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COMPARATIVE MORPHOLOGICAL, PROXIMATE, PHYTOCHEMICAL AND ANTI-OXIDANT STUDY OF OYSTER MUSHROOM CULTIVATED ON PAPER WASTE AND SAWDUST

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ABSTRACT

This study assessed the effects of sawdust (amended with wood shavings and rice bran) and paper waste on the growth, proximate composition, phytochemical content and anti-oxidant properties of *Pleurotus pulmonarius*. To reduce substrate acidity, 200 g of calcium carbonate was added, and after pasteurisation, the substrates were inoculated and incubated for an average of 24 days. *P. pulmonarius* grown on paper waste exhibited the fastest spawn running (18 days), pinhead formation (22 days) and fruiting body development (28 days). The largest pileus diameter (3.00 cm) and number of fruiting bodies (8) were observed on mushrooms grown on sawdust, while paper waste yielded the highest stipe length (2.73 cm) and fruiting body height (6.43 cm). Paper waste cultivation resulted in the highest sugar (0.30%), protein (29.77%) and ash content (50.58%), whereas sawdust resulted in the highest carbohydrate (52.18%) and moisture (69.70%). Sawdust also enhanced flavonoid, phenol, tannin and alkaloid levels. *P. pulmonarius* grown on paper waste showed superior anti-oxidant activity across all concentrations tested (50, 100, 150, 200 and 250 µg/mL). Both substrates influenced the proximate composition of the mushroom, with paper waste being optimal for growth and anti-oxidant activity, and sawdust better for phytochemical content.

Key words: *Pleurotus pulmonarius;* substrates; proximate composition; phytochemical constituent; anti-oxidant activity

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INTRODUCTION

Mushrooms are fungal fruiting structures that acquire their energy and growth ingredients from the biochemical degradation of their growth environment (Adebayo *et al*., 2014). They are low in energy and fat and are rich in vitamins, minerals, dietary fibre and protein (Nwachukwu *et al*., 2022). Furthermore, mushrooms have medicinal

values and promote good health as they are excellent sources of phytochemicals, proximate components and minerals (Okwulehie and Ogoke, 2013).

The genus *Pleurotus* comprises edible mushrooms belonging to class Basidiomycetes and order Agaricales. These mushrooms are widely distributed in both temperate and tropical regions (Chang and Miles, 2004). *Pleurotus pulmonarius*, also known as the Indian oyster, phenolic mushroom or lung oyster, is an edible white rot-fungus (WRF) (Jonathan *et al*., 2012). Mushrooms serve as a valuable source of vitamins, such as thiamine, riboflavin, niacin, biotin and ascorbic acid as well as various minerals (Isikhuemhen *et al*., 2009). Mushroom cultivation is considered an alternative way to reduce poverty in developing countries like Nigeria, as it involves low production costs with high profitability.

The conversion of agricultural wastes, such as sawdust and paper, into usable products can contribute to mitigating the environmental degradation they cause. Proper biotechnological processes in the environment can eliminate pollution and transform these wastes into beneficial byproducts (Milala *et al*., 2005). Many agricultural residues contain high concentrations of lignocellulosic compounds, which are difficult to handle and dispose of due to their chemical composition and properties during decomposition. In temperate and subtropical countries, agricultural economies are particularly focused on the use of cotton waste (including stem-leaf residues and gin trash), wheat straw, paper waste and sawdust from the timber industry. These materials are produced in large quantities, with post-harvest handling primarily involving burning or incorporation into the soil (Enibukun and Laba, 2019).

Research indicates that substrates used in mushroom production contain high levels of protein, lipids, carbohydrates and vitamins (Okwulehie *et al*., 2008). The nutritional composition and cultivation of *P. pulmonarius* are influenced by various substrates and environmental conditions, which affect the bioactive nutrients and vitamin contents of oyster mushrooms (Okwulehie *et al*., 2007; Oei, 2012). This study reports the morphology, proximate composition, phytochemical and anti-oxidant properties of *Pleurotus pulmonarius* cultivated on sawdust and paper waste.

MATERIALS AND METHODS

Preparation of Substrates

The substrates used were sawdust and paper waste. The sawdust was prepared by mixing with wood shavings and rice bran in ratio 3:2:1. Calcium carbonate ($CaCO_3$) weighing 200 g was added to the substrates in order to reduce the level of acidity. The substrates were then mixed thoroughly with water and heaped on a cemented floor to allow fermentation. This process, along with heat development, breaks down the chemical compounds into small components. Utmost care was taken to protect the substrates against rain or external moisture as it might introduce undesirable microbes. The mixed substrate was left on the cemented floor for 7 days and frequent turning and watering were done at an interval of 2 days so as to avoid drying up of substrates before bagging.

Bagging

Materials used for bagging of the compost include heat-resistant planting bags, medium pipe of about 2 inches, cotton wool, rubber band and pieces of paper. Compost was poured in the plastic bag about half-filled, while the plastic bag was folded to form

a rectangular shape at the base. The mouth of the planting bag was pushed through the pipe and then pulled out in order to tighten the bag. Opening of the bag was then plugged with a cotton wool, after which it was covered with a piece of paper and then fastened with a rubber band.

Sterilisation

The substrates was sterilised at 15 psi, 121 ^{0}C for 90 min (Kumari and Achal 2008). This was done to minimise or destroy the growth of other microorganisms that might compete with the growth of mushroom. Sterilisation took place in the evening. Bags were allowed to cool overnight and removed the next day ready for inoculation.

Inoculation

The spawn of the species of mushroom namely *Pleurotus pulmonarius* was obtained from Federal Institute of Industrial Research, Oshodi, Lagos state, Nigeria. The bottle containing the spawn was shaken vigorously so as to loosen the grains, then plug from the container was slightly flamed. Afterwards, the spawns were poured into the bag containing the compost and gently mixed together. This process continued until all the compost bags were inoculated.

Incubation

After inoculation, the spawned compost bags were kept in the dark and allowed to ramify, during which they grew into white cottony mycelia.

Fruiting and harvesting

After ramification of the compost, the planting bags were loosened and punctured to air, light and daily watering till the pin heads appeared. Thereafter, the height of fruiting bodies, stipe length and pileus diameter were measured with calibrated ruler. Matured mushrooms were harvested after 3 to 4 days of fruiting (Plates 1 and 2).

Process of harvesting involved the removal of the matured fruiting bodies from their substrates without destroying the substrate bags. Mature mushroom was held on their stipe below the pileus and close to the substrates level and was gradually pulled out. All fruiting bodies of a particular substrate bag were harvested at the same time since each bag had to be watered after harvest. This was done to enable the substrate to have moisture that enabled fruiting to occur again for harvest. Thereafter, fruiting bodies were air-dried, blended into powder and separately packaged for analysis.

Determination of proximate analysis

Proximate analysis (moisture and ash content) for the mushroom samples was determined using the AOAC procedures (AOAC, 1995). Protein content was determined following the method of Lowry (AOAC, 1995; Shiva *et al*., 2007). The total carbohydrate present in a sample was estimated by phenol- sulphuric acid method (AOAC 1995; Shiva *et al*., 2007).

Quantitative phytochemical screening

Determination of total phenolic content: The total phenol content in the methanolic extract of seed coat extract was determined spectrophotometrically using Folin-Ciocalteu method as described by Kujala *et al*. (2000).

Determination of flavanoids: The total flavonoids was determined using a colorimetric method, as described by Shiva *et al.* (2007).

Estimation of Alkaloids: Determination of alkaloids was done by using Harborne (1973) method.

Total tannin content (Folin-Ciocalteu colorimetric method): Each 0.5 mL of filtered sample was added with 3.75 mL of distilled water, and 0.25 mL of Folin-Ciocalteu reagent, and 0.5 mL of 35% sodium carbonate. The absorbance of each sample was measured at 725 nm using a spectrophotometer. The blank was prepared using the above reagents with distilled water instead of the sample (Chandran and Indira, 2016).

Qualitative phytochemical screening

The phytochemical analysis was determined using the methods of Saxena *et al*. (2012) and Rahman *et al.* (2017).

Phytochemical screening for phenols (ferric chloride test)

Each 2 mL of the filtered sample was mixed with 2 mL of 5% aqueous $FeCl_3$. The development of a blue colour indicated the presence of phenols.

Phytochemical screening for Phlobatannins

Each sample extract (2 mL) was boiled with 1% aqueous hydrochloric acid. The formation of a red precipitate confirmed the presence of phlobatannins.

Phytochemical screening for tannins (ferric chloride test)

The filtered sample (2 mL) was mixed with 10% alcoholic $FeCl_3$. The appearance of a black or brownish-blue colour indicated the presence of tannins.

Phytochemical screening for alkaloids (Dragendroff's test)

The filtered sample (2 mL) was dissolved in dilute hydrochloric acid and then filtered. The resulting filtrate was treated with Dragendroff's reagent (a solution of potassium bismuth iodide). The appearance of a red precipitate confirmed the presence of alkaloids.

Phytochemical screening for terpenoids (chloroform test)

The filtered sample (2 mL) was mixed with 0.5 mL of chloroform, 0.5 mL of acetic anhydride, and a few drops of concentrated sulfuric acid. The absence of a reddish-brown precipitate indicated that terpenoids were not present.

Phytochemical screening for saponin (foam test/frothing test)
The filtered sample (2 ml) was added with 4 mL of distilled water. The mixture was shaken vigorously; persistent foaming indicated the presence of saponins.

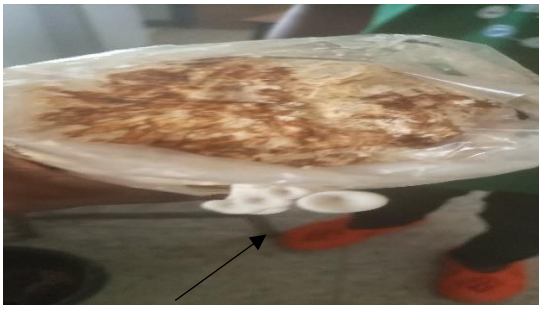
Phytochemical screening for flavonoids (alkaline reagent test)
Few drops of 20% NaOH was mixed with 2 mL of filtered sample resulting in the formation of an intense yellow colour. Then, a few drops of 70% diluted hydrochloric acid were added, and the yellow colour disappeared. This colour change confirmed the presence of flavonoids

Phytochemical screening for glycosides (Keller-Kiliani test)
The filtered sample (2 mL) was added with 0.5 mL glacial acetic acid, three drops of 1% aqueous $FeCl_3$ solution, and 0.5 mL concentrated H_2SO_4. The absence of a brown ring between the layers indicated that there were no cardiac steroidal glycosides present.

Determination of anti-oxidant activity
DPPH radical-scavenging activity: With minor modifications, the scavenging activity of *P. pulmonarius* extracts against the DPPH radical was evaluated using the Blois (1958) method.

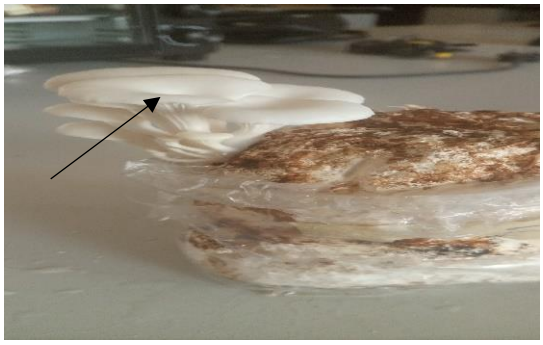
ABTS radical scavenging assay: For ABTS assay, the procedure followed the method of Arnao *et al*. (2001) with some modifications.

Data analysis
The Statistical Package for Social Science (SPSS version 16.0) programme was used to analyse the data that were collected, using the analysis of variance test. Means were separated using the Duncan's Multiple-Range Test (DMRT) at 5% level of probability.



A

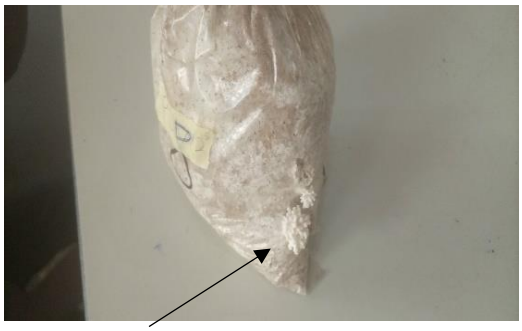
B



C

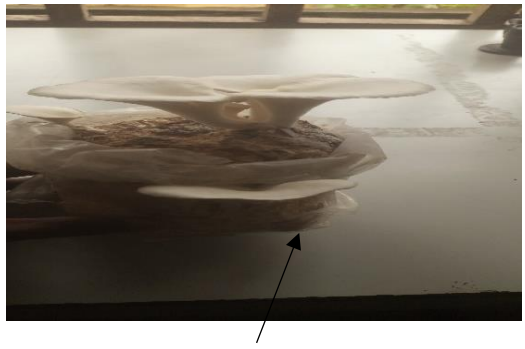
D

Plate 1: Sprouting of of *Pleurotus pulmonarius* on sawdust
A = 1 day after the appearance of pin head
B & D = 2 days after the appearance of pin head
C = 3 days after the appearance of pin head



A

B



C

Plate 2: Sprouting of *Pleurotus pulmonarius* on paper waste

A = Appearance of pin head
B = 2 days after the appearance of pin head
C = 3 days after the appearance of pin head

RESULTS

Spawn running and growth emergence of *Pleurotus pulmonarius* cultivated on sawdust and paper waste.

The spawn running and growth emergence of *Pleurotus pulmonarius* cultivated on sawdust and paper waste is shown in Table 1. *Pleurotus pulmonarius* grown on sawdust resulted in the fastest rate of mycelium growth, pin head appearance and formation of fruiting body while those cultivated on paper waste were the slowest.

Table 1: Spawn running and growth emergence of *Pleurotus pulmonarius* cultivated on sawdust and paper waste

Substrate	Spawn running (days)	Pin head appearance (days)	Fruiting body formation (days)
PpPW	18.00±0.58^{a}	22.00±1.15^{a}	28.00±1.15^{a}
PpSD	30.00±0.58^{b}	31.00±1.15^{b}	36.00±1.15^{b}
Total mean	24.00±2.71	26.50±2.14	32.00±1.93

Means with the same letter(s) are not significantly different ($p>0.05$)
PpPW= *Pleurotus pulmonarius* cultivated on paper waste
PpSD= *Pleurotus pulmonarius* cultivated on sawdust

Growth morphology of *Pleurotus pulmonarius* cultivated on sawdust and paper waste

Table 2 shows the comparative analysis of the growth morphology of *Pleurotus pulmonarius* cultivated on sawdust and paper waste. *P. pulmonarius* grown on paper waste showed the highest length of stipe and height of fruiting body while those cultivated on sawdust had the highest length of pileus diameter and larger number of fruiting bodies.

Table 2: Growth morphology of *Pleurotus pulmonarius* cultivated on sawdust and paper waste

Substrate	Pileus (cm)	Stipe (cm)	Height of fruiting body (cm)	Number of fruiting bodies
PpPW	2.37±0.09^{b}	2.73±0.15^{a}	6.43±0.37^{a}	3.00±0.58^{b}
PpSD	3.00±0.00^{a}	2.60±0.42^{a}	5.57±0.64^{b}	8.00±0.58^{a}
Mean	2.68±0.15	2.67±0.20	6.00±0.38	5.50±1.18

Means with the same letter(s) are not significantly different ($p>0.05$)
PpPW= *Pleurotus pulmonarius* cultivated on paper waste
PpSD= *Pleurotus pulmonarius* cultivated on sawdust

Effects of different substrates (sawdust and paper waste) on the proximate content of *Pleurotus pulmonarius*

The effects of different substrates (sawdust and paper waste) on the proximate content of *Pleurotus pulmonarius* is shown in Table 3. The highest content of sugar, protein and ash was recorded in *P. pulmonarius* cultivated on paper waste while *P. pulmonarius* grown on sawdust had the highest amount of carbohydrate and moisture.

Table 3: Effects of different substrates (sawdust and paper waste) on the proximate content of *Pleurotus pulmonarius*

Treatment	Sugar (%)	Carbohydrate(%)	Protein (%)	Ash (%)	Moisture (%)
PpPW	0.30±0.06[a]	23.87±0.04[b]	29.77±0.04[a]	50.48±0.05[a]	49.52±0.01[b]
PpSD	0.09±0.01[b]	52.18±0.05[a]	14.57±0.04[b]	30.30±0.17[b]	69.70±0.17[a]
Mean	0.20±0.05	38.03±6.33	22.17±3.40	40.39±4.51	59.61±4.51

Means with the same letter(s) are not significantly different (p>0.05)
PpPW= *Pleurotus pulmonarius* cultivated on paper waste
PpSD= *Pleurotus pulmonarius* cultivated on sawdust

Comparative quantitave phytochemical properties of *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Table 4 shows the comparative phytochemical properties of *Pleurotus pulmonarius* cultivated on paper waste and sawdust. *P. pulmonarius* grown on sawdust had a significantly higher flavonoid content compared to those cultivated on paper waste. *P. pulmonarius* grown on both substrates had the same phenol and tannin contents. *P. pulmonarius* grown on sawdust had a higher alkaloid content compared to those cultivated on paper waste.

Table 4: Comparative quantitative phytochemical properties of *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Subtrate	Flavonoid (mgQE/g)	Phenol (mg)	Tannin (mg/ml)	Alkaloid (%)
PpPW	251.70±0.40[b]	3.04±0.02[a]	1.04±0.02[a]	4.04±0.02[b]
PpSD	293.56±0.32[a]	3.04±0.01[a]	1.15±0.09[a]	4.80±0.46[a]
Total mean	272.63±9.36	3.04±0.01	1.10±0.05	4.42±0.27

Means with the same letter(s) are not significantly different (p>0.05)
PpPW= *Pleurotus pulmonarius* cultivated on paper waste
PpSD= *Pleurotus pulmonarius* cultivated on sawdust

Comparative qualitative phytochemical properties of *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Table 5 shows the qualitative phytochemical properties of *Pleurotus pulmonarius*. Flavonoid and phenol were present in the *P. pulmonarius* grown on both paper waste and sawdust. Tannin and alkaloid were also present but in lesser quantities when compared with the amount of flavonoid and phenol observed. Saponin was observed

to be abundantly present in the *P. Pleurotus* grown on sawdust compared to the amount obtained in those cultivated on paper waste. Terpenoids, phlobatanins and glycoside were absent in *P. pulmonarius* grown on both substrates.

Table 5: Comparative qualitative phytochemical properties of *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Phytochemical	PpPW	PpSD
Flavonoid	++	++
Phenol	++	++
Tannin	+	+
Alkaloid	+	+
Terpenoids	-	-
Phlobatanins	-	-
Saponin	+	++
Glycoside	-	-

Where + is present, ++ is abundantly present, and – is absent
PpPW= *Pleurotus pulmonarius* cultivated on paper waste
PpSD= *Pleurotus pulmonarius* cultivated on sawdust

Comparative anti-oxidants analysis of *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Figure 1 illustrates the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, which is a measure of anti-oxidant capacity, across various concentrations (50, 100, 150, 200 and 250 ug/ml) for three different treatments: Ascorbic (blue), *P. pulmonarius* cultivated on paper waste (orange) and *P. pulmonarius* cultivated on sawdust (gray).

At a concentration of 50, *P. pulmonarius* cultivated on sawdust resulted in the highest DPPH value of 81.3, followed by the *P. pulmonarius* cultivated on paper waste with a value of 74.5. The Ascorbic treatment showed a significantly lower anti-oxidant activity with a DPPH value of 33.33. At a concentration of 100, the trend was similar to the PPW treatment with the highest anti-oxidant activity at 82.27. The *P. pulmonarius* cultivated on sawdust treatment had a DPPH value of 66.1, which was lower than *P. pulmonarius* cultivated on paper waste. The Ascorbic treatment resulted in a very low DPPH value of 2.56.

At the concentration of 150, the *P. pulmonarius* cultivated on paper waste resulted in the highest anti-oxidant activity with a DPPH value of 77. The *P. pulmonarius* cultivated on sawdust treatment had a DPPH value of 56. The Ascorbic treatment had a DPPH value of 5.98. At the concentration of 200, the *P. pulmonarius* cultivated on paper waste resulted in a DPPH value of 59.1, which was lower than the paper concentrations but still the highest among the treatments at this concentration. The *P. pulmonarius* cultivated on sawdust resulted in a DPPH value of 54.4. The Ascorbic treatment resulted in a low DPPH value of 11.11. At the highest concentration of 250, the *P. pulmonarius* cultivated on paper waste resulted in the highest anti-oxidant activity with a DPPH value of 62.6, followed by Ascorbic

treatment with a DPPH value of 57.26. The lowest value was recorded in mushroom grown in sawdust.

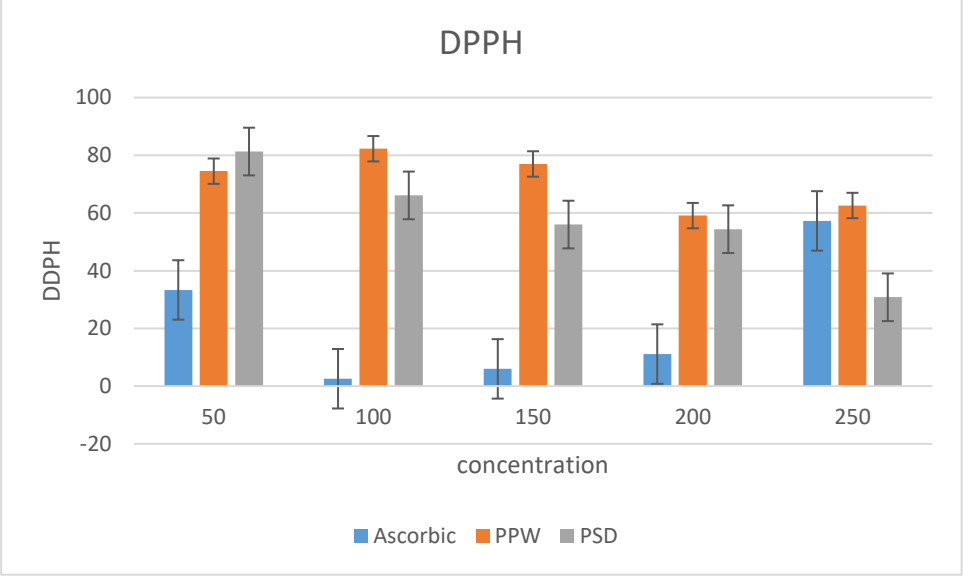


Figure 1: DPPH radical scavenging activity across various concentrations

ABTS Value for *Pleurotus pulmonarius* cultivated on paper waste and sawdust

Figure 2 shows that ABTS value for the *Pleurotus pulmonarius* cultivated on sawdust was higher (0.62) than that observed in *Pleurotus pulmonarius* grown on paper waste (0.60).

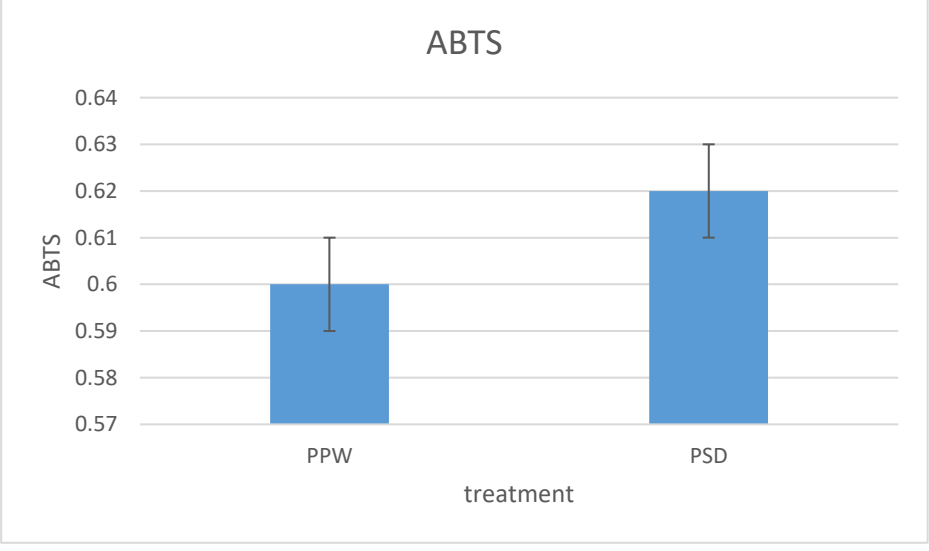


Figure 2 : Analysis of ABTS radical-scavenging activity across different treatments

DISCUSSION

Mushrooms are known as healthy foods throughout the world with proteins, vitamins, minerals, chitin, essential amino acids as well as low fat and calories (Valverde *et al*., 2015). The nutritional value of mushroom is comparable to foodstuffs such as corn, soybeans or beans. They are especially important foods with the basic amino acids they contain. Within the mushrooms, there are proteins at levels ranging from 5-49% of dry weight. In addition to protein, mushrooms also contain dietary fibres, minerals such as potassium, phosphorus iron, vitamins and carbohydrates (Valverde *et al*., 2015). This study showed that various compositions of *P.pulmonarius* can be affected based on the substrates used for cultivation.

Results of this study showed that substrate ramification was rapid in *P. pulmonarius* cultivated on paper waste, as well as the appearance of pin head and the formation of fruiting bodies. Tesfay *et al*. (2020) demostrated the effectiveness of paper waste in mushroom cultivation. *P. pulmonarius* grown on sawdust showed the highest number of fruiting bodies and largest pileus diameter while those cultivated on paper waste had the highest stipe length and height of fruiting body, which is in line with Enibukun and Laba (2019), who worked on the bioconversion of sawdust and paper treaded with edible fungus (*Pleurotus pulmonarius*).

The increase in the sugar content in *P. pulmonarius* cultivated on paper waste suggests that the nutrient composition of paper waste may enhance the accumulation of simple sugars as reported by Roy *et al.* (2015) who concluded that substrates with higher organic matter content could increase sugar content in mushroom. Conversely, *Pleurotus pulmonarius* cultivated on sawdust resulted in a significantly higher carbohydrate content. Obodai *et al*. (2003) reported that substrates rich in lignocellulosic materials, such as sawdust, generally increase carbohydrate levels in mushrooms.

The protein content was significantly higher in *Pleurotus pulmonarius* grown on paper waste. Levanon *et al*. (1993) reported that nitrogen-rich substrates, like paper waste, enhanced increased protein synthesis in mushrooms. The ash content, representing total minerals, was higher in *Pleurotus pulmonarius* cultivated on paper waste. This indicates that paper waste may contain more minerals or elements absorbed by the mushrooms, as reported by Manzi *et al*. (2001), who observed that the mineral content of mushrooms was influenced by the mineral composition of their substrate. *Pleurotus pulmonarius* cultivated on sawdust had a higher moisture content which may be as a result of the retention capability of sawdust substrate as suggested by Kalac (2013) who noted that the moisture content in mushroom is influenced by the water-holding capability of the substrate.

Also, *Pleurotus pulmonarius* grown on sawdust produced the highest amount of flavenoid, phenol, tannin and alkaloid. Chang and Miles (2004) reported that alkaloids have been examined in a variety of mushroom species. The quantitative phytochemical screening of *Pleurotus pulmonarius* has confirmed that these species of mushrooms are among those that generate alkaloids. These phytochemical components are largely responsible for therapeutic properties of mushrooms. Alkaloids on the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract and uterus have been linked to the beneficial medicinal qualities of several mushrooms. These alkaloids have been demonstrated to be useful against infections, malaria and malignant disorders (Akinbode *et al*., 2021). Anti-oxidants and

phenolic compounds exhibit a broad range of therapeutic actions, including anti-inflammatory, anti-cancer and anti-diabetic activities (Akinbode *et al*., 2021). One of the most varied classes of natural substances are flavonoids, which have been demonstrated to have a wide range of chemical and biological functions, such as the ability to scavenge free radicals and to have anti-viral, anti-allergic, anti-inflammatory and vasodilator effects (Akinbode *et al*., 2021).

Pleurotus pulmonarius cultivated on paper waste demonstrated a higher anti-oxidant activity across all tested concentrations (50, 100, 150, 200, and 250 µg/mL) compared to those cultivated on sawdust and the ascorbic acid. For instance, at 50 µg/mL, *Pleurotus pulmonarius* cultivated on paper waste showed a DPPH value of 81.3, whereas those on sawdust had 74.5; ascorbic acid had only 33.33. These findings are in line with Cheung *et al*. (2003), who reported significant anti-oxidant properties in different edible mushrooms. The higher anti-oxidant capacity of the mushrooms grown on paper waste was further demostrated by this study, indicating that the kind of substrate used can improve particular bioactive qualities.

CONCLUSION

This study highlights the significant impact of substrate composition on the growth and nutritional quality of *Pleurotus pulmonarius*. Paper waste proved to be a superior substrate in enhancing certain nutritional and bioactive properties of the mushroom, including protein content, sugar accumulation, mineral content and anti-oxidant activity. These findings suggest that paper waste, with its high nitrogen and organic matter content, is particularly effective in promoting the synthesis of proteins and simple sugars, as well as enhancing anti-oxidant properties, which are critical for the therapeutic potential of mushrooms. Conversely, sawdust, rich in lignocellulosic material, contributed to a higher carbohydrate content and moisture retention in the mushrooms, along with elevated levels of key phytochemicals like flavonoids, phenols, tannins and alkaloids, which are associated with various health benefits. The results underscore the importance of substrate selection in mushroom cultivation, as different substrates can significantly influence both the yield and nutritional profile of the mushrooms. This provides insights for optimising mushroom production for specific nutritional and medicinal purposes.

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