JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

CHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITIES OF TWO EDIBLE MUSHROOMS *(Termitomyces robustus* and *Lentinus squarrosulus)*

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doi: 10.15414/jmbfs.2016.5.5.416-423

ARTICLE INFO

Received 22. 5. 2015
Revised 19. 12. 2015
Accepted 4. 1. 2016
Published 1. 4. 2016

Regular article

OPEN ACCESS

ABSTRACT

The methanol extracts of two edible mushrooms; *Termitomyces robustus* (ewe) and *Lentinus squarrosulus* (erirokiro) were screened for phytochemicals of medicinal importance and the chemical profile investigated using standard analytical methods with the aim of assessing their health promoting properties. Both mushrooms tested positive to flavonoids, saponin, tannin and terpenoid but negative to steroid, anthraquinone and phlobatannin. The results of proximate compositions in % were; moisture contents (7.22 ± 0.07; 11.03 ± 0.21), crude protein (31.34 ± 0.01; 42.77 ± 0.57), ash (7.07 ± 0.04; 10.45 ± 0.43), crude fibre (4.07 ± 0.18; 9.48 ± 0.04), crude fat (3.71 ± 0.16; 6.76 ± 0.22), carbohydrate by difference (24.90 ±0.11; 41.27 ± 0.19), calorific value in kcal (331.55±3.41; 342.35±3.09), and total dietary fibre (10.21 ± 0.00; 11.68 ± 0.00). The anti-nutrient factors in mg/g were; tannin (3.25 ± 0.80; 7.40 ± 0.14) oxalate (1.53 ± 0.00; 1.71 ± 0.07), and phytate (1.48 ± 0.06; 1.94 ± 0.05). Mineral elements, vitamins, essential and non-essential amino acids in substantial quantities were detected in the mushrooms. The phenolic compounds identified and quantified were gallic acid, catechin,chlorogenic acid, caffeic acid, ellagic acid, epicatechin, rutin, isoquercitrin, quercitrin, quercetin and kaempferol.The mushrooms exhibited various antifungi and antimicrobial activities. The two mushrooms possessed good nutritional and chemical qualities and could be sources of many different nutraceuticals.

Keywords: Phytochemicals, proximate, flavonoids, Polyphenols, *Termitomycesrobustus, Lentinussquarrosulus*

INTRODUCTION

Mushrooms are the higher fungi which have long been used for food and medicinal purposes. Fresh and preserved mushrooms are consumed in many countries as a delicacy, particularly for their specific aroma and texture **(Pavel , 2012).**They have rich nutritional value with high protein content (up to 44.93%), vitamins, minerals, fibers, trace elements and low calories and lack cholesterol **(Hrudayanath and Sameer, 2014; Agahar- Murugkar and Subbulakshmi, 2005; Wani *et al*., 2010).** Wild mushrooms are becoming more and more important in our diet due to their value as food as well as their medical and nutraceutical values (**Chang and Miles, 2004**). Their global economic value is now increasing as a result of their nutritional, organoleptic, and pharmacological characteristics **(Diez and Alvarez, 2001; Solak *et al*., 2006).**The leading countries in mushroom production are China, US, Netherlands, India and Vietnam, according to recent FAO report **(FAO, 2014; Yaoqi *et al*., 2014**). Mushrooms production can alleviate poverty in rural communities and improve the diversification of agricultural production **(Godfrey *et al*., 2010)** as well as national economy if given its proper place especially now that mushrooms are being considered as an alternative food source to provide adequate nutrition for world's increasing population. Several researches have established some edible mushroom species as sources of physiological agents for medicinal applications, possessing antitumour, cardiovascular, antiviral, antibacterial and other activities **(Halpern and Miller, 2002; Wasser, 2002; Chang, 1996).** Each mushroom type produces a specific set of metabolites capable of dealing with the set of microbes that coexist in that specific environment (**Dembitsky, 2010**). It was reported by **Bobek *et al*., (1991)** that the consumption of a mushroom-containing diet prevented serum cholesterol increase at the end of the four week period and lowered by almost 40 % as compared with control groups which have not had mushroom in their diet. **Kabir and Kimura (1989)** reported that dietary mushrooms have reduced the blood pressure in rats. *Termitomyces robustus* is called 'Ewe'meaning "expand" among the Yorubas in western Nigeria. The mushroom caps, globular at first, expands and opens out to become almost flat. It has blackish brown colour. It is the most popular edible mushroom in Nigeria but remains underutilized due to traditional believes, myths or dietary habit. *Lentinus sqarrosulus*,called 'erirokiro' to describe its tough, leathery texture is whitish in colour and also common in the genus *Lentinus* **(Oso, 1976)**. Over the last decade, mushrooms have been studied as novel functional food, there has been many studies on the nutritional contents of different mushroom species globally, little or no work has been carried out on chemical qualities of so many species and there is a dearth of information about polyphenolic components of the species in Nigeria. The objective of this study was to investigate the chemical profile of two edible mushrooms, screen them for phytochemicals and use Reverse-Phase HPLC-DAD to quantify the polyphenols present.

MATERIAL AND METHODS

Samples preparation

The two species obtained from local markets in Nigeria were scraped and thoroughly cleaned with water to remove sand, cut into smaller pieces (both the pileus and stipes), oven dried at 60 ^{o}C, then ground and sieved to give 40 mm mesh size powder.

Preparation of extracts

The powdered mushrooms were subjected to a cold maceration process for 72 h with methanol and ethanol separately to obtain the alcoholic extracts and for 24 h to obtain the aqueous extracts and filtered. The extracts were concentrated under vacuum and evaporated using a rotary evaporator at low temperature (45^{o}C).

Phytochemical screening of the extracts

Phytochemical screening of the methanol extracts was carried out using qualitative tests for analyses of different constituents of plant materials according to the common phytochemical methods described by **Harborne (1973); Trease and Evans (1983)** and **Sofowora, (1993).**

Chemical analyses

Proximate composition (fat, crude fibre, and ash) was determined on dry basis by the standard method of Association of Official Analytical Chemist **(AOAC, 2006)**, the protein content was determined using the micro-Kjedahl method (N x 6.25) and the carbohydrate determination by difference **(AOAC, 2006).** Total dietary fibre (TDF) was determined to dried, fat-free sample according to Megazyme TDF Assay procedure, K-TDFR 05/12 (MEGAZYME INTERNATIONAL, IRELAND). The mineral elements were determined using Atomic Absorption Spectrophotometer **(Pearson, 1976),** vitamins by spectrophotometric methods (**Biesalski *et al*., 1986; Benderitter *et al*., 1998; Okwu and Josiah, 2006**), and antinutrients by titrimetry and spectrophotometric methods **(Makkar and Goodchild, 1996; Day and Underwood, 1986).**

Determination of antibacterial activity

The antibacterial activity of aqueous, methanolic and ethanolic extracts of the mushrooms against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* bacteria was evaluated by using agar well diffusion method **(Ahmad and Beg, 2001; Srinivasan *et al*., 2001)**. Plate count agar (PCA) plates were inoculated with 100 µl of standardized inoculum ($1.5x10^8$ CFU/ml) of each selected bacterium and spread with sterile swabs. Wells of 8 mm size diameter were made with sterile borer into agar plates containing the bacterial inoculum and the lower portion was sealed with a little molten agar medium. About 0.5 ml of each of the extracts was poured into a well of inoculated plates. Chemical antibiotics, Streptomycin sulphate (10 $ugml^{-1}$) was used as a positive control which was introduced into a well instead of plant extract. The solvents; deionized water, methanol or ethanol were used as negative controls, which were introduced into the wells instead of the extracts. The plates thus prepared were left at room temperature for ten min allowing the diffusion of the extracts into the agar **(Rios *et al*., 1988)**. After incubation for 24 h at 37 ^oC, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimetres.

Determination of antifungal activity

The antifungal activity of plant extracts was evaluated against food-associated fungi by using poisoned food technique. Potato dextrose agar (PDA), was weighed (39g) and dispersed in a litre of deionised water sterilized at 121 ^oC for 15 min, allowed to cool (45 ^oC) before pouring (20 ml) into separated dishes. The fungi; *Trichoderma rubrum* and *Aspergillus fumigatus* were inoculated on potato dextrose agar (PDA) plates and incubated for 25 ^oC for 72 h, to obtain young, actively growing colonies of moulds. 0.2 ml of each of the extract was mixed with 20 ml of cooled (45^oC) molten PDA medium and allowed to solidify at room temperature for 30 min. Thereafter 10 µl of fungal spores in distilled water was added at the centre of the solidified PDA plates. PDA plates with 10 $ug.ml^{-1}$ of bonlate were used as positive controls. PDA plates with the solvents; deionized water, methanol or ethanol were used as negative controls **(Georgii and Korting, 1991, McCutcheon *et al*., 1994)**. The inoculated plates were incubated at 25 ^oC and colony diameter measured and recorded after 3 days. Percentage mycelial growth inhibition (% MGI) was calculated as given below:

$$\% \text{ MGI} = \frac{\text{Mean dia. of fungal colony in control - Mean dia. of fungal colony in plant extract x 100}}{\text{Mean dia. of fungal colony in control}}$$

Determination of amino acid profile

About 20 g of each frozen fresh mushroom sample was weighed properly and minced by using 100 ml phosphate buffer supplemented with 2 % SDS (sodium dodecyl sulfate) by using philip household blender. The homogenate was filtered through double layered cheese cloth. The filtrate was subjected to ammonium sulphate salt precipitation method at 65 % saturation. The proteins were pelleted by centrifugation, concentrated by dialysis and then freez-dried for amino acid analysis. The 4.0 g protein isolate was hydrolyzed and evaporated in a rotary evaporator. The amino acid profile in the samples were determined using Technicon sequential multi-sample amino acid analyzer (TSM) **(Benitez, 1984).**

Quantification of phenolic compounds by high-performance liquid chromatography with diode-arraydetection

Reverse phase chromatographic analysis was carried out under gradient conditions using C_{18} column (4.6 mm x 150 mm) packed with 5µm diameter particles; the mobile phase was water containing 2 % acetic acid (A) and methanol (B), and the composition gradient was: 5 % of B until 2 min and changed to obtain 25 %, 40 %, 50 %, 60 %, 70 % and 100 % B at 10, 20, 30, 40, 50 and 60 min, respectively, following the method described by **Amaral *et al*., (2013).** Each sample extract was analyzed at a concentration of 20 mg/ml. The presence of eleven compounds was investigated for, *T. robust* and ten for *L.*

Phytochemical components

The screen of the methanol extracts of the samples showed the presence of some bioactive compounds (Tables 1). It is noteworthy that both mushrooms contained flavonoids, saponin and tannin but steroid, anthraquinone and phlobatannin were absent. Only *T.robustus* tested positive to alkaloid. The presence of flavonoids in both is well understood as it is in agreement with the previous literature that plants are major sources of phenolic compounds, which are synthesized as secondary metabolites during normal development in response to stress conditions, such as wounding and UV radiation among others **(Sies, 1997).** The alkaloids, saponnis and tannins play important roles in various antibiotics used in treating common pathogenic strains as reported by **Kubmarawa *et al*., (2007).**

Chemical composition

The result of the proximate analysis of the mushrooms on dry weight basis is presented in Table 2. The moisture content of *T. robustus* was significantly higher ($p \leq 0.05$) than that of *L. squarrosulus*. Fresh wet mushrooms have higher moisture contents which make them easily perishable. Excessive moisture content in raw materials favours the microbial growth and the decomposition of active compounds by hydrolysis. The moisture content of dried plants varies, depending on the prevailing local environment and length of storage. The protein content of *T. robustus* was significantly higher ($p \leq 0.05$) than that of *L. squarrosulus* and do not agree with the study carried out by **Jose and Kayode (2009)** which indicated that *L. squarrosulus* had higher protein than *T. robustus*. **Adejumo and Awosanya, (2005)** however, reported a higher percentage of crude protein and moisture for *Termitomyces mammiforms* than *Lentinus tigrinus.* Percentage of crude protein obtained in this study for *T. robustus* was higher than (33.8%) reported by **Aletor (1995)** for this same species. **Oboh and Shodehinde (2009)** obtained 28.60 % and 24.80 % for the pileus and stipes of *T. robustus* respectively. It has been reported that the protein content of mushrooms is affected by a number of factors. The development stage of mushroom is a significant factor affecting the protein content. In addition, the type of mushroom, the part sampled, the location as well as the substrates affect protein content **(Barros*et al*., 2007; Kalmiş *et al*., 2011)**. The whole mushrooms (pileus and stipes) were analysed in this work. Proteins are the building blocks of life. The body needs protein to repair and maintain itself. Since it was present in appreciable quantity in both mushrooms, nutritional power of these fungi as protein supplements cannot be ignored.

Both mushrooms contained higher ash, than crude fat, the same trend obtained by **Oboh and Shodehinde (2009),** and the ash content of the two compared favorably with percentage ash of 7.8 ± 0.6, 8.3 ± 0.0, and 7.3 ± 0.3 reported earlier for *Ganoderma spp., Omphalotus olearius* and *Hebeloma mesophaeum* respectively (**Aremu *et al*., 2009),** also with some other vegetables such as *Occimumgraticimum* (8.00 %) and *Hibiscus esculentus* (8.00 %) **(Akindahunsi and Salawu, 2005).** High ash content is an indication of high mineral content, a reflection of the mineral contents preserved in the food materials **(Antia *et al*., 2006)**. Mineral elements are essential for tissue functioning and a necessity in daily requirement for human nutrition. The result therefore suggests a high deposit of mineral elements in the mushrooms**.** *T. robustus* has higher crude fat than that of *L. squarrosulus*, the values obtained for the two however fell within the range of percentage crude fat obtained for some wild mushrooms in Turkey, which was 1.40 ± 3.00 – 10.58 ± 0.30 for *Cantharellus cibarius* Fr and *Lycoperdon perlatum* Pers., respectively **(Ahmet *et al*., 2009).** Dietary fats help in absorbing and retaining flavours, thus, increase the palatability of food **(Antia *et al*., 2006).** Although, a diet providing 1-2 % of its caloric of energy as fat is said to be sufficient to human beings; as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging **(Antia *et al*., 2006), Ononugbu (2002**) reported that vegetable fats and oil lower blood lipids thereby reducing occurrence of disease associated with damage of coronary artery. Plant lipids are useful as essential oils, spice, oleoresins and natural food colors and have been developed to products that work with diverse requirements, as culinary, medicinal and cosmetics **(Yadav and Tyagi, 2006).**

The *L. squarrosulus* in this study had significantly higher crude fibre than *T.robustus*, both had higher values than percentage crude fibre of 3.5 ± 0.2, 2.8 ± 0.5 and 3.2 ± 1.0 reported for *Ganoderma spp., Omphalotus olearius* and *Hebeloma mesophaeum* respectively by **Aremu *et al.* (2009).** The total dietary fibre values were higher in both mushrooms than crude fibre as expected. Carbohydrate by difference of *L. squarrosulus* was significantly higher ($p \leq 0.05$) than that of *T. robustus*, the lower carbohydrate level can not affect energy contribution due to the compensation from higher protein values in *T.robustus.* Therefore, there was no significant differences in calorific values of the two mushrooms. Carbohydrates are one such group of carbon compounds, which are essential to life. Almost all organisms use carbohydrates to exploit their rich supply of potential energy to maintain life. Calculated energy values of the *T.robustus* and *L. squarrosulus*, here were found to be lower than previous data

obtained for eight edible wild mushrooms in Turkey which varied from 367.88 kcal/100 g to 450.20 kcal/100 g on dry matter basis **(Ahmet *et al*., 2009).**
The macro elements; calcium, phosphorus, potassium, sodium and magnesium as well as the trace elements iron, copper and zinc were all in higher concentrations in *T. robusts* than *L. squarrosulus*. Lead, mercury and manganese were not detected in any of the mushrooms. Calcium is a major factor sustaining strong bones; it plays a part in blood clotting, muscle contraction and relaxation. **Muhsin, (2006)** reported Ca levels of 124 ppm in 1 g of dried *Lactarius delicious,* calcium and phosphorus are the minerals are abundant in the the bones. *Agaricus bisporus* was found to contain 110 mg/100g phosphorus **(Peter *et al.*, 2012).** Potassium is the most abundant element in both mushrooms, edible wild mushroom species have an average potassium content of 34,350 mg/kg on a dry basis, making them an important and valuable potassium source for the human diet **(Kalmiş *et al*., 2011).** Studies have revealed that the potassium concentration of mushrooms is relatively constant **(Vetter, 1994).** The sodium content of *Tricholoma terreum* was reported as 92.6-325 mg/kg on a dry basis **(Demirbaş, 2001; Vetter, 2003; Kalmiş *et al*., 2011),Vinhal,*et al*., 2012** also reported 255.34 and 613.03 mg/100g for sodium and potassium respectively in *Agaricus sylvaticus.* Sodium and potassium are important intracellular and extracellular cations respectively. Values obtained for magnesium in both mushrooms were lower than what was reported by **Ezeibekwe, *et al*., (2009)** for *Plerotus tuber-regium* (0.24 %) and *Auricularia auricular* (0.36 %). Magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis. **Ahmet, (2009)** obtained the range 30.20 ± 2.90 - 550.00 ± 15.00 in mg/kg for Fe in *Ramaria flava* and *Lycoperdon perlatum* Pers; and 15.20 ±1.10 - 330.00 ± 91 for Cu in *Craterellus cornucopioides* and *Armillaria mellea* respectively. Iron plays important role in the formation of haemoglobin **(Latunde-Dada, 2006)** and hence recommended for anaemic convalescence, copper is an essential micronutrient which functions as a biocatalyst required for body pigmentation in addition to iron. The trace metal contents of mushrooms are related to species of mushroom, collecting site of the sample, age of fruiting bodies and mycelium, as well as distance from sources of pollution **(Kalac *et al*., 1991)**. They are mainly affected by acidic and organic matter contents of the soil. This may be responsible for the wide difference in the Zn contents of the two mushrooms in this study. Zn content of 370.00 mg/kg dry weight was found in *R. flava* and 47.00 mg/kg dry weight in *L. perlatum* **(Ahmet, 2009).** Zinc is widespread among living organisms due to its biological significance and mushrooms are known as zinc accumulators **(Mendil, 2004).** Manganese is part of enzyme involved in urea formation, pyruvate metabolism and the galactotransferase of connective tissue biosynthesis **(Chandra, 1999),** but it was not detected in any of the mushrooms under study.
Vitamins A (carotene equivalent) and tocopherol E were detected in the two mushrooms investigated (Table 2). Vitamin A was found in significantly higher ($p \leq 0.05$) concentration in *T. robustus* but E in lower concentration than *L. squarrosulus.* Vitamin A is involved in immune function, vision, reproduction, and cellular communication **(Johnson and Russell, 2010; Solomons, 2006).** According to Mushroom and Health Report, common *Agaricus bisporus* contained 13.0 µg/100 Beta-carotene equivalent vitamin A (2 µg RE) **(Peter *et al*., 2012)** and *Agaricus sylvaticus* was also found to contain α-tocopherol of 0.020 mg/100 g **(Vinhal *et al*., 2012).**Vitamin E is an important vitamin required for the proper function of many organs in the body. It is also an antioxidant. This means it helps to slow down processes that damage cells **(Yun-Zhong et al., 2002)**. Water soluble vitamins; thiamine (B_1) and ascorbic acid (C) were also detected in the two mushrooms in appreciable quantities compared with vitamin C of 12.65 mg/100 g reported for *Agaricus sylvaticus* **(Vinhal *et al*., 2012)** and B_1 of 0.025 mg/100 g fresh weight found in *Agaricus bisporus* **(Peter *et al*., 2012).** All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy and also help the body metabolize fats and protein.

Tannin, oxalate and phytates were found in higher concentrations in *T.robustus* than *L. Squarrosulus.* phytate and oxalate concentrations obtained in the present study were lower compared to phytic acid content range of 160 mg/100 g in *T. robustus* to 360 mg/100 g in *C. cyathiformis* with a CV of 28.4 % and oxalate content range of 80 mg/100 g in *T. robustus* to 220 mg/100 g in *A. auricula* with a CV of 3.8 % obtained by **Aletor (1995)** from a research on some edible tropical species of mushrooms. Antinutrients are substances that bind enzymes or nutrients and inhibit the absorption of the nutrients. Tannins even at low levels inhibit digestive enzymes activities making their presence in food undesirable from a nutritional point of view **(El-Adawy *et al*., 2000).** However, tannins have been shown to give substantial protection against cancer of the lungs and stomach when ingested orally **(Yavelow *et al*., 1983).** Tannin and other phenols may play a role in fighting tooth decay by inhibiting the growth of bacteria that cause tooth decay **(Moles and Waterman, 1985).** Phytates, like tannins have also been found to interact with digestive processes in a beneficial way, slowing down the absorption of sugars and regulate insulin levels when present in small amounts in food, this is beneficial in the prevention and treatment of diabetes and hyperlipidemia (high blood fats) and phytic acid also acts as antioxidant **(Hawkins *et al*., 1993; Phillippy and Graf, 1997)**. Many researchers believe that dietary restriction cannot significantly reduce risk of stone formation because dietary oxalate was found to accounts for only 10-15% of the oxalate that was detected in the urine of individuals who formed calcium oxalate stones, **(Assimos and Holmes, 2000; Curhan, 1999; Parivar *et al.*, 1996; Hanson *et al*., 1989).** This result therefore suggests that the mushooms are safe for consumption.

Amino acid composition of the mushrooms

The result of the amino acid analysis of the protein isolates from the two mushrooms is presented in Table 3. All the essential and non-essential amino acids detected were in significantly higher ($p \leq 0.05$) concentrations in *T.robustus* than *L.squarrosulus* except leucine which was the highest amino acid found in *L. squarrosulus*. Both mushrooms contained most of the essential amino acids in substantial quantities and even exceeded protein requirement pattern **(FAO/WHO, 1991)** in some cases. Aspartic acid (0.37 %), arginine (0.21 %), alanine (0.09 %), proline (0.06 %), and tyrosine (0.19 %) content obtained for *L. squarrosulus* by **Sharma *et al.*, (2012)** were lower than the quantities found in the same species in this study. Glutamic acid was the highest amino acid in *T.robustus* in conformity with the work of **Nakalembe and Kabasa, (2013)** on *Termitomyces* species, followed by leucine while glutamic acid was second to leucine in *L. squarrosulus* contrary to the findings of **Dembitsky *et al.*, (2010)** that aspartic acid dominate other amino acids in *Boletus* species. The most important benefit of leucine is its capacity to maintain blood sugar level. It can also help in producing growth hormones, healing bones and skin, sustaining nitrogen balance and maintaining mental ability **(Anthony *et al.*, 2002).** Overall, the amino acid profiles were similar in both mushroom species though they belong to different families, also similar to the profile obtained from *P. leurotus* species except for norvaline and tryptophan found in small amounts in *P. ostreatus* and *P. Sajorcaju* **(Pornariya and Kanok-Orn, 2009).**

Phenolic compounds in the mushrooms

HPLC fingerprinting of *T. robustus* extract revealed the presence of the gallic acid (t_R = 11.57 min; peak 1), catechin (t_R = 16.09 min; peak 2), chlorogenic acid (t_R = 23.51 min; peak 3), caffeic acid (t_R = 25.06 min; peak 4), ellagic acid (t_R = 32.14 min; peak 5), epicatechin (t_R = 35.98 min; peak 6), rutin (t_R = 38.25 min; peak 7), isoquercitrin (t_R = 42.56 min; peak 8), quercetin (t_R = 45.01 min; peak 9), quercitrin (t_R = 48.93 min; peak 10) and kaempferol (t_R = 54.39 min; peak 11) (Fig. 1 and Table 4) while that of *Lentinus squarrosulus* extract revealed the presence of the gallic acid (t_R = 10.52 min; peak 1), catechin (t_R = 16.03 min; peak 2), chlorogenic acid (t_R = 22.53 min; peak 3), caffeic acid (t_R = 25.11 min; peak 4), ellagic acid (t_R = 32.15 min; peak 5), epicatechin (t_R = 35.09 min; peak 6), rutin (t_R = 39.64 min; peak 7), isoquercitrin (t_R = 42.95 min; peak 8), quercitrin (t_R = 45.10 min; peak 9) and quercetin (t_R = 48.72 min; peak 10) (Fig. 2 and Table 4).

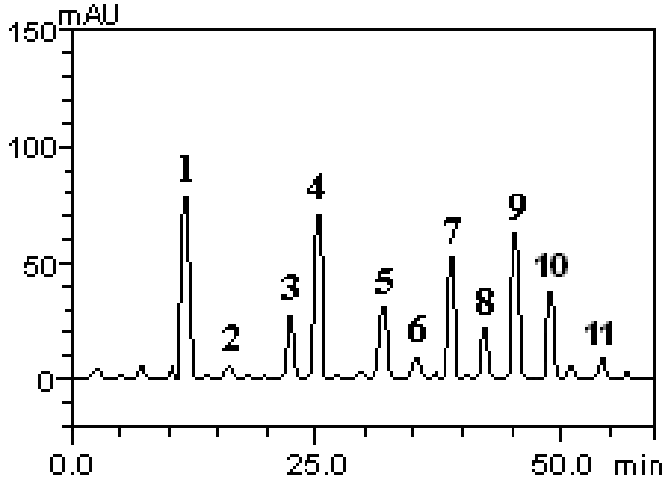

Figure 1 Reverse-phase high performance liquid chromatography profile of *Termitomyces robustus* extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), isoquercitrin (peak 8), quercitrin (peak 9), quercetin (peak 10) and kaempferol (peak 11).

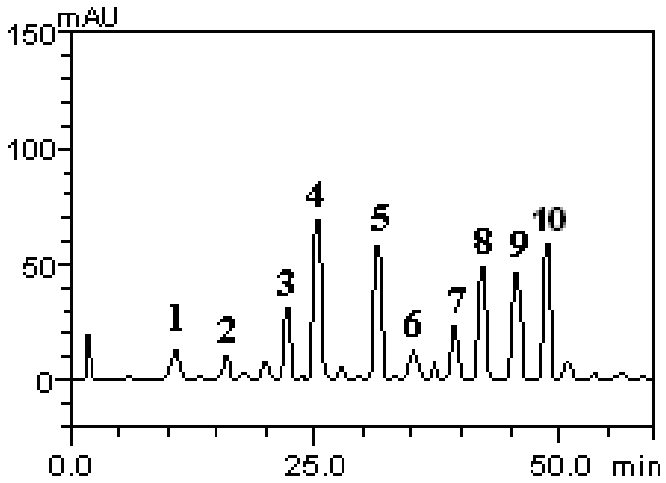

Figure 2 Reverse-phase high performance liquid chromatography profile of *L. squarrosulus* extract. Gallic acid (peak 1), catechin (peak 2), chlorogenic acid (peak 3), caffeic acid (peak 4), ellagic acid (peak 5), epicatechin (peak 6), rutin (peak 7), isoquercitrin (peak 8), quercitrin (peak 9) and quercetin (peak 10).

Gallic acid, caffeic acid, rutin, quercetin and isoquercitrin were present in both at different concentrations. Gallic acid, a benzoic acid derivative and caffeic acid, a cinnamic acid derivative belong to a phenolic acid class. Gallic acid concentration is significantly higher ($p \leq 0.05$) in *T. robust* than *L. squarrosulus*, ellagic and chlorogenic acids are significantly higher ($p \leq 0.05$) in *L. squarrosulus* but no significant difference in the caffeic acid concentrations of the two mushrooms. According to the studies published by **Kaur *et al.*, (2009)** and **Rasool *et al.*, (2010),** gallic acid possesses significant antioxidant activity and may protect the liver from the harmful effects of free radicals that are formed as a result of various metabolic processes in the body and inhibit the growth of human prostate cancer cells. Caffeic acid (3, 4-Dihydroxycinnamic Acid) is a naturally occurring substance found in many plants, including coffee beans but is entirely unrelated to caffeine and shares no stimulant activity with that of caffeine. Ellagic acid is a type of chemical found in a variety of fruits, berries and plants. Foods high in ellagic acid include raspberries, pomegranates, blackberries, pecans and walnuts. Structurally, chlorogenic acid is a combination of two molecules. It is a caffeic acid molecule bound to a quinic acid moiety, the combination is referred to as chlorogenic acid, and all three molecules can be bioactive after chlorogenic acid ingestion **(Jin *et al.*, *2012*).** Catechin, epicatechin, isoquercitrin and quercetin were in higher concentrations in *L.squarrosulus*, while rutin and quercitrin in lower concentrations than in *T.robustus.* Quercetin and kaempferol are in flavonol 3-O-glycosides class, quercetin, is a flavonol occurring in fruit and vegetable in food component with proven beneficial impact on health. Kaempferol was presnt in *T. robustus* but not found in *L.squarrosulus.* Isoquercitrin (2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-3-oxy-chromen-4-one) is quercetin 3-glucoside, while quercetin, is present in many plants, but for practical purposes it is derived from rutin (quercetin-3-O-rhamnoglucoside). Quercetin is one of the most potent antioxidants among polyphenols and has also been demonstrated to display the antiviral, antibacterial, anticarcinogenic and antiinflammatory effects **(Walle, 2004; Naidu *et al*., 2012).** Chemically, quercetin is closely related to rutin and quercitrin, two other flavonoids. Quercetin lacks a sugar molecule that is attached to these other flavonoids. When rutin and quercitrin are digested, intestinal bacteria remove the sugar molecule.

Antimicrobial Activities of Mushrooms

The antibacterial activities of aqueous solutions and alcoholic extracts of *T. robustus* (ewe) and *L. squarrosulus* (erirokiro) at concentrations 0.05 g/ml are presented in Figures 3- 5 with Streptomycin sulphate used as positive controls for antibacterial. None of the extracts showed activity against *P. aeruginosa* except ethanol extract of *L. squarrosulus. S. typhi* was also susceptible to an aqueous and methanol extracts of *L. squarrosulus*. Only alcoholic extracts of *T. robustus* and *L. Squarrosulus* showed inhibitory activities against *S. aureus.* The antimicrobial activities of the extracts against *P. aeruginosa* and *S. typhi* were similar, methanol extracts showed more effectiveness than ethanol, this may be due to the fact that they are gram negative organisms and they are physiologically related, this was noticed to be related to the work of **Abosi and Raseroka (2003)**.The extracts showed better antimicrobial activities against *S. aureus*, this may be linked with the composition of their cell wall as they are gram positive. Aqueous extract of *L. squarrosulus* was active against *S. typhi* but that of *T. robustus* was not. Both mushrooms showed activities against *S. aureus* but *T. robustus* showed no activity against *P. aeruginosa* and *S. typhi*. Generally, all the extracts of the two mushrooms exhibited weak antibacterial activities compared with the chemical antibiotics; streptomycin sulphate. The antifungal activities of the mushrooms against the selected pathogens are presented in Figures 6 - 7. These present the percentage mycelia growth inhibition of the sample extracts against filamentous fungi, *T. rubrum* and *A. fumigatus*. The results revealed methanol extracts of the samples had no inhibitory effects against any of the fungi in contrast with bonlate which was used as the positive control. *A. fumigatus* showed higher susceptibility to aqueous and ethanol extracts. The aqueous and ethanol extracts of the mushrooms demostrated quite appreciable antifungal activities when compared with bonlate. The high susceptibility displayed by the fungi to the ethanolic extracts of the samples suggests that the ethanolic extracts may be developed as antifungal drugs to treat infections caused by these organisms and preservatives for stored products like grains.

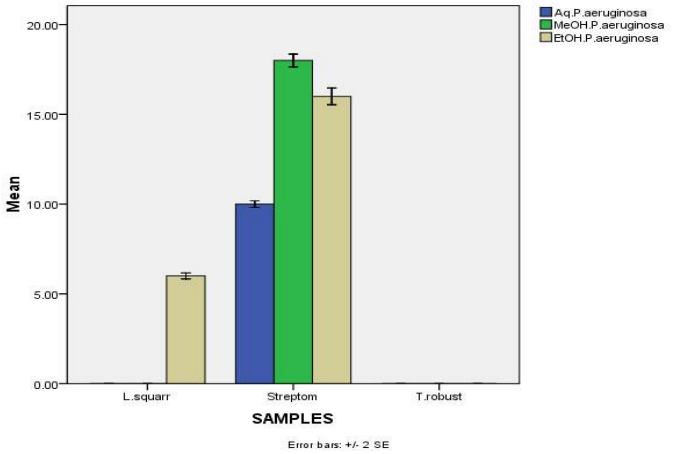

Figure 3 Antibacterial activities of extracts (0.05 g/cm^3) with Streptomycin sulphate as positive control against *Pseudomonas aeruginosa* at 24 h incubation

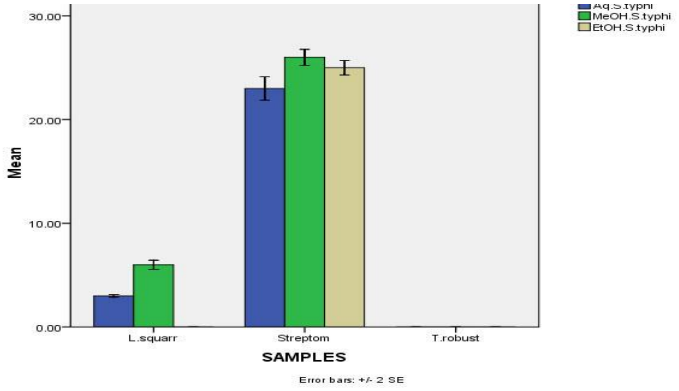

Figure 4 Antibacterial activities of extracts (0.05 g/cm^3) with Streptomycin sulphate as positive control against *Salmonella typhii* at 24 h incubation

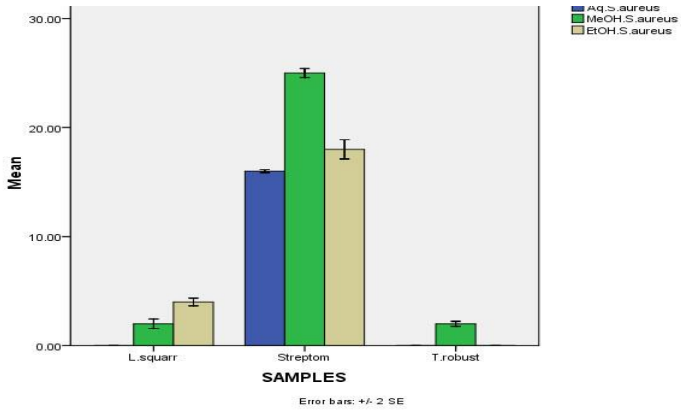

Figure 5 Antibacterial activities of extracts (0.05 g/cm^3) with Streptomycin sulphate as positive control against *Staphylococcus aureus* at 24 h incubation

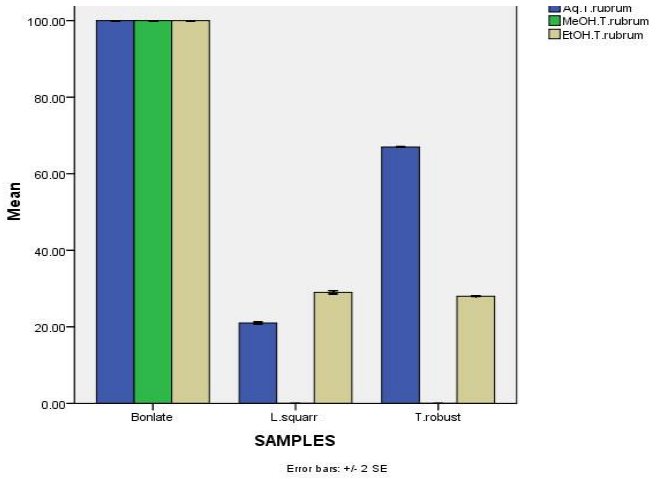

Figure 6 Antifungal activities of extracts (0.05 g/cm^3) with bonlate as positive control against *Trichoderma rubum* at 24 h incubation

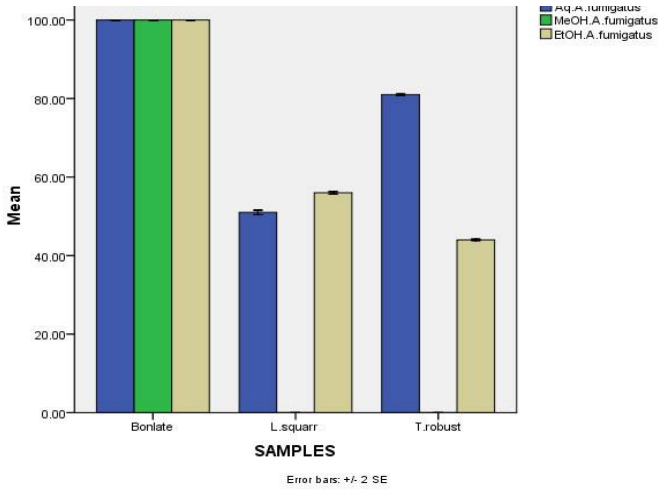

Figure 7 Antifungal activities of extracts (0.05 g/cm^3) with bonlate as positive control against *Aspetrillus fumigatus* at 72 h incubation

Figure 8 *Termitomyces robustus*

Figure 9 *Lentinus squarrosulus*

Table 1 Phytochemicals screening result

Phytochemicals	Mushrooms species	
	T.robustus	*L. squarrosulus*
Alkaloid	+	-
Saponin	+	+
Tannin	+	+
Flavonoid	+	+
Steroid	-	-
Terpenoid	+	+
Anthraquinone	-	-
Phlobatannin	-	-

\- = Absent
\+ = present

Table 2 Chemical compositions of the mushrooms

Compositions	Mushroom species	
	T. robustus	*L. squarrosulus*
Moisture	11.03±0.22b	7.22±0.07a
Crude protein	42.77±0.57b	31.24±0.02a
Ash	10.45±0.43b	7.07±0.04a
Crude fibre	4.07±0.18a	9.48±0.04b
Crude fat	6.76±0.22b	3.71±0.16a
Carbohydrate	24.90±0.11a	41.27±0.19b
Calorific Value (kcal)	331.55±3.41a	342.35±3.09a
Total dietary fibre	10.21±0.00a	11.68±0.00b
Sodium (mg/100g)	270.00 ± 0.08b	200.00 ± 0.03a
Potassium (mg/100g)	1460.00 ± 0.11b	800.00 ± 0.33a
Calcium (mg/100g)	60.00 ± 0.01b	40.00 ± 0.03a
Magnesium (mg/100g)	106.00 ± 0.05b	98.00 ± 0.04a
Zinc (mg/100g)	81.00 ± 0.03b	19.00 ± 0.02a
Copper (mg/100g)	0.90 ± 0.02b	0.20 ± 0.02a
Iron (mg/100g)	2.70 ± 0.01b	0.40 ± 0.01a
Phosphorus(mg/100g)	30.80 ± 0.01b	22.50 ± 0.02a
Manganese (mg/100g)	ND	ND
Lead (mg/100g)	ND	ND
Mercury (mg/100g)	ND	ND
Vitamin A(β-Carotene Equivalent, µg/g)	13.33 ± 0.02b	12.80 ± 0.01a
Thiamine (µg/g)	324.23 ± 0.02a	328.42 ± 0.00b
Ascorbic acid (µg/g)	14.22 ± 0.01b	11.61 ± 0.00a
Tocopherol (µg/g)	383.12 ± 0.02a	1191.39 ± 0.01b
Tannin (mg/g)	7.40 ± 0.14b	3.25 ± 0.80a
Oxalate (mg/g)	1.71 ± 0.07b	1.53 ± 0.00ab
Phytate (mg/g)	1.94 ± 0.05b	1.48 ± 0.06a

Values represent means of triplicate readings ± S.D. Values with the same superscript along the row are not significantly different ($p \geq 0.05$).

Table 3 Amino acid composition

Amino acids	Concentrations in the Mushroom species (g/100g)		
	T.robustus	*L.squarrosulus*	**RP %*
Histidine	2.33	1.96	1.9
Isoleucine	3.77	2.85	2.8
Leucine	9.07	16.99	6.6
Lysine	5.02	2.67	5.8
Methionine	1.28	0.73	
Phenylalanine	4.39	3.37	6.3a
Threonine	3.48	2.53	3.4
Valine	3.91	3.01	3.5
Arginine	5.01	3.62	
Aspartic acid	8.62	7.17	
Serine	2.39	2.03	
Glutamic acid	10.98	10.00	
Proline	2.75	2.14	
Glycine	4.33	3.00	
Alanine	3.33	3.23	
Cysteine	0.99	0.60	2.5b
Tryosine	3.02	2.06	

a = Phenylalanin with Tyrosine, b = Cystein with Methionine, **RP %*= Requirement Pattern in % protein (FAO/WHO 1991)

Table 4 Phenolic compounds in the mushrooms

Phenolic compounds	Concentrations in the Mushroom species (mg/g)	
	T.robustus	*L.squarrosulus*
Gallic acid	47.91±0.02b	7.93±0.02a
Chlorogenic acid	18.47±0.01a	20.61±0.03b
Caffeic acid	41.80±0.05a	41.09±0.02a
Ellagic acid	20.52±0.02a	38.67±0.02b
Catechin	3.64±0.01a	6.58±0.01b
Epicatechin	7.28±0.01a	8.23±0.01b
Rutin	36.94±0.01b	15.34±0.01a
Isoquercitrin	13.85±0.02a	30.71±0.02b
Quercitrin	40.96±0.02b	29.55±0.03a
Quercetin	26.11±0.01a	38.96±0.01b
Kaempferol	7.83±0.03b	0±0.00a

Values represent means of triplicate readings ± S.D. Values with the same superscript along the row are not significantly different ($p \geq 0.05$).

CONCLUSION

The nutritional quality of the mushrooms in terms of protein, vitamins and mineral elements revealed their potentials as nutritious foods. The reported health benefits of *Termitomyces robustus* and *Lentinus squarrosulus* could be related to the presence of their natural phytochemicals like phenolic acids, flavonoids, amino acids and vitamins which are known to possess antioxidant properties. They could be developed as functional foods for the prevention of degenerative diseases. Increase consumption of these mushrooms would seem to be of great health benefit. Large scale cultivation should therefore be encouraged not only for the nutraceutical potentials but food security as well as economic sustainability. Research into favourable conditions to domesticate the above mentioned mushrooms and the numerous wild species as well as government policies to promote production and trade are essential in order to maximise the poential of mushrooms as valuable natural resources for food, medicine and biochemicals for industrial purpose.

Acknowledgment: I am grateful to Mrs O. O. Crown , Mrs Oseni and Mr M. O. Oguntokun,, all in Federal University of Technology, Akure, ondo State, Nigeria for their assistance throughout the period of this study and every one, who in one way or the other has contributed to the successful completion of this work.

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