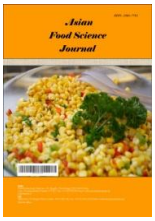

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Bioactivity of Mushroom (*Pleurotus pulmonaris*) Extract on Some Food Pathogens

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Authors' contributions

This work was carried out between both authors. Author VCW designed and supervised the research work and edited the manuscript. Author VOE performed the work and carried out the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

The interest in discovering and developing natural antimicrobial has significantly increased due to consumer preferences for food that are free of chemical preservatives while still microbiologically safe. One of the best sourced natural antimicrobials is certain mushrooms as many of them not only have nutraceutical functions but also possess antimicrobial properties. For this work, matured mushroom (*Pleurotus pulmonaris*) was harvested, clean, dried, and milled into powder. Bioactive extracts were done in ratios of 25 g +200 ml, 50 g + 200 ml, and 100 g + 200 ml both for water and ethanol extracts. The treatments were tested against Escherichia coli and Staphylococcus aureus. The results showed that Escherichia coli and Staphylococcus aureus were resistant to water extract of mushroom at the concentrations used. Ethanol extracts showed that sample C containing 50 g of milled mushroom compounds had the highest diameter of inhibitory zone of 1.8 cm for Escherichia coli and 1.3 cm for Staphylococcus aureus. Results also indicated samples B and D were not significantly different from each other though there were microbial inhibitions. This report concludes that further study should be carried out to determine the effective of the extract on field crops and stored produce.

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Keywords: Antimicrobial agents; mushroom extracts; food pathogens.

1. INTRODUCTION

Mushrooms are fruiting body of macro-fungus that are visible to the human eyes. Majority of species that forms fruiting body in its life cycle are classified under phylum Ascomycota and Basidiomycota [1,2]. Although, mushrooms vary in shape, size and functions, they are all composed of protein, fat, fibers, carbohydrates and mostly water [3]. Generally, mushrooms are composed of 16-85% carbohydrates, 0.2-87% lipids, 14-44% proteins, 1-10% RNA, 0.15-0.3% DNA and 1-29% ash [3]. Edible mushrooms are considered a healthy food because the average fat content is 4.0% and about 72% unsaturated fatty acids [3]. The bioactive substances found in mushrooms can be divided into secondary metabolites (acids, terpenoids, polyphenols, sesquiterpenes, alkaloids, lactones, sterols, metal chelating agents, nucleotide analogs, and vitamins), glycoproteins and polysaccharides [4]. The antimicrobial properties of various species of mushrooms have been shown to have a wide array of activities on microbial food pathogens. Recently however, these antimicrobial properties have become more and more attractive as natural solutions to crop pathogenic diseases which are safe for human consumption, unlike chemical antimicrobial agents which may sometimes be toxic [5]. To identify novel sources of mushroom antimicrobials, an initial screening process for mushroom species with antimicrobial properties is often based on the ecology of the region where the mushrooms grow or on local usage of the mushrooms [6,7,5,8,9]. Over 100 mushroom isolates from various Brazilian ecosystems have been screened and 13 isolates identified with antimicrobial activities [6]. Also, extensive work has been done on 30 edible wild mushroom species from West Bengal, India, and found that all their methanol extracts showed different levels of antimicrobial activities [9]. Investigations have also shown that using an agar well diffusion assay that *Ramaria flava*, a wild mushroom inhibited some Gram-positive bacteria [10]. *Clitocybe alexandri* and *Rhizopogon roseolus* mushrooms were found to have antimicrobial effects against certain microorganisms such as *E.coli* [11]. *Agaricus bisporus*, a popular culinary mushroom, showed strong inhibition of gram-positive bacteria [12]. Pathogens of crop diseases that are usually used for the evaluations of the antibacterial activities of mushroom extracts include *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Shigella spp*., and *Staphylococcus spp*. Some previous studies have indicated that the number of mushrooms with activities against Gram positive bacteria is much greater than that with activity against Gram-negative bacteria, which agreed with the review of [13]. This phenomenon was also noticed by various researchers [5] and [7], who studied 48 and 20 mushroom species, respectively. Extracts from cultured *Pleurotus ostreatus* [14], wild species of Pleurotus [15], and *Ganoderma spp*. [16] have demonstrated antifungal activities against various *Fusarium* spp a well-known pathogen of tropical crops. Some primary metabolites of mushrooms may also contribute to their antimicrobial properties. For example, the strong antimicrobial activity of some polypores resulted from high molecular-weight polysaccharides [17] and proteins [18,19].

Food security is essential for the continued existence and well-being of humanity. Food pathogens pose a constant threat to food security by negatively impacting on crop yield and causing enormous crop losses. Therefore, it is necessary to investigate ways of mitigating this situation using safe antibacterial and antifungal derivatives such as from mushrooms. Microbial pathogens do not only threaten crop yield but also pose a serious health hazard to humans. In order to reduce food poisoning, safe antimicrobial agents must be used. Increasing interest is now being shown in finding safe substances for fighting microbes that damage crop yield. This study is therefore focused on investigating the pathogen-inhibiting characteristics of ethanol and water extracts of oyster mushrooms *(Pleurotus pulmonaris*) at different concentrations on the growth of two common food pathogens *Escherichia coli* and *Staphylococcus aureus.*

2. MATERIALS AND METHODS

2.1 Experimental Laboratory

This study was carried out at the Microbiology and Fermentation Laboratory, Department of Food Science and Technology, Rivers State University of Science and Technology.

2.2 Source of Experimental Materials

The mushroom (*Pleurotus pulmonaris)* was obtained from the mushroom unit, Faculty of

Agriculture, University of Port Harcourt, while the pure cultures of *Staphylococcus aureus and Escherichia coli* were gotten from the Microbiology and Fermentation Laboratory, Department of Food Science and Technology, Rivers State University of Science and Technology and the cultures were properly identified [20,21].

2.3 Sub-Culturing and Confirmation of Bacteria Isolates

Cultures of *E. coli* and *Staph aureus* were carefully selected from the properly identified culture plates and were sub-cultured into a fresh prepared nutrient agar plate by streaking to get pure cultures. The cultures were then incubated at room temperature. For the confirmation of the isolates the following biochemical tests; oxidase test, mortality test, catalase test, indole and coagulase test were carried out [22]. Gram staining reaction was also carried out on the isolates [23], *E. coli* had a negative gram staining while *S. aureus* was positive.

2.4 Preparation of Mushroom Sample

Matured mushroom (pileus diameter of 4 – 4.5 cm) was collected from the Mushroom Unit, Faculty of Agriculture, University of Port Harcourt, Rivers state. The mushroom was then weighed and dried with convectional electric oven at 55^0C for 6 hours. The dried mushrooms were milled with a blender at a speed of 250 rpm and the milled mushroom was packed into a polythene and sealed.

2.5 Extraction of Active Compound

The method as modified by Patil and Rasika [24] was used in the experiment. Mushroom of various weight 25g, 50g and 100g were weighed in duplicates and each dissolved in either 200 ml of ethanol (99.5% laboratory grade) or 200 ml of distilled water for ethanol and water extraction, respectively. The solution was allowed to stand for 5 hours and filtered using Whatman filter paper number 4. The filtrate was concentrated using rotary evaporator thereafter 1ml of the extracts was then used to inoculate the plate containing the nutrient agar and the organisms.

2.6 Data Collection

Data was collected on the diameter of inhibitory zone for both *S. aureus* and *E. coli.*

2.7 Experimental Design and Data Analysis

Completely Randomized Design (CRD) consisting of 8 treatments replicated 3 times was used in the study. Data was analyzed statistically using analysis of variance (ANOVA).

3. RESULTS

The data presented in Table 1 shows the result of the biochemical test carried out to confirm the identity of the organisms to be used. Gram staining was positive for *Staph. aureus* and negative for *E. coli*, oxidase test was negative for both *Staph. aureus* and *E. coli*, motility test showed positive for *E. coli* and negative for *Staph.aureus*, catalyse test gave negative result to both organisms, test indicated that *E. coli* was positive while *Staph. aureus* was negative and coagulase test was positive for *Staph. aureus* and negative for *E. coli*.

From Table 2, it was observed that mushroom ethanol extract had an inhibitory effect on both *Staph. aureus* and *E. coli* while mushroom water extract had no inhibitory effect.

Fig. 1 shows the result of the bioactivity of mushroom ethanol extract and mushroom water extract on *Staphylococcus aureus.* Data showed that 25 ml of active mushroom ingredient had an inhibitory zone of 1.0 cm, 50 ml had an inhibitory zone of 1.3 cm and 100 ml had a diameter of 1.3 cm. No inhibitory effect was observed in the plates treated with water extract of the mushroom.

Fig. 2 records the result showed that the ethanol mushroom extract had am inhibitory effect on *E. coli* Proliferation. Treatment A with 25ml recorded an inhibitory diameter of 1.3cm, 50ml recorded 1.8cm diameter while 100ml of the extract had 1.8cm inhibitory zone. All concentrations of the water extracts was ineffective on *E. coli* as the Organisms developed resistance to the extract.

Table 3 presents the results of the control treatment using gram negative sensitivity antibiotic disc. From the table, ciproflozacin and peflozacin had the highest sensitivity followed by sparfloxacin and Amozacillin while the organisms were resistant to Septrin, Chloramphenicol, Augumentin, Gentamycin and Streptomycin.

Table 4 presents the result of the gram positive sensitivity antibiotic disc indicated that Ciproflozacin, Streptomycin and Septrin had the highest sensitivity while Zinnat, Peflozacin and Gentamycin had medium sensitivity and Amozacillin, Enthomysin and Ampiclox were ineffective.

Table 1. Biochemical test

Chemical test	*E. coli*	*S. aureus*
Gram staining	-	+
Oxidase	-	-
Motility	+	-
Catalyse	+	+
Indole	+	-
Coagulase	-	+

Key: + = positive, - = negative

Table 2. Effect of different extracts concentrations on the growth of *E. coli* and *S. aureus*

Extract conc. (ml)	*E. coli*	*S. aureus*
E-25	+	+
E- 50	+	+
E-100	+	+
W-25	-	-
W-50	-	-
W-100	-	-

Key: + = inhibited growth of organism; - = had no inhibitory effect on growth; E = Ethanol extract; W = Water extract

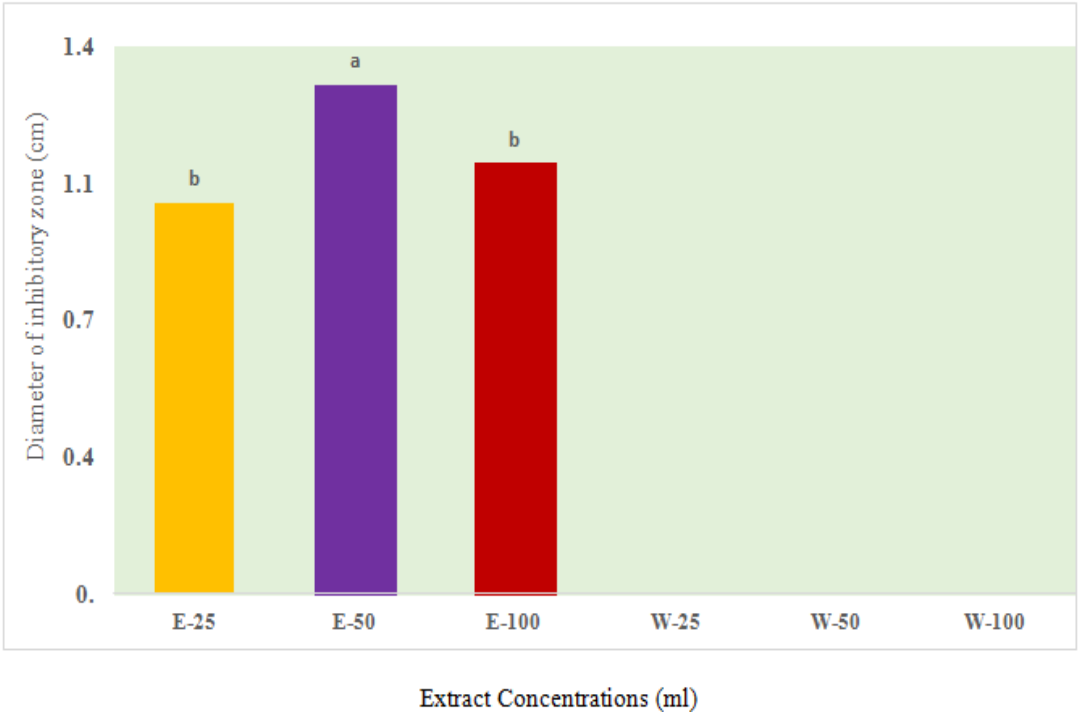

Fig. 1. Diameter of inhibitory zone of *Staphylococcus aureus*

Key: Ethanol extract (E) and Water extract (W) concentration in ml. Letters with the same superscript are not significantly different at 5% level of probability (P< 0.05)
level of probability (P< 0.05)

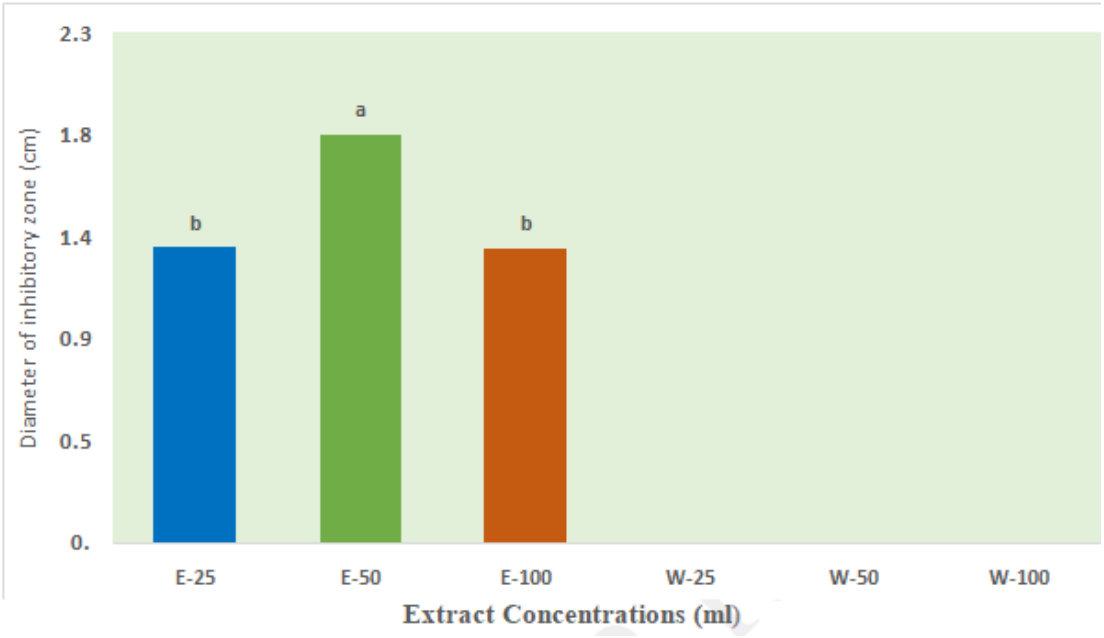

Fig. 2. Diameter of inhibitory zone of *Escherichia coli*

Key: Ethanol (E) and Water (W) extract concentration. Letters with the same superscript are not significantly different at 5% level of probability (P≤ 0.05)

Table 3. Control treatment using gram negative sensitivity antibiotics disc

Antibiotics	Organism (*E.coli*)	Range
Septrin	R	_
Chloramphenicol	R	_
sparfloxacin	S	++
Ciproflozacin	S	+++
Amozacillin	S	++
Augumentin	S	_
Gentamycin	R	_
Peflozacin	S	+++
Tarvid	S	+
Streptomycin	R	_

Key: +++ high sensitivity, ++ medium sensitivity, + low sensitivity, - resistant

Table 4. Control treatment using gram positive sensitivity antibiotics disc

Antibiotics	Organisms (*Staph.aureus*)	Range
Zinnat	S	++
Amozacillin	R	_
Rotadin	S	+
Coproflozacin	S	+++
Streptomycin	S	+++
Septrin	S	+++
Enthomysin	R	_
Peflozacin	S	++
Gentamycin	S	++
Ampiclox	R	_

Key: +++ high sensitivity, ++ medium sensitivity, + low sensitivity, - resistant

4. DISCUSSION

From the results obtained, the sensitivity profile of ethanol extract on *S. aureus* at different concentrations indicated that mushroom ethanol extract had a low inhibitory effect on *S. aureus* with inhibitory zone of 1.0 cm, 1.3 cm, and 1.1 cm. The result of the bioactivity of mushroom ethanol extract on *E. coli* indicated inhibitory zone of 1.3 cm against 25 ml, 1.8 cm against 50 ml, and 1.3 cm against 100 cm. However, the 50 ml concentrations of ethanol extract had the highest inhibitory effect on both organisms. It was effective on *E. coli* than on *S. aureus*. It was also observed from the study that the gram-negative bacteria was more susceptible to the ethanol extract than the gram- positive bacteria. All concentrations of the mushroom water extract were ineffective as it had no inhibitory effect on the tested organisms. The findings of the study agree with [25] that the antimicrobial compounds found in oyster mushrooms are capable of inhibiting pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*.

Results of ethanol extracts are similar to those reported for Moringa oleifera seeds with diameter of inhibition ranging between 1.0 cm and 1.7cm. The results also showed different levels of sensitivity for gram positive and gram- negative bacteria. These results are in agreement with the findings reported by [26]. The anti- microbial activity of mushroom extracts could be attributed to the presence of compounds such as terpenes, Confluentin and Griflorin [13]. However, Research have shown that the concentration of bioactive compounds directly influenced the anti-microbial capacity of mushroom extracts [27]. This finding is in agreement with the results obtained [27] who reported that the bioactive compounds present in mushrooms exhibited inhibitory activity on micro-organism. Such bioactive compounds include total phenols and flavonoids, as the various extract concentrations showed different levels of diameter of inhibition. The ethanol extract showed higher antimicrobial activity than the water extract, possibly as a result of the solubility of the bioactive compounds in ethanol than in water.

5. CONCLUSION

From the results obtained, ethanol extract of mushroom bioactive compound had inhibitory effect on *E. coli* and *S. aureus.* However, *E. coli* and *S. aureus* were resistant to mushroom water extract at the concentrations used. Mushroom components are gaining increasing interest in their application as antimicrobial agents in food safety. Since many mushrooms have been eaten safely for years, there is a significant advantage in using this multifunctional mushroom antimicrobial as alternatives to currently used antibiotics and synthetic food preservatives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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