789.
[19] A Fernandes; L Barros; A Martins; P Herbert; ICFR Ferreira; *Food Chem.*, **2015**, 169, 396-400.
[20] P Maftoun; H Johari; M Soltani; R Malik; NZ Othman; HAEl Enshasy. *Int. J. Biotech. Well. Indus.*, **2015**, 4, 67-83.
[21] FS Reis; L Barros; A Martins; ICFR Ferreira. *Food Chem. Toxicol*., **2012**, 50, 191-197.
[22] K Deepalakshmi; S Mirunalini. *J. Biochem. Tech*., **2014**, 5, 718-726.
[23] J Vetter. *Food chem.*, **1994**, 50, 277-279.
[24] A Synytsya; K Mickova; I Jablonsky; M Slukova; J Copikova. *Czech J. Food Sci.*, **2008**, 26, 441–446.
[25] SP Wasser; AL Weis. *J. Med. Mushrooms.*, **1999**, 1, 31-62.
[26] Y Patel; R Naraian; VK Singh. *World J. Fungal Plant Biol.*, **2012**, 3, 01-12.
[27] T Watanabe. *Nippon Rinsho*, **1969**, 27, 1759-61.
[28] YH Gu; G Sivam. *J. Med. Food*, **2006**, 9, 196-204.
[29] V Venkatakrishnana; R Shenbhagaramanb; V Kaviyarasanb; D Gunasundaric; K Radhikad; R Dandapania; LK Jagadishb. *J. Phytol.*, **2010**, 2, 22-28.
[30] S Maiti; SK Mallick; SK Bhutia; B Behera; M Mandal; TK Maiti. *Int. J. Med. Mushrooms*, **2011**, 13, 427-40.
[31] P Bobek; S Galbavy; L Ozdin. *Oncol. Rep.*, **1998**, 5, 727-730.
[32] H Tong; F Xia; K Feng; G Sun; X Gao; L Sun; R Jiang; D Tian; X Sun. *Bioresour. Technol.*, **2009**, 100, 1682-1686.
[33] C Bas; TH Kuyper; ME Noordeloos; EC Vellinga. Flora Agaricana Neerlandica- Critical monographs on families of agaricus and boleti occurring in the Netherlands, vol. 2, CRC Press, United States of America, **1990**.
[34] BE Lechner; JE Wright; E Alberto. *Mycologia*, **2004**, 96, 845-858.
[35] B Manjula. *Proc. Indian Acad. Sci. (Plant Sci.)*, **1983**, 92, 81-213.
[36] P Pradhan; AK Dutta; K Acharya. *Protoc. Exch*., **2015**, DOI: 10.1038/protex. 2015. 026.
[37] VL Singleton; JJ Rossi. *Am. J. Enol. Vitic.*, **1965**, 16, 144-158.
[38] M Nagata; I Yamashita. *J. Japan. Soc. Food Sci. Technol*., **1992**, 39, 925-928.
[39] S Khatua; AK Dutta; K Acharya. *PeerJ*, **2015**, 3:e810; DOI 10.7717/peerj.810.
[40] K Shimada; K Fujikawa; K Yahara; T Nakamura. *J. Agric. Food Chem.*, **1992**, 40, 945-948.
[41] P Prieto; M Pineda; M Aguilar. *Anal. Chem.*, **1999**, 269, 337-334.
[42] Anonymous, World Health Organization (WHO), Quality control methods for medicinal plant materials, WHO, Geneva, **1998**.
[43] K Acharya; M Bera; E Tarafder; A Dasgupta. *Der Pharmacia Lett*., **2015**, 7, 175-181.
[44] K Acharya; S Khatua; S Sahid. *Research J. Pharm. and Tech.*, **2015**, 8, 860-866.
[45] K Acharya, S Ghosh, S Khatua, P Mitra. *J. Verbr. Lebensm.*, **2016**, 11, 33-42.
[46] K Acharya; S Mukherjee; P Mitra. *Int. J. Pharmacog. Phytochem Res.*, **2015**, 7, 866-872.
[47] K Acharya; I Bera; S Khatua; M Rai. *Der Pharmacia Lett.*, **2015**, 7, 72-78.
[48] RE Beyer. *J. Bioenerg. Biomembr*,. **1994**, 26, 349-358.
[49] F Shahidi; PKJPD Wanasundara. *Crit. Rev. Food. Sci. Nutr.*, **1992**, 32, 67-103.