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# Pharmacognostic standardization of *Ganoderma lucidum*: A commercially explored medicinal mushroom

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**ABSTRACT**

*Ganoderma lucidum, commonly known as 'Reishi', has been used traditionally by the Chinese and Japanese in their curative herbal concoctions. A number of products from Ganoderma lucidum are commercially available in forms of extract powders and oral liquids, but the ever-growing demand of herbal bioactive medicines makes quality assurance of the starting material very important. The present study was undertaken to provide quality control standardization of Ganoderma lucidum based on microscopic, physico-chemical and chromatographic features and to partially screen its antioxidant activity. The physico-chemical parameters included loss on drying, ash value, fluorescence analysis which were determined by standard methods. Chromatographic parameters entailed the HPTLC and HPLC profiles of methanolic extract of G. lucidum, the $R_f$ values and the chromatographic peaks were noted as signature parameters for identification of the mushroom. Preliminary mycochemical analysis of the methanol extract indicated presence of flavonoid, phenol, and terpenoids. Quantitatively the major bioactive components in the extract were present in the following order: phenol> flavonoid> β-carotene~ lycopene> ascorbic acid in the methanolic extract. In terms of partially screening the antioxidant potential of the extract, DPPH radical scavenging activity ($EC_{50}$ value 666 µg) and total antioxidant capacity (18.64 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, HPLC, HPTLC, Medicinal mushroom, Physico-chemical evaluation, Quality control

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## INTRODUCTION

The use of mushrooms in ancient oriental therapies dates back at least to the Neolithic age. Contemporary research has validated the traditional knowledge on medicinal mushrooms. Recently studied medicinal actions of mushrooms included antioxidative [1-3], antidiabetic [4], antimicrobial [5], antiparasitic [6], antineoplastic [7, 8], antiulcer [9] and hepatoprotective [10, 11] effects. They make up a vast source of new powerful pharmaceutical products. Medicinal mushrooms are an unlimited source of bioactive polysaccharides, polysaccharide-protein complexes and high molecular weight and low molecular weight compounds [12]. Among different biologically active ingredients mushroom polysaccharides hold an elite position. Recent investigations established that mushroom polysaccharides prevent oncogenesis and act as a strong immunomodulator [13-17].

The mushroom *Ganoderma lucidum* holds an important place in a variety of Asian traditional medicine and well known as Reishi. In traditional Chinese medicine it is known as Lingzhi, in Korean medicine as Yeongji, in Taiwan

as Ling-Chih [18]. Yue et al 2008 [19], reported that extracts from different parts of fruit body except pileus possessed strong inhibitory activities on sarcoma-180 growth in mice model. Evidence shows that *G. lucidum* extracts may have a potential inhibitory effect on cancer metastasis through ERK ½ and $PI_3$ kinase activation [20]. Triterpenoids from this mushroom have been reported to possess hepatoprotective, antihypertensive, hypocholesterolemic, anti-tumour, antihistaminic effects, anti-angiogenic activity, effects on platelet aggregation and complement inhibition. Studies on *G. lucidum* polysaccharides functions have suggested that the anti-tumour effects through immunomodulation and antiangiogenesis [21].

A number of products made from *G. lucidum* are commercially available, mostly in the form of powdered extracts, oral liquids and capsules. In view of the diverse medicinal prospects of *G. lucidum*, and an increasing market demand, it is extremely important to ensure the quality of the raw drug, especially in the times of adulteration and substitution prevailing in the nature. The current study has been undertaken to evaluate the detailed pharmacognostic profile of the powdered fruitbodies of *G. lucidum*, which will be useful to pharmaceutical industries for the authentication of their commercial samples.

## MATERIALS AND METHODS

### Mushroom sample

Basidiocarps of *Ganoderma lucidum* were purchased from the village areas of Nadia district, West Bengal, India. Identity of this mushroom was authenticated in consultation with standard literature [22]. A reference specimen was also kept in the herbarium following the process Prakash *et al* (2015) [23]. Basidiocarps were dried properly by a field drier at 40°C for 24 hour to make them crispy and pulverized using an electric blender and sieved through 160 mesh and stored in an air-tight containers.

### Powder-microscopic, physicochemical, and organoleptic characterization of the powdered basidiocarps

Powdered sample was hydrated and macerated with 10% KOH and mounted on glass slide with glycerol. For effective results various stains were used to distinguish different cellular structures. The slide was then viewed under Leica DMLS microscope and images were captured at desired magnification. On the other hand, different characters like color, odor, taste, nature of the powdered sample were evaluated. Physico-chemical constants such as loss on drying, total ash [24], water holding capacity, swelling capacity [25] 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 drops of freshly prepared reagent solution was added to it, mixed by gently tilting the slide. Then the slide was placed inside the UV viewer chamber and viewed in short (254 nm) and long (365 nm) UV radiations. 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.

### Qualitative phytochemical screening of methanol soluble extract

The methanol soluble extract was subjected to various qualitative tests to detect the presence of organic constituents like saponins, terpenoids, phenolic and flavoroids using standard protocols [26].

### Quantitative estimation of some important phytochemicals from methanol soluble extract

The content of total phenolic compounds in extract was estimated using Folin-ciocalteu reagent [27]. Gallic acid as standard. The results were expressed as µg of gallic acid equivalents per mg of dry extract. Total flavonoid content was determined using aluminium nitrate and potassium acetate [28]. Quercetin (5–20 µg/ml) was used to obtain the standard curve. The results were expressed as µg of quercetin equivalents per mg of dry extract. β-carotene and lycopene were estimated using a standard protocol [29]. Ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye [30].

### HPTLC profile of methanol soluble extract

HPTLC, a micro analytical separation method, has important application in herbal drug analysis. Hence, a densitometric HPTLC analysis was performed to generate a characteristic fingerprinting profile of the extract. 2 µl of the sample solution was loaded on pre-coated TLC plate (Silica gel 60 F254 sheets 20×20 cm, 0.5 mm thickness, Merck Darmstadt, Germany) using CAMAG Automatic sampler. Plate was developed up to a distance of 85.0 mm

in CAMAG twin trough glass chamber pre-saturated with the mobile phase ethyl acetate: toluene (1:1 v/v) for 30 min. The plate was fixed in scanner stage and scanning was done at 200-450 nm.

**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°C. 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 [31].

**Antioxidant assay**

Total antioxidant capacity and DPPH radicals scavenging activity of methanolic extract of *G. lucidum* was analysed as described by Mitra *et al* (2014) [32]. 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).

## RESULTS AND DISCUSSION

**Microscopic characterisation**

The macerated powder showed many fragmented mycelia and spores (Figure 1). Hyphal system was found to be trimitic, with generative, thin-walled, branched, 3 - 4.5 µm wide, skeletal hyphae, yellowish brown in colour, thick-walled to solid, arboriform, skeletal stalks 3 - 4.5µm wide, with branches ending in flabelliform binding processes, binding hyphae were colourless, thik-walled, sinuous, branched, 1-2µm wide. Spores 8.5-11×6-7µm, ovoid or truncate, bitunicate, exospores hyaline and smooth. Endospores were echinulate, brownish, sometimes guttulated. Melzer reaction shows negative result, signifying its inamyloid nature. The microscopic data coincides with data present in the standard literature [22].

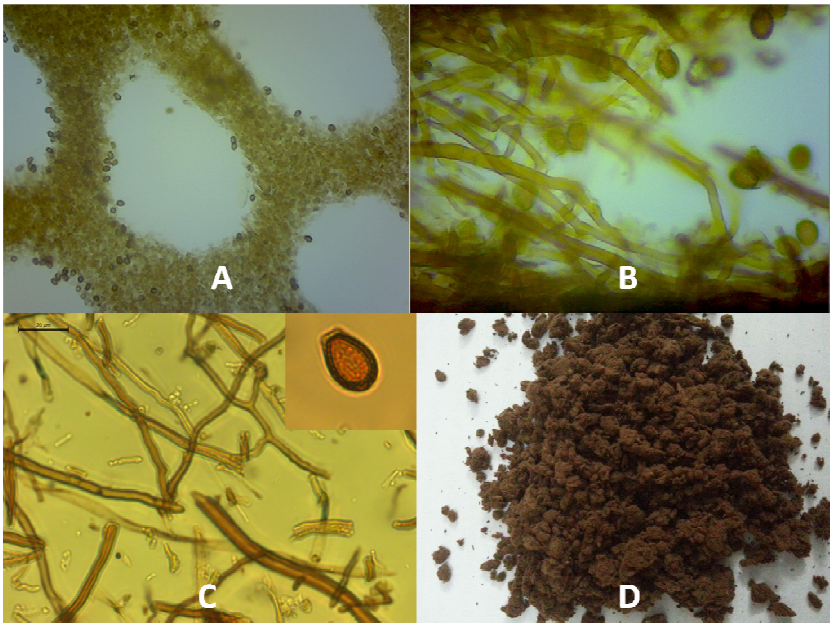


**Figure 1: Powder microscopy. A: Fragmented pore region of basidiocarp; B: Generative hyphae with spores C: Skeletal and binding hyphae; Spore magnified (C-inset); D: Sieved powder**

**Organoleptic and physicochemical characterisation**

Organoleptic study was conducted with the sieved powder. The powder was dark-brown in color, with chocolatey odor, tasteless and of granular 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 amount of moisture content. The moisture content of the drug is not too high, thus it could prevent microbial growth. Moisture content of the powder was determined by loss on drying method and the value was 19.403%. Determination of total ash is important in evaluation of purity of drug become it confirms the presence or absence of foreign inorganic matter. The total ash was found to be 7.64% w/w. The hydration properties of mushroom powder, including the solubility, swelling capacity are desirable to get beneficial physiological functional characteristics. Swelling capacity and water holding capacity of the powder were 271.43±10.0 ml/g and 1112.00±85.19 g/g respectively. The methanol soluble extractive value was 7.85%. The extract was brown in color and sticky in nature.

**Fluorescence analysis**

Fluorescence is useful in identification of authentic samples and recognizing adulterates and thus is an important pharmacognostic character. The drug was allowed to react with different reagents which resulted in fluorescence by various chemical constituents present in 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 [34, 35]. In the present study powder drug was treated with 16 different chemical reagents which gave characteristics color when seen under UV light (365 nm) and was compared with color observed under ordinary light (Table 1).

**Table 1: Fluorescence analysis of dry powder *Ganoderma lucidum***

| Sl No. | Reagent | Visible | UV | |
|---|---|---|---|---|
| | | | Long (365nm) | Short (254nm) |
| 1 | Powder as such | Brown | Black | Black |
| 2 | Hager's | Dark black | Black | Black |
| 3 | Mayer's | Dark brown | Black | Black |
| 4 | Dragendroff's | Dark brown | Black | Dark brown |
| 5 | Iodine solution | Dark brown | Black | Black |
| 6 | 1(N) $HNO_3$ | Dark brown | Black | Black |
| 7 | 50% $HNO_3$ | Reddish brown | Black | Brown |
| 8 | Phloroglucinol | Reddish brown | Black | Black |
| 9 | Barfoed reagent | Dark brown | Black | Black |
| 10 | Sodium nitroprusside | Dark brown | Black | Blackish brown |
| 11 | Ninhydrin | Dark brown | Black | Black |
| 12 | $FeCl_3$ | Dark brown | Black | Black |
| 13 | 1(N)NaOH | Black | Black | Black |
| 14 | Acetic acid | Dark brown | Black | Black |
| 15 | 1(N)HCl | Dark brown | Black | Black |
| 16 | Methanol | Dark brown | Black | Black |
| 17 | 1(N) NaOH in methanol | Dark brown | Black | Black |

**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 methanolic extract of the powder showed positive test for terpenoids, phenolics and flavonoids while absence of saponins (Table 2).

**Table 2: Qualitative chemical screening of methanol extract from *Ganoderma lucidum***

| Name of tested chemical | Reagent/Test | Nature of change | Result |
|---|---|---|---|
| Flavonoid | Ferric chloride | Green coloration | ++ |
| Phenol | Folin-ciocalteu | Bluish black coloration | ++ |
| Saponin | Shaking | No change | - |
| Terpenoids | Acetic anhydride with sulphuric acid | Reddish color | + |

*Note: + = Present in low amount, ++ = Present in moderate amount*

Table 3 summarises the total phenol, flavonoids, ascorbic acid, beta-carotene and lycopene content in the Methanolic extract of *Ganoderma lucidum*. Data shows that total phenols and flavonoids were the major antioxidant components, whereas, ascorbic acid, β- carotene and lycopene were found in vestigial amounts.

**Table 3: Bioactive phytochemicals in the methanol exract of *G. lucidum***

| Flavonoids | Total phenols | Ascorbic acid | β- carotene | Lycopene |
|---|---|---|---|---|
| (µg/mg) | | | | |
| 4.855±0.4 | 22.285 ± 0. 799 | 2.77 ± 0.416 | 0.106 ± 0.027 | 0.079 ± 0.005 |

## Chromatographic characterisation

HPTLC is an efficient and valuable technology for the preliminary separation and determination of constituents. The plate was scanned from 200 nm to 450 nm with 50 nm intervals. It has been observed in figure 2 that maximum number of peaks were detected at 300 nm. The respective peaks at 300 nm have been discussed in table 4. Figure 3 represents HPLC chromatogram of the extract consisting of 26 peaks excluding the solvent peaks. The retention times of each peak have been marked in the figure. The visualized peaks together with the HPTLC peaks may act as a specific fingerprint towards authentication of the crude samples.

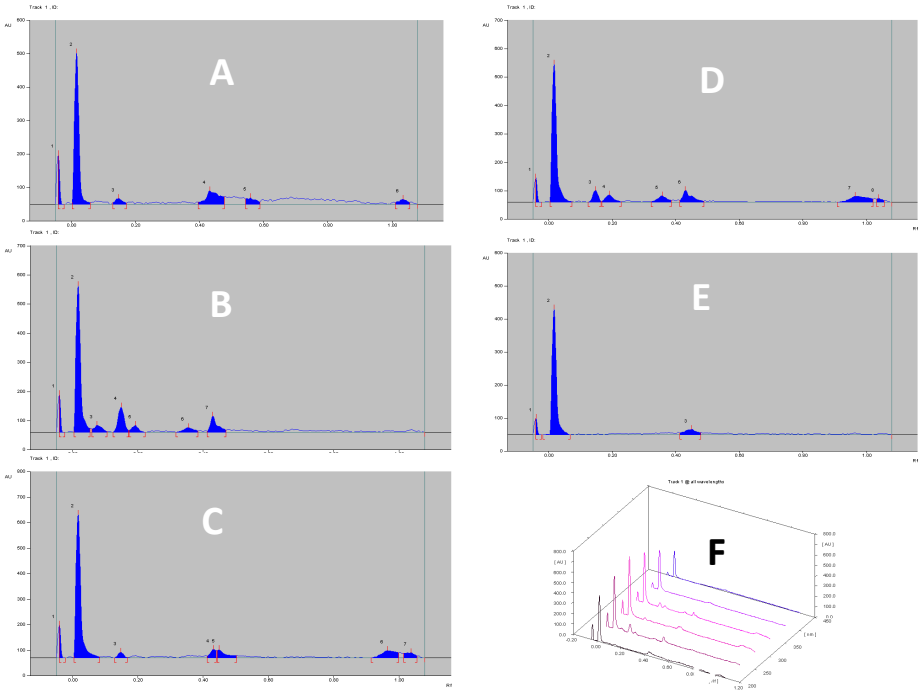


**Figure 2: HPTLC fingerprint of methanolic extract from *Ganoderma lucidum*. A: 200 nm B: 250nm C: 300 nm D: 350 nm E: 400 nm F: Chromatogram in all 5 wavelengths**

**Table 4: HPTLC Chromatogram at 300 nm of methanol extract from *Ganoderma lucidum***

| Peak no | $R_f$ | Max Height (AU) | Area (AU) |
|---|---|---|---|
| 1 | 0.13 | 22.4 | 264.2 |
| 2 | 0.41 | 32.9 | 424.9 |
| 3 | 0.44 | 31.7 | 611.4 |
| 4 | 0.92 | 28.2 | 909.5 |
| 5 | 1.02 | 19.8 | 425.6 |

## Antioxidant assay

DPPH assay has been widely used to test free radical scavenging ability of various food samples. In methanol solution DPPH produces violet color. Suitable reducing agent donate electron to DPPH and the solution loses color depending upon the number of electron taken up. Color changes from purple to yellow and the reduction capacity of $DPPH^{\cdot}$ is determined by decrease in its absorbance at 517 nm. Extract exhibited radical scavenging activity at the rate of 16.34%, 39.97% and 74.03% at 17.5, 87.5 and 175 µg concentrations respectively. $EC_{50}$ value was found to be at 666 µg which was much higher than that of ascorbic acid, a positive control.

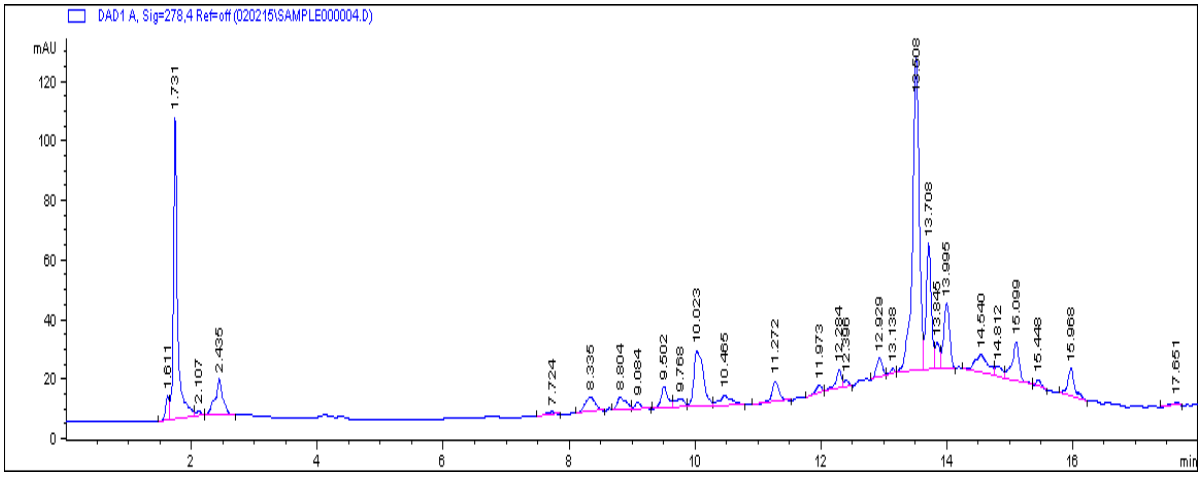


**Figure 3: Enlarged HPLC chromatogram of methanolic extract from *Ganoderma lucidum***

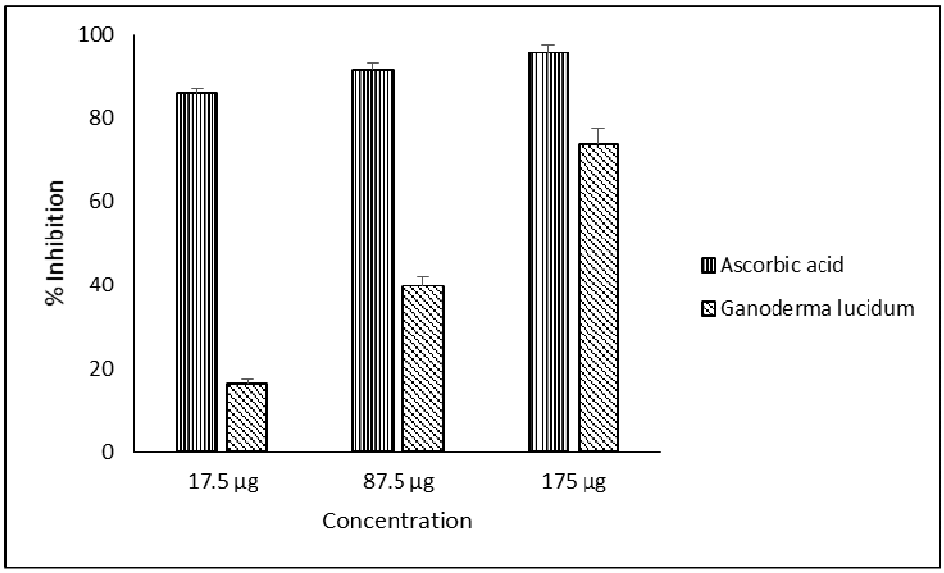


**Figure 4: DPPH radical scavenging activity of the methanolic extract of *G. lucidum* compared with standard. The values are the means of three separate experiments each in triplicate**

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 24.761 ± 2.973 µg AAE/ mg of extract total antioxidant activity.

## CONCLUSION

The study deals with the identification of salient microscopic and physico-chemical characters along with chromatographic fingerprints of a medicinally important mushroom *Ganoderma lucidum*, 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.

## REFERENCES

[1] K Acharya; S Chatterjee; S Ghosh. *Pharmacologyonline*, **2011**, 1, 440-450.

[2] S Chatterjee; GK Saha; K Acharya. *Pharmacologyonline*, **2011**, 3, 88-97.
[3] S Khatua; S Paul; K Acharya. *Res. J. Pharm. Technol.*, **2013**, 6, *5*, 496-505.
[4] G Biswas; K Acharya. *Int. J. Pharm. Pharm. Sci.*, **2013**, 5, *1*, 391-394.
[5] S Giri; G Biswas; P Pradhan; SC Mandal; K. Acharya**.** *Int. J. PharmTech Res.*, **2012**, 4, *4*, 1554-1560.
[6] S Mallick; A Dutta; S Dey; J Ghosh; D Mukherjee; SS Sultana; S Mandal; S Paloi; S Khatua; K Acharya; C Pal. *Exp. Parasitol.*, **2014**, 138, 9-17.
[7] G Biswas; S Chatterjee; K Acharya. *Dig. J. Nanomater. Bios.*, **2012**, 7, 185-191.
[8] S Chatterjee; G Biswas; S Chandra; GK Saha; K Acharya. *Bioprocess Biosyst. Eng.*, **2013**, 36, 101-107.
[9] A Chatterjee; S Khatua; S Chatterjee; S Paloi; S Mukherjee; A Mukherjee; K Acharya; SK Bandyopadhyay. *Glycoconjugate J.*, **2013**, 30, 759-768**.**
[10] G Biswas; S Sarkar; K Acharya. *Dig. J. Nanomater. Bios.*, **2011**, 6, 637-641.
[11] S Chatterjee; A Dey; R Datta; S Dey; K Acharya. *Int. J. PharmTech Res.*, **2011**, 3, 2162-2168**.**
[12] SP Wasser. *Appl. Microbiol. Biotechnol.*, **2011**, 89, 1323-1332.
[13] 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.
[14] AK Nandi; IK Sen; S Samanta; K Maity; KSP Devi; S Mukherjee; TK Maiti; K Achrya; SS Islam. *Carbohydr. Res.*, **2012**, 363, 43-50.
[15] S Samanta; K Maity; AK Nandi; IK Sen; KSP Devi; S Mukherjee; TK Maiti; K Acharya; SS Islam. *Carbohydr. Res.*, **2013**, 367, 33-40.
[16] AK Nandi; S Samanta; IK Sen; KSP Devi; TK Maiti; K Acharya; SS Islam. *Carbohydr. Polym.*, **2013**, 94, 918-926.
[17] AK Nandi; S Samanta; S Maity; IK Sen; S Khatua; KSP Devi; K Acharya; TK Maiti; SS Islam. *Carbohydr. Polym.*, **2014**, 99, 774-782.
[18] JC Mau; HC Lin; CC Chen. *J. Agric. Food Chem.*, **2002**, 50, *21*, 6072-6077.
[19] QG Yue; KP Fung; PC Leung; CB Lau. *Phytotherapy Res*, **2008**, 22, *10*, 1282-1291.
[20] CJ Weng; GC Yen. *Clinical and experimental Metastasis*, **2010**, 27,*5*, 361-369.
[21] B Boh; M Beroric; J Zhang; L Zhi Bin. *Biotechnol. Annual Rev.*, **2007**, 13, 265-301.
[22] JR Sharma. In Aphyllophorales of Himalaya, Botanical Survey of India, Kolkata, **2012**; pp. 173-174.
[23] P Pradhan; AK Dutta; K Acharya. *Protoc. Exch.*, **2015**, DOI: 10.1038/protex. 2015. 026.
[24] AK Tripathi; S Tripathy; NK Verma. *Der Pharm. Lettre*, **2014**, 6, *5*, 30-36.
[25] C Phat; H Li; D-U Lee; BK Moon; Y-B Yoo; C Lee. *J. Food Eng.*, **2015**, 145, 19-24.
[26] AZ Yusuf; A Zakir; Z Shemau; M Abdullahi; SA Halima. *J. Pharmacog. Phtytother.*, **2014**, 6, *2*, 10-16.
[27] VL Singleton; JA Rossi. *Am. J. Enol. Vitic*., **1965**, 16, 144-158.
[28] EA Adebayo; JK Oloke; AA Ayandele; CO Adegunlola. *J. Microbiol. Biotech. Res*., **2012**; 2(2): 366-374.
[29] M Nagata; I Yamashita. *Nippon Shokuhin Kogyo Gakkaishi*, **1992**, 39, *10*, 925-928.
[30] C Rekha; G Poornima; M Manasa; V Abhipsa; DJ Pavithra; KHT Vijay; KTR Prashith. *Chem. Sci. Trans*., **2012**, 1, *2*, 303-310.
[31] S Khatua; AK Dutta; K Acharya. *PeerJ*, **2015**; 3:e810; DOI 10.7717/peerj.810.
[32] P Mitra; NC Mandal; K Acharya. *Der Pharm. Lettre*, **2014**, 6, *5*, 92-98.
[33] MA Sonibare; OV Olatubosun. *Pharmacognosy J.*, **2015**, 7, *2*, 107-116.
[34] S Bhattacharya; MK Zaman. *Int. J. PharmTech Res.*, **2009**, 1, *2*, 292-298.