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Antimicrobial Activity and Nutraceutical Potential of Cultivated *Pleurotus ostreatus* (Jacq. Ex Fr.) P.Kumm and *Pleurotus sajor-caju* (Fr.) Singer in Ibadan

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Abstract The increase in the side effects of many synthetic antimicrobial agents and the incidence of multidrug resistance in bacteria has prompted scientists to research plant-based antimicrobial with therapeutic potential. Mushrooms have been shown to present such potential with high medicinal value. The antimicrobial activity and nutraceutical potential of two mushrooms namely *Pleurotus ostreatus* and *Pleurotus sajor-caju* were investigated. *P. ostreatus* was found to exhibit antagonistic activity in varying degrees against *S. aureus, E. coli, P. fluorescens, S. liquifaciens, S. marcescens, K. pneumonia,* and *P. mirabilis* with the diameter zones of inhibition ranging between 10.8±0.5 to 20.8±0.4 mm. The highest amount of Flavonoids (39.50±0.40%) was observed in the aqueous extract fractions of *Pleurotus sajor-caju* followed by Alkaloids in Ethyl acetate extract fraction of *Pleurotus ostreatus* (36.80±0.57) while the least was Tannins (0.09±0.00%) in Ethanol extract fraction of *Pleurotus sajor-caju*. The two mushrooms exhibited a concentrated dependent scavenger ability against Diphenyl-picrylhydrazyl (DPPH). Based on the result obtained, *Pleurotus ostreatus* have high inhibitory activity against pathogenic microorganisms, while the two mushrooms (*Pleurotus ostreatus* and *Pleurotus sajor-caju*) have high antioxidant capacity against free radicals which can serve as a good means of reducing the incidence of infection and high prevalence of malnutrition.

Keywords: *phytochemicals, antimicrobial, Pleurotus ostreatus, Pleurotus sajor-caju, proximate analysis*

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1. Introduction

Several compounds with good therapeutic potential have been discovered during the continuous search for natural sources of bioactive substances. Over the years, mushrooms have been used in traditional medicine to prevent and treat various diseases and are therefore rich in bioactive compounds [1,2]. Phenols, triterpeneoids, flavonoids, sterols, polysaccharides, polysaccharide proteins, and polypeptide complexes are among the many biologically active compounds found in mushrooms [3].

Extracts from Mushrooms have shown various pharmacological activities such as antiviral, antibacterial, antifungal, antitumor, immunomodulatory [4,5], and also antioxidant, antihypercholesterolemic, antidiabetic, hepatoprotective, anti-inflammatory, and anticholinesterase activities [6,7].

From "nutrition" and "pharmaceuticals" the term "nutraceutical" has been coined. De Felice believes that nutraceuticals are defined as, "a food or part of a food product which provides health benefits including the prevention and treatment of diseases" [8]. Nutraceutical products include dietary fibre polyunsaturated fatty acids PUFA, fish oil; proteins, peptides, amino acids, ketoacids, minerals such as antioxidating vitamins, and others glutathione, selenium, etc.

The genus Pleurotus is a collection of higher fungi (Basidiomycota) comprising about forty species. They're usually referred to as oyster mushrooms because their fruiting bodies are opened like oyster shells during metamorphosis. They're the third largest commercially cultivated mushrooms in the world, and they're one of the most commonly consumed mushrooms. In traditional medicine, *Pleurotus* species are used to treat several diseases such as diabetes, asthma, constipation, gastrointestinal disorders, nervous disorders, high cholesterol, cardiovascular disease, and so on. [9,10] Mushrooms are a healthy food with high protein content, vitamins such as B and Ds, minerals, low fat and cholesterol levels. These essential vitamins help to break down proteins, fats, and carbohydrates so that they can be an energy source for human consumption [11]. Therefore,

this study is designed to analyze the antimicrobial and nutraceutical potential of cultivated *Pleurotus ostreatus* and *Pleurotus sajor-caju* in Ibadan.

2. Materials and Methods

2.1. Collection of Samples

The fruit bodies of *Pleurotus ostreatus* and *Pleurotus sajor-caju* were bought from the Department of Botany University of Ibadan, Ibadan, and Forestry Research Institute of Nigeria (FRIN) respectively. They were oven-dried at 40^0C for 48 hours (two days) and ground into fine powder.

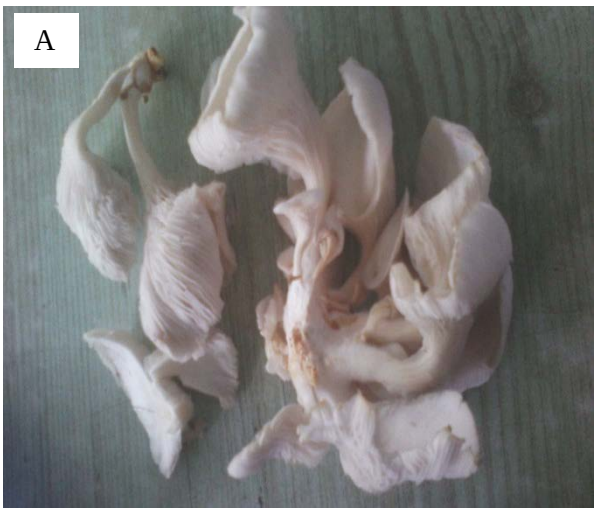

Figure 1. *Pleurotus ostreatus* (Jacq. ex Fr.) P. Kumm (A)

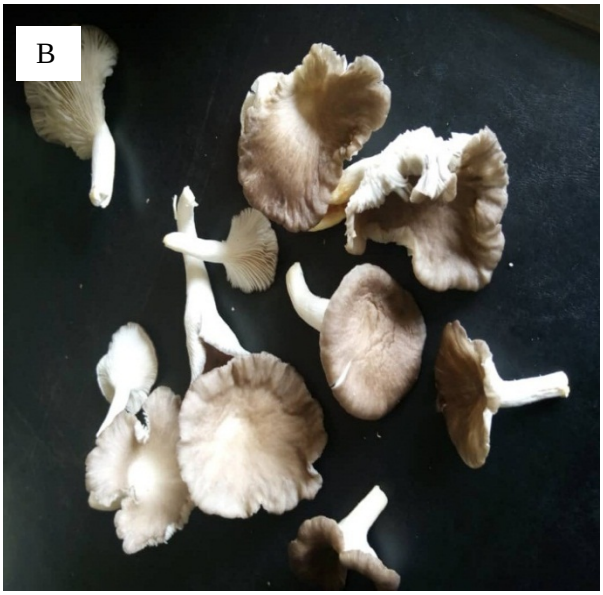

Figure 2. *Pleurotus sajor-caju* (Fr.) Singer (B)

2.1.1. Successive Exhaustive Extraction of the Mushrooms

This extraction method adopted in this study was successive extraction according to Das *et al*. [12] which involves solvents of increasing polarity from a non-polar solvent to a more polar solvent. For subsequent extractions of 1) N Hexane, 2) acetate, 3) ethanol, and 4) water, the following solvents have been used. The Extract fractions were concentrated using a rotary vacuum evaporator at 40-50°C and later stored in an opaque container at 40°C for further studies.

2.1.2. Phytochemical Analysis of the Mushrooms

The qualitative and quantitative phytochemical analysis was carried out according to Hussain *et al*. [13] on the extract fractions, for the following bioactive compounds: Flavonoids, steroids, glycosides, terpenoids, alkaloids, anthraquinones, saponins, cardiac glycosides, tannin, and Terpenoids.

2.1.3. Antimicrobial Activity of the Mushrooms

To determine the antimicrobial sensitivity of the extract fractions of the mushrooms, the agar well diffusion method has been used. With the help of a sterilizing cork borer, wells were produced on Agar's plates. Bacteria cultures were prepared in a nutrient medium with a density adjusted to 0.5 Mcfarland turbidity standards, and the final concentration of each bacterial culture was set at 10^5 cfu per mL. An aliquot of the test culture was uniformly distributed over the surface of the solidified agar to assess the antimicrobial efficacy of extract fractions. 100 µl of the extract fractions of each mushroom were loaded into the different wells. For bacteria, all preprogrammed plates with the appropriate extract and test organisms have been stored at 37°C for 24 hours. Zones of inhibition have been recorded in millimeters following incubation. All the tests were carried out in duplicate and their means were recorded [14].

2.1.4. Determination of Antioxidant Activity

The radical scavenging activities of the plant extracts against 2,2-Diphenyl-1-picryl hydroxyl radical (Sigma-Aldrich) were determined by UV spectrophotometry at 517 nm. The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0, and 5 mg/mL in methanol (Analar grade). Vitamin C was used as the antioxidant standard at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5 and 0.75 mg/mL. I mL of the extract was placed in a test tube, and 3 mL of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH [16]. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = {}^{Aa}\!/_{Ab} \times 100 \quad (1)$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the extract.

2.1.5. Statistical Analysis

The data have been summarised by percentages, means, and standard deviations. To assess the hypothesis that all means are equal, data were analysed using variance ANOVA. This was done using statistical packages for Social Science version 23 (SPSS 23). In addition, a post hoc test for Duncan multiple range testing has been performed. A 5% confidence interval has been used for all statistical tests.

3. Results and Discussion

The qualitative phytochemical screening of *Pleurotus ostreatus* is shown in Table 1. Tannins, flavonoids, alkaloids, and Phenols were present in Ethyl acetate, Ethanol, and aqueous extract fractions of *Pleurotus ostreatus* but absent in N-Hexane extract fraction; Anthraquinones, Cardiac glycoside, and Terpenoids were present in Ethyl acetate, Ethanol and N-Hexane extract fractions but are absent in Aqueous extract fraction. Steroids were present in N-Hexane, Ethanol, and Aqueous extract fractions but absent in Ethyl acetate extract fraction.

Table 1. Qualitative phytochemical content of different extract fractions of *Pleurotus ostreatus*

Phytochemicals	Extraction solvent			
	Hexane	Ethylacetate	Ethanol	Aqueous
Saponins	-	++	++	++
Tannins	-	+	+	+
Flavonoids	-	+	+	+
Cardiac glycosides	+	+	++	-
Anthraquinones	++	++	+	-
Terpenoids	++	+	+	-
Steroids	++	-	++	+
Alkaloids	-	+	+	+
Phenols	-	+	+	+

+ Present, ++ Strongly Present - Absent

Table 2. Qualitative phytochemical content of different extract fractions of *Pleurotus sajor-caju*

Phytochemicals	Extraction solvent			
	Hexane	Ethylacetate	Ethanol	Aqueous
Saponins	-	++	-	+
Tannins	-	+	+	+
Flavonoids	-	+	+	++
Cardiac glycosides	-	++	+	-
Anthraquinones	++	++	+	+
Terpenoids	++	++	+	+
Steroids	+	+	++	-
Alkaloids	-	-	+	-
Phenols	-	+	+	+

+ Present, ++ Strongly Present – Absent

The qualitative phytochemical screening of *Pleurotus sajor-caju* is shown in Table 2. *Pleurotus sajor-caju* contained Flavonoids, Tannins, and Phenols in Ethyl acetate, Ethanol, and aqueous extract fractions but were absent in N-Hexane extract fraction. Saponins were present in Ethyl acetate and Aqueous extract fractions but absent in N-Hexane and Ethanol extract fractions. Cardiac glycosides were present in the Ethanolic and Ethyl acetate extract fractions but absent in N-Hexane and Aqueous extract fractions. Steroids were present in N-Hexane, Ethanol, and Ethyl acetate extract fractions but absent in the aqueous extract fraction. Anthraquinones and Terpenoids were present in all the extract fractions, while Alkaloids were present in only the Ethanol extract fraction.

The study emphasizes the use of serial exhaustive extraction using various solvents based on their polarity from the polar (Aqueous) to non-polar (ethyl acetate) [12]. More phytochemicals have been recovered by this method compared to the conventional methods of extraction. Ammar *et al*. [16] in their report on the efficient extraction of organic and inorganic materials from plants, stated that polar solvents were proven to be effective at extracting these materials efficiently. It may be that the mushroom samples were composed of more polar chemical constituents as solvents, which resulted in a smaller extraction capacity compared to nonpolar solvents.

Aruwa *et al*. [7] observed that the activity of the extracts was a result of the presence of the phytochemicals and the result of the screening of the selected mushrooms shows the presence of bioactive components such as alkaloids, flavonoids, anthraquinones, terpenoids, cardiac glycosides, steroids and phenols. These bioactive compounds have been reported to be protective against microbial attack, and they are also an effective possible therapeutic agent.

The comparative phytochemical quantification results of *Pleurotus ostreatus* and *Pleurotus sajor-caju* extracts are shown in Table 3. The two mushrooms contain alkaloids ranging from 36.80±0.57 mm in Ethyl acetate fraction of *Pleurotus sajor-caju* to 17.80±0.28 mm in ethanol fraction of *Pleurotus ostreatus*; Flavonoid content ranged between 39.50±0.40 in aqueous fraction of *Pleurotus sajor-caju* to 19.40±0.00 mm in Ethyl acetate fraction of *Pleurotus ostreatus*; Terpenoids content in all the extract fractions of *Pleurotus sajor-caju* and *Pleurotus ostreatus* ranged from 3.00±0.00 mm to 0.80±0.00 mm, Saponins content ranged from 3.73±0.14 mm in ethanol fraction of *Pleurotus sajor-caju* to 1.14±0.42 mm in aqueous fraction of *Pleurotus ostreatus* while Tannins content ranged from 0.15±0.22 mm in N-Hexane fraction of *Pleurotus sajor-caju* to 0.09±0.00 mm in ethanol fraction of *Pleurotus sajor-caju*. *Pleurotus ostreatus* had the highest quantity of Alkaloids in the Ethyl acetate fraction when compared to the rest of the extract fractions.

The extract fractions of the tested mushrooms divulged various phytochemicals such as Cardiac glycosides, Saponins, Flavonoids, Phenols, Tannins, Steroids, Alkaloids, Anthraquinones, and Terpenoids in the *Pleurotus ostreatus* and *Pleurotus sajor-caju.* These phytochemicals have been discovered from some of the crude extraction methods, with a limited number of bioactive compounds, in previous studies. [17].

The aqueous fraction of *Pleurotus sajor-caju* had the highest percentage of Flavonoids followed by Alkaloids in the Ethyl acetate fraction of *Pleurotus ostreatus*. Alkaloids are medicinally denoted to be used as a local anesthetic [18] while Tannins were recorded as the least quantity in aqueous extract fraction of *Pleurotus ostreatus.* Tannins inhibited microbial multiplication by denaturing the enzymes involved in microbial metabolism [19].

Table 3. Quantitative phytochemical content of the different extract fraction of selected Mushrooms

Mushrooms	Solvents	Phytochemicals Alkaloids (%)	Flavonoids (%)	Terpenoids (%)	Saponins (%)	Tannins (%)
P.S	Hex.	-	-	1.61±0.28	-	-
	Ea	-	20.70±0.42	2.14±0.14	1.72±=0.12	0.15±0.22
	Ethanol	17.80±0.28	20.80±0.00	0.80±0.00	-	0.09±0.00
	Aqueous		39.50±0.40	0.92±0.14	1.14±0.42	0.09±0.13
P.O	Hex.	-	-	3.00±0.00	-	-
	Ea	36.80±0.57	19.40±0.00	1.51±0.42	3.40±0.00	0.11±0.14
	Ethanol	27.40±0.00	19.70±0.14	1.42±0.00	3.73±0.14	0.10±0.56
	Aqueous	19.60±0.28	20.00±0.57	-	3.62±0.28	0.10±0.00

Values are means ± Standard deviation of duplicate observations. Hex: n-hexane, Ea.: Ethylacetate, P.S: *Pleurotus sajor-caju* P.O: *Pleurotus ostreatus.*

Table 4 shows the antagonistic activity of *Pleurotus ostreatus* against selected tested bacteria of which the Ethyl acetate extract fraction shows a zone of inhibition of 11.8±0.3 mm and 18.5±0.7 mm against *P. fluorescens and S. liquifaciens* respectively. The Aqueous and Ethanolic fractions have zones of inhibition of 20.1±0.1 mm and 11.0±0.0 mm against *S. marcescens* and also the aqueous fraction shows a 17.3±0.3 mm zone of inhibition against *K. pneumonia.* The Ethyl acetate fraction has a zone of inhibition of 10.8±0.3 mm against *P. mirabilis*. The N-Hexane extract fraction shows no antagonistic activity against the tested bacteria. The antagonistic activity of *Pleurotus sajor-caju* extract fractions against indicator bacteria shows no zones of inhibitions

Table 4. Antagonistic activity of *Pleurotus ostreatus* against selected indicator organisms (mm±SD) using different extraction medium

Indicator organism	Extraction solvent Aqueous	Ethanolic	N-hexane	Ethylacetate
S. aureus	-	-	-	-
E. coli	-	-	-	-
P. fluorescens	-	-	-	11.8±0.3
S. liquifaciens				18.5±0.7
S. marcescens	20.1±0.1	11.0±0.0	-	-
K. pneumonia	17.3±0.3	-	-	-
P. mirabilis	-	-	-	10.8±0.3

Values are means ± Standard deviation of triplicate observations

The presence of a high quantity of Flavonoids and Alkaloids which have been documented to display antimicrobial activity [20] and confirmed by the work of Okafor *et al*. [21] established while the Ethyl acetate and aqueous extract fractions of the *Pleurotus ostreatus* have antimicrobial action against the tested indicator organisms. The inequalities in the level of antagonistic activity of the *Pleurotus ostreatus* extracts are due to the composition of the bioactive compounds, the technique of extraction, and the active ingredient existing in the edible mushroom [21].

The mechanism of action by which the bioactive component of the mushroom exerts antagonistic activity might be due to bacterial enzyme inhibition [22]. In addition, phytochemicals are said to inhibit the growth of bacteria by interfering with the cellular membrane and their metabolic processes, and also by modulating signal transduction or gene expression pathways. By combining with the cell membrane, it is claimed that saponins may exert some antibacterial activity and cause changes in cellular morphology which result in cell lysis [23].

Table 5. Antagonistic activity of *Pleurotus sajor-caju* extracts against selected indicator organisms (mm±SD) using different extraction medium

Indicator organism	Extraction solvent Aqueous	Ethanolic	N-hexane	Ethylacetate
S. aureus	-	-	-	-
E. coli	-	-	-	-
P. fluorescens	-	-	-	
S. liquifaciens	-	-	-	-
S. marcescens	-	-	-	-
K. pneumoniae	-	-	-	-
P. mirabilis	-	-	-	-

Table 6 shows the percentage scavenging behaviour of various fractions of *Pleurotus ostreatus* extract. There was a significant difference in the percentage scavenging activities of each medium when compared to the standard at 200 and 400 µg/ml concentrations though there was no difference between the scavenging activity of the extract in hexane and ethanol medium, also the extract in ethyl acetate and aqueous medium at 200 µg/ml and 400 µg/ml concentration. Compared with the standard, there was no significant difference in the percentage of salvage activities for each medium.

Table 6. Percentage scavenging activity of the different extract fractions of *Pleurotus ostreatus* on DPPH

Mushroom Conc. (µg/ml)	Extraction solvent Hex. (%)	Ea. (%)	Et. (%)	Aqueous. (%)	Standard (%)
200	36.26^{d}	42.19^{e}	41.81^{d}	37.30^{e}	96.97^{c}
400	36.84^{c}	43.92^{d}	46.16^{c}	50.36^{d}	97.00^{c}
600	36.95^{c}	46.54^{c}	47.30^{c}	67.15^{c}	97.02^{c}
800	64.73^{b}	48.65^{b}	47.71^{b}	67.71^{b}	97.18^{b}
1000	88.78^{a}	48.85^{a}	49.42^{a}	68.68^{a}	98.34^{a}

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P≤ 0.05 according to Duncan's multiple range tests. Conc: Concentration, Hex. Hexane, Ea: Ethyl acetate and Et: Ethanol, DPPH: 2,2-diphenyl-1-picrylhdrazyl.

The percentage scavenging activity of the different extracts of *Pleurotus sajor-caju* on DPPH is shown in Table 7. There is no significant difference in the mean percentage scavenging activities of *Pleurotus sajor-caju* extract in the entire medium including the standard at 1000 µg/ml. For the extract at 800 µg/ml concentration, there was no difference in the mean percentage scavenging activities in hexane, ethyl acetate, and aqueous medium when compared with the standard. At 600 µg/ml concentration, there was no difference in the mean percentage scavenging activities in hexane, ethyl acetate, and aqueous medium when compared with the standard. At 400 µg/ml concentration, there was a significant difference in the mean percentage scavenging activities of the extract in hexane, ethyl acetate, and aqueous medium when compared to the standard, though no difference was observed when mean percentage scavenging activities of ethanolic extract of the mushroom was compared to the standard. There was a significant difference in the mean percentage scavenging activities of the extract in hexane, ethyl acetate, ethanol, and aqueous medium when compared to the standard, though no difference exists between hexane, ethyl acetate, and aqueous medium of *Pleurotus sajor-caju* at 200 µg/ml concentration.

Table 7. Percentage scavenging activity of the different extract fractions of *Pleurotus sajor-caju* on DPPH

Mushroom Conc. (µg/ml)	Extraction solvent				Standard (%)
	Hex. (%)	Ea. (%)	Et. (%)	Aqueous. (%)	
200	38.65^{e}	30.43^{e}	24.42^{e}	33.08^{e}	96.97^{c}
400	42.82^{d}	31.20^{d}	30.87^{d}	37.33^{d}	97.00^{c}
600	48.93^{c}	34.89^{c}	36.16^{c}	47.00^{c}	97.02^{c}
800	49.16^{b}	35.55^{b}	40.92^{b}	57.28^{b}	97.18^{b}
1000	51.12^{a}	39.49^{a}	47.33^{a}	59.85^{a}	98.34^{a}

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P≤ 0.05 according to Duncan's multiple range tests. Conc: Concentration, Hex. Hexane, Ea: Ethyl acetate and Et: Ethanol, DPPH: 2,2-diphenyl-1-picrylhdrazyl.

The mushroom extracts possess significant antioxidant activity such that the extract is capable of inhibiting free radical formation and also possesses the ability to scavenge. The antioxidant system comprises two different types of functional components which are the first line and second line. The first line comprises of preventive antioxidant that acts by destroying the free radicals or by suppressing the formation of the free radicals while the second line of defense shows the DPPH radical scavenging activities [24]. It is important to note that the antioxidant activity of mushrooms contributes significantly to the nutraceutical properties and increases the nutritional value of mushrooms. Therefore, to reduce oxidative damage in the human body, mushrooms could be used as a possible remedy. The antioxidant induced the determination of the reduction capacity of DPPH radical at 517 nm [24].

The reduction in the absorbance was due to a reaction between an antioxidant molecule and radical by hydrogen donation, which is indicated by its color change from purple to yellow. Therefore, the scavenging and percentage inhibition of free radicals by antioxidants is assessed with DPPH [25].

4. Conclusion

Aqueous extract of the edible mushroom (*Pleurotus ostreatus*) may inhibit the growth of certain pathogenic bacteria and the possibility of developing antimicrobials from it is of great promise for the treatment of diseases. Due to their high antioxidant capacity against free radicals, *Pleurotus ostreatus* and *Pleurotus sajor-caju* can be used as functional foods.

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Competing Interests

The authors have declared that no competing interests exist.

Authors' Contributions

Samuel Temitope Ogunbanwo conceived the topic and designed the study; Eniola Oluwatomisin Akinbode and Gabriel Aruwa performed the research and managed the analyses of the study and wrote the protocol; Clementina Oyinkansola Adenipekun managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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