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Research Article

Antibacterial Effect of *Pleurotus ostreatus* on Selected Bacterial Isolates

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ABSTRACT

Pleurotus ostreatus, commonly known as oyster mushrooms, are edible fungi with a long history of culinary and medicinal use. This study was carried out to determine the anti-bacterial effect of *Pleurotus ostreatus* against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*. The bacterial isolates were collected from the Microbiology Laboratory of Caleb University, Lagos, Nigeria. The isolates were confirmed using biochemical tests. The antibacterial susceptibility pattern of the isolates to different antibiotics was determined using the disc diffusion technique. Extraction was carried out on dried and finely ground *Pleurotus ostreatus* using methanol and water as solvents. Susceptibility of the isolates to *Pleurotus ostreatus* was done using the agar well diffusion technique. The disc diffusion technique showed susceptibility and resistance of the bacterial isolates to different antibiotics. Methanol extracts of *Pleurotus ostreatus* showed higher anti-bacterial activity against the selected bacteria judging by the longer diameter of zones of inhibition. More studies should be carried out on *Pleurotus ostreatus* to extract and purify the individual bioactive components which could form the basis for the development of antimicrobial agents.

Keywords: Antibacterial, *Pleurotus ostreatus*, Bacterial isolate, Microbes

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INTRODUCTION

Pleurotus species belong to Agaricales and the family of Pleurotaceae (white spore oyster mushroom) and are distinguished by their colour and habitat. Most of them are saprophytic and rarely parasitic. The morphological characteristics of *Pleurotus* species are unstable due to varying agro-climatic conditions and different substrates used for cultivation (Kong, 2004). The taxonomical and phylogenic identification of *Pleurotus* species is quite complex, leading to its misidentification. The genus, species name, and anamorphic states of commercially important *Pleurotus* species were described by Guzman (2000).

Pleurotus species are commercially essential mushrooms and are widely cultivated throughout the world. The production of *Pleurotus* mushrooms alone accounts for around 25% of the total cultivated mushrooms globally (Raman *et al*., 2021). In America and Europe, *Pleurotus* species are considered special mushrooms, whereas, in Korea, their cultivation is economically profitable, and it is one of the highly consumed species (Knop *et al*., 2015). *Pleurotus* species are predominantly found in

tropical forests and often grow on fallen branches, dead and decaying tree stumps, and wet logs. Biographical studies have shown that the *Pleurotus* genus is among the more conspicuous fungi that induce wood decay in terrestrial ecosystems worldwide due to its formidable lignin-modifying enzymes, including laccase and versatile peroxidases (Raman *et al*., 2021). *Pleurotus ostreatus* can be grown easily due to their fast colonization nature on diversified agro-substrates and have a biological efficiency of 100%. *Pleurotus* mushrooms are rich in proteins, dietary fibre, essential amino acids, carbohydrates, water-soluble vitamins, and minerals (Ahmed *et al.,* 2016). These mushrooms are richly endowed with immense health significance. *Pleurotus* mushrooms are wielded as flavouring, aroma, and excellent preservation quality. Apart from their unique applications, *Pleurotus* mushrooms have impacted delicacies meaningfully and have demonstrated impressive nutritional and medicinal values (Raman *et al*., 2021).

Pleurotus ostreatus, commonly known as oyster mushrooms, are edible fungi with a long history of culinary and medicinal use. These mushrooms belong to the genus *Pleurotus* and are known for their fan-shaped caps and decurrent gills (Chang, & Miles, 2004). Besides their nutritional value, *Pleurotus ostreatus* has been proven to have antibacterial properties. The antibacterial potential of *Pleurotus ostreatus* has been the subject of interest. Antibiotic resistance has spread rapidly due to several factors, including the misuse and overuse of antibiotics in human and animal healthcare, inadequate infection control practices, and the ability of bacteria to acquire and transfer resistance genes (Ventola, 2015). The evolution and widespread of resistant bacteria have been observed across various settings, including hospitals, communities, and livestock farms. The rise in antibiotic resistance stages a significant threat to public health, and searching for alternative antimicrobial agents is important. *Pleurotus ostreatus* has shown promising results in fighting multidrug-resistant bacteria, suggesting their potential as good therapeutic agents or as a source for the development of novel antimicrobial drugs.

METHODOLOGY

Sample Collection

Sporophores of *Pleurotus ostreatus* with the molecular identification number: MW457626.1 was obtained from the Microbiology Laboratory. Isolates of *Escherichia coli, Salmonella* specie and *Staphylococcus aureus* were collected from the Microbiology Laboratory, Department of Microbiology, Caleb University. The bacterial isolates were confirmed using Gram reaction and biochemical tests.

Biochemical Tests

The following biochemical tests were carried out to confirm *Escherichia coli*. Indole production test, methyl red (MR) test, citrate utilization test, triple sugar iron (TSI) agar test.

For the confirmation of *Staphylococcus aureus,* the following biochemical tests were carried out; catalase test, coagulase test, and mannitol fermentation test.

The following biochemical tests were carried out for the confirmation of *Salmonella typhi*; citrate utilization test, Indole test, urea hydrolysis test, and methyl red (MR) test. It was also cultured in these differential media; triple sugar iron agar and lysine iron agar (Cheesbrough, 2003).

Preparation of *Pleurotus ostreatus*

The *Pleurotus ostreatus* was allowed to air dry in a shaded area and ground into fine powder. The ground powder was introduced into two Petri dishes which is half the total weight (16.7/2 = 8.3 g). The content was poured into two separate conical flasks labelled A and B and 100 ml of Ethanol was poured into A while 100 ml of water was poured into B. This setup was left for 4 days. After 4 days, the contents were separately sieved with the aid of a filter paper and a funnel. The sieved-out content was boiled on the constant temperature magnetic stirrer on low heat to get the active compounds.

Media Preparation

All media used were prepared according to the manufacturer's instructions.

Antimicrobial Susceptibility Test

Nine (9) freshly prepared plates of Mueller Hinton agar were used for antimicrobial susceptibility testing. Twenty-four (24) hour cultures of *Escherichia coli*, *Staphylococcus aureus,* and *salmonella typhi* were introduced into 5 ml of sterile distilled water in universal bottles using a syringe. Turbidity was measured and compared to MacFaland standard 0.5. A swab stick was used to dip into the universal bottles and swab on the surface of the Mueller Hinton agar plates (1 for each). With the aid the of a cork borer with 6 mm diameter, 2 holes each were made on three Mueller Hinton plates (one for each pathogen) labelled A (ethanol extract) and B (aqueous extract).

This was followed by introduction of 0.4ml of ethanol extract to point A and 0.4 ml of aqueous extract to point B on each agar plate (one for each bacterial isolate).

Gram-negative antibiotics impregnated multi disc were introduced to *E. coli* and *Salmonella typhi* plates while Gram-positive discs were introduced onto the *Staphylococcus* plate. The experimental setup was incubated at 35^{o}C for 18 – 24 hrs (NCCLS, 2003).

RESULTS

Biochemical Confirmation of Isolates

The result of the biochemical confirmation of the isolates is presented in Table 1. Biochemical tests confirmed the selected isolates to be *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus.* The antibiotics susceptibility tests show resistance of the selected isolates to different antibiotics as depicted in Table 2. All three organisms were resistant to Augmentin, cefotaxime, and imipenem. All three organisms were susceptible to ciprofloxacin, gentamicin and levofloxacin. *Escherichia coli* was resistant to aztreonam and amoxicillin/clavulanic acid. *Salmonella typhi* was resistant to aztreonam and cefuroxime. *Staphylococcus aureus* was resistant to cefotaxime, amoxicillin/clavulanic acid, and cefuroxime. The diameter of zones of inhibition produced by ethanol and aqueous extracts of *Pleurotus ostreatus* on selected bacteria is shown in Table 2. This result shows that the ethanol extract produced better zones of inhibition on the isolates than the aqueous extract. Generally, the zones of inhibition obtained from *Pleurotus ostreatus* extract surpassed those of conventional antibiotics therefore it could be a suitable replacement.

Table 1. Biochemical characterization of Isolates

Tests								
Urease	**Citrate**	**Catalase**	**Coagulase**	**Indole**	**Gram Reaction**	**Mannitol**	**Shape**	**Isolates**
−	−	+		+	−	−	Cocci	*Escherichia coli*
+		+	+	NA	−	−	Bacilli	*Salmonella* sp
+	+	+	−	NA	+	+	Cocci	*Staphylococcus aureus*

Keys: + = positive, − = negative, NA = not applicable

Table 2: Antibiotics Susceptibility Pattern of Isolates

S/N	Antibiotic/Extract	*Escherichia coli* (mm)	*Salmonella* sp (mm)	*Staphylococcus aureus* (mm)
1	NF (10 µg)	14	-	15
2	AT (30 µg)	0	0	13
3	AUG (5 µg)	0	0	0
4	CRO (5 µg)	14	4	15
5	OFX (5 µg)	3	14	13
6	GN (10 µg)	21	14	10
7	NA (30 µg)	11	-	18
8	LE (5 µg)	21	18	12
9	CXM (30 µg)	21	0	0
10	AMC (20/10 µg)	0	-	0
11	CTX (30 µg)	0	0	0
12	IMP (10 µg)	0	0	0
13	CIP (5 µg)	-	14	-
14	ERY (15 µg)	-	14	-
15	AZM (15 µg)	-	14	-
16	Ethanol extract	27	20	23
17	Aqueous extract	21	15	14

Key: CTX = cefotaxime, CXM = cefuroxime, CIP = ciprofloxacin, ERY = erythromycin, GN = gentamicin, OFX = ofloxacin, AUG = augmentin, NF = norfloxacin, CRO = ciprofloxacin, NA = nalidixic acid, IMP = imipenem, AZM azithromycin, AMC = amoxicillin/clavulanic acid, LE = levofloxacin, AT = Aztreonam, 0 = no zone of inhibitiom, - = was not applicable. Interpretation of the test results is based on the standard by [4]

DISCUSSION

Pleurotus species, commonly known as oyster mushrooms, have gained significant attention in recent years due to its various health-promoting properties. Among these properties, the antibacterial potential of *Pleurotus* has been extensively studied. Antibacterial resistance by bacteria as depicted in Table 2 has become an issue of growing concern in the health care industry. Emerging antibiotic resistance is currently acknowledged as one of the most significant public health problems with high mortality rates associated with multidrug-resistant bacterial infections (Rasheed *et al*., 2014). The selective pressures imposed by antimicrobial use, overuse, and misuse are driving the gradual increase in antibiotic resistance and leading to the emergence of multidrug-resistant bacterial strains. Previously treatable bacterial infections are now often untreatable or require the use of the last line of antibiotics (Wu *et al*., 2021). Antibiotic resistance in *E. coli, Salmonella typhi,* and *Staphylococcus aureus* is of particular concern because they are some of the most common pathogens of humans, and common causes of both community and hospital-acquired bacteraemia (Salvadori *et al*., 2004) as well as a cause of diarrhea (Kaper *et al*., 2004).

This study indicates that *Pleurotus* extracts possess bioactive compounds that can be used against a wide range of bacteria since both Gram-positive and Gram-negative bacteria are represented in the study. This study agrees with the work of Chang & Miles (2004) and Akyüz & Kirbag (2009). The bioactive compounds of *Pleurotus ostreatus* can target bacterial cell walls, enzymes, and virulence factors. Chang & Miles (2004) isolated a polysaccharide from *Pleurotus geesteranus* and found that it effectively stopped the growth of *Streptococcus mutans*, a bacterium associated with dental caries.

The antibacterial potential of *Pleurotus* can be applied in various fields. Huang *et al*. (2022) explored the use of *Pleurotus ostreatus* extracts as natural food preservatives to stop the growth of spoilage bacterial in freshly cut fruits and vegetables. Moreover, the antibacterial properties of *Pleurotus* extracts have shown potential in controlling bacterial infections in aquaculture systems, as demonstrated by Akyüz & Kirbag (2009).

Recent studies have highlighted the significant antibacterial potential of *Pleurotus* species. The bioactive compounds present in *Pleurotus* mushrooms exhibit a wide range of antibacterial activity against various bacterial pathogens. Until now, around 70 species of *Pleurotus* have been registered. The majority of these species are known for their medicinal value, in particularly, their antimicrobial properties (Akyuz & Kirbag, 2009; Mohamed & Farghaly, 2014). On the other hand, wild strains of some species belonging to the genus *Pleurotus*; *P. cystidiosus* show potential pharmacological potential and nutraceutical properties (Kalaw & Albinto, 2014), this will reflect the importance of these cultivated wild strains as an antimicrobial agent. Other species belonging to the genus *Pleurotus* have been tested by other researchers (Schillaci *et al*., 2013). Authors have studied the inhibitory effect of *P. eryngii* var. ferulae, *P. eryngii* var. eryngii, *P. nebrodensis,* and *P. eryngii* var. elaeoselini against four pathogenic bacterial: *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Interestingly, their results demonstrated that all *Pleurotus* species tested displayed antibacterial activity, especially *P. nebrodensis* extract, and this species was capable of inhibiting significantly the growth of *S. epidermidis* (Schillaci *et al*., 2013).

CONCLUSION

Three (3) bacterial isolates were selected for this study and were confirmed to be *Escherichia coli, Salmonella typhi*, and *Staphylococcus aureus* using biochemical tests. Antibiotic susceptibility tests demonstrated the susceptibility and resistance of the organisms to different antibiotics. Antibiotic susceptibility testing using the agar well diffusion technique shows the susceptibility of the isolates tested to ethanol and aqueous extracts of *Pleurotus ostreatus*.

The high level of bacterial susceptibility to *Pleurotus ostreatus* extracts offers significant potential for various applications. In the field of medicine, the antibacterial properties of *Pleurotus ostreatus* can be explored for the development of novel antimicrobial agents or as a substitute therapy for bacterial infections.

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