



Original Article

Comparative Study of the Phytochemicals in *Pleurotus Ostreatus* Cultivated on Different Composting Substrates of *Pycnanthus Angolensis*

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ABSTRACT

There are diverse beneficial health effect of mushrooms to humans, such as food, drugs and medicines, and it is cultivated worldwide on almost all agricultural and agro industrial residues. This work examined the quantities of phytochemicals present in *Pleurotus ostreatus* that was cultivated on compost and vermicompost prepared from wood dust substrates of *P. angolensis* with organic waste (cow dung, goat dung, sheep dung and poultry droppings) mixed at ratio 9:1 using stock pile method for a period of 70 days. Samples of harvested mushroom were analysed quantitatively using the standard method described by Nwosu (2011) for phytates, saponin, tannin, oxalates and alkaloids. Comparing the amount of phytochemicals in compost and vermicompost, the result showed that alkaloids have the highest amount of 0.27% in mushroom cultivated on composting substrate of the wood dust with cow dung compared to the least of 0.02% of tannins in mushroom cultivated on all the substrate mix. There is no significant difference in the quantities of these chemicals in *P. ostreatus* cultivated on both compost and vermicompost substrates at $P < 0.05$. Composting and vermicomposting of cultivating substrates reduced the quantities of all the phytochemicals in mushroom and are safe for dietary intake.

Key words: Compost, Cultivation, Mushroom, Phytochemicals, Substrates.

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INTRODUCTION

A large quantity of agricultural waste and lignocellulosic residues are produced through the activities of various industries. In Nigeria, these are either disposed off by burning, or dumped in sites where they can pose hazard to the environment and human health. Cultivation of saprophytic edible mushroom may be the only currently economical biotechnology for lignocellulose organic waste recycling that

combines the production of protein rich food with the reduction of environmental pollution (Obodai and Apetogbor, 2003). These can otherwise be used in the cultivation of edible and medicinal mushrooms.

Oyster mushroom (*Pleurotus species*) belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide following the *Agaricus*

bisporus (Sanchez, 2010). Most often, their mycelia are buried in the soil around the root of trees beneath leaf litters in the tissue of a tree trunk, on a fallen log of wood or in other nourishing substrates. Many genera of mushrooms are edible and rich in essential nutrients such as carbohydrates, proteins, vitamins, mineral, fat, fibres and various amino acids (Okwulehie and Odunze, 2004).

Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity (Fakoya and Akinyele, 2008). It has been established that they grow and fruit on various agricultural wastes (Moncaio *et al.*, 2005). One of the values of commercial cultivation of mushrooms is cost effectiveness, especially in a developing economy like Nigeria where nutritive foods are scarce and when available, are usually very costly. Labour is also not expensive. *Pleurotus* species can efficiently degrade agricultural wastes and grow at a wide range of temperatures (Sanchez, 2010). *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources. Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates (Badu *et al.*, 2011; Patil *et al.*, 2010). So mushrooms are known to contain other chemicals other than food nutrients. The level of different phytochemicals in cultivated mushrooms on such substrates must be determined so as to ascertain their safety for consumption.

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens. The name comes from the Greek word phyton, meaning plant. Some *phytochemicals* have been used as poisons and others as traditional medicine. As a term, *phytochemicals* are thought to be largely responsible for the protective health benefits of these plant-based foods and beverages, beyond those conferred by their vitamin and mineral contents. Antioxidants are substances that help fight the harmful effects of unstable molecules in your body called free radical. In some cases, phytochemicals produce antioxidant effects. Before a grower of mushroom could assume that any mushroom cultivated is edible, such should be properly identified and tested for quantitative and qualitative content of chemicals.

The aim of this research was to analyse the cultivated edible mushroom *Pleurotus ostreatus* on different substrates of *Pycnanthus angolensis* wood dusts for their phytochemical contents in order to note and compare their various concentrations and suitability for intake.

MATERIALS AND METHODS

Compost Preparation

The composting was prepared by passive pile method (Keith *et al.*, 2009). One kilogram of compost substrates was prepared by mixing wood waste of *P. angolensis* and each of the animal wastes; cow dung, goat dung, sheep dung and poultry droppings in the ratio of, 9:1 respectively and the control sample as 100% wood dust. The various samples were composted in a plastic bowl of 30cm depth for 70 days. The compost was kept at moisture of 65% and ambient room temperature and turned every 4 days.

Vermicomposting

Composting heaps of the various substrates were made inside plastic bowl for 10 days, after which earthworms, *Eudrillus eugiene* were introduced at the rate of 10 worms/kg of feed mix (substrate) for vermicompost preparation. It was kept wet to a moisture level of 70% for 60 days (Henamgee, 2003). The vermicompost formed completely gave the odour of moist soil.

Strain of *Pleurotus ostreatus* strain was obtained from Federal Institute of Industrial Research Oshodi (FIRO) Lagos, Nigeria. The cultures were preserved on 2 % malt extract agar slants at 4°C. Spawns were then made from this starter culture. These served as inoculum to inoculate larger containers (like bottles) with mother spawn, which were used to inoculate the various substrates of compost and vermicompost. Harvested mushroom were tested for phytochemicals.

Determination of phytochemicals of the harvested mushrooms

(i) Phytate/phytic acid determination

The method described by Nwosu (2011) was used. The phytic acid in the samples was precipitated with excess FeCl_3 after extraction of 1g of each sample with 100ml 0.5N HCl. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture containing equal portions (1ml) of conc. H_2SO_4 and 65% HClO_4 . The liberated phosphorus was measured colorimetrically

(Jenway 6051 Colorimeter) at 520nm after colour development with molybdate solution.

The percentage phytate was thus calculated:

$$\% \text{ Phytate} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where W = weight of sample used

au = absorbance of test sample

as = absorbance of standard phytate solution

C = Concentration of standard phytate solution

Vt = Total volume of extract

Va = Volume of extract analysed. The % phytic acid was calculated using the formula:

(ii) Saponins

This was done by the double solvent extraction gravimetric method (A.O.A.C., 2005). Two grams (2g) of the processed sample was mixed with 100ml of 20% aqueous ethanol solution and incubated for 12hours at a temperature of 55°C with constant agitation. After that, the mixture was filtered through whatman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30min and the extracts weighed together.

The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml) of diethyl ether was added to it. After mixing well, there was partition and the upper layer was discarded while the lower aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with drop wise addition of NaOH solution. Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of n-butanol. The combined extract (ppt) was washed with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin was then dried in an oven (Gallenkamp Hot box Oven) at 60°C (to remove any residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

Where W = Weight of sample used

W₁ = Weight of empty evaporation dish

W₂ = Weight of dish + saponin extract

(iii) Tannin

Tannin content of the sample was determined by Folin Denis Colorimetric method (Kirk and Sawyer, 1998). A measured weight (1g) of the processed sample was mixed with distilled water in the ratio of 1:10 (W/V). The mixture was agitated for 30min at room temperature and filtered to obtain the extract. A

standard tannic acid solution was prepared 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flasks to serve as standard and reagent blank respectively. Then 2ml of each of the sample extracts were put in their respective labeled flasks. The content of each flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5ml of saturated Na₂CO₃ solution. Thereafter each flask was diluted to the 50ml mark with distilled water and incubated for 90min at room temperature. Their absorbance was measured at 710nm in a colorimeter (Jenway 6051) with the reagent blank at zero. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va} \times D$$

(iv) Oxalate estimation/determination

This was carried out by the procedures described by Nwosu (2011). One gram (1g) of the sample was weighed into a 100ml beaker, 20ml of 0.30N HCl was added and warmed to (40-50°C) using magnetic hot plate and stirred for one hour. It was extracted three times with 20ml flask. The combined extract was diluted to 100ml mark of the volumetric flask. The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0ml of 5N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable know the alkaline regions. It was also made acid to phenolphthalein (3 drops of this indicator added, excess acid decolorizes solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% CaCl₂ was then added and the mixture allowed to stand for 3h after which it was then centrifuged at 300 rpm for 15min. The supernatants were discarded. 2ml of 3N H₂SO₄ was added to each tube and the precipitate dissolved by warming in a water bath (70 -80 °C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.01N KMnO₄ at room temperature until the first pink colour appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was then warmed to 70- 80 °C and titrated until a permanent pink colour that persisted for at least 30sec was attained. The percentage (%) oxalate content was thus calculated:

$$\% \text{ Oxalate} = \frac{100}{W} \times 0.00225 \times \text{Total titre}$$

Where W = Weight of sample used

(v) Alkaloids procedure

The alkaline precipitation gravimetric method (Inuwa *et al.*, 2011) was used. A measured weight (1g) of the sample was dispersed in 30ml of 10% acetic acid in ethanol solution. The mixture was shaken well and allowed to stand for 4h at room temperature. The mixture was shaken periodically at 30min interval. At the end of this period, the mixture was filtered through whatman No.42 grade of filter paper. The filtrate (extract) was concentrated by evaporation, to a quarter of its original volume. The extract was treated with dropwise addition of concentration NH₃ solution to precipitate the alkaloid. The dilution was done until the NH₃ was in excess. The alkaloid precipitate was removed by filtration using weighed whatman No.42 filter paper. After washing with 1% NH₄OH solution, the precipitate in the filter paper was dried at 60°C in an oven (Gallenkamp hot box oven) and weighed after cooling in a desiccator. The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where W1

= Weight of empty filter paper

W2 = Weight of filter paper + alkaloid precipitate

The phytochemical screening carried out on harvested *P. ostreatus* cultivated on the different substrates revealed the presence of biologically active compounds or substances. Tannins, phytates, saponins, oxalates and alkaloid were all present at varying quantities in mushroom cultivated on various composting and vermicomposting substrates. The highest amount of alkaloid of 0.27%, and maximum phytate content (0.08%) were found in mushroom cultivated on composting wood dust of *P. angolensis* and cow dung. Mushroom cultivated on vermicomposting wood dust of *P. angolensis* and Poultry droppings had the least phytate content of 0.06%. Mushroom cultivated on composting wood dust of *P. angolensis* and Poultry droppings contain 0.23% of alkaloid, while the same amount was observed in mushroom cultivated on vermicomposting wood dust of *P. angolensis* with sheep dung. The quantities of alkaloid were followed by saponin in all substrates. Composting reduced the quantity of phytochemicals. Some bioactive chemical compounds (such as saponins and tannins) are known to have therapeutic effects against microbes and parasites (Dei *et al.*, 2007). The amount of alkaloid varies significantly with different substrates used for cultivation. The highest alkaloid of 0.27% was observed in mushroom cultivated on substrates of wood and cow dung, compared to the least of 0.24% in those cultivated on the wood dust and sheep dung, and poultry droppings.

RESULTS AND DISCUSSION

Phytochemical screening of *Pleurotus ostreatus* on composting of wood dusts with different animal wastes

		Tannin %	Saponin %	Phytate %	Oxalate %	Alkaloid %
<i>P. angolensis</i> and Cow dung	Compost	0.02±0.01	0.14±0.03	0.08±0.00	0.03±0.05	0.27±0.02
	Vermicompost	0.02±0.01	0.13±0.02	0.08±0.03	0.03±0.03	0.26±0.02
<i>P. angolensis</i> and Goat dung	Compost	0.02±0.03	0.15±0.02	0.07±0.01	0.02±0.02	0.24±0.02
	Vermicompost	0.02±0.02	0.14±0.03	0.07±0.03	0.02±0.03	0.24±0.02
<i>P. angolensis</i> and Sheep dung	Compost	0.02±0.01	0.14±0.01	0.07±0.01	0.02±0.00	0.24±0.01
	Vermicompost	0.02±0.02	0.14±0.02	0.07±0.01	0.02±0.01	0.23±0.02
<i>P. angolensis</i> and Poultry droppings	Compost	0.02±0.01	0.14±0.01	0.07±0.01	0.02±0.01	0.23±0.01
	Vermicompost	0.02±0.02	0.13±0.02	0.06±0.02	0.02±0.02	0.24±0.02
<i>P. angolensis</i> not composted		0.02±0.02	0.15±0.01	0.08±0.02	0.03±0.02	0.29±0.02

Values are mean of triplicate measurements ± Standard Error of Mean (SEM) P<0.05

Though, there is no significant difference in quantities of phytates, tannins, saponin and

oxalates. It is known that saponin inhibits sodium ion efflux by blockage of the influx of

concentration in the cells, activating a sodium ion – calcium ion antiporter in cardiac muscles. The increase in calcium ion influx through this antiporter strengthens the contraction of cardiac muscles (Egwin *et al.*, 2011).

Composting and vermicomposting reduced the amount of phytochemical substance in mushroom. The maximum phytate content (0.08%) was lower than the reported for locust beans (0.10%) considered to be safe, although saponins have been shown to be highly toxic under experimental conditions, acute poisoning is relatively rare, both in man and animals. Tannin, content in all harvested mushroom was below the value observed in everyday legumes, these are considered to be safe and acceptable. The values for phytochemicals were generally low in all the samples studied, though it was lesser in mushroom cultivated on vermicomposted substrates. The result was similar to those observed in Ijeoma *et al.* (2015) in the work with three edible mushrooms. The phytate values ranged from 0.09 to 0.25mg/100g, Hydrocyanides (0.04 – 1.00mg/100g) and tannin (0.11 – 1.02mg/100g). This showed that the toxic effect of these substances may not be experienced by the consumer since there is further destruction of these substances during cooking. Phytate level of *Pleurotus sajor cajor* was 11.535 mg/g, this can be related to the study of Ndolovu and Afolayan in 2008 who consider the nutrient level and phytate contents of leaves, fruits and stem of *Corchorus olitorius*. It was discovered that the phytate level in *Corchorus olitorius* was 11.71 mg/g which could be compared with this present study where the highest of 8.0mg/g was observed.

Higher values of tannins (18.27%±0.30), phytates (2.43%±0.09), trypsin inhibitors (2.39%±0.11), saponins (1.26%±0.06) and oxalates (0.57%±0.06) were observed by Ogbe and Obeka, 2011 on *Ganoderma lucidium*. The low oxalate content of *Pleurotus ostreatus* on all the composted and vermicomposted substrates implied that it is less toxic and can be regarded as good food supplement. The results obtained in this study showed no significant difference in the phytate compositions of *P.ostreatus* cultivated on compost and vermicompost 0.07% on all substrates. These results are 100 times lower than the standard safe limit (22.10mg/100g).

This present study observed that tannin content of the mushroom species considered in this study are significantly low. This is lower

than the 15.15 of melon husk as reported by Ogbe and George (2012). Tannin content can be related to that of *Trametes vesicolor* which was 0.116 mg/g and of *Chrysophyllum africanum* 0.29 mg/100g studied by Christopher and Dosunmu (2011) who studied the chemical evaluation of proximate composition, ascorbic acid and anti-nutrient content of African star apple (*Chrysophyllum africanum*) fruit. Some of these phytochemicals (phytate, oxalate and tannins) can be reduced by proper processing of food (Akinyeye *et al.*, 2011).

CONCLUSION

The compositions observed in this study have shown the presence of some vital phytochemicals. The results showed that these edible mushroom varieties could be safe for consumption as their various phytochemical concentrations were found to be significantly lower than their World Health Organizations reported safe limits. The observed levels suggest that these mushrooms would be a good source of some natural antibiotics and antioxidants. Therefore, *Pleurotus ostreatus* harvested from all the composting and vermicomposting preparations of *Pycnanthus angolensis* are safe food for consumption.

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