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Nutraceutical and bioactive potential of *Macrocybe gigantea* through nutritional, phytochemical and GC-MS characterization

Gourab Kumar Barman[1], Milanti Kujur[1], Jyotsna Das[2], Dipanwita Saha[3] and *Aniruddha Saha[1]

[1]Molecular Plant Pathology and Fungal Biotechnology Laboratory, Department of Botany, University of North Bengal, West Bengal, India-734013
[2] Department of Botany, Alipurduar University, West Bengal, India-736122
[3]Plant Biotechnology Laboratory, Department of Biotechnology, University of North Bangal, West Bengal, India-734013
**Corresponding author*; E-mail: asahanbu@yahoo.co.in

ABSTRACT

Macrocybe gigantea is a giant edible mushroom traditionally consumed in India, but its bioactive potential remained underexplored. In this study, the species was identified by morphological and ITS-based molecular analysis, and its nutritional, phytochemical, and antimicrobial properties were evaluated. Nutritional analysis revealed appreciable carbohydrate (35.4 gm/100 gm DW) and protein (22.3 gm/100 gm DW) content, while mineral profiling indicated exceptionally high iron levels (196.5 mg/100 gm). Phytochemical screening showed a total phenolic content of 14 mg GAE/gm and flavonoid content of 3 mg QE/gm, which correlated with strong antioxidant activity (78% DPPH radical inhibition at 500 µg/mL). Antimicrobial assays demonstrated significant antifungal activity, particularly against *Curvularia lunata* (93.2% inhibition) and *Curvularia hominis* (93.2% inhibition). GC-MS analysis of the methanolic extract revealed bioactive metabolites, including fatty acid esters and sugar derivatives such as â-D-galactopyranoside and octadecadienoic acid derivatives, compounds known for antioxidant and antimicrobial effects. Overall, the findings highlight *M. gigantea* as a nutritionally rich mushroom with strong antioxidant and antifungal potential, supporting its ethnomedicinal use and suggesting promising applications in nutraceutical and pharmaceutical industries.

Keywords: Mushrooms, *Macrocybe gigantea*, ITS sequencing, antioxidant, antibacterial, minerals, GC-MS analysis

Macrocybe gigantea (Massee) Pegler and Lodge is a large, fleshy, edible macro-fungus belonging to the family Tricholomataceae under the order Agaricales (Pegler *et al.*, 1998; Kui *et al.*, 2021). However, some studies have also placed it under the family Biannulariaceae (Wijayawardene *et al.*, 2020). It typically occurs in warm, humid climates and is distributed across tropical and subtropical forests of Africa and Asia. In India, it has been reported from several regions including Karnataka (Pushpa *et al.*, 2014), Kerala (Manimohan *et al.*, 2007) and West Bengal (Khatua and Acharya, 2016).

The mushroom usually appears during the rainy season and grows either in clusters or groups. It is easily recognized by its large size, cream to greyish or ochraceous pileus, and white spores. Morphologically, the pileus ranges from convex to umbonate or depressed, and the stipe is white and fleshy. Microscopic analysis showed clamped hyphae, a key taxonomic trait distinguishing it from *Tricholoma* species, which possess clampless hyphae and are ectomycorrhizal in nature (Pegler *et al.*, 1998).

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Historically, this species was included in the genus *Tricholoma*. However, based on distinct morphological and molecular traits, Pegler *et al*. (1998) reclassified it under the newly defined genus *Macrocybe*. It has occasionally been misclassified as *Calocybe gigantea* due to its large basidiomata; however, *Calocybe* differs by possessing siderophilous granules in the basidia and displaying significant molecular differences (Devi and Sumbali, 2021).

M. gigantea is widely consumed in various parts of India. Its sweet taste and pleasant aroma have contributed to its popularity, and it is also employed as a traditional home remedy (Wang *et al*., 2004). It is rich in proteins, polysachharides, glycogen, fats, amino acids, and various vitamins (Pamitha and Latha, 2014). It also contains important minerals such as magnesium, zinc, and calcium (Liu *et al*., 2012), making it a valuable dietary component. In terms of pharmacological potential, *M. gigantea* is reported to exhibits strong antioxidant properties, including lipid peroxidation inhibition and superoxide radical scavenging (Banerjee *et al*., 2007; Gaur and Rao, 2016). It also shows antibacterial and antitumor activities (Giri *et al*., 2012). Notably, laccase enzymes extracted from its fresh fruiting bodies have been reported to inhibit HIV-1 reverse transcriptase with an IC_{50} of 2.21 µM (Wang and Ng, 2004), underscoring its significant therapeutic potential.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted in West Bengal, India, a region known for its tropical and subtropical climate supporting diverse fungal species. The mushroom was found growing at the base of *Delonix regia*, closely associated with the roots. The mushroom specimen were collected from Raja Rammahunpur, Darjeling, West Bengal, India (Latitude 26.708802, Longitude 88.355201). The area is characterized by high humidity, moderate rainfall, and organic matter-rich soil, providing an ideal environment for fungal growth.

Mushroom fruiting bodies were collected in May to August, 2024. Samples were photographed and characters were noted in their natural habitat, carefully harvested, and stored in sterile bags to minimize contamination. The specimens were then transported to the laboratory for further examination.

Pure Culture Isolation

Pure culture was obtained from the fruiting body following the tissue culture method (Chang and Miles, 2004). The outer surface of the basidiocarp was washed thoroughly under running tap water and surface-sterilized with 70% ethanol for 1 min, followed by rinsing in sterile distilled water. Small inner tissue blocks were aseptically excised from the junction of pileus and stipe using a sterile scalpel under laminar airflow. The tissue bits were placed on Potato Dextrose Agar (PDA) plates supplemented with Chloramphenicol (74 µl/100mL) to prevent bacterial contamination. Plates were incubated at 28±2°C in the dark.

Morphological study

Macroscopic features such as cap shape, size, color, gills, and stipe characteristics were documented using a camera. Microscopic features were analyzed by preparing slides stained with cotton blue to observe spores, basidia, and other structures under a light microscope.

Molecular Identification

Genomic DNA was extracted from fresh fruiting body using the CTAB method (Sharma *et al*., 2003). The internal transcribed spacer (ITS) region of 5.8s rDNA was amplified using universal primers ITS1 (5" -TCCGTAGGTGAACCTGCGG-3") and ITS4 (5"-TCCTCCGCTTATTGATATGC-3"). PCR products

were purified and sequenced commercially. The obtained sequence was deposited in NCBI GenBank (Accession No. OR554176).

Phylogenetic Analysis

The obtained ITS sequence was compared with related sequence using BLASTn against NCBI database. Multiple sequence alignment was performed with ClustalW in MEGA-11 software (Tamura *et al*., 2021). A phylogenetic tree was constructed using the Neighbor-Joining method (Saitou and Nei, 1987) with 1000 bootstrap replicates (Felsenstein's, 1985) to confirm the taxonomic position of *M. gigantea*.

Ethnobotanical Survey

Local villagers who frequently consume wild mushrooms including *Macrocybe gigantea* were interviewed for their knowledge about the mushroom. The interview was carried out were noted and cross verified by some other villagers. Participants were asked about their knowledge of the mushroom, including its local name, collection practices, preparation methods, and consumption. Verbal consent was obtained before the interviews.

Proximate analysis

Carbohydrate content was determined by the anthrone method (Yemm and willis, 1954) and protein content was estimated by the Lowry method (1951) using bovine serum albumin as a standard. Results were expressed as percentage dry weight (DW).

Mineral Analysis

Mineral composition was determined by Atomic Absorption Spectrophotometry (AAS). The analysis was outsourced to the Department of Agricultural Chemistry and Soil Science, Uttar Banga Krishi Vishwavidyalaya (UBKV), West Bengal, where standard protocols (AOAC, 2019) were followed to quantify essential minerals in the samples. Samples were digested using a nitric acid-perchloric acid mixture, and elements i.e. K, Ca, Mg, Na, Fe, Zn, Cu, Mn, Cr, Ni, Pb, and Cd were quantified. Results were expressed in mg per 100 g dry weight.

Preparation of Mushroom Extract

Following the protocol of Barros *et al*., 2007, methanol extract was prepared with little modifications. The collected fruiting bodies were washed, shade dried, and powdered. Ten grams of mushroom powder was soaked in 100 mL methanol and incubated in a shaking incubator for 18 h at room temperature. The extract was filtered through Whatman No. 1 filter paper and evaporated to dryness in a water bath. The dried extract was stored at 4p C until further use.

Phytochemical Estimation

Total phenolic content (TPC): Determined using the Folin-Ciocalteu reagent with gallic acid as standard; results expressed as mg gallic acid equivalent (GAE) per g extract (Kadam *et al*., 2013).

Total Flavonoid Content (TFC): Determined by the aluminium chloride colorimetric method (Atanassova *et al*., 2011) using quercetin as standard; results expressed as mg quercetin equivalent (QE) per g extract.

Antifungal assay

The dual culture approach was used to determine the antagonistic capability of macro fungi against phytopathogenic fungi (Dennis and Webster, 1971; Skidmore and Dickinson, 1976). The methanolic extract of *Macrocybe gigantea* was evaluated against phytopathogenic fungi (*Curvularia hominis*, *Curvularia lunata*, *Alternaria alternata*, *Fusarium equiseti*, *Fusarium oxysporum* and *Colletotrichum siamense*) obtained from Molecular plant pathology

and Fungal Biotechnology lab, Department of Botany, University of North Bengal, India. The dual culture technique was performed on PDA plates by placing a 5-mm mycelial disc of the test mushroom and the pathogen on opposite sides of the plate. Plates were incubated at 27±2°C for 8 days. Radial growth of the pathogens was measured along two perpendicular axes, and percentage growth inhibition (PGI) was calculated using the formula:

$$PGI = \frac{C - T}{C} \times 100$$

Where, C= radial growth of the pathogen in control and T= radial growth in the presence of the test isolate.

Antioxidant Activity

The antioxidant potential was evaluated by DPPH free radical scavenging assay (Brand-Williams *et al*., 1995). Different concentrations of extract (100-500 µg/mL) were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 min. Absorbance was measured at 517 nm, and percentage inhibition was calculated.

GC-MS Analysis for bioactive compounds

GC-MS analysis of the methanolic extract was performed at IIT Madras using an Agilent system equipped with an HP-5MS capillary column. Helium was used as the carrier gas, and the oven program was set from -60°C to 325°C. Mass spectra were compared with the NIST library for compound identification. Results were expressed as retention time (RT), compound name, molecular formula, and peak area percentage.

Statistical analysis

All experiments were conducted in triplicates, and the results are expressed as mean ± standard deviation (SD).

RESULTS

Morphological Characterization

Pileus 13-22 cm diameter, initially convex, later becoming applanate to plane at maturity; surface smooth to glabrous, creamish white to pale yellow in colour; margin incurved in young basidiomes, gradually straight to slight wavy at maturity. Stipe 18-22 × 4-6 cm, cylindrical, solid, wider base, central, circular in cross section; surface smooth to slightly fibrillose, concolourous with pileus (whitish to cream). Lamellae crowded to subcrowded, adnexed, whitish to cream in colour; edges smooth, entire; lamellulae present in 3-4 tiers. Spore print white (Fig 1).

Micro-morphological Characteristics

The basidia were clavate to cylindrical, four-spored, with prominent oil droplets, measuring 20.2-26.7 × 4.5-9.2 µm. Basidiospores were hyaline, thin walled, smooth, and broadly ellipsoid to subglobose, measuring 4.7-6.3 × 2.5-4.2 µm (Fig 2).

Phylogenetic Analysis

The ITS region of the collected sample was successfully amplified, producing a sequence of approximately 650 base pairs. BLAST analysis of query sequence (Accession No. OR554176) confirmed 99.53 % similarity to *Macrocybe gigantea* sequences available in GenBank (Accession No. MN197655). Phylogenetic analysis using the Neighbor-joining method placed the specimen within the *Macrocybe gigantea* clade with bootstrap support, confirming its taxonomic placement (Fig 3).

Ethnobotanical Observations

The traditional knowledge regarding the edibility of *Macrocybe gigantea* was recorded through interactions with local tribal and rural communities of Darjeeling district, West Bengal. The species is

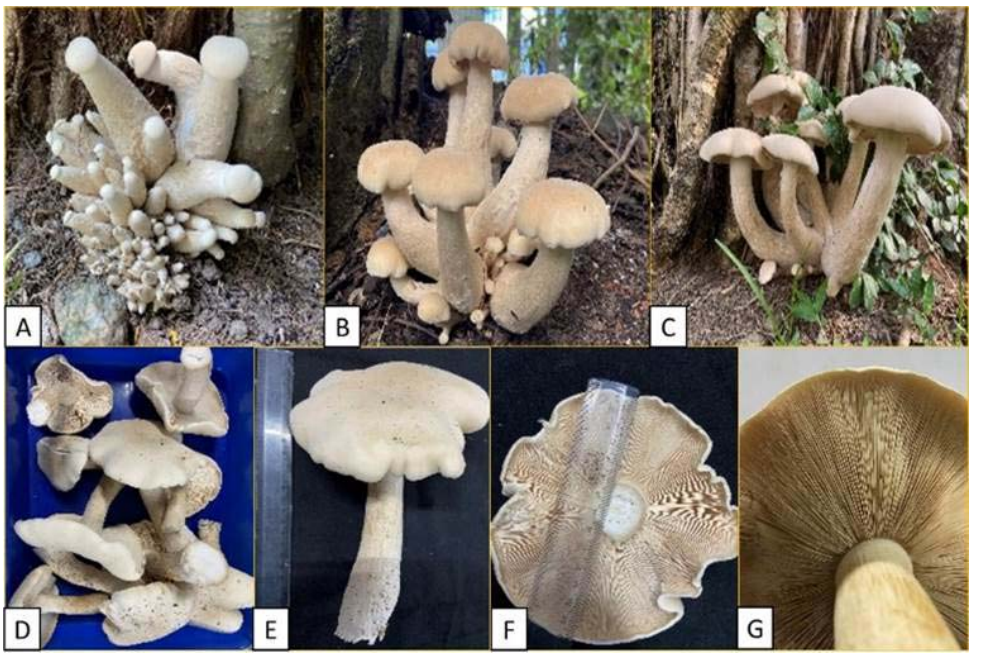

Fig. 1. Fruiting bodies of *Macrocybe gigantea* collected from natural habitat and examined morphologically: (A-C) clusters of mature basidiocarps growing on soil adjacent to the roots of *Delonix regia*; (D) freshly harvested basidiocarps; (E) whole fruiting body showing stipe and pileus; (F) pileus with ruler showing diameter; (G) gill structure (lamellae) on the ventral side of the pileus

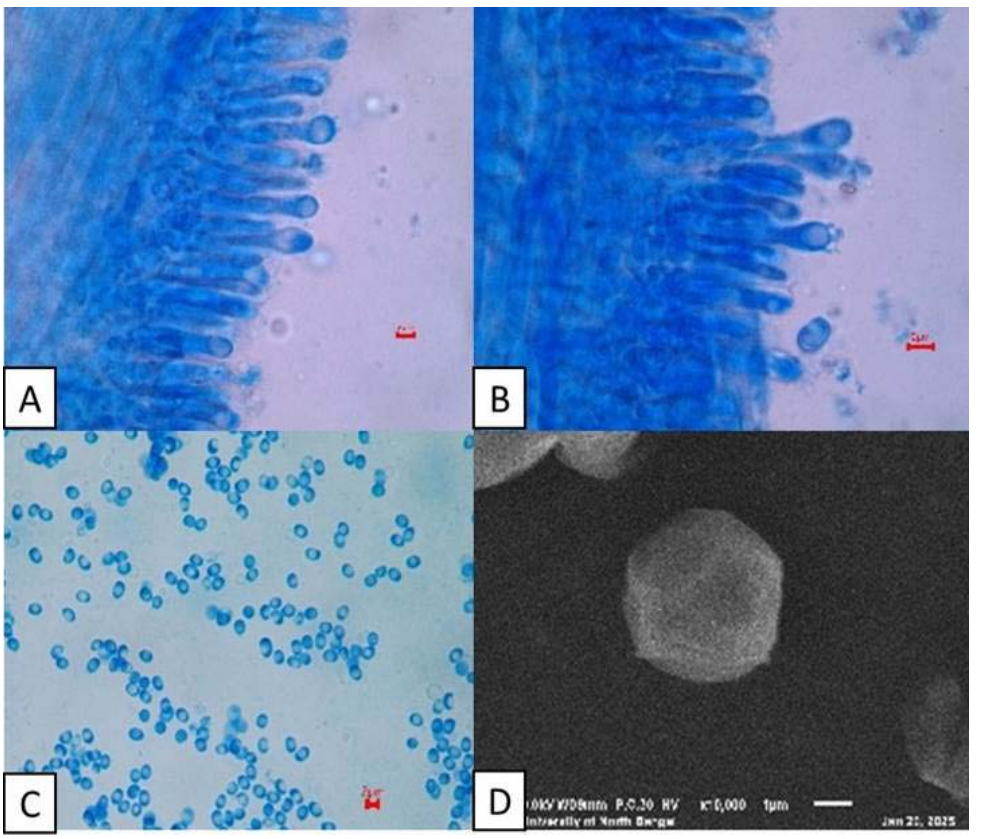

Fig. 2. Microscopic features of *Macrocybe gigantea*. (A-B) Basidia with sterigmata; (C) Basidiospores observed under light microscopy; (D) Basidiospores surface morphology under scanning electron microscopy (SEM). Scale bars= 2µm (A-C), 1 µm (D)

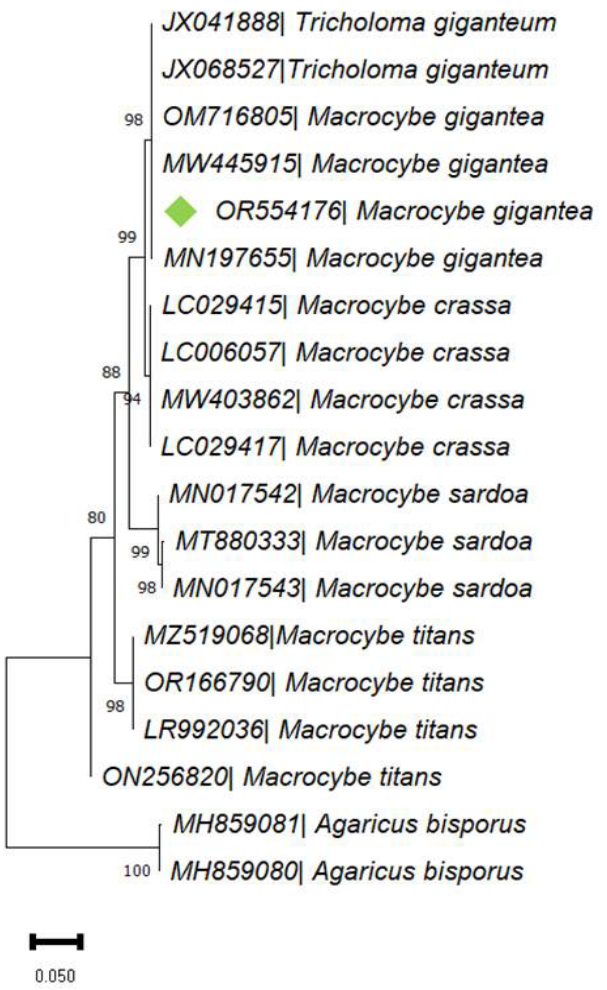

Fig. 3. Phylogenetic tree of *Macrocybe gigantea* based on ITS sequence data. The tree was constructed using the Neighbor-Joining method in MEGA11 with bootstrap analysis (1000 replicates). The query sequence, indicated by a light green label, clusters with reference sequences from NCBI, confirming its taxonomic placement within *Macrocybe gigantea*

regarded as a seasonal delicacy and is consumed as part of the traditional diet. After harvesting, the fresh fruiting bodies are thoroughly washed and chopped, and then cooked with locally available ingredients such as onion, garlic, chili, and tomato to prepare a curry (Fig 4). The preparation is usually consumed with rice as a staple food. Such practices not only confirm the edibility and cultural acceptance of *M. gigantea* but also highlight the ethnomycological value of this species in indigenous food traditions. Documentation of these practices validates the importance of wild mushrooms in nutritional security and their role in sustaining local knowledge systems.

Cultural studies

Tissue fragments aseptically excised from healthy fruiting bodies of *Macrocybe gigantea* successfully developed into pure mycelial cultures on PDA medium after 7-9 days of incubation at 28 ± 2°C (Fig 5). The colonies were initially white, cottony to fluffy, and gradually became denser with age. These pure mycelial cultures were further used for antifungal bioassays against phytopathogenic fungi.

Nutritional and phytochemical composition

Proximate analysis revealed appreciable levels of carbohydrates (35.4±2.4 gm/100gm Dry weight) and proteins (22.3±2.2 gm/100gm Dry weight). The

Fig. 4. Ethnobotanical documentation of *Macrocybe gigantea*: (A) Fresh mushroom fruiting bodies after washing; (B) sliced mushroom with onion, garlic, chili, and tomato before cooking; (C) prepared mushroom curry traditionally consumed by villagers

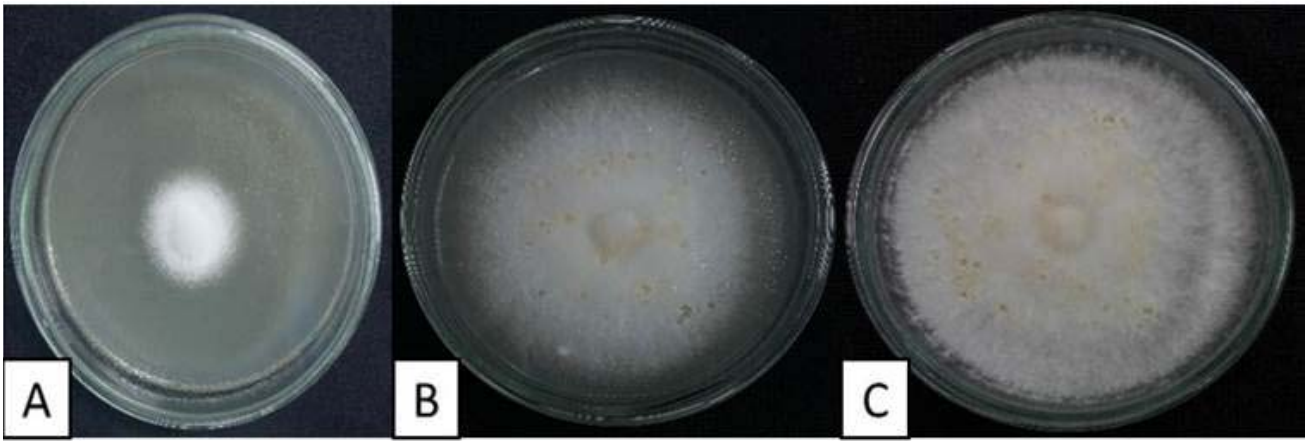

Fig. 5. Pure culture of *Macrocybe gigantea* on Potato Dextrose Agar (PDA) showing progressive mycelial growth: (A) 2 days, (B) 5 days, (C) 8 days of incubation at 28 ± 2°C

methanolic extract contained 14±1.2 mg GAE/gm phenolics and 3±0.2 mg QE/gm flavonoids (Table 1).

Table 1. Nutritional and phytochemical composition of *Macrocybe gigantea*

Parameter	Value (mean ± SD)
Carbohydrate	35.4±2.4 gm/100 gm DW
Protein	22.3±2.2 gm/100 gm DW
Total phenolic content	14±1.2 mg GAE/gm DW
Total flavonoid content	3±0.2 mg QE/gm DW
Antioxidant activity (DPPH)	78.14 % inhibition (500 µg/mL)

DW= dry weight; GAE= gallic acid equivalents; QE= quercetin equivalents; DPPH= 2,2-diphenyl-1-picrylhydrazyl

Mineral Composition

The mineral content of *Macrocybe gigantea* was quantitatively analysed and is presented in Table 2. The mushroom was found to be a rich source of essential minerals, with iron (196.5 mg/100 gm) recorded at the highest concentration. This was followed by magnesium (33.4 mg/100 gm), chromium (13.5 mg/100 gm), calcium (11.5 mg/100 gm), copper (9.8 mg/100 gm), zinc (7.3 mg/ 100 gm), and manganese (5.4 mg/100 gm).

Table 2. Mineral composition of *Macrocybe gigantea* (mg/ 100 gm dry weight).

Mineral Composition	Amount (mg/100gm)
Iron (Fe)	196.5
Magnesium (Mg)	33.4
Calcium (Ca)	11.5
Zinc (Zn)	7.3
Copper (Cu)	9.8
Manganese (Mn)	5.4
Chromium (Cr)	13.5

GC-MS Profiling

The methanolic extract of *Macrocybe gigantea* was subjected to GC-MS analysis, which revealed the presence of several bioactive compounds with diverse biological properties (Table 3). A total of seven major compounds were identified, representing different chemical classes such as fatty acids, esters, and sugar derivatives. Among them, β-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic methylboronate (34.16%) was the most abundant, followed by α-D-Glucofuranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,5-bis (butylboronate) (22.61%) and 2-Myristynoyl pantetheine (18.62%). Other notable

Table 3. Major compounds identified by GC-MS in methanolic extract of *Macrocybe gigantea*

Peak No	RT (min)	Peak Area (%)	Compound Name	Pub Chem CID	Molecular Formula	M.W. (g/mol)
1	4.405	5.47	3-hydroxy Dodecanoic acid	94216	$C_{12}H_{24}O_3$	216.32
2	6.332	18.62	2-Myristynoyl pantetheine	535560	$C_{25}H_{44}N_2O_5S$	484.7
3	26.181	6.56	Pentadecanoic acid, 13-methyl-, methyl ester	554151	$C_{17}H_{34}O_2$	270.4
4	30.282	9.69	7, 10- Octadecadienoic acid, methyl ester	5365663	$C_{19}H_{34}O_2$	294.5
5	30.420	2.90	6-Octadecenoic acid	11634	$C_{18}H_{34}O_2$	282.5
6	39.677	34.16	α-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic methylboronate	91696686	$C_{17}H_{37}BO_6Si_2$	404.5
7	39.935	22.61	α-D-Glucofuranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,5-bis (butylboronate)	101280625	$C_{17}H_{34}B_2O_6Si$	384.2

compounds included 7, 10- Octadecadienoic acid, methyl ester (9.69%), Pentadecanoic acid, 13-methyl-, methyl ester (6.56%), and 3-hydroxy Dodecanoic acid (5.47%), while 6-Octadecenoic acid (2.90%) was detected in the lowest concentration.

Antioxidant Activity

The methanolic extract of *M. gigantea* demonstrated concentration dependent DPPH radical scavenging activity, reaching 78.14 % inhibition at 500 µg/mL with an IC_{50} value of ~ 200 µg/mL (Fig 6).

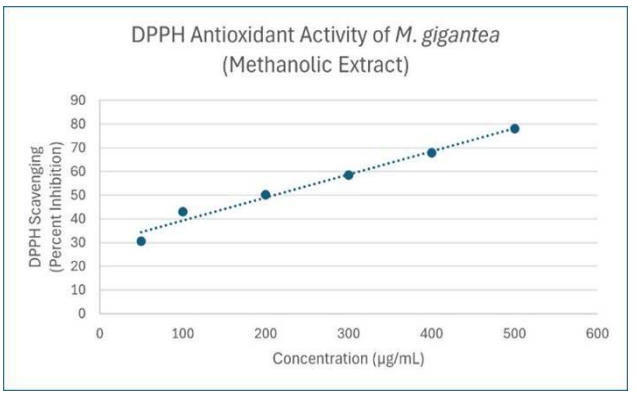

Fig. 6. DPPH antioxidant activity curve (% inhibition vs concentration)

Antifungal Activity

The antagonistic potential of *Macrocybe gigantea* against phytopathogenic fungi was evaluated using the dual culture assay (Fig 7 & 8). The pure culture of each phytopathogen was maintained separately as control to compare the radial growth with the dual culture plates. The mycelial growth of all test pathogen was significantly inhibited by the mushroom isolate, with the percent growth inhibition (PGI) ranging from 52.8% to 93.2% (Table 4).

The maximum inhibition was observed against *Curvularia lunata* (93.2%), followed by *Curvularia hominis* (75.2%) and *Fusarium equiseti* (62.9%). Moderate inhibition was recorded against *Alternaria alternata* (60.6%), while the lowest activity was noted against *Colletotrichum siamense* (52.8%). These results clearly indicate that *M. gigantea* possesses strong antifungal activity, particularly against *Curvularia* species. Whereas, in the dual culture assay with *Fusarium oxysporum*, the mushroom isolate failed to inhibit the growth of the pathogen. Instead, the pathogen exhibited vigorous growth covering almost the entire plate within 8 days, while the mushroom colony was restricted in size. No inhibition zone was observed, and the pathogen ultimately overgrew the mushroom culture, suggesting that the mushroom lacked antifungal activity against *F. oxysporum* under the tested conditions.

Table 4. Antifungal activity of *Macrocybe gigantea* against selected phytopathogenic fungi

Test Pathogen	Radial growth in control (cm) (in 8 days)	Radial growth in dual culture (cm) (in 8 days)	% inhibition
Curvularia lunata	8.9 ± 0.2	0.6 ± 0.4	93.2
Curvularia hominis	8.9 ± 0.1	2.2 ± 0.6	75.2
Fusarium equiseti	8.7 ± 0.3	3.1 ± 0.7	62.9
Fusarium oxysporum	8.9 ± 0.1	7.6 ± 0.4	14.6
Alternaria alternata	8.8 ± 0.2	3.4 ± 0.2	60.6
Colletotrichum siamense	8.6 ± 0.4	3.9 ± 0.3	52.8

Fig. 7. Pure culture of phytopathogenic fungi on PDA after 8 days of incubation at 28 ± 2°C: (A) *Curvularia lunata*, (B) *Curvularia hominis*, (C) *Alternaria alternata*, (D)*Fusarium oxysporum*, (E) *Fusarium equiseti,* (F) *Colletotrichum siamense*

DISCUSSIONS

The present study provides insights into nutritional, phytochemical, and antimicrobial potential of *Macrocybe gigantea*, a giant edible mushroom with ethnobotanical significance in eastern India. Morphological and molecular characterization confirmed its identity, supporting earlier reports that place this species within the Lyophyllaceae (Razaq *et al*., 2016). Nutritional profiling revealed appreciable levels of carbohydrate and protein, comparable to other cultivated mushrooms such as *Pleurotus sp*. and *Calocybe sp*. (Alam *et al*., 2008). The high protein content indicates its role as a sustainable plant-based

Fig. 8. Dual culture assay of *Macrocybe gigantea* against phytopathogenic fungi at different time intervals. (A-1, A-2, A-3) *Curvularia hominis*; (B-1, B-2, B-3) *Curvularia lunata*; (C-1, C-2, C-3) *Colletotrichum siamense*; (D-1, D-2, D-3) *Fusarium equiseti*; (E-1, E-2, E-3) *Alternaria alternata*; (F-1, F-2, F-3) *Fusarium oxysporum* observed at 2, 5, 8 days, respectively

protein source, while its notable iron content (196.5 mg/100gm) highlights its dietary relevance in addressing micronutrient deficiencies. Similar mineral enrichment has been reported in *Agaricus bisporus* and *Pleurotus ostreatus* (Abou raya *et al*., 2014), but the iron content of *M. gigantea* appears particularly high, suggesting its value as a functional food. The presence of phenolic (14 mg GAE/gm) and flavonoid (3 mg QE/gm) compounds, along with strong antioxidant activity (78% inhibition at 500 µg/mL), indicates the mushroom2 s potential to counteract oxidative stress. Previous studies on wild mushrooms demonstrated that phenolic-rich extracts show strong radical scavenging capacity (Barros *et al*., 2007), and the antioxidant profile of *M. gigantea* aligns well with these findings.

Antimicrobial assays demonstrated pronounced antifungal activity, with maximum inhibition against *Curvularia lunata* (93.2%) and *Curvularia hominis* (75.2%). Such strong activity suggests the presence of bioactive metabolites capable of suppressing pathogenic fungi. These findings agree with earlier reports on edible mushrooms showing antifungal activities. For example, Chen and Huang (2011) demonstrated that culture filtrates of *Lentinula edodes*, *Clitocybe nuda*, and *Ganoderma lucidum* strongly inhibited spore germination and mycelial growth of several phytopathogens such as *Colletotrichum higginsianum*, *Alternaria brassicicola*, and *Fusarium oxysporum*. Our observations with *M. gigantea* provide additional evidence that edible mushrooms harbour bioactive compounds with significant antifungal potential.

GC-MS analysis further confirmed the presence of diverse metabolites, including fatty acids like 6-octadecenoic acid, esters, and sugar derivatives such as octadecadienoic acid derivatives. These compounds are well known for their antioxidant, antimicrobial, and cytoprotective properties (Sardar, 2023; Karthikeyan *et al*., 2014). Together, these findings underline the nutritional, medicinal, and ethnobotanical significance of *M. gigantea*, validating its traditional use as an edible mushroom and suggesting its potential as a source of functional food ingredients and bioactive compounds for pharmaceutical applications.

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