NUTRIENT CONTENT AND ENZYMATIC ACTIVITY OF WILD EDIBLE MUSHROOMS IN MAMIT AND CHAMPHAI DISTRICTS, MIZORAM

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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MZU REGISTRATION No.: 1506016

Ph.D. REGISTRATION No.: MZU/Ph.D./1609 of 07.11.2020

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SCHOOL OF EARTH SCIENCE

&

NATURAL RESOURCE MANAGEMENT

FEBRUARY, 2026

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In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Environmental Science of Mizoram University, Aizawl

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SUPERVISOR'S CERTIFICATE

This is to certify that the Ph.D. Thesis entitled, "**Nutrient content and enzymatic activity of wild edible mushrooms in Mamit and Champhai districts, Mizoram**" is a bonafide work assigned to Mr. VL Thachunglura having Reg. No. MZU/Ph.D./1609 of 07.11.2020, Department of Environmental Science for partial fulfillment for the Degree of Doctor of Philosophy under Mizoram University.

The report submitted by the candidate is his own study and carried out by him and results embodied in the Thesis have not been submitted for award of any degree or any other institute or University of learning. It is recommended that this thesis be placed before the examiners for the award of the Degree of Doctor of Philosophy.

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DECLARATION

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FEBRUARY, 2026

I, VL Thachunglura, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute

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ACKNOWLEDGEMENT

My deepest appreciation goes to my supervisors, Prof. Prabhat Kumar Rai and Prof. John Zothanzama. The completion of this Ph.D. would not have been possible without their excellent guidance and support, right from the preparation of my synopsis to the final stages of writing this thesis. Their immense wisdom, patience, and wealth of experience have inspired and guided me throughout the course of my research.

I am grateful to the Head of the Department of Environmental Science, Mizoram University, for providing the necessary facilities during the course of my research. I also extend my sincere thanks to the faculty members and staff of the Department of Environmental Science for their encouragement and assistance in various aspects of my work.

I am equally thankful to the Department of Biotechnology and the Department of Chemistry, Mizoram University, for their support. I would like to express my heartfelt gratitude to my family, my labmates in Microbial Ecology Laboratory (ZZ Overgrow), and my friends, who stood by me and encouraged me at every step of this journey. A special note of thanks goes to Ms. Noreen Lalrinmawii and Dr. Zohmangaiha Chawngthu, whose help and support at every stage of my research have been invaluable.

To my dearest parents, Lalfakmawii and MS Dawngliana Pachuau, who always believed in me even when I doubted myself. This work is as much yours as mine. Above all, I owe everything to Almighty God, who has blessed me with wisdom, health, and strength to carry out and complete this research work.

Date: June, 2026 (VL THACHUNGLURA)

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1. INTRODUCTION

1.1. Introduction

The contemporary global landscape faces unprecedented challenges in sustaining the rising demand for food, driven by rapid population growth, urbanization, and environmental degradation. Ensuring food security has therefore become a critical priority, particularly in rural communities that, despite being central to agricultural production, remain highly vulnerable to hunger and malnutrition. In this sense, edible mushrooms stand out as a promising yet underutilized bioresource with immense potential to enhance food and nutritional security (Kumar et al., 2021; Ayimbila and Keawsompong, 2023; Ionescu et al., 2025).

The global fungal diversity remains vastly underexplored. In this respect, Hyde (2022) estimated global fungal diversity to range from 2.2 to 3.8 million species using algorithmic models, whereas 'high-throughput' sequencing approaches suggest an even larger estimate of 11.7–13.2 million species. However, only about 150,000 species have been formally described, representing just 4.5-5%% of the estimated total revealing the enormous gap in documentation. Consequently, the "Fungal Tree of Life (FTOL)" remains far from complete, with large portions of fungal diversity yet to be discovered, classified, and phylogenetically resolved (Wu et al., 2019; Zhou and May, 2022). Among these, 2,189 edible mushroom species were classified by Li et al. (2021), of which 2,006 are considered safe for consumption and 183 require processing or may induce allergic reactions.

For millennia, fruiting bodies of higher fungi have been collected as food due to their palatability and rich nutritional profile (Mattila et al., 2001; Hyde et al., 2019; Khumlianlal et al., 2022). Their strong association with human culture extends beyond diet, contributing biologically, economically, and environmentally to society, and positioning them as key components of sustainable development and public well-being (Hrudayanath and Sameer, 2015). Edible mushrooms are nutrient dense, containing high quality proteins, vitamins, minerals, and essential amino acids, while being low in fat

and calories (Manzi et al., 1999; Gençcelep et al., 2009; Kalač, 2009). Wild edible mushrooms are also rich in natural antioxidants (Elmastas et al., 2007; Chye et al., 2008; Kalyoncu, 2010) and thus serve as accessible sources of bioactive compounds that support a balanced and healthy diet.

Nutritionally, wild edible mushrooms supply essential dietary components required by the human body, including carbohydrates, proteins, lipids, vitamins, minerals, fiber, and water (Wang and Zhao, 2023). Medicinal mushroom species contain a wide array of bioactive compounds, including polysaccharides, terpenoids, phenolic compounds, lectins, and glycoproteins and volatile compounds, which exhibit potent anticancer, antioxidant, antidiabetic, anti-inflammatory, antimicrobial, hepatoprotective, neuroprotective, and immunomodulatory properties, enhancing metabolism, reducing obesity risk, and potentially slowing aging processes due to strong antioxidant activity (Łysakowska et al., 2023). Both wild and cultivated mushrooms are valued for antioxidant capacity derived from polyphenols, polysaccharides, carotenoids, vitamins, and minerals (Kozarski et al., 2015; Chun et al., 2021).

To meet growing demand, isolation of pure culture from mushrooms has become essential for reliable and large or field scale production (Liu et al., 2023). Pure cultures ensure genetic stability, improve yield, enhance resistance to disease, and maintain desired sensory qualities, contributing to consistent commercial viability (Kaur et al., 2011). Culturing wild mushrooms in vitro provide new opportunities for developing high quality food, nutraceuticals, and pharmaceutical products (Valverde et al., 2015; Lu et al., 2025). Considering population growth and rising food demand, the identification and domestication of new edible mushroom species has become increasingly crucial, which first requires rigorous confirmation of edibility, followed by evaluation of their cultivation potential and feasibility (Wangdi and Tamang, 2022).

Parallel to their nutritional value, mushrooms play a major role in ecological processes and biotechnology. Wood decaying fungi decompose lignified plant matter, recycling carbon and breaking down cellulose, hemicellulose and lignin (Blanchette, 1991; Wei and Dai, 2004). Their enzymatic activity supports ecosystem functioning while presenting industrial applications including biodegradation of pollutants, textile processing and organic waste conversion (El-Gendi et al., 2021; Bahram and Netherway, 2021). White-rot fungi can degrade persistent pollutants such as Polycyclic Aromatic

Hydrocarbons (PAHs), pharmaceuticals and pesticides, making them effective agents in bioremediation (Viswanath et al., 2014; Fukasawa, 2021). Interestingly, fungi produce over half of the world's industrial enzymes, including laccase, manganese peroxidase, lignin peroxidase, cellulases and xylanases (Huang et al., 2023). These industrial enzymes can be secreted extracellularly and harvested efficiently for large scale use (Kango et al., 2019; Bhadra et al., 2022; Huang et al., 2023). However, despite their benefits, mushrooms also pose food safety concerns due to heavy metal bioaccumulation.

Rapid industrialization and urbanization have increased heavy metal contamination across the environments (Rai, 2009; Shetty et al., 2024). When metals accumulate beyond safe thresholds, they disrupt metabolic processes and may cause illness, morbidity or even mortality in case of intense ecotoxic exposure (Jaishankar et al., 2014; Rai et al., 2019). Although bioremediation using fungi is highly efficient (Das, 2005; Kapahi and Sachdeva, 2017; Rai, 2022; Rai et al., 2023), the same biosorption ability can lead to heavy metal uptake in wild edible mushrooms (Ediriweera et al., 2022). Mushrooms often contain higher metal conctents than agricultural crops that sometimes exceed permissible limits (Kalac and Svoboda, 2000; Zhu et al., 2011; Liu et al., 2022). This creates a dual implication valuable for remediation, however on other side are potentially hazardous for consumption reinforcing the need for health risk assessment and monitoring to ensure food safety (Árvay et al., 2014; Singh and Nyau, 2020; Širić et al., 2022; Qin et al., 2024). Therefore, such human health risks assessments of wild edible mushrooms are most urgently needed rather than their utilization in bioremediation. To this end, species-specific uptake patterns and environmental conditions must therefore be considered when evaluating edible wild mushroom safety (Chen et al., 2009; Kokkoris et al., 2019).

Fungi play a fundamental role in ecosystem functioning by recycling carbon and mobilizing essential nutrients such as nitrogen, phosphorus, and trace biological elements. They support plant life through endophytic and mycorrhizal associations, thereby enhancing nutrient uptake, stress tolerance, and overall soil health (Kuehn et al., 2011; Naranjo-Ortiz and Gabaldon, 2019). Fungi bear mutualistic interactions with other organisms, facilitating nutrient exchange and ecological balance (Lee et al., 2013). Many species also grow on dead organic matter, acting as decomposers, parasites, or

symbionts, depending on environmental conditions (Aanen et al., 2002). Beyond their ecological importance, macrofungi have been used as traditional medicine for centuries, and for various purposes such as fermentation, enzyme production, and as a source of food (Corbu et al., 2023), and numerous historical and scientific records document their applications in traditional healing, cultivated production, antioxidant activity, and nutritional properties (Stamets and Zwickey, 2014; Öztürk et al., 2015; Li et al., 2016; Zhang et al., 2016; Joshi and Khaund, 2014; Jeitler et al., 2020; Ślusarczyk et al., 2021; Pouris et al., 2024). Their rich profile of bioactive compounds has contributed to widespread interest in therapeutic and dietary research.

Despite extensive global interest in mushrooms as food, medicine, and biotechnological resources, significant knowledge gaps still remain in documentation, assessment, and utilization of wild edible mushrooms. Such knowledge voids are more pronounced in regions of immense ecological impprtance like Mizoram, Northeast India, wherein fungal diversity is exceptionally rich, yet understudied**.** While thousands of edible mushroom species exist globally, only small fractions have been scientifically assessed for their nutritional composition and safety for consumption. Considering that approximately 150,000 fungal species have been formally described, edible mushrooms represent merely about 1.5% of their global diversity (Hyde, 2019; Li et al., 2021). This remarkably low proportion highlights the urgent need to identify, characterize, and evaluate additional wild edible mushroom species, particularly from underexplored regions of Mizoram, India where comprehensive documentation is lacking (Zothanzama et al., 2018). In this vein, it is worth mentioning that only 27 edible species of mushrooms were documented in previous studies (Lalrinawmi et al., 2017; Zothanzama et al., 2018). Many macrofungal species continue to be collected and consumed based solely on their associated traditional knowledge, often without standard taxonomic confirmation, which can result in accidental ingestion of poisonous mushrooms and highlights the critical need for scientific validation. Collecting data directly from local communities about which mushrooms they consume and how they use them is therefore essential, not only for preserving indigenous knowledge but also for identifying additional edible species that have not yet been formally documented in earlier studies.

Macrofungi are recognized as valuable nutritional resources, however their capacity to bioaccumulate heavy metals introduces potential health risks. Most existing

studies have focused on cultivated mushrooms or those collected from industrially impacted areas. In this vein, data on wild edible mushrooms from natural forest ecosystems, particularly regarding heavy metal contents, safety thresholds, and associated health risks remain limited. This knowledge gap is especially pronounced in Mizoram, where no systematic assessments of wild edible mushrooms have been documented in multiple taxonomy, nutritional, biochemical (e.g., antioxidant responses), and heavy metal exposure or eco-toxicity prospects.

To date, approximately 264 macrofungal taxa have been documented in Mizoram through morphological and molecular analyses (Zothanzama et al., 2016; Vabeikhokhei et al., 2019; Zohmangaiha et al., 2019; Chawngthu et al., 2021), with most studies focusing primarily on diversity and taxonomy. A systematic exploration that integrates nutritional composition, heavy metal quantification and health risk assessment, isolation of pure cultures, evaluation of enzymatic and antioxidant activities, and characterization of volatile compounds were scant in relation to Mizoram. Furthermore, reporting of ecologically and economically significant macrofungi is needed from such biodiversity rich study areas and selected sampling sites for detailed investigation. Such comprehensive studies would address critical gaps in "ethnomycological knowledge" help validate traditionally consumed species, and identify previously undocumented wild edible mushrooms. By expanding the formally recognized species pool, this study would contribute to food security, public health safety, nutritional enhancement, rural livelihood, and create new opportunities for biotechnological applications, thereby providing both ecological and socio-economic benefits.

1.2. Scope of research

This study aims to investigate wild edible mushrooms from Mamit and Champhai districts of Mizoram, a region recognised for its rich fungal diversity, yet still insufficiently documented in scientific literature. Previous works have reported the occurrence of wood rotting fungi and soil ectomycorrhizal fungi (Lalrinawmi et al., 2017; Zothanzama et al., 2018; Vabeikhokhei et al., 2019; Chawngthu et al., 2023) with few nutritional assessments studies (Zohmangaiha et al., 2023). However, comprehensive information on their identification, nutrient composition, heavy metal

bioaccumulation, antioxidant activity and volatile compounds remains limited. Therefore, the present research seeks to tie these gaps or pragmatically fill the existing knowledge voids through a multidisciplinary evaluation of selected wild mushrooms.

Specimens were collected randomly from various forest habitats and further identified based on macro- and micromorphological characteristics. In this respect, species presenting taxonomic ambiguity were further characterised by PCR amplification and sequencing of the ITS rDNA region (Zothanzama et al., 2016). Pure cultures of edible fungi were maintained on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) media that are in fact intended for the observation of cultural characteristics of the basidiomes. Nutritional profiling including protein, carbohydrate, fat, fibre, and mineral content, together with antioxidant assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), is intended to determine dietary and functional attributes.

Considering the metal accumulating nature of wild mushrooms, the contents of cadmium (Cd), lead (Pb), nickel (Ni), and manganese (Mn) need to be quantified, followed by estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI) and carcinogenic risk index (CRI) estimation to evaluate health risk exposure. Ligninolytic enzymes, including laccase, manganese peroxidase and lignin peroxidase can be assessed to determine their biodegradation potential and volatile metabolite analysis to characterise mushroom compounds for their commercial up-scalability. These pragmatic and panoramic aspects of wild edible mushrooms from the selected study areas in Mizoram are inextricably linked with rural livelihood and human well-being, thereby nurturing important “United-Nation Sustainable Development Goals (UN-SDGs)”.

In light of the above knowledge gaps, the present study aims to undertake a systematic documentation and characterization of wild edible mushrooms, while acknowledging that non-edible taxa locally referred to as Pachhia in Mizo (e.g., *Ganoderma* sp., *Trametes* sp., and many other taxa) also possess remarkable medicinal and biochemical attributes deserving scientific consideration. Moreover, the study includes several fungal species from Mizoram that are of particular

mycological significance, including taxa that are rare in India or occur only in limited quantities.

The study further covers biochemical antioxidant evaluation and ecotoxicological investigations, including health risk assessment, to assess both the therapeutic potential and safety of these macrofungi. In this sense, the present study intends to strengthen local knowledge, promote safe and sustainable utilisation, and open avenues for value addition and future applications of both edible and non-edible mushrooms. These multifaceted aspects of wild edible mushrooms is attempted to be pragmatically explored with following broad objectives.

1.3. Objective of the study

- To analyse macro-nutrient and micro-nutrient content of wild edible mushrooms.
- To isolate pure culture of wild edible mushrooms.
- To determine enzyme activity of pure cultures of wild edible mushrooms.

These objectives were further subdivided into biochemical (e.g., antioxidative potential and VOCs) ecotoxicity (specifically in relation to heavy metals) aspects to offer a state-of-art-knowledge on wild edible mushrooms of Mizoram, NE India, underlying in an Indo Burma global biodiversity hotspot.

2. LITERATURE REVIEW

The nutritional composition of mushrooms exhibits significant variation, influenced primarily by species type and developmental stage (Zakhary et al., 1983). This variability is further modulated by climatic and environmental factors, which critically affect fungal physiology and growth, with moisture and temperature being particularly influential (van Peer et al., 2009; Green et al., 2004; Talley et al., 2002). Moisture content is specifically a key quality parameter for fresh mushrooms, diminishes from initial levels and is dependent on harvest timing, maturation, ambient conditions during growth, and post harvest storage (Roy et al., 1993; Oluwafemi et al., 2016; Crisan and Sands, 1978).

The edibility of mushrooms is often difficult to determine due to high morphological similarity between edible and toxic species, regional variation in traditional knowledge, and limited documentation of consumption related evidence.According to Liu et al. (2018), the edibility of mushroom species can be evaluated based on documented case reports and the frequency of human consumption. Using this approach, mushroom species are recognised either as edible without reported adverse effects or as edible under specific conditions, depending on the available evidence. In this system, the number of case reports considered for each species reflects the extent of documented consumption, while the final edibility status (FES) represents the confirmed edibility assessment derived from these reports.

Wild edible mushrooms are recognized as valuable nutritional resources, particularly for their protein content, which is a crucial determinant of food quality (Captagun et al., 2010; Xu et al., 2011; Bano et al., 1988). These proteins comprise diverse fractions including albumins, globulins, glutelins, and prolamins (Bauer-Petrovska, 2001). Beyond fundamental nutrition, mushrooms are a significant reservoir of bioactive compounds (Breene, 1990; Cheung, 2010). Carbohydrates represent the predominant constituent of fruiting bodies, encompassing glucans, sugars, glycogen, and chitin, making edible mushrooms an essential source of this

nutrient (Ouzouni et al., 2009; Kalač, 2009; Kurztman, 1997).

Mineral composition in mushrooms is subject to considerable fluctuation, affected by both edaphic factors such as soil metal contents, pH, and organic matter and fungal characteristics, including morphology, biochemical makeup, and developmental stage (Garcia et al., 1998; Vetter, 1990; Valverde et al., 2015). Consequently, edible mushroom can be moderately rich in minerals and vitamins, presenting a natural and healthful food source (Mattila et al., 2001; Sadler, 2003). The analyses confirm that they contain appreciable amounts of calcium, iron, magnesium, manganese, potassium, and zinc, serving as a beneficial supplement to cereal based diets (Mallikarjuna et al., 2013; Chang and Buswell, 1996). Furthermore, their profile is characterized by low caloric and fat content, aligning with balanced dietary requirements (Manzi et al., 1999; Manzi et al., 2001; Tsai et al., 2008).

Regional assessments from Turkey Black Sea area consistently report high nutrient values in various wild mushroom species (Caglarlrmak et al., 2002; Ayaz et al., 2011; Okan et al., 2013). Extensive research across India underscores their nutritional significance, particularly for indigenous communities (Sargunam, 2012; Sudheep and Sridhar, 2014; Das et al., 2017). Studies from diverse locales, including the Western Ghats, West Bengal, Nagaland, and Meghalaya, document numerous edible species rich in protein and carbohydrates, often with substantial mineral content (Johnsy et al., 2011; Pradhan et al., 2010; Singha et al., 2017; Karun and Sridhar, 2017; Semwal et al., 2014; Agrahar-Murugkar and Subbulakshmi, 2005; Barua et al., 1998; Ao et al., 2016; Bhaben et al., 2011; Kumar et al., 2013; Joshi and Khaund, 2013). Specific analyses of species like *Schizophyllum commune* and *Lentinus edodes* from Northeast India confirm high protein and low fat levels (Longvah and Deosthale, 1998).

The isolation and pure culture of fungi are prerequisite steps for biochemical characterization, with techniques tailored to the source substratum (Finkelstein and Ball, 1991). Standard methods involve transferring sporophore tissue or spores onto nutrient media like ‘Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA)’ under aseptic conditions, with media specification

being critical for reproducible growth (Nevalainen et al., 2014). Fungal cultivation typically employs media rich in carbohydrates and nitrogen, with a pH of 5-6, and incubation at 25-30°C, though optimal conditions are species-specific (Stamets, 1993; Palacios-Cabrera et al., 2005; Daryaei et al., 2016). Pure cultures are maintained through sub-culturing and identified via morphological and microscopic examination (Gilna and Khaleel, 2011). Common challenges during isolation include contamination by bacteria, yeasts, or mites, the latter posing a significant risk to culture collections by spreading contaminants (Choi et al., 1999). Furthermore, wild edible mushrooms produce a diverse array of enzymes that are integral to their growth and development (Joshi and Khaund, 2014; Kuforiji and Fasidi, 2008), and their mycelial production in culture is influenced by media composition, including nitrogen, carbon, vitamin, and mineral sources (Assefa and Abate, 2020). Successful isolation and pure cultivation of wild strains have been demonstrated, enabling their taxonomic classification (Hoa et al., 2007).

Fungi contribute over half of the industrially applied enzymes and offer advantages such as high extracellular secretion, cost-effective large-scale yield and ease of purification (El-Gendi et al., 2021). Edible mushrooms are particularly valued for their high nutritional composition, minerals, vitamins, medicinal properties, and diverse bioactive metabolites while exhibiting notable capacity to bioaccumulate heavy metals (Maaloul et al., 2023; Niego et al., 2021; Elekes et al., 2010). They efficiently degrade lignocellulosic biomass due to secretion of ligninolytic enzymes including laccase, manganese peroxidase and lignin peroxidase (Civzele et al., 2023; Suryadi et al., 2022). Edible mushrooms continue to receive attention owing to their dual role as nutrient-rich foods and therapeutic resources containing valuable bioactive compounds (Mukhopadhyay and Guha, 2015; Wang et al., 2019; Finimundy et al., 2018).

Laccases (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) are multicopper oxidase enzymes capable of catalysing the oxidation of diverse phenolic substrates while simultaneously reducing molecular oxygen to water (Eriksson and Bermek, 2009). These enzymes oxidize a wide spectrum of compounds including *p*- and *o*-diphenols, polyphenols, polyamines, aryldiamines, aminophenols, hydroxyindoles, and even certain inorganic ions (Kunamneni et al., 2008). Laccases are widely

distributed across microorganisms, plants, and fungi (Malhotra and Alghuthaymi, 2022) and are integral to lignin degradation, functioning as key lignin-modifying enzymes that may be secreted constitutively or induced during fungal growth (Ding et al., 2014; Eggert et al., 1997). Although predominantly associated with white-rot fungi, their versatility and biotechnological relevance have been demonstrated in multiple studies (Reyes et al., 2021; Shraddha et al., 2011). Structurally, laccases typically contain 15–30% carbohydrate with a molecular mass ranging between 60–90 kDa (Shraddha et al., 2011). Owing to their ability to oxidize phenolic and non-phenolic substrates, laccases are widely applied across food, pharmaceutical, and environmental sectors, including in paper and pulp biobleaching, biosensing, biofuel cells, and as eco-friendly biocatalysts (Zerva et al., 2019; Moreno et al., 2019; Mayolo-Deloisa et al., 2022).

Manganese peroxidase (MnP) (EC 1.11.1.13; Mn^{2+}:H_2O_2 oxidoreductase) is an extracellular heme-containing enzyme classified under oxidoreductases, particularly those utilizing peroxides as electron acceptors. This enzyme participates in the H_2O_2-mediated oxidation of lignin-based compounds, where it initially converts Mn^{2+} to Mn^{3+}. The resulting Mn^{3+} ions are released from the enzyme surface and subsequently oxidize various phenolic substrates, including lignin model compounds and several types of organic pollutants. In natural systems, MnP contributes significantly to the depolymerization of plant lignin as a key component of the ligninolytic enzyme complex, making it one of the primary enzymes involved in lignin degradation. Due to this capability, MnP holds considerable promise in agricultural applications, particularly in the breakdown of lignocellulosic components such as cellulose, hemicellulose, and lignin (Xu et al., 2017; Gold et al., 1993; Zhou et al., 2013). Many mushrooms are known to synthesize lignin peroxidase (LiP) and manganese peroxidase, enabling them to utilize cellulose effectively as a carbon source (Xu et al., 2013).

Lignin peroxidase (LiP; EC 1.11.1.14) are heme-containing oxidoreductase enzymes responsible for the degradation of lignin and its derivatives. They play a key role in the ligninolytic cycle, facilitating the breakdown of the plant cell wall polymer lignin. LiPs utilize hydrogen peroxide (H_2O_2) as an electron acceptor to oxidize diverse substrates, forming reactive radical intermediates that initiate lignin

depolymerization (Becker and Wittmann, 2019). These highly oxidative enzymes are activated through H_2O_2-mediated oxidation, generating an intermediate capable of oxidizing phenols, aromatic amines, ethers, and polycyclic aromatic hydrocarbons (Kumar et al., 2020). Lignin degradation is extensively studied in white-rot fungi, particularly within the order Polyporales, where LiPs are recognized as among the most efficient ligninolytic enzymes (Sanchez-Ruiz et al., 2021). Wild mushrooms especially, white-rot fungi produce a suite of ligninolytic enzymes, including laccase (Lac; EC 1.10.3.2), manganese peroxidase (MnP; EC 1.11.1.13), and lignin peroxidase (LiP; EC 1.11.1.14). Together, these enzymes play a critical role in lignin biodegradation, enabling nutrient cycling, biomass conversion, and biotechnological applications such as bioremediation, biofuel production, and synthesis of value-added chemicals.

Bioremediation or associated phytoremediation have emerged as promising solutions for environmental pollution caused by heavy metals (Rai, 2011; Yan et al., 2020). Plants growing on marginal or degraded lands, as well as non-edible macrophytes in wetlands, are often preferred bioagents because they do not compete with agricultural land and do not impose direct health risks (Rai et al., 2024). Microorganisms, particularly fungi, have shown remarkable potential for remediation of heavy metals due to their ability to survive in diverse and extreme environments while secreting degradative enzymes. Fungal biomass has been utilized for mycoremediation, exploiting its enzymatic activity and metabolic adaptability to degrade persistent environmental pollutants, including heavy metals (Deshmukh et al., 2016; Kües, 2015; Okal et al., 2023). Mycoremediation has thus evolved as an eco-friendly, cost-effective, and sustainable approach to restore polluted soils and waters (Kulshreshtha et al, 2014; Akhtar and Mannan, 2020).

Fungi can remove heavy metals using live (growing) or dead biomass (El-Bondkly and El-Gendy, 2022). In live fungi, tolerance to toxic metals is required, as heavy metals can interfere with growth, physiology, and genetic machinery (Angon et al., 2023). Dead biomass relies primarily on cell wall functional groups for metal binding, without needing metabolic activity or oxidative stress tolerance (Kumar and Dwivedi, 2021). The fungal remediation of heavy metals works through three main mechanisms such as degradation by ligninolytic fungi, biosorption, and mycorrhizal

fungal degradation (Vishwakarma, 2019; Hu et al., 2021). Among these, biosorption has gained particular attention as a cost effective approach. In this process, fungal biomass attracts and binds dissolved metal ions via surface functional groups until equilibrium is achieved, enabling the removal of pollutants in a metabolism independent manner (Das, 2005; Dhankhar and Hooda, 2011).

The efficiency of heavy metal accumulation varies by fungal species, substrate composition, and environmental conditions, highlighting the importance of species-specific studies (Chen et al., 2009; Kokkoris et al., 2019; Ediriweera et al., 2022). Despite growing interest in mycoremediation, insufficient knowledge about the degradation and biosorption capabilities of diverse fungal species limits the targeted application of fungi for environmental restoration (Hyde et al., 2019). Moreover, the bioaccumulation of heavy metals in edible fungi remains a crucial area of research, as excessive accumulation can pose human health risks. The evaluation of health risks associated with mushroom consumption has gained increasing attention in the literature, particularly due to the ability of many species to accumulate heavy metals from their growing substrates. Several studies have reported that certain wild mushrooms contain elevated levels of toxic metals, including cadmium, lead, and mercury, which may exceed permissible limits and pose potential health hazards to consumers (Fu et al., 2020; Hu et al., 2017; Nowakowski et al., 2021). Therefore, health risk assessment is crucial for distinguishing nutritionally beneficial species from those that may be unsafe for long-term or frequent consumption. Understanding these dynamics is especially important in biodiversity hotspot regions, where mushroom diversity contributes not only to ecosystem services but also to the nutritional and socio-economic well-being of indigenous communities.

Extensive research on Basidiomycetes fungi has significantly expanded, primarily due to their potential utility in various biotechnological applications, playing a crucial role in modulating physiological functions within the human body (Shi et al., 2013; Reis et al., 2017; Gründemann et al., 2020). For centuries, mushrooms have been appreciated not only as a culinary delicacy but also for their potential role as functional foods that enhance antioxidant defenses and mitigate oxidative stress (Liuzzi et al., 2023). Their antioxidant capacity is largely attributed

to diverse bioactive molecules. Ergothioneine, a sulfur-containing amino acid, provides potent cellular protection, while polysaccharides, especially β-glucans, contribute to free radical neutralization and immunomodulatory effects. Essential vitamins, including ascorbic acid (C) and tocopherols (E), protect against oxidative damage, and carotenoids such as β-carotene and lycopene serve as singlet oxygen scavengers and inhibit lipid peroxidation (Cheah and Halliwell, 2021; Petraglia et al., 2023; Naim, 2024). More than 347 common volatile compounds (VCs) have been found in mushrooms, including aldehydes, ketones, alcohols, and sulfur-containing chemicals (Deng et al., 2023). Medicinal fungi contain bioactive compounds with pharmacological properties and have been harvested and utilized for centuries in Korea, China, Japan, and eastern Russia. (Mustafin et al., 2021: Niego et al., 2021).

Wild mushrooms contain diverse bioactive compounds with notable anticancer, antioxidant, antidiabetic and immunomodulatory properties, and their metabolites are known to enhance metabolic functions, reduce obesity, and delay cellular ageing due to the presence of potent antioxidant molecules (Łysakowska et al., 2023). Wild edible mushrooms have been traditionally utilized for medicinal purposes, and both wild and cultivated edible mushrooms are recognized for strong antioxidant potential attributed to compounds such as polysaccharides, polyphenols, vitamins, carotenoids, and essential minerals (Kozarski et al., 2015; Hyde et al., 2019),). Mushrooms also serve as a primary natural source of ergothioneine, a unique antioxidant amino acid that provides cellular protection against oxidative stress (Chun et al., 2021).

Antioxidant capacity is often evaluated using radical scavenging assays, and extract concentration typically shows a positive correlation with free radical slaking ability in ABTS assays, which are effective for assessing hydrogen-donating and chain-breaking antioxidants (Leong and Shui, 2002). Similarly, the DPPH assay is widely used to measure free radical neutralization capacity due to the stability of DPPH radicals (Baliyan et al., 2022). Wood-decaying basidiomycete responsible for white-rot and brown-rot in several tree species and has long been used in European traditional medicine for antipyretic, antirheumatic and antitussive purposes (Sułkowska-Ziaja et al., 2018). Species like *Laetiporus sulphureus* fruiting bodies contain valuable bioactive substances including high amounts of α-(1→3)-glucan

(Wiater et al., 2012). This species grows well on enriched sawdust substrates, and its extracts have shown potential as antioxidant and antimicrobial agents suitable for many foods and nutraceutical applications (Turkoglu et al., 2007; Pleszczyńska et al., 2013). Nevertheless, *Laetiporus sulphureus* remains under-studied despite its medicinal significance, nutritional composition, and cultivation feasibility. Global mushroom production has risen more than forty-fold since 1978, exceeding 43 million tons by 2020, with Asia, particularly China contributing more than 70% of total output (Royse et al., 2017). This increasing production reflects rising global interest in mushrooms as sustainable foods rich in bioactive compounds and antioxidants.

Research on *Ganoderma* has attracted significant attention due to its considerable potential applications in biotechnology (He et al., 2022). *Ganoderma* species undeniably offer valuable medicinal benefits, yet they concurrently pose a formidable threat to global plant, particularly affecting economically important trees and perennial crops in tropical regions (Luangharn et al., 2021; Karunarathna et al., 2024). Wood-decay enzymes produced by *Ganoderma* species are utilized in bioleaching and bioremediation processes. These enzymes aid in breaking down complex organic materials and pollutants, thus improving the effectiveness of environmental cleanup and recovery efforts (Loyd et al., 2018; Ipeaiyeda et al., 2020; Wang et al., 2021). *Ganoderma lucidum* mycelial pellets have been reported to have high potential as a naturally sustainable bioremediation system for treating domestic wastewater, particularly with high organic content (Hanafiah et al., 2019). *Ganoderma* species produce a wide variety of chemical compounds, including alkaloids, meroterpenoids, nucleobases, nucleosides, phenols, steroids, terpenoids, glycoproteins, polysaccharides, proteins, and triterpenes (Wachtel-Galor et al., 2012; Galappaththi et al., 2023).

Polysaccharides (ganoderans) and triterpenes (such as ganoderic acids, ganodermanondiol, ganodermanontriol, ganolucidic acid B, and lucidumol B) are the primary bioactive chemical constituents (Ferreira et al., 2015; Liu et al., 2016; Galappaththi et al., 2023). The biological activities of various *Ganoderma* species and their isolated compounds have been extensively studied (Osińska-Jaroszuk et al., 2014; Wange et al., 2020; Andrejč et al., 2022; Rijia et al., 2024), revealing that

Ganoderma species contains an array of biologically active compounds that improve the immune system and possess antioxidant, antitumor, anti-inflammatory, antifungal, and antimicrobial properties.

Studies on fungal diversity in Mizoram, North-East India remain relatively limited, particularly concerning taxonomic and biochemical characterisation. The first systematic exploration of fungi in this region was initiated by Bisht (2011) and Zothanzama (2011), marking an important step in documenting fungal wealth within the Indo-Burma biodiversity hotspot. Subsequent works focused mainly on the diversity and distribution of edible mushrooms (Zothanzama and Lalrinawmi 2015; Lalrinawmi et al., 2017), poisonous mushrooms (Zothanzama et al., 2018), soil macrofungi, and wood-rotting fungi (Lalrinawmi and Zothanzama 2016; Zothanzama et al., 2016; Lallawmsanga et al., 2016; Vabeikhokhei et al., 2019; Zohmangaiha et al., 2019; Chawngthu et al., 2023) and a brief report on the nutritional value of *Lentinula edodes* was provided by Zohmangaiha et al. (2023). However, research on their nutritional composition, mineral and heavy metal content, isolation of pure culture and their characteristics, health risk assessment of widely consumed species, as well as studies on antioxidant properties and volatile compound profiling, is still scarce. This highlights the necessity for further biochemical investigations to better understand the potential value and safety of wild mushrooms in Mizoram.

3. MATERIALS AND METHODS

3.1. Study site

The samples of wild edible mushrooms were collected in the fields from Mamit and Champhai Districts of Mizoram. Mamit District lies between 23.8377° N, 92.5396° E, located in northwestern Mizoram. It is characterized by hilly terrain and lush green forests, while Champhai District in the east is marked by its mountainous landscapes, steep slopes, and proximity to the India-Myanmar border, and lies between 23.6357° N, 93.1780° E (Figure 3.1).

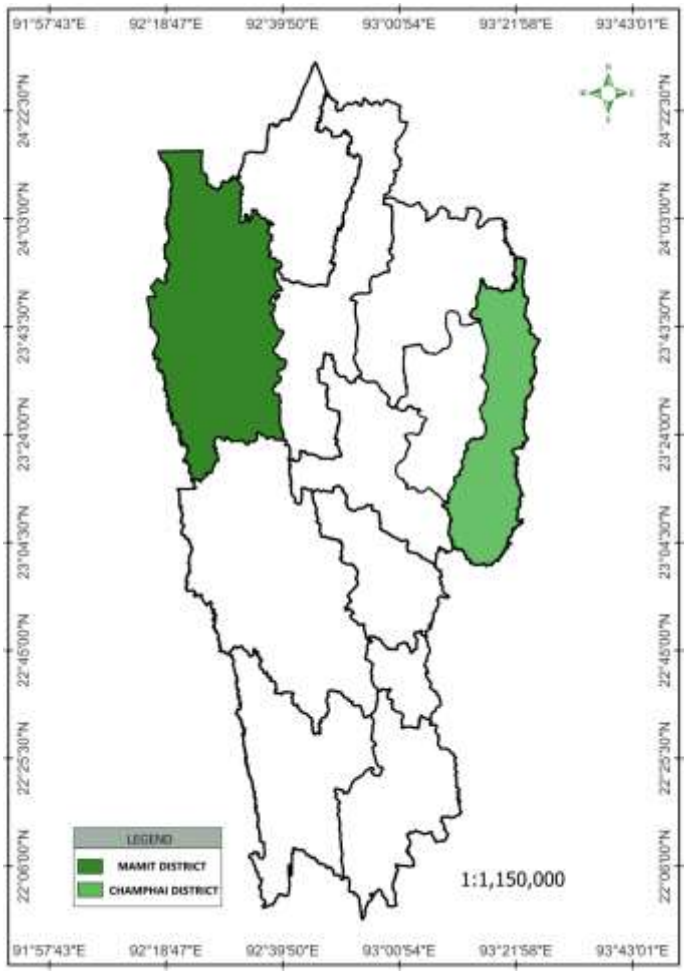

Figure 3.1: Map indicating study site (Mamit District and Champhai District)

3.2. Collection and identification of specimen

Basidiomes were collected randomly from their natural habitat within Mamit and Champhai District during 2020–2024 in accordance with Halling (1996) and Mueller et al (2004). The samples were identified *in situ* if possible and morphological observations such as pileus, lamellae, stipe, spore print and color were assessed from fresh samples (Largent and Stuntz, 1977; Lodge et al., 2004; Zothanzama, 2011) or else the specimens were retained for later identification. Colour descriptions were based on the Methuen Handbook of Colour (Kornerup and Wanscher, 1978). Photographs of the specimen were taken using digital imaging (Nikon D-5200) and the location of each collection site was recorded using Garmin GPS etrex10. These collected basidiomes were further wrapped in aluminum foil or waxed paper for the protection and moisture retention. The samples were labeled before transporting back to the laboratory for further analysis.

Microscopic characteristics were examined from both fresh and dried specimens using a light microscope (Carl ZEISS Axio Lab.A1) after sectioning and directly staining with 5% 'potassium hydroxide (KOH)', 'Lactophenol cotton blue (LPCB)' and 'Melzer's reagent' authenticated by referring to several authors. For certain species, molecular analyses were performed using 'Cetyltrimethylammonium Bromide (CTAB)' extraction method following Zothanzama et al. (2016) and references therein. A small amount of fungal tissue was homogenized in CTAB lysis buffer, incubated at 65°C for cell lysis, and purified with chloroform. The supernatant was transferred to a new tube, mixed with isopropanol, and incubated at -20°C to precipitate DNA. After centrifugation, the DNA pellet was washed with 70% ethanol, air-dried, and resuspended in 100 µL of sterile water.

'Polymerase Chain Reaction (PCR)' amplification targeted the 'Internal transcribed spacer (ITS)' region using primers ITS1-F and ITS4 (White et al., 1990). Reactions (25.5 µL) contained GoTaq Green MasterMix (Promega), primers (1 µL each, 5 µM), template DNA (1 µL), and nuclease-free water. Thermocycling conditions included initial denaturation (94°C, 5 min), 35 cycles of denaturation (94°C, 1 min), annealing (52°C, 1 minute), extension (72°C, 1 min), and final elongation (72°C, 5 min). Amplicons were visualized via agarose gel electrophoresis

and sequenced bidirectionally using Sanger sequencing. Consensus sequences were aligned in 'BioEdit' (Hall, 1999) and compared to GenBank entries via BLASTn (Altschul et al., 1990). Appropriate literature was further consulted for the identification of each specimen. To ensure accuracy in taxonomic position, the 'Index Fungorum' (http://indexfungorum.org) and 'MycoBank' (http://mycobank.org) database were consulted.

The edibility of the recorded mushroom species was assessed following the criteria outlined by Li et al. (2021). Edibility status was evaluated based on documented case reports and frequency of human consumption available in the literature. Using this approach, the majority of the species documented in the present study were recognised as edible, while a few species were considered edible under specific conditions. In this system, total reports refer to the number of documented case reports examined for each species, whereas the final edibility status (FES) represents the edibility designation assigned based on the compiled evidence.

3.3. Nutritional composition

Moisture, protein, fat, crude fiber and ash content of the selected mushroom samples were determined on a dry weight basis using the standard methods of the 'Association of Official Analytical Chemists' (AOAC, 2000). Total carbohydrate content was estimated following the method of Raghuramulu et al. (2003), while energy values (Kcal) were calculated using the equation proposed by Crisan and Sands (1978). All mushroom analyses were performed in triplicate on a dry-weight basis.

3.3.1. Processing of fungal specimen

Pertaining the evaluation of the proximate compostiton, the selected specimens were cleaned to remove some forest debris and wood substrates (without washing) and oven-dried at 45°C for 3 days to obtain a consistent weight. The drying was performed without separating the stipe and pileus. Once completely dried, the samples were ground into a fine powder and transferred into polythene bags. These bags were properly labeled, sealed tightly to prevent moisture absorption, and stored in a refrigerator at 4 °C until further analysis.

3.3.2. Moisture content

The moisture content was determined by drying the sample at 105 °C. 2 g was taken in a porcelain crucible and placed into a 'hot air oven (HAO)' at 105 ºC for 3 h. The sample was cooled in a desiccator, and the difference between the sample weight before and after drying was used to measure the moisture content percentage (AOAC, 2000). The process of heating and cooling was repeated till a constant weight was obtained.

"Moisture (%) = *Fresh weight* (g) – *dry weight* (g) */ fresh weight* (g) *x* 100"

3.3.3. Protein content

Protein content (calculated as total N × 6.25) was derived from the total nitrogen concentration, which was determined through the 'micro-Kjeldahl' method (AOAC, 2000). Oven-dried samples (0.5 g) were transferred into separate 30 mL Kjeldahl flasks, followed by the addition of 15 mL concentrated sulphuric acid (H_2SO_4). The mixtures were carefully heated in a fume hood until a clear greenish solution was obtained. After digestion, the flasks were cooled for 30 minutes, and 10 mL of distilled water was added to prevent solidification. The digested samples were then subjected to distillation, and 35 mL of the distillate was collected in a receiving flask. The distillate was titrated with 0.01 M hydrochloric acid (HCl) until the endpoint was indicated by the appearance of a pink coloration.

Protein (%) = *Percentage of nitrogen x* 6.25

3.3.4. Fat content

A 2–4 g portion of the moisture-free mushroom samples was placed in an extraction thimble and subjected to a 6 h Soxhlet extraction using petroleum ether as the solvent. After extraction, the solvent was evaporated, and the residue was dried in an oven at 80 °C. The thimble was then cooled to room temperature in a desiccator and weighed. The loss in thimble weight represented the amount of crude fat extracted and was expressed as the percentage of fat in the sample (AOAC, 2000).

Fat (%) = 100 (*weight of Soxhlet flask with extracted fat – weight. of empty Soxhlet flask*) */ Weight of sample*

3.3.5. Crude Fibre

The crude fibre content was determined using standard acid–alkali digestion followed by incineration. A 2 g portion of the mushroom samples was boiled with 1.25% dilute sulphuric acid (H_2SO_4), washed thoroughly with deionized water, and subsequently boiled with 1.25% dilute sodium hydroxide (NaOH). The residue remaining after these digestion steps was collected as crude fibre. After completion of the digestion cycle in the Fibretherm unit, the fibre bags were removed from the instrument, and the residues were transferred into pre-weighed crucibles (W_1). The crucibles were dried overnight at 80–100 °C and weighed (W_2). They were then incinerated in a muffle furnace at 600 °C for 2–3 h, cooled in a desiccator, and reweighed (W_3). The crude fibre content was calculated as the difference between W_2 and W_3 and expressed on a moisture- and fat-free basis (AOAC, 2000).

$$\text{Crude fibre (\%)} = [(W_2 - W_3) / W_1] \times 100.$$

3.3.6. Ash content

Empty crucibles with their lids were first placed in a muffle furnace at 550 °C overnight to remove any adhering impurities. The crucibles were then cooled in a desiccator for 30 minutes and weighed to the nearest 0.001 g. 2 g of dried mushroom sample was transferred into each crucible. The crucibles were initially heated over a low Bunsen flame with the lid half-covered until fumes ceased. Subsequently, the crucibles were placed in a muffle furnace and ashed at 550 °C overnight, with the lids kept aside to prevent loss of ash. After cooling in a desiccator, the crucibles and ash were weighed. The ashing process was repeated until two successive constant weights were obtained (AOAC, 2000).

Ash (%) = (*Weight of ash* / *Weight of sample*) × 100

3.3.7. Total Carbohydrate

The total carbohydrate content in the mushroom samples was estimated by difference

(Raghuramulu et al. 2003). It was calculated by subtracting the sum of all other proximate components from 100, using the following formula:

Total carbohydrates (%) = 100 − [*Moisture* (%) + *Protein* (%) + *Crude fibre* (%) + *Fat* (%) + *Ash* (%)].

3.3.7. Energy value

The energy value of the mushroom samples was estimated based on their proximate composition (Crisan and Sands, 1978).

Energy (kcal) = (2.62 × % *Protein*) + (8.37 × % *Fat*) + (4.2 × % *Carbohydrate*).

3.3.8. Mineral content of the sample

The mineral content of the mushroom samples was assessed using the ash obtained after incineration (AOAC, 2000). Minerals such as calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na) and zinc (Zn) were evaluated using an Atomic absorption spectrophotometer (Shimadzu AA-7000; Shimadzu Corporation, Japan) after dry ashing of the sample (Isildak et al., 2004) with detection of limits ranges between 0.01 to 0.09 ppm.

3.4. Isolation of pure culture from wild edible mushrooms

Following Thongklang *et al*. (2010), the specimens were cultured on different media containing 0.01% (w/v) chloramphenicol under aseptic conditions (surface serialization using 70% ethanol and 0.5% sodium hypochlorite) to inhibit bacterial growth and incubated at 28 ± 2 °C for 7–14 days, under dark condition. After incubation, the agar surface was fully covered with the white mycelium. The culture media used in this study included PDA, MEA, and SDA. PDA was prepared with potato infusion obtained from 200 g of boiled potatoes, supplemented with 20 g of dextrose and 15 g of agar per liter of distilled water. MEA consisted of 20 g malt extract, 1–2 g peptone, and 15 g agar per liter of distilled water, while SDA contained 40 g dextrose, 10 g peptone, and 15 g agar per liter of distilled water. These media were selected to support the growth and sporulation of different fungal species collected from Mizoram.

The mother cultures obtained were sub-cultured into a new plate containing the same agar (media). The pure cultures obtained were stored in refrigerator at 4 °C for further studies and they were sub-cultured periodically once in 2 to 3 months.

3.4.1. Culture characteristics

The fungal isolates were characterized on the basis characteristics such as size (in mm), shape, surface, elevation, colour of colony, consistency and other properties (Barnett and Hunter, 1972; Senanayake et al., 2019).

3.4.2. Enzymatic activity of wild edible mushrooms

The pure culture fungal isolate was cultivated in PD broth medium using the shake flask method at 150 rpm for 6 days at 28 ± 2 °C. The culture was then centrifuged at 10,000 rpm for 10 minutes, filtered through Whatman No. 1 filter paper, and the supernatant was assayed for enzyme activity.

Laccase activity was determined by following Eggert et al. (1997) monitoring the oxidation at 420 nm of 500 µM ABTS (2,2′-azino-bis-[3-ethylthiazoline-6-sulfonate]) buffered with 50 mM sodium tartrate buffer (pH 4.0). Enzyme units are given in mol of product formed per min (ϵ_{max}=3.6×10^4×M^{-1}×cm^{-1}). Manganese peroxidase (MnP) activity was assayed using 3-methyl-2-benzothiazolinone hydrazone (MBTH) in 1.0 mL of sodium acetate buffer (pH 4.5) containing 4.95 mM 3-dimethylaminobenzoic acid (DMAB) and 0.25 mM hydrogen peroxide. Oxidation of MBTH was monitored spectrophotometrically by measuring the increase in absorbance at 590 nm (ε_{590} = 53,000 M^{-1} cm^{-1}) (Castillo et al., 1994). One unit of MnP activity was defined as the amount of enzyme required to catalyze the formation of 1 µmol of green or purple product per minute under the assay conditions. Lignin peroxidase (LiP) activity was measured using veratryl alcohol (Ming and Kent, 1984) in 200 mM succinate buffer (pH 3.0) supplemented with 2 mM hydrogen peroxide. The oxidation of lignin peroxidase was monitored by the increase in absorbance at 310 nm (ε_{310} = 9,300 M^{-1}·cm^{-1}). One unit of MnP activity was defined as the amount of enzyme catalyzing the formation of 1 µmol of veratraldehyde per minute under the specified conditions.

3.5. Heavy metal and health risk analysis

For the analysis of heavy metals (Isildak et al., 2007; Gençcelep et al., 2009), 2 g of the dried mushroom sample was placed in a porcelain crucible and ashed in a muffle furnace at 550 °C for 18–20 hours. The resulting ash was treated with 1 mL of concentrated nitric acid (HNO_3) and heated for an additional 4 h to ensure complete combustion. Subsequently, 1 mL each of concentrated sulfuric acid (H_2SO_4), HNO_3, and hydrogen peroxide (H_2O_2) was added to the crucible and the mixture was filtered through a 0.2 μm syringe filter. Three blank samples were processed in the same manner. The filtrate was diluted with deionized water to a final volume of 10 mL.

The contents of cadmium (Cd), manganese (Mn), nickel (Ni), and lead (Pb) in the samples were determined using an Atomic Absorption Spectrometer (Shimadzu AA-7000; Shimadzu Corporation, Japan). Detection limits varied depending on coexisting substances in the samples.

3.5.1. Health risk assessment

A human health risk assessment was performed to evaluate the potential risks associated with the consumption of wild edible mushroom. In this assessment, a key indicator including the estimated daily intake, target hazard quotient, hazard index, and carcinogenic risk index or the incremental lifetime cancer risk were calculated to estimate both the non-carcinogenic and carcinogenic health risks.

Human health risk assessment indices/indicators such as ‘estimated daily intake (EDI)’ of the elements was calculated (Fu et al., 2020), which was in fact based on the daily intake of the respective food items, the averaged exposure time, and the average contents of each food sample.

$$‘\text{EDI} = \frac{\text{MC x IR x EF x ED}}{\text{ET x BW}}‘$$

where, MC is the element content in wild edible mushroom (mg/kg in dw), IR - food ingestion rate (6.6×10^{-3} kg/person/day). EF - exposure frequency (365 days/year), ED - exposure duration for an adult (70 years), ET - averaged exposure time (365 days/year × 70 year or 25550 days/year), and BW - mean body weight of consumers (60 kg).

In this context, another health indicator i.e., ‘Target hazard quotient (THQ)’

was used to evaluate the potential health risks of human consumption following the equation.

‘$\mathrm{THQ} = \frac{\mathrm{EDI}}{\mathrm{R}f\mathrm{D}}$ ‘

where, R_fD is the oral reference dose of the heavy metals in mg/kg/day (USEPA 2012; USEPA 2017).

The ‘Hazard Index (HI)’ is calculated by adding up the THQ values for each food type assessment element (Fu et al., 2020). The risk for chronic systemic effects is considered acceptable if the resulting HI is less than 1. However, if the HI equals or exceeds 1, it indicates possible long-term consumption risks associated with adverse non-carcinogenic health effects. In such cases, there is the potential for harm due to non-carcinogenic health effects.

$HI = THQ_{Mn} + TH_{Ni} + TH_{Pb} + TH_{Cd}$

‘Carcinigenic Risk Index (CRI)’ was calculated by multiplying the EDI by the corresponding oral cancer slope factor (CSF) (Hu et al., 2017). As a result, Cd and Pb were the metals assessed for ILCR values, as they are known carcinogens with established CSFs. The CSF values for Cd and Pb were provided by USEPA (2010). An ILCR value between 10^{-4} and 10^{-6} is generally considered an acceptable risk level. Values exceeding 10^{-4} indicate a potential carcinogenic risk, while a CRI/ILCR below 10^{-6} is considered negligible (Li et al., 2013; Hu et al., 2017).

3.6. Antioxidant activity

The antioxidant activity of the mushroom extracts was evaluated using ABTS and DPPH radical scavenging assays.

3.6.1. Extraction of specimen

For mycelium and fermentation broth, the tissue from the selected mushroom specimens was aseptically inoculated into ‘Potato Dextrose Broth (PDB)’ and incubated at 27 °C for 14 days. After incubation, the fermentation broth was filtered through Whatman filter paper to separate the mycelium. The mycelium was dried and ground into a fine powder, which was sequentially extracted using dichloromethane, ethyl acetate, and methanol, followed by filtration. The

fermentation broth was simultaneously extracted with dichloromethane and ethyl acetate, and the resulting organic and aqueous phases were separated using a separating funnel.

For direct fruiting bodies, fresh fruiting bodies were dried at 60 °C for 24 h to a constant weight and then ground into a fine powder. 10 g of each powdered fruiting body sample was mixed with 100 mL of methanol and stirred at 150 rpm for 24 h. The mixture was filtered through Whatman No. 4 filter paper, and the residue was subjected to a second extraction with an additional portion of methanol (Moro et al., 2012). All extracts were concentrated using a rotary evaporator, and the resulting crude extracts were stored at 4 °C for further experiments.

3.6.2. ABTS radical scavenging activity

The ABTS radical-scavenging activity of the extract was assessed using Re et al. (1999). Formation of the ABTS radical-cation (ABTS•+) was initiated through the reaction of 5 mL of ABTS stock solution with 5 mL of 2.45 mM potassium persulfate ($K_2S_2O_8$) solution, which was then kept in darkness at room temperature for a duration of 16 h. Prior to application, the solution underwent dilution with water to attain an absorbance of 0.700 ± 0.020 at 734 nm and was allowed to stabilize at 30°C. The mushroom extract was prepared at varying concentrations and further diluted with methanol to yield the sample solution. Subsequently, 20 µL of the sample solution was homogenised with 150 µL of ABTS• + solution, followed by a 6-minute incubation period at room temperature, with absorbance being measured at 734 nm. Blank samples were included in each analysis.

The ABTS scavenging ability was expressed as IC_{50} (µg/mL), and the inhibition percentage was calculated using the following formula:

ABTS scavenging activity (%) = $(A_0\text{-}A_1) / A_0 \times 100$

where,

A_0 is the absorbance of the control;

A_1 is the absorbance of the sample.

3.6.3. DPPH radical scavenging activity

The assessment of DPPH free radical scavenging capabilities was carried out utilizing the methodology proposed by Blois (1958) on 96-well microplates. The undiluted form of the mushrooms extracts obtained from mushrooms was adjusted to varying concentrations of 1000, 800, 600, 400, and 200 (µg/ mL) in methanol. Subsequently, 10 µL of 0.1 mM DPPH (dissolved in MeOH) was combined with 180 µL of the sample solutions at different concentrations to achieve a total volume of 190 µL. The mixtures were thoroughly blended in the 96-well plates, followed by an incubation period at 37°C for 30 minutes, and the absorbance was recorded at 515 nm using a UV spectrophotometer. The determination of the extract concentration necessary for 50% inhibition (IC_{50}) was performed to enable a comparison of scavenging activities. Ascorbic acid was used as a standard and positive control in this study.

The percentage of DPPH free radical scavenging activity was calculated as follows:

RSA on DPPH (%) = $[(A_{control} - A_{sample})/ (A_{control})] \times 100$

where:

A_{sample} = the absorbance of the extract/reference;

$A_{control}$ = the absorbance of the DPPH solution without the addition of extract.

3.7. Characterization of volatile compounds using GC-MS

The characterization of volatile compounds was done using GC-MS following the method of Dey et al. (2015). The GC-MS analysis was carried out using a Clarus 690 Perkin/Elmer (Auto system XL) Gas Chromatograph with a mass detector Turbo mass gold Perkin Elmer Turbomass 5.1 spectrometer and an Elite 1 (100% Dimethylpolysiloxane) capillary column measuring 123.5 M × 678 M. The instrument was fixed at an initial temperature of 40°C ramp 5°C/min to 115°C, hold 5 min, ramp 5°C/min to 140°C, hold 5 min, ramp 2°C/min to 210°C, hold 8 min, and maintained at this temperature for 3 min. At the end of this period, the oven temperature was increased up to 250°C, at the rate of 5°C/min, and maintained for 9 min. The injection port temperature was maintained at 250°C, while the flow rate of

Helium was kept at 1.5 mL/min. 70 eV was used as the ionisation voltage.

The samples were injected in a 10:1 split mode. The scan range for mass spectral scanning was set to 500-800 (m/z). The temperature of the ion source was ensured at 230°C, while the temperature of the interface was kept at 240°C. The MS start time was 3 min, and end time was 75 min with solvent cut time of 3 min. The spectrums obtained of volatile compounds detected the through GC-MS were compared and matched with NIST 17 online library Ver. 2.3.

4. RESULTS AND DISCUSSION

4.1.Identification of wild edible mushrooms

A total of 46 species of wild edible mushrooms (WEM) were identified based on a combination of morphological characteristics and molecular analyses. These species were distributed across 8 orders, 20 families, and 22 genera. These results were line with other studies such as those of Larinawmi et al. (2017) and Zothanzama et al. (2018). These studies earlier reported the occurrence of 27 wild edible mushroom species across different regions of Mizoram that were further elucidated on the basis of morphological characteristics. In the present study, a total of 46 edible mushroom species were identified, despite sampling being limited to only two districts, indicating a significant improvement in documentation and diversity assessment. Therefore, this study was an extension to earlier studies to offer knowledge advances. The complete list of 46 identified species, are presented in Table 4.1.

Table 4.1: List of wild edible mushrooms identified from Mamit and Champhai district

No	Species	Family (Order)	Collection Sites	Substrate
1	*Agaricus comtulus*	Agaricaceae (Agaricales)	Mamit	Soil
2	*Amanita jacksonii*	Amanitaceae (Agaricales)	Mamit	Soil
3	*Amanita vaginata*	Amanitaceae (Agaricales)	Mamit	Soil
4	*Auricularia auricula-judae*	Auriculariaceae (Auriculariales)	Mamit	Wood
5	*Auricularia delicata*	Auriculariaceae (Auriculariales)	Champhai	Wood

6	*Boletellus emodensis*	Boletaceae (Boletales)	Champhai	Soil
7	*Cantharellus cibarius*	Cantharellaceae (Cantharellales)	Mamit	Soil
8	*Cantharellus tropicalis*	Cantharellaceae (Cantharellales)	Mamit	Soil
9	*Coprinellus disseminatus*	Psathyrellaceae (Agaricales)	Mamit	Wood
10	*Dacrymyces spathularia*	Dacrymycetaceae (Dacrymycetales)	Mamit & Champhai	Wood
11	*Fistulina hepatica*	Fistulinaceae (Agaricales)	Mamit	Wood
12	*Lactifluus volemus*	Russulaceae (Russulales)	Mamit & Champhai	Soil
13	*Lactifluus corrugis*	Russulaceae (Russulales)	Mamit & Champhai	Soil
14	*Lactifluus dwaliensis*	Russulaceae (Russulales)	Champhai	Soil
15	*Lactifluus piperatus*	Russulaceae (Russulales)	Champhai	Soil
16	*Laetiporus sulphureus*	Laetiporaceae (Polyporales)	Mamit	Wood
17	*Lentinula lateritia*	Omphalotaceae (Agaricales)	Champhai	Wood
18	*Lentinus squarrosulus*	Polyporaceae (Polyporales)	Mamit & Champhai	Wood
19	*Lentinus tigrinus*	Polyporaceae (Polyporales)	Mamit	Wood
20	*Lentinus sajor-caju*	Polyporaceae (Polyporales)	Champhai	Wood
21	*Lentinus cladopus*	Polyporaceae	Champhai	Wood

		(Polyporales)		
22	*Lycoperdon pratense*	Lycoperdaceae (Agaricales)	Mamit	Soil
23	*Lycoperdon perlatum*	Lycoperdaceae (Agaricales)	Mamit	Soil
24	*Ramariopsis kunzei*	Clavariaceae (Agaricales)	Champhai	Soil
25	*Ramaria cystidiophora*	Gomphaceae (Gomphales)	Champhai	Soil
26	*Russula aurora*	Russulaceae (Russulales)	Mamit & Champhai	Soil
27	*Russula brevipes*	Russulaceae (Russulales)	Mamit & Champhai	Soil
28	*Russula compacta*	Russulaceae (Russulales)	Mamit & Champhai	Soil
29	*Russula subfragiliformis*	Russulaceae (Russulales)	Mamit & Champhai	Soil
30	*Russula rosea*	Russulaceae (Russulales)	Mamit & Champhai	Soil
31	*Russula cyanoxantha*	Russulaceae (Russulales)	Mamit & Champhai	Soil
32	*Russula sanguinea*	Russulaceae (Russulales)	Mamit & Champhai	Soil
33	*Russula crustosa*	Russulaceae (Russulales)	Mamit & Champhai	Soil
34	*Russula vesca*	Russulaceae (Russulales)	Mamit & Champhai	Soil
35	*Russula emetica*	Russulaceae (Russulales)	Mamit & Champhai	Soil
36	*Schizophyllum commune*	Schizophyllaceae (Agaricales)	Mamit & Champhai	Wood

37	*Strobilomyces strobilaceus*	Boletaceae (Boletales)	Champhai	Soil
38	*Termitomyces heimii*	Lyophyllaceae (Agaricales)	Mamit	Soil
39	*Termitomyces clypeatus*	Lyophyllaceae (Agaricales)	Mamit	Soil
40	*Tremella fuciformis*	Tremellaceae (Tremellales)	Mamit	Wood
41	*Pleurotus giganteus*	Pleurotaceae (Agaricales)	Mamit & Champhai	Wood
42	*Pleurotus djamor*	Pleurotaceae (Agaricales)	Mamit	Wood
43	*Pleurotus pulmonarius*	Pleurotaceae (Agaricales)	Mamit	Wood
44	*Pleurotus cystidiosus*	Pleurotaceae (Agaricales)	Mamit & Champhai	Wood
45	*Panus roseus*	Panaceae (Agaricales)	Mamit	Wood
46	*Volvariella taylorii*	Pluteaceae (Agaricales)	Mamit & Champhai	Soil

Morphological identification methods were used in general, however, few species of hidden taxonomic status were unveiled and validated through molecular analysis. The nucleotide sequences of the mushroom samples were compared against sequences in the GenBank database, leading to the identification of 11 representative fungal isolates from Mizoram. The GenBank accession numbers for the successfully sequenced samples were *Agaricus comtulus* (PX781190), *Auricularia delicata* (PV052801), *Lactifluus volemus* (PX781193), *Laetiporus sulphureus* (PP886450), *Lentinus squarrosulus* (PV052803), *Lycoperdon pratense* (PX781192), *Russula crustosa* (PX781191), *Pleurotus giganteus* (PX781194), *Pleurotus cystidiosus* (PX783212), *Panus roseus* (PX783211), and *Pleurotus pulmonarius* (PV052804).

Overall, these results confirm the accurate taxonomic identification of the collected mushroom specimens and further demonstrate the reliability and effectiveness of ITS sequencing as a molecular tool for distinguishing closely related fungal species.

The fruiting bodies of the 46 identified wild edible mushrooms collected from Mizoram are presented in Figures 4.1, 4.2, 4.3, and 4.4, illustrating the wide diversity of macrofungal forms, colors, textures, and characteristics observed during the study.

Figure 4.1: Fruiting bodies of wild edible mushrooms collected from Mizoram. A-E *Pleurotus giganteus*; F-I *Panus roseus*; J-L *Russula cyanoxantha*; M-O *Lycoperdon pratense*; P-Q *Agaricus comtulus*; R-S *Tremella fuciformis*; T *Russula crustosa*; U *Lycoperdon perlatum*

Figure 4.2: Fruiting bodies of wild edible mushrooms collected from Mizoram. A-C *Fistulina hepatica*; D-F *Cantharellus cibarius*; G-H *Lentinus sajor-caju*; I-L *Boletellus emodensis*; M-N *Coprinellus disseminatus*; O-P *Cantharellus tropicalis*; Q-R *Russula compacta*; S *Pleurotus pulmonarius*; T *Russula subfragiliformis*; U *Russula emetica*

Figure 4.3: Fruiting bodies of wild edible mushrooms collected from Mizoram. A *Russula aurora*; B *Volvariella taylorii*; C-E *Laetiporus sulphureus*; F-G *Schizophyllum commune*; H-I *Termitomyces heimii*; J *Ramaria cystidiophora*; K-L *Dacrymyces spathularia*; M *Lactifluus piperatus*; N *Lactifluus volemuss*; O-P *Russula sanguinea*; Q-R *Russula brevipes*; S-T *Russula vesca*

Figure 4.4: Fruitng bodies of Fruiting bodies of wild edible mushrooms collected from Mizoram. A-B *Strobilomyces strrobilaceus*; C-D *Amanita vaginata* E-G *Auricularia auricula-judae*; H *Auricularia delicata*; I *Lentinula lateritia*; J *Pleurotus djamor*; K *Lactifllus corrugis*; L *Lactifluus dwaliensis*; M-N *Amanita jacksonii*; O-Q *Lentinus squarrosulus*; R-S *Russula rosea*; T-U *Termitomyces clypeatus*; V *Lentinus tigrinus*; W *Lentinus cladopus*; X *Pleurotus cystidiosus*; Y *Ramariopsis kunzei*

Auricularia auricula-judae (Local name: Pu Vana Beng), *Auricularia delicata* (Local name: Pu Vana Beng), *Cantharellus tropicalis* (Local name: Maupa), *Lactifluus volemus* (Local name: Pa uithin), *Lactifluus dwaliensis* (Local name: Pa uithin), *Lactifluus corrugis* (Local name: Pa uithin), *Lactifluus piperatus* (Local name: Pa Lengvar), *Lentinula lateritia* (Local name: Pa Pal), *Lentinus squarrosulus* (Local name: Pa Chang), *Lentinus tigrinus* (Local name: Pa Hnahkhar), *Lentinus sajor-caju* (Local name: Pa Khangbun), *Ramariopsis kunzei* (Local name: Far Pa Var), *Ramaria cystidiophora* (Local name: Far Pa Eng), *Russula aurora* (Local name: Pa Lengvar), *Russula compacta* (Local name: Pa Lengvar), *Russula subfragiliformis* (Local name: Pa Lengsen), *Russula rosea* (Local name: Pa Lengsen), *Russula sanguinea* (Local name: Pa Lengsen) *Russula cyanoxantha* (Local name: Meihawl Pa), *Schizophyllum commune* (Local name: Pasi), *Termitomyces heimii* (Local name: Pasawntlung), *Termitomyces clypeatus* (Local name: Pasawntlung), *Pleurotus djamor* (Local name: Pa Chang Sen) *Pleurotus pulmonarius* (Local name: Pa chang hang), and *Volvariella taylorii* (Local name: Changel Pa) are consumed in certain parts of Mizoram.

With the increasing use of multilocus DNA sequencing and phylogenetic reconstruction, numerous taxa have undergone reclassification, with several species being transferred to different genera or reassigned to new families and orders. As a result, presenting the currently accepted higher level taxonomy is necessary to maintain systematic accuracy and ensure that the nomenclature reflects the most recent phylogenetic evidence.

To uphold taxonomic and nomenclatural integrity, all identifications and name validations were cross checked against “Index Fungorum, Species Fungorum, and MycoBank”, which are the internationally recognized databases for fungal taxonomy which help ensure that the identification of the 46 mushroom species is aligned with current fungal systematics.

Among the identified taxa, the order Agaricales was the most dominant, comprising 18 species (Figure 4.5). This was followed by the Russulales and Polyporales, which contributed fewer species but still represented significant portions of the fungal diversity. At the family level, Russulaceae was the most species-rich family, comprising 14 species. Polyporaceae and Pleurotaceae were the

next most represented families, each with 4 species, reflecting moderate family-level diversity within the studied ecosystem (Table 4.2).

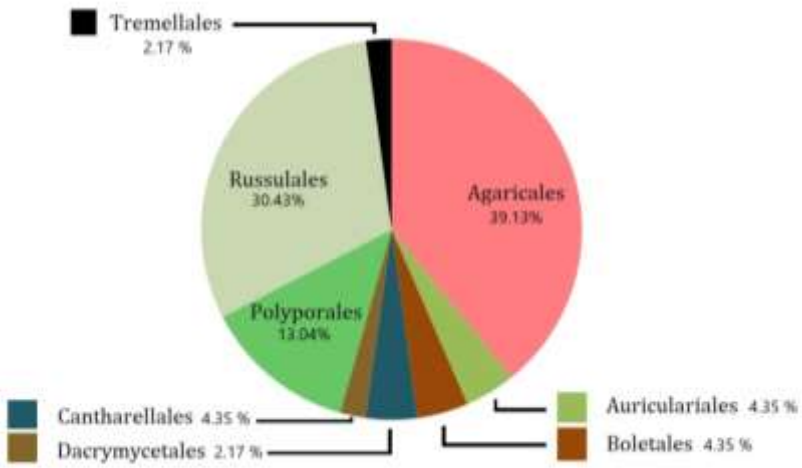

Figure 4.5: Order-wise percentage distribution of the identified wild edible mushroom species.

Table 4.2: Number of mushroom species recorded per family

Order	**Family**	**No. of Species**	**Distinct Features**
Agaricales	Agaricaceae	1	Characterized by free gills, central stipe, and frequent presence of an annulus; predominantly saprotrophic on soil or organic debris.
	Amanitaceae	2	Distinguished by the presence of a volva and universal veil remnants; mostly ectomycorrhizal with woody plants.
	Clavariaceae	2	Produces club- or coral-shaped basidiomes; mostly saprotrophic on soil or leaf litter; simple to intricately branched morphologies.
	Fistulinaceae	1	Moist basidiomes; mostly lignicolous, causing brown rot; produces separate tubes

			rather than a unified pore surface.
	Lycoperdaceae	2	Characterized by gasteroid (puffball-type) basidiomes; gleba transitions from firm to powdery at maturity; spores dispersed through an apical opening.
	Lyophyllaceae	2	Forms clustered, fleshy basidiomes; gilled hymenophore; exhibits ecological diversity including saprotrophic and mycorrhizal lifestyles.
	Pleurotaceae	4	Characterized by lateral or eccentric stipes and decurrent gills; lignicolous and saprotrophic; efficient decomposers of wood.
	Pluteaceae	1	Free gills and pink spore prints; saprotrophic on decaying wood and humus; basidiomes lack a volva but often possess a distinct pileus margin.
	Psathyrellaceae	1	Contains fragile basidiomes; many taxa exhibit autodigestion (deliquescence) of gills; saprotrophic on nutrient-rich substrates.
	Omphalotaceae	1	Basidiomes with strongly decurrent gills; saprotrophic on woody substrates; some taxa exhibit bioluminescence.
	Schizophyllaceae	1	Distinguished by tough, split gills capable of rehydration; highly resilient wood decomposers; biotechnologically significant hyphal system.

Auriculariales	Auriculariaceae	2	Comprises gelatinous, ear or cup shaped basidiomes; primarily wood decaying and saprotrophic; produces longitudinally septate basidia.
Boletales	Boletaceae	2	Poroid hymenophores; basidiomes typically fleshy; largely ectomycorrhizal and symbiotic with forest trees.
Cantharellales	Cantharellaceae	2	Possesses smooth, wrinkled, or ridge like hymenophores (false gills); forms obligate ectomycorrhizal associations; basidiomes often brightly pigmented.
Dacrymycetales	Dacrymycetaceae	1	Composed of yellow to orange, gelatinous fruiting bodies; characterized by tuning fork basidia; saprotrophic on decaying wood.
Russulales	Russulaceae	14	Brittle, chalky basidiomes due to sphaerocysts; ectomycorrhizal with diverse forest hosts; latex exudation common in some genera.
Polyporales	Laetiporaceae	1	Brightly colored, shelf-like polypores; primarily causes brown rot of wood; hymenophore poroid.
	Panaceae	1	Contains small, saprotrophic basidiomes with dark colored spores.
	Polyporaceae	4	Wood-decaying bracket fungi with tough, perennial to annual basidiomes; poroid hymenophore; major agents of white rot, decomposing lignin.

Tremellales	Tremellaceae	1	Produces gelatinous basidiomes; often parasitic on other fungi, particularly corticioid hosts; basidia are typically tremelloid and septate.

4.1.1. Edibiltiy status of the identified wild edible mushroom

According to Liu et al. (2018), most of the species identified in this study are classified as E1 (Edible, confirmed), while some fall under the E2 (Edible, confirmed but with conditions) in the final edibility status (FES), which are assigned based on case reports and consumption frequency. In this system, total reports indicate the number of case reports considered for each species. FES represents the confirmed edibility classification.

Among the identified taxa, 42 species are listed as E1, while 4 species are not considered safe for direct consumption and being listed as E2 (Table 4.3). Although some literature reports these species as edible, other studies have documented them as poisonous, highlighting the need for caution.

Table 4.3: List of identified taxa categorized according to the evidence-based edibility classification system [E1, E2, E3, P], highlighting the 4 species assigned to the E2 category.

Species	**E1**	**E2**	**E3**	**P**	**Total**	**FES**
Amanita vaginata	19	4	10	2	35	E2
Coprinellus disseminatus	5	4	1	0	10	E2
Lycoperdon pratense	7	1	1	0	9	E2
Lycoperdon perlatum	19	4	1	0	24	E2

Note: E1 = Edible, confirmed; E2 = Edible, confirmed but with conditions; E3 = Edible, unconfirmed; P = Poisonous.

Lycoperdon pratense and *Lycoperdon perlatum* are considered edible only at the young developmental stage, when the fruiting bodies contain white, firm gleba,

and are recognised as culinary favourites in several countries (Falandysz et al., 2012). *Amanita vaginata* is traditionally consumed as a delicacy in parts of the Mekong region of Asia (Mortimer et al., 2014). *Coprinellus disseminatus* has also been reported as edible and is consumed in various countries owing to its favourable nutritional value (Novaković et al., 2018; Fabros and Dulay, 2025); however, other studies have reported this species to be poisonous (Li et al., 2021). Therefore, these 4 species are classified under E2 in the Final Edibility Status.

4.2. Nutritional value of wild edible mushrooms

Among 46 identified edible mushroom species, 23 were analyzed for their proximate and mineral content. These species include *Auricularia delicata*, *Laetiporus sulphureus*, *Panus roseus*, *Lentinus squarrosulus*, *Pleurotus cystidiosus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, *Schizophyllum commune*, *Lactifluus volemus*, *Lactifluus corrugis*, *Dacrymyces spathularia*, *Auricularia auricula-judae*, *Cantharellus cibarius*, *Lactifluus piperatus*, *Lentinula lateritia*, *Russula crustosa*, *Russula cyanoxantha*, *Russula subfragiliformis*, *Termitomyces heimii*, *Tremella fuciformis*, *Volvariella taylorii*, *Pleurotus giganteus* and *Lycoperdon pratense*. The selection of these species was guided by availability of adequate sample quantity, consistency of occurrence across collection sites, and suitability for standardized biochemical analysis. Species that were rare, seasonally restricted, or available only in small quantities were excluded to ensure analytical reliability and reproducibility of results.

The proximate composition (macro-nutrient), including moisture, protein, fat, fibre, ash, carbohydrates (CHO), and caloric values, of the 23 wild edible mushroom species collected from the Mamit and Champhai districts is presented in Table 4.4.

Table 4.4: Proximate composition of wild edible mushrooms (g/100g | %) in dry weight basis

Mushroom specimen	**Moisture (%)**	**Protein (%)**	**Fat (%)**	**Fiber (%)**	**Ash (%)**	**CHO (%)**	**Caloric values (Kcal/100g)**

Auricularia delicata	12.39 ± 0.18	17.79 ± 1.01	1.96 ± 0.16	6.52 ± 0.20	6.31 ± 0.07	54.73 ± 1.46	293.3 ± 2.53
Laetiporus sulphureus	9.25 ± 0.19	21.29 ± 0.50	2.05 ± 0.14	7.01 ± 0.32	6.58 ± 0.31	54.17 ± 0.53	300.5 ± 1.96
Panus roseus	11.83 ± 0.12	36.46 ± 0.50	2.20 ± 0.09	7.99 ± 0.34	7.74 ± 0.18	33.77 ± 0.56	255.8 ± 1.49
Lentinus squarrosulus	10.24 ± 0.11	34.10 ± 0.87	2.67 ± 0.17	8.32 ± 0.54	7.18 ± 0.14	36.58 ± 1.00	268.7 ± 4.67
Pleurotus cystidiosus	10.19 ± 0.11	33.54 ± 1.34	2.15 ± 0.19	6.03 ± 0.19	5.84 ± 0.12	42.24 ± 1.14	278.0 ± 9.35
Pleurotus djamor	10.21 ± 0.04	32.96 ± 0.50	2.71 ± 0.16	8.29 ± 0.24	7.04 ± 0.41	38.35 ± 0.43	270.1 ± 3.45
Pleurotus pulmonarius	11.36 ± 1.11	34.12 ± 0.88	2.77 ± 0.13	8.21 ± 0.14	5.98 ± 0.12	38.19 ± 0.64	272.8 ± 1.36
Schizophyllum commune	8.06 ± 0.05	19.59 ± 0.47	1.93 ± 0.21	5.63 ± 0.67	6.99 ± 0.19	58.14 ± 1.45	310.7 ± 2.53
Lactifluus volemus	10.72 ± 0.16	33.25 ± 0.50	2.8 ± 0.07	11.95 ± 0.13	7.18 ± 0.06	34.02 ± 0.53	253.43 ± 1.38

Lactifluus corrugis	11.02 ± 0.07	34.10 ± 0.09	3.44 ± 0.04	9.03 ± 0.15	7.03 ± 0.10	35.37 ± 0.25	266.77 ± 0.64
Dacrymyces spathularia	12.28 ± 0.08	19.27 ± 0.06	2.10 ± 0.03	7.29 ± 0.05	6.08 ± 0.03	52.98 ± 0.08	291.42 ± 0.06
Auricularia auricula-judae	12.72 ± 0.08	16.33 ± 0.29	2.27 ± 0.04	5.94 ± 0.22	7.08 ± 0.11	55.64 ± 0.15	295.86 ± 1.09
Cantharellus cibarius	10.7 ± 0.03	23.33 ± 0.29	2.54 ± 0.05	8.05 ± 0.05	6.67 ± 0.1	48.69 ± 0.44	286.94 ± 0.74
Lactifluus piperatus	10.12 ± 0.03	26.54 ± 0.29	2.63 ± 0.02	6.63 ± 0.03	6.24 ± 0.05	47.82 ± 0.33	293.26 ± 0.42
Lentinula lateritia	11.17 ± 0.02	28.29 ± 0.58	2.69 ± 0.02	7.4 ± 0.02	6.91 ± 0.01	43.52 ± 0.57	279.44 ± 0.93
Russula crustosa	9.54 ± 0.05	25.95 ± 0.29	3.87 ± 0.04	7.08 ± 0.02	7 ± 0.07	46.57 ± 0.25	295.73 ± 0.42
Russula cyanoxantha	12.10 ± 0.03	16.04 ± 0.29	2.11 ± 0.03	5.97 ± 0.03	7.11 ± 0.07	56.63 ± 0.32	294.74 ± 2.30
Russula subfragiliformis	10.96 ± 0.04	25.66 ± 0.29	2.67 ± 0.03	6.98 ± 0.06	6.97 ± 0.02	46.94 ± 0.38	286.73 ± 1.04

Termitomyces heimii	12.71 ± 0.09	31.79 ± 0.29	2.14 ± 0.05	10.03 ± 0.38	7.39 ± 0.06	35.92 ± 0.45	252.15 ± 1.71
Tremella fuciformis	9.3 ± 0.14	26.83 ± 0.29	3.37 ± 0.19	7.22 ± 0.13	6.01 ± 0.06	47.27 ± 0.24	296 .91 ± 1.68
Volvariella taylorii	9.7 ± 0.05	32.08 ± 0.29	3.03 ± 0.04	5.7 ± 0.13	7.81 ± 0.10	41.67 ± 0.47	284.51 ± 1.04
Pleurotus giganteus	9.73 ± 0.09	23.04 ± 1.34	2.46 ± 0.09	7.98 ± 0.42	7.52 ± 0.09	49.19 ± 1.75	288.13 ± 1.63
Lycoperdon pratense	8.76 ± 0.07	16.33 ± 0.29	3.34 ± 0.05	7.35 ± 0.14	6.6 ± 0.08	57.43 ± 0.54	312 ± 1.32

Note: Each value is expressed in mean ± SEM, (n = 3)

The highest moisture content was recorded in *Auricularia auricula-judae* (12.72 g/100 g), whereas the lowest moisture content was observed in *Lycoperdon pratense* (8.76 g/100 g). Protein contents of the studied wild edible mushrooms were generally high and the maximum protein content was found in *Panus roseus* (36.46 g/100 g), while the minimum protein level was obtained in *Russula cyanoxantha* (16.04 g/100 g). With respect to fat content, *Russula crustosa* exhibited the highest value (3.87 g/100 g), whereas *Schizophyllum commune* showed the lowest fat content (1.93 g/100 g). The highest crude fibre content was recorded in *Lactifluus volemus* (11.95 g/100 g), while the lowest fibre content was observed in *Volvariella taylorii* (5.70 g/100 g). Ash content was observed highest in *Volvariella taylorii* (7.81 g/100 g) while it was recorded lowest in *Pleurotus cystidiosus* (5.84 g/100 g). The maximum carbohydrate content was recorded in *Russula cyanoxantha* (56.63 g/100

g), whereas the minimum carbohydrate level was noted in *Lactifluus volemus* (34.02 g/100 g). In terms of energy value, *Lycoperdon pratense* recorded the highest caloric content (312 kcal/100 g), while the lowest caloric value was observed in *Termitomyces heimii* (252.15 kcal/100 g).

Wild edible mushrooms respond to a range of environmental cues that regulate the timing and spatial occurrence of sexual reproduction and fruiting body formation, thereby optimising spore dispersal efficiency (Sakamoto, 2018). Moisture content is a key determinant of both physiological integrity and post harvest quality, as elevated moisture levels enhance susceptibility to microbial proliferation and enzymatic degradation (Johnsy et al., 2011). Fresh edible mushrooms generally consist of approximately 90% moisture and 10% dry matter, whereas dried mushrooms display an inverse composition, with about 90% dry matter and 10% moisture (Kurtzman, 1975; Crisan and Sands, 1978). Consequently, moisture content plays a central role in influencing the physical stability, chemical composition, and overall nutritional quality of wild mushrooms (Shams et al., 2022).

The nutritional composition of wild edible mushrooms is strongly influenced by substrate characteristics, environmental conditions, and developmental stage at harvest. Because mushrooms acquire nutrients directly from their growth substrates, variations in substrate composition can markedly affect levels of protein, carbohydrates, dietary fibre, minerals, and bioactive compounds (Kalac, 2016; Barros et al., 2007). In addition, environmental factors such as temperature, humidity, and seasonal availability, along with harvest maturity, contribute to substantial nutritional variability. As a result, significant differences in proximate composition may occur even within the same species across different habitats, seasons, and geographic regions.

Mushrooms are widely endowed with an average protein content of 23.80 g ± 9.82 g/100g dry weight that are known for having more naturally occurring bioactive proteins and peptides than most widely consumed vegetables (Zhou et al., 2020). In sense of present study, *Lentinus roseus, Lentinus squarrosulus, Pleurotus cystidiosus, Pleurotus djamor* and *Pleurotus pulmonarius* showed similar protein level which was in agreement with the results of Chang and Buswell (1996). Analysis of *Laetiporus sulphureus* by Teke et al. (2021) showed 8.62 g/100 g crude

protein which is substantially low in comparison to the protein content (21.29 g/100 g) obtained in the Indian sample of *Laetiporus sulphureus*. However, it is known that protein content varied widely according to type of mushrooms, the level of nutrient like nitrogen (Flegg and Maw 1977) and location (Zakhary et al. 1983).

Nwanze et al. (2006) documented considerably higher fat content in *Pleurotus pulmonarius* stipe (6.01 g/100 g) and pileus (6.56 g/100 g). Low results were found for fat content in agreement with several studies on different mushrooms species (Agrahar-Murugkar and Subbulakshmi, 2005; Pushpa and Purushothama, 2010). Mushrooms are low in fat but rich in essential unsaturated fatty acids which are necessary for human diet and health (Nakalembe et al., 2015). The low fat content in the studied wild edible mushrooms can be attributed to their high moisture contents. This high protein and low fat characteristic of the edible mushrooms has been previously reported (Lallawmsanga et al., 2016; Khumlianlal et al., 2022).

The studied mushrooms showed similar fiber content to earlier reports of Silva et al. (2002) for *Pleurotus pulmonarius*, Rai and Sohi (1988) for *Lentinus* species, Raman et al. (2020) for *Pleurotus djamor* and Luangharn et al. (2014) for *Laetiporus sulphureus*. Fibers in mushrooms can promote healthy digestion, aids in maintaining a healthy weight and supports heart health.

The value of ash content is a key to determine the minerals residue that remains after the organic matter has been completely combusted and ash itself does not provide direct macronutrients value of the mushrooms. However, it is an indication of the mineral content of the mushrooms. The obtained values of ash were similar to the report of Khan et al. (2013) for *Pleurotus djamor*, Okwulehie et al. (2014) for *Pleurotus pulmonarius*, Ao and Deb (2019) for *Schizophyllum commune* and *Laetiporus sulphureus* respectively. Wide variation in ash content can occurred due to the fruiting body of mushrooms (Kalmis et al. 2011) where young fruiting bodies contained higher content of ash than the matured ones.

Carbohydrate content appeared to be abundant in all tested samples and constitute the main nutritional value of mushroom. It have been observed that the obtained results of *Schizophyllum commune* is reasonably higher, when compared with previous studies (32.43 g/100 g) of Kumar et al. (2013) and seemed similar to those reports by Rampinelli et al. (2010) for *Pleurotus djamor* and Vishwakarma et

al. (2017) for *Pleurotus cystidiosus*. Conversely, wild edible mushrooms are a good source of carbohydrate and their contents varies greatly and generally range from 28.38 g/100 g to 84.48 g/100 g of dry weight (Wang et al. 2001; Pushpa and Purushothama 2010; Shin et al. 2007). Carbohydrates are an essential part of a healthy diet and provide an important source of energy for human body. Energy values were in agreement with the results of Manjunathan and Kaviyarasan (2010) and Nakalembe et al. (2015). However, energy values (kcal) of mushrooms were calculated based on protein, carbohydrate and fat contents. The carbohydrates, caloric values and other macro-nutrients contents are almost consistent with the values reported earlier (Ouzouni and Riganakos, 2007; Khan et al., 2013; Liu et al., 2016; Sifat et al., 2020; Jacinto-Azevedo et al., 2021). The mineral content (micro-nutrient) of the 23 collected wild edible mushrooms is presented in Table 4.5.

Table 4.5: Mineral contents of wild edible mushroom (mg/100g) in dried weight basis

Mushroom specimen	**Ca**	**Fe**	**K**	**Na**	**Zn**
Auricularia auricula judae	1.6 ± 0.03	5.4 ± 0.04	1190 ± 0.3	18.3 ± 0.04	6.2 ± 0.02
Cantharellus cibarius	5.6 ± 0.02	14.6 ± 0.03	2110 ± 0.2	31.3 ± 0.02	17.2 ± 0.03
Lactifluus piperatus	14.1 ± 0.04	22.4 ± 0.04	1760 ± 0.4	38.6 ± 0.02	31.5 ± 0.03
Lentinula lateritia	6.9 ± 0.03	6.7 ± 0.02	1470 ± 0.1	28.1 ± 0.04	18.1 ± 0.01
Russula crustosa	2.8 ± 0.04	14.3 ± 0.06	1230 ± 0.2	27.6 ± 0.03	17.1 ± 0.04
Russula cyanoxantha	3.5 ± 0.02	13.7 ± 0.02	1280 ± 0.2	29.7 ± 0.02	17.4 ± 0.03
Russula subfragiliformis	5.4 ± 0.04	15.7 ± 0.04	1470 ± 0.3	24.1 ± 0.02	11.8 ± 0.02
Termitomyces heimii	11.1 ± 0.02	26.8 ± 0.02	2240 ± 0.2	24.5 ± 0.02	26.5 ± 0.06

Tremella fuciformis	1.5 ± 0.04	3.5 ± 0.03	1160 ± 0.5	14.7 ± 0.04	4.7 ± 0.03
Volvariella taylorii	8.3 ± 0.03	17.6 ± 0.03	1890 ± 0.3	21.2 ± 0.05	25.7 ± 0.04
Pleurotus giganteus	16.522 ± 0.03	15.25 ± 0.02	1618.7 ± 0.3	14.313 ± 0.02	23.318 ± 0.04
Lycoperdon pratense	12.734 ± 0.05	16.72 ± 0.05	1312.9 ± 0.4	13.712 ± 0.04	12.803 ± 0.013
Auricularia delicata	5.744 ± 0.098	6.506 ± 0.088	1204.09 ± 3.33	17.928 ± 0.073	7.568 ± 0.057
Laetiporus sulphureus	17.961 ± 0.077	20.374 ± 0.064	1752.13 ± 4.07	15.758 ± 0.068	16.542 ± 0.097
Panus roseus	9.878 ± 0.041	11.482 ± 0.083	1252.33 ± 1.43	11.583 ± 0.039	18.195 ± 0.082
Lentinus squarrosulus	22.122 ± 0.072	21.691 ± 0.128	1925.50 ± 0.7	14.812 ± 0.068	22.161 ± 0.107
Pleurotus cystidiosus	19.516 ± 0.092	17.404 ± 0.066	1457.28 ± 1.32	12.419 ± 0.029	13.495 ± 0.029
Pleurotus djamor	14.443 ± 0.068	15.706 ± 0.065	1422.68 ± 0.68	14.56 ± 0.064	16.21 ± 0.029
Pleurotus pulmonarius	16.308 ± 0.078	14.334 ± 0.055	1821.74 ± 1.4	16.625 ± 0.082	8.541 ± 1.18
Schizophyllum commune	7.841 ± 0.095	7.022 ± 0.088	1642.39 ± 2.22	21.558 ± 0.084	3.697 ± 0.063
Lactifluus volemus	21.374 ± 0.064	10.286 ± 0.013	1241.78 ± 0.15	14.588 ± 0.012	24.494 ± 0.01
Lactifluus corrugis	16.929 ± 0.026	14.277 ± 0.037	1448.67 ± 0.77	12.664 ± 0.007	26.181 ± 0.022
Dacrymyces spathularia	3.878 ± 0.027	9.796 ± 0.027	1291.50 ± 0.38	13.155 ± 0.01	11.246 ± 0.023

Note: Each value is expressed in mean ± SEM, (n = 3)

From the mineral analysis, it was observed that potassium dominated the elemental composition, whereas sodium, zinc, and iron showed relatively similar contents (K >> Na ≈ Zn ≈ Fe > Ca), and calcium occurred at the lowest levels across the studied mushroom species. Calcium (Ca) content ranged widely, with the highest value recorded in *Lentinus squarrosulus* (22.12 mg/100 g), and closely followed by *Lactifluus volemus* (21.37 mg/100 g), whereas the lowest Ca content was observed in *Tremella fuciformis* (1.5 mg/100 g) and *Auricularia auricula-judae* (1.6 mg/100 g). Ca, however, exhibited the lowest mineral content, consistent with previous findings of Gałgowska and Pietrzak-Fiećko (2020).

Termitomyces heimii exhibited the highest Iron (Fe) content (26.8 mg/100 g), indicating its richness in this essential micronutrient, while the lowest Fe content was found in *Tremella fuciformis* (3.5 mg/100 g). Adequate Fe intake is vital for preventing anaemia, as this essential nutrient is required for haemoglobin synthesis, oxygen transport throughout the body, and reducing fatigue and weakness (Abbaspour et al., 2014). In support of this, Mallikarjuna et al. (2013) reported Fe contents ranging from 62.7 mg/kg to 353 mg/kg in both wild and cultivated mushroom species, which are substantially higher than the values observed in the present study. Similarly, Salihović et al. (2021) documented Fe levels between 12.72 mg/kg and 213.07 mg/kg, highlighting the variability of Fe accumulation among different mushroom taxa.

In the present study, the highest potassium (K) concentration was observed in *Termitomyces heimii* (2240 mg/kg), followed by *Cantharellus cibarius* (2110 mg/kg), highlighting the potential of these species as significant dietary sources of potassium. In contrast, the lowest K content was observed in *Tremella fuciformis* (1160 mg/100 g). K was the most abundant mineral across all studied mushroom species. Adequate potassium intake is important, as it helps reduce tension in blood vessels and can contribute to lowering blood pressure (Singh et al., 2025). Multiple K salts are also used therapeutically for various health indications, making dietary potassium a key factor in blood pressure management (McLean and Wang, 2021).

The sodium (Na) content was comparatively low in all species. In this vein, *Lactifluus piperatus* showed the highest Na level (38.6 mg/100 g), while the lowest value was noted in *Panus roseus* (11.58 mg/100 g). Sodium plays a crucial role in

maintaining fluid balance, nerve conduction, and muscle function; however, excessive intake can increase blood pressure and cardiovascular risk (Kazi et al., 2025). Therefore, the moderate Na content in these mushrooms suggests they are a favorable dietary source for maintaining electrolyte balance without contributing to sodium-related health issues (Grillo et al., 2019).

Lactifluus piperatus (31.5 mg/100 g) was found to be highest in zinc (Zn), followed by *Termitomyces heimii* (26.5 mg/100 g) and *Lactifluus corrugis* (26.18 mg/100 g). The lowest Zn content was recorded in *Schizophyllum commune* (3.7 mg/100 g). Zn is an essential micronutrient required for growth, immune function, and cellular processes. It plays a fundamental role in maintaining organ system health, supporting development, and facilitating tissue repair through a finely regulated balance of absorption and elimination in the body (Gu and Lin, 2010; Sangeetha et al., 2022). The Zn contents of previous studies were between 10.4 mg/kg to 93.8 mg/kg (Kakoti et al., 2021) and 56.5 mg/kg to 1132 mg/kg (López et al., 2022). It is also known that the cap contained higher Zn levels than in the stipe (Elekes et al., 2010). Zn as well as other essential minerals and metals are concentrated in the cap where the reproductive functions, including spore production take place.

Termitomyces heimii, *Lentinus squarrosulus*, and *Lactifluus piperatus* emerged as mineral rich species, while *Tremella fuciformis* and *Auricularia auricula-judae* showed comparatively lower mineral contents. The mineral profile of mushrooms is likewise highly variable and largely dependent on soil type, habitat conditions, and prevailing climatic factors. Wild mushrooms collected from urban or industrial areas contaminated with toxic substances may accumulate elevated concentrations of heavy metals, including nickel (Ni), cadmium (Cd), and lead (Pb), which can pose potential health risks to consumers (Haro et al., 2020). During growth, mushrooms possess a strong capacity to absorb and bioaccumulate mineral elements from their substrates, often incorporating them into functional organic complexes (Kalač, 2010). Mineral nutrients are indispensable for sustaining life and play crucial roles in enzymatic activity, metabolic regulation, cellular structure, and molecular signalling at biological, chemical, and molecular levels.

4.2.1. Comparisons with other foods

The mean proximate composition of the 23 wild edible mushroom species was 45.9% in carbohydrates, protein (26.47%), moisture (10.65%), fiber (7.5%), ash (6.83%), and fat (2.6%), with a mean caloric value of 283.8 Kcal/100 g. Wild edible mushrooms are increasingly recognized as nutrient-dense foods and can serve as alternative sources of protein, carbohydrates, and dietary fibre when compared with conventional foods such as chicken breast (Ahmad et al., 2018) and pumpkin seeds extract (*Cucurbita pepo*) (Elinge et al., 2012), as well as plant-based sources such as almonds (*Prunus dulcis*; nuts, almonds, whole, raw; FDC ID: 2346393, NDB Number: 12061) and chickpeas (*Cicer arietinum*; garbanzo beans, Bengal gram, dry; FDC ID: 2644282, NDB Number: 100311) (FoodData Central, 2025). The comparisons are presented in Table 4.6.

Table 4.6: Comparative proximate composition of wild edible mushrooms and selected foods (per 100 g in protein, fat, carbohydrate, fibre; Kcal/100g in energy)

Food item	**Protein**	**Fat**	**CHO**	**Fibre**	**Energy**
Mean of 23 edible mushrooms	26.47	2.60	45.90	7.50	283.82
Termitomyces heimii	31.79	2.14	35.92	10.03	252.15
Chicken breast	24.2	8.5	0.0	0.0	178
Pumpkin seeds	27.48	38.0	28.03	1.0	564
Almonds	21.4	51.1	20.0	10.8	626
Chickpeas	21.3	6.27	60.4	7.6	383

Notes: Values for wild edible mushrooms were derived from the present study. Nutritional data for almonds and chickpeas were obtained from USDA (United States Department of Agriculture) FoodData Central (2025), while the proximate composition values for chicken breast (Ahmad et al., 2018) and pumpkin seeds (Elinge et al., 2012) were compiled.

The 23 species mean protein content was 26.47%, which is comparable to or higher than that of chicken breast (24.2%), pumpkin seeds (27.48%), chickpeas (21.3%), and almonds (21.4%). In this regard, *Termitomyces heimii* exhibited a

protein content of 31.79%, surpassing both conventional protein sources and the 23 species mean value of wild edible mushroom. In contrast, the 23 species mean fat content was low (2.6%), considerably lower than chicken breast (8.5%), pumpkin seeds (38%), chickpeas (6.27%), and almonds (51.1%), while *Termitomyces heimii* showed an even lower fat content of 2.14%. Dietary fibre, an important component for human health, was also higher in mushrooms, with a 23 species mean of 7.5%, compared to chicken breast (0%), pumpkin seeds (1%), chickpeas (7.6%), and almonds (10.8%), while *Termitomyces heimii* contained 10.03%.

The 23 species mean carbohydrate content was 46.9%, providing moderate energy compared with chicken breast (0%), pumpkin seeds (28.03%), chickpeas (60.4%), and almonds (20%), with *Termitomyces heimii* slightly below the mean at 35.92%. These results highlight that wild edible mushrooms, including *T. heimii*, are a nutrient-rich food group, offering a combination of high protein, moderate carbohydrates, low fat, and significant dietary fibre, making them a valuable alternative or complement to conventional protein and carbohydrate sources.

The mean caloric value of the 23 wild edible mushroom species analysed was 283.82 kcal/100 g, indicating that mushrooms are generally low- to moderate-energy foods. Among the species studied, *Termitomyces heimii* recorded a comparatively lower caloric value (252.15 kcal/100 g), further emphasising the limited energy contribution of individual mushroom species. When compared with common animal and plant-based foods, mushrooms exhibit a markedly lower caloric density. For instance, chicken breast provides 178 kcal/100 g, representing a lean animal protein source, whereas plant-based foods such as chickpeas (383 kcal/100 g), pumpkin seeds (564 kcal/100 g), and almonds (626 kcal/100 g) are considerably more energy-dense, largely due to their higher carbohydrate and lipid contents. These observations are consistent with earlier reports demonstrating that mushrooms contain appreciable levels of protein and carbohydrates but minimal fat, resulting in a low caloric density. Consequently, mushrooms may be regarded as nutrient-rich, low-energy foods, making them particularly suitable for weight management and health-conscious dietary interventions (Sande et al., 2019; Pashaei et al., 2024; Mayirnao et al., 2025).

The mineral composition of wild edible mushrooms revealed appreciable

levels of essential macro- and micro-elements (Table 4.7).

Table 4.7: Comparative mineral composition of wild edible mushrooms and selected foods (mg/100g)

Food item	**Calcium**	**Iron**	**Potassium**	**Sodium**	**Zinc**
Mean of 23 edible mushrooms	10.7	13.98	1530.07	19.64	16.55
Termitomyces heimii	11.1	26.8	2240	24.5	26.5
Chicken breast	–	1.2	–	71	0.9
Pumpkin seeds	9.78	3.75	237.24	170	14.14
Almonds	254	3.74	733	<2.5	2.86
Chickpeas	111	5.09	1070	9	3.12

Notes: Values for wild edible mushrooms are derived from the present study. Data for chicken breast, almonds, and chickpeas were obtained from USDA (United States Department of Agriculture) FoodData Central (2025), while pumpkin seed and chicken breast proximate values were compiled from Ahmad et al. (2018) and Elinge et al. (2012). Dashes (–) indicate values not reported or not applicable.

The mean values of the 23 species showed moderate calcium (10.7 mg/100 g) and sodium (19.64 mg/100 g) contents, along with relatively higher concentrations of iron (13.98 mg/100 g), potassium (1530.07 mg/100 g), and zinc (16.55 mg/100 g). *Termitomyces heimii* contained high mineral contents, such as 11.1 mg/100 g of calcium, 26.8 mg/100 g of iron, 2240 mg/100 g of potassium, 24.5 mg/100 g of sodium, and 26.5 mg/100 g of zinc. These minerals play vital physiological roles in maintaining electrolyte balance, muscle contraction, nerve transmission, cardiovascular function, bone and teeth development, and the maintenance of cellular homeostasis (Jomova et al., 2022). In addition, adequate intake of essential minerals supports enzymatic reactions, strengthens the immune system, aids oxygen transport through haemoglobin formation, and contributes to antioxidant defence mechanisms that protect cells from oxidative stress. Their presence in wild edible mushrooms

therefore highlights the broader nutritional and health promoting potential of these species (Liuzzi et al., 2023).

When compared with conventional foods, wild edible mushrooms exhibited a competitive mineral composition. Potassium content was observed to be higher than chicken breast and pumpkin seeds and still comparable to almonds and chickpeas. Although calcium levels in mushrooms were lower than those in almonds and chickpeas. Overall, the mineral composition of wild edible mushrooms highlights their potential contribution to meeting daily mineral requirements and their value as nutritionally beneficial components of balanced human diets.

4.3. Isolation of pure culture from wild edible mushrooms

A total of 16 species of wild edible mushrooms were successfully cultured on various agar-based media to evaluate their mycelial growth and cultural characteristics. Potato Dextrose Agar (PDA) proved to be the most effective, supporting the optimal growth of 12 out of the 16 species. Sabouraud Dextrose Agar (SDA) supported the growth of 1 species, while Malt Extract Agar (MEA) was effective for 3 species, indicating some species-specific preferences for nutrient composition and growth conditions.

Based on their ecological characteristics, these 16 species were further classified into two main groups, 9 species were identified as wood rotting fungi, which predominantly colonize and decompose lignocellulosic substrates, while the remaining 7 species were categorized as soil-inhabiting mushrooms, which are typically saprotrophic on organic matter in the soil. Interestingly, the wood rotting fungi generally produced more vigorous and uniform mycelial growth, making them easier to obtain as pure cultures on the media used. This observation suggests that nutrient rich agar provides optimal conditions for lignocellulose degrading species, likely due to their efficient utilization of complex carbohydrates and ability to secrete extracellular enzymes such as cellulases and ligninases. In contrast, soil inhabiting mushrooms exhibited slower and sometimes irregular growth, reflecting their adaptation to heterogeneous substrates and reliance on specific organic matter components.

The pure cultures obtained includes species such as *Russula aurora*,

Fistulina hepatica, *Laetiporus sulphureus*, *Lentinus sajor-caju*, *Cantharellus cibarius*, *Pleurotus giganteus*, *Lentinus squarrosulus*, *Dacrymyces spathularia*, *Russula cyanoxantha*, *Cantharellus tropicalis*, *Schizophyllum commune*, *Russula crustosa*, *Pleurotus djamor*, *Auricularia auricula-judae*, *Pleurotus pulmonarius*, and *Lactifluus volemus*, as presented in Figure 4.6.

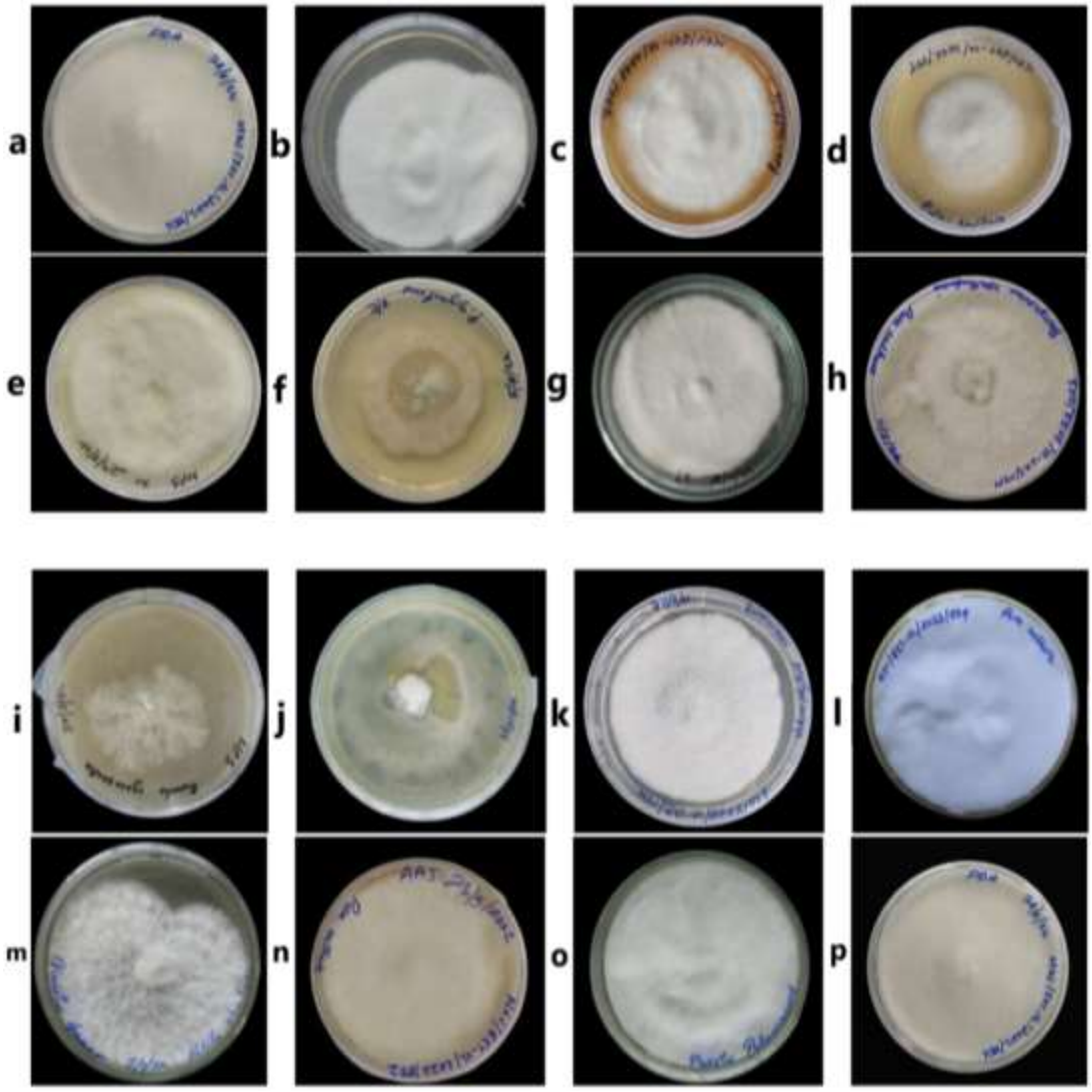

Figure 4.6: Pure cultures of wild edible mushrooms grown on various agar based media - a) *Russula aurora* (MEA); b) *Fistulina hepatica* (PDA); c) *Laetiporus sulphureus* (PDA); d) *Lentinus sajor-caju* (PDA); e) *Cantharellus cibarius* (PDA); f) *Pleurotus giganteus* (PDA);g) *Lentinus squarrosulus* (PDA); h) *Dacrymyces spathularia* (PDA); i) *Russula cyanoxantha* (MEA); j) *Cantharellus tropicalis* (MEA); k) *Schizophyllum commune* (PDA); l) *Russula crustosa* (SDA); m) *Pleurotus*

djamor (PDA); n) *Auricularia auricula-judae* (PDA); o) *Pleurotus pulmonarius* (PDA); p) *Lactifluus volemus* (PDA).

The radial growth of the 16 wild edible mushroom species was measured to assess their mycelial expansion (Figure 4.7).

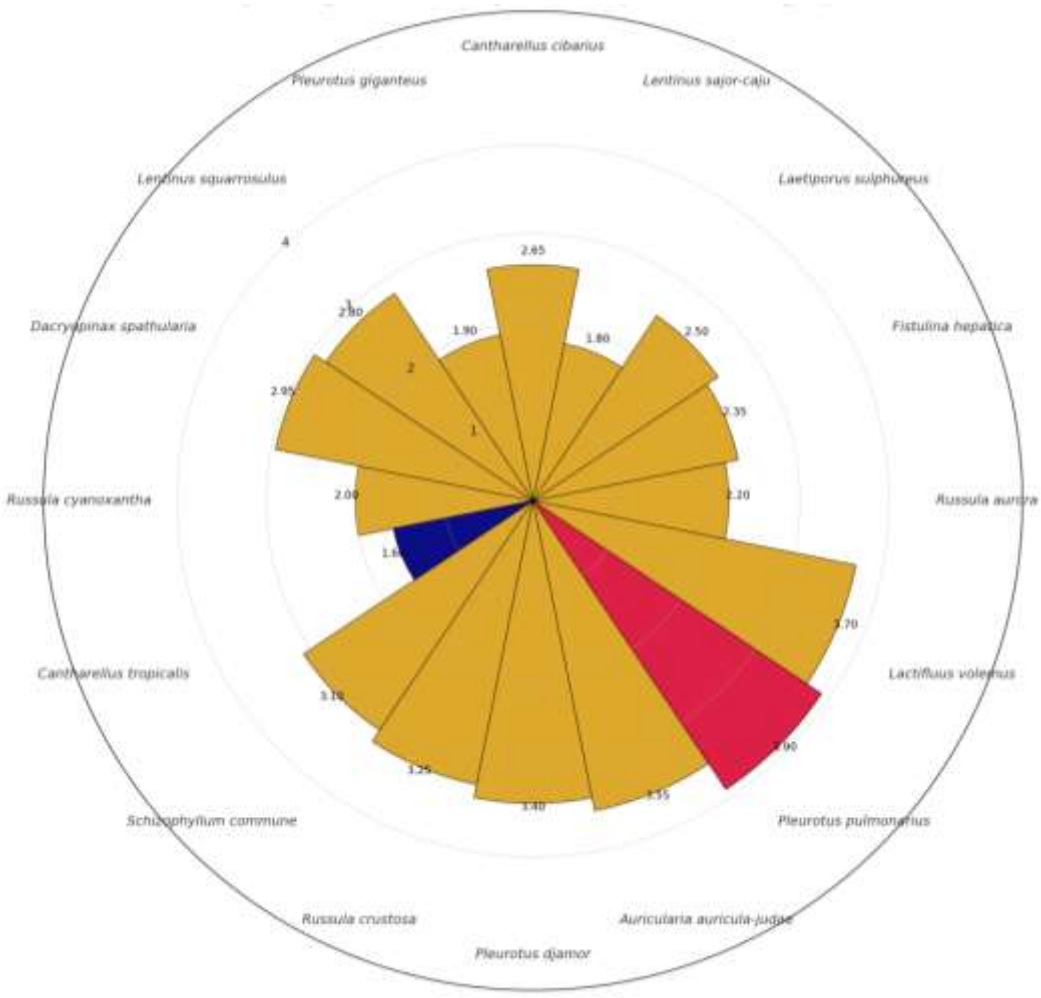

Figure 4.7: Radial growth of different culture on 7th day of incubation.

Cantharellus tropicalis exhibited the slowest growth with a radial expansion of 1.60 cm, followed closely by *Lentinus sajor-caju* (1.80 cm) and *Pleurotus giganteus* (1.90 cm). Moderate growth rates were observed in *Russula cyanoxantha* (2.00 cm), *Lactifluus volemus* (2.20 cm), *Fistulina hepatica* (2.35 cm), *Laetiporus sulphureus* (2.50 cm), *Cantharellus cibarius* (2.65 cm), *Lentinus squarrosulus* (2.80 cm), and *Dacrymyces spathularia* (2.95 cm). Faster mycelial expansion was recorded

in *Schizophyllum commune* (3.10 cm), *Russula crustosa* (3.25 cm), *Pleurotus djamor* (3.40 cm), *Auricularia auricula-judae* (3.55 cm), *Russula aurora* (3.70 cm), and *Pleurotus pulmonarius*, which exhibited the highest radial growth of 3.90 cm. These results indicate considerable variation in growth rates among species, reflecting differences in their adaptability and vigor under controlled culture conditions.

4.3.1. Culture characteristics of pure culture isolate

The cultural characteristics of the isolated wild edible mushroom species were evaluated under in vitro. Macroscopic observations were recorded based on colony morphology, including growth pattern, colour, texture, margin, elevation, surface characteristics, and overall form of the mycelium. Distinct variations in cultural behaviour were observed among the species, reflecting species-specific growth responses to the culture media. The detailed cultural characteristics of each isolate are presented in Table 4.8.

Table 4.8: Cultural characteristics of pure culture isolates of wild edible mushroom

Species	Observation
Cantharellus tropicalis	Slow growth; thin, sparse; white to pale cream; circular colony; smooth margin; flat elevation; delicate surface; poor aerial mycelium; full plate coverage slow.
Lentinus sajor-caju	White, dense cottony mycelium; circular colony; undulate margin; raised elevation; rough surface; compact aerial hyphae; moderate plate coverage.
Pleurotus giganteus	Fast-growing, thick cottony white mycelium; circular colony; slightly undulate margin; raised fluffy elevation; rough surface; rapid lateral spread.
Russula cyanoxantha	Slow growing, thin white mycelium; circular colony; smooth margin; flat to slightly raised elevation; uniform, compact surface; limited aerial growth.
Lactifluus volemus	Slow to moderate growth; compact white mycelium;

	circular colony; smooth margin; flat to slightly raised elevation; fine, even surface.
Fistulina hepatica	Moderate growth; white floccose mycelium; circular colony; regular margin; slightly raised elevation; uneven surface texture.
Laetiporus sulphureus	Moderate to fast growth; white, turning creamy; cottony mycelium; irregular margin with age; raised elevation; rough surface; faint concentric zonation present.
Cantharellus cibarius	Moderate growth; thin white to cream mycelium; circular colony; smooth margin; flat elevation; sparse aerial mycelium.
Lentinus squarrosulus	Fast growing, dense cottony white mycelium; circular initially, later irregular; undulate margin; raised elevation; rough surface; vigorous aerial growth.
Dacrymyces spathularia	Moderate growth; white (7–10 days) turning creamy; friable mycelium; concentric rings present; undulate margin; raised fluffy elevation; rough surface; circular initially, later irregular.
Schizophyllum commune	Fast growing mycelium; dense cottony white mycelium; circular colony; lobed to undulate margin; raised elevation; rough surface; rapid plate coverage.
Russula crustosa	Moderate to fast growth; compact white mycelium; circular colony; smooth margin; flat to slightly raised elevation; even, non-floccose surface.
Pleurotus djamor	Fast growing; profuse cottony white mycelium; circular colony; irregular margin at maturity; raised fluffy elevation; rough surface; rapid coverage.
Auricularia auricula-judae	Fast growing mycelium; thin to moderately dense white mycelium; circular colony; slightly undulate margin; flat to slightly raised elevation; velvety to moist surface.
Russula aurora	Fast growth; compact white to off-white mycelium;

	circular colony; smooth margin; slightly raised elevation; velvety, uniform surface.
Pleurotus pulmonarius	Very fast growing; dense cottony white mycelium; circular colony; undulate margin; raised elevation; rough surface; maximum radial expansion.

The culture characteristics can be influenced by several factors and may vary under different conditions. Factors such as the composition of the growth medium, temperature, pH, humidity, and nutrient availability can affect mycelial density, colour, texture, margin, elevation, and overall growth pattern (Lee et al., 2005; Shim et al., 2005). Consequently, the growth rate and appearance of the mycelium may differ among isolates even within the same species, reflecting both species-specific traits and environmental responses.

4.3.2. Enzyme activity of selected pure culture of wild edible mushroom

All the isolated pure cultures were initially screened qualitatively for enzymatic activity, and only those demonstrating higher efficiency were selected for subsequent quantitative analysis (Table 4.9). Based on this preliminary screening, seven species showing notable ligninolytic (ligninase) activity were selected for further investigation.

The selected species comprised *Lentinus sajor-caju*, *Schizophyllum commune*, *Pleurotus pulmonarius*, *Dacrymyces spathularia*, *Laetiporus sulphureus*, *Pleurotus djamor*, and *Cantharellus cibarius*. Among these, all species are wood rotting fungi, with the exception of *Cantharellus cibarius*, which is an ectomycorrhizal soil inhabiting macrofungi

Table 4.9: Enzymatic activity of pure culture isolates under submerged fermentation

Fungal species	**Laccase**	**Manganese peroxidase**	**Lignin peroxidase**

Lentinus sajor-caju	0.542 ± 0.019	0.151 ± 0.005	0.013 ± 0.001
Schizophyllum commune	0.681 ± 0.010	0.188 ± 0.004	0.015 ± 0.001
Pleurotus pulmonarius	0.090 ± 0.005	0.033 ± 0.002	0.152 ± 0.006
Dacrymyces spathularia	0.021 ± 0.002	0.007 ± 0.001	0.009 ± 0.002
Laetiporus sulphureus	0.042 ± 0.003	0.015 ± 0.002	0.029 ± 0.004
Pleurotus djamor	0.123 ± 0.005	0.040 ± 0.003	0.161 ± 0.008
Cantharellus cibarius	0.007 ± 0.002	0.002 ± 0.001	0.002 ± 0.001

Quantitative evaluation of ligninolytic enzyme production among the seven selected fungal species revealed considerable interspecific variation in laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) activities under submerged fermentation conditions. Among the isolates, *Schizophyllum commune* exhibited the highest laccase activity (0.681 ± 0.010 U/mL), indicating its strong potential as an efficient lignin degrader. This was closely followed by *Lentinus sajor-caju* (0.542 ± 0.019 U/mL), reflecting its moderate ligninolytic ability. *Cantharellus cibarius* recorded extremely low laccase activity (0.007 ± 0.002 U/mL), suggesting minimal involvement of this species in laccase-mediated lignin depolymerization. Similarly, significant differences were recorded for MnP activity, where *Schizophyllum commune* again emerged as the most potent producer (0.188 ± 0.004 U/mL), supporting its enzymatic versatility. *Dacrymyces spathularia* and *Cantharellus cibarius* showed the lowest MnP activities (0.007 ± 0.001 U/mL and 0.002 ± 0.001 U/mL, respectively), indicating limited peroxidase based lignin degradation potential.

Interestingly, LiP production patterns differed from that of laccase and MnP.

The highest LiP activities were observed in *Pleurotus djamor* (0.161 ± 0.008 U/mL) and *Pleurotus pulmonarius* (0.152 ± 0.006 U/mL), suggesting that *Pleurotus* species may rely more on peroxidase driven ligninolysis rather than laccase pathways. Other species produced comparatively lower LiP levels, highlighting species-specific enzyme regulation.

In the present study, *Schizophyllum commune* exhibited the highest laccase (0.681 ± 0.010 U/mL) and MnP (0.188 ± 0.004 U/mL), while *Pleurotus djamor* showed the highest LiP (0.161 ± 0.008 U/mL). For species common to both studies, Yogachitra (2019) reported much lower enzyme activities, including laccase (0.000431 U/mL), MnP (0.00006 U/mL), and LiP (0.000164 U/mL) in *Schizophyllum commune*, and similar trends in *Pleurotus pulmonarius* and *Pleurotus djamor*. The higher values in the present study are likely due to differences in cultivation method, measurement from culture filtrates, and optimized submerged fermentation conditions. However, Sumiati et al. (2022) reported significantly higher ligninolytic activities in all their isolates. Their isolate KRB-12 exhibited exceptionally high laccase (8244.72 U/mL), MnP (5486.49 U/mL), and LiP (3261.60 U/mL), far exceeding the enzyme yields obtained in our sample. The higher ligninolytic enzyme activity reported by Sumiati et al. (2022) may be attributed to the use of naturally wood decaying fungal isolates, which are ecologically adapted to actively degrade lignocellulose. Such strains typically express ligninolytic enzymes at elevated levels for survival and nutrient acquisition, resulting in significantly higher activities than those observed in cultured edible mushrooms.

Pukahuta et al. (2004) reported laccase activity of 0.121 U/mL in *Lentinus squarrosulus*, which closely corresponds with laccase levels observed in *Lentinus sajor-caju* and *Pleurotus* species in the present study. Nicolcioiu et al., (2014) extracted ligninolytic enzymes from *Laetiporus sulphureus* fruiting bodies using 70% hydroethanolic maceration, reporting 0.79 U/mL of laccase, 2.15 U/mL of manganese peroxidase, and 0.73 U/mL of lignin peroxidase. These activities exceed those observed in *Laetiporus sulphureus* mycelia, which is expected because fruiting bodies accumulate stored oxidative enzymes, whereas submerged cultures measure actively secreted enzymes during growth. Popa et al. (2016) reported a

laccase activity of 0.74 U/mL in *Laetiporus sulphureus* after 30 days of incubation in potato dextrose broth, whereas significantly lower activity was observed when cultured in Hwang medium, highlighting the critical role of culture medium in regulating enzyme production.

The present study validates that *Schizophyllum commune* is the most efficient laccase and MnP producer. It is well known that *Schizophyllum commune* is widely recognized as an efficient laccase-producing basidiomycete (Illuri et al., 2021; Kumar et al., 2022). It also synthesizes valuable metabolites such as schizophyllan and ethanol, along with lignocellulolytic enzymes including cellulase, xylanase, manganese peroxidase (MnP) and lignin peroxidase (LiP). Schizophyllan exhibits immunostimulatory, antibacterial, anticancer and anti-inflammatory properties, making it useful in food, pharmaceutical and oil recovery industries. Furthermore, *Schizophyllum commune* can degrade synthetic dyes and convert lignocellulosic biomass to bioethanol and additionally shows high β-glucosidase (54 nkat/mL) and xylanase activity (620 nkat/mL) (Veloz-Villavicencio et al., 2020).

Lallawmsanga et al. (2019) reported a remarkably high laccase activity of 66.32 U/mL in *Schizophyllum commune* from Mizoram, which is substantially higher than the values obtained in the present study. Such differences likely arise from methodological variations, including the use of laccase inducers in their study that can intensely enhance enzyme production. *Pleurotus* species dominates in LiP production, reflecting the functional diversity of ligninolytic systems. Such variation may be attributed to genetic differences, ecological adaptation, substrate specificity, and metabolic regulation. *Pleurotus* species were strong producers of ligninolytic enzymes, especially lignin peroxidase (LiP) along with laccase and MnP, enabling efficient degradation of azo dyes in liquid media. Their dye decolorization efficiency has been well documented, confirming high LiP activity under dye stress conditions (Verma and Madamwar, 2002; Laxmi and Nikam, 2015; Kunjadia et al., 2016).

In the screening of fungal enzymes, most of the soil fungi pure cultures did not show significant enzyme activity, likely because soil macrofungi are exposed to fewer lignocellulosic substrates in their natural environment, resulting in lower induction of enzyme production. *Cantharellus cibarius*, an ectomycorrhizal fungus,

exhibited low lignocellulolytic enzyme activity in the present study, which is consistent with its ecological role of forming symbiotic associations with host plants rather than actively decomposing lignocellulosic substrates. Nevertheless, Khaund and Joshi (2014) reported that *Cantharellus cibarius* showed high amylase activity but very low cellulase activity, indicating that while it contributes minimally to lignocellulose degradation and retains enzymatic potential for carbohydrate metabolism. In contrast, wood decaying fungi naturally colonize lignocellulosic materials and possess a well developed enzymatic system, which leads to higher enzyme synthesis and the results indicated that wood fungi generally exhibited greater enzyme production compared to soil fungi, making them more suitable for various biotechnological applications.

4.4. Heavy metals and health risk assessment

The metal content in the fruiting bodies of wild edible mushrooms varies widely and is largely species dependent, and it is influenced by many factors such as the sample collection site, the age of the fruiting bodies and mycelium, and the distance from potential sources of pollution (García et al., 2009; Falandysz and Borovička, 2013; Ndimele et al., 2017). The contents of heavy metals in the selected mushroom species evaluated in this study are presented in Table 4.10.

The contents of heavy metals (Mn, Ni, Pb, and Cd) varied among the analyzed fungal species. Manganese was the most abundant metal, with values ranging from 3.34 mg/kg in *Panus roseus* to 13.08 mg/kg in *Lycoperdon pratense*, resulting in a mean conctents of 7.02 mg/kg. Mn levels observed in our study appear lower when compared to the findings of Ouzouni et al. (2009), where Mn contents were reported to be within the range of 7.19 to 62.63 mg/kg.

Table 4.10: Heavy metals content in five selected wild edible mushroom

Fungal Species	**Mn (mg/kg)**	**Ni (mg/kg)**	**Pb (mg/kg)**	**Cd (mg/kg)**
Lactifluus volemus	7.78 ± 0.032	0.187 ± 0.003	0.337 ± 0.017	0.600 ± 0.011

Lycoperdon pratense	13.083 ± 0.072	0.440 ± 0.003	0.853 ± 0.013	0.737 ± 0.021
Fistulina hepatica	6.109 ± 0.007	0.058 ± 0.002	0.182 ± 0.002	0.003 ± 0.000
Schizophyllum commune	4.7695 ± 0.042	0.033 ± 0.002	0.054 ± 0.010	0.018 ± 0.001
Panus roseus	3.3445 ± 0.004	0.047 ± 0.000	0.034 ± 0.003	0.006 ± 0.001
MEAN VALUE	7.0172	0.1530	0.2920	0.2728

Note: Each value is expressed in mean ± SEM, (n = 3)

Nickel contents were comparatively low across all species, varying between 0.03 mg/kg in *Schizophyllum commune* and 0.44 mg/kg in *Lycoperdon pratense*. In this sense, another assessment by Chen et al. (2009) also observed Ni contents varying between 0.11 mg/kg and 1.35 mg/kg. In contrast, Zhu et al. (2011) reported higher levels, ranging from 0.76 mg/kg to 5.08 mg/kg, which were notably higher compared to the findings in our study.

Lead showed moderate accumulation, with the highest content recorded in *Lycoperdon pratense* (0.85 mg/kg) and the lowest in *Panus roseus* (0.03 mg/kg), yielding a mean of 0.29 mg/kg. There was clear variation in heavy metal conctents among the five selected wild edible mushroom species. Metal accumulation is strongly species-dependent, as reported in previous studies. For instance, Uzun et al. (2011) documented Pb levels ranging from <0.010 to 2.3 mg/kg across 45 mushroom species. Similarly, Kaya et al. (2011) observed no detectable Pb in 25 wild edible mushroom species. However, the remaining five species were observed to contain Pb between 0.32 and 3.10 mg/kg on a dry weight basis. These findings indicate that the uptake of metals by mushrooms is highly variable and influenced by species characteristics, supporting the variability observed in the present study.

Cadmium contents ranged from 0.003 mg/kg in *Fistulina hepatica* to 0.74 mg/kg in *Lycoperdon pratense*, with an overall mean value of 0.27 mg/kg. In this sense, Fu et al. (2020) also noted Cd contents ranging from 0.21 mg/kg to 20.5

mg/kg. On the contrary, our results showed lower levels of Cd. Long-term exposure to Cd, a toxic heavy metal found in various environmental sources can result in cancer, kidney damage, lung problems, and other human health risks (Chen et al., 2009; Charkiewicz et al., 2023). The overall metal abundance was in the order of Mn > Pb > Cd > Ni.

Lactifluus volemus is an ectomycorrhizal fungus widely distributed in the Champhai district and occurs with high species diversity, while *Lycoperdon pratense* is likewise a soil inhabiting fungus. Both taxa are soil inhabiting fungi, whereas the remaining three species (*Fistulina hepatica*, *Schizophylum commune* and *Panus roseus*) in the present study are wood decaying edible fungi. The accumulation of heavy metals varied among the analyzed fungal species, with *Lycoperdon pratense* exhibiting the highest overall metal contents. This species recorded the maximum levels of Mn (13.08 mg/kg), Ni (0.44 mg/kg), Pb (0.85 mg/kg), and Cd (0.74 mg/kg), indicating a greater bioaccumulation potential compared to the other taxa. In comparison with green plants, it is known that wild edible mushroom exhibit a greater capacity to accumulate heavy metals (Liu et al., 2024).

A study of 12 common edible and non edible mushroom species collected from unpolluted areas of the province of Ciudad Real, Central Spain, by Campos et al. (2009) showed that *Cantharellus cibarius*, an ectomycorrhizal fungus, accumulated the highest contents of heavy metals. A comparison between the study on heavy metal content in wood rotting fungi by Qin et al. (2024) and the findings of Svoboda et al. (2000) suggests that mushrooms associated with soil substrates tend to accumulate higher contents of heavy metals than wood decaying fungi. Focusing on the two ectomycorrhizal soil fungi, Keles and Gençcelep (2023) reported Cd, Mn, Ni, and Pb levels of 0.72 mg/kg, 6.38 mg/kg, 2.12 mg/kg, and 0.12 mg/kg respectively in *Lactifluus volemus* from Turkey. However, Ni content of 0.19 mg/kg is notably lower than their reported value.

The results of our study show comparable contents for these metals, indicating a similar pattern of accumulation. Additionally, Zsigmond et al. (2015) documented 0.87 mg/kg of Cd, 2.29 mg/kg of Ni, and 22.8 mg/kg of Mn in *Lactifluus volemus* from Romania, further supporting species-specific tendencies toward metal uptake. In the case of *Lycoperdon pratense*, Gençcelep et al. (2009)

reported Mn levels as high as 29 mg/kg, which is higher than the 13.14 mg/kg observed in the present study. Likewise, Uzun et al. (2011) recorded 53.8 mg/kg of Mn, 4.19 mg/kg of Ni, 0.27 mg/kg of Cd, and <0.01 mg/kg of Pb in *Lycoperdon pratense*, indicating regional variation as well as the strong metal accumulating capacity of this species compared to our findings. On the other hand, it appears that accumulation of these specific metals could be species dependent, and as has already been noticed by Kalac and Svoboda (2000). In the present study, soil inhabiting mushrooms were observed to accumulate higher contents of heavy metals than wood decaying species. This may be due to their direct and prolonged contact with the soil matrix, which serves as the primary reservoir of metal ions, allowing greater uptake and bioaccumulation through the mycelial network.

In general, heavy metal accumulation in macrofungi is a complex process influenced by both environmental factors, such as soil pH, temperature, metal availability, organic matter content, and physicochemical properties of the substrate, as well as intrinsic factors, including taxonomic affiliation, growth and developmental stage, and mycelial age (Chen et al., 2009; Kokkoris et al., 2019). Different mushroom species exhibit distinct accumulation patterns depending on their nutritional strategies and soil characteristics largely determine the availability and mobility of metal ions.

4.4.1. Health risk assessment in wild edible mushroom

To evaluate the potential health risks associated with heavy metals in the studied mushrooms, the estimated daily intake (EDI) of cadmium (Cd), manganese (Mn0, nickel (Ni), and lead (Pb) was calculated. The EDI represents the likely amount of each metal ingested through regular mushroom consumption. Five commonly consumed edible mushroom species were assessed for their heavy metal content and subsequently evaluated for potential health risks. Among these, *Lactifluus volemus* and *Schizophyllum commune* are particularly popular in Mizoram. The calculated values of EDI, target hazard quotient (THQ), and hazard index (HI) and carcinogenic risk index/incremental lifetime cancer risk (CRI/ILCR) for the mushroom samples are summarized in Table 4.11.

The estimated daily intake values for *Lactifluus volemus*, *Lycoperdon pratense*, *Fistulina hepatica*, *Schizophyllum commune*, and *Panus roseus* were found to be within the maximum tolerable daily intake limits established by the Food and Agriculture Organization/World Health Organization (FAO/WHO, 2011).

THQ indicates non-carcinogenic risk due to individual metals, a THQ < 1 suggests negligible risk. *Lycoperdon pratense* exhibited the highest THQ values for Mn (0.1) and Pb (0.027), highlighting a relatively higher non-carcinogenic risk from these metals in this particular species. *Fistulina hepatica* and *Panus roseus* had very low THQ values (THQ Cd: 0.0003 and 0.001, respectively), suggesting minimal hazard. Across metals, the relative hazard ranking based on THQ was Mn > Pb > Cd > Ni, consistent with the EDI trend. The THQ values obtained in the present study were considerably lower than those reported in wild mushrooms by Salihović et al. (2021) in Bosnia and Herzegovina and by Sun et al. (2017) in Yunnan Province, China. This further suggests that the concentrations of heavy metals in the analyzed mushrooms are low and are unlikely to cause adverse health effects, reflecting the unpolluted nature of the sampling sites.

Table 4.11: Health risk assessment of wild edible mushrooms from their respective heavy metal content

Parameter / Species	*Lactifluus volemus*	*Lycoperdon pratense*	*Fistulina hepatica*	*Schizophyllum commune*	*Panus roseus*
ESTIMATED DAILY INTAKE ($\times 10^{-3}$)					
Cd	0.067	0.083	0.0003	0.002	0.001
Mn	0.853	1.445	0.672	0.525	0.368
Ni	0.021	0.048	0.0064	0.004	0.005
Pb	0.036	0.094	0.020	0.006	0.004
TARGET HAZARD QUOTIENT					
Cd	0.067	0.083	0.0003	0.002	0.001
Mn	0.006	0.1	0.004	0.004	0.003
Ni	0.001	0.002	0.0000	0.0000	0.000
Pb	0.01	0.027	0.006	0.002	0.001

HAZARD INDEX					
HI	0.084	0.122	0.010	0.008	0.005
CARCINOGENIC RISK INDEX/ INCREMENTAL LIFETIME CANCER RISK					
Cd ($\times 10^{-4}$)	4.22	5.23	0.02	0.13	0.06
Pb ($\times 10^{-7}$)	3.06	7.99	1.7	0.51	0.34

The HI represents the cumulative non-carcinogenic risk from multiple metals. The highest HI was observed in *Lycoperdon pratense* (0.122), while the lowest HI was in *Panus roseus* (0.005). *Lactifluus volemus* showed an intermediate HI value (0.084), indicating a moderate cumulative risk. This ranking of HI correlates with the THQ values, suggesting that species with higher metal accumulation, particularly Mn and Pb, contribute more significantly to overall non-carcinogenic risk. All the HI values for the elements evaluated from the five edible mushroom species were found to be less than 1. This indicates no potential non-carcinogenic health risk to mushroom consumers. Previous studies have also shown that even within the same geographical area, certain mushroom species may exhibit safe metal levels whereas others may exceed permissible limits, posing health concerns (Sarikurkcu et al., 2020; Liu et al., 2022). Such variation largely depends on species-specific accumulation abilities and the environmental conditions of the collection site.

CRI (or ILCR) estimates the potential lifetime risk of developing cancer due to exposure to carcinogenic metals (Cd and Pb). For Cd, the highest CRI was recorded in *Lycoperdon pratense* (5.23×10^{-4}), and the lowest in *Fistulina hepatica* (0.02×10^{-4}. For Pb, the highest CRI was again in *Lycoperdon pratense* (7.99×10^{-7}), whereas the lowest was in *Panus roseus* (0.34×10^{-7}). Similarly, all ILCR values fell within the acceptable range (10^{-6}–10^{-4}) or below the negligible risk threshold ($<10^{-6}$) (Hu et al., 2017), indicating minimal carcinogenic risk associated with consumption. However, long-term intake of mushrooms with comparatively higher Cd levels should be approached with caution. Although Pb exhibited no concerning impact in terms of carcinogenic or non-carcinogenic risk, Cd showed a greater potential to

contribute to cancer risk, line up with previous findings (Fu et al., 2020; Liu et al., 2015).

The results indicate that *Lycoperdon pratense* consistently exhibits the highest EDI, THQ, HI, and CRI values across most metals, suggesting it accumulates heavy metals more efficiently than other species and may pose a higher health risk to consumers. Conversely, *Fistulina hepatica* and *Panus roseus* represent species with minimal heavy metal accumulation and negligible risk. It is well-known that some mushrooms, particularly puffballs (*Lycoperdon pratense*), tend to accumulate elevated concentrations of Mercury (Hg), Lead (Pb), Cadmium (Cd), and Arsenic (As), which may pose serious health risks especially due to Hg and As contamination (Nowakowski et al., 2021). The capacity of mushrooms to bioaccumulate heavy metals differs greatly among species. Hence, those containing heavy metals above permissible limits may pose significant carcinogenic risks (Orywal et al., 2021). Past studies have reported high metal accumulation in mushrooms growing in polluted environments, such as near busy highways, sewage sludge disposal sites, and industrial emission zones including urban areas (Das, 2005; Falandysz and Borovička, 2013; Kalac, 2013). In contrast, Mizoram is characterized by minimal industrial development and limited manufacturing activities. The mushroom samples in this study were collected from remote forested regions with little human interference. Therefore, the comparatively low heavy metal levels detected are likely attributed to the nature of these habitats and the absence of substantial pollution sources.

4.5. Antioxidant activity of selected wood rotting fungi

The antioxidant potential of the selected wood-rotting fungi was evaluated using DPPH and ABTS radical scavenging assays. *Laetiporus sulphureus* was subjected to multi-solvent extraction, where the mycelial were extracted with dichloromethane, ethyl acetate, and methanol, while the fermentation broth was extracted using dichloromethane and ethyl acetate. In addition, two *Ganoderma* species, *Ganoderma tropicum* and *Ganoderma australe* were processed through methanolic extraction. All the extracts were assessed within a concentration range of 200–1000 µg/mL to

determine their free radical scavenging efficiency under both DPPH and ABTS radical scavenging assays.

4.5.1. Antioxidant activity of edible wood rotting fungi *Laetiporus sulphureus*

The fruiting body of *Laetiporus sulphureus* collected from Mizoram vary considerably in size (6-15 cm in width and 10-28 cm in length). The context is thick, soft, and fleshy when hydrated, becoming brittle and chalky upon desiccation. Molecular analysis revealed that the specimen (PP886450) shares the highest sequence similarity (99.48%) with *Laetiporus sulphureus* voucher FPIA-DEP47 (PP203300). The other closely related sequences included *Laetiporus sp.* mkacc53788 (EU840634) at 97.37%, *Laetiporus sp.* 3296G (EU840625) at 97.21%, and *Laetiporus sp.* X1314 (KC595925) at 96.91%.

The ABTS radical inhibition increased steadily with concentration (200–1000 µg/mL), ranging between 40.2–53.98 % for the dichloromethane mycelial extract, 40.8–51.08 % for the ethyl acetate mycelial extract, and 40.34–52.0 % for the methanolic mycelial extract (Figure 4.8). A marginally higher scavenging potential was observed in the fermentation broth extracts, with dichloromethane and ethyl acetate recording 41.4–53.61 % and 42.18–54.35 %, respectively. Ascorbic acid is used as the positive control, showed markedly higher ABTS activity, achieving 94.97 % inhibition at 1000 µg/mL and an IC_{50} value of 532.4 µg/mL.

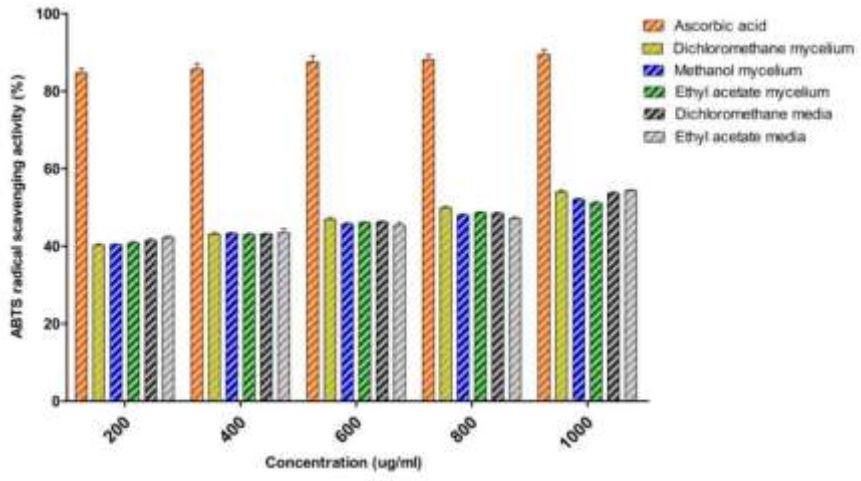

Figure 4.8: ABTS Radical Scavenging activities (%) of different solvent extracts of edible mushroom *Laetiporus sulphureus* at different concentration.

The ABTS radical inhibition results demonstrate the ability of the extracts to neutralize free radicals, suggesting their potential to prevent lipid oxidation by interrupting chain reactions. The IC_{50} values for the mycelial extracts were 596.9 µg/mL (dichloromethane), 579.7 µg/mL (ethyl acetate), and 602.0 µg/mL (methanol). The fermentation broth extracts presented IC_{50} values of 669.7 µg/mL (dichloromethane) and 774.7 µg/mL (ethyl acetate).

The DPPH radical scavenging assay is widely employed to predict antioxidant capacity, particularly through its ability to inhibit lipid peroxidation. The DPPH scavenging activities of the various extracts are presented in Figure 4.9. A concentration dependent increase in radical quenching was observed in the extracts. The dichloromethane, ethyl acetate, and methanolic extracts of the fungal mycelium exhibited scavenging activity within the range of 56.63–67.63%, 58.40–66.11%, and 60.80–70.16%, respectively, across a concentration range of 200–1000 µg/mL. Similarly, extracts obtained from the fermentation broth demonstrated elevated DPPH radical inhibition, where the dichloromethane extract showed 65.48–70.29% activity and the ethyl acetate extract exhibited 65.73–69.78% inhibition. This tendency aligns with the ABTS radical scavenging response, indicating strong free radical neutralizing potential of the studied solvent fractions.

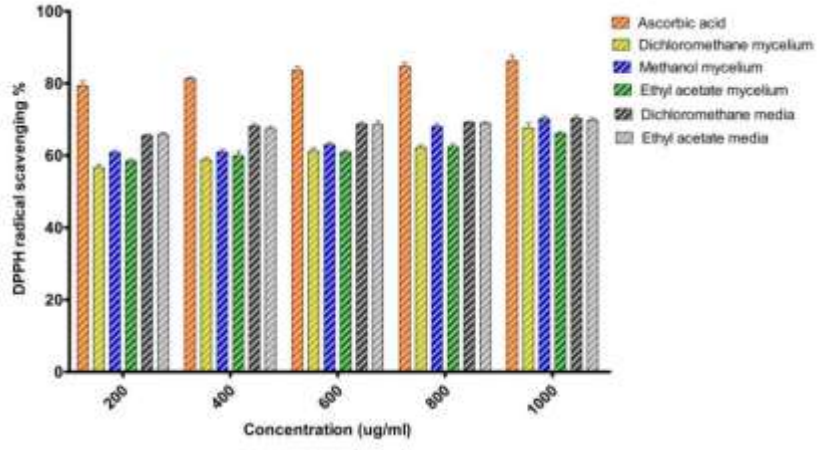

Figure 4.9: DPPH Radical Scavenging activities (%) of different solvent extracts of edible mushroom *Laetiporus sulphureus* at different concentration.

It has been widely observed that the different crude extracts obtained from the fermentation broth demonstrated slightly higher DPPH radical scavenging

activity compared to the mycelium extracts. Ascorbic acid (positive control) exhibited a DPPH radical scavenging activity of 86.27% at 1000 µg/mL, with an IC_{50} value of 525.4 µg/mL. For the fungal mycelium extracts, the IC_{50} values were 665.6 µg/mL for dichloromethane, 707.6 µg/mL for ethyl acetate, and 690.9 µg/mL for methanol extracts. While, the fermentation broth extracts showed lower IC_{50} values, with dichloromethane and ethyl acetate extracts showing IC_{50} values of 431.2 µg/mL and 468.9 µg/mL, respectively.

The dried mycelium and fermentation broth of *Laetiporus sulphureus* were extracted using dichloromethane, ethyl acetate, and methanol, and their antioxidant activities were subsequently evaluated. Among these, the dichloromethane extracts from both mycelium and fermentation broth exhibited the highest radical scavenging activity at 1000 µg/mL for both ABTS and DPPH assays. The results of the present study indicate that submerged culture of this edible mushroom is a suitable approach for obtaining significant antioxidant compounds. A slightly higher radical scavenging activity, reflecting the ability of compounds to neutralize harmful free radicals, was observed in the fermentation broth extracts compared to mycelium extracts, consistent with the findings of Börühan Çetin et al. (2021). In their study, among 130 fungal species, fermentation broth extracts from the majority of isolates (80.77%) exhibited greater scavenging activity than mycelial biomass extracts, suggesting that metabolites secreted into the culture medium by the growing fungus may be more potent than those retained in the mycelium. Similarly, Popa et al. (2016) reported that submerged culture extracts of *Laetiporus sulphureus* showed superior DPPH radical scavenging activity compared to extracts derived from the fruiting body. This enhanced activity may result from the increased production of bioactive compounds during fermentation. Fermentation processes are known to elevate levels of phenolic compounds, which contribute to antioxidant activity. In this sense, the fermentation has been shown to enhance the reducing power of soluble phenolics and overall antioxidant potential in various substrates (Zhao et al., 2021). Furthermore, the structural features of these compounds, particularly the number and position of hydroxyl groups, play a critical role in their antioxidant capacity, with a greater number of hydroxyl groups being associated with stronger radical scavenging activity (Charlton et al., 2023).

The study demonstrated that extracts from the mycelium and fermentation broth of *Laetiporus sulphureus* possess significant antioxidant activity, as shown by their ability to scavenge ABTS and DPPH radicals, with activity increasing with extract concentration. Fermentation broth extracts generally exhibited higher scavenging activity than mycelium extracts, with dichloromethane extracts showing the highest activity in both assays. These results are consistent with previous studies, indicating that submerged culture of *Laetiporus sulphureus* is an effective method for producing antioxidant compounds.

The ethanol extract of *Laetiporus sulphureus* showed strong DPPH scavenging activity, with 14–86% inhibition at 100–800 µg/mL (Turkoglu et al., 2007). In the present study, dichloromethane, ethyl acetate, and methanolic mycelial extracts inhibited DPPH by 56.63–70.16% (200–1000 µg/mL), while fermentation broth extracts showed slightly higher activity (65.48–70.29%), confirming the moderate to strong antioxidant capacity of the wild edible mushroom *Laetiporus sulphureus*. Although lower than ascorbic acid (>90%), this activity is notable for a natural extract, supporting its potential in functional foods and nutraceuticals (Kozarski et al., 2015; Brand-Williams et al., 1995). The greater performance of fermentation broth is likely due to extracellular bioactive metabolites (Börühan Çetin et al., 2021). While the antioxidant properties of fungal fruiting bodies are well-studied, bioactive metabolites from mycelium and fermentation media under submerged culture have received less attention (Elisashvili, 2012). Therefore, this study highlights *Laetiporus sulphureus* as a valuable natural source of antioxidant compounds, with potential applications in functional foods, contributing to food security and human well being. The findings provide a useful reference for future research on edible mushrooms from the Himalayan region.

4.5.2. Antioxidant activity of *Ganoderma tropicum* and *Ganoderma australe*

Ganoderma tropicum (GenBank Accession: PQ119874) is a wood-rotting Basidiomycete, taxonomically placed within Ganodermataceae, and is commonly found in tropical and subtropical forests. It forms sessile to stipitate basidiocarps, typically laccate, reddish-brown to dark brown, with a white margin when actively

growing. Double-walled spores with truncate apex and ornamented endospore layer are the characteristic of the species. It causes white-rot on fallen logs and tree stumps, contributing to degradation of lignin and nutrient cycling. The species is often grouped within the *Ganoderma lucidum* (*Ganoderma lingzhi*) *c*omplex, but can be distinguished through spore morphology and pileus texture.

Ganoderma australe (GenBank Accession: MG448604) is a white-rot fungus belonging to Ganodermataceae, widely distributed in tropical to temperate regions, and typically colonizes decaying hardwoods and standing trees, causing heart-rot/butt-rot, and plays a major role in lignocellulosic decomposition. Basidiocarps are perennial, woody to corky, often dark brown with a laccate crust, and become zonate with age. It possesses double-walled basidiospores with a brown endospore and a hyaline myxosporium, a key morphological characteristic for *Ganoderma*. The fruiting bodies of these two species are shown in Figure 4.10.

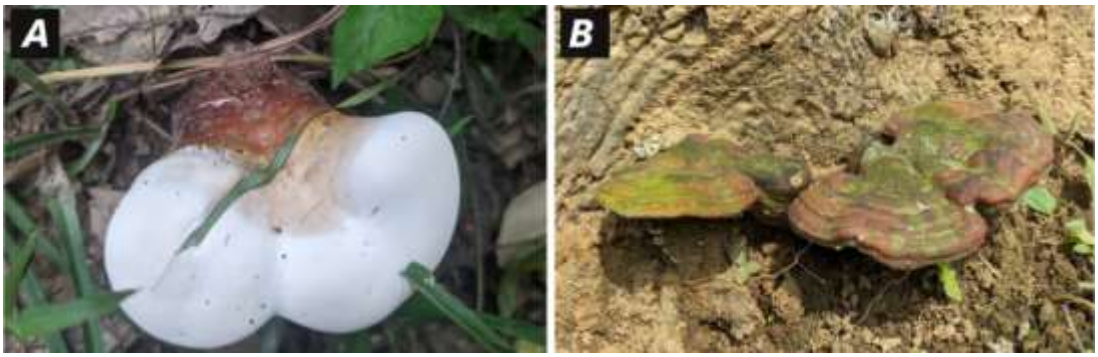

Figure 4.10: Basidiomes of (A) *Ganoderma tropicum* and (B) *Ganoderma australe*

The antioxidative potential of the methanolic extracts against oxidative damage induced by free radicals was evaluated through the DPPH scavenging assay. Results revealed that *Ganoderma tropicum* and *Ganoderma australe* possess in vitro antioxidant activity (Figure 4.11). Ascorbic acid was used as a positive control, and the IC_{50} values for *Ganoderma tropicum* and *Ganoderma australe* were 585.2 µg/mL and 608.7 µg/mL, respectively. The highest radical scavenging activity percentages of 74.91% and 77.47% were observed at a concentration of 1000 µg/mL for *Ganoderma tropicum* and *Ganoderma australe*, respectively. The scavenging activity of the extracts followed the sequence *Ganoderma australe* > *Ganoderma*

tropicum. Particularly, *Ganoderma australe* exhibited higher percentage of radical scavenging even at the lowest concentration (200 µg/mL).

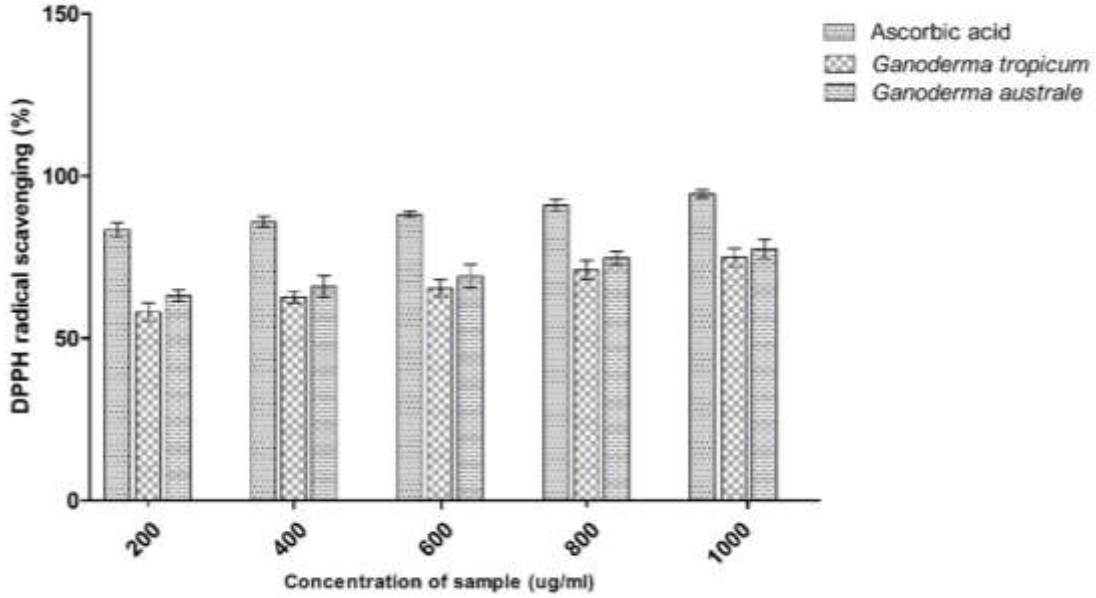

Figure 4.11: DPPH scavenging assay of *Ganoderma tropicum* and *Ganoderma australe*

The ABTS radical scavenging activity is presented in Figure 4.12. Both *Ganoderma tropicum* and *Ganoderma australe* demonstrated significant efficacy in scavenging the ABTS radical, with inhibition percentages at 1000 µg/mL of 58.79% and 74.15%, respectively. *Ganoderma australe* showed higher ABTS radical scavenging activity at 1000 µg/mL than *Ganoderma tropicum*. Variations in scavenging activity, observed up to 15%, may be attributed to environmental factors such as temperature, maturity stage, and collection site. Ascorbic acid exhibited an inhibition rate of 89.44%. The IC_{50} values for *Ganoderma tropicum* and *Ganoderma australe* were 831.7 µg/mL and 609.5 µg/mL, respectively.

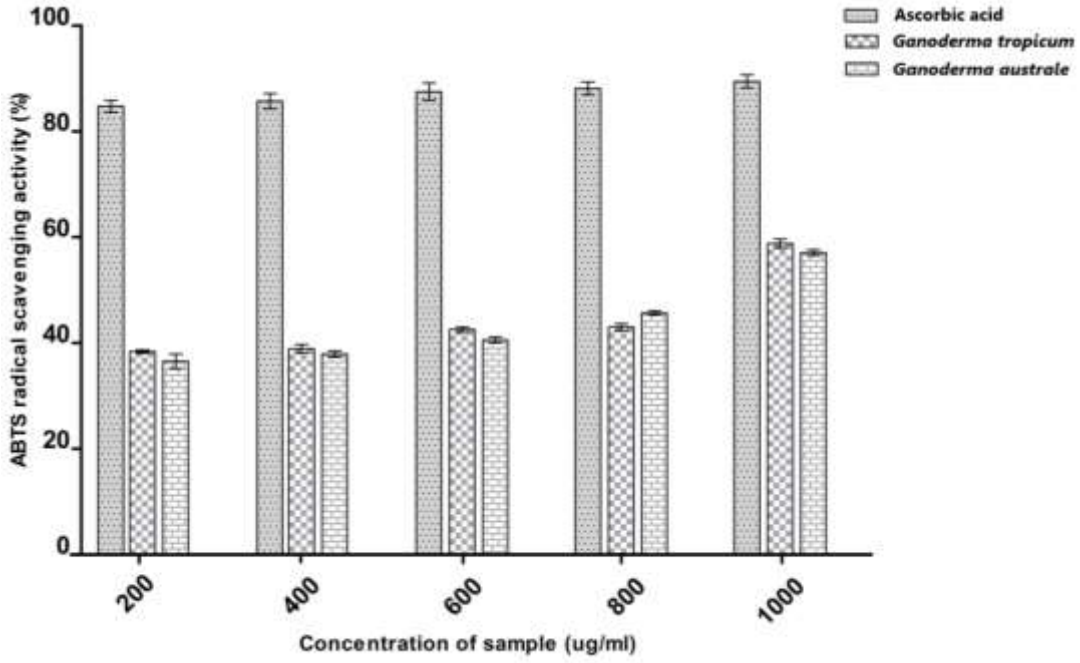

Figure 4.12: ABTS scavenging assay of *Ganoderma tropicum* and *Ganoderma australe*

These findings emphasize the potential of *Ganoderma* species as natural sources of antioxidants. The high radical scavenging activities observed suggest that these mushrooms could be valuable in developing therapeutic agents to combat oxidative stress-related diseases. A low IC_{50} value indicates strong antioxidant activity, as it reflects the smaller concentration of the mushroom extract required to inhibit 50% of free radicals (DPPH/ABTS) in the assay. Therefore, in the context of antioxidant potential, a lower IC_{50} is generally considered more effective. Variations in these results may be influenced by environmental conditions (temperature, collection site), species differences, maturity stage, and extraction methods (Darsih et al., 2019).

The methanolic extracts of *Ganoderma lucidum* mycelia cultivated on potato dextrose broth (PDB) and sweet corn media exhibited scavenging activities of 22.14 ± 3.25% and 12.84 ± 2.03%, respectively, at a concentration of 0.2 mg/mL, and the activity was attributed to polyphenols and triterpenoids (Darsih et al., 2019). *Ganoderma lucidum* also showed 91.69 ± 2.25% DPPH and 96.72 ± 0.38% ABTS

scavenging activity at 1000 µg/mL (Modi et al., 2014). Methanol extracts of *Ganoderma lucidum* presented an IC_{50} of 251.95 µg/mL in a DPPH assay (Hidayat and Fatmawati, 2019), which was better than that of the two species observed in the present study. Methanolic extracts of *Ganoderma tsugae* exhibited 88.4% DPPH scavenging activity at 5 mg/mL (Mau et al., 2005), while hot water extracts of *Ganoderma lucidum* demonstrated a significant radical scavenging effect, with an IC_{50} of 5.28 mg/mL (Abdullah et al., 2012).

Crude polysaccharides of *Ganoderma australe* showed IC_{50} values of 0.48 mg/mL (ABTS) and 0.36 mg/mL (DPPH) (Muñoz-Castiblanco et al., 2023), which are higher than the IC_{50} values observed for the methanolic extract of *Ganoderma australe* in the present study. Despite this, the results confirm that *Ganoderma australe* exhibits significant antioxidant activity. *Ganoderma adspersum* methanol extracts showed much lower IC_{50} values, with a DPPH IC_{50} of 21.45 ± 0.87 µg/mL and an ABTS IC_{50} of 25.71 ± 0.69 µg/mL (Chafouz et al., 2023). At 500 µg/mL, Bristy et al. (2022) reported the scavenging ability of the ethanolic extract of *Ganoderma tropicum* to be 87.01%, which is higher than the activity observed in our methanolic extract at 1000 µg/mL. The presence of antioxidant components, particularly phenolic compounds, underlies the observed radical scavenging capabilities.

The antioxidant activity in *Ganoderma* species is closely associated with phenolic compounds, whose hydroxyl groups play a critical role in neutralizing free radicals (Mathew et al., 2015). The strong radical scavenging effect observed in *Ganoderma australe* suggests that phenolic compounds may substantially contribute to its antioxidant mechanism. The variation in radical scavenging activities across different *Ganoderma* species underscores the promise of *Ganoderma australe* as a potent source of natural antioxidants, particularly for therapeutic applications targeting oxidative stress-related conditions.

4.6. Volatile compounds of selected wood rotting fungi

The methanolic extract chromatograms of the wood rotting fungi *Ganoderma tropicum* and *Ganoderma australe* are presented in Figure 4.13. The individual

compounds detected, along with their retention time (RT), peak area (%), molecular formula (MF), and molecular weight (MW), are summarized in Table 4.12 for *Ganoderma tropicum* and Table 4.13 for *Ganoderma australe*. The peak integration and identification were performed using the NIST 17 library (National Institute of Standards and Technology - Mass Spectral Library, 2017 Edition) database, and the resulting spectra were carefully compared with those of known reference compounds to ensure accurate compound identification.

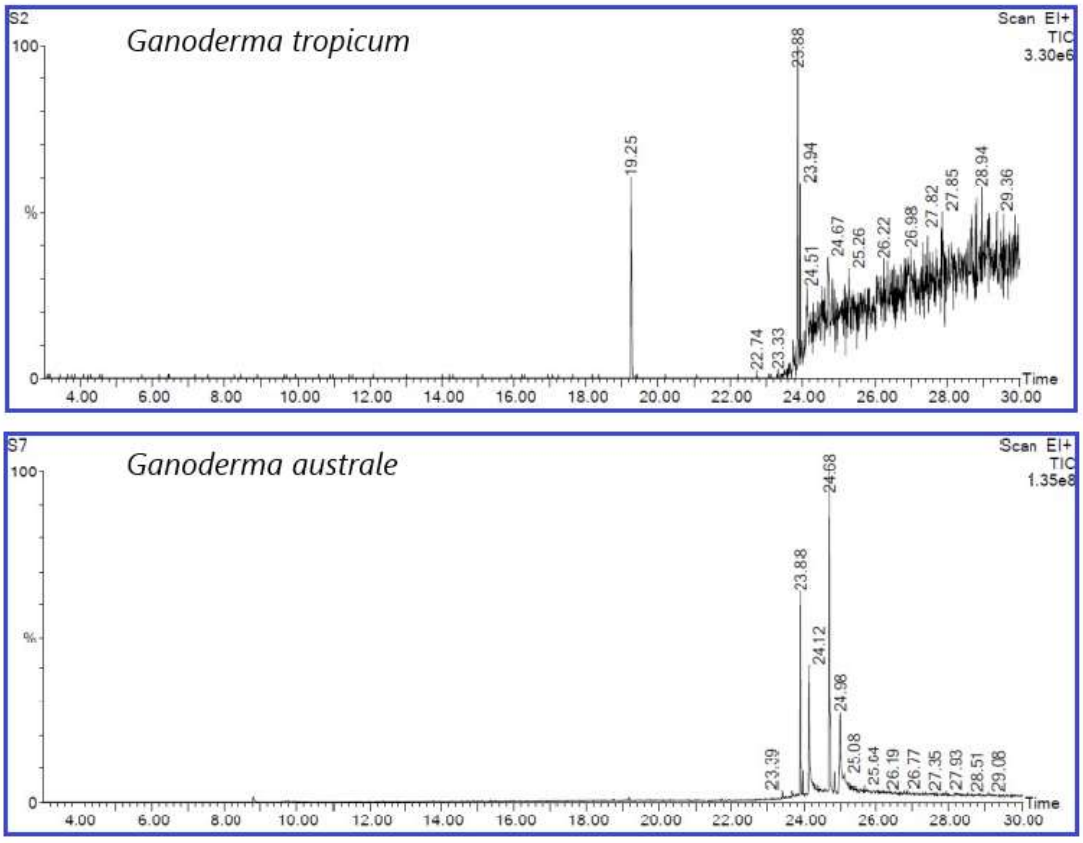

Figure 4.13: GC-MS chromatogram of the methanolic extract of wood rotting fungi *Ganoderma tropicum* and *Ganoderma australe*

GC-MS analysis of the methanolic extract of the wood rotting fungal species *Ganoderma tropicum* revealed 20 chromatographic peaks representing 8 unique bioactive compounds. The dominant compounds identified were Tetrasiloxane, Decamethyl-, which appeared repeatedly (No. 5: 1.805%, No. 6: 1.469%, No. 7: 2.421%, No. 8: 1.742%, No. 9: 2.662%, No. 13: 1.336%) with a total contribution of

11.44%, followed by Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-) (No. 10: 1.711%, No. 11: 1.989%, No. 14: 1.399%, No. 16: 1.963%, No. 19: 1.561%, No. 20: 1.729%) making a combined 10.35%.

Table 4.12: GC-MS profiling of the identified compound from the methanol extract of *Ganoderma tropicum*

No	RT	Area %	Name (Compound)	MW	MF
1	19.250	2.588	4-Fluro-2-Trifluromethylbenzoic acid, 3-Flurophenyl Ester	302	$C_{14}H_7O_2F_5$
2	23.882	2.622	2-Methylheptanoic acid	144	$C_8H_{16}O_2$
3	23.942	1.699	Tert-Butyl(Dimethyl)Siilyl-2-([Tert-Butyl(Silyl]oxy)-3-Flu	334	$C_{15}H_{31}O_3FSi_2$
4	24.142	2.115	2-Ethylhexanal Ethylene Glycolacetal	172	$C_{10}H_{20}O_2$
5	24.512	1.805	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
6	24.582	1.469	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
7	24.672	2.421	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
8	24.802	1.742	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
9	25.072	2.662	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
10	25.262	1.711	Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
11	25.403	1.989	Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
12	25.563	1.316	Spiro[2,4]Hept-5-ene.5-Trimethylsilylmethyl-1-Trimethylsilyl-	252	$C_{14}H_{28}Si_2$
13	25.693	1.336	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
14	25.783	1.399	Anthracene, (-Ethyl-9,10-	280	$C_{19}H_{24}Si$

			Dihydro-10-Trimethylsilyl-		
15	26.413	1.608	Spiro[2,4]Hept-5-ene.5-Trimethylsilylmethyl-1-Trimethylsilyl-	252	$C_{14}H_{28}Si_2$
16	26.903	1.963	Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
17	27.854	2.511	1,3-Dioxolane,2-Pentadecyl-	284	$C_{18}H_{36}O_2$
18	28.104	2.074	1,3-Dioxolane,2-Pentadecyl-	284	$C_{18}H_{36}O_2$
19	28.664	1.561	Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
20	29.114	1.729	Anthracene, (-Ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$

The other compounds present include 1,3-Dioxolane, 2-Pentadecyl- (No. 17: 2.511%, No. 18: 2.074% with a total of 4.585%), Spiro[2,4]Hept-5-ene trimethylsilyl derivatives (No. 12: 1.316%, No. 15: 1.608% with a total 2.924%), 2-Methylheptanoic acid (No. 2: 2.622%), 2-Ethylhexanal Ethylene Glycolacetal (No. 4: 2.115%), 4-Fluoro-2-Trifluoromethylbenzoic acid, 3-Fluorophenyl Ester (No. 1: 2.588%), and Tert-Butyl(Dimethyl)Silyl derivative (No. 3: 1.699%). The presence of siloxane-based compounds and anthracene derivatives suggests a chemically diverse metabolite profile *in Ganoderma tropicum*, indicating potential antioxidant, antimicrobial, and industrial relevance (Ahmad et al., 2024). The relatively high abundance of Tetrasiloxane, Decamethyl- and Anthracene derivatives reflects their possible biofunctional influence within the extract, while fatty acid and glycol-acetal groups contribute additional bioactive diversity.

Results of GC-MS study showed the presence of 13 different bioactive compounds in *Ganoderma australe* at different retention time such as 1-Methyloctadec-12-enoic acid, Methyl ester (6.847%), Tetrasiloxane, Decamethyl- (No. 11: 1.728%, No. 13: 1.5%, No. 16: 1.342% with a total of 4.57%), Antracene, 9-ethyl-9,10-Dihydro-10-Trimethylsilyl- (No. 17: 1.742%, No. 19: 1.416%, No. 20: 1.372% with a total 4.53%), 1,2,3,4,5-Cyclopentanepentol (3.934%), 2-Ethylhexanal

ethylene Glycol Acetal (No. 7: 1.594%, No. 8: 2.229% with a total of 3.823%), 5-(2-Methoxypropan-2-yl)-2-methyl-2-vinyltetrahydrofuran (No. 6: 1.865%, No. 9: 1.603% with a total of 3.468%), 2-Methylheptanoic acid (3.050 %), Antracene,9,10-Dihydro-9,10-Bis(Trimethylsilyl)- (No. 12: 1.329%, No. 18: 1.717% with a total area of 3.046%), 2,3-Dimethoxy-2-3-Dimethylbutane (2.194 %), Tert-butyldimethylsilil,3-{(Tert-Butyldimethylsilyl)Thio}Propanoate (1.972%), 4-Methyl-2,4-Bis(P-hydroxypheny)Pent-1-ene, 2TMS Derivative (1.502 %), 4-Deoxypyridoxine, 2Tms Derivative (1.478 %) and 1,3-Dioxolane, 2-Pentadecyl- (1.366%).

The analysis of bioactive substances in *Ganoderma australe* reveals a variety of chemical components with notable therapeutic benefits. The key compounds identified include Tetrasiloxane, Decamethyl-, 2-methylheptanoic acid, Antracene, 9-ethyl-9, 10-Dihydro-10-Trimethylsilyl- which highlight the properties of this white rot fungus, potentially offering advantages in health and wellness applications.

Table 4.13: GC-MS profiling of the identified compound from the methanolic extract of *Ganoderma australe*

No	RT	Area%	Name (Compound)	MW	MF
1	23.872	3.050	2-Methylheptanoic acid	144	$C_8H_{16}O_2$
2	23.942	1.972	Tert-butyldimethylsilil,3-{(Tert-Butyldimethylsilyl)Thio}Propanoate	334	$C_{15}H_{34}O_2SSi_2$
3	24.092	3.934	1,2,3,4,5-Cyclopentanepentol	150	$C_5H_{10}O_5$
4	24.682	6.847	11-Methyloctadec-12-enoic acid, Methyl ester	310	$C_{20}H_{38}O_2$
5	24.923	2.194	2,3-Dimethoxy-2-3-Dimethylbutane	146	$C_8H_{18}O_2$
6	25.033	1.865	5-(2-Methoxypropan-2-yl)-2-methyl-2-vinyltetrahydrofuran	184	$C_{11}H_{20}O_2$
7	25.083	1.594	2-Ethylhexanal ethylene Glycol	172	$C_{10}H_{20}O_2$

			Acetal		
8	25.323	2.229	2-Ethylhexanal ethylene Glycol Acetal	172	$C_{10}H_{20}O_2$
9	25.423	1.603	5-(2-Methoxypropan-2-yl)-2-methyl-2-vinyltetrahydrofuran	184	$C_{11}H_{20}O_2$
10	25.773	1.478	4-Deoxypyridoxine, 2Tms Derivative	297	$C_{14}H_{27}O_2NSi_2$
11	25.853	1.728	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
12	26.543	1.329	Antracene,9,10-Dihydro-9,10-Bis(Trimethylsilyl)-	324	$C_{20}H_{28}Si_2$
13	27.093	1.500	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
14	27.253	1.502	4-Methyl-2,4-Bis(P-hydroxypheny)Pent-1-ene, 2TMS Derivative	412	$C_{24}H_{36}O_2Si_2$
15	27.564	1.366	1,3-Dioxolane, 2-Pentadecyl-	284	$C_{18}H_{36}O_2$
16	27.784	1.342	Tetrasiloxane, Decamethyl-	310	$C_{10}H_{30}O_3Si_4$
17	27.964	1.742	Anthracene, 9-ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
18	28.284	1.717	Anthracene,9,10-Dihydro-9,10-Bis(Trimethylsilyl)-	324	$C_{20}H_{28}Si_2$
19	29.164	1.416	Anthracene, 9-ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$
20	29.554	1.372	Anthracene, 9-ethyl-9,10-Dihydro-10-Trimethylsilyl-	280	$C_{19}H_{24}Si$

2-Methylheptanoic acid is indeed a medium-chain fatty acid, composed of eight carbon atoms. They may have potential medicinal roles as an acidifier, arachidonic acid inhibitor, and catechol-O-methyltransferase inhibitor. It may also act as a methyl donor, inhibit methylguanidine, and increase aromatic amino acid decarboxylase activity and uric acid production (Yuvaraj et al., 2019; Kumar et al.,

2022). Both species contained 2-Methylheptanoic acid. 1,2,3,4,5-cyclopentanepentol may also exhibit intriguing biological properties (Angyal and Luttrell, 1970).

Liu et al. (2009) identified 28 volatile aroma compounds in *Ganoderma sinense*, while Wang et al. (2009) reported 26 volatile aroma compounds in the same species. Both studies highlighted ketones, alcohols, and lactones as the major components. In the mycelia of *Ganoderma lucidum*, 58 compounds were detected, with 1-octen-3-ol, ethanol, hexanal, 1-hexanol, sesquirosefuran, 3-octanol, and 3-octanone being the dominant volatile flavor compounds (Chen et al., 2010). Taşkın et al. (2012) identified 18 aroma compounds in *Ganoderma lucidum*. Among these, alcohols such as 1-octen-3-ol, 3-octanol, 1-octanol, and 2-ethyl-1-hexanol were detected as the major components, comprising approximately 48.05% of the total aroma. Despite being present in lower amounts, minor compounds collectively increase the overall bioactivity, adding to the intricate and varied therapeutic potential of *Ganoderma* species. The existence of common compounds like 2-Methylheptanoic acid implies similar bioactive profiles among various *Ganoderma* species, whereas unique compounds in each species highlight their individual therapeutic characteristics. This diversity in chemicals and bioactivity establishes *Ganoderma* species as valuable for medicinal and therapeutic purposes, calling for further research and the possibility of developing health promoting products.

SUMMARY AND CONCLUSION

The present study "Nutrient content and enzymatic activity of wild edible mushrooms in Mamit and Champhai districts, Mizoram," which was carried during 2020-2024 revealed interesting results? These results can be concluded in terms of different morphological, taxonomic, biochemical, and molecular aspects. The collected taxa were identified based on macro- and micromorphological characteristics and species with taxonomic ambiguity were further confirmed through molecular analysis. This study recorded a total of 46 wild edible mushroom species. In this respect, 23 species were further screened rationally and analysed for proximate nutritional composition and mineral content. These results confirmed the comparatively high levels of protein, fibre, essential minerals and other vital nutrients, highlighting their potential as valuable dietary resources.

In the present study, pure cultures were successfully isolated from sixteen edible mushrooms using various media, and seven of these were further evaluated for enzymatic activity. The studied fungal isolates exhibited lignocellulolytic enzyme activities, including laccase, manganese peroxidase, and lignin peroxidase. Although the enzyme activities were generally moderate rather than high, they were sufficient to indicate the biotechnological and industrial potential of these fungi, particularly in applications related to biomass degradation and value-added product development. In addition, wood rotting fungal species demonstrated greater sensitivity to growth conditions and yielded pure cultures more readily than soil-inhabiting edible mushrooms. This observation reflects the strong cultural adaptability and ease of laboratory establishment of wood-decaying fungi.

Assessment of heavy metal content and associated health risk was conducted on five edible species of wide dietary consumption. Herein, results underlie within safe permissible limits, confirming that these mushrooms are suitable for consumption. In this respect, safe consumption can be attributed to growth of selected mushrooms in relatively unpolluted environment study area with noindustriies and least anthropogenic sources of other metallic contaminants.

Furthermore, Antioxidant profiling was carried out on one edible species and two wood rotting fungi, while GC–MS analysis of volatile compounds from the wood rotting taxa revealed various bioactive metabolites with antioxidant and health-promoting properties, further supporting their medicinal potential. Henceforth, in addition to nutritional security, these antioxidants and metabolites can augment the primary health care of local indigenous people of Mizoram. Moreover, several wood rotting and soil macrofungi of significant mycological importance were identified, including first time reports for some taxa, contributing valuable new information to fungal diversity records. The findings reveal abundant and diverse macrofungal resources in Mizoram and emphasise the nutritional richness, antioxidant value, and enzymatic potential and bioprospecting prospects of wild mushrooms. Thus, present study expanded the horizon of scientific knowledge on morphological, biochemical, and molecular aspects wild edible mushrooms in Mizoram.

Furthermore, the outcomes of this research provide a strong foundation for future investigations on mushroom based nutraceuticals, functional foods, and biotechnological applications, as well as for the conservation and sustainable utilisation of macrofungal biodiversity. Given their high nutritional value, low caloric density, and health promoting properties, wild edible mushrooms can play a significant role in enhancing food and nutritional security, improving public health, and supporting rural livelihoods through community based collection, cultivation, and value addition. In a broader context, the sustainable exploitation of mushroom resources aligns with the United Nations Sustainable Development Goals, particularly those related to ‘zero hunger (SDG 2)’, ‘good health and well-being (SDG 3)’, ‘decent work and economic growth (SDG 8)’, and ‘responsible consumption and production (SDG 12)’. Thus, the present study not only contributes to regional mycological knowledge but also underscores the potential of wild mushrooms as valuable bioresources for sustainable development and socio-economic upliftment in Mizoram.

APPENDICES

Appendix 1: Publications in peer reviewed journal / book chapter

1. **Thachunglura, V.L.,** Zothanzama, J., Khumlianlal, J. & Rai, P.K. (2025). Evaluation of antioxidant activity of chicken polypore *Laetiporus sulphureus* (Bull.) Murrill. *New Zealand Journal of Botany* 63(5), 1431–1442. https://doi.org/10.1080/0028825X.2025.2495621
2. **Thachunglura, V.L.,** Zothanzama, J., Chawngthu, Z., Bochung, L., Vanlalmalsawmi, R. & Rai, P.K. (2025). Molecular identification and proximate composition of wild edible wood growing mushrooms of Mizoram, North East India for food safety and livelihood. *Journal of Applied and Natural Science* 17(1): 265–276. https://doi.org/10.31018/jans.v17i1.6338
3. **Thachunglura, V.L.,** Rai, P.K., Chawngthu, Z., Bochung, L., Vanlalhluna, P.C. & Zothanzama, J. (2025). Extended distribution of the rare basidiolichen *Sulzbacheromyces yunnanensis* (Lichenized Basidiomycota) from Mizoram, India. *Journal of Threatened Taxa* 17(4): 26887–26892. https://doi.org/10.11609/jott.9399.17.4.26887-26892
4. **Thachunglura, V.L.,** Chawngthu, Z., Rai, P.K. & Zothanzama, J. (2024). Evaluation of Nutritional Value, Heavy Metals Content, and Health Risk Assessment of Wild Edible Mushrooms in Mamit District, Mizoram, North East India. *International Journal of Plant and Environment* 10(3):79-85. https://doi.org/10.18811/ijpen.v10i03.08
5. **Thachunglura, V.L.,** Rai, P.K., Zothanzama, J., Bochung, L. & Chawngthu, Z. (2024). First report of *Caloboletus guanyui* (Boletaceae) in India: Taxonomic and phylogenetic insights from Mizoram. *Plant Science Today* https://doi.org/10.14719/pst.5046
6. **Thachunglura, V.L.,** Rai, P.K., Khumlianlal, J. & Zothanzama, J. (2024). A checklist of wild mushrooms in Mizoram, Northeast India. *Plant Pathology & Quarantine Journal of Fungal Systematics and Evolution* 16 (1), 125-142.

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7. **Thachunglura, V.L,** Rai, P. K., Chawngthu, Z., Lalzarzovi, S.T, Zothanzama, J., & Vanlalhluna, P. (2025). Heavy metals bioaccumulation in wild edible mushrooms of an Indo Burma biodiversity hotspot: Suitability assessment for safe consumption and food safety/security implications. *EQA - International Journal of Environmental Quality* 70, 66–75. https://doi.org/10.6092/issn.2281-4485/21806
8. **Thachunglura, V.L.,** Rai, P.K., Zohmangaiha, Z., Lalbiakmawia, B., Lalmuansangi, L., & Zothanzama, J. (2023). *Pleurotus giganteus* as a Valuable Source of Nutrients. *Indian Journal of Science and Technology* 16(sp1), 89–94. https://doi.org/10.17485/ijst/v16sp1.msc12
9. **Thachunglura, V.L.,** Rai, P.K., Chawngthu, Z., Renthlei, L., Vanlalmalsawmi, R., Bochung, L., Khumlianlal, J. & Zothanzama, J. (2024). Nutritional composition and mineral contents of common edible wild mushrooms from Mamit and Champhai Districts of Mizoram, India. *Food Agricultural Sciences and Technology* 10(1), 42-58.
10. **Thachunglura, V.L.,** Chawngthu, Z., Zothanzama, J., Lallawmkima, Lalbiakmawia, B., Khumlianlal, J. & Rai, P.K. (2023). Russulaceae of Ailawng forest with an emphasis on *Russula purpureoverrucosa* (Russulaceae): A first report for India. Science Vision 3, 41–47. https://doi.org/10.33493/scivis.23.03.01
11. **Thachunglura, V.L.,** Lalbiakmawia, B., Zohmangaiha, Renthlei, L., Rai, P.K. & Zothanzama, J. (2023). The edible fan-shaped jelly fungus *Dacryopinax spathularia*. In Land use and Bioresource Management for Sustainable Livelihood. O.P. Tripathi, S.T. Lalzarzovi, Lalnuntluanga and BP Mishra, Today & Tomorrow's Printers and Publishers - New Delhi, pp. 201-206.
12. Chawngthu, Z., **Thachunglura, V.L.,** Zothanzama, J. & Khumlianlal, J. (2024). Risk assessment of heavy metals in wild mushroom from Mizoram, India. *Environmental Pollution and Management* (Elsevier). https://doi.org/10.1016/j.epm.2024.09.001
13. Khumlianlal, J., Huidrom, S., Sharma, K. C., **Thachunglura, V.L.** & Indira, S. (2024). Proximate Analysis and Mineral Content of Wild Edible Mushrooms from Manipur, India. *International Journal of Current Microbiology and Applied Sciences* 13(3), 269–275. https://doi.org/10.20546/ijcmas.2024.1303.026
14. Renthlei, L., Zothanzama, J, Mishra, B.P., Chawngthu, Z., **Thachunglura, V.L.,**

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15. Vanlalmalsawmi, R., Zothanzama, J., **Thachunglura, V.L.,** Lalbiakmawia, B. & Lallawmkima (2023). Arbuscular Mycorrhizal Fungi in Mizo Bird's Eye Chilli (*Capsicum frutescens* L.) from Home Gardens in Aizawl. *International Journal of Ecology and Environmental Sciences* 49(6). https://doi.org/10.55863/ijees.2023.2992
16. Zohmangaiha, C., **Thachunglura, V.L.,** Lalnuntluanga & Zothanzama, J (2023). Morphological characterization and nutritional quality of *Lentinula edodes*. *Journal of Agriculture Food and Environment* 4(3): 9-12. https://doi.org/10.47440/JAFE.2023.4302
17. Chawngthu, Z., Tluanga, L., Zothanzama, J., **Thachunglura, V.L.,** Lalbiakmawia, B. & Renthlei, L. (2023). Wood rotting polyporales from the biodiversity reserves within the Indian subtropical habitat. *Indian Journal of Microbiology Research* 10(3), 140–148. https://doi.org/10.18231/j.ijmr.2023.025
18. Lallawmkima, **Thachunglura, V.L.,** Lalbiakmawia, B., Zothanzama, J. & Lalzarzovi S.T. (2023). Study of soil organic carbon on current jhum and jhum fallows from Rawpuichhip, Mamit District. *Science Vision* 3, 41–47. https://doi.org/10.33493/scivis.23.03.01
19. Vanlalmalsawmi, R., Lalnuntluanga, John Zothanzama, Lallawmkima Bochung, Benjamin Lalbiakmawia & **Thachunglura, V.L.** (2025). Soil Physicochemical Properties and its Relation to AMF Colonization in *Capsicum frutescens* L. across Seasonal Variation. *International Journal of Current Microbiology and Applied Sciences* 14(05): 33-38. https://doi.org/10.20546/ijcmas.2025.1405.004
20. Renthlei, L., Madhurima, **Thachunglura, V.L.,** Lalbiakmawia, B., Mishra, B.P. & Zothanzama, J. (2023). Identification of Wild Mushroom in Sub-tropical forest of Aizawl district, Mizoram. In Land use and Bioresource Management for Sustainable Livelihood. O.P. Tripathi, S.T. Lalzarzovi, Lalnuntluanga and BP Mishra, Today & Tomorrow's Printers and Publishers - New Delhi, pp. 111-117.
21. Lallawmkima, **Thachunglura, V.L.,** Vanlalmalsawmi, R., Lalmuansangi, Lalzarzovi, S.T. & Zothanzama, J. (2023) Soil chemical properties on current jhum and jhum fallows from Rawpuichhip, Mamit. In Land use and Bioresource

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Appendix 2: Lists of conference/ symposium/ seminar attended

1. Presented "**Wild Edible Mushrooms from Mizoram**" at the International Conference on Biodiversity, Biogeochemistry, and Ecosystem Sustainability in a Changing Environment, held from 14-16th June 2023 organized by Mizoram University and the Indian Ecological Society.

2. Presented "**Nutritional Analysis, Mineral Content, and Cultivation of *Pleurotus pulmonarius* from Mizoram**" at the 4th Mizoram Science Congress (National Seminar), held on 24-25th November 2022.
3. Presented "**Isolation, Health Benefits, and Cultural Characterization of *Dacryopinax spathularia***" at the Two-Day National Seminar on Land Use and Bioresource Management for Sustainable Livelihood, held on 27-28th March 2023 at Mizoram University.
4. Presented "**Into The Mycological Depths: Unravelling Fungal Research in Mizoram**," in the International Conference on Climate Change and natural Resource Management for Sustainable Development – ICNS, held on 13-15th March, 2024.
5. Presented "**The role of fungi in mitigating heavy metal pollution: Fact or Myth?**" in the International Seminar on Biodiversity Exploration and Documentation, Environment and Health, held on 20-21st November, 2024 at Mizoram University.
6. Presented "**PACHHIA: The Fungi Nobody Eats but Everybody Needs**" in the International Symposium on Climate Change and Adaptation, held on 11th December, 2025 at Mizoram University.

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DEPARTMENT : Environmental Science

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DATE OF ADMISSION : 07.11.2020

APPROVAL OF RESEARCH PROPOSAL:

1. DRC : 27.04.2021
2. BOS : 29.04.2021
3. SCHOOL BOARD : 05.05.2021
4. MZU REGISTRATION NO : 1506016
5. Ph.D. REGISTRATION NO & DATE : MZU/Ph.D./1609 of 07.11.2020
6. EXTENSION (IF ANY) : NIL

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ABSTRACT

NUTRIENT CONTENT AND ENZYMATIC ACTIVITY OF WILD EDIBLE MUSHROOMS IN MAMIT AND CHAMPHAI DISTRICTS, MIZORAM

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF ENVIRONMENTAL SCIENCE

SCHOOL OF EARTH SCIENCE

&

NATURAL RESOURCE MANAGEMENT

FEBRUARY, 2026

NUTRIENT CONTENT AND ENZYMATIC ACTIVITY OF WILD EDIBLE MUSHROOMS IN MAMIT AND CHAMPHAI DISTRICTS, MIZORAM

BY

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In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Environmental Science of Mizoram University, Aizawl

ABSTRACT

Wild edible mushrooms constitute an ecologically and nutritionally significant yet largely underutilized biological resource, with substantial potential to contribute to food and nutritional security, livelihood enhancement, and sustainable development, particularly in biodiversity rich but scientifically underexplored regions. Mizoram, situated in Northeast India within the Indo–Burma global biodiversity hotspot, is characterized by diverse forest ecosystems that harbour exceptionally rich macrofungal diversity (Zothanzama et al., 2011; Zothanzama et al., 2016; Chawngthu et al., 2023). Despite this, systematic scientific documentation and comprehensive evaluation of wild edible macrofungi from this region remain sparse. The present study undertakes a rigorous, multidisciplinary investigation of wild edible mushroom species collected from natural forest habitats of the Mamit and Champhai districts of Mizoram.

The present work was carried out under the following heads:

- To analyse macro-nutrient and micro-nutrient content of wild edible mushrooms.
- To isolate pure culture of wild edible mushrooms.
- To determine enzyme activity of pure cultures of wild edible mushrooms.

These objectives were further subdivided into biochemical (e.g., antioxidative potential and VOCs) ecotoxicity (specifically in relation to heavy metals) aspects to offer a state-of-art-knowledge on wild edible mushrooms of Mizoram, NE India, underlying in an Indo Burma global biodiversity hotspot.

A total of 46 species of wild edible mushroom were identified based on macro- and micro morphological characteristics. Taxa exhibiting taxonomic ambiguity or overlapping morphological characters were further resolved through molecular characterization using polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA,

followed by comparative sequence analysis with reference sequences in public databases. The identified taxa represent a wide taxonomic breadth and include *Agaricus comtulus*, *Amanita jacksonii*, *Amanita vaginata*, *Auricularia auricula-judae*, *Auricularia delicata*, *Boletellus emodensis*, *Cantharellus cibarius*, *Cantharellus tropicalis*, *Coprinellus disseminatus*, *Dacryopinax spathularia*, *Fistulina hepatica*, *Lactifluus volemus*, *Lactifluus corrugis*, *Lactifluus dwaliensis*, *Lactifluus piperatus*, *Laetiporus sulphureus*, *Lentinula lateritia*, *Lentinus squarrosulus*, *Lentinus tigrinus*, *Lentinus sajor-caju*, *Lentinus cladopus*, *Lycoperdon pratense*, *Lycoperdon perlatum*, *Ramariopsis kunzei*, *Ramaria cystidiophora*, *Russula aurora*, *Russula brevipes*, *Russula compacta*, *Russula subfragiliformis*, *Russula rosea*, *Russula cyanoxantha*, *Russula sanguinea*, *Russula crustosa*, *Russula vesca*, *Russula emetica*, *Schizophyllum commune*, *Strobilomyces strobilaceus*, *Termitomyces heimii*, *Termitomyces clypeatus*, *Tremella fuciformis*, *Pleurotus giganteus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, *Pleurotus cystidiosus*, *Panus roseus*, and *Volvariella taylorii*.

The edibility of the identified species was confirmed based on existing literature, particularly the comprehensive classification provided by Li et al. (2021).Most of the species identified in this study were classified as E1 (edible, confirmed), while a few fell under E2 (edible, confirmed but with conditions) in the final edibility status (FES). Among the identified taxa, 42 species were categorized as E1, whereas 4 species were classified as E2, indicating that they are not considered safe for direct consumption without proper processing or caution. The E2 species include *Amanita vaginata*, *Coprinellus disseminatus*, *Lycoperdon pratense*, and *Lycoperdon perlatum*.

The identified species were distributed across 8 orders, 20 families, and 22 genera. This represents a substantial expansion in the recorded diversity of wild edible mushrooms compared to earlier regional studies (Lalrinawmi et al., 2017; Zothanzama et al., 2018), which reported 27 species primarily identified through morphology. However, the present study documented a considerably higher number of edible taxa despite sampling being restricted to only two districts, Mamit and

Champhai. This increase reflects advancement in diversity assessment and documentation achieved through the integration of molecular tools with classical morphological approaches, thereby extending existing knowledge and providing a more comprehensive account of wild edible mushroom in Mizoram.

Out of the 46 species identified, 23 representative wild edible mushroom species were selected for the evaluation of their nutritional value based on their availability, abundance, presence of mature fruiting bodies, and broad ecological distribution across the study sites. The evaluated species included *Auricularia delicata*, *Laetiporus sulphureus*, *Panus roseus*, *Lentinus squarrosulus*, *Pleurotus cystidiosus*, *Pleurotus djamor*, *Pleurotus pulmonarius*, *Schizophyllum commune*, *Lactifluus volemus*, *Lactifluus corrugis*, *Dacryopinax spathularia*, *Auricularia auricula-judae*, *Cantharellus cibarius*, *Lactifluus piperatus*, *Lentinula lateritia*, *Russula crustosa*, *Russula cyanoxantha*, *Russula subfragiliformis*, *Termitomyces heimii*, *Tremella fuciformis*, *Volvariella taylorii*, *Pleurotus giganteus*, and *Lycoperdon pratense*. Comprehensive nutritional profiling was conducted to evaluate both macronutrient and micronutrient composition, including proteins, carbohydrates, fats, fibre, and essential mineral elements, thereby assessing their overall dietary and nutritional significance. The analysed wild edible mushroom species exhibited a favourable and nutritionally rich composition characterized by high protein and carbohydrate contents alongside low fat levels.

On average, the 23 species contained 26.47% protein, 45.90% carbohydrates, and only 2.60% fat, with a fibre content of 7.50%, contributing to digestive health. The mean energy value was 283.82 kcal per 100 g, indicating moderate caloric contribution while providing nutritionally dense macronutrients. In addition to their macronutrient profile, the mushrooms demonstrated a competitive mineral composition, containing mean contents of 10.7 mg/100g calcium (Ca), 13.98 mg/100g iron (Fe), 1530.07 mg/100g potassium (K), 19.64 mg/100g sodium (Na), and 16.55 mg/100g zinc (Zn). The high K-to-Na ratio suggests potential cardiovascular benefits, while appreciable levels of Fe and Zn support haemoglobin synthesis, immune function, and metabolic enzyme activity. Collectively, these

findings highlight the potential of wild edible mushrooms as low fat, protein rich, and mineral dense functional foods with significant dietary and nutritional relevance. In comparison with conventional food sources, the analysed wild edible mushrooms displayed a nutritionally competitive and well balanced mineral profile, supplying essential macro- and microelements, thereby substantiating their classification as nutritionally dense functional foods.

Pure cultures were successfully obtained from 16 species and were maintained under laboratory conditions to investigate their cultural characteristics, growth behaviour, and physiological attributes. The cultured species included *Russula aurora*, *Fistulina hepatica*, *Laetiporus sulphureus*, *Lentinus sajor-caju*, *Cantharellus cibarius*, *Pleurotus giganteus*, *Lentinus squarrosulus*, *Dacryopinax spathularia*, *Russula cyanoxantha*, *Cantharellus tropicalis*, *Schizophyllum commune*, *Russula crustosa*, *Pleurotus djamor*, *Auricularia auricula-judae*, *Pleurotus pulmonarius*, and *Lactifluus volemus*. Among the culture media evaluated, Potato Dextrose Agar (PDA) proved to be the most effective, supporting optimal mycelial growth in 12 out of the 16 species. Sabouraud Dextrose Agar (SDA) supported the growth of 1 species, while Malt Extract Agar (MEA) was effective for 3 species, indicating species-specific preferences for nutrient composition and growth conditions. Based on ecological characteristics, the cultured species were further categorized into two functional groups, where 9 species were identified as wood rotting fungi, which primarily colonize and decompose lignocellulosic substrates, while the remaining 7 species were classified as soil inhabiting mushrooms, typically saprotrophic on organic matter in forest soils. Particularly, wood rotting fungi generally exhibited more vigorous, uniform, and rapid mycelial growth under in vitro conditions, facilitating easier isolation and maintenance of pure cultures on the tested media.

These culture based studies also facilitated the evaluation of enzymatic potential, providing insight into the functional and biotechnological relevance of the investigated taxa. The present study demonstrates distinct species-specific variation in ligninolytic enzyme production among selected wild edible mushrooms under

submerged fermentation. The evaluated species include *Lentinus sajor-caju, Schizophyllum commune, Pleurotus pulmonarius, Dacryopinax spathularia, Laetiporus sulphureus, Pleurotus djamor,* and *Cantharellus cibarius*. *Schizophyllum commune* emerged as the most efficient producer of laccase and manganese peroxidase, whereas *Pleurotus djamor* exhibited the highest lignin peroxidase activity. Wood rotting fungi consistently showed higher ligninolytic potential compared to the ectomycorrhizal species *Cantharellus cibarius*, which displayed minimal enzyme activity, reflecting its non-saprotrophic ecological role. The observed differences are attributed to ecological adaptation, metabolic regulation, and substrate specialization, confirming that wood decaying edible mushrooms possess greater biotechnological potential for lignin degradation and related industrial applications.

In view of the well documented metal accumulating nature of wild mushrooms, the contents of selected heavy metals, namely cadmium (Cd), lead (Pb), nickel (Ni), and manganese (Mn), were quantified to assess potential contamination and accumulation levels in 5 representative wild edible mushroom species, namely *Lactifluus volemus, Lycoperdon pratense, Fistulina hepatica, Schizophyllum commune,* and *Panus roseus*. Human health risk assessment was subsequently performed using estimated daily intake (EDI), target hazard quotient (THQ), hazard index (HI), and incremental lifetime cancer risk or carcinogenic risk index (ILCR/CRI) to evaluate both non-carcinogenic and carcinogenic risks associated with mushroom consumption. The results indicated that the contents of the analysed heavy metals were generally low and remained within permissible safety limits, suggesting a minimal likelihood of adverse health effects and reflecting the relatively unpolluted nature of the sampling sites.

All ILCR values were within the acceptable risk range (10^{-6}–10^{-4}) or below the negligible risk threshold ($<10^{-6}$), as proposed by Hu et al. (2017), indicating minimal carcinogenic risk from the consumption of the analysed wild edible mushrooms. However, comparatively higher contents of Cd in certain species warrant caution with long term or frequent consumption, as Cd exhibited a greater

potential contribution to carcinogenic risk relative to other metals. Although Pb did not present significant carcinogenic or non-carcinogenic risk in the present assessment, interspecific variation in heavy metal bioaccumulation was evident, highlighting that the capacity of mushrooms to accumulate metals differs markedly among species. Consequently, taxa containing heavy metals above permissible limits may pose elevated health risks, particularly in contaminated environments (Orywal et al., 2021). Mizoram is characterized by limited industrialization and minimal anthropogenic disturbance. The mushroom samples analysed in this study were collected from remote forest ecosystems with low human interference; therefore, the comparatively low levels of heavy metals detected are likely attributable to the pristine nature of the habitats and the absence of significant pollution sources.

The study evaluated the antioxidant activity of three wood rotting fungal species using DPPH and ABTS radical scavenging assays. The edible mushroom *Laetiporus sulphureus* demonstrated moderate to strong antioxidant activity, with fermentation broth extracts showing higher scavenging potential than mycelial extracts. The inedible *Ganoderma* species also exhibited significant antioxidant capacity, with *Ganoderma australe* displaying stronger activity than *Ganoderma tropicum*. Volatile compound analysis via GC-MS was conducted on *Ganoderma tropicum* and *Ganoderma australe*, revealing bioactive constituents such as 2-methylheptanoic acid common to both, along with compounds like tetrasiloxanes and anthracene derivatives in *Ganoderma tropicum* and cyclopentanepentol in *Ganoderma australe*.

The present study addresses critical knowledge gaps by integrating nutritional profiling, antioxidant potential, ecotoxicological risk assessment, enzymatic activity, and culture based investigations of wild edible mushrooms from Mizoram. The findings are expected to scientifically validate traditionally consumed species, documenting taxa of high nutritional and functional value, and promote their safe and sustainable utilization. This research directly supports the Mizo community by providing scientific evidence for a culturally important food resource, with potential benefits for dietary diversification, nutritional improvement, and livelihood

enhancement. The study contributes to food and nutritional security, public health assurance, conservation of indigenous knowledge, and the medicinal and biotechnological potential of wild edible mushrooms, while aligning with key Sustainable Development Goals (SDGs).