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Antibacterial Activity of Ethanolic Extract of *Pleurotus ostreatus* on Selected Pathogenic Organisms

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Abstract
The antibacterial activity of an ethanolic extract of *Pleurotus ostreatus* in four various concentration (25, 50, 75 and 100 ml) was investigated against 3 species of bacteria: one Gram-positive bacteria (*Staphylococcus aurues*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the minimum inhibitory concentration. Data obtained on minimum inhibition concentration were arranged in one way analysis of variance. The result showed that 100 ml of *Pleurotus ostreatus* had the highest minimum inhibitory concentration on the bacteria especially on the multi-drug resistant bacteria *Pseudomonas aeruginosa* compared to other concentrations. *Pleurotus ostreatus* can be used in the treatment of the studied bacterial organisms especially for Gram-negative bacteria of *Pseudomonas aeruginosa* ATCC®27853which is a multi-drug resistance as a replacement for antibiotics.

Keywords: Oyster mushroom, *Pleurotus ostreatus,* antibiotic-resistant, antibacterial activity

Introduction

Infectious diseases account for a high proportion of health problems in most of the developing countries, especially in Nigeria. Many of these diseases are consequence of the food we eat either in raw or processed state. The use of conventional drugs and antimicrobials in the poultry has been banned in Europe but not in developing countries like Nigeria (Ekunseitan *et al.,* 2016). Although several antimicrobial agents have been synthesized chemically, an indiscriminate use of these commercial antimicrobial drugs has led to the development of resistance to the existing antibiotics by the microorganisms (Raghunath, 2008).

In recent times, there has been a positive interest in knowledge gathering of traditional/folklore medicine and documentation, this has led to use of natural plant resources not only as prophylaxis but curative in the control and treatment of various infections (Sibanda and Okoh, 2007). Moreover, antibiotics are sometimes associated with adverse effects on hosts like hypersensitivity and these side effects sometimes become much worse than cure. This situation has generated the need, and has provided the necessary impetus for a continuous search for novel antimicrobial agents from different natural biological sources (Cordell, 2000).

The medicinal properties of *Pleurotus spp* has been reported (Gregori *et al*., 2007 and tested for their pharmaceutical importance. Most of the work has been done using fruiting body (Iwalokun *et al*., 2007). Purification of the medicinally important compound(s) and their therapeutic mechanisms are still largely untouched. Search for an alternative drug from medicinal purpose will not only help as therapeutic armoury to control the bacterial disease but also minimize the side effects as the source is nutritious. This may be one of the reasons for the increased advocacy for the exclusion of antibiotics and hormone growth promoters in the rearing of poultry birds.

This study therefore, seeks to compare the efficacies of ethanolic extracts to check for the minimum inhibitory concentration of *Pleurotus ostreatus* against selected pathogenic organisms.

Materials and Methods

The research was carried out at the Animal Products Processing and feed quality laboratory, the College of Animal Science and Livestock Production while the antibacterial activity of both plants was carried out at Microbiology laboratory of College of Veterinary, Federal University of Agriculture Abeokuta. The experimental materials include *Pleurotus ostreatus* (oyster mushroom) which was obtained from a mushroom cultivation at Ibadan. Three bacteria species was used in the study: Gram positive (*Staphylococcusaureus*ATCC®25923) and two-gram negative bacteria (*Pseudomonas aeruginosa* ATCC®27853 *and Escherichia coli* ATCC® 25922) isolates.

Phytochemical test was carried on *Pleurotus ostreatu s*carried out using standard procedures to determine flavonoid, tannin, phytate, phenol, antioxidants, saponin, oxalate and carotenoid. A 1 kg of fresh oyster mushroom was soaked with 2litres of ethanol at a ratio of 1:2 and was left for 3 days (72 hours) for extraction. After 72 hours, the extract was sieved out using a muslin cloth. The extract was clarified by filtration through celite on water pump which was then concentrated in vacuo using a rotation evaporator.

Thereafter, 0.1 ml of each plant extract was incorporated into the medium (MacConkey agar and Nutrient agar).MacConkey agar medium was prepared by suspending 47 g in 1 litre of distilled water. This was brought to boil so as to dissolve completely and then sterilized by autoclaving at 121°C for 15minutes. After cooling, *Escherichia* coli (ATCC® 25922) were inoculated into the medium. *Escherichia coli* serves as a representative of Gram negative organisms and it is sensitive with no resistant plasmid, while nutrient agar medium was used in determination of the other bacteria. The medium was incubated at 37°C for24hrs. While the MIC for all the extracts was determined by using a modified agar- well diffusion method (Ubalua and Oti, 2008). Different concentration of each extract (100, 75, 50 and 25 mg/ml of *Pleurotus ostreatus*) were prepared on triplicate basis. The growth or otherwise of the sensitive organisms was visually assessed (Oxoid, 2011).

Data obtained in the study was arranged in One-way Analysis of variance in a completely randomised design (CRD) and futher expressed in Bar charts. Significant differences among treatments were determined using Duncan's Multiple Range Test (Duncan 1955) as contained in SAS (2010) package.

Results and Discussion

Table 1 shows the phytochemical screening of *Pleurotus ostreatus.* This reveals the presence of compounds documented and linked to antibacterial and anti-diarrhoeal activities (Ahmad *et al.*, 2006). Different inherent compounds like tannin, phenol and oxalate have been shown to display various mechanisms of actions such as increasing colonic water, inhibiting intestinal motility and inhibition of specific pathogenic organisms (Ahmad *et al.*, 2006).

Table 1: Phytochemical Screening of *Pleurotus ostreatus* (Oyster Mushroom)

Parameters	
Flavonoid mg/100g	0.03
Tannin mg/100g	26.00
Phytate mg/100g	100.00
Phenols GAE/g	0.23
Antioxidants mg/100g	0.07
Saponin mg/100g	0.20
Oxalate mg/100g	3.08
Carotenoid mg/100g	0.60

Figure I shows the Zone of inhibition of extracts of *Pleurotus ostreatus* (Oyster mushroom) on the selected pathogenic organisms. The different concentration of *Pleurotus ostreatus* (Oyster mushroom) revealed significant ($p<0.05$)inhibitory effect on all the three pathogenic microorganisms with the highest sensitivity observed in 100ml *Pleurotus ostreatus* (Oyster mushroom).

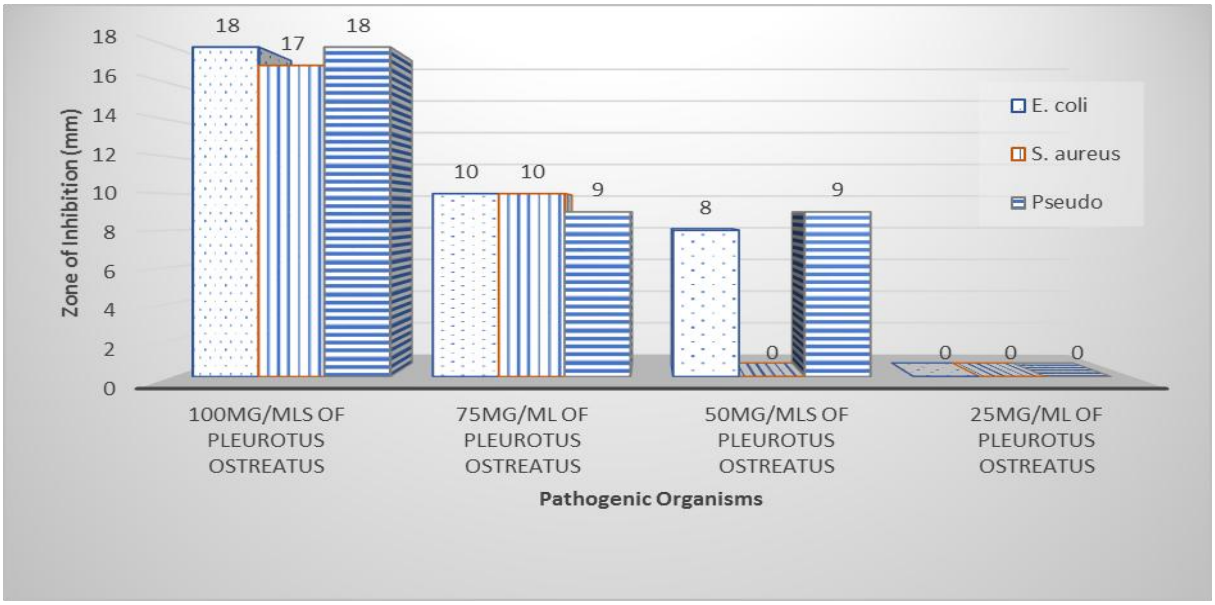

Fig. I: Zone of inhibition of extracts of *Pleurotus ostreatus* (Oyster mushroom) on the selected pathogenic organisms

This affirms reports of Hacioglu *et al.* (2011). This ability lies in the ability to alter resistance of bacterial organisms particularly antibiotic resistance genes that may be located on plasmids of these organisms. Ethanol extraction has been reported to be effective against several microorganisms (Jonathan and Fasidi, 2003; Hacioglu *et al.,* 2011) since most active components are generally water-insoluble, hence it is expected that low polarity organic solvents would yield more active extracts containing more inherent compounds which will invariably result in better mode of action against pathogens either *in vivo* or *in ovo.* The broad spectrum of antimicrobial activity obtained in the present study may be attributed to the presence of bioactive metabolites of various chemical types in mushroom compounds.

Conclusion

Pleurotus ostreatus can be used in the treatment of the diseases associated with selected pathogenic organisms especially for Gram-negative bacteria of *Pseudomonas aeruginosa* ATCC®27853which is a multi-drug resistant organism and give way for developing new therapeutic agents and scientifically validates its antimicrobial potentials.

References

Apata, D.F. (2009). Antibiotic Resistance in Poultry. *Intl J. of Poult. Sci.,* 8 (4): 404-408.

Cordell, G.A. (2000). Biodiversity and drug discovery symbiotic relationship. *Phytochem*, 55: 463-480.

Duncan, D.B. (1955). Multiple ranges and multiple F-Test. *Biometrics,* pp 1-42.

Ekunseitan, D.A., Yusuf, A.O., Olayinka, O.A., Ayoola, A.A. and Adegbenjo, A.A. (2016). Comparative study of two plants (*Lagenaria breviflora* and *Petiveria alliacea*) and their phytobiotic potential in poultry health. *Nig. J. of Ani. Prod.,* 43(1): 289-298.

Gregori. A., Svagel, M. and Pohleven, J. (2007). Cultivation techniques and medicinal properties of *Pleurotus specie. Food Tech. Biotechnol.,* 45: 238-249.

Hacioglu, N., Akata, I. and Dulger, B. (2011). Antimicrobial potential of *Xylaria polymorpha* (Pers.) Grev. *African J. of Microbiol. Res*., 5(6): 728-730.

Iwalokun, B.A., Usen, U.A., Otunba, A.A. and Olukoya, D.K. (2007). Comparative phytochemical evaluation antimicrobial and antioxidant properties of *Pleurotus ostreatus. Afri.J. on Biotech.* 6:1732-1739.

Oxoid, (2011). The Oxoid Manual of Culture Media, Ingredients and other Laboratory Services. 10th edition. Published by OXOID Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, England.

Raghunath, D. (2008). Emerging antibiotic resistance in bacteria with special reference to India. *J. Biosci*. 33(4): 593-603.

SAS (2010). Version 8, SAS, Institute Inc., Cary NC, USA.

Sibanda, T. and Okoh, A.I. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *Afr. J. Biotechnol*. 6 (25): 2886-2896.

Ubalua, A.O. and Oti, E. (2008). Evaluation of antimicrobial properties of some medicinal plants for fresh Cassava roots preservation. *Pakis. J. of Nutri.*, 7(5): 679-681.