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Phytochemical, Nutritional, Anti-Nutritional Evaluation and Antimicrobial Analysis of *Pleurotus Ostreatus* from Oshogbo in Osun State, Nigeria

M. E. Khan[1]*, I. G. Osigbemhe[2], Y. Sikiratu[1], I. N. Oyeza[1], K. O. Adebayo[3], H. Aliyu[1], S. O. Genevieve[1], H. N Aliyu[1], , A Alexander[4], M. K. Yahaya [5] , N. O. Ugbenyo[5] and A. M. Abdulazeez[5]

[1]Department of Chemistry,
Federal University Lokoja,
Kogi State,
Nigeria.

[2]Department of Chemistry,
Faculty of Physical Services,
University of Benin,
Benin City,
Nigeria.

[3]Department of Industrial Chemistry,
Federal University Lokoja,
Kogi State,
Nigeria.

[4]Department of Chemistry,
College of Education Hong,
Adamawa State,
Nigeria.

[5]Department of Chemistry,
Confluence University of Science and Technology Osara,
Kogi State,
Nigeria.

Email: khandora2000@gmail.com

ORCID ID: 0000- 0002-8374-478X

Abstract

The study was carried out to determine the nutritional constituents, anti-nutritional composition, antimicrobial activity and phytochemical composition of Pleurotus ostreatus. Samples were determined in triplicates to ascertain the nutritional quality of the mushrooms. The proximate analysis revealed that Pleurotus ostreatus contains moisture (27.0), Ash content (29.8) crude fibre (9.20), crude protein (28.74), crude fat (3.18), carbohydrate (2.08). Phytale (++), oxalate (+++) are the anti-nutritional factors detected. The screening of bioactive compounds confirmed the presence of carbohydrates,

Author for Correspondence

tannins, steroids, glycosides, flavonoids, and alkaloids. Antimicrobial analysis on several bacterial species tested revealed inhibitory effects against Streptococcus faecalis, Salmonella typhi, Escherichia coli, and Staphylococcus aureus. The inhibitory zones (ZI), the minimum inhibitory concentration (MIC),and the minimum bactericidal concentrations (MBC), for the tested microbes were:- S. aureaus ; 20mm, 25mm, and 16mm Bacillus subtilis 14mm, 17mm and 30mm, Pseudomonas aeruginosa 15.5mm, 20mm and 26mm, Salmonella typhi 10.5mm, 17.5mm and 12mm, mL respectively. Candida albican, the only fungus did not show any. The observed antimicrobial activities of the extracts may be due to the presence of these bioactive compounds. Pleurotus ostreatus *can therefore be used as an antimicrobial agent against some of the inhibitory bacteria. The findings of the nutritional analysis indicated that* Pleurotus ostreatus *contains essential and valuable nutrients that are beneficial to the human body.*

Keywords: phytochemcals, anti-nutrition and nutrition, anti-biological activity and *pleurotus ostreatus*

INTRODUCTION

Plants and other living things produce bioactive substances called phytochemicals to defend themselves (Kumar *et al.,* 2022). These bioactive substances, including phenolics, flavonoids, phytates, terpenoids, alkaloids, saponins, and others, have also been found to be abundant in edible mushrooms (Odiase-Omoighe and Agoreyo 2022; Ekute, 2019; Serrano *et al*., 2020).

As saprophytes, mushrooms are fungi that grow on dead and decomposing materials, such as manure and old, rotting logs, which are rich in organic content. They consist of some *Ascomycota* members as well as members of the *Basidiomycota.* In many civilizations, mushrooms are grown and consumed for their edible, delicate, and therapeutic qualities. They have also been used as a dietary supplement. Vitamins, proteins, carbs, lipids, amino acids, and minerals are all thought to be present in mushrooms. (Jiskani, 2011).

Edible mushrooms offer numerous nutritional health benefits, including antioxidants that help shield the body from harmful free radicals that contribute to conditions like heart disease and cancer. They also contain copper, which aids in the production of red blood cells, essential for delivering oxygen throughout the system (Becker, 2007; Guzmán-Rodríguez *et al.,* 2021).

Much research is needed especially on the nutritional and the anti-nutritional aspects and also the phytochemicals present (both qualitative and quantitative to attest for their traditional medicinal uses by testing them on some disease causative microbes).To determine their capacity as a source for the development of new drugs and to provide foundational material/information for future research on this species of mushroom, studies on bacteria and fungi are essential to understanding the various applications of traditional medicine (Ayoola *et al*., 2005; Edeoga *et al*., 2008).

Oyster mushrooms, or Pleurotus species, are fungi with recognizable fleshy fruiting bodies. Due to their exceptional flavor and taste, the Pleutorus genera are considered delicacy in various parts of the world (Shah *et a*l., 2004).

Among the genera of *Pleurotus*, *P. ostreatus* is the most popular and widely cultivated in different regions of the world. Mushrooms farmers usually make millions of dollars from this single species. They are found in the wild or cultivated artificially in mushroom farms. *P. ostreatus,* with English name "Mushroom", common (local) names oyster mushroom, is called

"*ero-usu"* in Igbo, "*kawanamankaza*" in Hausa, "*gigeiolu*" in Yoruba and "*awu*" in Igala.It is a *Basidiomycete* that is grown in temperate and subtropical climates worldwide. It belongs to the class *Agaricomycetes*, order *Agarcales*, and family *Pleurceae.* According to Jwanny *et al*. (1995), the mushroom can thrive on a variety of agro-industrial types. Based on published works of Fatih (2008) and Chang and Miles (1992), the stalk of *P. ostreaus* is typically white, short, and eccentric, and an individual sporocarp is a large structure with gray to grey-brown carp that grows to about 5 to 15 cm in its longest dimension. Determining the quality and amount of phytochemicals, antibacterial activity, proximate composition, and anti-nutritional components of *Pleutotus ostreatus* extract obtained from Oshogbo in Osun State is the goal of this study.

Figure 1: A labeled structure of *Pleurotus ostreatus*

MATERIALS AND METHODS

Collection and Identification of Pleurotus ostreatus

Fresh *Pleurotus ostreatus* samples were taken on June 23, 2023, at precisely 8:23 a.m. from Oshogbo in Osun State, Nigeria to the State University for identification. Dr. John Ayinde, a Botanists of Botany Department identified them and a sample was preserved for reference with Herbarium No. 0273.

Preparation

Fresh fruiting bodies of *P. ostreatus* samples were cut to smaller pieces and air-dried under shade. The air-dried mushroom samples were milled to powder using Power Deluxe® Electric Blender (Model: PDB-8231-G) and stored in air-tight polyethylene Zip-lock bags prior for analysis. Preparation of aqueous and ethanol extracts 10.00g of the mushrooms powder were marcerated separately in 100 mL of ethanol. That was left to stand for 72 hours and subsequently filtered to collect the filtrates for phytochemical screening. Qualitative and quantitative analyses of Phytochemicals were done using the methods of, Ezeonu and Ejikeme (2016); Nimenibo-Uadia *et al.* (2017).

Proximate Analysis
Using the standard procedure outlined by the Association of standard Analytical Chemists (AOAC), the samples' approximate composition was ascertained. Robert (2010) and AOAC (2006, 2015) conducted all analyses in triplicate using dry samples, and the findings were reported as average values.

Anti-nutritional analysis
To find anti-nutritional components (tannin, oxalate, and phytate) in different extracts, chemical tests were performed using a conventional protocol (Khan *et al.,* 2023; Sewell *et al*., 2016).

Antimicrobial screening

Culture Media
Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), nutrient agar (NA), and potato dextrose agar are among the culture substances that were used for sensitivity testing, determining the least inhibitory concentration (MIC), and determining the minimum bactericidal/fungicidal concentration (MBC). All substances were formulated following the manufacturer's instructions and autoclaved for 15 minutes at 121°C to sterilize them.

Determination of Inhibitory Activity (sensitivity test) of the extract using agar well diffusion method
Sterilized swab sticks were used to spread the standardized bacterial suspension onto Mueller Hinton agar plates. Each plate contained four wells created with a sterilized cork borer. The wells were labeled according to the different concentrations of the prepared extract -100, 50, 25, and 12.5 mg/mL. Approximately 0.2 mL of the extract was added to each well. The plates were left to be heated for about an hour to allow the extract to diffuse into the agar. The plates were subsequently incubated for 24 hours at 37°C. After incubation, the plates were examined for any signs of inhibition, known as the zone of inhibition, which is a clear area surrounding the wells where bacterial growth was absent. A clear ruler calibrated in millimeters was used to measure the diameters of the zones, and the measurements were recorded.

M.I.C Analysis
The minimum inhibitory concentration (MIC) of the extract was determined using a serial dilution approach with Mueller Hinton broth as the medium. The extract was diluted stepwise in test tubes containing Mueller Hinton broth, and each tube was inoculated with standardized bacterial cultures. After inoculation, the tubes were incubated for 24 hours at 37°C. At the end of the incubation period, the tubes were examined for bacterial growth, with turbidity used as an indicator. The MIC was defined as the lowest concentration in the series where no visible growth was observed (Khan, 2017).

MBC Analysis
The minimum bactericidal concentration (MBC) of the extract was determined based on the lowest (MIC) values. The test tubes that showed no turbidity (clear) in the MIC test were sampled with a sterilized wire loop, and a loopful was streaked onto sterile nutrient agar plates. The plates were then incubated at 37°C for 18 to 24 hours. After incubation, the plates were examined for the presence or absence of growth. This step helps to determine whether the extract has a bacteriostatic or bactericidal effect.
GC-MS analysis of methanol extracts

The methanol extracts were analyzed under the following experimental conditions using an Agilent Technologies 7890A GC and 5977B MSD: the gas chromatography oven temperature was initially set at 40°C and increased to 250°C at a rate of 5°C/min; the injection volume was 1 µL; the HP 5-MS capillary column, which was non-polar, had dimensions of 30 m in length, an internal diameter of 0.25 mm, and a film thickness of 0.25 µm. The flow rate of the mobile phase (helium as the carrier gas) was set to 1.0 mL/min. The results of the full scans of the samples were compared using the NIST Mass Spectral Library Search Program, covering a mass-to-charge range of 40 to 650 m/z.

RESULTS AND DISCUSSIONS

Table 1.0: ***Qualitative Phytochemical analysis on P.ostreatus***

S/N	TEST	ETHANOL
1	Flavanoids	+
2	Cardiac Glycoside	+
3	Tannins	+
4	Phlobatannins	-
5	Alkaloids a. Mayer test b. Wagner's test	+
6	Carbohydrate (Molish Test)	+
7	Resins	-
8	Steriod	-
9	Glycosides	-

Key: (+) signs = present, and (-) = Below Detection Limit (BDL)

The mushroom contains a high content of flavonoids, cardiac glycosides, carbohydrates, and alkaloids, as revealed by phytochemical analysis.

The quantitative analysis of *Pleurotus ostreatus* has shown that it contains a significant among of flavonoids and alkaloids that makes it a valuable medicinal plant with various human health benefits.

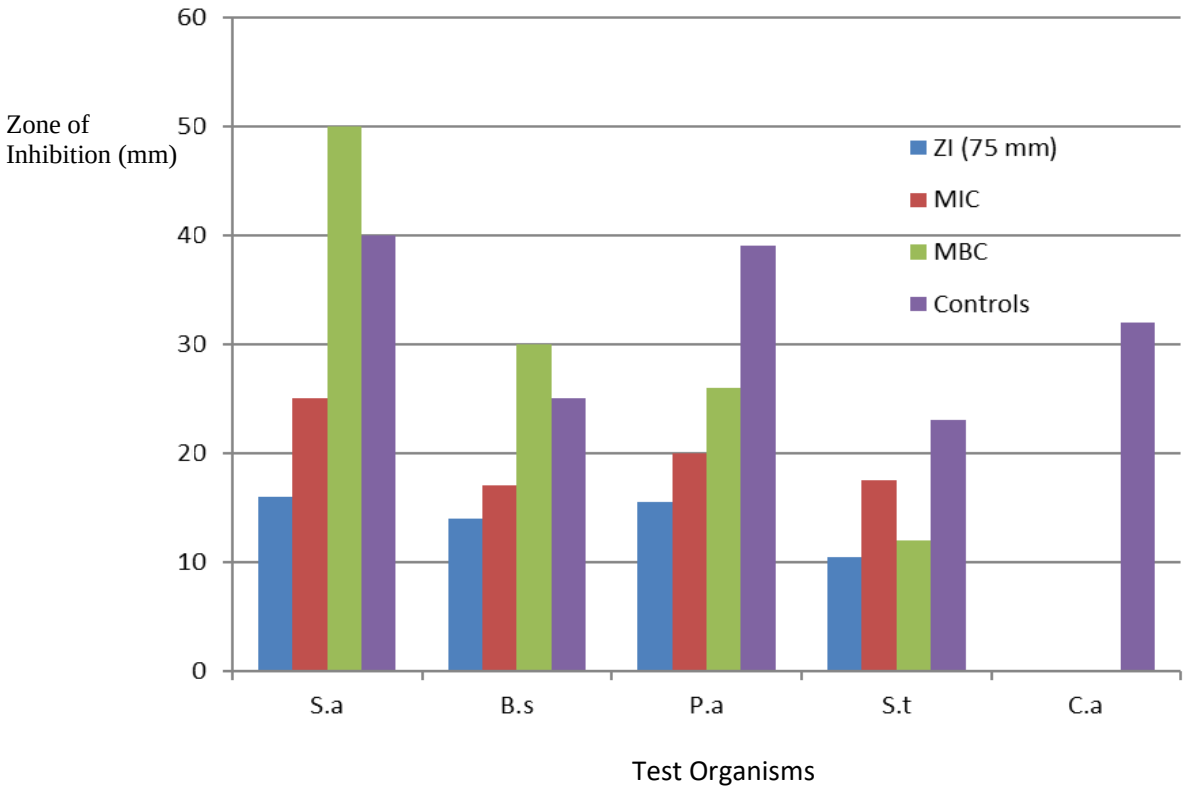
Table 2.0: Quantitative phytochemical composition of *P. otreatus*

Parameter	Qunty Pyh. of *P. ostreatus*
Terpenoid	2.1652±0.045
Total glycoside	0.0633±0.003
Flavonoid	0.052±0.002
Oxalates	0.0226±0.002
Phytates	0.1341±0.005
Tannins	0.1242±0.003
Phenol	0.5231±0.004
Saponin	0.4865±0.012
Alkaloids	0.5432 ±0.020

Determination of Inhibitory Activity of Ethanol extract of *Pleurotus ostreatus*

Ethanol extract of *Pleurotus ostreatus* showed some Inhibitory effects of all bacterial species tested, but no effect on the fungus, *Candida albican*. It thus, implies that it can be used as a precursor for the production of bacterial pharmaceuticals. From the obtained values, the MIC

and MBC, which are to check then minimum concentration of *p. ostreatus* that can kill 50% of the microbes and whether the fungus and bacteria were eradicated or only had their growth suppressed, were also carried out and there were inhibition of the various bacteria species, viz: *S. aureus* (16.0, 25.0, 50.0), *B. subtilis* (14.0, 17.0, 30.0), *P. aeroginosa* (15.5, 20.0, 26.0), *S. typhi* (10.5, 17.5, 12.0), respectively. There was no effect of the fungus.



Key; Test organisms: *S.a; Staphylococcus aureus, B.s; Bacillus subtilis, P. Pseudomonas aeroginosa. a, S.t. Salmonella typhi, C.a; Candida albican*

Proximate Composition of *Pleurotus ostreatus*

The result obtained for the proximate composition of dried *Pleurotus ostreatus* are mean of triplicate values given in standard deviation format.

Table 3.0: Proximate composition of *Pleurotus ostreatus*

Sample	% Moisture content	% Ash content	% Crude Fiber	% Crude protein	% Crude fat	% Carbohydrate
Dandelion of *Pleurotu sostreatus*	27.03±2.01	29.8 ±2.03	9.20 ±1.05	28.74±3.11	3.18±0.12	2.08 ± 0.02

The proximate evaluation of the sample was carried out with standard method and result showed that Ash content had the highest percentage of 29.8±2.03 whereas, carbohydrate had the least percentage of 2.08 ± 0.02.

Anti-Nutritional Factor of *Pleurotus ostreatus*

Table 4.0: Anti-nutritional Analysis of *Pleurotus ostreatus*

Sample	Phytate	Oxalate	Tannin
Dandelion of *Pleurotus ostreatus*	42.40 ± 2.12	77.86 ±6.32	186 ±4.23

The result for anti-nutritional content of mushroom is presented in table 6.0. The result confirm high presence of tannins and oxalate but moderate phytate. Proteins and other organic substances from our diet, such as amino acids and alkaloids, are bound to and precipitated by tannins, which are polyphenolic molecules that prevent human cells from using them. They also cause liver necrosis, lower blood pressure, lower serum cholesterol levels, speed up blood clotting, and alter immunological responses, among other physiological consequences. Oxalates can cause hyperoxaluria, inflammation, joint pains(arthritis, carpal tunnel syndrome) skin irritation, (eczema and acne), kidney stones and other urinary tract infections, (Khan *et al.*, 2023).

Table 5.0: *GC-MS Library compounds of Pleurotus ostreatus*

PK	RT (Mins)	Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
6.8073	6.8073	3-Bis (ethylthio) hexane	$C_{10}H_{22}S_2$	206.41	0.3389
1	23.0451	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.40	12.0276
2	24.6191	Hexadecanoic acid, propyl ester	$C_{19}H_{38}O_2$	298.51	1.0382
3	26.2621	Linoelaidic acid	$C_{18}H_{32}O_2$	280.45	37.8319
4	26.6819	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	8.4933
5	27.4733	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	2.2338
6	27.6141	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	266.46	1.7669
7	28.1759	Oleic Acid	$C_{18}H_{34}O_2$	282.46	0.4263
8	30.1401	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	13.3284
9	30.4215	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	3.2541
10	30.9242	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	9.2607

The mushroom's gas chromatography and mass spectrometry were conducted using a Varian 3800/4000 gas chromatograph mass spectrometer, which had an Agilent with a capillary column DB5ms (30.0m x 0.25mm, 0.25µm film thickness) and an EI as an ion source.

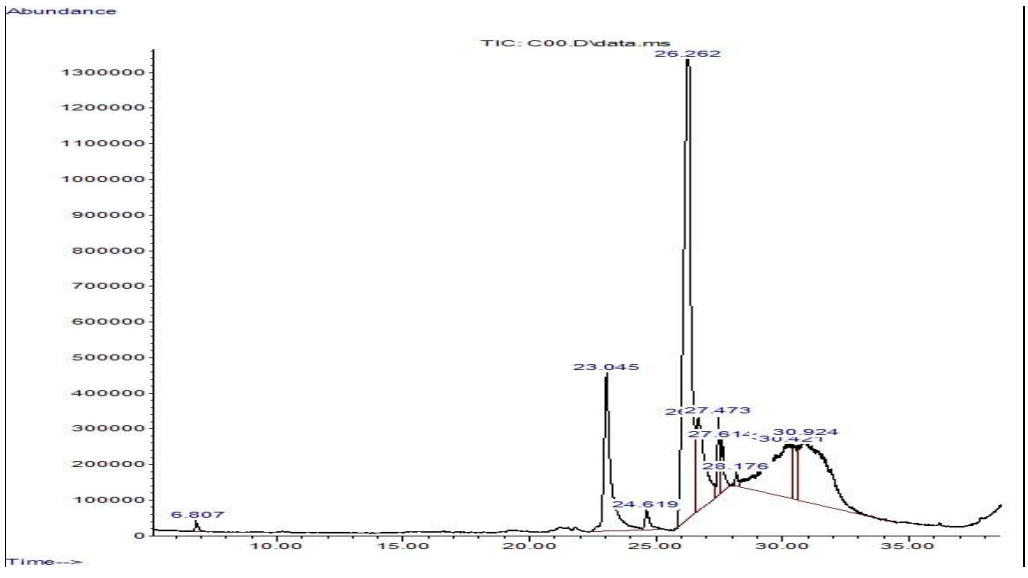


Figure 3: GC-MS Chromatogram of *P. ostreatus*

Since their use in pharmaceuticals is linked to enhanced target affinity, better metabolic stability, and higher stiffness to access bioactive conformation, octadecadienoic acids are often used components in drug design (Sardana *et al*., 2021). Thus, Hexadecanoic acid, propyl ester is not a bioactive compound, but could be used as a component in the synthesis of pharmaceuticals and also as an intermediate in organic synthesis (Chandra *et al*., 2017). Linoelaidic acid is the precursor in the synthesis of all prostaglandins, used by the human cardiovascular system. (Ku and Lin, 2015, and Rangsinth *et al*., 2023).The triglycerides in the molecules are metabolized into ketones that supply the brain cells and thus, prevent Alzheimer's and Parkinson's diseases. Numerous active ingredients were found in the ethanol extract of *Pleurotus ostreatus*, including alkaloids, flavonoids, cardiac glycoside, steroids, carbohydrates, and tannins. It also showed a reasonable antibacterial activity (Ching *et al*., 2013). A few nutrients, including moisture, ash content, crude fiber, crude protein, crude fat, and carbohydrates, as well as anti-nutrients like phytare and oxalate, were also found in this investigation. Therefore, despite its anti-nutritional deficiencies, Pleurotus ostreatus can improve a variety of nutritional and therapeutic qualities and improve human nutrition.

CONCLUSION

Base on the analyses carried out on *Pleurotus ostreatus,* the study showed that *P. ostreatus* is rich in medicinal and nutritional properties. The phytochemicals too in this mushroom makes it pertinent to include it in daily human diet to promote better health and reduce health risks, since it contains important high crude fiber, protein and ash content for the treatment of many medical issues.

Further screening is also recommended so as to identify compounds that are of essential drugs and nutrients to human. Analysis should be carried out on the toxic mineral composition and anti-nutritional composition. Food and agricultural organizations should raise awareness of the value of mushrooms because of their high crude fiber, protein, and ash content, which can be used to treat a variety of illnesses.

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