Phytochemical screening and anti-nutrient profile of an edible mushroom, *Termitomyces robustus* (Beeli) R. Heim in Kwara State, Nigeria

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Abstract: The phytochemical screening and anti-nutrient analysis of an edible mushroom, *Termitomyces robustus* purchased from a local market in Oro, Kwara State, Nigeria were carried out using standard methods to determine its relevance in ethno medicine and also determine the effect of the anti-nutrients on human health. The mushroom was screened for the absence or presence of phytochemical compounds such as saponins, alkaloids, steroids, flavonoids, terpenoids, glycosides, phenols, anthraquinones and phlobatannins. For the anti-nutrient composition, oxalate, phytate and cyanide contents were determined. The results of the qualitative phytochemical analysis of the mushroom showed the presence of saponins and flavonoids only. This result suggests that the mushroom may not be of immense therapeutic uses. The quantitative analysis of the phytochemical properties of the mushrooms showed saponins (3.51%) and flavonoids (18.58%). The results of the anti-nutrient analysis revealed that the mushroom contained 0.33mg/100g of oxalate, 0.15mg/100g of phytate and 29.07mg/100g of cyanide. The concentrations of the anti-nutrients were below levels considered harmful.
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1. Introduction

Mushrooms are fungi fruit – bodies which are typically produced above the ground on soil or on its food source. Mushrooms are saprophytes. They are most often applied to fungi (Basidiomycota, Agaricomycetes, order Boletales and family Boletaceae) that have stem (stipe), a cap (pileus) and gills (lamellae) on the other side of the cap. Mushrooms are found in areas with range of temperature 20 – 40^0C and grow well in agricultural wastes. They require a moderate rainfall and pH range of 3-10 for growth (Chang, 1991). Mushrooms are found growing on dead organic matter of plant origin, therefore, utilizing almost all plant materials as substrates. Mushrooms have been used as a valuable food source and as traditional medicine around the world, especially in Japan and China (Chang, 1991). Many health promoting substances e.g. antimicrobial, anticancer, antioxidant, cholesterol lowering property and immunostimulatory effects have been documented for some species of mushrooms (Adeduntan, 2014). *Boletus edulis* and *Lentinus edodes* have been reported to be antitumour and hypocholestronic agents respectively; *Lentinus erodes, Volvariella volvacea* and *Flammulina velutipes* are known to possess anticancerous properties and to lower blood pressure while *Polyporus officinalis* has been shown to be a purgative, to stop bleeding and a good remedy for chronic catarrh, tuberculosis, rheumatism, jaundice and dropsy (Bahl, 1998). *Auricularia auricula* is known to cure eye inflammation whereas *Auricularia polytrichia* produces antibacterial substances (Bahl, 1998). The ethno-medicinal uses of *Phallus aurantiacus*, *Pleurotus tuber-regium*, *Termitomyces microcarpus*, *Termitomyces robustus*, *Termitomyces globulus* and *Calvatia cyathiformis* had been amply described by (Oso, 1975). *Pleurotus tuber-regium* when ground with condiments like snail-fluid, pepper, onion and palm oil is used for curing ailments such as headache, stomach pains, fever, cold, small- pox, asthma and boils while *Termitomyces microcarpus* is employed in treating gonorrhoea (Oso, 1975).

Most people only consume mushrooms because of their unique flavor and not really for their nutritional or medicinal composition (Ezeibekwe *et al.,* 2009). Apart from the nutritional and medicinal values of some mushrooms, it has been reported that mushrooms contain anti-nutrient factors that may be detrimental to normal health functioning (Asuquo and Etim, 2011). In most cases, these anti-nutrients are commonly synthesized by plants to serve as a protective measure for them. If plants with high contents of these anti – nutrients are consumed, it leads to adverse health problems. Since mushrooms are widely accepted and locally available, an understanding of the chemical composition is of importance.

The objective of this study is to determine the phytochemical and anti-nutrient composition of an edible mushroom, *Termitomyces robustus* widely consumed in Kwara State, Nigeria.

2. Materials and method

2.1 Collection, identification and processing of samples

Fresh and matured samples of the edible mushroom were purchased from a local market in Oro, Kwara State, Nigeria in October, 2015. The species was authenticated at the Herbarium section of the Department of Plant Science and Biotechnology, Ekiti State University Ado Ekiti, Nigeria. The mushroom was washed with distilled water to remove any adhering soil or extraneous materials and was then sun dried until they became brittle. They were then ground into fine powder using a blender and then stored in air-tight bags at room temperature until analysis.

2.2 Qualitative determination of phytochemicals

The phytochemical components of the mushroom were determined using standard procedures (Sofowora, 1982; Trease and Evans, 1983).

Test for saponin

Two grams of sample were weighed in a beaker; 5ml of distilled water was added and heated to boil. Persisted foaming on warming was taken as an evidence for the presence of saponin.

Test for tannin

Two grams of sample were weighed and mixed with 10 ml of distilled water. The mixture was filtered and two drops of 5% ferric chloride ($FeCl_3$) was added to filtrate. Blue- black was taken as an indication of the presence of tannin.

Test for alkaloid

Two grams of sample were weighed in a beaker and it was extracted with 10 ml 2% hydrochloric acid by heating gently for about 5 minutes. The HCL extract was filtered with Whatman No 1 filter paper to have a clear solution and prevent false result. 2.5 ml of the filtrate was treated with few drops of Dragendoff's reagent. Appearance of precipitate indicated the presence of alkaloid in the extract.

Test for cardiac glycosides

Two grams of the sample was dissolved in 2 ml glacial acetic acid containing one drop of ferric chloride ($FeCl_3$). The solution was underplayed with 1.0 ml of concentrated sulphuric acid (H_2SO_4). A reddish brown colour at the interface indicates the presence of a steroidal ring, that is, a glycine portion of the cardiac glycosides (Keller-Killiani's test).

Test for flavonoid

Five ml of the diluted ammonia solution were added to a portion of aqueous filtrate of sample extract followed by the addition of concentrated sulphuric acid. Formation of yellow colour indicated the presence of flavonoid.

Test for phenol

Five grams of the powdered sample was mixed with 20 ml of H_2SO_4 in ethanol and heated for five minutes. 1 ml of the filtrate of the heated mixture and two drops of ferric chloride were mixed to observe green, blue or black coloration.

Test for phlobatannins

Deposition of a red precipitate when aqueous extract of the mushroom was boiled with 1% aqueous HCI acid was an evidence for the presence of phlobatannins.

2.3 Quantitative analysis

The quantitative amounts of the phytochemicals which were found in the mushroom extracts were determined using standard procedures as described by Obadoni and Ochuko (2001), Trease and Evans (2002) and Amakura *et al.* (2009).

2.4 Determination of anti-nutrient profile of the mushroom

Oxalate

The oxalate content of the sample was determined by the method described by (Alabi *et al.,* 2005). 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 (D'mello, 1982). 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^{o}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 (Aletor, 1995). 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 result of the phytochemical screening of the edible mushroom, *Termitomyces robustus* is presented in Table 1. The result showed that the mushroom contained only saponins and flavonoids. Other phytochemicals such as alkaloid, tannins, steroids, terpenoids, glycosides, phenols, anthraquinoues and phlobatannins were not detected in the mushroom. The quantitative analysis of the phytochemical properties of the mushroom showed saponin (3.51%) and flavonoids (18.58%) (Table 2). The results of the anti nutrients analyzed are shown in Table 3. The oxalate, phytate and cyanide contents were 0.33mg/100g, 0.15% and 29.07mg/100g respectively.

Table 1: **Qualitative assay of phytochemical components of *Termitomyces robustus***

Phytochemicals	Occurrence
Saponin	+
Alkaloid	-
Tannin	-
Steroid	-
Flavonoid	+
Terpenoid	-
Glycoside	-
Phenol	-
Anthraquinone	-
Phlobatannin	-

+ = Present, - = absent

Table 2: **Quantitative analysis of phytochemical components of *Termitomyces robustus***

Phytochemicals	% composition
Saponin	3.51± 0.00
Flavonoid	18.58 ± 0.00

Mean ± SD (n = 4)

Table 3: Antinutrient profile of *Termitomyces robustus*

Antinutrient	Composition (mg/100g)
Oxalate	0.326 ± 0.00
Cyanide	29.066 ± 0.01
Phytate (%)	0.145 ± 0.01

Mean ± SD (n = 4)

4. Discussion

The results of the qualitative phytochemical screening of the mushroom, *Termitomyces robustus* tends to agree with earlier work of Kayode *et al.* (2013) who detected saponins and flavonoids in oyster mushroom (*Pleurotus sajor –caju*) but did not detect anthraquinoues in the mushroom.

The saponin content quantified in this study (3.51%) compared favorably with the value obtained for *Pleurotus sajor-caju* (3.03%) by Kayode *et al.* (2013). However the value obtained for flavonoid in this studies is very high (18.58%) when compared with the values (0.06% and 0.925 – 4.795%) reported by Kayode *et al.* (2013) and Adeduntan (2005). respectively. Saponins are among various secondary plant metabolites with potent antifungal, anti-bacterial, anti-inflammatory and phytoprotectant properties which form barriers to microbial attack and in plant defense against herbivores (Papadopoulou, 1999). It has been reported that in plants, saponin may serve as anti-feedants and protect plant against microbes and fungal attack (Omotayo and Omoyeni, 2009). Saponin also lowers the cholesterol level, possesses anti-diabetic and anti carcinogenic activities as well as stimulating immune response (Edward, 2011). Flavonoids are the highly diversified plant pigments that are present in a wide range of fruits, vegetables, nuts and beverages. They are regularly consumed in the human diet and have various biological activities including anti-inflammatory, anti-cancer and anti-viral properties (Lee *et al.,* 2007). Clinical studies have also suggested that flavonoids have roles in cancer prevention (Romagnolo and Selmin, 2012).

The oxalate content observed in this study compares with the report of (Adeduntan, 2005) who reported oxalate content (mg/100g) of 0.225, 0.315 and 0.405 in *Auricularia judae*, *Xylaria hypoxylon* and *Trametes* vesicolor respectively. However, the oxalate content observed in this study is low when compared with 140.80mg/100g observed in *Oxyporus popilunus* (an inedible mushroom) by (Asuquo and Etim, 2011). The value for oxalate determined in *T. robustus* in this study was quite lower than 15-30 grams which is the reported lethal dose for oxalate (Hotz and Gibson, 2007). The phytate concentration (0.15%) observed in this study is low when compared with values reported in *Pleurotus tuber-regium* (Akindahunsi and Oyetayo, 2006). This value is also low when compared with green leafy vegetables whose phytate contents were found to be exceptionally high Akindahunsi (1999). The lethal dose of phytate is reported to be from 250-500mg/100g (Bushway *et al*., 1998). It has been stated that phytate could affect mineral bioavailability when it is 1% or more in diet (Fasidi and Kadiri, 1995). The total cyanide content observed in this study (29.07mg/100g) is relatively high when compared with values (0.07-0.10mg/100g) reported by (Akindahunsi and Oyetayo, 2006). It is however lower than the reported lethal dose of cyanide (35mg/kg) body weight (Enenbong, 2001).

Generally, the concentrations of the anti nutrients observed in *T. robustus* in this study are low and are unlikely to produce any undesirable effect on the

consumers. However, there is need to give more attention to the study of anti nutrients in mushrooms because at high concentrations, the anti nutrients can have adverse effect on digestibility as they can bind metal ions like calcium, zinc, iron, and other minerals thereby reducing their availability (FAO, 1990). High level of phytate can however be reduced by a number of processing methods like soaking, boiling and fermentation (FAO, 1990).

Conclusion

From the results of the analyses, the mushroom, *Termitomyces robustus* may not be of immense therapeutic use considering the absence of most of the phytochemicals. However, it is worthy of note that the mushroom is rich in flavonoids, indicating that it has good potentials for cancer prevention. The anti nutrients studied are below their reported lethal doses. Thus, they are unlikely to produce any undesirable effect on the consumer.

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