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Research Article
Nutri-toxicological Evaluation of *Pleurotus ostreatus* grown on Silk Cotton (*Ceiba pentandra*) and Bush Candle (*Canarium schweinfurthii*) Substrate

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Abstract

Background and Objective: Protein deficiency remains the major nutritional bane of people living in the developing and under-developed countries just because very few individuals can afford the rich-in-protein food. Optimum utilization of mushroom could therefore help to bridge the gap. This research work investigated the nutri-toxicological implication of utilizing edible mushroom (*Pleutotus ostreatus*) cultivated on tropical trees' sawdust as an alternative source of protein. **Materials and Methods:** The mushroom was cultivated on two tropical trees sawdust: Silk cotton tree (*Ceiba pentandra*) and bush candle tree (*Canarium schweinfurthii*). Various bioassays including minerals and proximate analysis were conducted to assess the mushroom's nutritional qualities while bio-assessments were conducted on the toxicological effects on experimental animals (albino rats) when used as the sole protein source. **Results:** The result of the proximate composition showed that the protein content was higher in *P. ostreatus* obtained from *C. pentandra* than *C. schweinfurthii* as 11.44±0.65 and 10.95±0.28, respectively while the calorific value was relatively high in the *P. ostreatus* obtained from the two substrates as 276.63±0.17 and 269.60±0.84 for *C. schweinfurthii* and *C. pentandra*, respectively. Mineral composition of the fresh *P. ostreatus* revealed that the calcium content of *P. ostreatus* obtained from *C. schweinfurthii* was higher compared to that of *C. pentandra* as 22.70 mg/100 g and 16.10 mg/100 g, respectively but the *P. ostreatus* obtained from *C. pentandra* had higher Phosphorus content of 7360.50 mg/100 g while that from *C. schweinfurthii* was as low as 2897.20 mg/100 g. It was also evident that all the clinical chemistry values of the rats fed with Diet Composed with Mushroom (DCWM) fall within the normal standard range while that of the negative control i.e., Diet Free of Protein (DFOP) deviated. **Conclusion:** *P. ostreatus* cultivated on these two indigenous substrates has no toxicological effect and is therefore recommended for consumption as an alternative cheap protein source.

Key words: *P. ostreatus*, *Canarium schweinfurthii*, *Ceiba pentandra*, protein, toxicological

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mushrooms are classified as vegetables in the food world but they are not technically plants[1]. They belong to the fungi kingdom. Although they are not vegetables, mushrooms provide several important nutrients[2]. Edible mushrooms are nutritionally endowed fungi mostly Basidiomycetes, they grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials[3-5]. They are prospective source of valuable food protein with the ability to effectively bio-convert various lignocellulosic materials to protein[6]. Mushrooms are cultured worldwide for their taste, nutritional attributes and potential application in industries[7].

Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They are often called 'boneless vegetarian meat' that contains 20-30% protein (dry weight) which is higher than those of vegetables and fruits and is of superior quality[8]. They are of more valuable source of protein than beef or fish. *Pleurotus* sp. is considered ideal for patient with hypertension and diabetics[9]. Mushroom falls between the best vegetables and animal protein source and are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals[10].

The protein value of mushrooms is twice as that of *Asparagus officinalis* and Potatoes (*Solanum tuberosum*) four times as that of tomatoes (*Solanum lycopersicum*) and carrots (*Daucus carota*) and six times as that of oranges (*Citrus sinensis*)[8]. The chemical and nutritive composition of various species of edible mushroom reported by previous workers have shown them as nutritious and highly comparable to exotic species[11,12]. The fiber, potassium and vitamin C content in mushrooms all contribute to cardiovascular health. Potassium and sodium work together in the body to help regulate blood pressure. Consuming mushrooms, which are high in potassium and low in sodium, helps to lower blood pressure and decrease the risk of high blood pressure and cardiovascular diseases[13]. Indiscriminate burning of sawdust in the saw-mills has been a major factor creating environmental pollution and this is capable of altering the bio-atmospheric compositions of the ecosystem. Therefore, this research work was designed to provide a better way of utilizing sawdust as substrate for mushroom cultivation instead of burning which is invariably translated into solving the problems of nutritional deficiency as well as providing information on the safety of consuming the mushrooms cultivated on sawdust of such tropical trees through investigation of the toxicological effect on experimental animals.

MATERIALS AND METHODS

Source of sample: This research work was carried out between March and November, 2017. Fresh wild *P. ostreatus* was obtained from Igodan Lisa neighborhood forest of Ondo state and was identified at the Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo state, Nigeria and authenticated at the Department of Microbiology, Federal University of Technology, Akure. Artificial cultivation of the mycelium was done on Potato Dextrose Agar (PDA) through a mycelium generation principle and subsequently inoculated into sterile sorghum seeds to produce the grain mother spawn, which was kept at 4°C until needed as stock culture.

Substrates preparation and sample cultivation: Trunks of two tropical trees: Silk cotton (*Ceiba pentandra*, (L.) Gaertn; Family: Bombacaceae) and bush candle (*Canarium schweinfurthii*, Engl; Family: Burseraceae) were obtained and authenticated at the Department of Forestry and Natural resources, Ondo State University of Science and Technology, Okitipupa. They were later processed into sawdust in a sawmill located at Igodan-Lisa. Unwanted materials such as stone, metals, pecks of wood and stick were removed from the sawdust. They were filled into transparent plastic poly bags of 200 g each and sterilized by autoclaving at 121°C for 15 min. Sterilized substrates were cooled and inoculated with the grain mother spawn. The inoculated substrates were incubated in a ventilated carton boxes ($1.0\times0.8\times0.5$ m) for 24 days at 25 ± 2°C after which the sprouting fruitbodies were harvested, oven dried at 55°C and pulverized using a 2 L BPA Free Jar Electric Mixer Blender.

Mineral compositions and proximate analysis: The mineral contents were determined using Atomic Absorption Spectrophotometer after mineralization while the phosphorus content was determined using colorimetric methods. The proximate analysis was carried out according to the method described in AOAC[14]. Kjeldah's method was used to determine the total nitrogen[15] while a factor of 6.25 was used to calculate the crude protein content.

Biochemical indices assay: Wistar strain albino rats between 2 and 2½ weeks were purchased from the Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo state, Nigeria and acclimatized for 2 weeks, during which period they were maintained *ad libitum* on conventional diet. The rats were subsequently divided into three treatment groups. Animals in group A were fed the Diet Free of Protein (DFOP), while animals in group B were fed the Diet Composed with soybean (DCWS) and animals in groups C were fed the Diet Composed with Mushroom (DCWM) as shown in Table 1. The feed and water were given *ad libitum* throughout the duration of feeding trial experiment. Daily feed intake and weekly change in body weight were monitored throughout the experiment. The experiment lasted for 30 days of feeding trials after which the albino rats were anaesthetized with chloroform and whole blood was collected into sample tubes containing EDTA as anti-coagulant and plain sample tubes for serum by decapitation. The serum was aspirated and further centrifuged at 3000 rpm for 30 min. The serum was refrigerated at 4°C until required for serum marker enzymes assay.

Statistical analysis: The result of the three replicates were pooled and expressed as mean deviation±SE. Data was analyzed using a one way analysis of variance (one-way ANOVA) and the Least Significance Difference test were carried out using Duncan's Multiple Range test at 5% level of significance[16] i.e., $p \leq 0.05$.

RESULTS

Proximate composition of *P. ostreatus* cultivated on the sawdust used as substrates: Table 2 shows the proximate composition of the cultivated *P. ostreatus* on sawdust of 2 different substrates. The protein content was higher in *C. pentandra* than *C. schweinfurthii* as 11.44±0.65 and 10.95±0.28, respectively. Also, the calorific value was relatively high in the *P. ostreatus* obtained from the 2 substrates as 276.63±0.17 and 269.60±0.84 for *C. schweinfurthii* and *C. pentandra*, respectively.

Mineral composition of *P. ostreatus* cultivated on the sawdust used as substrates: Table 3 provides the mineral composition of fresh oyster mushroom (*P. ostreatus*) grown on sawdust of two different tropical trees as substrates. The calcium content of *P. ostreatus* obtained from *C. schweinfurthii* was higher compared to that of *C. pentandra* as 22.70 mg/100 g and 16.10±0.00^a, respectively.

Table 1: Feed formulation for the evaluation of protein quality (g/1000 g)

	Diets		
Components	Diet free of protein (g)	Diet composed with soya bean (g)	Diet composed with mushroom (g)
Cellulose	40.0	40.0	40.0
Sucrose	100.0	100.0	100.0
Corn oil	40.0	40.0	40.0
Min. mix/Vit mix	50.0	50.0	50.0
Methionine	4.0	4.0	4.0
Soya bean meal	-	250.0	-
Mushroom	-	-	125.0
Corn starch	766.0	516.0	641.0
Total	1000.0	1000.0	1000.0

Table 2: Proximate composition of harvested *P. ostreatus* (mg/100 g dry matter) cultivated on two different substrates

	Substrates	
Proximate composition	*C. schweinfurthii*	*C. pentandra*
Moisture content (%)	11.15±0.16^b	10.10±0.65^{a,b}
Protein (%)	10.95±0.28^a	11.44±0.65^b
Fat (%)	9.47±0.41^b	7.68±0.61^a
Carbohydrate (%)	42.42±0.12^a	41.73±0.40^a
Crude fibre (%)	23.11±0.24^b	20.99±0.26^{a,b}
Ash content (%)	6.90±0.08^b	8.06±0.18^c
Calorific value (Kcal g^{-1})	276.63±0.17^b	269.60±0.84^a

*Values are means of triplicates±SD, samples carrying the same superscripts in the same row are not significantly different at p>0.05

Table 3: Mineral composition of harvested *P. ostreatus* (mg/100 g dry matter) cultivated on two different substrates

	Substrates	
Mineral composition of mushroom	*C. schweinfurthii*	*C. pentandra*
Calcium (Ca)	22.70±2.00^d	16.10±0.00^a
Iron (Fe)	0.19±0.11^b	0.38±0.17^c
Sodium (Na)	26.50±1.87^a	62.00±2.01^c
Potassium (K)	40.60±1.99^b	53.80±3.62^c
Phosphorus (P)	2897.20±16.45^a	7360.50±32.00^c

*Values are means of triplicates±SD, samples carrying the same superscripts in the same row are not significantly different at p>0.05

Chemical pathology of albino rats fed with different composed diets: Table 4 shows changes in the biochemical enzyme markers and the serum protein of albino rats fed with different composed feeds. There was a significant difference in the values of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in the negative control as compared with the positive control.

DISCUSSION

Proximate content of cultivated mushroom is presented in Table 2. Longvah and Deosthale[17] reported that amino acid configurations of mushrooms are comparable to some animal proteins, therefore, the mushrooms' proteins are important for

Table 4: Changes in clinical chemistry values or biochemical markers in rats fed with the different composed diets

Feeds	ALT (IU L^{-1})	AST (IU L^{-1})	ALP (IU L^{-1})	ALB (g L^{-1})	TPR (g L^{-1})	BLB (mg L^{-1}) Direct	BLB (mg L^{-1}) Total	GBL(g L^{-1})
DFOP	27.0±0.52[c]	23.0±0.19[e]	150.0±0.44[e]	33.0±0.39[c]	56.0±0.28[c]	0.3±0.11[c,d]	1.5±0.01[d]	20.0±0.31[d]
DCWS	24.0±0.22[a,b]	19.0±0.34[b]	144.0±0.20[c]	41.0±0.71[b]	66.0±0.32[a,b]	0.11±0.03[b]	0.9±0.13[a]	25.0±0.12[b]
DCWM	25.0±0.12[a]	18.0±0.25[a,b]	126.0±0.21[b]	37.0±0.45[b]	60.0±0.17[a]	0.09±0.03[a]	0.95±0.11[b]	23.0±0.38[a]

Values are means of triplicates±SD, samples carrying the same superscripts in the same column are not significantly different at p>0.05, DFOP: Diet free of protein (Negative control), DCWS: Diet composed with soybean (Positive control), DCWM: Diet composed with mushroom (Sample diet), ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline phosphatase, ALB: Albumin, TPR: Total protein, BLB: Bilirubin, GBL: Globulin

nutrition. In this study, the proximate composition of *P. ostreatus* obtained showed a higher value of protein content of 11.44±0.65 in *C. pentandra* than 10.95±0.28 in *C. schweinfurthii* which is similar to the work of Yilmaz *et al.*[18]. Also, these results can be compared with different mushroom fruit bodies' protein content that varied between[19] 8.6 and 42.5%.

Mineral content is also important for the nutritional value of mushrooms. The species provides a reasonable amount of minerals in comparison with vegetables[20]. The mineral composition ranged between 40.60 and 53.80 mg/100 g for potassium, 0.19 and 0.38 mg/100 g for Iron, 26.50 and 62.00 mg/100 g for Sodium, 16.10 and 22.70 mg/100 g for calcium, respectively. However, the result of calcium agreed with the finding of Deepalakshmi and Mirunalini *et al.*[21] that calcium content of *P. ostreatus* ranged between 2-35 mg/100 g, Also the phosphorus content of the mushroom ranged between 897.20 and 7360.50 mg/100 g which is slightly in agreement with the work of Patil *et al.*[22]. Phosphorous was also known to be the most abundant mineral element in the harvested mushroom and the relatively high phosphorus content was quite similar to that given by the Standard Tables of Food Composition in Japan[15] as 1061 mg/100 g and 1647.6 mg/100 g dry matter, respectively. The value of mineral content in *P. ostreatus* therefore is a reflection of the mineral composition of the substrate. Also, a variation in the proximate composition of the fruiting bodies harvested from various substrates used for the study was observed as the sawdust from *C. pentandra* produced mushroom with 11.44±0.65 crude protein content while the least was recorded in the sawdust of *C. schweinfurthii* as 10.95±0.28. However, *C. schweinfurthii* gave higher carbohydrate content of 42.42±0.12. The moisture content ranged from 10.10±0.65 and 11.15±0.16%, ash content 6.90±0.08 and 8.06±0.18, 20.99±0.26 and 23.11±0.24% crude fibre, 7.68±0.61-9.47±0.41% fat, 41.73±0.40 and 42.42±0.12% carbohydrates. This is similar to the report of Ola and Oboh[11] that carbohydrate content of *Pleurotus ostreatus* range between 40.60-53.30%. *Pleurotus ostreatus* offered prospects for reducing environmental pollution by converting lignocellulosic wastes/residues into protein rich biomass which serves as a source of an alternative protein for humans. Hence *P. ostreatus* has the capacity to bio-convert nutritionally valueless substance into high protein food as well as having biodegradability properties which would invariably increase soil fertility and bring about nutrient recycling and ecological stability[23].

Aspartate aminotransferase and Alanine aminotransferase are enzymes that are located in the liver cells and leak out and make their way into the general circulation when liver cells are injured or damaged and ALT is regarded as being a more specific indicator of liver inflammation[24].

The results obtained for liver function tests evidently revealed that all the clinical chemistry values of the rats fed with DCWM (Diet Composed with Mushroom) fall within the normal standard range. For alanine amino transferase, it was 25.0±0.2 IU L^{-1} while the normal standard is <35 IU L^{-1}. The aspartate aminotransferase value was 18.0±0.2 IU L^{-1} while the normal standard ranged[25] between 3.5-19 IU L^{-1}. The Alkaline phosphatase was also 126.0±0.2 and the standard normal[25] range is 44-147 IU L^{-1}. Albumin value was 37.0±0.2 and the standard normal value is between[25] 35 and 50 g L^{-1}. Also the total protein was 60.0±0.2 g L^{-1} while it falls within the range of 58-80 g L^{-1}, bilirubin (direct) was 0.09±0.0 mg L^{-1} and the standard range[25] is 0.0-0.3 mg dL^{-1}, Bilirubin (Total) was 0.95±0.1 mg dL^{-1} and the standard range[25] of 0.3-3.9 mg dL^{-1}, Globulin was 23.0±0.2 g L^{-1} while the standard range[25] is 20-45 g L^{-1}.

There was a significant increase in the level of serum AST and ALP of the albino rats fed with Diet Free of Protein (negative control) as compared Diet Composed with Soyabean (positive control) and Diet Composed with Mushroom, the increase in AST and ALP therefore implies a liver dysfunctioning as a result of the level of damage and injury inflicted on the liver tissues. This correlated with the report of Oboh and Akindahunsi[26] that an increase in serum levels of both transaminases (ALT and ALP) indicates a possible damage to the liver. Also, a decrease (below normal) in the serum level of the albumin and Total protein was observed in the rats fed with DFOP and this implies an insufficient protein intake by the albino rats according to the report of McPherson and Pincus[27] that the normal range is between 3.5 and 5.5 g dL^{-1}.

CONCLUSION

The *P. ostreatus* cultivated on sawdust of *C. schweinfurthii* and *C. pentandra* are of good nutritional value as they contain a level of protein and mineral compositions. Also, there was no known toxicological or side effect on the experimental animals. Therefore, those who cannot afford meats and other expensive protein sources should subscribe to the consumption of mushroom especially *Pleurotus* as it serves as alternative source of protein. Further studies on the medicinal values of the substrates as one of the determining factors of the mushroom's quality could therefore be recommended for future research work.

SIGNIFICANCE STATEMENT

This study discovered the nutritional potentials of mushroom that can be beneficial for man at large. It will also help the researchers to uncover the critical areas of nutrition and non-toxicological relevance of the trees substrates that many researchers were not able to explore. Thus a new theory on nutri-toxicological exploration may be arrived at.

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