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RESEARCH ARTICLE

Proximate composition and micro-nutritional value of three Russula species from Mizoram, India

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The present study aims to evaluate the nutritional properties and mineral content of wild edible mushrooms. The samples were collected during the monsoon season of 2020-2021 from three different sites in Aizawl District, Mizoram, India. The samples were cleaned to remove any debris and properly labelled. The specimens were identified using standard methods based on macroscopic and microscopic characteristics. Three samples were selected and oven-dried at 45–60 °C for three days in a hot air oven for their proximate analysis. The analysis revealed that the three species of wild edible mushrooms are high in protein (14.42–23.30 g/100g dry weight) and carbohydrates (54.84–58.71 g/100g dry weight), have a low-fat content, and contain significant amounts of essential minerals (5.53–7.19 g/100g dry weight). This study presents data on the nutritional properties and mineral composition of three widely consumed wild edible mushrooms that are commonly collected and consumed by the local people of Mizoram, India.

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Introduction

Edible mushrooms have been consumed by humans for centuries, used as food, medicine, and for ceremonial purposes.[1] They are a nutritious addition to diets, offering a rich content of protein, fiber, vitamins, and minerals, while being low in fat.[2-4] There has also been an increase in their consumption, both as part of regular diets and in the form of dietary supplements.[5] They contain antioxidants, such as ergothioneine and glutathione, which help combat oxidative stress and support overall health. Moreover, mushrooms have been found to contain bioactive compounds like beta-glucans and polysaccharides that can enhance the immune system and potentially have anti-cancer properties.[6-8]

Wild edible mushrooms have enormous promise for delivering long-term solutions in food supply and security. These mushrooms can help to alleviate food scarcity and play an important role in guaranteeing a steady food supply. They are especially important in rural communities, where they provide a variety of benefits that improve nutrition and economic stability.[3] Aside from their dietary benefits, wild edible mushrooms promote local livelihoods through foraging and selling, thus improving the general well-being and resilience of rural communities.

The genus *Russula* Pers., of the Russulaceae family, includes over 780 species with a worldwide distribution, making it the second largest genus within the Agaricomycetes class.[9] The rich and diverse ecosystems create an ideal environment for the growth of numerous fungal species, contributing to the unique ecological makeup of Mizoram[10-11] and several species of *Russula* have also been

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reported from various districts within Mizoram.[12-13] However, detailed information on the nutritional value of edible wild mushrooms in Mizoram remains limited and scattered. While some studies have reported on the nutritional value of a few species[3,14], they have found that most edible species in Mizoram possess good nutritional value, offering potential health benefits for the local population, many species remain unexplored regarding their nutritional properties, such as protein content, carbohydrates, fiber, fats, and trace minerals. Consequently, there is a pressing need for comprehensive research on the nutritional properties of wild edible mushrooms. This would enable local people to better utilize these mushrooms as a reliable and efficient food source.

Materials and methods

Collection, storage and identification of specimens

Fruiting bodies were collected from the Mizoram University campus (23.7338° N, 92.6680° E), Hlimen Forest (23.6705° N, 92.7123° E) and Lungleng Forest (23.6513° N, 92.6750° E) in Aizawl during the rainy seasons of 2021 and 2022. After the collection, the specimens were cleaned to remove debris, and then properly labeled. The collected specimens were identified using standard methods based on macroscopic and microscopic characteristics.[12,13,15-17] The specimens were oven-dried at 45–60 °C for three days in a Hot Air Oven (HOA). After oven-dried, the samples were ground into a fine powder and stored in a freezer at 4 °C before assessing their proximate composition.

Processing of specimen

The collected specimens were immediately cleaned with a sharp knife to remove some forest debris and wood substrates. The specimens were carefully labeled at the collection site and placed in air-tight containers before transporting back to the laboratory. The fleshy collected mushroom samples were oven-dried at 40 °C to 60 °C for three consecutive days and ground into a fine powder for further assessing proximate analysis.

Analytical methods

The analytical methods which include moisture content, fat content, protein, fiber and carbohydrates were evaluated using AOAC. Micro-nutritional properties, including minerals such as calcium (Ca), iron (Fe), manganese (Mn), potassium (K), magnesium (Mg), and zinc (Zn), were assessed using an atomic absorption spectrophotometer.

Moisture content

Chemists[18] 5 g of the samples was taken and initial weight was recorded first, and then kept in a hot air oven at 105°C till the constant weight was obtained. The moisture content was calculated with the formula:

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

Fat content

10 grams of the mushroom sample was extracted with petroleum ether in an extraction apparatus for 16 hours. The extract was then dried, cooled in desiccators and weighed and recorded the mass.[19]

Fat content was calculated with the following equation:

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

Protein content

Oven-dried samples of 0.5 g from each substrate were separately put into 30 mL Kjeldahl flask and 15 mL conc. sulphuric acid (H_2SO_4) added. The mixture was cautiously heated in a fume hood until a greenish-clear solution appeared. The digest was allowed to cool for 30 minutes and 10 mL of distilled water was added to prevent caking. The digested sample was distilled and 35 mL of the distillate was collected in a receiver flask. This was titrated with 0.01 M hydrochloric acid (HCl) until a pink color emerged. Percentage protein was calculated as percentage nitrogen x 6.25.

Fibre content

A total of 1g of the mushroom sample was allowed to boil with 1.25% diluted sulphuric acid (H_2SO_4), washed with water, and further boiled with 1.25% dilute sodium hydroxide (NaOH) and the remaining residue after digestion was taken as crude fibre. 1 gram of moisture and fat-free sample was weighed and kept in the fibre bags. The glass spacer was put into the bags. The bag in the sample carousel was loaded at the previewed positions (positions 1–12). The sample carousel was put into the glass container carefully. The glass container was placed axially on the previewed position of the hot plate. A method was created to estimate crude fiber. The programme was started in the fibretherm. After completion of the programme, the fibre bags were removed. The residue was transferred to weighed crucible (W_1) and drier overnight at 80 °C–100 °C and weighed (W_2). The crucible was heated in muffle furnace at 600 °C for 2 to 3 hours and then cooled in a desiccator and the weight of the crucible was taken after cooling (W_3).

Observations

Weight of the sample = W_1 g

Weight of the crucible + sample before heating at 600 °C = W_3 g

Weight of the crude fibre = (W_2 –W_3) g

Crude fibre (g %) = 100 - (moisture + fat) x weight of fibre / Weight of the sample taken (Moisture and fat free) (W_1)

Ash content

A total of 2g of the dried samples were weighed into crucible and ignited in a muffle furnace at 550 °C until white ash was obtained. The crucible was then transferred to a desiccator and left to cool and weighed. This process was repeated until two successive constant weights were obtained and the ash percentage was calculated using the following equation:

Ash % = (B–C)/A x 100

Where,

B = Weight of the crucible and sample before ashing

C = Weight of the crucible and sample after ashing.

A = Weight of the sample

Total carbohydrate content

The total carbohydrate content present in mushroom samples was estimated by subtracting total components except carbohydrates from 100g of mushroom sample following Crisan and Sands[20]

Carbohydrate (%) = 100 – (Protein + Fat + Fiber + Ash content + Moisture content).

Minerals

The ash was used to determine the minerals content in mushrooms. Mineral, such as calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), and zinc (Zn), were assessed using an atomic absorption spectrophotometer (AAS).[21]

Statistical analysis

All samples were evaluated in duplicate for mineral analysis triplicate for proximate composition, with results expressed as the mean ± standard deviation.

Results and discussion

The proximate composition was conducted on a dry weight basis and the collected specimens were identified as *Russula aurora* (Local name – Pa Leng, Specimen Voucher No – TPZ/18/033), *Russula compacta* (Local name – Pa Leng, Specimen Voucher No - TPZ/18/035) and *Russula cyanoxantha* (Local name – Pa Lengvar, Specimen Voucher No - TPZ/18/023). These three wild edible mushrooms were chosen due to their abundance in the Aizawl District and their proximate compositions are presented in Table 1.

Russula aurora has the highest value of moisture content at 13.26%, while *Russula cyanoxantha* has the lowest at 11.26 %. In terms of ash content, *Russula aurora* (7.19%) was found to be the highest and *Russula cyanoxantha* (5.53%) had the lowest ash content. *Russula aurora* contains the highest amount of fat with a value of 4.60%, followed by *Russula cyanoxantha* (3.44%) whereas *Russula compacta* (2.40%) had the lowest fat content among the three species. Regarding

Table 1. Proximate composition of selected wild mushroom from Aizawl District, Mizoram (g/100g in dried weight basis)

Species	Moisture	Ash	Fat	Crude fiber	Protein	Carbohydrate
Russula aurora	13.26 ± 0.27^a	7.19 ± 0.20^a	4.60 ± 0.06^a	6.49 ± 0.36^a	23.30 ± 0.46^a	54.84
Russula compacta	11.51 ± 0.4^b	6.79 ± 0.15^a	2.40 ± 0.19^b	4.80 ± 0.19^b	15.79 ± 0.90^b	58.71
Russula cyanoxantha	11.26 ± 0.21^b	5.53 ± 0.12^b	3.44 ± 0.09^b	7.51 ± 0.30^a	14.42 ± 0.18^b	57.84

Each value is expressed in mean ± deviation of three replicates
In each column, different letters mean significant differences between species ($p < 0.05$)

crude fiber, *Russula cyanoxantha* has the highest content at 7.51%, and *Russula compacta* has the lowest at 4.80%. *Russula aurora* has the maximum protein level of 23.30%, while *Russula cyanoxantha* has the minimum at 14.42%. Carbohydrates form the largest fraction of the proximate composition. For carbohydrates, *Russula compacta* has a maximum content of 58.71%, and *Russula aurora* has a minimum of 54.84%.

The average nutritional content of the three mushrooms in this study is as follows: Moisture content has a mean of 12.01%, ash content with 6.51%, fat content is 3.48%, crude fiber is 6.27%, protein levels average 17.84%, and carbohydrate content has a mean of 57.13%. When compared to other studies on *Russula* species, these findings align with previous reports,4,23 though some variations exist. Differences in nutritional content can occur due to species-specific traits and the ability of mushrooms to accumulate nutrients from their substrates, reflecting both species and source -dependent factors.

The nutritional value of mushrooms is intricately linked to moisture levels and environmental conditions. Various factors influence the nutritional profile of different mushroom species, such as growth conditions including soil type, climate, and nutrient availability.[22-23] Furthermore, species differences stemming from distinct genetic compositions play a significant role. As mushrooms mature and progress through their growth stages, their nutritional composition can alter based on their developmental stage.[24-25] Moreover, certain mushrooms form symbiotic partnerships with specific plants through mycorrhizal associations, which can enhance their nutrient absorption. This symbiotic relationship allows mushrooms to access nutrients from the plant, leading to a richer nutritional value.[26]

The mineral content of the three collected wild edible mushrooms is presented in Table 2. Potassium (K) is the most abundant mineral among the elements analyzed. Mushrooms contain significant amounts of potassium and calcium, making these key minerals.[27]

The maximum calcium content was found in Russula compacta (7.50 mg/kg), while the minimum was observed in *Russula cyanoxantha* (6.49 mg/kg). The high calcium content in these mushrooms may contribute to better bone health and metabolic functions, making them more beneficial for dietary calcium intake compared to species with lower calcium content.[28] For iron, *Russula cyanoxantha* exhibited the highest level at 5.46 mg/kg, while *Russula aurora* had the lowest level at 3.94 mg/kg. High iron content in these mushrooms can help prevent anaemia and support overall health.[29] Magnesium was most abundant in *Russula compacta* (4.09 mg/kg), whereas *Russula cyanoxantha* (3.44 mg/kg) had the lowest content. High potassium intake from these mushrooms can help control blood pressure and reduce the risk of cardiovascular diseases. It was observed that *Russula aurora* contained the highest potassium level (81.54 mg/kg), while *Russula compacta* had the lowest (54.73 mg/kg). Zinc levels were highest in *Russula cyanoxantha* with 2.49 mg/kg, while *Russula aurora* had the lowest with 1.22 mg/kg. High zinc levels are crucial for immune function and wound healing. Adequate zinc intake from these mushrooms can help support growth, development, and overall immune system health. Manganese also plays a role in antioxidant functions and the regulation of blood sugar levels,[30] *Russula compacta* had the highest manganese content, showing 23.75 mg/kg, and *Russula aurora* had the lowest with 19.72 mg/kg.

The mineral composition of mushrooms is reported to be largely influenced by the

Table 2. Mineral composition of selected wild mushrooms from Aizawl District, Mizoram (mg/kg in dried weight basis)

Species	Ca	Fe	Mg	K	Zn	Mn
Russula aurora	6.91 ± 0.02	3.94 ± 0.1	3.94 ± 0.04	81.54 ± 0.85	1.22 ± 0.29	19.72 ± 0.31
Russula compacta	7.5 ± 0.22	4.53 ± 0.45	4.09 ± 0.42	54.73 ± 0.71	1.65 ± 0.04	23.75 ± 0.15
Russula cyanoxantha	6.49 ± 0.17	5.46 ± 0.30	3.44 ± 0.1	61.36 ± 0.98	2.49 ± 0.17	21.54 ± 0.26

Each value is expressed in mean ± deviation of replicates
*Ca (calcium), Fe (iron), Mg (magnesium), K (potassium), Zn (zinc), Mn (manganese)

bioaccumulation of minerals through the growing mycelium from the substrate. Minerals are crucial for numerous bodily functions, including the formation of strong bones, the transmission of nerve impulses, and the regulation of muscle function. They also support metabolic processes and help maintain fluid balance. By fulfilling these essential roles, minerals contribute significantly to overall health, helping to prevent deficiencies and ensuring a long, healthy life.[31]

Macrofungi can accumulate high levels of mineral elements even in soil with low concentrations of metals. The concentration of minerals correlates directly with factors such as species, geographical region of growth, fruiting body maturation period, substrates, and proximity to pollution sources.[32-33] As a result, numerous mushrooms exhibit elevated levels of heavy metals, which can adversely affect human health upon consumption and potentially lead to severe health issues or even death. These heavy metals can cause a range of problems, including organ damage, neurological issues, and other serious health conditions, depending on the type and concentration of the metals involved.[34]

Conclusion

The three species studied are widely consumed and predominantly found in Champhai District. Many species of *Russula* are present in Mizoram and are commonly referred to by local names such as Pa lengsen, Pa lengvar, and Pa leng. Due to the similar characteristics among *Russula* species, some are difficult for locals to identify and share the same local name. For example, in the present study, the different species *R. aurora* and *R. compacta* are referred to by the same local name, similar to how *Lactifluus corrugis* and *L. volemus* share a name. These mushrooms are widely consumed and are rich in nutrients. The nutritional profiling of wild edible mushrooms demonstrates that these mushrooms are rich in vital elements, making them valuable additions to well-balanced diets. This rich fungal biodiversity not only expands dietary alternatives but also offers chances to include nutrient-dense foods into daily nutrition. The present study highlights the potential of mushrooms to greatly improve health and well-being, supporting both individual dietary needs and broader nutritional programs.

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