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AGRICULTURE | ORIGINAL ARTICLE

Effect of Different Substrates on Phenotype, Nutritional, Phytochemical Composition and Antioxidant Activity of *Pleurotus pulmonarius*

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ABSTRACT

Mushrooms have been an important food to man for millennia. Several species of *Pleurotus* have been cultivated successfully on different substrates including palm bunch, sawdust and corn cob. *Pleurotus pulmonarius* has also been cultivated but there is a gap in knowledge as to this effect. The sole aim of this study is to bridge the gap in knowledge as to the effect of different substrates on the phenotypic, nutritional, phytochemical and antioxidant activity of *P. pulmonarius*. Sawdust, palm bunches, corn cobs *and P. pulmonarius* were obtained from NIHORT in Imo and Ibadan respectively. Composting, spawn inoculation and incubation were carried out to culture the mushroom. Data analysis was done with GraphPad Prism 5. Proximate, phytochemical and antioxidant activity of the mushroom was conducted at IITA, Ibadan. Palm bunch produced the greatest number of fruiting bodies (36.00±15.50), making it the substrate yielding fruit with the highest fruit weight (216.00±62.92). Protein and moisture content was higher on palm bunch (25.61±0.11, 4.09±0.01 respectively), while carbohydrate was higher on sawdust (62.85±0.09). Mineral composition varied with the highest value observed for potassium on sawdust and Palm bunch (3.93±0.01, 3.90±0.03), there is therefore no significant difference at ($p \leq 0.05$). *P. pulmonarius* has low phytochemical composition, but its free radical scavenging ability against DPPH free radicals was high (73.57±0.27, on palm bunch and 28.22±0.39 on sawdust). ABTS* scavenging ability was the highest in palm bunch (38.45±0.25). These varying differences in the composition of *Pleurotus pulmonarius* grown on palm bunch, sawdust and corn cob ratify the hypothesis that substrates influenced its growth and composition

1. Introduction

Mushroom is a fungus with a fruiting body that bears spores; it is usually fleshy when freshly harvested. Mushroom is sometimes referred to as the Mother of all vegetables because it has been consumed as food since time immemorial (Ponmurugan *et al.*, 2007). Mushrooms usually grow in the wild, especially on decaying materials such as wood, food materials and plants. These materials where mushroom grow are called substrates (Waktola & Temesgen, 2018). Mushroom has been cultivated in many parts of the world including Africa and the species of mushroom mostly cultivated in Africa include; *Agaricus*sp, *Lenticus* sp and *Pleurotus* sp.

The first recorded cultivation of mushroom was in China around 600 A. D. with *Auricularia auricula*, the Wood Ear (Kues and Liu, 2000). This birthed mushroom cultivation and it has expanded amazingly over the past few decades (Kues and Liu, 2000). *Pleurotus* sp (Oyster mushroom) belong to the class Basidiomycetes and family Pleutaceae and this specie of mushroom is known to grow naturally in temperate and tropic regions(Sánchez, 2010). Apart from mushrooms being known to grow abundantly in the wild, they are capable of converting agro-waste into protein rich palatable food through enzymatic reactions(Waquas *et al.*, 2011). The cultivation of Oyster mushroom is not only commercially purposed but also as a therapeutic agent (Waquas *et al.*, 2011).

Cite This Article

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Mushroom serves as food and is known to contain sufficient amount of protein, carbohydrates, fat, minerals and vitamins(Kinge *et al.*, 2016). Research conducted by Iwalokun *et al*., (2007) showed that not only does mushroom (*Pleurotus ostreatus*) contain important diet components but also organic acids such as ascorbate and trace elements such as selenium. Mushroom are repositories for important phytochemicals such as phenol, saponin, alkaloid and flavonoid. Not only that these mushrooms have bioactive components, they have also shown important antioxidant activities(Ivanova *et al.*, 2014). *In vivo* and *in vitro* studies on edible mushroom have shown antioxidant, antitumor, antihypertensive and antiaging potentials (Iwalokun *et al.*, 2007). These bioactive compounds in mushroom mediate biological activities including the stimulation of interleukin-12, nitric oxide synthase activation, free radical scavenging and iron chelating properties (Kinge *et al.*, 2016). Mushrooms are increasingly being recognized as important food products due to their significant role in human health, nutrition and disease (Okhuoya *et al*., 2010). *Pleurotus pulmonarius* is a specie of mushroom recently brought into Nigeria. This specie of mushroom has been successfully cultivated and consumed in Nigeria with neither any idea of its nutritional composition nor its bioactive components or its therapeutic properties. This study aims to bridge this gap in knowledge.

From studies conducted on other species of mushroom, the substrates known to grow mushroom include Corn cob, Saw dust and even cotton waste amended with Banana leaves. It was shown that these substrates are capable of growing species of mushroom such as *Pleurotus ostreatus* and *Pleurotus florida*(Gerben *et al.*, 2000). Research conducted by Okhuoya et al., (2010) showed that *Auricularia polytricha* could grow on Palm oil wastes and even palm fronds. Since the cultivation of *Pleurotus pulmonarius*, there is currently no work showing the best substrate for its growth and yield. Kinge *et al*., (2016) argued that Oyster mushroom (*Pleurotus florida* and *Pleurotus ostreatus*) cultivated on Saw dust possesses better growth and nutritional properties than those cultivated on Corn cobs. Our research will show whether this new specie of mushroom can grow on some selected substrates including Corn cob, Palm bunch and Saw dust. The research will also show the substrate with the best fruit yield. Kinge *et al*., (2016) also argued in their research that substrate had an effect on the nutritional and bioactive components of mushroom. The limitation of mushroom research is that in phytochemical analysis, the presence or absence of these bioactive components are only indicated. There was no information as to the amount at which they were present. This work is structured to tackle this limitation as the quantitative presence of bioactive components was determined. Mushroom science has long been left unhandled as there is still a long gap of knowledge which needs to be covered by researchers. The research as to the recommendation of mushroom possessing important components for human body system development needs to be advanced. This will ensure that mushroom science is not forgotten. The aim of this research is to determine the best substrate suitable for the growth and yield of *Pleurotus pulmonarius* and to determine the phytochemical, antioxidant and nutritional composition of *Pleurotus pulmonarius*.

2. Materials and Methods

2.1. Study area and sample collection

The various samples used for this research were collected from Okigwe town in Imo State, Nigeria. The samples collected include; Saw dust, Palm bunch, Corn cob, and spawn for inoculation. Sawdust of *Gmelina aborea* was collected from the timber shops at Okigwe town in Okigwe L.G.A, Imo State, Nigeria. The Palm bunches and Corn cobs were sourced at Umuowa-Ibu village and National Horticultural Research Institute (NIHORT), Mbato premises, both located in Okigwe L.G.A, Imo State. The spawn of *P. pulmonarius* was obtained from the mushroom unit, NIHORT, Ibadan.

2.2. Composting

The method of Adebola *et al.*, (2016b) was used with slight modifications. Saw dust was moistened sufficiently while the Corn cob was chopped into small pieces before moistened. 1Kg each of the substrate was weighed and packed tightly in polythene bags. The neck of the bag was made with heat resistant PVC tubes and covered with a cotton plug. The bags containing each substrate was then sterilized by tyndalization and then allowed to cool to room temperature.

2.3. Spawn inoculation and incubation

After cooling, 10% w/w ratio of the spawn to the substrate was introduced in the bags through the neck aseptically and incubated for 28 days. Then the bags were then transferred into a dark room. After primordial initiation, the substrates were then transferred to a cropping house(Kinge *et al.*, 2016; Adebola *et al.*, 2016b).

2.4. Data collection

The fruiting bodies were harvested manually and the data collected include: number of fruiting bodies, fruit weight (g), width of pileus (cm), length of stipe (cm), number of days for full mycelia colonization, primordial initiation days, mycelia extension days and biological efficiencies (%). This data was then analyzed by GraphPad Prism Statistical Package. Among the phenotypic data collected, the proximate, phytochemical and antioxidant activity of the harvested mushroom were determined.

2.5. Data analysis

The proximate, phytochemical and antioxidant activity of *P. pulmonarius* was conducted at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

2.6. Preparation of sample for phytochemical analysis

The mushroom will be air dried, grinded into powder using a blender with stainless steel, blade and body and processed for phytochemical analysis(Adebola *et al.*, 2016b).

2.7. Preparation of methanolic extract

Methanolic extracts of the mushroom from different substrates were separately prepared following the method of Ibe *et al.*, (2014), by adding 25 ml of methanol to 0.5 g of samples contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 hour at room temperature. The mixtures were centrifuged at 3,000 rpm for 10 minutes, and then the supernatant were collected and stored at -4ºC for further analysis.

2.8. Phytochemical analysis

2.8.1. Determination of total alkaloids

The total alkaloid content of the samples were measured using 1,10-phenanthroline method described by Iwalokun *et al.*, (2007) with slight modifications. About 100 mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 minutes. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1 ml plant extract, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1 ml of 0.05 M of 1, 10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2ºC. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with distilled water). The values were expressed as mg/g of dry weight.

2.8.2. Determination of total flavonoids

The method of determination of total flavonoids is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415nm was read after 40 minutes. Blank samples was then prepared from 100 ml of the mushroom extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol will be measured under the same conditions. All determinations will be carried out in triplicates(Herin *et al.*, 2013).

2.8.3. Determination of total saponins

Total saponin was determined by the method described by Herin *et al.*, (2013). About 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 minutes at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 minutes. Then tubes were cooled in ice for 4 minutes and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

2.8.4. Determination of total tannins

Tannin content of samples was determined according to the method of Iwalokun *et al.*, (2007) as follows. Sample (0.1 g) was extracted with 5 ml of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. About 0.1 ml of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate (Na_2CO_3) solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material.

2.8.5. Determination of total phenolic contents (TPC)

The total phenol content of samples of methanolic extracts was determined according to the Folin–Ciocalteu method reported by Herin *et al.*, (2013). Briefly, for each sample, 300 µl of extract was dispensed into test tube (in duplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na2CO3 solution (7.5% w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µl of distilled water instead of sample extract. TPC was expressed as Gallic acid equivalent (GAE) in mg/g material.

2.9. Antioxidant activity determination

2.9.1. Estimation of DPPH free-radical-scavenging ability

The free-radical-scavenging ability y of the methanolic extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Ibe *et al.*, (2014) with slight modification. Briefly, for one sample, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60 µM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 minutes before the absorbance was taken at 517 nm. The decrease in absorbance of DPPH· on addition of test samples in relation to the control was used to calculate the percentage inhibition (%Inh.) following the equation: %Inh. = [(A517control –A517sample) ÷ A517control] x 100. The IC50, which stands for the concentration of extract required for 50% scavenging activity, was calculated from the dose-inhibition linear regression curve of each extract.

2.9.2. Estimation of ABTS radical-scavenging ability*

The ABTS* radical-scavenging ability of extract was determined according to the method described by Ibe *et al.*, (2014). The ABTS* radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with K2S2O8 (2.45 mM) in the dark for 16 hours at room temperature and adjusting the absorbance at 734

nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. The calibration equation for TEAC was Y = -0.0505x + 0.1954 (R2 = 0.9902).

2.10. Proximate analysis

2.10.1. Determination of moisture content

The method of Okigbo *et al.*, (2015) was employed using hot air drying oven. Empty clean crucible dish was dried in an oven at a temperature of 105°C for one hour and cooled in desiccators. Two grams (2 g) of the samples was weighed and put in a dish and heated overnight (12 hours). The dish will then be removed from the oven, cooled in desiccators and weighed. The moisture content was calculated using the following formula:

$$\% \text{ moisture content} = \frac{Loss\ in\ Weight}{Sample\ Weight} \times 100$$

2.10.2. Determination of ash content

The method of Okigbo *et al.*, (2015) was used to determine the ash content. Two grams of each dried sample was weighed into a pre-heated and cooled crucibles and incinerated in a muffle furnace at 200°C for four hours. The ash was then be cooled in desiccators and weighed. Ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{Weight\ of\ Ash}{Weight\ of\ Sample} \times 100$$

2.10.3. Determination of crude fat content

Two grams each of dried samples was weighed into a porous thimble and its mouth covered with cotton wool. The thimble will then be placed in an extraction chamber and then suspended above a receiving flask containing petroleum ether (BP. 40 – 60°C). The flask was then heated on hot mantle and the oil was extracted. The extraction continued for eight hours after which the thimble was removed from the Soxhlet and apparatus were reassembled and heated over a water bath, the flask containing the oil was then disconnected, cleaned up and placed in an oven at 100°C for thirty minutes. The flask was cooled in desiccators and weighed. The percentage crude lipid content was calculated using the following formula (Okigbo *et al.*, 2015):

$$\% \text{ Crude Fat} = \frac{Weight\ of\ Oil\ Extracted}{Weight\ of\ Sample} \times 100$$

2.10.4. Determination of crude protein content

Two grams of the samples was weighed into Kjeldahl digestion flask and catalyst mixture of $NaSO_4$, $CuSO_4$ and selenium oxide in (10:5:1) will be added to each sample which was followed by 10 cm^3 of concentrated H_2SO_4. The content in the flask was then heated in the Kjeldahl digestion flask for one and half hours, this is to ensure that digestion was completed. The flask was cooled and the content diluted with 10 ml distilled water. The diluted content was then filtered in to 100 ml volumetric flask and made up to the mark with distilled water. Exactly 10 cm_3 of the exact sample was taken into digestion flask and 20$cm^3$of 45% NaOH solution will be added to it. The content was then diluted to about 200cm^3 with distilled water and distilled using micro Kjeldahl distillation apparatus. The distillate was received into a flask containing 10cm^3 of boric acid solution indicator after the distillation after which the distillate was titrated with 0.01M HCl to the end point.

Crude protein was calculated using the following formula:

$$\% \text{ Crude protein} = \frac{TV \times C \times F \times V1}{W \times V2} \times 100$$

Where: TV = Titre Value of the acid, C = Concentration of acid used, V1 = Volume of the distilled water used for diluting the digest, V2 = Volume of the exact sample used for titration, W = weight of sample used, F = protein multiplication factor 0.0014 (Ooi *et al.*, 2012).

2.10.5. Determination of carbohydrate content

The total amount of carbohydrate in the sample was obtained by using the weight difference percentage. This was done by subtracting the percentage sum of the food nutrients (% crude protein, % crude fat, and ash) from 100% dry weight. Percentage carbohydrate was calculated using the formula below:

Carbohydrate % = 100 – (Crude Protein + Crude Fat + Ash) (Ooi *et al.*, 2012).

2.10.6. Determination of mineral ions composition

Analysis of minerals was be done through the washing of the samples, and drying the powdered preparation for two hours at 200°C and further drying at 500°C in the furnace until a constant weight is obtained. The samples were digested using nitric acid and made up to 50 ml with deionized water and then filtered. Sodium and potassium was determined using flame photometer model PFP7, clinical flame photometer Biochrom UK, Jenway, England. Atomic Absorption Spectrophotometer model AA320N Brain Scientific and Instrument England was used to determine copper, cobalt and cadmium. Three replicates of each were made (Ooi *et al.*, 2012).

3. Results

The phenotypic characteristics for *Pleurotus pulmonarius* grown on each substrate are presented in Figure 1. There were significant differences most especially for Palm bunch and other substrates.

Figure 2 shows the proximate composition including crude protein, crude fat, moisture content, ash and carbohydrate. There were significant differences ($p \leq 0.05$) with Palm bunch showing the highest for protein and moisture content. *Pleurotus pulmonarius* grown on sawdust has the highest fat and ash content. Mineral analysis was also conducted for the mushroom harvested from Palm bunch and Saw dust; there are no significant

differences (p≤0.05) in their phosphorous, calcium, magnesium and potassium content, overall, saw dust possessed the highest mineral content.

In the mineral content analysis of *Pleurotus pulmonarius* grown on different substrates shown in Figure 3, no significant differences (ns) were observed in phosphorus, calcium, magnesium, and potassium contents between the palm bunch and sawdust substrates. However, significant differences were observed in sodium, manganese, iron, copper, and zinc contents, with p-values ranging from 0.05 to 0.0001, indicating higher concentrations in mushrooms grown on sawdust for these minerals.

Figure 4 presents the Phytochemical analysis of *Pleurotus pulmonarius* showing there are significant differences (p≤0.05) in the phenolic, tannin, saponin, flavonoid and alkaloid content.

Figure 5 shows the antioxidant activity of *Pleurotus pulmonarius* showing the Free-radical-scavenging ability of the extract against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was relatively higher in Saw dust compared to Palm bunch. ABTS scavenging ability and the reducing power of the extracts were higher in Palm bunch than in Saw dust.

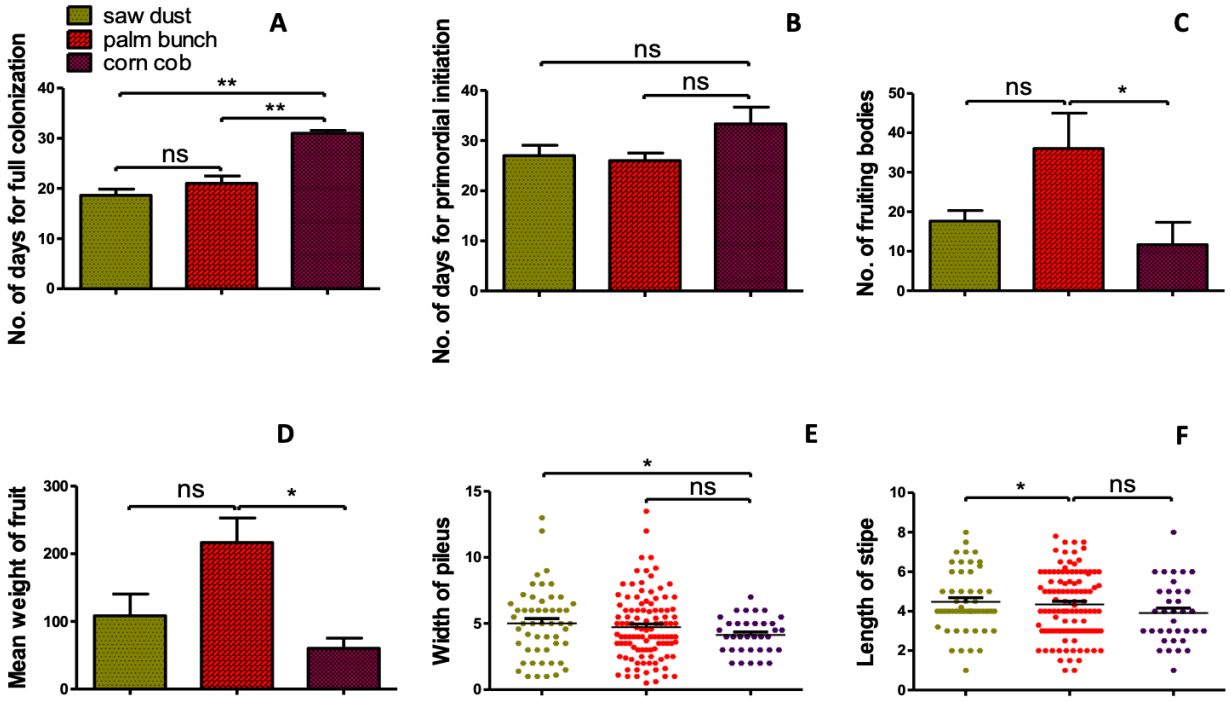

Figure 1. Phenotypic analysis of Pleurotus pulmonarius grown on different substrates. After days of incubation, (A) shows the number of days it took for the mycelia to colonize the substrate. (B) Shows the number of days for the first fruiting body called the pin to sprout, (C) shows the number of fruiting bodies grown on each substrate. (D) Shows the mean weight of fruit harvested from a particular substrate. (E) Shows the width of pileus of each fruiting body and (F) shows the length of stipe of each fruiting body. Data are from three independent experiments performed for each substrate. Error bar represents the standard error of the mean. The statistical method used for the analysis is the Paired Students' T test method at 95% level of significance (p≤0.05). No significant difference was represented by (ns) and (*) represents the range of p values obtained. (*) Represent 0.05, (**) represents 0.01. Data analysis and graphical presentation of data was done with GraphPad Prism 5.

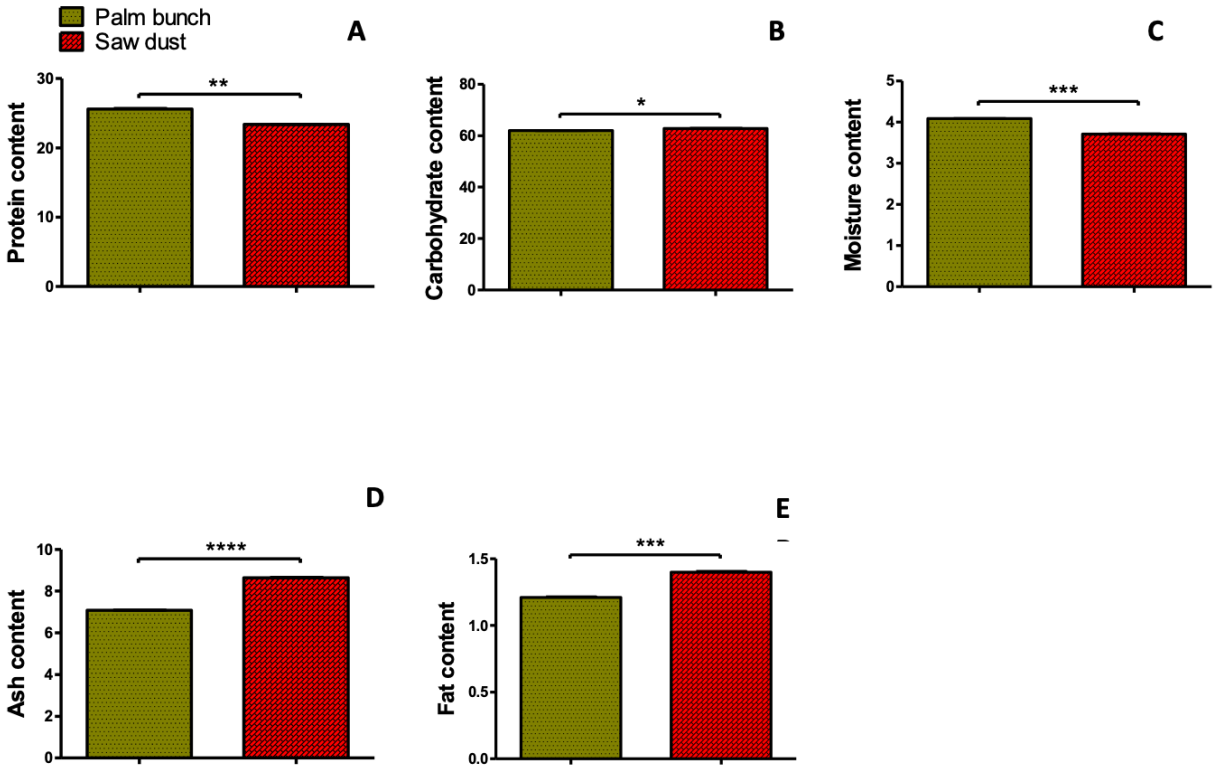

Figure 2. Proximate analysis of Pleurotus pulmonarius grown on different substrates. (A) Shows the amount of crude protein present in the harvested mushroom, (B) shows the crude carbohydrate content where (C) shows the moisture content of *Pleurotus pulnonarius*. (D) Shows the amount of ash and (E) represents the amount of fat present in the mushroom. Experiments for each substrate were performed in triplicates. Proximate content was measured in percentage (%). No significant difference was represented by (ns) and (*) represents p value of 0.05, (**) represents p value of 0.01, (***) represents p value of 0.001 and (****) represents p value of 0.0001. Statistical method used is the Paired Students' T test method at 95% level of significance.

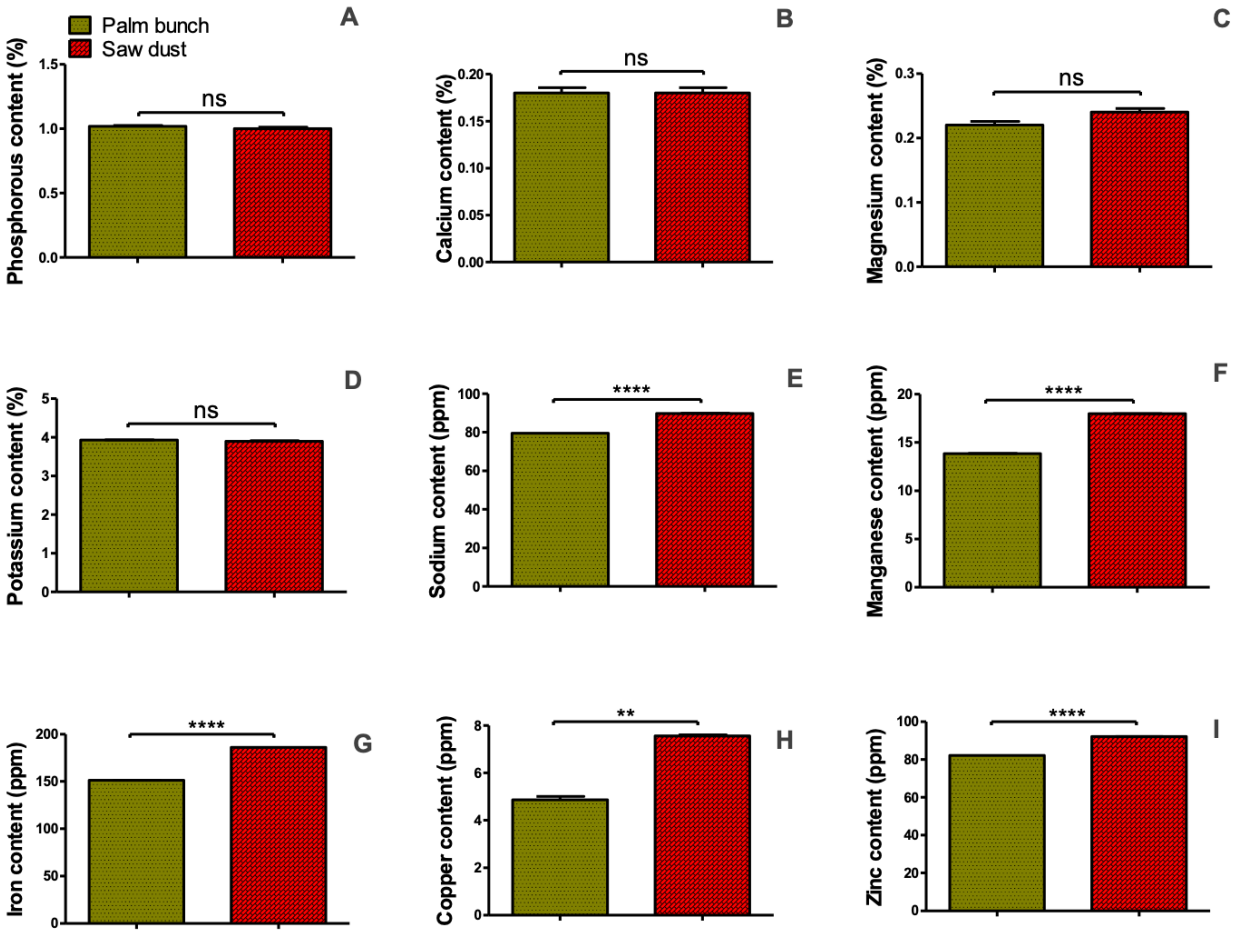

Figure 3. Mineral content of Pleurotus pulmonarius grown on different substrates. (A) shows the phosphorus content of *Pleurotus pulmonarius*, (B) shows the calcium content, (C) shows the magnesium content of the, (D) shows the potassium content, (E) shows the sodium content of *Pleurotus pulmonarius* measured in parts per million (PPM), (F) shows the manganese content also measured in PPM, (G) shows the iron content, (H) shows the copper content and (I) shows the zinc content of the mushroom. (G), (H) and (I) were also measured in PPM. Mineral analyses for each substrate were performed in triplicates. No significant difference was represented by (ns) and (*) represents p value of 0.05, (**) represents p value of 0.01, (***) represents p value of 0.001 and (****) represents p value of 0.0001. Statistical method used is the Paired Students' T test method at 95% level of significance.

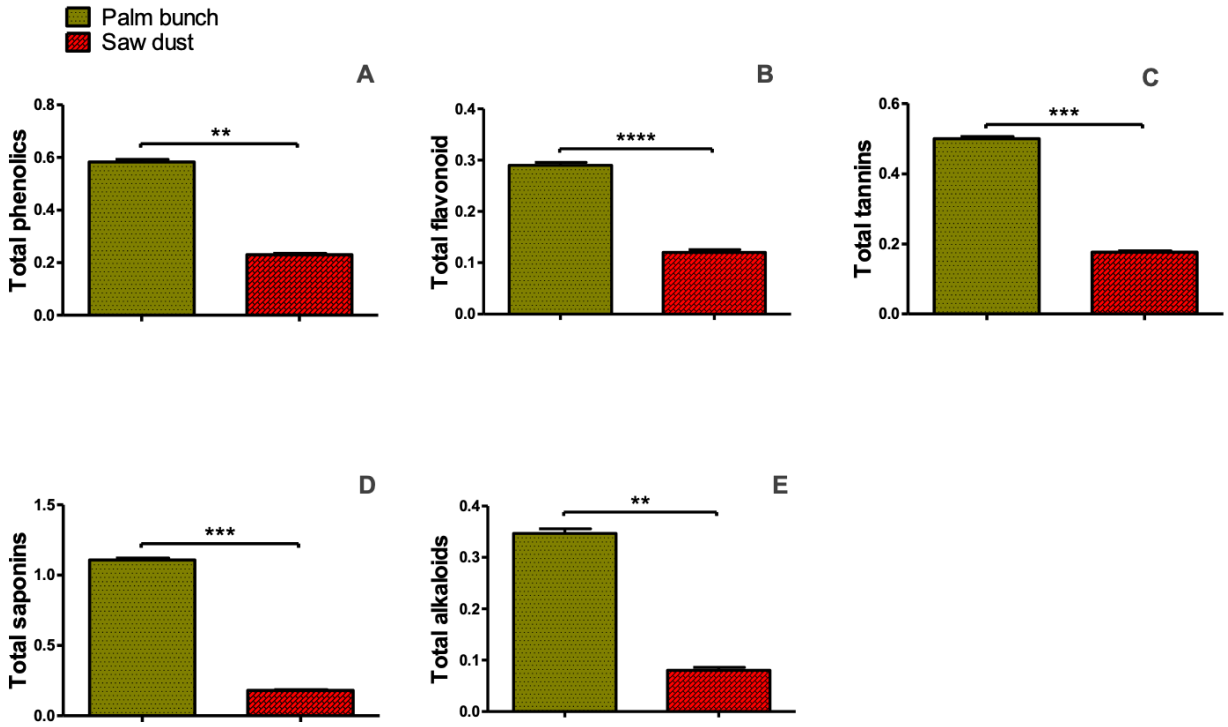

Figure 4. Phytochemical analysis of Pleurotus pulmonarius grown on different substrates. (A) Shows the total phenolics content, (B) shows the total flavonoid content, (C) shows the total tannin content (D) shows the total saponin content and (E) shows the total alkaloid content of *Pleurotus pulmonarius*. All phytochemicals analyzed were measured in mg/g. Experiments were done in triplicates. (*) represents p value of 0.05, (**) represents p value of 0.01, (***) represents p value of 0.001 and (****) represents p value of 0.0001. Statistical method used is the Paired Students' T test method at 95% level of significance.

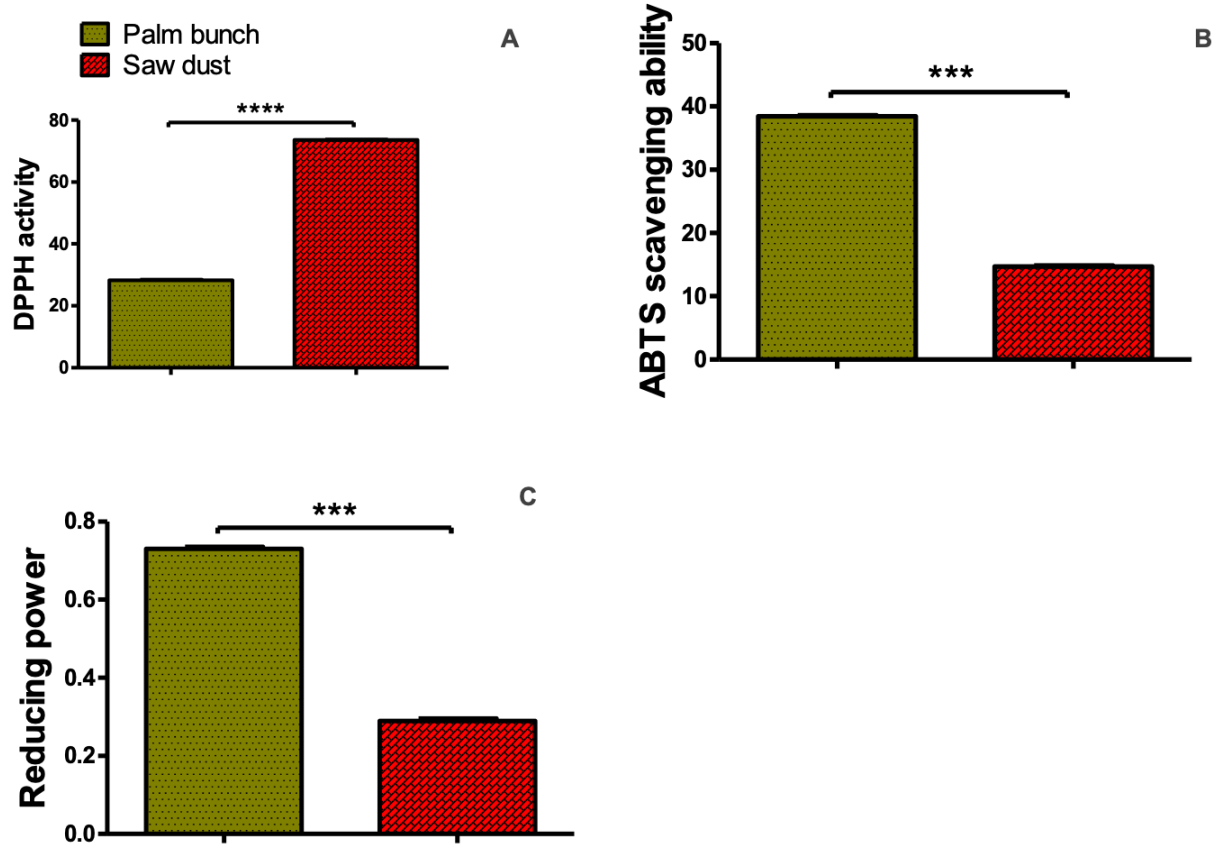

Figure 5. Antioxidant activity of Pleurotus pulmonarius grown on different substrates. (A) Shows the free-radical-scavenging ability of the extract of *Pleurotus pulmonarius* against 1, 1-diphenyl-2-picrylhydrazyl (DPPH* SC_{50}) measured in mg/ml, (B) shows the ABTS^{*+}radical-scavenging ability of extract measured in (mmol TEAC/g) and (C) shows the reducing power of extract of *Pleurotus pulmonarius* measured in mg GAE/g. TEAC stands for Trilox equivalent antioxidant activity, GAE stands for Gallic acid equivalent. Experiment to determine the antioxidant activities were done in triplicates. (*) represents p value of 0.05, (**) represents p value of 0.01, (***) represents p value of 0.001 and (****) represents p value of 0.0001. Statistical method used is the Paired Students' T test method at 95% level of significance.

4. Discussion

The results showed varying effects of different on the phenotypic characteristics of *Pleurotus pulmonarius*. The number of days for full colonization of the substrate explains the number of days it takes the mycelia of the mushroom to fully cover the substrate (Adebola *et al.*, 2016b). From the results shown, it was observed that the number of days for full colonization was shorter in Saw dust < Palm bunch < Corn cob (18.67±2.08, 21.00±2.65 and 31.00±1.00 respectively). From statistical analysis using the Student's T test, there was no significant difference ($p \leq 0.05$) for number of days for full colonization for Saw dust and Palm bunch. There was significant difference (at 95% level of significance) between the number of days it takes for full mycelia colonization for Palm bunch and Corn cob and also for Saw dust and Corn cob. Full mycelia colonization of mushroom on different substrate could be attributed to the composition of the substrate. Although, mushroom synthesizes the nutrients they need to grow, the particular nutrients required by mushroom include sugars, starch and lignin (Ahmad *et al.*, 2016). Saw dust contains 42% cellulose, 33% hemicelluloses and 35% lignin. Corn cob possesses 36% cellulose, 26% hemicelluloses and 17% lignin. Palm bunch has 44.2% cellulose, 33% hemicelluloses and 20.4% lignin (Nurul *et al.*, 2017). From the above listed results from various works showing the percentage composition of cellulose, hemicelluloses and lignin in Saw dust, Palm bunch and Corn cob, Saw dust possesses the highest amount of lignin which most mushroom utilize for growth. This therefore could be an explanation for the faster rate of full mycelia colonization seen in *Pleurotus pulmonarius* grown on saw dust.

After full mycelia colonization, pin heads start to develop; this stage is called the primordial stage. Though, it takes about 27 days for primordial initiation on Saw dust, 26 days on Palm bunch and 33 days on Corn cob. Statistically, there is no significant difference ($p \leq 0.05$). Palm bunch (36.00±15.52) yields more number of fruiting bodies than Saw dust (17.67±4.62) and Corn cob (11.67±9.87), using the student's T test, the number of fruiting bodies were significantly different at 95% level of significance for Palm bunch and Corn cob. All substrates produce statistically same fruit weight except for Palm bunch and Corn cob at 95% level of significance. Saw dust and palm bunch has no significant difference on the number of fruiting bodies of *Pleurotus pulmonarius*. This was also observed in the mean fruit weight. Saw dust and

corn cob showed significant difference (p≤0.05) in the width and length of pileus. It can therefore be inferred that though full colonization is faster on Saw dust. Palm bunch has a better yield as it possesses the highest number of fruiting bodies (36.00±15.52) and highest mean weight of fruit (216±62.92).

The proximate analysis of *Pleurotus pulmonarius* grown on different substrates were shown. Protein content in *Pleurotus pulmonarius* grown on Palm bunch is higher than that grown on Saw dust (25.61±0.11, 23.4±0.05 respectively) with statistics showing significant difference at p≤0.05. From the results obtained, it could be said that *Pleurotus pulmonarius* grown on Palm bunch and Corn cob are rich sources of proteins and compare favourably with meat, egg and milk (Adebola *et al.*, 2016b). Mushrooms are very useful for vegetarians because they contain some essential amino acids which are found in animal proteins (Wani *et al.*, 2015). Carbohydrate content and moisture content were also higher in *Pleurotus pulmonarius* grown on palm bunch at p≤0.05. Carbohydrate which is an important food constituent is present in sufficient amount in *Pleurotus pulmonarius*. *Pleurotus pulmonarius* should therefore be recommended by food organizations as an important food as it possesses high amount of carbohydrates and protein. Fat content was relatively low in both substrates.

The mineral content of *Pleurotus pulmonarius* includes phosphorous, calcium, magnesium, sodium, potassium, manganese, iron, copper and zinc. Potassium content is higher in percentage compared to other minerals (3.93% for *Pleurotus pulmonarius* grown on Palm bunch and 3.9% for *Pleurotus pulmonarius* grown on Saw dust). Calcium content was relatively low in mushroom harvested from both substrates (about 0.18% and there was no significant difference)

Phytochemical content was relatively low in *Pleurotus pulmonarius*. The amount of total saponin, alkaloid and other phytochemicals were significantly different (at 95% level of significance). The review of the biological activities of saponins showed that they exhibit antioxidant activities. Saponins also have haemolytic and hypolipidaemic activities as well as the ability to lower cancer risks and inhibit microscopic life forms. Pure alkaloids were also present, though no report of the antioxidant activity as yet and their derivatives have basic medicinal value due to the analgesic, antispasmodic and antibacterial properties they posses. *Pleurotus pulmonarius* could therefore be recommended as important food due to its high nutritional content but its medicinal value is relatively low.

DPPH radicals have been extensively used by many workers to investigate the scavenging activity of some natural components. This is due to its high sensitivity. This was employed to investigate the free-radical-scavenging ability of the mushroom. As antioxidants donate protons to this radical, the absorption decreases. The extent of the decrease in absorption is taken as a measure of the extent of radical scavenging (Ibe *et al.*, 2014). The SC_{50} value for *Pleurotus pulmonarius* grown on Saw dust caused the inhibition of DPPH absorbance (73.57±0.27). Since SC_{50} is a measure of inhibitory concentration, a lower SC_{50} is a reflection of greater antioxidant activity of the sample. *Pleurotus pulmonarius* grown on Saw dust has the highest inhibition potential (73.57±0.27 for Saw dust and 28.22±0.39 for Palm bunch). The amazing high antioxidant activity of *Pleurotus pulmonarius* grown on Saw dust could still be attributed to the crude composition of the substrate (Saw dust).

ABTS* scavenging ability which was reported as the trilox equivalent antioxidant activity (TEAC) is also shown. The results showed that there was significant difference (p≤0.05) in the order of palm bunch > saw dust (38.45±0.25, 14.71±0.24 respectively). Interestingly, DPPH was significantly higher in *Pleurotus pulmonarius* grown on Saw dust. This shows that substrate plays a major role in the composition of mushroom. It could therefore be inferred that *Pleurotus pulmonarius* grown on palm bunch exhibited a better ABTS* scavenging ability. The reason for varying antioxidant activity of *Pleurotus pulmonarius* grown on different substrates may be due to difference in methods even though *Pleurotus pulmonarius* grown on both substrates exhibited antioxidant capabilities.

ABTS assay is based on the inhibition of the absorbance of the radical cation. ABTS* which has a characteristic long wavelength adsorption spectrum. ABTS* radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involves H atom transfer; the reactions with ABTS* radicals involve electron transfer process (Boutaoui *et al*, 2018). The extensive use of DPPH for screening antioxidant activity assay may have been because it can accommodate many samples in a short period and it is sensitive enough to detect active ingredients at low concentration (Ezeonu & Ejikeme, 2016).

5. Conclusion and Future Recommendation

The result of this study concludes that there are strong and varying differences in the phenotypic, nutritional and bioactive components of *Pleurotus pulmonarius* grown on different substrates. Phenotypically, from the results obtained and shown in this study, *Pleurotus pulmonarius* grown on Palm bunch has better yield with regards to fruit weight and number of individual fruiting bodies obtainable. When cultivating *Pleurotus pulmonarius* for better yield, Palm bunch is the best substrate. This is controversial when looking for faster yield as *Pleurotus pulmonarius* grown on Saw dust require less number of days for full mycelia colonization. There is no significant difference (p≤0.05) in the primordial initiation stage for Saw dust and Palm bunch.

Pleurotus pulmonarius grown on Palm bunch has higher protein and moisture contents, while carbohydrate and fat content was higher in *Pleurotus pulmonarius* grown on Saw dust. It could be recommended that for the consumption of mushroom even though there is significant presence of proximate content, substrate influences the nutritional composition of *Pleurotus pulmonarius*.

There were also varying differences in their mineral content. Phosphorous and potassium were the highest minerals present. There were no significant differences in their presence when tested across different substrates. Phytochemical compositions were relatively low. Saponin content was the highest (1.11±0.02 for Palm bunch and 0.18±0.01 for Saw dust).

Interestingly, antioxidant activities of *Pleurotus pulmonarius* exhibited high DPPH free-radical-scavenging ability and TEAC. *Pleurotus pulmonarius* grown on Saw dust exhibited higher DPPH free radical scavenging ability while *Pleurotus pulmonarius* grown on Palm bunch exhibited higher ABTS* scavenging ability and reducing power in Saw dust. This proved that *Pleurotus pulmonarius* could be more effective in managing oxidative stress condition and its various complications.

The results of this study also suggest that for the commercialization and consumption of *Pleurotus pulmonarius*. Single substrate should not only be used as growth medium. *Pleurotus pulmonarius* grown on different substrate should be readily available in the market for consumers. This is because substrate plays a major role in the phenotypic, nutrition, phytochemical and antioxidant composition of *Pleurotus pulmonarius*.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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