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## Research Article

# NUTRITIONALAND BIOACTIVE EVALUATION OF *PLEUROTUSPULMONARIUS* (FREIS) QUELL.FRUITBODIES GROWN ONDIFFERENT WOOD LOGS

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**ABSTRACT**

This study was conducted to determine thenutritional and bioactive composition of *Pleurotus pulmonarius* fruit bodies cultivated on logs of *Dacryodesedulis, Mangiferaindica* and *Treculiaafricana.* Logs were cut into average length of 18cm with inoculation holes of 3cm × 15mm diameter using high speed drill (HSD) of 5 drill bit and allowed to decompose for 8months. During mushroom cultivation, logs were soaked in tap water for 24hrs and pasteurized at 80$^{o}$C in an improvised metallic drum (IMD) for 1hourusing cooking gas as heat source and allowed to cool overnight. Pure mycelium culture of *P. pulmonarius* was aseptically bulked in sorghum grains. 10g of grain based spawn was inserted into 2/3 of each hole by way of inoculation and sealed with sterile polybag for mycelium incubation. Polybags were cut open after spawn run following primordial initiation. Fruit bodies were harvested at maturity, sundried and packed in airtight container for further analysis. Data were analyzed using Analysis of Variance (ANOVA)and mean separation by DuncanMultiple Range Test (DMRT) at 5% levels of significance. Results indicated that *P. pulmonarius* fruit bodies harvested from various logs were significantly different (P<0.05) in their nutritional and bioactive compounds composition. Fruit body samples were rich in Protein,Carbohydrates, Sodium, Potassium and Calcium. It was also observed that fruit bodies contained significant amount of alkaloids, tannins and saponins;flavonoids and phenols could be useful in drug synthesis. Therefore adopting this technique in oyster mushroom cultivation would create the opportunity of harnessing waste of woods.



## INTRODUCTION

Mushrooms are unique biota which assemble their food by degrading enzymes and decompose the complex food materials present in the biomass where they grow (Chang, 2006). Oyster mushrooms can be grown on various substrates due to its strong enzymatic features. Differentsubstrates are used in each region depending on their availability (Cohen andHadar, 2002). Wheat straw, sawdust and other agricultural by-products resulting after processing of waste paper, Hazelnut and Tilia have been used in Oyster mushroom cultivation (Ukoima*et al.*, 2009a, 2009b,2009c, Yıldız*et al.*,2002); maize, corn, rice, elephant grass (Obodai*et al.,* 2003), sugarcane (Membrillo*et al.,* 2011), coffee (Gume*et al.,* 2013) have been examined as alternative substrates for its cultivation.

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These substrate materials are usually by-products from industries, households, agriculture etc, and are usually considered as wastes (Okwulehie *et al*., 2008). However, these wastes are actually resources in the wrong place at a particular time and mushroom cultivation can harness them for its own benefit (Chang, 2013). Kadiri and Aizai (2005) showed that *Lentinussubnudus* could be cultivated on wood logs of tropical trees. According to Hyunjong and Seung (2004), hard woods such as poplar, willow, beech, elm and alder are the most commonly used tree species in oyster mushroom cultivation. Oyster mushrooms do not grow well on Oak tree logs. Hyunjong and Seung (2004) reported that since mushroom feed primarily on sapwood, any tree trunk selected for inoculation must have a larger sap wood area. The lighter or outermost wood of a log is the sapwood and the darker or inner wood is the heartwood. The desirability of a food product does not necessarily bear any correlation to its nutritional values instead, its appearance, taste and aroma, sometimes can stimulate one's

appetite (Chang, 2013). Mushroom has been used as a food and medicine since immemorial due to its delicious taste and dietary qualities (Agraha-murugkar and subbulakshimi, 2005; Muhammad *et al.,* 2007). Mushrooms are also known for their medicinal properties; they are low in calories and are ideal food for diabetic and heart patients. Mushroom has qualities like lowering the blood cholesterol level, warding against cancer and invigorating hair growth. Tewari (2008) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins.*Pleurotus* species are good source of protein, vitamins and minerals (wahlid *et al.,* 2006; Chang, 2013). Mushroom protein is intermediate between that of animals and vegetables, but superior to most other foods, including milk and contains all the essential amino acids required by man (Purkayastha and Nayak, 2002; Chang and Miles, 2004; Kurtzman, 2009). Mushrooms contain appreciable quantities of crude fibres although little information exists on the total dietary fibre (TDF) content of mushrooms. Okwulehie*et al,* (2008) reported high crude protein and carbohydrate contents in *P.ostreatus* cultivated on different substrates. The world production of oyster mushroom is estimated to be 875,000 tons in 1997 (Chang, 1999). China was responsible for 87% of world supply, oyster mushroom is the easiest to produce and least expensive to grow. Most of the world's supply of oyster mushrooms today comes from commercial mushroom growers (Chang, 2011). For small-scale cultivation with limited budget, oyster mushroom is the clear choice for gaining entry into the mushroom industry (Muhammad *et al.,* 2007). This research work is aimed at determining the nutritional and bioactive compounds composition of *P. pulmonarius* cultivated on three wood logs.

## MATERIALS AND METHODS

### Source of Culture

Pure culture of *P. pulmonarius* (Fries) Quel.Was obtained from the laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

### Spawn Production

Spawn of *P. pulmonarius* was produced using sorghum grains. Grains were washed in tap water and soaked overnight. They were then boiled in water in the ratio of 1:1 (sorghum grain: water) using cooking gas for 15-20 minutes and drained of excess water. Completely drained sorghum grains were mixed with 4% (w/w) $CaCo_3$ and 2 % (w/w) $CaSO_4$ to optimize pH and prevent clumping of grains respectively as described by Muhammad *et al.* (2007). Grains were later stuffed into 35cl Lucozade bottles tightly plugged with cotton wool and sterilized in an autoclave at 121$^o$C for 30minutes. After sterilization, the bottles were allowed to cool,before they were inoculated with actively growing mycelium of *P. pulmonarius* by grain-to- grain transfer and incubated in the dark (27±2$^o$C) for 10-15days until the grains were fully colonized by mycelium (Shyam *et al.,* 2010).

### Preparation of Wood Logs (Substrates)

Average trees size of *T. africana, M. indica* and *D. edulis* were fell during the Hammattern season (winter) according to the recommendations of Oei (2003). Trees were cut into logs of 18cm using Electric wood saw (EWS); Model: Elect. 1710, Japan. Care was taken to ensure that the barks of the logs were not peeled off as instructed by (Hyunjong and Seung, 2004).

### Inoculation Holes

Holes of depth 3cm by 15mm diameter were made hexagonally on each log with high speed drills (HSD) of 5 drill bit in respect to log size. Average number of holes per log was determined by the formula (Stamets 2013; Nwoko, 2015).

$$NH = \frac{DL(cm)\ X\ LL(cm)}{6}$$

Where: NH= Number of holes

DL= Diameter of log(cm)
LL= Length of Log (cm)
6= Derived constant.

### Mushroom Cultivation

Logs were laid in open field for 8-9months in alternating rains and sun to allow for decomposition. Dry weight of logs (g/kg) were determined before they were soaked in water for 24hr. Logs were pasteurized at 80$^o$c in an improvised metallic drum (IMD) for 1hr using cooking gas as a local heat source and allowed to cool overnight (Canford, 2004; Nwoko *et al.,*2016).Log inoculation was done by inserting about 10g grain spawn of *P. pulmonarius* into 2/3 of the holes and subsequently sealing the logs with transparent polybags to avoid contaminants. Mycelium recovery and colonization were clearly visible after 24hrs; when fully colonized polythene bags were cut open to allow for fruiting (Hyunjong and Seung, 2004). Before pinhead initiation, white mycelium was visibly noticed on the cut ends of the logs. Light intensity and humidity of the air were increased to about 400 lux and 75% respectively. To achieve these, logs were watered at least morning and evening at the cropping room of the mushroom. Temperature was maintained at 27 ± 2$^0$C (Oei, 2003; Chen, 2004).

### Proximate Analysis

Proximate Analysis was carried out on each of the 3 mushroomsamples.Nutrients like carbohydrates, protein, fat ash, moisture and crude fiber contents were determined by using the methods outlined in the AOAC (1984). Protein determination was carried out using the Kjedahl method (AOAC, 1984). Fat determination was carried out using a Soxhlet apparatus (AOAC, 1984). Also determination of fiber content was done according to the enzymatic gravimetric method (AOAC, 1984).

### Determination of Minerals

Mineral compositions of dried mushroom samples were determined by wet-ashing method. The solutions of ash obtained from the samples were dissolved in a drop oftrioxonitrate (V) acid made up to 50ml with deionized water and analyzed for Calcium (Ca) and Magnesium (Mg) using vanadate ethyldiamine-tetra acetic acid (EDTA)

complexometric titration method according to MFA (1982). Sodium (Na) and Potassium (K) were estimated using flame photometer while Phosphorus (P) was determined using UV-visible spectrometer after making Ammonium vanado-molybdate at 436nm according to the established procedures of Perkin Elmer (1982).

**Table 1. Effect of log substrates on proximate composition (%) of *P. pulmonarius* fruit bodies**

| woodlogsubstrate | MC | ASH | Fat | Fibre | Protein | CHO | DM | $N_2$ |
|---|---|---|---|---|---|---|---|---|
| *D. edulis* | $2.63^{c}$ | $9.46^{a}$ | $2.69^{a}$ | $6.15^{c}$ | $37.17^{b}$ | $41.91^{c}$ | $97.38^{a}$ | $5.95^{b}$ |
| *M. indica* | $3.12^{a}$ | $7.0^{c}$ | $2.56^{c}$ | $2.29^{a}$ | $37.86^{a}$ | $43.11^{a}$ | $96.88^{c}$ | $6.06^{a}$ |
| *T. africana* | $2.81^{b}$ | $8.48^{b}$ | $2.59^{b}$ | $6.24^{b}$ | $37.68^{a}$ | $42.21^{b}$ | $97.19^{b}$ | $6.03^{a}$ |

Values are means of 3 replicates and values bearing the same letter are not significantly different (P≤0.05).
MC = Moisture content, CHO = Carbohydrate, DN = Dry matter

**Table 2. Mineral constituents (mg/100g) of *P. pulmonarius* fruit bodiesas affected by different log substrates**

| woodlog substrate | Na | K | Mg | Ca | P |
|---|---|---|---|---|---|
| *D. edulis* | $15.82^{a}$ | $172.23^{a}$ | $17.28^{a}$ | $127.40^{a}$ | $33.23^{a}$ |
| *M. indica* | $14.94^{b}$ | $171.18^{b}$ | $16.52^{c}$ | $126.46^{c}$ | $32.16^{c}$ |
| *T. africana* | $15.26^{c}$ | $171.67^{c}$ | $16.80^{b}$ | $126.79^{b}$ | $32.76^{b}$ |

Values are means of 3 replicates and means bearing the same letter are not significantly different (P≤0.05).

**Table 3. Effect of wood logs on bioactive compounds composition (%) of *P. pulmonarius* fruit bodies**

| woodlog substrate | Alkaloids. | Flavonoids. | Phenols | Tannins | Saponins |
|---|---|---|---|---|---|
| *D. edulis* | $4.07^{c}$ | $0.18^{c}$ | $0.94^{b}$ | $1.62^{b}$ | $2.52^{b}$ |
| *M. indica* | $4.16^{b}$ | $0.21^{b}$ | $0.93^{b}$ | $1.52^{c}$ | $2.55^{b}$ |
| *T. africana* | $4.34^{a}$ | $0.26^{a}$ | $1.05^{a}$ | $1.74^{a}$ | $2.63^{a}$ |

Values are means of 3 replicates and means bearing the same letter are not significant at (P<0.05).

### Determination of Percentage Bioactive Compounds

Percentage Alkaloids were determined by the methods of AOAC (1975) and Maxwell *et al.* (1995). Percentage Flavonoids, Saponins and Tannins were also determined by the procedures according to Cloupai-Abyazini (1994), Peng and Kobayashi (1995) while percentage Phenols were estimated by the method of Harbone (1988).

### Statistical Analysis

The data obtained were statistically analyzed using Analysis of Variance (ANOVA)mean separation and tests of significance were carried out by Duncan Multiple Range Test (DMRT) at P<0.05 (Steel and Torie, 1980).

## RESULTS AND DISCUSSION

Table 1 shows the proximate composition of *P. pulmonarius* as affected by different log substrates. The results of the moisture, ash, fat, fibre, protein, carbohydrate, dry matter and free nitrogen contents of *P. pulmonarius* fruit bodies cultivated on the *D.edulis*, *M. indica* and *T. africana* are significantly different P≤0.05. This shows that the mushroom is highly nutritious when grown on these logs. This also indicates the major reason why oyster mushrooms grow naturally on already degrading logs in the wild and sometimes, around homes (Oei, 2003; Chang, 2013). The relative high percentage of dry matter, carbohydrate and protein in the mushroom frut bodies cultivated on the log substrates conforms to the work of Marlow (2001) and Ukoima *et al.* (2009c). The high protein contents of the *P. pulmonarius*fruit bodies cultivated on the various logs confirms the assertion by several workers that mushroom protein is intermediate between that of animals and vegetables, but superior to most other foods, including milk and contains all the nine essential amino acids required by man (Parkayastha and Nayak, 2002; Chang and Miles 2004; Kurtzman, 2009). Low fat content of the mushroom shows that the mushroom could be good for people with cardiac problems This is in line with the reports of Okhuoya and Okigbo (1991), Okwulehie and Odunze (2004a), who maintained that mushrooms generally contain low-oil and fat, and because of the low content of oil and fat in mushrooms, they are recommended as good supplements for patients with cardiac problems.Table 2 represents the results of minerals compositions of *P. pulmonarius* grown on various logs. The results showed that the mushroom samples were significantly P≤0.05 rich in Sodium, Potassium, Magnesium, Calcium and Phosphorus. Potassium and Phosphorus contents were higher than other minerals analyzed and also higher in mushrooms harvested from *D. edulis* logs. In this study, Sodium was found to be the lowest among other minerals analyzed in the mushroom across all log substrates.The low Sodium content in mushrooms makes them ideal for persons with certain types of heart and kidney ailments (Quimio, 2004). The rich minerals contents in *P. pulmonarius* fruit bodies grown on the logs as observed in this study could be because the mushroom effectively utilized the high amount of nutrients present in the sapwood as reported by Hyunjong and Seung (2004).

These mineral values are higher than those reported by Adejumo andAwosanya (2004); Ogbo and Okhuoya (2006); Okwulehie *et al* (2007); Okoi and Iboh(2015). *D. edulis*gave the highest constituents of all the mineral nutrients analyzed while*M. indica*gave the lowest. The observed appreciable quantities of various mineral elements analyzed in the three mushroom samples indicates that these logs contain the corresponding nutrients in a relative amount since the nutritional composition of mushrooms depends on the substrate where they were grown. (Chang, 2013). Table 3represent the result of bioactive constituents of P. *pulmonarius* as affected by different log substrates are shown inTable3. Results show that

Alkaloids, flavonoides phenols, tannins and saponins were significantly different P≤0.05 at different quantities. Alkaloids were found in higher quantity than other bioactive compounds analyzed. Alkaloids have powerful effect in animal physiology and are important in pharmaceutical industries for drug manufacturing (Edeoga and Erieta, 2001). Edeoga and Erieta also (2001) also recorded that alkaloids are stimulants and acts by prolonging the action of several hormones. Flavonoids, phenols, tannins and saponinsconcentrations in *P. pulmonarius* fruit bodies cultivated on the different trees logs were higher than those reported by Okwulehie*et al.* (2007). Flavonoids act as ant-carcinogens, anti-bactarials (Hilang and Feraro,1992); saponins are implicated in the prevention of parasitic fungal diseases (Edeoga and Erieta, 2001) while tannins have been used as anti- tumor agents and perform a wide range of anti-infective actions (Haslam, 1996). The high concentrations of these important bioactive compounds in the fruit bodies of *P. pulmonarius* with respect to their various log substrates indicate that the trees may also contain the compounds in high amount. This also shows that these mushroom samples may be considered useful in the production of certain pharmaceutical chemicals (Okwulehie*et al*., 2007). The high concentrations of these compounds may also contribute to their taste, aroma and flavor, thereby increasing their nutritional, medicinal and food value. *Pleurotus pulmonarius* fruit bodies were successfully cultivated on the logs of *D. edulis*, *M. indica* and *T. africana.*Nutritional and bioactive compounds analysis of fruit bodies from different log substrates showed that they were rich in nutrients and could be of high pharmaceutical importance.Therefore, efforts should be made to determine the composition of other nutrients such as vitamins and amino acids of *P. pulmonarius* with respect to the same log substrates. Commercialization of log technique of mushroom cultivation should also be encouraged since log does not easily get spent and can be repeatedly used for a long period of time. Wherever log cultivation ofoyster mushroom is practiced, afforestation should be encouraged to avoid indiscriminate logging, which can lead to desertification.

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