MACRO-MORPHOLOGY AND NUTRITIONAL COMPOSITION OF WILD EDIBLE MUSHROOMS FROM ODUOHA-EMOHA, RIVERS STATE, NIGERIA.

[1]Chukunda, F. A., [2*]Emiri, U.N. and [3]Njoku-John, C.S
[1,3]Department of Forestry and Environment, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria. [2]Department of Crop/Soil Science, Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.
Correspondence: ucheemiri@gmail.com; +2348163640641

SUMMARY
The research was aimed at evaluating the macro-morphological and nutritional composition of four wild edible mushrooms collected from Oduoha-Emohua forest in Rivers State, Nigeria. The essence of this study is to enable rural consumers of wild edible mushrooms identify and ascertain the nutrient status of the mushrooms. The mushrooms were collected by random sampling. They were hand - picked, bagged in black polyethylene bags and kept in the refrigerator until when needed. The mushrooms were identified as Auricularia polytricha, Lentinus squarrosulus, Cantharellus cibarius and Pleurotus tuber-reguim. The colour of the mushrooms ranged from golden/yellow, black brown and cream white. The cap length of L. squarrosulus (4.20^{a}cm±1.80) was the highest while the least was C. cibarius (0.9cm±0.94). C. cibarius (1.83cm±09.4) had the highest stipe length while the least was L. squarrosulus (1.25cm ± 0.28). The fresh weight of P. tuber-regium (6.20cm±1.26) was the highest while the least was A. polytricha. The results on proximate composition showed that the mushrooms differed significantly (P≤0.05) from each other. The results of mineral and Vitamin C analysis showed significant (P≤0.05) differences in the mineral content of the mushrooms. The amount of calcium present in L. squarrosulus milligram per 100g of sample was significantly higher (80.5mg± 2.05). However, C. cibarius had more of phosphorus compared to other mineral elements. A. polytricha recorded highest value of potassium (58.8mg±4.05) whereas P. tuber-regium had more of magnesium (200.32mg±3.38) and L. squarrosulus had more of sodium (26.8mg±4.00). Documentation of the identity and nutrient status of these and other mushrooms is recommended to avoid loss of mushrooms and their potential benefits due to constant deforestation practices.

Keywords: Macro-morphology, nutritional composition, wild edible mushrooms, Oduoha-Emohua Forest.

Edible mushrooms are the fleshy and edible fruit bodies of several species of macro fungi (fungi which bear fruiting structures that are visible to the naked eye). They can appear either below (hypogeous) or above ground (epigeous) where they may be picked by hand (Chang *et al*., 1989). Edibility is defined to include absence of poisonous effects on humans, desirable taste and aroma (Matilda *et al*., 2000). Edible mushrooms are consumed for their nutritional value and occasionally, for their medicinal value. Mushrooms consumed by those practising folk medicine are known as medicinal mushrooms (Chang *et al*., 1989). While, hallucinogenic mushrooms (e.g. psilocybin mushrooms) occasionally consumed for recreational or religious purposes produce severe nausea and disorientation. Hence, they not commonly considered edible mushrooms (Boa, 2004).

Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivatable and common wild mushrooms are often available in markets, and those that are more difficult to obtain such as the prized truffle and matsutake may be collected on a smaller scale by private gatherers. Before assuming that any wild mushroom is edible, it should be identified. Accurate determination and proper identification of a species is the only safe way to ensure edibility, and the only safeguard against possible accident. Some mushrooms that are edible for most people can cause allergic reactions in some individuals, and old or improperly stored specimens can cause food poisoning (Jordan, 2016).

Deadly poisonous mushrooms that are frequently confused with edible mushrooms are responsible for many fatal poisonings and include several species of the *Amanita* genus, especially, *Amanita phalloides* or the 'death cap'. Species of mushrooms that are edible may be dangerous if grown in polluted locations where they can accumulate pollutants such as heavy metals (Kalac and Svoboda, 2000)

Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). All essential amino acids are present as well as water soluble vitamins and the essential minerals (Buigut, 2002). The protein value of mushrooms is twice as that of asparagus and potatoes, four times as that of tomatoes and carrots, and six times as that of oranges (Jiskani, 2001). They are also recommended to diabetic and anemic persons, owing to their low carbohydrate and high folic acid content. Some mushrooms are reputed to possess anti- allergic, anti-cholesterol, anti-tumor and anti-cancer (Jiskani, 2001).

Some mushrooms that are considered choice by some and toxic by others. In some cases, proper preparation can remove some or all of the toxins. *Amanita fulva* (Tawny Grisette) must be cooked before eating. *Amanita muscaria* is edible if parboiled to leach out toxins. Fresh mushrooms cause vomiting, twitching, drowsiness, and hallucinations due to the presence of muscimol. (Rubel and Arora, 2008). Mushroom consumption is relegated to the background and perceived as a delicacy for the poor and rural dwellers only. This may be on account of its wild habitat, poor knowledge of its edibility and nutritional constituents.

Hence, this study was initiated to identify the wild edible mushrooms found in Oduoha Emoha forest of Rivers State and determine their nutritional composition.

MATERIALS AND METHODS

Study Area

The field collection of mushroom samples was conducted at Oduoha-Emohua secondary forest in the rainforest zone of Rivers State. The area lies in the zone of humid tropical climate which has two seasons. The wet season extends from March to October and dry season extends from November to February. The mean annual temperature ranges from 21^0C and 29^0C (Chukunda *et al.,*2008). The laboratory studies were performed at the Department of Forestry and Environment, Rivers State University and Department of Plant Science and Biotechnology, University of Port Harcourt.

Collection and identification of wild edible mushrooms

The sporocarps of matured mushroom species were randomly collected from their natural habitat using scapel. Collection was done between May and June 2017. Mushroom samples collected were *Lentinus Squarrosulus, Auricularia Polytricha, Cantharellus cibarius and Pleurotus tuber-regium*. The photographs of the specimens were taken and the mushrooms were later taken to Mycology/Pathology laboratory Unit in the Department of Forestry and Environment, Rivers State University Nkpolu-Oroworukwo, Port Harcourt, where it was dried at 60^0C and preserved for further analysis. Identification of the samples of mushrooms was done macroscopically. Macro-morphological identification was based on habitat, colour, cap length, stipe length and fresh fruit body weights (Wasser 2007, Ukoima *et al*., 2009). The identification of the species was done according to systematic criteria described by Wasser and Weis (1999) and Wasser (2007).

Determination of morphological parameters

The mushroom cap length was determined by placing a transparent ruler across the center of the pileus of each harvested mushroom fruit body and to record the diameter of the cap, while the stripe length was determined by placing the ruler along the length of each fruit body stipe (Ukoima *et al.,* 2009). The fresh fruit body weight was determined by weighing each fresh fruit body immediately after harvest using a portable digital balance.

Proximate Compositions of Mushroom

Determination of protein content

Half gramme (0.5g) each of powdered *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber-regium* mushroom samples were extracted with 2% Sodium chloride in a water bath at 60^oC for one hour. The extract was filtered out and 3% copper acetate monohydrate was added to the filtrate to precipitate protein. The precipitated protein was the centrifuged and dissolved in 50 cm^3 of 0.01M NaOH. The quantity of protein in the alkaline solution was then determined using the Folin-phenol method (Kadiri and Fasidi, 1990). The nitrogen content was converted into protein by multiplying the percent nitrogen with

conversion factor (4.38), excluding the non-protein N coming from the chitin of the cell wall of fungi (Miles and Chang, 1997).

Determination of total carbohydrate

One gramme of each of *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber-regium*were was digested using 13 ml of 52% Perchloric acid diluted with water in the ratio of 270:100ml. One ml of the digest was pipetted into a test tube and 5ml of freshly prepared anthrone reagent was added, mixed and allowed to stand in a boiling water bath (Technotest 13539, Italy) for 12 minutes. The test tube and its content were then removed and cooled to room temperature. The absorbance of the sample mixture and standard was read at 630nm against the reagent blanks using a uv visible spectrophotometer. The total available carbohydrate content of the samples was calculated using the method of Osborne and Voogt (1978)

Total carbohydrate (% glucose) = $\frac{25 \times b}{a \times w}$

Where, W = weight (g) of *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* or *Pleurotus tuber-regium* fruit bodies, a = absorbance of standard, b = absorbance of mushroom sample

Determination of moisture content

The fresh fruit body weight of *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber-regium* were weighed using a chemical balance. These samples were then oven dried separately at 8^{o}C for 48 hours. The loss in weight obtained after drying was regarded as the moisture content (Manzi *et al.,* 1999).

Determination of crude fibre

Half gramme of samples of *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber-regium* mushroom were extracted for three hours with petroleum ether using a Soxhlet apparatus. The fat - free material was placed in a 200ml beaker and 50ml of 1.25% w/v. Sulphuric acid was added and covered with a watch glass. The content of the beaker was heated gently on a hot plate (Gehardt) for 30 minutes.

After acid hydrolysis, the content of the beaker was filtered under vacuum through a Buchner funnel fitted with filter paper and washed with boiling water until the washing was no longer acidic to litmus. The residue was washed back into the original flask using a wash bottle containing 1.25% Sodium hydroxide. This was boiled for 30 minutes covered with a watch glass.

The resulting insoluble material was transferred to a dried weighed ashless filter paper (What man No. 41) and washed thoroughly first with hot water and then with 15ml of ethanol (95%) by

volume. The filter paper and content were incinerated to ash at 50^0C in a muffle furnace for 1hr. The ash was allowed to cool and then weighed using the method of AOAC (2012). The weight of the ash was subtracted from the increase of weight on the paper due to the insoluble material and the difference reported as

Crude fibre (%) = $\frac{\text{weight of fibre}}{\text{weight of sample}} x\ 100$

Determination of lipids

Two grammes each of powdered samples were extracted using petroleum ether in a Soxhlet extractor for 4 hours. The extracts were evaporated to dryness in a weighed flask using a vacuum evaporator. The weighed flasks were dried in the oven at 80^oC for 2 hours and allowed to cool and re-weighed. The differences between the initial and final weights were regarded as the lipid content (Parent and Thoen, 1977).

Determination of ash content

Three grammes each of powdered mushroom samples of *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber-regium* were washed in a Gallenkamp furnace in previously ignited and cooled crucible of known weight at 550^oC for 6 hours. Fairly cooled crucibles were put in desiccators and weighed (Manzi *et al.*, 1999).

Mineral Assay

The atomic absorption method was employed for mineral element assay. Mineral elements of the mushrooms were determined by preparing solutions of their ashes. The quantitative measurement of each element; Iron (Fe), Magnesium (Mg), Sodium (Na), Calcium (Ca) and Potassium (K) was taken using GBC Avanta Ver 2.02 Atomic Absorption Spectrophotometer (Japan). Phosphorus was determined on the ash solution using the molybdenum blue method (AOAC, 2002).

Determination of vitamin C

The method of the Association of Analytical Chemists (AOAC, 2002) was used. Ten grammes of each mushroom sample was weighed, macerated and blended in a blender (Kenitone Model S0300B, China) to make100 ml juice. Fifty millilitres of the juice were measured into a 100 ml volumetric flask and 25 ml of 0.5 % of oxalic acid was added as a stabilizing agent and diluted to volume. Ten millilitres were pipetted into a 50 ml conical flask and titrated against 2, 6 – Dichlorophenol solution until a faint pink colour persisted for 15 seconds. The vitamin C content was calculated using the formula below:

Vitamin C (Ascorbic acid) mg/100 g sample = $\frac{VXT}{W}$

Where, V = volume (ml) of dye used for titration of aliquot of the juice; T = ascorbic acid equivalent of the dye (mg/ml), and W = quantity (g) of sample in aliquot titrated.

Data Analysis

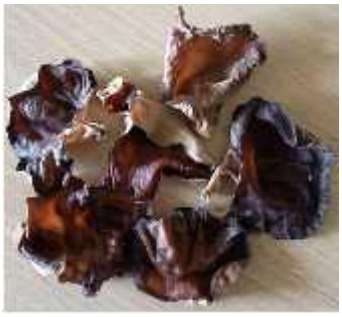
Data were statistically analyzed as described by Steel and Torrie (1980) using one-way analysis of variance. Significant differences were declared using Duncan Multiple Range Test (DMRT) at a probability of 5%.

RESULTS

Macro morphological characteristics of the four wild edible mushrooms

The results on the macro-morphological traits of the four wild and edible mushrooms (Plate 1) collected on dead wood from Oduoha-Emohua Forest, Rivers State are presented in Table 1. The cap colours of the mushrooms were either golden yellow, black, brown or cream white. The cap length, stipe length and fresh fruit body weight differed significantly ($P \leq 0.05$) from one another. Also, the shape of the mushroom cap differed from each other, and were either conically flat or laterally flat, while the margin pattern varies from smooth to rough depending on the type of mushroom.

Lentinus squarrosulus



Auricularia polytrich

Pleurotus tuber-regium

Cantharellus cibarius

Plate 1: Samples of mushrooms collected from Oduoha-Emohua Forest, Rivers State.

Table 1: Macro-morphological characteristics of four wild edible mushrooms from Oduoha- Emohua Forest, Rivers State.

Wild edible mushroom		Morphological characteristics							
Scientific name	Local name	Cap colour	Cap length (cm)	Stipe length (cm)	Fresh fruit body weight (g/kg)	Cap size (cm)	Shape of cap	Margin pattern	Surface pattern
Cantharellus Cibarius	Eru munulor	Golden/ yellow	$0.9^{d} \pm 0.94$	$1.83^{a} \pm 0.94$	$5.96^{b} \pm 0.34$	5	Conically flat	Smooth	Leathery
Auricularia polytricha	Eru nshi	Black	$1.35^{c} \pm 1.07$	$1.35^{c} \pm 1.07$	$3.88^{d} \pm 0.25$	5	Conically flat	Smooth	Leathery
Pleurotus tuber-regium	Eru obah	Brown	$2.25^{b} \pm 0.84$	$1.40^{b} \pm 1.05$	$6.20^{a} \pm 1.26$	6	Laterally flat	Rough	Leathery
Lentinus squarrosulus	Ateketelo	Cream/ white	$4.20^{a} \pm 1.80$	$1.25^{d} \pm 0.25$	$5.65^{c} \pm 2.05$	9	Laterally flat	Rough	Leathery

Means followed by the sane alphabets within each column are not Significantly different by DMRT ($P \leq 0.05$),

Proximate composition of the four wild edible mushrooms

The results on proximate composition of four wild edible mushrooms collected from Oduoha-Emohua forest is presented in Table 2. The results showed significant differences in the proximate composition of *Cantharellus cibarius, Auricularia, polytricha, Pleurotus tuber-regium* and *Lentinus squarrosulus* at $P \leq 0.05$. The moisture content was highest in *P. tuber- regium* (88.40%±0.02), followed by *L. squarrosulus* (70.0%±6.26), *C. cibarius* (31.53%±2.44) and least in *A. polytricha* (25.0±2.35). The total carbohydrate content was more in *L. squarrosulus* (50.00%±3.80) followed by *C.cibranus* (40.56%±6.40) whereas the crude protein was more in *L. squarrosulus* (22.82%±1.80) followed by *C. cibarius* (21.54%±2.44), *A. polytricha* (10.3%) and *P. tuber-regium* (10.3%). The crude fibre content of the four wild edible mushrooms differed significantly ($P \leq 0.05$) where *A. polytricha* had the highest value of 21.97%±0.03 followed by *C. cibarius* (10.03%±0.68) and the least was *P. tuber-regium* (6.38%±0.02). The presence of total ash in the wild edible mushroom was more in *P. tuber- regium* (10.02%±0.02) followed by *C. cibarius* (9.44%±2.57*)* and *L. squarrosulus* (7.52%±0.02) whereas the crude fat content of *L. squarrosulus* (6.3%±201) was the highest followed by *P. tuber-regium* (3.5%±0.30) and the least was *A. polytricha* (0.97%±0.28).

Table 2: Proximate Compositions of Four Wild Edible Mushrooms Collected from Oduoha- Emohua Forest (mg/100g)

Mushroom	Moisture content (%)	Total Carbohydrate (%)	Crude protein (%)	Crude fibre (%)	Total ash (%)	Crude Fat (%)
Cantharellus cibarius	$31.53^{c} \pm 2.44$	$40.56^{b} \pm 6.40$	$21.54^{b} \pm 2.44$	$10.03^{b} \pm 0.68$	$9.44^{b} \pm 2.57$	$2.91^{f} \pm 0.38$
Auricularia polytricha	$25.0^{d} \pm 2.35$	$38.48^{c} \pm 2.06$	$10.3^{c} \pm 0.26$	$21.97^{a} \pm 0.03$	$6.87^{d} \pm 2.60$	$0.97^{d} \pm 0.28$
Pleurotus tuber-regium	$88.4^{a} \pm 5.20$	$33.54^{d} \pm 3.20$	$10.5^{c} \pm 0.44$	$6.38^{d} \pm 0.02$	$10.02^{a} \pm 0.02$	$3.5^{b} \pm 0.30$
Lentinus squarrosulus	$70.0^{b} \pm 6.26$	$50.00^{a} \pm 1.80$	$22.82^{a} \pm 1.80$	$7.52^{c} \pm 2.05$	$7.52^{c} \pm 2.05$	$6.3^{a} \pm 2.01$

Means followed by the same superscript letters within each column are not significantly different by DMRT ($p \leq 0.05$),

Minerals and Vitamin C composition of the four wild edible mushrooms

The results of mineral and Vitamin C composition (Table 3) showed significant ($P \leq 0.05$) difference in the mineral content of the four wild edible mushrooms. The amount of calcium present in *Lentinus squarrosulus* per 100g of sample was significantly higher (80.5mg± 2.05) followed by *Auricularia polytricha* (60.7mg±0.03). However, *Catharellus cibarius* had more of phosphorus compared to other mineral elements. *A. polytricha* had more of potassium (58.8mg±4.05) whereas *P. tuber-regium* had more of magnesium (200.32mg±3.38) and *L. squarrolusis* had more of sodium (26.8mg±4.00).

Table 3: Minerals and Vitamin C content of four edible mushrooms from Oduoha-Emohua Forest in milligramme per 100 g of mushroom

Mushroom	Calcium	Iron	Sodium	Potassium	Magnesium	Phosphorus	Vitamin C
Cantharellus cibarius	$56.89^{c} \pm 0.08$	$30.09^{b} \pm 0.24$	$0.01^{d} \pm 0.04$	$45.37^{b} \pm 4.0$	$146.40^{b} \pm 2.26$	23.15a ± 0.52	$0.06^{a} \pm 0.01$
Auricularia polytricha	$60.7^{b} \pm 0.93$	$15.96^{d} \pm 0.15$	$0.85^{c} \pm 3.11$	$58.8^{a} \pm 4.05$	$115.79^{d} \pm 1.46$	$13.78^{c} \pm 0.65$	$0.03^{b} \pm 0.03$
Pleurotus tuber-regium	$51.90^{c} \pm 0.20$	$28.39^{c} \pm 0.28$	$15.96^{b} \pm 1.50$	$36.78^{c} \pm 0.26$	$200.32^{a} \pm 3.38$	$20.29^{b} \pm 0.20$	$0.02^{c} \pm 0.03$
Lentinus squarrosulus	$80.5^{a} \pm 2.5$	$36.2^{a} \pm 0.05$	$26.8^{a} \pm 4.00$	$100^{d} \pm 0.42$	$140^{c} \pm 2.13$	$10.30^{d} \pm 0.23$	$0.01^{d} \pm 0.02$

Means followed by the same superscript letters within each column are not significantly different by DMRT ($p \leq 0.05$)

DISCUSSION

Four wild edible mushrooms collected and identified using macro-morphological characteristics as *Lentinus squarosulus, Cantharellus cibarius, Auricularia polytricha* and *Pleurotus tuber- regium*. The occurrence of these mushroom species has been reported by several researchers (Oei, 2003; Ellioth, 1991).

The wild mushrooms collected from study area naturally grew on dead woods and damped soils. These results were not unexpected because the vegetation is a typical tropical rainforest, which supported their growth (Alofe, *et al*., 1996, Jonathan and Fasidi, 2003). The consumption of wild edible mushrooms is dependent on their availability in their local markets to augment for family income (Osemwegie *et al*., 2010). This report agreed with the findings of Odebode (2005) that mushrooms are used as food and medicine.

The results of proximate composition analysis showed that the four wild edible mushrooms are significantly ($P \leq 0.05$) rich in carbohydrate, moisture, crude proteins, lipids and total ash. This confirms the assertion of Akindahunsi and Oyetayo (2006) who reported that *Pleurotus tuberregium* was rich in protein, lipids, ash and total carbohydrate.

The wild mushrooms collected are very important food items especially, the *Pleurotus tuberregium* which is rich in proteins, high fibre content and low lipid, an indication that they are good mushrooms. In this study, the high fibre content observed among the three mushrooms agreed with the results obtained by Obodai (1992). The relative high percentage of carbohydrates in the four mushroom samples collected proved that they are nutritious and good for human consumption. This is in agreement with the report obtained by Marlow (2001). On the contrary, the high moisture content of the mushrooms obtained may be an indication that most fresh mushrooms cannot be kept for a longer time. This may be due to high water activity which favoured microbial growth (Aletor, 1995). The study on the proteins of the wild mushroom collected revealed that protein contents of mushrooms vary according to the genetic constitution and differences in growing medium (Samme *et al*., 2003; Adejumo and Awosanya, 2005). The results of present study agreed with the reports of Fasidi and Kadiri (1990) and Ola and Obah (2001) that some mushrooms, *V. volvacea* and *T. robusta* contain higher protein content.

The results of proximate values of the four edible species of mushrooms clearly indicate the potential for their use as sources of good quality food. The crude protein, ash and crude fiber values of most mushrooms compared favourably with and in some instances surpassed those reported for most legumes except groundnut and soybeans grown in West Africa (Aletor and Aladetimi, 1995).

The mineral levels, mainly potassium, phosphorous, sodium and iron in these mushrooms were higher than those reported for several cowpea varieties (Aletor and Aladetimi, 1995). Using this proximate analysis, the mineral and analytical food value as approximate indices of nutritional quality, it would appear that some of these mushrooms fall between most legumes and meat. In earlier studies, Gruen and Wong (1982) indicated that edible mushrooms were highly nutritional and compared favorably with meat, egg and milk. Some of the mushrooms are known to possess anti tumorigenic and hypo- cholesterolaemic agents, which suggests that mushrooms could hold special attraction for and may be recommended for people with cholesterol related ailments. The moisture contents of some of the mushrooms analyzed are high, indicating that mushrooms are highly perishable. High moisture contents promote susceptibility to microbial growth and enzyme activity. From results, the four mushroom species were rich in potassium, calcium, magnesium, iron and manganese. This is in agreement with the report of analysis of some cultivated mushrooms like *Agaricus bisporus*, *Lentinus edodes*, and *Pleurotus ostreatus* (Mattila *et al*., 2000). They were generally low in sodium, phosphorus and copper. Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others. From the study, it was observed that these four edible mushrooms hold tremendous promise in complementing the protein and mineral supply deficits prevalent in developing countries.

Mineral composition analysis indicated that the fruit bodies of the three mushrooms were rich in calcium, nitrogen, iron, sodium, potassium magnesium and phosphorus vitamin c. This is inconsonance with the findings of Adejumo and Awosanya (2004; Ukoima *et al.,* 2009). In this present study, the consumption of (100g) of mushrooms will give 27.09 mg of iron which is sufficient to meet the recommended daily allowance (RDA) for most growing school children and possibly reduce the incidence of nutrient deficiencies in their meal.

CONCLUSION AND RECOMMENDATIONS

Mushrooms can be used as source of alternative food in addition for fortification of diet for enhanced nutrition. The four wild edible mushrooms have proven to be rich sources of proteins, carbohydrates, low fat content, such as potassium, calcium, phosphorus and magnesium. However, sodium is relatively less in the mushroom species, hence is recommended for patients with hypertension. Further study on the phytochemical constituents of the mushrooms is recommended.

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