

ORIGINAL RESEARCH ARTICLE

Antifungal and bioactive potential of *Pleurotus ostreatus* cultivated on agro-waste substrates with molecular identification and functional characterizationOlagunju Johnson Adetuwo^{1*}, Soji Fakoya¹, Faith Naomi Ogundana², and
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Abstract

The rising incidence of antifungal resistance among clinically relevant pathogens such as *Candida albicans* and *Aspergillus fumigatus* necessitates the discovery of novel, sustainable bioactive compounds. Edible mushrooms are promising sources of antimicrobial agents; however, the influence of cultivation substrates on their antifungal potential remains insufficiently explored, particularly in tropical systems. This study evaluated the antifungal and bioactive properties of *Pleurotus ostreatus* cultivated on three agro-waste substrates (Gmelina sawdust, oil palm fruit fiber, and cassava peels) in Nigeria. Molecular identification using ITS, LSU, and RPB2 markers confirmed species identity. Extracts were evaluated for antifungal activity against *C. albicans* and *A. fumigatus*, alongside antibacterial and antioxidant assays. Results demonstrated that substrate type influenced mycochemical composition and antioxidant activity. Mushrooms cultivated on cassava peels gave the highest phenolic content and antioxidant activity, corresponding with enhanced antifungal efficacy. Ethanol extracts showed stronger antimicrobial activity and lower minimum inhibitory concentrations than aqueous extracts, indicating the role of moderately polar bioactive compounds. These findings establish a direct link between substrate composition, metabolite production, and antifungal activity, highlighting substrate-driven metabolic modulation as a key determinant of bioactivity. This study positions agro-waste cultivated *P. ostreatus* as a sustainable source of antifungal agents and supports substrate optimization as a scalable strategy for natural product discovery. Future integration of metabolomics and genome-guided approaches will be essential to elucidate active compounds and mechanisms of action.

Keywords: Antifungal activity; Antioxidant activity; *Aspergillus fumigatus*; *Candida albicans*; Microbiome; Natural products

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1. Introduction

Invasive fungal infections caused by opportunistic fungal pathogens such as *Candida albicans* and *Aspergillus fumigatus* remain a major global public health challenge. Over

the past two decades, the burden of fungal infections has increased substantially due to immunosuppression, organ transplantation, cancer chemotherapy, HIV/AIDS, prolonged hospitalization, and antimicrobial misuse. Antifungal resistance has emerged as a serious global threat, reducing the effectiveness of conventional antifungal drugs and increasing mortality rates. Azole-resistant *Aspergillus fumigatus* and multidrug-resistant *Candida* species are increasingly reported in both clinical and environmental settings.¹ Consequently, there is an urgent need for alternative and sustainable antifungal compounds from natural biological sources.²⁻⁴

Natural product discovery continues to represent one of the most reliable approaches for the development of antimicrobial therapeutics. Fungi, bacteria, plants, algae, and marine organisms synthesize diverse secondary metabolites with significant pharmacological activities.⁵⁻⁷ Among fungal resources, edible mushrooms have attracted substantial scientific interest because they contain biologically active compounds with antioxidant, antimicrobial, immunomodulatory, anti-inflammatory, anticancer, antiviral, and hepatoprotective properties.^{8,9} Mushrooms have been used traditionally for nutritional and medicinal purposes across different cultures for centuries.¹⁰⁻¹²

Pleurotus ostreatus, commonly referred to as the oyster mushroom, is one of the most widely cultivated edible mushrooms globally due to its adaptability, high nutritional value, ease of cultivation, and medicinal importance.¹³ The species possesses the capacity to degrade lignocellulosic materials efficiently through extracellular enzymes such as laccases, cellulases, manganese peroxidases, and lignin peroxidases.^{14,15} This enzymatic versatility allows *P. ostreatus* to grow effectively on agro-industrial wastes, including sawdust, cassava peels, rice straw, oil palm residues, sugarcane bagasse, banana leaves, and corn cobs.¹⁵

Agro-waste accumulation constitutes an environmental challenge in many developing countries, especially in tropical regions where agricultural activities generate large quantities of lignocellulosic residues annually. Improper disposal of agricultural wastes contributes to environmental pollution, greenhouse gas emissions, soil degradation, and microbial contamination.¹⁶ Converting these wastes into value-added products through mushroom biotechnology provides a sustainable strategy for waste management, environmental remediation, and functional food production.

Previous studies have shown that cultivation substrates significantly influence mushroom growth, nutritional composition, antioxidant capacity, and secondary

metabolite production.¹⁶⁻¹⁸ Environmental conditions, substrate nutrient composition, mineral availability, carbon-to-nitrogen ratio, and stress responses can regulate fungal metabolic pathways and alter bioactive compound biosynthesis. Phenolics, flavonoids, terpenoids, polysaccharides, alkaloids, and ergosterol derivatives are among the important metabolites associated with the medicinal properties of mushrooms.

Despite growing scientific interest in functional mushrooms, there remains limited information regarding the relationship between tropical agro-waste substrates and antifungal metabolite production in *P. ostreatus*. Many available studies focus mainly on nutritional composition without integrating molecular identification, antioxidant analysis, and antifungal evaluation. Furthermore, few studies explore the role of substrate-driven metabolic modulation within the framework of sustainable biotechnology and One Health.¹⁹

The One Health concept recognizes the interconnectedness of human health, environmental health, and food systems. Sustainable mushroom cultivation aligns strongly with One Health objectives by promoting environmental sustainability, reducing agricultural waste accumulation, improving food security, and contributing to antimicrobial discovery. Functional mushrooms cultivated on agro-waste materials may therefore provide both nutritional and therapeutic benefits while simultaneously supporting circular bioeconomy development.^{20,21}

The present study explored the antifungal and antioxidant properties of *P. ostreatus* cultivated on selected tropical agro-waste substrates, including Gmelina sawdust, cassava peels, and oil palm fruit fiber. Molecular identification using internal transcribed spacer (ITS), large subunit ribosomal RNA gene (LSU), and RNA polymerase II second-largest subunit gene (RPB2) markers was integrated with phytochemical, antioxidant, antimicrobial, and minimum inhibitory concentration (MIC) analyses to establish the relationship between substrate composition, metabolite accumulation, and bioactivity. The findings provide insight into sustainable fungal biotechnology and substrate optimization strategies for natural product discovery.

2. Materials and methods

2.1. Mushroom cultivation

P. ostreatus was cultivated in Okitipupa, Ondo State, Nigeria, which has a tropical climate suitable for mushroom cultivation. Three agro-waste substrates were used in this study: Gmelina sawdust, oil palm pressed fiber, and cassava

peels. The substrates were air-dried, milled, and sterilized at 121 °C for 15 min. Subsequently, 500 g of each substrate was packed into a treated plastic container fitted with a cover lid. After cooling, each substrate was inoculated with 5 g of spawn under aseptic conditions and incubated at 28 ± 2 °C until complete colonization and fruiting.

2.2. Molecular identification

Molecular characterization of the mushroom was performed to confirm the identity of the cultivated mushroom as *P. ostreatus*.²² DNA-based methods were employed to overcome the limitations of morphological identification and provide genetic evidence for species-level classification.

2.2.1. DNA extraction

Fresh mycelium was aseptically scraped from actively growing cultures on potato dextrose agar plates (Himedia Laboratories, India). Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method²² with slight modifications. Approximately 100 mg of mycelium was ground in liquid nitrogen and mixed with CTAB extraction buffer (Thermo Fisher Scientific, United States of America), followed by incubation at 65 °C for 30 min. The DNA was purified using chloroform-isoamyl alcohol (24:1) and precipitated with ethanol. DNA quality and integrity were assessed using a 1% agarose gel stained with ethidium bromide, whereas DNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, United States of America) based on the A260/A280 ratio.

2.2.2. Polymerase chain reaction amplification

Polymerase chain reaction (PCR; 7500 Fast DX, Thermo Fisher Scientific, United States of America) was used to amplify three genetic markers: ITS (ITS1–5.8S–ITS2), LSU (28S), and *RPB2*. The primer pairs ITS1/ITS4, LR0R/LR7, and fRPB2-5F/fRPB2-7cR were used to amplify ITS, LSU, and *RPB2* (Thermo Fisher Scientific, United States of America), respectively. PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. The amplification products were verified by electrophoresis on 1.5% agarose gels (DY-300 Electrophoresis Machine, FINLAB, Nigeria).

2.2.3. Sequencing and sequence analysis

The PCR products were purified and sequenced in both directions using the Sanger sequencing method at a certified sequencing facility (Nigeria Centre of Disease

Control Laboratory, Nigeria). Raw chromatogram files containing forward and reverse reads were visually examined to confirm peak quality and identify ambiguous base calls. Low-quality regions at the 5' and 3' ends of each read were trimmed prior to assembly.²¹ Reverse reads were converted to their reverse complements and aligned with the corresponding forward reads to generate high-confidence consensus sequences from the overlapping regions.²² The final consensus sequences were used for molecular identification using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBl=BlastSearch).

The sequences were compared with sequences in the NCBI GenBank database using BLASTn to confirm species identity.

2.2.4. Validation of molecular data

Species identity was considered confirmed when the query sequences showed ≥ 98% sequence identity to reference sequences in GenBank. The final consensus sequences were submitted to GenBank to obtain accession numbers.

2.3. Extraction of bioactive compounds

Dried mushroom samples were pulverized into a fine powder using a sterile electric grinder (6SM150T, Taizhou Lehu Export & Import Co., Ltd., China). A 20-g portion of each powdered sample was separately extracted with 200 mL of either 70% ethanol or distilled water at a solid-to-solvent ratio of 1:10 (w/v). Extraction was carried out at room temperature (25 ± 2 °C) for 24 h with continuous agitation at 150 rpm using an orbital shaker (201BS, Scitek Global, Nigeria). The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator (SCILBGEX, Scitek, Global, Nigeria) at 40 °C. The extracts were dried to a constant weight and stored at 4 °C until further analysis.⁶

2.4. Chromatographic profiling of bioactive compounds

Bioactive compounds in the *P. ostreatus* extracts were preliminarily profiled by high-performance liquid chromatography (HPLC). Briefly, dried samples were separately extracted with methanol using a Soxhlet apparatus (DL300, Shamboo Scientific, India). Extracts were filtered and concentrated under reduced pressure. HPLC was performed using a Shimadzu Nexera X2 system equipped with a UV-visible detector (Japan). Separation was performed using a reverse-phase C18 analytical column (4.6 × 250 mm; particle size: 5 µm). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile) under gradient elution

conditions. The flow rate was maintained at 1.0 mL/min with an injection volume of 20 µL, and detection was performed at 280 nm. Gallic acid, catechin, quercetin, and caffeic acid were used as reference standards for peak identification. HPLC was used primarily for preliminary chromatographic profiling of the bioactive compounds present in extracts of *P. ostreatus* cultivated on different substrates.

2.5. Antioxidant analysis

The antioxidant activity of the *P. ostreatus* extracts was evaluated using standard spectrophotometric assays.

2.5.1. DPPH radical-scavenging assay

Radical-scavenging activity (RSA) was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Extracts at concentrations of 5–15 mg/mL were mixed with 0.1 mM DPPH solution in methanol and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer (DR6000, HACH, United States of America). Ascorbic acid was used as the positive control.

Radical-scavenging activity was calculated as follows:

$$\text{RSA (\%)} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (1)$$

where A_{control} is the absorbance of the DPPH solution without the extract, and A_{sample} is the absorbance of the DPPH solution containing the extract.

The IC_{50} value was determined by plotting the percentage RSA against the extract concentration and calculating the concentration corresponding to 50% inhibition using nonlinear regression analysis.

2.5.2. Ferric reducing antioxidant power assay

Reducing power was assessed using the ferric reducing antioxidant power (FRAP) assay. The extracts were reacted with FRAP reagent (containing acetate buffer, 2, 4, 6-Tris(2-pyridyl)-s-triazine, and FeCl_3), and absorbance was measured at 593 nm after 10 min. FRAP values were calculated from separate ascorbic acid and Trolox calibration curves and expressed as mmol AAE/g and mmol TE/g of extract, respectively.

2.5.3. Total phenolic content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. The extracts were reacted with Folin-Ciocalteu reagent (Sigma-Adrich, Germany) and sodium carbonate, and absorbance was measured at 765 nm. The results were expressed as milligrams of gallic acid

equivalents (GAE) per gram of extract.

2.6. Antimicrobial assay

Antimicrobial activity was evaluated using the agar well diffusion method. The test organisms included *C. albicans*, *A. fumigatus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella* Typhi, and *Pseudomonas aeruginosa*. The microbial suspensions were adjusted to a 0.5 McFarland standard ($\sim 1 \times 10^6$ CFU/mL). Wells (6 mm diameter) were made in the agar plates, and 100 µL of each extract at a concentration of 5 mg/mL was introduced into each well. Fluconazole and ciprofloxacin were used as positive controls for fungi and bacteria, respectively, whereas 20% (v/v) ethanol was used as the negative control. The plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The zones of inhibition were measured in millimeters.

2.7. Minimum inhibitory concentration

Minimum inhibitory concentrations were determined using the broth microdilution method in accordance with the European Committee on Antimicrobial Susceptibility Testing guidelines.²³ Serial twofold dilutions of each extract (12.5–100 mg/mL) were prepared. The MIC was defined as the lowest concentration at which no visible microbial growth was observed after incubation.

2.8. Statistical Analysis

All experiments were performed in triplicate ($n = 3$). Data are presented as the mean $\pm$ standard deviation. Statistical significance was determined using one-way analysis of variance followed by Tukey's post hoc test. Differences with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Molecular confirmation of *P. ostreatus*

The nucleotide sequences obtained for *P. ostreatus* were deposited in GenBank for reference (accession number PX630178). Full sequences of the ITS region are shown below:

P. ostreatus, ITS region, 496 bp:

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AAACCACCTGTGAACCTTTTGATAGATCTGTGAAGT
CGTCTCTCAAGTCGTCAGACTTGGTTGCTGGCAG
TTCGACGTCTCGGTGTGACTACGCAGTCTATTTTA
CTTACACACCCCAAATGTATGTCTACGAATGTCAT
TTAATGGGCCTTGTGCCTTTAAACCATAATACAAC
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TTTCAACAACGGATCTCTTGGCTCTCGCATCGATG
 AAGAACGCAGCGAATGCGATAAGTAATGTGAATT
 GCAGAATTCAGTGAATCATCGAATCTTTGCACGCA
 CCTGCGCCCTTGGTATTCCGAGGTGCATGCCTGT
 TTGAGTGTCAATAAATCTCAAACCTCACTTTGGTT
 TCTTTCCAATTGTGATGTTTGGATTGTTGGGGGCT
 GCTGGCCTTGACAGGTGCGCTCCTCTTAAATGCAT
 TAGCAGGACTTCTCATTGCCTCTGCGCATGATGTG
 ATAATTATCACTCATCAATAGCACGCCTGAATAGA
 GTCACCG

Table 1. Molecular identification summary based on BLASTn analysis

Species	Locus	Identity (%)	Query coverage (%)
<i>P. ostreatus</i>	ITS	99.8	99.9
<i>P. ostreatus</i>	LSU	99.6	99
<i>P. ostreatus</i>	RPB2	99.1	98

Abbreviations: ITS: Internal transcribed spacer region; LSU: Large-subunit ribosomal RNA gene; RPB2: RNA polymerase II second-largest subunit gene.

Representative ITS sequences showed high similarity (>99%) with reference *P. ostreatus* strains deposited in GenBank (Table 1).

3.2. Substrate-dependent variation in mycochemical composition

Marked variation in mycochemical composition was observed across substrates. Alkaloids were the most abundant compounds, highest in cassava peels (~16–17 mg/100 g), followed by sawdust (~13–15 mg/100 g) and palm fiber (~11–12 mg/100 g). Tannins were significantly elevated in cassava peels (~9 mg/100 g), indicating enhanced secondary metabolism. Phenolics, flavonoids, and terpenoids showed moderate variation but were consistently higher in cassava peel substrate.

The mycochemical results in Figure 1 suggest that cassava peels strongly enhance bioactive compound biosynthesis.

3.3. HPLC profiling of phenolic compounds

Table 2 shows the HPLC tentative identification of phenolic compounds based on comparative retention behavior with standards. The relative abundance of the tentatively

identified phenolic peaks was higher in cassava-peel-derived samples.

3.4. Antioxidant activity

3.4.1. DPPH assay

The radical-scavenging activities of extracts derived from the cassava peels, oil palm fiber, and sawdust substrates were between 45.5% and 69.52%. The corresponding IC_{50} values were between 2.64 and 4.65 mg/mL, respectively, as shown in Table 3.

3.4.2. FRAP assay

Table 4 shows the FRAP values of *P. ostreatus* cultivated on different agro-waste substrates. The ascorbic acid equivalent values were 10.32 mmol/g for cassava peels, 10.25 mmol/g for sawdust, and 10.22 mmol/g for palm fiber. The cassava peel substrate produced the highest FRAP value.

Although the numerical differences in the FRAP values among the substrates were relatively small, the samples derived from the cassava peel substrate exhibited the highest antioxidant activity.

3.4.3. Total phenolic content

The extract derived from the cassava peel substrate had the highest total phenolic content, at 1.65 mg GAE/g, followed by the extracts derived from sawdust and oil palm fiber, at 1.52 and 1.50 mg GAE/g, respectively (Table 5). The corresponding catechin equivalent values were 10.76, 10.65, and 10.63 mg CAE/g, respectively.

3.5. Antimicrobial activity

The mushroom extracts exhibited broad-spectrum antimicrobial activity, with the ethanol extracts showing greater activity than the aqueous extracts, as shown in Table 6. The ethanolic extracts of *P. ostreatus* exhibited significantly greater antimicrobial activity than the aqueous extracts ($p < 0.05$), indicating enhanced extraction efficiency of moderately polar bioactive compounds.

The inhibition zones produced by the ethanolic extracts were 19.2 ± 0.7 mm against *S. aureus*, 18.5 ± 0.6 mm against *Escherichia coli*, 18.0 ± 0.6 mm against *S. Typhi*, 17.8 ± 0.5 mm against *C. albicans*, 18.2 ± 0.5 mm against *A. fumigatus*, 17.4 ± 0.5 mm against *K. pneumoniae*, and 16.5 ± 0.5 mm against *P. aeruginosa*. The aqueous extracts exhibited lower activity with inhibition zones ranging from 12.8 to 15.0 mm.

The inhibition of *C. albicans* and *A. fumigatus* indicates the antifungal potential of the extracts.

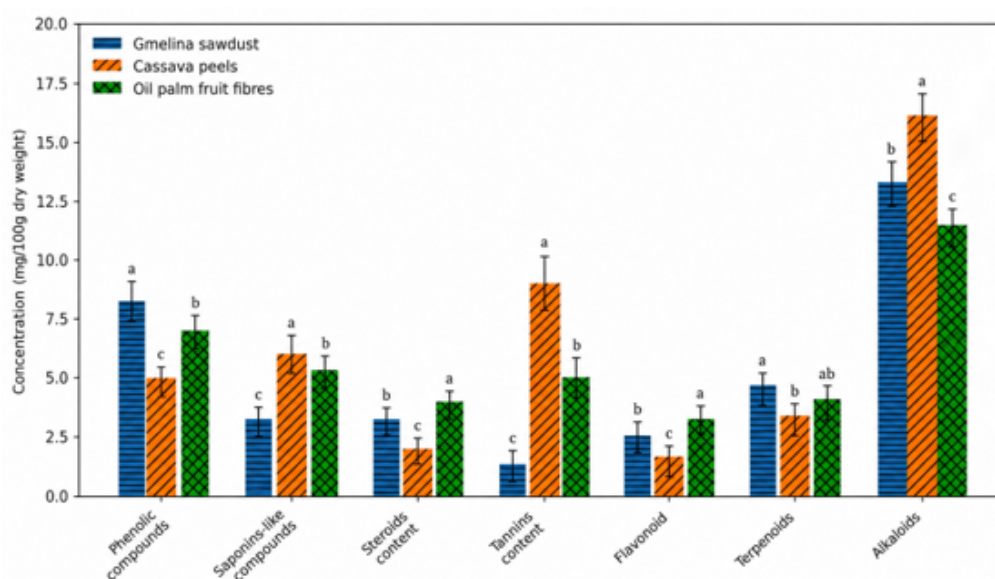


Figure 1. Mycochemical composition of *P. ostreatus* cultivated on Gmelina sawdust, cassava peels, and oil palm fruit fiber. Values are presented as mean $\pm$ SD ($n = 3$). Bars with different superscript letters within the same parameter are significantly different according to Tukey's post hoc test following one-way ANOVA ($p < 0.05$), whereas bars sharing at least one superscript letter are not significantly different.

Table 2. Tentatively identified phenolic compounds detected by high-performance liquid chromatography

Substrate	Relative abundance	Retention behavior	Compound
Cassava peel	High	Early	Gallic acid-like peak
Sawdust	Moderate	Moderate	Catechin-like peak
Cassava peel	High	Late	Quercetin-like peak

Note: The tentative identification was based on comparative retention behavior with standards.

Table 3. DPPH radical-scavenging activity at 5 mg/mL and IC_{50} values of *P. ostreatus* extracts

Substrate	RSA (%)	IC_{50} (mg/mL)
Sawdust	45.5 ± 0.01	4.65 ± 0.3
Cassava peels	69.52 ± 0.4	2.64 ± 0.1
Oil palm fiber	67.76 ± 0.3	2.82 ± 0.2

Note: The inverse relationship between RSA and IC_{50} values further supports the greater antioxidant capacity of the extracts derived from the cassava peel substrate.

Abbreviations: IC_{50} : Half maximal inhibitory concentration; RSA: Radical-scavenging activity.

3.6. Minimum inhibitory concentration

In Table 7 the ethanolic extract of *P. ostreatus*, the MIC was 25 mg/mL against *E. coli*, *S. aureus*, and *S. typhi* and 50 mg/mL against *K. pneumoniae*, *P. aeruginosa*, *C. albicans*, and

A. fumigatus. The aqueous extracts exhibited higher MIC values of 50–62.5 mg/mL, indicating lower antimicrobial potency.

The ethanolic extracts exhibited lower MIC values than the aqueous extracts, indicating greater antimicrobial potency. The relatively high MIC values observed in this study suggest moderate antimicrobial activity at the crude-extract level. However, crude mushroom extracts contain complex mixtures of metabolites, and purification of the active compounds may improve their efficacy.

3.7. Integrated bioactivity profile

Among the substrates evaluated, cassava peels produced extracts with the highest total phenolic content and antioxidant activity. The ethanol extracts exhibited greater antimicrobial activity than the aqueous extracts. The extracts also exhibited activity against *C. albicans* and *A. fumigatus*, indicating antifungal activity.

Table 4. FRAP values of *P. ostreatus* extracts at 5 mg/mL

Substrate	AAE (mmol/g)	Trolox (mmol TE/g)
Sawdust	10.25 ± 0.4	9.65 ± 0.2
Cassava peels	10.32 ± 0.2	9.74 ± 0.1
Oil palm fiber	10.22 ± 0.3	9.62 ± 0.4

Note: Both the ascorbic acid calibration curve and Trolox calibration curve were used to determine FRAP values.
Abbreviations: AAE: Ascorbic acid equivalent; FRAP: Ferric reducing antioxidant power; TE: Trolox equivalents.

Table 5. Total phenolic content of *P. ostreatus* extracts at 5 mg/mL

Substrate	mg GAE/g	mg CAE/g
Sawdust	1.52 ± 0.2	10.65 ± 0.2
Cassava peels	1.65 ± 0.4	10.76 ± 0.3
Oil palm fiber	1.50 ± 0.1	10.63 ± 0.1

Abbreviations: CAE: Catechin equivalents; GAE: Gallic acid equivalents.

Table 6. Antimicrobial activity of *P. ostreatus* extracts, expressed as inhibition-zone diameter at 5 mg/mL

Pathogen	Ethanol extract (mm)	Aqueous extract (mm)	Negative control (mm)	Positive control (mm)
<i>E. coli</i>	18.5 ± 0.6 ^b	14.2 ± 0.4 ^c	5.1 ± 0.4 ^d	25.1 ± 0.4 ^a
<i>S. aureus</i>	19.2 ± 0.7 ^b	15.0 ± 0.5 ^c	7.5 ± 0.5 ^d	27.5 ± 0.5 ^a
<i>K. pneumoniae</i>	17.4 ± 0.5 ^b	13.6 ± 0.3 ^c	4.6 ± 0.4 ^d	24.6 ± 0.3 ^a
<i>P. aeruginosa</i>	16.5 ± 0.5 ^b	12.8 ± 0.3 ^c	3.8 ± 0.4 ^d	23.8 ± 0.4 ^a
<i>S. typhi</i>	18.0 ± 0.6 ^b	14.0 ± 0.4 ^c	5.4 ± 0.5 ^d	25.4 ± 0.6 ^a
<i>C. albicans</i>	17.8 ± 0.5 ^b	13.7 ± 0.3 ^c	6.3 ± 0.4 ^d	26.3 ± 0.4 ^a
<i>A. fumigatus</i>	18.2 ± 0.5 ^b	14.5 ± 0.5 ^c	6.6 ± 0.5 ^d	25.8 ± 0.3 ^a

Notes: Data are presented as mean ± SD ($n = 3$). Within each row, values with different superscript letters (a–d) are significantly different (Tukey's post hoc test, $p < 0.05$); values sharing the same letter are not significantly different. The negative control was 20.0% (v/v) ethanol. Ciprofloxacin and fluconazole were used as positive controls for bacteria and fungi, respectively.

Table 7. Minimum inhibitory concentrations of *P. ostreatus* extracts against the test pathogens

Pathogen	Ethanol extract (mg/mL)	Aqueous extract (mg/mL)	Negative control	Positive control
<i>E. coli</i>	25	50	100	12.5
<i>S. aureus</i>	25	50	100	12.5
<i>K. pneumoniae</i>	50	50	100	25
<i>P. aeruginosa</i>	50	50	100	25
<i>S. typhi</i>	25	50	100	25
<i>C. albicans</i>	50	50	100	12.5
<i>A. fumigatus</i>	50	50	100	25

Notes: The negative control was 20.0% (v/v) ethanol. Ciprofloxacin and fluconazole were used as positive controls for bacteria and fungi, respectively.

4. Discussion

The emergence of antifungal-resistant strains of *C. albicans*, along with other pathogenic fungi, has raised significant concerns within the medical and scientific communities.^{24,25} This alarming trend underscores the urgent need for the development and exploration of alternative antifungal therapies, particularly those derived from natural sources. One promising candidate in this regard is the edible mushroom species *P. ostreatus*, commonly known as the oyster mushroom.²⁶

Research has shown that the bioactive compounds present in *P. ostreatus* possess potential antifungal properties, which could be instrumental in combating these resistant strains.²⁷⁻²⁹ As traditional antifungal agents become less effective due to increasing resistance, the investigation into naturally occurring substances offers a promising approach to addressing this challenge. Furthermore, *P. ostreatus* is readily available and has been used extensively in various culinary applications, making it a practical and accessible option for further research and potential therapeutic use.³⁰⁻³²

It is necessary to conduct comprehensive studies to ascertain the efficacy and mechanisms of action of the antifungal components found in *P. ostreatus*. This study demonstrates that agro-waste substrates influence metabolite production and antifungal activity in *P. ostreatus*. Although mushrooms are well established as sources of antimicrobial and antioxidant compounds³³⁻³⁵, the present findings suggest that cultivation substrate may also influence their mycochemical profiles. Cassava peels likely enhanced bioactivity because of their carbohydrate composition, mineral content, and lignocellulosic structure. Fungal metabolism is highly responsive to environmental conditions and nutrient availability.³⁵⁻³⁷ Nutrient-rich substrates can enhance the expression of genes associated with oxidative stress responses and secondary metabolism. Increased phenolic production may therefore represent an adaptive metabolic response that simultaneously enhances antioxidant and antimicrobial properties. Similar observations have been reported for *Pleurotus* species, where substrate variation significantly influences phytochemical profiles and biological activity.³⁸ The observed inhibition of *C. albicans* and *A. fumigatus* is consistent with previous reports describing antifungal activity of mushroom extracts.³⁹ The relatively higher efficacy of ethanol extracts compared to aqueous extracts suggests that moderately polar compounds, including phenolics, flavonoids, and terpenoids, are major contributors to antimicrobial activity.⁴⁰⁻⁴¹ These compounds have been reported to disrupt fungal cell membranes and interfere with essential cellular processes. The strong correlation between phenolic

content and antioxidant activity further supports the role of phenolic compounds as key contributors to bioactivity. Antioxidant properties may contribute to antifungal effects through redox-mediated mechanisms, although this was not directly investigated in the present study. Previous studies established these compounds to disrupt fungal cell membranes, interfere with ergosterol biosynthesis, and induce oxidative stress.⁴²⁻⁴⁴ The observed correlation between phenolic content and antioxidant activity further supports a mechanism that may involve redox-associated pathways, which may influence both pathogen survival and host immune responses.⁴⁵

The high antioxidant capacity observed, particularly in cassava peel-derived samples, further suggests a role in regulating oxidative stress during infection.⁴⁵ The combination of antioxidant and *in vitro* antifungal activities supports further investigation of *P. ostreatus* as a source of bioactive compounds. The findings are consistent with previous studies on oyster mushrooms cultivated on agro-waste substrates, where enhanced antimicrobial activity has been linked to increased phytochemical content.⁴⁶⁻⁴⁷ This highlights the importance of substrate optimization as a practical strategy for improving bioactive compound production.

Furthermore, agro-waste materials such as oil palm pressed fruit fibers, cassava peels, and sawdust represent low-cost and sustainable substrates that can enhance the functional value of cultivated mushrooms. This aligns with ongoing efforts in sustainable biotechnology and natural product discovery.⁴⁶

The limitations of the study include the absence of purified compound isolation, lack of targeted metabolomic profiling, absence of *in vivo* validation, and limited mechanistic characterization of antifungal activity.

5. Conclusion

This study demonstrates that substrate composition significantly influences the production of bioactive metabolites and antifungal activity in *P. ostreatus*. Among the substrates evaluated, cassava peels supported higher phenolic content, enhanced antioxidant capacity, and improved antifungal efficacy against *C. albicans* and *A. fumigatus*. The stronger activity observed in ethanol extracts compared to aqueous extracts further suggests that moderately polar compounds, including phenolics and related metabolites, contribute substantially to the observed bioactivity. These findings highlight the importance of substrate selection as a practical and cost-effective strategy for optimizing the functional and therapeutic potential of cultivated mushrooms. The use of agro-waste materials not only supports sustainable production systems but also

enhances the value of *P. ostreatus* as a source of bioactive compounds. Overall, this work provides evidence that agro-waste-driven cultivation can enhance antifungal potential in *P. ostreatus* and supports its application in sustainable natural product discovery and antimicrobial development.

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Conflict of interest

The authors declare that they have no competing interests.

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Methodology: Olagunju Johnson Adetuwo, Faith Naomi Ogundana, Titilayo Adejoke Abeegunrin

Supervision: Soji Fakoya

Writing–original draft: Olagunju Johnson Adetuwo

Writing–review and editing: Olagunju Johnson Adetuwo, Soji Fakoya

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

Further disclosure

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