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Research article

Influence of sorghum on proximate and phytochemical composition of cultivated edible mushroom [*Pleurotus florida* (Mont.) Singer]

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Abstract: The use of mushrooms as food is as old as civilization and the cultivation of edible mushrooms currently has greater importance in the diet of mankind. This research was undertaken with the aim of determining the influence of sorghum substrate on proximate and phytochemical quality of *Pleurotus florida*. It was cultivated using wood species; *Gmelina arborea* and *Ceiba pentandra* mixed with sorghum. The matured mushroom was harvested fresh, pulverized and analyzed according to standard procedures. The proximate quality in terms of ash, moisture, fibre, fat and protein content and the phytochemical factors such as oxalate, phytate, alkaloid and tannin were also examined. The result shows that ash content and moisture content are not significant ($P \geq 0.05$) in *Pleurotus florida* grown on *Gmelina arborea* substrates cultured with sorghum while protein, fibre and fat contents are significantly different at ($P \leq 0.05$). But the result of ash content, fibre content and fat contents are significant at ($P \leq 0.05$) in *Pleurotus florida* grown on *Ceiba pentandra* substrates cultured with sorghum while moisture and protein content are not significant ($P \geq 0.05$). The result of phytochemical revealed that oxalate, alkaloid and tannin are significant on *Pleurotus florida* grown on *Gmelina arborea* cultured with sorghum where phytate shows no significant difference. But there are also significant differences in phytate, alkaloid and tannin grown on *Ceiba pentandra* cultured with sorghum. These results, therefore, make these mushrooms popular to consume as good food sources and for other purposes.

Keywords: Substrate, Mushroom, *Pleurotus florida*, Proximate, Phytochemical.

INTRODUCTION

Mushrooms are a group of fleshy macroscopic fungi, which recently, as other fungi were introduced into the plant kingdom of cell wall and spores. Mushroom has been valued and treated throughout the world as a special kind of food and medicine for thousands of years (Lindequist *et al.*, 2005). There are many varieties of mushroom of which *Pleurotus*' are characterized by a white spore print, attached to gills, often with an eccentric stripe or no stripe at all. They are commonly known as "Oyster mushroom" (Miles & Chang, 1997). Oyster mushroom is one of the *Pleurotus* spp., and is commonly called Dhengri in India because of its oyster like shape. The genus *Pleurotus* belongs to family Tricholomataceae and has about 40 different species and 12 out of it are cultivated in different parts of the world. *Pleurotus* is efficient lignin-degrading mushroom which can grow well on different types of lignocellulosic materials such as wood (Azizi *et al.*, 1990). Cultivation of *Pleurotus* mushroom is a very simple and it requires low-cost production technology and this this gives consistent growth with high biological efficiency. Different species of *Pleurotus* spp. can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various regions of tropical countries (Sharma & Jandaik, 1985). In recent times, the cultivation of *Pleurotus* spp. had excelled next to *Agaricus bisporus* (J.E. Lange) Imbach throughout the world in terms of yield and production (Erkel, 1992). Among the *Pleurotus* spp. are; *Pleurotus sajor-caju* (Fr.) Singer which have been widely studied for the cultivation followed by *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm. Mushrooms have been considered as a delicacy from ancient times. It provides high nutritive value to the diet in the form of proteins, carbohydrates, essential salts and vitamins. As a food item, the nutritive value of mushrooms lies between that of meat and vegetables. Chang (1980) reported that the protein content of four popular edible mushrooms such as *Agaricus bisporus*, *Lentinus edodes* (Berk.) Singer, *Pleurotus* spp. and *Volvariella volvacea* (Bul.) Singer which are commercially cultivated in various countries, there fresh weight ranged from 1.75 to 3.63% respectively. Mushrooms are highly nutritious so they contain good quality proteins, vitamins and mineral (Khanna & Garcha, 1984). Mushrooms are low-calorie food with little fat and are highly suitable for obese persons with no starch and very low sugars, they can serve as medicinal food for diabetic patient (Bano, 1976). Edible mushroom-like *Pleurotus* are known to be among the largest of all fungi and so is said to exist in its natural habitat in a mycorrhyzal relationship with a tree, and this is one of the reasons why forest are often generous to mushroom hunters. Despite its nutritional value, mushroom cultivation is not widespread; many

mushrooms are considered to be healthy food because they contain large enough protein needs of the rural poor especially during the rains. It is also rich in some essential vitamins (B_1, B_2, and C) and essential minerals than most plants. They also have a low-fat content and hence high fibre content that enhances the digestion of food. They have some medicinal properties as in *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer, it is used to treat heart problems in the eastern part of Nigeria especially among the Igbos and Edos. It is also used in the treatment of asthma, cough and obesity. During the rainy seasons, different species of both edible and non-edible species usually grow on various natural substrates such as garden soil, decayed wood, termite nest, palm wastes, leaf litters, under the shade provided by cocoa, teak, coffee and rubber plantations. People in the villages (mushroom hunters) usually wake up early in the morning to look for wild edible mushrooms. The substrates used in different region depend on the locally available agricultural waste materials. This study was therefore conducted to investigate the improvement in proximate and phytochemical Composition of cultivated *Pleurotus florida* (Mont.) Singer grown on Sorghum as a substrate.

MATERIALS AND METHODS

Culture and cultivation

The pure cultures *i.e.* the mother of *Pleurotus florida* (Mont.) Singer was obtained from Forestry Research Institute of Nigeria, (FRIN), Ibadan and was sub-cultured at the Department of Microbiology Laboratory, Federal University of Technology, Akure, Nigeria.

Wood substrate

The substrates used for this experiment were collected at the point of conversion of the logs in Akure. The wood (converted to shavings), bark and the sawdust of *Ceiba pentandra* (L.) Gaertn. were collected from sawmill at Akad sawmill in Akure, Ondo State while others (*Gmelina arborea* Roxb. sawdust, shavings and bark) substrate was gotten at the Departmental Wood Workshop of Federal University of Technology, Akure, Ondo State and this helped to prevent mixing with other sawdust from another species of wood.

Spawn preparation

Spawn is considered to be the mushroom seed, which is the vegetative mycelium of mushrooms grown in a convenient medium. The *Pleurotus florida* spawn was sub-cultured using its mother spawn gotten from FRIN and this was done by putting the needed quantity of red sorghum into a basin, and was washed with clean water for about three times to remove the chaff, dust and other particles. The washed grains were then boiled between the periods of 30 to 40 minutes and thereafter was drained and spread on a sterilized laboratory table. The boiled grains were then put into the spawn bottles (transparent bottles) 2/3 of each spawn bottles were filled with grains mixed with calcium carbonate ($CaCO_3$). Calcium salt is used to adjust and buffer the pH of the substrate near 6. Additionally, it helps to avoid the formation of clumps either of the grains used for spawning or mushroom substrate and also helps to provide calcium which is an essential mineral macronutrient after which they were autoclaved using pressure pot for 50 minutes. The grains in the bottles were then inoculated with the mother spawn under aseptic condition after cooling and were left in the darkroom for at least two weeks to allow ramification at room temperature, although the ramification started the next day of inoculation and gradually for the whole two weeks. This was done repeatedly to acquire the quantity of planting spawn needed.

Substrate preparation

The materials used for the production of the substrate were from different wood species [*Ceiba pentandra* (L.) Gaertn and *Gmelina arborea* (Roxb)], of different particle sizes (sawdust and wood shavings) and the grinded bark of each wood species. These materials were thoroughly mixed together with calcium carbonate, then water was also added to them separately and was thoroughly mixed together. 200 g of the mixture was the weighed using weighing balance and poured inside white nylon bag tied with rubber band to avoid in-flow of air and passage of water. The bagged substrates were then arranged inside a black polythene bag, properly wrapped and were then put inside a pressure pot and was sterilized for about 1 hour, 30 minutes so as to remove any contaminant that may be present in the substrate (this process is referred to as Pasteurization).

Inoculation of substrate

After pasteurization, the substrates were then packed having sterilised the hands to be used, into a sterilized bucket with Ethanol (to avoid contamination of the substrate) for cooling. The bagged substrate were then arranged on the Laboratory table (having swapped the table with ethanol and cotton wool and the Bunsen burner was lightened up on the table so as to encourage a sterilized environment and each bag was then inoculated with the prepared planting spawns. The substrates were later transferred to the darkroom and covered with a black polythene sheet and were left in the mushroom house till the end of the ramification process.

Method of proximate analysis

Analysis for proximate contents of the dried powder of *Pleurotus florida* was done by methods described by the American Organization for Analytical Chemistry (AOAC, 2000). The sample was weighed (0.1 g) and was analyzed for Ash, Moisture contents, Protein content, Fiber content and Crude fats (lipids). The protein content was obtained by multiplying the nitrogen content by 6.25 and the carbohydrate content was obtained by the difference. Calorific values were obtained by multiplying the values of the crude protein, fat, and carbohydrate contents (except crude fibre) by their physiological fuel values.

Method of phytochemical analysis

The soluble organic solvent fractions (Methanol and N-butanol) of 2 g each were weighed and dissolve in 5mls distilled water while Ethyl acetate soluble fraction was dissolved by a 1 ml of ethyl acetate and made up to volume by adding 4mls of distilled water. The determination of Oxalate was calculated according to Day & Underwood (1986), Phytate was calculated by according to (Lucas & Markaka, 1975), while Alkaloids and Tannins were calculated according to (Herbone, 1976).

RESULTS AND DISCUSSION

The results of the analysis of proximate composition of *Pleurotus florida* were presented in table 1 below. It was discovered that results from this study shows that %ash and moisture content in all the *G. arborea* substrates (*G. arborea* back, shaving, or sawdust) were not significant. The percentage (%) ash ranges between 6.91% to 7.00% while that of moisture content ranges between 22.12% and 25.76 % respectively. The result from this study is similar to (Chang & Miles, 1993) which recorded a high value of moisture content in their study of about 90%. Therefore, the high level of moisture content in this study renders the mushroom highly perishable if not well preserved. The result of this study was higher to the report of (Shamaki *et al*., 2012) who reported a lower level of total ash of 5.93% in their study. Also, the result of this study was in agreement with that of (Adeduntan *et al*., 2014) who reported that there is no significant difference in ash content and moisture content when wheat was used as sources of spawn. The result of % protein, crude fiber and fat content in the *Pleurotus florida* shows significant differences. The higher was recorded in *Pleurotus florida* cultivated with *G. arborea* bark with 29.33% while the least was found in the one planted with sawdust with 25.00% respectively while in crude fiber, *P. florida* grown with sorghum the highest with sawdust, followed by bark and the least was found in wood shaving with 21.94%, 19.03% and 12.40% respectively. But in the result of fat content, it shows a high level of significant (Table 1) respectively. The fat content recorded from this study was higher to that of Shamaki *et al.*, (2012), which recorded low-fat content in their study of *G. lucidum* of about (2.60%). This shows the health benefits of this mushroom and stressing its nutritional value, it is reported that extract from this mushroom has cholesterol-lowering properties (Berger *et al*., 2011) hence, its antihypertensive potentials in humans (Mizuno, 2011).

Table 1. Effect of *Gmelina arborea* Roxb. substrates on proximate composition of *Pleurotus florida* (Mont.) Singer.

Substrate	Ash (%)	Moisture (%)	Protein (%)	Crude fibre (%)	Fat (%)
Gmelina arborea bark + sorghum	7.00±0.01^{a}	22.12±0.02^{a}	29.33±0.04^{a}	19.03±0.01^{b}	18.87±0.11^{c}
Gmelina arborea shaving + sorghum	7.21±0.02^{a}	22.13±0.02^{a}	27.41±0.01^{b}	12.40±0.01^{c}	21.99±0.01^{b}
Gmelina arborea sawdust + sorghum	6.91±0.01^{a}	25.76±0.02^{a}	25.00±0.58^{c}	21.94±0.01^{a}	22.50±1.75^{a}

Note: Mean values with the same superscript in a column are not significantly different at (P >0.05). Each value is a mean of three replicates ± standard deviation.

Table 2. Effect of *Ceiba pentandra* (L.) Gaertn substrates on proximate compositions of *Pleurotus florida* (Mont.) Singer.

Substrate	Ash (%)	Moisture (%)	Protein (%)	Crude Fibre (%)	Fat (%)
Ceiba pentandra Bark +sorghum	6.78±0.06^{c}	22.74±0.01^{a}	22.53±0.07^{c}	23.46±0.05^{b}	20.23±0.03^{a}
Ceiba pentandra Shaving + sorghum	10.04±0.01^{a}	23.51±0.01^{a}	31.14±0.01^{a}	20.95±0.01^{c}	20.38±0.01^{a}
Ceiba pentandra Sawdust + sorghum	8.03±0.01^{b}	21.37±0.02^{a}	27.89±0.01^{b}	26.84±0.01^{a}	17.10 ±0.15^{b}

Note: Mean values with the same superscript in a column are not significantly different at (P >0.05). Each value is a mean of three replicates ± standard deviation.

The results of the proximate compositions of *Pleurotus florida* when *Ceiba pentandra* was used as the substrate was shown in table 2 below respectively. It was discovered from the result that the ash content ranges from 6.78% to 10.04% and there were significant differences among the wood component used. It was discovered that the high value was recorded in *Ceiba pentandra* shaving (Table 2). From the result it was also discovered that only in moisture content alone is where we discovered no significant difference, it means that any of the wood substrates could be used as substrates. But in the case of protein, crude fiber and fat content of the mushroom, there were significant differences between the wood substrates used. It was also discovered that protein ranges from 22.53 to 31.14% and the result of this study is similar to (Adeduntan *et al.*, 2014) who reported 22-47% in their finding as well as similar to (Chang &

Mshigeni, 1996; Poppe, 2000; Kurtzman, 2005) who reported 20-40 % in their findings.

Table 3. Effect of *Gmelina arborea* Roxb. substrates on phytochemical contents of *Pleurotus florida* (Mont.) Singer.

Substrate	Oxalate (mg g^{-1})	Phytate (mg g^{-1})	Alkaloid (mg g^{-1})	Tannin (mg g^{-1})
Gmelina arborea bark + sorghum	0.67 ± 0.01^{a}	6.18 ± 0.01^{b}	3.26 ± 0.02^{c}	0.91 ± 0.01^{b}
Gmelina arborea shaving + sorghum	0.67 ± 0.01^{a}	7.83 ± 0.01^{a}	6.24 ± 0.02^{a}	1.21 ± 0.01^{a}
Gmelina arborea Sawdust + sorghum	0.57 ± 0.01^{b}	5.36 ± 0.01^{c}	6.03 ± 0.02^{b}	1.22 ± 0.02^{a}

Note: Mean values with the same superscript in a column are not significantly different at (P >0.05). Each value is a mean of three replicates ± standard deviation.

The results of the phytochemical analysis were reported in table 3 and 4 below. The analysis result was carried out on *Pleurotus florida* cultivated on *Gmelina arborea* and *Ceiba pentandra* substrates (shredded bark, wood shavings and sawdust) cultured with sorghum as source of spawn substrates and this revealed the level of anti-nutritional factor (Oxalate, Phytate, Alkaloid and Tannin) contained in the cultivated mushroom. From the results, it was discovered that there were no significant differences in oxalate in *Pleurotus florida* mushroom cultivated with *Gmelina arborea* but there are significant differences in phytates, alkaloids and tannins both in *G. arborea* and *Ceiba pentandra*.

Table 4. Effect of *Ceiba pentandra* (L.) Gaertn substrates on phytochemical contents of *Pleurotus florida* (Mont.) Singer.

Substrate	Oxalate (mg g^{-1})	Phytate (mg g^{-1})	Alkaloid (mg g^{-1})	Tannin (mg g^{-1})
Ceiba pentandra bark + sorghum	0.57 ± 0.01^{a}	11.13 ± 0.02^{a}	5.77 ± 0.05^{a}	1.21 ± 0.02^{c}
Ceiba pentandra shaving + sorghum	0.57 ± 0.01^{a}	7.01 ± 0.01^{c}	5.62 ± 0.02^{b}	2.58 ± 0.01^{a}
Ceiba pentandra sawdust + sorghum	0.50 ± 0.15^{b}	8.65 ± 0.02^{b}	2.94 ± 0.03^{c}	1.66 ± 0.04^{b}

Note: Mean values with the same superscript in a column are not significantly different at (P >0.05). Each value is a mean of three replicates ± standard deviation.

The maximum oxalate content of *Pleurotus florida* cultivated on *Gmelina arborea* substrates is 0.67 mg g^{-1} in the fruiting bodies harvested on the shredded bark and wood shaving cultured with sorghum as the spawn substrate whereas the least is 0.50 mg g^{-1} cultivated on the wood sawdust. The highest phytate content of *Pleurotus florida* cultivated is found on *Ceiba pentandra* fruiting bodies harvested on the sawdust of the wood cultured with sorghum with the value of 8.65 mg g^{-1} and the least is recorded with the fruiting bodies harvested on the wood *G. arborea* sawdust cultured with sorghum grain with the value of 5.36 mg g^{-1} respectively. But in case of alkaloid and tannin, the highest values of alkaloid were recorded in *G. arborea* wood shaving with the values of 6.24 mg g^{-1} while the least is recorded in *Ceiba pentandra* sawdust with the value of 2.94 mg g^{-1}, whereas the highest tannin was recorded in *C. pentandra* with 2.58 mg g^{-1} and the least is 0.91 mg g^{-1} which was found in *Gmelina arborea* wood bark. Oxalates are naturally occurring substances that are found in plants, animals, and as well as in humans (Odebiyi & Sofowora, 1978). In chemical terms, oxalates belong to a group of molecules called organic acid, and are routinely made by plants, animals, and humans (Adeduntan *et al*., 2014). Human bodies always contain oxalates, and her cells routinely convert other substances into oxalates (Okombo & Liebman, 2010). For example, vitamin C is one of the substances that our cells routinely convert into oxalates (Chai & Liebman, 2005). In addition to the oxalates that are made inside of our body, oxalates can arrive at our body from the outside and from certain foods that contains those (Adejumo & Awosanya, 2005). Oxalate content is not expected to be consumed excessively as much intake of it has the propensity to precipitate (or solidify) in the kidneys or in the urinary tract to form calcium oxalate crystals which contribute to the formation of kidney stones (Michael, 2002). Also, tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods (Jayaraman, 2011). Many tannin molecules have been shown to reduce the mutagenic activity of a number of mutagens. Tannins have also been reported by researcher to exert other physiological effects, such as it accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses (Chung *et al*., 2001). Therefore, the results of phytochemicals properties of this study is similar to (Shamaki *et al*., 2012; Adeduntan *et al*., 2014) which also reported lower phytochemicals in their studies.

CONCLUSION

The proximate composition and anti-nutritional factors of *Pleurotus florida* were analyzed from this research. Examples of proximate composition analyzed are ash, protein, moisture, fibre and the fat content of *Pleurotus florida*. Also, some of anti-nutritional analyses carried out are oxalate, Phytate, Alkaloid and Tannin in *Pleurotus florida*. It was discovered that all the anti-nutritional factors are at a bearable level even with those that are significantly high, as they are still within the range that could be consumed and will not result to any adverse effect. It also reflects in this study that proximate content contained in this mushroom could make the mushroom serve as a supplement to food nutrients.

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