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Nutritional Profile and Microbial Analysis of *Pleurotus ostreatus* Cultivated on Agricultural Residues

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Abstract:- ***Pleurotus ostreatus*** **is a widely cultivated edible mushroom, which offers significant nutritional and medicinal benefits. In this study, *P. ostreatus* was cultivated on various agricultural residues: sawdust, corncob, cassava peel, and plantain peel. The agricultural residues which served as substrates were further sun-dried, soaked in water, and packed into polyethylene bags; after which they were sterilized and inoculated with *P. ostreatus* spawn. The inoculated bags were then incubated at 25-30°C and 90% relative humidity for the period of 20 days. The nutritional profile, mineral content, and phytochemical composition of the substrates and cultivated mushrooms were analyzed. Furthermore, the microbial load of the substrates and mushrooms was assessed using standard microbiological methods. The obtained results revealed significant differences in nutrient content among substrates and mushrooms when compared statistically. Mushrooms exhibited higher protein (2.88 ± 0.02%), fiber (3.94 ± 0.01%), and mineral content compared to the agricultural residual substrates. The analysis revealed that mushrooms cultivated on plantain peel demonstrated the highest levels of flavonoids (4.18 ± 0.01 mg/kg) and alkaloids (4.07 ± 0.11 mg/kg). Also, Microbial analysis revealed a significant reduction in microbial load in the cultivated mushrooms compared to the substrates. These findings highlight the potential of *P. ostreatus* cultivated on agricultural residues as a sustainable and nutritious food source. The study further emphasizes the importance of selecting appropriate substrates to optimize mushroom quality and reduce potential microbial contamination. Hence, further research is needed to explore the impact of different cultivation techniques and environmental factors on the nutritional and medicinal properties of *P. ostreatus*.**

Keyword:- *Pleurotus ostreatus, Nutritional Profile, Mushroom, Bioactive Compounds, Agricultural Residues and Microbial Analysis.*

I. INTRODUCTION

The oyster mushroom, or Pleurotus ostreatus, is a commonly grown edible fungus valued for its nutritional content, possible biotechnological uses, and therapeutic qualities (Babu et al., 2020; Raman et al., 2020).

Remarkably, they hold significance in Asian nations, where there dietary intake as dietary supplements have increased due to their strong nutritional, culinary, and medicinal qualities. According to Raman et al. (2020), they are both a valuable source of revenue and a staple food.

Mushroom cultivation has gained significant attention in recent years, driven by its potential to contribute to sustainable agriculture, food security, and human health. Its cultivation on agricultural residues offers a sustainable approach to waste management and food production.

A variety of agricultural residues, including wheat straw, barley straw, rice straw, sawdust, banana leaves, and bean straw, can be used as substrates for cultivation (Tesfaw et al., 2015; Iqbal et al., 2016; Manimuthu et al., 2015). To enhance growth and yield, supplements like rice husk can be added to the substrates (Khare et al., 2010; Tesfaw et al., 2015). However, it's important to note that supplements can increase the risk of contamination and the reuse of supplemented substrates has been shown to improve growth and yield in subsequent cultivation cycles (Tesfaw et al., 2015).

Several studies have highlighted the nutritional value of oyster mushrooms. Tolera and Abera (2017) investigated the impact of different drying methods and osmotic pretreatments on the nutritional quality of dried mushroom slices. While drying methods significantly affected nutrient content, osmotic pretreatment was found to reduce protein, fat, and fiber content while increasing ash and carbohydrate content. Bashir et al. (2020) compared the proximate and mineral composition of oyster mushrooms dried using six different methods. Freeze drying was found to retain the highest levels of nutrients, while sun drying resulted in significant losses.

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Rashidi & Yang (2016) further explored the nutritional value and antioxidant properties of fresh grey oyster mushrooms, emphasizing their high fiber content, low-fat profile, and potent antioxidant activity. King et al. (2012) highlighted the role of dietary fiber in regulating blood glucose levels. The high fiber content of mushrooms, along with their magnesium content, contributes to their potential health benefits. Orywal et al. (2022) further emphasized the importance of magnesium in controlling insulin action and glucose metabolism.

Recent research has focused on optimizing oyster mushroom cultivation techniques to enhance yield and nutritional quality. Hutabarat et al. (2022) investigated the use of agro-industrial wastes as substrates for pink oyster mushroom cultivation, demonstrating that mycelium-treated substrates can be used for animal feed due to their increased protein content. Ganjikunta et al. (2020) explored the impact of different bran supplements on oyster mushroom growth and yield, finding that wheat bran significantly enhanced yield and biological efficiency. Hwang et al. (2015) investigated the use of agricultural waste from pruned fruit tree branches as a substrate for king oyster mushroom cultivation.

Torres-Martínez et al. (2022) highlighted the potential of *Pleurotus* spp. as a novel ingredient in meat and meat products to improve their nutritional and functional properties. Inci et al. (2022) explored the use of oil palm waste-based substrates as a sustainable alternative for oyster mushroom cultivation. Bulam et al. (2019) emphasized the various bioactive compounds present in oyster mushrooms, such as polysaccharides, peptides, and polyphenols, which contribute to their health-promoting properties. Kumar (2020) summarized the nutritional and medicinal properties of oyster mushrooms, highlighting their potential as functional foods. Zeng et al. (2023) investigated the impact of supplementing oyster mushroom cultivation substrates with *Codonopsis pilosula* stems and leaves, resulting in enhanced nutritional value and the accumulation of various bioactive compounds.

However, this study aimed to evaluate the nutritional profile and microbial quality of *P. ostreatus* cultivated on various agricultural residues: sawdust, corncob, cassava peel, and plantain peel.

II. MATERIALS AND METHODS

A. Study Area

The study was carried out at the University of Port Harcourt demonstration farm, the department of Microbiology Technology laboratory and Faculty of Pharmaceutical Sciences, University of Port Harcourt.

B. Collection of Substrate and oyster mushroom

Substrates for this study included sawdust, plantain peel, cassava peel, and corn cob. Sawdust was obtained from Rumosi Wood Mill, Port Harcourt. Plantain peel, cassava peel, and corn cob were obtained from different processing points within Port Harcourt metropolis and were taken to the mushroom unit of the University of Port Harcourt Demonstration Farm. The pure culture of the mushroom to be used for this study was obtained from the mushroom bank of the mushroom unit, University of Port Harcourt Demonstration Farm.

C. Cultivation of Oyster Mushroom

- *Preparation of Substrate*

Substrate samples were sun-dried Each substrate were soaked in water in different ratios, depending on substrate, but ensuring no water logs. The method used for mushroom cultivation was that of modified Stamets (2000). Five hundred grammes of each material were packed and sealed in polyethylene bags, and sterilised and inoculated with 10% (w/ w) of spawn in a sterile environment and later transferred to an incubation room for ramification. Each substrate was replicated five times.

- *Preparation of Culture Media*

All culture media were prepared according to the manufacturer's specifications.

Thirty-nine grams (39 g) of Potato Dextrose Agar (PDA) (containing 4.0 g potato extract, 20.0 g dextrose, and 15 g agar) and 50 g of Malt Extract Agar (MEA) (containing 30 g malt extract, 5 g mycological peptone, and 15 g agar) were added to 1 L of distilled water in two 2-liter flasks. The mixtures were then heated on a Bunsen burner to dissolve the agar. The solutions were autoclaved at 121°C for 15 minutes. Fifteen milliliters (15 ml) of each medium were then dispensed into Petri dishes.

D. Sample Collection

Pure cultures of the mushroom were maintained on potato dextrose agar (PDA) and malt extract agar (MEA) plates. These were inoculated with oyster mushroom cultures using a spatula and incubated at 25°C. Mycelial growths, in terms of diameter on the culture plate, was measured using a calibrator.

- *Inoculation*

The agricultural residues to be used as substrates were placed in heat-resistant polypropylene bags and sterilized with intermittent heating for three consecutive days. They were then allowed to cool to room temperature. Mycelium was removed from the culture bottle using a sterilized spoon and placed on the substrate. After wearing appropriate safety gear, the surface area of the substrate was sterilized with ethanol. Each experimental polypropylene bag was inoculated at the center of the substrate with 10 g of pure culture of *P. ostreatus* under aseptic conditions and sealed with a wrap. The bags were then incubated in a dark room at 25-30°C and 90% relative humidity for 20 days.

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➢ *Colonization of the Substrate*

The inoculated substrate went through colonization within a period of 3 weeks to 4 weeks.

➢ *Product Evaluation*

Mycelia run was assessed by recording the number of days' mushroom mycelia fully colonised a substrate bag.

➢ *Fruiting and Harvesting of Mushroom*

Substrate bags, when fully colonised with mycelia, was transferred to the fruiting room and opened to initiate fruiting, through sprinkling of water on the bags. Sporophores (fruiting bodies) was harvested by hand-twisting, weighed with electronic digital balance and dried in a fabricated solar dryer. When constant weight was observed, the dried samples were kept in air-tight envelops and taken to the laboratory for analysis proximate, phytochemical and microbial analysis as well as clinical trial.

➢ *Identification of the Samples*

Mushroom samples were identified and authenticated in Pharmacognosy and Phototherapy laboratory, Faculty of Pharmaceutical Sciences, University of Port Harcourt, where voucher specimens were deposited.

➢ *Proximate Analysis*

Before proximate analysis substrates and harvested mushrooms were dried at 60°C in oven until constant weight was obtained and grounded into powder for further nutrient analysis (AOAC, 2002).

➢ *Moisture Content Determination.*

At first, the weight of empty crucibles was dried, and 5g of sample was placed on them. The crucible was then placed in an air oven (thermostatically regulated) and dried at 105^0 degrees Celsius for 24 hours. After drying, the crucible was removed from the oven and chilled in a desiccator. It was then weighed using cover glass. The crucible was returned to the oven for 30 minutes of drying before being removed, cooled in a desiccator, and weighed. Drying, cooling, and weighing were repeated until the two successive weights matched (AOAC, 2002).

From these weights, the percentage of moisture in food samples was calculated as follows:

$$\% \text{ Moisture} = \frac{Loss\ of\ weight\ of\ sample}{Initial\ weight\ of\ sample}\ x\ 100$$

➢ *Fats Content Determination*

Following the standard AOAC method (AOAC, 2002), the samples' fat content was ascertained by transferring the dried sample that remained after the moisture determination to a thimble and plugging the top of the thimble with a wad of fat-free cotton. The thimble was then dropped into the fat extraction tube that was connected to a Soxhlet flask, which was filled with at least 75ml of anhydrous ether. The top of the fat extraction tube was connected to the condenser, and the sample was extracted for at least 16 hours on a water bath at 800C.

The thimble was taken out of the apparatus at the end of the extraction period, and the majority of the ether was distilled out by collecting it in a Soxhlet tube. When the tube was almost full, the ether was drained off. A tiny funnel with a cotton plug was used to pour the ether into a tiny, dry beaker once it had reached a little volume. Ether was used to thoroughly clean and filter the flask. After evaporating over a steam bath at low heat, the ether was dried for one hour at 1000C, chilled, and weighed. The ether-soluble substance in the sample was determined by the weight differential.

$$\% \text{ Crude fat} = \frac{Loss\ of\ ether\ soluble\ materials}{Weight\ of\ sample}\ x\ 100$$

➢ *Ash Content Determination*

The standard AOAC method was used to determine the samples' ash content (AOAC, 2003). This method determines the weight of the leftover ash after oxidizing all organic matter through incineration. In short, five grams (5 g) of the sample were burned and placed in a crucible-equipped muffle furnace at 550ºC for eight hours. This formula was used to calculate it:

$$\% \text{ ash} = \frac{Weight\ of\ ash}{Initial\ weight\ of\ sample}\ x\ 100$$

➢ *Crude Protein Determination*

The samples (0.2) g were transferred into Kjeldahl flask containing mixture of 3g of hydrated cupric sulphate + 20 ml sodium sulphate and 1 g of Conc. H_2SO_4 (N x 6.25) to digest the sample. After digestion was completed, the solution became clear and was cooled properly and then filled the solution with distilled water up to level of 100ml. 5 ml of digest was collected for distillation process and then 5 ml of NaOH was transferred into the distillation flask and then distilled for sometimes. The indicator, Boric Acid absorbed the liberated ammonia from distillation unit and this was finally titrated with standard acid, HCL until the solution colour changes to pink from green colour. The crude protein content was determined by using the formula:

$$\% \text{ crude protein} = \frac{T \times S \times 0.01}{A \times W} \times 6.25$$

Where:
T = Titre value;
S = Final digest solution;
A = Aliquot volume;
W = Sample weight

➢ *Crude Fibre Determination*

One (1) gram of the sample (W1) was boiled with 200 ml of a solution having 2.50 g of H_2SO_4 and then using a cotton cloth the solution was filtered and the residue obtained was again boiled with 200 ml of solution filled with 2.50 g of NAOH for another 30 minutes. The final residue obtained was then again filtered through cotton cloth and transferred into oven for drying and weighed (W2). The final residue formed was then converted into ashes in a muffle furnace and cooled in desiccator and weighed (W3);

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$$\% \text{ Crude fibre} = \frac{W2-W3}{W1} X\ 100$$

Where:
W1 = Weight of the sample
W2= Weight of the sample after drying in an oven
W3 = Weight of the sample after ashing in a muffle furnace

➢ *Total Carbohydrate Estimation*

Available carbohydrate content in the sample was determined following the method described by Ashraf et al. (2013). This was calculated as the difference obtained after subtracting the lipid, ash and fibre values from the total dry matter using the formula below**:**

$$\% \text{ Carbohydrate} = 100 - (a + b + c + d)$$

Where;
a = amount of crude protein
b = amount of crude lipid
c = amount of ash content
d = amount of crude fibre

E. Mineral Analysis

Ten (10) ml Nitric and 5 ml $HClO_4$ acid were added to a sample of 1 g in a digestion flask. The mixture was digested for 1 hr. The digested mixture was filtered. The filtrate was made up to 100 ml with distilled water. Mineral contents were determined by using a biochemical analyzer (Humalyzer 3000). Commercially available biochemical kit (Randox®) was used for biochemical assay (Akther et al., 2020).

➢ *Determination of Sodium (Na)*

Sodium ions are precipitated as a triple salt with magnesium and uranyl acetate. The remaining uranyl ions are then reacted with ferrocyanide in an acidic medium, resulting in the development of a brownish color. The intensity of this color, measured at a wavelength of 530 nm using a green filter (Hg 546), is inversely proportional to the concentration of sodium in the sample. The assay is conducted at room temperature with a light path of 1 cm.

$$\text{Sodium in } \frac{\text{mmol}}{\text{L}} = \frac{\text{(A) sample}}{\text{(A) Standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

➢ *Determination Of Calcium (Ca)*

The determination of calcium (Ca) is based on the principle that calcium ions form a violet complex with O-Cresol phthalein complex one in an alkaline medium. This colorimetric method does not require deproteinization. The assay involves measuring the absorbance at a wavelength of 570 nm, using a spectrophotometer set within the range of 550-590 nm, with the temperature maintained between 20-25°C or 37°C and a light path of 1 cm. The concentration of calcium in the sample, expressed in mg/dL, is calculated using the formula:

$$\text{Concentration in } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

➢ *Determination of Magnesium (Mg)*

The magnesium determination method is based on the specific binding of calmagite, a metallochromic indicator, with magnesium at an alkaline pH, resulting in a shift in the absorption wavelength of the formed complex. The chromophore's intensity is directly proportional to the magnesium concentration in the sample. Measurements are conducted at an assay wavelength of 520 nm, with an increase in absorbance observed within the 500-550 nm range, and a decrease in absorbance noted within the 570-650 nm range. The analysis is performed at a temperature of 20-25°C or 37°C, with a 1 cm light path, and measurements are taken against a reagent blank for accuracy

$$\text{Magnesium } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

➢ *Determination of Potassium (K^+)*

The determination of potassium (K^+) involves a reaction between sodium tetraphenyl boron and potassium ions, resulting in the formation of potassium tetraphenyl boron, which creates a fine turbidity. The intensity of this turbidity is directly proportional to the potassium concentration in the sample. The assay is conducted at room temperature, using a light path of 1 cm, with measurements taken at a wavelength of 630 nm (Hg 623) using a green filter.

$$\text{Potassium } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

➢ *Determination of Phosphorus (P)*

The determination of phosphorus concentration involves measuring the absorbance of the sample at a wavelength of 340 nm, or alternatively using filters at 334 nm or 365 nm. The measurement is conducted at a temperature range of 20-25°C or 37°C, with a light path of 1 cm, and is taken against a reagent blank. The phosphorus concentration in the sample, expressed in mg/dL, is calculated by comparing the absorbance of the sample (A) to that of the standard (A) using the formula:

$$\text{Phosphorus concentration } \frac{\text{mg}}{\text{dl}} = \frac{\text{(A) sample}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{\text{mg}}{\text{dl}})$$

➢ *Determination of Iron (Fe)*

The determination of iron (Fe) involves dissociating the iron from its transferrin-iron complex in a weakly acidic medium. The liberated iron is then reduced to its bivalent form using ascorbic acid. Ferrous ions react with Ferrozine to form a colored complex, with the intensity of the color being directly proportional to the iron concentration in the sample. The assay is conducted at a wavelength of 562 nm, with the temperature maintained between 15–25°C or at 37°C, using a light path of 1 cm. The iron concentration in the sample (in µg/dL) is calculated using the formula:

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$$\text{Iron in } \frac{\mu g}{dl} = \frac{\text{(A) sample - (A) sample blank}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{mg}{dl})$$

➢ *Determination of Zinc (Zn)*

The determination of Zinc (Zn) involves dissociating iron from its transferrin-iron complex in a weakly acidic medium, where the liberated iron is then reduced to the bivalent form using ascorbic acid. The resulting ferrous ions react with Ferrozine to form a colored complex, the intensity of which correlates with the iron concentration in the sample. The assay is conducted at a wavelength of 562 nm, with a temperature range of 37°C or 15-25°C and a light path of 1 cm. The zinc concentration in the sample is calculated using the formula:

$$\text{Zn in } \frac{\mu g}{dl} = \frac{\text{(A) sample - (A) sample blank}}{\text{(A) standard}} \times \text{Standard conc. } (\frac{mg}{dl})$$

F. Phytochemical Analysis

The methods of Talukdar, Choudhary, Chakraborty, & Dutta (2010) were employed in the evaluation of the mushroom in order to look for the presence of alkaloids, glycosides, tannins, flavonoids, Phytate, and saponins.

➢ *Saponins*

The froth test was used to identify the saponins. For this test, 1g of the sample was weighed into a conical flask. First, the liquid was boil for five minutes after adding ten milliliters of sterile distilled water. After the mixture was filtered, 10 ml of sterile distilled water and 2.5 ml of the filtrate were combined in a test vial. After the test container was capped with a cork, it was shaken forcefully for about thirty seconds. After then, it was allowed to stay in place for half an hour. The formation or appearance of honeycomb froth indicated the presence of saponins.

➢ *Tannins*

The presence of Tannins was determined by adding 3-4 droplets of a 10% ferric chloride solution to a portion of the extract that had been diluted with water. The presence of garlic tannins was confirmed by the blue colour, while the presence of catechol tannins was indicated by the green colour.

➢ *Alkaloids*

In order to determine the presence of alkaloids, 2 ml of extract was measured and transferred to a test tube, which was then treated with picric acid solution. The presence of alkaloids was indicated by the orange coloration.

➢ *Flavonoids*

Total flavonoid content of the samples was determined by following the Aluminum chloride method. Macro-fungi concentrate was mixed with $NaNO_2$ solution, distilled H_2O and $AlCl_3$ solution after 6 min. It was allowed to stand for 6 min, NaOH solution and distilled H_2O was added to the mixture to achieve the final volume. The mixture was vortexed extensively and stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of the sample.

➢ *Phytate*

Using the method outlined by Lucas and Markaka, referenced in Essien and Akpan (2014), phytic acid determination was used to extract and precipitate phytate. This comprises filling a 250 mL conical flask with 2g of the sample.

The samples were soaked in 100 mL of concentrated HCl for three hours in the conical flask before being filtered through double-layered filter paper. To provide or enhance appropriate acidity, distilled water (107 mL) was added to 50 mL of sample filtrate in a 250 mL beaker. Each sample solution was titrated with a standard iron chloride solution containing 0.00195 g iron/mL after adding 10 mL of a 0.3% ammoniumthiocyanate solution as an indicator. The end point was indicated by a brownish-yellow coloration that lasted for five minutes. The phytic acid % was computed.

➢ *Cyanogenic Glycoside*

The alkaline picrate approach of Onwuka (2005) was the technique employed. After adding 50 mL of distilled water to the samples (5g each) in conical flasks, they were left to stand overnight. After adding 4 mL of alkaline picrate to 1 mL of sample filtrate in a corked test tube, the mixture was incubated for 5 minutes in a water bath. Cyanides were present when the color changed from yellow to reddish brown after five minutes of incubation in a water bath. Prior to creating the cyanide standard curve, the absorbance of the samples was measured at 490 nm, as well as that of a blank that contained 1 mL of distilled water and 4 mL of an alkaline picrate solution.

III. MICROBIAL ANALYSIS

A. Preparation of Peptone Water

Peptone water was prepared by dissolving 15 grams of peptone powder in 1000 ml of distilled water in a sterile beaker. The solution was swirled to dissolve completely. 225 ml of the peptone water was measured and poured into a sterile conical flask for pre-enrichment of the organisms in the food sample. 9 ml of peptone water was pipetted into sterile test tubes and was properly covered with clean cotton wool before autoclaving at 121°C for 15 minutes.

B. Nutrient Agar, MacConkey Agar, and Potato Dextrose Agar

The media used were Nutrient Agar and MacConkey Agar for culturing bacterial colonies and Potato Dextrose Agar for isolating fungal colonies.

The media were prepared by dissolving 2.8 g of Nutrient Agar, 5.5 g of MacConkey Agar, and 3.6 g of Potato Dextrose Agar in 100 ml of distilled water, respectively. These solutions were brought to a boil to dissolve completely by heating over a Bunsen burner flame for 30 minutes.

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The media were sterilized at 121°C for 15 minutes using an autoclave at 15 psi. The sterilized media were allowed to cool to 45°C before being poured into sterile Petri dishes. The plates were allowed to set and solidify.

C. Mannitol Salt Agar

Mannitol Salt Agar is a microbiological medium used for the isolation of *Staphylococcus* species.

111 grams of mannitol salt powder was measured and dispensed into 1 liter of distilled water. The solution was swirled to dissolve before autoclaving at 121°C for 15 minutes at 15 psi. The medium was allowed to cool before dispensing 20 ml into each sterile Petri dish. It was then allowed to solidify before use.

D. Serial Dilution of Sample

25 grams of each food sample was introduced into 225 ml of peptone water in a stomacher bag and macerated to homogenize. Ten-fold serial dilutions were performed by pipetting 1 ml from the stock solution (10^{-1}) into a test tube containing 9 ml of peptone water labeled 10^{-2}. This process was repeated up to 10^{-5} in duplicate.

E. Enumeration of Total Heterotrophic Bacteria

An aliquot (0.1 ml) of the dilution was inoculated into a Petri dish containing Nutrient Agar for culturable heterotrophic bacteria. The inoculum was spread-plated with a bent glass rod. The plates were incubated at 28°C for 24 hours. The colonies that formed during this incubation were counted.

F. Enumeration of Total Heterotrophic Fungi

The medium used for the enumeration of total heterotrophic fungi was Potato Dextrose Agar. An aliquot (0.1 ml) of appropriate dilutions of samples was inoculated into Petri dishes containing Potato Dextrose Agar. The plates were incubated for 2-3 days at room temperature, and the colonies formed were counted.

G. Isolation Based on Cultural Characteristics

Using a sterile wire loop, a loopful of unique colonies that developed on the various medium plates were selected according to cultural traits (color, shape, size, elevation, margin, opacity, and appearance). A freshly made Nutrient Agar plate was edged with the inoculum, which was then streaked out over the medium's surface in one of many possible patterns. The wire loop was periodically heated to red-hot in order to create the streaking. For a whole day, the streaked plates were incubated at 37°C.

Thereafter, distinct colonies that developed from the streaked plates were transferred to agar slants and incubated at 37°C for 24 hours to obtain stock cultures.

H. Identification of Fungal Isolates

Identification of fungal isolates was based on morphological and microscopic characterization, such as mycelium, pigmentation, type of sporulating structures, and sexual reproduction (if present). They were examined using a hand lens to determine these morphological characteristics. Several colonies were selected from the incubated plates for identification using the wet mount method. A wet mount slide was prepared by transferring a small amount of the culture with an inoculating loop to a slide. This was covered with a coverslip and examined under low power (x10) or high power (x100) objectives.

I. Identification Tests of Bacterial Isolates

Isolates, after obtaining pure cultures, were further subjected to Gram staining and different biochemical tests. The different biochemical tests conducted to identify bacterial isolates are listed as follows:

J. Gram Staining Technique

A drop of sterile water was placed at the center of a clean glass slide. With the aid of a sterile wire loop, a discrete colony was smeared in the drop of water on a clean, grease-free glass slide and allowed to air dry. It was heat-fixed by passing it over a flame. The fixed smear was flooded with crystal violet stain for 1 minute and washed off with tap water. Lugol's iodine was then added and allowed to stay for 1 minute, followed by rinsing with tap water. The smear was decolorized with alcohol and rinsed immediately with tap water. The smear was then counterstained with safranin for 1 minute, washed off with tap water, and allowed to air dry at room temperature. The stained smear was examined under the microscope using the 100x objective with immersion oil. Gram-positive organisms retained the primary stain (blue or purple, crystal violet), while Gram-negative organisms picked up the red or pink stain of the safranin.

K. Biochemical Tests

Biochemical tests were carried out to identify the isolates. The biochemical tests conducted included catalase, oxidase, citrate, indole, methyl red, Voges-Proskauer, motility, coagulase, and triple sugar iron tests.

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IV. RESULTS

Table.1. Proximate Composition of Agricultural Residues (Sawdust, Corncob, Cassava Peel, and Plantain Peel) and Cultivated Oyster Mushrooms (*Pleurotus ostreatus*)

Composition	Sawdust	Corn Cub	Cassava Peel	Plantain Peel	*Pleurotus Ostreatus*
Moisture content (%)	54.50 ±1.15^{a}	16.50 ±1.15^{b}	8.60 ±1.15^{c}	7.35 ±1.14^{d}	89.43 ± 1.06^{e}
Ash content (%)	2.30 ± 0.02^{a}	2.85 ± 0.01^{b}	7.82 ± 0.00^{c}	11.32 ± 0.01^{d}	0.58 ± 0.01^{e}
Fat content (%)	0.10 ± 0.01^{a}	1.05 ± 0.03^{a}	5.40 ± 0.01^{a}	9.03 ± 0.66^{b}	0.34 ± 0.01^{e}
Fibre content (%)	39.84 ± 0.00^{a}	63.52 ± 0.00^{b}	38.27 ± 0.01^{c}	25.80 ± 0.01^{d}	3.94 ±0.01^{e}
Protein content (%)	1.75 ± 0.01^{a}	4.38 ± 0.00^{b}	4.38 ± 0.01^{c}	10.32 ± 0.10^{d}	2.88 ± 0.02^{e}
Carbohydrate content (%)	1.57 ± 0.01^{a}	11.70 ± 0.12^{b}	35.54 ± 0.01^{c}	35.40 ± 0.00^{c}	2.25 ± 0.01^{e}

Values are presented in mean ± SEM. N=3. Mean values with same alphabet on same row have no statistically significant difference at p≤0.05

Table 2: Phytochemical Composition of Substrates (Sawdust, corn cub, cassava peel and plantain peel) and *Pleurotus ostreatus* cultivated on Agricultural residue

Composition	Sawdust	Corn Cub	Cassava Peel	Plantain Peel	*Pleurotus Ostreatus*
Tannin (mg/kg)	0.60 ± 0.02^{a}	1.26 ± 0.11^{b}	0.32 ± 0.00^{c}	0.15 ± 0.01^{d}	0.53 ± 0.01^{a}
Flavonoid (mg/kg)	0.12 ± 0.01^{a}	1.72 ± 0.10^{b}	1.24 ± 0.01^{c}	5.86 ± 0.21^{d}	4.18 ± 0.01^{e}
Alkaloid (mg/kg)	0.40 ± 0.01^{a}	2.04 ± 0.01^{b}	11.68 ± 0.01^{c}	5.28 ± 0.01^{d}	4.07 ± 0.11^{e}
Phytate (mg/kg)	ND	9.77 ± 0.01^{a}	3.11 ± 0.30^{b}	2.44 ± 0.01^{c}	3.65 ± 0.01^{d}
Saponin (mg/kg)	2.50 ± 0.12^{a}	And ^{b}	10.50 ± 0.12^{c}	1.40 ± 0.12^{d}	1.02 ± 0.01^{e}
Cyanogenic glycoside (mg/kg)	0.17 ± 0.04^{a}	0.17 ± 0.04^{a}	2.00 ± 0.12^{b}	2.00 ± 0.12^{b}	0.17 ± 0.04^{a}
Oxalate (mg/kg)	0.90 ± 0.12^{a}	2.50 ± 0.12^{b}	3.20 ± 0.12^{c}	12.25 ± 0.03^{d}	0.55 ± 0.01^{e}

Values are presented in mean ± SEM. N=3. Mean values with same alphabet on same row have no statistically significant difference at p≤0.05

Table 3: Mineral Composition of Substrates (Sawdust, corn cub, cassava peel and plantain peel) and *Pleurotus ostreatus* cultivated on Agricultural residue

Composition	Sawdust	Corn Cub	Cassava Peel	Plantain Peel	*Pleurotus Ostreatus*
Ca (mg/kg)	45.95 ± 0.01^{a}	1.90 ± 0.12^{b}	162.92 ± 0.01^{c}	26.40 ± 0.01^{d}	7.79 ± 0.01^{e}
Mg (mg/kg)	146.75 ± 0.01^{a}	13.90 ± 0.12^{b}	319.15 ±0.01^{c}	ND	78.22 ± 0.01^{d}
Fe (mg/kg)	134.10 ± 0.12^{a}	93.95 ± 0.01^{b}	50.40 ± 0.12^{c}	ND	8.39 ± 0.01^{d}
Na (mg/kg)	24.85 ± 0.01^{a}	192.50 ± 0.12^{b}	3.68 ± 0.01^{c}	50.24 ± 0.01^{b}	58.02 ± 0.04^{d}
Zn (mg/kg)	7.45 ± 0.01^{a}	ND	0.33 ± 0.01^{b}	0.30 ± 0.01^{b}	2.61 ± 0.01^{c}
K (mg/kg)	3159.05 ± 0.01^{a}	4829.55 ± 0.01^{b}	676.91 ± 0.01c	648.85 ± 0.01^{d}	618.34 ± 0.01^{e}
P (mg/kg)	17.39 ± 0.01^{a}	18.13 ± 0.09^{b}	6.50 ± 0.12^{c}	11.17 ± 0.01^{d}	22.23 ± 0.01^{e}

Values are presented in mean ± SEM. N=3. Mean values with same alphabet on same row have no statistically significant difference at p≤0.05

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Table 4: Microbial Load in Substrates and Cultivated *Pleurotus ostreatus*

Sample	Total Heterotrophic Bacteria	Total Coliform count	Staphylococcus count	Total Fungi count
Sawdust	TNTC	-	3.0×10^5	2.0×10^5
Substrate				
Corncob	1.0×10^6	-	1.0×10^5	-
substrate				
Cassava	2.6×10^6	-	-	-
Substrates				
Plantain	5.5×10^6	-	-	-
substrate				
Pleurotus ostreatus	2.0×10^5	-	-	2.0×10^5

Table 5: Microbial isolates from residues (Sawdust, corn cub, cassava peel and plantain peel) and *Pleurotus ostreatus* cultivated on Agricultural residue

Sample	Probable Microorganisms
Sawdust	*Staphylococcus* sp, *Aspergillus* sp
Corncob	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Aspergillus* sp
Cassava	*Enterococcus* sp, *Staphylococcus* sp, *Pseudomonas* sp
Plantain	*Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp
Pleurotus ostreatus	*Bacillus* sp, *Aspergillus* sp

Table 4.5: Biochemical and Morphological Characterization of Bacteria Isolated from Substrates (Sawdust, corn cub, cassava peel and plantain peel) and *Pleurotus ostreatus* cultivated on Agricultural residue

Samples	Catalase	Citrate	Coagulase	Oxidase	Indole	Urease	Motility	Lactose	Glucose	Sucrose	MR	VP	TSIA				Elevation	Edge	Shape	Surfaces	Pigmentation	Cell shape	Gram reaction	Isolated Organism
													Butt	Slant	H_2S	Gas								
Sawdust substrate	+	-	+	-	-	+	-	+	+	+	+	+	Y	Y	-	+	Raised	Entire	Round	Smooth	Cream	cocci	+	*Staphylococcus* sp
Corncob substrate	-	-	-	-	-	+	+	-	-	-	-	+	Y	Y	-	-	Raised	Entire	Round	Smooth	Yellow	Rods	+	*Enterococcus* sp
	-	-	-	+	-	+	-	-	-	-	-	+	Y	Y	-	-	Flat	Entire	Round	Smooth	White	Rods	+	*Bacillus* sp
	+	-	+	-	-	+	-	+	+	+	+	+	Y	Y	-	+	Raised	Entire	Round	Smooth	Cream	cocci	+	*Staphylococcus* sp

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Plantain peel substrate	-	-	-	-	-	+	+	-	-	-	-	+	Y	Y	-	-	Raised	Entire	Round	Smooth	Yellow	Rods	+	*Enterococcus* sp
	-	-	-	+	-	+	-	-	-	-	-	+	Y	Y	-	-	Flat	Entire	Round	Smooth	White	Rods	+	*Bacillus* sp
	+	-	+	-	-	+	-	+	+	+	+	+	Y	Y	-	+	Raised	Entire	Round	Smooth	Cream	cocci	+	*Staphylococcus* sp
Cassava peel substrate	-	-	-	+	-	+	-	-	-	-	-	+	Y	Y	-	-	Flat	Entire	Round	Smooth	White	Rods	+	*Bacillus* sp
	+	-	+	-	-	+	-	+	+	+	+	+	Y	Y	-	+	Raised	Entire	Round	Smooth	Cream	cocci	+	*Staphylococcus* sp
Pleurotus ostreatus	-	-	-	+	-	+	-	-	-	-	-	+	Y	Y	-	-	Flat	Entire	Round	Smooth	White	Rods	+	*Bacillus* sp

V. DISCUSSION

The proximate composition analysis (see table 1) revealed significant variations in the nutrient profile between the agricultural residues and the cultivated *Pleurotus ostreatus*. The mushrooms exhibited a significantly higher moisture content (89.43%) compared to the substrates, which ranged from 7.35% (plantain peel) to 54.50% (sawdust) The significant differences observed in moisture content across the substrates and the oyster mushroom at $p \leq 0.05$ emphasize the impact of substrate selection on the water availability during cultivation and mushroom require a moist environment to thrive, as moisture facilitates enzymatic activity and nutrient absorption during growth (Patil et al., 2020). Conversely, the mushroom had a significantly lower ash content (0.58%) compared to the substrates, which ranged from 1.90% (corn cob) to 11.32% (plantain peel). While the fat content was relatively low across all samples, the fiber content was significantly higher in the substrates, particularly corncob (63.52%) and sawdust (39.84%). The protein content was notably higher in the mushrooms (2.88%) compared to the substrates. The carbohydrate content varied, with the substrates generally having higher levels than the mushrooms. The values obtained for the proximate composition was statistically significant at $p \leq 0.05$. Studies of Dimopoulou, et al. (2022) & Stilinovic, et al., (2020) also corroborates that Mushrooms possess nutritional value due to the various nutrients they contain, such as protein, including essential amino acids, essential fatty acids, carbohydrates, dietary fiber, vitamins, and minerals

The phytochemical analysis (see table 2) highlighted the presence of various bioactive compounds. The mushroom exhibited a higher content of flavonoids (4.18 mg/kg) compared to most substrates, except for plantain peel (5.86 mg/kg). Additionally, the mushroom contained significant levels of alkaloids (4.07 mg/kg), suggesting potential medicinal properties. The presence of phytate, a mineral-binding compound, was detected in both the substrates and the mushroom, with corn cob having the highest content (9.77 mg/kg) This conforms to the studies of (Chun et al., 2021). That revealed a plethora of bioactive compounds in mushrooms.

The mineral analysis (see table 3) indicated that the substrates, particularly cassava peel, were rich in certain minerals. Cassava peel had the highest calcium (162.92 mg/kg) and magnesium (319.15 mg/kg) content. Sawdust was the richest in iron (134.10 mg/kg) and zinc (7.45 mg/kg). However, the cultivated mushrooms also contained significant amounts of these minerals, suggesting their potential as a good source of essential nutrients.

The microbial load analysis (see table 4) revealed a high bacterial load in the substrates, particularly sawdust. The cultivation process effectively reduced the initial microbial contamination, resulting in a lower microbial load in the cultivated mushrooms.

The isolation of microorganisms confirmed the presence of various bacterial and fungal species, including potential pathogens like *Staphylococcus* sp. and *Enterococcus* sp. Strict hygiene practices are crucial to minimize the risk of foodborne illnesses. *Bacillus* sp. and *Aspergillus* sp. were also isolated, indicating their potential role in substrate decomposition and secondary metabolite production.

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VI. CONCLUSION

The results of this study demonstrate the potential of agricultural residues as substrates for the cultivation of *Pleurotus ostreatus*. The mushrooms cultivated on these substrates exhibited a good nutritional profile and were relatively low in microbial contamination. However, the high microbial load of the substrates underscores the importance of proper sterilization and hygienic practices during cultivation. Further research is needed to optimize the cultivation process and to explore the potential of these mushrooms as a functional food and in biotechnology.

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