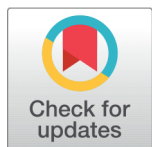


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Pleurotus giganteus as a Valuable Source of Nutrients

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Abstract

Objectives: The present study aims to identify and evaluate the chemical composition of young and matured sporocarps of *Pleurotus giganteus*. **Methods:** Wild edible mushroom, *Pleurotus giganteus* was collected during the monsoon season in 2021 from Reiek Tlang, Mizoram. The collected sample was cleaned, oven dried at 45°C and finely powdered and stored in a tightly stoppered bottle for further analysis. **Findings:** The nutrient profiles revealed that the sample is generally rich in protein and carbohydrates with low-fat content and contained considerable amounts of minerals but lower toxic metal contents and can be used as a natural food source without any health risk which is vital in supplementing nutrition to mankind. **Novelty:** This study provides information regarding the nutritive quality of *P. giganteus* and can be used as a reference for food compositional data.

Keywords: Mushrooms; Mizoram; Nutritional value; Fruit body; Nutrients; Molecular identification

1 Introduction

Fungi have become one of the main decomposers of recalcitrant organic matter and occur in diverse climates under different conditions⁽¹⁾. Mushroom is a general term used to describe the fruit bodies of fungi which belong either to Basidiomycetes or Ascomycetes. They usually grow on the trunks, soil, roots of trees as well as decaying woody materials and dead wood trees, and emerged more than a million years ago⁽²⁾.

Pleurotus giganteus is formerly known as *Lentinus giganteus* and it bears many structures that are atypical of *Lentinus* and its taxonomic position has long been unresolved. With the support of morphological and molecular evidence, *Lentinus giganteus* was placed in *Pleurotus*, rather than in *Lentinus*. It is one of the largest edible mushrooms and is typically found either in groups or solitary on buried rotten wood above the ground soil. It has a thick, radicate stipe and subdistant broad lamellae⁽³⁾. Wild edible mushrooms have been widely used as food since time immemorial and the consumption of edible wild mushrooms is increasing due to their high nutritional and culinary value⁽⁴⁾. Mushrooms or macrofungi obtained their nutrients through external digestion and absorption by the mycelium.

Nutritional values of genera pleurotoid were considered as a good source of nutrients⁽⁵⁾ and compatible with meat products⁽⁶⁾ due to the presence of high amount

of proteins, vitamins, minerals and they are capable of colonizing and degrading many lignocellulosic residues. However, the nutritional benefits of wild edible mushroom *P. giganteus* are still meagre and have not been completely harnessed possibly due to insufficient information on the viability of this mushroom.

Mizoram is gifted with diverse landforms and a variety of climatic conditions that are suitable for a varied range of fungal species^(7,8). However, no significant study has yet been undertaken on the nutritional value of the mycoflora in Mizoram. Wild growing edible mushroom species are frequently consumed and knowledge of their nutritional composition is essential. Therefore, the present study aimed to determine the proximate chemical composition of *Pleurotus giganteus* to assess its valuable nutritive constituents and this database can be used as a reference which thus necessitated this research.

2 Methodology

2.1 Sample collection and preparation

The sample was collected from a distance of potential pollution sources during the monsoon season in 2021 at Reiek Tlang, Mizoram (Figure 1). The region is a high rainfall area with dense forest and provides ideal atmospheric conditions for the growth of mushrooms. The collected sample was cleaned thoroughly to remove mud and other extraneous material and kept in an air-tight container before transporting it to the laboratory⁽⁹⁾. Without division into pileus and stipe, the whole fleshy collected mushroom sample was oven dried at 45°C and ground into a fine powder and stored in a tightly stoppered bottle for proximate analysis.

2.2 Molecular analysis

DNA was extracted from tissue removed from the inside of fruiting bodies using a CTAB extraction. A small amount of tissue from the inside of the fruiting body was added to a sterile 1.7 ml microcentrifuge tube with glass beads and 500 µL of CTAB lysis buffer. The centrifuge tube was then vortexed for a minute to homogenize lyse fungal cells. The tubes were then briefly centrifuged to move the larger tissue segments to the bottom of the tube and then the supernatant was transferred to another tube. The new microcentrifuge tube was then placed into a 65°C hot water bath to further lyse the cells. After 20 minutes, the tubes were removed and 500 µL of chloroform was added to the tube, mixed and then centrifuged at 13,000 rpm for 5 minutes. The top layer of the supernatant was transferred to a new microcentrifuge tube. The amount of liquid transferred was then measured and two-thirds of that amount was added to isopropanol stored in a -20°C freezer. The tube was then incubated for 5 minutes at room temperature before being centrifuged for 7 minutes at 15,000 rpm. The supernatant was removed and 500 µL of 70% ethanol was added. The tubes were centrifuged again at 15,000 rpm for 3 minutes and the supernatant was removed. The tubes were left open in a hood to allow the last of the ethanol to evaporate before the DNA pellet was re-suspended in 100 µL of sterile water.

PCR reactions were setup in 0.2 ml centrifuge tubes that contained 12.5 µl GoTaq Green Mastermix (Promega, Madison, WI), 9.5µl nuclease-free water, 1µl forward primer (5M), 1µl reverse primer (5M) and 1µl of fungal DNA template for a total reaction volume of 25.5µl. PCR was performed using primers ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3')⁽¹⁰⁾ with the following parameters; 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 52°C for 1 minute and 72°C for 1 minute with a final extension step of 72°C. PCR amplicons were verified by electrophoresis on a 1% agarose gel with SYBR green and visualized on a Gel Documentation System. Sequencing was performed using both primers by using Sanger sequencing using a DNA sequencer. A consensus sequence for contigs was trimmed and aligned using the Bioedit sequence alignment editor⁽¹¹⁾. A sequence was then compared to those in the GenBank database using the BLASTn⁽¹²⁾ search tool for similarities and submitted to Genbank. The sequences were then aligned with Clustal W⁽¹³⁾ and the phylogenetic tree was established using Maximum Likelihood in RaxML GUI software.

Phylogenetic analysis was conducted based on the ITS gene data using both Maximum Likelihood (ML) and Neighbor-Joining (NJ) approaches. ML and NJ searches were carried out using RaxML GUI. Alignment gaps were treated as missing data. NJ trees were constructed based on the total character differences and bootstrap values were calculated from 1,000 replications.

2.3 Proximate and mineral content analysis

In general, moisture, protein, fat, fibre and ash content were determined in accordance with the appropriate standard methods of the Association of Official Analytical Chemists⁽¹⁴⁾ and the contents of total carbohydrate and energy values were calculated using the conversion factors of Crisan and Sands⁽¹⁵⁾. Sample analysis was done in triplicates on a dry weight basis. Minerals (K, Na, Fe, Zn, Ca, Mn, Cu, Cd and Pb) were determined by using an atomic absorption spectrophotometer (Thermo Scientific iCE 3400 series, USA) after dry ashing of the sample⁽¹⁶⁾.

3 Results and Discussion

3.1 Molecular identification

The nucleotide sequence of the mushroom blasted against sequences from GenBank database revealed the identification of *Pleurotus giganteus*, a fungal isolate from Mizoram.

The ITS1-5.8S-ITS4 sequences of the fungal isolate was compared to 20 corresponding sequences of reference fungal taxa in the database and the list of species, voucher number, GenBank accession number and locality used for the analysis is given in Table 1 and phylogenetic tree in Figure 2.



Fig 1. Young and matured fruiting body of *Pleurotus giganteus*

Table 1. List of species, voucher, GenBank accession nos. and locality

Sl. no.	Species	Voucher/Strain	NCBI	Locality
1	<i>Pleurotus sp.</i>	DMS2021-8-7	OK643767	China
2	<i>Pleurotus giganteus</i>	MRNo556.	LC068800	Thailand
3	<i>Pleurotus giganteus</i>	MFLU08-1371	KP120919	Thailand
4	<i>Pleurotus giganteus</i>	FRI849	KX018294	China
5	<i>Pleurotus giganteus</i>	HMAS P1	KP793688	China
6	<i>Pleurotus giganteus</i>	NU-BOT-GN-PG-007	OM717958	
7	<i>Pleurotus giganteus</i>	P6	HM245789	China
8	<i>Pleurotus levi</i>	S.D. Russell iNaturalist # 91369573	OM972587	USA
9	<i>Pleurotus levi</i>	S.D. Russell MycoMap # 4187	ON245348	USA
10	<i>Pleurotus levi</i>	S.D. Russell Mushroom Observer # 465240	OM972617	USA
11	<i>Pleurotus levi</i>	S.D. Russell ONT iNaturalist # 132753423	OP643485	USA
12	<i>Pleurotus levi</i>	Mushroom Observer 382406	MW633064	USA
13	<i>Pleurotus dryisus</i>	ASI 2123	AY265823	Korea
14	<i>Pleurotus dryisus</i>	FLAS-F-61321	MH211881	USA
15	<i>Pleurotus sp.</i>	NOM6	KF415286	Nigeria
16	<i>Pleurotus cystidiosus</i>	Blaos clone A	DQ882570	Japan

Continued on next page

Table 1 continued

17	<i>Pleurotus cystidiosus</i>	JZ9	MG437337	India
18	<i>Pleurotus fuscusquamulosus</i>	EGDA-Pl23	MW915606	Egypt
19	<i>Pleurotus fuscusquamulosus</i>	-	KM111497	Tanzania
20	<i>Ceriporiopsis semisupina</i>	NHMM	MG719280	India

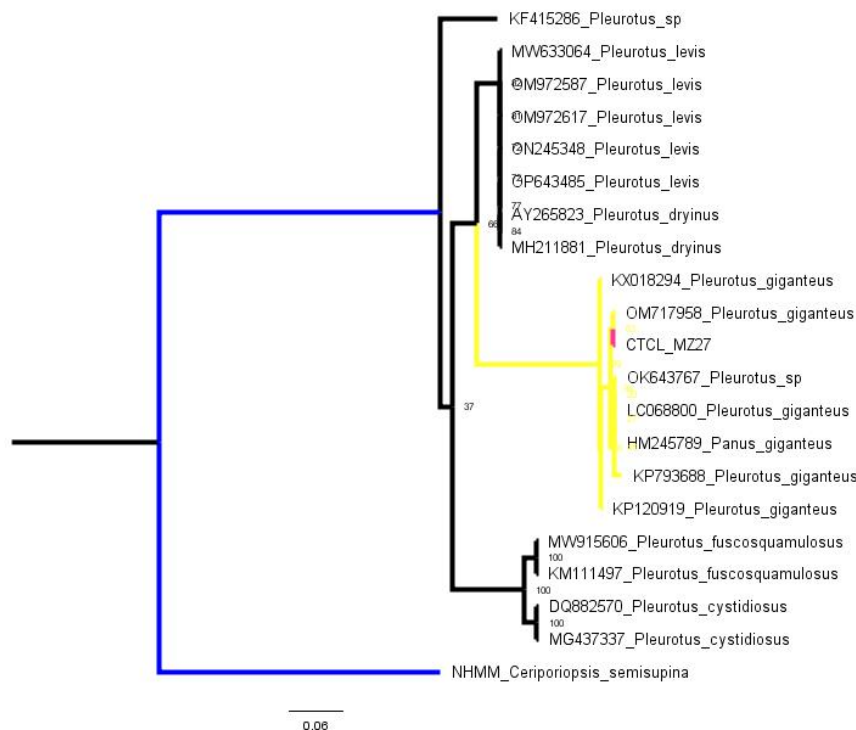


Fig 2. Phylogenetic tree

The evolutionary history was inferred by using the Maximum Likelihood method based on the GTR-GAMMA model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in raxmlGUI 2.0.

In the maximum Likelihood tree generated, the specimen, *Pleurotus giganteus* clustered with the related species with high support value. *Ceriporiopsis semisupina* was used as the Outgroup which is highlighted in blue colour. The fungal isolate collected from Mizoram, *Pleurotus giganteus* (CTCL_MZ27) is highlighted in red colour amongst the *Pleurotus giganteus* clade (indicated in yellow colour).

3.2 Proximate and mineral content analysis

The proximate and mineral content of the mushroom sample is given on a dry weight basis (Table 2).

This study revealed that the sample *Pleurotus giganteus* is a good source of protein (23.04%), carbohydrate (49.19%) and fibre (7.98%) and generally low in fat concentration (2.46%). Moisture content (9.73%) can be affected by environmental temperature, relative humidity during growth and storage. Moisture has no nutritional relevance but it is the most important factor which directly affects the nutrient contents of the mushrooms. The nutritional value of some edible *Pleurotus sp.* as well as other species have been documented on a dry weight basis^(17–19) which is fair close to the result obtained in this study. Phan *et al.*⁽³⁾ obtained 15.4% of protein, 3.7% of fat, 364 kcal/100 g of energy value, 33.3% of fibre and 67.2% carbohydrates in *P. giganteus*. The carbohydrate and fibre content is noticeably higher than the present value obtained. For instances Kalkoti *et al.*⁽²⁰⁾ obtained 16.52% protein and 38.62% of carbohydrate from *P. giganteus*. In this regard, it is clear that variation can occur in nutrient contents of wild edible mushrooms by their location, developmental stage, substrate as well as the type of mushrooms.

Mineral concentrations of *P. giganteus* were investigated and the data are given in Table 3. Except for chromium and lead, all the minerals were determined in the sample. The most abundant element was found to be K, followed by Na, Fe, Zn and Ca.

Table 2. Chemical composition of *P. giganteus* in a dried weight basis (g/100g) and energy value in Kcal/100g

Chemical composition	Amount
Moisture	9.73 ± 0.09g/100g
Protein	23.04 ± 1.34g/100g
Fat	2.46 ± 0.09g/100g
Crude Fibre	7.98 ± 0.42g/100g
Ash	7.52 ± 0.09 g/100g
Carbohydrate	49.19 ± 1.75 g/100g
Energy value	288.13 ± 3.63 kcal/100g

Data shown are means and standard deviations of triplicate determinations.

Regarding the microelements, manganese and copper were found at very low concentrations. Variations in the mineral content of the mushrooms can be caused by various environmental factors including soil quality, growth substrate and climate⁽²¹⁾.

Table 3. Total elemental compositions of *P. giganteus* in a dried weight basis (ppm) ND: Not detectable

Minerals	Concentrations (in ppm)
Potassium	1259.81
Sodium	217.15
Iron	63.28
Zinc	58.96
Calcium	18.64
Manganese	0.36
Copper	0.32
Cadmium	ND
Lead	ND

4 Conclusion

This study essentially helps to enhance the nutritional distribution of the mushroom *P. giganteus*. It contains a considerable amount of protein, carbohydrates and fibre, which serve as a good source of nutrients for the human body and can be consumed unreservedly without any health risk. High carbohydrate content can be a good source of carbohydrates for diabetic patients. They have a low-fat content making them ideal components in several diets and daily consumption. It also appears to be a good source of minerals and trace elements that are essential for human health. Nevertheless, inadequate information is available concerning the nutritional properties of wild edible mushrooms in Mizoram and further studies are needed to assess and document the nutritional database of edible macrofungi.

5 Declaration

Presented in 4th Mizoram Science Congress (MSC 2022) during 20th & 21st October 2022, organized by Mizoram Science, Technology and Innovation Council (MISTIC), Directorate of Science and Technology (DST) Mizoram, Govt. of Mizoram in collaboration with science NGOs in Mizoram such as Mizo Academy of Sciences (MAS), Mizoram Science Society (MSS), Science Teachers' Association, Mizoram (STAM), Geological Society of Mizoram (GSM), Mizoram Mathematics Society (MMS), Biodiversity and Nature Conservation Network (BIOCON) and Mizoram Information & Technology Society (MITS). The Organizers claim the peer review responsibility.

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