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Antimicrobial and Antioxidant Properties of *Pleurotus ostreatus* (Jacq: Fries) Cultivated on Different Tropical Woody Substrates

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Abstract

***Pleurotus ostreatus* (Jacq:Fries) was cultivated on three different tropical woody substrates viz: Cananium sp., *Pycnanthus angolensis* (Welw) and *Ceiba pentandra* (L) Gaertn. Total phenol, antioxidant and antimicrobial properties of extracts obtained from the artificially cultivated *P. ostreatus* were assessed using standard methods. Total phenol contents (TPC) ranging from 0.89 µg/g to 2.63 µg/g was obtained from the *P. ostreatus* extracts. *P. ostreatus* cultivated on *P. ongoleubis* had the highest TPC (2.63µg/ml). Moreover, *P. ostreatus* extracts displayed a good DPPH radical scavenging ability. The percentage scavenging activity against DPPH radicals ranged from 73.75% to 90.75%. *P. ostreatus* cultivated on *P. ongoleubis*, had the highest DPPH radical scavenging ability (90.17%) while the *P. ostreatus* cultivated on *C. pentandra* had the least (73.75%). The mushroom extracts were able to inhibit all the test organisms with zones of inhibition ranging from 5.33 mm to 20.33 mm. *P. ostreatus* cultivated on the *P. ongoleubis* exhibited the best inhibitory effect (18 mm to 20 mm) against *Staphylococcus aureus*. The study revealed that different woody substrate used in cultivating *P. ostreatus* has a significant effect on its antimicrobial and antioxidant properties. *P. ostreatus* cultivated on sawdust from *P. ongoleubis* exhibited better antimicrobial and antioxidant activities when compared with *P. ostreatus* cultivated on the other woody substrates, *C. pentandra* and *Cananium* sp.**

Keywords: Woody, substrate, antimicrobial, antioxidant, *P. ostreatus*, extract.

Introduction

Edible mushrooms have been part of human diet for centuries. The nutritional value of mushrooms is twice that of any vegetable or fruit (Sivrikaya et al., 2000). Mushrooms are known to be medically active in several therapies, such as antitumour, antibacterial, antiviral, haematological and immunomodulating treatments (Wasser, 2002; Lindequist et al., 2005). The therapeutic effect had been linked to the presence of bioactive compounds in mushrooms. Some of these bioactives include glycolipids, compounds derived from shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, sesquiterpenoids, and many other substances of different origins (Lorenzen and Anke, 1998; Wasser and Weis, 1999; Mizuno, 1999). Moreover, mushrooms are recognized as a good source of amino acids which play an important role in their nutritional property.

Pleurotus mushrooms, commonly known as oyster mushrooms, grow in the wild in tropical, subtropical and temperate regions and are easily artificially cultivated (Akindahunsi and Oyetayo, 2006; Chirinang and Intarapichet, 2009). They are healthy foods, low in calories and in fat, rich in protein, chitin, vitamins and minerals (La Guardia et al., 2012; Akindahunsi and Oyetayo, 2006; Manzi et al., 1999). Extract of P. ostreatus had been reported to alleviate hepatotoxicity induced by CCl4 in rats (Jayakumar et al., 2006). The ability of extract from P. ostreatus to protect major organs such as the liver, heart, and brain of aged rats against oxidative stress had also been reported (Jayakumar et al., 2006).

Several lignocellulosic wastes such as corncobs, sawdust, corn stover etc had been used in cultivating Pleurotus species (Ragunathan and Swaminathan, 2000). Preference for the wild mushrooms by some consumers is based on flavor, texture, nutri-

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tional, and pharmacological characteristics of these mushrooms (Manzi et al., 2001). The different substrates used in cultivating mushrooms do have effect on the functional, organoleptic and chemical properties of mushrooms.(Micheal et al., 2011). In the tropics, Pleurotus species usually grow on some woody substrates in the wild where children and women pick it for sale. It is therefore pertinent to investigate the effect of such substrates on some functional properties of Pleurotus species cultivated on such woody substrates. A preliminary investigation on the yield and proximate composition of Pleurotus ostreatus cultivated on some tropical woody substrates had been carried out. The present study therefore seeks to assess the antimicrobial and antioxidant properties of P. ostreatus cultivated on three different tropical woody substrates.

Materials and Methods

Preparation and cultivation of Pleurotus ostreatus on woody substrate

Substrates used in cultivating P. ostreatus were prepared from sawdust of tropical plants, Canarium sp., P. ongoleubis and C. pentandra mixed with water. The substrates were filled into polyethylene bags (800g/bag). The bags were then sealed, autoclaved, cooled and inoculated with the spawn of P. ostreatus collected from Federal Institute of Industrial Research Oshodi, Lagos, Nigeria. Sawdust substrate in bags were inoculated with approximately 2 g of spawn using surface spawning technique under laminar flow and incubated in a dark chamber.

The growth of mycelium in each bag was observed. When the mycelium fully covered the substrate, bags were kept open in the growing house for fruit body formation. The harvested mushroom sporocarps were air-dried after which they were ground to powder using grinding machine.

Preparation of Mushroom Extracts

The extracts from the oyster mushrooms cultivated on the different woody substrates were obtained using 95% ethanol, following the method of Tsai et al. (2007). The dried sample (10 g) was extracted with 100 ml ethanol at room temperature on a shaker at 150 rpm for 24 h and filtered through No. 4 Whatman filter paper. The residue was re-extracted twice and the filtrates were combined. The extract was evaporated almost to dryness in a rotary evaporator (Rotavapor R-114, Buchi) at 40 °C and then subjected to freeze drying (LYOVAC, GEA).

Total Phenolic Contents (TPC)

The total phenolic content was determined by Folin- Cioc^alteu method (Minussi et al.,2003). Sample solution (50 µl) was added to 50 µl of 7.5% sodium carbonate, 50µl distilled water mixed thoroughly and allowed to stand for 2 min. Then, 500 µl of 10% Folin-Cioc^alteu reagent (Folin:Methanol, 1:1, v/v) was added and the mixture was mixed well. After incubation for 40 min, the absorbance was read at 765 nm. A calibration curve was obtained using various concentrations of gallic acid. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAEs) per gram of dry sample.

Determination of Antimicrobial Activity of Extracts

The antimicrobial tests were carried out by the agar diffusion method (Schinor et al., 2007). The dried extracts were reconstituted with 30% dimethyl sulfoxide (DMSO). The bacteria, Klebsiella Pneumoniae,Salmonella typhi, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis, and yeast, Candida albicans used as indicator organisms were clinical isolates obtained from Don Bosco Health Centre Laboratory, Akure, Nigeria. The yeast, Saccharomyces cerevisiae was obtained from culture collection of Department of Microbiology, Federal University of Technology, Akure. The bacteria were cultivated on Nutrient Agar Medium at 30 ± 1 oC for 24 h while the fungal were cultivated on Yeast Malt Extract Agar at 26 ± 1oC for 48 to 72 h. Aliquot of culture (100 µl) was evenly spread on the surface of the solidified agar. Wells of 7 mm were bored in the agar with sterile cork borers. Extract dissolved in 30% dimethyl sulfoxide (DMSO) to the concentration of 10 to 40 mg/ml and filtered through 0.22 µm membrane filter was introduced into the wells. A 100 µl volume was placed in each well. The plates were incubated at 30 ± 1 oC for 24 h for bacteria while the fungi were incubated at 26 ± 1oC for 48 to 72 h. Tetracycline and streptomycin were used as standard antibacterial while nystatin was used as antifungal standard under the conditions specified for bacteria and fungi respectively. The sensitivity of the test organisms to the extracts is indicated by a clear zone of inhibition around the well containing the extracts and the diameter of the clear zone was taken as an index of the degree of sensitivity.

Minimum Inhibitory Concentration

The agar diffusion method described above was used to screen the antimicrobial effect of the different concentrations of extracts (10 to 40mg/ml). The tests were performed in three replicates.

Scavenging Effect of Extracts on DPPH Radicals

Radical scavenging potential of extracts obtained from P. ostreatus cultivated on different tropical woody substrates was assessed using an ethanolic solution of the "stable" free radical, DPPH•. The method of Blois (1958) was used in studying the effect of these extracts on DPPH• radicals with some modifications. A solution of DPPH• (0.5 mmol/L) in ethanol and 0.05 mol/L acetate buffer (pH 5.5) was prepared. Extracts in solution (0.1 ml) at different concentrations was mixed with 2 ml of acetate buffer, 1.9 ml of absolute ethanol and 1 ml DPPH• solution. The mixture was shaken immediately after adding DPPH• and allowed to stand at room temperature in dark for 30 min. The decrease in absorbance at 517 nm was measured using a UNICO 2100 spectrophotometer. BHT was used as positive control and the sample solution without DPPH• was used as blank. The radical scavenging activity was measured as a decrease in

absorbance of DPPH.

Statistical Data Analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way analysis of variance and means were compared by Duncan's multiple range tests (SPSS 18 version). Differences were considered significant at $p \leq 0.05$.

Results and Discussion

P. ostreatus (oyster mushroom) is a common edible mushroom known all over the world. It is one of the more commonly sought wild mushrooms, though it can also be cultivated on straw and other media. The present study reports the antimicrobial and antioxidant properties of P. ostreatus cultivated on different tropical woody substrates.

Total phenol contents (TPC) ranging from 0.89 µg/g and 2.63µg/g was obtained for extracts of P. ostreatus cultivated on different woody substrates (Fig. 1). P. ostreatus cultivated on P. ongoleubis had the highest TPC (2.63 µg/ml). Phenolics have been reported as strong antioxidants capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Amic et al., 2003). Furthermore, phenolics 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 adhensins (Cowan, 1999).

All the microorganisms were inhibited by ethanolic extracts obtained from cultivated P. ostreatus. The zone of inhibition ranged from 5.33 mm to 20.33 mm (Table 1).The mushroom extracts cultivated on P. ongoleubis substrate exhibited a better antimicrobial against almost all the organisms except Pseudomonas aeruginosa and Bacillus subtilis. However, Staphylococcus sp. was found to be most susceptible to the antimicrobial activity of extracts from P. ostreatus. The zones of inhibition of extract obtained from P. ostreatus cultivated on Cananium sp., C. pentandra and P. ongoleubis on S. aureus were 18.33mm, 18.00mm and 20.33mm respectively. The minimum inhibitory concentrations (MIC) of the extracts on the test organisms ranged from 2.5mg/ml to 20mg/ml (Table 2). The highest MIC (20mg/ml) was recorded against K. pneumonia and P. aeruginosa. With an increasing number of bacteria developing resistance to commercial antibiotics, extracts and derivatives from mushrooms hold great promise for novel medicine in modern times (Chikara, 1992; Mizuno et al., 1995).

P. ostreatus extracts displayed a good DPPH radical scavenging ability thereby expanding its nutraceutical values. The results revealed that all the extracts were able to scavenge DPPH radical. P. ostreatus cultivated on P. ongoleubis, had the highest DPPH radical scavenging ability while P. ostreatus cultivated on C. pentandra had the least (Fig. 2). The DPPH scavenging effect of P. ostreatus cultivated on P. ongoleubis was higher and significantly different than extracts obtained from the other woody substrates. Antioxidants are important compounds that defend our body against free radicals and mushrooms are rich sources of these antioxidants (Pala and Wani, 2011). Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells (Baillie et al., 2009).

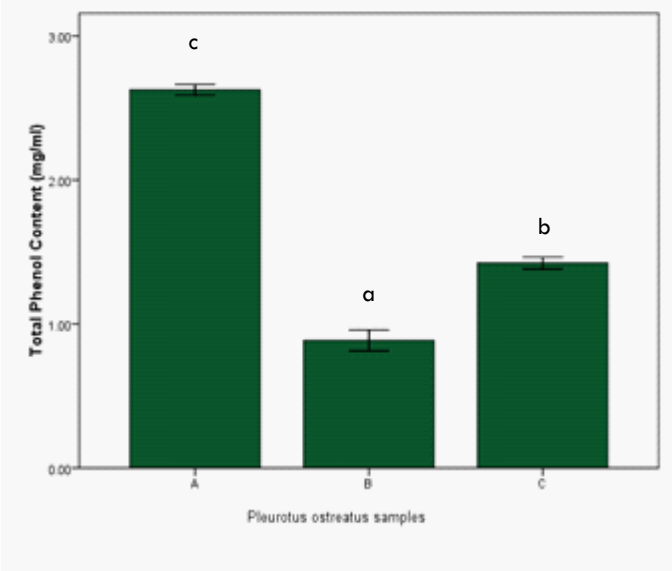

Figure 1. Total phenolic contents (mg/ml) of Pleurotus ostreatus cultivated on different tropical woody substrates. Bars with different superscript are significantly different ($P \leq 0.05$). A: *Pleurotus ostreatus* cultivated on *Pycnanthus ongoleubis*, B: *Pleurotus ostreatus* cultivated on *Ceiba pentandra*, C: *Pleurotus ostreatus* cultivated on *Cananium* sp.

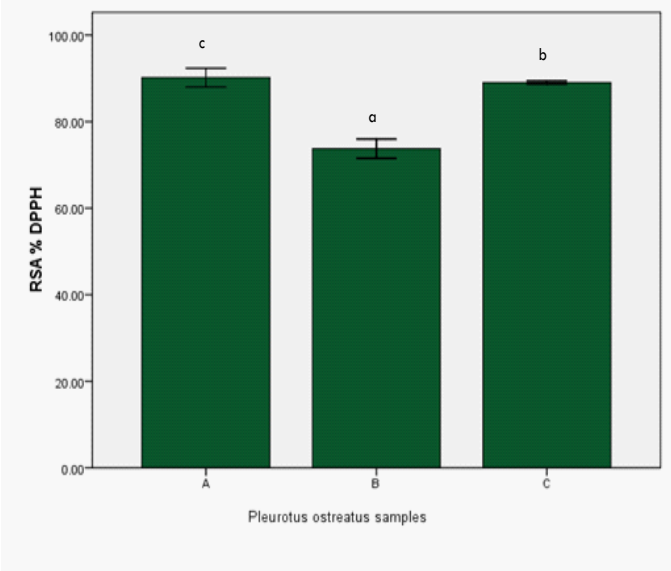

Figure 2. Percentage Radical scavenging activity (RSA) of Pleurotus ostreatus cultivated on the different woody substrates on DPPH Radicals. Bars with different superscript are significantly different ($P \leq 0.05$). A: *Pleurotus ostreatus* cultivated on *Pycnanthus ongoleubis*, B: *Pleurotus ostreatus* cultivated on *Ceiba pentandra*, C: *Pleurotus ostreatus* cultivated on *Cananium* sp.

Table 1. Zones of inhibition of extracts of *P. ostreatus* against clinical isolates.

Organisms	Zone of Inhibition (mm) – 40 mg/ml					
	A	B	C	STR	TET	Nys
Klebsiella pneumoniae	7.67±1.53	5.33±0.58	5.33±1.15	NI	14.33±1.155	NT
Salmonella tyhii	2.67±0.58	12.33±1.53	13.00±0.00	12.33±1.155	NI	NT
Staphylococcus aureus	20.3±1.5	18.00±1.00	18.33±0.58	20.00±1.00	14.00±1.00	NT
Proteus mirabilis	13.00±1.00	13.33±1.53	13.67±1.15	6.00±1.00	16.00±1.00	NT
Escherichia coli	11.33±0.58	12.10±1.00	11.67±1.16	9.67±0.577	NI	NT
Psuedomonas aeruginosa	11.67±1.53	9.67±0.58	13.00±1.00	9.33±0.577	NI	NT
Bacillus subtilis	12.33±1.16	14.00±1.73	10.00±1.00	10.67±0.577	NI	NT
Candida albicans	10.33±0.577	7.00±1.00	7.67±0.577	NT	NT	5.02±0.05
Saccharomyces cerevisae	12.33±2.082	9.67±0.577	11.67±1.53	NT	NT	10.26±1.53

Values are means of triplicate±SD. **A:** *Pleurotus ostreatus* cultivated on *Pycnanthus ongoleubis*, **B**: *Pleurotusostreatus* cultivated on *Ceiba pentandra*, **C**: *Pleurotus ostreatus* ultivated on *Cananium sp.*, **STR**= Streptomycin. **TET**= Tetracyclin, **Nys**=Nystatin, **NI** = no inhibition, **NT**=Not Determined.

Table 2. Minimum inhibitory concentration (mg/ml) of Pleurotus ostreatus extracts against the clinical bacterial isolates.

Organisms	MIC(mg/ml)		
	A	B	C
Klebsiella pneumoniae	20	20	20
Salmonella tyhii	12.5	12.5	15
Staphylococcus aureus	2.5	3.125	3.125
Proteus mirabilis	5.0	6.25	6.25
Escherichia coli	10	12.5	12.5
Pseudomonas aeruginosa	10	20	10
Bacillus subtilis	15	15	20

Values are means of replicate (n=3). **A:** *Pleurotus ostreatus* cultivated on *Pycnanthus ongoleubis*, **B**: *Pleurotusostreatus* cultivated on *Ceiba pentandra*, **C**: *Pleurotus ostreatus* cultivated on *Cananium* sp.

Conclusively, this study established the effect of different tropical woody substrates on the antioxidant and antimicrobial properties of P. ostreatus. P. ostreatus cultivated on sawdust from P. ongoleubis exhibited better antimicrobial and antioxidant activities when compared with P. ostreatus cultivated on the other woody substrates, C. pentandra and Cananium sp. Hence, P. ongoleubis will be a good substrate that will enhance the nutraceutical properties of P. ostreatus. Nutraceutical industries can therefore exploit sawdust from sawmills as substrates for the cultivation of P. ostreatus.

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