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Nutritional and Bioactive Compounds Evaluation of *Pleurotus pulmonarius* (Freis) Quell Fruit bodies Grown on Different Wood Logs in Abia State, Nigeria

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Abstract

This study was conducted to determine the nutritional and bioactive compounds composition of *Pleurotus pulmonarius* fruit bodies cultivated on tree logs of *Dacryodes edulis, Mangifera indica* and *Treculia africana*. Pure mycelium culture of *P. pulmonarius* was aseptically bulked in sorghum grains. Logs were cut into average length of 18 cm with inoculation holes of 3 cm × 15 mm 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 24 hrs and pasteurized at 80°C in an improvised metallic drum (IMD) for 1hour; using cooking gas as heat source and allowed to cool overnight. 10 g 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, sun-dried ground and packed in airtight container prior to further analysis. Data were analyzed using Analysis of Variance (ANOVA) and mean separation by Duncan Multiple Range Test (DMRT) while levels of significance were determined at 5%. Results indicate that *P. pulmonarius* fruit bodies harvested from various tree logs were significantly different $p<0.05$ in their nutritional and bioactive compounds composition. Fruit body samples were rich in protein, carbohydrates, Na, K, and Ca. It was also observed that fruit bodies contained significant amount of Alkaloids, Tannins and Saponins; and could be useful in drug synthesis. Therefore, adopting this technique in oyster mushroom cultivation would lead to more jobs creation and food security; but this must be done with careful regulations to avoid indiscriminate felling of trees.

Keywords: *Pleurotus pulmonarius*; Logs; Nutrients; Bioactive; Fruit bodies

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 [1]. Oyster mushrooms can be grown on various substrates due to its strong enzymatic features. Different substrates are used in each region depending on their availability [2]. Wheat straw, sawdust and other agricultural by-products resulting after processing of waste paper, Hazelnut and Tilia have been used in Oyster mushroom cultivation; maize, corn, rice, elephant grass, sugarcane, coffee Gume have been examined as alternative substrates for its cultivation [3-6]. These substrate materials are usually by-products from industries, households, agriculture etc, and are usually considered as wastes [7]. However, these wastes are actually resources in the wrong place at a particular time and mushroom cultivation can harness them for its own benefit [8].

Kadiri and Aizai showed that *Lentinus subnudus* could be cultivated on wood logs of tropical trees [9]. According to Hyunjong and Seung [10], hard woods such as poplar, willow, beech, elm and alder are the most commonly used tree species in oyster mushroom cultivation. He noted that unlike shiitake, Oyster mushrooms do not grow well on Oak tree logs. Hyunjong and Seung 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 [8]. Mushroom has been used as a food and medicine by different civilizations since immemorial time, due to its delicious taste and dietetic qualities [11,12]. 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 [13] reported that the fresh mushroom contains about 85% to 90% moisture, 3% protein, 4% carbohydrates, 0.3% to 0.4% fats and 1% minerals and vitamins. *Pleurotus* species are good source of protein, vitamins and minerals [8,14]. 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 [15-17]. Mushrooms contain appreciable quantities of crude fibres although little information exists on the total dietary fibre (TDF) content of mushrooms. Okwulehie [18] 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 [19]. 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. For small-scale cultivation with limited budget, oyster mushroom is the clear choice for gaining entry into the mushroom industry [12].

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This work aims to determine 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 pruduced 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 15-20mins 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 [12]. Grains were later stuffed into 35cl Lucozade bottles tightly plugged with cotton wool and sterilized in an autoclave at 121°C for 30 mins. 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 (at 27 ± 2°C) for 10-15 days until the grains were fully colonized by mycelium [20].

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. Trees were cut into logs of 18 cm 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 [10].

Inoculation holes

Holes of depth 3 cm by 15 mm 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, according to Stamets [21, 22].

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 8-9 months in alternating rains and sun to allow for decomposition. Dry weight of logs (g/kg) was determined before they were soaked in water for 24 hrs. Logs were pasteurized at 80°C in an improvised metallic drum (IMD) for 1hr using cooking gas as a local heat source and allowed to cool overnight, as recommended by Canford and Nwoko [23].

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 24 hrs; when fully colonized polythene bags were cut open to allow for fruiting [10]. 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 and t cropping room of the mushroom house was flooded with water. Temperature was maintained at 27 ± 2°C [24,25]. Pinheads of *P. pulmonarus* were first noticed in *D. edulis* logs followed by *T. africana* and then *M. indica* logs after 9, 10 and 12 days of inoculation respectively. Mushrooms were harvested as soon as the fruit-bodies were fully matured [7].

Proximate analysis

Proximate Analysis was carried out on each of the 3 mushroom samples. Nutrients like carbohydrates, protein, fat ash, moisture and crude fiber contents were determined by using the methods outlined in the AOAC [26]. Protein determination was carried out using the Kjedahl method. Fat determination was carried out using a Soxhlet apparatus. Also, determination of fiber content was done according to the enzymatic gravimetric method [26].

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 of trioxonitrate (V) acid made up to 50 ml with deionized water and analyzed for Calcium (Ca) and Magnesium (Mg) using vanadate ethyldiamine-tetra acetic acid (EDTA) complexometric titration method according to MFA [27]. 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 [28].

Determination of percentage bioactive compounds

Percentage Alkaloids were determined by the methods of AOAC and Maxwell [29,30]. Percentage Flavonoids, Saponins and Tannins were also determined by the procedures according to Cloupai-Abyazini, Peng and Kobayashi [31], while percentage Phenols were estimated by the method of Harborn [32].

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 $p<0.05$ [33].

Results and Discussion

Results and discussion of the work on the nutritional and bioactive compounds evaluation of *P. pulmonarius* fruit bodies grown on different wood logs are presented below.

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 [8,24]. The relative high percentage of dry matter, carbohydrate and protein in the mushroom fruit bodies cultivated on the log substrates conforms to the work of Marlow and Ukaima [34]. The high protein contents of the *P. pulmonarius* fruit bodies cultivated on the various logs confirms the assertion by several workers that mushroom protein

Log Substrate	MC	ASH	Fat	Fibre	Protein	CHO	DM	N2
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 1: Effect of log substrates on proximate composition (%) of *P. pulmonarius* fruit bodies.

Log 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 2: Mineral constituents (mg/100 g) of *P. pulmonarius* fruit bodies as affected by different log substrates.

Log 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).					

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

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 [15-17]. 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 [35,36], 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 analysed and also higher in mushrooms harvested from *D. edulis* logs. In this study, Sodium was found to be the lowest among other minerals analysed 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 [37].

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 [10]. These mineral values are higher than those reported by Adejumo and Awosanya; Ogbo and Okhuoya, Okwulehie, Okoi and Iboh [38-40]. *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 [8].

Bioactive constituents of P. *pulmonarius* as affected by different log substrates are shown in Table 3. 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 [41]. Rambeli and Menini [42] reported that alkaloids are stimulants and acts by prolonging the action of several hormones. Flavonoids, phenols, tannins and saponins concentrations in *P. pulmonarius* fruit bodies cultivated on the different trees logs were higher than those reported by Okwulehie [38]. Flavonoids act as ant-carcinogens, anti-bactarials [43]; saponins are implicated in the prevention of parasitic fungal diseases while tannins have been used as anti-tumor agents and perform a wide range of anti-infective actions [44,45]. 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 [39]. The high concentrations of these compounds may also contribute to their taste, aroma and flavor, thereby increasing their nutritional, medicinal and food value.

Conclusion and Recommendations

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 of oyster mushroom is practiced, afforestation should be encouraged to avoid indiscriminate logging, which can lead to desertification.

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