Original Research Article

PHYTOCHEMICAL CONTENT AND ANTIBACTERIAL EFFECT OF ETHANOLIC AND METHANOLIC EXTRACTS OF *Pleurotus ostreatus*MUSHROOM

ABSTRACT

Aims: Microbial resistance is a threat to the successful treatment of microbial infections. Mushrooms are known to possess antimicrobial and antioxidant potential which could be inhibitory to some pathogenic organisms.The aim was to assess the phytochemicals and antimicrobial potential of *Pleurotus ostreatus* extracts on some pathogenic organisms.

Methodology: The study was conducted at the Bells University of Technology, Nigeria, between December 2019 and August 2020. A 10% dried powder of *Pleurotus ostreatus* was extracted in absolute ethanol and methanol and evaporated in a water bath at 50^{o}C. Extracts were reconstituted in dimethyl Sulfoxide (DMSO) at 12.5, 25, 50, and 100% respectively. Antibiotic effects of extracts were tested on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Staphylococcus saprophyticus* by agar well diffusion method and compared with standard antibiotic discs. DMSO and the solvents served as positive and negative controls. Ethanolic extract was subjected to Gas Chromatography-Mass Spectrometer.

Results: The extracts exhibited varying inhibitory effects on the organisms except on *E coli* at 12.5% ethanolic extract. The inhibition zones increased with extract concentration from 2-12mm and 4-14mm in ethanolic and methanolic extracts respectively. The inhibitory effect of standard antibiotics was higher 17-25mm except for erythromycin (13-14mm). Test organisms were resistant to Beta Lactams (augmentin, cefuroxime, and ceftazidime) and sensitive to Aminoglycoside (gentamicin), Fluoroquinolones (ofloxacin), Ciprofloxacin, and erythromycin. GC/MS revealed the presence of 30organic compounds, amongst them were sugar, sugar alcohols, alkaloids, amines, fatty acids, esters,

aldehydes, and alcohols. The most abundant were oleic acid (33.75%) and 9-octadecenoic acid (Z)-2-hydroxy-1- (21.21%). Phytochemicals are heterocyclic compounds found in natural products.

Conclusion:*P. ostreatus* has been revealed to possess phytochemicals of medical relevance with potentialfor pharmacological application. The implication is that *Pleurotusostreatus* extracts could serve as a lead to novel drug discoveries which could enhance health and nutritional well-being.

Keywords*: Antibacterial, Extracts, Inhibition, Organisms, Phytochemicals, Pleurotus ostreatus, solvents*

1. INTRODUCTION

Edible mushrooms have long been recognized as an important food source with therapeutic benefits due to their nutritional value and immense health and tonic attributes. Specific bioactive compounds with medical relevance found in mushrooms include polysaccharides, tri-terpenoids, low molecular weight proteins, glycoproteins, and immunomodulation compounds (Willem, 2015). Medicinal mushrooms have been reported to be rich sources of natural antimicrobial, antioxidant, antiviral, anti-inflammatory and many secondary metabolites which have been exploited in traditional medicine (Gregori *et al.,* 2007; Ashagrie*et al*., 2015).

The oyster mushroom, *P. ostreatus* is a common edible mushroom cultivated commercially in Nigeria for food and economic benefits and have been used all over the world for its nutritional value and medicinal properties. Both the fruiting body and the mycelium contain compounds with a wide range of antimicrobial activity and are potential sources of phenol-degrading enzymes (Thillaimaharani*et al*., 2013).Antimicrobial drugs have long been used for prophylactic and therapeutic purposes, but the growing number of drug-

resistant bacteria poses an increasing threat to the effectiveness of existing antibiotics. High usage and abuse of synthetic antibiotics have led to the emergence of multidrug-resistant pathogens.The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics is continuously increasing.The world is facing significant challenges in modern healthcare delivery due to the inefficiency of some antimicrobial agents in fighting infections. There is an increasing needto search for new antimicrobial substances, especially from natural sources like bacteria, fungi, and plants. Natural products constitute a major source of drugs. Mushrooms have been reported to possess various bioactive compounds like terpenoids, flavonoids, tannins, alkaloids, and polysaccharides. These useful secondary metabolites remain largely untapped for medicinal purposes. Therefore, the research was to assess the phytochemical and antimicrobial potential of ethanolic and methanolic extracts of *Pleurotus ostreatus* against some Gram-positive (*Staphylococcus aureus,* and *Staphylococcus saprophyticus*) and Gram-negative (*Escherichia coli,* and *Pseudomonas aeruginosa*) microorganisms.

2. MATERIAL AND METHODS

2.1 Mushroom

The freshfruiting body of *Pleurotus ostreatus* was procured from the Federal Institute of Industrial Research Oshodi, Lagos, Nigeria.

2.2 Test organisms

Four test organisms employed were *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* and *Staphylococcus saprophyticus* (Gram-positive). The organisms were collected from the Microbiology Laboratory of the Department of Biological Sciences, Bells University of Technology Ota, Nigeria.

2.3 Standard Antibiotics

Standard antibiotics tested were Cefixime (CXM), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Ciprofloxacin (CPR), Ceftazidime (CAZ), Cefuroxime (CRX), Gentamicin (GEN) and Erythromycin (ERY).

2.4 Preparation of mushroom extract

The extraction process was carried out using absolute methanol and ethanol as solvents according to (Anyakorah *et al*., 2022). The fruiting body of *Pleurotus ostreatus* was properly rinsed with 2.5% potassium hydroxide and sterile distilled water,cut into smaller pieces and oven dried at 50℃ for 72 hrs. The dried mushroom was pulverized in a blender and the powdered samples were stored in an airtight container. Forty grams (40g) of the mushroom sample was extracted with 400ml of absolute methanol and ethanol respectively. The mixture was filtered with Whatman (No.1) filter paper. The filtrate was evaporated in a water bath at 50℃. The extracts were mixed with DMSO and stored at 5^{o}C in a refrigerator.

2.5 Screening of extracts for antibacterial activity

Agar well diffusion method was used for the antibacterial screening of mushroom extracts (Hemashenpagam and Selvaraj, 2010). While the agar disc diffusion method was employed for theantibacterial activity of standard antibiotics. A 24 h old test organism was adjusted to 0.5 McFarland standard and streaked onto solidified Mueller-Hinton agar plates with a swab stick.Six wells of 6 mm were made using a sterile cork borer. Four of the wells were filled with 100µl of 12.5%, 25%, 50%, and 100% concentration of prepared extract. DMSO was used as diluent to achieve the different concentrations(Anyakorah *et al*., 2022}.The fifth and sixth wells contained the solvent and DMSO which served as the positive and negative controls respectively. Incubation was at 37 ± 2□C for 24 hours in the incubation chamber. The growth inhibition zone was determined by measuring the diameter of the zone of inhibition(Hemashenpagam and Selvaraj, 2010).

GC-MS analysis of the mushroom ethanolic extract

A 10% extract in deionized water was sonicated in an ultrasonic cleaner at 40^{o}C for 40 mins. The sonicated aqueous extract was centrifuged at 4000 rpm for 10 mins. After centrifugation, the extract was filtered through filter paper (Whatman No. 1) and stored at a temperature of 5°C in a refrigerator for

subsequent experiments. A gas chromatograph–mass spectrometer (GC–MS) (Shimadzu, QP2010SE) performing at a 1:1 injection ratio (total volume 4 ml) was used to determine the phytochemicals present in the ethanolic solution of the mushroom extract. The phytochemicals were identified using software having a database incorporated into the GC–MS machine (Essien, 2019).

RESULTS

The ethanolic and methanolic extracts of *Pleurotus ostreatus* revealed varying degrees of inhibition at all tested concentrations except for *Escherichia coli* which showed no inhibition at 12.5 % ethanolic extract; this observation could be attributed to the low concentration (Figure 1).

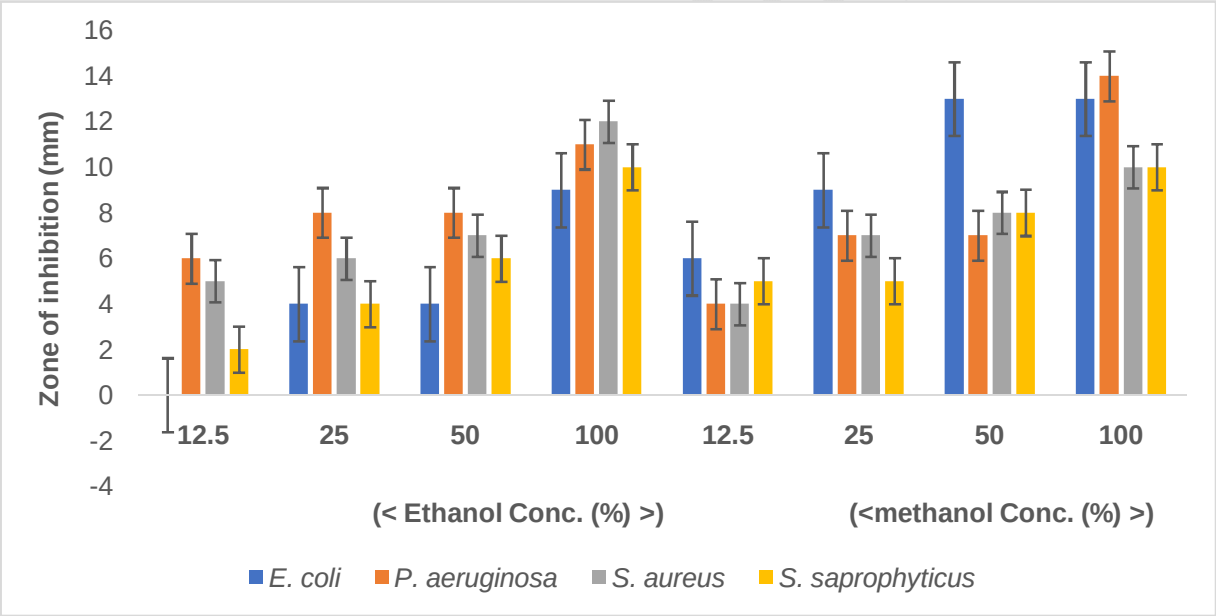

Fig. 1: Antibacterial activity of ethanolic and methanolic extracts of *Pleurotus ostreatus on E. coli, P. aeruginosa, S. aureus, and S. saprophyticus*

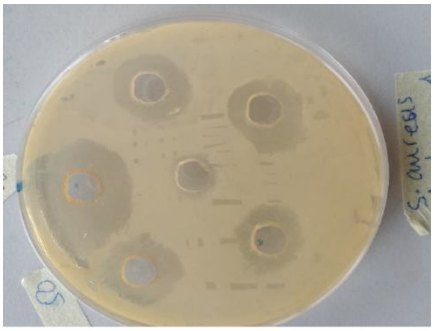

Plate 1: Antibacterial activity of Methanolic extract on*Staphylococcus aureus*

Plate 2: Antibacterial activity of standard antibiotics on*P. aeruginosa*

The inhibitory effect of the solvent extracts increased with the concentration of the extract, and zones of inhibition ranged from 2- 12 mm and 4- 14 mm in ethanolic and methanolic extracts respectively. The methanolic extract had higher zones of inhibition than the ethanolic extract. The result of this research is similar to Bawadekji*et al*. (2017) who observed that *P. ostreatus* extracts exhibited important zones of inhibition on *P. aeruginosa* and *S. aureus* although some zones of inhibition in this study were lower compared to other studies. Chowdhury *et al* (2015) reported zones of inhibition from 7 ± 0.2 to 20 ± 0.1 mm while [4] Thillaimaharani*et al.* (2013) reported 23 mm, and 20mm against *Streptococcus sp*. and *Epidermophyton floccosum* respectively. The differences in inhibition could be attributed to species and cultural differences as mushrooms belonging to the same specie could differ in nutritional and phenotypic attributes due to environmental and ecological conditions. However, this observation is contrary to Thillaimaharani*et al.* (2013) who reported maximum antibacterial and antifungal activity with ethanolic extract of *Pleurotus florida*. Ahmed *et al*. (2015) observed that water extracts of *P. ostreatus* were most inhibitory compared to alcohol solvents and the most sensitive bacteria were *Staphylococcus aureus* and *Escherichia coli*. The water extract contained an antimicrobial compound 3-(2-aminopheny1thio)-3-hydroxypropanoic acid which had a minimum inhibitory concentration of 30 µg/mL and 20 µg/mL against the growth of fungi and bacteria. Mohd *et al.* (2012) observed that in extraction with various solvents, water had the highest evaluation yield compared to ethanol, ethyl acetate, and hexane. These differences could be due to the different relative polarities of the various solvents.

The antibacterial activity of standard antibiotics on test organisms revealed that inhibition was dependent on the type of antibiotic (Figures 2, 3 and Plate 2).

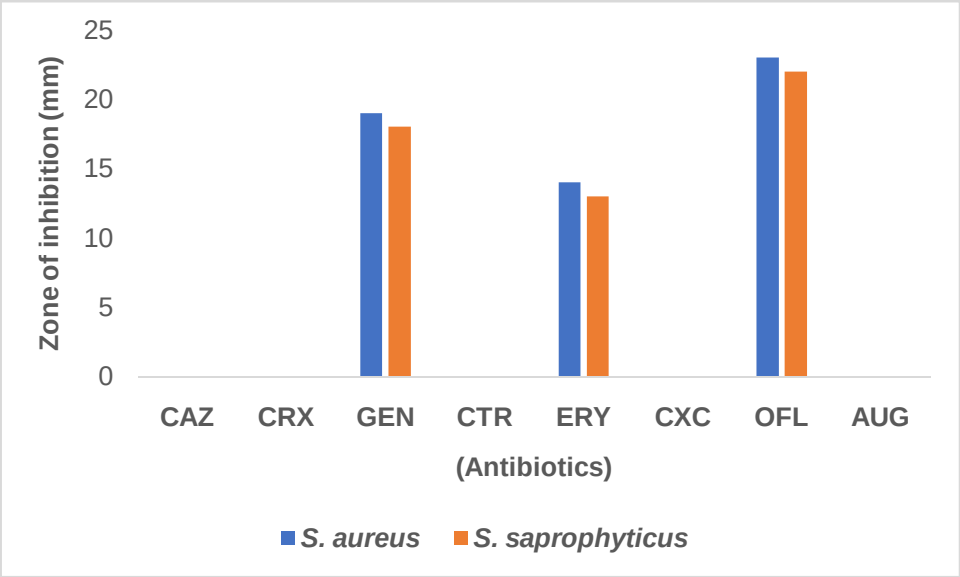

Figure 2: **Antibacterial activity of Standard antibiotics on *S. aureus* and *S. saprophyticus***

Legend: CAZ- Ceftazidime (30 µg), CRX- Cefuroxime (30µg), GEN- Gentamicin (30µg), CTR-Ceftriaxone,ERY- Erythromycin, CXC- Cloxacillin,OFL-Ofloxacin (5µg), AUG-Augmentin (30µg).

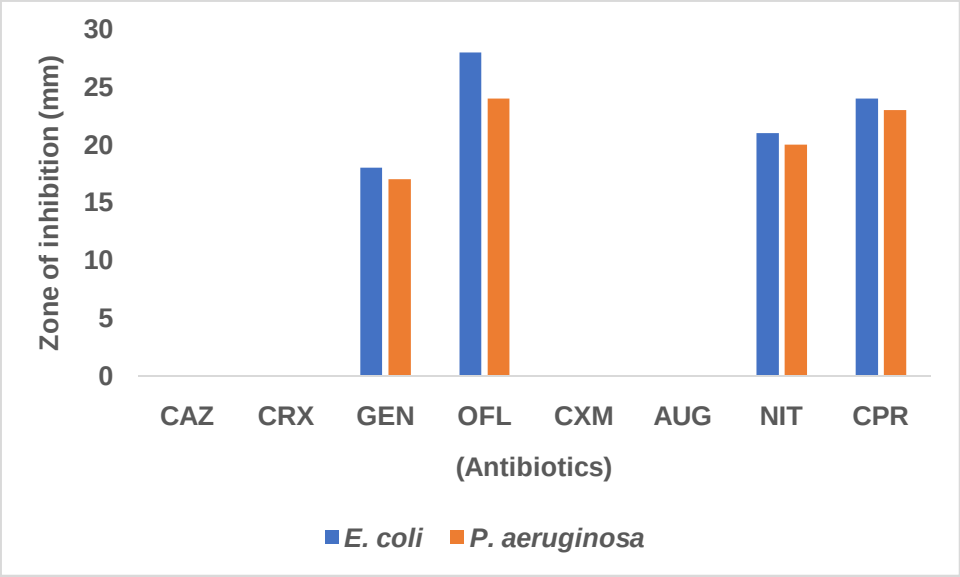

Figure 3: **Antibacterial activity of Standard antibiotics on *E. coli* and *P. aeruginosa***

Legend: CAZ- Ceftazidime (30 µg), CRX- Cefuroxime (30µg), GEN- Gentamicin (30µg), OFL-Ofloxacin (5µg),CXM- Cefixime (10µg), AUG-Augmentin (30µg),NIT- Nitrofurantoin (30µg), CPR- Ciprofloxacin.

Both Gram positive (*Staphylococcus aureus* and *Staphylococcus saprophyticus*) and Gram negative organisms (*E. coli* and *Pseudomonas aeruginosa*) were resistant to ceftaxidime, cefuroxime, ceftriazone, cloxacillin, and augmentin.Gram-positive organisms (*S. aureus* and *S. saprophyticus*) were sensitive to gentamicin, erythromycin, and ofloxacinbut resistant to□-lactams antibiotics. The Gram-negative organisms (*E. coli* and *P. aeruginosa*) were sensitive to gentamicin, ofloxacin, nitrofurantoin and ciprofloxacin but resistant to augmentin, cefuroxime, ceftazidime, and cefixime.

The GC-MS revealed the presence of thirty (30) organic compounds (Figure 4 and Table 1).

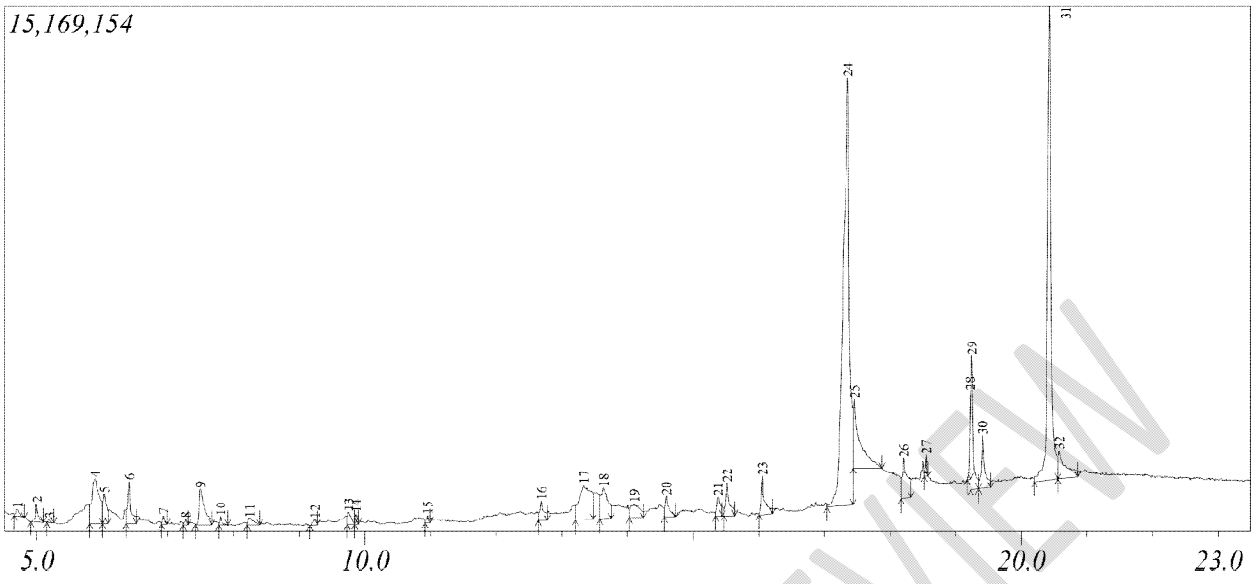

Fig. 4: GC MS analysis of an ethanolic extract of *Pleurotus ostreatus* using GC-MS-QP2010 SHIMADZU, showing 30 identified organic compounds in varying amounts.

Table 1: Gas chromatography–mass spectrometry results showing the phytochemicals, together with their corresponding peak areas, found in the *Pleurotus ostreatus* extract

Peak	Retention time (min)	Peak area (%)	Name of compound
1	4.699	0.32	5-Hexen-2-ol, 5-methyl-
2	4.993	0.57	2-Oxepanone, 7-methyl-
3	5.189	0.10	1-Methoxy-3-hydroxymethylheptane
4	5.881	4.16	Methyl 2-0-benzyl-d-arabinofuranoside
5	6.030	1.33	Erythritol
6	6.406	1.62	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
7	6.928	0.20	1H-Azepin-1-amine, hexahydro-
8	7.271	0.16	1-Methoxy-3-hydromethyloctane
9	7.492	2.33	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy
10	7.798	0.22	Deoxyspergualin
11	8.237	0.48	d-Mannitol, 1,4-anhydro-
12	9.232	0.27	d-Mannitol, 1,4-anhydro
13	9.752	0.59	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hex
14	9.867	0.27	1H-Pyrazole-1-acetonitrile, 4,5-dihydro-3--

15	10.957	0.24	trans-2-undecenoic acid
16	12.688	0.92	Pentanedioic acid, 3-ethyl-3-methyl-, bis(1-
17	13.331	4.79	2-Deoxy-D-galactose
18	13.641	2.50	alpha-D-Glucopyranoside, 0-alpha-D-gh
19	14.099	1.53	trans-2-undecenoic acid
20	15.595	0.95	Hydrazinecarboxamide, 2-(3-methylcyclobe
21	15.375	0.88	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahyde
22	15.518	1.46	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahyde
23	16.051	1.54	n-Hexadecanoic acid
24	17.359	33.75	Oleic Acid
25	17.461	5.41	9,12-Octadecadienoic acid(Z,Z)-
26	18.207	2.49	13-Tetradecenal
27	18.552	0.37	8-Methyl-6-nonenamide
28	19.224	1.50	(Z)6,(9-Pentadecadien-1-ol
29	19.252	3.19	Z,Z-3,13-Octadecedien-1-ol
30	19.417	2.18	Hexadecanoic acid, 2-hydroxy-1-(hydroxyn
31	20.432	21.21	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hyd
32	20.583	2.47	n-Propyl 9-tetradecenoate

The major compounds detected were oleic acid (peak 24, 33.75%)followed by 9-octadecenoic acid (Z)-2-hydroxy-1- (peak 31, 21.21%), then 2-deoxy-D-galactose (peak 17 and 4, 4.79%) and Methyl 2-O-benzyl-d-arabinofuranoside (peak 4,4.16%) (Table 1). Other fatty acids and their alcohols include, 9,12-Octadecadienoic acid(Z,Z)-(peak 25, 5.41%); Z,Z-3,13-Octadecedien-1-ol (peak 29, 3.19%); n-Hexadecanoic acid (peak 23, 1.54%), (Z)6,(9)-Pentadecadien-1-ol (peak 28, 1.50%), trans-2-undecenoic acid (peak 19, 1.53%), 9-Octadecenoic acid (Z)-,2-hydroxy-1-(peak 31, 21.21%) and Hexadecanoic acid-2-hydroxy- 1-(peak 30, 2.18%). The retention time for the chemicals ranged between 4.699- 20.583 mins.

Discussion

Both the Gram –ve bacteria (*E. coli* and *Pseudomonas*)and Gram +vebacteria (*S. aureus* and *S. saprophyticus*)were sensitive to gentamicinand ofloxacin. Ofloxacin is a broad-spectrum fluoroquinolone antibiotic known to inhibit DNA replication by inhibiting bacterial DNA topoisomerase and DNA-gyrase (Thai *et al*., 2020). Gentamicin is bactericidal broad spectrum aminoglycoside antibiotic that inhibits bacterial protein synthesis by binding to 30S ribosomes. Both Gram +ve and Gram -ve bacteria were found to be resistant to the beta lactam antibiotics. The indication is that all the test organisms have developed resistance to the beta lactam antibiotics used in this study and this has serious implication for treatment of diseases caused by these organisms. The beta lactam antibiotics function by inhibiting the synthesis of bacterial cell wall (peptidoglycan). The resistance to beta lactam by many bacteria has been attributed to modification of porins (permeability barrier) and of targets (low affinity of PBP's for the drug), production of inactivating enzymes (beta lactamases) and inhibition of release of autolytic enzymes. Bacteria have developed sophisticated genetic mechanisms to adapt to treatments with novel beta-lactam antibiotics (Heesemann, 1993).

The result of phytochemical analysis by GC-MS revealed that ethanolic extract of *Pleurotus ostreatus* have very informative structures which are of medical relevance. Most of the compounds are structural fragments found as part or component of bioactive compounds. Generally, the extract contained sugar, sugar alcohols, alkaloids, amines, fatty acids, esters, methyl esters, fatty alcohols, aldehydes alcohols, *etc*., some of the fatty acids like Trans- 2 – undecenoic, 9-octdecenoic acid (Z)-2-hydroxy-1- are unsaturated fatty acids. The analysis is similar to the work of Deepalakshmi and Mirunalini (2015) who revealed the presence of alkaloids, steroids, terpenoids, phenols carbohydrates and protein in ethanolic extract of *P. ostreatus* and concluded that *P. ostreatus* is a novel mushroom with various bioactive compounds with pharmacological importance. Monounsaturated and polyunsaturated fats are known to lower the risk of heart diseases, stroke and lower bad LDL cholesterol levels while increasing good HDL. Fatty acids are also reported to influence other disease conditions such as type 2 diabetes, inflammatory diseases and cancer (Philip, 2015). Thillaimaharani*et al.* (2013) also reported ethanol extract of *P florida* exhibited good antioxidant activity (230 µg equivalent of BHT/g), strong reducing power inhibition (79.24%) and high phenolic content (6.25 mg gallic acid/g of dry extract). The results provided evidence that the ethanolic extracts of *P. florida, an oyster mushroom* might indeed be potential sources of natural

antioxidant and antimicrobial agents. β-carotene–linoleic acid and ORAC assays of *P. ostreatus*, alsoshowed high antioxidant activity(Elijah *et al.* 2018).

Oyster mushrooms are known to impart a delicate briny savouryflavour to foods. *P ostreatus* from this analysis contained fatty alcohol which could be produced from hydrogenation of fatty acid and methyl ester and is a major contributor of odor in mushrooms. Other pleasant odor producing compounds identified were at peak 26: 13-tetradecenal (2.49%) and Peak 6 (1.62%) 2, 5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), a derivative of furan used in flavor and perfume industry. Furaneol has a butenolide structure motif like ascorbic acid with antioxidant activity comparable to ascorbic acid. Ascorbic acid and its derivatives exhibit diverse biological activities with potent antioxidant, antitumor and antiviral properties (Andrijana *et al.,* 2019).

Sugar and sugar containing derivatives were among the compounds found in *P. ostreatus* and these include: 2-deoxy-D-galactose (4.79%), Methyl-2-0-benzyl-ol-arabinofuranoside (4.16%), D-Glucopyranoside,o-alpha-(2.50%). Sugar alcohols, erythritol (1.33%) and mannitol were identified as components of ethanolic mushroom extract. Sugar alcohols are normally used as a sugar substitute in diet and health oriented foods especially in diets for diabetics.

Small amount of Deoxyspergualin (0.22%) was identified. Deoxyspergualin belongs to a class of antitumor/antibiotic agent used as immunosuppressant for the treatment of rejection crisis in cell transplantation (Harrison *et al.,* 2007). Also found were pyranone and pyranone moieties, unsaturated ester, 4H-Pyran-4-one-2, 3-dihydroxy-6-methyl (2.33%) at Peak 9. The Pyranone moieties are reported to exhibit biological activities like antimicrobial, antitumor and antileukamic properties (Fairlamb *et al*., 2004). However, Iwalokun*et al.* (2007) on phytochemical analyses of *P. ostreatus* reported low to moderate levels of terpenoids, tannins, steroidal glycosides and carbohydrates while flavonoids, cyanogenic glycosides were not detected. The differences in result of phytochemical analysis could be as a result of the solvent used for extraction. Relative polarity of solvents is known to influence the rate of extraction. Acetone and petroleum ether with relative polarity of 0.355 and 0.117 respectively were used by the Iwalokun et al, (2007) while ethanol with polarity of 0.654 was used for this study. Rahimah *et al.* (2019) reported that ethanolic extractions seem to be the most active preparation for *P. ostreatus* phytochemical detection as flavonoid, phenolic compounds, tannin, saponin, alkaloids, and steroids were detected in the

fresh plant's materials (FPM), dry plant's materials (DPM), 70% ethanolic extract (EE70) and also in the 96% ethanolic extract (EE96). Alkaloid, however was not identified by the Mayer Reagent in the FPM and DPM. The DPM and EE70 seemed to have the highest amount of saponin based on the foam output. Meanwhile, steroids and flavonoids were detected at a higher level in the EE96, based on the strength of visible color and triterpenoids and quinones could not be identified. He also reported differences in the phytochemical screening of samples could be due to the influence of water content and polarity of the active substances. Ethanol is a more polar solvent than acetone. The polar active substances seem to be more soluble in the ethanol (EE70) than the ethanol (EE96). The higher the bioactive substances in the preparation, the more significant the bio-therapeutic effects.

Conclusion

The ethanolic and methanolic extracts of *Pleurotus ostreatus* exhibited antibacterial activity against test organisms except on *Escherichia coli* at 12.5 % ethanolic extract. Methanolic extract was more inhibitory and had higher zones of inhibition than ethanolic extract. Comparatively the fluoroquinolones, aminoglycoside and sulfonamide antibiotics were most inhibitory on test organisms than the mushroom extracts. Ethanolic extract of *Pleurotus ostreatus* revealed the presence of thirty different chemical components in varying quantities. These include sugar, sugar alcohols, alkaloids, amines, fatty acids and their methyl esters, fatty alcohols, aldehydes alcohols, esters etc., The most abundant were oleic acid and 9-octdecenoic acid (Z)-2-hydroxy-1-. Most of these compounds are heterocyclic compounds with mainly nitrogen and oxygen heteroatoms and are found in many natural products as antioxidants, pigment, flavonoids, antibiotics and vitamins. The implication of this finding is that some of these compounds can serve as lead to drug discoveries as they are of medical importance and could enhance health and nutritional wellbeing. *Pleurotus ostreatus* possess phytochemicals of medical relevance and has great potential for pharmacological application.

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