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Research Article

Pharmacognostic Standardization and Free Radical Scavenging Evaluation of *Meripilus giganteus* (Pers.) P. Karst., A Potential Medicinal Mushroom

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ABSTRACT

Meripileus giganteus (Meripilaceae), also known as “giant polypore” is considered as a functional food and source of health promoting medicines. However, scientific parameters for identification and assertion of purity of the exact fungal material are not yet available. In this backdrop, the present investigation was undertaken to determine pharmacognostic properties of this wild mushroom. Different requisite parameters including organoleptic characters, microscopic studies and fluorescence behaviour of powdered crude drug were analyzed. In addition, phytochemical screening, HPLC finger print profiling and antioxidant activity of methanol extract were also evaluated. Microscopic studies revealed the presence of monomitic hyphae with simple septa; clavate basidia with a basal clamp; ellipsoid to sub-globose spores etc. Furthermore, phytochemical analysis revealed that the methanol fraction was enriched with several myco-constituents such as phenol, flavonoids, ascorbic acid, β-carotene and lycopene; while the chromatogram identified 11 peaks which might be of phenolic compounds. Besides, the extract displayed strong antioxidant potentiality as evident by low EC_{50} value in DPPH radical scavenging activity (0.4 mg/ml) and high ascorbic acid equivalent (AAE) antioxidant capacity (27.5 µg AAE/mg of extract). In summary, this is the first report on the pharmacognostic studies of *M. giganteus* which will be beneficial as referential information for correct identification and characterization of crude drug.

Keywords: Antioxidant property, Giant polypore, HPLC, Physico-chemical evaluation, Quality assessment.

INTRODUCTION

Natural medicines play an important role in health care programmes because synthetic drugs often cause adverse effects. However, obstacle behind global acceptance of herbal medicines is the lack of documentation and quality control measures[1]. In this context, the subject area of pharmacognosy has gained immense importance being efficient for identification of crude drugs of natural origin for authentication. Over the course of history, this old phramacy science has expanded considerably beyond traditional techniques such as macroscopic and microscopic identification. In recent years, it also includes study of ethnomedicine, ethnopharmacology, phytochemical analysis, isolation of active principles and biological activity[2-4]. Oxidation is essential in many living organisms for production of energy. However, modern lifestyle and increase of average longevity gradually result in oxidative stress which is the key reason for growing incidence of diseases especially cancer, rheumatoid arthritis, atherosclerosis etc[5]. Antioxidants has property to reduce the stress and could be used to help human body from oxidative damage induced ailments. Though, several commercial synthetic antioxidants are available in market such as butylatedhyroxyanisole (BHA), butylatedhydroxytoluene (BHT), andtert-butylhydroquinone (TBHQ) but they have been restricted for use due to toxic effects. Therefore, investigation for natural alternative sources has been increased rapidly in which macrofungi are considered as a strong candidate[6,7]. Mushrooms have been valued by human kind as a medicinal resource as they are scientifically proved to be a source of physiological beneficial medicine. Recent studies have established multi-purpose application of several mushrooms including *Astraeus hygrometricus* (Pers.) Morgan[8], *Entoloma lividoalbum* (Kühner & Romagn.) Kubička[9,10], *Lentinula edodes* (Berk.) Pegler[11], *Pleurotus flabellatus* Sacc.[12], *Pleurotus florida* (Mont.) Singer[13], *Pleurotus ostreatus* (Jacq.) P. Kumm.[14], *Pleurotus sajor-caju* (Fr.) Singer[15], *Ramaria aurea* (Schaeff.) Quél.[16], *Macrocybe crassa* (Sacc.) Pegler & Lodge[17], *Russula albonigra* (Krombh.) Fr.[18], *Russula senceis* S. Imai[19], *Termitomyces clypeatus* R. Heim[20], *Termitomyces eurrhizus* (Berk.) R. Heim[21], *Termitomyces medius* R. Heim & Grassé[22] etc. *Meripileus giganteus* (Pers.: Pers.) Karst. is also a popular medicinal mushroom that originates from Europe and North America. This edible macrofungi is a member of family Meripilaceae, order Polyporales, phylum Basidiomycycota and commonly known as “giant polypore” or “black staining polypore”. Recent investigation on screening pharmacological activities have expressed significant biological activities such as antioxidant, antibacterial,

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cytotoxic, neurotoxic, hemolytic and immunomodulation potentialities[23,24]. However, no pharmacognostic study has been carried out on this mushroom. Hence, the present work was conducted to evaluate pharmacognostic parameters of *M. giganteus* with a view to establish standards for its identity, quality, purity and chemical composition. Additionally, antioxidant activity of the macrofungi using *in vitro* assays was also determined for future application.

MATERIALS AND METHODS

Collection

Basidiocarps of *M. giganteus* were collected from market of Darjeeling, West Bengal, India. A representative specimen (Accession no: CUH AM053) was deposited at the CUH herbarium following the method of Pradhan *et al*[25]. For powder analysis basidiocarps were dried properly by a field drier at 40°C for one day to make them crispy. Dried fruit bodies were pulverized using an electric blender, sieved through 160 mesh and stored in an air tight container.

Microscopic evaluation of powdered basidiocarps

Powdered sample was hydrated and macerated with 10% KOH and mounted on glass slide with glycerol. For effective results various stains (Congo red, Melzer's reagent) were used to distinguish different cellular structure. Photomicrographs were taken using compound binocular microscope having sensor aided digital camera and computer attachment (Leica DMLS). Different organoleptic characters like colour, odour, taste and nature of powdered samples were evaluated.

Fluorescence analysis

Fluorescence analysis was determined using standard Pharmacopoeial method[26]. A small quantity of dried sieved mushroom powder was placed on a grease free clean microscopic slide and 1-2 drop of freshly prepared reagent solutions were added, mixed and waited for 1-2 minutes. The slide was further placed inside the UV chamber and viewed under daylight, short (254 nm) and long (365 nm) UV radiations. The colour observed by application of different reagents in different radiations were recorded.

Preparation of methanol extract

Dried powdered fruiting bodies (5 g) were extracted by stirring with 100 ml of methanol for overnight and subsequently separated by Whatman filter paper. The residue was then re-extracted with 30 ml of methanol and the combined methanolic extracts were evaporated at 40°C (Rotavapor R3 Büchi, Switzerland) to reduce volume. The methanolic fraction was stored at -20°C in dark bottle until analysis, for no more than 1 month. Percentage yield and organoleptic parameters of the extract were recorded.

Quantitative estimation of myco-chemicals

The content of total phenolic compounds in extract was estimated using Folin-Ciocalteu reagent and gallic acid as standard. The results were expressed as µg of gallic acid equivalents per mg of dry extract[27]. Total flavonoid content was determined using aluminium nitrate and potassium acetate. Quercetin (5–20 µg/ml) was used to calculate the standard curve. The results were expressed as µg of quercetin equivalents per mg of dry extract[28]. β-carotene and lycopene were estimated by measuring absorbance at 453, 505 and 663 nm[29]. Ascorbic acid was determined by titration against 2, 6-dichlorophenol indophenol dye[30].

High performance thin layer chromatography (HPLC) fingerprinting

The extract was filtered through 0.2 µm filter paper and 20 µl filtrate was loaded on 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[19].

DPPH radical scavenging assay

Hydrogen atom or electron donation abilities of the methanol extract and a pure compound, ascorbic acid, were measured using purple coloured methanol solution of DPPH. Various concentrations of extract (0.5, 1 and 1.5 mg/ml) were added to 2 ml of 0.004% methanol solution of DPPH (w/v). After 30 min incubation period at room temperature in dark, the absorbance was read against a methanol blank at 517 nm[31]. EC_{50} value is the effective concentration at which DPPH radicals were scavenged by 50%. Degree of scavenging was calculated by the following equation: Scavenging effect (%) = $\{(A0-A1)/A0\} \times 100$, Where A0 and A1 were the absorbance of control and absorbance in presence of sample respectively.

Determination of total antioxidant capacity by phosphomolybdenum method

The assay was carried out as described by Prieto *et al*[32] with little modification. 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). Blank was prepared by adding 0.3 ml water and 3 ml reagent solution. Tubes were capped and incubated at 95ºC for 90 min. Samples were cooled at room temperature and absorbance was measured at 695 nm against blank. Concentrations of ascorbic acid (1 – 30 µg) were used to obtain a standard curve. Total antioxidant activity was expressed as the number of ascorbic acid equivalents (AAE)/mg of extract.

RESULTS AND DISCUSSION

Microscopic evaluation of powdered basidiocarps

After passing through sieve, the dried powder appeared greyish yellow coloured, pungent in smell, tasteless and granular in texture. Besides, the powder was macerated with HNO_3 and KOH individually and micro-morphological characters were observed. General description along with measurements of hyphae, basidia and spores are given below: Hyphae: monomitic, with simple septa, thin-walled, 4.69-6.13 µm wide; cystidia absent; basidia: clavate, 4-sterigmate, 21.7-33.56 x 6.13-7.81 µm, with a basal clamp; spores: 6.25-9.375 µ x 5.94-6.13 µm, ellipsoid to sub-globose, exposure hyaline, smooth and thin-walled (Figure 1). Melzer's reaction showed negative result signifying that the spores were non-amyloid in nature.

Table 1: Fluorescence analysis of dry powder from *Meripilus giganteus*.

Sr No.	Reagent	Visible light	UV light	
			Long (365nm)	Short (254nm)
1.	Powder as such	Light yellow	Dark grey	Creamish green
2.	Hager's	Lemon yellow	Greenish brown	Greenish yellow
3.	Mayer's	Straw yellow	Dark greyish brown	Yellow
4.	Dragendroff's	Yellowish orange	Blackish brown	Yellowish green
5.	Iodine solution	Orangish yellow	Black	Dark greenish brown
6.	1(N) HNO_3	Yellow	Brown	Pale green
7.	50% HNO_3	Greyish yellow	Greyish brown	Greenish yellow
8.	Phloroglucinol	Dark straw yellow	Dark brown	Greenish grey
9.	Barfoed	Olive green	Greyish brown	Light greenish brown
10.	Sodium nitroprusside	Straw yellow	Light greyish brown	Light green
11.	H_2O	Straw yellow	Greyish brown	Light green
12.	$FeCl_3$	Light yellow	Dark brown	Olive green
13.	1(N)NaOH	Pale yellow	Brown	Light yellow
14.	1(N) HCl	Yellow	Light brown	Greenish yellow
15.	Methanol	Pale yellow	Brown	Light yellow
16.	1(N) NaOH in methanol	Yellow	Dark greyish brown	Yellowish green

A

B

C

D

Figure 1: Macroscopic and microscopic characters of *Meripilus giganteus* A: Dried basidiocarps B: Fruit body in powder form C: Mycelia D: Basidium along with basidiospores.

Fluorescence analysis

Some atoms and molecules have property of absorption of light at a particular wavelength and subsequently emit light of longer wavelength, the phenomenon is called fluorescence. It is an important parameter for pharmacognostic evaluation in identification of authentic samples and recognition of adulterants. Various chemical constituents may exist in the crude drug that exhibit fluorescence in UV light. However, if the constituents are not fluorescent they often can be converted to fluorescent compounds by applying different reagents. As a result, the

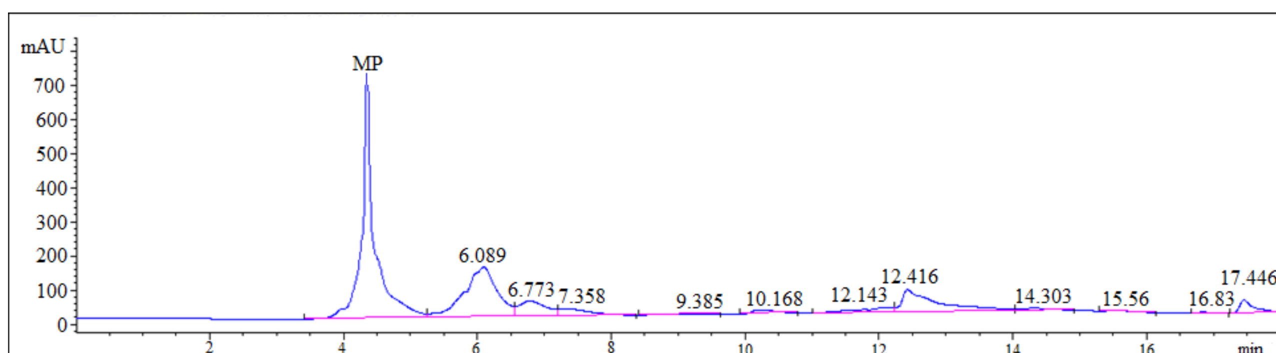

Figure 2: HPLC chromatogram of methanol extract from *Meripilus giganteus* (MP: Mobile phase).

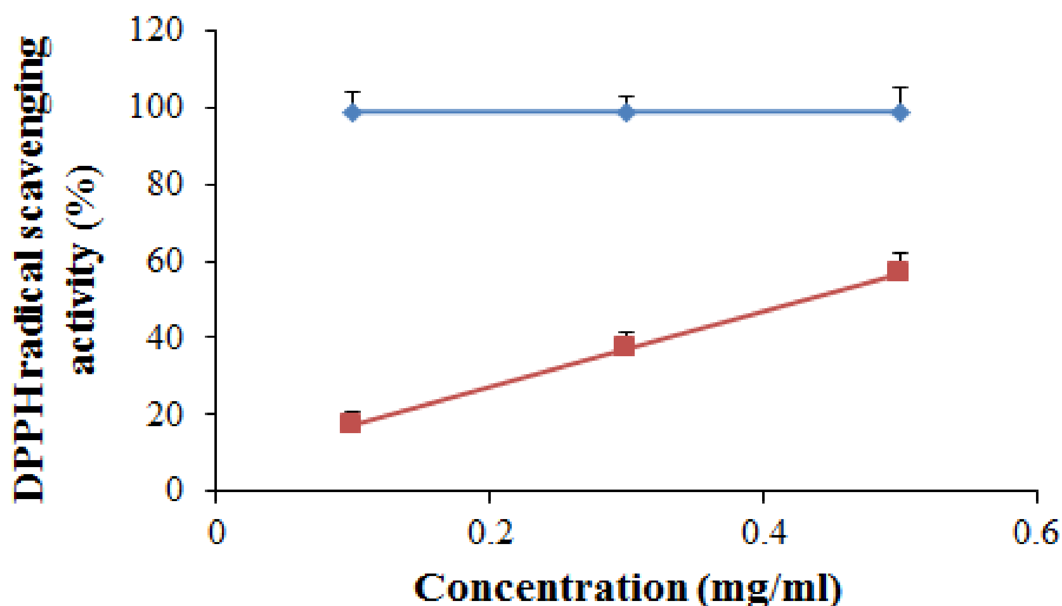

Figure 3: DPPH radical scavenging activity of methanol extract from *Meripilus giganteus.*

analysis displayed an array of colours that could be employed by which crude drugs are often assessed qualitatively[33,34]. In the present study powder drug was treated with 16 different chemical reagents which gave characteristic colours when observed under UV light (254 nm, 365 nm) and was compared with colour detected under visible light (Table 1).

Quantitative estimation of myco-chemicals

It has been demonstrated that most of the components in mushroom fruiting bodies are highly polar[35]. Thus in the present study, methanol was chosen for preparation of a fraction with extractive yield of 7.4±0.53% where the formulation appeared yellow in colour. Furthermore, the extract was subjected for quantitative analysis of different bioactive chemicals using standard protocols. The fraction was found to contain phenol as much as 5±0.88 µg gallic acid equivalent/mg of dry extract. Total flavonoid content was determined by using quercetin as standard. The extract contained flavonoid as 3.75±0.09 µg quercetin equivalent/mg of extract. Very negligible amount of β-carotene and lycopene were found such as 0.072±0.006 µg/mg and 0.028±0.003 µg/mg of the extract respectively. Ascorbic acid was found in the higher amount i.e. 3.19±1.06 µg/mg of extract.

HPLC fingerprinting

HPLC is an efficient and valuable technology for the preliminary separation and determination of constituents. Separation of bioactive compounds from the methanol extract was carried out using HPLC (Figure 2). The fingerprint showed presence of 11 peaks with Rt values of 6.089 min, area 5036.026; Rt 6.773 min, area 1276.359; Rt 7.358 min, area 672.097; Rt 9.385 min, area 116.535; Rt 10.168 min, area 229.576; Rt 12.143 min, area 512.061; Rt 12.416 min, area 2450.631; Rt 14.303 min, area 242.871; Rt 15.56 min, area 80.047; Rt 16.830 min, area 39.381; Rt 17.446 min, area 547.075.

DPPH radical scavenging assay

DPPH radical scavenging assay is the most commonly used and easy method to assess of antioxidant capacity. It is a stable N_2-centered free radical which gain stability after accepting an electron/hydrogen. In methanol solution DPPH produces violet colour which is changed to yellow, depending upon the number of electron taken up. Change in colour is determined by decrease in its absorbance at 517 nm. The degree of scavenging can be used to magnify hydrogen-donating ability of antioxidants. In the present study, DPPH radical scavenging activity of the methanol extract from *M. giganteus* was determined following the

assay which was found to be directly correlated with concentration of the sample (Figure 3). The fraction exhibited radical scavenging activity at the rate of 17.39%, 37.2% and 56.76% at 0.1, 0.3 and 0.5 mg/ml concentrations respectively. EC_{50} value was found to be at 0.4±0.093 mg/ml which was much higher than that of ascorbic acid, a positive control i.e. 0.004 mg/ml. Result demonstrated higher activity of the extract than that of *Macrocybe crassa*[17], although it was lower than *Laetiporus sulphureus*[36].

Determination of total antioxidant capacity by phosphomolybdenum method

Phosphomolybdenum method is a good procedure for evaluation of total antioxidant capacity. The assay is based on reduction of Mo (VI) to Mo (V) by antioxidant compound and formation of green phosphate/Mo (V) complex at acidic pH. Total antioxidant capacity of the fraction was investigated and compared against ascorbic acid. The extract presented 27.5±5.83 µg AAE/ mg of extract total antioxidant activity. The fraction presented higher activity than methanol extract of *Grifola frondosa*[37].

CONCLUSION

The present work provides pharmacognostic information on basis of modern techniques for correct identification and standardization of *M. giganteus* powder. Several suitable parameters including microscopic features, organoleptic characters, myco-chemical investigation and HPLC analysis were evaluated which would be helpful for establishing the pharmacopeia standards. Moreover, antioxidant activity of methanol extract from the mushroom was also investigated in terms of DPPH radical inhibitory action and total antioxidant capacity. Thus this study is useful for authentication and development of nutraceuticals from the mushroom as it provides information concerning pharmacognosy and bioactivity respectively.

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