

Article

## Evaluation of Antimicrobial Activity of *Pleurotus ostreatus* on Selected Multi-Drug Resistant Bacteria and Fungus

Zainab G. Aljassim<sup>1\*</sup>, Haitham M. Kadhim<sup>2</sup>, Abbas S. Al-mizraqchi<sup>3</sup>

<sup>1</sup>Pharmacology department, College of Dentistry, Baghdad University, Iraq.

<sup>2</sup>Pharmacology & Toxicology department, College of Pharmacy, Al-Nahrain University, Iraq.

<sup>3</sup>Medical microbiology department, College of Dentistry, Baghdad University, Iraq.

\*Correspondence: : haitham7424@yahoo.com

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### Abstract

*Pleurotus ostreatus*, or oyster mushroom, is a common edible wild mushroom characterized by its high nutritional values and promising diverse biological activities. It contains many bioactive components which have been found to possess several therapeutic functions. Because of the rising threat of treating serious and resistant infections, there is a developing need to discover new treatment strategies and compounds that can effectively eradicate infections. This study aims to evaluate and measure the antimicrobial activity and the minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) of *Pleurotus ostreatus* methanol and aqueous crude extracts on Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli*, Mutans Streptococci, *Lactobacillus*, and *Candida albicans*. The antimicrobial activity of mushroom extracts was evaluated against ten purified microbial isolates using agar disc diffusion assay and minimum bactericidal and fungicidal concentration assay. The results show that *P. ostreatus* methanol and aqueous crude extracts revealed antibacterial activity against the tested microorganisms and formed growth inhibition zones. Methanol crude extract shows more potent growth inhibition than the aqueous extract and in lower concentrations. This study shows that the tested oyster mushroom extracts have antimicrobial effects on different pathogens. Methanol crude extract of *P. ostreatus* revealed more powerful antibacterial and antifungal activity than the aqueous crude extract.

**Keywords:** Oyster mushroom, Antimicrobial activity, Methanol crude extract.

### Introduction

Oyster mushroom (*Pleurotus ostreatus*) is an edible mushroom that is a commonly widespread wild mushroom with high nutritional and promising biomedical values<sup>1</sup>. This mushroom can grow in many places. Its mycelia is found to kill and digest nematodes, which is thought to be how the mushroom gets nitrogen.<sup>2</sup> It contains terpenes, lactones, amino acids, and carbohydrates, providing valuable aromas and flavors to their fruiting body and mycelia.<sup>3</sup> Thus, it is used commercially for food such as in soup preparation, stir-fry recipes with soy sauce or eaten stuffed.<sup>4</sup> Several bioactive compounds, namely polysaccharides, lipopolysaccharides, proteins, peptides, glycoproteins, nucleosides, triterpenoids, lectins, lipids and their

derivatives, have been extracted from *P. ostreatus* for investigation purposes<sup>5</sup>. Several studies have shown that it has antitumor, immune-modulatory, antioxidant, anticancer, anti-inflammatory, anti-arthritic, antigenotoxic, hypo-cholesterolaemic, anti-hyperglycaemic, anti-hypertensive, antiplatelet aggregating, antiviral and antimicrobial activities<sup>6,7</sup>. The fruiting bodies of *P. ostreatus* contain an active alkaline skeletal  $\beta$ -D Glucan (pleuran), phenolic and tannin constituents that can be extracted. These chemicals were found to have antibacterial activity with several mechanisms, including cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions. Both alcoholic and aqueous extracts were tested and showed a promising natural antimicrobial agent that needs more evaluation for antimicrobial therapy. *P. ostreatus* extracts contain many bioactive chemicals; among these,  $\beta$ -D Glucan (pleuran), phenolic and tannin compounds might be the components responsible for the enjoyable antimicrobial activity<sup>6,8</sup>. A recent study showed a successful biosynthesis of stable gold nanoparticles (AuNPs) using edible mushroom *P. ostreatus* extracellular filtrate. This study showed that the biosynthesized AuNPs have more anticancer and synergist antimicrobial activity than the commercial AuNPs alone<sup>9</sup>. Another recent study showed that *P. ostreatus* reveals significant antibacterial efficacy on the Extended-spectrum beta-lactamase (ESBL) positive strains tested bacteria. The MBC values of the oyster extract are present in the range of 10 – 21mg/ml<sup>10</sup>. A study in 2013 using different alcoholic extracts (petroleum ether, acetone, and methanol) of *P. ostreatus* revealed excellent antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* species. *P. ostreatus* was also very effective against fungal pathogens<sup>11</sup>. This study aims to evaluate and measure the antimicrobial activity of *P. ostreatus*, methanol and aqueous crude extracts on Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli*, Mutans Streptococci, *Lactobacillus*, and *Candida albicans*. Also, it aims to determine the minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) for each tested microorganism.

## Materials and Methods

### *Isolation, purification, Identification and activation of bacterial and fungal Strains*

About 70-80 microbial strains were obtained from clinical specimens. They were isolated using a suitable medium and identified by gram stain, colony morphology, biochemical tests (Coagulase and catalase tests) and API-20E test strips (from bioMerieux, Inc.). Ten selected purified strains of *S. aureus*, *P. aeruginosa*, *E. coli*, Mutans Streptococci, *Lactobacillus*, and *C. albicans* were chosen for the study, which mainly showed the highest values of resistance to traditional antimicrobial medication. Clinical specimens were acquired from the teaching laboratories of Medical City Hospital in Baghdad. A selective colony of each microorganism was transferred from their agar plates to 10ml of sterile Brain Heart Infusion Broth (BHI-B) and incubated for 24 hours aerobically at 37 °C. The purity of the isolates was checked. This process was done according to<sup>12</sup>. To activate inoculums, 0.1 ml of the pure isolates were added to 10 ml of sterile BHI-B and followed by incubation aerobically for 18 hours. At 37°C for activation before each experiment<sup>13</sup>. The Disc diffusion test determined resistant strains of bacteria to different traditional antimicrobial medications. An antibiotic susceptibility test was performed to choose the most resistant strains for the study. All culture media were sterilized by autoclave at 121°C and 15 pounds/inch<sup>2</sup> pressure for 15 minutes. A hot air oven sterilized all cleaned glass wares at 180°C for 1 hr. The benches and the laboratory floor were disinfected by bleaching antiseptic solution (Fas).

### *P. ostreatus cultivation, extraction, preparation*

*P. ostreatus* mycelia are grown and maintained on potato dextrose agar medium (PDA), which consisted of 4 g/L potato extract, 20 g/L dextrose, and 20 g/L agar in the College of Agriculture/ Baghdad University. They then transferred to a spawning medium, a mixture of cereal grains, calcium carbonate, and calcium sulfate (100: 2: 1) (wt:wt:wt), and incubated at 22°C for 21 days. The spawns were then inoculated into around 1 Kg of a rice straw medium in plastic bags (24 × 60 cm) fitted with a cotton plug. The bags were sterilized with an autoclave for 40 min at 121°C under 1.5 × atmospheric pressure before spawning inoculation. The inoculated bags were incubated in a dark mushroom house at 18°C with 95% humidity and exposed to light for 2 h daily. The bags were ventilated with fresh air for 1 h daily until the mycelia fully penetrated the substrate. The bags were opened after 15 days to allow the production of fruiting bodies<sup>14,15</sup>. Ten Kilos of dried fruiting bodies of *P. ostreatus* were used and ground into a fine powder via an electrical grinder. The powder was then dissolved with two consecutive solvents, methanol 70% and distilled water, with a ratio of 1:5 W/V (100 g of powder/500 ml of solvent) and left on a hot plate and magnetic stirrer at 40°C. The extract was filtered with Whatman paper No.1. Extracts were evaporated with vacuum at 40°C to supply a crude extract. This extraction procedure is called the cold process (maceration)<sup>16</sup>. The entire job was done within the Basic Science department laboratory of post-graduate studies at the University of Baghdad/ College of Dentistry. The extracted substance was weighed and stored in sterile glass dishes, labeled and kept in the refrigerator<sup>17</sup>. The stock solution of methanol and aqueous extracts was prepared, centrifuged for 10 minutes, and then filtered by 0.2 millipore filters. The stock was kept at -20°C until used. A serial dilutions concentration was prepared to be applied on the tested microorganisms (100, 50, 25, 12.5, 6.25, and 3.125 mg/ml) using dimethyl sulfoxide (DMSO), and distilled water for methanol and aqueous extracts, respectively.

### *Evaluation of Antimicrobial Activity*

In this experiment, the agar diffusion technique was applied to study the antimicrobial effects of *P. ostreatus* against the isolates spread on the Brain Heart Infusion Agar (BHI-A) medium. Ten microbial isolates were used from each type. They were cultured on the agar plates for 24 h at 37°C. The microbial inoculum was transferred using a sterile cotton swab, and the microbial concentration was equal to  $1.5 \times 10^8$  CFU/ml. Holes of 5 mm were punctured using a sterilized cork porer into BHI-A agar plates. Each well was filled with 50 µl of methanol and aqueous *P. ostreatus* extract with different serial concentrations. Dimethyl sulfoxide (DMSO) and distilled water were negative controls. Then, the plates were incubated at 37°C for 24 h; later, inhibition zones were measured in millimeters<sup>18,19</sup>.

### *Determination of minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)*

To determine the MBC and MFC, a method was conducted using different dilutions of *P. ostreatus* methanol and aqueous crude extracts in concentration according to the results from the previous step of evaluation of antimicrobial activity assay. The values were determined according to<sup>20</sup>. Ten bacterial isolates were used. Pure culture of a specified microorganism grown overnight, then diluted in growth-BHI broth to a concentration around  $1 \times 10^5$  cfu/ml. Five dilutions of the antimicrobial test substance are prepared and mixed with the broth containing the tested microorganisms. A positive and negative control tube was included for every test microorganism. The tube plates are then incubated at 37°C for 24 h. Dilutions were cultured on a BHI-A agar plate. The MBC and MFC represent the lowest

concentrations of antibacterial and antifungal agents, respectively, required to kill a particular microorganism.

### Statistical Analyses

Processing of the data was carried out using SPSS V.25 For statistical analysis and using the Excel Program for Figures. Data included the calculation of mean, standard deviation (SD) and standard error. Analyses of variance (ANOVA) and t-tests were used between different groups. The confidence limit was accepted at 95% (P= 0.05). The Probability (P-value) is as follows: \*P<0.001 High significant, \*\*P<0.05 Significant, and \*\*\*P>0.05 Non-Significant.

### Results

*P. ostreatus* methanol and aqueous crude extracts revealed antibacterial activity against all the tested microorganisms, and growth inhibition zones were formed. Between 100 and 3.125 mg/ml, different concentrations were used in a serial dilution using an agar well diffusion assay tested on the BHI agar plates and incubated with the tested microorganisms. The diameter of the inhibition zone was found to increase as the concentrations of the extracts increased. The results revealed that the methanol crude extract showed more potent growth inhibition than the aqueous crude extract and at lower concentrations. T- Test was used to perform a comparison among different concentrations of each of *P. ostreatus* methanol and aqueous crude extracts, and the differences for all the microorganisms tested can be demonstrated in Table (1) and Table (2) and Figure (1) and Figure (2). In addition, a t-test was used to compare the extracts at the same concentration for the different pathogens. For the methanol extract, the difference was highly significant (P<0.001) at the 12.5 and 25 mg/ml concentrations and significant (P<0.05) at the 50 and 100 mg/ml concentrations. There was no zone of inhibition at the lower concentrations of 6.25 and 3.125 mg/ml between the different pathogens at the same concentration. For the aqueous extract, the difference was highly significant (P<0.001) at the 100 mg/ml concentration, significant (P<0.05) at the 50 mg/ml concentration and non-significant (P >0.05) at the 25 mg/ml concentration, in addition, there was no zone of inhibition at the lower concentrations of 12.5, 6.25 and 3.125 mg/ml between the different pathogens at the same concentration. *P. ostreatus* extracts revealed bactericidal and fungicidal activity against the tested microorganisms. *P. ostreatus* methanol crude extract killed the highest number of microbial isolates of MRSA at a concentration of 25 mg/ml. It killed the highest number of microbial isolates of *P. aeruginosa*, *E. coli*, MS, *Lactobacillus*, and *C. albicans* at a concentration of 30 mg/ml, as shown in table (3). *P. ostreatus* aqueous crude extract killed the highest number of MRSA, MS, and *Lactobacillus* microbial isolates at a concentration of 55 mg/ml. It killed the highest number of microbial isolates of *P. aeruginosa*, *E.coli*, and *C. albicans* at a 60 mg/ml concentration, as shown in Table (4). The MBC range for the methanol crude extract was shown to be between (25-30) mg/ml for *E. coli* and between (20-30) mg/ml for the rest of the tested bacteria. The MFC for candida was also between (20-30) mg/ml. The MBC range for the aqueous crude extract was shown to be between (50-60) mg/ml for all of the tested bacteria, and the MFC for candida was in the same range.

Microorganism	Mean diameter of inhibition zones (mm) ±SD							No. of isolates	P-value
	Control	Concentration mg/ml							
		3.125	6.25	12.5	25	50	100		
MRSA	0.0±0.0	0.0±0.0	0.0±0.0	11.8±1.93	15.9±3.10	21.2±2.25	26.9±2.07	10	0.000*
<i>P. aeruginosa</i>	0.0±0.0	0.0±0.0	0.0±0.0	10.2±1.61	16.4±2.06	22.5±2.36	26.3±2.35	10	0.000*

<i>E. coli</i>	0.0±0.0	0.0±0.0	0.0±0.0	7.3±2.90	13.1±1.85	19.6±2.17	25.9±3.24	10	0.000*
MS	0.0±0.0	0.0±0.0	0.0±0.0	9.9±1.54	14±1.88	19.6±2.22	24.4±3.77	10	0.000*
<i>Lactobacillus</i>	0.0±0.0	0.0±0.0	0.0±0.0	6.8±1.03	11.1±2.33	18.4±2.91	22.5±3.59	10	0.000*
<i>C. albicans</i>	0.0±0.0	0.0±0.0	0.0±0.0	8.8± 1.22	16.7±3.97	19.9±3.44	25.1±3.07	10	0.000*
<i>P-value</i>				0.00*	0.00*	0.017**	0.033**		-

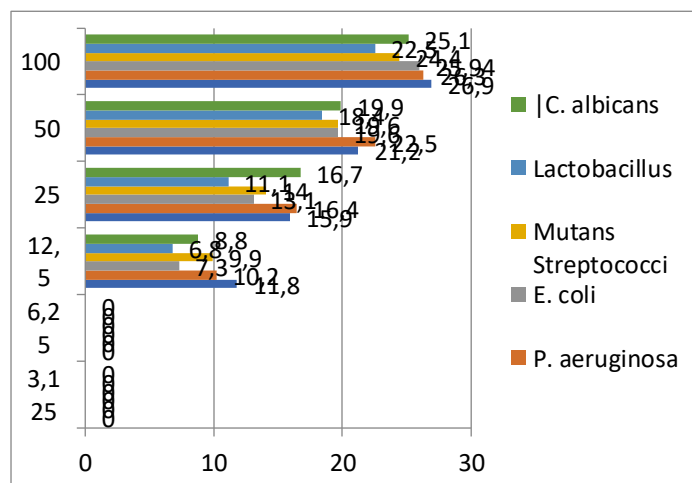
P. ostreatus: Pleurotus ostreatus, SD: Standard deviation, \*P<0.001: High significant, \*\*P<0.05: Significant, \*\*\*P>0.05: Non significant, MRSA: Methicillin-resistant Staphylococcus aureus, P. aeruginosa : Pseudomonas aeruginosa, E. coli: Escherichia coli, MS: Mutans Streptococci, C. albicans: Candida albicans.

**Table 1. Antimicrobial activity of P. ostreatus methanol crude extract on different pathogens.**

Microorganism	Mean diameter of inhibition zones (mm)±SD							No. of isolates	P-value
	Control	Concentration mg/ml							
		3.125	6.25	12.5	25	50	100		
MRSA	0	0.0±0.0	0.0±0.0	0.0±0.0	8.8±3.93	16.8±3.08	21.9±2.42	10	0.000*
<i>P. aeruginosa</i>	0	0.0±0.0	0.0±0.0	0.0±0.0	8.2±4.49	15.4±3.09	24.3±3.36	10	0.000*
<i>E. coli</i>	0	0.0±0.0	0.0±0.0	0.0±0.0	8.1±3.38	14.3±2.45	22.2±4.36	10	0.000*
MS	0	0.0±0.0	0.0±0.0	0.0±0.0	8±3.29	13±3.26	17.5±4.45	10	0.000*
<i>Lactobacillus</i>	0	0.0±0.0	0.0±0.0	0.0±0.0	9±2.10	12.9±3.47	20.3±2.62	10	0.000*
<i>C. albicans</i>	0	0.0±0.0	0.0±0.0	0.0±0.0	8.6±1.86	11.8±2.74	18.7±2.26	10	0.000*
<i>P-value</i>	-	-	-	-	0.979***	0.010**	0.00*	-	-

P. ostreatus: Pleurotus ostreatus, SD: Standard deviation, \*P<0.001: High significant, \*\*P<0.05: Significant, \*\*\*P>0.05: Non significant, MRSA: Methicillin-resistant Staphylococcus aureus, P. aeruginosa : Pseudomonas aeruginosa, E. coli: Escherichia coli, MS: Mutans Streptococci, C. albicans: Candida albicans.

**Table 2. Antimicrobial activity of P. ostreatus aqueous crude extract on different pathogens.**



**Figure 1. Antimicrobial activity of P. ostreatus methanol crude extract on different pathogens.**

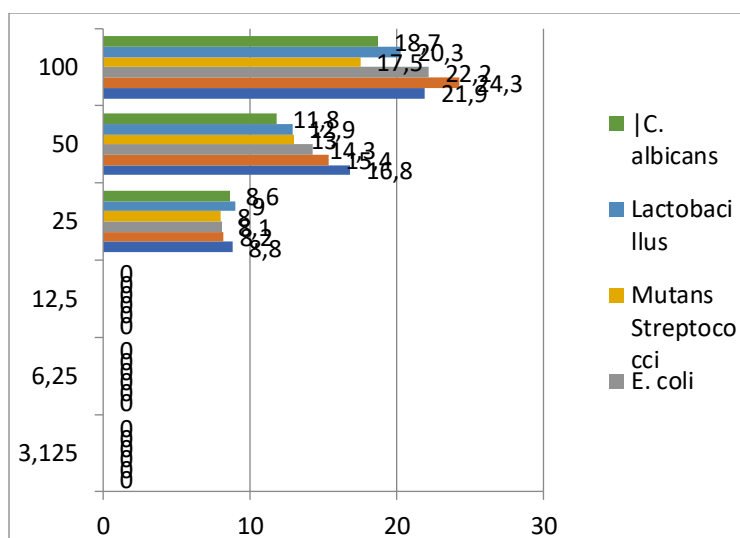


Figure 2. Antimicrobial activity of *P. ostreatus* aqueous crude extract on different pathogens.

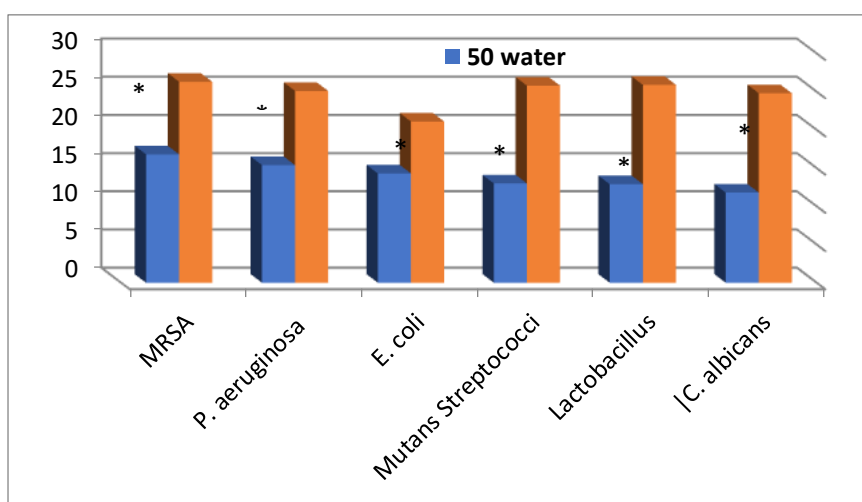


Figure 3. Comparison between the antimicrobial activity of methanol and aqueous crude extracts of *P. ostreatus* at 50 mg/ml concentrations. Red columns refer to the methanol extract, and blue columns refer to the aqueous extract.

Microorganism	No. of isolates killed within the MBC						
	Concentration mg/ml					Control	No. of isolates
	30	25	20	15	10		
MRSA	3	5	2	0	0	0	10
<i>P. aeruginosa</i>	5	3	2	0	0	0	10
<i>E. coli</i>	7	3	0	0	0	0	10
MS	6	2	2	0	0	0	10
<i>Lactobacillus</i>	6	3	1	0	0	0	10
<i>C. albicans</i>	7	1	2	0	0	0	10

*P. ostreatus*: Pleurotus ostreatus, MRSA: Methicillin-resistant Staphylococcus aureus, *P. aeruginosa* : Pseudomonas aeruginosa, *E. coli*: Escherichia coli, MS: Mutans Streptococci, *C. albicans*: Candida albicans.

Table 3. Isolates killed within the MBC for *P. ostreatus* methanol crude extract on different pathogens.

Microorganism	No. of isolates killed within the MBC						
	Concentration mg/ml					Control	No. of isolates
	60	55	50	45	40		
MRSA	3	6	1	0	0	0	10
<i>P. aeruginosa</i>	5	3	2	0	0	0	10
<i>E. coli</i>	5	4	1	0	0	0	10
MS	2	6	2	0	0	0	10
<i>Lactobacillus</i>	4	5	1	0	0	0	10
<i>C. albicans</i>	5	3	2	0	0	0	10

P. ostreatus: Pleurotus ostreatus, MRSA: Methicillin-resistant Staphylococcus aureus, P. aeruginosa : Pseudomonas aeruginosa, E. coli: Escherichia coli, MS: Mutans Streptococci, C. albicans: Candida albicans.

**Table 4.** Isolates killed within the MBC for *P. ostreatus* aqueous crude extract on different pathogens.

## Discussion

A growing medical problem nowadays is antibiotic resistance. The rise in resistance and the ongoing bacterial adaptation to antimicrobials is mainly due to the persistent misuse and overuse of antibiotics by humans and animals. This leads to difficulties in treating severe microbial infections and is directed toward discovering new treatment options to cure the present resistant strains. Natural materials are a rich source for finding many compounds with multiple actions. Pleurotus ostreatus is a non-toxic, edible mushroom that has been proven to be medicinally valuable by many studies. It has been shown to have antioxidant<sup>21</sup>, anti-inflammatory<sup>22</sup>, anti-hypertensive<sup>23</sup>, cholesterol-lowering<sup>24</sup>, cardiovascular diseases prevention, liver protective<sup>25</sup>, anti-fibrotic<sup>26</sup>, anti-diabetic<sup>27</sup>, anticancer<sup>21</sup>, antimicrobial<sup>8</sup>, antiviral (Pan et al) activities. A number of previous studies show that oyster mushroom contains many compounds of nutritional value including essential amino acids, glycopeptides, vitamins such as thiamine, riboflavin, niacin, biotin, and ascorbic acid, various carbohydrates such as pentoses, methyl-pentoses, hexoses, amino sugars, sugar alcohols, sugar acids, polysaccharides, glycogen,  $\beta$ -glucan, saturated and unsaturated fatty acids, macro and microelements such as potassium, phosphorus, sodium, calcium, magnesium, copper, zinc, and iron, crude fibers<sup>28,29</sup>. The extraction process takes the active compounds from cell bodies and mycelium by dissolving active compounds that can then be extracted. The type of solution used can determine the nature of extracted compounds<sup>30</sup>. The results of extractions of Pleurotus using water and methanol show a brown paste yield. The contents of different mushrooms, in both their fruiting body and mycelium, were studied previously by many researchers in different parts of the world and proved to be a rich source of natural antimicrobial promising effects<sup>31,32,33</sup>.  $\beta$ -glucan possesses antibacterial properties, whereas the cell wall glucans are well known for their immunomodulatory properties, and many of the externalized secondary metabolites can combat bacteria, fungi, and viruses. The results of this present study indicate that both the methanol and aqueous crude extracts have antibacterial and antifungal activity against all the tested microorganisms. The methanol crude extract inhibits microbial growth at lower concentrations than the aqueous one. A recent TLC study showed the bioactive content of the methanol extractions of different Pleurotus species, including Pleurotus ostreatus. The TLC results were shown to belong to the terpenoid group of compounds<sup>34</sup>. The study included investigating antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA). The result of that study, together with previous research carried out by<sup>35</sup>, indicates that oyster mushrooms can produce secondary metabolites such as terpenoids as their most rich and varying secondary metabolite compound. The mechanism of antibacterial activity of the terpenoid compound from the TLC results is still unknown. However, according to<sup>36</sup>, Terpenoids have lipophilic

properties and can easily interact with the bacterial wall, interfering with the biosynthesis of its components. Furthermore, these compounds enter the bacterial cell and may interfere with protein synthesis and DNA replication. Another previous study by <sup>37</sup> uses IR, NMR, and mass spectrometric analysis to characterize purified antimicrobial compounds from *P. ostreatus* water extract. Their results identified the presence of 3-(2-aminophenylthio)-3-hydroxypropanoic acid with MIC of (20 µg/mL) against two bacterial strains and (30 µg/mL) against two fungal strains. The study suggested that the presence of the amino phenylthio group has played an essential role in the antimicrobial activity observed. The water extracts from *P. ostreatus* contain antimicrobial compounds that are effective against a broad spectrum of bacteria and fungi used in that study, including *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes*, *S. dysenteriae*, *S. enterica*, *C. albicans*, *C. humicola*, *T. cutaneum*, *A. fumigatus*, *A. flavus*, *A. terreus*, *D. rostrata*, and *C. clavate*. Because of the proven antimicrobial activity, it is worthwhile to exploit and search for the potential of this new compound and its components in treating infectious bacteria and fungi diseases. More studies are needed to identify the mode of action of *P. ostreatus* extracted compounds.

### Conclusion

The way the world deals with antibiotics has to change due to the growing threats of microbial resistance and treatment of some severe infections. There is an urgent need to discover new treatment options to cure multi-drug resistant strains. This study shows that the tested oyster mushroom extracts have promising antimicrobial effects on different pathogens. Methanol crude extract of *P. ostreatus* revealed a more powerful antibacterial and antifungal activity than the aqueous one. More studies should be performed to identify and utilize the components of *P. ostreatus* methanol extract to be used medicinally.

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