Assessment of Proximate and Antioxidants Profile oF *Pleurotus ostreatus* (Jacq) P. Kumm. Cultivated on Selected Medicinal Plant Leaves

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Abstract: Oyster mushrooms can synthesize great number of minerals, and secondary metabolites that possess antioxidant activities which play important role in maintaining human health. This study investigated the proximate, mineral and antioxidant properties of *Pleurotus ostreatus* cultivated on medicinal leaves such as Neem leaves (*Azadirachta indica*), Moringa leaves (*Moringa oleifera*), Lemon grass (*Cymbopogon citratus*), Scent leaves (*Ocimum gratissimum*) and oil palm fibre as control. Spawns of *P. ostreatus* were cultivated on medicinal leaves (substrates), Proximate and mineral content were determine using standard chemical and spectrophotometric method. Results showed that the proximate composition (%) of *P. ostreatus* cultivated on *A. indica*, *M. oleifera* and *C. citratus* had highest values of 15.20 ± 0.09^{d}, 17.35 ± 0.42^{e} and 4.47 ± 0.06^{c} for moisture, protein, and lipid contents respectively while highest 45.90 ± 0.53^{a}, 22.30 ± 0.43^{b} and 15.97 ± 0.11^{c} were recorded for carbohydrate, crude fibre and total ash contents respectively in *P. ostreatus* cultivated on oil palm. Mineral composition (mg/100g) revealed that *P. ostreatus* cultivated on *M. oleifera* had highest 11.12, 17.87, 8.22 and 7.34 for Sodium, Calcium, Iron and Zinc respectively. Total phenolic, vitamin C content, Ferric Reducing ability, hydroxyl radicals scavenging ability, Iron chelating ability, Nitric oxide (%) activities, free radical scavenging activity and ABTS scavenging ability had highest values of 33.348mg, 91.252 mg/100g, 2.808 mg/ml, 82.014% 42.616%, 72.180%, 67.256% and 0.024 mg/ml respectively in aqueous extracts while flavonoids contents had highest 0.804mg in ethanoic extracts of *P. ostreatus* cultivated on *M. oleifera* leaves. It can be concluded that substrates used in this study improved the nutraceutical properties of *P. ostreatus.*

Key word: *Pleurotus ostreatus*, medicinal plant leaves, proximate, mineral content and antioxidant

INTRODUCTION

Pleurotus is an edible mushroom that may be known as the oyster, abalone, or tree mushroom, and are among the most commercially cultivated mushrooms in the world (Mahari *et al*., 2020). They're regarded as functional foods which give health benefits. Mushrooms such as *Pleurotus ostreatus* are widely consumed for their therapeutic and nutritional benefits, and have been reported to contain significant levels of minerals in addition to bioactive compounds that exert antioxidant effects, similar as antitumour, antibacterial, antiviral, haematological and immunomodulating treatments (Vetter, 2023) the remedial effect had been linked to the presence of bioactive composites in mushrooms. Some of these bioactives include glycolipids, composites deduced from shikimic acid, sweet phenols, adipose acid derivations, polyacetylamine, polyketides, sesquiterpenoids, and numerous other substances of different origins. Mushroom secrete extracellular enzymes to digest organic materials to take up nutrient from their substrates (Raman *et al*., 2021), and are suitable to synthesize a great number of secondary metabolites that retain antioxidant conditioning (Ling *et al*., 2017), This has helped to prevent diseases associated with oxidative stress such as inflammation, cancer, aging, cardiovascular diseases, diabetes, and a variety of others (Shaida, 2019). Oxidative stress on mortal health have come a serious issue to contend with, most people now calculate on traditional drug for their primary health care requirements, and utmost of this remedy involves the use of factory excerpts and their active factors. Oxidative stress is a well-known miracle caused by an imbalance between conformation and junking of reactive micro-molecules similar as reactive oxygen species (ROS) (Bhatia *et al*., 2014). These reactive motes may be produced in all mammalian cells through normal cellular metabolism or activation of membrane-bound enzymes in response to exogenous

stimulants (Li and Shah, 2015). Antioxidants play an important part in maintaining mortal health due to their capability to scavenge free revolutionaries in the bodies (Vetter, 2023), thus, consumption of supplemented antioxidants in our diets similar as mushrooms is veritably important to offer acceptable security. Presently, researchers are looking for natural sources of substances which have the capability to capture free revolutionaries, to stimulate the vulnerable system and bring numerous other health benefits. The different substrates used in cultivating mushrooms do have effect on the functional, organoleptic and chemical parcels of mushrooms (Mihai *et al*., 2022). The primary aim of this study was to assess the proximate, mineral and antioxidant properties of *Pleurotus ostreatus* cultivated on some medicinal leaves such as, Neem leaves (*Azadirachta indica*), Moringa leaves (*Moringa oleifera*), Lemon grass (*Cymbopogon citratus*), Scent leaves (*Ocimum gratissimum*) and oil palm fibre as control.

MATERIALS AND METHODS

Preparation of substrates: The substrates (Neem leaves (*Azadirachta indica*), Moringa leaves (*Moringa oleifera*), Lemon grass (*Cymbopogon citratus*) Scent leaves (*Ocimum gratissimum*) and oil palm fibre) were collected from Owena Ondo State, South Western part of Nigeria. They were sundried and broken into smaller pieces using mortar and pestle. It was further pulverized into fine powdered using a mill machine (5657 HAAN 1 TYPE ZM1, Retsch GmbH, Haan, Germany). The substrates were moistened with water to a concentration of 60%. Five (5) hundred grams (500 g) of the substrate was packed into polypropylene bags and sealed with paper with the aid of polyvinyl rings. The bags were then autoclaved for 15minutes at 121 ^{o} C (Fasoranti *et al*., 2019).

Source of Spawn: Spawns of *Pleurotus ostreatus* were obtained from Federal Institute of Industrial Research (FIIRO), Oshodi Lagos, Nigeria.

Cultivation of Pleurotus ostreatus: Substrates were prepared and allowed to cool over-night and then inoculated with 5 g of *Pleurotus ostreatus* spawn. The bags were then kept in the dark with 85% relative humidity for 38 days for spawn run. The bags were then uncapped and transferred to the fruiting room to allow the normal development of the fruit bodies. The harvested mushrooms were air-dried at room temperature (27 ^{o}C) for ten days, ground into powder according to the method of Ashraf *et al.* (2013).

Determination of proximate and mineral content of cultivated P. ostreatus: The proximate analysis of the cultivated *P. ostreatus* were carried out according to the method of AOAC (2005). The minerals of the mushroom were analyzed using the samples obtained by ashing at 550 ^{o}C and dissolving in HCl (25 ml) and 5 % lanthanum chloride (2 ml), boiled, filtered and make it up to standard volume with deionized water (AOAC 2005). Na, K, Mg, Ca, Fe and Zn were determined with a Buck Atomic Absorption Spectrometer (Buck Scientific, Model 200A/200, Inc. East Norwalk, Connecticut, U.S.A).

Moisture content determination: Moisture amount was determined by keeping weighed quantity of sample in a thermostat - controlled oven at 80 ^{o}C for 2 hours. The dry weight of each sample was taken on a weighing balance. The percentage of the moisture content and dry mater was then calculated by the formula as presented below:

$$\text{Moisture content (\%)} = \frac{\text{Change in weight(intial}-\text{final weight)}}{\text{intial weight before drying}} \times 100$$

Dry Matter (%) = 100 – Moisture (%)

Determination of protein: The sample was weighed into a standard 250 ml flask containing 2 g of copper sulphate, 2 g of sodium sulphate and 5ml of Sulphuric acid. The digestion flask was placed on the heating mantle and was heated at 50 ^{o}C for 2 h. On cooling, the solution was transferred to a 100 ml standard flask and titrated against hydrochloric acid and standard solution of Sodium hydroxide (NaOH)

solution using methyl orange dissolved in 25 ml of 4 % boric acid as indicator until a yellow colour was obtained. A blank titrated was also done in the same method. The crude protein was calculated using the formula below:

$$\text{Crude protein (\%)} = \frac{(Va - Vb\ X\ 1.4007)}{W\ X\ 100} \times 6.25$$

Determination of crude lipid: About 5 g of the sample was weighed into the thimble and dried in an oven at 102 ^{o}C for 5 h. On cooling, the thimble was placed in a soxhlet extractor; 90 ml of petroleum ether (a solvent) was used for the extraction. The unit for extraction was assembled over a water bath. After extraction, the solvent was evaporated for recovery, the contents left after evaporation was dried in the oven at 102 ^{o}C for 2 h. On cooling, the flask and its contents were weighed respectively. The crude lipid was then calculated using the formula below:

$$\text{Crude lipid (\%)} = (W2 - W1) \times \frac{100}{S}$$

Where W1 = Weight of the empty flask, W2 = Weight of flask and content, S = Weight of sample

Total carbohydrate estimation: Available carbohydrate content in the sample was determined. This was calculated as the difference obtained after subtracting the lipid, ash and fiber values from the total dry matter using the formula below:

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 fiber

Determination of crude fiber: The samples were weighed and 200 ml of 5 HCl was transferred to a beaker. The solution was heated in a water bath at 90 ^{o}C for 2 h, it was filtered and washed back into a beaker with 200 ml of NaOH solution and reheated for 2 h at the same temperature. The mixture was filtered, washed thoroughly with hot water, alcohol and ether followed by drying at 120 ^{o}C. On cooling, the mixture was weighed, ignited in a muffle, cooled in a desiccator and weighed again. The loss in weight was recorded as the crude fiber for both samples.

Determination of total ash: Total ash content was determined by igniting previously dried sample in a muffle furnace at 500 °C for 4 h. The ash content was calculated by the equation below:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of dried sample}} \times 100$$

Preparation of mushrooms extracts: Mushroom sample (8 g) was soaked in 400 ml of sterile distilled water and 400ml of ethanol respectively and kept for three days with occasional shaking. Each portion was then filtered using Whatman filter paper No 1. The filtrates were concentrated and the extracts (residues) were stored at 4 °C in a sterile container for future use Handa *et al*. (2008).

Antioxidant activity of P. ostreatus: Flavonoid content, total phenol content, vitamin C, FRAP, OH* radical scavenging ability, Fe^{2+}chelating ability, nitric oxide scavenging ability, DPPH radical scavenging, ABTS* scavenging ability were analysed according to technique described by Oyetayo *et al*. (2009).

Determination of total flavonoids: Total flavonoid content was determined as described by Jia *et al*. (1998). A portion (0.25 ml) of various extracts was diluted with 1.25 ml of distilled water. A portion (75 µl) of a 5% $NaNO_2$ solution were added and after 6 min 150 µl of a 10% $AlCl_3.H_2O$ were added and mixed. After 5 min, 0.5 ml of 1 M NaOH was added. The absorbance was measured immediately against the reagent blank at 510 nm. Catechin was used as the reference standard. The total flavonoid content is expressed as mg of catechin equivalents (CAE)/ g of extract.

Determination of total phenolics: The total phenolics in various mushroom extracts were measured according to the method of Singleton and Rossi (1965) with some modifications. One mL of the sample was mixed with 1 ml of Folin- Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated Na_2CO_3 (35 %) was added to the mixture and it was made up to 10 ml by adding deionized distilled water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured at 725 nm against the reagent blank. Gallic acid was used as the reference

standard. The total phenolic content is expressed as mg of gallic acid equivalents (GAE)/g of extract.

Determination of vitamin C: The AOAC (1995), 2, 6-dichlorophenol titrimetric method was used to determine vitamin C. About 2 g of the sample was extracted by homogenizing in acetic acid solution. Vitamin C standard solution was prepared by dissolving 50mg standard ascorbic acid tablet in 100 ml volumetric flask with water. The solution was filtered and 10 ml of the clear filtrate was added into a conical flask in which 2.5 ml acetone has been added. This was titrated with indophenol dye solution (2, 6 dichlorophenol indophenol) for 15 seconds. The procedure was followed for the standard as well. Calculation:

mg ascorbic acid/1g sample = C ×V× (DF/WT)

Where: C = mg ascorbic acid 1 ml dye, V = volume of dye used for titration of diluted sample, DF = dilution factor, WT = weight of sample (g)

Ferric reducing antioxidant power (FRAP): FRAP assay was done according to the method of Dong *et al*. (2014). The oxidant in the FRAP assay was prepared by mixing 2,4,6-tri(2-pyridyl)-s-triazine (10 mM in 40 mM HCl, 2.5 ml), acetate buffer (0.3 M pH 3.6, 25 ml), and 2.5 ml of $FeCl_3 6H_2O$ (20 mM). The combination prepared by mixing all these reagents is known as FRAP reagent. An amount of 1.8 ml of freshly prepared FRAP reagent, 100 ml of ethanoic extract and 100 ml of distilled water was added to the test tube. The tubes were then incubated at 37 °C for 30 minutes and absorbance was taken at 595 nm in spectrophotometer (Perkin Elmer UV/VIS).

Hydroxyl radical scavenging ability: The determination of scavenging effect on hydroxyl radical was carried out as described in Oyetayo *et al*. (2009). The reaction mixture in a final volume of 1.0 ml, containing 0.4 ml of 20 mmol/ml sodium phosphate buffer (pH 7.4), 0.1 ml of 0.125–2 mg/ml extracts, 0.1 ml of 60 nmol/L deoxyribose, 0.1 ml of 10 mmol/L hydrogen peroxide, 0.1 ml of 1 mmol/L ferric chloride, 0.1 ml of 1.04 mmol/L EDTA and 0.1 ml of 2 mmol/L ascorbic acid was incubated at 37 °C for 1 hour. Solutions of $FeCl_2$ and ascorbic acid were made up immediately before use in de-ionised water. The reaction was stopped by adding 1 ml of 17 mmol/L thiobarbituric acid (TBA) and 1 ml of 17 69 mmol/L trichloroacetic acid (TCA). The mixture was boiled for 15 min, cooled in ice and then the absorbance measured at 532 nm using a UNICO 2100 spectrophotometer (As). BHT was used as positive control while distilled water in place of extracts or BHT was used as blank (Ab) and the sample solution without adding deoxyribose as sample blank (Asb).

$$\text{Scavenging activity (\%)} = \frac{\text{Ab}-(\text{As}-\text{Asb})}{\text{Ab}} \times 100$$

Where Ab, As and Asb are the absorbance at 532 nm of the blank, the extract or BHT and the sample blank without deoxyribose, respectively.

Ferrous ion chelating ability: The ability of the mushroom extract to chelate ferrous ions was estimated by the method of Dinis *et al*. (1994). Briefly, 2 ml of various concentrations of the extracts in methanol were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml). The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage inhibition of ferrozine-Fe2+ complex formation was calculated as [(A0-A1)/A0] ×100, where, A0 was the absorbance of the control, and A1 of the mixture containing the extract or the absorbance of a standard solution.

Nitric Oxide scavenging assay: Mushroom extract was dissolved in distilled water for quantification. Sodium Nitroprusside (5 mM) in standard phosphate buffer saline (0.025 m, pH 7.4) was incubated with different concentration of methanol extract and the tubes were incubated at 29 ^{o}C for 3 h. Control experiment was conducted in an identical manner without the test compounds but with same amount of buffer. Sodium nitroprus side in aqueous solution at physiological pH spontaneously generates Nitric oxide which interacts with oxygen to produce Nitrite ions. After 3 hours, the

incubated samples were diluted with 1 ml of Griess reagent. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthylethylenediamine hydrochloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standardized in comparison to methanol extract. The percentage inhibition was calculated using following formula and graph was plotted compared to standard.

DPPH radical scavenging activity: The scavenging effect of mushroom extract on DPPH radicals was determined according to the method of Shimada *et al*. (1992). Various concentrations of the mushroom sample (4 ml) were mixed with 1 mL of ethanoic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. Absorbance of the radical without antioxidant was used as control. The amount of sample necessary to decrease the absorbance of DPPH by 50 % (EC50) was calculated graphically. The percentage inhibition was calculated according to the formula: (A0-A1)/A0] ×100, where, A0 was the absorbance of the control and A1 was the absorbance of the sample.

ABTS radical cation scavenging activity: The ABTS radical cation scavenging activity was performed with slight modifications described by Re *et al*. (1999). The ABTS radicals were produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Prior to use, the solution was diluted with ethanol to get an absorbance of 0.700 ± 0.025 at 734 nm. Free radical scavenging activity was assessed by mixing 10 µl of test sample with 1.0 mL of ABTS working standard in a micro cuvette. The decrease in absorbance was measured exactly after 6 min. The percentage inhibition was calculated according to the formula: [(A0-A1)/A0] ×100, where, A0 was the absorbance of the control, and A1 was the absorbance of the sample.

Statistical analysis: The data obtained were statistically analyzed using Analysis of Variance (ANOVA) mean separation and tests of significance were carried out by Duncan's New Multiple Range Test (DMRT) at $p<0.05$.

RESULTS

The proximate composition (%) of *P. ostreatus* cultivated on *A. indica* had highest moisture composition (15.20%) while oil palm fibre had least (4.30%). *P. ostreatus* cultivated on *M. oleifera* had highest crude protein (17.35%) while Oil palm fibre had least (7.90%). Furthermore, highest lipid (4.47%) composition was recorded in *P. ostreatus* cultivated on *C. citratus*. *P. ostreatus* cultivated on oil palm fibre had highest carbohydrate composition (45.90%) and *A. indica* had least (38.33%). *P. ostreatus* cultivated on oil palm fibre had highest crude fibre content (22.30%) while least (18.91%) was found in *M. oleifera*. The highest (15.97%) total ash contents were detected from *P. ostreatus* cultivated on oil palm fibre while least (8.26%) was found in *P. ostreatus* cultivated on *M. oleifera* (Table 1). Mineral composition (mg/100g) of *P. ostreatus* cultivated on different medicinal plants revealed that *P. ostreatus* cultivated on *M. oleifera* had highest values of 11.12, 17.87, 8.22 and 7.34 for Sodium, Calcium, Iron and Zinc respectively, *O. gratissimum* had highest (21.24) and (12.33) for Potassium and Magnesium respectively, oil palm fibre had least for all the minerals (Table 2).

The antioxidant properties of *P. ostreatus* cultivated on the selected medicinal plants revealed flavonoids contents had highest (0.804mg) in ethanoic extracts of *P. ostreatus* cultivated on *O. gratissimum* and least (0.083mg) occurred in aqueous extracts of *P. ostreatus* cultivated on oil palm fibre (Figure 1). The total phenolic contents had highest (33.348mg) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (2.864mg) occurred in ethanoic extract of *P.*

ostreatus cultivated on oil palm fibre (Figure 2). Vitamin C composition had highest (91.252 mg/100g) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (6.312mg/100g) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 3). Ferric Reducing ability had highest (2.808 mg/ml) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and *A. indica* and least (0.284 mg/ml) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 4). The hydroxyl radicals scavenging ability of *P. ostreatus* had highest (82.014%) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (24.612%) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 5). Iron chelating ability of *P. ostreatus* had highest (42.616%) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (3.523%) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 6). Nitric oxide (%) activities of *P. ostreatus* had highest (72.180%) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (4.806%) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 7). The free radical scavenging activity of *P. ostreatus* had highest (67.256%) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and least (3.723%) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 8). ABTS scavenging ability of *P. ostreatus* had highest (0.024 mg/ml) in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* and *A. indica* and least (0.004 mg/ml) occurred in ethanoic extract of *P. ostreatus* cultivated on oil palm fibre (Figure 9).

Table 1: Proximate content of *P. ostreatus* cultivated on medicinal leaves

Substrates	Moisture content	Protein	Lipid	Carbohydrate	Crude fibre	Total ash
A. indica	15.20±0.09^{d}	14.19±0.38^{d}	3.12±0.20^{c}	38.33±0.19^{a}	20.48±0.33^{b}	8.68±0.14^{a}
M. oleifera	10.91±0.14^{c}	17.35±0.42^{e}	3.93±0.15^{c}	40.64±0.22^{a}	18.91±0.13^{b}	8.26±0.12^{a}
O. gratissimum	11.89±0.33^{c}	12.94±0.21^{d}	3.87±0.17^{c}	39.26±0.06^{a}	19.30±0.18^{b}	12.74±0.04^{c}
C. citratus	7.30±0.09^{a}	9.71±0.03^{a}	4.47±0.06^{c}	42.10±0.17^{a}	21.60±0.30^{b}	14.82±0.34^{c}
Oil palm fibre	4.30±0.15^{a}	7.90±0.23^{a}	3.63±0.23^{c}	45.90±0.53^{a}	22.30±0.43^{b}	15.97±0.11^{c}

Key: Data are represented as mean ± standard deviation, n=3 with the same superscript down the row are not significantly different ($p<0.05$)

Table 2: Mineral composition of *P. ostreatus* cultivated on medicinal leaves (mg/100g)

Substrates	Sodium	Potassium	Calcium	Magnesium	Iron	Zinc
A. indica	5.63±0.05^{a}	12.52±0.65^{c}	5.36±0.11^{a}	6.23±0.43^{d}	3.54±0.55^{d}	4.76±0.29^{a}
M. oleifera	11.12±0.45^{c}	20.32±0.36^{b}	17.87±0.21^{b}	11.45±0.50^{a}	8.22±0.10^{b}	7.34±0.06^{a}
O. gratissimum	8.93±0.11^{a}	21.24±0.05^{b}	15.06±0.14^{b}	12.33±0.13^{a}	5.65±0.56^{d}	4.86±0.15^{a}
C. citratus	6.44±0.56^{a}	11.42±0.44^{c}	7.15±0.22^{a}	4.23±0.34^{d}	2.44±0.61^{c}	2.13±0.20^{c}
Oil palm fibre	2.28±0.23^{b}	9.59±0.18^{c}	0.95±0.32^{c}	1.12±0.43^{c}	1.1±0.06^{c}	0.23±0.43^{c}

Data are represented as mean ± standard deviation, n=3 with the same superscript down the row are not significantly different ($p<0.05$).

Plate 1: Mushroom spawn

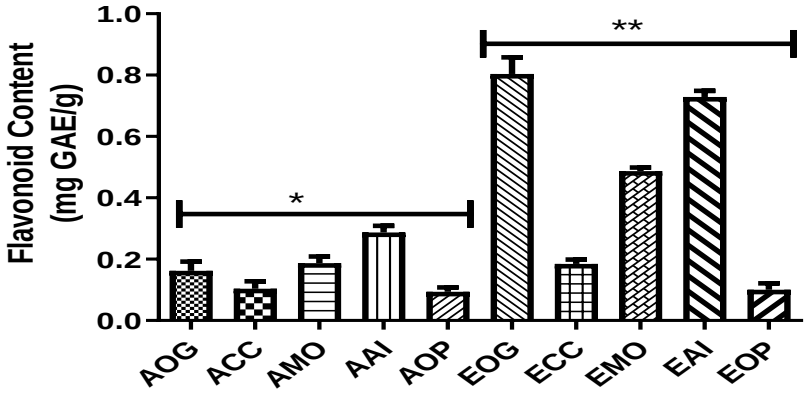

Figure 1: Flavonoid content of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **AOG =: Aqueous extracts of *O gratissimum*, ACC = Aqueous extracts of *C. citratus,* AMO = Aqueous extracts of *M. oleifera,* AAI = Aqueous extracts of *A. indica* AOP = Aqueous extracts of oil palm fibre. EOG = Ethanoic extracts of *O. gratissimum,* ECC = Ethanoic extracts of *C. citratus*, EMO= Ethanoic extracts of *M. oleifera,* EAI = Ethanoic extracts of *A. indica* EOP = Ethanoic extracts of oil palm fibre.

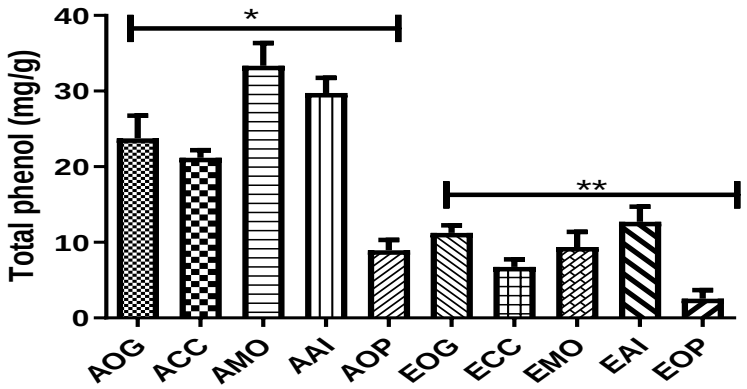

Figure 2: Total phenol content of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

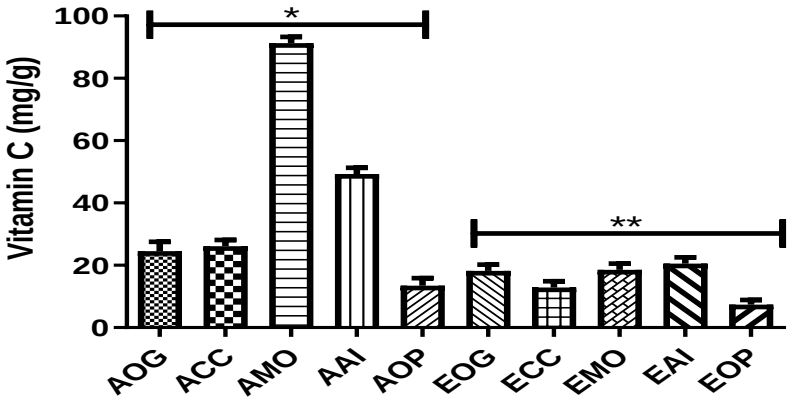

Figure 3: Vitamin C content of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

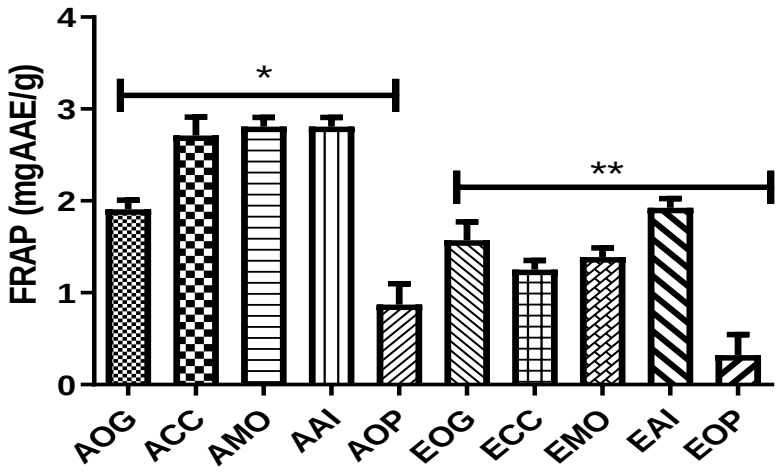

Figure 4: FRAP content of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

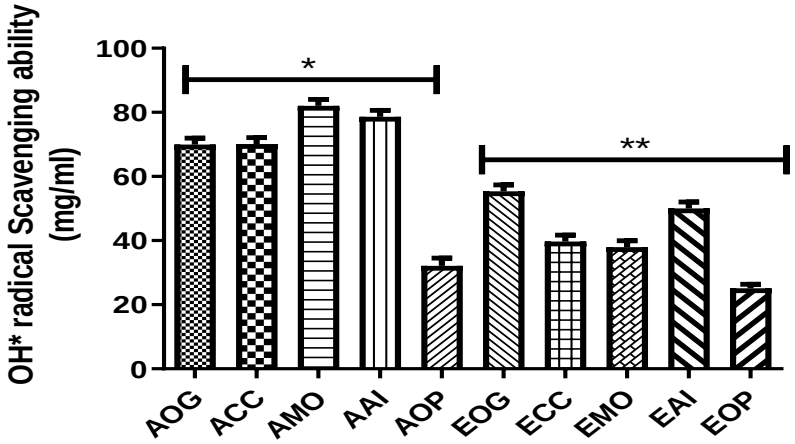

Figure 5: OH^* radical scavenging ability of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

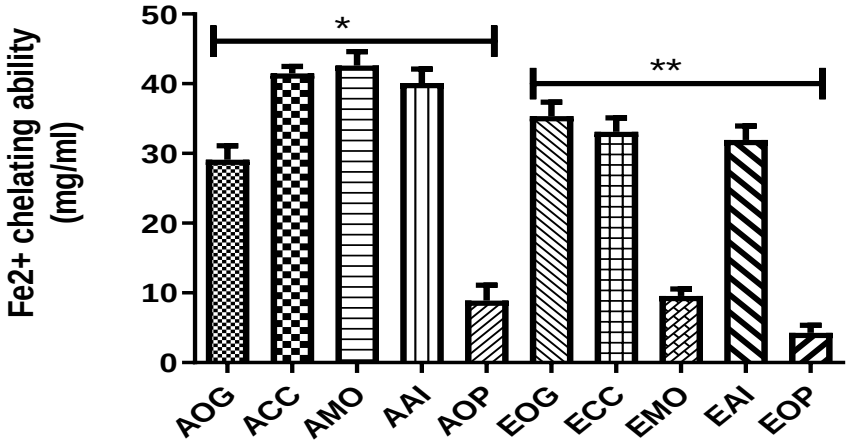

Figure 6: Fe^{2+} chelating ability of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

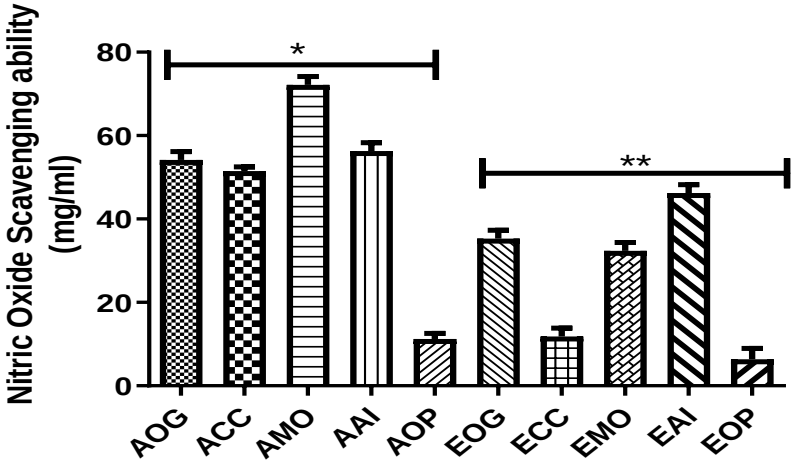

Figure 7: Nitric oxide scavenging ability of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

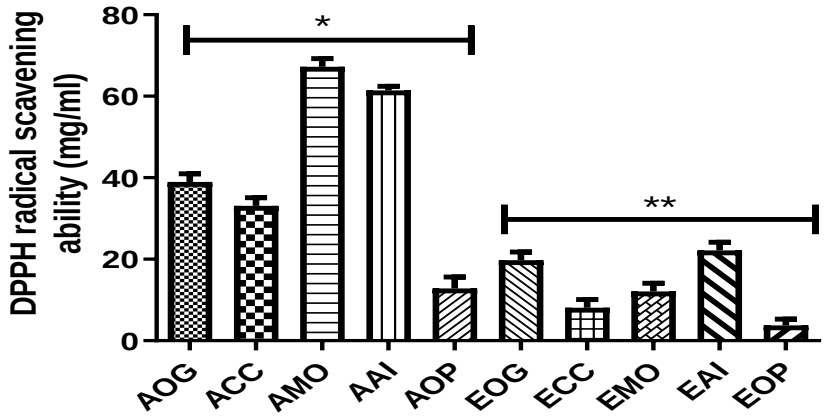

Figure 8: DPPH radical scavenging ability of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

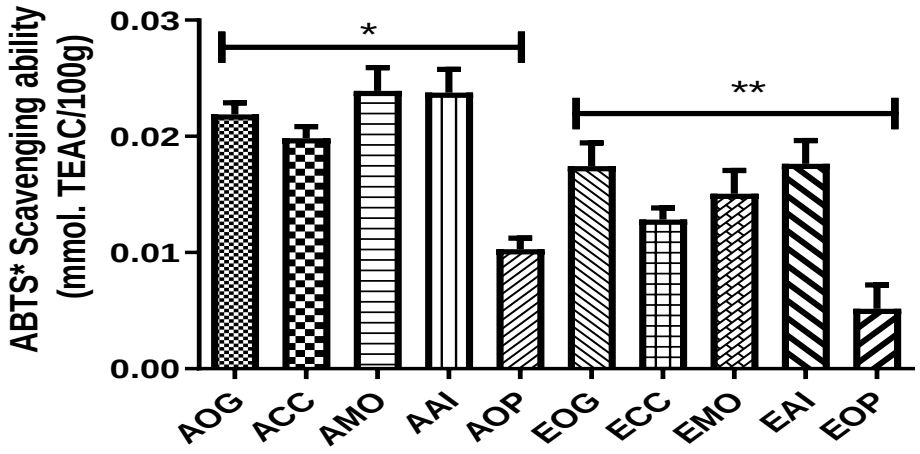

Figure 9: ABTS* Scavenging ability of *P. ostreatus* cultivated on medicinal leaves
Note: * is significantly at (p>0.05) different from **

DISCUSSION

Mushrooms have been found to possess abilities to absorb nutrients which can improve its nutritional value. The result obtained revealed high content of carbohydrate, crude fibre and total ash, this is in agreement with results obtained by Oyetayo and Ariyo (2013), carbohydrate and protein content obtained in this study were higher than that of (Agbagwa *et al*., 2020) where he cultivated *P. ostreatus* on three agro wastes. Low lipid content in this study is similar to the report of (Fosoranti *et al*., 2019) where he cultivated Pleurotus spp. on substrate fortified with Selenium. The high values of protein and carbohydrates on the cultivated *P. ostreatus* were similar to results reported by Nwoko *et al*. (2017). This observation may likely be due to the biosorption potentials of *P. ostreatus* on the various substrates used.

The mineral content of *P. ostreatus* cultivated on *M. oleifera* had highest values for sodium, magnesium, iron and zinc, while *P. ostreatus* cultivated on *O. gratissimum* had highest for potassium and calcium, and those cultivated on oil palm fibre had the least minerals content. Potassium was reported as the most abundant mineral elements in this study, this is similar to the result of (Agbagwa *et al*., 2020), although other minerals obtained in this study were lower. The variations in the levels of elemental composition of these mushrooms may be due to the accumulation and adsorption of these elements from the substrates. Study have shown that mineral elements assist and support physiological, biochemical and metabolic processes (Piska *et al*., 2017). Nevertheless, there is dearth of information on the mineral and vitamin compositions of *P. ostreatus* grown on sawdust, wood ash and cassava bran.

Antioxidant components are responsible for defending our body against free radicals, and it is known that low levels of antioxidants cause oxidative stress and may damage or kill cells (Sharifi-Rad *et al*., 2020), The result obtained revealed that flavonoid content was highest in ethanoic extracts of *P. ostreatus* cultivated on *M. oleifera* compared to its aqueous extracts, the presence of flavonoid in this study has been is reported in different edible mushrooms, e.g., *myricetin, chrysin, catechin, hesperetin, naringenin, naringin, formometin, biochanin, pyrogallol, resveratrol, quercetin, rutin, kaempferol* (Ferreira *et al*., 2009). Total phenol content, vitamin C, FRAP, OH^{*}, Fe^{2+} chelating ability, nitric oxide, DPPH and ABTS also had highest values in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* leaves, it was reported by (Abddullah *et al*., 2012) that at 2.0 mg/m concentration of various mushrooms extracts studied, the aqueous extracts scavenging potential against the OH were all above 50% except for the extract of *Auricularia auricula*, which recorded 39.5%, it was observed that alkaline extracts exhibited lower scavenging activities when compared to aqueous extracts. Also, this is further supported by (Karaman *et al*., 2019) that extracts from the fruiting bodies of two edible mushrooms, *Coprinus comatus* and *C. truncorum* were highly effective in neutralizing OH radicals.

DPPH scavenging ability had highest values in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* leaves, this was different from the report of (Mleczek *et al*., 2018) who reported that *Dictyophora indusjiata* methanolic extract at a concentration of 6.4 mg/mL showed 92.1% DPPH radical scavenging activity, while 63.3–67.8% scavenging activity was recorded with extracts of other mushroom specialties prepared in methanol, variation from this study may be due to different species of mushroom, environmental conditions, cultivation methods, stages of development when harvested, and genetic variation among the strains. Extracts of *P. ostreatus* cultivated on oil palm fibre had least values. The results indicated that good antioxidant activity was observed in aqueous extract of *P. ostreatus* cultivated on medicinal leaves, especially *M. oleifera.* This observation is in

agreement with the result of Effiong *et al*. (2024) who reported that water was the most appropriate solvent for extraction, in her comparison of five extracting solvent on *P. ostreatus*. Similarly, Zhang *et al*. (2012) revealed that the fruiting bodies of *P. ostreatus* exhibited stronger DPPH and superoxide anion radical scavenging activity with increased concentration. ABTS* scavenging ability had highest values in aqueous extracts of *P. ostreatus* cultivated on *M. oleifera* leaves, this is similar to the report of Gargano *et al*. (2017) who worked on methanol extracts made from various mushroom species and observed that the antioxidant potential of aqueous extracts was dependent upon the concentration used; however, lower doses were required to eliminate all the ABTS+ radicals. Aqueous extracts prepared using *Boletus edulis*, *Lentinus edodes*, and *Amanita cesarea* exhibited a very high ABTS+ scavenging capacity that ranged between 85.8% and 92% at significantly low concentrations of 0.14 mg/ml. Also, (Panda *et al*., 2020) differently observed that acidic extracts showed greater scavenging activities compared to the alkaline and aqueous extracts. The primary challenge encountered in this phase maintaining the optimal moisture content (60%) in the substrates and ensuring their sterilization posed significant challenges. Incorrect moisture levels could inhibit mycelial growth, while inadequate sterilization could lead to contamination.

A controlled environment was established for adjusting the moisture content, utilizing precise measurements of water added to the substrates. The substrates were then packed into polypropylene bags and sealed appropriately. Autoclaving at 121°C for 15 minutes, as per Fasoranti *et al*. (2019), ensured the substrates were free from contaminants. Post-autoclaving, the substrates were allowed to cool overnight to prevent thermal shock to the inoculated spawn.

The inoculation process required maintaining sterility to prevent contamination, and creating optimal incubation conditions (dark environment with 85% relative humidity) was essential for successful spawn run and fruit body development. The inoculation process was conducted in a laminar flow hood to maintain sterility. The spawn was carefully measured (5 g) and inoculated into the cooled substrates. For incubation, a dedicated dark room with controlled humidity (85%) was set up using humidifiers and monitoring devices to ensure consistent environmental conditions for 38 days, facilitating a successful spawn run.

Post-harvest, drying the mushrooms at room temperature (27°C) for ten days while preventing mold growth and ensuring complete drying was challenging.

The harvested mushrooms were spread out thinly on clean drying racks to ensure adequate air circulation. Regular monitoring was performed to detect and prevent mold growth. After drying, the mushrooms were ground into powder following the method of Ashraf *et al.* (2013) to ensure uniformity for subsequent analyses.

The successful implementation of this project was achieved through meticulous planning, adherence to protocols, and the adoption of systematic solutions to overcome various barriers.

CONCLUSION

The findings of this study established the effect of different medicinal leaves as substrates on the yield, proximate, mineral content and antioxidant content of *P. ostreatus. P. ostreatus* cultivated on *M. oliefera* exhibited better mineral and antioxidant activities compared with *P. ostreatus* cultivated on the other substrates. Hence, *M. oliefera* may be a good substrate that can enhance the yield and nutraceutical properties of *P. ostreatus*. The findings therefore underscore the significant influence of cultivation substrates on the nutritional and antioxidant properties of *P ostreatus*, providing valuable insights for future research and commercial mushroom cultivation practices.

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