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Optimization of Cultivation Parameters and Evaluation of Nutritional and In-Vitro Antioxidant Properties of Macrocybe gigantean

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ABSTRACT

Background: *Macrocybe gigantea* is an edible wild mushroom with potential nutritional and bioactive value. The present study aimed to isolate, identify and optimize the cultivation parameters of M. gigantea and to evaluate its nutritional composition, phytochemical constituents and in-vitro antioxidant activity. **Methods:** Mushroom was identified using morphological characteristics and ITS rDNA partial sequencing followed by BLAST analysis. Growth media, pH, temperature and different lignocellulosic substrates were evaluated for mycelial growth and yield. Proximate composition (dry weight basis), phytochemical screening and antioxidant activity were analysed using standard methods including DPPH, FRAP and reducing power assays. **Results:** The collected mushroom was identified as *Macrocybe gigantea* based on microscopic examination and ITS rDNA sequencing, which showed 99.12% similarity (93% query coverage; E-value 0.0; max score 1112) in BLAST analysis. Paddy straw showed the highest biological efficiency (84.7±1.1%) compared with other substrates. Nutritional composition analysis on a dry weight basis revealed a high protein content (47.34±3.28%), followed by a substantial proportion of carbohydrates (37.21±1.86%), and a comparatively low fat content (3.04±0.24%). Additionally, it is a valuable source of vitamins B, C, and D. Phytochemical screening revealed the presence of phenolics, flavonoids and other bioactive compounds. The mushroom ethanol extract exhibited better antioxidant activity with an IC_{50} value of DPPH (57.07±1.71 µg/ml), ABTS (39.18 µg/ml±1.18) and phosphomolybdenum assay (IC_{50}: 45.22±1.36 µg/ml). **Conclusion:** *Macrocybe gigantea* demonstrated high biological efficiency on paddy straw and exhibited appreciable nutritional value along with significant in-vitro antioxidant activity, particularly in the ethanol extract. Further in-vivo studies are required to validate its potential health benefits.

Introduction

Mushrooms are members of the class Basidiomycota and order Agaricales. Unlike green plants, they do not have chlorophyll, which is necessary for making their own food. For

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growth and development, they require prepared foods such as smaller, broken-down forms of cellulose, starch, and lignin (Stamets, 2000). According to taxonomy, the vast majority of mushroom species belong to the kingdom Fungi and either the Ascomycota or Basidiomycota groups. The basidiocarp, or fruiting bodies, of the macrofungi are large enough to be seen without glasses and are simple to pluck with the hand. Mushrooms appeal to different people in different ways. They may serve as a source for scientists developing new drugs, and they are works of art for artists. There are numerous traditional methods for testing these fungi; however, they are not very accurate (Fabros *et al.*, 2022, Lian *et al.*, 2024, Pathak *et al.*, 2022).

Due to their inherent simplicity, phenotypic diversity, and evolutionary convergence, fungi systematics have been demonstrated to offer limited utility in terms of morphological information (Ayimbila and Keawsompong, 2023). Some deadly toxic mushrooms look like food species based on their appearance. For example, the poisonous and lethal *Amanita phalloides* may be confused with the edible *Volvariella volvacea*. Therefore, in order to prevent any potential danger from consuming hazardous mushrooms, it is crucial to appropriately identify them (Ortiz-Letechipia *et al.*, 2024).

Mushrooms possess filamentous spores and mycelia with limited phenotypic traits for reliable differentiation among closely related species (Łysakowska *et al.*, 2023). Therefore, molecular techniques, particularly ITS rDNA-based markers, provide rapid and accurate identification of wild mushroom specimens and ensure the correct species is used as inoculum (Giusti *et al.*, 2021, Rofeal *et al.*, 2022).

Edible mushrooms are an excellent source of high-quality protein, minerals, and vitamins, making them important for human nutrition (Chang, 1996, Dar *et al.*, 2024). Several factors such as spawn quality, growing media, pH, temperature, humidity, and light intensity significantly influence mushroom growth and productivity (Chun *et al.*, 2021, Ferdousi *et al.*, 2020, Jayaraman *et al.*, 2024).

Researchers are increasingly interested in evaluating the in-vitro antioxidant activity of mushroom extracts to determine their bioactive potential (Arulmozhi *et al.*, 2024, Chun *et al.*, 2021). Although *Macrocybe gigantea* is recognized as an edible wild mushroom and is traditionally consumed in several regions, comprehensive scientific studies on this species remain limited (Peiris *et al.*, 2024). Most previous reports have focused either on basic taxonomic description or general compositional analysis, with limited information on systematic cultivation optimization, standardized nutritional profiling on a dry weight basis, and comparative in-vitro antioxidant evaluation (Lakshmikanthan *et al.*, 2026). Furthermore, integrated studies combining molecular authentication with growth parameter optimization and bioactivity assessment are scarce. Therefore, a detailed scientific investigation is necessary to validate its cultivation potential and nutritional significance (Mwangi *et al.*, 2022). However, limited information is available on the antioxidant potential of *Macrocybe gigantea*, which highlights the need for the present investigation.

In the present study, ITS rDNA sequencing was employed to molecularly identify *Macrocybe gigantea* through PCR amplification and sequence comparison using BLAST analysis. Molecular authentication provides reliable confirmation beyond morphological identification and supports accurate classification of wild edible mushrooms. Furthermore, different growth media, pH levels and temperature conditions were systematically evaluated to optimize mycelial growth. The mushroom was cultivated using various agricultural residues as substrates, and its nutritional composition, phytochemical constituents and *in-vitro* antioxidant activity were assessed. Although *Macrocybe gigantea* is recognized as an edible species, comprehensive studies integrating molecular identification, cultivation optimization and bioactive evaluation remain limited. Therefore, this study aimed to provide a comprehensive assessment of cultivation

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parameters, nutritional quality and antioxidant potential of *Macrocybe gigantea*, contributing baseline data for its scientific validation and utilization.

Materials and Methods

Organisms

Fruiting bodies of *Macrocybe gigantea* were collected in monsoon season of the year 2022 from the campus of Shrimathi Devkuvar Nanalal Bhat Vaishnav College for Women, Chrompet, Chennai, Tamil Nadu, India with approximate coordinates of 12.9560° N, 80.1453° E. The species was found growing in clusters on humus-rich soil in open grassy habitat. Specimens were identified based on macroscopic and microscopic characteristics using standard mycological keys. A voucher specimen was deposited in the Department of Plant biology and Plant Biotechnology herbarium under accession number SDNB-PBPB-2022-224.

Morphological and molecular identification

Colony morphology of *Macrocybe gigantea* was observed on potato dextrose agar (PDA), and over 50 microscopic characteristics per isolate were examined using a compound microscope. Macroscopic features of the basidiomata, including pileus and stipe color and spore print, were recorded. Specimens were preserved in 70% ethanol and treated with 5% KOH or Melzer's reagent for microscopic analysis. Moreover, rehydrated basidiocarps were sectioned (10-20µm), stained with methyl blue, and examined at 40× magnification. Spore prints were prepared on paper, stained with cotton blue, and spore dimensions measured using a calibrated ocular micrometer (Ortiz-Letechipia *et al.*, 2024). For molecular identification, basidiospores were suspended in sterile water, adjusted to 10^5 spores/ml, and inoculated onto PDA. After incubation at 25 °C, germinated colonies were subcultured for genomic DNA extraction using the CTAB method (Vilas *et al.*, 2020). DNA quality was confirmed by agarose gel electrophoresis. The ITS region was amplified using ITS1 and ITS4 primers and sequenced via sanger Sequencing (Adeniyi *et al.*, 2018). Sequences were aligned using ClustalW, identified through BLAST against the NCBI GenBank database, and a phylogenetic tree was constructed using MEGA 11. The amplified ITS rDNA PCR product was purified and sequenced. The obtained sequence was compared with reference sequences available in the NCBI GenBank database using BLAST, and species identity was confirmed based on highest sequence similarity.

Physiological studies on mycelial growth

The mycelial growth of *Macrocybe gigantea* was assessed on different nutrient media PDA, Czapek Dox, and mushroom complete medium by inoculating 5 mm mycelial discs into Petri dishes (pH 7) and incubating at 25 °C. Biomass production was evaluated in both solid and liquid media. To study pH effects, cultures were grown in PDB at pH levels ranging from 5 to 9 for 15 days, with biomass measured at 5-day intervals by drying the harvested mycelium. Temperature effects were tested at 15, 20, 25, 30, and 35 °C under optimal pH, and biomass was recorded after 5, 10, and 15 days of incubation. Each treatment was performed in triplicate (n=3), and results are presented as mean ± standard deviation.

Cultivation of Macrocybe gigantea

High-quality spawn of *Macrocybe gigantea* was produced by inoculating sterilized grains (sorghum, ragi, wheat, maize, millet) whose moisture content was adjusted using sterile water and verified by the hand-squeeze method to 45–55% moisture, with 2 g/kg calcium carbonate added, then incubated at 30±2 °C for 12±3 days. The substrate was pasteurized by hot water treatment at 70–80 °C for 1 hour and allowed to cool to room temperature before spawning. Spawn was used to inoculate pasteurized substrates (sugarcane bagasse, paddy straw, wood shelves, groundnut shells) maintained at 65–70% moisture, which was achieved by adding measured sterile water and confirmed by the hand-squeeze test (release of a few drops on pressing), layered in polypropylene bags with 125 g spawn per 0.5 kg substrate. All the experiments were performed in triplicates (n=3). Bags were incubated at 25–35 °C

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and 80–90% humidity for 10–15 days until full colonization. After opening, a 1-inch layer of sterile vermicompost was applied to induce pinning, which appeared in 20–25 days, followed by fruiting within 9–12 days. Subsequent flushes were harvested over 50–60 days under controlled temperature (30–35 °C) and humidity (85–90%), and biological efficiency was calculated using following formula:

Biological efficiency

$$= \frac{Total\ fresh\ weight\ of\ the\ basidiomata}{Total\ dry\ weight\ of\ the\ substrate} \times 100$$

Determination of the nutritive profile of Macrocybe gigantea

Moisture content was determined in fresh mushrooms by repeated drying at 100–105 °C until constant weight. All other experiments were conducted using dried fruit bodies of *Macrocybe gigantea* that were ground into a fine powder. All other experiments were conducted using dried fruit bodies of *Macrocybe gigantea* that were ground into a fine powder. The levels of protein, fat, fiber, ash, and carbohydrates were determined following standard methods described earlier (Alam *et al.*, 2008). Crude fats contents (volatile oil, acid value, saponification value, iodine values) and Vitamins B1, B2, B3, C, D, and E were quantified as per the methods of Association of Official Analytical Chemists (Association of Official Analytical Chemist, 1990). This comprehensive analysis provided a detailed nutritional profile of *Macrocybe gigantea*.

Phytochemical analysis of Macrocybe gigantea

The extract of *Macrocybe gigantea* was screened for the presence of major phytochemical groups, including alkaloids, flavonoids, phenolics, saponins, and tannins. All qualitative tests were performed following the standardized procedures of Hayat Hayat *et al.* (2024). Additionally, the total flavonoid content (TFC) and total phenolic content (TPC) were quantified using the methods described by Hayat *et al.* (2024), ensuring accuracy and reproducibility of the results.

Invitro* antioxidant potentials of *Macrocybe gigantea

Antioxidant activity of *Macrocybe gigantea* extracts was assessed using DPPH, $ABTS^{+}$, and phosphomolybdenum assays at 20–100 µg/ml concentrations. DPPH and $ABTS^{+}$ scavenging was measured by absorbance changes, using quercetin and ascorbic acid as standards. Total antioxidant capacity was evaluated via phosphomolybdenum assay after heating with reagent at 95 °C. Absorbance was measured using a UV–Vis spectrophotometer at the following wavelengths: DPPH (517 nm), ABTS (734 nm) and Phosphomolybdnum assay (695 nm). Moreover, trolox and ascorbic acid were used as reference standards. Standard calibration curves were also prepared using different concentrations (10–100 µg/ml), and results were expressed as µmol Trolox equivalents (TE)/g dry weight. All tests were done in triplicates (n=3) and analysed statistically.

Data analysis

All experiments were performed in triplicate (n=3), and results are expressed as mean±standard deviation. Statistical significance among treatments was analysed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test at P-value < 0.05 using SPSS software.

Results

Morphology taxonomy studies of Macrocybe gigantea

Macrocybe gigantea mycelial growth is spherical on PDA. The colonies grow quickly, with thick, flat structures, and white, irregular edges before covering the entire plate in 10 to 15 days. The hyphae have several clamp connections and are hyaline and septate. The hyphal width is 7.3 µm on average. In 1912, Massee first characterized *Macrocybe gigantea* (Massee) Pegler & Lodge comb. nov. as *Tricholoma giganteum*. With a pileus (top) that is 25–37 cm in diameter and convex when young and widely convex when old, the basidiocarp (fruiting body) is big. The surface has an incurved margin, is white at the edge, and splits when dried. The pale yellow, sinuous lamellae (gills) come in different lengths. The stipe, or stem, is cylindrical, fibrillose-striate, and

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12–17 cm tall with a diameter of 4–7 cm. Thin-walled hyphae that range in diameter from 3 to 5 µm, inflate up to 16 µm, and are clamped together make up the context (flesh), which is up to 3 cm thick at the disc. Spores measure 5.2–6.9 × 4.2–5.1 µm, and the spore print is white (**Figure 1**).

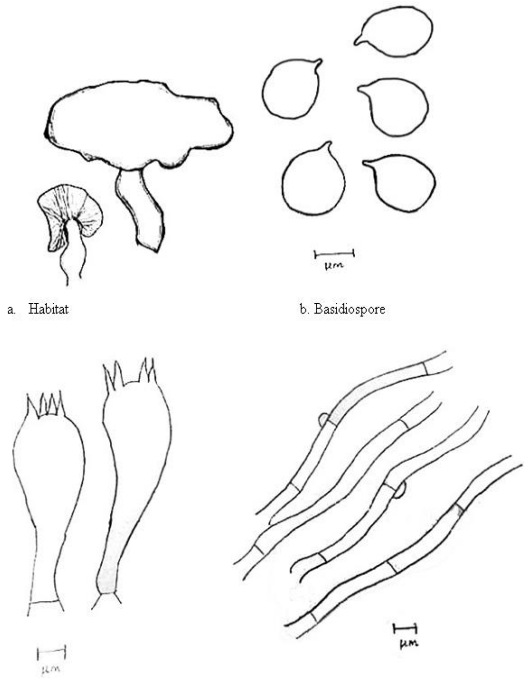


Figure 1. Morphological and taxonomical studies of *Macrocybe gigantean*.

Molecular identification of Macrocybe gigantea

The nucleotide sequence analysis method was used to identify the isolates of mushrooms down to the species level. Moreover, the PCR product was electrophoresed using a specific primer to amplify the rRNA gene. The presence of bands with a molecular size of 676 base pairs was demonstrated in the data, confirming the test accuracy and applicability to real fungus. The ITS sequence was aligned using BLAST against the NCBI GenBank database, and subsequently, submitted to GenBank under the accession number OQ644634.1. The sequence showed 99.12% similarity with *Macrocybe gigantea*, with 93% query coverage, an E-value of 0.0, and a maximum score of 1112. The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model. The robustness of the tree topology was evaluated using bootstrap analysis with 500 replicates, and the corresponding bootstrap values were indicated at the branch nodes. The phylogenetic tree (**Figure 2**) was constructed with Tricholomopsis scabra as the outgroup to ensure proper rooting and reliable inference of evolutionary relationships among the analyzed taxa.

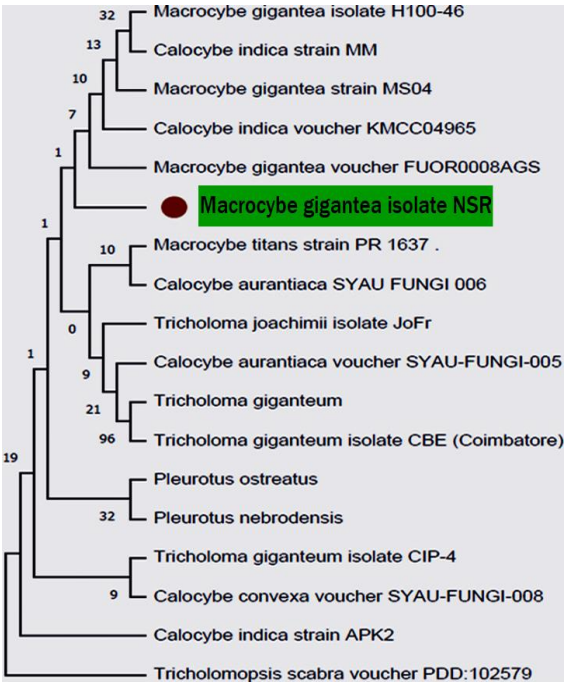


Figure 2. Phylogenetic tree of *Macrocybe gigantean*.

Impact of culture media on mycelial growth

The growth of *Macrocybe gigantea* was evaluated over a 15-day period on three different solid and liquid culture media. The results, presented in **Table 1**, revealed significant differences ($P<0.05$) in both mycelial radial growth (in cm) and biomass production (dry weight) among all the media tested. PDA showed significantly ($P<0.05$) higher mycelial growth and biomass production compared to MCM and CDA. MCM also differed significantly from CDA, exhibiting moderate growth and biomass yield, while CDA recorded the lowest values. Overall, all three media were statistically different from each other ($P<0.05$), with PDA performing best, followed by MCM and then CDA.

Impact of pH and temperature on biomass growth

The effects of pH (5.0–9.0) and temperature

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(15–35 °C) on the biomass production of *Macrocybe gigantea* in PDA medium are presented in **Figures 3** and **4**. Biomass production varied significantly (P<0.05) with changes in pH, with maximum dry weight recorded at pH 6.5 (9.3± 0.5 g/l), which was significantly higher than all other pH levels. This was followed by pH 6.0 and 7.0, while markedly reduced biomass was observed under more acidic and alkaline conditions, with the lowest value at pH 9.0 (2.3±0.3 g/l). Similarly, temperature significantly influenced mycelial growth (P<0.05), with maximum biomass obtained at 30°C (9.8±0.6 g/l), differing significantly from other temperatures tested. Biomass progressively increased from 15 °C (4.0±0.7 g/l) to 30 °C and declined thereafter, indicating reduced growth at both lower and higher temperatures.

Table 1. Impact of culture media on mycelial growth of *Macrocybe gigantean*.

Media	Solid media				Liquid media
	Day 6 (mm)	Day 9 (mm)	Day 12 (mm)	Day 15 (mm)	Dry weight (g/l)
Potato dextrose agar (PDA)	18 ± 3	38 ± 4	54 ± 6	73 ± 5^{a}	12.76 ± 1.43^{a}
Malt extract agar (MCM)	15 ± 2	32 ± 5	42 ± 5	65 ± 3^{b}	8.87 ± 0.65^{b}
Czapek dox agar (CDA)	12 ± 2	23 ± 3	31 ± 2	49 ± 4^{c}	6.32 ± 0.36^{c}

Values are mean±SD. Means with the same letters of superscript are not significantly different according to Tukey's post hoc (P<0.05).

Evaluation of different substrates for spawn, spawn run, and biological efficiency

For spawn production, sorghum proved to be the most suitable substrate, requiring a significantly shorter time (P<0.05) for complete colonization (11±1 days), whereas wheat required the longest duration (19±2 days), as illustrated in **Figure 5**. The effect of different lignocellulosic substrates on mycelial colonization, primordia initiation, basidiocarp formation, and biological efficiency of *Macrocybe gigantea* is presented in **Table 2**. Complete mycelial colonization occurred significantly faster (P<0.05) on paddy straw (20±1 days), followed by sugarcane bagasse (24±2 days), while wood shelves (28±1 days) and broken groundnut shell (30±3 days) required a longer duration and did not differ significantly from each other. Primordia formation was also earliest on paddy straw (28±2 days), significantly differing (P<0.05) from sugarcane bagasse (35±2 days), wood shelves (37±3 days), and broken groundnut shell (40±2 days). Similarly, basidiocarp formation was observed first on paddy straw (35±2 days), whereas sugarcane bagasse (41±2 days), wood shelves (43±2 days), and broken groundnut shell (42±2 days) showed delayed development. Biological efficiency varied significantly (P<0.05) among substrates, with paddy straw recording the highest efficiency (84.7±1.1%), followed by sugarcane bagasse (37.5±0.8%), wood shelves (17.5±0.3%), and broken groundnut shell (7.5±1.1%). Overall, paddy straw proved to be the most suitable substrate for rapid colonization and higher biological efficiency of *Macrocybe gigantea*.

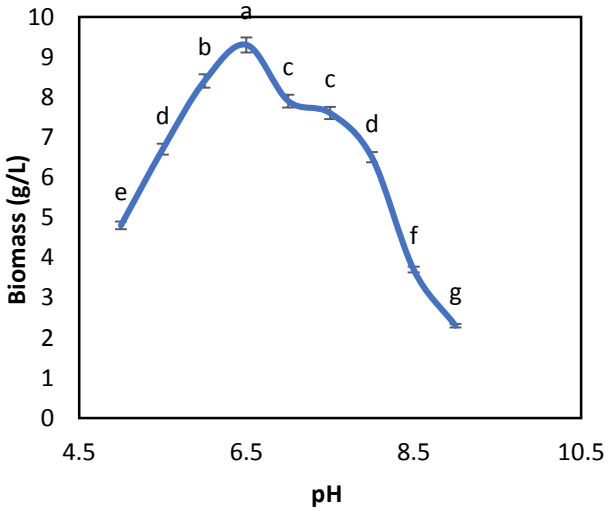


Figure 3. Impact of various pH on biomass growth of *Macrocybe gigantean*. Value showing same letters of are not significantly different according to Tukey's post hoc (P<0.05).

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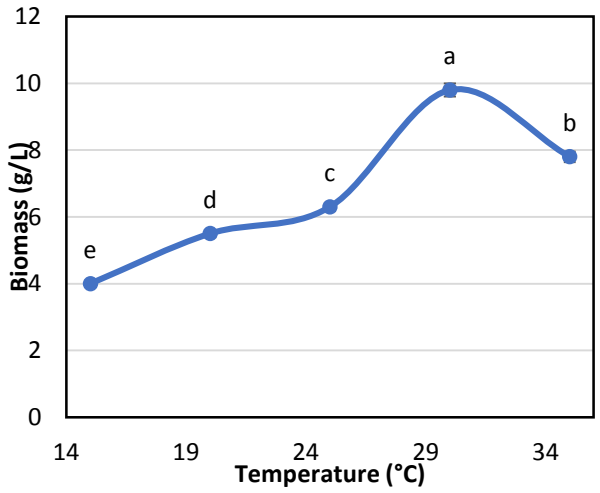


Figure 4. Impact of different temperatures on biomass growth of *Macrocybe gigantean.* Value showing same letters are not significantly different according to Tukey's post hoc (P<0.05).

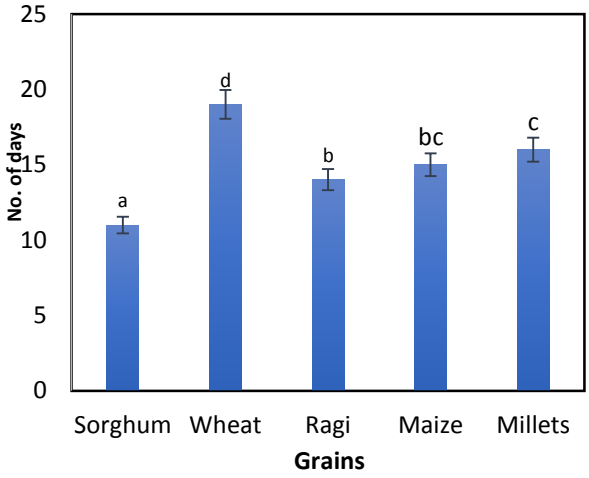


Figure 5. Evaluation of different substrates for spawn production. Values sharing the same superscript letters are not significantly different according to Tukey's post hoc test (P < 0.05).

Table 2. Spawn run parameters of *Macrocybe gigantea* on different substrates and biological efficiency.

Variable \ Substrate used	Paddy Straw	Sugarcane bagasse	Wood shelves	Broken groundnut shell
Weight of substrate (kg)	1	1	1	1
Complete mycelial cover (day)	20 ± 1^{a}	24 ± 2^{b}	28 ± 1^{c}	30 ± 3^{c}
Casing material vermin compost	20.5±1.0	24.5±2.0	28.5±1.0	30.5±3.0
Primordia formation (days)	28 ± 2^{a}	35 ± 2^{b}	37 ± 3^{bc}	40 ± 2^{c}
Basidio carp formation (day)	35 ± 2^{a}	41 ± 2^{b}	43 ± 2^{b}	42 ± 2^{b}
1st Flush (g)	600±25	250±18	175±9	75±4
2st Flush (g)	247±18	125±11	-	-
3st Flush (g)	-	-	-	-
Pileus breadth (cm)	8.7±0.7	7.5±1.1	6.8±0.4	4.5±1.1
Stipe length (cm)	14.5±1.2	14.0±0.9	8.0±0.6	5.0±0.6
Total yield (g)	847 ± 11^{a}	375 ± 8^{b}	175 ± 3^{c}	75 ± 11^{d}
Biological efficiency (%)	84.7 ± 1.1^{a}	37.5 ± 0.8^{b}	$17.5\%\pm0.3^{c}$	7.5 ± 1.1^{d}

"–" indicates no yield. Values are expressed as mean ± SD. Means sharing the same superscript letters are not significantly different according to Tukey's post hoc test (P<0.05).

Nutritive profile of Macrocybe gigantea

The results comprise phytochemicals, minerals, vitamins, fatty acids, and amino acids in the surrounding area. The nutritional components of the dried fruiting body of *Macrocybe gigantea* are listed in dietary values of *Macrocybe gigantea* extract (**Table 3**). Fresh mushrooms contain approximately 88±4% moisture, with the remaining 12% of their fresh weight comprising other nutritional constituents. The proximate analysis of *Macrocybe gigantea* revealed a nutritionally good profile characterized by a high protein content (47.34±3.28%) and comparatively low total fat (3.04%), underscoring its potential as a lean protein source. Total carbohydrates constituted 37.21±1.86% of the composition, of which 8.93±0.24% were non-reducing sugars, suggesting a notable proportion of soluble

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carbohydrates. The crude fibre content was 15.63±3.21%, while total ash accounted for 6.23±0.95%, reflecting a measurable mineral contribution. Micronutrient evaluation demonstrated the presence of vitamin B (4.37±0.25%), vitamin C (2.78±0.18%), and vitamin D (0.987±0.02%). In contrast, vitamins A and E were not detected under the conditions of analysis.

Phytochemical properties

The phytochemical profile of *Macrocybe gigantea* extracts made with different solvents is compiled in **Table 4**. Alkaloids, saponins, glycosides, proteins, carbohydrates, phenols, terpenoids, and flavonoids were among the substances found; amino acids were not present in any of the extracts. The sole substance present in the aqueous extract was glycosides, which had the most varied profile. The varied profile was reported in the ethanol extract of alkaloids, proteins, carbohydrates, phenols, terpenoids, and flavonoids. Hexane extract of glycosides, amino acids, phenols, and terpenoids are not present in *Macrocybe gigantea* while alkaloids, proteins, phenols, and flavonoids were present in the diethyl ether extract. The chloroform extract of alkaloids, proteins, and flavonoids were all present in *Macrocybe gigantea* (**Table 4**). The total flavonoids and phenolic content in *Macrocybe gigantea* contain 33 µg/mL of flavonoids and 9.64±0.33 mg/g of phenolic content in ethanol extract.

Antioxidant properties

Antioxidants are molecules capable of donating electrons to neutralize free radicals, and thereby, prevent oxidative damage. The antioxidant activity of *Macrocybe gigantea* extracts was evaluated using DPPH, ABTS, and phosphomolybdenum assays, and the IC_{50} values (µg/ml) are presented in **Table 5** as mean±SD, with different superscript letters indicating significant differences according to Tukey's post hoc test (P<0.05). Ascorbic acid was used as the standard and exhibited the lowest IC_{50} values in all assays, confirming its superior antioxidant potency. In the DPPH assay (**Figure 6**), the ethanol extract showed significantly higher activity (57.07±1.71 µg/ml) compared to diethyl ether, chloroform, hexane, and aqueous extracts. Similarly, in the ABTS assay (**Figure 7**), the ethanol extract demonstrated strong antioxidant activity (39.18±1.18 µg/ml), followed by the aqueous extract, while chloroform and hexane extracts recorded higher IC_{50} values, indicating lower activity. In the phosphomolybdenum assay (**Figure 8**), the ethanol extract again exhibited superior antioxidant potential (45.22±1.36 µg/ml), whereas hexane showed the least activity. Overall, significant differences were observed among the extracts (P<0.05), and the results consistently indicate that ethanol is the most effective solvent for extracting antioxidant compounds from *Macrocybe gigantea*, highlighting the influence of solvent polarity on bioactive compound recovery.

Table 3. Nutritive profile of *Macrocybe gigantean.*

Contents	Percentage of dry weight
Macronutrients	
Moisture content (Fresh mushroom)	88±4
Protein	47.34±3.28
Carbohydrate	37.21±1.86
Fat	3.04±0.24
Fibre	15.63±3.21
Ash content	6.23±0.95
Non reducing sugar	8.93±0.24
Vitamins	
A	NIL (0 %)
B	4.37±0.25
C	2.78±0.18
D	0.987±0.02
E	NIL (0 %)

Discussion

Macrocybe gigantea is classified in the genus *Macrocybe* because it differs from other species due to the abundance of clamp connections and the lack of siderophilous granules in the basidia. With a diameter of up to 37 cm, the huge basidiocarp is solitary to few and has pale-yellow lamellae, a cylindrical smooth stipe, and a smooth, crackling white surface. Although *Calocybe* and *Macrocybe* have certain physical similarities (Galappaththi *et al.*, 2022), *Macrocybe gigantea* is unique because of its large basidiomata, squamulose stipe surface,

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and profusion of refractive pseudocystidia (Roy *et al.*, 2022). Given the frequent misidentification of *Macrocybe* species as *Calocybe* due to overlapping morphological traits, the combined morpho-molecular approach adopted in the present study strengthened taxonomic reliability. Molecular confirmation supported the morphological identification, minimizing ambiguity in species delimitation.

Table 4. Screening of phytochemical analysis *of Macrocybe gigantea* using various solvents.

Phytoconstituents	Aqueous	Ethanol	Diethyl ether	Hexane	Chloroform
Alkaloids	-	+	+	+	+
Saponins	-	-	-	+	-
Glycosides	+	-	-	-	-
Carbohydrates	-	+	-	+	-
Proteins	-	+	+	+	+
Amino acids	-	-	-	-	-
Phenols	-	+	+	-	-
Terpenoids	-	+	-	-	-
Flavonoids	-	+	+	+	+

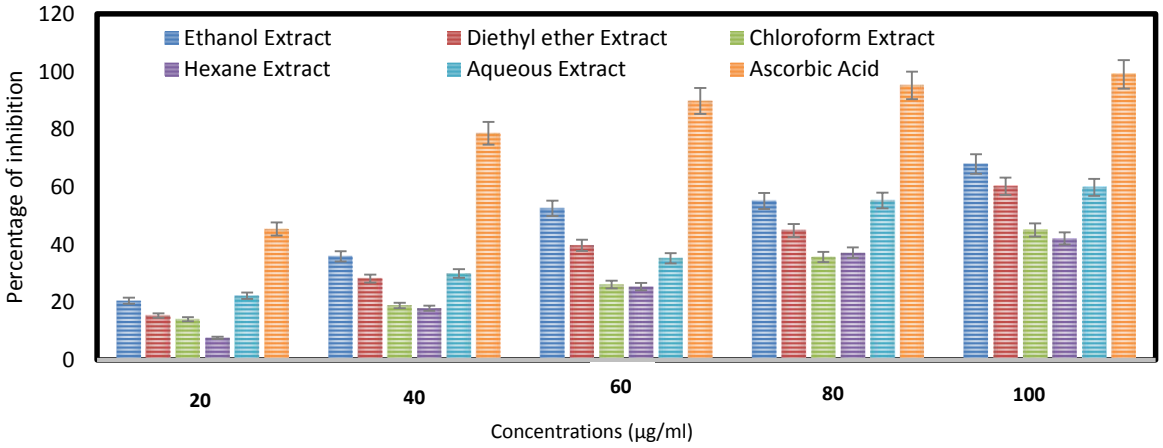


Figure 6. DPPH radical scavenging ability of mushroom extracts of *Macrocybe gigantean.*

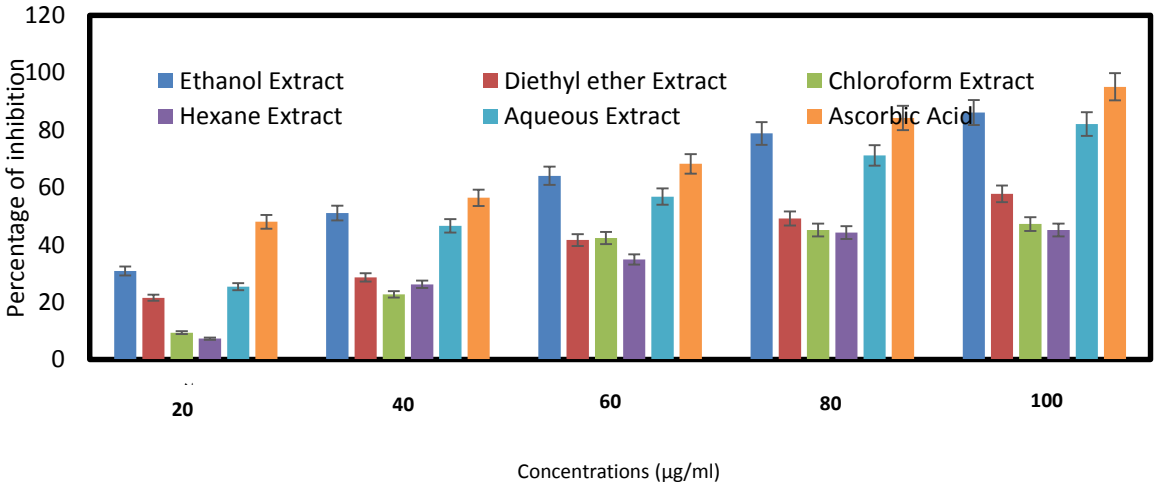


Figure 7. ABTS radical scavenging ability of mushroom extracts of *Macrocybe gigantean.*

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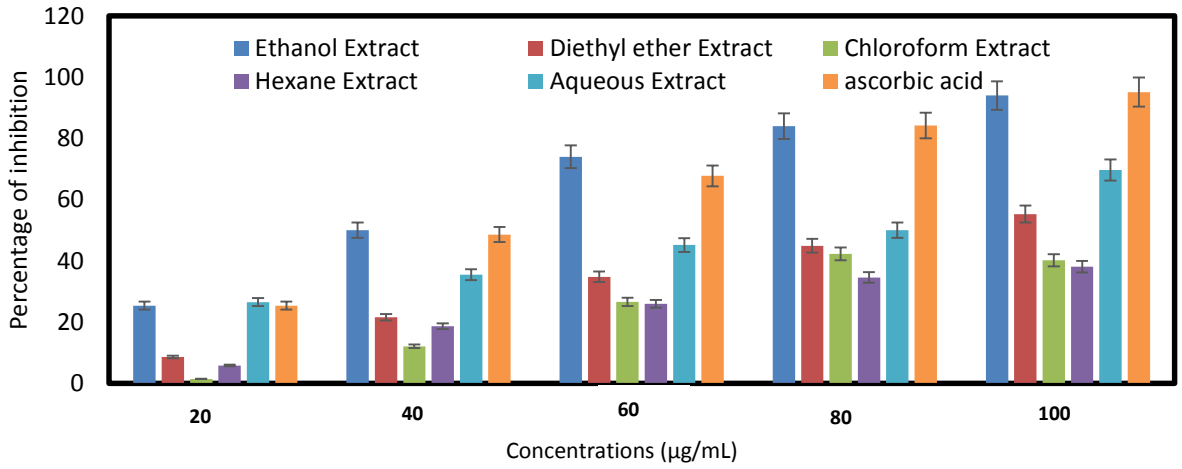


Figure 8. Phosphomolybdenum assay of mushroom extracts of *Macrocybe gigantean.*

Table 5. IC_{50} (µg/ml)values of various extract against antioxidant assays

Assays	Ethanol extract	Diethyl ether extract	Chloroform extract	Hexane extract	Aqueous extract	Ascorbic acid
DPPH	57.07 ± 1.71^{b}	89.14 ± 2.67^{d}	112.70 ± 3.38^{e}	114.58 ± 3.44^{e}	66.60 ± 2.00^{c}	27.70 ± 0.83^{a}
ABTS	39.18 ± 1.18^{b}	81.41 ± 2.44^{d}	105.88 ± 3.18^{e}	110.79 ± 3.32^{e}	53.74 ± 1.61^{c}	25.09 ± 0.75^{a}
Phosphomolybdenum assay	45.22 ± 1.36^{b}	89.42 ± 2.68^{d}	111.18 ± 3.34^{e}	122.62 ± 3.68^{f}	66.02 ± 1.98^{c}	32.46 ± 0.97^{a}

Values are expressed as mean ± SD. Means sharing the same superscript letters are not significantly different according to Tukey's post hoc test ($P < 0.05$).

The present study provides the first integrated evaluation of the nutritional, phytochemical and antioxidant potential of *Macrocybe gigantea* collected from the study region. The observed variations in growth conditions, antioxidant activity and phytochemical composition highlight the species' adaptability and potential nutritional relevance (Lakshmikanthan *et al.*, 2025). The growth conditions of *Macrocybe gigantea* in this study are consistent with outcomes from previous studies on a range of fungal species. According to the research by Vilas *et al.* (Vilas *et al.*, 2020), *Macrocybe gigantea* have more growth of colonies and biomass production than other fungal species including *Pleurotus* sp. Simultaneously, PDA medium was reported to have the highest growth of mycelial development and biomass production in *Pleurotus* sp. The results of this study were in line with those of Akshaya *et al.* (2021), who also found that PDA facilitated superior fungal growth in comparison to other media. This medium might be the ideal choice for cultivating *Macrocybe gigantea* in order to generate biomass on a big scale, as the fungus thrives on PDA and PDB. Aditya and Jarial (2022) reported that Hypsizygus ulmarius produced maximum biomass on PDA media, which had greater therapeutic potential due to its active compounds Aditya and Jarial (2022). The culture medium has a significant impact on the mycelial growth and biomass production of *Macrocybe gigantea.* The ideal growing conditions for *Macrocybe gigantea* for scientific and industrial applications, particularly those involving the production of bioactive chemicals, are improved by the results. The results corroborated previous studies showing that pH 6 supports biomass buildup and optimal development for a range of fungi, including *Lentinus swartzii* (Dulay *et al.*, 2021), *Hypsizygus ulmarius* (Aditya and Jarial, 2023), *Volvariella volvacea* (Abon *et al.*,

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2020). The present study found that temperature has a major effect on biomass output and mycelial growth. 30°C was the ideal temperature for *Macrocybe gigantea*, which is in line with earlier research on other fungi including *Calocybe Indica* (Alam *et al.*, 2010) and *Lentinus swartzii* (Dulay *et al.*, 2021). The optimal growth at specific pH and temperature reflects the ecological adaptation of the species to tropical climatic conditions. These parameters are typical of lignocellulose-degrading basidiomycetes and indicate strong enzymatic efficiency in converting plant-derived substrates into fungal biomass. Such growth characteristics highlight the species' suitability for controlled cultivation and industrial biomass production.

Substrate evaluation demonstrated that sorghum promoted fastest spawn colonization, while paddy straw was particularly suitable for fruiting due to its porous structure and favorable carbon - nitrogen balance, supporting efficient fruit body formation and yield stability (84.7±1.1%), consistent with its favorable lignocellulosic composition. Additionally, sorghum produced the fastest mycelial colonization and the highest spawn growth, while wheat produced the slowest pinhead development (Sağir and Yildiz, 2004). The differences in colonization rates among substrates could be explained by variations in the moisture content during boiling. Numerous research, reported with many varieties showed maximum growth in *Pleurotus ostreatus* (Narh *et al.*, 2011) and *Pleurotus florida* (Karpagavalli *et al.*, 2024); they identified sorghum as the perfect spawn substrate; nevertheless, the sustainability of cultivating it with agroforestry products is being examined. Furthermore, Kalaw *et al.* discovered that rice straw was preferred in this investigation (Kalaw *et al.*, 2021). It broke down the agro wastes through mushroom enzymes easily and allowed air to enter because of its fibrous structure (Jayaraman *et al.*, 2024), although wheat straw had the biggest impact on mycelial growth (Yin *et al.*, 2025).

These days, mushrooms' secondary metabolites are expanding rapidly because of their wide range of biological activities. The use of functional foods for their benefits has so greatly increased as a result of awareness of their safety and lack of adverse effects (Ayyankalai *et al.*, 2025, Bhambri *et al.*, 2022). The nutritional profile observed in the present study supports the role of *Macrocybe gigantea* as a protein-rich and low-fat food source. The coexistence of essential macronutrients and bioactive phytochemicals indicates its dual significance as both a dietary component and a potential nutraceutical resource. Mushrooms are known to contain diverse carbohydrates, including mono- and disaccharides, glucans, glycogen, sugar alcohols, and chitin. Additionally, their appreciable fiber content contributes to digestive health by facilitating the elimination of waste and toxins, thereby promoting overall well-being (Vetter, 2023). In the present study, *Macrocybe gigantea* exhibited a notably high protein content (47.34±3.28%), suggesting the probable presence of essential and non-essential amino acids, which are commonly abundant in edible mushrooms. Beyond proteins, carbohydrates and fiber, mushrooms serve as valuable sources of essential vitamins and minerals. However, detailed amino acid profiling was not undertaken in this study which warrants further investigation. Collectively, these findings highlight the nutritional potential of *Macrocybe gigantea* and emphasize the importance of promoting its cultivation and consumption in local communities to ensure sustainable production and year-round availability.

The present findings demonstrate that *Macrocybe gigantea* possesses significant antioxidant potential, with marked differences among solvent extracts ($P<0.05$). The ethanol extract consistently exhibited the lowest IC50 values in DPPH, ABTS, and phosphomolybdenum assays, indicating superior radical scavenging and total antioxidant capacity, while hexane showed the least activity. The enhanced efficacy of the ethanol extract highlights the importance of solvent polarity in efficiently extracting phenolic and flavonoid compounds responsible for antioxidant activity. These results are consistent with the report of a study (Sasidhara and Thirunalasundari, 2014), supporting the view that ethanolic extraction is optimal for recovering multifunctional antioxidant

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constituents from edible mushrooms. The results align with previous studies on various mushrooms such as *Pleurotus ostreatus* (Effiong *et al.*, 2024), *Inonotus obliquus, Grifola frondosa, Ganoderma lucidum. Ganoderma tsugae, Lentinula edodes, Trametes versicolor, and Hericium erinaceus* (Sharpe *et al.*, 2021), *Lentinus edodes*, *Agaricus blazei* (Da *et al.*, 2011), which consistently reported superior antioxidant performance of ethanol and methanol extracts compared to ethyl acetate and hexane (Dip *et al.*, 2024). As emphasized by Kaprasob *et al.*, using multiple assays like DPPH and ABTS is essential due to differing mechanisms electron transfer and hydrogen atom transfer, offering a comprehensive assessment (Kaprasob *et al.*, 2022). Cumulatively, these results underscore *Macrocybe gigantea*, particularly its ethanol extract, as a potent natural antioxidant source. The strong antioxidant activity observed may be attributed to the combined presence of phenolics, flavonoids and other bioactive metabolites detected in the phytochemical screening.

Despite generating valuable baseline data on the nutritional, phytochemical, and antioxidant properties of *Macrocybe gigantea*, certain limitations of the present study should be considered when interpreting the findings. The antioxidant potential was evaluated solely through in vitro assays, which, although informative, may not accurately reflect biological activity under in vivo conditions. Furthermore, the phytochemical analysis was primarily qualitative, and advanced analytical techniques were not employed to precisely identify and quantify the individual bioactive compounds responsible for the observed activity. The absence of toxicological assessment also limits conclusions regarding safety for therapeutic application. Therefore, future studies incorporating detailed compound characterization, *in vivo* validation, and toxicity evaluation are essential to more comprehensively substantiate the nutraceutical and therapeutic relevance of this species.

Conclusion

The present study demonstrates that *Macrocybe gigantea* can be successfully cultivated under optimized conditions and exhibits appreciable nutritional value and in-vitro antioxidant activity. Paddy straw was identified as the most suitable substrate for higher biological efficiency. These findings suggest that this mushroom may serve as a valuable edible resource; however, further *in-vivo* and clinical studies are required to substantiate its potential health benefits.

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Authors' contributions

Saravanan N performed the experiments and drafted the manuscript. Rajagopal S and Ramalingam S conceived and supervised the study. Kumar P and Jagadeesan M contributed to data analysis and interpretation. Srinivasan MK assisted with experimental validation, literature review, and manuscript editing.

Conflict of interest

The authors declare no conflict of interest.

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