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Nutritional composition, antioxidant activity, antibacterial activity, GC-MS based metabolite profile of *Pleurotus giganteus* (Berk.)

G.K. Barman[1], S. Saha[1], M. Kujur[1], D. Saha[2] and A. Saha[1]*

[1]*Department of Botany, Molecular Plant Pathology and Fungal Biotechnology Laboratory, University of North Bengal, Darjeeling-734 013, India*
[2]*Department of Biotechnology, Plant Biotechnology Laboratory, University of North Bengal, Darjeeling-734 013, India*

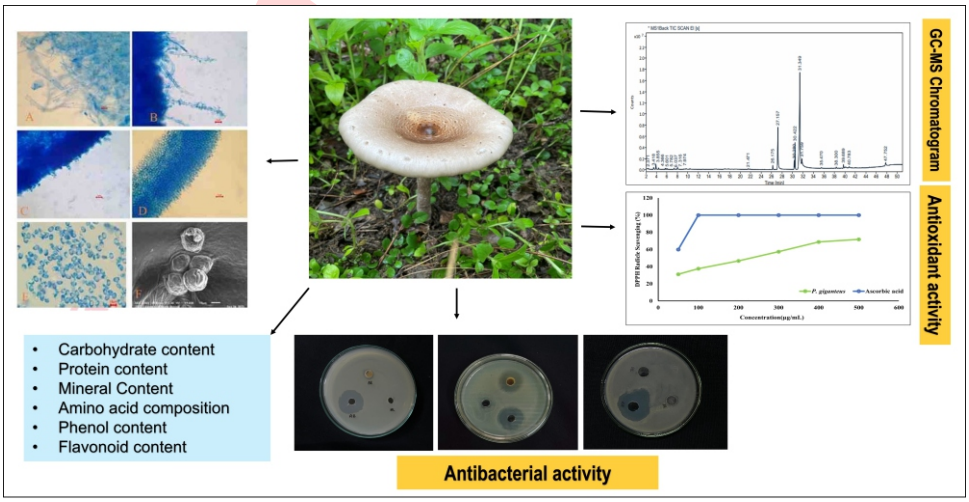
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***Corresponding Author Email:** asahanbu@yahoo.co.in ***ORCiD:** https://orcid.org/0000-0002-5659-4771

Abstract

Aim: This study aimed to evaluate the nutritional composition, antioxidative and antibacterial properties of the wild myco-food *Pleurotus giganteus*. The study is relevant to nutritional and medicinal properties of *P. giganteus* and its perspective applications in functional food, nutraceutical and pharmaceutical industries.

Methodology: *P. giganteus* was characterised morphological and molecularly. The nutritional properties and element contents were analysed from the fruiting body of *P. giganteus*. Three bacterial strains (*Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus subtilis*) were used to check the antibacterial activity of *P. giganteus*.



Results: *Pleurotus giganteus* was characterised on basis of morpho-molecular techniques. High nutritional value contents like carbohydrate (32.4 g 100 g^{-1} d.wt.) and high protein (25.3 g 100 g^{-1} d.wt.) and elements like potassium, zinc and magnesium were found in *Pleurotus giganteus*. Phenol (17.7mg GAE g^{-1}) and flavonoid (9.56 mg QE g^{-1}) contents were recorded in high concentration, along with strong DPPH radical scavenging activity (IC_{50}-235.01 µg m l^{-1}). The maximum antibacterial activity was showed by *Salmonella typhimurium*. Twenty bioactive compounds were detected through GC-MS analysis, among them the maximum area was shown by cis-13-octadecenoic acid, followed byn-hexadecanoic acid and ergosterol.

Interpretation: *P. giganteus* is a nutritionally rich edible mushroom with strong antioxidant and antibacterial potential.It is a reservoir of natural compounds that will be an alternative for synthetic drugs.

Key words: Antibacterial, DPPH, ITS, *Pleurotus giganteus*

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Introduction

Modern dietary habits are increasingly shifting towards healthy and nutritious food. Edible mushrooms are considered a premier choice as it is packed with several nutritional components, including proteins, dietary fibers, vitamins and trace minerals (Lesa *et al.,* 2022). Beyond their nutritional profile, they are rich in diverse health promoting bioactive compounds. Due to the presence of these unique myco-compounds, mushrooms have various biological activities, like antioxidant, antibacterial, anticancer, antidiabetic and anti-inflammatory (Saha *et al.,* 2026). Therefore, edible mushrooms are increasingly recognised as next-generation functional foods.

The genus Pleurotus, commonly known as the oyster mushroom, belongs to the family Pleurotaceae within the order Agaricales (Stastny *et al.,* 2022). These macro-fungi play an important role in forest ecosystems as they possess remarkable ability to biodegrade wood. Beyond their ecological significance, majority of the species of oyster mushroom are edible. Traditionally, they are harvested by tribal and indigenous communities from wild as a delicious myco-food due to its robust taste and flavour (Saha *et al.,* 2023; Siric *et al.,* 2022). *Pleurotus* species are rich in proteins, dietary fibre, vitamins and essential minerals. Additionally, it is also a source of diverse myco-chemicals including phenolic acids, flavonoids, alkaloids, terpenoids and so on. *Pleurotus* sp. is not only valuable for their nutritional supremacy, but also for their diverse pharmacological properties, including antioxidant, antidiabetic, antitumor, anticancer, antibacterial and anti-inflammatory (Saha *et al.,* 2023).

Pleurotus giganteus (Berk.) Karunarathna and K.D. Hyde, known as the giant oyster mushroom, is one of the important wild edible mushrooms of sub-Himalayan West Bengal, India. The mushroom is edible in a few Asian countries like China, Malaysia, Vietnam, Laos and Thailand (Phan *et al.,* 2019). However, comprehensive information regarding its nutritional contents, myco-chemical profiling and biological activities is scanty. Therefore, the present study aimed to evaluate the nutritional composition, myco-chemical profiling, antioxidant and antibacterial activities of *P. giganteus.* Furthermore, GC-MS analysis was performed to identify the bioactive metabolites present in this mushroom. To ensure accurate taxonomic identification, the collected specimen was characterized using both morphological observations and ITS-based molecular phylogenetic analysis.

Materials and Methods

Sample collection: The fruiting bodies of *Pleurotus giganteus* were collected from the tea garden of Kumargram, Alipurduar district, West Bengal, India from May to August, 2024. Samples are carefully harvested, placed in sterile polyethylene bags to prevent contamination and transported to the laboratory for further analysis.

Morphological study: Macroscopic features like pileus shape and color, lamellae arrangement, stipe features and overall morphology were studied. The microscopic structures such as basidiospores, basidia and hyphal elements were examined under a compound light microscope.

Molecular identification: Genomic DNA was isolated from fresh fruiting bodies using the C-TAB extraction protocol as described by Sharma *et al.* (2003). The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC -3'). Amplified PCR products were purified and commercially sequenced. The obtained nucleotide sequence was submitted to the GenBank database (Accession No. Or554131).

Phylogenetic analysis: The ITS sequence generated in this study was compared with related sequences available in the NCBI database usingBLASTn. Multiple sequence alignment was performed using Clustal Wimplemented in MEGA11 software. A phylogenetic tree was constructed following the Neighbor-Joining method with 1000 bootstrap replicates to confirm the taxonomic placement of *P. giganteus*.

Preparation of mushroom extract: Methanolic extract of the mushroom was prepared following the method of Barros *et al.* (2007) with minor modifications. Collected fruiting bodies were thoroughly washed, shade-dried and ground into a fine powder. Ten grams of powdered sample was extracted with 100 ml methanol and incubated in a shaking incubator at room temperature for 18 hrs. The extract was filtered through a Whatman No. 1 filter paper and concentrated to dryness in a water bath. The dried extract was stored at 4ºC until further analysis.

Proximate composition: Carbohydrate content was estimated using the anthrone method (Yemm and Willis, 1954), while protein content was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as a reference standard. The results were expressed as percentage of dry weight.

Estimation of phytochemicals

Total phenolic content: Total phenolic content was quantified using the Folin-Ciocalteu reagent, with gallic acid employed as the calibration standard. The phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (Kadam et al., 2013).

Total flavonoid content: Total Flavonoid content in the methanolic extract was quantified following the aluminium chloride colorimetric method (Atanassova *et al.*, 2011). Quercetin was used as a reference compound for calibration. The flavonoid content was calculated from the standard curve and expressed as milligrams of quercetin equivalent (QE) per gram of extract.

Antioxidant activity: The antioxidant capacity of the mushroom extract was assessed by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assayas described by Brand-Williams *et*

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Fig. 1: Macro-morphological features of wild edible mushroom *Pleurotus giganteus*. (A) Fruiting body showing funnel-shaped pileus in natural habitat; (B) Lateral view showing stipe and lamellae arrangement and © Fresh specimen with stipe length measurement.

al. (1995). Various concentrations of the extract (100-500 µg ml^{-1}) were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 min at room temperature. The absorbance was recorded at 517 nm on a spectrophotometer. The percentage radical scavenging activity was calculated by the following formula:

$$\% \text{ Scavenging} = \frac{\textit{Control Absorbance-Sample Absorbance}}{\textit{Control Absorbance}} \times 100$$

The results were expressed as percentage inhibition against extract concentration, and IC_{50} values (concentration required to inhibit 50% of DPPH radicals) were determined.

Mineral analysis: Mineral content of the samples was analysed using Atomic Absorption Spectrophotometry (AAS). The analysis was conducted at the Department of Agricultural Chemistry and Soil Science, Uttar Banga Krishi Vishwavidyalaya (UBKV), West Bengal, following standard Mallikarjuna *et al.* (2013) procedures. Dried samples were digested with nitric acid-perchloric acid mixture, and essential minerals such as potassium, calcium, magnesium, sodium, iron, zinc, copper and manganese were quantified. Mineral concentration was expressed as milligrams per 100 g of dry weight.

Amino acid profiling: The amino acid composition of dried powdered mushroom sample was analysed at Guwahati Biotech Park, Assam, using high-performance liquid chromatography (HPLC) with pre-column derivatization.

GC-MS Analysis: Gas chromatography-mass spectrometry (GC-MS) analysis of methanolic extract was carried out at IIT Madras using an Agilent GC-MS system fitted with an HP-5MS capillary column. Helium was employed as a carrier gasand the oven temperature was programmed from -60ºC to 325ºC. Mass spectra obtained were matched with those in the NIST library for compound identification. Identified compounds were reported along with their retention time, molecular formula, compound name and peak area percentage.

Antibacterial assay: The antibacterial assay was performed to assess the antibacterial potential of the methanolic extract of *Pleurotus giganteus* against selected pathogenic bacteria associated with various human diseases. The antibacterial potential of the methanolic mushroom extract was evaluated against *Bacillus subtilis* (ATCC®11774™), *Staphylococcus aureus* (ATCC®11632™) and *Salmonella typhimurium* (ATCC®25241™) using the agar well diffusion technique (Manandhar *et al.,* 2019). The bacterial strains were obtained in Kwik-Stick™ form from Microbiologics, USA and preserved on nutrient agar slants at 4ºC until commencement of experiment. Fresh bacterial suspensions were uniformly spread onto nutrient agar plates using sterile swabs. Wells of 7 mm diameter were aseptically punched into the agar and loaded with 100 µl of methanolic mushroom extract (sample, SA) Methanol was used as the negative control (CN), while streptomycin (10 µg ml^{-1}) served as a positive control. The inoculated plates were incubated at 37ºC for 24 hrs, thereafter the antibacterial activity was assessed by measuring the diameter of inhibition zones in millimetres.

Statistical analyses: All experimental analyses were carried out in triplicate. The data obtained were presented as mean values along with their corresponding standard deviation (mean ± SD).

Results and Discussion

The fruiting bodies of *Pleurotus giganteus* were large, fleshy and centrally stipitate (Fig. 1). The pileus diameter ranged approximately 6.4 to 30.7 cm, exhibiting initially convex to plano-convex shape, which gradually became centrally depressed upon maturation. The pileus surface was smooth to slightly scaly, with coloration varying from dark brown at the centre to pale brown or greyish-orange toward margins, showing gradual discoloration with advancing age. Margins were thin and slightly incurved in young basidiocarps, later becoming expanded or uplifted at maturity. Lamellae were densely arranged, decurrent in nature, and comprised five distinct series of lamellulae. The gills appeared white to cream at early stages and gradually turned pale ochraceous as the mushroom aged. The spore print obtained on a dark background was white in colour. The stipe was

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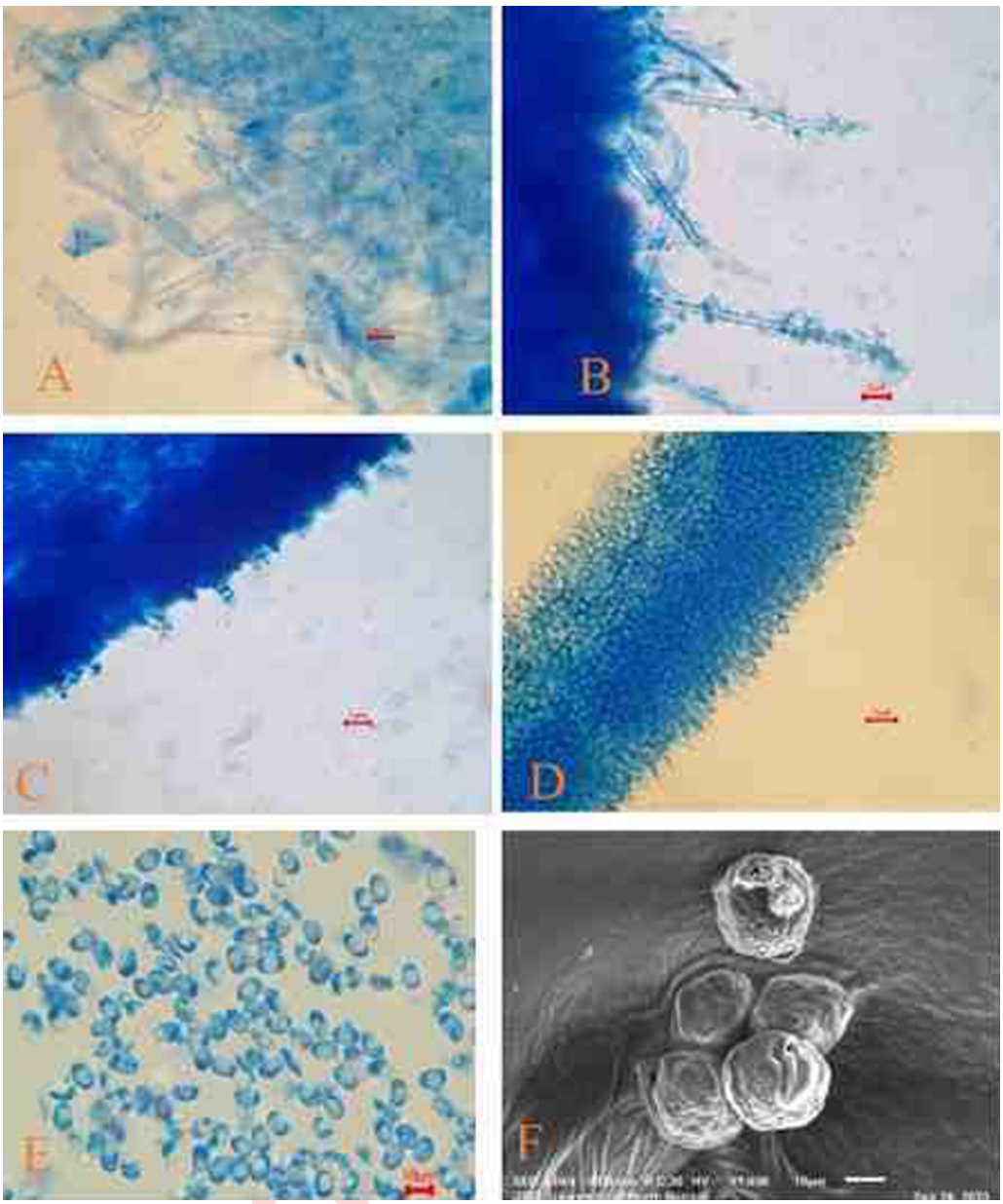


Fig. 2: Scanning electron microscopic illustration of *Pleurotus giganteus* (A) Skeletal hyphae, (B) Generative hyphae, (C) Basidia with Sterigmata, (D) Cheilocystidia, (E) Spores under Light Microscope and (F) Spores.

centrally positioned to slightly eccentric, measuring approximately 5-20 cm in length and 0.7-2 cm in thickness. It was cylindrical, solid and fibrous in texture, concolorous or slightly comparedto the pileus surface. The odour was mild and agreeable, while taste ranged from mildly sweet to neutral. Basidiospores measured 6-8 ×3-4 µm, were ellipsoid to oblong in shape, smooth and thin-walled (Fig. 2). Basidia were clavate, measuring 25-35 × 5-7 µm, and bore four sterigmata. Cheilocystidia were present, hyaline, measuring 12-34 × 5-12 µm. The macro- and micro-morphological characteristics observed in the present study were consistent with thediagnostic features reported for *P. giganteus* as described by Chen *et al.* (2016).

The internal transcribed spacer (ITS) region of the collected specimen was successfully amplified, yielding a nucleotide sequence of approximately 650 base pairs. BLASTn comparison of the generated sequence (GenBank Accession No. OR554131) revealed a 99.84% sequence similarity with reference sequences deposited in GenBank (Accession No. MG746393). Phylogenetic reconstruction based on the

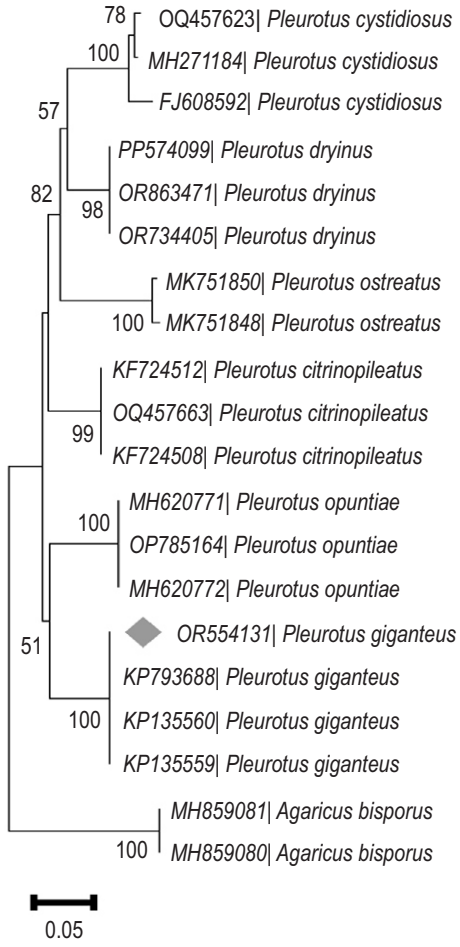


Fig. 3: Phylogenetic tree of *Pleurotus giganteus* based on ITS sequence data. The tree was constructed using the Neighbor-Joining method in MEGA11 with bootstrap analysis (1000 replicates). The query sequence, indicated by a green label, clusters with reference sequences from NCBI, confirming its taxonomic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

Table 1: Nutritional and phytochemical composition of *Pleurotus giganteus*

Parameters	Concentration
Carbohydrate	32.4±2.4 g 100 g^{-1} d.wt.
Protein	25.3±3.1 g 100 g^{-1} d.wt.
Total phenolic content	17.7±1.1 mg GAE g^{-1} d.wt.
Total flavonoid content	9.56±0.2 mg QE g^{-1} d.wt.
Antioxidant activity (DPPH)	71.8 % inhibition (500 µg ml^{-1})

GAE= gallic acid equivalents; QE= quercetin equivalents; DPPH= 2,2-diphenyl-1-picrylhydrazyl. Values are mean±S.D.

Table 2: Mineral composition of *Pleurotus giganteus* (mg 100 g^{-1} d. wt.)

Minerals	Composition (mg 100 g^{-1})
Potassium (K)	2963 ± 35
Iron (Fe)	46.5 ± 0.8
Magnesium (Mg)	168 ± 3.5
Calcium (Ca)	58 ± 1.6
Zinc (Zn)	90 ± 2.4
Copper (Cu)	3.9 ± 0.12
Manganese (Mn)	1.8 ± 0.06
Sodium (Na)	65 ± 1.5

Table 3: Amino acid composition of *Pleurotus giganteus* fruiting bodies

Amino Acids	Concentration (µmol l^{-1})
Phosphoserine (PhSer)	242.761 ± 4.2
Taurine (Taur)	2798.894 ± 42.0
Aromatic Amino Acid (AAAA)	29.939 ± 0.6
Proline (Pro)	70.855 ± 1.3
Glycine (Gly)	9.564 ± 0.18
Phenylalanine (Phe)	5.748 ± 0.11
Beta-Aminoisobutyric Acid (BAIBA)	6.882 ± 0.13

Neighbor-joining method clustered the isolate within the *Pleurotus giganteus* clade with strong bootstrap support, thereby confirming its taxonomic identity (Fig. 3). A similar ITS-based molecular phylogenetic approach was employed by Saha *et al.* (2026), for authentication of *Agrocybe broadwayi*. Proximate analysis indicated that *P. giganteus* indicated that moderate amount of carbohydrates (32.2±2.4 g 100 g^{-1} d.wt.) was present in it as compared to the previous studies of Ouali *et al.* (2023), who recorded 63.62% carbohydrate in *P. pulmonarius.*

The protein content (25.3±3.1g 100 g^{-1} d.wt.) in the test mushroom was found to be higher as compared to the protein content (13.91%) in *P. pulmonarius.* thereafter, the high protein and moderate carbohydrate of the tested mushroom makes it a valuable dietary food. The methanolic extract was used for myco-chemical analysis. It was found that the extract contained 17.7±1.1 mg gallic acid equivalent (GAE) g^{-1} extract of total phenol and 9.56±0.2 mg quercetin equivalent (QE) g^{-1} of total flavonoids (Table1). The extract showed a dose-dependent DPPH scavenging activity (Table 1; Fig. 4). The maximum inhibition of 71.8 % was observed at a concentration of 500 µg ml^{-1}. It was also noted that IC_{50} value of the extract was 235.01 µg ml^{-1}. These result is considerably lower than the reports of Biswas *et al.* (2024), where they recorded 640 to 710µg ml^{-1}. IC_{50} values for Amanita konajensis against DPPH radicals, indicating a strong antioxidant activity of the studied mushroom.

Mineral analysis of *P. giganteus* revealed the presence of both macro- and micro-elements in appreciable concentrations, supporting its value as a nutritionally rich edible mushroom (Table 2). Potassium was the most abundant mineral (2963 mg 100 g^{-1}), followed by magnesium (168 mg 100 g^{-1}), zinc (90 mg 100 g^{-1}), sodium (65 mg 100 g^{-1}), calcium (58 mg 100 g^{-1}), and iron (46.5 mg 100 g^{-1}). Trace levels of copper (3.9 mg 100 g^{-1}) and manganese

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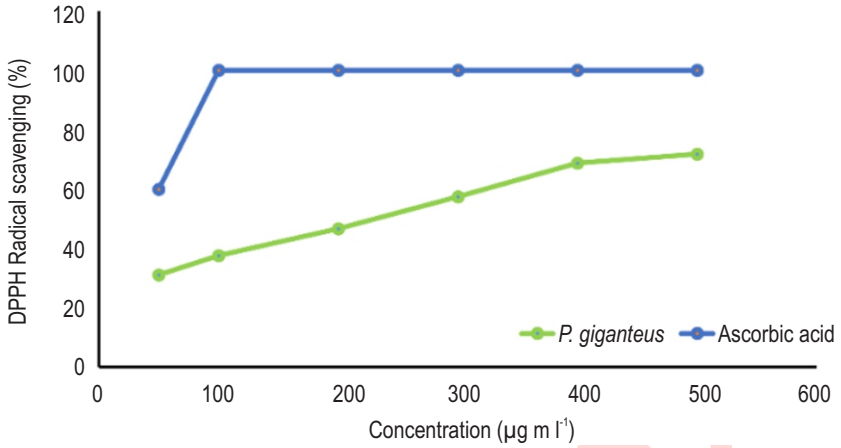


Fig. 4: Antioxidant activity of methanolic extract of *Pleurotus giganteus* compared with ascorbic acid standard quantified DPPH assay. Values are mean of three replicates ± S.D.

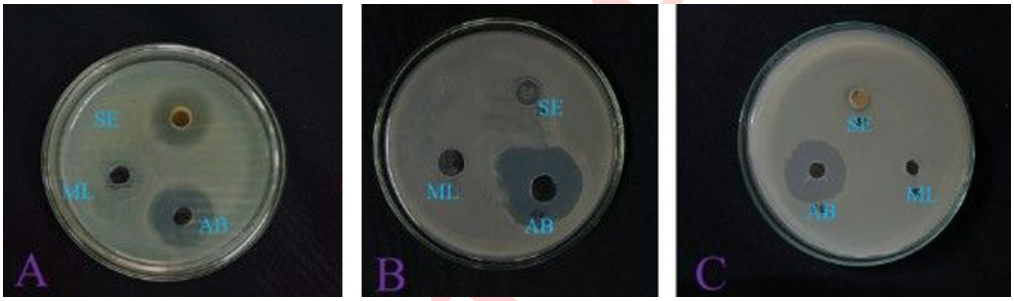


Fig. 5: Antibacterial activity of methanolic extract of *Pleurotus giganteus* against (A) *Salmonella typhimurium,* (B) *Bacillus subtilis* (C) *Staphylococcus aureus* by agar well diffusion method. Each plate contains three wells: SA- test sample (*P. gigantea* extract), ML- negative control (methanol), and AB- positive control (streptomycin). Clear inhibition zones indicate bacterial growth suppression by mushroom extract and standard antibacterial agentstreptomycin.

(1.8 mg 100 g^{-1}) were also detected. Mineral profiling revealed high concentrations of potassium, magnesium and zinc, aligning with the mineral richness typically associated with edible mushrooms (Adebayo *et al.,* 2014). These findings further support the nutritional importance of *P. giganteus* as a rich dietary source of essential minerals. Amino acid profiling of *P. giganteus* demonstrated the presence of several nutritionally and physiologically important amino acids, highlighting its dietary significance (Table 3). Among the identified compounds, taurine was detected in high concentration (2798.894 µmol l^{-1}). Moderate to lower concentrations of other amino acids, such as phosphoserine (242.761 µmol l^{-1}), proline (70.855 µmol l^{-1})and glycine (9.564 µmol l^{-1}) were recorded. Additionally, aromatic amino acids such as tyrosine (29.939 µmol l^{-1}) and phenylalanine (5.748 µmol l^{-1}), along with β-aminoisobutyric acid (6.882 µmol l^{-1}) were also present in detectable trace. The presence of essential amino acids and bioactive amino derivatives, such as taurine and proline, further enhances the mushroom's nutritional profile, contributing to human health benefits including neuromodulation and antioxidant defence (Bach *et al.,* 2017).

GC-MS analysis of the methanolic extract of *P. giganteus* resulted in the identification of 20 bioactive compounds (Table 4). These compounds were detected at varying retention times andrelative peak areas, reflecting differences in their abundance within the extract. The predominant compound was cis-13-Octadecenoic acid (51.24 %), followed by n-hexadecanoic acid (25.61 %), 9-octadecenoic acid (Z)-, methyl ester (6.83 %), ergosterol (2.53 %), glycerine (1.61 %), 9,12-octadecadienoic acid (Z, Z)-, methyl ester (1.61 %) and octadecanoic acid (1.36%). Several additional compounds, including heterocyclic compounds (2-pyrolidine, 5-ethyl-2 (5H)-furanone), alcohols, esters and organic acids, were also identified.

The presence of fatty acids and sterols suggest the nutraceutical potential of *P. giganteus.* Notably, the presence of

Table 4: Major compounds identified by GC-MS in methanolic extract of *Pleurotus giganteus*.

RT (min)	Peak Area (%)	Compounds	PubChem CID	Molecular Formula	Molecular weight (g mol^{-1})
2.371	0.19	Cyclododecanone, thiosemicarbazone	690106	$C_{13}H_{25}N_3S$	255.428
3.418	0.88	1,2-Cyclopentanedione	566657	$C_5H_6O_2$	98.1
3.855	1.66	Glycerin	753	$C_3H_8O_3$	92.09
4.366	0.52	5-Ethyl-2(5H)-furanone	16997	$C_6H_8O_2$	112.13
5.601	0.34	2-Pyrrolidinone	12025	C_4H_7NO	85.10
5.782	0.28	D-Alanine, N-propargyloxycarbonyl-,isohexyl ester	91712731	$C_{13}H_{21}NO_4$	255.31
6.037	0.28	Binapacryl	10234	$C_{15}H_{18}N_2O_6$	322.31
7.316	0.27	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	119838	$C_6H_8O_4$	144.12
7.974	0.58	5- Methoxypyrrolidin-2-one	181561	$C_5H_9NO_2$	115.13
26.175	1.76	Hexadecanoic acid methyl ester	8181	$C_{17}H_{34}O_2$	270.5
27.157	25.61	n-Hexadecanoic acid	985	$C_{16}H_{32}O_2$	256.42
30.280	1.61	9, 12-Octadecadienoic acid (Z,Z)-, methyl ester	—	$C_{19}H_{34}O_2$	294.4721
30.422	6.83	9-Octadecenoic acid (Z)-, methyl ester	8202	$C_{19}H_{36}O_2$	296.5
31.349	51.24	Cis-13-Octadecenoic acid	5312441	$C_{18}H_{34}O_2$	282.5
31.759	1.36	Octadecanoic acid	5281	$C_{18}H_{36}O_2$	284.5
35.470	0.73	6-Octadecenoic acid	11634	$C_{18}H_{34}O_2$	282.5
38.300	0.73	10, 12, 14-Nonacosatriynoic acid	—	$C_{29}H_{46}O_2$	426.674
39.689	1.53	β-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate	91696686	$C_{17}H_{37}BO_6Si_2$	404.5
40.783	0.69	2,3-Dihydroxypropyl elaidate	57483218	$C_{21}H_{40}O_4$	356.5
47.752	2.53	Ergosterol	444679	$C_{28}H_{44}O$	396.6

Table 5: Antibacterial activity of methanolic extract of *Pleurotus giganteus* against selected bacterial strains, expressed as zone of inhibition (mm)

Bacterial strains	Zone of inhibition (mm)
Gram positive: *Bacillus subtilis*	7.14 mm
Staphylococcus aureus	8.12 mm
Gram Negative: *Salmonella typhimurium*	14.5 mm

ergosterol, a fungal sterol and precursor of vitamin D_2, is of particular importance. Fatty acids such as oleic, linoleic, palmitic and stearic acids are well recognized for their antioxidant, antimicrobial and anti-inflammatory properties (Piska *et al.,* 2017; Ribeiro *et al.*, 2009). The methanolic extract of *Pleurotus giganteu*s exhibited antibacterial activity against the studied bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Salmonella typhimurium*) (Fig. 5). The antibacterial activity was measured in terms of inhibition *Salmonella typhimurium* was the most susceptible bacterium (14.5 mm) among the tested strains. However, inhibitory effect of the extract was found very low against *Staphylococcus aureus* (8.12 mm) and *Bacillus subtilis* (7.14 mm) under the experimental conditions. Thus, the extract exhibited the strongest antibacterial activity against *S. typhimurium.*

The standard antibiotic streptomycin (positive control) produced comparatively larger zones of inhibition, whereas methanol used as the negative control did not show any antibacterial activity in their study also displayed antibacterial activity of oyster mushroom (*P. ostreatus*) against various bacterial strains (*Staphylococcus aureus, Escherichia coli* and *Neisseria gonorrhoeae*). Therefore, the antibacterial activity of the studied mushroom makes it a valuable functional food not only for their nutritional attributes but, also for their health-beneficials properties. The present study provides valuable insights into the nutritional composition, bioactive constituents and biological properties of *Pleurotus giganteus.* The findings highlight the significance of this edible mushroom as a natural source of health-promoting compounds and support its potential utilization in functional food and nutraceutical applications.

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Authors' contribution: G.K. Barman: Conceived and designed the study, performed the experiments and drafted the manuscript; **S. Saha**: Contributed to methodology development and experimental work; **M. Kujur:** Assisted in some experimental work; **D. Saha:** Provided supervision and critical revision of the manuscript; **A. Saha:** Contributed to overall supervision, project administration and funding acquisition.

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