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EFFECTS OF SUBSTRATES ON THE PROXIMATE AND PHYTOCHEMICAL ANALYSIS OF *Pleurotus tuber regium*(Fries) Singer

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ABSTRACT
The effect of sclerotium and three different substrates on the proximate and phytochemical composition of Pleurotus tuber- regium (Fries) Singer was investigated. The result showed that Pleurotus tuber- regium grown from the three substrate namely : cassava peel, oil palm husk and banana peel with its sclerotium is nutricious. The highest values of moisture content and protein, were in cassava peel (7.90%) and oil palm (30.49%) while sclerotium fruiting bodies had the highest values for ash (3.76%) and crude fibre (5.16%) and sclerotium after fruiting bodies had the highest values for crude fiber (6.73%)and carbohydrate (64.33%) while the lowest values in moisture was in oil palm (5.92%), in protein and ash, lowest values were in sclerotium after fruiting bodies (18.66%), (1.59%) respectively, sclerotium after fruiting body had lowest values in protein (18.66%) and ash (1.59%). Banana leaves has lowest value in fat (0.81%), sclerotium fruiting bodies had lowest value in crude fiber (2.47%) and oil palm husk had lowest value in carbohydrate (52.43%). There were significant differences on all the samples. Saponins, tannins, steroids, flavonoids, glycoside, phenols were present in all the samples while alkaloid and phlobatannins were absent in all the samples. This work shows that Pleurotus tuber- regium can supply rich nutrient that can support good functioning of both human and animal system .

Keywords: Fruiting bodies, Mushroom, Phytochemicals , *Pleurotus tuber regium*, Proximate, Substrates.

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INTRODUCTION

Mushrooms are non-timber forest products which are often found as saprophytes on soil, open fields, farm lands wood and roadsides. Mushrooms are popularly consumed as delicacy by Nigerians and many other countries of the world (Gregori *et al*., 2007; Zhang *et al*., 2010; Gucia *et al*., 2011; Nnorom *et al*., 2013). They are important constituents of minor forest produce and a non-timber forest product, that grow on the most abundant bio molecule of this biosphere, that is, cellulose. They are widespread in nature and they remained the earliest form of fungi known to mankind. Mushrooms were long appreciated due to their flavor, texture, taste, and some for medicinal attributes, others for their aesthetic attributes like shapes, sizes, colours for which they are referred to as flowers of the fall. Mushrooms are fruit bodies of macroscopic, filamentous and epigeal fungi, they are made up of hyphea which forms interwoven web of tissues known as mycelium in the substrate upon which the fungus feeds (Adedayo *et al.*, 2010).

Onuoha *et al*., (2009) defined mushroom as macro fungus with definitive fruiting body and large enough to be seen with the naked eyes. Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium. Mushrooms grow naturally in Nigeria during the early and late rainy seasons (Gbolagade *et al*., 2006). Man's attention is usually attracted to mushrooms by the unusual shape of their fruit bodies which suddenly appear after rain in striking numbers in field and woodlands (Onuoha, 2007).

Edible mushrooms like *Pleurotus* are known to be among the largest of fungi. *Pleurotus tuber-regium* is a tropical sclerotial mushroom (Fasidi and Olorunmaiye, 1994). Being sclerotial the mushroom produces a sclerotium or underground tuber, as well as a mushroom, fruiting bodies then emerge from the sclerotium. Both the sclerotium and the fruiting bodies are edible (Oranusi *et al*., 2014).

The sclerotium is spherical to ovoid and can be quite large, up to 30 cm or larger in diameter. It is dark brown on the outside and white on the inside. They are generally referred to as 'oyster mushrooms because of their naturally occurring flavour. Edible oyster mushrooms are excellent delicacies in many regions of the world (Iwase *et al*., 2000; Jonathan *et al*., 2008). In Nigeria, the most popular edible oyster mushroom is *P. tuber-regium* (Gbolagade, 2006) and commonly eaten especially in the eastern part. This fungus usually grows wild on decomposing wood during the rainy season where it always produces sclerotium during unfavourable weather condition.

Cultivation of oyster mushroom has been universally recognized because of its nutritional value and minimal technology. This type of mushroom obtain its nutrient by decomposing various agricultural by-products due to its saprophytic nature. *Pleurotus tuber-regium* has a history of economic importance in Africa as food and as a medicinal mushroom. Many therapeutic substances e.g anti-microbial, anti-viral , anti-neoplatic, anti-oxidant, anti-inflammatory, hepatoprotective, anti-diabetic, anti-tumor,anti-cancer,cholesterol lowering properties, anti-parasitic, anti-aging, anti-mutagenic among others (Olagbemide and Ogunnusi,2015 ; El Bohi *et al*., 2005; Venkatakrishnana *et al* .,2010; Keyhani *et al*., 2007; Chen *et al.* 2012 ; Refaie *et al*., 2009; Adebayo *et al*., 2012; Nozaki *et al*., 2008; Ramkumar *et al*.,2010). Despite the medicinal, nutritional and economic importance of *Pleurotus tuber-regium* indicated by various authors and researchers, there is still less awareness on its importance. The objective of this study was to determine the relative nutritive qualities and Phytochemicals present in *Pleurotus tuber regium* as affected by the three different substrates and its sclerotium.

MATERIAL AND METHOD

The experiment was conducted in Plant Physiology Laboratory of the department of Plant- Biology, University of Ilorin, Kwara State, Nigeria between August and November 2015.

Collection of material

Sclerotia used for this study were collected from a local trader in Ondo Town, Ondo State . The substrates used : oil palm husk, cassava peel and banana leaves were gotten within Ilorin, Kwara State.

Preparation of the substrates for cultivation

500g of each substrate was used. The oil palm fiber was soaked in water over night in order to melt the remaining oil in the fibre, the cassava peel was sun-dried for three days to reduce moisture and microbial activities, the dried cassava peels was then macerated and the dried banana leaves was cut into small sizes and soaked in water overnight in order to allow water penetration. The substrates were then composed that is mixed with water until adequate moisture content was obtained, the moisture content is attained when handful of the substrate is squeezed and no water drips out. The substrates were packed in polytene bag tied with rubber band leaving small opening and beaten with wood so that the substrate can mold together. The bagged substrates are then sterilized using autoclave at 121 pressure for I hour. After sterilization, the bags were allowed to cool down in the laboratory at room temperature. The sclerotium was soaked in water over night in order to be soft and easy to cut. It was then cut into 50g and inoculated in to the substrates bags and then properly watered to create a humid environment normally required for fructification. Whole (un-cut) sclerotium weighing 500g was soaked in water over night in order to absorb moisture and easy fructification and placed on a flat surface and then watered daily to produce fruiting bodies.

Phytochemical and Proximate Screening

This involved phytochemical and proximate screening of both sclerotium and the fruiting bodies of *Pleurotus tuber–regium* . The samples were analyzed for Proximate and Phytochemical by the methods of AOAC (2003) and conducted in Chemistry Laboratory of Nigerian Stored Product Research Institute Asadam Road, Ilorin, Kwara State.

STATISTICAL ANALYSIS

The data collected were subjected to statistical analysis by using Statistical Package for Social Sciences (SPSS; Version16.0)

RESULTS AND DISSCUSSION

Fruiting bodies were observed forming on the wet sclerotium third day after wetting which was preceeded by mycelia colonization. Three weeks after inoculation of the substrates, it was observed that white mycelia colonized all the substrates. Fruiting bodies were first observed on the banana leaves five week later. Two weeks after, fruiting bodies was observed on oil palm husk and cassava peel. Within five days the fruiting bodies were finally mature and harvested before the pilus curve.

Table 1 below shows the Proximate analysis of the fruit bodies of *Pleurotus tuber- regium.* The samples were analyzed for moisture, protein, fat, ash, fiber and carbohydrate. Cassava peel has the highest moisture content (7.90%) while oil palm husk has lowest(5.92%) moisture content. Protein was highest (30.49%) in oil palm husk while sclerotium fruiting bodies has lowest value(18.66%). Sclerotium fruiting bodies has highest in ash and fat content (3.76% and 5.16%) respectively while sclerotium after producing fruiting bodies has the lowest (1.59%) in ash and banana leaves has lowest value in fat content(0.81%). Crude fat is low in all the samples. The highest values for crude fibre and carbohydrate were obtained in sclerotium after producing fruiting bodies (6.73% and 64.33%) while the lowest content were in sclerotium fruiting body(2.47%) and oil palm husk(52.43%) respectively. Carbohydrate and protein content were high in all the samples. There were significant differences for all the nutrient analysed.

Table 2 below shows the Phytochemical analysis of the fruiting bodies of *Pleurotus tuber regium.* The result showed that saponins , tannins ,steroids, flavonoids , glycoside, phenols were present in all the samples while alkaloid and phlobatannins are absent in all the samples. The result also showed that terpenoids are present in sclerotium fruiting bodies, banana peel and oil palm husk but absent in cassava peel and sclerotium after producing fruiting bodies. Anthraquinones are present in sclerotium fruiting bodies and banana peel and absent in oil palm husk, cassava peel and sclerotium after producing fruiting bodies.

Table 1: Proximate Analysis On The Mushroom

Substrate	Moisture%	Protein%	Ash%	Fat%	Crude fiber%	Carbohydrate %
Banana leaves	6.06^{d}	25.22^{b}	3.23^{b}	0.81^{c}	6.13^{b}	58.56^{c}
Oil palm husk	5.92^{e}	30.49^{a}	2.93^{c}	2.71^{b}	6.03^{b}	52.43^{d}
Cassava peel	7.90^{a}	23.92^{c}	2.49^{d}	0.86^{c}	5.02^{c}	59.82^{b}
Sclerotium (After fruit bodies)	7.79^{b}	18.66^{e}	1.59^{e}	0.89^{c}	6.73^{a}	64.33^{a}
Sclerotium fruit bodies	6.33^{c}	22.24^{d}	3.76^{a}	5.16^{a}	2.47^{d}	60.05^{b}

Means followed by the same letter within row for each substrate are not significantly different at $P < 0.05$.

Table 2: Phytochemical test on the mushroom

SAMPLE/TEST	A	B	C	D	E
Saponins	+	+	+	+	+
Alkaloid	–	–	–	–	–
Tannins	+	+	+	+	+
Steroids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Terpenoids	+	+	+	–	–
Glycoside	+	+	+	+	+
Phenols	+	+	+	+	+
Anthraquinones	+	+	–	–	–
Phlobatannins	–	–	–	–	–

A=Sclerotium fruit body ,B=Banana peel, C=oil palm husk,D=cassava peel, E=sclerotium; + mean positive; - mean negative

DISCUSSION

Pleurotus tuber-regium is a tuberous species which is usually tough and requires sufficient chewing before it could be swallowed and as such people who could not afford meat normally cherish this mushroom. The sclerotium itself could be grind and used as thickners in soup. They fall between the best vegetables and animal protein source and are considered as source of proteins, fats, carbohydrates, amino acids and vitamins (Jiskani, 2001).

Results of the proximate analysis of mushrooms in this study showed that they contain carbohydrate, protein, crude fibre, crude fat, moisure and ash. *Pleurotus tuber-regium* contain high carbohydrate and appreciable high protein this agree with the report of Eze *et al*., (2014) who also observe the same. The result obtained indicated that carbohydrate content in this work is higher than that reported by Ikewuchi and Ikewuchi (2008). The high protein and carbohydrate content is a proof of their being highly nutricious and may be adequate to meet the nutritional requirement of human and animals. It has being reported that protein content of mushrooms is twice that of vegetables, four times that of oranges and significantly higher than that of wheat (Okwulehie and Odunze, 2004).

The considerable high amount of crude fibre obtained from this study agrees that the mushroom has high medicinal importance as opined by Jiskani,(2001) that *Pleurotus tuber regium* is an anti-cancer and anti-diabetic mushroom. Fibre is also known as anti-tumorigenic and hypocholestrolaemic agent (Okoro and Achuba, 2012). This implies that this mushroom may be recommended for people with cholesterol related problems (Chihara, 1993).

Ash represent the mineral left after feed are burnt in oxygen. It is used as a measure of mineral content of any sample (Agomuo, 2011). This is why the fruiting bodies directly from the sclerotium has more ash content and least value from sclerotium after producing fruit bodies because the fruiting bodies grown from the sclerotium has extracted most of the ash.

Cassava peel substrate has highest moisture due to the fact that the peel retain more water than the other substrate and also the sclerotium after fruiting bodies has the next high moisture content. The moderate moisture content suggested that great care must be taken in mushroom handling and preservation because the moisture content increase mushroom susceptibility to microbial infections and this makes it easy for mushroom contamination when not in a sterile environment.

Saponins, tannins, steroids, flavonoids, glycoside and phenols are present in all the samples were also reported by ikewuchi and ikewuchi (2008). The presence of Phenolics compounds may be responsible for the strong antioxidant activity of the mushroom (Mbaebie, 2012). Tannins reduce blood cholesterol (Basu *et al.*, 2007). Saponins have been shown to possess a hemolytic effect on red blood cells. It also has cytotoxic effects and beneficial in its cholesterol lowering ability (Agomuo, 2011), reduce cancer risk, immunity booster, reduce bone loss, anti-oxidant among others. Steroid possess pharmacological actions (reduced elevated cholesterol level). Presence of glycoside help in heart failure regulation, diuretic and many other medicinal properties. This agrees with (Chang, 1994; Fasidi and Kadiri, 1994; Bilal *et al*., 2010; Dreyfuss and Chapela, 1994 ; Teow, 1997; Chang and Buswell, 1996) among others that confirmed the medicinal values of *Pleurotus tuber regium* in their various reports.

CONCLUSION

In conclusion, the presence of nutritive and phytochemicals in *Pleurotus tuber-regium*, as revealed in this study has shown that *Pleurotus tuber-regium* may be an alternative source of essential nutrient. It is hereby recommended that mushroom production should be encouraged in order to ensure regular availability of essential nutrients in diet.

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