



Bioactive, nutritional and heavy metal constituents of some edible mushrooms found in Abia State of Nigeria

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ABSTRACT

The study was designed to study the phytochemical properties, mineral analysis, proximate analysis and heavy metals compositions of four edible and non-edible species of mushrooms (*Cheimonophyllum candidissimus*, *Pleurotus* sp., *Russula* sp. and *Auricularia* sp.). Results revealed that the bioactive constituents of the mushrooms were as follows; Alkaloids (0.12±0.02-1.01±0.03%), Tannins (0.44±0.09-1.38±0.6%), Phenols, (0.13±0.01-0.26±0.00%), Saponins (0.14±0.03-0.32±0.04%), Flavonoids (0.08±0.02-0.34±0.02%). The result of proximate composition indicated that the mushroom contained 5.17±0.06-12.28±0.16% protein, 0.16±0.02-0.67±0.02% fats, 1.06±0.03-8.49±0.03% fibre and 62.06±0.52-80.01±4.71% carbohydrate. The mineral compositions of the mushrooms were as follows, Calcium (81.49±2.32-914±2.32 mg/100 g), Magnesium (8±1.39-24±2.40 mg/100 g), Potassium (64.54±0.43-164.54±1.23 mg/100 g), Sodium (9.47±0.12-30.97±0.16 mg/100 g) and Phosphorus (22.19±0.57-53.2±0.44 mg/100 g). Heavy metals concentration indicated Cadmium (0.7-0.94 ppm), Zinc (27.82-70.98 ppm), Lead (0.66-2.86 ppm) and Copper (1.8-22.32 ppm). The results obtained indicate that mushrooms are good sources of phytochemicals, proximate components and minerals needed for maintenance of good health, and can also be incorporated in the manufacturing of drugs. However, the heavy metals obtained from the mushrooms indicate that when consumed high quantity may cause liver or kidney damage; death may result.

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INTRODUCTION

Mushrooms are fungi fruit-bodies which spontaneously appear in forests and farm lands in great quantities after rain. The natural substrata of mushrooms include logs of wood; decomposing agro-wastes, decomposing animal wastes, and soil where they obtain their nutrients through external digestion and absorption by the mycelium. There are edible and poisonous mushrooms and both categories possess nutritional and medicinal values. Mushrooms have been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia and cancer. In the 16th century,

herbalist John Gerard recommended *Auricularia auricula-judae* for curing sore throat. He recommended the preparation of a liquid extract of the mushroom by boiling the fruit bodies in milk, or leaving them steeped in beer, which would then be sipped slowly in order to cure sore throat. It is used as blood tonic, and had been reported in Ghana that this same *Auricularia auricula-judae*, was used to cure eye disease and Jaundice when boiled in milk (Netravathi et al., 2006; Shashirekha and Rajarathnam, 2011). These functional characteristics are mainly due to their chemical composition (Netravathi et al., 2006; Shashirekha and Rajarathnam, 2011). According to Guca et al. (2011), edible wild mushrooms can contain in the flesh, a spectrum of mineral macro- and micronutrients, non-essential trace elements and

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problematic heavy metals. Edible mushrooms are regarded as epicurean delicacy. Rambelli and Menini (1983) reported that mushrooms are appreciated for their good taste and nutritional value and are used in soups as meat supplement as well as seasoners. In Nigeria, the rural dwellers consume mushrooms as delicacies in soups and as ingredients for seasoning or part of the local melon cake (a local snack called 'usu' in Igbo). For instance, the sclerotia of *Pleurotus tuber-regium* is used as thickener, as well as in preparing melon cake ('usu'). Bano (1993) reported that mushrooms are rich in protein contents, almost twice that of vegetables and four times that of oranges. Also, they have been shown to be rich in free amino acids, fiber, vitamins and minerals with low fat and carbohydrate content (Rambelli and Menin 1983). Mushrooms are good sources of vitamins like riboflavin, biotin and thiamine (Bano, 1993).

Organisms require trace amounts of some heavy metals, including iron, cobalt, copper, manganese, chromium and zinc. Excessive levels of these metals, however, can be detrimental to organisms. Other heavy metals such as cadmium and lead have no known beneficial effect on organisms (Ouzouni et al., 2009). The ability of mushroom species to bio-accumulate minerals from the growth medium into the fruiting body are well documented (Rajaratnan et al., 1998; Kalac, 2010). The fruiting bodies of higher mushrooms are relatively rich in mineral constituents (Schmitt et al., 1977; Varo et al., 1980; Vetter, 1990) due to some ecological as well as genetic factors (yet unknown). Biological factors such as species of mushrooms, morphological part of fruiting body, developmental stages and age of mycelium, biochemical composition and interval between the fructifications affect mineral accumulation in macro fungi (Garcia, 1998; Mustafa et al., 2005).

Iron, copper, manganese, zinc (trace elements), lead, cadmium and nickel (toxic metals) were chosen as representatives, whose levels in the environment represent a reliable index of environmental pollution (Mustafa et al., 2005). Minerals such as iron, copper, zinc and manganese are essential metals since they play an important role in biological systems, whereas lead and cadmium are non-essential metals as they are toxic, even in traces (Schroeder, 1973). The essential metals can also produce toxic effects when the metals intake is excessively elevated (Mustafa et al., 2005). The present investigation was focused on the analysis of four edible and non-edible species of mushrooms (*Cheimonophyllum candidissimum*, *Pleurotus* sp., *Russula* sp. and *Auricularia* sp.). The results obtained from the study were used to discuss the essential as well as trace elements in edible mushrooms, together with the limits of their toxic metals.

MATERIALS AND METHODS

Fresh and fleshy mushrooms (*Cheimonophyllum*

candidissimum, *Pleurotus* sp., *Russula* sp. and *Auricularia* sp.) (Figures 1-4); were purchased from Ndioru local market in Ikwano Local Government Area of Abia State, while some were obtained from bushes around Umudike, Umuahia Abia State in June, 2012. The samples were carefully separated into pileus and stipes and sun-dried until they became brittle. This is as opposed to oven-drying at 40°C employed by Okwulehie and Odunze, (2004b).

Preparation of samples for analysis

The dried mushrooms were ground to fine powder using Corona blender. (Landers), Model Y. CIA. S.A. 0897. Preservation of the specimen was done in specimen bottle at room temperature in the laboratory of Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, and clean dry bottles were used to store the samples differently.

Phytochemical content determination

Test for alkaloids (Harborne, 1973)

About 5.0 g of the dry powdered sample of the mushroom was placed into a 100 ml conical flask, containing 2 ml of 5% H₂SO₄ in ethanol. The mixture was heated to boiling in a water bath, left to cool and then tested for the presence of alkaloids. Two (2) ml of the filtrate of the heated samples were used to test for colour change using 2 drops of Mayer's reagent for yellow precipitate and 2 drops of Wangner's reagent for reddish-brown precipitate.

Test for flavonoids

5 ml of dilute solution was added to 5 ml of aqueous filtrate of each sample. To this mixture, about 2 drops of H₂SO₄ was added and observed for yellow colouration which would disappear on storage (Harborne 1973).

Test for tannin (Pearson, 1976)

5.0 g of dry powdered sample was boiled in 20 ml distilled water in a water bath. On cooling a drop of ferric chloride was added and observed for a brownish green or a blue-black colouration.

Test of saponins (Harborne, 1973)

About 2 g of the dry powdered sample was boiled in 20 ml of distilled water in a bath after cooling, the boiled



Figure 1. *Cheimonophyllum candidissimus*.



Figure 2. *Pleurotus* sp.



Figure 3. *Russula* sp.



Figure 4. *Auricularia* sp.

mixture was filtered. 10 ml of the filtrate was mixed with 5 ml distilled water and shaken vigorously for a stable froth. Three drops of olive oil were added to the frothing solution and the formation of an emulsion confirmed the presence of saponins.

Test for phenol (Harborne, 1988)

5 g of the powdered sample was mixed with 20 ml of H₂SO₄ in ethanol and heated for 5 min. 1 ml of the filtrate of the heated mixture and 2 drops of neutral ferric chloride were mixed to observed green, blue or black colouration.

Quantitative estimation of the phytochemicals

Alkaloids

5 g of the dry powdered sample was used to determine the alkaloids contents of the mushroom following the method of Harborne (1973). The alkaloid was expressed in percentage.

$$\% \text{ Alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Flavonoids

5 g of the dry powdered sample was used to determine the flavonoids content according Boham and Kocipai (1994). The sample was mixed with 100 ml of 2 M HCl at room temperature. The solution was boiled for 30 min with water bath, cool and filtered. 20 ml of ethyl acetate was added to the filtrate and was filtered again with a weighed filter paper. The filter paper was oven dried, cool and weighed again.

$$\% \text{ Flavonoids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Tannin

The method of Okeke and Elekwa (2002) was employed for tannin determination using 5 g of the sample shaken with 50 ml of H₂O and was left to stand for 30 min. The solution was filtered and 2 ml of the filtrates was introduced into a test tube and 3 ml of 0.1 M FeCl₃ and 2 ml of potassium ferrocyanide were added. Addition of 46 ml of water was done. It was filtered again and 1 ml of the filtrate was used to read the absorbance at 710 nm within

10 min using a spectrophotometer.

Saponins

Saponins determination was carried out using Harborne (1973) method. 5 g of the sample was boiled with 100 ml of 20% ethanol in a water bath for 1.30 min and filtered while still hot. The filtrate was collected and heated for 30 min in 40 ml of ether, and then poured into a separating funnel, thereafter the lower part of the filtrate in the separating funnel was collected and 60 ml of n-butanol was added and the upper layer/part was collect while the lower part was discarded. The filtrate was evaporated to dryness using steam bath at 70°C in an oven, and then cooled and weighed.

Phenolic content

The total phenol content was determined using Harborne (1973) method. The fat free 0.2 g sample was boiled for 15 min with 50 ml of ether for phenol extraction. 5 ml of the extract was pipetted into a 50 ml flask and 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were added and made up to mark, and was allowed to react for 30 min for color development. The absorbance of the solution was read at 505 nm wave length using a spectrophotometer.

Proximate analysis

Moisture content

5.0 g of the powdered dry samples was placed in clean dry glass Petri dishes of known weight. Next, the samples were placed in an electric oven at 15°C and allowed to dry for about 6–8 h (La Guardia et al., 2005; Konuk et al., 2006). The oven dried samples were weighed and placed back in the oven for 1 h for further heating. The samples were then weighed again and this process was repeated until the weight became constant. The percentage moisture content was calculated as follows.

$$\% \text{ Moisture} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Ash content

One gram of the dry sample was used. This value was obtained by weighing the sample before and after burning it at 50°C overnight (Mattila et al., 2002).

Crude fiber

The total fiber content was determined by the Weende method (AOAC, 1980). 5.0 g of the sample was placed in 250 ml beaker and was hydrolyzed by adding 20 ml of 25% sulphuric acid and was boiled under control for about 30 min on a hot plate. The mixture was filtered through a piece of clean white cloth, then rinsed with hot distilled water. The residue was again boiled with 50 ml of 2.5% sodium hydroxide (NaOH) for 30 min, and was then filtered and rinsed with distilled water. Finally, the residue was collected and transferred into a crucible and was dried in the oven to a constant weight. The weight of the fiber was calculated and expressed as a percentage of crude fiber as follow.

$$\text{Crude fiber} = \frac{\text{Weight of fiber}}{\text{Weight of sample}} \times 100$$

Protein content

The protein content of the samples was determined using the Macro-Kjeldahl method. 1.0 g of the dry powdered sample was digested with 5 ml of concentrated H₂SO₄ to which was added a tablet of selenium catalyst in a fume cupboard. The digest was made up to mark in a 250 ml volumetric flask. 10 ml of the digest was distilled and titrated with 0.2 NH₂SO₄. The crude protein was therefore equaled to the N multiplied by a conversion factor, 6.25 (La Guardia et al., 2005).

Lipid content

The fats and oils content of the sample were determined following the Twisselman method using a diethyl-ether as solvent (AOAC, 1980). 5.0 g of the dry mushroom sample was introduced into an ether-extracting thimble and placed on a soxhlet reflux flask connected to a round bottomed flask of known weight. This was placed on a heating marital filled with about 250 ml of petroleum ether. The oil was extracted by a reflux system. After a series of refluxes, a clear solution was obtained in the flask, and then the sample was removed from the flask. Further heating separated the ether from the extraction oil. The round-bottomed flask containing the oil was finally dried in an oven at 70°C and determination by gravimetric method was done and expressed as a percentage of the sample used.

$$\% \text{ Fat and oil} = \frac{\text{Weight of fiber}}{\text{Weight of sample}} \times 100$$

Mineral element determination

The levels of the mineral elements (calcium, phosphorus, sodium, magnesium, potassium and nitrogen) were determined using the wet digestion extraction methods as describe by Nivozamsky et al. (1983).

Heavy metals analyses

Preparation of selenium

1 L of sulfuric acid were mixed with 3.5 g of selenium powder and heated in a sturdy Pyrex glass container on a hot plate at high temperature until the solution became clear. The selenium was dissolved into the sulfuric acid at a temperature of about 280°C and after the it has dissolved, the hot plate was turn off and the solution was allowed cool down.

The levels of copper, lead, cadmium and zinc were determined using nitrogen, phosphorous and multi-elemental digestion method as described by Nivozamsky et al. (1983).

RESULTS

The results of phytochemical, proximate, minerals and heavy metals composition of the four mushrooms, namely: *C. candidissimus*, *Pleurotus* sp., *Russula*, sp. and *Auricularia* sp. are presented in Tables 1-4.

The results of the phytochemical compositions of the mushrooms are presented in Table 1. The result shows that all the mushrooms contain alkaloids, flavonoids, phenols, saponins and tamins in varying quantities. However, the highest percentage of alkaloids content is 1.01±0.03% obtained in *C. candidissimus*. The highest content of tannins was obtained from *Russula* sp. and the lowest from *Pleurotus* sp. Phenols content from *C. candidissimus* was the highest and the least was obtained from *Russula* sp. The saponins content of *C. candidissimus* was the highest and the lowest was from *Russula* sp. Flavonoids contents were significantly higher in *C. candidissimus* and the least was from *Auricularia* sp.

The result of the proximate composition of the mushrooms is summarized in Table 2. The highest protein content of the mushrooms was obtained from *C. candidissimus* with and the lowest protein occurred in *Pleurotus* sp. The fat content obtained from *C. candidissimus* was highest and the lowest was obtained from *Russula* sp. The ash content of the *C. candidissimus* was higher than that of *Pleurotus* sp which showed lower content. Carbohydrate from *Pleurotus* sp. was significantly higher than that of *C. candidissimus* which was the lowest.

Table 1. Phytochemical composition of mushrooms.

Sample	Alkaloids (%)	Tannins (%)	Phenols (%)	Saponins (%)	Flavonoids (%)
<i>C. candidissimus</i>	1.01±0.03	0.45±0.01	0.26±0.01	0.32±0.04	0.34±0.02
<i>Pleurotus</i> sp.	0.34±0.09	0.44±0.09	0.19±0.01	0.24±0.02	0.27±0.02
<i>Russula</i> sp.	0.23±0.02	1.38±0.67	0.13±0.01	0.14±0.03	0.13±0.02
<i>Auricularia</i> sp.	0.22±0.01	0.57±0.01	0.18±0.01	0.15±0.02	0.08±0.02

Table 2. Proximate composition of mushrooms.

Sample	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
<i>C. candidissimus</i>	12.28±0.16	0.67±0.02	8.49±0.03	6.05±0.07	10.13±6.36	62.06±0.52
<i>Pleurotus</i> sp.	5.17±0.06	0.19±0.02	4.52±0.25	3.84±0.12	9.27±0.15	80.01±4.71
<i>Russula</i> sp.	8.01±0.11	0.16±0.02	2.51±0.03	4.59±0.49	9.26±0.12	75.49±0.27
<i>Auricularia</i> sp.	10.75±0.12	0.32±0.02	1.06±0.06	4.08±0.38	12.06±0.04	71.74±0.29

Table 3. Minerals composition of mushrooms.

Sample	Ca (Mg/100 g)	Mg (Mg/100 g)	K (Mg/100 g)	Na (Mg/100 g)	P (Mg/100 g)
<i>C. candidissimus</i>	81.49±2.32	13.6±1.39	112.14±0.24	9.47±0.12	43.9±0.47
<i>Pleurotus</i> sp.	114.91±2.32	12.8±1.39	164.54±1.23	11.87±0.09	26.29±2.62
<i>Russula</i> sp.	100.2±4.01	8±1.39	124.14±0.24	20.37±0.06	26.29±0.24
<i>Auricularia</i> sp.	124.25±4.0	24±2.40	112.94±0.24	30.97±0.16	53.2±0.44

Table 4. Heavy metal concentration of mushrooms

Sample	Cd (ppm)	Zn (ppm)	Pb (ppm)	Cu (ppm)
<i>C. candidissimus</i> ,	0.85	70.10	2.10	28.32
<i>Pleurotus</i> sp.	0.86	33.6	0.66	9.62
<i>Russula</i> sp.	0.7	29.4	1.9	21.8
<i>Auricularia</i> sp.	0.92	70.98	2.86	2.24

The result of the minerals composition of the mushrooms is summarized in Table 3. The calcium content obtained from *Auricularia* sp. was appreciably the highest while the lowest was found in *C. candidissimus*. Magnesium content of *Auricularia* sp. was the highest and *Russula* sp. had the least. Sodium content was highest in *Auricularia* sp. Phosphorus content was highest in *Auricularia* sp. and the lowest in *Russula* sp.

The results of the heavy metals' concentrations of mushrooms are summarized in Table 4.

The highest content of Zinc was found in *Auricularia* sp. Lead content of *Auricularia* sp. was the highest. While for Copper, the least was from *Russula* sp. (1.8 ppm).

DISCUSSION

Generally, the results obtained indicate the presence of phytochemicals in all the samples but in varying levels. Alkaloids, tannins, saponins and phenols are considered to be anti-nutrients because they have been reported to cause deleterious effects when consumed in large quantities. However some of the phytochemicals contained in the mushrooms have been shown to have useful application. Edeoga and Erita (2001) observed powerful effect of alkaloids in animal physiology and showed their considerable pharmacological activities. Alkaloids and their synthetic derivatives are used as

basic medicinal agents for analgesic antispasmodic and bactericidal effect (Stary, 1998). Also, phenols are useful as they form the main constituents of most antiseptics and disinfectants. Thus the presence of phenolic compounds in the mushrooms species may be the reason for the antifungal, antiseptic and therapeutic properties (Gill, 1992).

The presence of flavonoids in the mushrooms indicates their medicinal value too. Flavonoids have antioxidants properties against free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu, 2004).

The high content of saponin in the mushroom is useful in medicinal and pharmaceutical industry due to its foaming ability that produces frothy effect in the food industry. Tannin concentration detected in the mushrooms have been found to possess' astringent properties, which hasten the healing of wounds and inflamed mucous membrane (Okwu, 2004).

The proximate analyses indicated the presence of proteins, fat and carbohydrate in all the samples. Mushrooms are good source of protein and carbohydrate which are of great demand in both man and animals. They serve as source of life and are better energy source, and they will be suitable for diet formulation.

The mushrooms were known to contain calcium, potassium, magnesium, phosphorus and sodium and these elements are very important in human nutrition. They are required in repairing worn-out cells, strong bone and teeth, building blood cells and maintaining osmotic balance (WHO, 1996).

The results in Table 4 indicate the concentration of heavy metal content in the mushrooms. Cadmium is an highly toxic element, its presence at elevated levels in soil and drinking water is a threat to food safety and human health (Geng et al., 2005). They affect biological activities and have detrimental effect on digestive, respiratory and immune system.

High concentration of zinc is widely used throughout the world in medicines, foods and in industries for preventing corrosion. Zinc is one of the most important mineral our body needs due to the fact that it is highly associated with protein and carbohydrate foods. Zinc is also used in medicines that treat rashes, acne, dandruff and athlete's foot.

Lead is known to be harmful in nature, serves as lead compounds in drug discovery and paints. Lead can cause kidney damage, miscarriages, anemia and rise in blood pressure. Copper is a trace element that is essential for human health. Cadmium and lead are known as principal toxic elements since they inhibit many vital processes. They can be taken up directly from water and, to some extent, from air and dietary food. These elements also have a tendency to accumulate in both plants and animals (Demirbas, 2001). Low heavy metals (Cd or Pb) were detected in the fruiting bodies studied. However, the

samples used in this study had acceptable concentration level according to FAO/WHO (1976) standards for Cd and Pb. According to FAO/WHO (1989) standards, acceptable intakes of Cd and Pb for an adult are 0.42-0.49 and 1.5-1.75 mg/week, respectively. The trace element concentrations in mushrooms are generally species-dependent (Kalac and Svoboda, 2000) and are hardly affected by the pH or organic matter content of the soil. Falandysz et al. (2008) reported that Cd and Pb could be considered limiting metals in edible mushrooms. As shown in Table 4, *C. candidissimus* contained 2.10 and 0.85 ppm of Pb and Cd, respectively. These are well over the FAO/WHO recommended safe values. In other words, the Cd and Pb concentrations of the samples were above the tolerance limits established by the FAO/WHO. Hence, this species is better recommended for uses other than consumption.

Studies are on going to ascertain the nutritive mineral and vitamins of these mushroom species to know their food and medicinal values so as to be fully exploited for enhancement of life and mankind.

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