# ANTIMICROBIAL ACTIVITY OF THE CRUDE POLYSACCHARIDE EXTRACTS OF THE EDIBLE MUSHROOM PLEUROTUS OSTREATUS (JACQ FR.) KUMM BEFORE AND AFTER BOILING.

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## ABSTRACT

The antimicrobial effects of the crude polysaccharide extract of the mushroom, *Pleurotus ostreatus* on some clinical isolates were investigated using the agar well diffusion method. Ethanol polysaccharide extracts of raw *P. ostreatus* showed sizeable inhibition zones of 15mm, 15mm, 16mm, 18mm and 12mm on *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans* respectively. The ethanol extract of boiled *P. ostreatus* also showed sizeable inhibition zones of 11mm, 12mm and 6mm on *Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* respectively. The aqueous extracts of the raw *P. ostreatus* showed inhibition zone of 5mm against *Pseudomonas aeruginosa* while aqueous extract of boiled *P. ostreatus* showed no observable zones of inhibition against the test organisms. The minimum inhibition concentration determined by the agar well diffusion technique for the ethanol extracts of raw *P. ostreatus* were 25mg/ml, 50mg/ml, 50mg/ml, 50mg/ml and 25mg/ml for *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans* respectively while minimum inhibition concentration for the ethanol extract of boiled *P. ostreatus* were 25mg/ml, 50mg/ml, 50mg/ml, 50mg/ml and 100mg/ml for *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans* respectively. The outcome of this study showed that antimicrobial activity using extracts of *P. ostreatus* has potential.

**Keywords:** antimicrobial, polysaccharide, extract, edible mushroom, clinical isolates

## INTRODUCTION

Mushrooms, because of their special fragrance and texture have been used for many years in oriental culture as tea and nutritional food (Manzi *et al*., 1999). The scientific community in searching for new therapeutic alternatives has studied many kinds of mushrooms and has found variable therapeutic activities such as anti-carcinogenic, anti-inflammatory, immunosuppressor and antibiotic among others (Asfors and Ley, 1993; Mizuno, 1999). It has been known for many years that selected mushrooms of higher Basidiomycetes origin are effective against certain ailments and infections and this has stirred a growing interest in such mushrooms from industry, media and scientific community (Wasser, 2002).

*Pleurotus ostreatus* is a Basidiomycete found in the temperate and subtropical forest throughout the world. In Nigeria, it is found in the wild on decaying logs and tree trunks. This mushroom is edible and thus safe to eat. It is highly consumed in Nigeria. It is used to spice soup and fried as meat (Iwalokun *et al*., 2007). The mushroom is known to contain B-vitamins such as niacin, flavin and pyridoxine.

Researchers have shown antimicrobial activity of several mushrooms (Gezer *et al.*, 2006 and Turkoglu *et al*., 2007). In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists into searching for new antimicrobial substances from various sources which are the good source of novel antimicrobial chemotherapeutic agents (Karaman *et al*., 2003). Extracts from fruiting bodies of vari-

ous mushrooms have been reported for antimicrobial activity against a wide range of infectious bacteria (Dugler *et al.*, 2002).

There's no doubt that edible mushrooms nutritionally sound as a tasteful food source for most people and can be a significant dietary component for vegetarians. Nevertheless, the edible mushrooms are consumed as raw food in most of the western countries whereas in countries like Nigeria, most of the food items are boiled, cooked and consumed (Iwalokun *et al*., 2007). This brings up the question of whether it retains its biological activity after being cooked and can they be considered as a functional food? Based on this problem, the present work is carried out to evaluate the antimicrobial activities of crude polysaccharide extracts from fruiting body of *Pleurotus ostreatus* before and after boiling.

## MATERIALS AND METHODS

### Collection and identification of mushroom samples

Samples of *Pleurotus ostreatus* used in this investigation were collected from a mango tree located in a farmland in Ugbowo, Benin City, Edo State. It was identified using the standard descriptions of Zoberi (1973).

### Preparation of mushroom extract

The mushroom sample was divided into two, one part was shade dried at room temperature for 7 days to prevent loss of bioactive components while the other part was boiled for 45mins at 100°C, which was later dried at room temperature. The dried samples were ground into powder using both milling machine and electronic blender. Twenty grammes (20g) portion of the powdered samples were soaked in 180ml of absolute ethanol for 24hrs and stirred every 6 hours using different sterile glass rods and then filtered through Whatman No. 1 filter paper to yield the crude ethanolic extract. Same was done to get the aqueous polysaccharide extract. The filtrates were concentrated using the rotary evaporator at 40°C until a paste was formed.

### Antimicrobial screening tests

Clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Candida albicans* obtained from the Department of Microbiology, University of Benin Teaching Hospital, Ugbowo, Benin City, Edo State, Nigeria were used in the screening tests. The identities of the isolates were confirmed by their cultural, morphological and biochemical characteristics (Cheesebrough, 2000).

Antimicrobial activity of the extracts of *Pleurotus ostreatus* was determined by agar well diffusion method (Ebi and Ofoefule, 1997). Solutions of the extracts were prepared by dissolving 0.5g of the extracts in 3ml of normal saline in test tubes and the resultant solution diluted to a concentration of 100mg/ml by the addition of 2ml of normal saline (Mizuno, 1999). Twenty-eight (28) grammes of nutrient agar was dissolved in 100ml of distilled water and sterilized at 121°C for 15mins. Fifteen milliliter (15ml) of nutrient agar was then dispensed into labeled Petridishes and was allowed to cool for some minutes. Potato dextrose agar (PDA) was used for the fungus. Plates were then seeded with 0.2ml of standardized broth cultures of the bacteria and fungus. The surfaces of the seeded media were allowed to dry for 30mins. Wells of 6.00mm in diameter and 8.00mm deep were aseptically made in the seeded agar plates using sterile cork borers (Azoro, 2002). The mushroom extracts (0.1ml) were then filled into the wells of agar plates directly. Bacteria and fungi control wells contained 0.1ml of ciprofloxacin and ketoconazole respectively. The agar plates were left on the bench for 45mins before incubation to allow diffusion of the extract into the agar. Plates seeded with the fungus were incubated at 28° C for 48hrs while those plates seeded with bacteria cultures were incubated at 37°C for 18hrs. At the end of the incubation period, the inhibition zones formed on the medium were evaluated in millimeter (mm).

### Determination of minimum inhibitory concentration

Analysis of the minimum inhibitory concentration (MIC) was carried out according to the standards recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). This test was carried out for the ethanol extract of both raw and boiled *Pleurotus ostreatus*. A two-fold dilu-

tion of each of the extracts in normal saline was prepared. Agar well diffusion as described by Prescott *et al*. (2002) was employed. Four different concentrations of the extracts were prepared. These are 100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml and 0.1ml of each concentration of the extracts was dispensed into each wells using calibrated dropper. The plates were left on the bench for 45 minutes before incubation to allow diffusion of the extract into agar. Subsequently, the plates seeded with bacteria culture were incubated at 37°C for 18-24 hrs while those seeded with the fungus were incubated at 28° C for 48hrs. The minimum concentration that showed inhibition was taken as minimum inhibitory concentration (MIC).

**Sensitivity of test microorganisms to antimicrobial drugs.**

Sensitivity of the microorganisms to antibiotics was tested using commercially available reference discs containing the antibiotics. The discs were placed on nutrient agar plates already seeded with the test organisms and were incubated. The plates seeded with bacterial culture were incubated at 37°C for 18-24 hrs while those seeded with the fungus were incubated at 28°C for 48hrs. After which their different zones of inhibition were recorded in mm.

## RESULTS AND DISCUSSION

Antimicrobial screening of the stock concentration of the different crude polysaccharide extracts of *P. ostreatus* showed observable zones of inhibition (Table 1) using the agar well diffusion method. Ethanol extract (100mg/ml) of raw *P. ostreatus* exhibited antimicrobial activity on the test organisms. The most susceptible was *K. pneumonia* for ethanol extract of both raw and boiled *P. ostreatus* with a diameter of 18 and 12mm respectively (Table 1). The ethanol extract of boiled *P.ostreatus* showed no antibacterial activity against *P. aeruginosa* and *S. aureus* but in the raw extract, clear zones were observed of 15 and 16mm respectively (Table 1). Against *E. coli*, ethanol extracts of both raw and boiled showed anti-bacterial activity but the activity range was less in boiled extract (11mm) when compared to raw extract (15mm). This might be due to the influence of temperature that affected the compound which is responsible for the activity. In the present study, the ethanol extracts of both raw and boiled *P. ostreatus* showed antifungal activity against *Candida albicans*. The aqueous extract of both raw and boiled showed no significant difference ($P>0.05$) (as in *P. aeruginosa*) or no antimicrobial activity.

**TABLE 1: Inhibition zone diameter (mm) of the crude polysaccharide extract of raw and boiled Pleurotus ostreatus against test microorganisms**

| Crude polysaccharide extract | Concentration mg/ml | *E. coli* | *P. aeruginosa* | *S. aureus* | *K.pneumonia* | *C.albicans* |
|---|---|---|---|---|---|---|
| Ethanol extract of raw *P. ostreatus* | 100 | 15 | 15 | 16 | 18 | 12 |
| Aqueous extract of raw *P.ostreatus* | 100 | - | 5 | - | - | - |
| Ethanol extract of boiled *P. ostreatus* | 100 | 11 | - | - | 12 | 6 |
| Aqueous extract of boiled *P. ostreatus* | 100 | - | - | - | - | - |
| Ciprofloxacin | 5µg | 12 | 14 | 17 | 15 | - |
| Ketoconazole | 2µg | NT | NT | NT | NT | 16 |

*No Inhibition, NT- Not tested, mm- Millimeter*

Minimum inhibitory concentration (MIC) of the crude polysaccharide ethanol extract of raw *P.ostreatus* determined by the agar well diffusion method (Table 2) were found to be 25mg/ml, 50mg/ml, 50mg/ml. 50mg/ml, 25mg/ml on *E.coli, P.aeruginosa, S.aureus, K. pneumonia* and *C. albicans* respectively while that of boiled *P. ostreatus* were found to be 25mg/ml, 50mg/ml, 50mg/ml, 50mg/ml, 100mg/ml on *E.coli, P. aeruginosa, S.aureus, K. pneumonia* and *C. albicans* respectively (Table 3).

**TABLE 2: Minimum Inhibitory Concentration (MIC) of the crude ethanol polysaccharide extract of Raw *Pleurotus ostreatus* against test microorganisms**

| Concentration mg/ml | Dilution | *E.coli* | *P.aeruginosa* | *S. aureus* | *K.pneumonia* | *C.albicans* |
|---|---|---|---|---|---|---|
| 100 | $2^{0}$ | 15 | 12 | 16 | 17 | 14 |
| 50 | $2^{-1}$ | 11 | 9 | 13 | 12 | 11 |
| 25 | $2^{-2}$ | 6 | - | - | - | 6 |
| 12.5 | $2^{-3}$ | - | - | - | - | - |

*MIC- lowest concentration that showed observable zone of inhibition (mm). No zone of Inhibition*

**TABLE 3: Minimum Inhibitory concentration (MIC) of the crude ethanol polysaccharide extract of boiled *Pleurotus ostreatus* against test organisms**

| Concentration mg/ml | Dilution | *E.coli* | *P.aeruginosa* | *S. aureus* | *K.pneumonia* | *C.albicans* |
|---|---|---|---|---|---|---|
| 100 | $2^0$ | 11 | 10 | 13 | 10 | 7 |
| 50 | $2^1$ | 8 | 6 | 10 | 5 | - |
| 25 | $2^2$ | 6 | - | - | - | - |
| 12.5 | $2^3$ | - | - | - | - | - |

*MIC-Lowest concentration that showed observable zone of inhibition (mm). No zone of inhibition*

The present work has revealed the antimicrobial potency of the ethanol extract of this edible mushroom, *Pleurotus ostreatus*. Ethanol extracts of raw and boiled *P. ostreatus* were observed to inhibit gram positive and gram-negative bacteria as well as the fungus tested *in vitro* (Tables 2 & 3 ) to suggest that *P. ostreatus* has a broad-spectrum antibacterial and antifungal activity. Similar antimicrobial potentials have been observed in the culture extracts of *Irpex lacteus* (Rosa *et al.*, 2003) *Agrocybe* sp. and juice of *L. edodes* (Kuznetsov *et al.*, 2005). Antimicrobial potencies of the extracts from mushrooms such as *Cuminum cyminum, Carum carvi, Coriandum sativum* and *Foeniculum vulgare* have also been reported with activity against bacterial pathogens such as *Pseudomonas, Klebsiella, and E. coli* as observed for *P. ostreatus* in this study. Similar observation was made in ethanol extract of a polyporous mushroom, *Laetiporous sulphureus* (Bull). Murill (Turkoglu *et al.*, 2007).

The ethanol extract of raw *P. ostreatus* inhibited all the test organisms, the most susceptible organism was *Klebsiella pneumonia* which was inhibited by ethanol extracts of both raw and boiled *P. ostreatus*, having an inhibition zone of 18mm and 12mm respectively (Tables 2 & 3).

Ethanol extract of the boiled mushroom inhibited the growth of *Escherichia coli, Klebsiella pneumonia* and *Candida albicans.* The fact that the boiled mushroom extract inhibited the growth of some of the organisms shows that boiling did not reduce or dilute all the medicinal properties. This indeed has confirmed the fact that *P. ostreatus* can be considered a "functional food" because the result has shown that it contains some components which can affect one or more identified functions of the body whether it is consumed raw or boiled.

The ethanol extracts of raw and boiled *P. ostreatus* also exhibited antifungal activity by inhibiting the fungus, *Candida albicans.* The aqueous extract of raw and boiled *P. ostreatus* exhibited very little or no antimicrobial activity. This shows that the aqueous extract did not yield enough concentration of the active components that can inhibit these organisms, thus, making ethanol, a better extracting agent.

It is noteworthy that the spectrum of antimicrobial activity of the crude ethanol polysaccharide extract of both raw and boiled is comparable to that of commercial antimicrobial drugs. Sensitivity test carried out (Table 4) showed that most of the microorganisms showed resistance towards these antimicrobials.

**TABLE 4**: Sensitivity of Microorganisms to Antimicrobial drugs

| Organisms/antimicrobial drugs | N | E | T | GN | PEF | CPX | S | SXT |
|---|---|---|---|---|---|---|---|---|
| *E.coli* | NT | NT | 13 | 11 | 10 | 20 | - | - |
| *P. aeruginosa* | NT | NT | 14 | 16 | 13 | 14 | - | - |
| *S. aureus* | NT | - | NT | 15 | 10 | NT | NT | - |
| *K. pneumonia* | NT | NT | 10 | 13 | - | - | - | - |
| *C. albicans* | 19 | NT | NT | NT | NT | NT | NT | - |

*N- Nystatin(10µg), T- Tarivid(10µg), GN- Gentamycin (10µg), SXT- Septrin(30µg), E- Erthromycin (10µg), PEF-Pefloxacin(30µg), CPX- Ciprofloxacin (10µg), S-Streptomycin (30µg). No zone of inhibition NT- Not tested*

The antimicrobial activity of *P. ostreatus* confirms studies that mushrooms possess antimicrobial effects (Hur *at el.,* 2004). The outcome of this study shows that antimicrobial activity using extracts of *P. ostreatus* has potential and more research needs to be done in this area to determine the exact bioactive components of these extracts.

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