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Antioxidant and Antimicrobial Activities of Oyster Mushroom

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Abstract Four species of oyster mushroom *(Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus pulmonarius and Pleurotus populinus*) were evaluated for their antimicrobial and antioxidant capacity (total antioxidant activity). The total antioxidant activity differed significantly. Mean total antioxidant activities were (*P.ostreatus* = 35.36±0.01mm, *P.sajor-caju* = 32.26±0.02mm, *P.pulmonarius* = 28.86±0.01mm, *P.populinus* = 26.65±0.01mm). Antimicrobial activities of the extracts against *Bacillus cereus, Streptococcus agalactiae, Agrobacterium vitis, Pseudomonas aeruginosa, Escherichia coli and Shigella dysenteriae* were investigated. Antimicrobial activities of the oyster mushroom extracts against *Bacillus cereus, Streptococcus agalactiae, Agrobacterium, Pseudomonas aeruginosa, Escherichia coli and Shigella dysenteriae* were examined by agar well diffusion method and zones of inhibition varied for different organisms but zones of inhibition were highest in *P.ostreatus* and *P.sajor-caju* for all tested organisms except in *E.coli* and *S.dysentriae* where *P.pulmonarius* and *P.populinus* had higher zones. *P.ostreatus* and *P.sajor-caju* were not significantly different against all tested microorganisms but were found to be significantly different ($p \geq 0.05$) from *P.pulmonarius* and *P.populinus* against *B.cereus, E.coli* and *S.dysenteriae*. *P.pulmonarius* and *P.populinus* were not significantly different in their inhibition against all tested microorganisms.

Keywords: *mushrooms, antimicrobial potentials, inhibition, antioxidant*

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1. Introduction

1.1. Background of Study

Mushrooms are macro fungi with distinctive fruiting bodies, which are usually fleshy and edible, hypogeous or epigeous, large enough to be seen with the naked eye, and picked by hand [1]. Mushrooms have been widely used very often as delicious and nutritious foods [2]. Some mushrooms serve as food because of their nutrient contents while some have been used extensively in traditional medicine [3]. Mushrooms are considered as a functional food, which can provide health benefits beyond the traditional nutrients they contain. Pleurotus is a genus of gilled mushroom.

The genus Pleurotus comprises about 40 species that are commonly referred to as"oyster mushrooms". *Pleurotus* genus includes *P. ostreatus, P. sajor caju, P. florida, P. flabellatus, P. highbing 51, P. cystidiosus, P. sapidus, P. eryngii, P. tuberegium, P. ulmarium, P. pulmonarius, P. citrinopileatus, P. geesteranus, P.populinus* and other some of which are of a special consideration due to their high nutritional values and medicinal importance [4]. Oyster mushroom (Pleurotus species) is the third largest commercially produced mushroom in the world [5].

Medically, the species *P. ostreatus* have been reported to decrease cholesterol levels [6]. The carpophore of the mushroom is also a potential source of lignin and phenol degrading enzymes [7]. It is also used industrially as a bioremediator [8,9]. In recent times they have also attracted great attention as a source of bioactive metabolites for the development of drugs and nutraceuticals [10,11]. Some of them have also been found to be a source of secondary metabolites such as phenolic compounds, flavonoids, terpenoids, sterols, ascorbic acid, ergothioneine and carotenoids [12]. They exhibit high antioxidant properties and they therefore ward off cancers, HIV-1 AIDS and other viral ailments; they are antimutagenic, anti-tumoral and can be used to manage cardiovascular disorders [12]. Scientific research has shown that high amounts of antioxidants may prevent oxidative stress caused by free radicals which lead to cell damage, generation of cancer cell and brain cell aging [13]. Oxidative stress might occur as a result of either the presence of oxidation agents, decrease in the level of antioxidants or both factors. Under such circumstances, reactive oxygen species (ROS) and free radicals are produced as harmful by-products of oxidation process. Many investigations from different region of the world confirmed that the *Pleurotus* mushroom having high nutrition also contains various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectins and nucleotides which have been isolated and identified

from the fruit body, mycelium and culture broth of mushrooms and are shown to have promising biological effects [3].

Infectious diseases account for a high proportion of health problems in most of the developing countries. Although several antimicrobial agents have been synthesized chemically, indiscriminate use of commercial antimicrobial drugs has led to the development of resistance to the existing antibiotics by the microorganisms. The spread of such drug resistant pathogens is becoming one of the most serious threats to successful treatment of microbial diseases. Natural antimicrobials can be derived from different plant parts, various animal tissues or from microorganisms [14,15]. In recent times, there has been a renewed interest in traditional medicine and emphasis has been placed on the use of natural plant materials in the control and treatment of various infections and diseases, resulting in an increase in the demand for more drugs from plant sources. More recently, in addition to medicinal plants; research on drugs derived from fungal sources has also gained interest from researchers [16]. Many fungi, including mushrooms contain dozens of active constituents that together combine to give the mushrooms their therapeutic value [17,18]. Mushrooms have been reported to contain dietary fibres, β-glucans, chitin, pectinous substances, natural antibiotics, phenolic compounds, flavonoids and several other secondary metabolites [19]. Both fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. Several studies report the efficacy of different mushroom extracts against several microorganisms [9,19,20].

There is underutilization of oyster mushroom as food in Nigeria which could be because people do not know much about its biological function beyond provision of basic nutrient. The resistance of the existing antibiotics by the microorganisms and the spread of such drug resistant pathogens has generated the need and provided the necessary impetus for a continuous search of a novel antimicrobial agent from different natural sources. The objectives of this paper are to evaluate the antimicrobial potential and antioxidant activity of some species of oyster mushroom.

2. Materials and Methods

2.1. Collection of Sample and Storage

The samples *(Pleurotus ostreatus, Pleurotus pulmonarius, Pleurotus sajor caju and Pleurotus populinus)* grown on blend of sawdust and corncob were purchased from Dilomat farms and services, Rivers State University of Science and Technology, Faculty of Agriculture, Portharcourt, Nigeria. The mushrooms were dried in an oven at 40℃ and pulverized, packaged in air tight containers and then stored at ambient condition for subsequent analysis.

2.2. Preparation of the Extract

The modified methods [21] were employed in the extraction process. The solvent n-hexane was used for the extraction. Four hundred grams (400 g) of the powdered sample were weighed and soaked in 2000 mL n-hexane (98% BDH). The mixtures were kept in air-tight containers and left for 72 hrs at room temperature. Residue was removed using double layer muslin cloth and further filtration was done using WhattMan No 1 filter paper (24 cm). The extract was recovered from the filtrate using soxhlet extractor.

2.3. Evaluation of Antimicrobial Activity

Agar well diffusion techniques as described by [22] who adopted for the study. Mueller Hinton agar plates (MHA oxoid) England, were inoculated with 0.1 mL of an over night broth culture of each bacteria isolate (Equivalent to 3 x 107cfu/ml) MF (Mcfarl and standard) in sterile Petri-dish. The seeded plates were rocked for uniform distribution of isolates and allowed to set. Holes were bored on the plates by using standard sterile cork borer of 6 mm diameters and equal volumes of the extract (100µl of 25% solution in water) were transferred into the well with the aid of micropipette. The experiments were carried out in triplicate. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract. The plates were incubated at 37℃ for 24 h until marked decline in the potency of the mushroom extract to inhibit the growth of the test isolates was observed. Zone of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded.

2.4. Antioxidant Activity Determination

This was carried out using lipid peroxidation method in tissue homogenate with little modification of the method [23]. A weighed sample of oyster mushroom, 0.5g was homogenized in 19.5mL of potassium chloride solution, and the homogenate was stored at a temp of -4℃. To a first centrifuge tube, 2.0ml of the homogenate was added to 0.2ml of distilled water; 2.0ml of homogenate, 0.1ml of ascorbic acid solution and 0.1ml of mohr salt solution into second centrifuge tube; to the third centrifuge tube, same components as have been added to the second tube was added in addition to 1ml of trichloroacetic acid solution.

The three centrifuge tubes were placed in a water bath at 37℃ for 10 minutes and 1ml of trichloroacetic acid solution was added to the first and second tube. It was then centrifuged for 10 minutes at 3000rpm. 2ml of supernatant was poured into three clean test tubes and 1ml of thiobarbituric acid was added and placed in a boiling water bath for 10 minutes and was allowed to cool in ice to room temperature. The absorbance of the supernatant was then read at 532nm for the samples

2.5. Statistical Analysis of Experimental Data

The data obtained were analyzed by using Analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS), version 20.0 using results presented as Mean ± Standard deviation. Duncan multiple

range test (level of significance of P=0.05) was performed to evaluate the level of differences among means of the different samples. Statistical significance was accepted at $p \leq 0.05$ [24].

Plate 1. Pleurotus Sajor-Caju

Plate 2. Pleurotus Pulmonarius

Plate 3. Plate Pleurotus Ostreatus

Plate 4. Pleurotus Populinus

3. Result and Discussion

3.1. Antimicrobial Activity

This study evaluated the antimicrobial capacity of four different Oyster mushroom species (*Pleurotus ostreatus, Pleurotus sajor-caju, Pleurotus pulmonarius, Pleurotuspopulinus*). The antimicrobial potentials of extracts of oyster mushroom species were estimated from their ability to inhibit different pathogenic micro-organisms. Table 1 shows antimicrobial activities of the extract of the various mushrooms. Their zones of inhibition were in the order of *Pleurotus ostreatus* (14.00±1.00mm) >*Pleurotus sajor-caju* (13.00±1.00mm)> *Pleurotus pulmonarius* (11.00±1.00mm) = *Pleurotus populinus* (11.00±1.00mm) for the gram positive organism, *Bacillus cereus.* There was no significant difference between *Pleurotus ostreatus* and *Pleurotus sajor-caju*with respect to their effect on this organism while they significantly differ from *Pleurotus pulmonarius* and *Pleurotus populinus* which again were found not to be significantly different from each other. Based on this result, *Pleurotus ostreatus* and *Pleurotus sajor-caju* will be most effective when used as antimicrobial agent against this organism (*B. cereus*).

For *Streptococcus agalactiae*, a similar trend was again observed with *Pleurotus ostreatus* and *Pleurotus sajor-caju* havig the highest value (12.00±1.00mm) followed by *Pleurotus pulmonarius* (11.00±1.00mm) while *Pleurotus populinus* had the least value (10.00±1.00mm). There was no significant difference between all the species extracts (*P.ostreatus, P.sajor-caju, P.pulmonarius, P.populinus*) implying that their efficacy against this microorganism based on this result will not be significantly different (though *Pleurotus ostreatus* and *Pleurotus sajor-caju* was slightly more effective). For the gram negative organism (Agrobacterium), *Pleurotus sajor-caju* had the highest zone of inhibition (12.00±1.00mm) followed by *Pleurotus ostreatus* and *Pleurotus pulmonarius* that both had (11.00±1.00mm) zone of inhibition while *Pleurotus populinus* had the least zone of inhibition (10.00±1.00mm) implying that it inhibited the microorganism the least. There was no significant difference between the extracts implying that any of the extracts will do a similar job in terms of inhibition of the organism even through *Pleurotus sajor-caju* will be slightly more effective followed by *Pleurotus ostreatus* and *Pleurotus pulmonarius.*

For the gram negative organism (*Pseudomonas) aeruginosa, Pleurotus sajor-caju* had the highest zone of inhibition (11.33±1.53mm), followed by *Pleurotus ostreatus* (11.00±1.00mm) with both *Pleurotus pulmonarius* and *Pleurotus populinus* having least zone (10.00±1.00mm). *Pleurotus sajor-caju* and *Pleurotus ostreatus* were not significantly different but where significantly different from *Pleurotus pulmonarius* and *Pleurotus populinus* which were not significantly different from each other. This result implies that the efficacy of *P.sajor-caju and P.ostreatus* will be similar with respect to this microorganism but their difference (in effectivenss) with *P.pulmonarius* and *P.populinus* will be noticeable (significantly different). *Pleurotus sajor-caju* and

Pleurotus ostreatus will be preferred as antimicrobial source of this microorganism.

For *Escherichia coli, P.pulmonarius* and *P.populinus* proved more effective with inhibition zone of (3.00±1.00mm) while both *P.ostreatus and P.sajor-caju* had no observed zone of inhibition. *P.pulmonarius* and *P.populinus* had no significant difference and is low when compared to inhibition against other tested microorganisms. This suggests that *E.coli* is highly resistant to these extracts and extracts of these mushrooms may not be suitable for preventing or treating against infection of this pathogen. No zone of inhibition was observed for both *P.ostreatus* and *P.sajor-caju* meaning that E.coli is not sensitive to these extracts at all.

For *Shigella dysenteriae, P.ostreatus* and *P.sajor-caju* showed no zone of inhibition (0.00±0.00mm) and were not significantly different from the other. *P.pulmonarius* and *P.populinus* showed small zone of inhibition (2.00±1.00mm and 3.00±1.00mm) but were significantly different from *P.ostreatus* and *P.sajor-caju* but not significantly different between each other. It is interesting to note that the gram positive bacteria showed more sensitivity than gram negative bacteria. This is in agreement with the findings of other researchers [25]. It is interesting to note that the pathogenic microorganism, *Pseudomonas aeruginosa*, which is resistant to conventional synthetic antibiotics like gentamycin and tetracycline was found to show susceptibility to all the extracts. Mushrooms produce various antiviral, antifungal compounds to survive in the wild against competing or pathogenic agents [26,27]. Also observed in this study is that there were variations in the degree of antimicrobial activities of mushrooms. This result is in agreement with the reports of [28,29]. The broad spectrum activity of mushrooms was also brought to light as the extracts of mushrooms showed inhibitory effects on isolates used for this investigation. This suggests that the bioactive products which are contained in mushrooms are in concentrations which exhibit varying degrees of antimicrobial activity. The observed antimicrobial properties could be due to the presence of alkaloids and flavonoids which have been shown to possess antimicrobial properties [30]. The variations in the antimicrobial activities of mushrooms may be due to the differences in their bioactive compositions or concentrations, methods of extraction and mechanism of action of active ingredients in these edible mushrooms [31].

Table 1. Antimicrobial Activities of Four Oyster Mushroom Species (mm)

SAMPLES	*B.cereus*	*S.agala*	*Agrobact*	*P.aeruginosa*	E.coli	S.dysen
PO	14.00^{a} ±1.00	12.00^{a} ±1.00	11.00^{a} ±1.00	11.00^{a} ±1.00	0.00^{b} ±0.00	0.00^{b} ±0.00
PSC	13.00^{a} ±1.00	12.00^{a} ±1.00	12.00^{a} ±1.00	11.33^{a} ±1.53	0.00^{b} ±0.00	0.00^{b}±0.00
PP	11.00^{b} ±1.00	11.00^{a} ±1.00	11.00^{a} ±1.00	10.00^{a} ±1.00	3.00^{a} ±1.00	2.00^{a} ±1.00
PPL	11.00^{b} ±1.00	10.00^{a} ±1.00	10.00^{a} ±1.00	10.00^{a} ±1.00	3.00^{a} ±1.00	3.00^{a} ±1.00
LSD	**2.306004**	**2.306004**	**2.306004**	**2.306004**	-	-

Mean ± standard deviation of triplicate determination.
Means of the same superscript on the column are not significantly different ($p \leq 0.05$).

Key:
PO = *Pleurotus ostreatus*
PSC = *Pleurorotus sajor-caju*
PP = *Pleurotus pulmonarius*
PPL= *Pleurotus populinus*

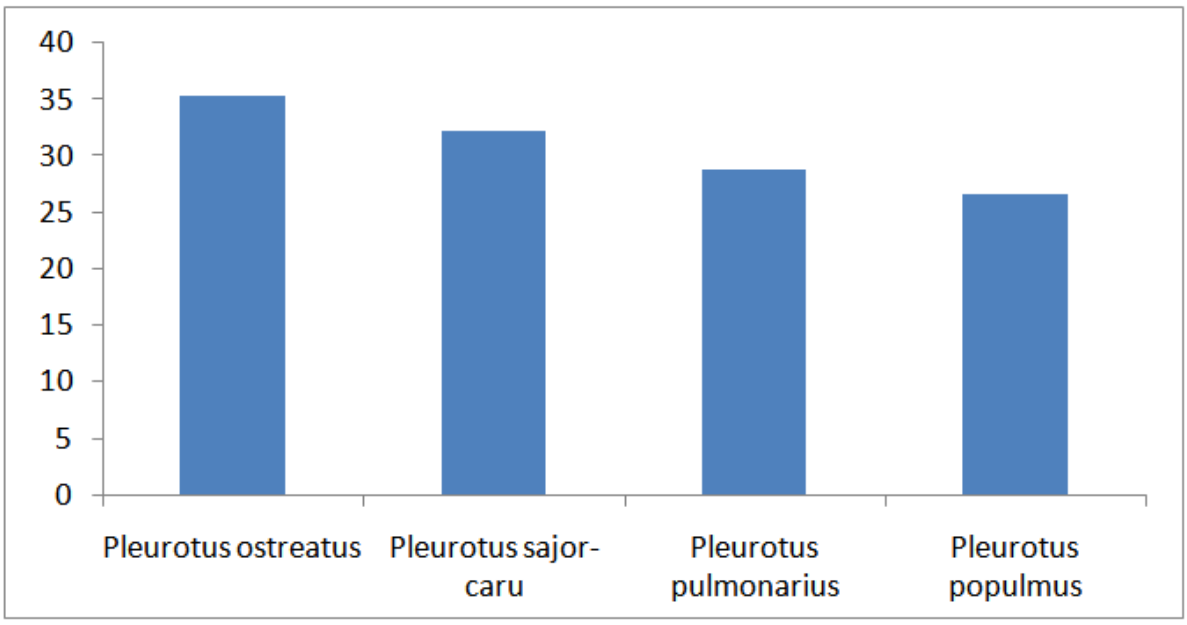

Figure 1. Total Antioxidant in oyster mushroom varieties

3.2. Antioxidant Capacity

The antioxidant capacity is a way of depicting the effect of reducing compounds in the mushroom extract. The observed total antioxidant activity can be attributed to the presence of phytochemicals. Bioactive compounds found in edible mushroom are known to play a vital role in promoting health. A significantly higher total antioxidant capacity in Figure 1 was observed in *Pleurotus ostreatus* (35.36±0.10µM) followed by *Pleurotus sajor-caju* (32.26±0.015 µM). The total antioxidant capacity (contents) ranges from 26.64 to35.36 µM with *Pleurotus ostreatus* showing the highest antioxidant activity (35.36µM), followed by *Pleurotus sajor* caju with antioxidant capacity of 32.26µM, followed by *Pleurotus pulmonarius* with antioxidant capacity of 28.86µM, while *Pleurotus populinus* with antioxidant capacity of 26.64µM.They might be used directly in enhancement of antioxidant defenses through dietary supplementation to reduce the level of oxidative stress. These antioxidants play a vital role in medicinal properties of the mushrooms. These mushrooms can be therefore harnessed in the management of oxidative stress induced diseases [32,33].

4. Conclusion

The comprehensive information made available by this study shows that the tested oyster mushroom extracts possesses many promising therapeutic properties at varying amounts. Based on the results obtained from the analysis, it can be concluded that the n-hexane extracts of these edible mushrooms (*Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus sajor-caju*, *Pleurotus populinus)* possessed a broad-spectrum antimicrobial activities and thus the potential of developing antimicrobials from them appears rewarding. They can also be used as functional foods (serve as a rich source of natural rich antioxidant food for the enhancement of the immune system against oxidative damage.) since they have significant antioxidant activity and they can be used as easily accessible source of natural antioxidants and as possible food supplement and in pharmaceutical industry considering the lingering threat of multi-drug resistance.

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