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Phytochemical Screening, Proximate Composition and Mineral Activities of Four Selected Nigerian Mushrooms

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ABSTRACT: The study examined the phytochemical screening, proximate composition and mineral activities of four selected mushrooms. Four fungi (*Calocybe indica, Pleurotus pulmonarius, Auricularia sp*, and *Pleurotus ostreatus*) were collected and phytochemical, proximate and mineral contents were determined. Results showed that moisture content was observed to be highest in *Auricularia sp*. (46.8 %), *C.indica* (46.8 %) protein (10.31 %), ash (2.72 %), fat (3.2 %), fibre (3.7 %) and CHO (38.36 %) were observed to be highest in *P. ostreatus*, while ash was observed to be lowest in *C. indica* (2.0 %). Potassium (0.33 %), calcium (0.14 %), manganese (0.28 %) and phosphorus (0.31 %) were observed to be highest in *P. ostreatus*, sodium (0.15 %) was observed to have highest value in *P. pulmonariu*s, while sodium (0.3 %) was lowest in *C. indica*. For macromineral iron (70.65 %), zinc (16.53 %), magnesium (6.39 %) and copper (3.54 %) were observed to be highest in *P. ostreatus*, while copper (1.83 %) was observed to be lowest in *C. indica*. Phytochemical screening revealed that alkaloid (0.54 %), phenol (0.35 %) and saponin (0.47 %) were highest in *P. ostreatus*, while tannin was observed to have the highest values in *P. ostreatus* (0.07 %) and *P. pulmonarius* (0.07 %). These bioactive compounds and nutritional contents in *C. indica, P. pulmonarius, Auricularia sp*, and *P. ostreatus* may be responsible for their nutritional and therapeutic uses. These results therefore not only make mushrooms popular to consume as food sources but also make them valuable in drug development.

Keywords: Phytochemical Screening; Proximate Composition; Mineral Activities, Fungi

Introduction

Mushroom has been defined as 'a macro-fungus with a distinctive fruiting body, which can be hypogenous or epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 2009). They are documented as being rich in proteins, minerals, vitamins while they are low in lipids (Pathak, 2007). Documented literature indicates that mushrooms have photochemicals and other compounds which are strong antioxidants (Fang, 2002; Liu, 2004**)**. Phenolic compounds, alkaloids, saponins, flavonoids, tannins, sterols, triterpenes, coumarins and cyanogenic glycosides have been detected in wild mushrooms analyzed in Sudan and in Nigeria (Adebayo, 2012; Egwim, 2011; Ehssan and Saadabi, 2012). The compounds seem to mop the free radicals generated in the normal natural metabolism of aerobic cells, mostly in the form of reactive oxygen species (ROS). These include superoxide (O_2-) and hydroxyl (OH^-) radicals among several others.

Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Barja, 2004). Once in circulation, most of the free radicals are neutralized by cellular antioxidant defense enzymes e.g. Superoxide dismutase (SOD) or catalase (CAT). Non-enzymatic molecules like ascorbic acid and carotenoids are reported to be present in mushrooms and they also act as antioxidants (Fang, 2002; Isabel, 2004). Maintenance of equilibrium between free radicals production and antioxidant defenses is an essential condition for normal organism functioning (Hollman and Arts, 2000). The disequilibrium, excess free radicals in the system, is known as oxidative stress. It interferes with cell integrity hence normal functioning is altered leading to many stress-related diseases like cancers and diabetes. Mushroom nutriceuticals describe a new class of compounds extractable from either the mycelium or fruit body of mushrooms and embodies both their nutritional and medicinal features. They are consumed as a dietary

supplement which has potential therapeutic applications (Chang and Miles, 1992). Musroom nutraceuticals are enriched food materials which are used for maintenance of healthy diet. These are part of a meal (Chang and Miles, 2009). Infusion of mushrooms has been used to prevent beriberi. In addition, the decoction has been used for the treatment of abscesses and wounds (Yu, 2009).

Mushrooms have continued to generate a lot of interest particularly in its consumption as food, in the cure of diseases, in bioremediation and as important items of commerce in Nigeria and all over the world. The increased interest in consumption of mushrooms as food stems from their nutritional, antioxidant and therapeutic values. Studies have shown that tropical mushrooms are highly rich in proteins, minerals, vitamins, crude fiber and carbohydrate with low fat and oil content. The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than that of wheat. The high level of vitamins in mushrooms particularly vitamin C and D has been reported as responsible for its antioxidative activity. Mushrooms contains also an appreciable quantities of crude fibres, although, little information exist on Total Dietary Fibre (TDF) content of mushrooms. The crude fibre content values reported from many studies suggest that mushrooms are potential sources of dietary fibre. Mushrooms generally contain low fat and oil content. Because of the low fat and oil content, they are recommended as good source of food supplement for patients with cardiac problems or at risk with lipid induced disorders.

Despite the many studies on nutrients and minerals contents of different mushroom species globally, little or no work has been carried out on the antioxidant activity in edible species such as *Calocybe indica, Pleurotus pulmonarius, Auricularia sp.* and *Pleurotus ostreatus* in parts of Nigeria. Also there are some edible species of mushrooms which are yet to be exploited and it is to this end that the present work is aimed at investigating the proximate composition, mineral activities and the phytochemical content of four selected Nigerian mushrooms.

Materials and methods

Fungi used: The fungi used for this study were *Calocybe indica, Pleurotus pulmonarius, Auricularia sp.* and *Pleurotus ostreatus,* which were obtained from the laboratory collection of Federal Institute of Industrial Research, Oshodi, (FIIRO), Lagos.

Proximate and mineral element content determination: Samples were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 18th Edition, 2005). All analysis was carried out in duplicate. Calcium, potassium, sodium and phosphorus determination were carried out using Spectrophotometric method.

Quantitative determinations of phytochemicals

Tannin: A sample of 0.2 g was measured into a 50 ml beaker 20 ml of 50 % methanol was added and covered with paraffin and placed in a water bath at 77-80 °C for 1 h. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered Whatman No 41 filter paper into a 100 ml volumetric flask, 20 ml water added, 2.5 ml Folin-Denis reagent and 10 ml of 17 % Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20 min. The bluish–green colour will develop at the end of range 0-10 ppm were treated similarly as 1 ml sample above. The absorbance of the tannic acid standard solutions as well as samples were read after color development on a spectronic 21D spectrophotometer at a wavelength of 760nm. % tannin was calculated using the formula below:

$$\% \text{ Tannin} = \frac{\text{absorbance of sample} \times \text{average gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Alkaloids: This is a distillation and titrimetric procedure 2 g of finely ground sample was weighed into a 100 ml beaker and 20 ml of 80 % absolute alcohol added to give a smooth paste. The mixture was transferred to a 250 ml flask and more alcohol added to make up to 100 ml and 1 g magnesium oxide added. The mixture was digested in a boiling water bath for 1.5 h under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a small Buchner funnel. The residue was returned to the flask and re-digested for 30min with 50 ml alcohol after which the alcohol will be evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10 % HCl was added. The whole solution was later transferred into a 250 ml volumetric flask 5 ml of zinc acetate solution and 5 ml of potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution.

The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filtrate was transferred into a separation flask and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10 ml hot distilled water and transferred into a Kjeldahl tube with the addition of 0.20 g sucrose and 10 ml Conc. H_2SO4 and 0.02 g selenium for digestion to a colourless solution to determine %N by Kjeldahl distillation method. %Nitrogen got is converted to % total alkaloid by multiplying by a factor of 3.26 i.e. % Total alkaloid = % N × 3.26. % Alkaloids = %N ×3.26

Saponin: The Spectrophotometric method of Brunner (1984) was used for saponin analysis. One gram (1 g) of finely ground sample was weighed into a 250 ml beaker and 100 ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter the mixture was filtered through a Whatman No1 filter paper into a 100 ml beaker and 20 ml of 40 % saturated solution of magnesium carbonate was added. The mixture obtained with saturated magnesium carbonate $MgCO_3$ was again filtered through a Whatman No1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50 ml volumetric flask and 2 ml of 5 % ferric chloride ($FeCl_3$) solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0-10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ solution as done for 1 ml sample above. The absorbance of the sample as well as standard saponin solutions were read after colour development in a Jenway V6300 spectrophotometer at a wavelength of 380 nm.

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

Steroids: A sample of 0.50 g was weighed into a 100 ml beaker. 20 ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 min on a shaker. The whole mixture was later filtered through a Whatman No.1 filter paper into another dry clean 100 ml conical flask/beaker. The resultant residue was repeatedly treated with chloroform-methanol mixture until free of steroids. 1 ml of the filtrate was pipetted into a 30 ml test tube and 5 ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37-40 °C for 90 min. It was cooled to room temperature and 10 ml of petroleum ether added followed by the addition of 5 ml distilled water. This was evaporated to dryness on the water bath. 6 ml of Liebermann Burchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620 nm on a Spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4 mg/ml were prepared from 100 mg/ml stock steroid solution and treated similarly like sample as above. % steroid was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Phenol: A sample of 0.20 g was weighed into a 50 ml beaker. 20 ml of acetone was added and homogenize properly for 1 hr to prevent lumping. The mixture was filtered through a Whatman No.1 filter paper into a 100 ml volumetric flask using acetone to rinse and made up to mark with distilled water with thorough mixing. 1 ml of sample extract was pipetted into 50 ml volumetric flask, 20 ml water added, 3 ml of phosphomolybdic acid added followed by the addition of 5 ml of 23 % Na_2CO_3 and mixed thoroughly, made up to mark with distilled water and allowed to stand for 10 min to develop bluish-green colour. Standard phenol of concentration range 0-10 mg/ml were prepared from 100 mg/l stock phenol solution from Sigma-Aldrich chemicals, U.S.A. The absorbance of sample as well as that of standard concentration of phenol were read on a digital spectrophotometer at a wavelength of 510 nm.The percentage phenol is calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

Data analysis: Descriptive analysis such as mean, standard deviation and bar chart as well as inferential statistics such as correlation analysis were carried out using Statistical Analysis (SAS) Software Package.

Results

Figure 1 shows that protein contents for the four selected mushroom values were 8.6 %, 8.1 %, 10.5 % and 10.1 %. The fat levels for all the selected mushrooms are quite low (2.7 %, 2.4 %, 3.3 % and 3.1 %) as the fibre contents (2.8 %, 2.4 %, 3.8 % and 3.6 %) and the ash levels (2.1 %, 2.0 %, 3.0 % and 2.4 %) whereas the moisture contents which are 46.8 %, 46.8 %, 40.9 % and 42.8 % are on high range as the CHO levels of 36.9 %, 37.8 %, 38.8 % and 38.0 (Figure 2).

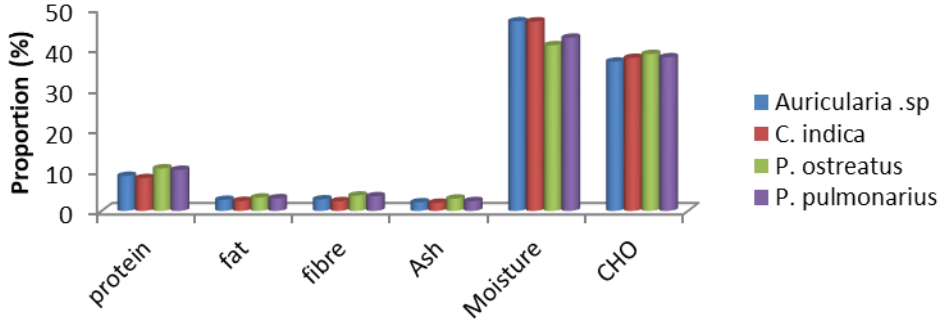

Figure1: Proximate composition of *P. ostreatus, Auricularia .sp, C. indica and P. pulmonarius*

Figure 2 shows that among the selected mushrooms, Na content was high for *C. indica* specie (0.29 %) and was low for other selected mushroom species (0.08 %, 1.12 % and 0.11 %). K content was very high for *P.ostreatus* specie (0.35 %), Ca contents were low (0.11 %, 0.11 %, 0.14 % and 0.14 %). The above results for macrominerals showed that Mg contents and P contents were high; (0.23 %, 0.23 %, 0.28 % and 0.27 %) and (0.27 %, 0.27 %, 0.32 % and 0.30 %) respectively.

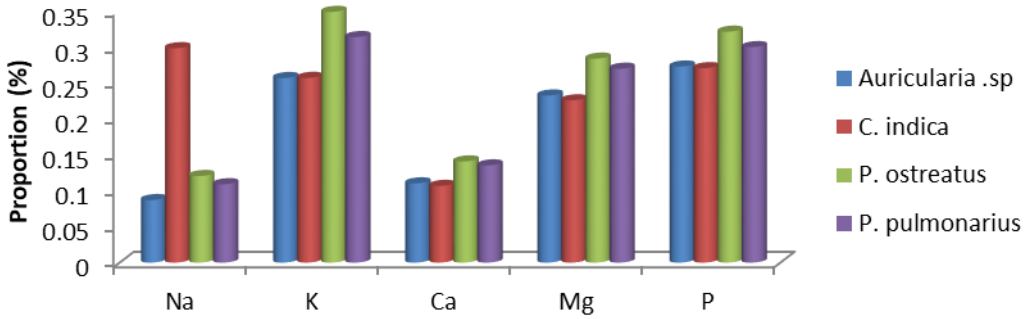

Figure 2: Macrominerals composition of *P. ostreatus, Auricularia .sp, C. indica* and *P. pulmonarius*

Figure 3 shows that preliminary phytochemical analysis revealed the presence of alkaloid, phenolic compounds, saponin, steroids and tannins in aqueous and methanolic extracts of the selected mushroom species. From the graphical analysis, it shows that alkaloid is more prevalent in *P. pulmonarius* than other mushroom species examined in the study. Also, there were prevalence of alkaloid in *Auricularia sp*. and *P.ostreatus* species, phenol in *P. pulmonarius* and Saponin in *P. ostreatus* and *Auricularia sp*. respectively.

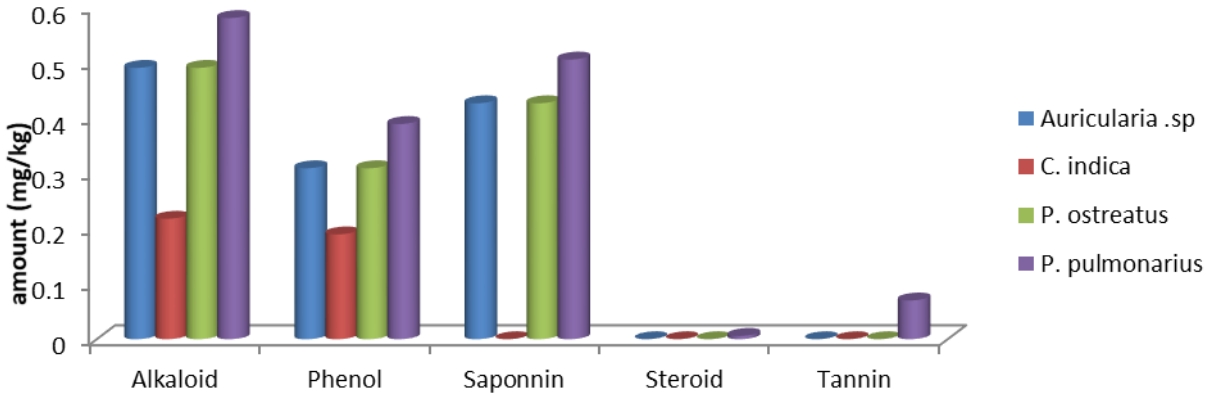

Figure 3: Phytochemicals composition of *P. ostreatus, Auricularia .sp, C. indica and P. pulmonarius*

Figure 4 shows that among the selected mushrooms, Fe (70.65%), Zn (16.53%), Mn (6.39%) and Cu (3.54%) were observed to be highest in *P.ostreatus.* Fe (57.5%), Zn (14.2%), Mn (4.13%) and Cu (1.83%) were observed to be low in *C.indica*.

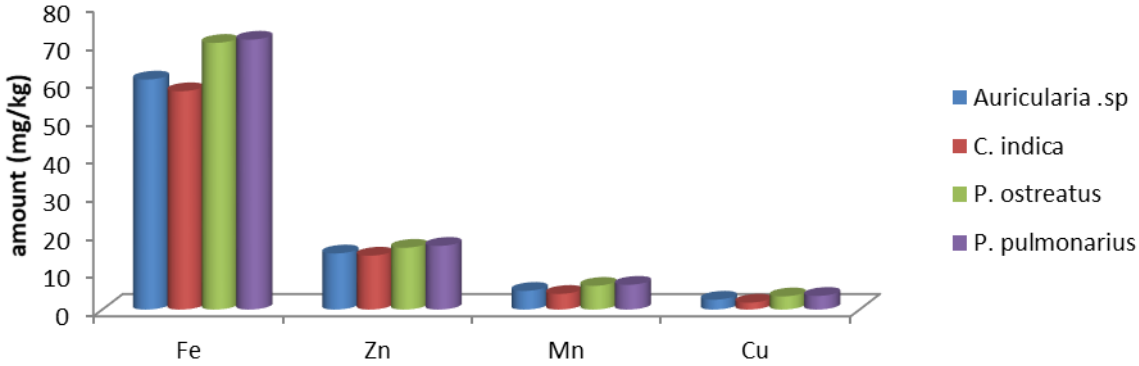

Figure 4: Microminerals composition of *P. ostreatus, Auricularia .sp, C. indica* and *P. pulmonarius*

Proximate macromineral, micromineral and phytochemical contents of P. pulmonarius: The result in Table 1 shows the proximate composition, macromineral and micromineral contents as well as the phytochemical

properties of *P.pulmonarius*. The proximate analysis of the mushroom sample (*P. pulmonarius)* revealed protein content (9.33 % and 1.42), fat (2.89 % and 0.49), fibre (3.15 % and 0.78), ash (2.38 % and 0.5), moisture (44.34 % and 3.95) and CHO by difference (37.85 % and 1.35) on dry weight basis respectively. The result of macro mineral analysis shows sodium (015 % and 0.22), potassium (0.29 % and 0.05), calcium (0.12 % and 0.02), magnesium (0.25 % and 0.03) and phosphorus (0.29 % and 0.03); these values were lower compared with the values of the micro minerals as follows; iron (64.84 % and 8.64), zinc (15.51 % and 1.76), manganese (5.46 % and 1.51) and copper (2.88 % and 1.16). Also, Table 1 shows the phytochemical properties of *P. pulmonarius.* It is observed from the result that saponin is the predominant anti-nutrient followed by phenol and tannin. The quantitative analysis of the phytochemical properties of the mushroom species showed alkaloids (0.4 %), phenol (0.28 %), saponin (0.45 %), steroids (0.01 %) and tannin (0.07 %). Saponin seems to be the most predominant phytochemical in the mushroom species as obtained in the values of the phytochemicals.

Table 1: Proximate, macromineral, micromineral and phytochemical contents of *P. pulmonarius*

Variable	Mean	Std Dev	Std Error	Coeff of Variation	Range	Minimum	Maximum	N
Proximate (%)								
Protein	9.33	1.42	0.35	15.21	4.18	6.97	11.15	16
Fat	2.89	0.49	0.12	16.97	1.46	2.15	3.61	16
Fibre	3.15	0.78	0.2	24.88	2.18	2.03	4.21	16
Ash	2.38	0.5	0.12	20.94	1.61	1.75	3.36	16
Moisture	44.34	3.95	0.99	8.91	12.69	37.46	50.15	16
CHO	37.85	1.35	0.34	3.57	4.11	36.51	40.62	16
Macrominerals (%)								
Na	0.15	0.22	0.05	142.1	0.91	0.06	0.97	16
K	0.29	0.05	0.01	16.1	0.13	0.22	0.36	16
Ca	0.12	0.02	0.01	16.49	0.06	0.09	0.15	16
Mg	0.25	0.03	0.01	12.41	0.09	0.21	0.3	16
P	0.29	0.03	0.01	9.71	0.1	0.24	0.34	16
Microminerals (mg/kg)								
Fe	64.84	8.64	2.16	13.32	24.1	49.7	73.8	16
Zn	15.51	1.76	0.44	11.32	5.9	12.3	18.2	16
Mn	5.46	1.51	0.38	27.59	4.7	2.8	7.5	16
Cu	2.88	1.16	0.29	40.27	3.6	1.1	4.7	16
Phytochemicals (mg/Kg)								
Alkaloids	0.4	0.16	0.05	40.23	0.39	0.2	0.58	10
Phenol	0.28	0.08	0.03	29.53	0.22	0.18	0.39	10
Saponin	0.45	0.04	0.02	9.02	0.08	0.43	0.51	6
Steroids	0.01	0	0	5.94	0	0.01	0.01	2
Tannin	0.07	0	0	5.98	0.01	0.07	0.07	2

Proximate, macromineral, micromineral and phytochemical contents of Auricularia sp.: The result in Table 2 shows the proximate properties of *Auricularia sp*., macro and micro minerals as well as the phytochemical properties of the mushroom. The proximate analysis of the mushroom revealed protein content (8.64 % and 1.82), fat (2.71 % and 0.63), fibre (2.84 % and 0.92), ash (2.1 % and 0.38), moisture (46.85 % and 3.8) and CHO by difference (36.88 % and 0.12) on dry weight basis respectively. The result of macromineral analysis shows sodium (0.09% and 0.03), potassium (0.26 % and 0.04), calcium (0.11 % and 0.03), magnesium (0.23 % and 0.03) and phosphorus (0.27 % and 0.03); these values were lower compared with the values of the microminerals as follow; iron (60.58 % and 12.44), zinc (14.78 % and 2.63), manganese (4.93 % and 2.18) and copper (2.6 % and 1.63). Also, Table 2 shows the phytochemical properties of *Auricularia sp*. It is observed from the result that saponin is the predominant anti-nutrient followed by phenol and tannin. The quantitative analysis of the phytochemical properties of the mushroom species showed alkaloids (0.49 %), phenol (0.31 %), saponin (0.43 %). Alkaloid seems to be the most predominant phytochemical in the mushroom species as obtained in the values of the phytochemicals. The proximate analysis of the mushroom samples revealed protein content (8.08 % and 0.29), fat (2.45 % and 0.14), fibre (2.39 % and 0.33), ash (1.99 % and 0.09), moisture (46.8 % and 1.05) and CHO by difference (37.8 % and 0.38) on dry weight basis respectively. The result of macromineral analysis shows sodium (0.3 % and 0.45), potassium (0.26 % and 0.01), calcium (0.11 % and 0.01), magnesium (0.23 % and 0.01) and phosphorus (0.27 % and 0.01); these values were lower compared with the values of the microminerals as follow; iron (57.5 % and 4.74), zinc (14.2 % and 0.61), manganese (4.13 % and 0.69) and copper (1.83 % and 0.29).

Table 2: Proximate, macromineral, micromineral and phytochemical contents of *Auricularia sp*

Variable	Mean	Std Dev	Std Error	Coeff of Var	Range	Minimum	Maximum	N
Protein	8.64	1.82	0.91	21.09	3.3	6.97	10.27	4
Fat	2.71	0.63	0.31	23.14	1.11	2.15	3.26	4
Fibre	2.84	0.92	0.46	32.46	1.62	2.03	3.65	4
Ash	2.1	0.38	0.19	18.21	0.69	1.75	2.44	4
Moisture	46.85	3.8	1.9	8.11	6.61	43.54	50.15	4
CHO	36.88	0.12	0.06	0.32	0.27	36.74	37.01	4
Na	0.09	0.03	0.02	35.93	0.06	0.06	0.12	4
K	0.26	0.04	0.02	14.07	0.06	0.22	0.29	4
Ca	0.11	0.03	0.01	23.34	0.05	0.09	0.13	4
Mg	0.23	0.03	0.01	12.18	0.05	0.21	0.26	4
P	0.27	0.03	0.02	11.81	0.06	0.24	0.3	4
Fe	60.58	12.44	6.22	20.54	21.8	49.7	71.5	4
Zn	14.78	2.63	1.32	17.83	4.9	12.3	17.2	4
Mn	4.93	2.18	1.09	44.19	4.1	2.8	6.9	4
Cu	2.6	1.63	0.81	62.57	3.1	1.1	4.2	4
Alkaloids	0.49	0	0	0.43	0	0.49	0.49	2
Phenol	0.31	0	0	0.92	0	0.31	0.31	2
Saponin	0.43	0	0	0.5	0	0.43	0.43	2
Steroids	.	.	.	.	.	.	.	0
Tannin	.	.	.	.	.	.	.	0

Also, Table 3 shows the phytochemical properties of *C.indica*. It is observed from the result that saponin is the predominant anti-nutrient followed by phenol and tannin. The quantitative analysis of the phytochemical properties of the mushroom species showed alkaloids (0.22%), phenol (0.19%). Alkaloids seems to be the most predominant phytochemical in the mushroom species as obtained in the values of the phytochemicals

Table 3: Proximate, macromineral, micromineral and phytochemical contents of *Calocybe indica*

Variable	Mean	Std Dev	Std Error	Coeff. of Var	Range	Minimum	Maximum	N
Protein	8.08	0.29	0.14	3.55	0.59	7.78	8.37	4
Fat	2.45	0.14	0.07	5.81	0.27	2.31	2.58	4
Fibre	2.39	0.33	0.16	13.81	0.59	2.09	2.68	4
Ash	1.99	0.09	0.05	4.73	0.19	1.89	2.08	4
Moisture	46.8	1.05	0.52	2.24	2.14	46.23	48.37	4
CHO	37.8	0.38	0.19	1.02	0.77	37.44	38.21	4
Na	0.3	0.45	0.22	149.73	0.9	0.07	0.97	4
K	0.26	0.01	0.01	4.55	0.02	0.25	0.27	4
Ca	0.11	0.01	0.01	10.64	0.02	0.1	0.12	4
Mg	0.23	0.01	0.01	5.54	0.02	0.21	0.24	4
P	0.27	0.01	0.01	4.14	0.02	0.26	0.28	4
Fe	57.5	4.74	2.37	8.24	8.5	53.2	61.7	4
Zn	14.2	0.61	0.3	4.26	1.3	13.6	14.9	4
Mn	4.13	0.69	0.35	16.84	1.5	3.4	4.9	4
Cu	1.83	0.29	0.14	15.74	0.7	1.5	2.2	4
Alkaloids	0.22	0.02	0.01	10.55	0.04	0.2	0.24	4
Phenol	0.19	0.01	0.01	6.65	0.03	0.18	0.2	4
Saponin	.	.	.	.	.	.	.	0
Steroids	.	.	.	.	.	.	.	0
Tannin	.	.	.	.	.	.	.	0

Proximate, macromineral, micromineral and phytochemical contents of Pluerotus ostreatus: The result in Table 4 shows the proximate properties of *P. ostreatus* macro and microminerals as well as the phytochemical properties of the mushroom. The proximate analysis of the mushroom samples revealed protein content (10.31 % and 0.77), fat (3.2 % and 0.31), fibre (3.7 % and 0.42), ash (2.72 % and 0.45), moisture (41.85 % and 3.56) and CHO by difference (38.36 % and 1.74) on dry weight basis respectively. The result of macromineral analysis shows sodium (0.11 % and 0.01), potassium (0.33 % and 0.03), calcium (0.14 % and 0.01), magnesium (0.28 % and 0.02) and phosphorus (0.31 % and 0.02); these values were lower compared with

the values of the microminerals as follows: iron (70.65 % and 2.06), zinc (16.53 % and 1), manganese (6.39 % and 0.67) and copper (3.54 % and 0.73).

Table 4 also shows the phytochemical properties of *P. ostreatus*. It is observed from the result that saponin is the predominant anti-nutrient followed by phenol and tannin. The quantitative analysis of the phytochemical properties of the mushroom species showed alkaloids (0.54 %), phenol (0.35 %), saponin (0.47 %), steroids (0.01 %) and tannin (0.07 %). Alkaloids seems to be the most predominant phytochemical in the mushroom species as obtained in the values of the phytochemicals.

Table 4: Proximate, macromineral, micromineral and phytochemical content of *Pluerotus ostreatus*

Variable	Mean	Std Dev	Std Error	Coeff of Variation	Range	Minimum	Maximum	N
Protein	10.31	0.77	0.27	7.44	1.86	9.29	11.15	8
Fat	3.2	0.31	0.11	9.84	0.75	2.86	3.61	8
Fibre	3.7	0.42	0.15	11.4	0.96	3.25	4.21	8
Ash	2.72	0.45	0.16	16.52	1.13	2.23	3.36	8
Moisture	41.85	3.56	1.26	8.51	8.28	37.46	45.74	8
CHO	38.36	1.74	0.61	4.53	4.11	36.51	40.62	8
Na	0.11	0.01	0	8.6	0.03	0.1	0.13	8
K	0.33	0.03	0.01	9.66	0.08	0.28	0.36	8
Ca	0.14	0.01	0	4.85	0.02	0.13	0.15	8
Mg	0.28	0.02	0.01	7.17	0.05	0.25	0.3	8
P	0.31	0.02	0.01	6	0.05	0.29	0.34	8
Fe	70.65	2.06	0.73	2.91	5.4	68.4	73.8	8
Zn	16.53	1	0.35	6.07	2.8	15.4	18.2	8
Mn	6.39	0.67	0.24	10.57	1.9	5.6	7.5	8
Cu	3.54	0.73	0.26	20.55	2.1	2.6	4.7	8
Alkaloids	0.54	0.05	0.03	9.71	0.09	0.49	0.58	4
Phenol	0.35	0.05	0.02	13.26	0.09	0.31	0.39	4
Saponin	0.47	0.05	0.02	9.79	0.08	0.43	0.51	4
Steroids	0.01	0	0	5.94	0	0.01	0.01	2
Tannin	0.07	0	0	5.98	0.01	0.07	0.07	2

The result in Table 5 shows the phytochemical constituents (alkaloids, tannin, phlobatannin, saponin, flavonoids, anthraquinones, steroids, terpenes, cardenolides, phenol, chalcones and cardiac glycosides) that are present in *Auricularia sp, Calocybe indica, P. ostreatus* and *P. pulmonarius* at varying degrees. The table shows that alkaloids is present in an appreciable amount in all the selected mushroom species of *Auricularia sp., Calocybe indica, P. ostreatus* and *P. pulmonarius*. Tannin is present in an appreciable amount in *Plueurotus pulmonarius* and *Pluerotus ostreatus*, but present in a moderate amount in *Calocybe indica*, and in a minute amount in *Auricularia sp*., Phlobatannin is present in a minute of amount in all the selected mushroom species of *Auricularia sp., Calocybe indica, P. ostreatus* and *P. pulmonarius*. Saponin is present in an appreciable amount in *Pleurotus pulmonarius* and *Pluerotus ostreatus* but present in a moderate amount in *Calocybe indica* and *Auricularia sp*.

Flavonoids is completely absent in *Calocybe indica* ,present in a moderate amount in *Pleurotus ostereatus*, but present in a minute amount *Pluerotus pulmonarius* and *Auricularia sp*. Anthraquinones is present in a moderate amount in *Calocybe indica*, completely absent in *Pluerotus pulmonarius* and *Auricularia sp*., but present in a minute amount in *Pluerotus ostreatus*. Steroids is present in a minute amount in *calocybe indica* and *Pluerotus ostreatus* ,present in an appreciable amount in *Pluerotus pulmonarius* and present in a moderate amount in *Auricularia sp*. Terpenes is completely absent in *Calocybe indica* ,present in a moderate amount in *Pleurotus pulmonarius* and *Auricularia sp*., and present in a minute amount in *Pluerotus ostreatus*. Cardenolides is completely absent in *Calocybe indica*, and *Pluerotus ostreatus*, but present in a minute amount in *Pleurotus pulmonarius* and *Auricularia sp*. Phenol is present in an appreciable amount in all selected mushrooms species of *Auricularia sp., Calocybe indica, P.ostreatus* and *P.pulmonarius*. Chalcones is completely absent in *Calocybe indica* but present in a minute amount in *Auricularia sp., P. ostreatus* and *P. pulmonarius*. Finally, cardiac gylcosides is present in a minute amount in *Calocybe indica* and also present in a moderate amount in *Auricularia sp., P. ostreatus* and *P. pulmonarius*.

Table 5: Secondary metabolites present in *Auricularia sp,. Cylocybe indica, P. ostreatus* and *P. pulmonarius*

Phytochemical	***C. indica***	***P. pulmonarius***	***Auricularia sp.***	***P. ostreatus***
Alkaloids	+++	+++	+++	+++
Tannin	++	+++	+	+++
Phlobatannin	+	+	+	+
Saponin	++	+++	++	+++
Flavonoids	-	+	+	++
Anthraquinones	++	-	-	+
Steroids	+	+++	++	+
Terpenes	-	++	++	+
Cardenolides	-	+	+	-
Phenol	+++	+++	+++	+++
Chalcones	-	+	+	+
Cardiac glycosides	+	++	++	++

Key: +++ Present in an appreciable amount; ++ Present in a moderate amount; + Present in a minute amount;
- Completely absent

Table 6 shows the Pearson's Correlation Coefficient between the proximate contents, macro and micro mineral compounds and phytochemical contents of the selected mushroom species. Correlation was tested at $P \leq 0.05$. From the results, there was significantly positive correlation between protein and fat ($r = 0.98$; $P \leq 0.05$), protein and fibre ($r = 0.99$; $P \leq 0.05$), protein and ash ($r = 0.83$; $P \leq 0.05$) and protein and CHO ($r = 0.57$; $P \leq 0.05$). However, protein has negative correlation with moisture ($r = - 0.57$; $P \leq 0.05$). The study also revealed that fat has strong positive correlation with fibre ($r = 0.99$; $P \leq 0.05$), ash ($r = 0.83$ and CHO ($r = 0.61$; $P \leq 0.05$); $P \leq 0.05$) while very strong negative relationship exist between fat and moisture ($r = - 0.95$; $P \leq 0.05$). The results of the analysis above also shows significant positive correlation between fibre and ash ($r = 0.83$; $P \leq 0.05$). The study reveals significant positive relation between fat and CHO ($r = 0.58$; $P \leq 0.05$) while fibre has negative correlation with moisture ($r = - 0.93$; $P \leq 0.05$). The results of Table 6 also shows the correlation between the macromineral contents present in the selected mushroom species. From the analysis, there was weak negative correlation between Na and K ($r = - 0.16$; $P \leq 0.05$), Na and Ca ($r = - 0.23$; $P \leq 0.05$), Na and Mg ($r = - 0.22$; $P \leq 0.05$), Na and P ($r = - 0.17$; $P \leq 0.05$). Also, there was significant positive correlation between K and Ca ($r = 0.91$; $P \leq 0.05$), K and Mg ($r = 0.098$; $P \leq 0.05$),K and P ($r = 0.94$; $P \leq 0.05$) and finally, there was significant positive correlation between Ca and Mg ($r = 0.95$; $P \leq 0.05$), Ca and P ($r = 0.96$; $P \leq 0.05$), and the results of the analysis reveal significant positive correlation between Mg and P ($r = 0.97$; $P \leq 0.05$). The findings from Table 6 also shows the correlation coefficient between the micromineral contents of the selected mushroom species. The results show that there was significant positive correlation between Fe and Zn ($r = 0.96$; $P \leq 0.05$), Fe and Mn ($r = 0.98$; $P \leq 0.05$), Fe and Cu ($r = 0.92$; $P \leq 0.05$), Zn and Mn ($r = 0.99$; $P \leq 0.05$), Zn and Cu ($r = 0.98$; $P \leq 0.05$) and there was significant relationship between Mn and Cu ($r = 0.98$; $P \leq 0.05$). The correlation analysis in Table 5 reveals that there was significant positive correlation between Alkaloids and Phenol ($r = 0.99$; $P \leq 0.05$) on the selected mushroom species.

Discussion

The study examined the proximate composition, phytochemical screening and mineral activities of some selected mushrooms namely *Calocybe indica, Pleurotus pulmonarius, Auricularia sp.* and *Pleurotus oestreatus*. The fat and protein contents in these mushrooms are similar to the results obtained by Fasidi and Kadiri (2004) and this proved that mushrooms are nutritious and good for human consumption. The high moisture content accounts for its short shelf life as it deteriorates easily after harvest if preservative measures are not employed. This high water content promotes susceptibility to microbial growth and enzymes activities. However, moisture content of mushroom depends on their harvesting time, maturation period and environmental conditions such as humidity and temperature in growing period and storage conditions (Crisan and Sands, 2008). The crude fibre content obtained from this study suggests that this mushroom is a potential source of dietary fibre (roughages). High level of fibre is known as anti-tumorigenic and hypocholestrolaemic agent (Okoro and Achuba, 2012). This implies that this mushroom may be recommended for people with cholesterol related problems (Chihara, 2003). Low crude fat recorded from this study in comparison to protein suggests that this mushroom could be recommended as good source of food supplement for patient with cardiac problems or at risk with lipid induced disorders.

Table 6: Pearson's correlation of proximate properties, minerals compounds and phytochemical contents of *Auricularia sp*., *Cylocybe indica*, *P.ostreatus* and *P.pulmonarius*

	Protein	Fat	Fibre	Ash	Moisture	CHO	Na	K	Ca	Mg	P	Fe	Zn	Mn	Cu	Alkaloids	Phenol	Saponin
Protein	1	0.98 <.0001	0.99 <.0001	0.83 <.0001	-0.94 <.0001	0.57 0.0216	-0.17 0.5298	0.93 <.0001	0.96 <.0001	0.97 <.0001	0.96 <.0001	0.95 <.0001	0.93 <.0001	0.93 <.0001	0.92 <.0001	0.38 0.2828	0.37 0.2928	0.08 0.879
Fat		1	0.99 <.0001	0.83 <.0001	-0.95 <.0001	0.61 0.0113	-0.22 0.421	0.89 <.0001	0.95 <.0001	0.96 <.0001	0.96 <.0001	0.92 <.0001	0.87 <.0001	0.89 <.0001	0.86 <.0001	0.39 0.2614	0.37 0.2928	-0.01 0.9903
Fibre			1	0.83 <.0001	-0.93 <.0001	0.58 0.0189	-0.26 0.3235	0.91 <.0001	0.97 <.0001	0.97 <.0001	0.96 <.0001	0.95 <.0001	0.89 <.0001	0.91 <.0001	0.88 <.0001	0.46 0.1831	0.44 0.1996	0.08 0.8869
Ash				1	-0.82 0.0001	0.56 0.0247	-0.16 0.5612	0.89 <.0001	0.82 <.0001	0.86 <.0001	0.88 <.0001	0.75 0.0008	0.66 0.0051	0.70 0.0027	0.67 0.0044	0.35 0.3235	0.30 0.3958	-0.18 0.7368
Moisture					1	-0.81 0.0002	0.04 0.8763	-0.91 <.0001	-0.88 <.0001	-0.95 <.0001	-0.95 <.0001	-0.82 0.0001	-0.78 0.0004	-0.77 0.0004	-0.76 0.0006	-0.22 0.5365	-0.18 0.6117	0.17 0.7457
CHO						1	-0.03 0.9045	0.64 0.0074	0.50 0.0511	0.67 0.0048	0.65 0.0063	0.38 0.152	0.35 0.1854	0.32 0.231	0.32 0.2324	-0.07 0.8423	-0.16 0.6504	-0.55 0.2569
Na							1	-0.16 0.5455	-0.23 0.3869	-0.22 0.4216	-0.17 0.5386	-0.25 0.3511	-0.16 0.5434	-0.21 0.4249	-0.15 0.5743	-0.32 0.3712	-0.28 0.425	0.14 0.7912
K								1	0.91 <.0001	0.98 <.0001	0.94 <.0001	0.86 <.0001	0.83 <.0001	0.84 <.0001	0.83 <.0001	0.30 0.4066	0.26 0.4594	-0.09 0.8615
Ca									1	0.95 <.0001	0.96 <.0001	0.97 <.0001	0.90 <.0001	0.92 <.0001	0.87 <.0001	0.36 0.3035	0.38 0.2763	0.26 0.6226
Mg										1	0.97 <.0001	0.91 <.0001	0.87 <.0001	0.88 <.0001	0.86 <.0001	0.36 0.3019	0.34 0.34	-0.04 0.9437
P											1	0.90 <.0001	0.82 <.0001	0.84 <.0001	0.80 0.0002	0.29 0.4235	0.27 0.4557	-0.02 0.97
Fe												1	0.96 <.0001	0.98 <.0001	0.92 <.0001	0.38 0.2856	0.42 0.2322	0.41 0.4205
Zn													1	0.99 <.0001	0.98 <.0001	0.24 0.4977	0.29 0.4102	0.38 0.4515
Mn														1	0.98 <.0001	0.38 0.2799	0.42 0.2258	0.41 0.4246
Cu															1	0.40 0.2567	0.42 0.2287	0.26 0.6171
Alkaloids																1	0.99 <.0001	1.00 <.0001
Phenol																	1	1.00 <.0001
Saponin																		1

The analysis of mineral elements of these mushrooms revealed appreciable concentrations of calcium, magnesium, potassium and phosphorus while iron and zinc were in lower concentrations. This observation is similar to studies by Crisan and Sands (2008); Chang and Miles (2004) who reported that the most common minerals in mushrooms are potassium, phosphorous, sodium, calcium and magnesium. Phytochemical analysis which revealed the presence of saponins, alkaloids, tannins, phenol and steroids is similar to the study of Schneider and Wolfling (2004) that saponins inhibit sodium ions efflux by blockage of the influx of concentration in the cells activating a sodium-calcium ions anti-porter in cardiac muscle and the increase in calcium ions influx through this anti-porter strengthens the contraction of heart muscles. Saponins can also inhibit the growth of cancer cells, boost immune system and energy, lower cholesterol, act as natural anti-inflammatory, antibiotic, and anti-oxidant, and can reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physicochemical interaction (Aberoumand, 2012; DeSilva, 2013) and have haemolytic activity (Khalil and Eladawy, 2004). Tannin concentration detected in mushrooms has been found to possess astringent properties, which hasten the healing of wounds and inflamed mucous membrane (Okwu, 2004).

Alkaloids have been reported to have stimulating effects and act as tropical anaesthetic in ophthalmology, powerful pain relievers, antipuretic action (Edeoga and Eriata, 2001). The presence of alkaloids explains that the mushroom may have antibacterial activity as explained by Idowu (2003) that alkaloids have antibacterial activity.These bioactive compounds together with the mineral activities obtained in *C. indica, P. pulmonarius, Auricularia sp. and P. oestreatus* together with their nutrient contents may be responsible for human nutritional and therapeutic uses. These results therefore not only make these mushrooms popular to consume as good food sources but also make them valuable in drug development. Alkaloids isolated from species of mushrooms usually show high value for its phytochemical screening and this study confirms it to be one of the major phytochemical in mushrooms. The presence of alkaloids and saponins in many mushroom species have also been reported by Yang, (2003); Beecher, (2003); Redhead, (2001) to be of great value. The medicinal uses of these mushrooms were also reported by these researchers. This value may be due to the presence of the secondary metabolites in these mushrooms. For example, some alkaloids in mushrooms have been found to be carcinogenic, mutagenic and teratogenic while others cause abnormal sperm and male sterility. Sterols in these mushrooms have been found to exhibit antibacterial activity (Barros *et al.*, 2007). The nutritional importance of a given food depends on the nutrients composition (Aletor and Omodara, 2004). The steroids and tannin contents were lower than other phytochemical contents in the selected mushroom species suggesting that they are less complex in nutritional purpose than other minerals thereby preventing efficient absorption by their body systems (Aletor and Omodara, 2004). This is in support of the findings of Jiskani (2001); Sadler (2003); Moore and Chi (2005) who that mushrooms have nutritional attributes and have potential applications in human and other animals. Wang and Zhao (2023) indicated that mushrooms are highly nutritional and compared favourably with meat and milk; will be a good supplement to cereals or carbohydrate meals (Chang and Buswell, 2006) and a significant dietary component for vegetarians (Breene, 2000).

The presence of these essential nutrients and minerals found in these species implies that it can be utilized for its medicinal values in healthcare delivery systems. Potassium and calcium are important in stimulating action potential across nerve endings, and also to enhance heart contractile rate (Jeremy, 2007). Iron is highly required physiologically for heme formation and to enhance oxygen carrying capacity of red blood cells. Zinc is an important requirement in protein synthesis, normal body development and recovery from illnesses. It is a co-factor in the function of the enzyme carbonic anhydrase required for carbon dioxide transport and as part of peptidases needed for protein digestion (Muhammad, 2011); it is also a necessary part of DNA for cell division and synthesis hence its importance in wound healing. Calcium is the major component of bone and assists teeth development (Brody, 2004). Magnesium is an essential cofactor in many enzymatic reactions in intermediary metabolism (Akpanabiater, 2008). Calcium and phosphorus are directly involved in the development and maintenance of the skeletal system and participate in several physiological processes and plays an important role in muscle contraction, blood clot formation, and nerve impulse transmission, the maintenance of cell integrity and acid-base equilibrium, and activation of several important enzymes. Phosphorus is an important constituent of nucleic acids and cell membranes, and is directly involved in all energy-producing cellular reactions. Finally, this study shows that the selected mushroom species has a great potential in complementing protein and minerals deficiencies prevalent in the developing countries. It should be incorporated into our diets in order to improve its quality and thereby improve the overall health and general wellbeing of people.

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