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Proximate Analysis and Mineral Content of Wild Edible Mushrooms from Manipur, India

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A B S T R A C T

Integrating mushrooms into diets supports sustainable development goals by increasing nutritional variety, lowering environmental impact compared to livestock cultivation, strengthening local economies, and improving food security. The present study aimed to assess the nutritional value and mineral content of five edible wild mushrooms belonging to the family *Russulaceae*. In Manipur, wild mushrooms are primarily gathered and highly valued as a traditional nutritious food among the rural poor. Despite this, their nutritional characteristics have not been adequately studied and documented. *Lactifluus dwaliensis*, *Lactifluus volemus*, *Russula rosea*, *Russula nigricans*, and *Russula virescens* collected from Churachandpur, Manipur were initially identified based on morphological and molecular analysis. The nutrient analysis of the wild edible mushrooms collected revealed that they are an excellent source of protein and carbohydrates, while containing low amounts of fat. These mushrooms also showed high levels of essential minerals that are essential for human nutrition and overall health.

Introduction

Mushrooms are fleshy, spore bearing fruiting bodies of fungi which usually appear spontaneously in forests and farmlands in great quantities after rain. It is a well-known fact that mushrooms are an integral part of the diet of various ethnic groups across different states in India. They have broad cultural acceptance and constitute a traditionally very important nutritious food. Wild edible mushrooms serve as a nutritious addition to diets due to their rich content of protein, fiber, vitamins, and minerals, while being low in fats (Assemie and Abaya, 2022; Khumlianlal *et al.*, 2022; Thachunglura *et al.*, 2023a). They are indeed recognized as an excellent source of a variety of bioactive compounds that are known to have a potential source of antioxidant and

antimicrobial properties (Gebreyohannes *et al.,* 2019; Podkowa *et al.,* 2021). In regions where livelihood options may be limited, collecting and selling cultivated as well as wild edible mushrooms can provide an additional source of income for rural families (Bandara *et al.,* 2021; Thachunglura *et al.,* 2024). Wild edible mushrooms contain a variety of nutrients and bioactive compounds that can be utilized to create food supplements, offering potential benefits such as anti-diabetic, cardiovascular, and immune-modulating properties (Altaf *et al.,* 2020). The combination of nutritional benefits and economic opportunities makes wild edible mushrooms a valuable resource for addressing food insecurity and poverty in many regions. The Indo-Burma biodiversity hotspot, which includes the region of Manipur in Northeast India, indeed experiences high humidity during the monsoon season, making it conducive to the growth of various fungi species. The fungi are essential, yet little understood and often overlooked components of healthy ecosystems (Zothanzama, 2011; Chawngthu *et al.,* 2023). This region is known for its rich biodiversity, including a wide variety of fungi, yet it remains relatively under-researched compared to other parts of the world. The family Russulaceae is known for their diversity and ecological importance in forest ecosystems (Wang *et al.,* 2015; Pérez-Moreno *et al.,* 2021). They are also consumed as food in various cultures around the world (Appolinaire *et al.,* 2016). However, detailed studies on the nutritional composition and antioxidant potential of *Russula* species, particularly those found in North East India are still lacking. Several species from the Russulaceae family have also been documented in the neighboring states such as Mizoram, Nagaland, and Meghalaya (Ao *et al.,* 2016; Kalita *et al.,* 2016; Thachunglura *et al.,* 2023b). Some prior studies have examined the nutritional values of wild and cultivated mushrooms in Northeast India (Ao and Deb, 2019; Kakoti *et al.,* 2021; Khumlianlal *et al.,* 2023). There are many different types of wild edible mushrooms that have not been fully characterized in terms of their nutritional value and mineral content. Gaining a better understanding of these properties could have important implications for dietary purposes.

Materials and Methods

Collection, Storage and Samples Preparation

The mature fruiting bodies of the wild edible mushrooms were collected from Churachandpur districts of Manipur. Each fruiting body was handpicked from their natural habitats and cleaned from soil substrates. Four of the collected samples namely *Lactifluus dwaliensis, Lactifluus volemus, Russula rosea* and *Russula virescens* were identified by employing a combination of macro-morphological characteristics and molecular analysis while *Russula nigricans* was identified based on morphological characteristic (Das, 2003; Shimono *et al.,* 2007; Yu and Liang, 2022; Chun-Ying *et al.,* 2020).

Total DNA was extracted from mushroom tissue using CTAB method for molecular identification (Doyle, 1987). The ITS (Internal Transcribed Spacer) region was amplified using primers (ITS1 and ITS4) and PCR reactions in a Bio-Rad C1000 Touch PCR Thermal Cycler for 3 minutes at 95°C, followed by 35 cycles of denaturation for 1 minute at 95°C, annealing for 0.5 minute at 58°C, and extension for 2 minutes at 72°C. Purified standard amplicons were sequenced in both directions.

To find the sequence with the highest similarity, the obtained sequences were edited with BioEdit and compared with sequences in the NCBI GenBank database using BLAST searches. The identified sample were ground into a fine powder and stored in fresh plastic polythene bags at 4 °C until further analysis.

Proximate composition analysis

The proximate composition of the mushrooms was expressed on a percentage dry weight basis. Contents of moisture, total ash, and crude fat were determined using standard proximate analysis methods (AOAC, 1990) while crude fiber was determined using AOAC (2000) standard method. Protein was estimated by Bradford method and the content of total carbohydrate was calculated as differences as follows:

Total carbohydrates (%) = 100 − [moisture (%) + crude fiber (%) + crude fat (%) + ash (%)].

Minerals analysis

Analysis for Sodium (Na), Calcium (Ca), Potassium (K), Phosphorus (P), Magnesium (Mg), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), was carried out using (ICP-OES) Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) (Sharif *et al.,* 2016).

Statistical analysis

Statistical analyzes were conducted using Graph Pad Prism version 5 for Windows. All the determinations were carried out in triplicate and data were presented as mean ± standard deviation.

Results and Discussion

The consensus sequences of the collected mushrooms have been recorded in GenBank with the following accession numbers: PP469736 for *Lactifluus dwaliensis*, PP469737 for *Lactifluus volemus*, PP469738 for *Russula rosea*, and PP469739 for *Russula virescens*. The identified mushroom specimens have been deposited at the Microbial Resource Division of IBSD in Imphal.

Nutritional value

The proximate compositions of the studied wild edible mushrooms, *Lactifluus dwaliensis*, *Lactifluus volemus*, *Russula rosea*, *Russula nigricans* and *Russula virescens* are shown in Table 1.

Edible mushrooms have become an important solution to the problem of protein deficiency that can arise from diets that rely heavily on cereals. Additionally, edible mushrooms are highly regarded for their nutritional content and positive impact on health, making them a high-quality dietary choice (Manzi *et al.,* 1999; Agrahar-Murugkar and Subbulakshmi, 2005; Thachunglura *et al.,* 2023b).

Mushroom is considered to be a complete, health food and suitable for all age groups. The nutritional value of edible mushroom is affected by numerous factors such as species, stage of development and environmental condition (Bellettini *et al.,* 2019).

Fresh edible mushrooms generally contain around 90% moisture (Crisan and Sands, 1978). In the present study, all the mushrooms contained high moisture content ranging from 82.18% in *Russula rosea* to 93.36% in *Russula virescens*. The ash content ranged from 5.8% in *Russula rosea* to 8.95% in *Russula virescens*, suggesting that the studied mushrooms contain essential minerals of nutritional significance. The fat content in five species of mushroom under study ranges from 1.39 % in *Russula virescens* to 10.78% in *Lactifluus dwaliensis*. Mushrooms maintain low-fat levels mainly because of their abundant moisture content. The mushrooms studied in this study contained between 80% and 90% moisture, which substantially lowered the amount of fat present.

In the present study, mushrooms were found to contain a significant amount of fiber, with *Russula nigricans* (36.74%) having the highest concentrations and *Russula* virescens (9.84%) having the least amount of fiber. This emphasizes the importance of fiber, a key component of carbohydrates, in mushrooms, which improves their nutritional profile and supports a healthy human diet (Ao and Deb, 2019).

Mushrooms are an excellent source of protein for vegetarians as they contain all essential amino acids required to meet adult nutritional needs (Valverde *et al.,* 2015). Protein content of mushrooms varied from 12.32% in *Russula nigricans* to 25.74% in *Russula virescens*. The nutritional value of mushrooms including protein content can be affected by several factors such as substratum composition, pileus size, maturity, variations in genetic structure and other environmental factors (Bernas *et al.,* 2006). Carbohydrate constitutes the largest fraction of mushroom dry matter ranging from 39.09% in *Russula nigricans* to 54.08% in *Russula virescens.*

In contrast to our samples, Kostic *et al.,* (2020) reported that *Russula rosea* and *Russula nigricans* had 12.2% and 19.33% of protein, as well as 82.03% and 75.26% of carbohydrates, respectively. The carbohydrate content appears particularly higher in *Russula rosea* and *Russula nigricans* compared to our present study. This demonstrates the variability in nutritional content even among mushrooms of the same species.

The nutritional contents of the mushrooms were both species and source dependent and their ability to accumulate nutrients from the substrates. In the dried fruiting bodies, carbohydrate was the major constituent, followed by protein, fiber, ash, and fat. Compared to the other mushroom species in this study, *Russula virescens* had the highest concentrations of protein, carbohydrate, ash and moisture content, *Lactifluus dwaliensis* had the highest fat content and second to *Russula virescens* in protein, ash and moisture content. *Russula nigricans* had the highest crude fiber content and the least carbohydrate. The high ash content of *Russula virescens* and *Lactifluus dwaliensis* may contribute to their rich minerals content. Overall, the results indicate that the mushrooms studied are good sources of proteins, crude fiber, carbohydrate, though different nutritional composition richness varies by species.

Table.1 Methods parameters

View Direction	Radial	Axial
UV exposure time	15	15
UV RF Power	1150	1150
UV Neb Gas Flow	0.5	0.5
VIS Exposure Time	5	5
VIS RF Power	1150	1150
VIS Neb Gas Flow	0.5	0.5
Cool Gas Flow Rate	12	12
Aux Gas Flow Rate	0.5	0.5

Table.2 Proximate analysis of collected wild edible mushrooms

Mushroom	Moisture	Ash	Crude fat	Crude fiber	Protein	Carbohydrate
Lactifluus dwaliensis	90.16 ± 0.950	7.74 ± 0.01	10.78 ± 0.090	12.40 ± 0.016	23.85 ± 0.06	45.23
Lactifluus volemus	84.79 ± 0.85	6.8 ± 0.05	5.9 ± 0.05	14.1 ± 0.05	20.8 ± 0.05	43
Russula rosea	82.18 ± 1.12	5.8 ± 0.03	1.97 ± 0.13	20.02 ± 0.05	20.01 ± 0.0	52.21
Russula nigricans	88.97 ± 0.67	6.37 ± 0.03	5.55 ± 0.03	36.74 ± 0.204	12.32 ± 0.0	39.09
Russula virescens	93.36 ± 0.86	8.95 ± 0.09	1.39 ± 0.03	9.84 ± 0.04	25.74 ± 0.02	54.08

Each Value is expressed in mean ± SD, (n = 3)

Table.3 Mineral content of collected wild edible mushrooms

Mushroom	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Lactifluus dwaliensis	1.42 ± 0.01	0.65 ± 0.22	26.58 ± 0.28	112.09 ± 0.19	1.91 ± 0.01	0.33 ± 0.03	3.03 ± 0.17	0	1.86 ± 0.08
Lactifluus volemus	1.7 ± 0.01	0.37 ± 0.2	6.65 ± 0.2	128.35 ± 0.35	1.66 ± 0.32	0.24 ± 0.0	2.90 ± 0.15	0	1.1 ± 0.07
Russula rosea	2.25 ± 0.01	0.37±0.1	5.24 ± 0.22	120.56 ± 0.49	1.56 ± 0.13	0.24 ± 0.13	2.28 ± 0.01	0	1.54 ± 0.08
Russula nigricans	2.03 ± 0.15	0.42 ± 0.19	26.65 ± 0.35	126.63 ± 0.21	1.81 ± 0.03	0.31 ± 0.01	2.79 ± 0.11	0	0.81 ± 0.03
Russula virescens	2.04 ± 0.21	0.44 ± 0.016	21.42 ± 0.20	157.73 ± 0.29	2.57 ± 0.08	0.52 ± 0.02	2.94 ± 0.07	0	1.17 ± 0.04

Results are presented as mean ± SD (n=3). Each value is expressed in ppm

Figure.1 A) *Lactifluus dwaliensis,* B) *Lactifluus volemus,* C) *Russula rosea,* D) *Russula nigricans* and E) *Russula virescens*

Mineral Content

Minerals are vital for human nutrition, as they are necessary for various life processes (Gałgowska and Pietrzak-Fiećko, 2020). They support a variety of physiological activities, including bone strength, immunological function, and enzyme activity. Incorporating edible mushrooms into diet provides a natural and balanced intake of important minerals, which promotes general health (Bell *et al.,* 2022).

Mushrooms are an excellent source of important minerals. The mineral composition of mushrooms is reported to be largely influenced by the bioaccumulation of minerals through the growing mycelium from the substrate (Chang and Hayes, 1978; Chang and Miles, 2004). The results of the present study to determine the mineral content in the dried samples of wild edible species of *Lactifluus dwaliensis*, *Lactifluus volemus*, *Russula rosea*, *Russula virescens* and *Russula nigricans* are summarized in Table 2. Minerals are micronutrients that are essential for the proper functioning of the body.

The results indicated that potassium was found to be the most abundant element and ranged from 112.0 mg/g in *Lactifluus dwaliensis* to 157.73 mg/g in *Russula virescens*. Amongst the evaluated species, *Lactifluus dwaliensis* and *Russula virescens* possesses the highest concentration of three essential minerals each which positively correlate with their high ash content as compared to other species. *Lactifluus dwaliensis* possess highest concentration of Cu, Na and Zn while *Russula virescens* possess highest concentration of K, Mg, and Mn. Amongst the minerals analyzed, K was found to be the most abundant, followed by Fe, Na, Mg, Ca, Zn, Cu and Mn, while P was present in a negligible quantity. Most of the major minerals required for the body are present in all of the studied wild edible mushrooms.

The nutritional and mineral compositions of five wild edible mushrooms from the family of Russulaceae have shown potential as sources of sustenance, especially in rural areas of North East India. However, it is imperative to note that the nutrient content varies among the studied species, and therefore, due diligence must be exercised in the identification process, which can be complicated given the intricate task of distinguishing between different *Russula* species.

Further research on the antioxidant properties, antimicrobial activity, and nutritional profiles of various wild edible mushrooms in Manipur is essential to comprehensively understand both the potential benefits and ecological significance of these fungi.

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Author Contribution

Joshua Khumlianlal: Investigation, formal analysis, writing—original draft. Surmani Huidrom: Validation, methodology, writing—reviewing. K. Chandradev Sharma:—Formal analysis, writing—review and editing. V. L. Thachunglura: Investigation, writing—reviewing. Sarangthem Indira: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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