

# Biochemical Profiling and Nutritional Assessment Of *Calocybe Indica* Cultivated Under Bihar Conditions

Anamika Kumari<sup>1</sup> and Vinay Bhushan Kumar<sup>2\*</sup>

<sup>1</sup>University Department of Botany, Patliputra University, Patna

<sup>2</sup>Department of Botany, T.P.S. College, Patliputra University, Patna

\*Corresponding author: [vinaykumar10121976@gmail.com](mailto:vinaykumar10121976@gmail.com)

## ABSTRACT

*Calocybe indica* (milky mushroom) is an edible tropical mushroom widely recognised for its high nutritional quality, ease of cultivation, and diverse bioactive constituents. The present study was undertaken to evaluate the biochemical composition and phytochemical profile of *C. indica* cultivated at T. P. S. College, Patna, Bihar, India. Fresh fruiting bodies were harvested, dried, powdered, and analysed using standard biochemical methods. Protein and carbohydrate contents were estimated by the Lowry and phenol-sulphuric acid methods, respectively, while total phenols, flavonoids, alkaloids, and tannins were determined using established phytochemical assays. The results revealed that *C. indica* contained  $23.56 \pm 0.42\%$  protein,  $34.97 \pm 0.44\%$  carbohydrate,  $8.10 \pm 0.31$  mg GAE/g total phenols,  $3.74 \pm 0.19$  mg QE/g flavonoids,  $3.36 \pm 0.14\%$  alkaloids, and  $4.39 \pm 0.21$  mg/g tannins. The appreciable protein content together with the presence of phenolic compounds, flavonoids, alkaloids, and tannins indicates that *C. indica* is a nutritionally rich mushroom with promising antioxidant and functional properties. These findings support its potential use as a functional food and nutraceutical ingredient while reinforcing its importance as an economically valuable edible mushroom suitable for commercial cultivation in tropical and subtropical regions. Further investigations on the isolation and characterisation of its bioactive compounds may facilitate the development of mushroom-based therapeutic and value-added food products.

**Keywords:** *Calocybe indica*; milky mushroom; biochemical composition; phytochemicals; protein; carbohydrates; phenolic compounds; functional food; antioxidants.

## 1. INTRODUCTION

Edible mushrooms have attracted growing scientific and commercial interest due to their nutritional richness, medicinal potential, and adaptability to sustainable agricultural systems. As naturally occurring macrofungi, mushrooms produce visible fruiting bodies above the soil surface and reproduce through spores. Their life cycle consists of two distinct developmental stages: the vegetative phase, characterised by the growth of mycelia, and the reproductive phase, during which fruiting bodies are formed for spore production (Sánchez, 2017). Globally, nearly 14,000 mushroom species have been identified, of which approximately 2,000 are edible and around 200 are cultivated commercially for food and therapeutic purposes (Mleczek et al., 2018; Garofalo et al., 2017). Owing to their unique flavour, desirable texture, and high nutritional value, edible mushrooms have become increasingly recognised as functional foods that contribute to both nutrition and disease prevention.

Among cultivated edible mushrooms, *Calocybe indica* (P. & C.), commonly known as the milky mushroom, has emerged as one of the most promising tropical mushroom species. Originally described by Purkayastha and Chandra (1974), this mushroom was first identified in West Bengal, India, where its large white fruiting bodies and distinctive morphology attracted considerable attention (Purkayastha & Chandra, 1974). The species was later recognised as having significant cultivation potential because of its adaptability to tropical environments, leading to further studies on its biology and commercial production (Pandey & Tewari, 1993). The common local name "Dudh Chatta" reflects its characteristic milky white appearance, while it is also referred to as the white summer mushroom because it thrives under warm climatic conditions (Krishnamoorthy, 1995). Unlike many commercially cultivated mushrooms that require relatively cool temperatures, *C. indica* performs well under high-temperature conditions, making it particularly suitable for tropical and subtropical regions.

Natural populations of *C. indica* have been reported from different regions of India, particularly the plains of Tamil Nadu and Rajasthan, where fruiting generally occurs during the monsoon months between May and August after sufficient rainfall (Doshi et al., 1989; Krishnamoorthy, 1995). The species predominantly grows in grassland ecosystems as a saprophytic fungus that decomposes organic matter, although occasional ectomycorrhizal

associations have also been suggested (Subbaiah & Balan, 2015). These ecological characteristics, combined with its broad environmental adaptability, have contributed to its increasing importance in commercial mushroom cultivation.

The cultivation history of *C. indica* demonstrates its gradual transition from a naturally occurring mushroom to a commercially important crop. Artificial cultivation was initiated in West Bengal during the mid-1970s, after which production technologies were progressively refined (Anurag, 2017). A breakthrough occurred when Krishnamoorthy successfully rediscovered the species in Tamil Nadu and standardised cultivation techniques suitable for commercial-scale production, enabling wider adoption across southern India (Krishnamoorthy, 1997; Anurag, 2017). Today, the mushroom is cultivated extensively in Karnataka, Tamil Nadu, Kerala, and Andhra Pradesh and has become one of the leading cultivated mushrooms after button, oyster, and paddy straw mushrooms (Krishnamoorthy, 1997). Its popularity among growers is primarily attributed to its simple cultivation practices, relatively low production costs, attractive fruiting bodies, extended postharvest shelf life, and ability to utilise a wide range of lignocellulosic agricultural residues as substrates (Chelladurai et al., 2021; Maurya et al., 2019). Paddy straw has consistently been identified as one of the most suitable substrates because it promotes vigorous mycelial growth and higher biological efficiency (Maurya et al., 2019).

The growing importance of *C. indica* is also associated with its remarkable nutritional composition. Mushrooms have long been regarded as valuable dietary components because they provide high-quality proteins while containing relatively low levels of fat. Consequently, they are often referred to as the "poor man's protein," particularly in developing countries where access to animal-derived protein sources may be limited or economically constrained (Pandey, 2004). Studies have demonstrated that *C. indica* contains substantial amounts of proteins, carbohydrates, dietary fibre, lipids, essential amino acids, vitamins, and minerals, making it nutritionally comparable to other commercially cultivated edible mushrooms (Alam et al., 2008). Mature fruiting bodies generally contain higher protein concentrations than immature stages, further enhancing their nutritional significance (NahuAlam et al., 2018).

Beyond its nutritional value, *C. indica* possesses an extensive array of bioactive compounds that contribute to its functional and medicinal properties. These include phenolic compounds, flavonoids, alkaloids, steroids, terpenes, chalcones, tannins, glycosides, anthraquinones, saponins, and other phytochemicals known to exhibit biological activity (Ajiboye et al., 2013; Concepta et al., 2021). In addition, the mushroom contains nutritionally important compounds such as  $\beta$ -glucans, proteins, dietary fibre, vitamins, and essential minerals (Chelladurai et al., 2021; Maurya et al., 2019). The major mineral constituents include potassium, sodium, and phosphorus, while the amino acid profile comprises all essential amino acids, with glycine reported as one of the predominant components (NahuAlam et al., 2018). Its carbohydrate content ranges from 37–48% on a dry weight basis, protein content from 20–25%, dietary fibre from 13–24%, and lipid content remains comparatively low at approximately 4–5%, making it particularly suitable for health-conscious consumers (Alam et al., 2008).

Scientific investigations have increasingly focused on the pharmacological properties of *C. indica*. Experimental studies have demonstrated that extracts prepared from this mushroom possess considerable antioxidant potential, including free radical scavenging activity, reducing power, metal chelation, and hydrogen peroxide scavenging capacity (Mirunalini et al., 2012; Babu & Rao, 2013). Interestingly, comparative studies have indicated that the stipe exhibits higher flavonoid content, total phenolic concentration, chelating activity, and hydrogen peroxide scavenging ability than the pileus, highlighting differences in bioactive compound distribution within the fruiting body (Meghna et al., 2022). Furthermore, the antioxidant compounds present in *C. indica* may reduce oxidative stress induced by reactive oxygen species (ROS), thereby potentially lowering the risk of ageing, cardiovascular disorders, infections, and carcinogenesis (Chelladurai et al., 2014).

In addition to antioxidant properties, *C. indica* has demonstrated considerable therapeutic potential through multiple biological mechanisms. The mushroom contains biologically active polysaccharides, including  $\beta$ -glucans, mannans, chitin, hemicellulose, galactans, and xylans, together with polyphenolic compounds such as flavonoids, alkaloids, and triterpenoids, which have been associated with antioxidant, antidiabetic, anticancer, and anti-lipid peroxidation activities (Mirunalini et al., 2012; Ghosh & Acharya, 2022). Proteins and glucans isolated from *C. indica* have also shown immunostimulatory properties by enhancing the activity of natural killer cells, thereby strengthening immune defence mechanisms against abnormal and cancerous cells (Ghosh & Acharya, 2022). Moreover, fungal immunomodulatory proteins (FIPs) present in mushrooms have emerged as an important class of bioactive molecules capable of regulating immune responses, while extracts of milky mushroom have demonstrated antioxidant, antimicrobial, anticancer, and immunomodulatory activities in several experimental studies (Anusiya et al., 2021).

The versatility of *C. indica* extends beyond its nutritional and medicinal attributes. Owing to its favourable biochemical composition, low fat content, high dietary fibre, and alkaline ash, the mushroom is considered

beneficial for individuals suffering from hyperacidity, constipation, and lifestyle-related disorders (NahuAlam et al., 2018). Consequently, the applications of mushrooms have expanded beyond conventional dietary use to include functional foods, nutraceuticals, pharmaceutical products, and traditional medicinal formulations (Anju & Ukkuru, 2016). The increasing consumer demand for naturally derived health-promoting foods has further accelerated research into the cultivation, nutritional composition, therapeutic properties, and industrial utilisation of *C. indica*.

Considering its excellent cultivation characteristics, balanced nutritional profile, abundance of bioactive compounds, and broad spectrum of biological activities, *Calocybe indica* has emerged as an important edible mushroom with significant commercial and therapeutic value. Continued research on this species is expected to strengthen its applications in food science, nutrition, biotechnology, and pharmaceutical development. Therefore, this paper provides a comprehensive overview of the origin, cultivation, nutritional composition, bioactive constituents, health-promoting properties, and potential industrial applications of *Calocybe indica*, emphasising its importance as a functional food and a valuable natural resource for improving human health.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Material

Fresh fruiting bodies of *Calocybe indica* were cultivated at T. P. S. College, Patna, Bihar, India, under controlled cultivation conditions using standard mushroom cultivation practices. Mature and healthy fruiting bodies were harvested at the appropriate stage of development and cleaned to remove adhering substrate particles and other extraneous materials. The harvested mushrooms were sliced into small pieces and oven-dried at 40°C for 4–5 days until a constant weight was obtained. The dried samples were then ground into a fine powder using a laboratory grinder and passed through a fine mesh sieve to ensure uniform particle size. The powdered samples were stored in clean, airtight containers at room temperature until further biochemical and phytochemical analyses.

### 2.2 Estimation of Proteins by Lowry's Method

Protein estimation was performed following the method described by Lowry et al. (1951) using the Folin-Ciocalteu reagent. Working standards were prepared by diluting 10 ml of stock solution (1 mg/ml bovine serum albumin). Aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml of working standards were pipetted and made up to 1 ml with distilled water. To each tube, 5 ml of reagent C (alkaline copper reagent) was added with thorough mixing. Subsequently, 0.5 ml of reagent D (diluted Folin-Ciocalteu reagent) was added with vigorous mixing. The samples were incubated at room temperature for 30 minutes, and absorbance was recorded at 660 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). The protein content was calculated from a standard curve prepared using bovine serum albumin.

### 2.3 Estimation of Carbohydrates by Phenol-Sulphuric Acid Method

Total carbohydrate content was estimated using the phenol-sulphuric acid method as described by Subbaiah and Balan (2015). The powdered mushroom samples were extracted with distilled water and neutralised with sodium carbonate until effervescence ceased. The volume was made up to 100 ml and centrifuged at 3000 rpm for 10 minutes. Working standards (0, 0.2, 0.4, 0.6, 0.8, 1.0 ml) were prepared from a glucose stock solution (100 µg/ml), with volumes made up to 1 ml using distilled water. To each tube, 1 ml of 5% phenol solution and 5 ml of concentrated sulfuric acid were added sequentially. The tubes were placed in a water bath at 25–30°C for 20 minutes. Absorbance was measured at 490 nm against a reagent blank. Carbohydrate content was calculated from a standard glucose calibration curve.

### 2.4 Estimation of Phytochemicals

#### 2.4.1 Estimation of Phenols (Brunner, 1984)

**Extraction:** One gram of powdered sample was taken in a 100 ml beaker, and 15 ml of acidified methanol (methanol: HCl, 99:1) was added. The mixture was shaken for 30 minutes at room temperature. The supernatant was decanted, and the extraction was repeated three times with fresh acidified methanol. The pooled supernatant was centrifuged at 6000 rpm for 15 minutes, filtered through Whatman No. 1 filter paper, and the volume was made up to 50 ml with acidified methanol in a volumetric flask.

**Total Phenolic Content:** Working standards of gallic acid (0.1, 0.2, 0.3, 0.4, 0.5 ml) were prepared, and 0.5 ml of Folin-Ciocalteu reagent was added to each tube. Then, 2 ml of sodium carbonate solution (20%) was added, and the mixture was incubated at 37°C for 1 hour. The absorbance was measured at 760 nm. Total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g dry weight.

#### 2.4.2 Estimation of Flavonoids (Chang et al., 2002)

Flavonoid content was determined using the aluminium chloride colourimetric method. Aliquots of 0.5, 1.0, 1.5, and 2.0 ml of working standard (quercetin, 1 mg/ml) were taken and made up to 5 ml with distilled water. To each tube, 0.3 ml of 5% sodium nitrite was added and incubated for 6 minutes at room temperature. Then, 0.6 ml of 10% aluminium chloride was added and vortexed for 10 minutes. Finally, 2 mL of 1 M sodium hydroxide was added, and the volume was made up to 10 mL with distilled water. The absorbance was measured at 413 nm. Total flavonoid content was expressed as quercetin equivalents (QE) in mg/g dry weight.

#### 2.4.3 Estimation of Alkaloids (Harborne, 1984)

Alkaloid content was determined using the gravimetric method. Five grams of ground sample was weighed, and 50 ml of 10% acetic acid in ethanol was added. The mixture was filtered, and concentrated ammonium hydroxide was added dropwise to the filtrate to precipitate alkaloids. The precipitate was collected on pre-weighed filter paper and dried in an oven at 60°C for 30 minutes. The weight of alkaloids was calculated using the formula:

$$\% \text{ Alkaloids} = (W_2 - W_1) / W \times 100$$

Where:

- W = Weight of sample (g)
- W<sub>1</sub> = Weight of empty filter paper (g)
- W<sub>2</sub> = Weight of filter paper plus precipitate (g)

#### 2.5 Estimation of Tannins

Tannin content was estimated following the method described by Udu-Ibiam et al. (2014). Five-tenths gram (0.5 g) of powdered mushroom was transferred to a 250 ml conical flask. Seventy-five millilitres of distilled water was added, and the mixture was heated for 30 minutes. The extract was centrifuged at 2000 rpm for 20 minutes, and the supernatant was collected in a 100 ml volumetric flask with volume adjustment. To 1 ml of the mushroom extract, 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml with water. The mixture was shaken well, and absorbance was recorded at 700 nm after 30 minutes. Tannic acid was used as the standard.

#### 2.6 Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD). The data recorded from the biochemical compound analyses were statistically analysed using Completely Randomised Design (CRD) following the procedures suggested by Gomez and Gomez (1984).

### 3. RESULTS

#### 3.1 Protein Content

The protein content of *Calocybe indica*, determined using the Lowry method, was 23.56 ± 0.42% (Table 1). This value falls within the reported protein range of 20–25% for *C. indica* documented by Alam et al. (2008) and NahuAlam et al. (2018). The relatively high protein concentration indicates that *C. indica* is a nutritionally rich edible mushroom and can serve as an important dietary protein source, particularly for vegetarian populations and communities with limited access to animal-based protein.

#### 3.2 Carbohydrate Content

The total carbohydrate content estimated by the phenol–sulphuric acid method was 34.97 ± 0.44%. Although slightly lower than some previously reported values, it remains comparable with the carbohydrate composition reported for cultivated edible mushrooms (Alam et al., 2008). The carbohydrate fraction contributes substantially to the caloric value of the mushroom and includes structural polysaccharides that are associated with dietary fibre and functional health benefits.

### 3.3 Total Phenolic Content

The total phenolic content of *Calocybe indica* was  $8.10 \pm 0.31$  mg GAE/g (gallic acid equivalents per gram of dry weight). The presence of a considerable quantity of phenolic compounds indicates that the mushroom possesses notable antioxidant potential. These phenolic constituents are capable of scavenging free radicals and may contribute to reducing oxidative stress, thereby supporting the use of *C. indica* as a functional food with health-promoting properties.

**Table 1. Biochemical composition of *Calocybe indica***

| Parameter     | Mean $\pm$ SD    | Unit                   |
|---------------|------------------|------------------------|
| Protein       | $23.56 \pm 0.42$ | %                      |
| Carbohydrate  | $34.97 \pm 0.44$ | %                      |
| Total phenols | $8.10 \pm 0.31$  | mg GAE g <sup>-1</sup> |
| Flavonoids    | $3.74 \pm 0.19$  | mg QE g <sup>-1</sup>  |
| Alkaloids     | $3.36 \pm 0.14$  | %                      |
| Tannins       | $4.39 \pm 0.21$  | mg g <sup>-1</sup>     |

Values represent mean  $\pm$  SD of three independent replicates.

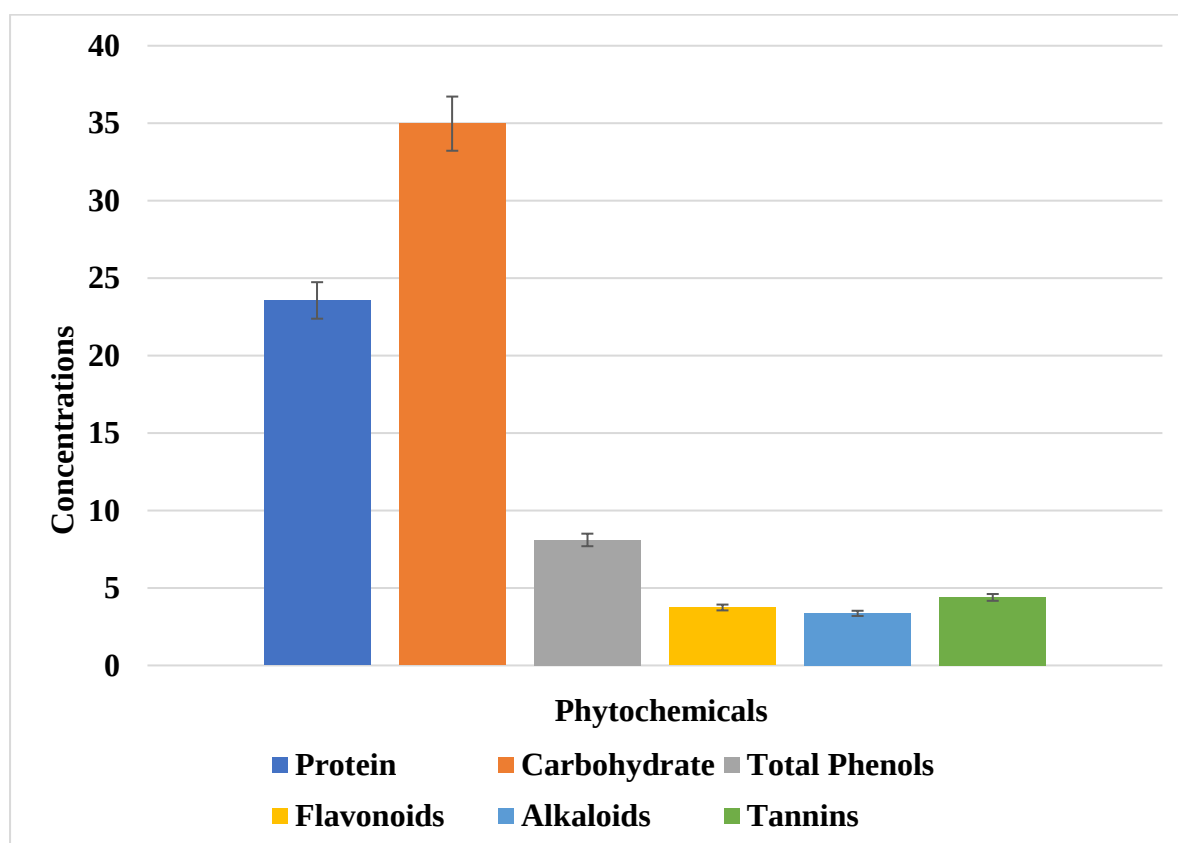


**Figure 1.** Proximate composition and phytochemical profile of *Calocybe indica*. Values represent protein, carbohydrate, total phenols, flavonoids, alkaloids, and tannins determined in the mushroom sample. Protein and carbohydrate are expressed as percentage (%), total phenols as mg gallic acid equivalents per gram (mg GAE/g), flavonoids as mg quercetin equivalents per gram (mg QE/g), and tannins as mg/g.

### 3.4 Flavonoid Content

The flavonoid content determined by the aluminium chloride colourimetric assay was  $3.74 \pm 0.19$  mg QE/g (quercetin equivalents per gram of dry weight). Flavonoids are important phytochemicals known for their antioxidant, anti-inflammatory, and antimicrobial activities. The observed flavonoid concentration further supports the nutraceutical significance of *C. indica* and its potential application in functional food formulations.

### 3.5 Alkaloid Content

The alkaloid content of *Calocybe indica*, determined by the gravimetric method, was  $3.36 \pm 0.14\%$ . Alkaloids are biologically active secondary metabolites that exhibit a wide range of pharmacological properties, including antimicrobial, analgesic, and anti-inflammatory effects. The presence of these compounds enhances the medicinal value of *C. indica* and supports its traditional therapeutic applications.

### 3.6 Tannin Content

The tannin content was recorded as  $4.39 \pm 0.21$  mg/g. Tannins are polyphenolic compounds recognised for their antioxidant and antimicrobial properties. The moderate tannin concentration observed in the present study suggests that *C. indica* contains sufficient levels of these beneficial compounds while remaining suitable for dietary consumption without significantly affecting nutrient bioavailability.

## 4. DISCUSSION

### 4.1 Protein Content

The protein content of *Calocybe indica* determined in the present study was  $23.56 \pm 0.42\%$ , indicating that this mushroom is a rich source of dietary protein. The obtained value falls within the protein range (20–25%) previously reported for *C. indica* by Alam et al. (2008) and NahuAlam et al. (2018). The high protein concentration confirms the nutritional importance of this mushroom, particularly for vegetarian populations and communities where animal protein is either limited or expensive. Consequently, *C. indica* continues to justify the popular description of mushrooms as the "poor man's protein" due to their high-quality protein and affordability (Pandey, 2004).

Compared with other commercially cultivated mushrooms, *C. indica* exhibits protein levels comparable to *Agaricus bisporus* (20–25%) and slightly higher than the average values reported for *Pleurotus* species (15–25%) and *Volvariella volvacea* (18–22%) (Alam et al., 2008; Ghosh & Acharya, 2022). In addition, the presence of all essential amino acids, with glycine reported as the predominant amino acid, further enhances its nutritional quality (NahuAlam et al., 2018).

### 4.2 Carbohydrate Content

The total carbohydrate content of *Calocybe indica* was  $34.97 \pm 0.44\%$ , which is slightly lower than the upper values reported by Alam et al. (2008) but remains within the nutritional range reported for cultivated edible mushrooms. Carbohydrates constitute a major component of mushroom dry matter and are primarily present as structural polysaccharides, including chitin,  $\beta$ -glucans, hemicellulose, and other non-starch polysaccharides (Subbaiah & Balan, 2015).

These polysaccharides not only contribute to the caloric value of the mushroom but also provide several physiological benefits.  $\beta$ -glucans isolated from *C. indica* have been reported to possess immunomodulatory activity by stimulating macrophages and natural killer cells, thereby enhancing host defence mechanisms and exhibiting potential anticancer effects (Ghosh & Acharya, 2022). Therefore, the carbohydrate fraction contributes substantially to both the nutritional and therapeutic value of *C. indica*.

### 4.3 Total Phenolic and Flavonoid Content

The total phenolic content recorded in the present investigation was  $8.10 \pm 0.31$  mg GAE/g, whereas the flavonoid content was  $3.74 \pm 0.19$  mg QE/g. These findings indicate that *C. indica* contains appreciable quantities of natural antioxidant compounds capable of scavenging reactive oxygen species.

Phenolic compounds are recognised as primary antioxidants in mushrooms and contribute significantly to reducing oxidative stress associated with chronic disorders such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer (Concepta et al., 2021). Earlier investigations by Mirunalini et al. (2012) and Babu and Rao (2013) demonstrated that *C. indica* possesses superior antioxidant activity compared with

*Agaricus bisporus*, mainly because of its higher phenolic concentration and greater free radical scavenging capacity.

Similarly, flavonoids represent an important class of phytochemicals with antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. The flavonoid level observed in this study further supports the medicinal significance of *C. indica* and its suitability as a functional food ingredient (Meghna et al., 2022).

#### 4.4 Alkaloid and Tannin Content

The alkaloid content of *Calocybe indica* was  $3.36 \pm 0.14\%$ , while the tannin content was  $4.39 \pm 0.21$  mg/g. These phytochemicals are known to contribute significantly to the medicinal properties of edible mushrooms.

Alkaloids possess diverse biological activities, including antimicrobial, anti-inflammatory, antioxidant, analgesic, and immunomodulatory effects (Concepta et al., 2021). Their presence in *C. indica* supports earlier reports describing the mushroom as a potential source of therapeutic compounds for pharmaceutical applications.

Likewise, tannins contribute antioxidant and antimicrobial properties while exhibiting moderate astringent effects. The tannin concentration obtained in this study is comparable to values reported for edible mushrooms by Udu-Ibiam et al. (2014). Since the concentration remains within acceptable dietary limits, it is unlikely to adversely affect nutrient bioavailability while still providing beneficial biological activities (Meghna et al., 2022).

#### 4.5 Nutritional and Functional Significance

The biochemical composition observed in the present study demonstrates that *Calocybe indica* is a nutritionally balanced edible mushroom containing substantial amounts of proteins, carbohydrates, and health-promoting phytochemicals. The combination of high-quality proteins with antioxidant compounds such as phenolics, flavonoids, alkaloids, and tannins enhances its value as a functional food.

In addition to these compounds, previous studies have reported that *C. indica* contains essential minerals, including potassium, sodium, phosphorus, magnesium, and calcium, which are important for maintaining electrolyte balance, bone metabolism, enzymatic activity, and cardiovascular function (NahuAlam et al., 2018). The mushroom also possesses low lipid content (4–5%) and relatively high dietary fibre, making it suitable for individuals requiring healthy, low-fat diets.

#### 4.6 Therapeutic Potential

The nutritional and phytochemical profile of *C. indica* strongly supports its medicinal importance. The presence of  $\beta$ -glucans, proteins, phenolics, flavonoids, alkaloids, and tannins contributes to multiple biological activities, including antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and anticancer effects. Previous investigations have demonstrated that proteins and glucans isolated from *C. indica* stimulate natural killer cell activity and enhance immune responses, thereby contributing to the body's defence against infections and cancer cells (Ghosh & Acharya, 2022).

Moreover, the high antioxidant capacity associated with phenolic and flavonoid compounds may reduce oxidative stress and protect against chronic metabolic disorders. The alkaline ash content and dietary fibre further make this mushroom beneficial for individuals suffering from hyperacidity, constipation, and digestive disorders (NahuAlam et al., 2018).

#### 4.7 Significance for Commercial Cultivation

The biochemical characteristics observed in this study reinforce the commercial importance of *Calocybe indica*. Its relatively high protein content (23.56%), appreciable carbohydrate content (34.97%), and abundance of antioxidant phytochemicals make it an attractive candidate for nutraceutical and functional food industries.

In addition to its nutritional superiority, *C. indica* offers several cultivation advantages, including rapid growth, adaptation to tropical climates, prolonged shelf life, and efficient utilisation of agricultural residues such as paddy straw (Krishnamoorthy, 1995). These characteristics reduce production costs while increasing profitability for mushroom growers. Furthermore, consumer preference for functional foods enriched with natural antioxidants continues to increase the market demand for *C. indica* (Subbaiah & Balan, 2015).

#### 4.8 Comparison with Other Cultivated Mushrooms

The nutritional composition of *Calocybe indica* compares favourably with other commercially cultivated mushrooms (Table 2). The protein content (23.56%) is comparable to *Agaricus bisporus* and exceeds the average reported values for *Pleurotus* species and *Volvariella volvacea* (Alam et al., 2008; Ghosh & Acharya, 2022).

Although the carbohydrate content (34.97%) is slightly lower than some reported values, it remains within the normal range for edible mushrooms and provides adequate dietary energy.

The comparative analysis reveals that *C. indica* offers nutritional value comparable to or exceeding that of other cultivated mushrooms, supporting its promotion as a valuable food source. Overall, the present findings confirm that *Calocybe indica* combines high nutritional quality with significant phytochemical richness, supporting its use as a functional food, nutraceutical ingredient, and commercially valuable edible mushroom. The observed biochemical profile is consistent with previous reports and further strengthens its potential for widespread cultivation and utilisation in food and pharmaceutical industries.

**Table 2: Comparative Nutritional Composition of Cultivated Mushrooms**

| Parameter        | <i>C. indica</i> | <i>Pleurotus</i> spp. | <i>Volvariella volvacea</i> | <i>Agaricus bisporus</i> |
|------------------|------------------|-----------------------|-----------------------------|--------------------------|
| Protein (%)      | 23.56            | 15-25                 | 18-22                       | 20-25                    |
| Carbohydrate (%) | 34.97            | 35-45                 | 30-40                       | 35-45                    |
| Lipid (%)        | 4-5              | 2-5                   | 3-5                         | 2-4                      |
| Fibre (%)        | 13-24            | 10-20                 | 12-18                       | 10-15                    |

(Sources: Alam et al., 2008; Ghosh & Acharya, 2022; Subbaiah & Balan, 2015)

## 5. CONCLUSION

The present study highlights the nutritional and biochemical significance of *Calocybe indica*, confirming its value as a functional edible mushroom with promising health-promoting properties. The biochemical analysis revealed that *C. indica* contained  $23.56 \pm 0.42\%$  protein, demonstrating that it is an excellent source of dietary protein and can serve as an affordable alternative protein source, particularly in vegetarian diets and regions where access to animal protein is limited. The mushroom also exhibited a carbohydrate content of  $34.97 \pm 0.44\%$ , indicating its contribution to dietary energy while providing beneficial polysaccharides and dietary fibre. Furthermore, the presence of  $8.10 \pm 0.31$  mg GAE/g total phenolic compounds and  $3.74 \pm 0.19$  mg QE/g flavonoids reflects a substantial antioxidant capacity that may help protect against oxidative stress and related chronic diseases. In addition, the detection of  $3.36 \pm 0.14\%$  alkaloids and  $4.39 \pm 0.21$  mg/g tannins suggests that *C. indica* possesses diverse phytochemicals with potential antimicrobial, anti-inflammatory, and pharmacological activities.

Overall, the findings demonstrate that *Calocybe indica* is not only a nutritious edible mushroom but also an important source of bioactive compounds that contribute to its functional and therapeutic potential. Its balanced nutritional composition, coupled with its antioxidant-rich phytochemical profile, supports its growing importance as a functional food, nutraceutical ingredient, and medicinal mushroom. Considering its ease of cultivation, adaptability to tropical climatic conditions, and utilisation of agricultural residues as substrates, *C. indica* represents a sustainable crop with significant economic and nutritional value. Therefore, wider cultivation, consumption, and scientific exploration of this mushroom could contribute to improved food security, human nutrition, and the development of novel health-promoting products.

## Acknowledgements

The authors express their sincere gratitude to the University Department of Botany, Patliputra University, Patna, and the Department of Botany, T.P.S. College, Patliputra University, Patna, for providing the necessary academic support, laboratory facilities, and institutional infrastructure to carry out this research. The authors are also thankful to the commercial mushroom cultivation units in Tamil Nadu, India, for providing fresh fruiting bodies of *Calocybe indica* used in this investigation. The valuable technical assistance provided by laboratory staff during sample preparation and biochemical analyses is gratefully acknowledged.

## Funding

The authors declare that no external funding was received for this research.

## Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

### Declaration of Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

### References

- Ajiboye, B. O., Ibukun, E. O., Edobor, O., Ojo, A. O., & Onikanni, S. A. (2013). Qualitative and quantitative analysis of phytochemicals in *Senecio bialfrae* leaf. *International Journal of Inventions in Pharmaceutical Sciences*, 1(5), 428–432.
- Alam, N., Amin, R., Khan, A., Ara, I., Shim, M. J., Lee, M. W., & Lee, T. S. (2008). Nutritional analysis of cultivated mushrooms in Bangladesh—*Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Pleurotus florida* and *Calocybe indica*. *Mycobiology*, 36(4), 228–232. <https://doi.org/10.4489/MYCO.2008.36.4.228>
- Anju, R. P., & Ukkuru, M. (2016). Health impact and medicinal properties of nutritionally edible milky mushroom (*Calocybe indica*). *International Journal of Advanced Engineering Research and Science*, 3, 236–932.
- Anurag, K. (2017). Commercial production of milky mushroom (*Calocybe indica*) in India. *Journal of Mushroom Research*, 5(1), 23–28.
- Anusiya, G., Gowthama Prabu, U., Yamini, N. V., Sivarajasekar, N., Rambabu, K., Bharath, G., & Banat, F. (2021). A review of the therapeutic and biological effects of edible and wild mushrooms. *Bioengineered*, 12(2), 11239–11268. <https://doi.org/10.1080/21655979.2021.2001181>
- Babu, D. R., & Rao, G. N. (2013). Antioxidant properties and electrochemical behaviour of cultivated commercial Indian edible mushrooms. *Journal of Food Science and Technology*, 50(2), 301–308.
- Bijalwan, A., Bahuguna, K., Vasishth, A., Singh, A., Chaudhary, S., Tyagi, A., Thakur, M. P., Thakur, T. K., Dobriyal, M., Kaushal, R., et al. (2020). Insights of medicinal mushroom (*Ganoderma lucidum*): Prospects and potential in India. *Biodiversity International Journal*, 4(5), 202–209.
- Brunner, G. (1984). *Methods of plant analysis*. Springer-Verlag.
- Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colourimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178–182.
- Chaturvedi, V. K., Agarwal, S., Gupta, K. K., Ramteke, P. W., & Singh, M. P. (2018). Medicinal mushroom: Boon for therapeutic applications. *3 Biotech*, 8, 334. <https://doi.org/10.1007/s13205-018-1358-0>
- Chelladurai, G., Mohan, J. R., & Sasirekhamani, M. (2014). In vitro cultivation technology and nutritional status of milky mushroom (*Calocybe indica*). *African Journal of Biotechnology*, 13(39), 3901–3906.
- Chelladurai, G., Yadav, T. K., & Pathak, R. K. (2021). Chemical composition and nutritional value of paddy straw milky mushroom (*Calocybe indica*). *Nature Environment and Pollution Technology*, 20(3), 1157–1164.
- Concepta, N. W., Sitati, K., Ogila, O., Waihenya, R. W., & Ochola, L. A. (2021). Phytochemical profile and antimicrobial activities of edible mushroom *Termitomyces striatus*. *Evidence-Based Complementary and Alternative Medicine*, 2021, 3025848.
- Doshi, A., Sharma, S. S., & Trivedi, S. (1989). Natural occurrence of *Calocybe indica* in Rajasthan. *Mushroom Science*, 12(1), 517–520.
- Garofalo, C., Osimani, A., Milanović, V., Taccari, M., Cardinali, F., Aquilanti, L., & Clementi, F. (2017). The microbiota of marketed processed edible insects as revealed by high-throughput sequencing. *Food Microbiology*, 62, 15–22. <https://doi.org/10.1016/j.fm.2016.09.012>
- Ghosh, S., & Acharya, K. (2022). Milky mushroom: A healthy nutritious diet. *Food Research International*, 156, Article 111113. <https://doi.org/10.1016/j.foodres.2022.111113>
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons.
- Harborne, J. B. (1984). *Phytochemical methods: A guide to modern techniques of plant analysis* (2nd ed.). Chapman & Hall.
- Krishnamoorthy, A. S. (1995). Rediscovery and commercial cultivation of *Calocybe indica*. *Indian Journal of Mushrooms*, 23(1), 1–5.
- Krishnamoorthy, A. S. (1997). Cultivation techniques for milky mushroom. *Indian Journal of Mushrooms*, 25(2), 1–4.
- Lowry, O. H., Rosebrough, N. T., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275.
- Maurya, A. K., John, V., Murmu, R., & Simon, S. (2019). Impact of different substrates for spawn production and production of milky mushroom (*Calocybe indica*). *International Journal of Pharma and Bio Sciences*, 10, 5–10.

- Meghna, S., Bains, A., Chawla, P., Fogarasi, M., & Fogarasi, S. (2022). The current status, bioactivity, food, and pharmaceutical approaches of *Calocybe indica*: A review. *Antioxidants*, 11(6), 1145. <https://doi.org/10.3390/antiox11061145>
- Mirunalini, S., Dhamodharan, G., & Deepalakshmi, K. (2012). Antioxidant potential and current cultivation aspects of an edible milky mushroom—*Calocybe indica*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4, 137–143.
- Mleczek, M., Rzymiski, P., Budka, A., Siwulski, M., Jasińska, A., Kalač, P., & Niedzielski, P. (2018). Elemental characteristics of mushroom species cultivated in China and Poland. *Journal of Food Composition and Analysis*, 66, 168–178. <https://doi.org/10.1016/j.jfca.2017.12.005>
- NahuAlam, S., Ahmed, K., & Rahman, M. (2018). Nutritional composition of milky mushroom. *Journal of Food Science and Nutrition*, 6(2), 78–84.
- Pandey, R. K. (2004). Nutritional importance of mushrooms. *Mushroom Research*, 13(1), 1–4.
- Pandey, R. K., & Tewari, R. P. (1993). Cultivation of *Calocybe indica* in India. *Mushroom Research*, 2(1), 23–26.
- Purkayastha, R. P., & Chandra, A. (1974). New species of edible mushroom from India. *Transactions of the British Mycological Society*, 62(2), 415–418.
- Sánchez, C. (2017). Bioactives from mushrooms and their application. In *Food Bioactives* (pp. 23–57). Springer. [https://doi.org/10.1007/978-3-319-51639-4\\_2](https://doi.org/10.1007/978-3-319-51639-4_2)
- Subbaiah, K. A., & Balan, V. (2015). A comprehensive review of tropical milky white mushroom (*Calocybe indica* P. & C.). *Mycobiology*, 43(3), 184–194.
- Udu-Ibiam, O. E., Ogbu, O., Ibiam, U. A., Nnachi, A. U., Agah, M. V., Ukaegbu, C. O., Chukwu, O. S., Agumah, N. B., & Ogbu, K. I. (2014). Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences*, 9(3), 86–91.
- Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible mushrooms: Improving human health and promoting quality of life. *International Journal of Microbiology*, 2015, Article 376387. <https://doi.org/10.1155/2015/376387>