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COAST
JOURNAL OF THE FACULTY OF SCIENCE
Volume 2, Issue 1. June, 2020 ISSN: 2714-3716
Coast, J. Fac. Sci. 2 (1): 317 - 325

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EFFECTS OF OYSTER MUSHROOMS (*Pleurotus pulmonarius* AND *Pleurotus ostreatus*) CULTIVATED ON TROPICAL AGRO-WASTE

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Abstract
This research was borne out of finding alternatives to antibiotics. In this study, *Pleurotus ostreatus* and *Pleurotus pulmonarius* were cultivated using cassava peels and sawdust of *Tectonia grandis.* The mushrooms utilized the lignocellulose in the agro-wastes to grow within 30 days and the antimicrobial potency of the harvested mushrooms was investigated. *P. ostreatus* and *pulmonarius* extracts were assayed against seven pathogenic bacteria: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Aeromonas hydrophilia* using Kirby Bauer's agar well diffusion. The result of sensitivity test showed that all test organisms except *A hydrophilia* were susceptible to the ethanoilic extract of *P. ostreatus* while most were resistant to *P. pulmonarius*. The *In vitro* bioassay revealed that the aqueous extract of *P. ostreatus* inhibited *S. aureus*, *K. Pneumoniae, E. coli, P. aeruginosa*, *B. subtilis* and *S. pyogenes* with inhibition zones of 18.00 ± 0.24 mm, 16.00 ± 0.15 mm, 14.00 ± 0.05 mm, 14.50 ± 0.12 mm, 15.10 ± 0.20 mm and 17.00 ± 0.22 mm respectively. Phytochemical assays showed that *P. ostreatus* contained some essential phytochemicals which include; alkaloids, tannins, saponins and phenol of which some were present in *P. pulmonarius*. The results obtained suggest that *P. ostreatus* grown on the mixture of these two substrates (cassava peels and sawdust of *T. grandis)* possessed broader antimicrobial spectrum against a vast numbers of medically implicated organisms used, hence future research work is required to consolidate the potency of mushrooms cultivated on these substrates.
Keywords: *Pleurotus ostreatus*, antimicrobial, pathogenic, agro-wastes, phytochemicals.

Introduction

Historically, mushrooms have long been thought to possess medicinal value, especially in traditional Chinese medicine (Oyetayo, 2011). They have been studied in the modern medical research since 1960s, where most studies use extracts, rather than whole mushrooms. Only a few specific extracts have been tested for efficacy in laboratory research. *Pleurotus* species have been used by the people all over the world for their nutritional, medicinal and other beneficial values. Oyster mushrooms are a good source of dietary fibre and other valuable nutrients. The fruiting body of the mushroom is also a potential source of lignin and phenol degrading enzymes and hence the agro industrial solid residues can be utilized for the cultivation of *Pleurotus* spp and this will serve as an environmental protection strategy. Mushrooms play many vital roles which are of great benefit to both man and nature; these include their suitability as food, as tonics and medicines, and also in the bioconversion of waste organic materials (Maria *et al.,* 2017).

Mushrooms have been evaluated for their nutritional status based on the chemical composition. Cultivated and wild mushrooms contain reasonable amount of proteins, carbohydrates, minerals, fibres and vitamins (Stamets, 2000). Mushrooms are low in calories, sodium, fats, and cholesterol (Chang, 1996). Certain mushrooms enjoy usage as therapeutics in folk medicines, such as traditional Chinese medicine (Peterson, 2008). The United States National Cancer Institute has chosen mushrooms as a source of new drugs for the treatment of cancer and the ethno-medicinal value of many edible mushrooms have been reported by many researchers (Asuquo and Etim, 2011). Oyster mushrooms are preferred by many people for their delicacy taste, mild yet chewy texture and unique aroma. The world trade of these mushrooms shows an increasing pattern and gives promising opportunity for the traders (Chang, 2001).

This trend happens not only because of high demand from consumers but also the ability to apply cheap cultivation strategies. They are found growing naturally on certain rotten woody material. They have a wide range of temperature adaptability and substrate utilization. They have been cultivated in large amounts by using lignocelluloses materials such as sawdust, paddy straw, wheat straw and cotton and proven to be successfully cultivated on banana pseudostem, Bahia grass, bamboo leaves, lawn grasses (Garuba *et al.,* 2017) yam peelings (*Dioscorea* sp.), cassava peelings (*Manihot* sp.), wild grass (*Pennisetum* sp.) corn stover (*Zea mays*) and oil palm (*Elaeis guineensis*) fruit fibers. The objectives of this study are to investigate the presence of antimicrobial agents in oyster mushrooms and also to determine the efficacy of different extracts of *Pleurotus* species against common clinical isolates of medical importance.

Materials and Methods

Spawn Collection

Spawn of two different species of oyster mushroom were used for this research work and they are: *Pleurotus ostreatus* and *P. pulmonarius.* They were collected from the Federal Institute of Industrial Research, Oshodi (FIIRO) in Lagos, identified and authenticated in the Research Laboratory of the Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa.

Cultivation of mushroom

The oyster mushrooms were cultivated using cassava peels and saw dusts of teak wood (*Tectonia grandis)* as the substrates using modified methods of Fakoya and Akinyele, (2008).

Preparation of the cassava peels and sawdust substrate

The dried cassava peels were soaked in a bowl overnight, approximately 2% of $CaCO_3$ of the total weight of the cassava peels was added while soaking. On the second day, the water was drained from the cassava peels, while the cassava peels were spread on a clean floor and allowed to dry. After drying fairly, the peels were mixed with the sorted sawdust of teak wood (*Tectonia grandis*) at ratio 1:1 and packed into polythene bags and sterilized at 121^{0}C for 4 hrs. After sterilization, the substrates were allowed to cool and were then inoculated with the spawn of *P. ostreatus* and

P. pulmonarius separately. Bags were then arranged in a dark room and were incubated at 25°C.

Cultivation

Fully sterilized substrates were allowed to cool and inoculated with spawn. This was followed by incubation in a woody and ventilated box of 2.0 m x 1.0 m x 0.5 m at 25±2°C. The relative humidity, temperature and light intensity were monitored and controlled for the period of cultivation while parameters like length of stipe, diameter of pileus, and biomass of the fruit bodies were monitored and observed.

Drying of harvested mushrooms samples

The harvested mushroom samples were washed and sun-dried for about 98 hours to remove the water content and were pulverized into powdery form.

Extraction of antimicrobial agents from the mushroom samples

Ethanol Extraction Method

Ten grams of the powdered mushroom samples were weighed into 100 ml, 50% ethanol and was allowed to be fully soaked for 72 hrs. After 72hrs, it was filtered and concentrated. The extract was collected in a bottled and stored at refrigerator temperature (4°C) for further use.

Acetone Extraction Method

This was carried out as it was in the case of ethanol extraction method. The extract was also collected in bottle and stored at refrigerator temperature (4°C) for further use as well.

Aqueous Extraction Method

This was also done like the previous two methods only that in this case, the water did not dry up, so there was no need for adding extra water. The solution was filtered like in the case of ethanol and acetone extraction and concentrated extract was equally stored at refrigerator temperature (4°C).

Collection of test organisms

Bacterial isolates used for this study were collected from the Ondo State Specialist Hospital, Okitipupa and authenticated at the Department of Biological Sciences (Microbiology research laboratory), Ondo State University of Science and Technology, Okitipupa. The cultures were checked for purity and maintained on Nutrient Agar slant stored at 4°C. The test organisms are: *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli* and *Aeromonas hydrophilia.*

Sensitivity Test

Using Kirby Bauer's method, nutrient agar plates were prepared. Fresh inoculums of the test organisms were added to the media using pour-plate method. The assay was carried out using the method of Prescott *et al.* (2005)

Determination of Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was determined using dilution susceptibility test described by Prescott *et al.* (2005).

Assays for Phytochemicals

The following bioactive compounds were assayed for: tannis, phenolics, saponins, flavoniods, steroid and phlo-batannins using the method described by Fakoya *et al.* (2013).

Statistical analysis

Quantitative data were expressed as mean ± standard deviation. Statistical evaluation of the data was performed using one-way analysis of variance followed by Duncan's multiple range test at 5% level of significance i.e. $P < 0.05$. (Zar, 1984)

Results

Table 1 below shows the diameter of inhibition and the antibacterial activities of oyster mushroom extracts on test organisms used for the study. The standard antibiotic used displayed well pronounced inhibitory effect against *Bacillus subtilis* with 23.00 mm diameter as the zone of inhibition among all the tested organisms while ethanol extract of *P. ostreatus* showed the highest diameter of

Table 1: Antibacterial activities of mushroom extracts on test organisms

Diameter of Inhibition zones							
Test Organisms	Standard antibiotics			Mushroom extracts			
	Ampicillin	EPA	APA	QPA	EPB	APB	QPB
S. aureus	20.0 ± 0.12^{c}	18.0 ± 0.20^{b}	13.0 ± 0.11^{d}	11.0 ± 0.00^{e}	0.0±0.00	0.0±0.00	12.0 ± 0.11^{d}
K. pneumoniae	20.4 ± 0.14^{c}	$16.0 \pm 0.18^{a,b}$	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
E.coli	13.0 ± 0.20^{d}	14.0 ± 0.11^{a}	12.0 ± 0.10^{d}	12.0 ± 0.00^{d}	0.0±0.00	0.0±0.00	0.0±0.00
P. aeruginosa	$16.0 \pm 0.15^{a,b}$	14.5 ± 0.10^{a}	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
B. subtilis	23.0 ± 0.22^{c}	15.1 ± 0.14^{a}	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
A.hydrophilia	14.0 ± 0.16^{a}	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
S. pyogenes	19.0 ± 0.11^{b}	$17.0 \pm 0.00^{a,b}$	0.0±0.00	12.0 ± 0.10^{d}	0.0±0.00	0.0±0.00	0.0±0.00

Values are means of triplicates ±SD, Samples carrying the same superscripts in the same row are not significantly different at ($p<0.05$)

KEY- EPA: Ethanol extract of *P. ostreatus* EPB: Ethanol extract of *P. pulmonarius*
APA: Acetone extract of *P. ostreatus* APB: Acetone extract of *P. pulmonarius*
QPA: Aqueous extract of *P. ostreatus* QPB: Aqueous extract of *P. pulmonarius*

18.00 mm zone of inhibition against *Staphylococcus aureus* among all the isolates (Plate 1) and 13.00 mm exhibited against *Staphylococcus aureus* by *P. pulmonarius* extract. Tables 2 and 3 show the result of the phytochemical tests carried out on the mushroom samples. The result shows that *P. ostreatus* contain alkaloids, flavonoids, triterpenoids, saponins, phenol compounds, protein etc. For the minimum inhibitory concentration, all the tested organisms were able to grow at the concentration of 6.25mg/ ml of *Pleurotus ostreatus* as shown in Table 4. None of the tested isolate could grow at concentrations of 100 mg/ml and 50 mg/ml. *Staphylococcus aureus, Streptococcus pyogenes* and *Bacillus subtilis* could not grow at concentration of 25 mg/ml. Also all test isolates except *Bacillus subtilis* were able to grow at concentration of 12.5 mg/ml.

Discussion

Oyster mushroom is an easily cultivatable mushroom that colonizes various crop residues as substrates. *Pleurotus* species are able to degrade and convert lignocellulosic compounds into protein-rich biomass (Mamiro and Mamiro, 2011) and help in managing agro-wastes whose disposal has become a problem (Das and Mukherjee, 2007). It demonstrated higher colonization rates, improved earliness, and sporophore yield on different agro wastes compared to other cultivated mushroom genera (Philippoussis *et al.*, 2001). Its cultivation has increased greatly during the last few decades throughout the world due to its ability to adapt varied agro-climatic conditions (Karuppuraj, *et al.,* 2014). In addition to that, *P. ostreatus* growth on different agricultural wastes can provide more food and decrease different crop residues. The results of the antimicrobial assay of the mushroom

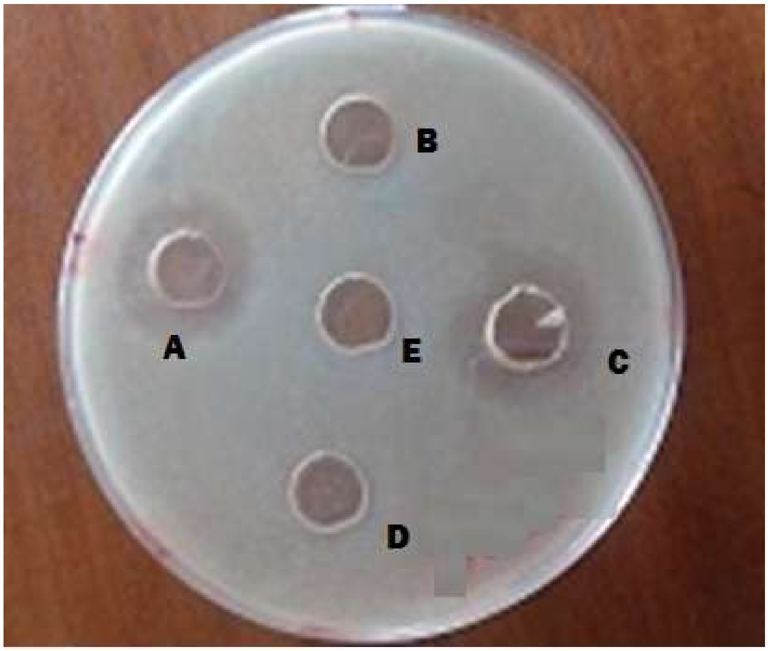

Plate 1: Zone of inhibition of mushroom extracts on *Staphylococcus aureus*

A= Well containing Ethanol extract of *P. ostreatus* B= Well containing Acetone extract of *P. ostreatus*
C= Well containing positive control (Ampicillin) D= Well containing Aqueous extracts of *P. ostreatus*
E= Well containing negative control (blank)

Table 2: Phytochemical Screening of *P. ostreatus*

Phytochemicals	Ethanol extract	Aqueous extract
Alkaloids	+	+
Flavonoids	-	-
Triterpenoids	+	-
Saponins	+	+
Phenol	+	+
Tannins	+	+
Protein	+	+
Carbohydrates	+	+
Glycosides	+	-

KEY: + Means present, - Means absent

Table 3: Phytochemical Screening of *P. pulmonarius*

Phytochemicals	Ethanol extract	Aqueous extract
Phenol	-	-
Tannins	-	-
Flavonoids	-	-
Protein	+	+
Carbohydrates	+	+
Alkaloids	-	-
Saponins	+	+
Steroid	+	-
Glycosides	-	-

Key: + Mean present - Means absent.

Table 4: Minimum Inhibitory Concentration of mushroom extract

	Different concentration of extract					
Test Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Control
S. aureus	-	-	-	+	+	+
S. pyogenes	-	-	-	+	+	+
K. pneumoniae	-	-	-	+	+	+
E. coli	-	-	+	+	+	+
P. aeruginosa	-	-	+	+	+	+
B. subtilis	-	-	+	-	+	+

Key: - Means no growth, + Means there is growth.

extracts in this study indicated that these mushrooms were able to exhibit antimicrobial activities against some of the tested microorganisms. The ethanolic extract of *P. ostreatus* showed a broad-spectrum activity against six (6) of the tested organisms (*Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Streptococcus pyogenes.* Table 1 shows the extracts inhibited the growth of the organisms as indicated by the zones of inhibition around the well. The tested isolates were resistant to most of the extracts of *P. pulmonarius* apart from the aqueous extract that shows inhibition against *Staphylococcus aureus* and *Escherichia coli.* The aqueous extract of *P. ostreatus* also showed sensitivity against some of the clinical isolates (*S. aureus, Pseudomonas aeruginosa, and Streptococcus* *pyogenes*). The results obtained in the antimicrobial tests of the mushroom extracts show that the effectiveness of each mushroom extract varies with mode of extraction as some isolates showed resistance to some of these extracts. However, many studies have been conducted to test the ability of *oyster mushrooms* to grow on different agro wastes. Examples of those agro wastes are rice straw, sawdust, (Ananbeh and Almomany, 2008). Mixing agro wastes at different ratios enhances the productivity of *P. ostreatus and P. pulmonarius* as demonstrated in this study and this agrees with the findings of Al-Momany and Ananbeh, 2011).

It was observed that the oyster mushrooms cultivated grow faster on sawdust supplemented with sorghum grains than on cassava peels that have no supplement. Substrates that are used in cultivating mushrooms have effect on the chemical, functional, and organoleptic characteristics of mushrooms (Oyetayo and Ariyo, 2013).

P. ostreatus and *P. pulmonarius* are primarily consumed for its nutritive value and used industrially as a bioremediator (Lakshmi, 2004). This study revealed the antimicrobial properties of the mushrooms whereby both extracts were observed to inhibit Gram positive and Gram-negative bacteria. This is in line with the study of Mavoungou *et al.* (1987) who had earlier reported that *Pleurotus ostreatus* has a broad-spectrum antibacterial activity.

The observed antimicrobial constituents of these mushrooms may also elicit antibacterial activity as found in many medicinal plants with mechanisms of actions characterized by cell membrane lyses, inhibition of protein synthesis, proteolytic enzymes and microbial adhesion (Cowan, 1999). The inhibitory response of tested organisms to the extracts of *P. ostreatus* and *P. pulmonarius* implied that strains variation of mushrooms may determine the susceptibility of bacteria to the antimicrobial agent from mushrooms. This is evident in the study that *P. ostreatus* being bactericidal to *S. aureus (*high degree of inhibition) *and P. pulmonarius* being bacteriostatic (static effects of inhibition). However, the observed zones of inhibition of *P. ostreatus* extract against *Bacillus subtilis* agrees with the findings of Wolff *et al.* (2000). The reported variations in the antimicrobial outcomes of these macrofungi may be connected with the differences in their microbial composition and concentrations (Ramesh and Pattar, 2010)

Mushrooms are regarded as non-sources of flavonoids. The absence of flavonoids in mushrooms may be of biological advantage in their various ecological niches since these bioactive compounds inhibit activities involved their pigmentation, growth and development (Mattila *et al.,* 2001). Finally, appreciable amount of extract and thus all its bioactive compounds can be recovered from the mushrooms used in this research, if its optimum growth conditions are known. This may further enhance its medicinal and market values. Based on the results of this study, *P. ostreatus and P. pulmonarius* an edible oyster mushroom possessed a broad-spectrum antibacterial activity.

Conclusion

This study conducted with common mushrooms in Nigeria reveals the potency of *Pleurotus ostreatus* and *Pleurotus pulmonarius* as potential antimicrobial agents with broad spectrum activities. The later was sensitive to two bacterial isolates used for the assay, while the former shows inhibition against 90% of the bacterial isolates used for the study. This can serve as a means of controlling infections associated with some of these isolates. However, this present study indicates that the mushroom contains antimicrobial compounds that can be further developed as phytomedicine for the treatment of infection that may be caused by the selected clinical bacteria. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development.

In conclusion, the action of extracts upon the antimicrobial models justified its usefulness in herbal formulation; hence, further isolations, identification, characterization and purification of the bioactive compounds responsible for biological activity are needed to enhance the exploitation of the bioactive compounds in the mushroom.

Acknowledgment

Ondo State University of Science and Technology is acknowledged for providing a conducive environment for this research work to be carried out.

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