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COAST
JOURNAL OF THE SCHOOL OF SCIENCE
Volume 4, Issue 2. November, 2022 ISSN: 2714-3716
Coast, J. Sch. Sci. 4 (2): 791 - 800

NUTRITIONAL, PHYTOCHEMICAL, AND ANTI-OXIDANT VALUES OF MUSHROOMS FROM IKALE AND ILAJE COMMUNITIES OF ONDO STATE, NIGERIA

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Abstract

Mushrooms are macro-fungi found growing in soil, decomposing wood, and agricultural wastes. Many edible mushrooms are either cultivated or harvested from the wild and served both as food and medicines in many parts of the world. Five mushrooms (*Auricularia auricular-judae, Ganoderma lucidum*, *Pleurotus ostreatus, Trametes ochracea* and *T. betulina*) collected from Ikale and Ilaje communities were assessed for their nutritional values, phytochemical constituents, and antioxidant properties. Chemical analyses were performed according to standard analytic methods. Free radical scavenging activities were determined with DPPH, ABTS, and reactions with ferrous sulphate and estimations from Ascorbic Acid standard curve. *T. betulina* had the highest flavonoid content (65.56mg/100g) while *P. ostreatus* had the least (27.45mg/100g). Phenolics were highest (128.13mg/100g) in *P. ostreatus* and lowest (30.58mg/100g) in *T. ochracea*. In terms of tannin content, *P. ostreatus* ranked highest (10.95mg/100g) and *A. auricular-judae* had the least (5.64mg/100g). However, oxalate was highest (0.81mg/100g) in *A. auricular-judae* and lowest (0.23mg/100g) in *P. ostreatus*. Of the four tests (DPPH, ABTS, FRAP, and TAC) performed for antioxidant activities, *P. ostreatus* was found to be the best radical scavenger with DPPH, FRAP and TAC while *T. betulina* gave the best result with ABTS. The mushrooms could be used as dietary supplements for energy and blood supply because of the carbohydrate and iron composition. However, toxicity studies are recommended on these mushrooms especially *T. betulina* (although considered inedible) which had flavonoid content that was significantly higher than the WHO safe limit.

Keywords: Mushroom, Phytochemical, Anti-oxidant, Ondo State, Nigeria

Introduction

Mushrooms are generally fleshy saprophytic fungi (belonging to the Phyla Basidiomycota and Ascomycota) with stipe-like stem, pileus in the form of cap, and lamellae (gills) on the underside of the cap (Kirk *et al.,* 2008; Hussein *et al.,* 2014). Their bodies grow under the ground (hypogenous) or above (epigenous) on different substrates including soil, living or decomposing wood, litter, and agricultural wastes (Boa, 2004; Edet *et al.,* 2016).

Edible mushrooms have found uses both as foods and medicines in humans' diets. They are rich sources of protein, lipids, amino acids, glycogen, vitamins and mineral

elements (Okhuoya *et al.*, 2010) and are also reported to have bioactive properties against common ailments such as asthma, smallpox, dysentery, stomach upset, cardiovascular diseases (Jonathan, 2002; Guillamon *et al.,* 2010). Some have been found to have anti-viral, anti-diabetic, anti-tumour, hypotensive, and immuno-modulatory activities (Omar *et al.,* 2011; Deeppalaksshmi and Sankaran, 2014). However, certain mushrooms that are considered edible by some people can cause allergic reactions in others (Afiukwa, 2013). The bioremediation potentials of a Nigerian white-rot fungus has been assessed by Adenipekun (2008).

The Yoruba people of Southwestern Nigeria have recognized different species of mushrooms not only as delicacies but also as part of traditional beliefs and myths (Oso, 1975, 1977b). For example, the white portion of the sclerotium of *Pleurotus tuber-regium* serves as a good replacement of melon in okra or vegetable soup. The legend and Yoruba folklore about the origins and traditional medicine applications of some mushrooms has been elaborately reported by Oso (1977a, b). In traditional medicine, a mixture of the sclerotium of *Pleurotus tuber-regium*, leaves of *Amaranthus viridis, Peperomia pellucida Kalanchoe crenata,* and *Lonchocarpus cyanescens* together with body fluid of snail, and palm oil is prepared by traditional doctors as oral remedies for protection against evil attacks (Oso, 1975).

Free radicals are reactive species resulting from the donation (to other substances) of oxygen formed during the essential metabolic processes in the human body or from external sources including air pollutants, cigarette smoking, radiation, pesticides, industrial solvents, and drugs (Bagchi and Puri, 1998). Hydroxyl, superoxide, nitric oxide, and lipid peroxyl radicals have been implicated in some clinical conditions such as brain disorder, ocular haemorrhage, respiratory distress, cancer, reproductive difficulty, gastrointestinal infections, and myocardial breach-induced stroke (Langseth, 1996). Antioxidants have been shown to neutralize free radicals and reduce their damaging effects in the body. Vitamins E, C (nutrients), and β-carotene (precursor of vitamin A) are important in the fight against oxidative stress in the body.

Apart from vegetables, meat, fish, poultry, spices, and fruits that are food sources of antioxidant vitamins (Naveen *et al.,* 2011), canthaxanthim (a non-nutrient antioxidant) from mushrooms have been assessed to be important in disease prevention and maintenance of good health (Langseth, 1996). Phenolic acids (in soya beans and red wine), polyphenols (in green/black tea and olives), phenolic esters (in coffee) and bio-flavonoids (in citrus and other fruits) are major anti-nutrient antioxidants. Medicinal plants that are rich in tannins, coumarins and flavonoids are also good antioxidants (Kahkonen *et al.,* 1999). Some mineral elements (copper, manganese, zinc and selenium) are major components of a defensive system of enzymes which decrease the deleterious oxidants in human tissues (Bagchi and Puri, 1998).

Wild mushrooms, which are valued among rural and semi-urban dwellers, are collected in Nigeria, mostly during raining season, for subsistence and also for sale in major markets (Ogundana and Fagade, 1982). However, biological facts about the mushrooms collected and consumed in Ikale and Ilaje communities of Ondo State is scarce, resulting in the dearth of information on the nutritional and antioxidant values of these macro-fungi. This study, therefore, describes the nutritive, phytochemical, and antioxidant values of wild mushrooms from Ikale and Ilaje communities of Ondo State, Nigeria. The aim is to provide information on the collection and

nutritional values of these macro-fungi among the Ikale and Ilaje people of Ondo State.

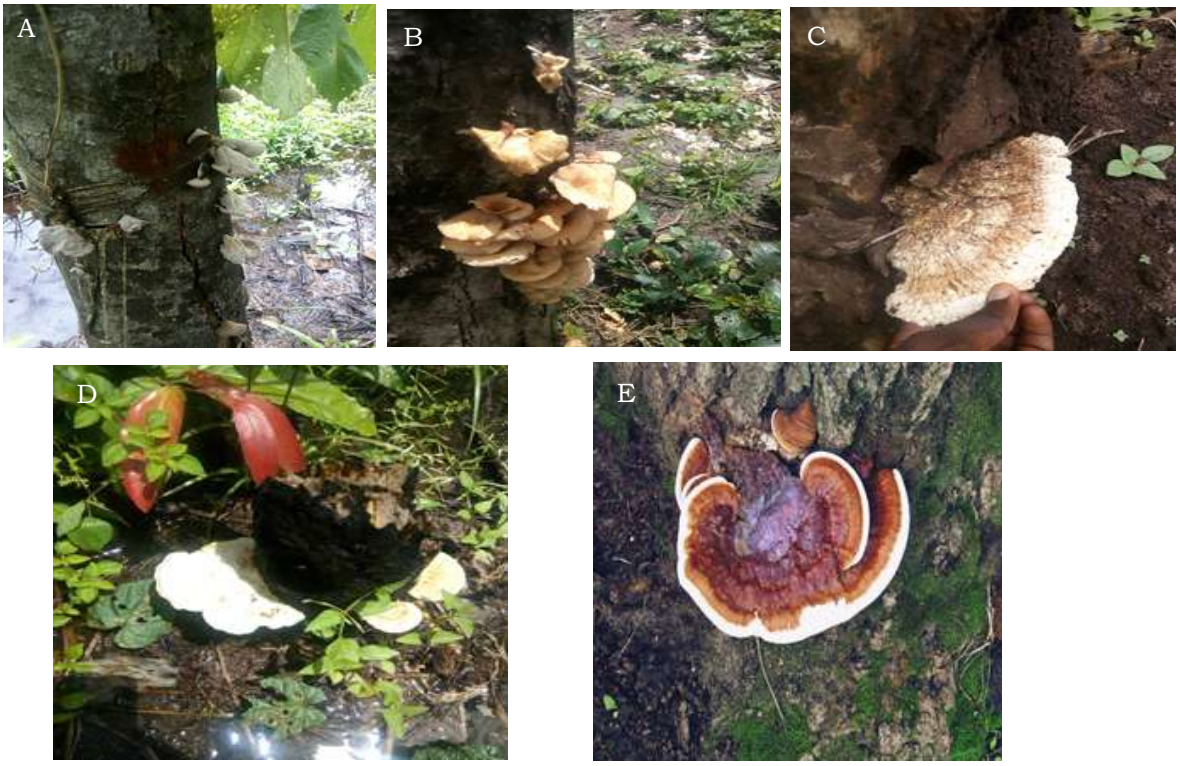
Materials and Methods

Study Areas

The Ikale people are found in Okitipupa (6° 32' 52" N, 4° 41' 56" E) and Irele (6° 29' 0" N, 4° 52' 0" E) LGAs of Ondo State; their language (Ikale) is considered as a dialect of the Yoruba language. The Ikales engage in agricultural businesses of palm oil, vegetables, plantain, cassava, and timber. The Ilajes, on the other hand, are a distinct migratory coastal group residing in Ilaje Local Government (6° 12' 11" N, 4° 46' 4" E) and also found among Apoi and Arogbo-Ijaws in Ese-Odo Local Government. The Ilajes speak the Ilaje language, which is also considered to be a dialect of the Yoruba language. The occupational activities of the Ilajes include farming, fishing, canoe construction, fish trap making, mat making, and trading.

Collection of Mushrooms

The Ikale and Ilaje indigenes were interviewed to determine their knowledge and utilization of mushrooms for subsistence or for commercial purposes. Direct questioning (interview) was employed to elicit information on the collection of wild mushrooms from their localities. Five (5) mushroom species (*Auricularia auricular-judae, Ganoderma lucidum, Pleurotus ostreatus, Trametes betulina* and *T. ochracea*) (Plates 1A-E) were collected (from the trunk of living and dead trees and soil) very early in the morning from farmlands in Ikale and Ilaje communities of Ondo State.



Plates 1A-E: Mushroom samples collected for this study. A, *Auricularia auricular-judae*. B, *Pleurotus ostreatus*. C, *Trametes betulina*. D, *T. ochracea*. E, *Ganoderma lucidum* (Photo by: O.F. Aduleye).

Preparation and Storage of Samples

The mushrooms were removed from the decaying wood and soil, thoroughly washed with distilled water to remove soil and other contaminants. They were oven-dried in the laboratory at 40°C. The dried mushrooms were subsequently ground into fine powder using blender. The powdered samples were stored in polythene bag and labeled accordingly for analysis.

Phytochemical Analysis

Total Phenolic Content (TPC) of the samples was determined using the modified Folin-Ciocalteu phenol reagent method (Kim *et al.*, 2003). Total Flavonoid Content (TFC) was determined using the method of Park *et al.* (2008). Tannin Content (TC) was determined according to the method described by Maga (1982). Oxalate content (OC) was assessed following the method of Day and Underwood (1986).

Proximate Analysis

The methods described in AOAC (2005) were used to analyse the proximate composition of the powdered mushrooms for crude protein, fibre, fat, and ash while carbohydrate was calculated by subtracting the sum of the values of the other nutrients from 100.

Mineral Element Analysis

The dried mushroom samples were screened for mineral components. Sodium (Na), Calcium (Ca), Magnesium (Mg), Phosphorus (P), Potassium (K), Zinc (Zn), and Iron (Fe) were assessed according to AOAC method (2005).

Free Radical Scavenging Capacity

2, 2-diphenyl-1-pictrylhydrazyl (DPPH) assay

The antioxidant activity of the powdered mushroom samples was determined using 2,2, diphenyl-1-picryl hydrazyl (DPPH) assay. Ten grams (10 g) of powdered sample was extracted in 100 ml of methanol to give an extract concentration of 100 mg/ml; 0.2 ml of extract was added to 2.8 ml of freshly prepared 20 mg/dm^3 DPPH in methanol and incubated for 20 minutes in the dark at room temperature. Absorbance was determined using UV/VIS SHIMADZU brand UVmin1240 spectrophotometer at 517 nm. Methanol only was used as the blank to adjust the spectrophotometer to zero absorbance whereas DPPH in methanol was used as the control. DPPH is a commercially available stable free radical which is purple in colour. Antioxidant molecules present in samples, when incubated, react with DPPH and convert it into di-phenyl hydrazine which is yellow in colour. The degree of decolourization was expressed as the % of DPPH radicals scavenged (% inhibition of DPPH) when the absorbance is measured with a spectrophotometer, and calculated as follows (Brand-Williams *et al.,* 1995):

% Inhibition = [1 – (Asample / Acontrol)] x 100

Acontrol is the Absorbance for control and Asample is the Absorbance for test sample.

Total Antioxidant Capacity (TAC) determination

One gram of the powdered mushroom samples was mixed with 1 ml of reagent (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid). The mixture was incubated in a boiling water bath for 90 minutes at 95°C. The samples were allowed to cool at room temperature, before the absorbance was measured at 695 nm and the total antioxidant capacity was calculated from ascorbic acid standard curve.

2,2-azobis-3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging ability

ABTS scavenging activity of the powdered mushroom samples was evaluated using the method described by Re *et al.* (1999). The working standard solution was prepared by mixing two stock solutions of 7 mM ABTS and 2.4 mM of potassium persulphate in equal quantity. The prepared solution was allowed

to react for 12 hours at room temperature in the dark. The resulting solution was further diluted by mixing 1 ml of ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 units at 734 nm after 7 minutes. The percentage inhibition was calculated as follows:

% ABTS inhibition = Abs (control) – Abs (sample)/Abs (control) x 100.

Where Abs (control) is the absorbance of ABTS radical + methanol, and Abs (sample) is the absorbance of ABTS radical + sample (extract or standard).

Ferric Reducing Antioxidant Power (FRAP) determination

The reducing power of the powdered mushroom samples was evaluated using the method of Benzie and Strain (1996). The reaction mixture contained 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the samples and standards prepared in various concentrations (0.025-0.5 mg/ml) in distilled water. The mixture was incubated for 20 minutes at 50°C and 2.5 ml of TCA (10% w/v) was added to it. It was then centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicated higher reducing power of the mushroom samples.

Data Analysis

Data were entered into Microsoft Excel spreadsheet (Microsoft Corporation) and analyzed using simple descriptive statistics (frequency and percentages). Values are presented as means of two determinations.

Results

Proximate Compositions

Figure 1 shows the proximate compositions of the mushroom samples. Carbohydrate was highest in *T. betulina* (55.98%) and lowest in *A. auricular-judae* (52.37%). Protein was found to be highest in *A. auricular-judae* (23.80%) and least in *G. lucidum* (16.30%). The fibre content of *G. lucidum* was highest (13.10%) and least in *A. auricular-judae* (8.8%). Fat was found to be highest in *T. ochracea* (2.25%) and least in *G. lucidum* (1.25). Ash content of *P. ostreatus* was the highest (9.12%) while it was lowest in *T. betulina* (5.4%). The moisture content varied between 5.83% in *P. ostreatus* and 6.89% in *G. lucidum*.

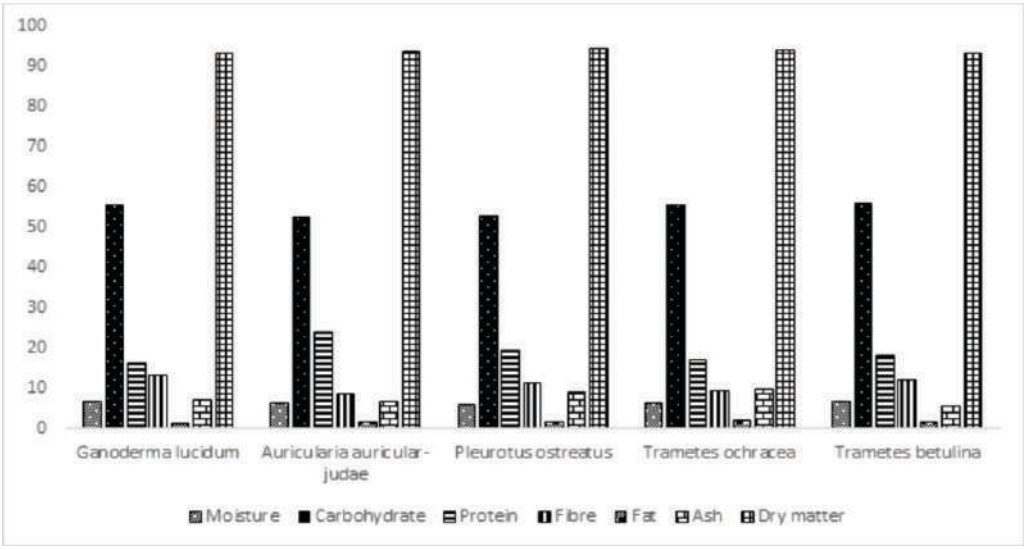


Figure 1: Proximate compositions of the mushroom samples.

Mineral Element Compositions

The mineral element compositions in the mushroom samples are presented in Figure 2. The proportions of Sodium, Calcium, Magnesium for some of the mushroom samples, and Phosphorus (for *G. lucidum*) are not shown on the graph because the values are very low. However, Sodium was found to be more in *A. auricular-judae* (0.11mg/100g) and least in *T. ochracea* and *T. betulina* (0.023mg/100g each). Potassium was found to be 2.175mg/100g in *A. auricular-judae* and 0.042mg/100g in *G. lucidum.* The Phosphorus content ranged from 6.63mg/100g in *G. lucidum* to 35.53mg/100g in *A. auricular-judae.* Calcium and Magnesium were generally low in all the mushroom samples. Iron content varied between 94mg/100g in *G. lucidum* and 480.50mg/100g in *T. ochracea.* Zinc ranged from 94.50mg/100g in *P. ostreatus* to 132mg/100g in *A. auricular-judae.*

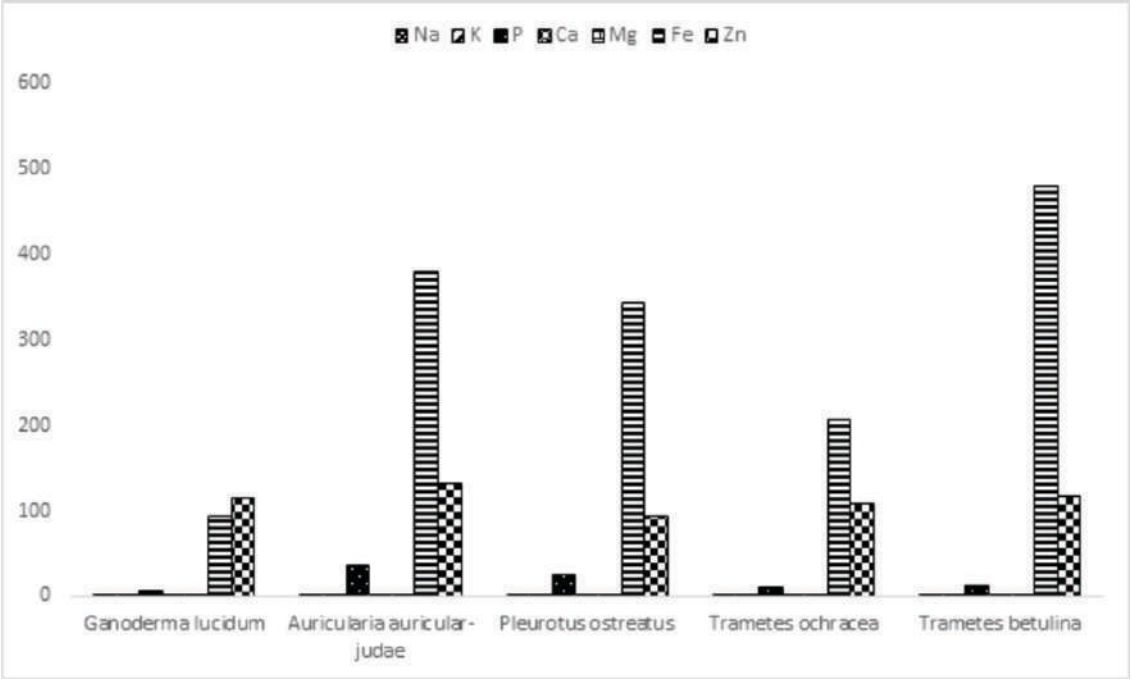


Figure 2: Mineral elements compositions of the mushroom samples

Phytochemical constituents

Table 1 shows the phytochemical constituents of the mushroom samples used in this study. Flavonoid was highest in *T. betulina* (65.56mg/100g) followed by *G. lucidum* (41.60mg/100g) and *A. auricular-judae* (40.98mg/100g). It was low in *T. ochracea* (37.95mg/100g) and lowest in *P. ostreatus* (27.45mg/100g). However, *P. ostreatus* contained the highest (128.13mg/100g) phenolic content, distantly followed by *A. auricular-judae* (51.28mg/100g), with *G. lucidum, T. betulina* and *T. ochracea* having 45.74mg/100g, 42.85mg/100g and 30.58mg/100g respectively. Tannin was generally low in all the mushroom samples; the concentration was highest (10.95mg/100g) in *P. ostreatus* and lowest (4.64mg/100g) in *A. auricular-judae.* Oxalate was found to be generally low but highest (0.8mg/100g) in *A. auricular-judae* and lowest (0.25mg/100g) in *P. ostreatus.*

Table 1: Phytochemical compositions of five mushrooms from Ikale and Ilaje communities of Ondo State

Mushroom	Flavonoids	Phenolics	Tannins	Oxalate
Auricularia auricular -judae	40.98	51.28	5.64	0.81
Ganoderma lucidum	41.60	45.74	6.80	0.72
Pleurotus ostreatus	27.45	128.13	10.95	0.23
Trametes betulina	65.56	42.85	5.92	0.32
Trametes ochracea	37.95	30.58	6.11	0.41

Values are means of duplicate determination (mg/100g)

Anti-oxidant properties

The reducing power and scavenging ability of the mushroom samples are shown in Table 2. The % inhibition of DPPH radicals was highest (65.19%) with *P. ostreatus* followed by *G. lucidum* (51.44%) and *A. auricular-judae* (50.37%). *T. ochracea* inhibited the DPPH radicals by 48.32% while the lowest inhibition was recorded for *T. betulina* (44.82%). Similarly, *P. ostreatus* had the highest (106.30mgFe^{2+}/100g) ferric reducing antioxidant power distantly followed by *A. auricular-judae* (92.76 mgFe^{2+}/100g) and *G. lucidum* (72.81 mgFe^{2+}/100g). It was however low in *T. betulina* (63.06 mgFe^{2+}/100g) and lowest (54.32 mgFe^{2+}/100g) in *T. ochracea*. The total antioxidant capacity was found to be 34.33mgAEE/100g in *P. ostreatus*, closely followed by *A. auricular-judae* (30.99 mgAEE/100g). It was 24.52 mgAEE/100g with *T. betulina*, 22.08 mgAEE/100g with *G. lucidum* and 19.19 mgAEE/100g with *T. ochracea*. With ABTS, *T. betulina* had the best activity (1.41mgTE/100g) followed by *P. ostreatus* (1.27 mgTE/100g) and *G. lucidum* (1.10 mgTE/100g). The antioxidant activity was generally low in *T. ochracea* (0.24 mgTE/100g) and in *A. auricular-judae* (0.13 mgTE/100g).

Table 2: Antioxidant activities of five mushrooms from Ikale and Ilaje communities of Ondo State

Mushroom	DPPH	FRAP	TAC	ABTS
Auricularia auricular -judae	50.37	92.76	30.99	0.13
Ganoderma lucidum	51.44	72.81	22.08	1.10
Pleurotus ostreatus	65.19	106.30	34.33	1.27
Trametes betulina	44.82	63.06	24.52	1.41
Trametes ochracea	48.32	54.32	19.19	0.24

Values are averages of two replicates. DPPH = 2,2-diphenyl-1-picrylhydrazyl (% inhibition). FRAP = Ferric Reducing Antioxidant Power (mg Fe2+/100g, Ferrous sulphate equivalent). TAC = Total Antioxidant capacity (mg AAE/100g, Ascorbic Acid Equivalent). ABTS = 2, 2,- azobis-3-ethylbenzothiazoline-6-sulfonate (mg TE/100g, Trolox equivalent)

Discussion

The moisture contents of the sampled mushrooms were generally low. The findings agreed with Keles *et al.* (2011) who found the dry matter in 24 wild edible mushrooms from Turkey to range between 91-95%. However, this is in contrast with the study of Adebayo (2011) who held that mushrooms have high moisture content. This conflict could be due to the fact that mushrooms

collected at full maturity have high dry matter and low moisture content (Fasidi and Kadiri, 1993). Carbohydrate, protein, and fibre were significantly present in the mushrooms studied. *G. lucidum* is an edible woody-brown color saprotrophic fungus that lives on dead or dying trees and old stumps or logs. High carbohydrate in *G. lucidum* could be a good source of energy for the body, as carbohydrate strengthens the body muscles. *A. auricula-judae* (wood ear/black wood ear/black fungus/jelly ear/tree ear), is a species of edible fungus found worldwide. Protein in *A. auricula-judae* is needed for wound-healing and as prevention against infections. *T. ochracea* (bracket fungi) grow on standing and fallen dead wood of deciduous trees, and are far too tough to be considered edible. However, fibre in *T. betulina* and *T. ochracea* could help to lessen the effects of constipation and haemorrhoid (Uzoma, 2013).

Although Phosphorus was present in the mushrooms in low quantities, Iron and Zinc were of considerable proportions in all the samples. Phosphorus in conjunction with Calcium is important in the formation of teeth (Artemis, 2011), and with vitamins in the treatment of arthritis (Holt, 2011; Gbadamosi and Oloyede, 2014). The high Iron content in the samples suggest that the mushrooms could be good sources of blood supplements in the prevention of anaemia (Gbadamosi and Otobo, 2013). Zinc (at 50mg/day) is also important in the management of rheumatoid arthritis (Holt, 2011). This can easily be achieved by diligently consuming the mushrooms.

Flavonoids have chelating properties and are known to have anti-inflammatory and anti-tumour activities (Izzi *et al.,* 2012). The flavonoid content in all the samples was lower than the WHO (2003) safe limit of 52.02mg/100g except in *T. betulina* that was significantly higher. *T. betulina* (formerly *Lenzites betulina*) is a species of inedible fungus. However, research has shown that it has several medicinal properties, including antioxidant, antimicrobial, antitumor, and immunosuppressive activities. Similarly, the samples had oxalate content that were far below the WHO (2003) tolerable standard of 105mg/100g. Phenolics and tannins were also within the dietary/nutrient reference values. Phenols prevent the formation of free radicals, as there are correlations between phenolic content and antioxidant activity (Iyawe *et al.,* 2011). The DPPH and FRAP values reported for *P. ostreatus* from Turkey (Keles *et al.,* 2011) were significantly higher than those found in the present study. *P. ostreatus* (oyster mushroom or oyster fungus) is a common edible mushroom (Patel *et al.,* 2012). It was first cultivated in Germany as a subsistence measure during World War I and is now grown commercially around the world for food.

Conclusions

High carbohydrate and iron contents found in the sampled mushrooms are indicative of their possible uses as dietary supplements for energy and blood supply. However, toxicity studies are recommended on these mushrooms.

Acknowledgement: The authors are grateful to Dr. S. Fakoya (Mycologist/Mushroom Biologist) for the authentication of the mushroom samples, and the anonymous reviewers for their useful suggestions.

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