

Biochemical and Nutritional Profiling of Wild *Auricularia Polytricha* from Bhopal, India.

Dr. R. K. Tenguria¹, Umesh Sikarware²

^{1,2}Govt. M.V.M., Bhopal

Abstract—Despite the growing interest in the pharmacological and nutritional properties of *Auricularia polytricha*, comprehensive biochemical datasets on uncultivated, wild populations in the Indian subcontinent remain significantly fragmented. Current literature heavily emphasizes commercial strains, overlooking the genetic and nutritional potential of native ecotypes. This study aimed to quantify the macromolecular composition (proteins, carbohydrates, and lipids) and characterize the qualitative and quantitative phenolic profiles of wild *Auricularia polytricha* fruiting bodies naturally occurring in Bhopal, Madhya Pradesh, India. Fresh fungal specimens were collected from Swarn Jayanti Park, cryopreserved, lyophilized, and pulverized. Aqueous and methanolic extracts were prepared to conduct qualitative phytochemical screening. Proximate analysis evaluated total soluble proteins, carbohydrates, and crude lipids using standard modified protocols, while Total Phenolic Content (TPC) was quantified utilizing the Folin-Ciocalteu colorimetric method. Proximate analysis revealed a highly favourable nutritional profile consisting of 10.28% total soluble protein, 9.31% total soluble carbohydrates, and a characteristically low crude lipid content of 4.0%. Phytochemical screening confirmed the robust presence of terpenoids, alkaloids, and flavonoids, though tannins and anthraquinones were undetected. Furthermore, the quantitative assay established a competitive Total Phenolic Content of 1.27 mg GAE/g of dry extract. The wild *Auricularia polytricha* ecotype native to Central India demonstrates a lean, nutrient-dense profile with potent antioxidant properties. These baseline findings strongly position this regionally adapted strain as a highly promising candidate for nutraceutical applications, functional food development, and future conservation or domestication programs.

Index Terms—*Auricularia polytricha*, wild mushrooms, proximate analysis, phytochemical screening, total phenolic content, and functional foods.

I. INTRODUCTION

Fungi represent one of the most diverse and essential kingdoms on Earth, playing pivotal roles in ecosystem dynamics. These organisms decompose organic matter, cycle nutrients, form symbiotic partnerships with plants, and even influence global climate patterns through carbon turnover [1].

Despite their ecological importance, fungi remain vastly underexplored, as estimates suggest there could be 2.2 to 6 million fungus species, yet fewer than 150,000 have been formally described, meaning over 95% remain undocumented [2] in this fascinating kingdom, mushrooms emerge as highly specialized, ephemeral fruiting bodies and are primarily classified within the division Basidiomycota and Ascomycota. Their evolutionary history extends back over 450 million years, supported by fossils from the Devonian period. Although taxonomists have identified approximately 1,600 macrofungal species, estimates suggest that up to 150,000 exist worldwide, indicating vast untapped diversity. However, only approximately 100 species are commonly used for culinary or medicinal purposes. These fungi occupy diverse habitats, from moist forest floors and decaying wood to grasslands and alpine tundra, where they play crucial ecological roles [3], [4].

Within the diverse group, genus *Auricularia*, commonly known as wood ear, jelly ear, or tree ear, comprises ear-shaped basidiomycetous fungi that grow saprophytically on decaying hardwoods and are among the most widely cultivated and consumed edible mushrooms worldwide [5], [6]. Beyond their nutritional value, *Auricularia* spp. contains polysaccharides, phenolics, and other bioactive compounds and exhibit antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, and cardioprotective activities [7], [8]. In Asia and increasingly elsewhere, these mushrooms are

incorporated into traditional medical systems and functional foods, and within the Indian subcontinent, they hold significant ethnomycological importance among indigenous communities and are now being promoted as potential nutraceutical crops [9].

Despite this long history of use and the growing body of work on the biology, pharmacology, and cultivation of *Auricularia* spp., detailed information on the proximate composition and baseline phytochemical profile of uncultivated, native wild populations remains fragmented and geographically biased [10], [11]. Available reports from different countries describe moisture, protein, fat, fiber, and ash contents and broadly indicate the presence of phenolics, flavonoids, and other secondary metabolites. However, much of the contemporary literature emphasizes defined commercial strains grown under standardized cultivation systems, frequently overlooking the unique biochemical diversity and nutritional potential inherent in wild, region-specific ecotypes [11], [12], [13].

For native Indian *Auricularia* germplasm, in particular, comprehensive datasets integrating macromolecular composition (proteins, carbohydrates, and lipids) with qualitative and quantitative phenolic profiling of wild-foraged fruiting bodies remain scarce. This is a critical oversight, as these naturally occurring populations represent valuable, uncharacterized genetic resources with immense potential for functional food development. The present study investigates the wild mushroom, *Auricularia polytricha*, identified in our earlier molecular and metagenomic study, whose fruiting bodies naturally occur in open fields and forested areas in and around Bhopal (Madhya Pradesh, India), with the specific objective of quantifying their macromolecular breakdown (proteins, carbohydrates, and lipids) and characterizing their phenolic profile in both qualitative and quantitative terms. By establishing this biochemical baseline for a wild, regionally adapted Indian population, the work aims to support future assessments of its nutritional relevance, functional food potential, and suitability for conservation or domestication programmes.

II. MATERIALS AND METHODS

2.1. Study Area and Sample Collection

Fresh fruiting bodies of wild *Auricularia polytricha* (Figure 1) were collected from Swarn Jayanti Park,

Kolar Road, Bhopal, Madhya Pradesh, India (23° 15' 0" N, 77° 25' 0" E). Sampling was conducted during the monsoon season of 2025. Specimens were harvested aseptically at the base, sealed in sterile containers, and transported to the laboratory under continuous cold-chain conditions to prevent enzymatic degradation of bioactive compounds.



Fig. 1: Field photograph of wild *Auricularia polytricha* fruiting bodies in their natural microhabitat, collected from Swarn Jayanti Park, Bhopal, Madhya Pradesh (India).

2.2. Preparation of Mushroom Extracts

Upon arrival, macroscopic debris was manually removed, and the fresh samples were temporarily cryopreserved at -20°C , followed by lyophilization to ensure the absolute preservation of heat-sensitive metabolites. The desiccated samples were subsequently pulverized into a uniform fine powder. For extraction, 1 g of the powder was homogenized in 10 mL of analytical-grade methanol or sterile distilled water (supplemented with 1–2% DMSO to facilitate the solubility of non-polar constituents). The resulting 100 mg/mL stock solutions were utilized for all subsequent quantitative and qualitative analyses.

2.3. Qualitative Phytochemical Screening

The detection of primary secondary metabolites in the structural extracts was conducted following standard biochemical protocols [14]. The methanolic and aqueous extracts were systematically screened for the presence of alkaloids (Dragendorff's reagent), flavonoids (lead acetate test), glycosides (Benedict's test), tannins (ferric chloride test), saponins (froth test), terpenoids (Salkowski's test), and anthraquinones (ammoniacal chloroform test). The results were recorded based on visual colorimetric changes and specific precipitate formations.

2.4. Proximate Composition Analysis

All standard biochemical assays described herein were slightly modified from their foundational protocols to optimize extraction efficiency and reagent interactions specifically for the complex structural matrix of wild fungal tissues under local laboratory environmental conditions.

2.4.1 Protein Quantification: Total soluble protein content was estimated using the standard Lowry method [15]. Following the reagent reactions, absorbance was measured spectrophotometrically at 750 nm. Protein concentration was extrapolated from a standard calibration curve generated using bovine serum albumin (BSA; 0–200 µg/mL).

2.4.2 Carbohydrate Quantification: Total soluble carbohydrates were quantified using the anthrone method [16]. The extracts were reacted with acidic anthrone reagent at 100°C for 10 minutes. The absorbance of the resulting complex was measured at 620 nm, utilizing D-glucose (10–200 µg/mL) as the reference standard.

2.4.3 Total Lipid Extraction: Crude lipid content was evaluated gravimetrically using a Soxhlet apparatus [17]. Five grams of pre-dried sample (105°C for 2 h) were subjected to continuous extraction with petroleum ether at a reflux rate of 5-6 cycles per hour for 6-8 hours. Following solvent recovery via rotary evaporation, the total lipid yield was calculated as a percentage of the initial dry sample weight.

2.5. Determination of Total Phenolic Content (TPC)

The total phenolic content was quantified using the established Folin-Ciocalteu colorimetric method [18]. Briefly, the mushroom extract was reacted with the Folin-Ciocalteu reagent under alkaline conditions, facilitated by the addition of 7.5% sodium carbonate. Following 30-min incubation in the dark at room temperature, the absorbance of the resulting molybdenum-tungsten blue complex was measured spectrophotometrically at 760 nm. The TPC was calculated using a gallic acid calibration curve (0.01–0.05 mg/mL) and expressed as milligrams of gallic acid equivalents per gram of dry extract weight (mg GAE/g dw).

III. RESULTS AND DISCUSSION

3.1. Qualitative Phytochemical Profiling

Testing the methanolic and aqueous extracts of the wild *Auricularia polytricha* mushrooms revealed a rich supply of beneficial natural compounds, which are known as secondary metabolites. Both extraction methods tested strongly positive for terpenoids and alkaloids, while tannins and anthraquinones were completely absent (Table 1), suggesting that they may require highly specific targeted solvents for successful extraction [19], [20].

Table 1: Phytochemical Composition and Intensity of Mushroom Extracts

Secondary Metabolite	Methanol Extract	Water Extract
Alkaloids	4+	4+
Flavonoids	4+	2+
Terpenoids	5+	5+
Glycosides	–	1+
Saponins	–	1+
Tannins	–	–
Anthraquinones	–	–

Note: 1+ = very low; 2+ = low; 3+ = moderate; 4+ = high; 5+ = very high and – = absent

The tests also showed that different solvents pull out different nutrients. For example, methanol was best for extracting flavonoids [21], whereas water was better for isolating completely hydrophilic molecules, such as glycosides and saponins [22], [23]. The exceptionally high presence of terpenoids in both extracts is a major finding, demonstrating their consistent recovery regardless of solvent polarity [24]. Because these specific classes of compounds are widely known to possess strong health and medicinal benefits, this wild native ecotype shows great promise as a highly functional, health-boosting food ingredient.

3.2. Proximate Composition and Nutritional Evaluation

The nutritional breakdown of the wild *Auricularia polytricha* samples demonstrated a lean and highly favorable diet profile that matched the quality of premium edible mushrooms. Total soluble protein was the most abundant macronutrient, recorded at 10.28% (102.80 mg/g dry weight), and the calibration curve plot used for analysis is depicted in Figure 2. This substantial protein yield makes this wild strain an excellent alternative non-animal protein source that

aligns with the established nutritional range for diverse wild and edible mushroom taxa [25].

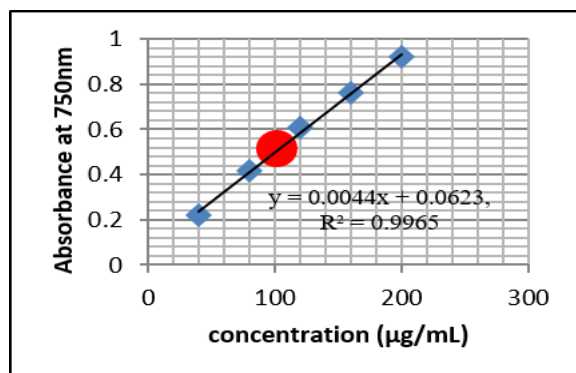


Fig. 2: Linear standard curve for the Lowry protein assay ($y = 0.0044x + 0.0623$, $R^2 = 0.9965$). The absorbance of the *Auricularia polytricha* sample fell within the validated linear range, ensuring accurate quantification.

Total soluble carbohydrate content followed closely at 9.31% (93.05 mg/g dry weight) (Figure 3), falling perfectly within the standard carbohydrate spectrum typical of edible species [26]. Conversely, the fat (crude lipid) content remained characteristically low at just 4.0% (Table 2), which exactly matches the species average reported across edible mushrooms [26], [27]. This specific combination of nutrients, which includes solid protein yields, moderate carbohydrates that provide dietary fiber, and minimal fat, perfectly aligns with the modern consumer demand for low-calorie, nutrient-dense foods.

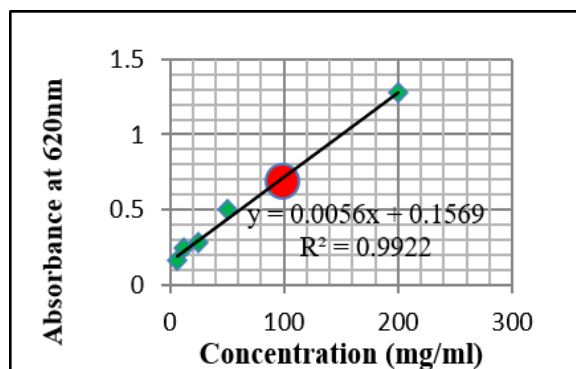


Fig. 3: Linear standard curve for anthrone carbohydrate assay ($y = 0.0056x + 0.1569$, $R^2 = 0.9922$). The absorbances of both the control and *Auricularia polytricha* samples fell within the validated linear range, ensuring accurate quantification.

Table 2: Serial solvent extractions established reproducible recovery across replicates.

Sample	Initial Weight (g)	Post-Extraction Weight (g)	Lipid Yield (%)
Replicate 1	1.000	0.960	4.0
Replicate 2	1.000	0.959	4.1
Replicate 3	1.000	0.961	3.9
Mean	1.000	0.960	4.0
Fungal Biomass	[1.000]	[0.960]	[4.0]

3.3. Total Phenolic Content (TPC)

Phenolic compounds are natural plant-based chemicals that give mushrooms their antioxidant power, which is their ability to fight harmful free radicals in the body. Quantitative testing revealed a total phenolic content of 1.27 mg GAE/g of dry extract (Figure 4).

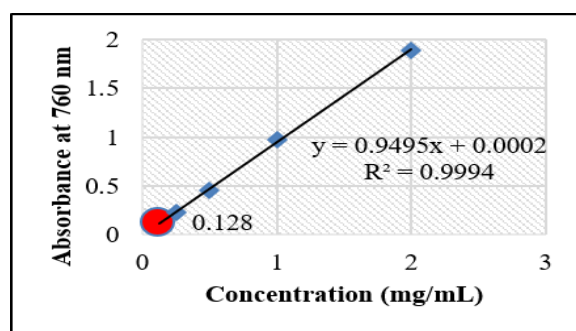


Fig. 4: Linear standard curve for the Folin-Ciocalteu phenolic assay ($y = 0.9495x + 0.0002$, $R^2 = 0.9994$). The *Auricularia polytricha* sample absorbance of 0.51 corresponded precisely to 0.127 mg/mL within the validated linear range.

This yield sits comfortably within the established benchmark of 1 to 6 mg GAE/g for valuable commercial edible mushrooms [28]. The accumulation of these phenolics, alongside the previously identified flavonoids, provides strong biochemical proof of the antioxidant abilities of the mushroom. This solid phenolic baseline not only justifies further medical and pharmaceutical research on this specific wild mushroom but also reinforces its immediate value as a healthy, antioxidant-rich dietary staple.

IV. SUMMARY & CONCLUSION

This study successfully established a comprehensive biochemical and nutritional baseline for wild

Auricularia polytricha ecotypes native to the Bhopal region of Central India. Proximate analysis revealed a highly favorable macromolecular profile, characterized by substantial protein (10.28%) and moderate carbohydrate (9.31%) yields, alongside characteristically low lipid content (4.0%). Furthermore, qualitative and quantitative phytochemical evaluations highlighted a potent secondary metabolite profile, rich in terpenoids, alkaloids, and flavonoids, complemented by a competitive total phenolic content of 1.27 mg GAE/g. Collectively, these findings demonstrate that this uncultivated, regionally adapted strain possesses immense nutritional value and robust antioxidant capacity. This work not only highlights the critical need to explore wild fungal biodiversity over standard commercial strains but also strongly positions these native Indian *Auricularia polytricha* populations as highly promising candidates for functional food development, nutraceutical applications, and future commercial domestication programs.

REFERENCE

- [1] Devkota, S., Fang, W., Arunachalam, K., Phyto, K. M. M., and Shakya, B., "Systematic review of fungi, their diversity and role in ecosystem services from the Far Eastern Himalayan Landscape (FHL)," *Heliyon*, vol. 9, no. 1, Art. no. e12756, 2023, doi: 10.1016/j.heliyon.2022.e12756.
- [2] Hawksworth, D. L., and Lücking, R., "Fungal diversity revisited: 2.2 to 3.8 million species," *Microbiology Spectrum*, vol. 5, no. 4, 2017.
- [3] Naranjo-Ortiz, M. A., and Gabaldón, T., "Fungal evolution: Diversity, taxonomy and phylogeny of the Fungi," *Biological Reviews of the Cambridge Philosophical Society*, vol. 94, no. 6, pp. 2101–2137, 2019, doi: 10.1111/brv.12550.
- [4] Kumar, K., Mehra, R., Guiné, R. P., Lima, M. J., Kumar, N., Kaushik, R., and Kumar, H., "Edible mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects," *Foods*, vol. 10, no. 12, Art. no. 2996, 2021.
- [5] Liu, E., Ji, Y., Zhang, F., Liu, B., and Meng, X., "Review on *Auricularia auricula-judae* as a functional food: Growth, chemical composition, and biological activities," *Journal of Agricultural and Food Chemistry*, vol. 69, no. 6, pp. 1739–1750, 2021.
- [6] Islam, T., Ganesan, K., and Xu, B., "Insights into health-promoting effects of Jew's ear (*Auricularia auricula-judae*)," *Trends in Food Science & Technology*, vol. 114, pp. 552–569, 2021, doi: 10.1016/j.tifs.2021.06.017.
- [7] Oli, A. N., Edeh, P. A., Al-Mosawi, R. M., Mbachu, N. A., Al-Dahmashi, H. O., Al-Khafaji, N. S., Ekuma, U. O., Okezie, U. M., and Saki, M., "Evaluation of the phytoconstituents of *Auricularia auricula-judae* mushroom and antimicrobial activity of its protein extract," *European Journal of Integrative Medicine*, vol. 38, Art. no. 101176, 2020, doi: 10.1016/j.eujim.2020.101176.
- [8] Jamtsho, T., and Wangchuk, P., "*Auricularia auricula-judae* (Bull.): Major bioactive compounds, health benefits, and pharmacological activities," in *Edible and Medicinal Mushrooms of the Himalayas*. CRC Press, 2023, pp. 19–43.
- [9] Giri, S., Paul, P., and Pradhan, P., "Ethnomycological perception towards wood ear fungi (*Auricularia* spp.) in and around the Indian Subcontinent," *Asian Journal of Forestry*, vol. 9, no. 1, pp. 82–96, 2025.
- [10] Mutuku, S. N., Lang'at, E. K., Osoro, E. K., Imbenzi, P. S., and Omolo, J. O., "Proximate composition, mineral element profile and antioxidant properties of the edible Kenyan mushroom, *Auricularia polytricha*," *Journal of Scientific and Innovative Research*, vol. 11, no. 3, pp. 51–55, 2022.
- [11] Ade-Ogunnowo, F. E., Adejoye, O. D., and Adesokan, K., "Phytochemical screening, proximate composition and mineral activities of four selected Nigerian mushrooms," *African Scientist*, vol. 25, no. 2, pp. 124–134, 2024.
- [12] Devi, M. B., and Singh, N. I., "Evaluation of proximate nutritional value of edible *Auricularia delicata* and *A. polytricha* from Manipur," *Journal of Mycopathological Research*, vol. 49, no. 1, pp. 199–200, 2011.
- [13] Regis, M. A. J., Geösel, A., and Geösel, R. M., "Cultivation of *Auricularia* species: A review of the history, health benefits, principles, practices, environmental conditions, research methods, and

- recent trends,” *Sydowia*, vol. 76, pp. 21–33, 2023.
- [14] Harborne, J. B., *Comparative Phytochemistry*. 1965.
- [15] Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M. L., and Farr, A. L., “The quantitative histochemistry of brain: I. Chemical methods,” *Journal of Biological Chemistry*, vol. 207, no. 1, pp. 1–17, 1954.
- [16] Yemm, E. W., and Willis, A., “The estimation of carbohydrates in plant extracts by anthrone,” *Biochemical Journal*, vol. 57, no. 3, p. 508, 1954.
- [17] Craig, L. C., Gregory, J. D., and Hausmann, W., “Versatile laboratory concentration device,” *Analytical Chemistry*, vol. 22, no. 11, p. 1462, 1950.
- [18] Singleton, V. L., and Rossi, J. A., Jr., “Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents,” *American Journal of Enology and Viticulture*, vol. 16, no. 3, pp. 144–158, 1965.
- [19] Senthilkumar, V. C., and Bhadusha, N., “Preliminary qualitative and quantitative phytochemical analysis from *Brachypterum scandens*,” *Research Journal of Medical Sciences*, vol. 17, pp. 109–113, 2023.
- [20] Srivastava, M., Kumari, M., Karn, S. K., Bhambri, A., Mahale, V. G., and Mahale, S., “Submerged cultivation and phytochemical analysis of medicinal mushrooms (*Trametes* sp.),” *Frontiers in Fungal Biology*, vol. 5, Art. no. 1414349, 2024, doi: 10.3389/ffunb.2024.1414349.
- [21] Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., and Ju, Y. H., “Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*,” *Journal of Food and Drug Analysis*, vol. 22, no. 3, p. 296, 2013, doi: 10.1016/j.jfda.2013.11.001.
- [22] Rahimah, S. B., Djunaedi, D. D., Soeroto, A. Y., and Bisri, T., “The phytochemical screening, total phenolic contents and antioxidant activities in vitro of white oyster mushroom (*Pleurotus ostreatus*) preparations,” *Open Access Macedonian Journal of Medical Sciences*, vol. 7, no. 15, pp. 2404–2412, 2019, doi: 10.3889/oamjms.2019.741.
- [23] Nortjie, E., Basitere, M., Moyo, D., and Nyamukamba, P., “Extraction methods, quantitative and qualitative phytochemical screening of medicinal plants for antimicrobial textiles: A review,” *Plants*, vol. 11, no. 15, Art. no. 2011, 2022, doi: 10.3390/plants11152011.
- [24] Torres-Martínez, B. D. M., Vargas-Sánchez, R. D., Ibarra-Arias, F. J., Ibarra-Torres, E. V., Torrescano-Urrutia, G. R., and Sánchez-Escalante, A., “Effect of extraction solvent on chemical composition, physicochemical and biological properties of edible mushrooms extracts,” *TIP Revista Especializada en Ciencias Químico-Biológicas*, vol. 24, 2021.
- [25] Ayimbila, F., and Keawsompong, S., “Nutritional quality and biological application of mushroom protein as a novel protein alternative,” *Current Nutrition Reports*, vol. 12, no. 2, p. 290, 2023, doi: 10.1007/s13668-023-00468-x.
- [26] Yu, Q., Guo, M., Zhang, B., Wu, H., Zhang, Y., and Zhang, L., “Analysis of nutritional composition in 23 kinds of edible fungi,” *Journal of Food Quality*, vol. 2020, Art. no. 8821315, 2020, doi: 10.1155/2020/8821315.
- [27] Tan, R. Y., Ilham, Z., Wan-Mohtar, W. A. A. Q. I., Halim-Lim, S. A., Ahmad Usulidin, S. R., Ahmad, R., and Adlim, M., “Mushroom oils: A review of their production, composition, and potential applications,” *Heliyon*, vol. 10, no. 11, Art. no. e31594, 2024, doi: 10.1016/j.heliyon.2024.e31594.
- [28] Palacios, I., Lozano, M., Moro, C., D’Arrigo, M., Rostagno, M., Martínez, J., García-Lafuente, A., Guzmán, E., and Villares, A., “Antioxidant properties of phenolic compounds occurring in edible mushrooms,” *Food Chemistry*, vol. 128, no. 3, pp. 674–678, 2011, doi: 10.1016/j.foodchem.2011.03.085.