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Pharmaceutical and Nutritional Prospects of Two Wild Macro-Fungi Found in Nigeria

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Abstract: The bioactive, proximate and vitamin composition of two wild macro fungi, found in Nigeria were investigated. The macro fungi, Schizophyllum commune, (split gill mushroom) and *Polyporus* sp.(bracket fungi) were obtained growing on dead logs of mango (Mangifera indica) and Iroko (Chlorophora excelsa), respectively. The fruit-bodies of the fungi were harvested fresh, dried, ground and analysed using Association of Analytical Chemists methods and other standard methods. The results of the analyses showed that the macro-fungi contained the bioactive compounds, alkaloids flavonoids, phenols, saponins and tannins in varying quantities. The fungi are equally rich in proteins, vitamins and mineral elements. The alkaloids contents of the two macro-fungi, *S. commune* and *Polyporus* are 0.015 and 0.013 mg, respectively. Flavonoids occurred more in *S. commune*, (3.8 mg100 g^{-1}) than in *Polyporus* sp.(0.60 mg 100 g^{-1}). While there is more saponins in *Polyporus* sp. (2.50 mg) than in *S. commune* (0.40 mg 100 mg^{-1}). Furthermore phenols and tannins occurred more in *S. commune* (0.70 and 0.70 mg) than in *Polyporus* sp. (0.081 and 0.32 mg), respectively. On the other hand, *Polyporus* sp. contained the highest amount of ash (8.22%), fat (2.04%), crude protein (14.00%) crude fibre (0.068), carbohydrates (85%) and moisture (95%) than *S. commune* that had 7.46, 1.28, 9.63, 0.044 81.59 and 91.80% of the compounds, respectively. *S. commune* is richer in ascorbic acid (0.49 mg) and niacin (1.30 mg) than *Polyporus* sp. 0.37 and 0.48 mg, respectively; whereas riboflavin and thiamin of Polyporus sp (0.54 and 0.47 mg) appeared more than those of *S. commune* with 0.22 and 0.28 mg, respectively, The mineral elements of *S. commune* of calcium (1.50 mg), potassium (3.63 mg) and sodium (0.38 mg) are higher than those of *Polyporus* sp. of calcium (1.401 mg), potassium (2.69 mg) and sodium (0.25 mg), while the magnesium (0.79 mg), nitrogen (2.24 mg) and phosphorus (0.25 mg) of *Polyporus* sp. are higher than those of *S. commune,* respectively. The result obtained from the analyses of the 2 macro fungi has been discussed in relation to their prospects for medicinal purposes.

Key words: Wild macro-fungi, bioactive compounds, proximate analysis, pharmaceutical prospects, Nigeria

INTRODUCTION

In present times, mushrooms have continued to generate a lot interest. These interests are mainly in the areas of the implication of mushrooms as food (Chang, 1980) in the cure of diseases (Rambelli and Menini, 1983; Oei, 1991; Buswell and Chang, 1993; Stamets, 1993) in bioremediation and as important item of commerce (Smith, 1972; Stamets, 1993).

The increased interest in the consumption of mushrooms as food stems from their nutritional and therapeutic values. Most mushrooms are very important nutritionally. According to Aletor (1995), Fasidi (1996), Okwulehie and Odunze (2004a) tropical mushrooms are rich in protein, minerals and vitamins. 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 (Aletor, 1990). It is not surprising therefore, Okwulehie and Odunze (2004a) reported that the increased demand for mushrooms could be contingent upon the phenomenal rise in the unit costs of the conventional sources of animal proteins such as beef, pork, chicken and fish as opined by Aletor (1995).

Mushrooms contain appreciable quantities of crude fibres. Although little information exists on the Total Dietary Fibre (TDF) content of mushroom, the crude fibre contents values reported by many authors, suggest that mushrooms are potential sources of dietary fibres (Crisan and Sands, 1978; Kurasawa *et al*., 1982).

According to Oso (1977), Okwulehie and Odunze (2004a) mushrooms generally contain low fat and oil content. Because of the low content of mushrooms, they are recommended as good food supplement for patients with cardiac problems.

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The vitamins content of many mushrooms have been investigated and results of such investigations show that they are rich in vitamins C, B1, B2, B3 and vitamins D, (Bano and Rajarathnam, 1981; Okwulehie and Odunze, 2004a). Since vitamins are essential in the diet of man and conventional sources of vitamins are scarce (Aletor, 1995) it is pertinent therefore that attempts made to increase the list of the sources of cheap vitamins, is not misdirected.

Fasidi and Kadiri (1990), Aletor (1995) and Fasidi (1996) have also reported that tropical mushrooms are rich in minerals nutrients and carbohydrates. From the fore going, it is wise to recommend mushrooms as alternative nutrient-rich "bush meat" in the diet of Nigerians and to encourage researches geared towards discovering nutrient-rich species.

Mushrooms have been discovered to have therapeutic values. Bushwell and Chang (1993) reported that mushrooms have antitumoural, anticancer, anticholesterol and anti-hemorrhage effects. The considerable pharmacological activities of mushrooms make them of interest in pharmaceutical industries for the development of drugs. Most bioactive compounds which play essential roles in human and animal physiology have been found in many mushrooms. According to Okwulehi and Odunze (2004b) Auricularia auricular, Pleurotus squarrosulus and Russula sp has been found to contain appreciable amounts of Alkaloids, phenols ,Saponins and Flavonoids. According to Sotowara (1993) the plants widely used as in traditional medicine, contain in one or more of its parts potent bioactive compounds which are precusors for useful drug synthesis.

Alkaloids have powerful effects in animal physiology and are of interest in pharmaceutical industries for drug manufacture (Edeoga and Eriata, 2001). According to Rambelli and Menini (1983) alkaloids are stimulants and act by prolonging the action of several hormones.

Flavonoids have been reported to be useful in the treatment of some physiological disorders and diseases. According to Higland and Ferraro (1993) flavonones which are anti-oxidants are used to combat carcinogenesis and ageing processes, Similarly flavonoids have been reported to have anti-bacterial functions (Dokara,1995).

Saponins which are characterized by their bitter tastes, have been implicated in the prevention of parasitic fungi diseases (Bidwell, 1979) and protection of grains from attack by weevils and related animal parasites (Rioz and Chaudhary, 1993).

Tannins also inhibit pathogenic fungi and also reduce the rate of grazing on plants by animals that feed of plants. They also affect many human physiological activities such as stimulation of phagocyte cells, host-mediated anti-tumour activities and a wide range of anti-infective actions (Haslam, 1996).

The present research is aimed at investigating if the mushrooms under investigation contain the compounds mentioned above and how much. This is in addition to the ascertaining how rich in nutrient the mushrooms are. This would enable the authors suggest how much prospects exist in the use of the mushrooms investigated, as food and as medicinal raw material in drug synthesis.

MATERIALS AND METHODS

Sample collection, identification and preparation for analysis: The 2 wild macro-fungi, namely, *Schizophyllum commune* (split gill mushroom) and *Polyporus* sp. (Bracket fungus), used for the investigation were collected from logs of mango (*Mangifera indica*) tree and Iroko (*Chlorophora excelsa*) tree, respectively, from different parts of Abia State. They were harvested fresh and fleshy in the month of August, 2006 and were identified by Dr. I.A. Okwujiako; a mycologist in the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike, Abia State.

The macro-fungi were prepared for analysis by drying the frui-tbodies in the Selecta model oven at 104°C for 4 h, following the method of Latiff *et al.* (1996). The *Polyporus* sp. was dried longer because of its thick and fleshy nature.

The dried specimens were broken into smaller pieces before grinding into fine powder using a Thomas Willey milling machine (Okwulehie and Odunze, 2004a).The dried and powdered samples were dispensed into air-tight bottles and kept in a cool dry place until required for the analysis.

Determination of percentage bioactive compounds

Alkaloids: For quantitative estimation of alkaloids, the method of Maxwell *et al.* (1995) was followed. The alkaloids were extracted from 20 g of each of the dried powdered mushroom samples using 100 mL of 10% acetic acid which was left to stand for 4 h. The extracts were filtered to remove cellular debris and then concentrated to a quarter of the original volume. To this concentrate, 1% NH4O was added drop-wise until no precipitate was formed. The alkaloids thus obtained were dried to a const-ant weight at 65°C in an oven. The weights were used to calculate the percentage alkaloids using the formula

$$\text{Percentage alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

Determination of percentage flavonoids and saponins: The percentage flavonoids and saponins were determined following the methods of Bohn and Abyazani (1994) and Peng and Kobayashi (1995). The percentages were calculated as above.

Determination of phenols: To determine the percentage phenols in the test macro-fungi, the method of Harborne (1973) was followed. Two grams of the sample was defatted with 100 mL of diethyl ether for 2 h using the Soxlet apparatus. The fat-free sample was boiled with 50 mL of petroleum ether for 15 min to extract the phenolic component. Five milliliters of the extract was pipetted into which 10 mL of distilled water was added. Two milliliter of ammonium Hydroxide (NH4OH0) solution and 5 mL concentrated amyl-alcohol were also added and the solution was made up to mark and left for 30 min for colour development. The absorbance of the solution was read off at 505nm wavelength using a spectrophotometer.

Determination of tannins: To determine the tannins components of the macro-fungi the method of Okeke and Elekwa (2003) was followed. In a test tube containing 0.5 g of the sample, 10 mL of 2M HCl was added. This was shaken for 5 min and transferred into a volumetric flask and made up 50 mL. The mixture was filtered and 5 mL of the filtrate was introduced into a test tube. Three milliliters of 0.1M $FeCl_3$ in 0.1N HCl and 3 mL of 0.008M of potassium ferrocynide ($K3Fe[CN]_3$) were added. The absorbance was read at 720 nm within 10 min.

Proximate analysis: The percentage moisture and ash contents of the dry samples were obtained oven dehydration at 98°C for 5 h and then weighing the incinerated residue obtained at 600°C after 2 h (Leori-Guzman *et al*., 1997; AOAC, 1989).

Crude fibre content was determined by Weende method (AOAC, 1989) while total nitrogen content determined using the micro-Kjeldahl method of the OAAC. The crude protein content was calculated using a conversion factor of 6.25. The total carbohydrate content was estimated as the remainder after accounting for the ash, crude fibre, protein and fat contents (Miller and Tobin, 1980). Similarly the gross food energy was estimated following the method of Osborne and Voogt, using the equation

$$\text{Fe (in grams calories)} = (\%CP \times 4 + (\%Fat \times 9) + (\%CHO \times 4)$$

Where:
FE = Food Energy
CP = Crude Protein
CHO = Carbohydrate

Determination of vitamins: Vitamins were determined using spectrophotometric method, according to AOAC (1989). The powdered macro fungi samples were extracted by EDTA/TCA extraction method and the extracts were read off at different wave lengths.

Determination of mineral elements: The determinations of the mineral elements in the dry samples were done by following the wet digestion extraction methods described by Udo, Ojiwole and Adeyemi. The powdered samples were sieved and 0.2 g of each put into a 25 mL round bottomed flask. The samples were digested using 5 mL nitric acid (HNO3) and 2 mL perchloric acid (HClO4). The solution was filtered after adding 15 mL of distilled water into a 50 mL volumetric flask and the volumes made up to mark with more distilled water. The minerals in the digested samples were then determined by atomic absorption spectrophotometer following the development of colour with ammonium molybdate.

RESULTS AND DISCUSSION

The result of the bioactive compound composition of the *Schizophyllum commune* and *Polyporus* sp. is summarized in Table 1. Alkaliods, flavonoids, phenols, saponins and tannins were all detected in the two fungi. The quantities however, varied with the fungus species; for example, *S. commune* yielded more flavonoids (3.80%), phenols (0.70%0 and tannins (0.70%) than *Polyporus* sp. with 0.60% flavonoids, 0.081% phenols and 0.32% tannins; while *Polyporus* sp. contained higher amounts of saponins of 2.50% than *S. commune* (0.40%). *S. commune* and *Polyporus* sp. contained almost the same quantity of alkaloids of 0.015 and 0.013%, respectively. The presence of these bioactive compounds in the two fungi suggests that they have medicinal potentials. This inference is drawn from the fact that most of the plant parts uses in the treatment of diseases have traces of alkaloids. For example, Ralula *et al.* (1994) detected alkaloids in Euphobia species used as purgative. Similarly *Momordica charanta* and *Azadirachta indica* used in the cure of malaria contain alkaloids (Herborne, 1973 ; Haslam, 1998). Godwin and Mercer (1972) reported that saponins, in low concentrations are toxic to animals mainly because they cause haemolysis of red blood cells while Zhang reported that saponins derived from *Marsdenia* species caused infertility in rats. These reports further confirm the medicinal potentials of the fungi under investigation. *Polyporus* species however, appear to have more of this potential since it contains more saponins than *S. commune* According Godwin and Mercer (1972) flavonoids are phenolic glycosides which exist in vivo as

Table 1: Bioactive compounds composition of S*chizophyllum commune* and *Polypous* sp. investigated (percentage dry basis)

Bioactive compound	*Schizophyllumcommune*	*Polyporus* sp.
Alkaloids	0.015	0.013
Flavooids	3.80	0.60
Phenols	0.70	0.081
Saponins	0.40	2.50
Tannins	0.70	0.32

Values are means of three replicates

glycosides. Okwulehie and Odunze (2004b) detected large quantities of flavonoids in *Pleurotus tuber-regium.* This mushroom is consumed mainly for its flavour. It is possible that the flavonoids are responsible for the flavouring properties of the sclerotium of P. *tuber-regium*. Incidentally S. *commune* which yielded more flavonoids (3.80%) than *Polyporus* sp. (0.60%) is edible with good flavour, while *Polyporus* sp. is not edible. The low content of flavonoids and high content of saponins in the *Polyporus* sp. may be part of the reasons for its inedibility.

Results of the proximate analysis and vitamins content of the macro-fungi are presented in Table 2 and 3. The two macro-fungi are rich in crude protein which is 14. 00% in *Polyporus* sp. and 9.63% in *S. commune.* Similarly the ash, fat, crude fibre and moisture contents of the two macro-fungi are 7.46, 1.28, 0.044 and 91.8% for *S. commune* and 8.22, 2.04, 0.068 and 95.0% for *Polyporus* sp., respectively. Their carbohydrate contents are 81.59 and 85.67%, respectively, while their energy values are 376.40 and 414.36 cal g^{-1}, respectively.

The two fungi species also contain ascorbic acid, niacin, riboflavin and thiamin. There is not much variation in the ascorbic acid, riboflavin and thiamin contents. The values of these vitamins for *S.commune* are 0.49, 0.22 and 0.28% while the values for *Polyporus* sp.are 0.37, 0.54 and 0.47%. The niacin content of *S. commune* is 1.30% and that of *Polyporus* sp. is 0.48%.

Table 2: proximate composition of Schizophyllum commune and *Polyporus* sp. investigated (percentage)

Analysis	*S. Commune*	*Polyporus* sp.
Ash	7.46	8.22
Fat	1.28	2.04
Crude protean	9.63	14.00
Crude fibre	0.044	0.068
Carbohydrate	81.59	85.67
Moisture	91.80	95.0
Energy value	376.40	414.36

Values are means of three replicates

Table 3: Vitamin composition of S. *commune and Polyporus* sp. investigated (mg100 g^{-1} dry wt basis)

Vitamins	*S. Commune*	*Polyporus* sp.
Ascorbic and (vit. c)	0.490	0.370
Niacin ($vitb_3$)	1.30	0.48
Riboflavin($vitB_2$)	0.22	0.54
Thiamin ($vitB_1$)	0.28	0.47

Values are means of three replicates

Table 4: Mineral Clements composition of S. *commune and Polyorus* sp. investigated (mg^{-1}100g dry wt basis)

Mineral element	*S. commune*	*Polyporus* sp.
Calcium(Ca)	1.501	1.401
Magnesium(Mg)	0.73	0.790
Nitrogen (N)	1.54	2.24
Phosmphorys (P)	0.145	0.288
Sodum (Na)	0.375	0.250

Values are means of three replicates

The result of the proximate and vitamin analyses of the two macro-fungi clearly shows that they are rich in nutrients and hence qualify as good food materials. Earlier studies have indicated that mushrooms are rich sources of nutrients and compare favourably with meat, egg and milk (Gruen and Wong, 1982; Zakhary *et al*., 1983; Aletor and Aladetimi, 1989; Okwulehie and Odunze, 2004). The results also show that the two fungi are rich Sources of mineral elements. *S. commune* yielded 3.63% potassium, 1.54% nitrogen, 1.50% calcium 0.73% magnesium, 0.15% phosphorus and 0.38% sodium, while *Polyporus* sp. contains 2.69% potassium, 2.24% nitrogen, 1.40% calcium, 0.795 magnesium, 0.25% phosphorus and 0.25% sodium Table 4.

The trend of the mineral elements in the two fungi appears the same. For example potassium content is high in each case followed by nitrogen and calcium. Similarly phosphorus, sodium and magnesium are low.

Generally the two macro-fungi are rich in nutrients including vitamins and minerals elements. Unfortunately *Polyporus* fungi which is a bracket fungus is not edible while *S. commune* is edible in Nigeria and Malaysia (Latiff *et al*., 1996) although Kuo (2003) considers it to be non-edible. According to Kuo (2003) *S. commune* is too small and leathery to be considered of culinary value, the present authors how ever present a contrary view since the mushroom is rich in nutrients, moreover there are much smaller mushrooms such as *Flammulina velutipes* that are still relished as food.

The high contents of the bioactive composition of the two macro-fungi species makes them potentially useful pharmaceutically. The pharmaceutical potentials of *S. commune* is further strengthened by the reports of Qui and Lui (2000) who reported that *S.commune* is pharmacologically important because of the presence of schizophyllan, a polysaccharide which has a considerable anti-cancer activity. From the present findings it can be conceived that the two macro-fungi investigated hold tremendous potentials as raw materials for drug manufacture in addition to potentials of *S. commune* as rich source of proteins, vitamins and mineral elements needed in the diets of Nigerians.

Further researches is however, required in the characterization of the bioactive components.

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