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Evaluation of the Bioactive Composition of Some Wild Mushrooms Found in Abia State of Nigeria

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Abstract: **The study was designed to evaluate the phytochemical composition, vitamins, minerals and heavy metal compositions of four wild species of mushrooms (*Steriumcomplicatum, Laetiporussulphhureas*, *Pleurotus species* and *Lentinussquarrosulus*). Results of the investigation revealed that the bioactive constituents of the mushrooms were as follows; Cyanide (2.78±0.00-4.39±0.01), Flavonoids (5.76±0.01-6.21+0.01%), Tannins (4.21±0.00-6.71±0.01%), Saponin (0.11±0.01-0.15±0.00%). The results of vitamins composition indicated that the mushrooms contained; vitamin A (1.06±0.00-2.11±0.01mg/100g), Vitamin B_1 (1.18±0.01-2.11±0.01mg/100g), Vitamin C (30.8±0.00-68±0.00mg/100g). The mineral compositions of the mushrooms were as follows; Magnesium (6.13±0.00-8.38±0.05mg/100g), Calcium (1.79±0.05-3.73±0.05mg/100g), Sodium (17.45±0.05-26.85±0.05mg/100g), and Phosphorus (26.25±0.05-41.1±0.1mg/100g). Heavy metals concentration indicated Lead (5.73±0.01-49±0.00ppm), Iron (49.7±0.10-73.55±0.05ppm), Manganese (14.7±0.00-28.8±0.10ppm) and Zinc (15.75±0.05-20.25±0.05ppm). The results obtained indicate that mushrooms are good sources of phytochemicals, vitamins, minerals needed for maintenance of good health and can also be incorporated in the production of drugs. Similarly, the heavy metals obtained from the mushrooms indicate that when consumed in high quantity may cause damage to the liver or kidney; death may result.**

Keywords: **Evaluation, Wild Mushrooms, Nutrients, Bioactive.**

1.INTRODUCTION

Mushroom is a general term used mainly for the fruiting body of macro fungi of the *Ascomycota* and *Basidiomycota* and represents only a short reproductive stage in their life cycle (Das, 2010). 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 animal wastes, and soil where they obtain their nutrients through external digestion and absorption by the mycelium. Mushroom can be epigeous or hypogeous, large enough to be seen with the unaided eyes and can be picked by hand (Chang and Miles, 1992).There are edible and poisonous mushrooms and both categories possess nutrient and medicinal values.

Mushrooms have a long association with human kind and provide profound biological and economic impact. From ancient times, wild mushrooms have been consumed by man as delicacy probably because of their taste and pleasing flavour (Das, 2010).They have rich nutritional value with high content 0f proteins, vitamins, minerals, fibres, trace elements and low or no calories and cholesterol (Wani*et al.,* 2010). Mushrooms are edible and they are common ingredients in soups and salads and can also be served as a side dish. The nutritional value of fried mushrooms depends on the type of the agricultural waste used for its production. White mushrooms contain 26 calories of energy per hundred gram of fruiting body. These have 43% carbohydrates, less than 1% of fat, 3.9% protein and are also rich riboflavin, niacin and pantothenic acid (Robinson, 2011). In Nigeria, mushrooms are consumed not only as food but also used for their medicinal values. 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 instances, the sclerotia of *Pluerotustuberregiums* used as thickener as well as preparing melon cake (*usu*). The food and Agriculture Organization recognized mushrooms as food contributing protein nutrition to the countries depending largely on cereals (FAO, 2011). Oyster mushrooms are rich source of proteins minerals (Caglarirmak, 2007). The desirability of a food product does not necessarily bear any correlation to its nutritional value, instead its appearance, taste and aroma stimulates ones appetite (preference). In addition to nutritional values, mushrooms have some major unique colour, tastes, aroma and texture characteristics which attract their consumption by humans (Shuting, 2004).

Mushrooms have been reported as therapeutic foods, useful in preventing diseases such as hypertension, hyper-cholesterolemia and cancer. In the 16th century, herbalist John Gerard recommended *Auriculariaauricular 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. Many of them have been used in folk medicine for thousands of years. Some of them are nutriceuticals (natural food having potential value in maintaining good health and boosting immune system of the human body) while others can produce potent nutriceuticals (compounds that have medicinal and nutritional attributes and are consumed as medicines in the form of capsules or tablets but not as food (Elmastas*et al.,* 2007). Mushrooms are known to be rich sources of various bioactive substances like antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti tumour, cyto-toxic, anti-diabetic compounds (Lindequist*et al.,* 2005). In developing countries like India with rich biodiversity, mushrooms are a bone for progress in the field of food, medicine and unemployment because of

several nutriceuticals and medicinal mushrooms that have been found to be useful towards human health development as food, medicine, minerals and drugs among others (Wani*et al.,* 2010). In Ghana, it has been reported that *Auriculariaauriculajudae* 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).

According to Guci*aet al.,* (2011), edible wild mushrooms contain micro nutrients, non-essential trace elements. Many wild edible and poisonous mushroom species have been known to accumulate great concentration of heavy metals while edible and poisonous mushroom has been found to be affected by environmental and fungal factor (Falandysz, 2003). Environmental factors such as organic matter amount, p^H and metal concentrations in soil and fungal factor. Organisms require trace amounts of some heavy metals including iron, cobalt, copper, manganese, chromium and zinc. Minerals such as iron, copper, zinc and manganese are essential metals since they play an important role in biological systems.

2.MATERIALS AND METHODS

Fresh and fleshy mushrooms (*Stereumco++mplicatum*, *Laetiporussulphureas*, *Pluerotusspecie* and *Lentinussqua-rrorulus*) were collected from different localities in Umuahia, Abia State in August 2014. The species of mushroom were dried at 50^{o}c for 48 hours in the oven following a modified method of (Efe, 2007).

Preparation of Samples for Analysis

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

Phytochemical Analysis

Tannins Determination

The method of Swain (1979) was used for the determination of tannin contents. About 0.2g of finely ground sample was measured into a 50ml beaker. 2ml of 50% methanol was added and covered with paraffin and placed in a water bath at 77- 80^{o}C for 1hour and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No. 1 filter paper into a 100ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. One (1ml) of sample extract was pipette into 50ml volumetric flask, 20ml distilled water, and 2.5ml Folin Denis Reagent and 10ml of 17% Na_2CO_3 was added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20mins when a bluish-green colouration developed. Standard tannic acid solutions of range 0-10ppm were treated similarly as 1ml of sample above. The absorbance of the tannic acid standard solutions as well as samples was read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760nm.

Percentage tannin was calculated using the formula

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Cyanide Determination

The method of Onwuka (2005) was used for the determination of cyanide. One (1) ml of the sample filtrate was poured in the corked test tube and four (4ml) of alkaline picrate was added and incubated in a water bath for 5min. After colour development (reddish brown colour), the absorbance of the corked test tube was read in the spectrophotometer at 490nm. Also the absorbance of the blank containing only 1ml distilled water was read and 4ml alkaline picrate solution was added. Extrapolate of the cyanide content was done from the cyanide standard curve.

Preparation of Cyanide Standard Curve

Different concentrations of KCN solution containing 5 to 50g cyanide was prepared in a 500ml conical flask. Twenty-five (25ml) of HCl was added. The different prepared cyanide standard curve was used to determine the concentrations.

Saponin Determination

The spectrophotometer method of Brunner (1984) was used for saponin determination. One (1g) of the finely ground sample was weighed into a 250ml beaker and 100ml isobutyl alcohol was added. The mixture was shaken on an Udy shaker for 5hrs. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of magnesium carbonate added. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatman No. 1 filter paper to obtain a clear colourless solution. One (1ml) of the colourless solution was pipette into 5ml volumetric flask and 2ml of 5% $FeCl_3$ solution was added and made up to mark with diluted water. It was allowed to stand for 30mins for blood red colour to develop. 0-10ppm standard saponin solutions was prepared from saponin stock solution. The standard solutions was treated similarly with 2ml of 5% FeCl solution as done for 1ml sample 3 above. The absorbance of the sample as well as standard saponin solutions was read after colour development on a spectronic 21D spectrophotometer at a wavelength of 380nm.

Percentage saponin was calculated using the formula.

$$\text{Saponin (\%)} = \frac{\text{Absorbance of sample} \times \text{average gradient} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Flavonoid Determination

This was determined according to the method of Harbone (1973). Five (5) grams of the sample was boiled in 50ml of 2m HCl solution for 30 min under reflux. It was allowed to cool and then filtered through Whatman No. 42 filter paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with drop. The

flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoid in the sample.

Vitamin Determination

Vitamin B_1 (Thyamine)

The method of Onwuka (2005) was used for vitamin B1 determination. Two (2g) of sample was weighed out and 50ml of alcoholic NaOH was added and allowed to stand for few minutes before being filtered out. Ten (10ml) of filtrate was measured out and 10ml of potassium dichromate added. The absorbance was read at 430nm.

Calculation of vitamin B1 was done as follows

$$Weight\ of\ sample = \frac{100 \times au \times C \times VF \times D}{as \times Va}$$

Where:

au = absorbance of sample, as = absorbance of stand,

C = conc. of standard (mg/l), VF = total volume of extract, Va = volume of extract analyzed, D = dilution factor where applicable.

Vitamin C Determination

The method of Onwuka (2005) was used for vitamin c determination. Five (5g) of the sample was used for the determination.

The Vitamin C content was calculated based on the relationship that 1ml 0.01m CuSO4 = 0.88mg Vitamin C.

Therefore

$$\text{vitamin C Mg}/100\text{g} = \frac{100 \times 0.08 \times (T.B) \times vt}{W \times VA}$$

Where:

W = Weight of sample, T = Titre value of sample,

B= Titre value of blank, Vt = Total extract volume,

Va = volume of extract titrate

Vitamin A

The method of the association of Vitamin Chemist (Kirkand Sawyer, 1998) was employed. A measured weight (5.0g) of the processed sample was dispersed in 30ml of absolute alcohol 3ml of 5% KOH solution was added to it and boiled under reflux for 30mins. After cooling rapidly in running water, 30ml of distilled water was added to it and the mixture was transferred into a

separation funnel. Three portion of 50ml of ether was used to wash the mixture thus extracting the vitamin A, the lower layer (aqueous) was discarded while the vitamin A extract was washed with a 50ml distilled water. Care was taken to avoid formation of emulsion. The extract was then evaporated to dryness and dissolved in 10ml of isoprophylalcohol and the absorbance of the vitamin A extract was also measured at 325nm. The vitamin A content was calculated using the relationship below

$$vitamin\ A\ mg/100g = \frac{100 \times au \times C}{W \times as}$$

Where:

W = Weight of sample, au = Absorbance of sample,

as = Absorbance of standard, C = Concentration of standard (mg/L).

Mineral Determination

The levels of the mineral content (P, Ca, Mg and Na) was done following the dry ash extraction methods (Jams, 1995), Kirk and Sawyer, (1998), Udoh and Oguwale (1986). A measured weight (5.0g) of the sample was burnt to ashes (as in ash determination). The resulting ash was dissolved in 5mls in a volume flask. This extracts was used in specific analysis for the different mineral elements.

Heavy Metal Analysis

The method used by Onwuka (2005). The dried sample was weighed into crucible and place in muffle furnace at room temperature and the temperature raised between 200-3000^{o}c over one hour. The furnace temperature was finally raised to 500^{o}c to complete the ash process. The ash was then dissolved in hot 10% HCl or HNO3 filter and diluted to required volume in standard flask with deionized water or dilute acid (0.01m HCl or HNO_3). The crucible was cooled to room temperature and 1 or 2 drops of deionized water, HNO3 or HCl was added. Dried at 120^{o}C and the ash process was completed in furnace at 500^{o}c. Thereafter, the extract was taken to the AAS (atomic absorption spectrophotometer) for the determination of the micro elements (Pb, Fe, Mn and Zn).

3.RESULTS

The results of phytochemicals, vitamins, minerals and heavy metals composition of the four (4) wild mushrooms namely *Stereumcomplicatum*, *Laetiporussulphureas*, *Pleurotus species* and *Lentinussquarrosulus* are presented in table 1-4 below.

Table 1: Phytochemical Composition of Mushrooms

Sample	HCN%	FLA%	TAN%	SAP%
Stereumcomplicatum	2.78±0.000	6.205±0.005	4.21±0.000	0.12±0.000
Laetiporussulphureas	3.87±0.000	5.81±0.01	5.405±0.005	0.135±0.005
Pleurotus specie	3.865±0.005	6.105±0.005	6.705±0.005	0.105±0.005
Lentinussquarrosulus	4.385±0.005	5.755±0.005	6.655±0.005	0.15±0.000

Values are means of three replicates

The results of phytochemical composition of the mushrooms are presented in Table 1. The result shows that all the mushrooms contain cyanide, flavonoid, tannin and saponin in varying quantities. However, the highest level

of cyanide contents was obtained in *Lentinussquarrorulus*. The highest content of flavonoid was obtained from *Stererumcomplicatum* and the lowest from *Lentinussquarrosulus*. The tannin content of *Plerotus species* was the highest and the lowest from *Stereumcomplicatum*.

Table 2: Vitamin Composition of Mushrooms (mg/100g)

Sample	Vit. A	Vit. B1	Vit. C
Stereumcomplicatum	2.11±0.01	2.105±0.005	30.8±0.000
Laetiporussulphureas	1.845±0.005	1.375±0.005	44.05±0.05
Pleurotus species	1.06±0.000	1.175±0.005	52.75±0.05
Lentinussquarrosulus	1.16±0.000	1.205±0.000	68.2±0.000

Values are means of three replicates

The results of the vitamins composition as summarized in Table 2. The highest vitamin content was found in *S. complicatum* and the least in *Pletuous species.* Vitamin B_1 content of *S.* complicatum was the highest and *L. squarrosulus* was the lowest. Vitamin C was significantly higher in *L. squarrosulus* and the least was *Pleurotus species*

Table 3: Mineral Composition of Mushrooms

Sample	Mg (mg/100g)	Ca (mg/100g)	Na (mg/100g)	K (mg/100g)
Stereumcomplicatum	6.13±0.000	3.725±0.05	26.75±0.5	41.1±0.1
Laetiporussulphureas	6.825±0.05	3.105±0.05	26.85±0.5	28.2±0.1
Pleurotus species	8.375±0.05	1.785±0.05	20.5±0.000	26.25±0.05
Lentinussquarrosulus	7.48±0.000	2.405±0.005	17.45±0.05	27.45±0.05

Values are means of three replicates

The results of the mineral composition as summarized in Table 3. The highest magnesium was obtained in *Pleurotus species* and the lowest in S.*complicatum*. Calcium content of *S. complicatum* was the highest and *Pleurotus species* was the least. Sodium was significantly higher in *L. sulphureas* and the least in *L. squarrosulus*. *S.complicatum* was the highest in phosphorus and *Pleurotus species* was the least. The mushrooms were known to contain magnesium, calcium, sodium and phosphorus

Table 4: Heavy Metals Composition of Mushrooms

Sample	Pb (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Stereumcomplicatum	9.0±0.000	49.7±0.1	28.8±0.1	19.15±0.05
Laetiporussulphureas	5.725±0.005	69.05±0.05	22.45±0.05	20.25±0.05
Pleurotus species	28.85±0.05	73.55±0.05	14.7±0.000	15.75±0.05
Lentinussquarrosulus	49.3±0.00	68.05±0.05	22.6±0.000	18.8±0.1

Values are means of three replicates

The result of heavy metal composition of the mushrooms as summarised in Table 4. *L. squarrosulus* contains the highest contents of lead while *L. sulphureas* contains the lowest. Iron content of *Pleurotus species* was the highest and *S. complicatum* contains the least number of iron. The highest manganese was obtained in *S. complicatum* and the lowest in *Pleurotus specie*. Zinc was significantly higher in *L. sulphureas* and the least in *Pleurotus specie*. The result indicates that the mushrooms contain higher concentration of heavy metal content

4.DISCUSSION

The results obtained indicate the presence of phytochemicals in all the samples but in varying levels. Flavonoids in the mushroom indicate their medicinal value of having antioxidant properties against free radical scavengers which prevent oxidative cell damage and have strong anti-cancer activity (Okwu, 2004). The high content of saponin in the mushroom is useful in pharmaceutical industry due to its foaming ability that produces frothy effects in the food industry. Tannin concentration detected in the mushrooms has been found to possess astringent properties which hasten the healing of wound and in flamed mucous membrane (Okwu, 2004).

Vitamins contained in this mushrooms are the perfect food for everyone. Vitamin B_1 control the release of energy from carbohydrate which is needed for the normal functioning of the brain, these elements is 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).

Metals such as iron, copper, zinc and manganese are essential metals since they play an important role in biological systems. Lead and cadmium are toxic, even in traces. The essential metals can also produce toxic effects when the metal intake is excessively elevated (Tuxen*et al*., 2007). High concentration of zinc is widely used throughout the world in medicine, foods and industries for preventing corrosion. Zinc is the most important mineral our body needs due to the fact that it is highly associated with protein and carbohydrate. Lead is known to be harmful in nature, serve as lead compounds in drug discovery and paints. Lead can cause kidney damage, miscarriage, anemia and rise in blood pressure. It can be deducted that some of the mushroom species contains variable higher concentration of some of this heavy metals. However, the trace element concentrations in mushroom are generally species dependent (Kalac and Svoboda, 2001) and are also affected by the pH or organic matter content of the soil. Therefore, the heavy metal concentration of the mushroom was above the tolerance limits established by FAO/WHO. Hence this species are recommended not to be consumed in excess or for other uses than consumption.

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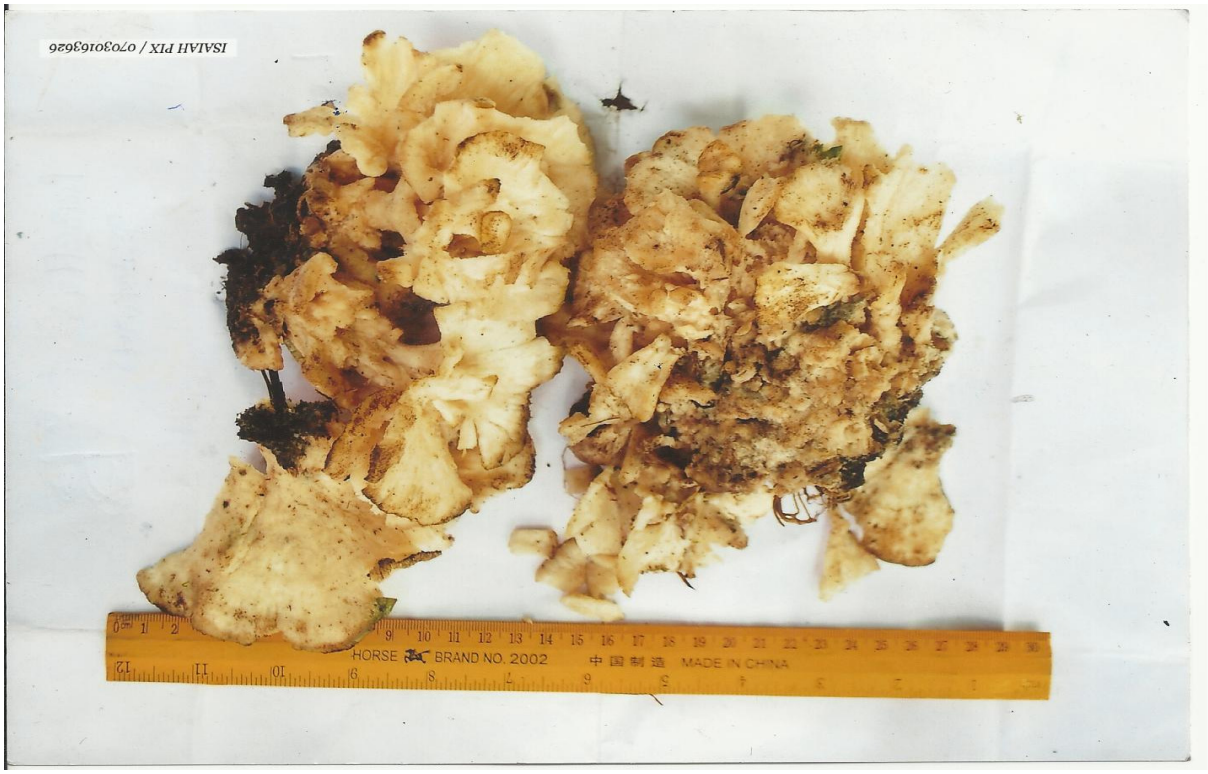
Stereumcomplicatum



Laetiporussulphureas

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