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Distribution of Phytochemicals and Some Anti-nutrients in Selected Edible Mushrooms in Ekiti State, Nigeria

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Abstract

The present study was carried out to determine the phytochemical and anti nutritional composition of some selected edible mushrooms collected in Ekiti State, Nigeria such as *Pleurotus sajor-caju*, *Termitomyces robustus*, *Lentinus squarosullus*, *Termitomyces microcarpous*, *Termitomyces clypeatus*, *Lentinus tuber-regium* and *Polyporus* sp. The results of phytochemical analyses revealed the presence of phytochemicals such as alkaloids (0.03-0.17%), saponin (0.03-0.12%), total phenols (0.12-4.32%), flavonoids (0.20-6.04%) and tannins (0.04-2.60%). The least concentration of all the phytochemicals was found in *Polyporus* sp. Results of anti nutrient screening revealed the presence of phytate (0.17-0.34mg/g), oxalate (1.87-4.04mg/g) and cyanide (0.05-0.27mg/g). Furthermore, results revealed significant differences in the phytochemical as well as anti nutritional composition of the mushrooms though some species had similar compositions. The obtained values of phytochemicals and anti nutrients were significantly lower in all the mushroom species compared to their toxic levels according to World Health Organization stipulated safe limits. Thus, the study suggests that all the mushroom species are very safe for consumption while the presence of alkaloids, saponins, phenols and flavonoids indicate medicinal potentials. Hence, screening and characterization of the secondary metabolites are required.

Keywords: phytochemicals, anti nutrients, edible mushrooms, Ekiti State

1. Introduction

Mushrooms are regarded as macro fungi with distinctive fruiting bodies which can either be epigeous or hypogeous and large enough to be seen with the naked eyes and to be picked by hand.^[1] Mushrooms are saprophytes. They include members of the Basidiomycota and some members of the Ascomycota. They are important constituents of minor forest produce that grow on the most abundant biomolecule of the biosphere and cellulose.^[2] Mushrooms offer tremendous applications as they can be used as food and medicines besides their ecological roles. They represent one of the world's greatest untapped resources of nutrition and palatable food of the future.^[2]

Mushrooms have been consumed for centuries as food or food supplement due to their delicacy, taste and flavour.^[3] They have been proven to possess good quality of protein, unsaturated fatty acids, fibres, minerals and vital vitamins that we need in our daily diet.^[4] According to,^[5] edible mushrooms contain a spectrum of minerals both macro and micronutrients and non-essential trace elements. They are used traditionally to cure certain diseases.^[6] Their polysaccharide content is used as anti-cancer drug. Research reports had it that mushrooms have been used to combat HIV effectively.^[7,8] Biologically active compounds from mushrooms possess anti-fungal, anti-bacterial, anti-oxidant and anti-viral properties and have been used as insecticides and nematicides as well.^[2] It has been reported that mushrooms have been used as medicine in China since 100 A.D. but it was only in the 1960 that scientists investigated the basic active principles of mushrooms which are health promoting.^[9] They have been reported to possess anti-allergic, anticholesterol, anti-tumour and anti-cancer properties.^[10] Mushrooms have been used to treat diseases/conditions such as renal failure, epilepsy, wounds, skin diseases, heart ailments arthritis, cholera, diarrhoea, dysentery, liver

diseases, gall bladder diseases among others.^[11] Despite the nutritional and medicinal values of some mushrooms, it has been reported that mushrooms contain anti-nutrients that may have adverse effect on normal health functioning.^[12] On most cases, these anti-nutrients are commonly synthesized by plants to serve as a protective measure for them. However, if plants with high contents of these anti nutrients are consumed, it may lead to adverse health problems.

The present study was designed to determine the bioactive compounds and anti nutrients present in some edible mushrooms in Ekiti State, Nigeria.

2. Materials and Methods

2.1 Phytochemical Screening

The phytochemical constituents of the mushroom extracts such as alkaloids, saponins, phenols, flavonoids and tannins were identified by standard procedures.^[13,14]

Test for Alkaloids

Dragendroff's test: Little amount of the sample was treated with the Dragendroff's reagent; the appearance of reddish brown precipitate indicated the presence of alkaloids.

Test for Saponin

Foam test: To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

Test for Phenols

Ferric chloride test: A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride (FeCl₃) and 3 drops of potassium Ferro cyanide were added. Formation of blue or green color showed the presence of polyphenols.

Tests for Flavonoids

Alkaline Test: To 3 ml of the extract, few magnesium ribbons are dipped and concentrated Hydrochloric acid was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

Tests for Tannins

A fraction of the extract was dissolved in water and then it was subjected to water bath at 37°C for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

2.2 Quantitative Analysis

The quantitative amounts of the phytochemicals which were found in the mushroom extracts were determined using standard procedures as described by.^[15,16,17]

2.3 Determination of Anti-nutrients

Oxalate

The oxalate content of the sample was determined by the method described by.^[18] 0.05g of the sample was weighed into a test tube and 10ml of acetate was added and placed in a water bath and boiled for 3 minutes. This was filtered and 3ml of the filtrate with 0.1 ml of diluted ammonia was shaken in a test tube. The presence of a yellow colouration in the lower layer indicates the presence of oxalate.

Hydrogen Cyanide

The cyanide content of the samples was determined enzymatically using the method of ^[19] 5g of sample was introduced into 300ml volumetric flask containing 160ml of 0.1M phosphoric acid and homogenized for 15 minutes at low speed and made up to the mark. The solution was centrifuged at 10,000 rpm (revolutions per minute) for 30 minutes. The supernatant was transferred into a screw cap bottle and stored at 40C. 5ml aliquot of the extract was transferred into quick fit stoppered test tube containing 0.4ml of 0.2M phosphate buffer pH 7.0. 10ml of diluted linamarase enzyme was added. The tube was incubated at 30°C for 15 minutes and the reaction was stopped by

addition of 0.2M NaOH (0.6ml). The absorbance of the solution was measured using spectrophotometer at 450nm against blank.

Phytate

Phytate was determined using the method described and modified by.^[20] This involved measuring the phosphorus in the sample aliquot after the sample was extracted with 0.5m HCl and later digested in 60% perchloric acid and trioxonitrate V acid. The absorbance of the solution was read at 700nm and matched in with the calibration curve of the standards.

3. Results

The results of the qualitative and quantitative phytochemical analyses of the mushrooms are presented in Tables 1 and 2 respectively. The results showed that all the mushrooms contained alkaloids, saponin, phenols, flavonoids and tannins in varying quantities. The highest content of alkaloid (0.17%) was obtained in *L. tuber-regium* and the lowest (0.03%) in *P. sajour-caju* and *L. squarosullus*. The highest percentage of saponin (0.12%) was obtained in *T. robustus* while the least (0.03%) was obtained in *T. microcarpous*, *T. clypeatus*, *L. tuber-regium* and *Polyporus* sp. The total phenol content (4.32%) was highest in *T. clypeatus* while the least (0.12%) was obtained in *Polyporus* sp. The flavonoid content of *T. microcarpous* (6.04%) was significantly higher than the others and the least (0.02%) was obtained in *Polyporus* sp. *L. squarosullus* and *L. tuber-regium* had similar contents of flavonoids. The highest contents of tannins (2.60% and 2.52%) were obtained in *T. robustus* and *T. clypeatus* respectively while the least was obtained in *Polyporus* sp.

Some anti nutrients (phytate, oxalate and cyanide) were determined and the results are presented in Table 3. The least phytate (0.17mg/g) and oxalate content (1.87mg/g) were observed in *T. robustus* while the least cyanide content was obtained in *L. squarosullus* (0.05mg/g), *P. sajour-caju* (0.06mg/g) and *T. clypeatus* (0.06mg/g). The highest content of phytate (0.34mg/g), oxalate (4.05mg/g) and cyanide (0.27mg/g) were observed in *T. clypeatus*, *Polyporus* sp. and *T. microcarpous* respectively. Similarities were observed in the phytate content of *P. sajour-caju*, *L. squarosullus* and *Polyporus* sp. as well as *T. robustus* and *T. microcarpous*.

Table 1: Qualitative phytochemical composition of the mushrooms

Mushrooms	Alkaloids	Saponins	Total phenols	Flavonoids	Tannins
<i>P.sajor-caju</i>	+	+	+	+	+
<i>T. robustus</i>	+	+	+	+	+
<i>L. squarosullus</i>	+	+	+	+	+
<i>T. microcarpous</i>	+	+	+	+	+
<i>T. clypeatus</i>	+	+	+	+	+
<i>L. tuber-regium</i>	+	+	+	+	+
<i>Polyporus</i> sp.	+	+	+	+	+

+ = Present

Table 2: Quantitative phytochemical composition (%) of the mushrooms

Mushrooms	Alkaloids	Saponins	Total phenols	Flavonoids	Tannins
<i>P.sajor-caju</i>	0.03 ^d	0.09 ^c	3.44 ^c	0.19 ^c	1.14 ^c
<i>T. robustus</i>	0.09 ^c	0.12 ^a	4.14 ^b	0.04 ^{cd}	2.60 ^a
<i>L. squarosullus</i>	0.03 ^d	0.10 ^b	3.83 ^c	0.06 ^{cd}	1.31 ^{bc}
<i>T. microcarpous</i>	0.12 ^b	0.03 ^d	2.23 ^d	6.04 ^a	1.43 ^b
<i>T. clypeatus</i>	0.12 ^b	0.03 ^d	4.32 ^a	5.32 ^b	2.52 ^a
<i>L. tuber-regium</i>	0.17 ^a	0.03 ^d	1.61 ^e	0.05 ^{cd}	0.62 ^d
<i>Polyporus</i> sp.	0.07 ^c	0.04 ^d	0.12 ^f	0.02 ^d	0.04 ^e

Means with the same letters within columns are not significantly different at $p < 0.05$

Table 3: Anti nutrient profile (mg/g) of the mushrooms

Mushroom	Phytate	Oxalate	Cyanide
<i>P.sajor-caju</i>	0.24 ^b	2.53 ^d	0.06 ^d
<i>T. robustus</i>	0.17 ^c	1.87 ^g	0.15 ^{bc}
<i>L. squarosullus</i>	0.22 ^b	2.94 ^c	0.05 ^d
<i>T. microcarpous</i>	0.17 ^c	2.33 ^c	0.27 ^a
<i>T. clypeatus</i>	0.34 ^a	2.05 ^f	0.06 ^d
<i>L. tuber-regium</i>	0.23 ^b	3.05 ^b	0.18 ^b
<i>Polyporus sp.</i>	0.24 ^b	4.05 ^a	0.14 ^c

Means with the same letters within columns are not significantly different at $p < 0.05$

4. Discussion

The results obtained in the present study indicate the presence of phytochemicals and anti nutrients in varying quantities among the mushrooms. The range obtained for alkaloids and saponins in the present study is lower than values reported for some edible mushrooms by.^[21] The values of tannins and phenols in the studied mushrooms (except *Polyporus sp.*) were however higher than the values reported by the same authors. Flavonoid content of the mushrooms (except *T. robustus* and *T. clypeatus*) compared favourably with the range reported by the authors. The alkaloid contents observed in the present study are lower than the value (0.37%) reported for an edible mushroom, *Auricularia polytrichia* by.^[22] while values obtained for *T. microcarpous*, *T. clypeatus* and *L. tuber-regium* are comparable to the value (0.15%) reported for *Pleurotus oestratus* by the same authors. The flavonoid content of *T. microcarpous*, *T. clypeatus* in the present study are higher than reported values (1.76% and 1.36%) in two wild mushrooms, *Daldinia concentrica* and *Pheolus schweinitzii* respectively by^[23] while flavonoid contents of the other mushrooms are lower. Alkaloids, tannins, saponins and phenols are considered as anti nutrients because they have been reported to cause deleterious effects when consumed in large quantities.^[24] The phytochemical composition of the studied mushrooms are lower than the WHO maximum permissible limits of 48.50mg/100g for saponins, 52.03mg/100g for flavonoids and 61.00mg/100g for alkaloids.^[24] These results suggest that the mushrooms could be safe for consumption. Some of the phytochemicals contained in the mushrooms have been shown to have useful applications. Alkaloids have been reported to have powerful effect in animal physiology and their considerable pharmacological activities have been revealed.^[25] Saponins have been reported to inhibit Na⁺ efflux by blockage of the influx of concentrations in the cell, activating Na⁺ - Ca⁺ antiporter in cardiac muscles. The increase in Ca⁺ influx through this antiporter strengthens the contraction of the heart muscles.^[26] The presence of saponins in the mushrooms is useful in medicinal and pharmaceutical industry due to its foaming ability that produces frothy effect in the food industry. Phenols are also useful as they form the constituents of most antiseptics and disinfectants. It has been reported that phenolic compounds are antioxidants and they exhibit a wide range of spectrum of medicinal properties such as anti cancer, anti- inflammatory and diabetic effects.^[27] Flavonoids have also been reported to possess a broad spectrum of chemical and biological activities including radical scavenging properties antiallergenic, antiviral, anti-inflammatory and vasodilating actions.^[28]

The phytate values obtained in the present study are higher than values reported for edible mushrooms,^[29] comparable to values reported by^[30] in some selected edible mushrooms. The phytate contents of the studied mushrooms are much lower than the standard safe limit of 22.10 mg/100g.^[24] indicating that the mushrooms are highly safe with toxicities associated with phytate concentration. The oxalate values observed in this study are higher than the values (0.22 and 0.41%) reported for two edible mushrooms in Ebonyi State, Nigeria by.^[22] The oxalate contents observed in this study are higher than values

(0.225mg/100g, 0.315 mg/100g and 0.405mg/100g) reported by^[31] for *Auricularia jundae*, *Xylaria hypoxylon* and *Trametes vesicolor* respectively. However, the oxalate content observed in this study is lower than 140.80mg/100g observed in *Oxyporus popilunus* (an inedible mushroom) by.^[12] The oxalate contents of the studied mushrooms are quite lower compared to World Health Organization tolerable limit (105 mg/100g), indicating their safety for consumption. The total cyanide content observed in this study for *P.sajor-caju*, *L.squarosullus* and *T.clypeatus* fall within the range (0.07-0.10mg/100g) reported by^[32] while the content in the other mushrooms are higher than the range reported by the same authors. All the studied mushroom species are safe for consumption as their cyanide contents fall below the reported lethal dose (35mg/kg) body weight.^[33]

5. Conclusion

Results of the present study have revealed the presence of vital phytochemicals and some anti nutrients in the selected mushroom species. Also, the results showed that the concentrations of these phytochemicals and anti nutrients are quite lower than the WHO reported safe limits, suggesting that the mushrooms could be safe for consumption. Furthermore, the phytochemicals present in the selected mushrooms could be an accessible source of natural antioxidant and antibiotics. Hence, isolation and characterization of pharmacologically active metabolites from these mushrooms is required.

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