

Full Length Research Paper

# Comparative phytochemical evaluation, antimicrobial and antioxidant properties of *Pleurotus ostreatus*

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This study scientifically examined the phytochemistry, antioxidant and antimicrobial potencies of two organic extracts of *Pleurotus ostreatus*. Generally, both extracts were effective against 89.8% of the isolates tested with *Bacillus subtilis* (7.6 – 7.8 mm), *Escherichia coli* (7.6 – 8.2 mm) and *Saccharomyces cerevisiae* (10.5 – 10.8 mm) exhibiting highest gram  $\pm$ ve, gram  $-$ ve and fungal susceptibilities by agar well diffusion method, respectively. However, petroleum ether extract (PE) exhibited greater anti-gram negative bacterial activity than the acetone extract (AE) and further produced growth inhibition of these isolates in broth. Compared to PE, the acetone extract elicited higher total phenolic content and *in vitro* antioxidant capacity. Phytochemical analyses of the extracts revealed low to moderate levels of terpenoids, tannins, steroidal glycosides and carbohydrates, while flavonoids, alkaloids and cyanogenic glycosides were not detected. The results indicate that *P. ostreatus* possesses antimicrobial and antioxidant potentials.

**Key words:** *Pleurotus ostreatus*, organic extracts, antimicrobial activity, antioxidant capacity, phytochemistry.

## INTRODUCTION

Edible mushrooms are nutritionally endowed fungi (mostly Basidiomycetes) that grow naturally on the trunks, leaves and roots of trees as well as decaying woody materials (Chang and Miles, 1992; Stamets, 2000; Lindequist et al., 2005). Scientific explorations of Shiitake mushrooms such as *Lentinus edodes*, Maitake mushrooms such as *Grifola frondosa*, chanterelles such as *Chaterellus carius*, white button mushrooms such as *Agaricus bisporus*, and oyster mushrooms have shown that they serve as repositories of B-vitamins such as niacin, flavin and pyridoxine (Solomko and Eliseeva, 1988); organic acids such as ascorbate, shikimate, malate and fumarate; carbohydrates such as the  $\beta$ -glucans; monoterpenoid and diterpenoid lipids; proteins such as hydrophobins and trace elements such as selenium (Dikeman et al., 2005; Valentao et al., 2005). These substances have been found through several *in vitro* and *in vivo* studies to be responsible for the antimicrobial, antioxidant, antitumor, antihypertensive and antiaging potentials of edible mushrooms

(Lindequist et al., 2005). The recognition of *Ganoderma lucidum* also called reishi as an antioxidant mushroom is due to its phenolic, terpenoids and polysaccharide polypeptide contents (Miller et al., 2000; Mau et al., 2002; Valentao et al., 2005). These bioactive compounds mediate biological activities including stimulation of interleukin-12 production, nitric oxide synthase activation, free radical scavenging and iron chelating properties (Cui et al., 2005; Acharya et al., 2005).

*Pleurotus ostreatus* is among the edible mushrooms consumed in Nigeria. It is used as spice in vegetable soups and also fried to serve as meat (Annon, 2004). The mushroom is credited to be the third largest macrofungus cultivated for food and industrial purposes worldwide. Nutritionally, the mushroom has been found to contain vitamins B1 (thiamin), B2 (riboflavin), B5 (niacin), B6 (pyridoxine) and B7 (biotin) (Solomko and Eliseeva, 1988).

The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes (Fountoulakis et al., 2002). While from clinical viewpoint, Bobeck and Galbavy (1999) showed that *P. ostreatus* elicited hypocholesterolemic and atherogenesis inhibition functions in rabbits and rat courtesy of its mycelial secretory products.

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However, unlike the fruiting bodies of few other edible mushrooms such as *L. edodes*, *G. fondosa* and *G. lucidum* known for exhibiting antibacterial and antifungal activity *in vitro*, there is lack of information on the microbicidal properties of *P. ostreatus* coupled with inadequate data on its phytochemistry. It is hypothesized that knowledge of the phytoconstituents of *P. ostreatus* would provide an insight into its biological functions beyond nutrition when consumed. In the present study, organic mycelia extracts of *P. ostreatus* were phytochemically analysed and tested for antioxidant function *in vitro*. The antimicrobial activity of these extracts was also examined.

## MATERIALS AND METHODS

### *Pleurotus ostreatus*

Strains of *P. ostreatus* sold as edible mushrooms in Lagos metropolis were brought at different locations in Lagos. They were identified at the Department of Pharmacognosy of the College of Medicine University of Lagos. A voucher specimen (PPL 001 - 002), was deposited in the Herbarium section at DANIFOL Biotechnology Consult Laboratory. The fruiting bodies, carefully removed from the hyphae were weighed then dried at 40°C for 24 h. The dried fruiting body samples were weighed and ground into powder prior to extraction.

### Preparation of *Pleurotus ostreatus* extracts

Dried samples of fruiting body powder (3.5 g each) were separately extracted with 100 ml each of petroleum ether (20 – 80°C) and 80% acetone for 2 h using soxhlet apparatus. The residual solvent was removed by evaporation at 40°C for 24 h *in vacuo* using a rotatory evaporator. The resulting organic extracts were further reconstituted to different concentrations (0 – 100% v/v) with 0.1% Tween-20 in phosphate buffered saline (pH 7.2) followed by storage in sterile capped bottles under refrigeration condition (4°C) prior to use for subsequent assays.

### Phytochemical analysis of *Pleurotus ostreatus* extracts

The phytoconstituents present in the organic extracts were determined qualitatively according to Sofowora (1993), Trease and Evans (1989) and Harbone (1973) as well as by thin layer chromatography (TLC). In TLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic-water (100 : 10 : 10) as the solvent system. The developed plates were then sprayed with vanillin solution (1% (w/v) in 50% phosphoric acid) for steroid detection, Dragendorff's reagent for alkaloid detection and sodium metaperiodate (0.1%) followed by ethanolic benzidine for glucose detection. Nicotinic acid, cholesterol D-glucose and tannic acid at 1% solution were prepared accordingly and used as standards in the TLC assay. The TLC results were further used to validate the presence of tannins based on positive reaction (brownish green – blue black coloration) with 0.1% FeCl<sub>3</sub>, alkaloids based on positive reaction (brown coloration) with Dragendorff's reagent (Trease and Evans, 1989; Sofowora, 1993), steroids based on positive reactions (violet to blue or green) with acetic anhydride and H<sub>2</sub>SO<sub>4</sub>, steroidal glycosides by Keller-Killani test and cyanogenic glycoside based red coloration of picrate paper (Harbone, 1973; Trease and Evans, 1993). The observation of persistent frothing in distilled water (2 ml) by 1% standard saponin solution (3 ml) follow-

ed by formation of emulsion with olive oil (0.5 ml) was used to indicate the presence of saponin in the extract (Trease and Evans, 1989).

### Total phenolic content determination

The total phenolic compounds present in the extracts were determined spectrophotometrically at 750 nm as described by Lee et al. (2001) using Folin-Ciocalteu pheno reagent. Standard solutions of gallic acid (20 – 200 mg/ml) were used to prepare a standard curve. The total phenolic contents of the extracts are expressed in milligrams per ml of gallic equivalents (GAE) of triplicate measurements.

### Antioxidant activity determination

The antioxidant activity of the *P. ostreatus* extracts was determined via the 2,2 – diphenylpicrylhydrazyl radical neutralization assay as described by Brand-Williams et al. (1995). Extracts were also tested as antioxidants using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay (Cano et al., 1998). In both assays, 50 µl of each extract was added to with 950 µl of the free radical methanolic solution and stirred continuously for 15 min for DPPH or 10 min for ABTS. Decreases in absorbance were respectively measured at 414 and 515 nm for ABTS and DPPH systems, respectively, and compared to that of infusion made from green tea a well-known antioxidant food (Cao et al., 1996). The infusion was prepared by boiling 1 g green tea bag with 100 ml of deionised water for 5 min. Antioxidant activity of the each extract was expressed as vitamin C equivalent antioxidant capacity (VCEAC) in millimolar (mM) by extrapolation from the standard curve. Two – fold serial dilutions of L-ascorbic acid (Aldrich, Germany) in deionised water to a final concentration range of 0.75 – 6.0 mM were used to prepare the calibration curve.

### Microorganisms

The microorganisms to which the antimicrobial properties of the organic extracts of *P. ostreatus* were tested were obtained from the Departments of Biotechnology and Molecular Biology of the Nigerian Institute of Medical Research (NIMR), Yaba-Lagos. The bacterial isolates were multidrug resistant determined by disc diffusion assay according to Iwalokun et al. (2004) (Table 1). They were recovered as viable isolates by subculturing their stock cultures in 16% glycerol-trypticase soy (TSB) or brain heart infusion broth (BHI) at –20°C or malt extract medium. The lactobacilli were recovered by growth in Mann Rogossa Sharpe (MRS) medium under anaerobic conditions (De Mann et al., 1960). Other bacterial isolates were recovered by growth on trypticase soy agar or blood agar under aerobic conditions at 37°C for 24 h. Aerobic growth at 30°C for 24 h on potato dextrose agar (PDA) was the basis for the recovery of *Saccharomyces cerevisiae* and *Candida* isolates tested. The recovered microbes were gram stained to confirm their gram reaction status and speciated according to Cowan and Steel (1974). The gram-negative organisms include *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Haemophilus influenza*, while the gram-positive isolates were *Bacillus licheniformis*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Lactobacillus acidophilus*.

Strains of *Candida albicans* ATCC 1880 and *Sacharomyces cerevisiae* were also tested to validate the antibiotic and extract susceptibility assays of the fungal isolates tested.

### Antimicrobial testing of *Pleurotus ostreatus* extracts

*P. ostreatus* extracts were tested for antimicrobial activity by agar – well diffusion technique (Akpata and Akinrimisi, 1977) with a little

**Table 1.** Bacterial isolates and antibiotic resistance profiles.

@Isolates	N	Antibiotic resistance
<i>Salmonella typhi</i>	5	Cot, Col, Tet, Str, Chl, Amp
<i>Staphylococcus aureus</i>	8	Str, Amp, Pen, Amo, Aug
<i>Staphylococcus epidermidis</i>	4	Str, Amp, Amo, Pen
<i>Staphylococcus aureus</i>	2	Str, Amp, Amo, Aug, Pen, Met
<i>Escherichia coli</i>	10	Cot, Col, Chl, Str, Tet
<i>Haemophilus influenzae</i>	4	Ery, Str, Cot, Amo, Amp, Tet
<i>Proteus spp</i>	5	Amp, Tet, Cot, Col, Str, Cot
<i>Pseudomonas aeruginosa</i>	6	Str, Ery, Tet, Kan, Col, Cot, Amo
<i>Klebsiella pneumoniae</i>	4	Amp, Tet, Str, TMP-STX
† <i>Lactobacillus acidophilus</i>	8	Tet, Col

@Isolates were grouped based on their display of 100% resistance to antibiotics by disk diffusion assay following identification according to Cowan and Steel (1974). N = number of isolates tested; Str = streptomycin; Ery = erythromycin; Aug = augmentin; Cef = cefotaxime; Cro = ceftriaxone; Tet = tetracycline; Amp = ampicillin; Amo = amoxicillin; Kan = kanamycin; Gen = gentamicin; Chl = chloramphenicol; Col = colistin sulphate; cot = cotrimoxazole; and TMP-STX = Trimetoprim – sulphamethoxazole. †The antibiotic resistance patterns of the isolates except *L. acidophilus* were given as previously described (Iwalokun et al, 2004).

modification. An overnight culture of each microbial isolate was emulsified with nutrient broth to a turbidity that was equivalent to 0.5 McFarland ( $10^8$  cfu/ml). 100  $\mu$ l of each standard inoculum was then streaked on nutrient agar and PDA at  $10^5$  cfu/ml per spot to attain a confluent growth (Bauer et al., 1966). Wells were made on the agar using a sterile cork borer and filled with 100  $\mu$ l *P. ostreatus* organic extract. The plates were incubated accordingly as described previously. Standard strain of *E. coli* ATCC 25922 was used as control organism for bacterial assays. Bacteria and fungal control wells contained 100  $\mu$ l of ciprofloxacin and fluconazole, respectively, at 5  $\mu$ g per well. A well containing 100  $\mu$ l of Tween-20-PBS solution (pH 7.2) was generally used as a positive control well in every assay. Growth inhibition was measured as diameters of inhibitory zones in the nearest 0.1 mm.

#### Minimum inhibitory concentration (MIC)

The MICs of the organic extracts were determined by broth dilution method according to Alade and Irobi (1993) with a little modification. The extract was serially diluted with normal saline (0.85 g/dl) to 5 – 50 mg/ml preparations dispensed (1.0 ml) into test tubes containing 1.0 ml of nutrient or potato dextrose broth. Each sensitive bacterial or fungal isolate (100  $\mu$ l of  $10^8$ cfu/ml) was inoculated into the test tubes. The tubes were mixed, covered with cotton wool and incubated appropriately as previously described. Thereafter, the tubes were then examined for microbial growth. The MIC was defined as the minimum concentration of the extract that did not allow any visible growth or turbidity of the organism in broth. MIC<sub>50</sub> refers to concentration (%) of *P. ostreatus* required to prevent the growth of 50% of organisms tested. While MIC<sub>90</sub> oil extract concentration, which inhibits the growth of 90% of the organisms tested.

## RESULTS

The present study has revealed the antibacterial and antifungal activity of petroleum – ether (PE) and acetone

(AE) extracts of *P. ostreatus* fruiting body against multi drug resistant bacterial pathogens presented in Table 1. Data presented in Table 2 showed the fruiting body wet and dry weights per *P. ostreatus* strain analyzed ranged from 1.18 – 1.37 g (mean weight  $1.27 \pm 0.038$  g) and 0.126 – 0.174 g (mean weight  $0.152 \pm 0.0025$  g), respectively. The petroleum and acetone extract yields ranged from 3.32 – 3.40 mg / g dry mycelial weight yielding an average of  $3.36 \pm 0.01$  mg/g dry weight.

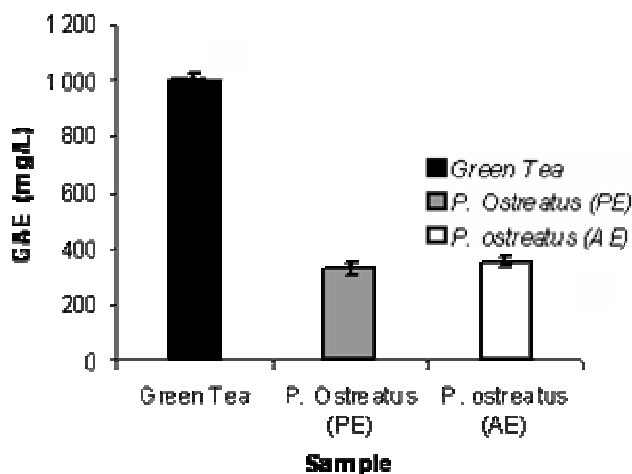
Phytochemical analysis revealed the presence of terpenoids, tannins, steroidal glycosides and carbohydrates in both extracts but with higher terpenoids contents in the PE fraction. Cyanogenic glycosides, alkaloids, flavonoids and anthraquinones were not detected in both extracts. Total phenol contents of 325.7 and 352.8 mg/L GAE were elicited by PE and AE extracts, respectively, and were further found to be significantly ( $P < 0.05$ ) lower than 1005.3 mg/L GAE ( $P < 0.05$ ) in the green tea infusion used as standard (Figure 1).

Preliminary antimicrobial testing of petroleum ether extract of *P. ostreatus* by agar-well diffusion produced zones of growth inhibition of  $3.0 - 7.8 \pm (0.1 - 0.9)$  mm for the gram positive bacteria,  $5.0 - 8.2 \pm (0.1 - 0.7)$  mm for the gram negative bacteria and  $8.1 - 10.8 \pm (0.1 - 1.6)$  mm for the fungal isolates tested (Table 3). Among the susceptible gram-negative bacteria tested, inhibitory zones due to petroleum extract were higher than those of the acetone extract.

Among the gram-positive bacteria tested, local isolates of *L. acidophilus* (2 of 8) and *B. subtilis* (5 of 5) were observed to be the least [ $3.0-3.2 \pm (0.1 - 0.2)$  mm] and most [ $7.6 - 7.8 \pm (0.2-0.8)$  mm] sensitive strains in both organic extracts. In gram-negative bacteria, local isolates of *E.*

**Table 2.** Oil yield and phytochemical analysis of extracts of *Pleurotus ostreatus* mycelium.

Parameter	Amount	
Fruiting body wet weight (g) / strain	1.18 – 1.37 (1.27 ± 0.038)	
Fruiting body dry weight (g) / strain	0.126 – 0.174 (0.152 ± 0.0025)	
Extract yield (mg/g dry weight)	3.32 – 3.40 (3.36±0.01)	
Phytoconstituents		
	PE	AE
Terpenoids	±±	±
Tanins	±	±
Steroidal glycosides	±	±
Carbohydrates	±	±
Cyanogenic glycosides	-	-

**Figure 1.** Total phenolic content of *Pleurotus ostreatus* extracts compared to green tea. AE = acetone extract; PE = petroleum ether extract.

*coli* and *H. influenzae* exhibited the highest [7.6 – 8.2 ± (0.7 – 0.8) mm] and lowest [5.0 - 5.5 ± (0.5-0.7) mm] susceptibilities, respectively. Furthermore, highest susceptibility [10.5 - 10.8 ± (1.2-1.6) mm] by *S. cerevisiae* strains (4 of 4) and lowest susceptibility [8.0-8.1 ± (0.1-0.3) mm] by *C. albicans* ATCC1880 were observed among the fungal isolates. On the whole, 79 (89.8%) of the 88 isolates tested showed sensitivity to acetone and petroleum ether extracts of *P. ostreatus* (Table 3).

The petroleum ether extract of *P. ostreatus* further showed stronger inhibition of these organisms in broth compared to the acetone extract (results not shown) with MICs of 78.3 – 100.0 ± (0 – 4.1)% extract for gram positive bacteria with *Staphylococcus* sp. having mean MIC<sub>50</sub> and MIC<sub>90</sub> of 71.7 ± 2.9 and 79.2 ± 8.8%, respectively. The two sensitive *L. acidophilus* were inhibited with MIC<sub>90</sub> of 100% extract, equivalent to the MIC (Table 4). The inhibition of gram-negative bacteria: *S. typhi* and *E. coli*

by PE was observed at lower MICs (72.5 – 75.0%), MIC<sub>50</sub> (66.3 – 67.5%) and MIC<sub>90</sub> (71.3 – 72.5%). The PE also caused growth inhibition of the fungal isolates tested in potato dextrose medium with MICs of 70.0 ± 3.5% and 67.5 ± 3.4% for *Candida* sp. and *S. cerevisiae*, respectively (Table 4).

Antioxidant activity of the extracts by DPPH and ABTS methods revealed a disparate VCEACs of 3.6 – 3.8 mM for PE and 4.1 – 4.4 mM for AE ( $P > 0.05$ ) compared to 6.2 – 6.4 mM in the green tea infusion ( $P < 0.05$ ) (Figure 2).

## DISCUSSION

*P. ostreatus*, an oyster mushroom is primarily consumed for its nutritive value and used industrially as a bioremediator (Solomko and Eliseeva, 1988; Fountoulakis et al., 2002; Tsioulpas et al., 2002). The present study has further revealed the antimicrobial potency of the oil of the macrofungus extracted with petroleum ether and acetone. Both extracts were observed to inhibit gram positive and gram-negative bacteria as well as fungi tested *in vitro* to suggest that *P. ostreatus* has a broad-spectrum antibacterial and antifungal activity. Similar antimicrobial potentials have been observed in the culture extracts of *Irpex lacteus* (Rosa et al., 2003) *Agrocybe* sp. (Kavanagh et al., 1950; Mavoungou et al., 1987), and juice of *L. edodes* (Kuznetsov et al., 2005). Antimicrobial potencies of the essential oils from mushrooms such as *Cuminum cymimum*, *carum carvi*, *Coriandum sativum* and *Foeniculum vulgare* have also been reported (Iacobellis et al., 2005) with activity against bacterial pathogens such as *Pseudomonas*, *Klebsiella*, *Salmonella* and *E. coli* as observed for *P. ostreatus* in this study.

The observed disparity in the the susceptibilities of gram-ve bacteria tested with petroleum ether extract eliciting greater effect provides an indication that the organic solvents used have varying abilities to extract bioactive substances from *P. ostreatus*. This is further evidenced

**Table 3.** Preliminary antimicrobial testing of *Pleurotus ostreatus* extracts. Inhibition zone diameter (mm ± SD)\*

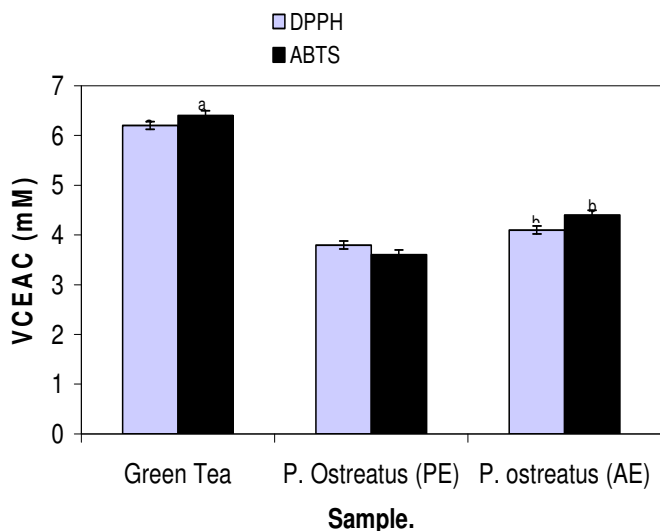
@Isolates	<i>Pleurotus ostreatus</i>		Standard drugs <sup>#</sup>	0.1% (v/v) Tween-20
	PE	AE		
<b>Gram - positive bacteria</b>				
<i>Staphylococcus aureus</i> ATCC 25923	7.5 ± 0.1	7.6 ± 0.3	27.5 ± 0.7	G
<i>Staphylococcus aureus</i> (8)	7.2 ± 0.9	7.1 ± 0.7	27.7 ± 0.9	G
<i>Staphylococcus aureus</i> (2)	7.0 ± 0.8	7.0 ± 0.6	28.1 ± 0.4	G
<i>Staphylococcus epidermidis</i> (4)	7.1 ± 0.8	7.0 ± 0.7	29.1 ± 0.5	G
<i>Bacillus subtilis</i> ATCC 6633	7.1 ± 0.1	7.2 ± 0.2	27.8 ± 0.3	G
<i>Bacillus subtilis</i> (5)	7.8 ± 0.2	7.6 ± 0.8	28.4 ± 0.6	G
<i>Bacillus licheniformis</i> (10)	7.7 ± 0.2	7.7 ± 0.4	27.8 ± 0.4	G
† <i>Lactobacillus acidophilus</i> (2)	3.0 ± 0.2	3.2 ± 0.1	28.9 ± 0.4	G
<b>Gram –negative bacteria</b>				
<i>Salmonella typhi</i> ATCC 13391	7.5 ± 0.1	7.2 ± 0.4	28.1 ± 0.1	G
<i>Salmonella typhi</i> (5)	7.2 ± 0.3	7.0 ± 0.1	28.4 ± 1.3	G
<i>Escherichia coli</i> ATCC25922	7.0 ± 0.0	7.0 ± 0.0	29.5 ± 0.1	G
<i>Escherichia coli</i> (10)	8.2 ± 0.7	7.6 ± 0.8	28.5 ± 1.0	G
<i>Proteus</i> sp. (3)	6.9 ± 0.5	6.5 ± 0.6	27.8 ± 0.7	G
<i>Klebsiella pneumoniae</i> (4)	7.1 ± 0.2	7.0 ± 0.1	28.0 ± 0.1	G
†† <i>Pseudomonas aeruginosa</i> (3)	5.6 ± 0.4	5.2 ± 0.2	28.2 ± 0.6	G
<i>Haemophilus influenzae</i> (4)	5.5 ± 0.5	5.0 ± 0.7	27.7 ± 0.9	G
<b>Fungi isolates</b>				
<i>Candida albicans</i> ATCC 1880	8.1 ± 0.1	8.0 ± 0.3	28.4 ± 0.0	G
<i>Candida</i> sp. (10)	8.2 ± 0.3	8.3 ± 0.1	28.2 ± 0.7	G
<i>Saccharomyces cerevisiae</i> (4)	10.8 ± 1.2	10.5 ± 1.6	28.8 ± 0.6	G

Figures in parentheses represent the number of isolates tested; † All and two of the tested *L. acidophilus* strains were sensitive to ciprofloxacin and *P. ostreatus*, respectively. †† Three (3) of the ciprofloxacin sensitive *Pseudomonas aeruginosa* isolates (6 Of 6) were also sensitive to *P. ostreatus*. G = Bacterial growth around the in phosphate buffered 0.1% Tween-20 control wells; # = growth inhibition zones for ciprofloxacin (5µg per well) and fluconazole (5µg per well) used as standard drugs. \*The diameters of zone of inhibition were expressed in millimeter (mm) as mean ± standard deviation. @Seventy-nine (89.8%) of the 88 isolates showed sensitivity to *P. ostreatus*. PE = Petroleum ether extract; AE = Acetone extract.

**Table 4.** Minimum inhibitory concentrations determinations of *Pleurotus ostreatus* petroleum ether extract against multidrug resistant bacteria and yeasts.

Isolates	MIC (% extract)		MIC
	MIC <sub>50</sub>	MIC <sub>90</sub>	
<b>Gram - positive bacteria</b>			
<i>Staphylococcus aureus</i> (15)	71.7 ± 2.9	79.2 ± 8.8 <sup>a</sup>	80.3 ± 4.1 <sup>a</sup>
<i>Bacillus</i> sp. (16)	70.8 ± 5.2	74.5 ± 4.3	78.3 ± 3.8 <sup>ab</sup>
<i>Lactobacillus acidophilus</i> (2)	ND	100.0 ± 0.0	100.0 ± 0.0
<b>Gram –negative bacteria</b>			
<i>Salmonella typhi</i> (10)	66.3 ± 4.8	72.5 ± 6.5 <sup>a</sup>	75.0 ± 5.1 <sup>a</sup>
<i>Escherichia coli</i> (15)	67.5 ± 2.9	71.3 ± 7.5	71.3 ± 7.5
<i>Proteus</i> sp.	83.8 ± 4.8	90.1 ± 4.1 <sup>a</sup>	92.6 ± 4.4 <sup>a</sup>
<i>Pseudomonas aeruginosa</i> (3)	91.7 ± 2.9	100.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>
<i>Klebsiella pneumoniae</i> (4)	75.0 ± 4.1	80.0 ± 4.1 <sup>a</sup>	88.6 ± 2.6 <sup>ab</sup>
<i>Haemophilus influenzae</i> (2)	88.1 ± 2.4	95.0 ± 4.1	100.0 ± 0.0 <sup>a</sup>
Fungi isolates <i>Candida</i> sp. (10)	65.0 ± 5.0	70.0 ± 3.5 <sup>a</sup>	70.0 ± 3.5 <sup>a</sup>
<i>Saccharomyces cerevisiae</i> (4)	62.7 ± 3.8	65.0 ± 4.2	67.5 ± 3.4 <sup>a</sup>

Data are presented as mean ± SD of experiments in duplicates. Figures in parentheses indicate the number of isolates tested. in duplicates. Disparity between mean values was analyzed by student's t- test. <sup>a</sup>P < 0.05 ( MIC<sub>50</sub> vs. MIC<sub>90</sub> or MIC); <sup>b</sup>P < 0.05 (MIC<sub>90</sub> vs. MIC); P < 0.05 = Significant).



**Figure 2.** Antioxidant capacity of *P. ostreatus* extracts compared to green tea. Data are mean  $\pm$  SEM of 3 determinations; AE and PE = Acetone and petroleum ether extract, respectively. <sup>a</sup>P < 0.05 (Green tea vs *P. ostreatus*) <sup>b</sup>P > 0.05 (*P. ostreatus* – AE vs PE).

by the different levels of terpenoids and phenolics observed in the two organic extracts. A clue that the petroleum extract may have greater antibacterial potential was provided by the observed MIC<sub>50</sub> and MIC<sub>90</sub> inhibitory concentrations of the extract against *S. aureus*, *S. typhi* and *Candida* isolates tested. Similar antibacterial characteristics have been observed in fluoroquinolones such as ciprofloxacin (Iwalokun et al., 2001) and medicinal plants such as *Ocimum gratissimum* (Iwalokun et al., 2003). Meanwhile, terpenoids have been implicated as the phytoconstituents responsible for the antibacterial activity of mushrooms including *Cuminum cyminum* and *Carum carvi* (Iacobellis et al., 2005). The observed phenolic and tannin constituents of *P. ostreatus* may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesins (Cowan, 1999).

However, the observed insensitivity of 6 of 8 *L. acidophilus* and 3 of 6 *P. aeruginosa* isolates tested to *P. ostreatus* extracts implies that strain variation may determine susceptibility of bacteria to antibacterial agents from mushrooms. A recent study by Kuznetsov et al. (2005) also revealed the insensitivity of Bifidobacteria and Lactobacilli to the antibacterial action of *L. edodes* juice. However, the observed inhibition of the *S. cerevisiae* strains tested by *P. ostreatus* agrees with the findings of Hwang et al. (2000) in which *Phellinus* sp. were found to halt the growth of *S. cerevisiae* with an IC<sub>50</sub> value of 76  $\mu$ g/ml via chitin synthase inhibition. These observations may confer ecological and metabolic benefits to edible mushrooms since lactic acid bacteria may act synergistically to

improve the nutritional quality and shelf life of associated fermented foods (Okamura-Matsui et al., 2001) and yeast contamination has been implicated in the decay of mushrooms (Middlehoven, 2004). However, the observed growth inhibition halos (5.5 – 10.1 mm) produced by *P. ostreatus* were lower than > 12.0 mm halos produced by *I. lateus* as reported by Rosa et al. (2003) but comparable to halos produced by *Agaricus* sp., *Gloedopus thelephoroides* and *Coprinus* sp. reported by the same workers.

The reported variations in the antimicrobial outcomes of these macrofungus may not be unconnected with their species and strain variations, differences in their microbicidal composition and concentrations, in the method of extraction and mechanism of actions of active principles in these edible mushrooms. Antibacterial agents such as agrocybin from *Agrocybe* sp (Kavanagh et al., 1950) and marasmic acid from *Marasmius conigenus* have been observed to elicit activity at microgram per ml concentrations. Guo et al. (2005) recently isolated trichogin, an antifungal protein from *Tricholoma giganteum* and found the protein exhibiting HIV-1 reverse transcriptase inhibition with an IC<sub>50</sub> of 83 nM.

Other bioactive antimicrobial compounds that have also been isolated from edible basidiomycetes include glaucopines from *Sarcodon glaucopus*, pleuromutilin and chanterellins from chanterelle mushrooms (Curini et al., 2005; Kavanagh et al., 1950).

These substances masquerade as phenolics and terpenoids (Mothana et al., 2000), which were also detected in *P. ostreatus*. Therefore, there is no doubt that the future determination of the structure and functions of the phenolic and terpenoid constituents of *P. ostreatus* would further validate its antimicrobial potentials.

The present study also observed *P. ostreatus* to elicit antioxidant capacity using the DPPH and ABTS methods thereby expanding its nutraceutical values. However, the observed VCEAC (3.6 – 4.4 mM) for *P. ostreatus* is lower than values reported for some selected Indian medicinal mushrooms including *G. lucidum* by Lakshmi (2004) and Button mushrooms by Miller et al. (2000) with antioxidant capacity mostly attributed to their phenolic contents, the antioxidant properties of polysaccharide and polysaccharide peptides of *G. lucidum* being an exception (You and Lin, 2003). However, the observed higher phenolic contents and antioxidant capacity in the acetone extract of *P. ostreatus* compared to the petroleum extract provides an indication for a superiority of acetone over petroleum ether in the exudation of antioxidant substances from *P. ostreatus*.

Flavonoids, which are also phenolic compounds with antioxidant activity (Mau et al., 2002; Gil et al., 2000) were not detected in *P. ostreatus*. Our observation agrees with findings of Mattila et al. (2001) and according to USDA, mushrooms are regarded as non-sources of flavonoids. The absence of flavonoids in mushrooms may be of biological advantage in their various ecological nic-

hes since these bioactive compounds inhibit enzyme activities (e.g. tyrosinase) involved in their pigmentation, growth and development (Xie et al., 2003).

The low and varied phenolic contents of *P. ostreatus*, could also emanate from the differences in the mycelial wet and wet weights of the tested in this study. This may be attributed to the variations in their growth conditions since they were not cultivated in the same location. The works of Nwanze et al. (2005a, 2005b) on *Lentinus squarrosulus* and *Psathyrella atroumbonata* showed that both media composition and oil type influence the mycelial biomass of these mushrooms. Therefore, appreciable amount of extract and thus phenolic compounds and terpenoids can be recovered from *P. ostreatus* if its optimum growth condition is known. This may further enhance its medicinal and market values.

Based on the results of this study, it can be concluded *P. ostreatus*, an edible oyster mushroom has antioxidant potentials and possess a broad-spectrum antibacterial and antifungal activity, optimisable by multiple organic mycelia extraction. Further investigations that would identify the active phenolic and terpenoid compounds and determine the optimal mycelial growth conditions of *P. ostratus* are necessary.

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