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Nutritive value of the potential macrofungi *Ganoderma applanatum* (Pers.) Pat. from Shivamogga District- Karnataka, India

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ABSTRACT

Numerous species of wild growing mushrooms are widely consumed as a delicacy in central Asia and eastern Europe. Credible evaluation of their nutritional value has so far been limited due to fragmentary knowledge of their composition and mainly due to the very limited information on the availability of their constituents. An attempt is made to determine the proximates, nutritive value and elemental composition of Ganoderma applanatum (Pers.) Pat. a wood-inhabiting wild macro fungi, grown in two distinct geographical regions ARF (Agumbe Reserve Forest) and SWLS (Shettihalli Wildlife Sanctuary) was analyzed by using Atomic Absorption Spectroscopy. The results of the proximate analysis showed both samples were rich in carbohydrates, protein and crude fiber and low in ash content and least content of fat. Among macronutrients the ARF sample recorded maximum values of sodium, potassium and magnesium where as the phosphorus and calcium were found to be same. The average values of manganese, copper and zinc were more in SWLS sample compared to ARF sample. The heavy metals lead and cadmium were found to be absent. The ARF sample showed higher amount of basic composition and macronutrients compared to the SWLS sample. The ARF is an evergreen forest with rich humus; high rain fall favors the luxurious growth of macro fungi while the SWLS is a dry deciduous forest.

Key words: Proximates, Mineral contents, Nutritive value, Wild macro fungi, *Ganoderma applanatum*

INTRODUCTION

Mushrooms are saprophytes. They include members of the basidiomycetes and some members of the Ascomycota. Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They fall between the best vegetables and animal protein source. Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals [1].

Wild edible mushrooms are traditionally used by many Asian countries as food and medicine [2-3] and are becoming more and more important in our diet for their nutritional characteristics. Some edible mushroom species are sources of physiological agents for medicinal applications, processing antitumor, cardiovascular, antiviral, antibacterial and other activities [4-6]. Each mushroom type produces specific metabolites capable of dealing with the set of microbes that coexist in that specific environment .Fruiting bodies of mushrooms are appreciated not only for texture and flavor but also for their chemical and nutritional characteristics [7]. Mushrooms are valuable healthy and nutritious food, low in calories and high in vegetable proteins, vitamins, iron, zinc, selenium, sodium, chitin,

fibres and minerals [8-11]. Mushroom is about 16.5 % dry matter out of which 7.4 % is crude fiber, 14.6 % is crude protein and 4.48 % is fat and oil [12].

Numerous species (more than 2000) of mushrooms exist in nature; however, only a few are used as food. The knowledge of the nutritional value of wild growing mushrooms has been limited when compared with vegetables. Compared to green plants, mushrooms can build up large concentrations of some heavy metals, such as lead, cadmium and mercury and a great effort has been made to evaluate the possible danger to human health from the ingestion of mushroom [13-14]. Lead, cadmium, iron, copper, manganese, zinc, cobalt, chromium, nickel, magnesium, aluminum, tin and arsenic were chosen as representative trace metals whose levels in the environment represent a reliable index of environmental pollution. Metals such as iron, copper, manganese and zinc are essential metals since they play a vital role in biological systems, where as lead and cadmium are non-essential metals as they are toxic even in traces [15]. The essential metals also produce toxic effects when the metal intake is excessively elevated.

Therefore it is important to determine the basic composition and analysis of macro and micro nutrients present in the wild mushrooms. However, data on the diversity of mycoflora in Asian continent are very scarce and fragmentary, covering mainly fungi of phytopalogical importance macro fungi. *Ganoderma* was acclaimed as a divine herb that could bestow longevity. It was also deemed as an elixir and wellbeing. This might be the case when certain mushrooms were treated as objects of worship on as objects of mysteries describing them as celestial herbs processing panaceal properties.

The genus *Ganoderma* belongs to the family Ganodermataceae within the Basidiomycetes ('higher fungi'). Its members possess a trimitic hyphal system, which consists of binding, skeletal and generative hyphae. Most of the *Ganoderma* species cause a white rot, but they can degrade the woody cell walls in a number of ways, including selective delignification and simultaneous rot [16-17]. *Ganoderma applanatum* (Pers.) Pat. is wide spread in the northern hemisphere. It has a broad host spectrum, mainly consisting of deciduous genera, e.g., *Acer, Fagus, Tilia, Populus, Plantanus, Quercus, Aesculus, Betula, Alnus* and Salix but also occasionally including conifers such as Abies and Piecea. It commonly causes a root and butt-rot but being confined mainly to trees with dysfunctional xylem associated with large wounds on the roots, it is regarded as predominantly saprophytic [18].

The number of existing fungi worldwide has been estimated to 1.5 million species [19]. One-thirds of the fungal diversity of the globe exists in India and of this, only 50 % are characterized until now [20]. Fungi are not only beautiful but play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine [21] food industry, textiles, bioremediation, natural cycling, in recycling nutrients and decomposing the dead organic matter in soil and litter as biofertilizers [22] and many other ways. Studies on the taxonomy and diversity of macro fungi are gaining importance, as many macro fungi are becoming extinct and facing threat of extinction because of habitat destruction.

The aim of the present study was to determine proximates, nutritive value and elemental composition of *G. applanatum* by using Atomic Absorption Spectroscopy.

MATERIALS AND METHODS

Study area

The study area Shivamogga district (fig. 1), is one of the richest floristic area, located in between 130 27' and 140 39' N lat and 740 38' and 760 34' E long with a wide range of ecosystems and species diversity. The average rainfall is 140 cm, temp is 25^0 C and RH is 60 to 100 %. The macro fungal diversity is very rich due to litter decomposition [23]. The samples were collected at two distinct geographical regions ARF (Agumbe Reserve Forest) and SWLS (Shettihalli Wildlife Sanctuary), the site of collection in ARF is 13^0 31' 11.04" N and 75^0 05' 18.43" E and in SWLS is 13^0 53' 33.27" N and 75^0 22' 16.30" E (fig. 1)

Fig 1. The study area showing the site of collection of samples – ARF and SWLS

Sample preparation

Ganoderma applanatum is widely distributed in moist deciduous to evergreen forests and forest plantations and occurs solitary or in large imbricate clusters on decaying wood at butt region of trees or on stumps. The sporocarp was dull grey, grey-brown to brown, fan shaped, Somewhat convex, the fruiting body was perennial, sessile and hard woody in texture i.e. 5.7-6.2cm broad and 5.5-11 cm thick margins irregular often furrowed having flesh up to 6-7cm thick and was brown tough and corky in appearance. Pores: white in colour, quickly turned brown on getting injury, multi-seried tubes present, 3.7-14mm long, having a separation of a thin layer tissue; tubes and pores turns black on treatment with KOH. The color, odour and other apparent properties of the macro fungi, vegetation were noted in the field and also the photographs of specimen were taken in the field (fig. 2).Identification was done by comparing their morphological and anatomical characteristics and also through the electronic data on identification keys of mushrooms [24].

Fresh samples after removing the external material such as mud, bush, soil etc. by washing with demineralized water were air dried between filter paper. Approximately 5 g of sample was taken immediately for moisture determination and the remaining sample was grind to powder. The powder was used for the determination of proximates, nutritive value and elemental composition. The analysis was carried out in the Department of Applied Botany, Kuvempu University, Shanakaraghatta and the Central Coffee Research Institute (CCRI), Baalehonnur, Chikmagalur district of Karnataka, India.

PROXIMATE ANALYSIS

Determination of ash content

10 g of sample was weighed in a crucible, was heated over a low flame till all the material charred, followed by heating in a furnace for 3-5 h at 600^0 C. It was cooled in a desicator and weighed to ensure the completion of ashing. It was repeated till the weight become constant (ash become white or grayish white). Weight of the ash gives the ash content [25].

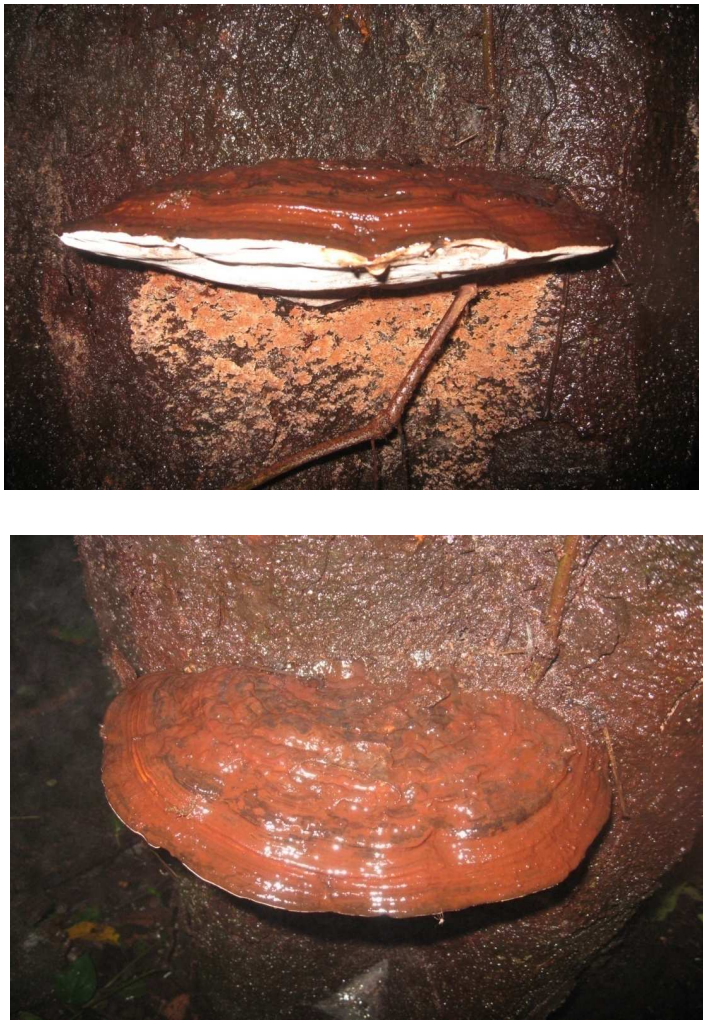


Fig 2. Showing the samples of *Ganoderma applanatum* with substratum

Determination of moisture content

The sample was taken into a flat bottom dish and kept overnight in a hot air oven at 100-110^{o} C and weighed. The loss in weight was regarded as a measure of moisture content [25].

Determination of crude fat

Crude fat was determined by extracting 2 g moisture free sample with petroleum ether in a Soxhlet extraction heating the flask on sandbar for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petroleum ether [26], it was filtered using Whatman no. 41 filter paper and the filtrate was evaporated in a pre- weighed beaker. Increase in weight of beaker gave the crude fat [25].

Determination of Crude fiber

The crude fiber was determined by treating the moisture and fat free material with dilute acid, then with 1.25 % alkali, thus initiating the gastric and intestinal action in process of digestion. The 2 g of this sample was treated with 200 mL of 1.25 % H_2SO_4. After filtration and washing, the residue was treated with 1.25 % NaOH. It was filtered, washed with hot water and then 1 % HNO_3 and again with water. The residue was ignited and the ash was weighed, loss in weight gave the content of crude fiber [26].

Determination of Crude Protein

The crude protein content of the sample was determined by the micro Kjeldhal method [27] in which the sample was digested with a known quantity of concentrated sulphuric acid in the Kjeltec digestion apparatus. The digested material was distilled after the incubation of alkali. The released ammonia was collected in 4 % boric acid in the 1002 Kjeltec Automatic Distilling unit. The resultant boric acid, now contained the ammonia released from the digested material, was then titrated against 0.1 N HCl manually. The nitrogen content thus determined was multiplied by a factor of 6.25 to arrive at the amount of crude protein.

Determination of Total Carbohydrates

The amount of total carbohydrate was calculated with the following formula [28].

Total carbohydrates (% fresh weight) = 100 – (% moisture + % ash + % crude protein + % crude fat)

The energy values were evaluated using the standard formula [29].

Energy values (Cal/100 g) = (2.62 x % protein) + (8.37 x % fat) + (4.2 x % carbohydrate).

Elemental analysis

The macronutrients sodium and potassium were analyzed by Flame Photometer- Jenway-PFP-7 FPM Compressor Unit- 122. The phosphorus was analyzed by Jenway 6300 Spectrophotometer.

The micronutrients calcium, magnesium, zinc, copper, manganese, lead and cadmium were analyzed by Atomic Absorption Spectra GBC 932 AA/AAS.

Determination of Macronutrients

Determination of Sodium and Potassium

The concentration of sodium and potassium were determined with the help of flame photometer using separate standards of sodium and potassium. The yellow colored solution was aspirated at the wavelength of flame photometer to detect the concentration of sodium and potassium. Finally the percentage of sodium and potassium were calculated with the help of following formula:

$$\% \text{ of Na/ K} = \frac{\text{Graph ppm}}{10^6} \times \text{Dilution factor} \times \frac{\text{Volume of sample digestion made}}{\text{Weight of the macrofungal sample}} \times 100$$

Determination of Phosphorous

Orthophosphate (phosphorous) present in the macro fungi was determined by vanado molybdate yellow colour method [30]. The 5 ml of aliquot of macrofungal digested was taken in 50 ml volumetric flask and mixed with 10 ml vanado molybdate reagent. Having thoroughly mixed the final volume was adjusted to 50 ml by distilled water. After 30 min the developed yellow colour was measured on a spectrophotometer at 470 nm. The concentration of phosphorous was calculated with the help of standard graph. The percentage of phosphorous is calculated with the help of following formula

$$\% \text{ of P} = \frac{\text{Graph ppm}}{10^6} \times \frac{\text{Vol. of digestion made}}{\text{Aliquot}} \times \frac{\text{Volume of sample digestion made}}{\text{Weight of the macrofungal sample}} \times 100$$

Determination of Calcium and Magnesium

One ml of aliquot macrofungal digested material was taken in 50 ml by volumetric flask, final volume was adjusted to 50 ml by adding distill water. The presence of calcium and magnesium were determined at the wavelength 422.7 and 228.2 nm of AAS respectively. The percentage of calcium and magnesium were calculated with the help of following formula [30].

$$\% \text{ of Ca/Mg} = \frac{\text{Graph ppm}}{10^6} \times \text{Dilution factor} \times \frac{\text{Volume of sample digestion made}}{\text{Weight of the macrofungal sample}} \times 100$$

Determination of Micronutrients

The 2 ml of digested samples were taken and diluted to 50 ml and the sample was aspirated of at the wavelength of 213.9, 324.75, and 279.5 of AAS to detect concentration of Zn, Cu and Mn respectively. Finally, the values of micronutrients are expressed in ppm by the help of following formula [30].

$$\text{ppm of Zn/Cu/Mn} = \frac{\text{ppm}}{1000} \times \text{Dilution factor} \times \frac{\text{Volume of sample digestion made}}{\text{Weight of the macrofungal sample}} \times 100$$

Analysis of Lead and Cadmium

The 2 ml of digested samples were taken and diluted to 100 ml. The presence of lead and cadmium were detected with the help of AAS by aspirating the sample at the wavelength of 217 nm and 228 nm with appropriate lamps. The ppm of lead and cadmium were calculated by the help of following formula [30].

$$\text{ppm of Pb/Cd} = \frac{\text{ppm}}{1000} \times \text{Dilution factor} \times \frac{\text{Volume of sample digestion made}}{\text{Weight of the macrofungal sample}} \times 100$$

Statistical analysis

Proximate and elemental analysis was carried out three times for each parameter of a macrofungal sample. Hence, we got three replicates (n = 3) from which the mean and standard deviation (SD) are derived.

RESULTS AND DISCUSSION

Proximate analysis

When the nutritional value of mushrooms is evaluated, perhaps the most important factor is their dry matter/ moisture content, which directly affects the nutrient content of mushrooms [31].

The result of proximate and elemental analysis of *G.applanatum*, a wild macrofungi collected from two different places of Western Ghats region of Karnataka are depicted in Tables 1-3. The two regions are Agumbe Reserve Forest (ARF) and Shettihalli Wildlife Sanctuary (SWLS).

Table 1: Proximate analysis of SWLS and ARF samples in a dry weight (d.w) basis

Sl No.	Factors (Proximates in %)	Sample	
		SWLS	ARF
		Mean± SD	Mean ± SD
i	Ash	2.30 ± .10	2.51 ± 0.11
ii	Moisture	42.67 ± 0.58	54.67 ± 0.58
iii	Crude fat	0.50 ± 0.03	0.54 ± 0.01
iv	Crude fiber	14.49 ± 0.05	16.18 ± 0.30
v	Crude protein	11.10 ± 0.20	11.56 ± 0.32
vi	Total Carbohydrates	41.80 ± 0.61	30.72 ± 0.47
vii	Nutritive value in Cal/100 g	208.83 ± 3.26	163.87 ± 2.06

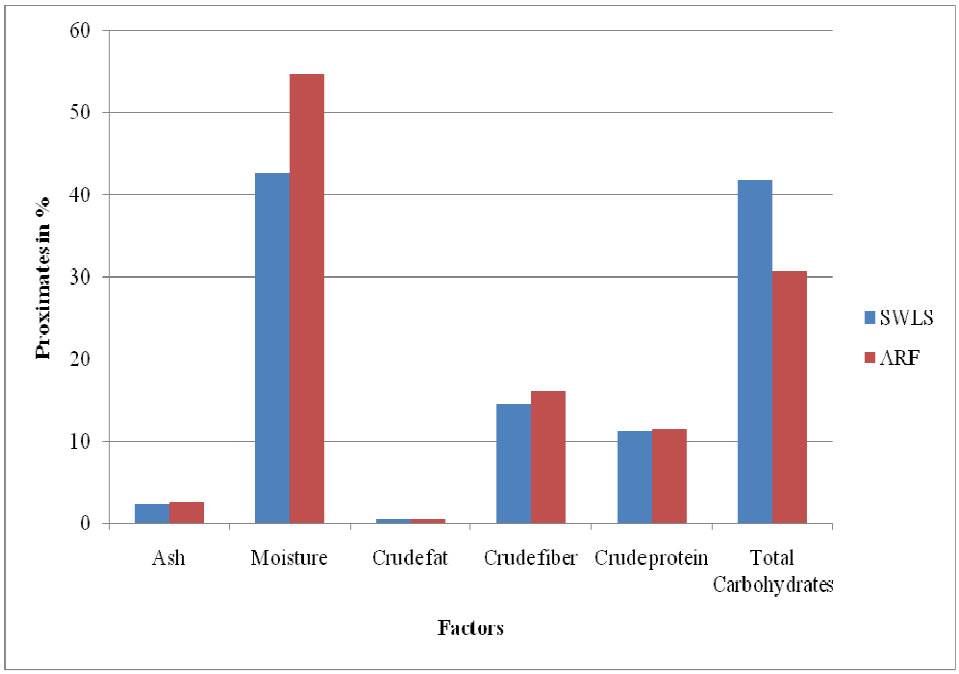
As recorded in the table 1 the moisture content of the ARF sample is found to be high (54.67) compared to SWLS sample (42.67).

Crude fat in mushrooms includes several classes of lipid compounds, free fatty acids, mono, di, and triglycerides, sterols, esters and phospholipids [28]. Various species are especially high in ergosterol, which is the precursor of vitamin D2 (ergocalciferol) [32].

The crude fat content of both ARF and SWLS sample were very less and are 0.54 and 0.50 respectively.The ash and fiber content of the ARF sample were found to be 2.51 and 16.18 respectively which are lesser than the above said content of SWLS sample 2.30 and 14.49 respectively.

The crude protein content (11.56) of the ARF sample is found to be higher compared to SWLS sample (11.10), while the total carbohydrate content (41.80) of the SWLS sample is higher when compared to ARF sample (11.56). Carbohydrates usually account for the prevailing component of fruiting bodies.

The results of the proximate analysis showed that both samples were rich in carbohydrate, protein and fiber content and less amount of ash and very least amount of fat (Graph 1).



Graph 1. Variation in the proximate composition of SWLS and ARF samples

Analysis of macro and micro-nutrients

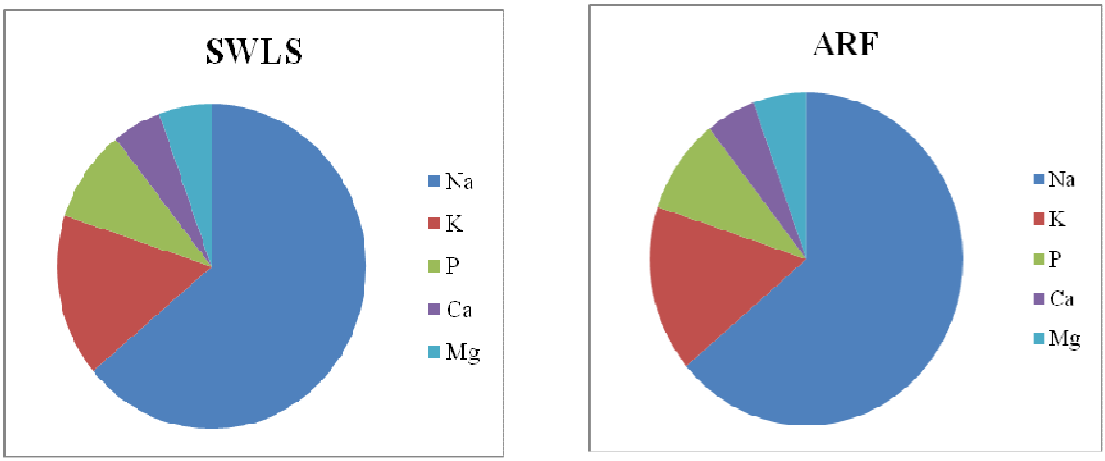
Although metal contents in fungi have been reported, attention has been paid to edible species [33-34]. Because of lack of data on metal contents in wood-inhabiting fungi from other countries in central Asia, it is difficult to compare the data with other regions.

The trace metal content of mushrooms are related to species of mushroom, collecting site of the sample, age of fruiting bodies and mycelium distance from sources of pollution [35] and remaining affected by acidic and organic matter content of the soil and also has been affected by environmental factors such as climate change and growing conditions[36].

The macronutrients *viz*., nitrogen, potassium, calcium and magnesium were analyzed by Flame Photometer- Jenway-PFP-7 FPM COMPRESSOR UNIT- 122, phosphorus by Jenway 6300 Spectrophotometer and were depicted in the Table 2.

Table 2: The Macronutrient analysis of SWLS and ARF samples

Sl No.	Factors (Macronutrients in %)	Sample	
		SWLS	ARF
		Mean± SD	Mean ± SD
i	Na	1.78 ± 0.003	1.86 ± 0.003
ii	K	0.43 ± 0.02	0.47 ± 0.03
iii	P	0.27 ± 0.01	0.27 ± 0.00
iv	Ca	0.15 ± 0.03	0.15 ± 0.02
v	Mg	0.15 ± 0.02	0.16 ± 0.02



Graph 2. Variation in the Macronutrient content of SWLS and ARF samples

The study reveals that the sample contains moderate amount of nutrients. Among macronutrients analyzed, nitrogen found to be high in both the samples. The ARF and SWLS sample contains 1.86 % and 1.78 % of nitrogen respectively. Subsequently followed by potassium, the highest percentage of potassium 0.47 was recorded in the ARF sample and 0.43 in SWLS sample. Na and K take part in ionic balance of the human body and maintain tissue excitability. Because of the solubility of salts, Na plays an important role in the transport of metabolites. K is of importance as a diuretic.

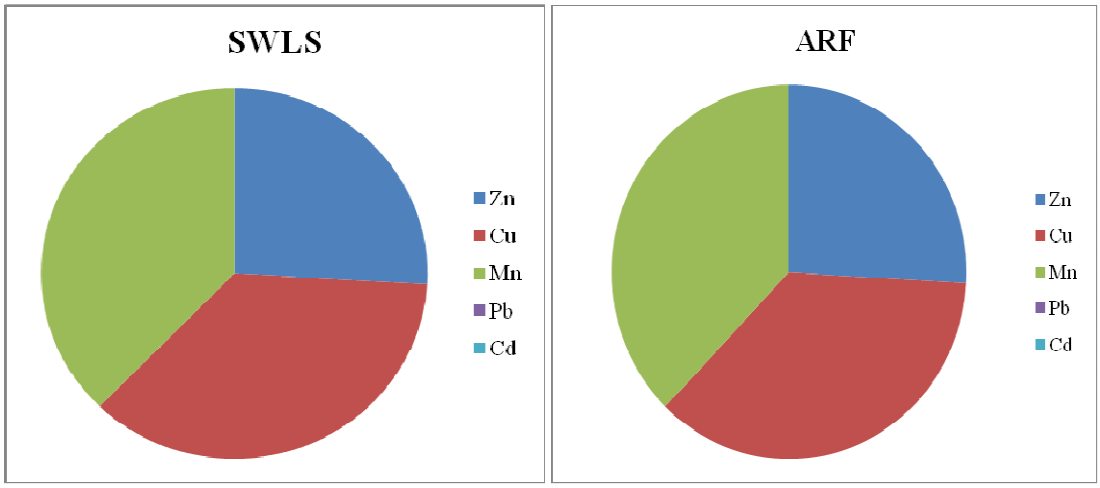
The content of phosphorus and calcium were found to be same in both the samples i.e., 0.27 % and 0.15 % respectively. Ca constitutes a large proportion of the bone, human blood and extracellular fluid; it is necessary for the normal functioning of cardiac muscles, blood coagulation and milk clotting and the regulation of cell permeability. It also plays an important role in nerve impulse transmission and in the mechanism of neuromuscular system.

The presence of magnesium is found to be 0.16 % in ARF sample and 0.15 % in SWLS sample which is almost similar. In humans, Mg is required in the plasma and extracellular fluid, where it helps to maintain osmotic equilibrium. It is required in many enzyme catalyzed reactions, especially those in which nucleotides participate where the reactive species is the magnesium salt, e.g., $MgATP^{2-}$. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system.

The micronutrients like zinc, manganese and copper were analyzed by Atomic Absorption Spectra GBC 932 AA/AAS and were depicted in the table 3.

Table 3: The micronutrient analysis of SWLS and ARF samples

Sl No.	Factors	(Micronutrients in ppm)	Sample	
			SWLS	ARF
			Mean± SD	Mean ± SD
i	Zn		60.77 ± 0.45	58.83 ± 0.31
ii	Cu		90.77 ± 0.40	82.47 ± 0.15
iii	Mn		94.73 ± 0.35	85.57± 0.06
iv	Pb		-----	-----
v	Cd		-----	-----



Graph 3. Variation in the micronutrient content of SWLS and ARF samples

Among micronutrients analyzed in ppm the manganese found to be high and it is higher in SWLS sample 94.73 compared to ARF sample 85.57, followed by copper i.e., 90.77 in SWLS sample and 82.47 in ARF sample. The presence of zinc is found to be high in SWLS sample 60.77 compared to ARF sample 58.33 but the heavy metals lead and cadmium were absent in both samples. Mn is essential for haemoglobin formation [37]. Cu is also a component of many enzyme systems such as cytochrome oxidase, lysyl oxidase and ceruloplasmin, an iron oxidizing enzyme in blood [38]. The observation of anaemia in Cu deficiency may probably be related to its role in facilitating iron absorpation and in the incorporation of iron into haemoglobin [39]. Zn is a component of many metalloenzymes, including some enzymes which play a central role in nucleic acid metabolism [40]. In addition, Zn is a membrane stabilizer and a stimulator of the immune response [41]. Its deficiency leads to impaired growth and malnutrition [42].

As compared with vegetable, mushrooms proved to be good sources of many mineral elements. The main constituents in the mushroom ash were K and P (totally 60 %) [43]. Hence, low ash (2.3 % - 2.51%) content in samples of *G.applanatum* can be attributed to their low K (0.43 % - 0.43%) and P (0.27 %) content and the found results in this study are in agreement with those reported in the literature [44], that the fungi belonging to Polyporus family that grow very slowly and are poor in potassium content (0-1 %) on dry weight.

CONCLUSION

The credible evaluation of the nutritional value of wild macrofungi has so far been limited, due to the fragmentary knowledge of its composition and mainly due to the poor information on the bioavailability of their constituents.

From this study, it was observed that, the wild macro fungi *G.applanatum* hold tremendous promise in complementing the protein and mineral supply deficits prevalent in developing countries. Because it has good food sources in terms of protein, carbohydrate, crude fiber, energy values, lesser amount of ash and least amount of crude fat content. The obtained results are in agreement with reports in the literature regarding the nutritive values of mushrooms. To satisfactory meet nutritional needs, combination with other food stuffs is recommended.

There is a need for ethnomycological and fungal conservation studies. In the long term, it is anticipated that some of the more valuable edible forest fungi will be able to be grown using suitable host trees in agroforestry systems. However, before this can be achieved, a small number of target fungi will need to be identified for commercialization, culture and inoculation systems developed for local conditions.

The ARF is a evergreen forest with rich humus, high rain fall, epiphytic and edaphic conditions favors the luxurious growth of macrofungi, while the SWLS is a dry deciduous forest. This may be the reason for variation in proximate composition and metal content profile of the two analyzed samples.

This study encourages further investigation of different available and less reported food sources such as related species of mushrooms from developing parts of the globe with rich forest resources, high biodiversity and agrarian economy.

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