IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS FROM SELECTED FAMILIES OF AGARICALES FOUND IN CHAMPHAI DISTRICT, MIZORAM, INDIA

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IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS FROM SELECTED FAMILIES OF AGARICALES FOUND IN CHAMPHAI DISTRICT, MIZORAM, INDIA

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Submitted

In partial fulfilment of the requirement of the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl.

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CERTIFICATE

This is to certify that Mr. **C. David Malsawmtluanga** has submitted the thesis entitled "**Identification and quantification of mycotoxins from selected families of agaricales found in champhai district, Mizoram, India**" under my supervision, for the requirement of the award of the Degree of Doctor of Philosophy in the Departmentof Botany, Mizoram University, Aizawl. The work is authentic, content of the thesis is the original work of the Research Scholar, and the nature and the presentation of thework are the first of its kind in Mizoram.

It is further certified that no portion(s) or part(s) of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute.

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April, 2024

I **C. David Malsawmtluanga**, 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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Date: (**C. David Malsawmtluanga**

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LIST OF ABBREVIATIONS AND UNITS

Units:

°C	Degrees Celsius
g	Grams
h	Hours
ha	Hectare
kg	Kilograms
Kcal	Kilocalories
km	Kilometers
m	Meters
mg	Milligrams
min	Minutes
mL	Milliliters
mm	Millimeters
ng	Nanograms
nm	Nanometers
rpm	Revolutions Per Minute
sq.km	Square Kilometers
sq. m	Square meter
sq. mm	Square millimeter
sq. yd.	Square yard
sq.ft.	Square foot
sq.in.	Square inch
sq.mi.	Square mile
µL	Microliters
µg	Micrograms
µL	Microliters
µM	Micromolar

%	Percentage
v/v	Volume/Volume
w/v	Weight/Volume

Abbreviations:

ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AD	Anno Domini
AFLP	Amplified Fragment Length Polymorphism
AAS	Atomic Absorption Spectrophotometry
ANOVA	Analysis of Variance
BHT	Butylated Hydroxy Toluene
CADC	Chakma Autonomous District Council
CTAB	Cetyltrimethylammonium Bromide
DAD	Diode Array Detector
DNA	Deoxyribonucleic Acid
DMRTs	Duncan's Multiple Range Comparisons
DPPH	2,2-Diphenyl-1-picrylhydrazyl
dw	Dry Weight
E	East
EC	Electrochemical
fw	Fresh Weight
GAE	Gallic Acid Equivalent
GPT-3	Generative Pre-trained Transformer 3
HCl	Hydrochloric Acid
HPLC	High-Performance Liquid Chromatography
IC_{50}	Half-maximal inhibitory concentration
ISSR	Inter-Simple Sequence Repeat
ITS	Internal Transcribed Spacer

Lat.	Latitude
LD50	Lethal Dose 50%
LGA	Lamarckian Genetic Algorithm
LOD	Limit of Detection
LOQ	Limit of Quantification
SPSS	Statistical Package for the Social Services
MADC	Mara Autonomous District Council
MIRSAC	Mizoram Remote Sensing Application Centre
ML	Maximum Likelihood
MSL	Mean Sea Level
MS-TOF	Mass Spectrometry-Time of Flight
N	North
NaHCO3	Sodium Bicarbonate
NaNO2	Sodium Nitrite
NaOH	Sodium Hydroxide
NJ	Neighbour-Joining:
NMDS	Non-metric Multidimensional Scaling
OATP1B	Organic Anion-Transporting Octapeptide 1B
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Photo Diode Array
PDB	Protein Data Bank
PDBQT	PDB with Charges and Atoms in Charges
ppm	Parts Per Million
ppt	Parts Per Trillion
q	Quarter
QE	Quercetin Equivalent
RAPD	Random Amplified Polymorphic DNA

R. D	Rural Development
RNA	Ribonucleic Acid
R	Range
RBC	Red Blood Cell
RSD	Relative Standard Deviation
SD	Standard Deviation
SE	Standard Error
SED	Standard Error of Difference
Sil.	Silhouette
Sipl.	Silhouette Place
SIT	Shade Intolerance
Sqrt	Square Root
SPSS	Statistical Package for the Social Services
SSR	Simple Sequence Repeat
TBC	Total Bacterial Count
TIA	Trunk Intensity Area
TKN	Total Kjeldahl Nitrogen
TM	Trunk Moss
TP	Total Phenolic
TPC	Total Phenolic Content
TTH	Total Trunk Height
TWH	Total Wall Height
UV	Ultraviolet
UV-vis	Ultraviolet-Visible
WEMs	Wild Edible Mushrooms
ZRS:	Zero-Resistance States

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CHAPTER I

1. Introduction

1.1 General Introduction

Fungi, a diverse group of eukaryotic microorganisms, play a crucial role in shaping the ecological balance of our planet (Alexopoulos et al., 1996). With a vast array of forms and functions, fungi inhabit a myriad of environments, from the depths of the ocean to the summits of mountains, and even within the complex ecosystems of our own bodies (Hawksworth, 1991). Despite their often-overlooked presence, fungi are essential for the sustenance of life, participating in nutrient cycling, symbiotic relationships, and numerous industrial applications (Webster and Weber, 2007).

1.2 Diversity of Fungi

The kingdom Fungi comprises a vast diversity of organisms, estimated to include over2 million species (Hawksworth and Lucking, 2017), although only a fraction of them have been formally identified. Fungi exhibit a wide range of morphologies, from microscopic unicellular yeasts to elaborate multicellular structures like mushrooms (Kirk et al., 2001). They are classified into several phyla, with the most well-known being Ascomycota and Basidiomycota, which include many of the familiar mushroom-forming species (James et al., 2006). Additionally, Zygomycota, Chytridiomycota, and Glomeromycota contribute to the fungal kingdom's richness (Blackwell, 2011). This diversity is not only reflected in their physical characteristics but also in their life strategies, including saprophytic, parasitic, and mutualistic lifestyles (Hibbett et al., 2007).

1.3 Fungal Biology: Structure and Function

Fungi share common cellular structures with other eukaryotes but possess unique features that set them apart (Gow et al., 2017). The fungal cell wall, composed of chitin, provides structural support and protection (Lengeler et al., 2000). Unlike plants, fungi lack chlorophyll and thus cannot photosynthesize (Gooday, 1995).

Instead, they obtain nutrients through the absorption of organic matter, often secreting enzymes to break down complex compounds into simpler molecules (Bennett and Lasure, 1991). This saprophytic lifestyle makes fungi pivotal in decomposing dead organic material and recycling essential nutrients in ecosystems (Boddy, 2000).

The life cycle of fungi is characterized by a reproductive strategy involving both sexual and asexual reproduction (Bennett and Lasure, 1991). Asexual reproduction commonly occurs through the formation of spores, which can disperse through the air, water, or other vectors (Alexopoulos et al., 1996). Sexual reproduction involves the fusion of specialized reproductive cells, leading to the formation of durable structures such as spore-bearing structures or fruiting bodies (Money, 2016).

1.4 Ecological Roles of Fungi

Fungi exert a profound influence on ecosystems, participating in nutrient cycling, symbiotic relationships, and influencing plant and animal communities (Wardle et al., 2004). As decomposers, fungi break down complex organic compounds into simpler forms, releasing nutrients that can be utilized by plants and other organisms (Boddy, 2001). This crucial role in nutrient cycling contributes to the sustainability of terrestrial ecosystems.

Mycorrhizal associations, symbiotic relationships between fungi and plants, are integral for both partners (Smith and Read, 2010). The mycorrhizal fungus extends the plant's root system, increasing its access to water and nutrients, while the plant provides the fungus with carbohydrates produced through photosynthesis. This mutualistic relationship is fundamental for the growth and health of many plant species.

Moreover, fungi engage in diverse interactions with animals, ranging from mutualistic to pathogenic (Gange et al., 2013). Some fungi form symbiotic relationships with insects, providing nutrients in exchange for dispersal assistance, while others can cause diseases in plants, animals, and humans. Understanding these ecological roles is essential for appreciating the intricate web of interactions

that fungi establish within ecosystems.

1.5 Economic and Industrial Significance

Fungi have extensive economic and industrial relevance (Money, 2016), contributing to various sectors. The food and beverage industry relies on fungi for the production of bread, cheese, and fermented beverages (Steinkraus, 1997). In medicine, fungi are the source of antibiotics, immunosuppressants, and other pharmaceuticals (Demain and Fang, 2000). Industrial processes, such as bioremediation and biofuel production, harness the unique metabolic capabilities of fungi (Lange, 2001). Furthermore, fungi play a crucial role in agriculture by forming mycorrhizal associations with plants, enhancing nutrient uptake and promoting plant growth (Smith and Read, 2010).

1.6 Mushrooms: Distinctive Fungal Structures

Among the diverse forms within the fungal kingdom, mushrooms stand out as distinctive structures, capturing the imagination with their intricate shapes and ecological significance. Mushrooms, belonging mainly to the phyla Basidiomycota and Ascomycota, represent a subset of fungi characterized by their conspicuous fruiting bodies. These fruiting bodies, often colloquially referred to as "mushrooms," play a vital role in the life cycle of these fungi and have garnered attention for their cultural, culinary, and medicinal importance.

Basidiomycota and Ascomycota are the primary phyla associated with mushroom formation (James et al., 2006). Mushrooms belonging to these phyla exhibit a wide range of shapes, sizes, and colors, from the classic cap-and-stem structure to more elaborate and exotic forms.

The mushroom life cycle begins with the germination of spores, which are released from specialized structures known as basidia or asci, depending on the phylum. Under favorable conditions, these spores germinate and give rise to hyphae, the thread-like structures that make up the main body of the fungus. The mycelium, a network of intertwined hyphae, then grows and spreads through the substrate, whether it be soil, decaying wood, or other organic matter (Bennett and Lasure,

1991).

Mushrooms are the reproductive structures that emerge from the mycelium when environmental conditions are suitable. They are designed to release spores into the surrounding environment, ensuring the dispersal of the fungal species. The cap of the mushroom holds the spore-producing surfaces, and the stem elevates the cap, facilitating the release of spores. This strategy ensures the survival and propagation ofthe fungal species in various habitats.

The ecological role of mushrooms extends beyond their reproductive function. They often play a crucial role in nutrient cycling and symbiotic relationships within ecosystems. Additionally, mushrooms contribute to the decomposition of organic matter, recycling nutrients and fostering soil health (Boddy, 2001).

Beyond their ecological significance, mushrooms have garnered attention for their diverse uses in human culture. Culinary enthusiasts prize certain mushroom species for their unique flavors and textures, incorporating them into various dishes. Moreover, mushrooms have been employed in traditional medicine for centuries, with some species exhibiting medicinal properties (Money, 2016). Recent scientific research has explored the potential of mushrooms in pharmacology, revealing compounds with antimicrobial, anti-inflammatory, and anticancer properties (Chang and Wasser, 2012).

Mushrooms, with their captivating structures and ecological roles, represent a fascinating aspect of fungal diversity. Their contributions to nutrient cycling, symbiotic relationships, and human culture underscore the intricate interplay between fungi and the broader ecosystem. This brief exploration sets the stage for further investigation into the specific characteristics and significance of mushrooms within the fungal kingdom.

1.7 Wild Edible Mushrooms (WEMs) and Poisonous Mushrooms

Wild Edible mushrooms (WEMs), classified as fleshy fruiting bodies of macrofungi, possess significant nutritional value, as acknowledged by Das et al. (2014). These mushrooms serve as a vital economic resource for tribal communities

and are considered essential forest products, as noted by Tibuhwa (2013). Commonly consumed mushroom varieties include *Agaricus bisporus*, *A. blazei*, *A. subrufescens*, *Lentinula edodes*, *L. polychrous*, *Pleurotus ostreatus*, *P. sajor-caju*, *Auricularia* sp., *Flammulina velutipes*, among others (Valverde et al., 2015). Mushrooms are renowned for their high protein content, mineral abundance, and a range of vitamins, including vitamins D, K, and occasionally A and C. Notably, they offer dietary fiber with low fat content, predominantly composed of unsaturated fatty acids (Srikram and Supapvanich, 2016).

The nutritional significance of mushrooms extends to their contribution of dietary fibers with low-calorie content, making them valuable additions to a balanced diet (Sanmee et al., 2003). Amino acids present in mushrooms further emphasize their nutritional value, acting as reliable indicators of the food's nutritive quality (Ming et al., 2014). Simultaneously, mushrooms are recognized for their role in breaking down organic matter and participating in various biological processes. They contain an array of minerals, essential and non-essential trace elements, and may accumulate problematic heavy metals. Factors influencing the level of metal accumulation in mushrooms include organic matter quantity, soil pH, metal concentrations in the soil, fungal factors like mushroom species, morphological part of the fruiting body, developmental stages, age of mycelium, biochemical composition, and the interval between fructifications (Gursoy et al., 2009).

The traditional use of medicinal mushrooms in ancient therapies, as highlighted by Wasser (2011), has gained authenticity through contemporary research in ethnomycology. Over the last few decades, substantial progress has been made in demonstrating the effectiveness and novelty of compounds derived from various mushroom types. Present-day clinical practices in countries such as China, Japan, Korea, and Russia heavily rely on preparations derived from mushrooms (Reshetnikovet al., 2004; Van Griensven 2009; Wasser 2010).

In ancient oriental traditions, mushrooms, particularly *Ganoderma lucidum* (Ling Zhi or Reishi) and *Lentinus edodes* (Shiitake), held significant importance. Various mushroom species, including *Inonotus obliquus* (Chaga), *Fomitopsis officinalis*

(Wood Conk or Agaricon), *Piptoporus betulinus* (Birch Polypore), and *Fomes fomentarius* (Poder, 2005), were employed in the treatment of gastrointestinal disorders, cancer, bronchial asthma, and other ailments. *Psilocybe* and *Amanita muscaria* were also historically used for medicinal purposes in different regions (Wasser and Weis, 1999; Van Griensven 2009; Wasser 2010).

Mushrooms serve as a rich source of bioactive compounds, such as phenolic and flavonoids, with benefits to human health (Heleno et al., 2015; Srikram and Supapvanich, 2016). Their antioxidant properties, rooted in phenolic content, contribute to their overall health benefits (Srikram and Suriyan, 2016). Additionally, mushrooms exhibit antimicrobial (Alves et al., 2012; Stojkovic et al., 2013) and antitumor (Popovic et al., 2013) properties. Organic acids, polyphenols, fatty acids, polysaccharides, tocopherols, and proteins are frequently reported as bioactive constituents in mushrooms (Stojkovic et al., 2017). The bioactivity of tocopherols can prevent diseases related to increased free radical formation and oxidative stress (Ferreira et al., 2009; Stojkovic et al., 2017). Polyunsaturated fatty acids (PUFA), essential for cell signaling and gene expression regulation, also exhibit various biological effects, including antioxidant, antitumor, antimutagenic, and antibacterial activities (Stojkovic et al., 2017). Given the correlation between free radical formation and diseases, the exploration for novel sources of natural antioxidants remains a significant focus in cancer chemoprevention (Stojkovic et al., 2017).

Mushrooms, with their diverse shapes, colors, and sizes, hold a mystique that captivates foragers and nature enthusiasts. However, hidden among the edible mushrooms lies a potentially deadly secret – poisonous mushrooms. *Amanita phalloides*, commonly known as the Death Cap, stands out as one of the deadliest mushrooms worldwide. Other hazardous species include *Amanita muscaria* (Fly Agaric), *Galerina marginata*, and *Cortinarius spp*., each harboring potent toxins that can cause severe illness or death if ingested (Benjamin, 1995).

The toxic effects of mushrooms are primarily attributed to various chemical compounds, each with distinct mechanisms of action. *Amanita phalloides*, for instance, contains potent hepatotoxins known as amatoxins. These compounds

disrupt cellular function by inhibiting RNA polymerase II, leading to severe liver and kidney damage. (Wieland, 1986). Muscimol and ibotenic acid in *Amanita muscaria* produce hallucinogenic effects but can lead to poisoning with symptoms ranging from nausea to seizures (Rumack and Spoerke, 1994).

One of the significant challenges associated with poisonous mushrooms is accurate identification. Visual similarities between toxic and edible species can deceive even experienced foragers. Accurate identification requires careful attention to details such as cap characteristics, gill structure, spore color, and the presence of a veil. Molecular techniques, including DNA analysis, have become invaluable in distinguishing between closely related species and ensuring accurate identification.

Public awareness campaigns, mycological education programs, and collaboration between enthusiasts and experts are crucial components of minimizing the risks associated with wild mushroom foraging. The enjoyment of wild edible mushrooms should be accompanied by a thorough understanding of local fungal diversity and a commitment to responsible harvesting practices.

In conclusion, the world of mushrooms encompasses not only ecological intricacies and culinary delights but also the imperative to distinguish between the bounty of theedible and the potential dangers of the poisonous. A harmonious interaction with mushrooms requires knowledge, respect, and a deep appreciation for the delicate balance between the benefits and risks they present.

1.8 Molecular technique for identification of Mushrooms

The field of mycology has undergone a transformative shift with the integration of molecular techniques, offering rapid and precise identification of mushrooms through a biotechnological approach. Lee et al. (2006), Moreau et al. (2006), Urbanelli et al. (2007), and Lian et al. (2008) have been at the forefront of introducing these advanced molecular methods. The advent of modern technology, particularly DNA-based techniques, has revolutionized our understanding of microbial diversity within natural ecosystems (Ward et al., 1990; Tuckwell et al., 2005).

DNA-based polymerase chain reaction (PCR) and taxon-specific primers, developed by Mullis and Faloona in 1987, have played a pivotal role in the accurate detection and study of fungi. These techniques provide a powerful tool for researchers and mycologists to delve into the intricate world of mushroom diversity in a systematic manner. The internal transcribed spacer (ITS) region, commonly utilized for fungi identification at the species level, has emerged as a reliable molecular marker (Sanchez-Ballesteros et al., 2000).

Molecular fingerprinting stands out as an efficient tool for analyzing genetic variations across different organisms. Its significance lies in its ability to exhibit high levels of detectable polymorphism and independence from environmental parameters (Zervakis et al., 2001). Various molecular markers, such as inter-simple sequence repeat (ISSR), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD), have become commonplace for molecular characterization in plants, animals, fungi, and other organisms (Mei et al., 2014).

The utilization of ISSR, SSR, AFLP, and RAPD markers has enhanced the precision and depth of molecular characterization. These markers provide valuable insights intothe genetic makeup of mushrooms, allowing for a more comprehensive understandingof their diversity and evolutionary relationships. Researchers leverage these markers to explore the intricacies of mushroom populations, unraveling patterns of genetic variation and relatedness.

The adoption of molecular techniques in mycology represents a paradigm shift, offering not only efficient identification but also a deeper comprehension of the complex interactions within fungal communities. This biotechnological approach contributes to the conservation of biodiversity, aids in ecological studies, and facilitates the identification of novel fungal species. As technology continues toadvance, molecular tools will undoubtedly play an increasingly integral role in unraveling the mysteries of the fungal kingdom.

1.9 High-Performance Liquid Chromatography (HPLC) for detection and

quantification of mycotoxins.

In recent years, the analytical landscape in mycology has witnessed a profound transformation, particularly in the detection and quantification of toxic compounds within mushrooms. Among the diverse array of mushroom toxins, amatoxins stand out due to their potent hepatotoxic effects and lethality. The need for precise and reliable analytical techniques for the detection of amatoxins has become increasingly crucial in the realms of food safety, clinical diagnostics, and forensic mycology. High- Performance Liquid Chromatography (HPLC) has emerged as a powerful and versatile tool, offering unparalleled sensitivity and accuracy in the identification and quantification of amatoxins.

HPLC, a sophisticated chromatographic technique, has become the method of choice for the detection of amatoxins due to its ability to provide high resolution, sensitivity, and specificity. This technique relies on the separation of components within a sample based on their interactions with a stationary phase and a mobile phase, allowing for the precise quantification of individual compounds. In the context of amatoxin detection, HPLC is particularly well-suited for its capability to discern and quantify multiple amatoxin variants present in complex matrices with high precision.

The principle behind HPLC involves the use of a liquid mobile phase to carry the sample through a column packed with a stationary phase. As the components in the sample interact with the stationary phase to varying degrees, they separate and elute from the column at different times, enabling their individual detection. This separation is enhanced by the use of specific detectors, such as ultraviolet (UV) or fluorescence detectors, which provide sensitivity to the unique spectral characteristics of amatoxins.

In the context of mycology, HPLC has demonstrated its efficacy in amatoxin detectionby offering advantages such as rapid analysis, low detection limits, and the ability to differentiate between amatoxin variants. Furthermore, HPLC is amenable to quantitative analysis, enabling the determination of toxin

concentrations in complex biological samples.

1.10 In silico molecular docking for the discovery of potent antidotes against Mushroom Poisoning.

Molecular docking plays an important role in the rational design drugs and is helpful in elucidating key features of ligand/receptor interactions (Garcia et al., 2014). The importance of in silico molecular docking in the context of antidote discovery for mushroom poisoning becomes evident when considering the intricate molecular mechanisms underlying toxicity. Amanita toxins, for instance, exert their devastating effects by inhibiting RNA polymerase II, disrupting protein synthesis and leading to organ failure. Traditional experimental approaches to identify antidotes involve screening vast libraries of compounds, a process that can be both time-consuming and costly. In silico molecular docking accelerates this process by virtually screening large databases of compounds against specific target proteins, providing a more efficient and cost-effective means of identifying potential antidotes.

The methodology of in silico molecular docking involves the use of computer algorithms to predict the binding affinity and orientation of a ligand within the active site of a target protein (Schleinkofer et al., 2006). The accuracy of these predictions is continually improving with advancements in computational algorithms and increased understanding of protein-ligand interactions. By leveraging molecular docking simulations, researchers can assess the strength of interactions, estimate bindingenergies, and prioritize candidate antidotes for further experimental validation.

In the realm of mushroom poisoning, in silico molecular docking has already demonstrated its utility in identifying potential antidotes against specific toxins. For example, studies have employed molecular docking simulations to identify compounds capable of disrupting the binding of amatoxins to RNA polymerase II, potentially preventing their toxic effects (Garcia et al., 2015). This approach provides a streamlined and efficient means of narrowing down candidate

compounds, significantly expediting the drug discovery process.

1.11 Objectives of the proposed research

1. To investigate the diversity and factors influencing the growth and distributionof wild edible mushrooms (WEMs) in Champhai district.

2. To analyse the proximate composition, mineral content and antioxidantactivities of wild edible mushrooms.

3. To identify and quantify mycotoxins present in wild mushrooms of Champhaidistrict.

4. In silico molecular docking of potent antidodes for treatment of Amatoxins.

CHAPTER II

2. Review of literature

2.1. Ecology and Diversity of Wild Mushrooms

A mushroom, defined as a macrofungus with distinctive fruiting bodies visible to the naked eye, has transitioned from being classified under the Kingdom Plantae to the Kingdom Fungi due to its unique morphological features. Comprising major phyla like Basidiomycota and Ascomycota, mushrooms reproduce through spores that germinate into filamentous mycelia. These mycelia, unseen without magnification, form primary and secondary mycelia through plasmogamy, accumulating nutrients from the substrate. The mushrooms undergo two life cycle phases: vegetative growth, involving the linear expansion of mycelia that break down complex substrates for nutrient absorption, and reproductive growth, characterized by the emergence of fruiting bodies when exposed to specific environmental cues such as low temperature, high humidity, and oxygen.

In the reproductive phase, mycelial growth halts, and fruiting bodies, including caps and stems, develop from pins. Spores housed in the gills are released for the next generation. This transition signifies the manifestation of mushrooms as a distinctive fungal group forming conspicuous sporocarps (Ferris et al., 2000). Overall, mushrooms play a vital ecological role, showcasing a dynamic life cycle influenced by environmental factors, substrate availability, and unique reproductive strategies.

Wild mushrooms play an important role in ecological process. Most of them are symbiotically associated with trees and sustains the growth of indigenous and commercial plantations of tropical forests (Zotti et al., 2013). The saprobic WEMs are very crucial in nutrient recycling and also providing economical benefits to rural/tribal people by collecting them from wild and selling in the markets (Apshahana et al., 2018). The number of fungi on Earth is currently estimated about 15,00,000 and only 14,000 were recognized as mushroom species (Hawksworth,

2001; Schmit and Mueller, 2007; Kirk et al., 2008) but the recorded WEMs is about 1154, which is only8.24% of the estimated mushroom species (Boa, 2004).

In their latest findings, Megersa et al. (2017) documented 49 fungal taxa over three years of collections in the Degaga natural forest of Ethiopia. Furthermore, a collection of 64 macrofungal species was obtained within a single rainy season. Notably, certaintaxa, such as *Agaricus spp*., *Agrocybe spp*., and *Calvatia spp*., could only be identified at the genus level. Additionally, some species remained unidentified, suggesting the potential existence of novel species yet to be recognized by science (Dejene et al., 2017). Reports on the diversity of wild mushrooms are scarce, a situation attributed to the absence of adequate research infrastructure and a shortage of fungal taxonomists and ecology specialists (Sitotaw et al., 2015; Megersa et al., 2017; Osarenkhoe et al.,2014). Consequently, fungi are notably absent from the country's biodiversity database (IBC, 2005). Despite the limited scientific understanding of fungal diversity, the harvesting and utilization of wild mushrooms persist as a customary practice among various tribes in the country (Tuno, 2001; Semwal et al., 2014).

The investigation into the variety and ecological dynamics of wild mushrooms in the riparian zone of Lake Kivu, Rwanda (Munyaneza et al., 2017), offers valuable insights into the fungal communities within this region. This overview summarizes key findings related to the prevalent order, diversity metrics, and the impact of habitat structure on the distribution of mushroom species. One noteworthy discovery from the study is the prevalence of the Agaricales order, constituting a substantial portion (81%) of the species collected. Agaricales is a diverse order known for its varied ecological roles and encompasses a range of mushroom species. This dominance aligns with global patterns, as Agaricales frequently stands out in assessments of fungal diversity (Smith and Singer, 1964). The computed diversity metrics, such as the total order/family ratio (0.31), family/genus ratio (0.65), and genus/species ratio (0.63), serve as indications of the considerable family and generic diversity within the mushroom collections. These ratios underscore the diverse fungal assemblage present in the riparian zone of Lake Kivu. Elevated

ratios typically indicate a diverse and mixed fungal community, contributing to the overall biodiversity of the ecosystem (Magurran, 1988).

Buba et al. (2018) conducted a comprehensive study on the ecology of edible mushrooms in the Nigerian savannah, aiming to provide insights for their optimal exploitation. The research revealed significant findings regarding mushroom species diversity, microhabitats, and their temporal variations. They identified 31 different edible mushroom species in the study area, predominantly belonging to the families Agaricaceae, Lyophyllaceae, and Polyporaceae. This taxonomic distribution aligns with global trends, showcasing the prevalence of these families in mushroom diversity studies (Geml et al., 2008). The study identified 18 different microhabitats supporting the growth of edible mushrooms. These included arable lands, fallow lands, soils around dead tree stumps, woods, and various living tree species. The microhabitat specificity provides valuable information for understanding the ecological niches of these mushrooms. Notably, *Parkia biglobosa* emerged as the microhabitat with the highest species richness (15) and species diversity (Shannon Diversity Index, SDI: 2.54). *Tamarindus indica* and decaying wood also showed significant diversity, emphasizing the impact of specific microhabitats on mushroom populations. Temporal variations were observed in mushroom species presence, with 21 species in 2016, 24 in 2017, and 13 species common to both years. Although differences were not statistically significant (p-value = 0.961), these temporal patterns provide valuable insights into the dynamics of mushroom populations over time.

The Chinese have identified 966 edible mushrooms and 576 medicinal species (Dai etal., 2009), of which around seventy species can be cultivated and eighteen species canbe cultivated commercially. In 2011 study conducted by Xu *et al.,* the focus was on exploring the diversity and population biology of wild mushrooms in southwestern China. Their findings not only revealed extensive diversity but also provided evidence of cryptic speciation, genetic differentiation, and geographic structuring within the studied region. They highlighted the occurrence of cryptic speciation and genetic differentiation among wild mushrooms in southwestern

China. This discovery emphasizes the genetic complexity and evolutionary processes within the fungal populations of this ecologically diverse region. The study reported a remarkable statistic - over 600 out of 2,000 edible fungal species worldwide occur in southwestern China. This high abundance of edible fungal species underscores the economic and cultural significance of wild mushrooms in the region. Some of the economically important mushrooms identified in this region include *Thelephora ganbajun* M. Zang, *Tricholoma matsutake* (S. Ito & S. Imai) Singer, and various species of *Russula*. These mushrooms hold economic value, likely contributing to local livelihoods through consumption and trade.

The number of mushroom species documented in India is about 1,200, out of which 300–315 species are considered edible (Thiribhuvanamala, 2011). Several mushroomsthat have been reported from India include *Auricularia*, *Ganoderma* and *Cordyceps* (Singh et al., 2007, 2008 and 2009). Furthermore, Rattan (1977) reported 197 species of *Resupinate aphyllophorales* from Northwest Himalayas region. In the year 1989, fifty-five taxa of *Russula* and twenty-six taxa of *Lactarius* were described by Atri andSaini. In addition, fifty mushrooms from Amarkantak in Madhya Pradesh weredescribed including major genus *Agaricus, Amanita, Boletus, Ganoderma, Macrolepiota, Nyctalis, Russula* and *Termitomyces* (Dwivedi et al., 2012). Two taxa of genus *Amanita* Pers. were reported for the first time in India such as *Amanita griseofolia* Zhu L. Yang and *A. hemibapha* var. *ochracea* Zhu L. Yang. Senthilarasu (2014) reported a total of 178 species in 68 genera (including 21 new species and two new genera *Chlorolepiota* and *Singerina*) belonging to 23 families and 5 orders (Agaricales, Boletales, Cantharellales, Polyporales and Russulales) have been described from Maharashtra.

The Indian state of Jammu and Kashmir, situated in the northwest Himalayas, possesses a rich and untapped reservoir of macrofungal diversity owing to its diverse climatic and topographic conditions. These factors create a favorable environment forthe abundant growth of a diverse array of fungi.

A significant body of research on mushrooms in the northwestern Himalayas has beenpresented by Atri and Saini (1989), who conducted a comprehensive review of

Russulaceae worldwide, encompassing its Indian components. Their work includes thedescription of numerous mushroom species, such as *Russula* and *Lactarius* (Atri et al., 1991a), *Agaricus campestris* (Atri et al., 1991b), *Termitomyces* (Atri et al., 1995), *Agaricales* and *Gasteromycetes* (Atri et al., 1995), and *Lepiota* (Atri et al., 1996). Atri et al. (1997) further delved into the taxonomy, distribution, ecology, and edibility of thirty taxa within the genus Russula, introducing new records from India.

The Garhwal Himalaya spans a vast range of altitudes, from approximately 350 metersto 7,817 meters above sea level, exhibiting a remarkable variation in climatic conditions. This region encompasses diverse climates, including the warm and humid Terai belt on one end and a cold desert bordering Tibet on the other. As a result of this climatic diversity, the Garhwal Himalaya is endowed with a rich and varied array of mushrooms. Notably, some mushrooms with medicinal significance have been collected and studied in the Garhwal region, as highlighted by Vishwakarma et al. (2011). Among these species are *Ganoderma lucidum*, *Agaricus campestris*, *Hydnum repandum*, *Coprinus comatus*, *Morchella esculenta*, and *Cantharellus cibarius*.

In India, the northeastern region stands out as a high-rainfall area, encompassing some of the wettest areas globally. The monsoon season, occurring from June to October, brings about elevated humidity levels, creating optimal atmospheric conditions for the flourishing of numerous saprophytes, including mushrooms (Choudhary et al., 2015).

According to Sarma et al., 2010 study in the Assam state of the northeastern part of India, the predominant macrofungi species include *Ganoderma lucidum* (100%), followed by *Cantharellus tubaeformis* (83.33%) and *Agaricus bisporus* (83.33%). Other notable species with a prevalence of 66.66% include *Schizophyllum commune*, *Auricularia delicata*, *Boletus luteus*, *Cantharellus cibarius*, *Lycoperdon cladopus*, and *Termitomyces clypeatus*. Species with a 33.33% prevalence include *Auricularia auricula*, *Lentinus edodes*, *Laetiporus sulphureus*, *Morchella esculanta*, *Termitomyces mammiformies*, *Auricularia polytricha*, *Agaricus*

silvaticus, Calvatia gigantea, Lentinus sajor-caju, Lentinus ostreatus, and *Tricholoma terreum*. Lastly, species with a 16.66% prevalence include *Agaricus campestris, Boletus edulis*, *Lenzites betulina*, *Lycoperdon pyriforme, Termitomyces robustus,* and *Termitomyces microcarpus*.

The forests of Nagaland host a diverse array of mushrooms, greatly enjoyed by the local population. A recent study by Tanti et al. (2011) identified thirteen macrofungi, belonging to nine genera and six families, being offered in the market of Kohima town in Nagaland. *Termitomyces* stands out as the most popular among edible mushrooms, prized for its distinctive and delicate flavor. This particular mushroom variety is typically found during the rainy season, specifically from May to July. *Termitomyces R.* Heim belongs to a genus of termitophilous fungi that live symbiotically within termite nests, particularly with species of the *Macroterminae* (Isoptera). This symbiotic relationship has been studied by researchers such as Batra and Batra (1979), Bels and Pataragetvit (1982), Heim (1977), and Rouland-Lefevre et al., (2002).

Mizoram diverse topography and climatic conditions provide an ideal habitat for a myriad of wild mushroom species. A comprehensive examination of the available literature indicates that, in botanical terms, Mizoram has not been thoroughly investigated, with only a few collections made in the past. Moreover, our understanding of the ecology and diversity of wild mushrooms in Mizoram, in general, is insufficient. Researchers have documented numerous genera and species, showcasing the region's potential as a hotspot for fungal diversity. Lalrinawmi et al., 2017 recorded Twenty-seven (27) species of edible mushrooms growing in both soil and wood. They reported wood inhabiting fungi viz. *Schizophyllum commune* (pasi), *Lentinula lateritia* (pa pal), *Auricularia auricular-judae* (Pu Vana beng), *Lentinus tigrinus* (pa hnahkhar), *L. polychrous* (pa chang/pa puanveng) and soil growing fungiviz., *Lactifluus corrugis* (pa uithin), *Lactarius piperatus* (pa lengvar), *Macrolepiota dolichaula* (pa se-ek), *Russula subfragiliformis* (pa lengsen), *Termotomyces heimii* (pasawntlung), *Volvariella taylorii* (changel pa) are the edible species known to the local community. Zarzoliana et al., 2020 surveyed the Eastern

part of Mizoram, unveiling aremarkable collection of 17 distinct species of WEMs. These mushrooms span a variety of families, showcasing the rich fungal diversity in the region. The identified specimens hail from families such as Russulaceae, Polyporaceae, Boletaceae, Lyophyllaceae, Agaricaceae, Auriculariaceae, Marasmiaceae, Gomphaceae, Schizophyllaceae, and Elaphomycetaceae.

2.2. Proximate composition and mineral content of WEMs

WEMs have been an integral part of human diets and traditional medicine in various cultures for centuries. As interest in natural and sustainable food sources grows, theproximate and mineral contents of these mushrooms have garnered attention due to their potential nutritional and health benefits. Proximate analysis provides insights intothe essential macronutrients, while mineral content elucidates the microelemental composition, both crucial aspects for understanding the nutritional value of WEMs.

Proximate analysis encompasses the determination of moisture, protein, fat, ash, and carbohydrates in a food sample, providing a comprehensive overview of its nutritionalcomposition. WEMs have been found to exhibit significant variability in these proximate componentsIn addition to proximate analysis, the mineral content of WEMs has been extensively investigated, shedding light on their potential contribution to essential elements in the human diet. Mushrooms are known to accumulate various minerals, including potassium, phosphorus, magnesium, calcium, iron, zinc, and selenium. These minerals play crucial roles in maintaining physiological functions andpreventing nutritional deficiencies.

Understanding the proximate and mineral contents of WEMs is vital not only for assessing their nutritional value but also for promoting their integration into modern diets and culinary practices. As consumers increasingly seek diverse and sustainable food options, WEMs present an intriguing avenue for exploration, combining both unique flavors and potential health benefits.

Currently, there is a growing fascination with mushrooms. This fascination primarily revolves around their potential uses, including their role as a source of

food (Chang, 1980), their potential in treating diseases (Rambelli and Menini, 1983; Bushwell and Chang, 1993), their application in bioremediation, and their significance in commercial endeavors (Smith, 1972; Stamets, 1993).

According to Aletor (1995), Fasidi (1996), Okwulehie, and Odunze (2004), tropical mushrooms are high in protein, minerals, and vitamins. Mushroom protein concentration has been claimed to be twice that of vegetables, four times that of citrus, and much greater than wheat (Baano, 1993; Aletor, 1990). It is not unexpected that Okwulehie and Odunze (2004) claimed that the increased demand for mushrooms could be reliant on the remarkable growth in the unit costs of conventional sources of animal proteins such as beef, hog, chicken, and fish, as opined by Aletor (1995).

Mushrooms exhibit a low dry matter content, ranging from 60-140 g/kg (Cheung, 1998). This finding aligns with the research of Bano and Rajarathnam (Bano, 1988), who documented a substantial moisture percentage of 81.8-94.8% in edible mushrooms. The variation in moisture content is influenced by factors such as mushroom species and various parameters associated with harvest, growth, and storage conditions. The high moisture content contributes to the short shelf life of fresh mushrooms (Guillamón, 2010). According to Rajarathnam and Sashirekha (2003), mushrooms generally consist of 63% carbohydrates, 25% protein, 4% fat, and 8% minerals represented by ash on a dry-weight basis.

In the composition of mushroom fruiting bodies, the predominant component is carbohydrate, with the majority existing in polymeric forms such as glucan and hemicellulose. Notably, starch is absent in this context. Monosaccharides, particularly glucose, mannitol, and trehalose, are the primary forms present, along with their derivatives and oligosaccharide groups. Typically, glucose and trehalose contents are relatively low, measured in grams per 100 grams of dry matter. Mannitol, which plays a role in volume growth and firmness of fruiting bodies, varies in quantity among different mushroom species (Barros, 2007).

Mushrooms differ from plants in that their reserve polysaccharide is glycogen, not

starch. This glycogen content typically ranges from 5-10% of dry matter. Mushroom cell walls, constituting up to 80% of dry matter, primarily consist of chitin, a water- insoluble structural polysaccharide. Chitin, while contributing to the firmness of the cell walls, is indigestible for humans and restricts the availability of other mushroom components (Kalač, 2009).

In addition to chitin, mushrooms contain a substantial amount of dietary fiber. Guillamón's research revealed significant variability in dietary fiber content among mushroom species (Guillamón, 2010). For example, in the Boletus group, *Agrocybe aegerita*, *A. bisporus*, *Pleurotus eryngii*, and *osteratus*, total fiber ranged from 5.5-42.6% of dry matter. β-glucans, the main fiber polysaccharides along with chitin, varied in composition. Mushrooms exhibit higher levels of insoluble dietary fiber (2.28-8.99 g/100 g edible weight) compared to soluble dietary fiber (0.32-2.20 g/100 g edible weight). β-glucans, constituting 4-13% of the total fiber, vary in their proportions depending on the mushroom species (Manzi et al., 2004). β-glucans are considered functional compounds with immunomodulatory properties, influencing both humoral and cellular immunity. Due to their anti-infective, anti-cytotoxic, antimutagenic, anti-tumorogenic, and anticoagulant properties, β-glucans hold promise as pharmacological agents (Wakshull et al., 1999).

Protein is the major component next to carbohydrates in mushrooms. Wide variations occurin the content of crude protein because not only the species of mushroom differ largely but also different converting factors are used based on the determination by Kjeldahl method. Although many researchers widely used the Nitrogen converting factor of 6.25 to calculate crude protein in mushrooms, Rajarathnam & Sashirekha (2003) and Barros et al. (2007), used a factorof 4.38 by considering the high proportion of non-protein nitrogen, mainly in chitin. To avoid overestimating the content of crudeprotein, Bauer-Petrovska (2001) recommended a specific converting factor of 4.16. Also, the distribution of proteins within a fruiting body is not even and changes in protein content remain unclear with the development of a fruiting body (Kalač, 2009). Albumins and globulins are the prevailing proteins of *Boletus edulis* and *Cantbaraellus cibarius* (Rajarathnam and

Sashirekha, 2003).

The majority of the essential amino acids are found in the proteins found in mushrooms. However, there aren't many necessary sulfur-containing and aromatic amino acids. Twenty percent or so of the total nitrogen is made up of free amino acids. Despite having little substance, they have a significant impact on how mushrooms taste. In *T. portentosum* and *T. terreum*, glutamic acid and alanine were discovered tobe the two most prevalent free amino acids (Diez and Alvarez, 2001).

Since edible mushrooms have a low-fat content, a high biological value, and the ability to improve people's health, they are regarded as a healthy food source (Dimitrijevic, et al., 2019). According to Oso (1977), because of the low content of fats by mushroom,they are recommended as good food supplement for patients with cardiac problems.

Mushrooms exhibit a considerable presence of various mineral elements. According to Manzi et al (2001), the ash content in mushrooms constitutes approximately 6-10.5% of dry matter, a finding corroborated by Kalač (2009), who reported a range of 5-12%. The primary components in the ash include potassium, phosphorus, magnesium, calcium, copper, iron, and zinc (Kalač, 2009; Guillamón, 2010). In the fruiting body, potassium distribution is uneven, with concentrations decreasing in the order: cap > stipe > spore-forming part > spores. Additionally, certain species contain germanium, known for its capacity to sustain human vitality (Rajarathnam and Sashirekha, 2003).

Mushrooms possess a distinctive ability to gather minerals present in their growth environment. While this trait can be advantageous for obtaining desired minerals in significant amounts, it also poses a risk when toxic elements are accumulated. Fungi can amass potassium and phosphorus in their fruiting bodies, with concentrations reaching 20-40 times and 10-50 times higher than those in the underlying substrates, respectively. Conversely, mushrooms growing in heavily polluted regions or certain species with a propensity for accumulation may exhibit substantially elevated levels of harmful elements, sometimes exceeding those in

substrates by one or two orders of magnitude. The accumulation of trace heavy metals in mushrooms, particularly toxic elements like cadmium, lead, mercury, chromium, arsenic, silver, and tin, has garneredsignificant attention.

2.3. Phytochemical and Antioxidant activities of WEMs

Mushrooms contain a diverse array of secondary metabolite compounds that contribute to various biological activities. Beyond serving as a nutrient-rich food source, they also function as non-toxic drugs with physiological benefits, as highlighted by Mirfat et al., (2010). Plant metabolite compounds are typically categorized into two groups: primary metabolites and secondary metabolites. Primarymetabolites are produced in limited quantities and play a crucial role in the growth and life processes of organisms (Nofiani, 2008). On the other hand, secondary metabolites are compounds generated by organisms as a defense mechanism against extreme environmental conditions or potential predatory threats. Although secondary metabolites are not directly involved in growth, they can transform into primary metabolites under stressful conditions (Nofiani, 2008). These secondary metabolite compounds, characterized by their bioactivity, serve as protective agents for plants, shielding them from pests and diseases in their environment.

Mushrooms accumulate a diverse range of secondary metabolites, such as phenolic compounds, polyketides, terpenes, and steroids. These substances exhibit antioxidant properties and possess significant pharmacological applications, contributing to the maintenance of consumer health (Barros et al., 2007). Phenolic compounds, recognized as vital secondary metabolites, possess redox potential that enables them to function as antioxidants, as reported by Soobrattee et al., (2005). These compounds exhibit a broad spectrum of medicinal benefits, including anti-cancer, anti-diabetic, and anti-inflammatory properties, as highlighted by Nagavani et al., (2010). The ability of phenolic compounds to scavenge free radicals is attributed to the presence of hydroxyl groups within them, as noted by Hatano et al., (1989). Flavonoids also contribute to antioxidant activity by scavenging free radicals in the body, providing protection against diseases such as cancer, arthritis,

Type II diabetes mellitus, and exhibiting anti-inflammatory, antiallergenic, antiviral, and vasodilating actions, as reported by Lee and Shibumoto (2002), Parajuli et al., (2012), and Pereira et al., (2009).

Antioxidants are essential for good health and are our first line of defense against free radical damage. With greater exposure to free radicals, the requirement for antioxidants becomes even more crucial. Antioxidants are chemical compounds that protect cells from damage from free radicals (Ferreira et al., 2007; Oyetayo, 2007; Puttaraju et al., 2006), which are produced exogenous or exogenously taken from foodthat's slows the progression of chronic diseases. Under normal circumstances, the antioxidant defense system in the human body can easily overcome any free radicals that are formed (Capelli and Cysewski, 2007). Free radicals are molecules that are unstable because they lose their electrons. They take electrons from other molecules or cells in our body to stabilize their charge (Gupita and Rahayuni, 2012), but if there is an excess of free radicals in the body, the process of taking electrons from body cells will occur, causing cell damage that can lead to premature aging and, according to Oscar et al., (2020), can prompt the emergence of various degenerative and chronic diseases. he free radicals that damage body cells can be neutralized with antioxidant compounds (Iorio, 2007) and these additional antioxidant compounds are generally obtained from plants.

Several works are focussed on the phytochemicals and antioxidant activity of WEMs globally, among the bioactive molecules, phenolic acids have received special attention because they have been reported to be the primary antioxidant properties of mushrooms (Barros et al., 2007). Yildiz et al., (2015) determined the content of phenolic derivatives in the following four species of mushrooms: *Ganoderma lucidum*, *Morchella esculenta*, *Lentinula edodes*, and *Hericium erinaceus*. The concentration of phenolic compounds, calculated as the amount of gallic acid, was found to be the highest in *G. lucidum* over 26.00 mg/g dry weight (d.w.). A comparable amount wasfound in *M. esculenta* (25.00 mg/g d.w.); while in *L. edodes* and *H. erinaceus*, the content did not exceed 10.00 mg/g d.w. and was estimated as 7.25 and 5.81 mg/g d.w., respectively. The amount of phenolic

derivatives in the following mushroom species was compared by Muszyńska et al. (2013): *Pleurotus ostreatus, Cantharellus cibarius, Armillaria mellea, Imleria badia* (*Boletus badius*), *Boletus edulis*, and *Lactarius deliciosus*. The largest total amount of phenolic derivatives, according to the authors, was found in *I. badia* (48.25 mg/kg d.w.); in the other species, the amounts ranged from 6.00 to 19.72 mg/kg d.w.

Some writers classify flavonoids as phenolic substances (Tungmunnithum et al., 2018). However, due to their vast quantity (about 8000), it's reasonable to define flavonoids separately. Flavonoids exert antioxidant activity due to the presence of hydroxyl groups, as is the case with phenolic compounds (Pietta, 2000). Kosanić et al., (2012) examined the favonoid content of three mushroom species: *Boletus aestivalis, B. edulis*, and *Leccinum carpini*. The analysis of acetone and methanol extracts revealed that acetone extracts contained more favonoid, specifically rutin. The amount of flavonoids in the extracts of *B. edulis*, *B. aestivalis*, and *L. carpini* was 4.93 µg/mg, 3.20 µg/mg, and 1.86 µg/mg, respectively. In one of their works, Butkhup et al. (2018) examined the flavonoid content of several mushroom species collected fromnortheastern Thailand in one of their studies. *Russula luteotacta* (2.09 mg/g d.w.), *Termitomyces fuliginosus* (2.19 mg/g d.w.), T. clypeatus (5.10 mg/g d.w.), *V. volvacea* (3.45 mg/g d.w.), and *Letinus polychrous* (2.27 mg/g d.w.) were discovered to have the highest concentration of these compounds, expressed as catechin.

Herawati et al., 2021 reported that edible wood fungus species like *A. auricula* contain specific secondary metabolites, including flavonoids, triterpenoids, saponins, andtannins. However, they do not contain steroids, alkaloids, carotenoids, or coumarin. On the other hand, *S. commune*, another edible wood fungus, contains flavonoids, steroids, tannins, and coumarin, but lacks triterpenoids, alkaloids, carotenoids, or saponins. They also reported that antioxidant test results, determined through IC_{50} calculations, reveal that *A. auricula* has an IC_{50} value of 499.25 g/mL, *S. commune* has a value of 121.37 g/mL. These findings from the research suggest that edible wood mushroom extracts contain various phytochemicals, demonstrating

potential antioxidant activity.

Mishra et al. (2013) found antioxidant activity in *Pleurotus citrinopileatus, P. djamor, P. flabellatus, P. eryngii, P. florida, Pleurotus ostreatus, P. sajorcaju,* and *Hypsizygus ulmarius*, with DPPH radical-scavenging activities ranging from 13.63 to 69.67 percent and chelating activities ranging from 60.25 to 82.7 percent. Hasnat et al. (2014) found that the ethanol and aqueous extracts of *Russula virescens* had dose- dependent DPPH radical scavenging activity. The aqueous extract had a scavenging potential of 12.67-81.13%, whereas the ethanol extract had an activity of 5.30-52.62%.The *R. virescens* extract was evaluated at concentrations ranging from 0.125 to 2.0 mg/mL. The aqueous extract had an effective concentration (EC_{50}) of 0.22 mg/mL, while the ethanol extract had an EC_{50} of 1.90 mg/mL. The two extracts had much higher scavenging potential than ascorbic acid, which demonstrated 95.90% activity at 2.0 mg/mL. Furthermore, Gąsecka et al., 2018 reported that at a concentration of 1 mg/mL, extracts of *Auricularia auricula-judae* (commonly known as black and red ear mushrooms) prepared in methanol showed 100% DPPH radical scavenging activity, whereas the *Tremella fuciformis* (snow mushrooms) methanol extract showed94.5% DPPH radical scavenging activity at a concentration of 0.4 mg/mL.

Zhenyu et al., (2012) investigated the antioxidant properties of mushroom extracts using a variety of samples. They discovered that the scavenging activity against the $ABTS^+$ radical increased steadily as the sample concentration increased. At a dosage of 20 mg/mL, mushroom extracts demonstrated the highest rate of radical scavenging, ranging from 18.54% to 100%. Among all the extracts, *Russula vinosa* Lindblad acid extracts had the highest antioxidant activity. Gargano et al., (2017) worked with methanol extracts from various mushroom species and discovered that the antioxidant capacity of aqueous extracts was proportional to the concentration utilized; however, lower amounts were necessary to remove all $ABTS^+$ radicals. Aqueous extracts of *Boletus edulis, Lentinus edodes,* and *Amanita cesarea* showed strong $ABTS^+$ scavenging ability, ranging from 85.8% to 92% at low doses of 0.14 mg/mL. The aqueous extracts of *Morchella esculenta, Agaricus bisporus,*

Lactarius deliciosus, and*Pleurotus* sp. were reported to have an intermediate radical scavenging capacity ranging from 26% to 60%, while the lowest potential was identified with the aqueousextract of *Cantharellus cibarius*, which was 10.6%.

2.4.Identification and Quantification of mushrooms toxins

The identification and quantification of mushroom toxins has long been an important area in the fields of toxicology and food safety. Mushrooms are widely favored for their culinary and medicinal value; however, the presence of potentially lethal toxins in some species poses a substantial challenge in ensuring their safe consumption (Liu et al., 2023). However, there are a number of recognized mushroom toxins with specific, and sometimes deadly, effects: α-amanitin, phallotoxin, orellanin, muscarine, monomethylhydrazine, coprine, ibotenic acid, muscimol, arabitol, bolesatine, ergotamine (FDA, 2008).

For the past 300 years, morphological identification has served as a crucial tool for identifying, classifying, and conducting phylogenetic analyses of fungi (Seifert et al., 2007). However, identifying fungi within the Kingdom using morphological parameters is challenging due to the absence of distinct morphological features and the prevalence of microscopic species (Schoch et al., 2012). Morphological character- based identification also has inherent limitations, contributing to false identifications, parallel evolution, phenotypic plasticity, homoplasy (Judd et al., 2002), and difficulties in selecting morphologically cryptic taxa (Schoch et al., 2012).

Due to the constant impact of environmental conditions on mushroom morphologicalfeatures, DNA technology provides a more reliable and environment-independent means of identification (Liu et al., 2013). These DNA-based technologies have successfully enhanced our understanding of phylogenetic relationships and microorganism diversity in natural ecosystems. The data obtained from DNA are openly accessible and are considered highly consistent for the entire community, surpassing reliance on culture-derived data alone (Tuckwell et al., 2005). Notably, theapplication of ribosomal RNA genes and protein-coding genes

has recently proven instrumental in resolving phylogenetic relationships within the fungal phylogeny (Liuet al., 2013).

The internal transcribed spacer region (ITS1 and ITS2) of ribosomal DNA is extensively utilized in phylogenetic analyses across various organisms, including animals, fungi, and plants (Liu et al., 2013). The ITS region, particularly ITS1 and ITS2, accumulates mutations more rapidly than 5.8S, 18S, and 28S rRNAs, making itthe most valuable region for molecular systematics (Liu et al., 2013). In a study by Liuet al. (2013) on 20 Chinese *Pleurotus ostreatus* strains, the length of the ITS1–5.8S– ITS2 region ranged from 638 to 640 bp. The size of the ITS1 and ITS2 regions varied among strains, ranging from 235 to 237 bp and 181 to 182 bp, respectively. However,the sequence of 5.8S rDNA was consistent at 154 bp across all studied strains (Liu et al., 2013). This suggests that the 5.8S rDNA sequence is conserved within the genus *Pleurotus*, while variations, including substitution, insertion, or deletion polymorphisms, allow for clear differentiation between the strains (Liu et al., 2013). A recent study on Pleurotus eryngii strains reported sequence lengths ranging from 518 to 616 bp, including differences in the ITS1 and ITS2 regions spanning from 214to 222 bp and 145 to 236 bp, respectively (Alam et al., 2009).

Numerous studies have been conducted to detect amatoxins in wild mushrooms using different analytical techniques, including RP-HPLC with UV-detection (Enjalbert et al., 1992), DAD (Kaya et al., 2012), MS-TOF (Sgambelluri et al., 2014), and DAD-MS (Garcia et al., 2015), either amperometric or coulometric, can be very attractive due to its specificity and sensitivity for the detection of easily oxidizable compounds in food, environmental, and biological samples (Maio et al., 2022; Sontag et al., 2019).

In 2023, Liu and colleagues developed a robust method integrating solid-phase extraction (SPE) purification and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to detect and quantify five amatoxins and two tryptamines (psilocybin and bufotenine) in mushrooms from China. They found that all seven toxins showed strong linearities (R^2>0.99) within the optimized

concentration range. Bufotenine, psilocybin, and amatoxins had limits of detection (LODs) of 2.0, 5.0, and 10 μg/kg, and limits of quantification (LOQs) of 5.0, 10, and 20 μg/kg, respectively. Using dried shiitake mushroom powder as the matrix, the recoveries for the two tryptamines ranged from 80.6% to 117%, with relative standarddeviations (RSDs) between 1.73% and 5.98%. Amatoxin recoveries varied from 71.8% to 115%, with RSDs ranging from 2.14% to 9.92% at three concentration levels. The study identified Amanita fuligineoides as containing α-amanitin, β-amanitin, and phalloidin in quantities of 607, 377, and 69.0 mg/kg, respectively. Another specimen, labeled as Tricholomataceae, exhibited a psilocybin concentration of 12.6 mg/kg.

Barbosa et al., (2022) used HPLC-UV-EC and HPLC-DAD-MS to analyze amanitin in wild mushrooms from Inner Center Region of Portugal. After validating the analyticalprocedures, the limit of detection for α-amanitin was 55μg mL^{-1} (UV) and 62 μg/mL(EC), while for β-amanitin it was 64μg mL^{-1} (UV) and 24μg mL^{-1} (EC). The intra- andinter-day precision differences were less than 13%, and the recovery ratios were between 89% and 117%. The devised method was successfully applied to fourteen Amanita species and found that amanitin were only identified at *A. phalloides* with amounts of 280μg g^{-1} and 290μg g^{-1} for β-amanitin and α-amanitin with EC detector, and 288μg g^{-1} and 280μg g^{-1} for UV detector.

Garcia et al., 2014 investigated amatoxins and phallotoxins in *Amanita phalloides* from Vinhais and Mogadouro (Portugal) using liquid chromatography-diode array and mass spectrometry detection (LC-DAD-MS). They quantified the toxin α-amanitin through RP-HPLC and DAD in caps, stipes, and volva. The study revealed that caps of *A. phalloides* from Vinhais and Mogadouro contained 783.94 ± 2.66 μg g^{-1} and 666.00 ± 1.45 μg g^{-1}, respectively. Stipes from Vinhais and Mogadouro had concentrations of 323.48 ± 4.03 μg g^{-1} and 302.53 ± 2.78 μg g^{-1}, respectively. The lowest concentrations were observed in volva from Vinhais (103.87 ± 1.84 μg g^{-1}) and Mogadouro (73.16 ± 1.66 μg g^{-1}).

Zhang et al., (2005) recently isolated lethal mushroom species, *Amanita exitialis* Zhu L. Yang and T.H. Li, from Guangdong Province, China, and successfully cultured

forthe first time. Identity confirmation involved sequencing the internal transcribed spacer regions of the nuclear ribosomal DNA and comparing them with a typical fruiting body. They reported that the analysis of amatoxins in pure cultures via HPLC- MS revealed production levels of 728.3 ± 43.8 µg/g (dry matter) of a-Amanitin and60.0 ± 20.7 µg/g (dry matter) of β -Amanitin, constituting approximately 10% of the yield from fruiting bodies.

2.5. In silico molecular docking for potent antidode of mushroom toxins.

In the quest for efficient antidotes against mushroom poisoning, the integration of in silico molecular docking has emerged as a powerful tool, revolutionizing the early stages of drug discovery. This innovative approach utilizes computational algorithms to simulate the binding interactions between potential antidotes and specific toxins found in mushrooms.

One of the key strengths of in silico molecular docking lies in its ability to rapidly assess and predict the binding affinities between antidote candidates and the molecular targets within the toxic compounds. This process aids researchers in identifying promising antidotes by evaluating their potential to neutralize the harmful effects of mushroom toxins.

While in silico molecular docking serves as a crucial initial step in the drug discovery journey, it is important to note that experimental validation remains imperative. However, by harnessing the power of computational approaches, researchers can significantly enhance the efficiency and success rate of identifying potential antidotes for mushroom poisoning. As technology advances, the synergy between in silico methods and experimental validation hold great promise for accelerating the development of life-saving antidotes in the fight against mushroom toxicity.

Unfortunately, no specific antidote for mushroom poisonings, particularly those involving amatoxins, has not been established. As a result, amatoxin poisoning is commonly associated with a poor prognosis, primarily attributed to the risk of liver or kidney failure. Various treatments have been employed following human

intoxications with *Amanita phalloides*, encompassing hormones (insulin, growth hormone, and glucagon), steroids, vitamin C, vitamin E, cimetidine, α-lipoic acid, antibiotics (benzylpenicillin, ceftazidime), N-acetylcysteine, and silybin. Among these, only benzylpenicillin, ceftazidime, N-acetylcysteine, and silybin have demonstrated some level of therapeutic efficacy; however, the mortality rate remains exceptionally high (Poucheret et al., 2010). Survival hinges largely on the extent of liver damage, the pace of hepatic regeneration, and the management of complications that may arise during the course of treatment for intoxication (Koda-Kimble et al., 2012). Liver transplantation is considered a last resort; nevertheless, it remains a crucial intervention in patients experiencing fulminant hepatic failure (Broussard et al., 2001;Pinson et al., 1990).

Considering the primary toxicity mechanism of amatoxins, characterized by the inhibition of RNAP II activity, an optimal therapeutic strategy against *Amanita* sp. intoxications would involve displacing and/or competing with amatoxins for binding to RNAP II without compromising its normal transcription activity. In this study, our objective was to identify an effective antidote for *Amanita* mushroom poisonings. Weemployed an innovative approach of in silico methods, focusing on the binding of α- amanitin to RNAP II and screening clinical drugs that exhibit bioisosterism with amatoxins. Bioisosterism was assessed through in silico models to evaluate potential competition and displacement from the amatoxins binding site on RNAP II. This methodology aimed to provide insights into a promising antidote for countering the toxic effects of *Amanita* mushroom poisonings.

CHAPTER III

3. Study area

3.1. A brief information about Mizoram

Mizoram, situated in the northeastern region along the eastern border of the country inthe southern part of north-eastern India, is distinguished by hilly terrain covered with varying degrees of forest density. Covering a geographical expanse of 21,081 sq.km, the state is positioned between 21° 58' & 24° 35' N Latitude and 92° 15' & 93° 20' E Longitude, with the Tropic of Cancer intersecting at 23° 30' N latitude. Its strategic significance is underscored by its proximity to the international boundaries, sharing 404 km with Myanmar to the east and south, 318 km with Bangladesh to the west, andbordering neighboring states such as Assam (123 km), Manipur (95 km), and Tripura(66 km) (Anon., 2009).

Comprising eleven districts, namely Aizawl, Lunglei, Champhai, Kolasib, Mamit, Serchhip, Khawzawl, Saitual, Hnahthial, Lawngtlai, and Saiha. Lunglei holds the distinction of being the largest district with an area of 4538 sq km, while Kolasib is the smallest at 1382.51 sq km. Lawngtlai and Saiha districts deviate from the administrative norm, housing two Autonomous District Councils each. Lawngtlai hosts the Lai Autonomous District Council (LADC) and the Chakma Autonomous District Council (CADC) with headquarters at Lawngtlai and Chawngte (Kamalanagar) respectively. The third autonomous district council, Mara Autonomous District Council (MADC), is located in Saiha district, with its administrative center in Saiha town. These autonomous regions function in accordance with the provisions outlined in the Sixth Schedule of the Constitution of India.

As of the 2011 census, Mizoram has a total population of 1,091,014, with 552,339 being male and 538,675 females. The literacy rate, based on the 2011 population census and statistics compiled by the Economics & Statistics Department, Governmentof Mizoram, stands at 91.58% (Anon., 2011). The main occupation of

the people is agriculture.

As per the State Forest Report, Mizoram spans a total geographical area of 21,081 sq.km. Within this expanse, a substantial 88.93% is covered by forests, comprising 138 sq. km of very dense forest, 5,858 sq. km of moderately dense forest, and 12,752 sq. km of open forest. Notably, there has been a decrease of 306 sq. km in forest cover compared to the State Forest Report of 2013, attributed to factors such as shifting cultivation and other biotic pressures on forest lands (Anon., 2015).

3.1.1. Physiography

The physiography Mizoram can be broadly categorized into hills and valleys. The hilly terrains are diverse, encompassing High hills characterized by undulating landscapes with an average altitude above 1300 m (msl), Medium hills ranging between 500 m and 1300 m in altitude, and Low hills with altitudes below 500 m above msl. The highest point, reaching 2,200 m, is observed in the Blue Mountains, notably Phawngpui. These hills align in a north-south direction, running parallel to each other,with valleys interspersed between them. Throughout the western part of the state, dissected hills and hillocks prominently shape the topography of most river valleys.

3.1.2. Geology

The geology of Mizoram is characterized by a repetitive sequence of arenaceous (sandstone) and argillaceous (clay) sediments, which underwent folding into approximately NNW-SSE trending longitudinal plugging anticlines and synclines. Geologically, two prominent groups, Surma and Barail, are identified, and the formations can be broadly classified as Bokabil, Bhuban, and Barail. The Surma grouprocks are visible in the western part of the state, displaying ridge and valley features and a trellis drainage pattern. This unit is distinguished by its dominance of trend linesand can be differentiated from the Barail group rocks found in the eastern part of the state. The Barail group exhibits a dendritic drainage pattern and denuded hills orientedin various directions. In the northeastern corner along the border with Myanmar, the rocks exhibit a north-linear trend, forming sub-parallel mountain

ranges and a valley-type topography. This geological feature is attributed to the alternation of hard stone and soft shale beds within the Barail group.

3.1.3. Drainage

Mizoram is traversed by a network of rivers, streams, and rivulets, each exhibiting varied patterns and lengths. The majority of these drainage lines originate in the central region of the state, flowing either north or south, guided by the north-south trending ridges. The valleys, often narrow, have been sculpted in softer formations, and the rivers, in certain locations, create deep gorges, cutting across the prominent ridges to form water gaps. Waterfalls are common in the upper courses of the rivers. The controlled course of drainage, influenced by parallel ranges, results in trellis, dendritic,and parallel drainage patterns of ephemeral and consequent types.

In the northern part of Mizoram, large rivers such as Tlawng (with its tributaries Teirei and Tut), Tuivawl, Tuirial, Langkaih, and Tuivai drain the region, eventually converging into the Tuiruang River in the Cachar plains of Assam. The southern part is drained by significant rivers like Chhimtuipui (also known as Kolodyne), accompanied by its tributaries Mat, Tuichang, Tiau, and Tuipui. Additionally, the river Khawthlangtuipui, with tributaries Kawrpui, Tuichawng, Kau, and De, drains the southwestern part of the state, eventually flowing into Bangladesh. These rivers, alongwith others, delineate their respective watersheds, contributing to a total of 25 watersheds throughout Mizoram.

3.1.4. Climate

Mizoram experiences a moderate climate due to its tropical location, avoiding extremes of heat and cold throughout the year. The region is directly influenced by the south-west monsoon, resulting in a considerable amount of rainfall. The climate is characterized as humid tropical, featuring a short winter, a lengthy summer, and substantial rainfall.

The highest temperatures are typically observed in May, June, and July, with a subsequent decrease in temperature upon the onset of the monsoon. As the

monsoon rains persist, temperatures continue to decline, reaching their minimum in December and January. During autumn, temperatures usually range between 18°C to 25°C, while winter temperatures typically fall between 11°C to 23°C, and summer temperatures range from 21°C to 31°C.

Heavy rainfall occurs from May to September, with an average annual rainfall of 250 cm. The northwestern part of the state receives the highest rainfall, exceeding 350 cm annually. Rainfall increases southward due to higher humidity levels. For instance, Aizawl, situated at 23°44'N and 92°43'E, receives about 208 cm of rainfall, while Lunglei (22°05'N and 92°04'E) records as much as 350 cm.

3.1.5. Vegetation/Forest Types

The forest or vegetation cover of North-east India has been extensively studied by notable botanists and forest officers such as Hooker (1872-1897), Kanjilal et al. (1934- 40), Champion and Seth (1968), Rao and Panigrahi (1961). However, comprehensive studies specifically focusing on the forest types of Mizoram have been limited, with only sporadic investigations by researchers such as Deb and Dutta (1987), Singh (1997), Lalramnghinglova and Jha (1997). Drawing upon these fragmented studies, along with field observations and collections, Singh et al. (2002) have classified the forests of Mizoram into the following types:

1. Tropical Wet Evergreen Forest
2. Montane Sub-tropical Forest
3. Temperate Forests
4. Bamboo Forests
5. Quercus Forests
6. Jhumland (Shifting Cultivation Areas).

3.1.6. Soil

The predominant soils in Mizoram mainly consist of loose sedimentary formations, characterized by their youthful, undeveloped, and sandy nature. Additionally, there are soils derived from sedimentary deposits exhibiting a red, loamy texture, often containing a substantial amount of laterite. These soils exhibit high acidity levels and are deficient in potash and phosphorus. However, in undisturbed soils, nitrogen content is notably elevated due to the accumulation of organic matter. The soils found in the valleys are more substantial, as they have been transported by rainfall from higher elevations. Mizoram's soils can be categorized into three orders according to soil taxonomy: Entisols, Inceptisols, and Ultisols.

3.2. Background of Champhai District

Champhai district is one of the eleven districts of Mizoram state in India.The district of Champhai (23° 28’ 28” N, 93° 19’ 32” E) is located in eastern Mizoram bounded by the two districts of Aizawl and Serchhip in the west, the state of Manipur in the north and Myanmar in the east. The district occupies an area of 3,185.83 km2 (1,230.06 sq mi). Champhai town is the administrative headquarters of the district (Champhai District: Census 2011). Champhai meaning ‘flat land’derived its name from the vast paddy field situated below the foot hill of the Champhai town. The district is dominated by the scheduled tribe (98.19%) recognized by the Constitution of India (Directorate of Census Operations, Mizoram 2011:7, 10 and 11). In the present-day scenario, Mizo and English are the principal languages and Christianity isthe dominant religion.

3.2.1. A brief History of Champhai

During the period from 1500 to 1800 AD, the Mizo people, who initially migrated from the east to Mizoram, belonged to the Fenngo clan, and their villages were namedafter their clan, such as Chawnchhim and Lawihmun. Ancient Mizo heritage in Champhai is evident through practices like erecting large stones, Chhura Farep, Chawngchilhi kai, Mura puk, and Sikpui lung.

In 1864, Vanhnuailiana arrived in Champhai with his group, establishing himself as the chief of Lawihmun, while his mother became the chief of Chawnchhim. In

1871, after Vanhnuailiana's passing, his wife Rolianpuii took over as the chief of Chawnchhim.

On February 17, 1872, Cachar Deputy Commissioner and General Bourcher visited Lawihmun for a treaty with Vanhnuailiana. However, finding no inhabitants, they proceeded to Chawnchhim the next day, where a treaty was made with the elders of Ropuiliani. In 1879, Vanhnuailiana's sons attempted to settle in the western part of Mizoram, leading to the Chhak leh Thlang Indo, a significant war between the east andthe west. Subsequently, the British Military occupied Chawnchhim.

In 1909, H.W.G. Cole, the 11th Deputy Commissioner of Mizoram, implemented a unique administration in Champhai, where chiefs became members of the Panchayat, taking turns as chairman. Noteworthy figures in Champhai include Thenzawl PasalthaChengaia, who played a key role in gun license matters.

Champhai has a rich history with various Mizo clans, such as Hmar, Ralte, Sailo, and Lusei, each occupying the region in succession. The British influence became evidentwhen Lt. Col.T.H. Lewin reclaimed Mary Winchester from a Mizo chief in 1872, and Captain Cookesly raised the British flag in Champhai on February 17, 1872. Subsequently, on February 18, 1872, Gen. Bourchear and Cachar D.C. Edger visited Champhai to negotiate a treaty with the local chiefs and elders.

3.2.2. Subdivision and Blocks

Champhai district is divided into two Rural Development Blocks, namely Champhai and Khawbung. The district encompasses five Legislative Assembly constituencies: Champhai North, East Tuipui, Lengteng, Tuichang, and Champhai South. Within this district, there are a total of eighty-eight inhabited villages. Among these, seventy-six are classified as revenue villages, and sixty-seven have established village councils.

3.2.3. Demography

In the 2011 census, the population of Champhai was 125,745, with 63,388 males and 62,357 females. In the 2001 census, the population was 108,392, comprising

55,756 males and 52,636 females (https://champ hai.nic.in/about-district/). Of the total population in the 2011 census, 38.59% resided in urban regions, totaling 48,529 individuals. The sex ratio in urban areas of Champhai district was 999 females per 1000 males, as per the 2011 census data. The average literacy rate in Champhai district,according to the 2011 census, was 96.69%. The literacy rates for males and females were 97.67% and 95.72%, respectively. In terms of rural areas, 61.41% of the Champhai district's population lived in villages, totaling 77,216 individuals. The sex ratio in rural areas was 974 females per 1000 males. Considering the child sex ratio, there were 968 girls per 1000 boys. The literacy rate in rural areas of Champhai districtwas 95.41% based on the 2011 census data.

3.2.4. Climate

Champhai District enjoys a pleasant climate, with winter temperatures ranging from 10°C to 20°C and summer temperatures averaging between 15°C and 30°C. The district experiences pre-monsoon rains from March to May, followed by the regular south-west monsoon lasting from June to October. The monthly average rainfall is 254 centimeters, contributing to an annual average rainfall of 1,814 millimeters (MIRSAC,2012). The cultivation of crops in the region is predominantly rain-fed, relying on themonsoon rainfall.

3.2.5. Agriculture

While shifting cultivation remains prevalent in the agricultural practices of the study area, there is a noteworthy presence of horticultural plantations, particularly for crops such as passion fruit and grapes. Commercial production of grape wine, marketed under the brand name "Zo-wine," has become a significant economic contributor, offering socio-economic sustenance to farmers engaged in grape cultivation. The region is recognized as the "Rice bowl of Mizoram" due to extensive wet ricecultivation stretching across the eastern fringe of the district.

3.2.6. Selected Study Sites

In this research, a comprehensive examination of wild mushrooms was undertaken,

focusing on six specific forest locations within Champhai district. These selected forests, namely Zote, Tlangsam, Ngur, Mualkawi, Khuangleng, and Vangchhia villages, were chosen as study sites for the purpose of collecting diverse wild mushroom specimens. The investigation aimed to explore the mycological diversity and distribution in these distinct forest ecosystems, contributing valuable insights intothe wild mushroom flora of the Champhai district.

(a) Zote Village

Zote (23.49° Lat. & 93.35° Long.) is a small locality located about 10 km in the northof Champhai town of Mizoram. Zote are one of the clans of the Hmar people who arebelieved to first settle here so the name of the village came to be known as "Zote". Though it is a small village, it has rich archaeological remains of a long-time span starting from neolithic to historical periods. The archaeological remains of this villageare found existed in the form of neolith, caves, Sikpui lung (megalith), and petroglyphs. At present various clans such as Hmar, Ralte, Khawlhring, Renthlei, Hualngo, Sailo, and Pawi inhabit this village and there are 330 households. The mainreligion of the people is Christianity. The main occupation of the villagers is jhum cultivation and the main crops are rice, potato, mustard, onion and chilli, etc.

(b) Tlangsam Village

Tlangsam is a Village in Champhai Block in Champhai District of Mizoram State, India. It is located 5 KM towards East from District head quarters Champhai. 4 KM from N Champhai. 84 KM from State capital Aizawl. The latitude 23.46° and longitude 93.36° are the geocoordinate of the Tlangsam.

(c) Ngur Village

Ngur (23.54° Lat. & 93.37° Long.) village is located in Champhai sub-division of Champhai district in Mizoram, India. It is situated 14 km away from Champhai, which is both district & sub-district headquarter of Ngur village. Ngur has a total population of 1,674 peoples, out of which male population is 826 while female population is 848. Literacy rate of ngur village is 75.09% out of which 73.49% males

and 76.65% females are literate. There are about 335 houses in ngur village.

(d) Mualkawi Village

Mualkawi (23.41° Lat. & 93.33° Long.) is a village in the Champhai district of Mizoram, India. It is situated 11km away from district headquarter Champhai. According to the 2011 census of India, Mualkawi has 128 households. The effective literacy rate (i.e., the literacy rate of population excluding children aged 6 and below) is 97.94%. Mualkawi has a total population of 664 peoples, out of which male population is 335 while female population is 329.

(e) Khuangleng Village

Khuangleng (23.31° Lat. & 93.31° Long.) is a village in the Champhai district of Mizoram, India. It is located in the Khawbung R.D. Block. It is situated 39km away from sub-district headquarter Khawbung (tehsildar office) and 40km away from district headquarter Champhai. According to the 2011 census of India, Khuangleng has 334 households. Khuangleng has a total population of 1,686 peoples, out of which male population is 846 while female population is 840. Literacy rate of khuangleng village is 80.25% out of which 80.26% males and 80.24% females are literate.

(f) Vangchhia Village

Vangchhia (23.12° Lat. & 93.20° Long.) is a village in the Champhai District of Mizoram (Fig.2). It is situated about 60km southeast of the Champhai District headquarters, lying on the Indo-Myanmar border. According to the 2011 census of India, Vangchhia has 153 households. Vangchhia has a total population of 837 peoples, out of which male population is 424 while female population is 413. Literacy rate of vangchhia village is 77.54% out of which 78.54% males and 76.51% females are literate. There are about 153 houses in vangchhia village. The 171 menhir stones in the village became Mizoram's first protected archaeological site in 2012.

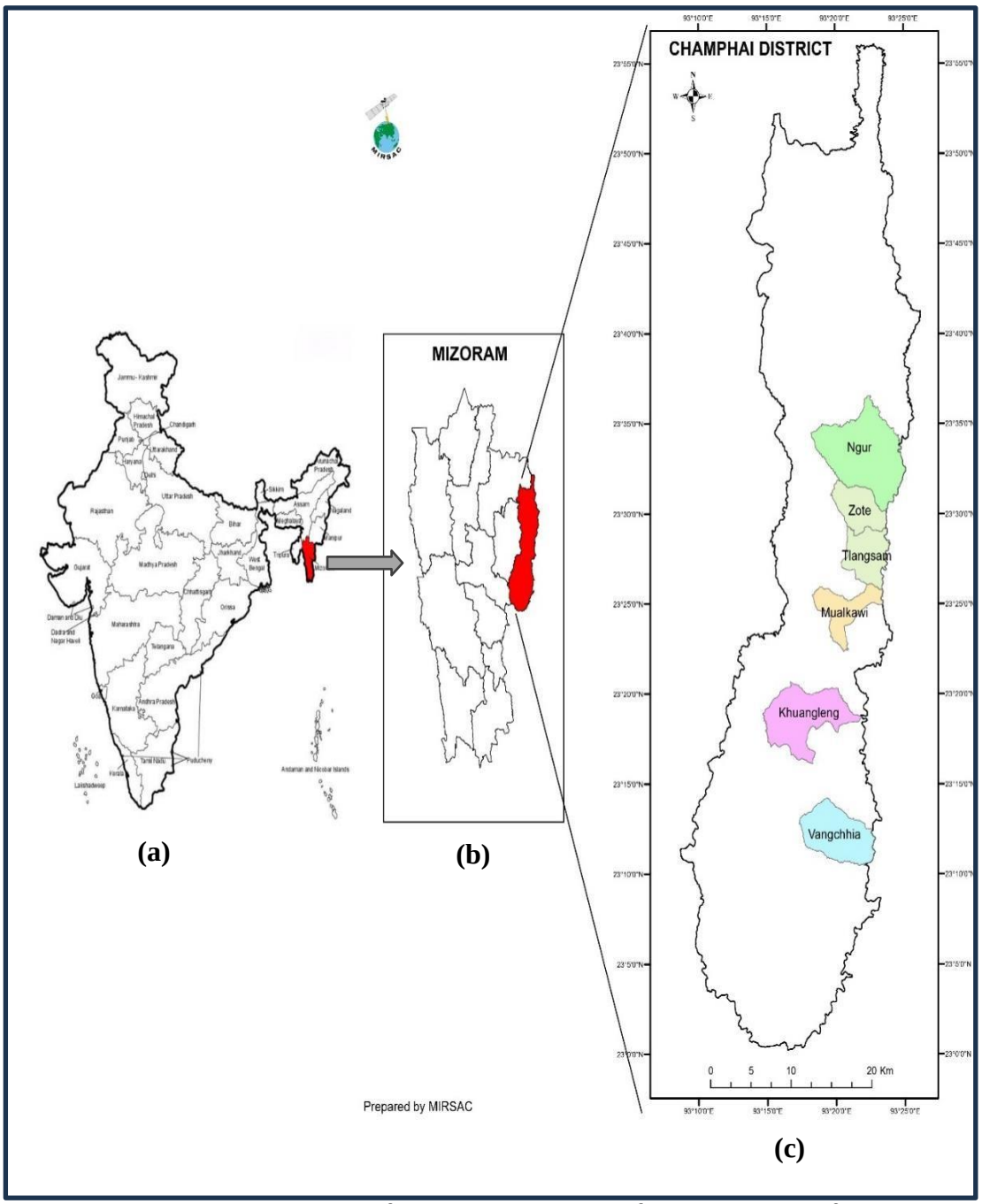


Figure 3.1. Geographical map of study area (a) Map of India (b) Map of Mizoram (c) Champhai district showing study sites.

(a) **(b)**

(c) **(d)**

(e) **(f)**

Figure 3.2. Photos showing villages of the study sites (a) Zote (b) Tlangsam (c) Ngur (d) Mualkawi (e) Khuangleng (f) Vangchhia.

CHAPTER IV

4. Diversity of Wild Edible Mushrooms (WEMs) and factors influencing their growth and distribution in Champhai District.

4.1 Introduction

Mushrooms play a vital role in the forest ecosystem due to their diverse relationships with forest trees, including edibility, toxicity, psychoactivity, mycorrhizal partnerships, and parasitism (Pérez-Moreno, 2021, Zotti, et al., 2013). These relationships make them a significant source of research interest and economic value. Mushrooms serve as a food source for local communities and mycorrhizal partners of host trees, and some species contain bioactive compounds with medicinal potential (Kalač, 2016). Although the flora of this region has been extensively studied, research on macrofungal diversity, a critical aspect of the ecosystem, has been largely ignored. The survey was conducted during the rainy season of 2017-2019 in six different locations, including mountains, grasslands, and forest areas of the Champhai forest. A total of 18 wild edible mushroom species were identified, with *Agaricus*, *Russula*, *Schizophyllum*, *Termitomyces*, and *Polypores* being the dominant genera.

Fungi belong to a diverse group of eukaryotes that play critical biological roles in forest ecosystems. Understanding their presence in plant communities, as well as their ecological functions and impacts on nature, is essential (Courty et al, 2010). Mushrooms, also known as macrofungi, are classified as members of the phyla Ascomycota and Basidiomycota. They are typically saprophytic and are capable of decomposing wood by breaking down cellulose and other polymers. The term "macro fungi" refers to a group of fungi that produce prominent sporocarps and includes "gilled fungi," "jelly fungi," "coral fungus," "stink fungi," "bracket fungi," "puffballs," "truffles," and "birds' nest" (Enow, 2013). Macrofungal diversity, including community diversity, is an essential component of global diversity, which is a crucial part of fungal diversity. Fruiting body mushrooms thrive only under specific conditions, including geographic location, elevation, temperature, humidity, light, pH

The diversity of mushrooms in India faces a serious threat, and while western countries have conducted extensive research on mushrooms, tropical countries, particularly India, have not. The Agaricaceae family is the most recorded mushroom species by Indian mycologists, mostly represented by the genus *Agaricus* from various states of India. According to Manoharachary et al., (2005), India has a third of fungal diversity, and only 50% of 1.5 million fungi have been characterized until now. Manoharacharyet al., (2005) reported the therapeutic applications of various species of mushrooms, including *Lentinus* sp., *Pleurotus* sp., *Schizophyllum* sp., *Pisolithus* sp., *Ganoderma lucidum*, *Agaricus bisporus*, *A. campestris*, *Pleurotus* sp., and *Termitomyces heimii*. Senthilarasu (2014) reported a total of 178 species in 68 genera (including 21 new species and two new genera *Chlorolepiota* and *Singerina*) belonging to 23 families and 5 orders (Agaricales, Boletales, Cantharellales, Polyporales and Russulales) have been described from Maharashtra. Northeastern region of India has a diverse range oftree and other woody plant species in its forests, and a diversified mycoflora is associated with the biodiversity of trees and other plants. Champhai District of Mizoram state is home to a variety of edible and non-edible wild mushroom species, and this study aimed to examine the diversity and pattern of occurrence of WEMs along altitudinal gradient in the forests of Champhai district, Mizoram, India.

The above information provides a good background on the importance of mushrooms in forest ecosystems, there are still several areas where further research is needed. One research gap is the lack of information on the impact of altitude and chemical properties of the soil on the occurrence and distribution of WEMs in different parts ofIndia, including Champhai district of Mizoram state. There is also a need for more detailed studies on the ecological roles of different types of mushrooms in forest ecosystems, particularly in terms of their interactions with other organisms and their impact on nutrient cycling and decomposition. Additionally, while there have been some reports of the medicinal properties of certain mushroom species, more research is needed to fully explore their potential in this area. Therefore, this study aims to fillthese gaps by investigating the impact of environmental factors on the occurrence of WEMs in the forests of Champhai district and by providing a more detailed

understanding of the ecological functions and potential medicinal properties of different types of mushrooms.

4.2. Methodology

4.2.1. Sampling Design and preparation

Six distinct forests, specifically Zote, Ngur, Mualkawi, Tlangsam, Vangchhia, and Khuangleng villages in Champhai district of Mizoram, India were selected for sampling during 2017-2019. These forests were selected because they are the primarysources of WEMs sold in the markets of Champhai. The sampling sites were selectedbased on a preliminary survey of each forest to locate areas with a diverse array of WEMs. Random sampling method was used to survey a total of 1.5-hectare area (10m x 10m) with 150 total plots of 0.01 ha each in six selected forest communities that occurred along three distinct altitudinal gradients: (a) lower altitude forest (Altitudinal zone 1 = 825m<1050m) (*N* = 50), (b) mid altitude forests (Altitudinal zone 2 = 1050m>1350m) (*N* = 50), and (c) higher altitude forests (Altitudinal zone 3 = 1350m>1820 m) (*N* = 50). To gather data on mushroom species diversity, stratified random sampling using quadrat method was employed. Mushrooms were collected from various habitats such as grazing fields, decaying wood, soils, and termite moundsin the forested areas, following Vishniac's methods (1977). Field characteristics, including habit, habitat, colour, measurements, and photographs, were documented foridentification purposes, based on fresh materials.

4.2.2. Quantitative Analysis

Quantitative data on species density, frequency, and abundance were collected for all the study sites, utilizing the methodology described by Curtis and McIntosh in 1950. *(a) Density:* The calculation of species density was based on determining the numerical strength of each individual species.

$$\text{Density} = \frac{\text{Total number of individuals of species in all quadrats}}{\text{Total number of quadrats studied}} \text{ X } 100$$

(b) Frequency: Frequency is the proportion or percentage of plots in which a particular

species is present. It is calculated by the following formula.

$$\text{Frequency (\%)} = \frac{\text{Number of quadrats in which the species occured}}{\text{Total number of quadrats studied}} \text{X } 100$$

(c) Abundance: Species abundance refers to the number of individuals of different species within a community, expressed per unit area. It can be quantified using the following equation.

$$\text{Abundance (\%)} = \frac{\text{Total number of individual of a species in all quadrats}}{\text{Total number of quadrats in which the species occured}} \text{X } 100$$

4.2.3. Species diversity indices

Species diversity indices were calculated using PAST software (Version 4.0.3) (Hammer et al, 2001) using the following methods as shown in **Table 4.1** and rarefaction curve and species accumulation curve were analyzed (Gotelli and Colwell, 2011) and plotted using RStudio (V 4.2.3) using packages "iNext" (Hsieh et al., 2016), "BiodiversityR" (Kindt & Coe, 2005), "vegan" (Oksanen et al., 2020), and "ggplot2" (Wickham, 2016).

Table 4.1. List of diversity indices and formulas used for the study.

Diversity indices	Formula	Description
Shannon diversity index (H')	$H' = \sum_{i=1}^{s} pi.Inpi$	S = No. of species in that area; *pi* = proportion of individuals found in the i^{th} species; *In* = natural logarithm
Simpson's index of diversity (1-D)	$D = \sum_{i=1}^{S} \frac{ni(ni-1)}{N(N-1)}$	N = Total number of the individuals in each sample; ni = No. of individuals of species in that area
Pielou's index (J)	$J = 1 - \sum_{i=1}^{S} \left(\frac{ni(ni-1)}{N(N-1)}\right)$	S = the number of different genera in a sample

Fisher's alpha index (α)	$S = \propto \cdot \ln\left(1 + \frac{N}{\propto}\right)$	
Margalef's index (Dmg)	$Dmg = \frac{(S-1)}{\ln(N)}$	

4.2.4. Distribution of WEMs along altitudinal gradient.

The study investigated the distribution of mushrooms along the altitudinal gradient using non-metric multidimensional scaling (NMDS). Cluster analysis and ordination was used to analyse and confirm the existence of ecologically significant clusters among our sampled plots selected for the study. The Bray-Curtis distance matrix was first calculated using the abundance data. The Bray-Curtis distance matrix was then clustered using hierarchical Ward's minimal variance, which creates clusters with the least variance based on the criteria for a least squares linear model (Legendre and Legendre, 2012). Then, the same distance matrix was used to coordinate our sampled locations using the nonmetric multidimensional scaling method (NMDS) which is an indirect gradient analysis technique and represents the pairwise dissimilarity among sites in a low dimensional space as closely as possible. The ordination was evaluated by calculating the ordination values (R^2) using Shepard's diagram or stress plot (Roberts, 2020). The analysis was conducted using the "Vegan" and "ggplot2" packages in RStudio (Version 4.2.3).

4.2.5. Effect of temperature and rainfall on fruiting of selected WEMs

Six permanent locations were selected across study sites in Champhai district, and sampling was conducted continuously from 2017 to 2019. The sampling approach incorporated both qualitative and quantitative methods, following the recommendations of Arnolds (1981). Observations were made monthly during the fruiting season of WEMs and more frequently at the peak production periods. Weather-related data, including total rainfall and daily mean temperatures, were considered in the study and obtained from the nearest weather station to the designated study areas.

4.2.6. Habitat and distribution of WEMs

Various indigenous mushrooms found in the six designated forests of Champhai were investigated over a three-year period (2017-2019). Morphologically distinct WEMs growing in diverse habitats within each plot were observed and recorded monthly from June to September. The identification of associated trees was conducted in collaboration with local residents and referenced against the herbarium at Mizoram University (MZUH).

4.2.7 Collection and Analysis of Soil Samples

Throughout the study years, soil samples were obtained from six different locations within the study area and subjected to analysis to assess their physical and chemical characteristics. The parameters examined included soil moisture, determined through the oven dry method, pH measured using a digital pH meter (SYSTRONICS-335), Total Kjeldahl Nitrogen (TKN) determined via the Kjeldahl method (1883), Soil Organic Carbon content assessed using the rapid titration method (Walkley and Black 1934), and available phosphorus measured through Olsen's method using a spectrophotometer (Olsen et al., 1954). The same research methodology and parameters were replicated in the subsequent year during the specific growing seasons of WEMs to enhance the depth of the research data. Soil sample collection occurred between the months of June to September in each study period. Results were given with an average of three iterations. Statistical analysis was performed using the Statistical Package for the Social Services (SPSS) for Windows statistical software package (SPSS Inc., 2005). One-way analysis of variance (ANOVA) was used in data analysis followed by Duncan's multiple range tests for comparison of statistical significance ($P<0.05$).

(a) Soil pH Procedure: 10g of freshly collected soil sample were taken in a beaker containing 50ml of distilled water. The soil water mixtures were stirred for 20 minutes on a magnetic stirrer. The solutions were left overnight and the pH readings were taken with the help of pH meter.

(b) Soil moisture content (Hot air oven method) Procedure: 10g of freshly collected soil sample were kept in a hot air oven at 105^{o}C for 24 hours and the oven-

dried soil are weighed again. For each sample, three replicates were maintained. The percentage moisture content is calculated by the following formula,

$$\text{Moisture content } (\%) = \frac{W_1 - W_2}{W_1} X\ 100$$

Where, W_1 = Initial weight

W_2 = Final weight

(c) Soil organic carbon (Walkey and Black method 1934)

The soil, previously dried in an oven, was finely ground until it completely passed through a 0.2mm sieve (80-mesh). A 0.5g sample of this ground soil was placed at the base of a dry 500ml conical flask. Subsequently, 10ml of 1N potassium dichromate was added to the flask, and gentle swirling ensured the dispersion of the soil in the dichromate solution. The flask, positioned on an asbestos sheet, received a cautious addition of 20ml of concentrated sulphuric acid from a measuring cylinder, with gentle swirling performed 2-3 times. The flask was then left undisturbed for 30 minutes. To achieve a distinct titration endpoint, 200ml of distilled water and 10ml of ortho-phosphoric acid were added. Following the addition of 1ml of diphenylamine indicator, the contents were titrated with ferrous ammonium sulfate solution until the color transitioned from blue-violet to green. Simultaneously, a blank titration was conducted without the presence of soil. The soil organic carbon content and organic matter was calculated by the following formula,

$$\text{Organic carbon } (\%) = \frac{10(\text{B} - \text{T})}{B} X\ 0.03\ X \frac{100}{S}$$

Where,

B = Volume of ferrous ammonium sulfate solution required for blank titration in ml.

T = Volume of ferrous ammonium sulfate solution required for soil sample in ml.

S = Wt. of soil in gram.

(d) Organic matter (%) = % Organic carbon x 1.724.

(e) Total Nitrogen Procedure:

(i) Digestion: Weighing 5g of the soil sample, it was carefully transferred to a digestion tube. Subsequently, 10-15ml of concentrated sulfuric acid (H_2SO_4) and 5-7g of a catalyst mixture were added to the sample. The digestion tubes were then placed in the Digester, and the digestion block was heated to 410°C until the sample's color turned colorless or displayed a light green hue.

(ii) Distillation: The main AC power and the rear side of the distillation unit were activated, with the distilled water tap set to the ON position. The power in the control panel was then switched on. Taking the digested sample in the large digestion tube (DTL), 10ml of distilled water was added and thoroughly shaken. The DTL, now containing the sample, was loaded into the Distillation Unit using the slider mechanism.

In a 250ml conical flask placed at the receiver end, 25ml of 40% boric acid (with 3 drops of Methyl red and 3 drops of Bromocresol green) was prepared. Using the control panel, 40ml of 40% NaOH was added. The timer was set to 20 seconds on the upper button. When the process concluded, the boric acid turned colorless. After the READY signal illuminated, the tap water inlet was opened for condensation. The necessary distillation time was then set at 6 minutes on the lower button. Initiating the process by pressing the run key on the lower button, the steam automatically ceased after the designated time, and the condensation tap water inlet was closed. The conical flask, now containing the boric acid, was removed from the receiver end, making the sample ready for titration.

(iii) Titration. The solution of Boric acid was titrated against with 0.1N HCl. Or 0.1N H_2SO_4 until the Boric acid turned pink. The burette reading was taken and the percentage of Nitrogen was calculated with the help of the formula,

$$\% \text{ of } N2 = \frac{14X\text{Normality of acid X Titrant value X } 100}{\text{Sample weight X } 1000}$$

(f) Available Phosphorus (Olsen's Method) Procedure: Preparation of extractant:

Adding 2.5g of soil to a 250ml conical flask, along with 50ml of an extracting solution

($NaHCO_3$), the mixture was subjected to shaking for 30 minutes using appropriate shakers. The resulting suspension was then filtered through Whatman filter paper No40. To achieve a clear filtrate, activated carbon, free of phosphorous, was introduced. Prior to pouring the suspension into the funnel, the flask underwent another round of shaking.

Colour development:

An aliquot of 5ml extract was transferred into a 25ml volumetric flask, where 5ml of Dickman's and Bray's reagent was added drop by drop with continuous shaking until the effervescence from CO_2 evolution ceased. The neck of the flask was rinsed, and the contents were diluted to approximately 22ml (Acidification should be checked; if pH is below 5.0, acidify with 5N H_2SO_4 to reach pH 5.0). Subsequently, 1ml of dilute $SnCl_2$ was introduced, and the volume was adjusted to the mark. The color remained stable for 24 hours, with maximum intensity achieved within 10 minutes at 660nm ($SnCl_2$ solution: 2.5g in 100ml glycerol, heated in a water bath for mixing).

Preparation of standard curve:

To create a standard curve, various concentrations of phosphorus (1, 2, 3, 4, 5, and 10ml) from a 2ppm phosphorus solution were measured and placed in 25ml volumetric flasks. The standard phosphorus concentrations ranged from 0.08µg/ml to 0.80µg/ml and were analyzed using a spectrophotometer at 660nm. The curve was then constructed, with colorimeter readings plotted on the vertical axis and the corresponding phosphorus amounts (in µgP/ml) on the horizontal axis

Calculation:

Olsen's phosphorus (mg/kg) = RxV/vx1Sx (2.24x106 /106)

= Rx (50/5) x (1/2.5) x2.24

= ugPx8.96

Where, V = Total volume of extractant (50ml) v = Volume of aliquot taken for analysis (5ml)

S = Wt. of soil (2.5g)

R = Wt. of the aliquot in ug (from standard)

4.3. Results and discussion

4.3.1. Density, Frequency and Abundance of WEMs

According to the results, *Schizophyllum commune* had the highest density among all the edible wild mushrooms identified, with 3.01 and 2.6 individuals per hectare in Zote and Mualkawi forests, respectively. *Lactifluus corrugis* followed with the second-highest density of 0.72 and 0.55 individuals per hectare in Mualkawi and Zote forests, respectively. *Lactarius piperatus* had a density of 0.39 individuals per hectare in Zote forest and 0.37 individuals per hectare in Ngur forest. The species with the lowest density was *Russula virescens*, with only 0.02 individuals per hectare in both Zote and Tlangsam forests (**Table 4.2**) (**Figure 4.1a**).

The species *Lactifluus corrugis* had the highest frequency of occurrence with 47.33% and 36.67% in Mualkawi and Zote forests, respectively. This was followed by *Lactarius piperatus* with 32.67% in Zote forest and 32% in Ngur forest. *Russula subfragilliformis* had a frequency of 27.33% in Zote forest and 22% in Ngur forest (**Table 4.2**) (**Figure 4.1b**).

According to the results, the most abundant species in the studied forests were *Schizophyllum commune*, with 21.52 and 21.50 occurrences in Zote and Ngur woods, respectively. *Termitomyces heimii* had the next highest prevalence, with 1.91 and 1.90 occurrences in Zote and Mulkawi forests, respectively. *Macrolepiota dolichaula* had the third highest prevalence, with 1.55 and 1.43 occurrences in Zote and Mualkawi forests, respectively (**Table 4.2**).

The results indicate that the studied forests in the area have a variety of edible wild mushrooms with varying densities, frequencies, and occurrences. *Schizophyllum commune, Lactifluus corrugis, Lactarius piperatus, Russula subfragilliformis, Termitomyces heimii,* and *Macrolepiota dolichaula* were identified as the most common edible mushroom species in the studied forests. The high density of *Schizophyllum commune* could make it a valuable resource for local consumption and potential commercial purposes. Meanwhile, the high frequency of occurrence of

Lactifluus corrugis and *Lactarius piperatus* indicates their consistent presence in the studied forests, making them viable sources for regular harvesting.

However, it is important to note that the harvesting and consumption of wild mushrooms require proper identification and knowledge to avoid poisoning and ensure sustainable collection practices. Additionally, the effects of environmental factors on the abundance and distribution of these mushroom species need to be further investigated to ensure their long-term preservation and utilization.

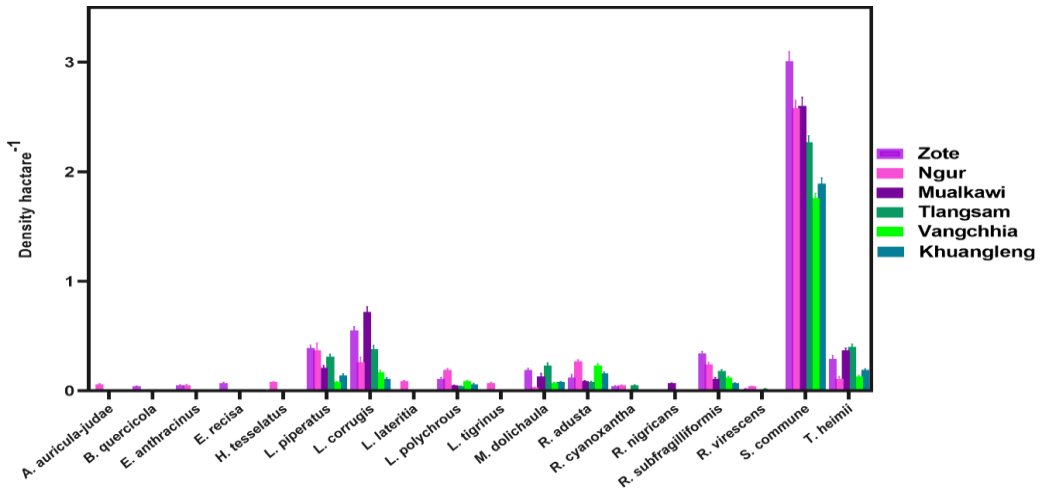


Figure 4.1a. Density per hectare of WEMs from six forests of Champhai district,Mizoram.

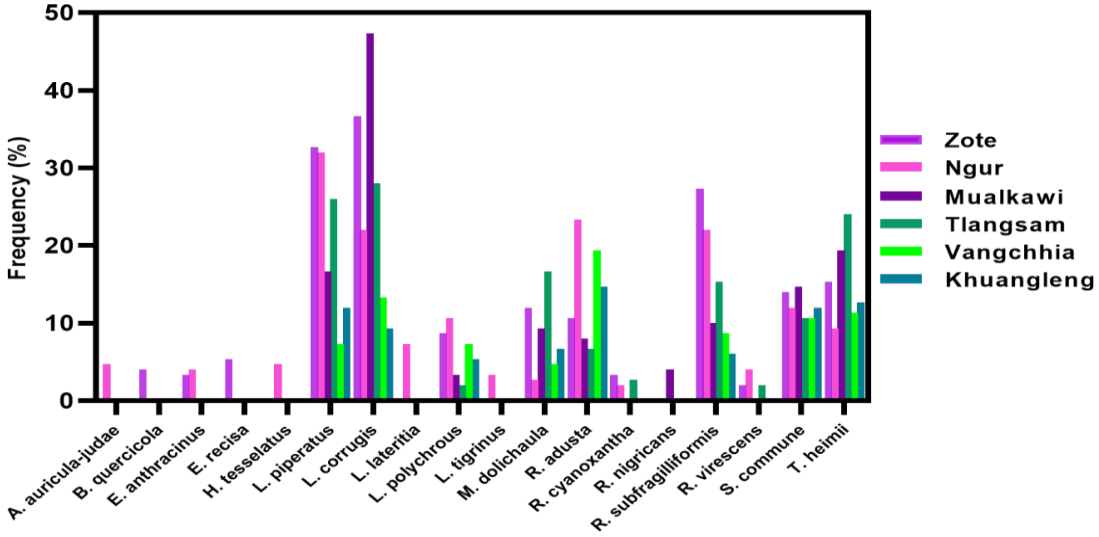


Figure 4.1b. Frequency (%) of WEMs from Champhai district, Mizoram.

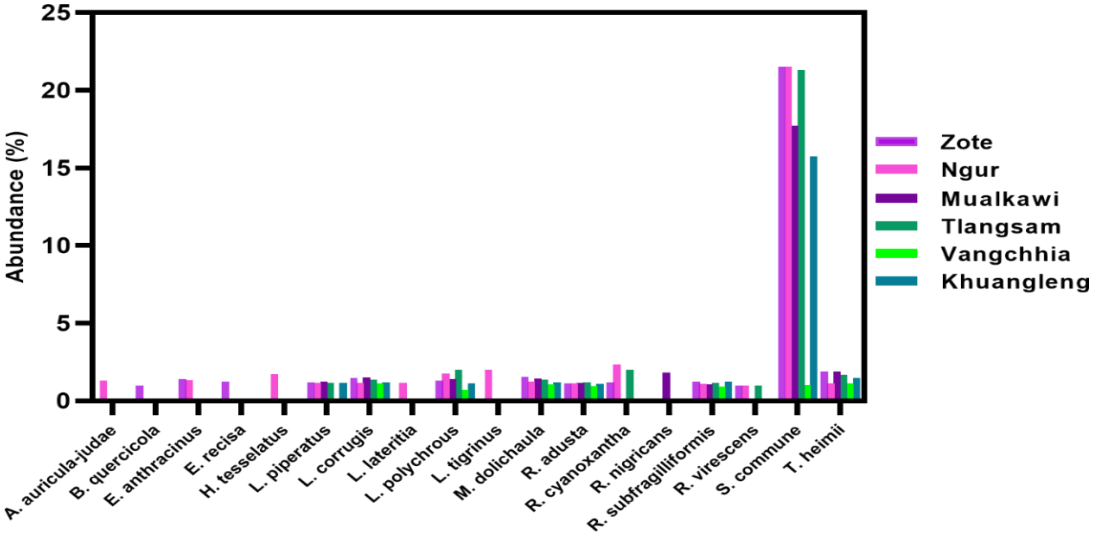


Figure 4.1c. Abundance (%) of WEMs from Champhai district, Mizoram.

Table 4.2. Density, frequency, and abundance of WEMs from six forests of Champhai district of Mizoram.

		Zote			Ngur			Mualkawi			Tlangsam			Vangchhia			Khuangleng		
	Scientific Name	**D/ha**	**F (%)**	**Ab.**	**D/ha**	**F (%)**	**Ab.**	**D/ha**	**F (%)**	**Ab.**	**D/ha**	**F (%)**	**Ab.**	**D/ha**	**F (%)**	**Ab.**	**D/ha**	**F (%)**	**Ab.**
1	*Auriclularia auricula-judae*	NF	NF	NF	0.06	4.67	1.29	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
2	*Boletus quercicola*	0.04	4	1	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
3	*Elaphomyces anthracinus*	0.05	3.33	1.4	0.05	4	1.33	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
4	*Exidia recisa*	0.07	5.33	1.25	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
5	*Hypsizygus tesselatus*	NF	NF	NF	0.08	4.67	1.71	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
6	*Lactarius piperatus*	0.39	32.67	1.2	0.37	32	1.17	0.21	16.67	1.24	0.31	26.00	1.18	0.08	7.33	NF	0.14	12.00	1.17
7	*Lactifluus corrugis*	0.55	36.67	1.49	0.26	22	1.18	0.72	47.33	1.52	0.38	28.00	1.36	0.17	13.33	1.12	0.11	9.33	1.21
8	*Lentinula lateritia*	NF	NF	NF	0.09	7.33	1.18	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
9	*Lentinus polychrous*	0.11	8.67	1.31	0.19	10.67	1.75	0.05	3.33	1.40	0.04	2.00	2.00	0.09	7.33	0.70	0.06	5.33	1.13
10	*Lentinus tigrinus*	NF	NF	NF	0.07	3.33	2	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF	NF
11	*Macrolepiota dolichaula*	0.19	12	1.55	0.03	2.67	1.25	0.13	9.33	1.43	0.23	16.67	1.36	0.07	4.67	1.05	0.08	6.67	1.20
12	*Russula adusta*	0.12	10.67	1.13	0.27	23.33	1.14	0.09	8.00	1.17	0.08	6.67	1.20	0.23	19.33	0.97	0.16	14.67	1.09
13	*Russula cyanoxanth*	0.04	3.33	1.2	0.05	2	2.33	NF	NF	NF	0.05	2.67	2.00	NF	NF	NF	NF	NF	NF
14	*Russula nigricans*	NF	NF	NF	NF	NF	NF	0.07	4.00	1.83	NF	NF	NF	NF	NF	NF	NF	NF	NF
15	*Russula subfragilliformis*	0.34	27.33	1.24	0.24	22	1.09	0.11	10.00	1.07	0.18	15.33	1.17	0.12	8.67	0.91	0.07	6.00	1.22
16	*Russula virescens*	0.02	2	1	0.04	4	1	NF	NF	NF	0.02	2.00	1.00	NF	NF	NF	0.00	NF	NF
17	*Schizophyllum commune*	3.01	14	21.52	2.58	12	21.5	2.60	14.67	17.73	2.27	10.67	21.31	1.76	10.67	1.03	1.89	12.00	15.72
18	*Termitomyces heimii*	0.29	15.33	1.91	0.11	9.33	1.14	0.37	19.33	1.90	0.40	24.00	1.67	0.13	11.33	1.14	0.19	12.67	1.47

*D=Density, ha=hectare, F=Frequency, Ab.= Abundance, NF=Not found

4.3.2 Diversity and composition of WEMs from Champhai district.

In the forests of Champhai district, a total of 18 wild edible mushroom species were identified, categorized into nine families. The most commonly found family was Russulaceae, comprising seven species, followed by Auriculariaceae, Lyophyllaceae, and Polyporaceae, each with two species. Boletaceae, Elaphomycetatceae, Marasmiaceae, and Schizophyllaceae had one species each. Notably, these findings align with those of Panda et al., (2019), who identified Russulaceae as the most dominant family among WEMs. Similar results were also documented in Champhai district, where a study by Ralte et al., (2020) reported the identification of 17 WEMs,with Russulaceae emerging as the predominant family.

Table 4.3 provides detailed information on different WEMs found in Champhai forests. Among the species identified, *Auriclularia auricula-judae*, *Hypsizygus tesselatus*, *Lentinula lateritia*, and *Lentinus tigrinus* were exclusive to Ngur forest. *Russula cyanoxantha*, *Russula virescens*, and *Elaphomyces anthracinus* were present in the forests of Zote, Ngur, and Tlangsam. *Russula nigricans* was found only in the Mualkawi forest. *Termitomyces heimii*, *Macrolepiota dolichaula*, *Russula adusta*, *Russula subfragilliformis*, *Lactarius piperatus*, *Lactifluus corrugis*, *Lentinus polychrous*, *Russula adusta*, and *Russula subfragilliformis* were found in all thesampled sites (**Figure 4.2**).

Table 4.3 List of WEMs found in Champhai district of Mizoram along with their habitats.

Sl. No	Scientific Name	Family	Habitat	Accession No.
1	*Auriclularia auricula-judae* (Bull.) J. Schröt.	Auriculariaceae	Dead and decaying wood	MZUBBOT0658
2	*Boletus quercicola* (Vassilkov) Singer	Boletaceae	Soil	MZUBOT0687
3	*Elaphomyces anthracinus* Vittad.	Elaphomycetatceae	Soil	MZUBOT0489
4	*Exidia recisa* (Ditmar) Fr.	Auriculariaceae	Dead and decaying wood	MZUBOT0549
5	*Hypsizygus tesselatus* (Bull.) Singer	Lyophyllaceae	Dead	MZUBOT0577

			and decaying wood	
6	*Lactarius piperatus* (L.) Roussel	Russulaceae	Soil	MZUBOT0337
7	*Lactifluus corrugis* (Peck) Kuntze	Russulaceae	Soil	MZUBOT0367
8	*Lentinula lateritia* (Berk.) Pegler	Marasmiaceae	Dead and decaying wood	MZUBOT0833
9	*Lentinus polychrous* Lév.	Polyporaceae	Dead and decaying wood	MZUBOT0777
10	*Lentinus tigrinus* (Bull.) Fr.	Polyporaceae	Dead and decaying wood	MZUBOT0454
11	*Macrolepiota dolichaula* (Berk. & Broome)	Agaricaceae	Soil	MZUBOT0312
12	*Russula adusta* (Pers.) Fr.	Russulaceae	Soil	MZUBOT0642
13	*Russula cyanoxantha* (Schaeff.) Fr.	Russulaceae	Soil	MZUBOT0563
14	*Russula nigricans* (Bull.) Fr.	Russulaceae	Soil	MZUBOT0414
15	*Russula subfragilliformis* Murrill	Russulaceae	Soil	MZUBOT0812
16	*Russula virescens* (Schaeff.) Fr.	Russulaceae	Soil	MZUBOT0767
17	*Schizophyllum commune* Fr.	Schizophyllaceae	Dead and decaying wood	MZUBOT0378
18	*Termitomyces heimii* Natarajan	Lypophyllaceae	Termite mound	MZUBOT0465

The study reported a total of 3,500 individuals of WEMs across 1.5 hectare, with a total species richness of 18 that belonged to 9 families, 13 genera and 18 species (**Table 4.4**). From the **Figure 4.3a & 4.3b,** the results of the species accumulation curve showed that after a sample size of 135, the cumulative species richness exhibited a gradual slope, indicating that the sampling size taken for this research

was adequate for the study of WEMs diversity in Champhai district (Bhutia, et al., 2019). Based on the species diversity indices, it can be observed that the Ngur forest had the highest species richness with 15 species, followed by Zote forest with 13 species. The Pielou's evenness (J) ranged from 0.36 to 0.44, with the highest value seen in Tlangsam and Vangchhia forests, indicating that the distribution of species among the forests was relatively even. The Shannon diversity index (H') ranged from 1.18 to 1.68, with the highest value observed in Ngur forest, suggesting that this forest had the highest diversity of species. Although the species richness of Ngur and Zote forests was higher, the species evenness was less than that of Tlangsam and Vangchhia forests. This could be due to the forests in Tlangsam and Vangchhia were recently protected by local non-government organization, and their settlement areas were close to their forests. Meanwhile, Simpson's index (1-D) ranged from 0.49 to 3.56, with the highest value seen in Ngur forest and the lowest in Khuangleng and Vangchhia forests, indicating that Ngur forest had the lowest dominance and Khuangleng and Vangchhia forests had the highest dominance (**Table 4.4)**. Overall, the study revealed that the forests in the area had a diverse range of WEMs, with varying levels of species richness and diversity.

Table 4.4 Diversity indices of WEMs from six forests of Champhai district of Mizoram.

Parameters	**All**	**Zote**	**Ngur**	**Mualkawi**	**Tlangsam**	**Vangchhia**	**Khuangleng**
Total sampled area(ha)	1.5	0.25	0.25	0.25	0.25	0.25	0.25
Total species richness	18	13	15	9	10	8	8
Total genus richness	13	10	11	7	7	7	7
Total family richness	9	8	8	4	5	5	5

Total individual counted	3500	783	672	652	592	396	405
Shannon index (H')	1.42	1.57	1.68	1.36	1.49	1.26	1.18
Simpson index (1-D)	3.56	0.64	0.65	0.60	0.64	0.54	0.49
Pielou's evenness (J)	0.41	0.37	0.36	0.43	0.44	0.44	0.41
Margalef's index (Dmg)	1.49	1.80	2.15	1.24	1.41	1.17	1.17
Fisher's alpha index (α)	1.83	2.214	2.721	1.477	1.709	1.42	1.413

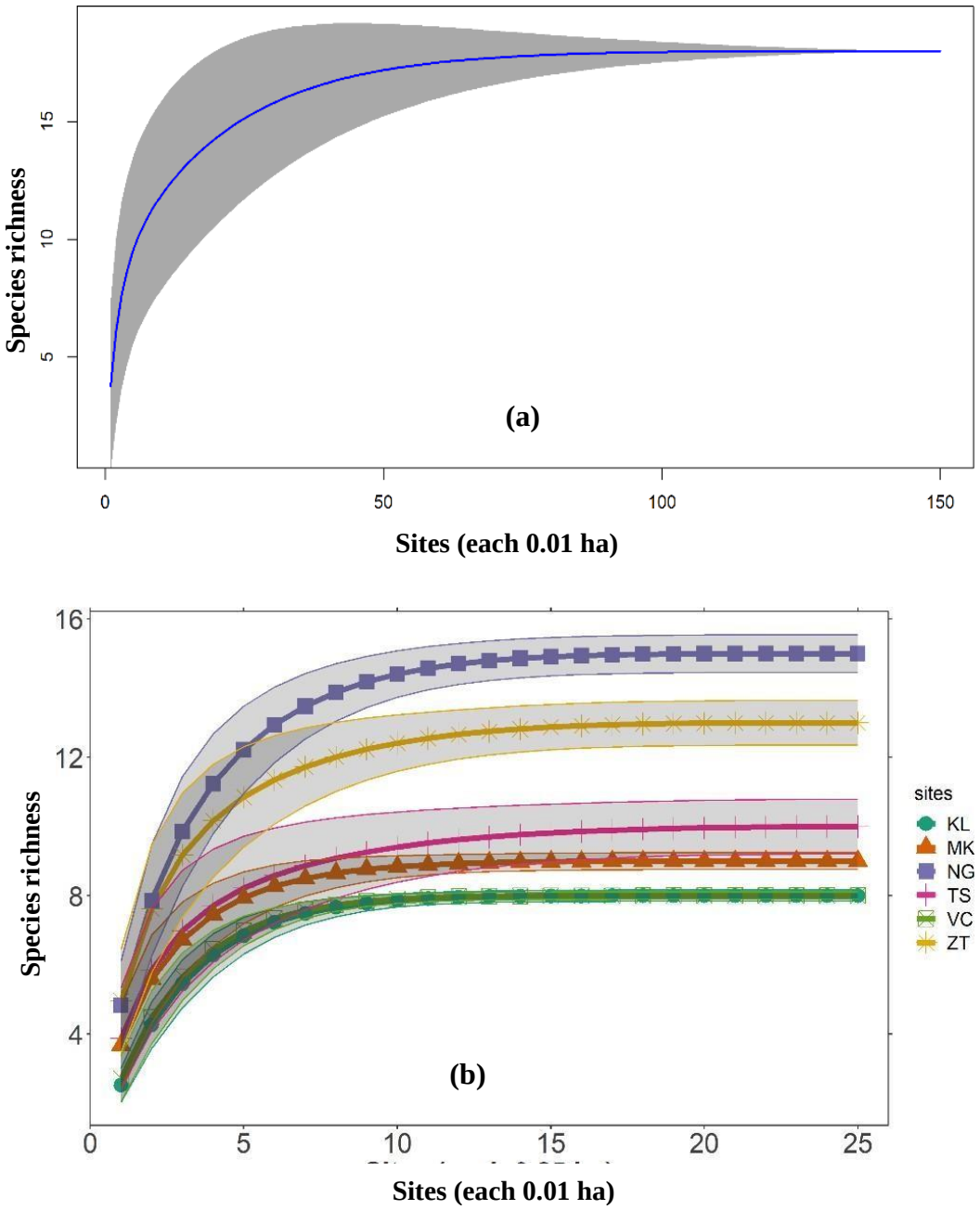


Figure 4.3. Species accumulation curve based on rarefaction method. **(a)** Whole WEMs population; **(b)** six different forests: KL = Khuangleng, MK = Mualkawi, NG = Ngur, TS = Tlangsam, VC = Vangchhia, ZT = Zote of Champhai district, Mizoram.

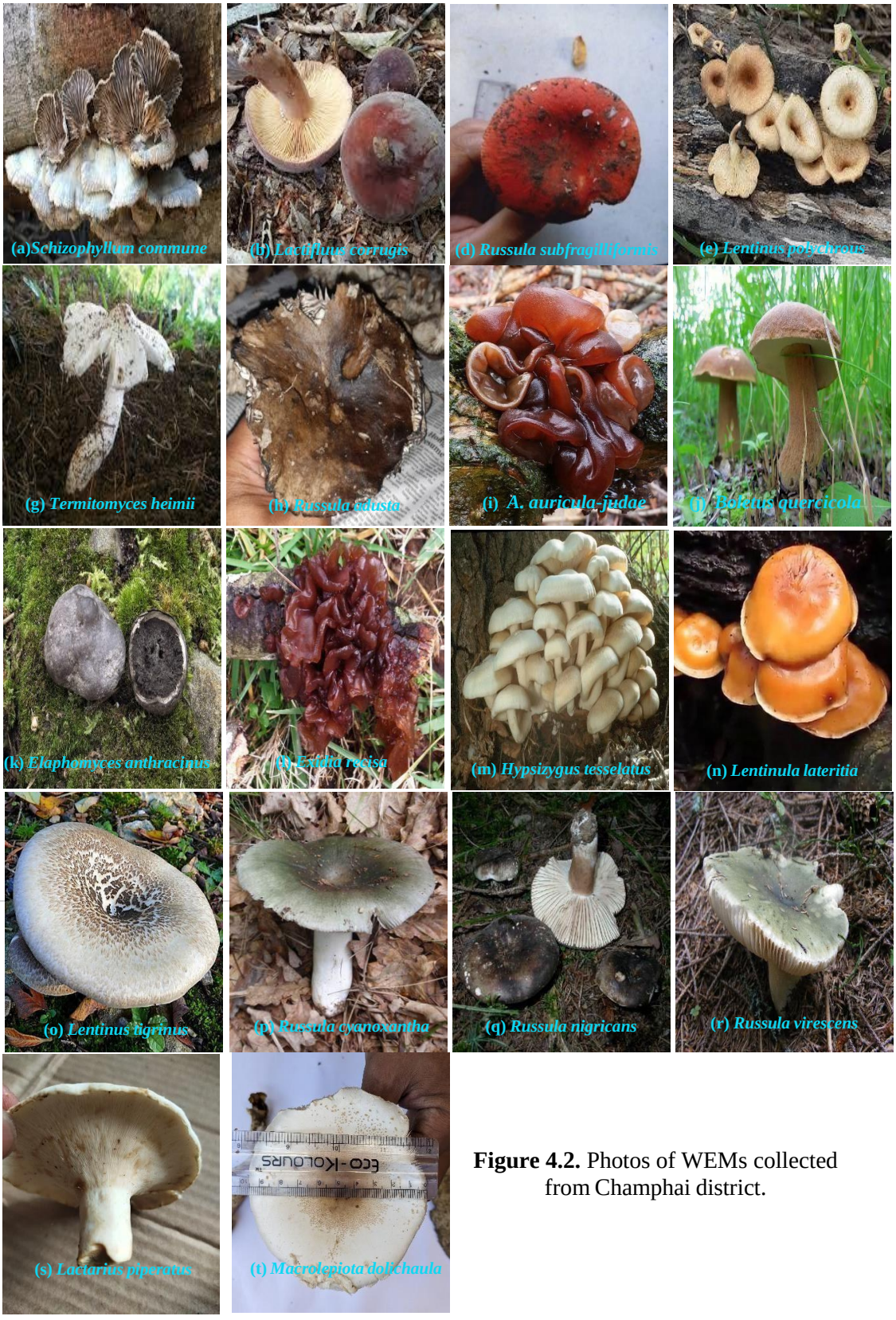


Figure 4.2. Photos of WEMs collected from Champhai district.

4.3.3 Determination of Species richness of WEMs using rarefaction curve.

The forests in Champhai are known for their high level of diversity in WEMs, but there is little information available about the differences in species richness between rare and abundant species of WEMs. The study found that the diversity of WEMs, which indicates species richness, varied across the area studied. However, some forests, suchas Ngur and Zote, had a significant number of species with estimated counts of 15 and13, respectively. In contrast, other areas, including Tlangsam, Mualkawi, Vangchhia, and Khuangleng, had a limited estimated species count of less than 10, as determined through extrapolation using rarefaction analysis (**Figure 4.4**). It is noteworthy that all sites showed comparable levels of diversity among dominant species. Therefore, the diversity variance was primarily due to differences in the number of uncommon species (Hofhansl et al., 2019). The differing species richness between WEMs at different altitudes can be attributed to a variety of factors. We believed that the frequently reported climatic variables, namely precipitation, temperature, and their interaction with their natural habitat (Sharma et al., 2019, Tambe et al., 2011), were the primary factors for varying WEMs richness along the Champhai District's altitudinal gradient.

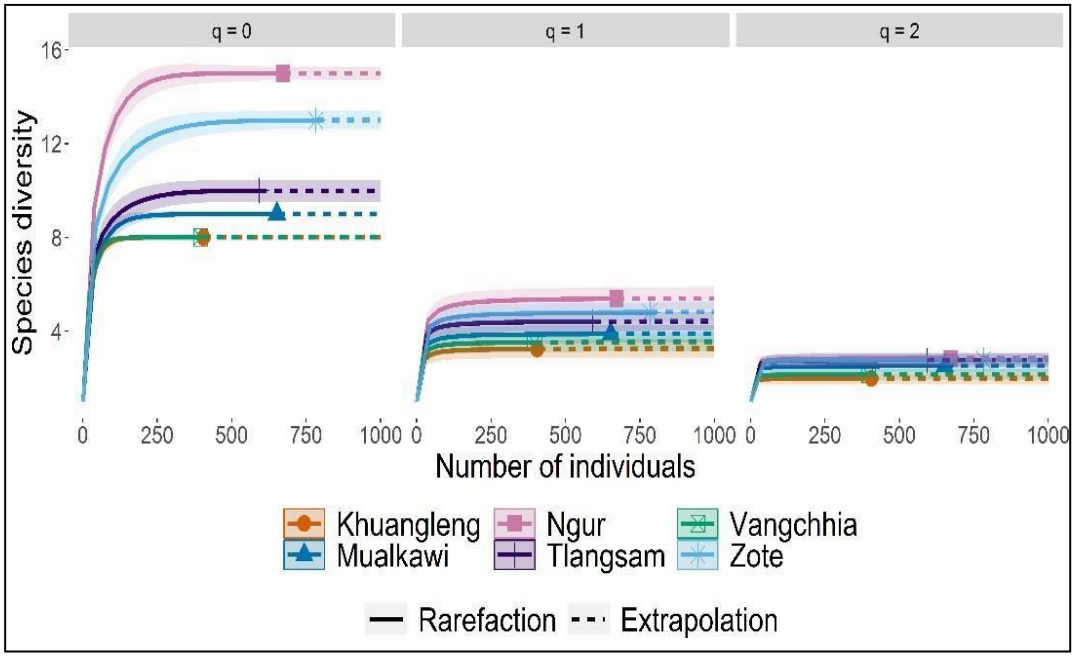
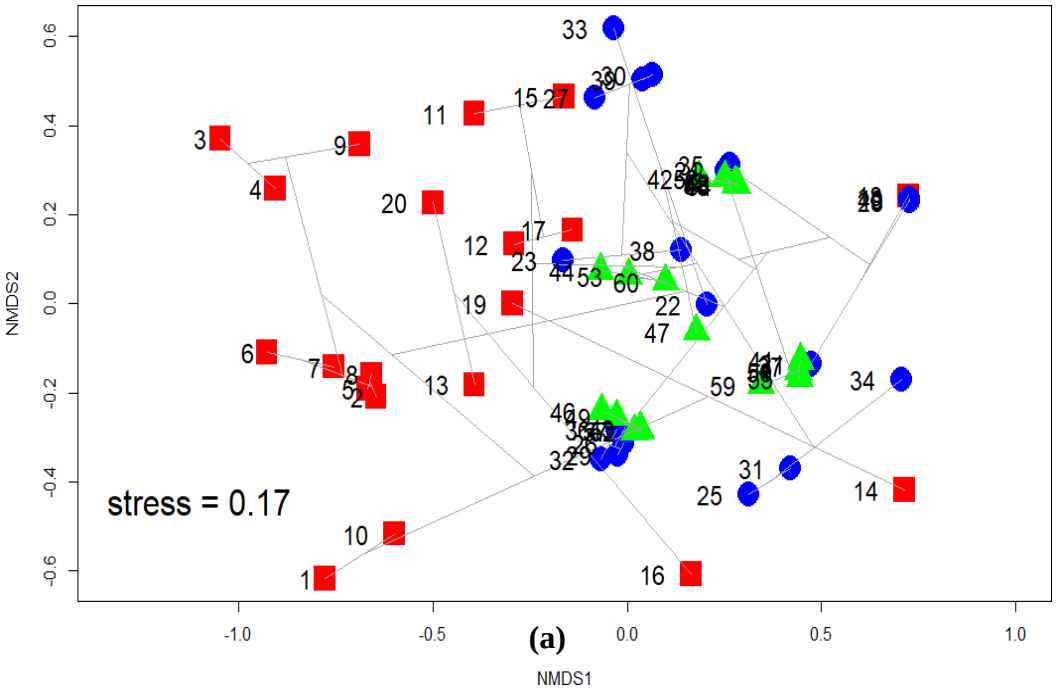


Figure 4.4. Rarefaction analysis using Hill ‘s numbers (0, 1, and 2) of the wild edible mushroom species from six forests in Champhai district. The Hill ‘s number q=0 represents the species richness; the Hill’s number q=1 represents the effective number of rare species

and the Hill ‘s number q=2 represents the effective number of abundant species. The solid lines represent the interpolation with the observed data and the dotted lines represent the extrapolation to 1,000 individuals. Shaded areas represent 95 % confidence intervals ($p < 0.05$).

4.3.4. Distribution of WEMs along altitudinal gradient

Three altitudinal zones (Altitudinal zone 1 = 845m<1050m, Altitudinal zone 2 = 1050m>1350m and Altitudinal zone 3 = 1350m>1850m) could be formed based on the cluster analysis of wild edible mushroom at different sites, although some sites deflected from its designated categories (**Figure 4.5a**). Similar groups were found from the result of NMDS (stress = 0.17) when superimposed with a cluster dendrogram (**Figure 4.5b**). Final Kruskal's stress of 0.17 after three iterations, indicated a highly accurate fit ($R^2 = 0.99$) (Séleck, et al., 2013) **Figure 4.5c**. Three altitudinal zones weredepicted by both NMDS and cluster analysis, however some sites at different altitudesharbours similar species. This indicate that altitude alone was not the sole factor in determining the distribution of wild edible mushroom.



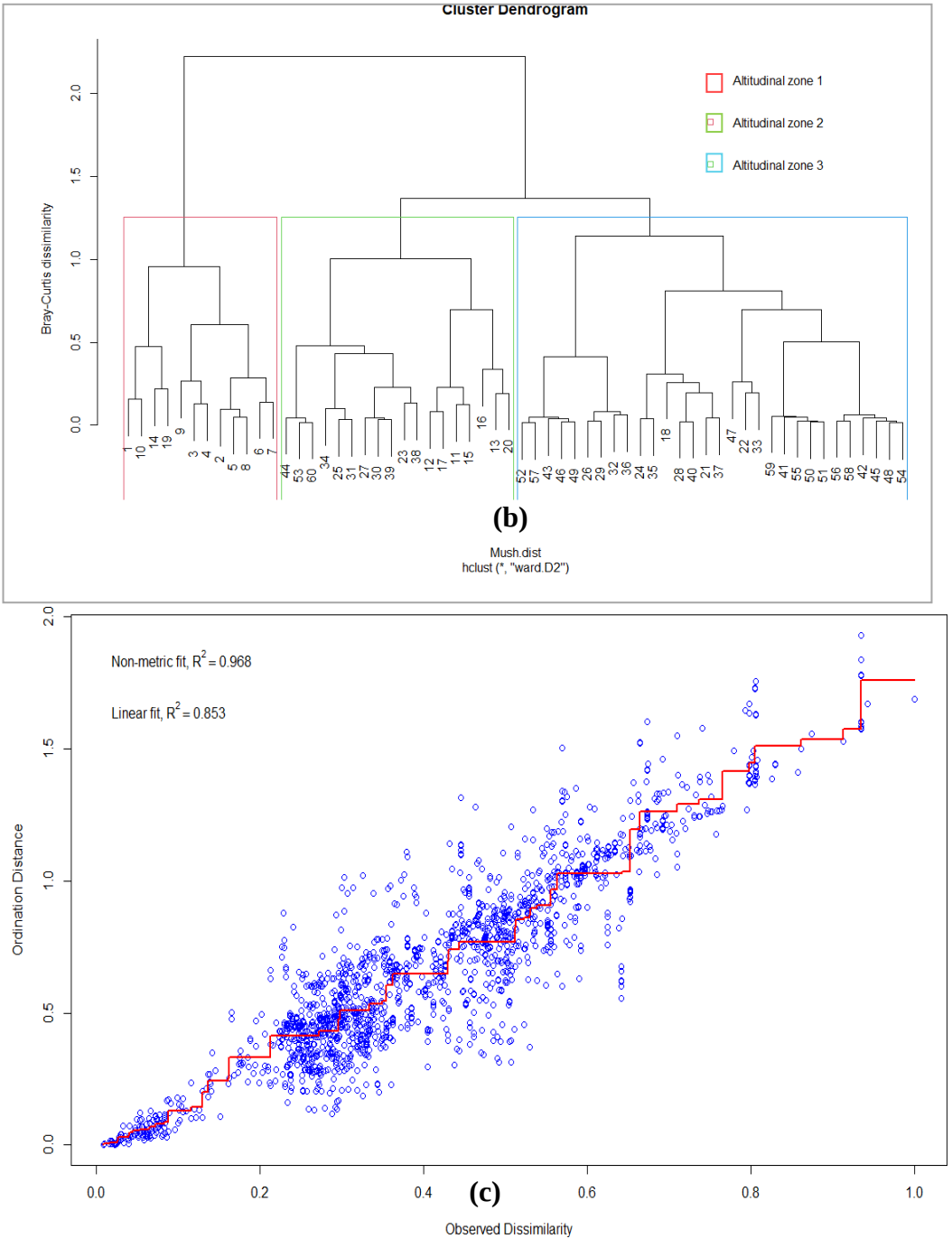


Figure 4.5. Nonmetric Multidimensional scaling **(a)** NMDS ordination superimposed with the result of cluster analysis, more similar plots are grouped closer to another, the red, green and blue dots represent altitudinal zone 1, altitudinal zone 2 and altitudinal zone 3 forest; **(b)** Hierarchical clustering using ward's algorithm, the *y*-axis represent the Bray-Curtis shared among each cluster; **(c)** Kruskal's stress with the goodness of fit (R^2) from the NMDS result.

The findings of our study revealed that certain common WEMs, such as *Lactifluus corrugis*, exhibited growth within an altitude range of 845 to 1561 meters, with an average altitude of 1384.3 meters. *Lactifluus piperatus* was identified at elevations ranging from 1125 to 1815 meters (mean altitude = 1401.7 meters). *Russula subfragilliformis* was predominantly located at altitudes ranging from 1074 to 1808 meters (mean altitude = 1403.9 meters), while *Russula adusta* favored elevations between 1350 and 1820 meters (mean altitude = 1533.2 meters). In contrast, *Termitomyces heimii* and *Macrolepiota dolichaula* demonstrated a preference for significantly lower altitudes, ranging from 825 to 1265 meters (mean altitude = 972.4meters) and 885 to 1289 meters (mean altitude = 1020 meters), respectively (**Table 4.5** and **Figure 4.6**). This discovery corresponds with earlier research by Ratiknyo et al. (2018), which indicated an initial increase in termite species numbers at altitudes between 800-900 meters, followed by a subsequent decrease in population of termites due to gradual decrease in temperature. In summary, it can be inferred that the majority of the studied WEMs exhibited a preference for higher altitudes (mean altitude > 1000 meters), except for *T. heimii*, which had a mean altitude of 972.4 meters. These findings align with the observations of Payton (1993), who attributed high species diversity at higher altitudes to factors like low temperature, high relative humidity, and soil moisture, influencing the vegetation types on mountain slopes. This emphasizes the significance of considering altitude variations and their connection to associated plant communities when comprehending the microhabitat specificity of WEMs.

Table 4.5. Different altitude ranges where selected WEMs were comonly found in Champhai district, Mizoram.

Sl. No	Name of the species	Altitudes (m)	Mean attitude (m)
1	*Lactifluus corrugis*	845 to 1561	1384.3 (m)
2	*Lactifluus piperatus*	1125 to 1815	1401.7 (m)
3	*Russula subfragilliformis*	1074 to 1808	1403.9 (m)
4	*Russula adusta*	1350 to 1820	1533.2 (h)
5	*Termitomyces heimii*	825 to 1265	972.4 (l)
6	*Macrolepiota dolichaula*	885 to 1289	1020.0 (l)

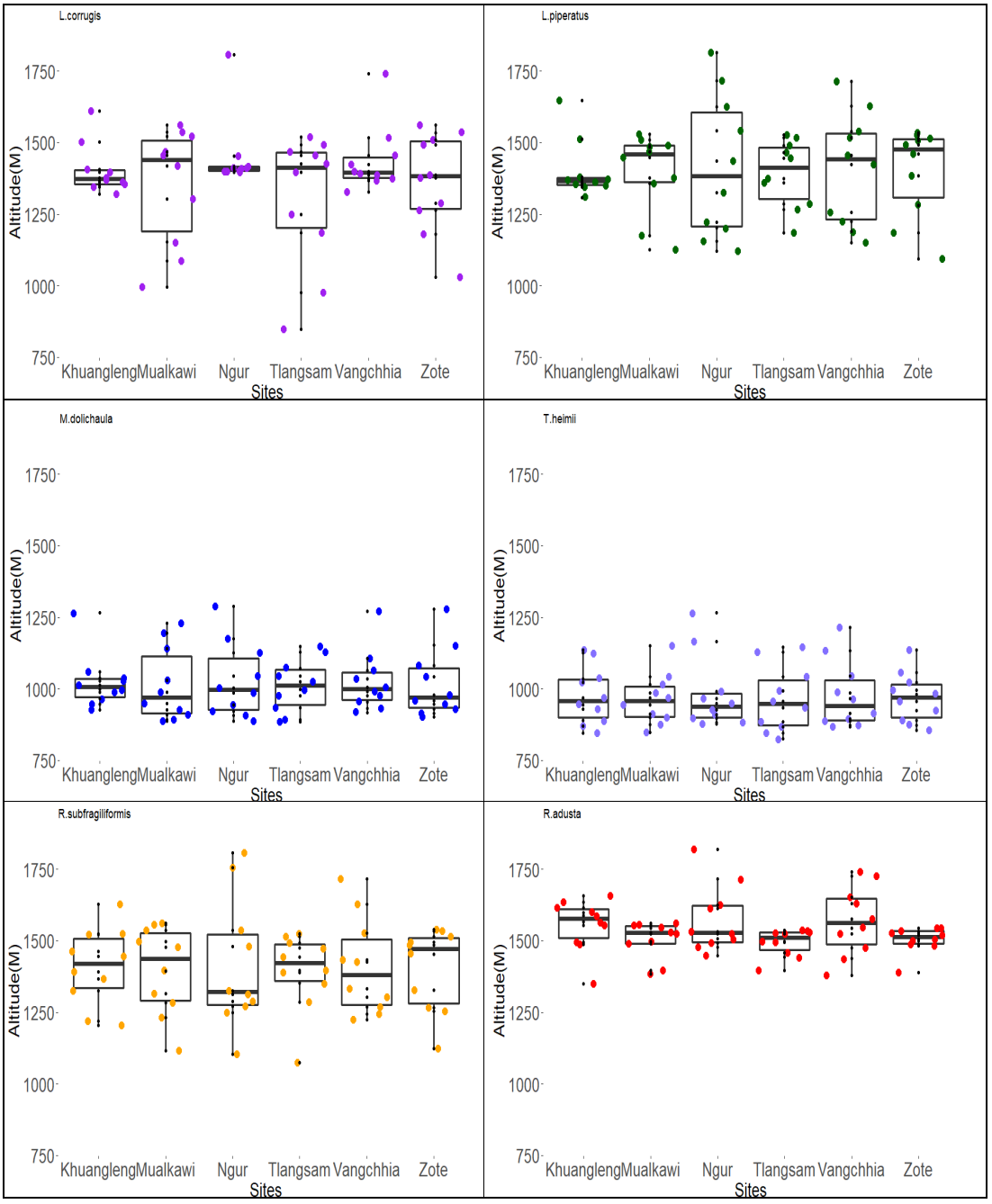


Figure 4.6. Box plots with jitter points showing the preferred altitudinal ranges of some common WEMs in Champhai district of Mizoram. The horizontal line crossing the box in the center represents the median. The vertical line outside the box represents the maximum and minimum values.

4.3.5. Effect of temperature and rainfall on fruiting of selected WEMs

The results of samplings carried out in the six locations during three years (2017-2019)of observation are summerized in **Table 4.6**. Within the studied plots from six

forests, *Schizophyllum commune* had the highest individual number *ie*., 859 followed by *Lactifluus corrugis* (592), *Lactarius piperatus* (348), *Russula subfragilliformis* (285), *Macrolepiota dolichaula* (102), *Lentinus polychrous* (85), *Termitomyces heimii* (64) and *Russula adusta* had the lowest number of individuals with only 56 individuals.

The greatest number of species was observed in 2018 in the month of June with a total number of 293 individuals and the smallest in 2017 in the month of October with 12 individual species. **Figure 4.7** shows the mean temperature and total rainfall data recorded each month during the observation period, along with the number of WEMs species found in each location. Only graph with monsoon season (May-October) were plotted beacause maximum number of species were recorded when temperatures were mild and rain abundant (mean 23.4°C and 262.5 mm, respectively). In winter (November-February), when it was colder and rainfall was generally less abundant (mean 19.4°C and 18 mm), no species were found except for *S. commune*.

These findings align with prior research, which asserted that a significant annual rainfall is essential for the fungal mycelium to produce fruiting bodies (Salerni et al., 2002; Dijk et al., 2003; Enow et al., 2013, Djik). Similar observations were noted in a study where a higher number of mushroom species were documented during the rainy season, particularly in the months of July, August, and September. Conversely, macrofungal species were entirely absent in January and November. The researchers also noted the presence of certain woody macrofungi throughout the entire year (Vishwakarma et al., 2017; Buba et al., 2018).

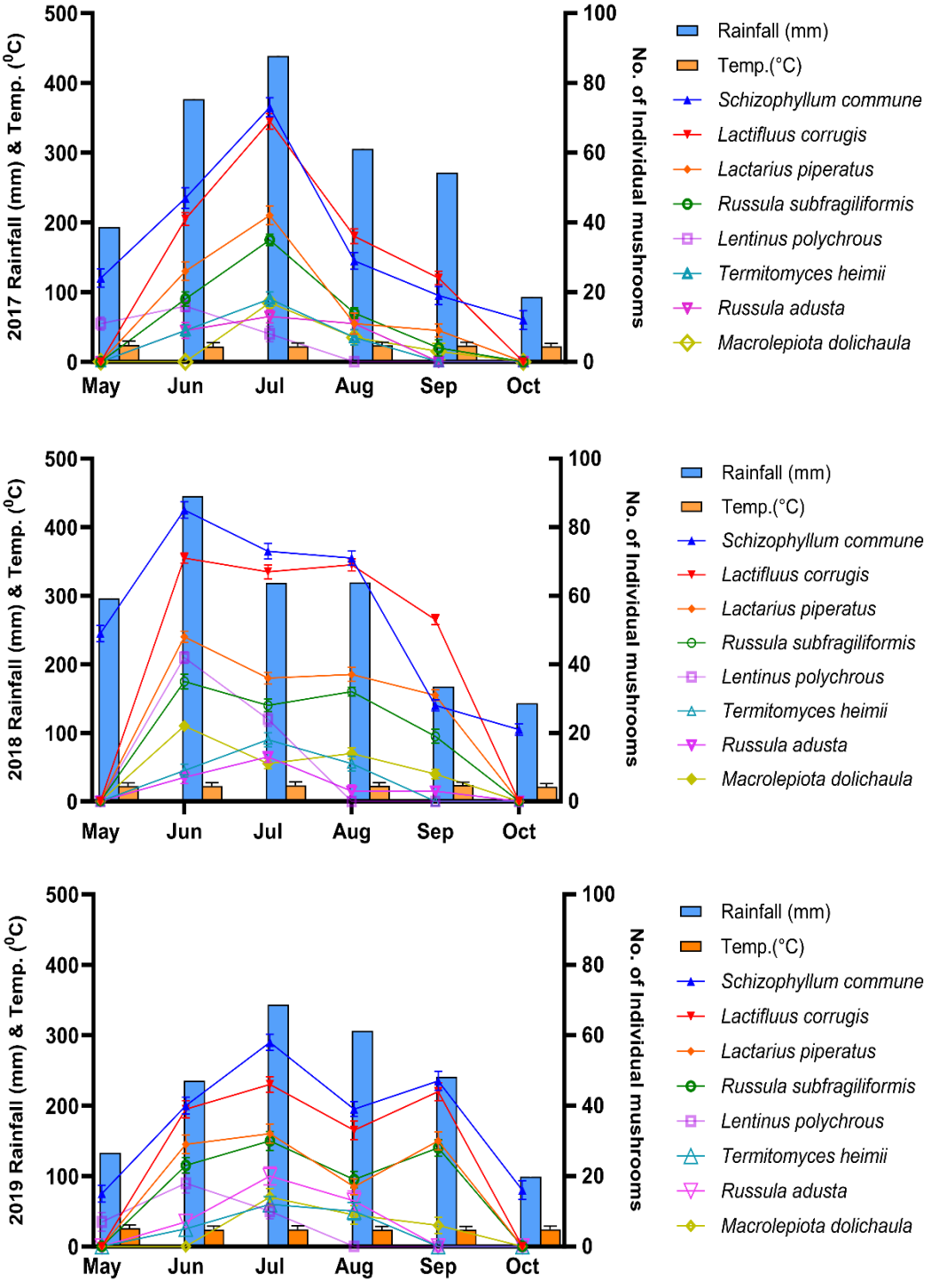


Figure 4.7. Relationship between rainfall, temperature with occurrence of common WEMs (2017 - 2019)

Table 4.6. Rainfall (mm), average day time temp. (°C) and No. of individual of selected WEMs studied during 2017-2019 for the month of May to October

	2017						2018						2019						
Months	May	Jun	Jul	Aug	Sep	Oct	May	Jun	Jul	Aug	Sep	Oct	May	Jun	Jul	Aug	Sep	Oct	
Rainfall (mm)	192.9	376.3	438.7	305.1	271.2	92.9	295.9	445.4	318.4	319.1	167.6	142.9	133.3	235.1	343.6	306.3	241.0	99.2	**Total Individual**
Average day time temp.(°C)	24.13	22.15	22.45	23.75	23.42	22.41	22.2	22.55	23.45	22.75	23.7	21.4	26.05	24.05	24.55	24.15	24.25	24.45	
Schizophyllum commune	24	47	73	29	19	12	54	85	73	71	68	61	35	40	58	39	47	24	859
Lactifluus corrugis	0	41	69	36	24	0	0	71	67	69	53	0	0	39	46	33	44	0	592
lactarius piperatus	0	26	42	11	9	0	0	48	36	37	31	0	0	29	32	17	30	0	348
Russula subfragiliformis	0	18	35	14	4	0	0	35	28	32	19	0	0	23	30	19	28	0	285
Lentinus polychrous	11	16	8	0	0	0	0	32	0	0	0	0	0	18	0	0	0	0	85
Termitomyces heimii	0	9	18	7	0	0	0	0	18	0	0	0	0	0	12	0	0	0	64
Russula adusta	0	9	13	11	0	0	0	0	13	0	0	0	0	0	10	0	0	0	56
Macrolepiota dolichaula	0	0	17	7	3	0	0	22	11	14	8	0	0	0	11	0	9	0	102
Total Individual by month	35	166	275	115	59	12	54	293	246	223	179	61	35	149	199	108	158	24	

4.3.6. Soil analysis

Following the computation of averages in the physico-chemical analysis of soil over a span of three years, it has been determined that the soil moisture content (%) for the selected WEMs ranges from 11.41±0.039 to 13.48±0.073 (**Table 4.7**), aligning with Kosol's (2022) earlier findings of moisture levels ranging between 11.51 to 13.5%. Notably, the highest moisture content was recorded during the rainy season, with the species *T. heimii* exhibiting the highest value (13.48±0.073), followed by *M. dolichaula* (12.42±0.626), *R. subfragilliformis* (11.45±0.059), *R. adusta* (11.44±0.049), *L. corrugis* (11.43±0.85), and the lowest observed in *L. piperatus* (11.41 ± 0.039) as shown in **figure 4.8a**.

The pH findings indicate that samples obtained from six distinct sites where selected WEMs were harvested exhibit an acidic nature. The soil reaction, as measured by pH, ranges from 4.53±0.186 to 6.57±0.148 (**Table 4.7**), closely resembling previous research results of 4.20 to 5.23 (Ambrosio et al., 2019; Gil-Martínez, 2020; Kosol, 2022). The highest pH values were observed for *T. heimii* (6.57±0.148), followed by *M. dolichaula* (5.78±0.173), *R. adusta* (5±0.188), *L. corrugis* (4.75±0.153), *R. subfragilliformis* (4.58±0.179), with the lowest pH recorded for *L. piperatus* (4.53±0.186) as shown in **figure 4.8b**. The elevated acidity in the forest soil can be attributed to the accumulation of organic acids resulting from vegetation decay and the decomposition of various litter types on the forest floor. Additionally, the acidity may be influenced by the inherently acidic nature of broad leaves. The presence of microorganisms in the forest soil could contribute carbon dioxide, thereby further intensifying its acidity.

The nitrogen analysis revealed a range in total nitrogen (%) content from 0.73±0.036 to 0.89±0.017. *T. heimii* exhibited the highest total nitrogen content at 0.89±0.017, followed by *L. corrugis* (0.85±0.057), *M. dolichaula* (0.79±0.024), *R. subfragilliformis* (0.78±0.022), *L. piperatus* (0.77±0.042), with the lowest observed in *R. adusta* at 0.73±0.036, as depicted in **Figure 4.8c** and **Table 4.7**.

The available phosphorus levels remained consistently low throughout the study period, ranging from 4.54±0.054 mg kg^{-1} to 11.84±0.064 mg kg^{-1}, consistent with

findings by Gezer & Kaygusuz (2015), who reported a range of 3.64 to 10.58 mg kg^{-1}. *T. heimii* recorded the highest phosphorus content at 11.84±0.064 mg kg^{-1}, while the lowest was observed in *R. adusta* at 4.54±0.054 mg kg^{-1}, as depicted in **Figure 4.8d** and **Table 4.7**. Arunachalam et al. (1998) have previously reported a heightened input of phosphorus through litter during the winter and spring seasons in 7-, 13-, and 16-years old forest regrowth in a humid subtropical region.

The study consistently identified high levels of Soil Organic Carbon content and organic matter throughout the entire research period. Specifically, the Organic Carbon content (%) and organic matter (%) reached their peak with *T. heimii* at 5.35±0.064 and 9.22±0.031, respectively, while the lowest values were recorded for *R. adusta* at 4.06±0.056 and 7±0.293, as illustrated in **Table 4.7** and **Figure 4.8e** and **Figure 4.8f**. Our results are supported by the results of De Bruyn and Conacher (1990) who reported that the higher amount of N, P and K in termite mound compared to surrounding soils was due to the cumulative effect of organic matter by the termites in their mound. The accumulation of the organic matter in the termite mound increases plant macronutrients such as nitrogen, phosphorus and potassium.

The heightened accumulation of organic carbon in the surface layer is attributed to the sluggish microbial decomposition of litter in acidic soils, a phenomenon previously documented by Nayak and Srivastava (1995) in humid sub- tropical soils under shifting cultivation in northeast India. However, the soil organic carbon content and nitrogen content in our study were notably higher than those reported in earlier studies by Gil-Martínez (2020).

Table 4.7. Soil physico-chemical properties of selected WEMs of Champhai district, Mizoram.

WEMs	Moisture content (%)	Soil reaction (pH)	Carbon content (%)	Organic matter (%)	Total Nitrogen (%)	Phosphorous (mg/kg)
Lactarius piperatus	11.41 ± 0.19^{c}	4.53±0.18^{f}	4.15±0.23^{d}	7.15±0.029^{d}	0.77±0.42^{cd}	6.67±0.24^{e}
Lactifluus corrugis	11.43±0.24^{c}	4.75±0.15^{d}	5.42±0.67^{a}	9.34±0.028^{a}	0.85±0.57^{ab}	7.23±0.26^{c}
Macrolepiota dolichaula	12.42±0.09^{b}	5.78±0.17^{b}	4.84±0.34^{c}	8.34±0.124^{c}	0.79±0.24^{bc}	9.64±0.19^{b}
Russula adusta	11.44±0.15^{c}	5.00±0.18^{c}	4.06±0.56^{e}	7.00±0.293^{e}	0.73±0.36^{d}	4.54±0.22^{f}
Russula subfragilliformis	11.45±0.17^{c}	4.58±0.17^{e}	4.87±0.15^{c}	8.4±0.022^{c}	0.78±0.22^{cd}	6.75±0.31^{d}
Termitomyces heimii	13.48±0.11^{a}	6.57±0.14^{a}	5.35±0.64^{b}	9.22±0.31^{b}	0.89±0.17^{a}	11.84±0.28^{a}

*Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs) ($P<0.05$).

**Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=3)

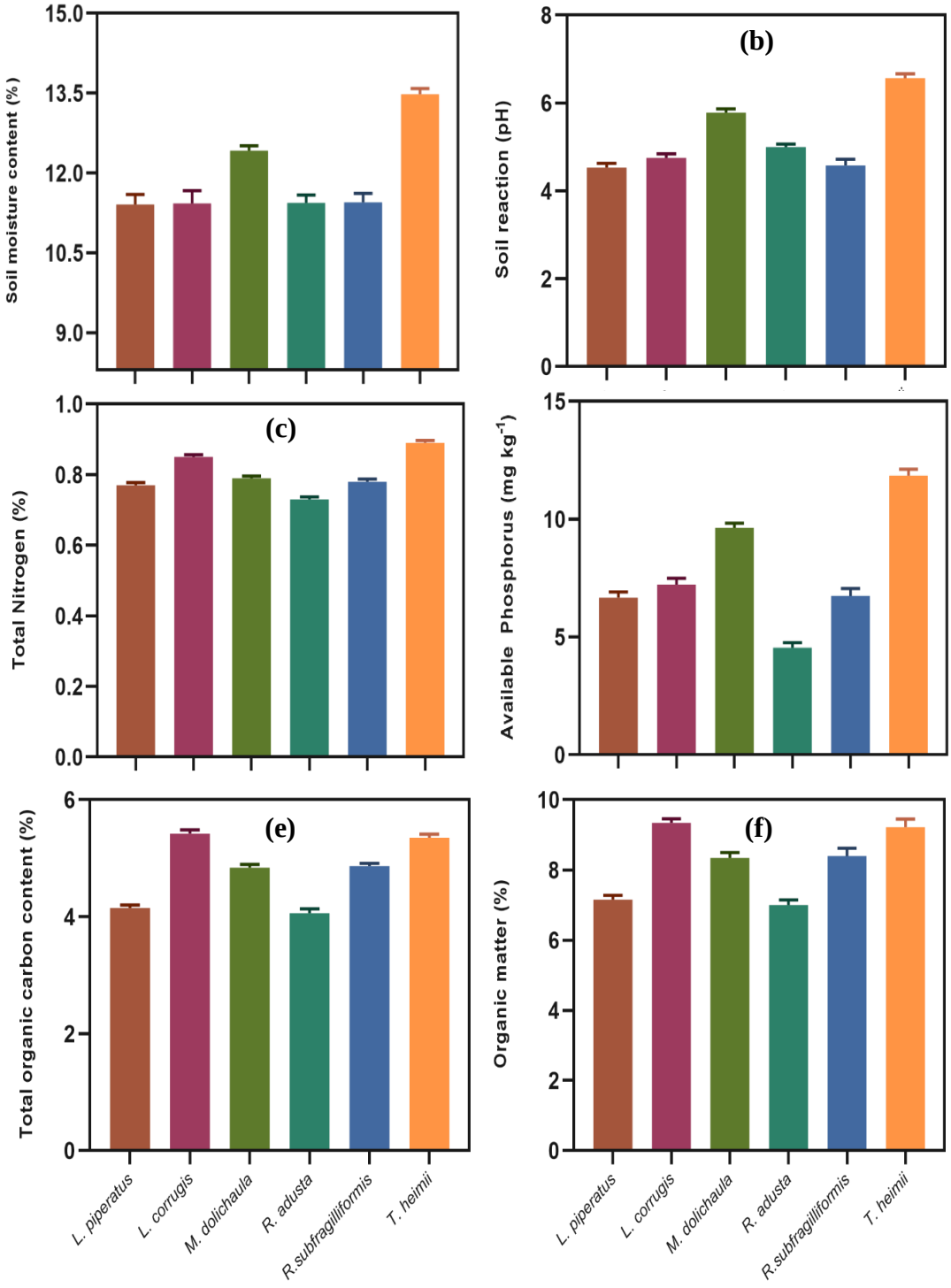


Figure 4.8. Soil physico-chemical properties of some selected WEMs from Champhai district. **(a)** Soil moisture content (%); **(b)** Soil reaction (pH); **(c)** Total nitrogen (%); (**d)** Available phosphorous (mg/kg); **(e)** Total organic carbon (%); **(f)** Organic matter (%).

4.3.7 Selected WEMs and their associated trees.

Selected WEMs and their corresponding trees were represented in a non-metric multidimensional scaling (NMDS) graph, as illustrated in **Figure 4.8a**, showcasing thevarious tree species or habitats where specific wild edible mushrooms commonly thrived across distinct sites. Utilizing a Bray-Curtis distance dissimilarity matrix (with stress = 0.08 and R2= 0.993) derived from six diverse forests, NMDS coordinates weregenerated, as depicted in **Figure 4.8b** and **Figure 4.9**.

The ordination plot revealed a strong association between trees such as *Lithocarpus dealbatus* (Fagaceae) and WEMs like *L. corrugis*, *L. piperatus*, and *R. subfragiliformis*. *Q. serrata* (Fagaceae) exhibited the highest WEMs association, particularly with *L. piperatus*, followed by *R. subfragiliformis*, *L. corrugis*, and a few species of *R. adusta*. Additionally, WEMs like *L. corrugis*, *L. piperatus*, *R. subfragiliformis*, and *R. adusta* were found in proximity to *C. trabuloides* (Fagaceae).

A noteworthy discovery was that all studied WEMs were located near *P. kesiya* (Pinaceae) within the study area. *L. pachyphyllus* (Fagaceae) demonstrated a close association with WEMs such as *R. subfragiliformis*, *L. corrugis*, *L. piperatus*, *R. adusta*, and *M. dolichaula*. *M. dolichaula* exclusively inhabited grazing fields, and their associated grasses are detailed in **Table 4.8**. Similar finding was observed that these mushrooms preferred to grow on grasslands and disturbed places (Rizal et al., 2017). Our results agree with Gucia et al., (2011) who stated that *Macrolepiota* species are saprobes that grow alone at forest edges and grassland. Similar results were observed for *T. heimii*, which exclusively thrived on termite mounds.

The connection between specific tree species and WEMs, as illustrated in the non-metric multidimensional scaling (NMDS) graph, underscores the intricate relationship these fungi share with their surrounding ecosystem. Our findings indicate a notable association between the majority of collected WEMs and tree families like Fagaceae and Pinaceae, prevalent in tropical and sub-tropical forests. This aligns with previous studies that also highlight the affinity of *Lactifluus*,

Lactarius, *Multifurca*, and *Russula* species with trees from Betulaceae (e.g., *Betula*, *Carpinus*, *Corylus*), Fagaceae (e.g., *Castanea*, *Fagus*, *Quercus*), Pinaceae (e.g., *Abies*, *Picea*, *Pinus*), and Cistaceae (e.g., *Cistus*, *Halimium*) in European and North American regions (Comandini et al., 2006; Van de Putte 2012; Leonardi et al., 2016; Leonardi et al. 2020). In the Asian context, these species are predominantly associated with Dipterocarpaceae (e.g., *Dipterocarpus*, *Shorea*) and Fagaceae (e.g., *Castanopsis*, *Lithocarpus*) (Le, 2007; Vande Putte, 2012).

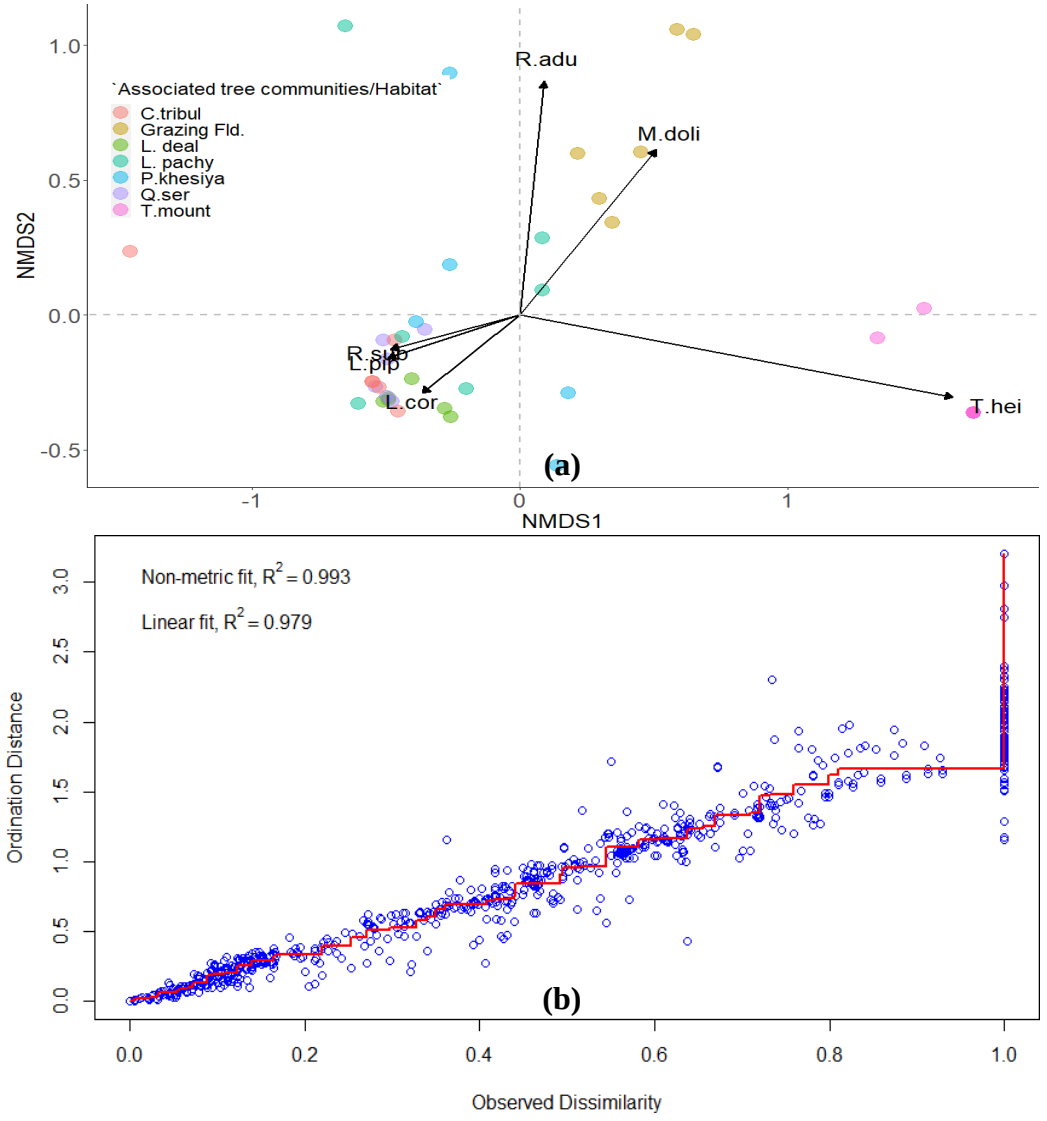


Figure 4.9. Non-Metric Dimensional Scaling **(a)** selected WEMs and their associated trees from Champhai district, Mizoram. **(b)** Stress plot.

Table 4.8. Common species of grass found in grazing fields where *Macrolepiota dolichaula* were commonly found in Champhai district, Mizoram.

Sl. No	Name of the species	Family
1	*Brachiaria semiundulata* (Hochstetter) Stapf.	Poaceae
2	*Digitaria ischaemum* (Schreb.) Muhl.	Poaceae
3	*Digitaria violascens* Link Hort. Berol.	Poaceae
4	*Eragrostis curvula* (Schrad.) Nees	Poaceae
5	*Oplismenus burmanii* (Retz.) P. Beauv.	Poaceae
6	*Setaria verticillata* (L.) P. Beauv., Essai Agrist.	Poaceae
7	*Themeda arundinacea* (Roxb.) A. Camus	Poaceae
8	*Themeda caudata* (Nees ex Hook. & Arn.) A. Camus.	Poaceae

(a) ***Castanopsis trabuloides*** **(Thing sia)**

(b) ***Lithocarpus dealbatus*** **(Fah)**

(c) ***Lithocarpus pachyphyllus*** **(Ṭhil)**

(d) ***Pinus kesiya*** **(Far)**

(e) ***Quercus serrata***

(f) **Grazing field**

(g) **Termite mound**

Figure 4.9. Photos showing different associated trees and habitat of selected WEMs from Champhai district, Mizoram.

4.4 Conclusions

In conclusion, this study provides the baseline information on diversity, the distribution, ecological preferences, and associated factors of wild edible mushrooms (WEMs) which has yielded valuable insights. The altitudinal gradient played a crucial role in determining the habitat preferences of various WEMs. For instance, *Lactifluus corrugis* thrived in altitudes ranging from 845 to 1561 meters (mean altitude = 1384.3 meters), forming associations with plant communities like *L. dealbatus*, *C. trabuloides*, and *Q. serrata* and exhibiting specific soil properties (**Table 4.7**). This underscores the importance of considering altitude variations and associated plant communities in understanding the microhabitat specificity of WEMs.

Furthermore, the influence of temperature and rainfall on the fruiting patterns of

selected WEMs was evident, with the highest abundance observed during the monsoon season characterized by mild temperatures and abundant rainfall. This temporal correlation highlights the intricate relationship between environmental factors and theflourishing of WEMs.

The soil analysis revealed distinct patterns in moisture content, pH, and nutrient composition, emphasizing the significance of soil characteristics in supporting the growth of WEMs. The association between specific tree species and WEMs, as depicted in the non-metric multidimensional scaling (NMDS) graph, further emphasizes the interconnectedness of these fungi with their surrounding ecosystem.

Overall, this study contributes to our understanding of the intricate ecological relationships that govern the distribution and growth of wild edible mushrooms. The identified microhabitats, altitudinal preferences, and associations with specific plant communities underscore the need for holistic considerations in the conservation and sustainable harvesting of these valuable natural resources.

CHAPTER V

5. Proximate analysis, mineral contents, and antioxidant activities of selected WEMs from Champhai District, Mizoram.

5.1 Introduction

In many cultures, mushrooms are referred to as "vegetable meat" and are highly regarded as culinary delights (Das et al., 2021). They are thought to be the most underutilized source of nutrient-rich meals, nonetheless (Tibuhwa, 2013). The focus of research into the nutritional value of mushrooms has recently switched to the studyof the physiologically active chemicals that are found in mushrooms because these substances also have medical implications for consumer health (Stojanova et al., 2021). Protein, sugar, glycogens, lipids, vitamins, amino acids, and crude fibers are allessential elements that the body needs to function normally. It can be used as food to combat malnutrition in developing countries such as India (Pandey et al., 2018).

Antioxidants are essential to our health because they act as our first line of defense against free radical damage. They are chemical compounds that protect cells from freeradical damage and are produced exogenously or obtained from food to slow the progression of chronic diseases (Bellettini et al, 2019). Antioxidant activity in commonWEMs has recently been discovered, and it is closely related to their total phenolic and polyphenol content (Palacios et al., 2011). In addition to various components with inhibitory and immunological efficacy, mushrooms include antioxidant bioactive compounds like flavonoids, phenolics, polysaccharides, glycosides, phenols, tocopherols, ascorbic acid, and organic acids. These biomolecules perform essential functions like removing free radicals from the body and preventing the development of cancer (Mwangi et al., 2022).

Among the bioactive molecules, phenolic acids have received special attention because they have been reported to be the primary antioxidant properties of mushrooms (Barros et al., 2007). Several *in vitro* studies on various mushrooms revealed that mushrooms with high phenolic content had a high antioxidant effect

(Bach et al., 2019). In addition to phenols, flavonoids are also anticarcinogenic, anti-mutagenic, and cardioprotective, as well as having antioxidant properties (Barros et al., 2008). There are currently 17 WEMs species listed in the Champhai district ofMizoram, India (Ralte et al., 2020). However, despite being a highly prized state delicacy, there have been no reports of the antioxidant and nutritional value. Theobjective of this study was to determine the nutritional value, mineral contents, and antioxidant activity of eight common WEMs found in Champhai District, Mizoram, India *viz., Lactarius piperatus*, *Lactifluus corrugis*, *Lentinus polychrous, Macrolepiotadolichaula*, *Russula adusta, R. subfragiliformis, Schizophyllum commune* and *Termitocytes heimii.* (**Table 5.1**).

Table 5.1. General information about the selected WEMs from Champhai district.

Sl. No	Scientific Name	Family	Habitats	Source forests	Growth stages
1	*Lactarius piperatus* (L.) Roussel	Russulaceae	Soil	Zote, Tlangsam, Ngur, Mualkawi, Vangchhia	Fruiting
2	*Lactifluus corrugis* (Peck) Kuntze	Russulaceae	Soil	Zote, Ngur, Tlangsam, Mualkawi, Vangchhia	Fruiting
3	*Lentinus polychrous* Lév.	Polyporaceae	Dead & decaying wood	Ngur, Zote, Tlangsam, Mualkawi	Fruiting
4	*Macrolepiota dolichaula* (Berk. & Broome)	Agaricaceae	Soil	Zote, Vangchhia, Khuangleng, Ngur	Fruiting
5	*Russula adusta* (Pers.) Fr.	Russulaceae	Soil	Vangchhia, Khuangleng, Ngur	Fruiting
6	*Russula subfragilliformis* Murrill	Russulaceae	Soil	Zote, Ngur, Tlangsam, Mualkawi	Fruiting
7	*Schizophyllum commune* Fr.	Schizophyllaceae	Dead & decaying wood	Zote, Ngur, Tlangsam, vangchhia	Fruiting
8	*Termitomyces heimii* Natarajan	Lypophyllaceae	Termite mound	Mualkawi	Fruiting

5.2 Methodology

5.2.1 Sample collection, preparation and identification

Višhniac recommendations were followed when collecting mushrooms (Vishniac et al., 1978). After drying in a hot air oven (40–50 ^{0}C), it was ground with mortar and pastel which was used for further testing. The specimens were identified using standard mushroom identification manuals such as www.mushroomexpert.com, www.mycokey.com, mycobank.org, and www.fungusid.com, as well as relevant literature and anatomical aspects (Verma et al., 2013).

5.2.2 Sample extract preparation

Each 5 g sample of finely dried powdered mushrooms was extracted with 50 mL of 80% methanol at 150 rpm using a magnetic stirrer for 24 h at 25 ^{0}C before being filtered through Whatman No. 1 filter paper. Filtrates were collected and the residue was re-extracted with 50 mL of methanol, centrifuged at 5000 rpm for 10 minutes, andevaporated before being stored at -20 °C until needed.

5.2.3 Proximate analysis

The Association of Official Analytical Chemists methods (AOAC 2005) were followed to determine the proximate analysis of the investigated WEMs. To determine moisture content, the samples were dried in an oven at 70 ^{0}C until constant weight were obtained. Crude fiber and ash content was determined by weighing the residue after burning at 550 ^{0}C for 3 h in muffle furnace. Crude fat content was determine using Soxhlet apparatus by extracting known weight of mushrooms powdered sample with petroleum ether. Total carbohydrate was determined following Anthrone method (Plummer, 1990). Total protein was determined according to the method of Lowry et al., (1951). The total energy contentwas determined by the value obtained for protein, carbohydrates, and fats multiplied by 4.00, 4.00, and 9.00 respectively, and adding up the values (AOAC., 2005).

5.2.4 DPPH free radical scavenging activity

The DPPH free radical scavenging activities of mushroom samples were determined

using the method of Muthoni et al., (2020). with slight modification. In brief, 0.1 mMDPPH solution in methanol was prepared, and 1 mL of this solution was added to 2 mL of different concentrations of mushroom extracts (10-100 µg mL^{-1}). The mixture was vigorously shaken and allowed to stand in the dark at room temperature for 30 minutes. The absorbance was then measured in a UV-vis spectrophotometer at 517 nm. The following equation was used to calculate the ability to scavenge the DPPH free radical: DPPH scavenge (%) = (A0 – A1)/A0 × 100%, where A1 is the absorbance value of the solution with different samples and A_0 is the absorbance value of the DPPH solution without samples.

5.2.5 ABTS radical scavenging activity

The ABTS activity of each mushroom extract was determined using a modified method of Seal (2014). For the formation of green-colored ABTS, an equal proportion (1_v:1_v) of ABTS (7 mM) mixed with K_2S_2O (2.45 mM) was left in the dark for 16-18h. The ABTS solution was further diluted with methanol (1_v:1_v) to produce a working solution with the absorbance of 0.700±0.005 at 745 nm. The resulting ABTS solution was then mixed with 1mL of mushroom extracts or standard (BHT) at varying concentrations (10 -100 µg mL^{-1}) and allowed to stand in the dark for 7 min and the absorbance was measured at 745 nm. The following equation was used to calculate the percentage inhibition of samples and standards: ABTS scavenge (%) = $(A_0 – A_1)/A_0 \times 100\%$, where A_1 is the absorbance value of the solution with different samples and $A_0$is the absorbance value of the ABTS solution without samples.

5.2.6 Determination of total phenolic content

The total phenolic content (TPC) of the mushroom sample was determined by Folin- ciocalteau method with slight modification (Kujala et al., 2000). Briefly, 1 mL of the sample, 0.5 mL of Folin-ciocalteau (1:10) reagent was added. After 5 min, 1.5mL of sodium carbonate (20%) was added and incubated at room temperature for 60 min. The absorbance was measured at 765 nm. Different concentrations of Gallic acid (20- 100 µg/mL) were used in the same manner for calibration of the standard curve, and quantification was done in terms of mg

equivalent of Gallic Acid (mg GAE g^{-1} dw).

5.2.7 Determination of total Flavonoid content

Using quercetin as a standard, the total flavonoid concentration was assessed using aluminium chloride ($AlCl_3$) with a small modification (Saeed et al., 2012). To that 4 mL of distilled water was combined with 1 mL of standard quercetin solution (20–100 µg/mL), then 0.03 mL of $NaNO_2$ (5%) was added. 0.03 mL of $AlCl_3$ (10%) was added after five minutes at 25 °C. 0.2 mL (1 mM) NaOH was added after 5 min intervals. The final volume was made to 10 mL with distilled water, and the absorbance was measured at 510 nm. A quercetin standard curve was used to calculate the flavonoid contents and distilled water was used as blank. Results of total flavonoid contents wereexpressed in quercetin equivalent (mg QE g^{-1} dw) and calculated from the prepared standard curve.

5.2.8 Determination of mineral content

Determination of mineral elements in the dry samples were done following the wet digestion extraction method (William, 1984). The powdered samples (0.2 g) were digested using 5 mL nitric acid (HNO_3) and 2 mL perchloric acid ($HClO_4$). The solution was filtered after adding 15 mL of distilled water into a 50 mL volumetric flask and the volume was made up to mark with distilled water. The minerals in the digested samples were then determined by AAS following the development of colourwith ammonium molybdate.

5.2.9 Statistical analysis

All the results were expressed in means of five replicates (mean ± SD) and analyzed by one-way analysis of variance (ANOVA) followed by a post hoc test using Duncan's multiple range tests for comparison of statistical significance (P<0.05). Pearson's correlation coefficients were calculated in order to measure the linear correlation between variables. All statistical calculations were performed using R-Studio (V.4.1.2) and SPSS (V.16) statistical software. The packages 'ggplot2', 'factoextra' and 'FactoMineR' were used for plotting graphs using R-Studio

(V.4.1.2).

5.3 Results and discussion

5.3.1 Proximate analysis

The proximate composition of the mushroom samples is shown in **Table 5.2**, with values expressed on dry weight (dw) basis. But the moisture content was determined on fresh weight (fw) basis. The range of variance for several parameters is as follows: 85.37- 93.19% for moisture content, of 5.67 to 15.33% for ash, 33.16- 9.16% for fiber, 19.53- 64.01g $100g^{-1}$ dw for protein, 53.53-6.17g $100g^{-1}$ dw for carbohydrates; and 5.83-2.24g $100g^{-1}$ dw for fat.

The total moisture content of WEMs was found to be highest in *R. adusta* (93.19%) and lowest in *S. commune* (85.37%) in the current study. Our findings are nearly identical to those of recent studies that reported moisture content greater than 80% (Jacinto-Azevedo et al., 2021). Many edible mushrooms benefit from moisture contentin terms of texture, palatability, and shelf life (Reis et al., 2012). As a result, the nutritional value of edible mushrooms was concerned with their dry matter content and specific composition, which can range from 3-15% in fresh mushroom (Ouzouni et al., 2009). Our study revealed that the dry matter contents ranged from 6.81% in *R. adusta* to 14.63% in *S. commune*, indicating that they have a high nutritional value.

The total ash content of selected WEMs was in the range of 5.67% to 15.33%, which was higher than the finding of Petridis et al., (2009). The highest ash content (%) was observed in *L. corrugis* (15.33%), followed by *L. polychrous* (14.93%), *M. dolichaula* (14.35%), *R. subfragiliformis* (13.62%), *R. adusta* (11.3%), *S. commune* (7.84%), *L. piperatus* (6.89%), and the lowest ash content was observed in *T. heimii* (5.67%). The primary components of mushroom ash are potassium and phosphorus (Mattila et al., 2001). *T. heimii* had a total ash content of 5.67%, which was significantly lower than the finding of Johnsy et al., (2011). In contrast, Barros et al (2008). reported that the ash content of wild and cultivated mushrooms ranged from 7.07 to 16.48%, which agrees with the findings in our study.

Evaluation of crude fiber revealed that *L. corrugis* (33.16%) had the highest percentage, followed by *M. dolichaula* (29.57%), *L. polychrous* (28.39%), *R. subfragiliformis* (20.43%), *R. adusta* (16.83%), *S. commune* (13.55%), *L. piperatus* (12.07%), and the least content of crude fiber was observed in *T. heimii* (9.16%), significant difference was observed among the species. Fresh mushrooms had a high amount of fiber, which may account for their relatively high ash content (Cheung, 2010). The crude fiber content of selected mushrooms ranged from 9.16 to 33.16%, which was nearly similar to the finding of Teke et al., (2021) and Chittaragi et al., (2014).

Protein are the most abundant macronutrients and was highest in *T. heimii* (64.01g $100g^{-1}$ dw), while, *L. piperatus* recorded the lowest content (19.53g $100g^{-1}$ dw). High level of protein was also observed in *L. corrugis* (34.46g $100g^{-1}$ dw), *R. subfragiliformis* (31.25g $100g^{-1}$ dw), *R. adusta* (29.40g $100g^{-1}$ dw) and *S. commune* (26.38g $100g^{-1}$ dw). However, no significant difference ($p \leq 0.05$) was found in proteincontent of *L. polychrous* and *S. commune* (both of which are wood growing). The protein content of WEMs is significantly richer than that of commercial leafy vegetables such as cabbage (1.8g $100g^{-1}$), cauliflower (2.6g $100g^{-1}$), potato (1.62g $100g^{-1}$), and spinach (2.00g $100g^{-1}$) (Gopalan et al., 1971). This demonstrated that mushrooms are richer in protein than green vegetables. The total protein content of the WEMs was found to be richer than the carbohydrate content previously studied (Manziet al., 2001).

Carbohydrates content of the eight WEMs varies from 6.17g $100g^{-1}$ dw (*S. commune*) to 53.53g $100g^{-1}$ dw (*M. dolichaula*) with significant difference among the species ($p>0.05$). The results are in accordance with both bioavailable (sugars, glycogen, andstarch) and non-bioavailable (chitin, hemicellulose, pectic materials, -glucans, and mannans) carbohydrates. Almost all the studied species had similar range of carbohydrate content to other studies on edible mushrooms (Ao & Deb, 2019).

The evaluation of total fat content from selected WEMs revealed that *R. subfragiliformis* had the highest fat content (6.21g $100g^{-1}$ dw) and the lowest fat

content was observed in *S. commune* (2.24g $100g^{-1}$ dw), which was nearly identical to the results reported by Petridis et al., (2009). The species of genera *Lactifluus*, *Macrolepiota* and *Termitomyces* had no significant difference in fat content ($p \leq 0.05$).Since edible mushrooms have a low-fat content, a high biological value, and the ability to improve people's health, they are regarded as a healthy food source (Dimitrijevic etal., 2019).

The highest energy content/calorific value was observed in *T. heimii* 369.57 (Kcal $100g^{-1}$ dw), while *S. commune* showed the lowest calorific value 150.9 (Kcal $100g^{-1}$ dw). Total energy in *T. heimii* was 369.57 Kcal $100g^{-1}$ dw, which was higher than the reported value from three different *Termitomyces* species (255.57 to 263.08 Kcal $100g^{-1}$ dw) (Nakalembe et al., 2015). The low energy content of *L. polychrous* (157.54 Kcal $100g^{-1}$ dw) and *S. commune* (150.9 Kcal $100g^{-1}$ dw) could be attributed to their low-fat contents.

Our findings revealed that among the eight edible mushrooms studied, protein and carbohydrate were the main components, while fat and ash contents were the lowest, implying that the mushrooms studied are a good source of protein, carbohydrates, andlow fat. As a result, they have high nutritional significance and are ideal for low-caloriediets.

5.3.2 Mineral contents

The metal concentrations were all calculated on a dry weight basis. Potassium was found to be the most abundant mineral among the eight WEMs, with concentrations ranging from 2411.59mg $100g^{-1}$ in *L. polychrous* to 1033.29mg $100g^{-1}$ in *T. heimii*. Phosphorus levels in *R. adusta* and *T. heimii* ranged from 185.29mg $100g^{-1}$ to 834.3mg$100g^{-1}$, respectively. Mangesium levels in *L. polychrous* were found to be highest (328.51mg 100 g) and lowest (136.71mg $100g^{-1}$) in *T. heimii*. Significant differences among the species were observed in phosphorous, potassium and mangesium. Among the eight edible mushrooms, *M. dolichaula* had the highest calcium content (126.46mg$100g^{-1}$) and *L. piperatus* had the lowest calcium content (32.34mg $100g^{-1}$). Iron was found to be in the range of 12.04 to 74.70mg $100g^{-1}$ in *T. heimii* Tand *L. corrugis*, while zinc was found to be in the range of 2.83 to

10.8mg $100g^{-1}$ in *T. heimii* and *L. polychrous*. The lowest mineral found was copper, with *M. dolichaula* 5.78mg $100g^{-1}$ having the highest copper concentration and *T. heimii* 1.06mg $100g^{-1}$ having the lowestcontent **(Table 5.3)**.

Potassium, phosphorous, and magnesium had been reported to be the most abundant minerals in edible mushrooms, but iron and zinc are scarce (Chang & Hayes, 1978). The most abundant minerals in all of the studied WEMs were potassium, phosphorous, and magnesium; a similar finding was reported by Genccelep et al., (2009). Iron, zinc and copper were the traced elements and their concentrations in WEMs were similar to the finding of Mallikarjuna et al., (2013).

Table 5.2. Proximate composition of selected WEMs from Champhai district, Mizoram.

Sl. No	Scientific Name	Moisture content (%)	Total Ash (%)	Crude Fiber (%)	Total Protein (g $100g^{-1}$)	Total Carbohydrates (g $100g^{-1}$)	Fat (g $100g^{-1}$)	Energy (Kcal $100g^{-1}$)
1	*L. corrugis*	91.3 ± 0.27^{b}	15.33 ± 0.24^{a}	33.16 ± 0.06^{a}	34.46 ± 0.19^{b}	45.47 ± 0.22^{b}	3.56 ± 0.23^{c}	351.96
2	*L. piperatus*	88.82 ± 0.25^{c}	6.89 ± 0.07^{f}	12.07 ± 0.05^{g}	19.53 ± 0.20^{g}	10.47 ± 0.21^{f}	5.14 ± 0.16^{b}	166.64
3	*L. polychrous*	86.19 ± 1.90^{e}	14.93 ± 0.06^{a}	28.39 ± 0.15^{c}	25.83 ± 0.14^{e}	7.88 ± 0.15^{g}	2.46 ± 0.23^{d}	157.54
4	*M. dolichaula*	89.36 ± 0.31^{c}	14.35 ± 0.24^{b}	29.57 ± 0.10^{b}	22.63 ± 0.30^{f}	53.53 ± 0.15^{a}	3.27 ± 0.25^{c}	333.62
5	*R. adusta*	93.19 ± 0.31^{a}	11.13 ± 0.15^{d}	16.83 ± 0.11^{e}	29.40 ± 0.27^{d}	34.21 ± 0.21^{d}	5.83 ± 0.15^{a}	307.59
6	*R.subfragiliformis*	90.34 ± 0.49^{b}	13.62 ± 0.14^{c}	20.43 ± 0.19^{d}	31.25 ± 0.26^{c}	37.54 ± 0.22^{c}	6.21 ± 0.24^{a}	330.28
7	*S. commune*	85.37 ± 0.29^{e}	7.84 ± 0.05^{e}	13.55 ± 0.02^{f}	26.38 ± 0.28^{e}	6.17 ± 0.22^{h}	2.24 ± 0.14^{d}	150.9
8	*T. heimii*	87.19 ± 0.23^{d}	5.67 ± 0.03^{g}	9.16 ± 0.11^{h}	64.01 ± 0.10^{a}	20.86 ± 0.20^{e}	3.35 ± 0.23^{c}	369.57

*Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs) ($P<0.05$).

**Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=5)

Table 5.3. Macro and micro elements concentrations (mg $100g^{-1}$ in dry weight) in selected WEMs from Champhai district, Mizoram.

Sl. No	Scientific Name	Iron (Fe)	Calcium (Ca)	Zinc (Zn)	Copper (Cu)	Potassium (K)	Magnesium (Mg)	Phosphorus (P)
1	*L.corrugis*	74.7 ± 1.85^{a}	101.03 ± 6.03^{c}	8.99 ± 1.89^{ab}	3.09 ± 0.54^{bc}	1870.45 ± 8.90^{d}	200.50 ± 2.50^{b}	237.44 ± 1.29^{f}
2	*L.piperatus*	16.5 ± 0.35^{f}	32.34 ± 2.64^{e}	4.41 ± 1.76^{bc}	1.95 ± 0.37^{cd}	2261.84 ± 6.35^{b}	152.09 ± 1.88^{d}	312.07 ± 1.48^{c}
3	*L.polychrous*	65.6 ± 1.27^{b}	126.46 ± 3.01^{a}	10.8 ± 1.67^{a}	4.41 ± 0.41^{ab}	2411.59 ± 3.70^{a}	328.51 ± 2.50^{a}	332.39 ± 2.06^{b}
4	*M.dolichaula*	54.8 ± 2.86^{c}	130.16 ± 2.86^{a}	7.82 ± 1.78^{bc}	5.78 ± 0.66^{a}	1613.88 ± 5.82^{e}	139.04 ± 1.88^{e}	264.28 ± 0.76^{e}
5	*R. adusta*	24.02 ± 0.83^{e}	88.65 ± 1.70^{d}	5.42 ± 1.82^{bc}	1.45 ± 0.19^{d}	1274.29 ± 7.27^{g}	142.11 ± 1.88^{e}	185.29 ± 0.87^{h}
6	*R.subfragiliformis*	31.54 ± 2.01^{d}	112.14 ± 2.13^{b}	6.41 ± 2.11^{bc}	2.06 ± 0.55^{cd}	1417.66 ± 5.46^{f}	175.42 ± 1.56^{c}	211.85 ± 2.27^{g}
7	*S. commune*	14.56 ± 1.43^{f}	38.54 ± 2.25^{e}	5.07 ± 1.46^{bc}	2.00 ± 0.52^{cd}	2214.04 ± 9.82^{c}	157.84 ± 3.44^{d}	302.32 ± 1.39^{d}
8	*T. heimii*	12.04 ± 1.38^{f}	34.37 ± 3.02^{e}	2.83 ± 1.01^{c}	1.06 ± 0.25^{d}	1033.29 ± 9.57^{h}	136.71 ± 1.69^{e}	834.30 ± 1.28^{a}

*Means followed by different letters within same column are significantly different according to Duncan's multiple range comparisons (DMRTs) ($P<0.05$).

**Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=5)

5.3.3 Principal Component Analysis.

The multivariate data information (nutrients and minerals) contained in the studied WEMs was analysed using Principal Component Analysis (PCA). The Eigenvalues were observed to be 6.58, 3.92, 2.09 and 0.82 for the first four principal components which measure the variation retained by the principal components. To determine how much principal components was to be considered, the percentage of variance from screeplot **Fig. 5.1d** was evaluated. The first four major components accounted for 95.8% of the variance. In PC1 (47%), the variables like ash, crude fiber, zinc, calcium, Iron, copper and magnesium were positively correlated whereas protein and phosphorous were negatively correlated. The second, PC2 (28%) showed positive correlation between moisture, carbohydrate and energy, and negative correlation with potassium and magnesium. PC3 (14.9%) depicted positive correlation between protein and phosphorous but negatively with fat and moisture. The fourth principal component, PC4 (5.9%), correlated positively with magnesium and fat while negatively with copper and carbohydrate **(Figure 5.1c)**. The biplot of individuals and variables in PC1 and PC2 of **Figure 5.1a** depicted that *L. polychrous* had high content of K, Mg and Zn. Whereas, *T. heimii* had high content of P and protein. Similarly, in PC3 and PC4 of **Figure 5.1b**, *M. dolichaula* showed high Cu and carbohydrate contents.

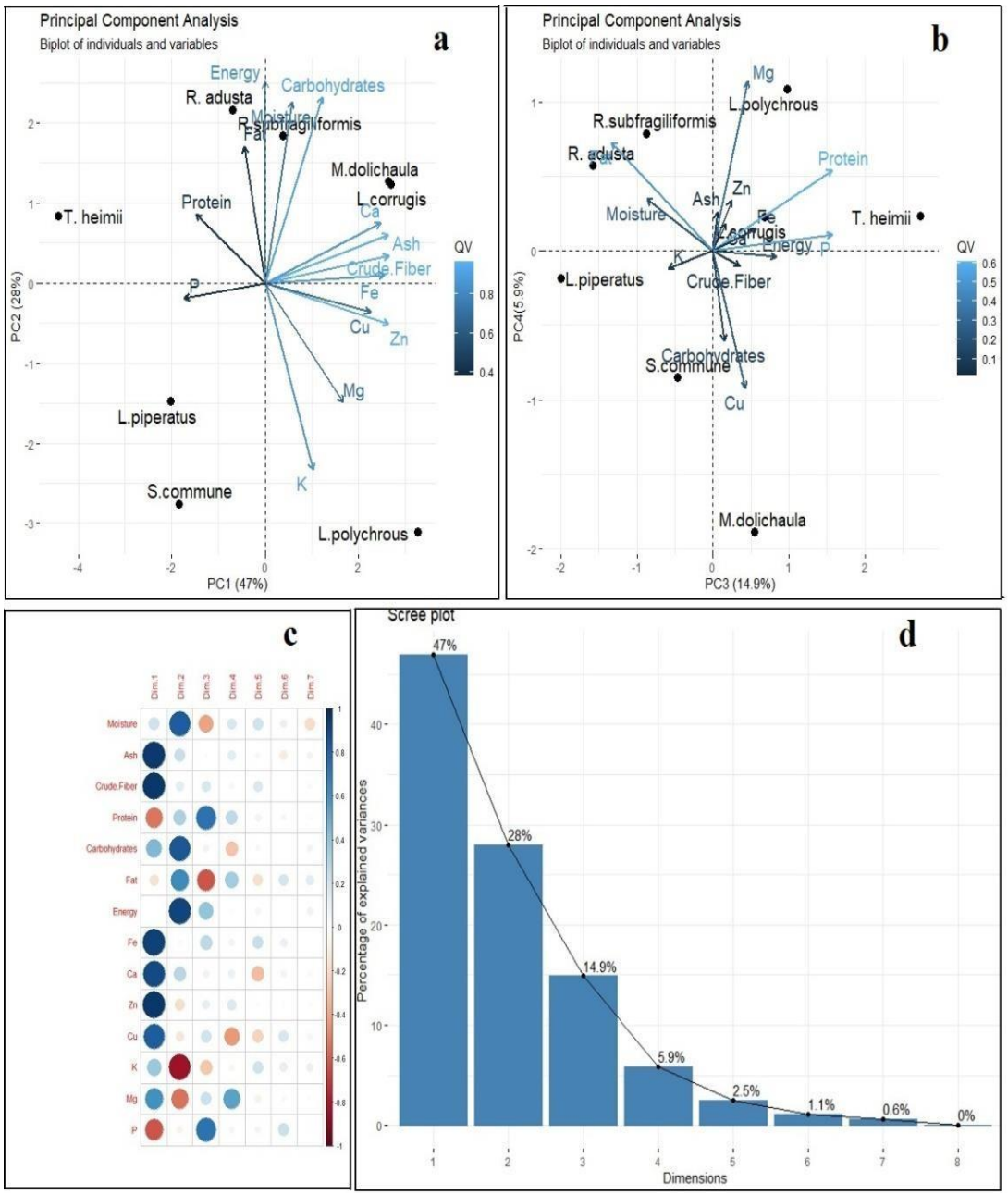


Figure 5.1. Principal Component Analysis (PCA) of WEMs with nutrients and minerals composition, (a) Biplot of individuals and variables (PC1 & PC2)**,** (b) Biplot of individuals and variables (PC3 & PC4)**,** (c) Principal components and their relation with variables, (d) Contribution of each principal component to total variance.

5.3.4 Determination of DPPH and ABTS free radical scavenging activities

The result of the DPPH radical scavenging activity of the eight WEMs extracts and standard BHT was concentrations dependent. The result indicated that *S. commune* had the strongest DPPH radical scavenging activity (69.45%) with an IC_{50} value of 31.12±0.29 µg/mL and *R. adusta* had the weakest DPPH radical scavenging activity (35.14%) with an IC_{50} value of 144.51±0.25 µg/mL as compared to standard Butylated Hydroxy Toluene (91.52%) with an IC_{50} of 12.55 µg/mL as shown in **Table 5.4** & **Figure 5.2a**.

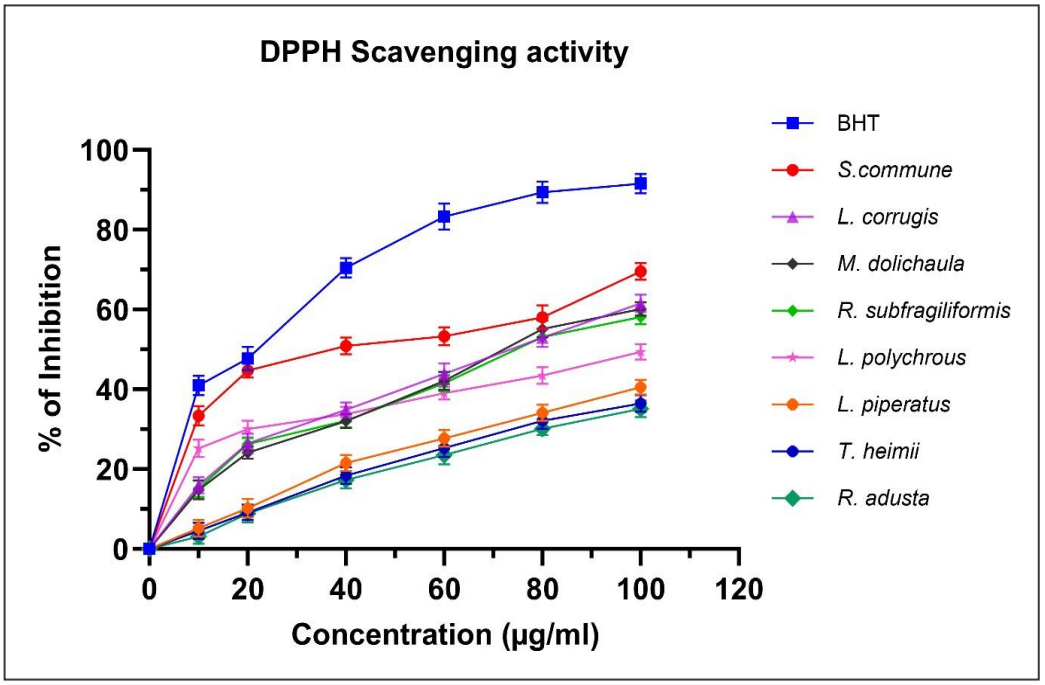
The percentage of ABTS free radical scavenging activity was highest in *S. commune* (87.23%) at a concentration of 100 µg/mL with an IC_{50} value of 42.2 µg/mL and *R. adusta* showed the weakest scavenging activity (52.12%) with an IC_{50} value of 86.55 µg/mL. The standard BHT was 95.2% with IC_{50} of 22.86 µg/mL as shown in **Table 5.4** & **Figure 5.2b**.

Table 5.4. Evaluation of antioxidant activities (DPPH and ABTS), total phenol and total flavonoid contents of selected WEMs from Champhai district, Mizoram.

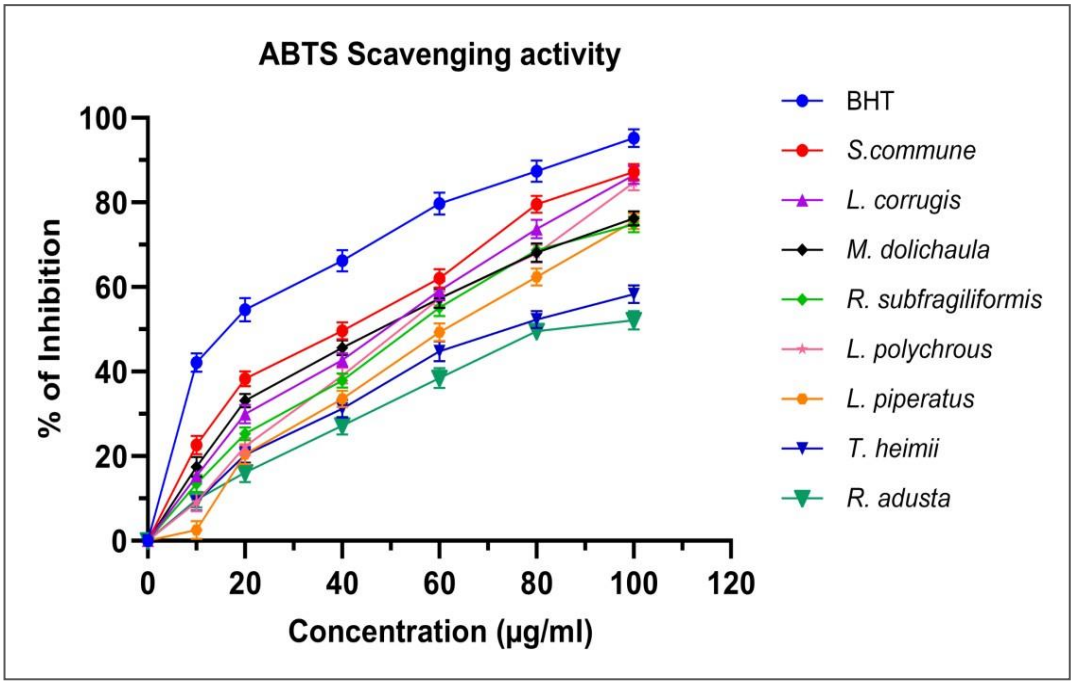
Sl.No.	Mushroom Species	DPPH IC_{50}±SD(µg/ml)	ABTS IC_{50}±SD(µg/ml)	TPC (mgGAE/g±SD)	TFC (mgQE/g±SD)
1	BHT	12.55±0.24^{i}	22.86±0.13^{h}	-	-
2	*L. corrugis*	77.01±0.20^{g}	50.14±0.25^{f}	17.5±0.45^{b}	4.83±0.56^{b}
3	*L. piperatus*	125.2±0.25^{c}	63.84±0.23^{c}	6.83±0.69^{d}	0.67±0.20^{d}
4	*L. polychrous*	103.8±0.45^{d}	55.02±0.22^{e}	13.17±0.55^{c}	0.97±0.20^{d}
5	*M. dolichaula*	80.11±0.29^{f}	54.74±0.36^{e}	16.21±0.81^{b}	2.13±0.41^{c}
6	*R. adusta*	144.5±0.25^{a}	86.55±0.30^{a}	3.07±0.28^{e}	0.09±0.03^{d}
7	*R.subfragiliformis*	83.06±0.23^{e}	58.63±0.25^{d}	15.74±0.74^{b}	1.07±0.12^{d}
8	*S. commune*	31.12±0.29^{h}	42.2±0.36^{g}	19.55±0.85^{a}	7.17±0.40^{a}
9	*T. heimii*	141.5±0.51^{b}	77.22±0.36^{b}	4.78±0.55^{e}	0.35±0.14^{d}

**Means followed by different letters within same column are significantly different according to Duncan's multiplerange comparisons (DMRTs) (P<0.05).*

***Means followed by same letter are not significantly different. Each value was represented as means ± SD (n=5)*



(a)



(b)

Figure 5.2. Comparisons between the percentages of radical scavenging activityof WEMs extract with positive control BHT**,** (a) DPPH scavenging activity**,** (b) ABTS scavenging activity.

5.3.5. Total phenolic and flavonoid content

Numerous studies have reported that the antioxidant activity of mushrooms and plants extracts are directly correlated to their phenolic and flavonoid content (Caprioli et al., 2019). Total phenolic contents in different mushroom extracts were determined from the linear regression curve of standard Gallic acid as shown in **Figure 5.3a** and were expressed as mg GAE g^{-1} extract. The total phenol content of the eight WEMs varies from 19.55 mg GAE g^{-1} in *S. commune*, and lowest 3.07 mg GAE g^{-1} extract in *R. adusta*. The species belonging to the genera *Schizophyllum*, *Lentinus* and *Lactarius* showed significant difference between their species, but no significant difference was observed among the species of the genus *Lactifluus* and *Macrolepiota*, also between *Russula* and *Termitomyces* (**Table 5.4**).

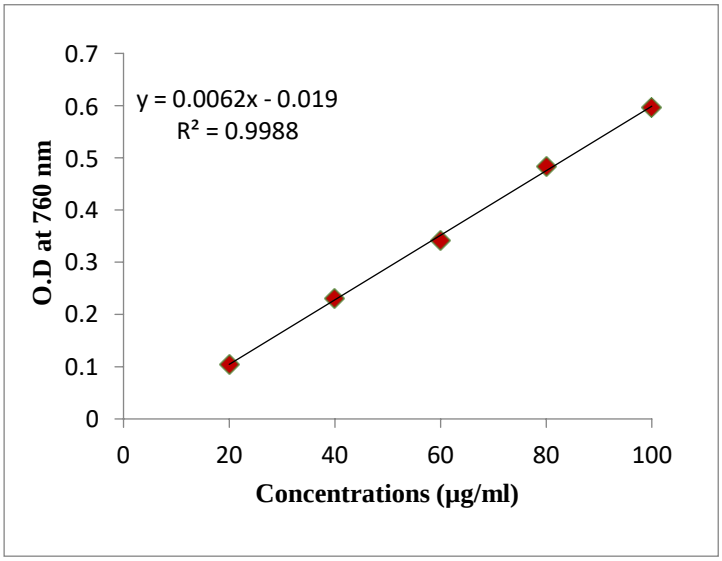


Figure 5.3a. Standard curve of Gallic acid.

The total flavonoid from methanolic extract of the eight samples was determined from the linear regression curve of standard Quercetin acid. *S. commune* (7.17 mg QE g^{-1} extract) had the highest total flavonoid and the least was *R. adusta* (0.09 mg GAE g^{-1} extract) as shown in **Table 5.4**. Significant difference was observed between the species of the genus *Schizophyllum*, *Lactifluus* and *Macrolepiota*. But no significant difference was observed among the genus *Lactarius*, *Russula* and *Termitomyces*.

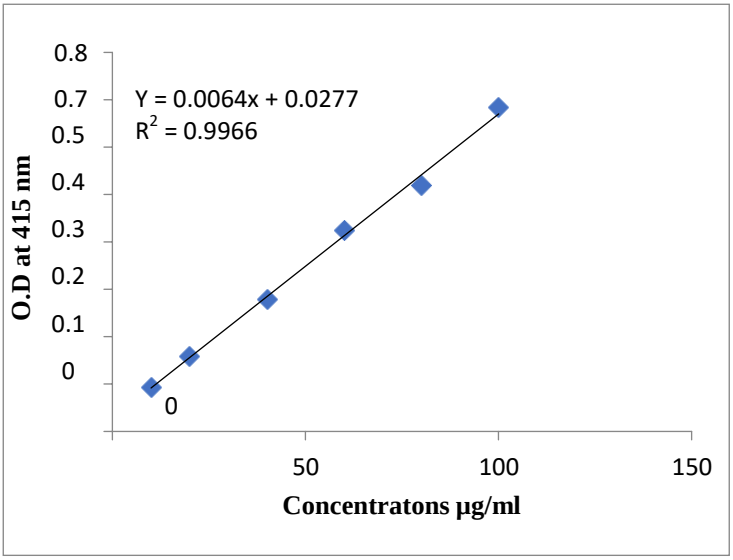


Figure 5.3b. Standard curve of Quercetin.

The results of the DPPH and ABTS radical scavenging activity showed that all of the mushroom's extracts were able to scavenge the free radical in a dose-response manner, which was consistent with the findings of Cheung et al., (2003). Strong DPPH and ABTS radical scavenging activity was observed in mushroom extracts containing more phenols than flavonoid, as phenols had a greater capacity to donate hydrogen toscavenge DPPH radicals. This suggested that phenolic compounds were the main antioxidant components present in mushroom extracts, which contributed to their highantioxidant activity (Butkhup et al., 2018). It can also be stated that climatic condition, geographical location and time of collection can influence total phenolic, flavonoid and nutritional composition of WEMs.

5.3.6. Pearson's correlation coefficient between antioxidant activity with total phenol and flavonoid contents

The correlation coefficient was used to measure the degree or strength between the total phenolic content (TPC), total flavonoid content (TFC) of mushroom extracts withDPPH, ABTS. Correlations were divided into categories based on the value of the strength to which the two variables are related: very strong (1.0 - 0.80), strong (0.79 -0.60), moderate (0.59 - 0.40), weak (0.39 - 0.20), and very weak (0.19 - 0.00) suggested by Evans (1996). Very strong correlation was observed between phenol

with DPPH and TPC (R=0.990) and ABTS (R=0.862), while TFC and DPPH (R=0.761) showed strong correlation and a moderate correlation were observed between flavonoid andABTS (R=0.687) as shown in **Table 5.5 & Figure 5.3**.

Table 5.5. Pearson's correlation coefficients between antioxidant activity, total phenol and flavonoid content.

Antioxidant assays	**Total phenol**		**Total flavonoid**	
	R value	P- Value	R value	P value
DPPH radical scavenging activity	0.990**	0.000	0.761*	0.006
ABTS radical scavenging activity	0.862**	0.006	0.687	0.060

***Correlation is significant at the 0.05 level (2-tailed)

****Correlation is significant at the 0.01 level (2-tailed)

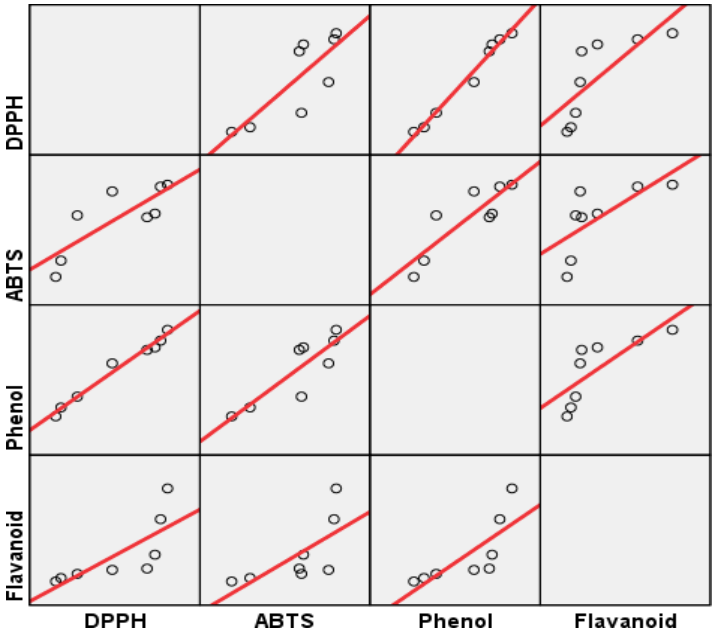


Figure 5.3. Scatter plot with linear regression line showing Pearson's correlation coefficientsbetween antioxidant activities, total phenol and flavonoid content.

5.4 Conclusions

The present study contributes to the nutritional characterization and antioxidant properties of WEMs from Champhai district, Mizoram, India. The high level of biological activity displayed by the extracts suggest the presence of a wide range of bioactive molecules, igniting the hunt for new bioactive substances that might be useful in many applications. Since time immemorial, different varieties of wild mushrooms have been a traditional food for the Mizo people. These mushrooms were collected in the wild, with no attempt made to cultivate them for commercial purposes. As a result, a nutritional database for these mushrooms has been created. The study of eight WEMs revealed that they had good antioxidant properties as well as an adequate amount of various nutrients and minerals. WEMs products, in the form of pure compounds or extracts with antioxidant activity, may help the endogenous defense system of the body. The results of this study can therefore be used to promote local consumption of mushrooms as functional foods and for commercial purposes, which could generate large employment opportunities for the locals to alleviate poverty and fight against malnutrition while also promoting their habitat preservation. It is worth noting that this is the first report on the proximate composition and antioxidant properties of WEMs from Mizoram, India.

CHAPTER VI

6. Identification and quantification of α-amanitin and muscarine using HPLC-PDA from wild mushrooms of Champhai district, Mizoram, India.

6.1. Introduction

The nutritional and medicinal properties of mushrooms have made them a subject of significant interest in the food and pharmaceutical industries (Kalač, 2013; Wasser, 2002; Valverde et al., 2015). Out of the estimated 140,000 mushroom species found globally, approximately 3,000 have been identified as edible, and about 700 are knownto offer health benefits (Chang & Wasser, 2012). Mizoram, a state in India, is home toa wide variety of mushrooms, which are found throughout the region. Local diets ofteninclude wild mushrooms that are safe to eat. Moreover, these mushrooms are often harvested by foragers for commercial purposes. Nevertheless, there is a risk of misidentifying poisonous mushrooms as edible ones, which can result in severe illnessor even death (Leudang et al., 2017; Parnmen et al., 2016). Mushroom poisoning, or mycetism, caused by the ingestion of mushrooms containing amatoxins is responsible for more than 90% of mushroom-related fatalities. More than 10 cases of fatality frommushroom poisoning have been reported in Mizoram, India, over the past ten years (2013-2023). The actual incidence of these poisonings may be higher than reported due to a significant number of cases that go unreported (Enjalbert et al., 2002; Karlson- Stiber et al., 2003). The severity of mushroom poisoning is influenced by various factors, such as the geographic location, growth conditions, amount of toxin ingested, and the individual's genetic profile (Erden et al., 2013). Several commonly found wildmushroom species have been identified as poisonous, including *Amanita*, *Cantharocybe*, *Chlorophyllum*, *Entoloma*, *Inocybe*, *Leccinellum*, *Russula*, and*Xerocomus* (Tawatsin et al., 2018).

Mushroom toxins can be classified into several categories, including cyclopeptides, orellanine, monomethylhydrazine, disulfiram-like reaction, muscarine, hallucinogenic indoles, isoxazoles, and gastrointestinal (GI) irritants (Berger &

Guss, 2002). α- amanitin is one of several toxic compounds found in mushrooms known as amatoxins.

Other amatoxins include beta-, gamma-, and epsilon-amanitin, as well as amanin and amanullin. Alpha-amanitin is a bicyclic octapeptide with a significant toxicity effect (**Figure 6.1**). Certain mushroom species such as Amanita (*A. phalloides*, *A. verna*, *A. virosa*, *A. bisporigera*, *A. ocreata*, *A. tenuifolia*), as well as Galerina species and *Conocybe filaris*, contain sufficient amounts of alpha-amanitin to cause liver damage and potentially fatal poisoning in adults. The oral LD_{50} of alpha-amanitin in humans is approximately 0.1mg/kg (Luo et al., 2012). Amatoxins are powerful inhibitors of RNA polymerase II, an essential enzyme for protein synthesis, particularly in liver cells (Bushnell et al., 2002). Identifying an antidote for mushroom poisoning remains a challenging task, and there is a critical need for innovative therapies to effectively treat affected patients (Garcia et al., 2015; Arici et al., 2020). Amatoxins are extremely lethal, and despite the various methods that have been developed to quantify them, there is still a need for the development of highly sensitive, selective, simple, fast, and accessible methods to measure amatoxins in different matrices such as wild mushrooms, urine, and serum.

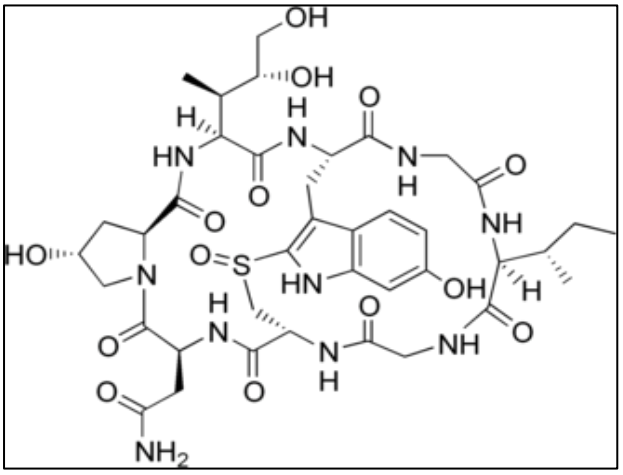


Figure 6.1. Chemical structure of α- Amanitin.

Muscarine is the primary toxin found in fungi belonging to the genera Inocybe and Clitocybe, and is also present in combination with isoxazole derivatives in certain species of the Amanita genus, such as *Amanita pantherina* and *Amanita muscaria*. It

was first isolated from *A. muscaria* by Schmiedeberg (1869). (**Figure 2**). Muscarine binds to acetylcholine receptors and triggers a distinctive set of symptoms, including excessive sweating, tear production, and slowing of the heart rate (bradycardia). These symptoms usually appear rapidly, within two hours of consuming the mushroom. Themuscarine toxin is prevalent in the basidiomata (fruiting bodies) of various Inocybe species collected from North America and Europe. Although poisonings caused by theconsumption of the aforementioned mushrooms are rarely fatal, it is still crucial to identify them promptly and begin medical treatment as soon as possible (Brvar et al., 2006).

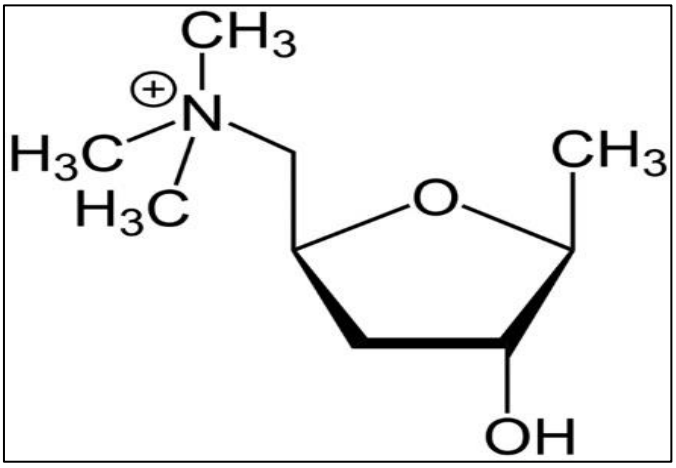


Figure 6.2. Chemical structure of Muscarine.

Numerous studies have been conducted to detect amatoxins in wild mushrooms using different analytical techniques, including RP-HPLC with UV detection (Enjalbert et al., 1992), DAD (Kaya et al., 2012), MS-TOF (Sgambelluri et al., 2014), and DAD- MS (Garcia et al., 2015). As far as we know, there has been no research conducted todetermine the toxin content of various *Amanita* species found in Mizoram, India. In light of the significance of identifying and measuring the levels of highly toxic amatoxins in mushroom samples, we present a straightforward, fast, consistent, and precise technique utilizing HPLC-PDA (High Performance Liquid Chromatography- Photo Diode Array) for the detection and quantification of α-amanitin and muscarine in wild mushrooms gathered from Champhai district of Mizoram.

6.2. Methodology

6.2.1. Reagents and Chemicals

All the reagents and solvents used in this quantification were of analytical grade. Thestandard α-amanitin, standard muscarine and sodium acetate were acquired from sigma-Aldrich (Steinheim, Germany). HPLC-grade methanol and acetonitrile were obtained from Rankem® (Pune, India) and ultrapure water was supplied by a milli-Qwater purification apparatus (Himedia, Mumbai, Maharashtra). All solutions preparedfor HPLC were filtered using a 0.45mm nylon filter.

6.2.2. Working solution, calibration curve for standard

The α-amanitin and muscarine stock solutions were prepared by diluting each standardto a concentration of 1.0 mg mL^{-1} using methanol. Standard solutions were further diluted with 1 mL of the mobile phase, which consisted of 0.05M ammonium acetate (pH 5.5) with acetonitrile (90:10 v/v). The resulting concentrations for α-amanitin andmuscarine in the standard solutions were 5, 10, 20, 40, and 80 μg mL^{-1} respectively. Allstock solutions were then stored in the dark at -20 °C until they were ready for use.

6.2.3. Mushrooms collection

To establish and validate the analytical method using HPLC-PDA, different mushroom samples were collected from the harvesting sites identified by villagers who had previously encountered poisonous mushrooms (**Figure 6.3**). These sites correspondedto recent incident reports, including fatal poisonings in Vangchhia (1 death and multiple hospitalizations, June 2016), and Ngur (1 death and other cases of indigestion, June 2018). After collecting the mushroom samples, they were adequately scrubbed, dried at 40°C for 48 h, and stored at −20°C to avoid degradation. Oyster mushroom samples (*Pleurotus ostreatus*) were purchased from local markets to serve as blank samples.

Amanita bisporigera *Amanita pantherina* *Amanita virosa* *Amanita pseudoporphyria*

Rusulla virescens *Macrolapiota dolichaula* *Turbinellus floccosus* *Cantharellus tropicalis*

Boletus hortonii *Hydnum rufescens* *Pleurotus ostreatus*

(a) **(b)**

Figure 6.3. Pictures of **(a)** some wild mushrooms collected from Champhai district, Mizoram India **(b)** *Pleurotus ostreatus* used as blank sample in this work.

6.2.4 Molecular analysis

DNA was extracted from tissue found within fruiting bodies using a CTAB extractionmethod (White et al., 1990). Initially, the fruiting body tissue was placed in a sterile 1.7 ml microcentrifuge tube along with glass beads and 500 µL of CTAB lysis buffer.To break down the fungal cells, the tube was vortexed for one minute. After a brief centrifugation to settle larger tissue fragments, the supernatant was carefully transferred to a new tube. This tube underwent further cell lysis in a 65°C hot water bath for 20 minutes. Following this, 500 µL of chloroform was added, and the tube was mixed and centrifuged for 5 minutes at 13,000 rpm. The resulting supernatant wasthen transferred to a fresh microcentrifuge tube. Subsequently, two-thirds of the transferred liquid was mixed with isopropanol and left to incubate at room temperature for 5 minutes before undergoing centrifugation for 7 minutes at 15,000 rpm. After discarding the supernatant, 500 µL of 70% ethanol was added, and the tube was centrifuged again for three minutes at 15,000 rpm. Finally, the supernatant was removed, and the tubes were left open to allow ethanol to evaporate. The resulting DNA pellet was re-suspended in 100 µL of sterile water. For PCR reactions, 25.5 µL of reaction mixture, consisting of GoTaq Green Mastermix, nuclease-free water, forward and reverse primers, and fungal DNA template, was prepared in 0.2 ml centrifuge tubes.

PCR was conducted utilizing the ITS1-F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC-3') primers under the following conditions: initial denaturation at 94^0C for 5 minutes, followed by 35 cycles of denaturation at 94^0C for 1 minute, annealing at 52^0C for 1 minute, and extension at 72^0C for 1 minute, with a final extension step at 72^0C. The resulting PCR amplicons were assessed via electrophoresis on a 1% agarose gel stained with SYBR green and then visualized using a Gel Documentation System. Sequencing was accomplished using both primers via Sanger sequencing on DNA sequencers.

Subsequently, a consensus sequence for contigs was trimmed and aligned using the Bioedit sequence alignment editor (Hall, 1999). The aligned sequence was compared to those in the GenBank database using the BLASTn search tool to identify

similarities and was subsequently submitted to GenBank (Altschul et al., 1990). Alignment of sequences was performed using ClustalW and a phylogenetic tree was constructed using the Maximum Likelihood method implemented in MEGA11 software (Tamura et al., 2021). Phylogenetic analysis was conducted based on the ITS gene data utilizingboth Maximum Likelihood (ML) and Neighbour-Joining (NJ) approaches. ML and NJsearches were conducted using MEGA11, treating alignment gaps as missing data. NJ trees were constructed based on overall character differences, and bootstrap values were computed from 1,000 replications.

6.2.5. Sample preparation and extraction procedure

Ten grams of mushroom samples were combined with 60 mL of an extraction solvent composed of methanol, water, and 0.01 mol/L HCl in a 5:4:1 (v/v/v) ratio, following the method proposed by Barbosa et al. (2022). The extraction process utilized a Borosil 3840020 Extraction Apparatus Soxhlet, complete with an Allihn Condenser and a 200ml flask. The extraction proceeded for a specified duration, after which the supernatant was collected, filtered using a 0.45 µm disposable filter holder, and evaporated to dryness at 50–55 °C. The resulting residue was reconstituted in 10 mL of a mobile phase. Solid-phase extraction (SPE) of α-amanitin and muscarine was conducted using Oasis® PRIME HLB cartridges. One mL of the previously prepared solution was loaded onto an Oasis® PRIME HLB extraction cartridge without sorbent preconditioning. Subsequently, the cartridge underwent a wash with 1 mL of 5% methanol, and elution was carried out using 1 mL of acetonitrile: methanol (9:1). The eluate was collected, thoroughly dried to a solid residue under a slight nitrogen flow at room temperature, and then reconstituted with 1 mL of mobile phase. Finally, 50 µL of the reconstituted solution was injected into the chromatographic system (**Figure6.4**).

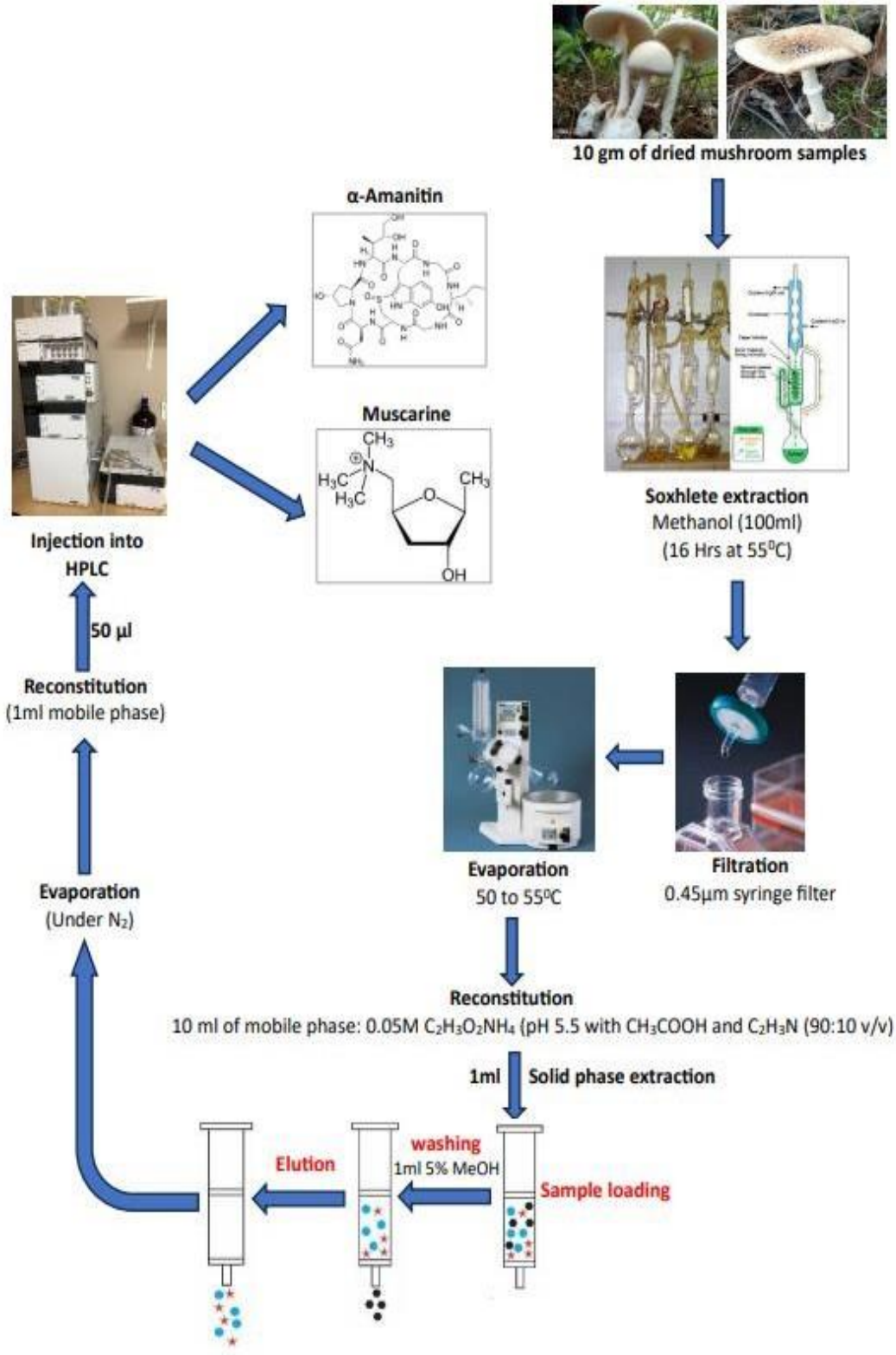


Figure 6.4. Flow chart showing the proposed sample treatment and analysis for HPLC-PDA.

6.2.6. Chromatographic system and condition

Quantification of α-Amanitin and muscarine was performed using Shimadzu HPLC-(Kyoto, Japan) chromatography comprising PDA(SPD-M20A), LC (LC-20AD) pump, autosampler (SIL-20AC). The chromatographic separation was done using a reverse-phase C-18 (250 mm× 4.5 mm). The mobile phase was composed of 0.05M ammonium acetate (pH 5.5 with acetic acid and acetonitrile (90:10 v/v). Then, it was filtered through a 0.45 µm syringe filter and degassed. Isocratic elution was applied ata flow rate of 1.5 mL min^{-1}, and the injection volume was 50 µL for all samples.

6.2.7. Method Validation

The International Conference on Harmonization rules were followed during the analytical validation (ICH., 2005). Linearity, selectivity, precision, limit of detection (LOD), limit of quantification (LOQ), recovery and matrix effects were taken into consideration for validation.

6.2.7.1. Linearity

Using the linear least squares methodology, a regression line was calculated from theplot of peak area vs analyte concentration of the seven standard solutions (5,10, 20, 40and 80 µg/mL).

6.2.7.2. Selectivity

The selectivity was determined by comparing chromatograms of standard α-amanitin and muscarine. An extract of blank mushroom samples spiked with α-amanitin and muscarine under optimised chromatographic conditions to exclude interferences that could co-elute with α-amanitin and muscarine. A lack of interference was defined as the absence of discernible interfering peaks at the α-amanitin and muscarine retentiontime.

6.2.7.3. Precision

Repeatability (intra-day precision) and intermediate precision (inter-day precision)

were used to determine the method's level of precision. On the same day, the repeatability was evaluated by assessing three levels of different standard solutions: low (5 g mL^{-1}), medium (20 g mL^{-1}), and high (80 g mL^{-1}). The intermediate precision was assessed by analyzing these standard solutions in triplicate on three distinct days. Results were expressed as standard deviation (SD) and relative standard deviation (RSD).

6.2.7.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

According to Equations (1) and (2), LOD and LOQ were calculated based on the calibration curve's slope (S) and the response's least standard deviation (σ). The outcomes of LOD and LOQ were subsequently verified.

$$LOD = \sigma/S \times 3.3 \quad \text{----------} \quad (1)$$

$$LOQ = \sigma/S \times 10 \quad \text{----------} \quad (2)$$

6.2.7.5. Recovery and Matrix Effect

The matrix effect and recovery (n = 6) were studied at three distinct concentrations: 5,20, and 80 µg ml^{-1}. The recovery was calculated by comparing the response of the recovered analyte in the spiked blank matrix to that of standard solutions. The calibration graphs obtained from standard solutions of α-amanitin and muscarine at the same concentration and the calibration graph obtained from spiking Oyster mushroom (*Pleurotus ostreatus*) samples with known amounts of the compounds were compared using the formula matrix effect (%) = (m_{QC}/m_{ST}) x100. m_{QC} is the slope of fortified Oyster mushroom samples, and m_{ST} is the slope of standard solutions of α- amanitin and muscarine.

6.3. Results and Discussion

6.3.1. Optimization of sample extraction

The retrieval of the desired substances from a biological matrix or a sample of mushrooms is crucial when creating an analytical technique as it will impact the overall levels of sensitivity, selectivity, and precision (Nováková *et al.,* 2009).

Various techniques have been documented for the extraction of toxins from mushroom samples. For instance, α-amanitin and phalloidin have been successfully extracted using acidic conditions, such as 0.1% trifluoroacetic acid in methanol (Ahmed et al., 2010), 0.5% acetic acid in methanol (Clarke et al., 2012), and 2.5% formic acid in acetonitrile (Chung et al., 2007), methanol (Rittgen et al., 2008), methanol/water (MeOH/H_2O; 1:1) (Zhang et al., 2005 & Tang et al., 2016), and acidified methanol/water (MeOH/H_2O/(HCl or HCOOH) 0.01 M; 5:4:1) by introducing mineral or organic acids (Enjalbert et al., 1999 & Hallen et al., 2003). Consequently, the choice of extraction solvents described in the literature varies depending on the specific mushroom toxins (Yoshioka et al., 2014).

In the present work, the mushroom samples were grounded and extracted using soxhlet apparatus before injecting into HPLC-PDA. First, the mushrooms sample were homogenized with aqueous methanolic solutions. Next, the obtained solution underwent a streamlined refinement process known as one-step PRIME. This approach encompasses evaluating the solution based on several factors, including its overall effectiveness, robustness, potential enhancements, matrix effects, and user-friendliness. The utilization of Oasis® PRIME HLB has been documented as an effective method for extracting and purifying these toxins from biological samples (Gouveia et al., 2020). The findings showed increased extraction capacity with recoveries between 90% and 100% (n = 6). The optimised pre-treatment approach employing the Oasis® PRIME HLB technology yields excellent recoveries with little matrix interference.

6.3.2. Optimization of the chromatographic conditions

The chromatographic separation was conducted using an isocratic method on a reverse-phase C-18 column (250 mm × 4.5 mm). Various parameters such as the composition and ratio of the mobile phase, flow rate, and column temperature were carefully optimized to achieve a symmetrical peak shape and maximum intensity. Mobile phases consisting of phosphate or citrate buffers have been employed, despite the potential risk of precipitation that can jeopardize the integrity of HPLC valves (Garcia et al., 2015 & Leite et al., 2013). Consequently, to address the

precipitation issue and ensure the stability of HPLC valves, we opted for an acetate buffer (pH 5.5)combined with acetonitrile in a ratio of 90:10 v/v. The mobile phase was delivered at a flow rate of 1.5 mL min^{-1}, and the total analysis time for the experiment was 25 minutes. Extensive optimization was conducted on the HPLC-PDA chromatographic conditions to achieve an efficient separation and accurate identification of α-amanitinsand muscarine in wild mushroom samples.

6.3.3 Molecular Identification

The nucleotide sequence of the mushroom was blasted to sequences from the GenBank database, resulting in the identification of *Amanita virosa*, *Amanita bisporigera*, *Amanita pantherina* and *Amanita pseudoporphyria* from Mizoram. The ITS1-5.8S- ITS4 sequences of the fungal isolate was compared to corresponding sequences of reference fungal taxa in the database and the list of species, voucher number, GenBank accession number and locality used for the analysis were also given (**Table 6.1a, 6.1b,6.1c** and **6.1d**).

Table 6.1a. List of species, voucher, GenBank accession nos. and locality of *Amanitavirosa.*

S/No	Species	Voucher/Strain	NCBI	Locality
1	*Amanita virosa*	157.AV.2.1	MG516218	Netherland
2	*Amanita virosa*	HMJAU23304	KJ466431	China
3	*Amanita virosa*	H:6003959	GU373492	Finland
4	*Amanita virosa*	HKAS90176	MH508650	China
5	*Amanita virosa*	MHHNU 8621	KY472227	China
6	*Amanita virosa*	LIP:0402243	OM451556	Spain
7	*Amanita ocreata*	LG 437	GQ486874	USA
8	*Amanita ocreata*	HKAS79686	KJ466381	China
9	*Amanita ocreata*	NAMA 1998-005	MK580759	USA
10	*Amanita amerivirosa*	RET 636-8	MG966421	USA
11	*Amanita amerivirosa*	RET 492-10	KX868057	USA
12	*Amanita amerivirosa*	RET 735-5	MW817633	Canada
13	*Amanita verna*	PC:HR56.31	OM451534	Spain
14	*Amanita verna*	AH:31842	OM451519	Spain
15	*Amanita volvata*	OMS-010	LC667040	Japan
16	*Aminata sp.*	A.sp1_C_LEB	MT888036	Lebanon

Table 6.1b. List of species, voucher, GenBank accession nos. and locality of *Amanita bisporigera.*

Sl. No	Species	Voucher/Strain	NCBI	Locality
1	*Amanita bisporigera*	RET 909-7	OR026670	USA
2	*Amanita bisporigera*	S.D. Russell iNaturalist # 27477129	MZ668253	USA
3	*Amanita bisporigera*		AY550243	USA
4	*Amanita bisporigera*	S.D. Russell MycoMap	MZ668025	USA
5	*Amanita bisporigera*	JMP0001	EU819411	USA
6	*Amanita bisporigera*	RET 632-7	KX827614	Canada
7	*Amanita bisporigera*	MHHNU 7224	KU311692	Canada
8	*Amanita Sp.*	FLAS-F-68562	OM672853	USA
9	*Amanita pallidorosea*	LE 296427	KJ739814	Russia
10	*Amanita pallidorosea*	RET 406-5	KX270316	India
11	*Amanita pallidorosea*	MHHNU 6838	FJ176734	China
12	*Amanita pallidorosea*	KA12-1144	KF245916	S. Korea
13	*Amanita subjunquillea*	Var.alba	FJ375332	China
15	*Amanita bisporigera*	S.D. Russell ONT iNaturalist 129621602	OP549215	USA
16	*Amanita subjunquillea*	ANT211	MH998628	Pakistan
17	*Amanita subjunquillea*	HMJAU59714	OR468732	China
18	*Amanita subjunquillea*	HMAS 253775	OR058522	China
19	*Amanita cokeri*	712	MK580746	USA

Table 6.1c. List of species, voucher, GenBank accession nos. and locality of *Amanitapantherina*

Sl. No	Species	Voucher/Strain	NCBI	Locality
1	*Amanita pantherina*	LS08	KR456156	China
2	*Amanita pantherina*	OMS-009	LC667039	Japan
3	*Amanita pantherina*	TNS-F-4491(TNS); LEM980134	AB096044	Nepal
4	*Amanita pantherina*	>72-24	OQ430774	China
5	*Amanita pantherina*	110117MFBPL0169	MW554376	China
6	*Amanita pantherina*	88	JN182878	China
7	*Amanita pantherina*	13024	MG367248	China

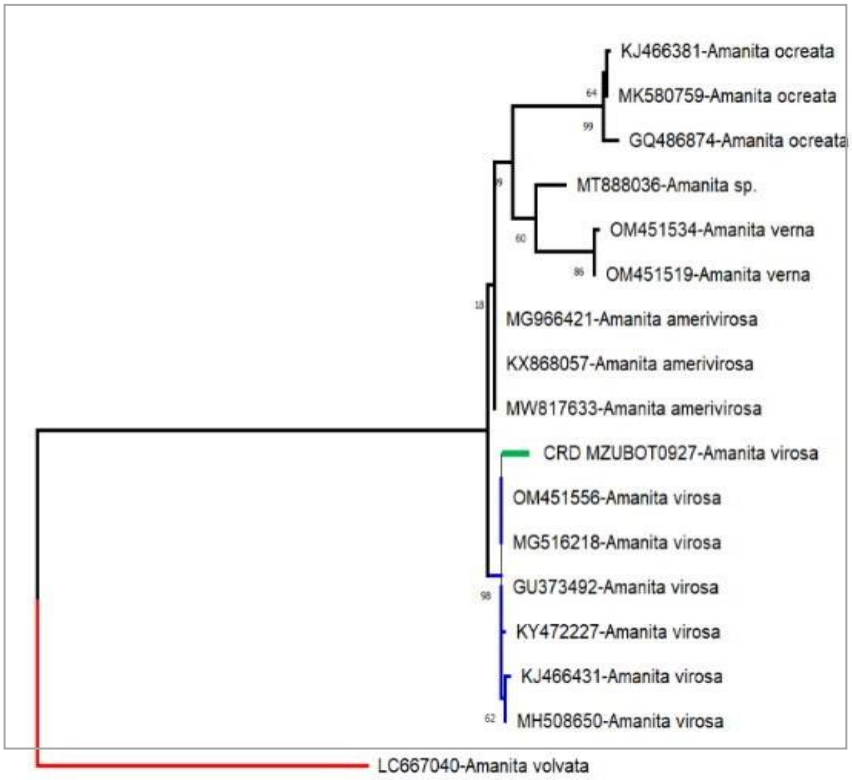
8	*Amanita pantherina*	45929(NY)	AB080784	Japan
9	*Amanita gemmata*	UBC:F19752	HQ604823	Canada
10	*Amanita gemmata*	UBC:F19764	HQ604824	Canada
11	*Amanita ibotengutake*	KA13-1204	KR673677	S.Korea
12	*Amanita ibotengutake*	KA12-1339	KF017937	S.Korea
13	*Amanita alpinicola*	Mushroom Observer #413217	ON950098	USA
14	*Amanita alpinicola*	CA FUNDIS iNaturalist # 174797375	OR783526	USA
15	*Amanita alpinicola*	CA FUNDIS iNaturalist # 174796747	OR783522	USA
16	*Amanita frostiana*	ACAD21078F mushroomobserver.org/475595	OL741520	Canada
17	*Amanita frostiana*	RET 547-2	KP313581	Canada
18	*Amanita ibotengutake*	KA13-1204	KR673677	S.Korea
19	*Amanita ibotengutake*	KA12-1339	KF017937	S.Korea

Table 6.1d. List of species, voucher, GenBank accession nos. and locality of *Amanita pseudoporphyria*

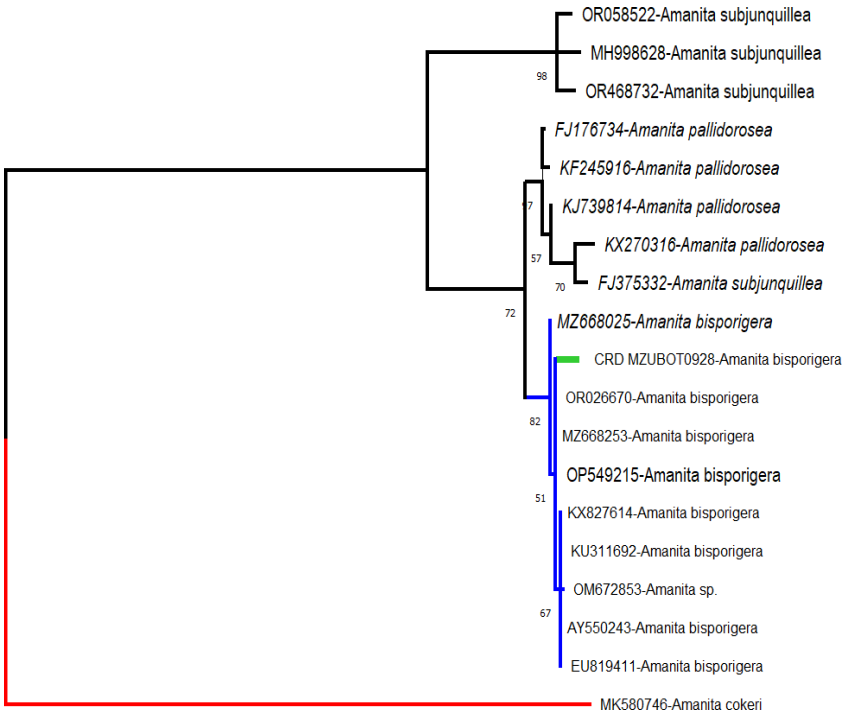
Sl. No	Species	Voucher/Strain	NCBI	Locality
1	*Amanita pseudoporphyria*	rpr-344	MW374150	China
2	*Amanita pseudoporphyria*	WM41 (HGASMF01-13548)	MZ092714	China
3	*Amanita pseudoporphyria*	BZ_N10	KY747456	Thailand
4	*Amanita pseudoporphyria*	HKAS56984	KC429050	China
5	*Amanita pseudoporphyria*	ut-m0001397	MW979501	USA
6	*Amanita neo-ovoidea*	RET 359-1	KX270315	China
7	*Amanita neo-ovoidea*	A10	FJ441040	China
8	*Amanita neo-ovoidea*	MHHNU 30952	KU497539	China
9	*Amanita neo-ovoidea*	AH20171017-01	MK279377	China
10	*Amanita proxima*	S14	KY606974	Italy
11	*Amanita proxima*	Ret290-10-col8	MH727195	Italy
12	*Amanita proxima*	S15	KY606975	Italy
13	*Amanita proxima*	Ret290-10-col9	MH727194	Italy
14	*Amanita heishidingensis*	HKAS81484	KJ922999	China

15	*Amanita heishidingensis*	HKAS82282	KJ922997	China
16	*Amanita atkinsoniana*	D. Russell MycoMap # 6689	MN906195	USA
17	*Amanita atkinsoniana*	S.D. Russell MycoMap # 6689	MZ668014	USA
18	*Amanita atkinsoniana*	Taxon 285	MW899480	USA
19	*Amanita albocreata*	OMDL K. Canan iNaturalist 176490186	OR824669	USA

The phylogenetic tree produced depicted *Amanita virosa*, *Amanita bisporigera*, *Amanita pantherina* and *Amanita pseudoporphyria* grouping closely with its related species, supported by a high confidence value. *A. volvata*, *A. cokeri* and *A. albocreata* served as the outgroup and was highlighted in red. Notably, the fungal isolate obtainedfrom Mizoram, identified as *Amanita virosa* (**Figure 6.5a**), *Amanita bisporigera* (**Figure 6.5b**), was highlighted in green within the *A. virosa* and *A. bisporigera* clade, which was marked in blue. Similarly, *Amanita pantherina* (**Figure 6.5c**) and *Amanita pseudoporphyria* (**Figure 6.5d**) was highlighted in orange within the *A. virosa* and *A.bisporigera* clade, which was marked in blue.



(a)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97
KJ739814-Amanita pallidorosea
KX270316-Amanita pallidorosea
57
70
FJ375332-Amanita subjunquillea
72
MZ668025-Amanita bisporigera
CRD MZUBOT0928-Amanita bisporigera
OR026670-Amanita bisporigera
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MZ668253-Amanita bisporigera
OP549215-Amanita bisporigera
51
KX827614-Amanita bisporigera
KU311692-Amanita bisporigera
OM672853-Amanita sp.
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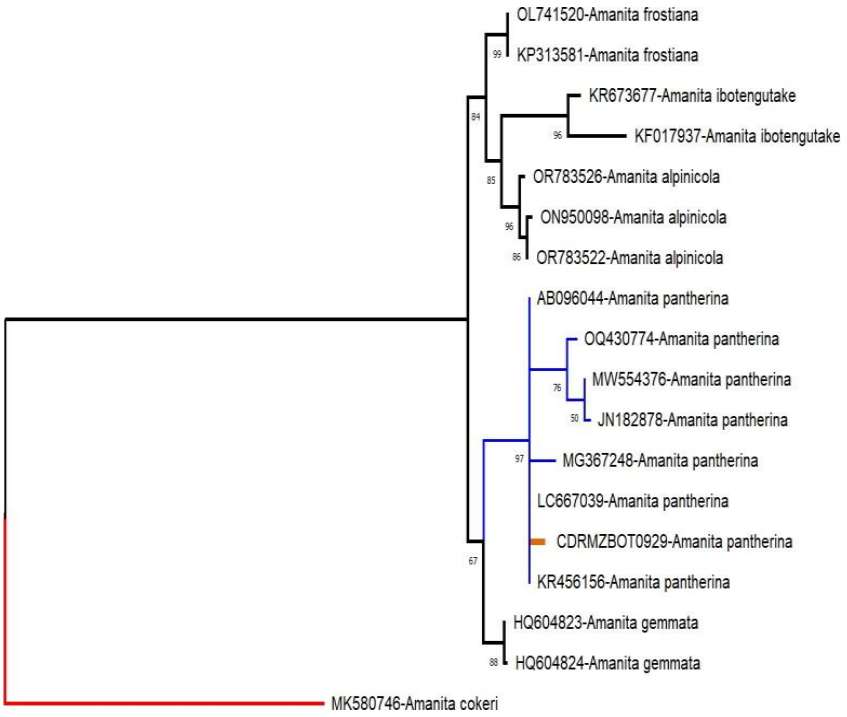
EU819411-Amanita bisporigera
MK580746-Amanita cokeri

(b)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KF017937-Amanita ibotengutake
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OR783526-Amanita alpinicola
96
ON950098-Amanita alpinicola
86
OR783522-Amanita alpinicola
AB096044-Amanita pantherina
OQ430774-Amanita pantherina
76
MW554376-Amanita pantherina
50
JN182878-Amanita pantherina
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MG367248-Amanita pantherina
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CDRMZBOT0929-Amanita pantherina
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KR456156-Amanita pantherina
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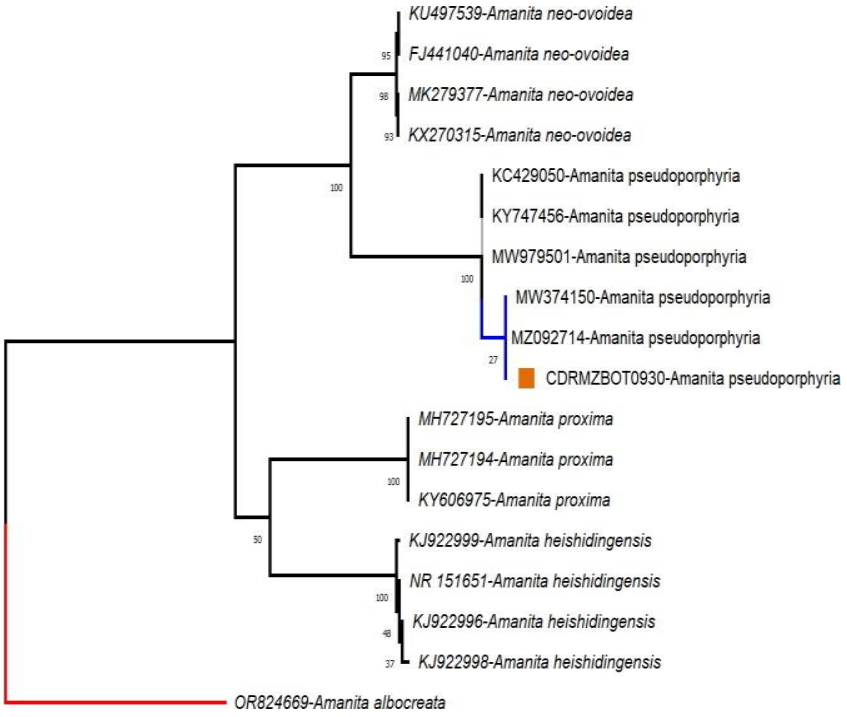
88
HQ604824-Amanita gemmata
MK580746-Amanita cokeri

(c)



(d)

Figure 6.5. Phylogenetic Tree of **(a)** *Amanita virosa* **(b)** *Amanita bisporigera* **(c)** *Amanita pantherina* (d) *Amanita pseudoporphyria.*

6.3.4. Validation of the Method

After the optimization process, the α-amanitin compound demonstrated a retention time of approximately 14 minutes, while muscarine exhibited a retention time of around 9 minutes. There were no interfering peaks at the α-amanitin and muscarine retention times in blank Oyster mushroom samples injected with α -amanitin and muscarine.

The calibration curves, constructed using standard solutions containing six different concentrations of α-amanitin and muscarine, demonstrated linearity within the range of 5 to 80 µg mL^{-1} (**Figure 6.6a & 6.6b**). The correlation coefficients obtained were deemed appropriate and are listed in **Table 6.2**.

Table 6.2. Calibration curve parameters of α-Amanitin and Muscarine; regression equation and coefficient (R^2) are expressed as the mean of 3 calibration curves.

Compound	Range (µg mL^{-1})	Linearity (R^2)	Linear Regression Equation
α – Amanitin ($C_{39}H_{54}N_{10}O_{14}S$)	5.0 – 80.0	0.9947	y = 12199x + 48509
Muscarine ($C_9H_{20}NO_2^+$)	5.0 – 80.0	0.9952	y = 12940x - 14343

The equation of the calibration curve follows the general form of y = mx + b, where y represents the peak area, m denotes the slope of the curve, and b signifies the intercept.

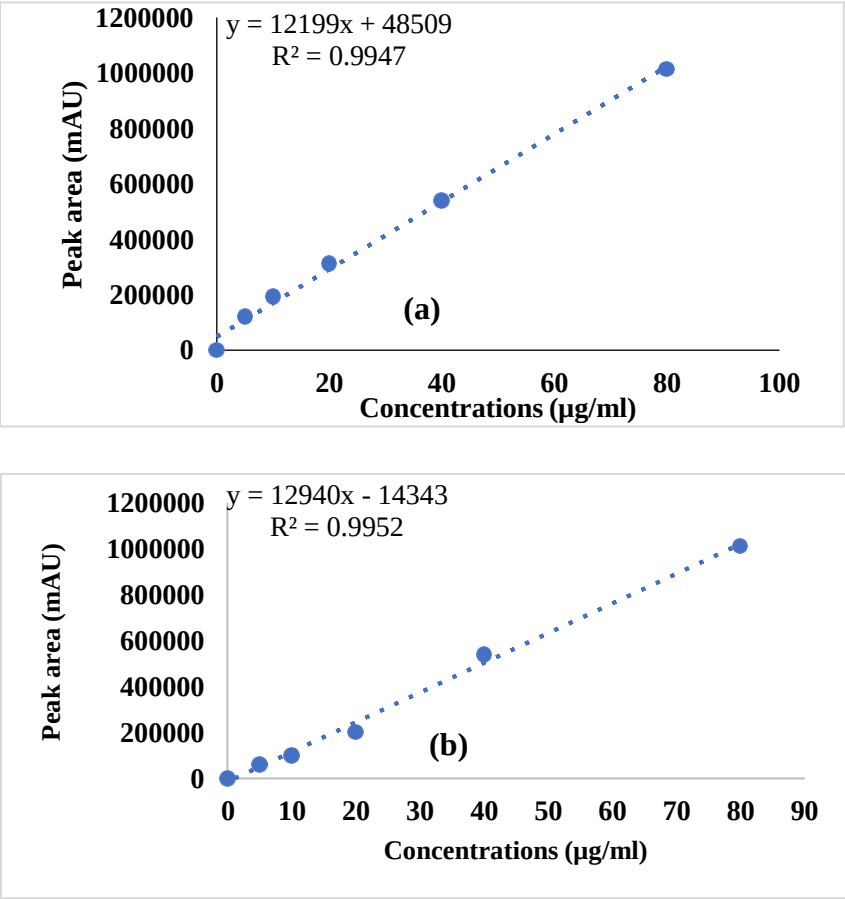


Figure 6.6. Standard graph of **(a)** α–Amanitin and **(b)** Muscarine

The estimated LOD and LOQ values of HPLC-PDA detection for α-amanitin and muscarine are shown in **Table 6.3**. For PDA detection, the LOD and LOQ for muscarine are lower than those for α-amanitin. It should be noted that the LOD and LOQ obtained in this study were derived using the calibration curve, which resulted inmore cautious estimates.

Table 6.3. Limit of Detection (LOD) and Limit of Quantification (LOQ) for mushroom extracts.

Matrix	**Mushroom toxin**	**LOD (ng/g)**	**LOQ (ng/g)**	**Method**
Mushroom tissue	α-Amanitin	88	210	HPLC-PDA
	Muscarine	65	185	HPLC-PDA

The total recovery and matrix effect of the HPLC-PDA assay of α-amanitin and muscarine for Oyster mushroom extracts, expressed as a percentage of recovery and relative standard deviation (%RSD). Recovery of α-amanitin and muscarine varied from 98.6% to 127.6%, with an RSD of < 6%, as demonstrated. The matrix effect wasassessed by comparing the slopes of calibration curves obtained after processing spiked Oyster mushroom samples with standard solutions of α-amanitin and muscarine at the same concentration. The results ranged from 93.5% to 95.6% for low, medium, and high concentrations of α-amanitin and muscarine, respectively (**Table 6.4**), with a6% RSD for -amanitin and a 9% RSD for muscarine.

Table 6.4. Recovery and matrix effect values for α-amanitin and muscarine analyzedby HPLC-PDA.

Compound	**Detection**	**Concentration (µg/ml)**	**Recovery (%) (RSD)**	**Matrix Effect (%) (RSD)**
α-Amanitin	PDA	5	104.5 (3.7)	96.5 (7.9)

		20	112.9 (4.5)	
		80	127.6 (5.4)	
Muscarine	PDA	5	98.6 (1.6)	98.3 (8.5)
		20	100.3 (2.8)	
		80	107.8 (3.9)	

The intra-day and inter-day precision for α-amanitin and muscarine in mushroom matrices ranged from 0.15% to 10.12% (**Table 6.5**), which was well within the desired limit (RSD 15%). According to the results compiled in **Tables 6.4 and 6.5**, this method demonstrated good precision and accuracy for measurements of α-amanitin and muscarine obtainedfrom samples of mushrooms.

Table 6.5. Intra- and inter-day precision for values for α-amanitin and muscarine analyzed by HPLC-PDA.

Compound	Detection	Concentration (µg/ml)	Precision (%RSD)	
			Intra-day	Inter-day
α-Amanitin	PDA	5	1.68	8.91
		20	0.89	6.63
		80	0.15	4.65
Muscarine	PDA	5	3.89	10.12
		20	1.54	7.65
		80	0.64	5.78

6.3.5. Method Application

Upon successful development and validation of the HPLC-PDA method, it was subsequently utilized to quantify the levels of α-amanitin (**Figure 6.7**) and muscarine (**Figure 6.8**) in ten different wild mushroom samples collected from study forests in Champhai. The results of the analysis revealed the presence of α-

amanitin only in the samples obtained from *Amanita virosa* (1.17 mg g^{-1}) and *Amanita bisporigera* (1.91 mg g^{-1}) mushroom species. On the other hand, Muscarine were present in mushroom samples of *Amanita pantherina* (0.007 mg g^{-1}) and *Amanita muscaria* (0.045 mg g^{-1}) as shown in **Table 6.6.**

Table 6.6. Peak area, retention time and final concentration of the α-amanitin and muscarine in mushroom samples.

S.No.	**α-Amanitin @ 302 nm**	**Peak area**	**Retention Time (min)**	**Concentration (µg/ml)**	**Concentration (mg/g)**
1	*Amanita pantherina*	ND	ND	ND	ND
2	*Amanita pseudoporphyria*	ND	ND	ND	ND
3	*Amanita polypyramis*	723 00	14.859	1.95	1.17
4	*Amanita bisporigera*	87340	14.720	3.18	1.91
5	*Macrolapiota doulichaula*	ND	ND	ND	ND
6	*Rusulla virescens*	ND	ND	ND	ND
7	*Turbinellus floccosus*	ND	ND	ND	ND
8	*Hydnum rufescens*	ND	ND	ND	ND
9	*Boletus hortonii*	ND	ND	ND	ND
10	*Cantharellus tropicalis*	ND	ND	ND	ND
S.No.	**Muscarine @ 302 nm**	**Peak area**	**Retention Time (min)**	**Concentration (µg/ml)**	**Concentration (mg /g)**
1	*Amanita pantherina*	16007	9.59	0.13	0.007
2	*Amanita pseudoporphyria*	15314	9.366	0.08	0.045
3	*Amanita polypyramis*	ND	ND	ND	ND
4	*Amanita bisporigera*	ND	ND	ND	ND
5	*Macrolapiota doulichaula*	ND	ND	ND	ND

6	*Rusulla virescens*	ND	ND	ND	ND
7	*Turbinellus floccosus*	ND	ND	ND	ND
8	*Hydnum rufescens*	ND	ND	ND	ND
9	*Boletus hortonii*	ND	ND	ND	ND
10	*Cantharellus tropicalis*	ND	ND	ND	ND

ND: Not Detected

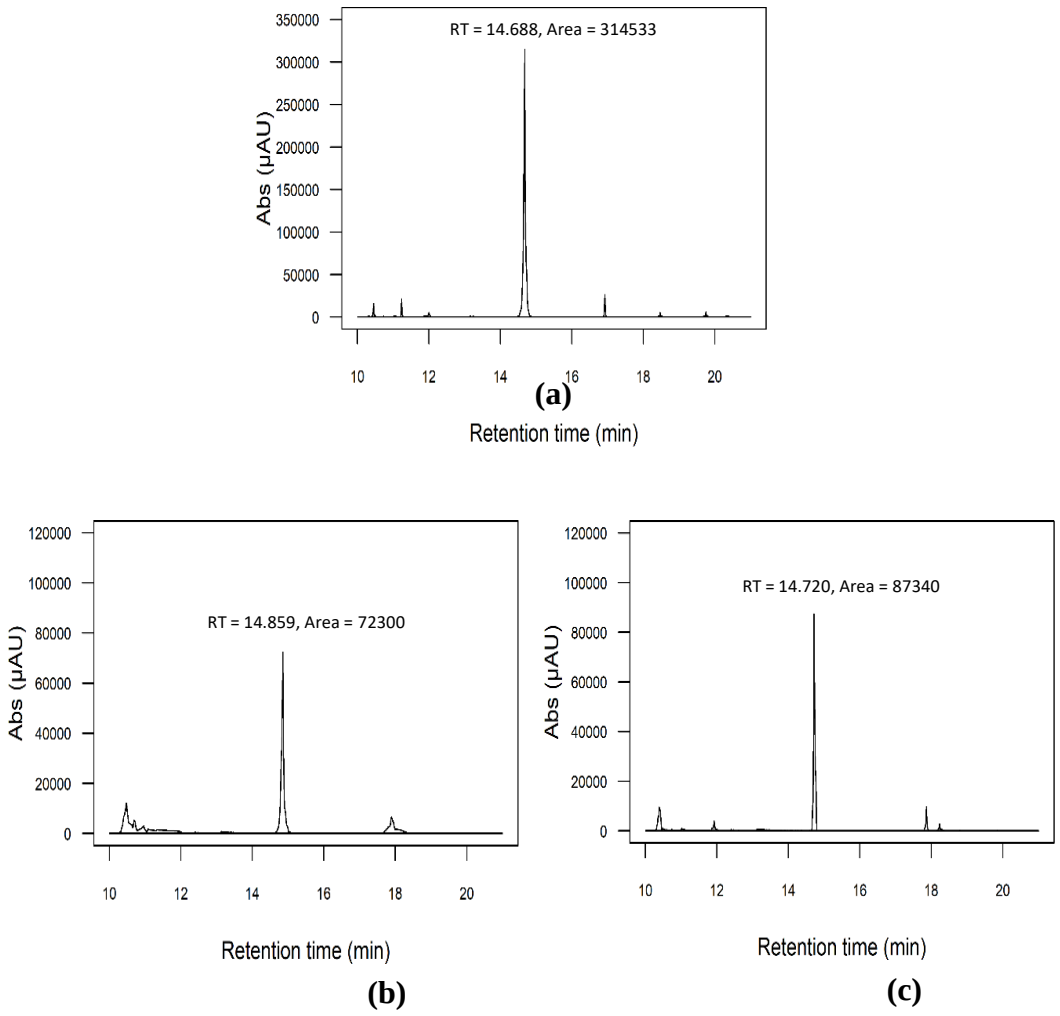


Figure 6.7. HPLC-PDA chromatogram of: **(a)** standard α-Amanitin **(b)** *Amanita virosa* and **(c)** *Amanita bisporigera* observed at 302 nm, with the separation of peaks of α-Amanitin.

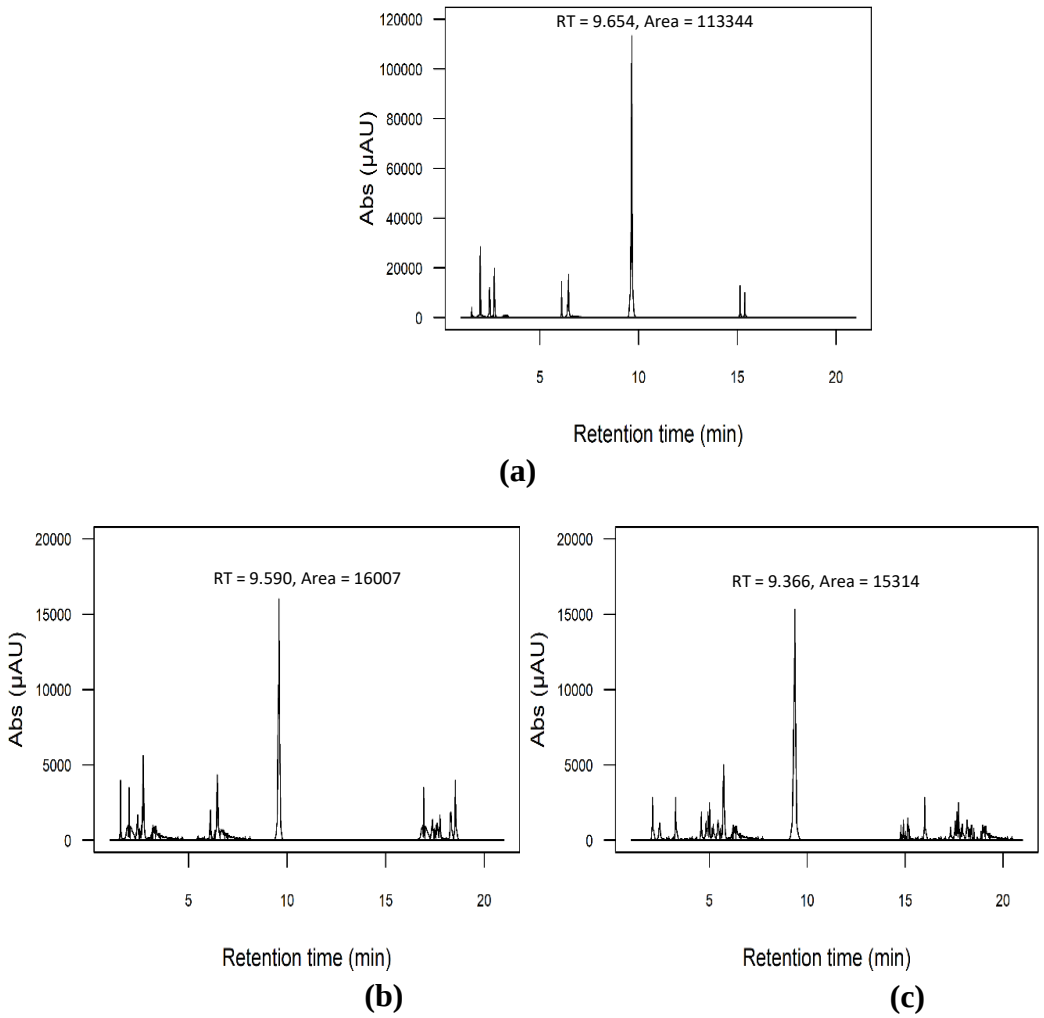


Figure 6.8. HPLC-PDA chromatogram of: **(a)** standard muscarine **(b)** *Amanita pantherina*, **(c)** *Amanita pseudoporphyria* observed at 302 nm, with theseparation of peaks of Muscarine.

6.4. Conclusion

In this study, we successfully developed and validated an HPLC-PDA method for theidentification and quantification of α-amanitin and muscarine in wild mushroom samples collected from different forests in Champhai district, Mizoram, India. The presence of both α-amanitin and muscarine was detected in the samples obtained from*Amanita polypyramis* and *Amanita bisporigera* mushroom species, highlighting the potential risk of consuming these mushrooms without proper identification. On the other hand, *Amanita pantherina* and *Amanita pseudoporphyria* samples only

showed the presence of trace amount of muscarine. The findings of this study emphasize the importance of identifying and quantifying toxic compounds in wild mushroom samples to prevent potential poisoning incidents. The developed HPLC-PDA method provides a valuable tool for assessing the safety of mushroom consumption and can contribute to public health protection a. Further research and monitoring are necessaryto expand the scope of analysis to include a wider range of mushroom species and geographical locations. Additionally, the identification of additional toxins and the development of new methodologies for their detection and quantification would contribute to a more comprehensive understanding of mushroom toxicity, research oneffective antidote and enhance public safety.

Chapter VII

7. In silico molecular docking of potent antidotes for treatment ofAmatoxins.

7.1 Introduction

The deadly Amanita mushroom species *Amanita phalloides* (Death Cap), *Amanita verna* (White Deadly Amanita), *Amanita virosa*, and *Amanita bisporigera* (Destroying Angel) are responsible for 90-95% of mushroom-related fatalities (Enjalbert et al., 2002). The extreme fatality of amanita poisoning is due to the presence of potent toxins such as cyclic octapeptides. Amatoxins are cyclic octapeptides, with α-amanitin causing severe liver and kidney injury (Garcia et al, 2015). Toxins contained in these species consist of nine defined members: α-amanitin, β-amanitin, γ-amanitin, ε- amanitin, amanin, amanin amide, amanullin, amanullinic acid, and proamanullin(Vetter, 1998). From these, α-amanitin accounts for about 40% of the amatoxins. Specific properties characterize these toxins. They are thermostable and are not removed by boiling and discarding water or by any form of cooking or drying, and belong to the most violent poisons from higher fungi: only one medium-size amatoxin- containing specimen contains from 10 to 12 mg amatoxins, a potential lethal dose for human adults (Lethal Dose: LD_{50} of 0.1-0.5 mg/kg body weight) (Wieland, 1984).

After ingestion poisonous Amanita mushrooms, amatoxins are readily absorbed and accumulate in the liver upon uptake via an organic anion-transporting octapeptide (OATP1B transporter protein located in the sinusoidal membrane of hepatocytes (Letschert et al., 2006), which is the same transport system for biliary acids. The mainmolecular mechanism of toxicity is their strong inhibition of RNAP II (Lindell et al., 1970) which is responsible for the transcription of the precursor of messenger RNA. This in turn causes inhibition of DNA and protein synthesis processes and leads to cell death (Wieland, 1983). Since liver toxicity depends on amanitin hepatocyte uptake, pharmacological blockade of the OATP1B transporters has been investigated as a treatment strategy for mushroom poisoning (Letschert et al.,

2006). However, other toxic mechanisms have been suggested, namely oxidative stress, which may play a critical role (Leist et al., 1997; Zheleva, 2013; Zheleva et al., 2007). In addition, α- amanitin may also act synergically with endogenous cytokines (e.g., tumor necrosis factor-α) to promote apoptosis (Leist et al.,1997).

RNAP II consists of a 10-subunit core enzyme and a peripheral heterodimer of subunits Rpb4 and Rpb7 (Rpb4/7 subcomplex). The core enzyme comprises subunits Rpb1, Rpb2, Rpb3, Rpb5, Rpb6, Rpb8, Rpb9, Rpb10, Rpb11 and Rpb12 (Cramer et al., 2008). The active site is located in the interface between the core subunits Rpb1 and Rpb2. Recently a crystal structure of α-amanitin with yeast RNAP II was published revealing several key molecular interactions that may contribute to inhibition of RNAP II (**Figure 7.1**) (Bushnell et al., 2002). Multiple relevant interactions between α-amanitin with RNAP II are located in the bridge helix and in adjacent part of Rpb1. Based on this α-amanitin may block translocation by interactingwith bridge helix and preventing the conformational changes of the TL and consequently transcriptional elongation (Gong et al., 2004).

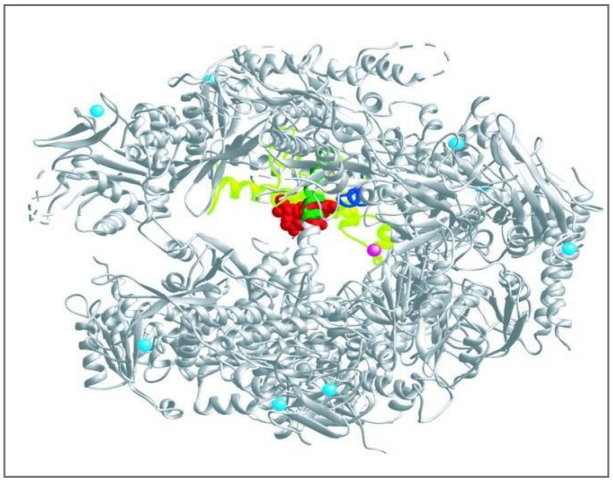


Figure 7.1. Ribbons representation of the RNAP II structure binding with α-amanitin. The active site magnesium is magenta, the region of Rpb1 around α-amanitin is light green and dark green (bridge helix), the region of Rpb2 near α-amanitin is dark blue, and α-amanitin is red (Source: Bushnell et al., 2002).

Unfortunately, so far, no consensual antidote for mushroom poisonings has been

found, and therefore, amatoxin poisoning is generally associated with a poor outcome, mainly due to liver or kidney failure. Several treatments have been used after human intoxications with *A. phalloides*, including hormones (e.g., insulin, growth hormone, and glucagon), steroids, vitamin C, vitamin E, cimetidine, α-lipoic acid, antibiotics (benzylpenicillin, ceftazidime), N-acetylcysteine, and silybin. Of the previous, only benzylpenicillin, ceftazidime, N-acetylcysteine, and silybin proved to have some degree of therapeutic efficacy, though the death rate remains extremely high (Poucheret et al., 2010). The survival of individuals depends largely on the severity ofliver damage, the rate of hepatic regeneration, and the management of complications that may develop during the intoxication treatment course (Koda-Kimble et al. ,2012). Liver transplantation is considered a last resort; however, it remains the cornerstone of treatment in patients with fulminant hepatic failure (Broussard et al., 2001; Pinson et al., 1990).

Considering the main toxicity mechanism of amatoxins (i.e., the inhibition of OATP1B and RNAP II activity), the ideal therapeutic approach against mushrooms intoxications would be to displace and/or compete with the amatoxins binding to OATP1B and RNAP II without impairing its normal transcription activity. Therefore,in the present study, we aimed to validate an effective antidote for *Amanita* mushroom poisonings, the mode of interaction of α-amanitin and five antidotes (Rifampin, Prednisolone, Cyclosporine, Polymycin B and Silibinin) with OATP1B and RNAP II,using in silico molecular docking method.

7.2 Methodology

Molecular docking plays an important role in the rational design drugs and is helpful in elucidating key features of ligand-receptor interactions. This in silico method allows predicting the preferred orientation of putative antidotes when bound to OATP1B and RNAP II, forming a complex with overall minimum energy. Molecular docking studies were performed on Rifampin, Prednisolone, Cyclosporine, Polymycin B and Silibinin according to previous methods (Garcia et al., 2014) as follows.

7.2.1 Obtaining crystal structure of Proteins and ligands for docking

The crystal structure of OATP1B and RNAP II complexed with α-amanitin (Protein Data Bank entry 8PGO and 3CQZ) was used to obtain the starting structures for the molecular docking (Bushnell et al., 2002), and only subunits 1B3 (**Figure 7.2a**) and Rpb1 (**Figure 7.2b**) were used. The optimized 1B3 subunits were docked with Rifampin, Prednisolone, Cyclosporine, Silibinin and Rpb2 with polymyxin B obtained from PubChem data bank and their detailed are shown in **Table 7.2 and Figure 7.9**.

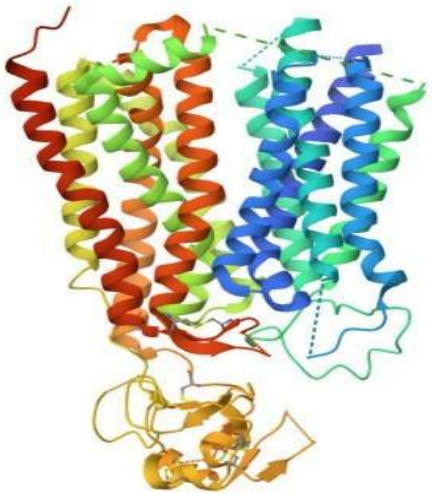


Figure 7.2a. Crystal structure of OATP represented by sub-unit 1B3 (Photo Courtesy: ProteinDataBank)

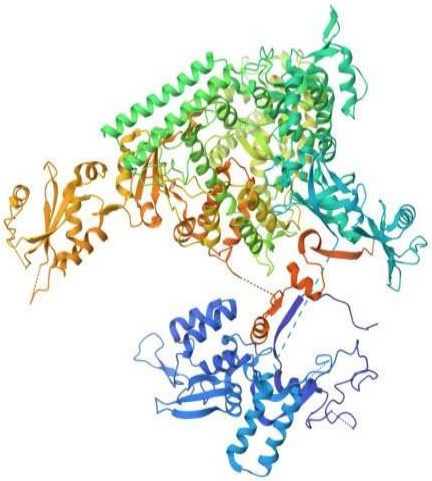


Figure 7.2b. Crystal structure of RNAPII represented by sub-unit Rpb1 (Photo Courtesy: ProteinDataBank, www.pdb.org))

7.2.2 Preparation of macromolecule/proteins and ligands for docking

In the realm of molecular docking studies, the meticulous preparation of protein and ligand structures is a pivotal process. AutoDock Tools, a user-friendly graphical interface for the AutoDock suite, facilitates this preparation through a comprehensive step-by-step procedure (Haddad et al., 2020).Commencing with the installation of AutoDock Tools, researchers gain access to a versatile toolset. Once installed, the focus shifts to the protein structure. The three- dimensional protein structure, acquired in PDB format, is meticulously examined for completeness. Attention is given to the presence of missing atoms or residues. The subsequent addition of hydrogens to the structure is complemented by the assignment of charges

using the Gasteiger method. Atom types are then meticulously assigned to ensure accuracy. Completeness checks are conducted, hydrogens are added, and charges are assigned. Molecular conversion tool (e.g., OpenBabel) were used to convert the PDB files to the required PDBQT format with hydrogen atoms and chargesincluded.

7.2.3 Preparations of Ligands for Docking

With the protein prepared, the 2D structures (.mol) of all compounds were obtained from Pubchem database (**Figure 7.3**). All the compounds (**a**–**e**) are converted to 3D structure (.pdb) using Chem3D 16.0. The 3D coordinates (.pdb) of each molecule wereloaded on to Chem3D for energy minimization.

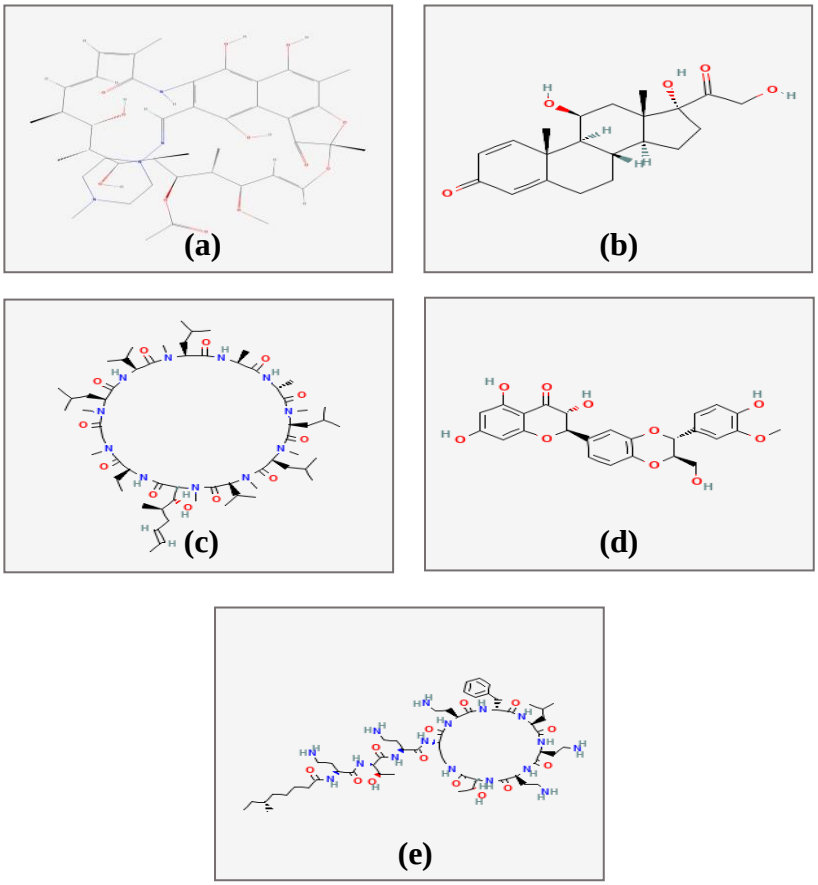


Figure 7.3. Structure of proposed antidode used as ligand for analysis of moleculardocking; (a) Rifampin (b) Prednisolone (c) Cyclosporine (d) Cyclosporine and (e) Polymycin B (Photo courtesy: www.pubchem.ncbi.nlm.nih.gov)

7.2.4. Molecular docking Analysis (AutoDock vina 4)

The docking procedure was made with the AutoDock 4 program (Morris et al. 2009; Mowry et al. 2013). This automated docking program uses a grid-based method for energy calculation of the flexible ligand in complex with a rigid protein. The program performs several runs in each docking experiment. Each run provides one predicted binding mode. The Lamarckian genetic algorithm (LGA) was used in all docking calculations.

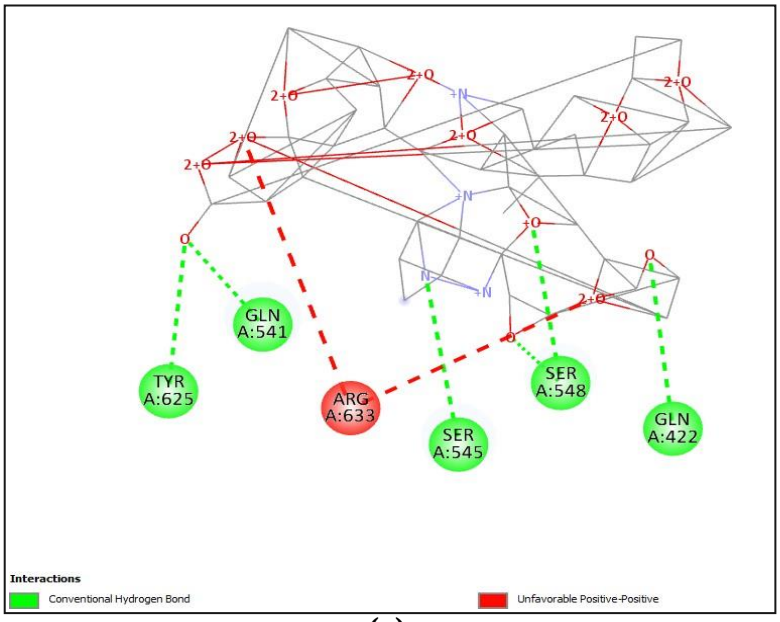
The grid point along the x, y, and z axes was centred on the proposed proteins (receptors), which was large enough to cover all possible rotations of the ligands. The distance between two connecting grid points was 0.375 Å. The docking process was performed in 500 LGA runs. The population was 150, the maximum number of generations was 27,000 and the maximum number of energy evaluations was 2.5 ×106. After complete execution of AutoDock, conformations of ligands in complex with the receptor were obtained, which were finally ranked on the basis of binding energy. The ligands with lowest binding energy were chosen and the model structures were viewed using BIOVIA Discovery Studio client 2021 visualizer (Trott et al., 2010).

7.3 Results and Discussions

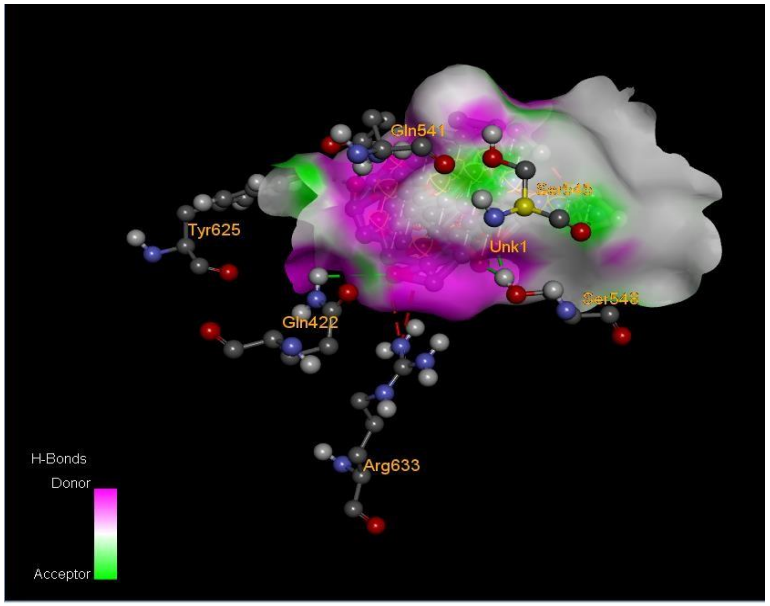
7.3.1 Binding mode predictions of Rifampin to OATP1B3

Rifampicin, also referred to as rifampin, is an ansamycin antibiotic employed for the treatment of various bacterial infections, including tuberculosis (TB), *Mycobacterium avium* complex, leprosy, and Legionnaires' disease (Sensi 1983). Typically, it is administered in combination with other antibiotics, except for specific cases: when recommended as a primary treatment for latent TB infection and as post-exposure prophylaxis to prevent *Haemophilus influenzae* type b and meningococcal disease in individuals exposed to these bacteria (Sterling et al., 2020). Prior to initiating prolonged treatment, it is advisable to assess liver enzymes and blood counts in the individual. Rifampicin can be administered orally or intravenously. **Figures 7.4a** and **Figure 7.4b** illustrate the relative orientation of rifampin and crucial residues within the OATP1B3/rifampin binding complex,

utilizing the structure with the lowest root-mean-square deviation (RMSD). Rifampin engages with residues Tyr625, Gln422, Gln541, Ser545, and Ser548 through conventional hydrogen bonding, while dipole-dipole interaction occurs between rifampin and Arg633. The binding free energy reached its lowest point at -12.79 (Kcal/mol) after 9 runs, with an inhibitory constant of 453.23 pM and a reference RMSD of 203, as indicated in **Table7.1**.



(a)



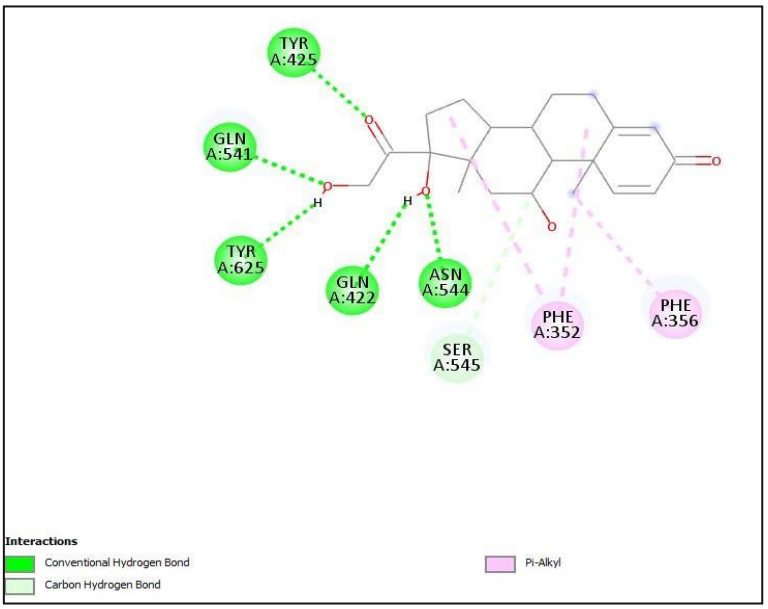
(b)

Figure 7.4. 2D and 3D interactions structure of molecular docking of rifampinand OATP1B3

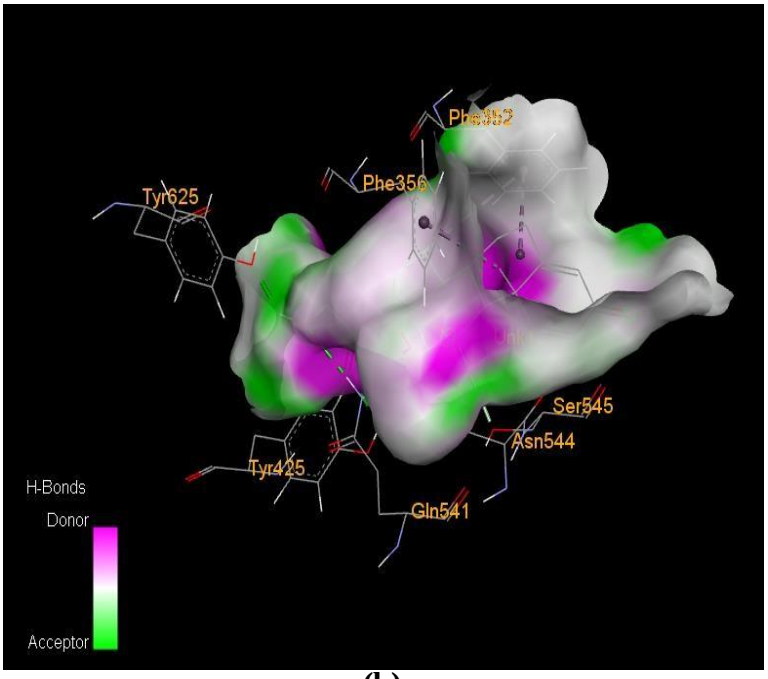
7.3.2 Binding mode predictions of Prednisolone to OATP1B3

Prednisolone, classified as a corticosteroid and a steroid hormone, is utilized in the treatment of specific allergies, inflammatory conditions, autoimmune disorders, and cancers (Stuart et al., 2009). Conditions such as adrenocortical insufficiency, elevated blood calcium levels, rheumatoid arthritis, dermatitis, eye inflammation, asthma, and multiple sclerosis are among those addressed by prednisolone. The administration of this medication can be oral, intravenous, topical as a skin cream, or through eye drops.

The OATP1B3 binding analysis revealed the lowest observed binding free energy values for prednisolone at -8.46 after 108 runs. The inhibitory constant was determinedto be 629.49nM, with a reference RMSD of 203.94, as detailed in **Table 7.1**. Prednisolone demonstrated interactions with active sites on OATP1B3, specifically Tyr425, Gln541, Asn544, and Tyr625 through conventional hydrogen bonding. Additionally, interactions were observed with Phe352 and Phe356 involving pi-alkyl groups, and with Ser545 through a carbon hydrogen bond, as depicted in **Figure 7.5a**and **Figure 7.5b**.



(a)



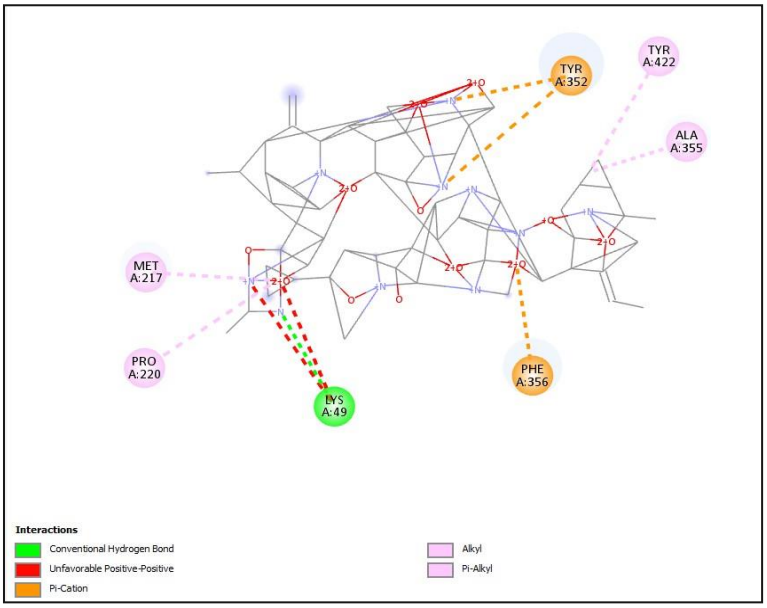
(b)

Figure 7.5. 2D and 3D interactions of molecular docking of prednisolone and OATP1B3

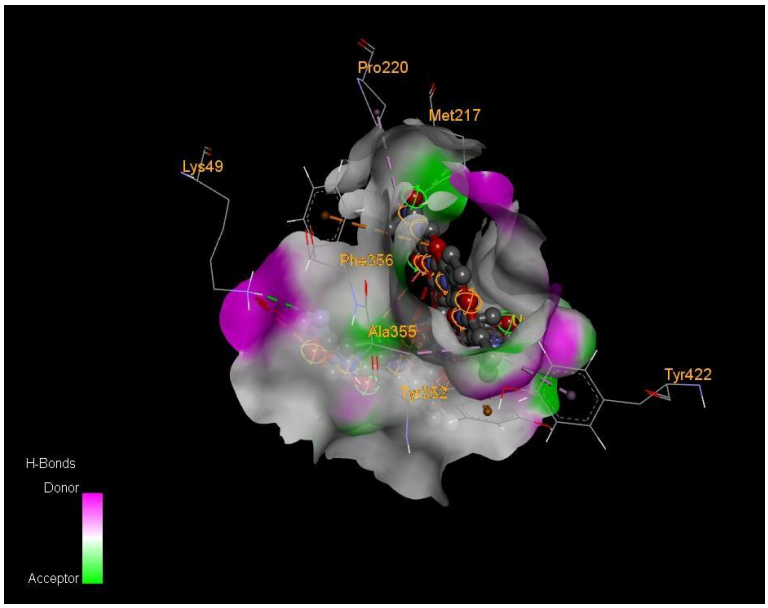
7.3.3 Binding mode predictions of Cyclosporine to OATP1B3

Cyclosporine, also known as cyclosporin, serves as a calcineurin inhibitor, functioning as an immunosuppressant medication. Its primary applications include the treatment and prevention of graft-versus-host disease in bone marrow transplantation, as well as the prevention of rejection in kidney, heart, and liver transplants. In the United States, it is also approved for addressing rheumatoid arthritis and psoriasis, persistent nummular keratitis following adenoviral keratoconjunctivitis (Reinhard, 2000), and as eye drops for managing dry eyes resulting from Sjögren's syndrome and meibomian gland dysfunction.

The OATP1B3/cyclosporine complex analysis reveals specific residues contributing to the binding energy, illustrated in Figure 7.6a and Figure 7.6b. Lys49 forms a conventional hydrogen bond with cyclosporine, while interactions involving alkyl groups include Tyr422, Ala355, Met217, and Pro220. Additionally, a pi-carbon bondis observed with Tyr352 and Phe356. The lowest binding energy, recorded at -8.03 kcal/mol, occurred after 125 runs, with an inhibitory constant of 1.29 uM and areference RMSD of 207.14, as outlined in **Table 7.1**.



(a)



(b)

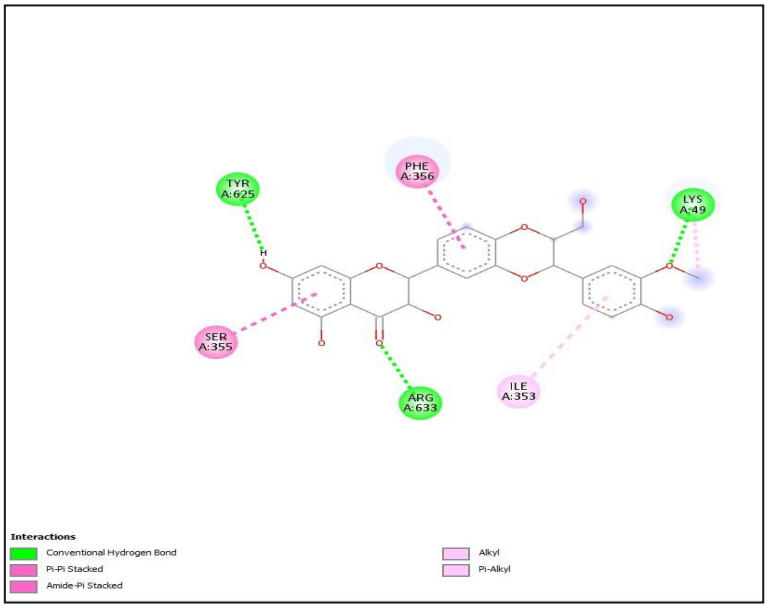
Figure 7.6. 2-D and 3-D interactions of molecular docking of Cyclosporine and OATP1B3

7.3.4 Binding mode predictions of Silibinin to OATP1B3

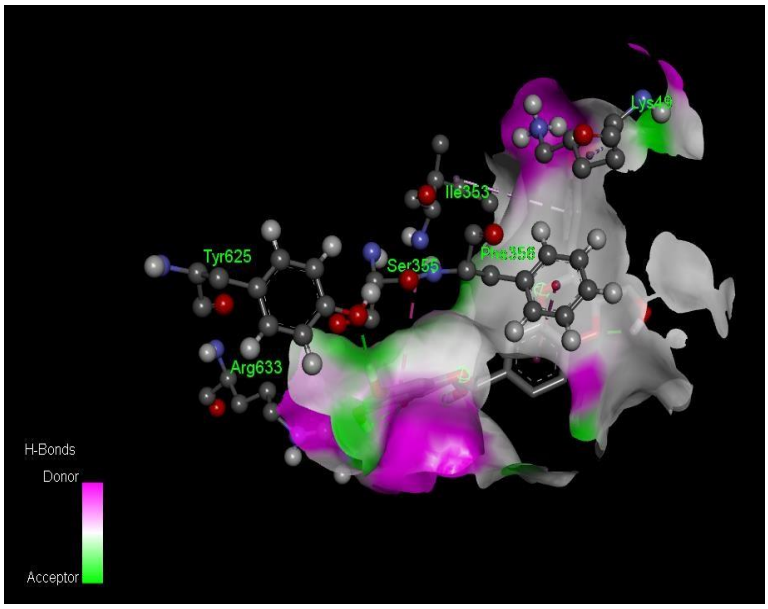
Silibinin, also referred to as silybin, is the primary active compound derived from Silybum, the generic name of the plant commonly known as milk thistle. It constitutes a major component of silymarin, that includes a mixture of flavonolignans such as silibinin, isosilibinin, silychristin, silidianin, and others. Silibinin is utilized both in its pure form as a medication and more commonly as a

key ingredient in herbal supplements derived from milk thistle. In specific cases, the water-soluble form of silibinin, known as silibinin-C-2',3-dihydrogensuccinate disodium salt, is employed for approved drug preparations and parenteral applications. This formulation is particularly used in the treatment of Amanita mushroom poisoning.

Silibinin, with a docking score of -6.92 kcal/mol achieved in 61 runs, exhibits an inhibitory constant of 8.46 µM and a reference RMSD of 208.33 (**Table 7.1**). The interaction pattern involves the formation of conventional hydrogen bonds with Lys49, Arg633, and Tyr625. Additionally, silibinin engages in pi-stacking interactions with Phe356 and Ser355. Furthermore, it establishes interactions with the alkyl group Ile353, as depicted in **Figure 7.7a** and **Figure 7.7b**.



(a)



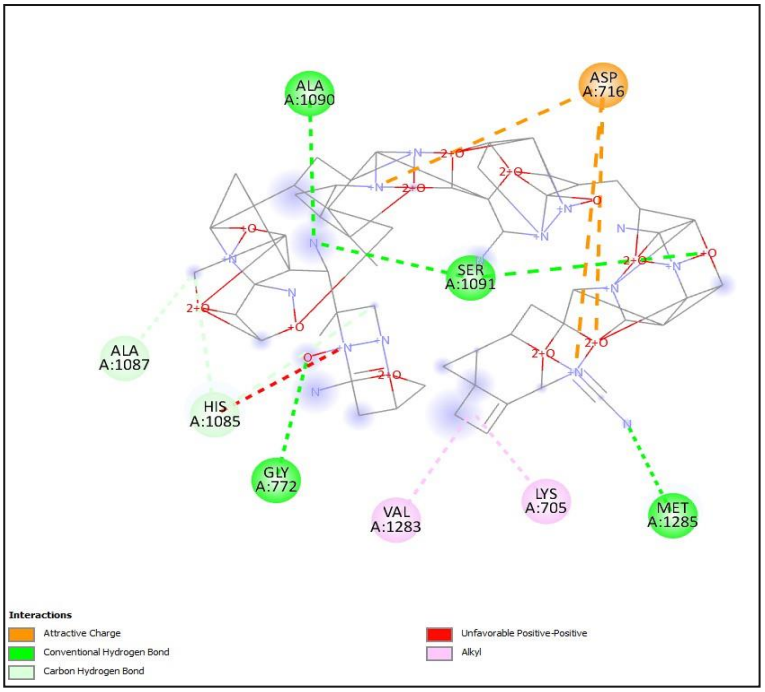
(b)

Figure 7.7. 2-D and 3-D interactions of molecular docking of Silibinin and OATP1B3

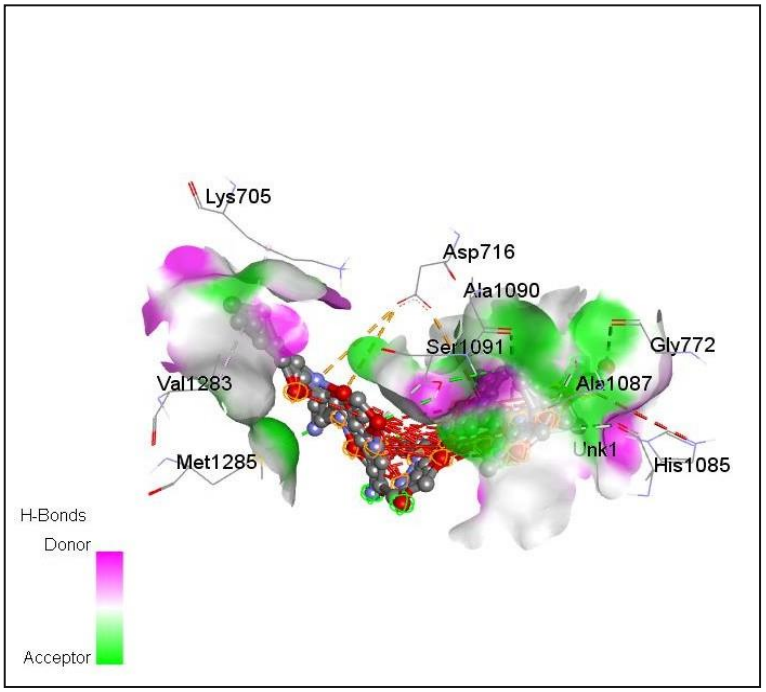
7.3.5 Binding mode predictions of Polymycin B to RNAP II

Polymyxin B, sold under the brand name Poly-Rx among others, is an antibiotic used to treat meningitis, pneumonia, sepsis, and urinary tract infections. While it is useful for many Gram-negative infections, it is not useful for Gram positive infections. It can be given by injection into a vein, muscle, or cerebrospinal fluid or inhaled. The injectable form is generally only used if other options are not available (Bennett et al.,2009). It is also available as the combination's bacitracin/polymyxin B and neomycin/polymyxin B/bacitracin for use on the skin (Woo & Robinson 2015).

The polymyxin B interacts with RNAP II had the lowest free binding energy of -7.58 Kcal/mol observed at 52 runs. The inhibitory constant was calculated as 2.80 µM with 120.1 reference RMSD as shown in **Table 7.1**. The polymycin B forms conventional hydrogen bond with Gly772, Ala1090, Ser1091 and Met1285; carbon – hydrogen bond with His1085 and Ala1087, and also interact with Lys705 and Val1283 alkyl group. It also formed an attractive charge with Asp716 (**Figure 7.8a** and **Figure 7.8b**).



(a)



(b)

Figure 7.8. 2-D and 3-D interactions of molecular docking of Polymycin B and RNAP II

Table 7.1. Binding affinity values of proposed antidodes to OATP1B3 and RNAP II proteins.

Protein name	Protein ID	Ligand name	Runs	Binding free energy (Kcal/mol)	Reference RMSD	Inhibition constant (Ki)	Amino acid involved in interaction
OATP1B3	8PGO	Rifampin	9	-12.79	203.4	453.23 pM	Gln422, Gln541, Ser545, Ser548, Tyr625 Arg633
OATP1B3	8PGO	Prednisolone	108	-8.46	203.94	629.49 nM	Phe352, Phe356, Gln422, Tyr425, Gln541, Asn544, Ser545Tyr625
OATP1B1	8PHW	Cyclosporine	125	-8.03	207.14	1.29 µM	Lys49, Met217, Pro220, Tyr352, Ala355, Phe356, Tyr422
OATP1B3	8PG0	Silibinin	52	-7.58	120.1	2.80 µM	Lys49, Ile353, Ser355, Phe356, Tyr625, Arg633
RNAP II	3CQZ	Polymycin B	61	-6.92	208.33	8.46 µM	Lys705, Asp716, Gly772, His1085, Ala1087, Ala1090, Ser1091, Val1283, Met1285

Table 7.2. Details of proposed antidodes for in silico molecular docking.

Name of proposed antidodes	Trade Name	Common Uses	Availability in India	Price (Rs.)
Rifadin	Rifampin	Antibiotic, Tuberculosis and Leprosy	Available	20-450 (MedIndia)
Prednisolone	Wysolone	Allergic, Asthma, Rheumatic disorder	Available	11-41 (MedIndia)
Cyclosporine	Neoral, Restasis	Immuno-suppressant, arthritis, eye-drop	Available	100-400 (MedIndia)
Silibinin	Silymarin	Fatty liver, liver-cirrhosis	Available	2000-3000 (MedIndia)
Polymycin B	Miepoly-B	Antibiotic, Pneumonia, meningitis, urinary infection	Available	500-2000 (MedIndia)

Figure 7.9. Photos of proposed antidodes for in silico molecular docking.

7.4 Conclusions

The present work reports the discovery of what we believe will be effective antidote for amatoxins poisoning: Rifampin, Prednisolone, Cyclosporine, Polymycin B and Silibinin. The present study provides unequivocal in silico evidence that the proposed antidotes give a potent protection against α-amanitin-induced toxicity, by interfering with its main mechanism of toxicity, the inhibition of RNAP II activity. Outstandingly, the in-silico studies on OATP1B and RNAP II were shown to be of outmost importance in the development process, and allow the suggestion of immediate use of the antidote in addition to the current therapeutic measures, as the proposed antidotes are a therapeutic drug with a well-established clinical use.

Herein, we have applied the same in silico methodology to a peptide with similar composition and molecular weight of amatoxins, and confirmed its ability to displace α-amanitin from RNAP II. Two valuable findings could be observed in silico: (1) proposed antidotes binding site is located in the same interface of α-amanitin, which can prevent the binding of the toxin; (2) proposed antidotes does not interact with bridge helix residues allowing the transcription process. Therefore, the proposed antidotes binding location on OATP1B and RNAP II can potentially protect OATP1B and RNAP II from the α-amanitin-induced impairment. In fact, competition between these antidotes and α-amanitin and/or displacement of α-amanitin from OATP1B and RNAP II by these antidotes can occur depending on the affinity of each molecule for the OATP1B and RNAP II binding site. Further in vivo model being needed to prove the applicability of this in silico results.

Chapter VIII

Summary and conclusion

Chapter 4 presents comprehensive findings on the density, frequency, abundance, diversity, distribution, and ecological factors influencing wild edible mushrooms (WEMs) in the Champhai district of Mizoram, India.

The study identified *Schizophyllum commune* with the highest density, *Lactifluus corrugis* with the highest frequency, and *Schizophyllum commune*, *Termitomyces heimii*, and *Macrolepiota dolichaula* as the most abundant species. The forests exhibited varying densities, frequencies, and occurrences of WEMs, emphasizing the potential value of *Schizophyllum commune* for local consumption and commercial purposes.

A total of 18 WEMs species across nine families were identified, with Russulaceae being the most dominant family. Different habitats in Champhai forests hosted exclusive WEMs, emphasizing the varied ecological niches within the region.

The study explored the diversity of WEMs across different altitudes, revealing varying species richness and depicting three distinct zones. However, some sites at different altitudes harbored similar species, suggesting that altitude alone did not solely determine WEMs distribution.

The research demonstrated a correlation between temperature, rainfall, and the fruiting patterns of selected WEMs. Species richness peaked during the monsoon season, emphasizing the importance of environmental factors in WEMs abundance.

A non-metric multidimensional scaling (NMDS) graph illustrated associations between selected WEMs and specific tree species or habitats. Different WEMs showed preferences for particular tree species, highlighting the interconnectedness of fungi with their surrounding ecosystem.

Soil analysis revealed varying moisture content, pH levels, and nutrient composition across different WEMs. Altitudinal preferences and habitat associations were

evident, emphasizing the significance of soil characteristics in supporting WEMs growth.

The chapter concludes that altitude, temperature, rainfall, and soil characteristics playcrucial roles in determining the density, diversity, and distribution of WEMs in the Champhai district. The findings underscore the importance of considering ecological factors for the conservation and sustainable utilization of these valuable forest resources.

In **Chapter 5**, the proximate analysis of wild edible mushrooms (WEMs) fromChamphai district, Mizoram, India is presented. The composition of mushroom samples, including moisture content, ash, fiber, protein, carbohydrates, fat, and dry matter, is detailed. The study found variations in these parameters among different WEMs species, highlighting the nutritional diversity of wild mushrooms.

The proximate composition of WEMs, expressed on a dry weight basis, revealed a range of variance for moisture content (85.37-93.19%), ash (5.67-15.33%), fiber (9.16-33.16%), protein (19.53-64.01 g/100 g dw), carbohydrates (6.17-53.53 g/100 g dw), and fat (2.24-5.83 g/100 g dw). The study emphasized the significance of dry matter content in assessing the nutritional value of mushrooms.

R. adusta exhibited the highest moisture content (93.19%), while *S. commune* had the lowest (85.37%). The study indicated that moisture content contributes to the texture, palatability, and shelf life of mushrooms. The total ash content varied from 5.67% to 15.33%, with *L. corrugis* having the highest (15.33%) and *T. heimii* the lowest (5.67%). The ash content primarily consists of potassium and phosphorus. *L. corrugis* had the highest crude fiber (33.16%), emphasizing the variability in fiber content among species. The study noted the influence of fiber on the relatively high ash content in fresh mushrooms. Protein, the most abundant macronutrient, ranged from 19.53 g/100 g dw (*L. piperatus*) to 64.01 g/100 g dw (*T. heimii*). WEMs exhibited higher protein content compared to commercial leafy vegetables. Carbohydrate content varied from 6.17 g/100 g dw (*S. commune*) to 53.53 g/100 g dw (*M. dolichaula*). The study highlighted the bioavailable and non-bioavailable carbohydrates in WEMs.

Total fat content ranged from 2.24 g/100 g dw (*S. commune*) to 6.21 g/100 g dw (*R. subfragiliformis*). Edible mushrooms, known for their low-fat content, contribute to a healthy diet. *T. heimii* exhibited the highest energy content (369.57 Kcal/100 g dw), while *S. commune* showed the lowest (150.9 Kcal/100 g dw).

Metal concentrations, including potassium, phosphorus, magnesium, calcium, iron, zinc, and copper, were determined on a dry weight basis. Potassium was the most abundant mineral among WEMs. Principal Component Analysis (PCA) was employed to analyze the multivariate data of nutrients and minerals. The first four principal components accounted for 95.8% of the variance, revealing correlations and differences among various components.

DPPH and ABTS free radical scavenging activities were assessed for eight WEMs. *S. commune* demonstrated the strongest DPPH and ABTS activities, indicating significant antioxidant potential.

Total phenolic content ranged from 3.07 mg GAE/g in *R. adusta* to 19.55 mg GAE/g in *S. commune*. *S. commune* also exhibited the highest total flavonoid content (7.17 mg QE/g).

The correlation analysis showed a very strong correlation between phenol content and antioxidant activities, emphasizing the role of phenols in the antioxidant properties of WEMs.

The study concludes that WEMs from Champhai district possess significant nutritional value, including proteins, carbohydrates, minerals, and antioxidants. The findings support the potential use of these mushrooms for both local consumption and commercial purposes, promoting their nutritional significance and potential economic benefits for the region.

In **Chapter 6**, the study focused on developing and validating an HPLC-PDA method for the identification and quantification of α-amanitin and muscarine in wild mushrooms from Champhai district, Mizoram, India. The optimization process included sample extraction using Oasis® PRIME HLB technology and chromatographic separation with careful parameter adjustments.

Molecular identification through nucleotide sequence comparison revealed species like *Amanita virosa*, *Amanita bisporigera*, *Amanita pantherina*, and *Amanita pseudoporphyria*. The validation process included calibration curves, LOD and LOQ values, recovery, matrix effect, and precision assessments, demonstrating the reliability of the HPLC-PDA method.

Application of the method to ten wild mushroom samples identified the presence of α-amanitin in *Amanita virosa* and *Amanita bisporigera*, and muscarine in *Amanita pantherina* and *Amanita muscaria*. The study concluded by highlighting the significance of identifying and quantifying toxic compounds to prevent poisoning incidents. The developed method provides a valuable tool for assessing mushroom safety, with a call for further research and monitoring to enhance public safety and understanding of mushroom toxicity.

Chapter 7 outlines the main toxicity mechanisms of amatoxins, focusing on their inhibition of organic anion-transporting octapeptide (OATP1B) and RNA polymeraseII (RNAP II) activities. Liver toxicity, a major consequence of amatoxin poisoning, is associated with amanitin hepatocyte uptake. Several treatments have been explored, but there is no consensus on an antidote, making amatoxin poisoning often fatal.

To address this, the study aims to validate effective antidotes, specifically Rifampin, Prednisolone, Cyclosporine, Polymycin B, and Silibinin, using in silico molecular docking methods. Molecular docking involves predicting the preferred orientation of the antidotes when bound to OATP1B and RNAP II, forming a complex with overall minimum energy.

The methodology section explains the molecular docking process, starting with obtaining crystal structures of proteins (OATP1B and RNAP II) and ligands (antidotes) from the Protein Data Bank. AutoDock Tools is used for the meticulous preparation of protein and ligand structures, and AutoDock Vina 4 is employed for thedocking analysis.

Results and discussions present the binding mode predictions for each antidote, detailing their interactions with OATP1B3 and RNAP II. The findings suggest

potential efficacy in displacing α-amanitin and preventing its binding to OATP1B and

RNAP II. The study concludes that these proposed antidotes may offer potent protection against α-amanitin-induced toxicity, with in silico evidence supporting theirtherapeutic potential. The proposed antidotes have well-established clinical use, and further in vivo studies are recommended to validate the in-silico results.

References

Ahmed, W.H.A., Gonmori, K., Suzuki, M., Watanabe, K., Suzuki, O. (2010). Simultaneous analysis of alpha-amanitin, beta-amanitin, and phalloidin in toxic mushrooms by liquid chromatography coupled to time-of-flight mass spectrometry. *Forensic Toxicol*. 28: 69–76. https://doi.org/10.1007/s11419-009-0089-7

Alam, N., Shim, M.J., Lee, M.W., Shin, P.G., Yoo, Y.B., Lee, T.S. (2009). Phylogenetic relationship in different commercial strains of *Pleurotus nebrodensis* based on ITS sequence and RAPD. *Mycobiology.* 37(3):183-188.

Aletor, V.A. (1995). Compositional studies on edible tropical species of mushrooms. *Food Chem*. 54(3): 265-268. https://doi.org/10.1016/0308-8146(95)00044-J

Aletor, V.A. (1990). Anti–nutritional factors in some Nigerian feedstuffs, herbage byproducts, crop residue and browse plants. A monograph prepared for the presidential Task Force on Alternative Formulation of Livestock Feed Products Development. Quality Evaluation and Health Implications. Cabinet Office, Lagos. Nigeria

Alexopoulos, C.J., Mims, C.W. and Blackwell, M. (1996). Introductory Mycology. FourthEdition. John Wiley & Sons Inc., New York.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*. 215(3): 403–410.

Alves, M., Ferreira, I., Dias, J., Teixeira, V., Martins, A. and Pintado, M. (2012). A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta medica*. 78(16): 1707-1718. https://doi.org/10.1055/s-0032-1315370

Ambrosio, E., Marescotti, P., Benucci, G.M.N., Cecchi, G., Brancucci, M., Zotti, M. and Mariotti, M.G. (2019). Can the soil geology and chemistry analysis of a site predict the geographic origin of wild edible mushrooms (Porcini group)?.*Acta Mycologica*. 54(2).

Anon. (2015). India State of Forest Report. Forest Survey of India, Ministry

of Environmental & Forests, Govt. of India, Dehradun, India. 34- 44.

Anon. (2011) *State Forest Report.* (2011). Published by Forest Survey of India,Dehradun. 187.

Anon. (2009). India State of Forest Report (Ministry of Environment & Forests), Govt.of India, Kaulagarh Road, Dehradun India.

Ao, T., Deb, C.R. (2019). Nutritional and antioxidant potential of some WEMs of Nagaland, India. *J Food Sci Technol*. 56(2):1084–9.

A.O.A.C. (2005). Official methods of analysis. 18th ed. Washington DC: *Associationof Officiating Analytical Chemists*.

Arici, M.A., Sahin, A., Cavdar, Z., Ergur, B.U., Ural, C., Akokay, P., Kalkan, S., Tuncok, Y. (2020). Effects of resveratrol on alpha-amanitin induced nephrotoxicity in BALB/c mice. *Hum. Exp. Toxicol*. 39: 328–337. https://doi.org/10.1177/0960327119888271

Arnolds, E. (1981). Ecology and coenology of macrofungi in grasslands and moist heathlands in Drenthe, the Netherlands. Vol. 1 Biblthca mycol. 83. J. eramer,Vaduz.

Arunachalam, A., Maithani, K., Pandey, H.N. and Tripathi R.S. (1998). Fine litter and nutrient dynamics during forest regrowth in humid sub-tropics of north- eastern India. *Forest Ecology and Management.* 110:209-219.

Atri, N.S., Saini, S.S. (1989). Family Russulaceae Roze-a review. In: Plant Science Research in India. Trivedi ML, Gill GS, Saini SS (Eds.), NewDelhi, India: Today and Tomorrow's Printers and Publishers. 115-125.

Atri, N.S., Saini, S.S., Mann, D.K. (1991a). Further studies on north-west Indian Agricaceae-Systematics of *Lactarius deliciosus* (Fr.) S.F. Gray.*Geobios NewReport*. 10: 106-111.

Atri, N.S., Saini, S.S., Gupta, A.K. (1991b). Systematic studies on *Agaricus campestris* (L.) Fr. *Geobios New Report*. 10: 32-37.

Atri, N.S., Saini, S.S., Kaul, G. (1995). Taxonomic studies on North Indian Agarics- The genus *Termitomyces*. *Mushroom Research*, 4: 7-10.

Atri, N.S., Saini, S.S., Kaur, G. (1996). Three species of agarics from Patiala. *Mushroom Research*. 5: 77-80.

Atri, N.S., Saini, S.S., Saini, M.K. (1997). Studies on genus *Russula* Pers. from

NorthWestern Himalaya. *Mushroom Research*. 6(l): 1-6.

Bach, F., Zielinski, A.A.F., Helm, C.V., Maciel, G.M., Pedro, A.C., Stafussa, A.P., Avila, S., Haminiuk, C.W.I. (2019). Bio compounds of edible mushrooms: invitro antioxidant and antimicrobial activities. *LWT*. 107:214–20.

Bano, Z. and Rajarathnam, S. (1988). Pleurotus mushroom. CRC Critical Reviews in *Food Science and Nutrition*. 27: 87.

Barbosa, I., Domingues, C., Barbosa, R.M. and Ramos, F. (2022). Amanitins in WildMushrooms: The Development of HPLC-UV-EC and HPLC-DAD-MS Methods for Food Safety Purposes. *Foods*, 11(23): 3929.

Barros, L., Baptista, P., Correira, D.M., Casa, S., Oliveira, B., Ferreira I.C.F.R. (2007). Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chem*. 105: 140.

Barros, L., Cruz, T., Baptista, P., Estevinho, L.M., Ferreira, I.C.F.R. (2008). Wild andcommercial mushrooms as source of nutrients and nutraceuticals. *Food ChemToxicol*. 46(8):2742–7.

Batra, L.R., Batra, S.W.T. (1979). Termite-fungus mutualism. In: Insect Fungus Symbiosis: Nutrition, Mutualism and Commensalism. BatraLR (Ed.), New York: Allanheld Osmun. 117-163.

Bauer-Petrovska, B. (2001). Protein fractions in edible Macedonian mushrooms. *European FoodResearch and Technology*. 212: 469.

Bellettini, M.B., Fiorda, F.A., Maieves, H.A., Teixeira, G.L., Ávila, S., Hornung, P.S., Junior, A.M., Ribani, R.H. (2019). Factors affecting mushroom *Pleurotus* sp.*Saudi J Biol Sci*. 26(4):633–46.

Bels, P., Pataragetvit, S. (1982). Edible mushrooms in Thailand, cultivated by termites. In: Tropical Mushrooms: Biological Nature andCultivated Methods. Chang ST, Quimio TH (Eds.), Hong Kong: Chinese University Press. 445-461.

Benjamin, D.R. (1995). Mushrooms: poisons and panaceas. WH Freeman and Co..

Bennet, J.W. and Lasure, L.L. (1991). Appendix B growth media. *More Gene Manipulations in Fungi*. 445.

Bennett, J.E., Dolin, R., Blaser, M.J., Mandell, G.L. (2009). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-Book. *Elsevier*

Health Sciences. 469. ISBN 9781437720600.

Berger, K.J., Guss, D.A. (2005). Mycotoxins revisited: Part I. *J. Emerg. Med*. 28: 53–62. https://doi.org/10.1016/j.jemermed.2004.08.013

Bhutia, Y., Gudasalamani, R., Ganesan, R., Saha, S. (2019). Assessing Forest structure and composition along the altitudinal gradient in the state of Sikkim, Eastern Himalayas, India. *Forests*. 10(8): 633. https://doi.org/10.3390/f10080633.

Blackwell, M. (2011). The Fungi: 1, 2, 3… 5.1 million species?. *American journal ofbotany*, 98(3): 426-438.

Boddy, L. (2000). Interspecific combative interactions between wood-decaying basidiomycetes. *FEMS microbiology ecology*. 31(3): 185-194.

Boddy, L. (2001). Fungal community ecology and wood decomposition processes in angiosperms: from standing tree to complete decay of coarse woody debris. *Ecological Bulletins*. 43-56.

Broussard, C.N., Aggarwal, A., Lacey, S.R. et al. (2001). Mushroom poisoning–fromdiarrhea to liver transplantation. *Am J Gastroenterol.* 96(11): 3195–3198.

Brvar, M., Možina, M., Bunc, M. (2006). Prolonged psychosis after *Amanita muscaria* ingestion. *Wien Klin Wochenschr*. 118: 294–297. https://doi.org/10.1007/s00508-006-0581-6

Buba, T., Agbo, V. and Abdullahi, A. (2018). The ecology of edible mushrooms of the Nigerian savannah: towards their optimal exploitation. *Journal of Applied Biosciences*. 132: 13439-13451.

Bushnell, D. A., Cramer, P., Kornberg, R. D. (2002). Structural basis of transcription:alpha-amanitin-RNAP II cocrystal at 2.8 A resolution. *Proc. Natl. Acad. Sci* U S A. 99 (2002): 1218-1222.

Bushwell, Y.A., & Chang, S.T. (1993). Edible mushroom attributes and applications in genetics and breeding of edible mushrooms. Gordon and Breanch Science Publishers, USA, 297-334.

Butkhup, L., Samappito, W., Jorjong, S. (2018). Evaluation of bio-activities and phenolic contents of wild edible mushrooms from northeastern Thailand. *Food Sci Biotechnol.* 27:193–202. https ://doi.org/10.1007/s10068-017-

0237-5

Capelli, Bob, Gerald Cysewski. (2007). Natural Astaxanthin. The world'sbest kept health secret. Cyanotech Corporation, Kailua-Kina.

Caprioli, G., Lupidi, G., Maggi, F. (2019). Comparison of chemical composition and antioxidant activities of two Winter savory subspecies (*Satureja montana* subsp. *variegata* and *Satureja montana* subsp. *montana*) cultivated in Northern Italy. Nat Prod Res. 33(21):3143–7.

Champhai district. Government of Mizoram, India. (2021). https://champ hai.nic.in/about-district/. Accessed 03 Jan 2021.

Champhai District: Census (2011) data. *census2011.co.in*. Retrieved 18 May 2018.

Champion, H.G. and Seth, S.K. (1968). A Revised Survey of Forest Types of India. FRI, Dehradun, pp: 404.

Chang, S., Hayes, W. (1978). The biology and cultivation of edible mushrooms. 1st ed. Academic Press.

Chang, S.T. and Wasser, S.P. (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International journalof medicinal mushrooms*. 14(2).

Cheung, L.M., Cheung, P.C.K., Ooi, V.E.C. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem*. 81(2):249–55.

Cheung, P.C.K. (1998). Plasma and hepatic cholesterol levels and fecal neutral sterolexcretion are altered in hamsters fed straw mushroom diets. *Journal Nutrition*. 128: 1512.

Cheung, P.C.K. (2010). The nutritional and health benefits of mushrooms. *Nutr Bull*. 35(4):292–9.

Chittaragi, A., Naika, R., Vinayaka, K. (2014). Nutritive value of few wild mushrooms from the western Ghats of Shivamogga district, Karnataka, India. *Asian J of Pharm Clin Res*. 1:50–3.

Choudhary, M., Devi, R., Datta, A., Kumar, A. and Jat, H.S. (2015). Diversity of WEMs in indian subcontinent and its neighboring countries. *Recent Advances in Biology and Medicine*. 1: 69-76.

Chung, W.C., Tso, S.C., Sze, S.T. (2007). Separation of polar mushroom toxins by mixed mode hydrophilic and ionic interaction liquid chromatography-

electrospray ionization-mass spectrometry. *J. Chromatogr. Sci*. 45: 104–111.

Clarke, D.B., Lloyd, A.S., Robb, P. (2012). Application of liquid chromatography coupled to time-of-flight mass spectrometry separation for rapid assessment of toxins in *Amanita* mushrooms. *Anal. Methods*. 4: 1298–1309. https://doi.org/10.1039/C2AY05575A

Comandini, O., Contu, M., Rinaldi, A.C. (2006). An overview of *Cistus* ectomycorrhizal fungi. *Mycorrhiza* 16: 381–395.

Courty, P.E., Buée, M., Diedhiou, AG., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, MP., Uroz, S., Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: new perspectives and emerging concepts. *Soil Biol. Biochem*. 1;42(5): 679-98. https://doi.org/10.1016/j.soilbio.2009.12.006.

Cramer, P., Armache, K. J., Baumli, S., Benkert, S., Brueckner, F., Buchen, C., et al. (2008). Structure of eukaryotic RNA polymerases. *Annu. Rev. Biophys*. 37 (2008): 337-352.

Curtis J.T., Mcintosh., R.P. (1950). The interrelations of certain analytic and syntheticphytosociological characters. *Ecology.* 31(3): 434-55.

Dai, Y.C., Yang, Z.L., Cui, B.K., et al. (2009). Species diversity and utilization of medicinal mushrooms and fungi in China (Review). *International Journal ofMedicinal Mushrooms*. 11: 287-302.

Das, A.K., Nanda, P.K., Dandapat, P., Bandyopadhyay, S., Gullón, P., Sivaraman, G.K., McClements, D.J., Gullon, B., Lorenzo, J.M. (2021). Mushrooms as functional ingredients for development of healthier and more sustainable muscle Foods: a flexitarian approach. *Molecules*. 23;26(9):2463.

Das, K.A.B.I.T.A., Lamo, A.L.B.I.N.U.S., Paul, D. and Jha, L.K. (2014). Ethnomycological knowledge on wild edible mushroom of Khasi tribes of Meghalaya, North-Eastern India. *European Academic Research*. 2(3): 3433-3443.

Deb, D.B. and Dutta, R.M. (1987). A contribution to the flora of Mizoram. *J. Econ.Tax. Bot.* 10(1):21-61.

De Bruyn, L.L., and Conacher, A.J., (1990). The role of termites and ants in soil

modification-a review. *Soil research*. 28(1): 55-93.

Dejene, T., Oria-de-Rueda, J.A. and Martín-Pinto, P. (2017). Wild mushrooms in Ethiopia: A review and synthesis for future perspective. *Forest Systems*. 26(1): eR02-eR02.

Demain, A.L. and Fang, A. (2000). The natural functions of secondary metabolites. *History of modern biotechnology I*. 1-39.

Diez, A.A. and Alvarez, A. (2001). Compositional and nutritional studies on two wildedible mushrooms from north west Spain. *Food Chemistry*. 75: 417.

Dimitrijevic, M.V., Mitic, V.D., Nikolic, J.S., Djordjevic, A.S., Mutic, J.J., Stankov Jovanovic, V.P. (2019). First report about mineral content, fatty acids composition and biological activities of four wild edible mushrooms. *Chem Biodivers*. 16(2): e1800492.

Dijk, H.V., Onguene, N.A., Kuyper, T.W. (2003). Knowledge and utilization of ediblemushrooms by local populations of the rain forest of South Cameroon. *Ambio*.32:19-23.

Dwivedi, S., Tiwari, M. K., Chauhan, U. K., & Pandey, A. K. (2012). Biodiversity of mushrooms of Amarkantak Biosphere Reserve Forest of Central India. *Int. J. Pharm. Life. Sci.* 3(1).

Enjalbert, F., Gallion, C., Jehl, F., Monteil, H., Faulstich, H. (1992). Simultaneous Assay for Amatoxins and Phallotoxins in *Amanita Phalloides* Fr by High-Performance Liquid-Chromatography. *J. Chromatogr*. 598: 227–236. https://doi.org/10.1016/0021-9673(92)85052-u

Enjalbert, F., Cassanas, G., Salhi, S.L., Guinchard, C., Chaumont, J.P. (1999). Distribution of the amatoxins and phallotoxins in *Amanita phalloides*. Influence of the tissues and the collection site. *Comptes Rendus Acad. Sci*. III.322: 855–862.

Enjalbert, F., Rapior, S., Nouguier-Soule, J., Guillon, S., Amouroux, N., Cabot, C. (2002). Treatment of amatoxin poisoning: 20-year retrospective analysis. *J. Toxicol. Clin. Toxicol*. 40 (2002): 715-757.

Enow, E. (2013). Diversity and distribution of macrofungi (mushrooms) in the MountCameroon Region. *J. ecol. nat. environ*. 5: 318–334. https://doi.org/ 10.5897/JENE2013.0379

Erden, A., Esmeray, K., Karagoz, H., et al. (2013). Acute liver failure caused by mushroom poisoning: A case report and review of the literature. *Int. Med. Case Rep. J*. 6: 85–90. https://doi.org/10.2147/IMCRJ.S53773

Evans, R.H. (1996). An analysis of criterion variable reliability in conjoint analysis. *Percept Mot Skills*. 82(3):988–90.

Fasidi, I.O. (1996). Studies on Volvariella esculenta (Mass) Singer: cultivation on agricultural wastes and proximate composition of stored mushrooms. *Food Chem.* 55(2), 161-163. https://doi.org/10.1016/0308-8146(95)00082-8

F.D.A. (2008). Mushroom Toxins. Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural toxin. 2; 218-230.

Ferreira, I.C., Barros, L. and Abreu, R. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*. 16(12): 1543-1560.

Ferreira, I.C.F.R., Baptista, P., Vilas-Boas, M., Barros, L. (2007). Free-Scavenging capacity and reducing power of wild edible mushrooms from north-east Portugal: individual cap and stipe activity. *Food chemistry.* 100: 1511-1516.

Gange, A.C., Mohammad, A.B., Damialis, A. and Gange, E.G. (2013). Mushroom phenological changes: A role for resource availability?. *Proceedings of the National Academy of Sciences*. 110(5): E333-E334.

Garcia, J., Carvalho, A.T., Dourado, D.F., Baptista, P., de Lourdes Bastos, M.,Carvalho, F. (2014). New in silico insights into the inhibition of RNAP II by alpha-amanitin and the protective effect mediated by effective antidotes. *J Mol Graph Model.* 51: 120–127. https://doi.org/10.1016/j.jmgm.2014.05.002

Garcia, J., Costa, V.M., Baptista, P., et al. (2015). Quantification of alpha-amanitin in biological samples by HPLC using simultaneous UV-diode array and electrochemical detection. *J. Chromatogr. B-Anal. Technol. Biomed. Life Sci*.997: 85–95. https://doi.org/10.1016/j.jchromb.2015.06.001

Garcia, J., Costa, V.M., Carvalho, A.T., Silvestre, R., Duarte, J.A., Dourado, D.F., Arbo, M.D., Baltazar, T., Dinis-Oliveira, R.J., Baptista, P. and de Lourdes Bastos, M. (2015). A breakthrough on *Amanita phalloides* poisoning: an effective antidotal effect by polymyxin B. *Archives of toxicology*. 89: 2305- 2323. https://doi.org/10.1007/s00204-015-1582-x

Garcia, J., Costa, V.M., Baptista, P., et al. (2015). Quantification of alpha-amanitin in biological samples by HPLC using simultaneous UV-diode array and electrochemical detection. *J. Chromatogr. B-Anal. Technol. Biomed. Life Sci*.997: 85–95. https://doi.org/10.1016/j.jchromb.2015.06.001

Garcia, J., Oliveira, A., de Pinho, P.G., Freitas, V., Carvalho, A., Baptista, P., Pereira,E., de Lourdes Bastos, M. and Carvalho, F. (2015). Determination of amatoxins and phallotoxins in *Amanita phalloides* mushrooms from northeastern Portugal by HPLC-DAD-MS. *Mycologia*. *107*(4): 679-687.

Gargano, M.L., van Griensven, L.J.L.D., Isikhuemhen, O.S., Lindequist, U., Venturella, G., Wasser, S.P., Zervakis, G.I. (2017). Medicinal mushrooms: Valuable biological resources of high exploitation potential. *Plant Biosyst*. 151: 548-565, 10.1080/11263504.2017.1301590

Gąsecka, M., Siwulski, M., Mleczek, M. (2018). Evaluation of bioactive compounds content and antioxidant properties of soil-growing and wood-growing edible mushrooms. *J. Food Process. Preserv.* 42: 1-10. 10.1111/jfpp.13386

Gençcelep, H., Uzun, Y., Tunçtürk, Y., Demirel, K. (2009). Determination of mineral contents of wild-grown edible mushrooms. *Food Chem*. 113(4):1033–6.

Gezer, K. and Kaygusuz, O. (2015). Soil and habitat characteristics of various species of mushroom growing wild in the Gireniz Valley, Turkey. *Oxidation Communications*. 38(1): 389-397.

Gil-Martínez, M., Navarro-Fernández, C.M., Murillo, J.M., Domínguez, M.T. and Marañón, T. (2020). Trace elements and C and N isotope composition in two mushroom species from a mine-spill contaminated site. *Scientific Reports*. 10(1): 6434.

Gong, X.Q., Nedialkov, Y.A., Burton, Z.F. (2004). Alpha-amanitin blocks translocation by human RNAP II. *J. Biol. Chem*. 279(2004): 27422-27427.

Gooday, G.W. (1995). The dynamics of hyphal growth. *Mycological Research*. 99(4): 385-394.

Gopalan, C., Rama, Sastri, B.V., Balasubramanian, SC. (1971). Nutritive value of Indian foods. Hyderabad: Indian Council of Medical Research.

Gotelli, N.J., Colwell, R.K. (2011). Estimating species richness. *Biol. Divers. Front. Meas. Assess*.12: 39–54.

Gouveia, F., Bicker, J., Santos, J. (2020). Development, validation and application ofa new HPLC-DAD method for simultaneous quantification of apixaban, dabigatran, edoxaban and rivaroxaban in human plasma. *J. Pharm. Biomed. Anal*. 181: 113109. https://doi.org/10.1016/j.jpba.2020.113109

Gow, N.A., Latge, J.P. and Munro, C.A. (2017). The fungal cell wall: structure, biosynthesis, and function. *Microbiology spectrum*. 5(3): 10-1128.

Gucia, M., Jarzyńska, G., Kojta, A.K. and Falandysz, J. (2012). Temporal variability in 20 chemical elements content of Parasol Mushroom (Macrolepiota procera) collected from two sites over a few years. *Journal of EnvironmentalScience and Health, Part B. 47*(2): 81-88.

Guillamón, E., García-Lafuente, A., Lozano, M., D´Arrigo, M., Rostagno, M.A., Ana Villares A., Martínez, J.A. (2010). Edible mushrooms: Role in the preventionof cardiovascular diseases. *Fitoterapia*. 81: 715.

Gupita, C.N., Rahayuni, A. (2012). Pengaruh berbagai Ph sari buah dan suhupasteurisasi terhadap aktivitas antioksidan dan tingkat penerimaansari kulit buah manggis. *J Nut Coll*. 1(1): 209-215. DOI:10.14710/jnc.v1i1.428 [Indonesian]

Gursoy, N., Sarikurkcu, C., Cengiz, M. and Solak, M.H. (2009). Antioxidant activities, metal contents, total phenolics and flavonoids of seven Morchella species. *Food and Chemical Toxicology*. 47(9): 2381-2388. https://doi.org/10.1016/j.fct.2009.06.032

Haddad, Y., Adam, V., & Heger, Z. (2020). Ten quick tips for homology modeling of high-resolution protein 3D structures. *PLoS computational biology*. 16(4): e1007449. https://doi.org/10.1371/ journal. pcbi.1007449

Hall, I.R., Lyon, A.J.E., Wang, Y., Sinclair, L. (1998). Ectomycorrhizal fungi with edible fruiting bodies 2. *Boletus edulis*. *Econ. Bot*. 52(1): 44–56.

Hall, T. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series*.141:95–98.

Hallen, H.E., Watling, R., Adams, G.C. (2003). Taxonomy and toxicity of

Conocybe lactea and related species. *Mycol. Res.*107: 969–979.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *PaleontologiaElectronica*. 4 (1): 9.

Hasnat, M.A., Pervin, M., Debnath, T., Lim, B.O. (2014). DNA protection, total phenolics and antioxidant potential of the mushroom *Russula virescens*. *J. Food Biochem*. 38: 6-17, 10.1111/jfbc.12019

Hatano, T., Edamatsu, R., Hiramatsu, M., Moti, A., Fujita, Y. et al. (1989). Effects oftannins and related polyphenols on superoxide anion radical, and on 1, 1- diphenyl-2-picrylhydrazyl radical. *Chemical and Pharmaceutical Bulletin*. 37: 2016-2021.

Hawksworth, D.L. (1991). The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological research*, 95(6): 641-655.

Hawksworth, D.L. and Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum*, *5*(4): 10-1128.

Heim, R. (1977). Termites at Champignons. Paris: Societe Nouvella Des Editions Boubee.

Heleno, S.A., Barros, L., Martins, A., Morales, P., Fernandez-Ruiz, V., Glamoclija, J., Sokovic, M. and Ferreira, I.C. (2015). Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms. *LWT-Food Science and Technology*. 63(2): 799-806. https://doi.org/10.1016/j.lwt.2015.04.028

Herawati, E., Ramadhan, R., Ariyani, F., Marjenah, M., Kusuma, I.W., Suwinarti, W., Mardji, D., Amirta, R. and Arung, E.T. (2021). Phytochemical screening and antioxidant activity of wild mushrooms growing in tropical regions. *Biodiversitas Journal of Biological Diversity*. 22(11).

Hibbett, D.S., Binder, M., Bischoff, J.F., Blackwell, M., Cannon, P.F., Eriksson, O.E.,Huhndorf, S., James, T., Kirk, P.M., Lücking, R. and Lumbsch, H.T. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological research*. 111(5): 509-547.

Hofhansl, F., Chacón-Madrigal, E., Morera, A., Silla, F., Huber, W., Weissenhofer, A., Wanek, W. (2019). Diversity and composition of tropical forest plant

communities in the Golfo Dulce region. *Acta ZooBot Austria*. 156: 31-46.

Hooker, J.D. (1872-1897). *The Flora of the British India.* Vol. 1-7. London.

Hsieh, T.C., Ma, K.H., and Chao, A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*. 7(12): 1451-1456.

I.B.C. (2005). National biodiversity strategy and action plan. December, 2005.Addis Ababa, Ethiopia.

I.C.H. (2005). ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and Methodology. *International Conference Harmon*. 1994: 17.

Iorio, E.L. (2007). The measurement of oxidative stress. International observatory of oxidative stress, free radicals and antioxidant systems. Special supplement to Bulletin.

Jacinto-Azevedo, B., Valderrama, N., Henríquez, K., Aranda, M., Aqueveque, P. (2021). Nutritional value and biological properties of Chilean wild and commercial edible mushrooms. *Food Chem*. 356:129651.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J. and Lumbsch, H.T. (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*, 443(7113): 818-822.

Johnsy, G., Sargunam, S., Dinesh, M., Kaviyarasan, V. (2011). Nutritive value of edible wild mushrooms collected from the Western Ghats of Kanyakumari District. *Bot Res Int*. (4):69–74.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., Donoghue, M.J. (2002). Plant Systematics: A Phylogenetic Approach, Second Edition. Sinauer Associates, Inc., Sunderland:1-39

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*. 113: 9.

Kalaˇc, P. (2013). A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J. Sci. Food Agric*. 93: 209–218.

Kalac, P. (2016). Edible mushrooms: chemical composition and nutritional value. *Academic Press*; Jan 30. https://doi.org/10.1016/B978-0-12-391946-7.00006-4

Kanjilal, U.N., Kanjilal, P.C., Das, A., De, R.N. and Bor, N.L. (1934-1940). Flora ofAssam. Vols. 1-5. Govt. Press, Shillong.

Karlson-Stiber, C., Persson, H. (2003). Cytotoxic fungi—An overview. *Toxicon*. 42: 339–349. https://doi.org/10.1016/s0041-0101(03)00238-1

Kaya, E., Karahan, S., Hanci, M. (2012). The Measurement of Alpha Amanitin Levels Using Hplc Method in *Amanita Phalloides* from Düzce Province. *Duzce Med. J*. 14: 15–17.

Kindt, R., Coe, R. (2005). BiodiversityR: A package for community ecology and metapopulation dynamics in R. Available online at: http://www.ucl.ac.uk/~rkindt/biodiversityR/index.html

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2001). Dictionary of the fungi9th edn. *CABI, Wallingford*.

Kjeldahl, J. (1883). A new method for the determination of nitrogen in organic matter. *Z. Anal. Chem*. 22:366.

Koda-Kimble, M.A., Alldredge, B.K., Corelli, R.L., Ernst, M.E. (2012). Koda-Kimbleand young's applied therapeutics: the clinical use of drugs. Wolters Kluwer Health/Lippincott Williams & Wilkins, Baltimore

Kosanić, M., Ranković, B., Dašić, M. (2012). Mushrooms as possible antioxidant andantimicrobial agents. *Iran J Pharm Res.* 11:1095–1102.

Kosol, S. (2022). Species diversityand ecology of edible wild-mushroom at Ban Boonjam community forest, Phrae Province. วารสารวจไมเ้ มองไทย. 3 (1): 38-46 (2562)

Kujala, T.S., Loponen, J.M., Klika, K.D., Pihlaja, K. (2000). Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds.*J Agric Food Chem*. 48(11):5338–42.

Lalramnghinglova, H. and Jha, L.K. (1997). Forest resources- an overview *In* L.K. Jha, *Natural Resources management.1. Mizoram.* 203-253. A.P.H. PublishingCorporation, New Delhi.

Lalrinawmi, H., Vabeikhokhei, J.M.C., Zothanzama, J., Zohmangaiha. (2017). Ediblemushrooms of Mizoram. *Science vision*. 17 (3), 172—181.

Lange, O.L. (2001). Photosynthesis of soil-crust biota as dependent on environmental factors. In *Biological soil crusts: structure, function, and management*. 217- 240.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, PA., Mcwilliam, H., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*.23(21):2947–2948.

Le, H.T. (2007). *Biodiversity of the genus Lactarius (Basidiomycota) in northern Thailand*. PhD thesis, Chiang Mai University, Thailand.

Lee, J.S., Lim, M.O., Cho, K.Y., Cho, J.H., Chang, S.Y., Nam, D.H. (2006). Identification of medicinal mushroom species based on nuclear large subunitrDNA sequences. *J Microbiol.* 44(1): 29-34.

Lee, K. G. and Shibamoto, T. (2002). Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *Journal of agricultural and food chemistry*. 50(17): 4947-4952.

Lengeler K.B., Davidson R.C., D'Souza C., Harashima T., Shen W.C., Wang P., Pan X., Waugh M., Heitman J. (2000). Signal transduction cascades regulating fungal development and virulence. *Microbiol. Mol. Biol. Rev.* 64:746–785.

Legendre, P., Legendre, L.F. (2012). Numerical Ecology. Elsevier: Amsterdam, The Netherlands, 2012; Volume 24.

Leist, M., Gantner, F., Naumann, H. et al. (1997). Tumor necrosis factor induced apoptosis during the poisoning of mice with hepatotoxins. *Gastroenterology*.112(3):923.

Leite, M., Freitas, A., Azul, A.M., et al. (2013). Development, optimization and application of an analytical methodology by ultra performance liquid chromatography-tandem mass spectrometry for determination of amanitins in urine and liver samples. *Anal. Chim. Acta*. 799: 77–87. https://doi.org/10.1016/j.aca.2013.08.044

Leonardi, M., Comandini, O., Rinaldi, A.C. (2016). Peering into the Mediterranean black box: *Lactifluus rugatus* ectomycorrhizas on *Cistus*. *IMA Fungus* 7: 275–284.

Leonardi, M., Furtado, A.N.M., Comandini, O., et al. (2020). *Halimium* as an

ectomycorrhizal symbiont: new records and an appreciation of known fungaldiversity. *Mycological Progress* 19: 1495–1509.

Letschert, K., Faulstich, H., Keller, D., Keppler, D. (2006). Molecular characterizationand inhibition of amanitin uptake into human hepatocytes. *Toxicol Sci*. 91 (2006): 140-149.

Leudang, S., Sikaphan, S., Parnmen, S., et al. (2017). DNA-based identification of gastrointestinal irritant mushrooms in the genus Chlorophyllum: A food poisoning case in Thailand. *Journal of Health Research*. 31(1): 41-49.

Li, X., Wang, Z., Wang, L., Walid, E., Zhang, H. (2012). In vitro antioxidant and anti- proliferation activities of polysaccharides from various extracts of different mushrooms. *Int. J. Mol. Sci*. 13: 5801-5817. 10.3390/ijms13055801

Lian, B., Zang, J., Hou, W., Yuan, S., Smith, D.L. (2008). PCR-based sensitive detection of the edible fungus Boletus edulis from rDNA ITS sequences. *Electron J Biotechnol*. 11(3): 1-8. http://dx.doi.org/10.4067/S0717-34582008000300011

Lindell, T. J., Weinberg, F., Morris, P. W., Roeder, R. G., Rutter, W. J. (1970). Specific inhibition of nuclear RNAP II by alpha-amanitin. *Science*. 170 (1970): 447- 449.

Li, X., Wang, Z., Wang, L., Walid, E., & Zhang, H. (2012). In vitro antioxidant and anti-proliferation activities of polysaccharides from various extracts of different mushrooms. International Journal of Molecular Sciences, 13(5), 5801-5817.

Liu, L.Q., Chen, J.Z., Fu, W.S. and Tang, C.Y. (2023). Determination of amanita peptide and tryptamine toxins in wild mushrooms by high performance liquid chromatography-tandem mass spectrometry. *Chinese Journal of Chromatography*. 41(11): 976-985.

Liu, Y., Wang, S., Yin, Y., Xu, R. (2013). Evaluation of genetic diversity of Chinese *Pleurotus ostreatus* cultivars using DNA sequencing technology. *Ann Microbiol*. 63:571-576.

Lowry, O., Rosebrough, N., Farr, A., Randall, R. (1951). Protein measurement with the folin phenol reagent. *J Biol Chem*. 193(1):256–75.

Luo et al. (2012). Ribosomal biosynthesis of α-amanitin in *Galerina marginata*. *Fung. Genet. Biol*. 49 (2012): 123-129. https://doi.org/10.1016/j.fgb.2011.12.005

Maio, M., Di Fiore, C., Iannone, A., Carriera, F., Notardonato, I., Avino, P. (2022). Review of the Analytical Methods Based on HPLC-Electrochemical Detection Coupling for the Evaluation of Organic Compounds of Nutritionaland Environmental Interest. *Analytica.* 3: 54–78.

Mallikarjuna, S.E., Ranjini, A., Haware, D.J., Vijayalakshmi, M.R., Shashirekha, M.N., Rajarathnam, S. (2013). Mineral composition of four edible mushrooms. *J Chem*. 2013:1–5.

Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, TS., Rawat, S., Johri, BN. (2005). Fungal biodiversity: distribution, conservationand prospecting of fungi from India. *Curr. Sci*. 10, 58-71.

Manzi, P., Aguzzi, A., Pizzoferrato, L. (2001). Nutritional value of mushrooms widelyconsumed in Italy. *Food Chem*. 73(3):321–5.

Manzi, P., Marconi, S., Aguzzi, A., Pizzoferrato, L. (2004). Commercial mushrooms:nutritional quality and effect of cooking. *Food Chemistry*. 84: 201.

Mattila, P., Könkö, K., Eurola, M., Pihlava, J.M., Astola, J., Vahteristo, L., Hietaniemi, V., Kumpulainen, J., Valtonen, M., Pilronen, V. (2001). Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. JAgric Food Chem. 49(5):2343–8.

Megersa, S., Gure, A., Feleke, S., Alemu, M. (2017). Macrofungi species richness anddiversity in Dagaga and Gambo plantation and natural forests of Arsi forest enterprise, Oromia, Ethiopia. *IJIR*. 3(1): 1681 -1686.

Mei, Z.Q., Fu, S.Y., Yu, H.Q., Yang, L.Q., Duan, C.G., Liu, X.Y., Gong, S., Fu, J.J. (2014). Genetic characterization and authentication of Dimocarpus longan Lour. using an improved RAPD technique. *Genet Mol Res.* 13(1): 1447-1455.http://dx.doi.org/10.4238/2014.March.6.3

Ming, T., Li, J., Huo, P., Wei, Y. and Chen, X. (2014). Analysis of free amino acids in Russula griseocarnosa harvested at different stages of maturity using iTRAQ®-LC-MS/MS. *Food analytical methods*. 7: 1816-

182https://doi.org/10.1007/s12161-014-9817-7

Mirfat, A.H.S., Noorlidah, A., Vikineswary, S. (2010). Scavenging activity of *Schizophyllum commune* extracts and its correlation to total phenolic content.*J Trop Agric Food Sci.* 38 (2): 231-238.

Mishra, K.K., Pal, R.S., Arunkumar, R., Chandrashekara, C., Jain, S.K. and Bhatt, J.C. (2013). Antioxidant properties of different edible mushroom species and increased bioconversion efficiency of *Pleurotus eryngii* using locally available casing materials. *Food chemistry*. 138(2-3): 1557-1563.

Money, N.P. (2016). Are mushrooms medicinal?. *Fungal biology*. 120(4): 449-453.

Moreau, P.A., Peintner, U., Gardes, M. (2006). Phylogeny of the ectomycorrhizal mushroom genus Alnicola (Basidiomycota, Cortinariaceae) based on rDNA sequences with special emphasis on host specificity and morphologicalcharacters. *Mol Phylogenet Evol.* 38(3): 794-807. https://doi.org/10.1016/j.ympev.2005.10.008

Morris, G.M., Huey, R., Lindstrom, W., et al. (2009). AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 30(16):2785–2791.

Mowry, J.B., Spyker, D.A., Cantilena, L.R. Jr., Bailey, J.E., Ford, M. (2013). 2012 annual report of the american association of poison control centers' national poison data system (NPDS): 30th annual report. *Clin Toxicol.* 51(10): 949– 1229.

Mullis, K.B., Faloona, F.A. (1987). Specific synthesis of DNA in vitro via a polymerase- catalyzed chain reaction. *Methods Enzymol.* 155: 335. https://doi.org/10.1016/0076-6879(87)55023-6

Munyaneza, E., Bizuru, E., Degreef, J. (2017). Diversity and ecology of wild mushrooms of Riparian zone of Lake Kivu, Rwanda (Doctoral dissertation, University of Rwanda).

Muszyńska, B., Sułkowska-Ziaja, K., Ekiert, H. (2013a). Phenolic acids in selected edible basidiomycota species: *Armillaria mellea*, *Boletus badius*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus* and *Pleurotus ostreatus*. *Acta Sci Pol Hortorum Cultus.* 12:107–116.

Muthoni G.B., Machocho, A.K., Mwihia, S.K., Ngugi, M.P. (2020). In vitro

antioxidant activities of methanolic extracts of *Caesalpinia volkensii* Harms., *Vernonia lasiopus* O. Hoffm., and *Acacia hockii* de wild. *Evid-Based Complementary Altern Med*. 2020:1–10.

Mwangi, R.W., Macharia, J.M., Wagara, I.N., Bence, R.L. (2022). The antioxidant potential of different edible and medicinal mushrooms. *Biomed Pharmacother*. 147:112621.

Nagavani, V., Madhavi, Y., Rao, D. B., Rao, P. K. and Rao, T. R. (2010). Free radical scavenging activity and qualitative analysis of polyphenols by RP-HPLC in the flowers of *Couroupita guianensis* Abul. *Electronic Journal of Environmental, Agricultural & Food Chemistry*. 9(9): 1471-1484.

Nayak, D.C. and Srivastava R. (1995). Soils of shifting cultivated areas in Arunachal Pradesh and their suitability for land use planning. *Journal of India Society of Soil Science.* 43:246-251.

Nakalembe, I., Kabasa, J.D., Olila, D. (2015). Comparative nutrient composition of selected Wild edible mushroom from two agro-ecological zones, Uganda. *Springerplus*. 4(1):433.

Nofiani, R. (2008). Urgensi dan mekanisme biosisntesis metabolit sekundermikroba laut. *Jurnal Natur Indonesia.* 10 (2): 120-125. DOI:10.31258/jnat.10.2.120-125

Nováková, L., Vlcková, H. (2009). A review of current trends and advances in modern bio-analytical methods: Chromatography and sample preparation. *Anal. Chim. Acta*. 656: 8–35. https://doi.org/10.1016/j.aca.2009.10.004

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H. (2022). vegan: Community Ecology Package. R package version 2.6-1. Available online at:https://CRAN.R-project.org/package=vegan

Okwulehie, I.C., Odunze, E.I. (2004). Evaluation of the nutritional value of some tropical edible mushrooms.

Olsen, S.R., Cole, C.V., Watanabe, F.S. and Dean, L.A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dep. of Agric. Circ. 939.

Osarenkhoe, O., John, O., Theophilus, D. (2014). Ethnomycological conspectus of

West African mushrooms: An AwarenessDocument. *Advances in Microbiology.* 4: 39-54.

Oscar, S., Antonio, C., Marina, G., Elsa, R., Gabriel, V. (2020). Phytochemical screening, antioxidant activity, and in Vitro biological evaluation ofleaf extracts of *Hyptis suaveolens* (L.) Poit. *South Afr J Biol.* 128: 62-66. DOI: 10.1016/j.sajb.2019.10.016

Oso, B.A., (1977). *Pleurotus tuber-regium* from Nigeria. *Mycologia*. 69(2): 271-279.

Oyetayo, F. L. (2007). Potential antioxidant properties of Nigerian edible mushrooms. *Agro Food Industry, Hi-Tech.* 18: 44–45.

Ouzouni, P.K., Petridis, D., Koller, W.D., Riganakos, K.A. (2009). Nutritional value and metal content of Wild edible mushrooms collected from West Macedoniaand Epirus, Greece. *Food Chem.* 115(4):1575–80.

Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M.A., Martínez, J.A., Garcia-Lafuente, A., Guillamon, E., Villares, A. (2011). Antioxidant properties of phenolic compounds occurring in edible mushrooms. *Food Chem*. 128(3):674–8.

Panda, M.K., Thotai, H.N., Sahu, S.C., Tayung, K. (2019). Wild edible mushrooms of Northern Odisha, India: data on distribution and utilization by ethnic communities. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*. 5(2): 248-268.

Pandey, V.V., Kumari, A., Kumar, M., Saxena, J., Kainthola, C., Pandey, A. (2018). Mushroom cultivation: Substantial key to food security. *J. Appl. Nat. Sci*. 10(4):1325–31.

Parajuli, S., Pun, N. T., Parajuli, S. and Jamarkattel-Pandit, N. (2012). Antioxidant Activity, Total Phenol and Flavanoid Contents in Some Selected Medicinal Plants of Nepal. *Journal of Health and Allied Sciences*. 2(1): 27-31.

Parnmen, S., Nooron, N., Leudang, S., et al. (2021). Foodborne illness caused by muscarine-containing mushrooms and identification of mushroom remnants using phylogenetics and LC-MS/MS. *Food Control*.128: 108-182.

Rajarathnam, S. and Sashirekha, M.N. (2003). Mushrooms and truffles / Use of wild mushroom. In Trugo L & Finglas PM. *Encyclopedia of food sciences*

and nutrition. Elsevier Science Ltd. 4048.

Rambelli, A., Menini, U. G. (1983). Manual on mushroom cultivation. https://www.cabidigitallibrary.org/doi/full/10.5555/19840510646

Rao, R.S. and Panigarhi, G. (1961). Distribution of vegetational types and their dominant species in Eastern Himalaya. *J. Indian Bot. Soc.* 40:274-285.

Ratiknyo, H., Ahmad, I. and Budiyanto, B.H. (2018). Diversity and abundance of termites along altitudinal gradient and slopes in Mount Slamet, Central Java,

Indonesia. *Biodiversitas Journal of Biological Diversity*. 19(5): 1649-1658.

Rattan, S. S. (1977). The resupinate Aphyllophorales of the North Western Himalayas. Bib. Myco. 60: 427.

Reinhard, T. (2000). "Lokales Cyclosporin A bei Nummuli nach Keratoconjunctivitis epidemica Eine Pilotstudie - Springer". *Der Ophthalmologe*. 97(11): 764– 768. doi:10.1007/s003470070025

Reis, F.S., Barros, L., Martins, A., Ferreira, I.C.F.R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food Chem Toxicol*. 50(2):191–7.

Reshetnikov, S.V., Wasser, S.P. and Tan, K.K. (2004). Higher Basidomycetes as a source of antitumor and immunostimulating polisacharides. Review. *Int. J. ofMedical Mushrooms*.6(2):185-190.

Rittgen, J., Putz, M., Pyell, U. (2008). Identification of toxic oligopeptides in *Amanita* fungi employing capillary electrophoresiselectrospray ionization-mass spectrometry with positive and negative ion detection. *Electrophoresis*. 29: 2094–2100. https://doi.org/10.1002/elps.200800055

Rizal, L.M., Hyde, K.D., Karunarathna, S.C., Chukeatirote, E. and Chamyuang, S. (2017). Additions to the Knowledge of Macrolepiota (Agaricales, Basidiomycota) in Northern Thailand. *Bhutan Journal of Natural Resourcesand Development*, *4*(1): 12-21.

Roberts, D.W. (2020). Comparison of distance-based and model-based ordinations. *Ecology*. 101(1):1–12. https://www.jstor.org/stable/26870929

Rouland-Lefevre, C., Diouf, M.N., Brauman, A, et al., (2002). Phylogenetic

relationships in *Termitomyces* (family Agaricaceae) based on thenucleotide sequence of ITS: a first approach to elucidate the evolutionary history of thesymbiosis between fungus growing termitesand their fungi. *Molecular Phylogenetics and Evolution*. 22: 423-429.

Rumack, B.H. and Spoerke, D.G. (1994). Handbook of mushroom poisoning: diagnosis and treatment. CRC press.

Saeed, N., Khan, M.R., Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern Med*. 12(1):221.

Salerni, E., Laganà, A., Perini, C., Loppi, S. and Dominics, V.D. (2002). Effects of temperature and rainfall on fruiting of macrofungi in oak forests of the Mediterranean area. *Israel Journal of Plant Sciences*. 50(3): 189-198.

Sánchez-Ballesteros, J., González, V., Salazar, O., Acero, J., Portal, M.A., Julián, M., Rubio, V., Bill, G.F., Platas, G., Mochales, S., Peláez, F. (2000). Phylogenetic study of Hypoxylon and related genera based on ribosomal ITS sequences. *Mycologia*. 92: 964-977. https://doi.org/10.1080/00275514.2000.12061240

Sanmee, R., Dell, B., Lumyong, P., Izumori, K. and Lumyong, S. (2003). Nutritive value of popular wild edible mushrooms from northern Thailand. *Food chemistry*. 82(4): 527-532.

Sarma, T.C., Sarma, I., Patiri, B.N. (2010). Wild edible mushrooms used by some ethnic tribes of western Assam. *The Bioscan*. 3(Specialissue): 613-625.

Schleinkofer, K., Wang, T. and Wade, R.C. (2006). Molecular docking. Encycl Ref Genomics Proteomics. *Mol Med*. 443: 1149-1153. https://doi.org/10.1007/3-540-29623-9_3820

Schmiedeberg, O. (1869). Das Muscarin: das giftige Alkaloid des Fliegenpilzes (*Agaricus muscarius* L.): seine Darstellung, chemischen Eigenschaften, physiologischen Wirkungen, toxicologische Bedeutung und sein Verhältnisszur Pilzvergiftung im allgemeinen. *FCW Vogel*.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. (2012). Fungal Barcoding Consortium (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode

marker for Fungi. *Proc Natl Acad Sci* USA.109:6241-624.

Seal, T. (2014). Antioxidant activities of some wild vegetables of north-eastern regionin india and effect of solvent extraction system. *Int J Pharm Pharm Sci*. 6(5):315–9.

Seifert, K.A., Samson, R.A., DeWaard, J.R., Houbraken, J., Levesque, C.A., Moncalvo, J.M., Hebert, P.D.N. (2007). Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. *Proc Natl Acad Sci.* 104:3901-3906.

Séleck, M., Bizoux, J.P., Colinet, G., Faucon, M.P., Guillaume, A., Meerts, P., Piqueray, J., Mahy, G. (2013). Chemical soil factors influencing plant assemblages along copper-cobalt gradients: implications for conservation andrestoration. *Plant and soil*. 373: 455-69.

Semwal, K.C., Lemma, H., Dhyani, A., Equar, G., Amhare, S. (2014). Mushroom: Nature›s Treasure in Ethiopia. *MEJS.* 6(2): 138-147.

Senthilarasu, G. (2014). Diversity of agarics (gilled mushrooms) of Maharashtra. *India.Curr. Res. Environ*. 4(1): 58-78. https://doi.org/10.5943/cream/4/1/5

Sensi, P. (1983). History of the development of rifampin. *Reviews of Infectious Diseases*. 5 (Suppl 3): S402–S406. doi: 10.1093/clinids/5.supplement_3. s402. JSTOR 4453138. PMID 6635432.

Sgambelluri, R.M., Epis, S., Sassera, D., Luo, H., Angelos, E.R., Walton, J.D. (2014). Profiling of Amatoxins and Phallotoxins in the Genus *Lepiota* by Liquid Chromatography Combined with UV Absorbance and Mass Spectrometry. *Toxins*. 6: 2336–2347. https://doi.org/10.3390/toxins6082336

Sharma, N., Behera, M.D., Das, A.P., Panda, R.M. (2019). Plant richness pattern in an elevation gradient in the Eastern Himalaya. *Biodivers. Conserv*. 28: 2085– 2104. https://doi.org/10.1007/s10531-019-01699-7.

Singh, M. P., Singh, B. S. and Dey, S. (2002). Flora of Mizoram.Vol. I. shiva Offset Press, Dehradun, India.

Singh, R. P., Pachauri, V., Verma, R. C., & Mishra, K. K. (2008). Caterpillar fungus (Cordyceps sinensis)-a review.

Singh, R. P., Rupesh, K. A., & Pachauri, V. I. N. E. E. T. A. (2009). Cordyceps

sinensis: Their collection, characterization and medicinal componenets. In Proceedings of the 5th International medicinal mushroom conference. Sept. 5-8.

Singh, R. P., Verma, R. C., Arora, R. K., Mishra, K. K., Bhanu, C., & Singh, M. (2007). Medicinal mushrooms of Uttaranchal with reference to *Ganoderma*, *Auricularia* and *Cordyceps sinensis*. Mushroom Biology and Biotechnolgy, eds Rai RD, Singh SK, Yadav MC and Tewari RP. Mushroom Society of India, 321-324.

Sitotaw, R., Mulat, A., Abate, D. (2015). Morphological andmolecular studies on *Termitomyces* species of Mengedistrict, Assosa Zone, Northwest Ethiopia. *Sci TechnolArts Res J*. 4: 49-57. https://doi.org/10.4314/star.v4i4.7

Smith, S.E. and Read, D.J. (2010). *Mycorrhizal symbiosis*. Academic press.

Sontag, G., Pinto, M.I., Noronha, J.P., Burrows, H.D. (2019). Analysis of Food by High Performance Liquid Chromatography Coupled with Coulometric Detection and Related Techniques: A Review. *J. Agric. Food Chem*. 67: 4113–4144.

Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I. and Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular mechanisms ofmutagenesis*. 579(1-2): 200-213.

SPSS Inc.: SPSS for Windows Release 14.0.1. SPSS Inc. Chicago, IL, USA, 2005.

Srikram, A. and Supapvanich, S. (2016). Proximate compositions and bioactive compounds of edible wild and cultivated mushrooms from Northeast Thailand. *Agriculture and Natural Resources.* 50(6): 432-436.

Steinkraus, K.H. (1997). Classification of fermented foods: worldwide review of household fermentation techniques. *Food Control*. 8(5-6): 311-317.

Stamets, P. (1983). Growing gourmet and medicinal mushrooms. Ten speed press. Hong Kong.

Sterling, T.R., Njie, G., Zenner, D., Cohn, D.L., Reves, R., Ahmed, A., et al. (2020). Guidelines for the Treatment of Latent Tuberculosis Infection: Recommendations from the National Tuberculosis Controllers Association and CDC, 2020. MMWR. Recommendations and Reports. *Centers for*

Disease Control, Atlanta, GA, USA. 69 (1): 1–11.

Stojanova, M., Pantić, M., Karadelev, M., Čuleva, B., Nikšić, M. (2021). Antioxidantpotential of extracts of three mushroom species collected from the Republic of North Macedonia. *J Food Process Preserv*. 45(2): e15155.

Stojković, D., Reis, F.S., Ferreira, I.C., Barros, L., Glamočlija, J., Ćirić, A., Nikolić, M., Stević, T., Giveli, A. and Soković, M. (2013). Tirmania pinoyi: Chemical composition, in vitro antioxidant and antibacterial activities and in situ control of Staphylococcus aureus in chicken soup. *Food research international*. 53(1): 56-62. https://doi.org/10.1016/j.foodres.2013.03.046

Stojković, D.S., Kovačević-Grujičić, N., Reis, F.S., Davidović, S., Barros, L., Popović, J., Petrović, I., Pavić, A., Glamočlija, J., Ćirić, A. and Stevanović,

M. (2017). Chemical composition of the mushroom Meripilus giganteus Karst. and bioactive properties of its methanolic extract. *LWT-Food Science and Technology*. 79: 454-462. https://doi.org/10.1016/j.lwt.2017.01.045

Stuart, M.C., Kouimtzi, M., Hill, S.R., eds. (2009). WHO Model Formulary 2008. *World Health Organization*. 53–54.

Tambe, S., Arrawatia, M.L., Sharma, N. (2011). Assessing the Priorities for Sustainable Forest Management in the Sikkim Himalaya, India: A Remote Sensing Based Approach. *J. Indian Soc. Remote Sens*. 39: 555–564. https://doi.org/10.1007/s12524-011-0110-6.

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*. 38(7): 3022-7. https://doi.org/10.1093/molbev/msab120

Tang, S.S., Zhou, Q., He, Z.M. (2016). Cyclopeptide toxins of lethal amanitas: Compositions, distribution and phylogenetic implication. *Toxicon*. 120: 78– 88.

Tanti, B., Lisha, G., Chandra, S.G. (2011). Wild edible fungal resources used by ethnictribes of Nagaland, India. *Indian Journal of TraditionalKnowledge*. 10(3): 521-515.

Teke, A., Bi, M., Ndam, L., Kinge, T. (2021). Nutrient and mineral components of Wild edible mushroom from the Kilum-Ijim forest, Cameroon. *Afr J Food*

Sci. 15(4):152–61.

Thiribhuvanamala, G. U. R. U. D. E. V. A. N., Prakasam, V., Chandrasekar, G., Sakthivel, K., Veeralakshmi, S., Velazhahan, R., & Kalaiselvi, G. (2011, October). Biodiversity, conservation and utilization of mushroom flora from the Western Ghats region of India. In Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7). 155-164.

Tibuhwa, D.D. (2013). Wild mushroom-an underutilized healthy food resource and income generator: experience from Tanzania rural areas. *Journal of ethnobiology and ethnomedicine*. 9:1-14.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efcient optimization, and multithreading. *Journal of Computational Chemistry*. 31: 455–461.

Tuckwell, D.S., Nicholson, M.J., McSweeney, C.S., Theodorou, M.K., Brookman, J.L. (2005) The rapid assignment of ruminal fungi to presumptive genera using ITS1 and ITS2 RNA secondary structures to produce group-specific fingerprints. *Microbiology.*151: 1557-1567. https://doi.org/10.1099/mic.0.27689-0

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines.* 5: 93. https://doi.org/10.3390/medicines5030093

Tuno, N. (2001). Mushroom utilization by the Majangir, an Ethiopian tribe. *Mycologist*. 15(2): 78-79. https://doi.org/10.1016/S0269-915X(01)80087-2 Urbanelli, S., Della Rosa, V., Punelli, F., Porretta, D., Reverberi, M., Fabbri, A.A.,

Fanelli, C. (2007) DNA-fingerprinting (AFLP and RFLP) for genotypic identification in species of the *Pleurotus eryngii* complex. *Appl Microbiol Biotechnol.* 74(3): 592-600. https://doi.org/10.1007/s00253-006-0684-z

Valverde, M.E., Hernández-Pérez, T. and Paredes-López, O. (2015). Edible mushrooms: improving human health and promoting quality life. *International journal of microbiology*. 2015.

Van de Putte, K. (2012). *Hidden diversity exposed: A case study of Lactifluus volemussensu lato*. PhD thesis, Biology, Ghent University, Ghent.

Van Griensven, L.J. (2009). Culinary-medicinal mushrooms: Must action be taken?. *International Journal of Medicinal Mushrooms*. 11(3): 281. https://doi.org/10.1615/IntJMedMushr.v11.i3.70

Verma, B., Prasad, P., Sahu, K. (2013). Mushrooms: edible and medicinal: cultivation, conservation, strain improvement with their marketing. *Daya publishinghouse*.

Vetter, J. (1988). Toxins of *Amanita phalloides*. *Toxicon*. 36 (1998): 13-24.

Vishniac, HS. (1977). Methods for the study of terrestrial microalgae and cyanobacteria. *In CRC Handbook of Microbiology*. 257-272. CRC Press.

Vishniac, H.S., Largent, D.L., Thiers, H.D., Largent, D., Johnson, D., Watling, R. (1978). How to identify mushrooms to genus. I: macroscopic features. *Bulletin of the torrey botanical Club*. 105(3):240.

Vishwakarma, M.P., Bhatt, R.P., Gairola, S. (2011). Some medicinal mushrooms of Garhwal Himalaya, Uttarakhand, India. *InternationalJournal of Medicinal and Aromatic Plants*. 1(1): 33-40.

Vishwakarma, P., Singh, P. and Tripathi, N.N. (2017). Diversity of macrofungi and its distribution pattern of Gorakhpur District, Uttar Pradesh, India. *Studies in Fungi*, *2*(1): 92-105.

Walkley, A. and Black, I.A. (1934). An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci*. 37: 29-37.

Wakshull, E., Brunke-Reese, D., Lindermuth, J., Fisette, L., Nathans, R.S., et al. (1999). PGG-glucan, asoluble beta-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kappa B-like factor in human PMN: evidence for a glycosphingolipidbeta-(1,3)-glucan receptor. *Immunopharmacology*. 41(1999): 89.

Ward, D.M., Weller, R., Bateson, M.M. (1990). 16S rRNA sequences reveal numerousuncultured microorganisms in a natural community. *Nature*. 344: 63-65.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., Van Der Putten, W.H.

and Wall, D.H. (2004). Ecological linkages between aboveground and belowground biota. *Science*. 304(5677): 1629-1633.

Wasser, S.P. and Weis, A.L. (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Critical Reviews™ in Immunology*. 19(1).

Wasser, S.P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60, 258– 274. https://doi.org/10.1007/s00253-002-1076-7

Wasser, S.P. (2010). Medicinal mushroom science: history, current status, future trends, and unsolved problems. *International journal of medicinal mushrooms*. 12(1). https://doi.org/10.1615/IntJMedMushr.v12.i1.10

Wasser, S.P. (2011). Current findings, future trends, and unsolved problems in studiesof medicinal mushrooms. *Applied microbiology and biotechnology*. 89: 1323-1332. https://doi.org/10.1007/s00253-010-3067-4

Webster, J. and Weber, R. (2007). Introduction to fungi. Cambridge university press.

White, T.J., Bruns, T., Lee, S.J., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide tomethods and applications*. 18(1): 315-22.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4. Available online at: https://ggplot2.tidyverse.org

Wieland, T. (1986). Non-Toxic Peptides from Amanita phalloides. In: Peptides of Poisonous Amanita Mushrooms. *Springer Series in Molecular Biology*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-71295-1_9

Wieland, T. (1984). Progress in Tryptophan and Serotonin Research. New York, Walter de Gruyter & Co, Berlin.

Wieland, T. (1983). The toxic peptides from Amanita mushrooms. *Int J Pept Protein Res*. 22 (1983): 257-276.

William, S. (1984). Official methods of analysis of the association of official analyticalchemists. *Association of Official Analytical Chemists*, Inc.

Woo, T.M., Robinson, M.V. (2015). Pharmacotherapeutics For Advanced Practice

Nurse Prescribers. *F.A. Davis*. 651. ISBN 9780803645813.

Xu, J., Yang, Z.L., Zhang, Y., Yu, Z. and Zhang, K. (2011). diversity and population biology of wild mushrooms from southwestern china. In Mushroom biology and mushroom products. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products, Arcachon, France, 4-7 October, 2011. Volume 1. Oral presentations (pp. 77-90). Institut National de laRecherche Agronomique (INRA).

Yang, Z.L. (2002). On wild mushroom resources and their utilization in Yunnan Province, Southwest China. *Journal of Natural Resources.* 17: 463–469.

Yildiz, O., Can, Z., Laghari AQ et al. (2015). Wild edible mushrooms as a natural source of phenolics and antioxidants. *J Food Biochem.* 39:148–154.

Yoshioka, N., Akamatsu, S., Mitsuhashi, T., et al. (2014). A simple method for the simultaneous determination of mushroom toxins by liquid chromatography- time-of-flight mass spectrometry. *Forensic Toxicol*. 32: 89–96.

Zervakis, G., Philippoussis, A., Ioannidou, S., Dlamantopoulou, P. (2001). Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates. *Folia Microbiol.*46(3): 231-234. https://doi.org/10.1007/BF02818539

Zhang, P., Chen, Z., Hu, J., Wei, B., Zhang, Z. and Hu, W. (2005). Production and characterization of amanitin toxins from a pure culture of *Amanita exitialis*. *FEMS Microbiology Letters*. 252(2): 223-228.

Zheleva, A. (2013). Phenoxyl radicals formation might contribute to severe toxicity of mushrooms toxin alpha-amanitin-an electron paramagnetic resonance study.*TJS.* 11(1): 33–38.

Zheleva, A., Tolekova, A., Zhelev, M., Uzunova, V., Platikanova, M., Gadzheva, V. (2007). Free radical reactions might contribute to severe alpha amanitin hepatotoxicity-a hypothesis. *Med Hypotheses.* 69(2): 361–36.

Zotti, M., Persiani, A.M., Ambrosio, E., Vizzini, A., Venturella, G., Donnini, D., Angelini, P., Di Piazza, S., Pavarino, M., Lunghini, D. and Venanzoni, R. (2013). Macrofungi as ecosystem resources: Conservation versus exploitation. *Plant Biosys*. 147(1): 219-225.

BRIEF BIO-DATA OF THE CANDIDATE

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Research Interest: Mycology, Amatoxins, Nutrition, Antioxidant, Phytochemistry.

PERSONAL DETAILS

Father's Name	:	C. Lalzuiliana (L)
Mother's Name	:	Kaphrangi
Date Of Birth	:	29th January, 1987
Gender	:	Male
Marital Status	:	Married
Nationality	:	Indian
Languages Known	:	English, Mizo
Permanent Address	:	D-114, Vengthlang, Champhai district, Mizoram-796321
Present Address	:	LV/D-10, Leitan Ramthar, Durtlang, Mizoram-796001

Qualification	Passing Year	University/ Institution	Percentage/ Grade	Division
HSLC (X)	2004	Govt. Champhai High School	49	Third
HSSLC (XII) (Physics, Chemistry, Biology)	2007	Govt. G.M. Higher Secondary School, Champhai	54.6	Second
B. Sc (Botany, Zoology, Chemistry)	2008	Pachhunga University College, Aizawl	69.75	First
M.Sc (Botany)	2010	Mizoram University, Tanhril	64.44	First
CCC	2022	National Institute of Electronicsand Information Technology (NIELIT), Aizawl	A	-

List of publications

1. Malsawmtluanga, C.D., Lalbiaknunga, J., Lalawmpuia and Laldinkima, C. (2024). Detection and quantification of Amatoxin in wild mushrooms from North-East India using HPLC-PDA method for food safety purposes. *Toxicon.* 251:108134.

2. Malsawmtluanga, C.D., Lalbiaknunga, J., Thangliankhup, K. and Lalrinmuana. (2023). Proximate analysis, mineral contents, and antioxidant activities of wild edible mushrooms from India. *International journal of medicinal mushrooms.*25(8): 73-85.

3. Phairong, M.M., Lalnunmawia, F., Lalbiaknunga, J. and Malsawmtluanga, D. (2022). Preliminary phytochemicals screening and estimation of total nutritional content in different varieties of mizo chilli (*Capsicum frutescens*). *Res. Jr. of Agril. Sci.*13(5):1350-135.

List of Presentations

1. Presented paper on "Study on allelopathic effect of selected weeds on *Zea mays* and *Phaseolus vulgaris*" in *Science and technology for shaping the future of Mizoram,Mizoram Science Congress,* 2016.

2. Presented paper on "Proximate analysis and Nutritional composition of eight common WEMs of champhai district" at *National conference on Biodiversity, Conservation and Utilization of Natural Resources with reference to Northe East India (BCUNRNEI)* (30-31 March, 2017).

3. Presented paper on "Ecology and diversity of wild edible mushrooms in Champhai district of Mizoram, India" at the national conference on *Recent advances in plant biology with special references to north-east, India,* organized by Departmentof Botany, School of Life Sciences, Mizoram University from april 20-21, 2023.

4. Presented paper on "Deadly amatoxin: detection and quantification of α-amanitin in wild mushrooms using HPLC-PDA method for food safety purposes" at *international conference on conservation of biodiversity in genomic era,* held at Pachhunga University College, Aizawl during 22-23, march 2024 and won first prize.

Conferences/ Seminars/ Workshops Participated

1. Participated in National workshop on "Statistical and computing method for life science data analysis" organized by The Biological Anthropology Unit, Indian Statistical Institute, Kolkata (05-10, March, 2018) at Department of Botany, Mizoram University.

2. Participated in "International faculty development programme on mathematical modelling of biosystems with special focus on epidemiology" held at Mizoram University during 22-27 August, 2022.

3. Participated in national seminar on "Biodiversity, conservation, utilization and commercialization of medicinal and aromatic plants" held during february 08-09, 2024organized by Department of Botany, Mizoram University.

4. Attended "Two-Days Hands-On Workshop on Geographic Information System (GIS) using QGIS" on 16^{th} – 17^{th} November, 2023 organized by Developmental Biology & Herpetology Laboratory, Department of Zoology.

5. Attended two days "Skill development program on organic farming for farmers and entrepreneurs of north east hilly (NEH) region, India" jointly organized by ICAR-National Bureau of Agriculturally Important Microorganism (NBAIM), Kushmaur, Mau, U.P. -275103 and Department of Botany, School of Life Sciences, Mizoram University, Aizawl during 27-28 March, 2024.

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DEGREE : Ph.D

DEPARTMENT : Botany

TITLE OF THESIS :**Identification and quantification of mycotoxins from selected families of agaricales found in champhai district, Mizoram, India.**

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ABSTRACT

IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS FROM SELECTED FAMILIES OF AGARICALES FOUND IN CHAMPHAI DISTRICT, MIZORAM, INDIA

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

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DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

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ABSTRACT

IDENTIFICATION AND QUANTIFICATION OF MYCOTOXIN FROM SELECTED FAMILIES OF AGARICALES FOUND IN CHAMPHAI DISTRICT, MIZORAM, INDIA

BY

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Submitted

In partial fulfilment of the requirement of the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl.

Abstract

The fungal realm, encompassing a diverse array of organisms with varied forms and functions, plays a pivotal role in shaping the ecological equilibrium of our planet. Despite their often-overlooked presence, fungi are essential for life, contributing to nutrient cycling, symbiotic relationships, and diverse industrial applications. This thesis delves into the intricate domain of wild edible mushrooms (WEMs), concentrating on their diversity, biology, ecological roles, economic importance, and the unique structures of mushrooms. Specifically, it explores the nutritional value and antioxidant properties of WEMs, the potential hazards associated with poisonous mushrooms, and the application of molecular techniques in mycology. Before this investigation, a comprehensive study of WEMs in Champhai district, Mizoram, had not been conducted. This gap included an examination of WEMs diversity, determination of their nutritional value, assessment of antioxidant activities, identification of poisonous mushrooms, and the discovery of effective antidotes for mushroom poisoning. Therefore, the present study titled "Identification and quantification of mycotoxins from selected families of Agaricales found in Champhai district, Mizoram, India" was designed with the following objectives.

1. To investigate the diversity and factors influencing the growth and distribution of wild edible mushrooms (WEMs) in Champhai district.

2. To analyse the proximate composition, mineral content and antioxidant activities of wild edible mushrooms.

3. To identify and quantify mycotoxins present in wild mushrooms of Champhai district.

4. In silico molecular docking of potent antidodes for treatment of Amatoxins.

The fruiting bodies of mushrooms specimen were collected from Champhai district, located 196 kilometers (23.4454 ^{0}N and 93.1780 ^{0}E) from Aizawl, Mizoram, India. The district occupies an area of 3,185.83 km2. For this study, six different forests (Zote, Tlangsam, Ngur, Mualkawi, Khuangleng and Vangchhia forests) were selected for the collection of WEMs during 2018-2020. These forests were selected because

they are the primary sources of WEMs sold in the markets of Champhai. The sampling sites were selected based on a preliminary survey of each forest to locate areas with a diverse array of WEMs. Random sampling method was used to survey a total of 1.5-hectare area (10m x 10m) with 150 total plots of 0.01 ha each in six selected forest communities that occurred along altitudinal gradient from 800-1800 meters.

To gather data on mushroom species diversity, stratified random sampling using quadrat method was employed. Mushrooms were collected from various habitats such as grazing fields, decaying wood, soils, and termite mounds in the forested areas. Field characteristics, including habit, habitat, colour, measurements, and photographs, were documented for identification purposes, based on fresh materials. A total of 3500 individual WEMs belonging to 9 families and 18 genera were recorded. Russulaceae emerges as the most commonly found family, comprising seven species. The Ngur forest emerges with the highest species richness (15 species), followed by Zote forest (13 species). Pielou's evenness (J) and Shannon diversity index (H') provide insights into the distribution and diversity of species among the forests, emphasizing Ngur forest's richness and diversity.

Altitudinal variations significantly influence the distribution of common WEMs. Species like *Lactifluus corrugis*, *Lactifluus piperatus*, *Russula subfragilliformis*, *Russula adusta*, *Termitomyces heimii*, and *Macrolepiota dolichaula* exhibit preferences for higher altitudes (mean altitude > 1000 meters), with *T. heimii* being an exception, thriving at a mean altitude of 972.4 meters. This observation aligns with prior research attributing high species diversity at higher altitudes to factors such as low temperature, high relative humidity, and soil moisture.

The study correlates WEMs fruiting with temperature and rainfall patterns over a three-year period. The abundance of *Schizophyllum commune*, *Lactifluus corrugis*, *Lactarius piperatus*, *Russula subfragilliformis*, *Macrolepiota dolichaula*, *Lentinus polychrous*, *Termitomyces heimii*, and *Russula adusta* varies across the six locations. The peak number of species is observed in 2018 during June, coinciding with the monsoon season characterized by mild temperatures (mean 23.4°C) and abundant rainfall (262.5 mm). Winter months, characterized by colder temperatures and less

abundant rainfall, see a decline in species presence, emphasizing the role of environmental factors in WEM fruiting.

The physico-chemical analysis of soil across three years provides valuable insights into the soil conditions conducive to WEMs growth. The soil in the selected WEMs harvesting sites is characterized by its acidic nature, with moisture content ranging from 11.41% to 13.48%. pH levels range from 4.53 to 6.57, indicating an acidic reaction. Nitrogen content ranges from 0.73% to 0.89%, while available phosphorus levels remain consistently low. Soil organic carbon content and organic matter are notably high, contributing to a conducive environment for WEMs.

The non-metric multidimensional scaling (NMDS) graph illustrates the association between selected WEMs and specific tree species across diverse forests. The ordination plot reveals strong associations between certain trees, such as *Lithocarpus dealbatus* and *Quercus serrata*, and WEMs like *Lactifluus corrugis*, *Lactarius piperatus*, *Russula subfragiliformis*, and *Russula adusta*. The connection between specific tree species and WEMs underscores the intricate relationship between these fungi and their surrounding ecosystem, providing valuable insights into their microhabitat specificity.

Eight common WEMs were selected for their proximate composition, mineral contents and antioxidant activities using standard protocols. The nutritional values were evaluated on a dry weight basis, and the mushrooms showed high total protein (19.53±0.20-64.01±0.10 g/100g) and total carbohydrate (6.17±0.22-53.53±0.15 g/100g) contents, but low-fat contents (2.24±0.14-5.83±0.15 g/100g). The most abundant mineral in all the wild edible mushrooms was potassium, which ranges from 2411.59±3.70-1033.29±9.57 mg/100g. In the principal component analysis between multivariate data information (nutrients and minerals), 95.8% of the variance was retained by the first four principal components. The first and second principal components were related to most of the nutrient and mineral contents. The methanolic extracts on selected mushrooms revealed that they all have good antioxidant capacity with DPPH (IC_{50}=31±0.29-144±0.25 µg/ml) and ABTS (IC_{50}=42.2±0.36-86.55±0.30 µg/ml). Strong DPPH and ABTS radical scavenging activity were observed in

mushroom extracts containing more phenols than flavonoid, which suggested that phenolic compounds might be the main antioxidant components present in mushroom extracts, which contributed to their high antioxidant activity. The current study revealed that wild edible mushrooms could be a good source of nutritional and antioxidant components. The results of this study can therefore be used to promote local consumption of mushrooms as functional foods and for commercial purposes while also promoting their habitat preservation. It is worth noting that this was the first study on the proximate composition, mineral content and antioxidant properties of Mizoram's wild edible mushrooms.

Misidentification and ingestion of poisonous mushrooms is the main factors leading to foodborne death in Mizoram, north-eastern part of India. Due to its morbidity and mortality, mushroom poisoning continues to be a severe food safety and health concern in several regions of the world. More than 10 cases of fatality from mushroom poisoning have been reported in Mizoram, India, over the past ten years (2013-2023). The present study aims at the identification and quantification of toxic compounds, α-amanitin and muscarine, in wild mushroom species from Champhai district, Mizoram, India using HPLC-PDA. The results of the analysis revealed the presence of α-amanitin only in the samples obtained from *Amanita virosa* (1.17 mg g^{-1}) and *Amanita bisporigera* (1.19 mg g^{-1}) species. On the other hand, Muscarine were present in *Amanita pantherina* (0.007mg g^{-1}) followed by *Amanita pseudoporphyria* (0.045mg g^{-1}), emphasizing the need for proper identification before consumption. The optimized chromatographic conditions and calibration curves demonstrated the effectiveness of the HPLC-PDA method in quantifying the toxins. To summarize, the analytical approach could assist food safety assessment and contribute to food-health security because it is quick, simple, sensitive, accurate, and identifies α-amanitin and muscarine in any mushroom sample.

Mushroom poisonings, particularly those induced by *Amanita* species, represent a serious health threat with high fatality rates. This study focuses on elucidating the binding interactions between potential antidotes and crucial proteins, namely Organic Anion-Transporting Octapeptide (OATP1B3) and RNA Polymerase II (RNAP II),

associated with the toxicity of *Amanita* mushrooms. The explored compounds include Rifampin, Prednisolone, Cyclosporine, Silibinin, and Polymyxin B.

Rifampin, an ansamycin antibiotic, is widely used for bacterial infections, notably tuberculosis. Molecular docking studies revealed a robust binding affinity between Rifampin and OATP1B3. The complex's three-dimensional orientation, showcased conventional hydrogen bonding with key residues Tyr625, Gln422, Gln541, Ser545, and Ser548. Dipole-dipole interactions with Arg633 further underscored the binding. The binding free energy reached a notable low of -12.79 Kcal/mol after 9 runs, indicating a strong inhibitory potential (Table 7.1).

Prednisolone, a corticosteroid with diverse medical applications, exhibited promising interactions with OATP1B3. The binding analysis revealed a lowest observed binding free energy of -8.46 Kcal/mol after 108 runs. Noteworthy interactions included conventional hydrogen bonding with Tyr425, Gln541, Asn544, and Tyr625. Pi-alkyl group interactions with Phe352 and Phe356, along with a carbon-hydrogen bond with Ser545, further highlighted Prednisolone's potential efficacy as an antidote.

Cyclosporine, renowned as an immunosuppressant, displayed intricate interactions with OATP1B3. The binding analysis revealed a low of -8.03 Kcal/mol after 125 runs. Key interactions included a conventional hydrogen bond with Lys49, alkyl group interactions with Tyr422, Ala355, Met217, and Pro220, and a pi-carbon bond with Tyr352 and Phe356. The results suggested a nuanced molecular dance, emphasizing Cyclosporine's potential as an antidote.

Silibinin, derived from milk thistle, showcased its potential in the treatment of Amanita mushroom poisoning. With a docking score of -6.92 Kcal/mol achieved in 61 runs, Silibinin exhibited inhibitory constants of 8.46 µM. Conventional hydrogen bonds with Lys49, Arg633, and Tyr625, along with pi-stacking interactions with Phe356 and Ser355, unveiled a molecular ballet that hinted at Silibinin's efficacy.

Polymyxin B, an antibiotic with a focus on Gram-negative infections, revealed promising interactions with RNAP II. The lowest free binding energy of -7.58 Kcal/mol, observed at 52 runs, indicated substantial binding affinity. Forming conventional hydrogen bonds with Gly772, Ala1090, Ser1091, and Met1285, along

with carbon-hydrogen bonds with His1085 and Ala1087, Polymyxin B's harmonious bond with RNAP II suggested potential therapeutic efficacy. Additional interactions with Lys705 and Val1283 alkyl group, along with an attractive charge with Asp716, further enhanced its appeal. This in silico exploration into the binding modes of potential antidotes provides a detailed understanding of their interactions with crucial proteins implicated in *Amanita* mushroom poisoning. The results underscore the promise of Rifampin, Prednisolone, Cyclosporine, Silibinin, and Polymyxin B as therapeutic interventions, offering a foundation for further in vivo validations. These findings pave the way for targeted antidote development, addressing a critical gap in the treatment of *Amanita* mushroom poisonings.

This comprehensive study on WEMs in Champhai district, Mizoram, delves into their diversity, nutritional value, antioxidant properties, and potential hazards associated with poisonous species. With a focus on promoting local consumption and commercial use, the research identifies specific mushroom families and their associations with tree species, emphasizing microhabitat specificity. Additionally, the study addresses the critical issue of mushroom poisoning in the region, quantifying toxic compounds and proposing potential antidotes through molecular docking studies. Overall, the findings contribute to both scientific knowledge and the well-being of the local community by providing insights into sustainable mushroom utilization and safety measures.