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Evaluation of the efficiency of the extract from three species belonging to oyster mushrooms, Pleurotus spp, against some pathogenic bacteria

Zeena Salah Al-jbouri[1*], Ahmed Kareem Abdulrazzaq[2], Majida Hadi Alsaady[1], wasan Luay Tareq[2]

[1] *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*
[2]*Ministry of Agriculture, Plant Protection Directorate*

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Abstract

This study aimed to evaluate the efficacy of secondary metabolites extracted from the Spent Mushroom Substrate (SMS) of three oyster mushroom species (*Pleurotus ostreatus*, *P. florida*, and *P. sapidus*) to inhibit the growth of pathogenic bacteria. The extracts were prepared using methanol 85%, ethanol 85%, and aqueous solvents at two concentrations: 50 µg/mL and 100 µg/mL. The antimicrobial activity of these extracts was tested against two pathogenic bacterial strains, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, using an agar-well diffusion assay to determine the minimal inhibitory concentration (MIC). The results showed varying degrees of antibacterial activity among the SMS extracts. At high concentrations (100 µg/mL), a methanolic extract of *P. ostreatus* exhibited the highest activity, with inhibition zones of 22.5 mm against *S. aureus* and 20.3 mm against *P. aeruginosa*. *P. florida* showed inhibition zones of 21.8 mm against *S. aureus* and 20.7 mm against *P. aeruginosa*. *P. sapidus* displayed moderate antibacterial activity, with inhibition zones of 20.3 mm against *S. aureus* and 20.1 mm against *P. aeruginosa*. Other extracts also showed varying inhibition for both bacterial species. On the other hand, the cold aqueous extract recorded the lowest inhibition zone; *P. ostreatus* recorded an inhibition of 12.2 and 14.7 mm for *S. aureus* and *P. aeruginosa* strains, respectively, while *P. florida* recorded 10.2 and 10.6 mm, respectively, and *P. sapidus* recorded 9.2 and 9.4 mm, respectively. These findings suggest that methanolic extracts of SMS from *P. ostreatus* exhibited the most potent antimicrobial activity, highlighting its potential as a natural antimicrobial agent.

Keywords: Oyster mushroom; Spent mushroom species; Antibacterial

((تقييم كفاءة مستخلص ثلاث انواع من الفطر المحاري *Pleurotus* spp. اتجاه البكتريا *Staphylococcus aureus* المرضية و *Pseudomonas aeruginosa*))

زينـة صـلاح نـوري[1]*, أحمد كريم عبدالرزاق [2], ماجدة هادي مهدي[1] , وسن لؤي طارق[2]

[1]قسم الأحياء، كلية العلوم، جامعة بغداد، العراق

[2]وزارة الزراعة، مديرية وقاية النبات

الخلاصة:

أجريت هذه الدراسة لتقييم فاعلية مواد الايض الثانوية المستخرجة من الوسط الغذائي النافذ (SMS) لثلاثة أنواع من فطر المحاري *Pleurotus ostreatus* و *P. florida* و *P. sapidus* لتثبيط نمو البكتيريا

*Email: zeina.s@sc.uobaghdad.edu.iq

الممرضة, تم تحضير المستخلصات باستخدام الميثانول تركيز 85% والايثانول 85% ومستخلص الماء الحار والبارد واستخدم تركيزين للمستخلصات انفة الذكر 50 و100 مايكروغرام لكل ليتر. ومن ثم تم اختبار النشاط المضاد لسلالتي البكتريا الممرضة *Pseudomonas aeruginosa* و*Staphylococcal aureus* , باستخدام تقانة الانتشار للوسط الغذائي لتحديد الحد الاعلى للتركيز المثبط, وكشفت نتائج الاختبار لمستخلصات الوسط الغذائي النافذ ان التثبيط كان بدرجات متفاوتة بين سلالتي للبكتيريا المستهدفة بالدراسة. عند التركيز العالي (100) أظهر المستخلص الميثانولي للفطر *P. ostreatus* تأثيرا أكبر ضد البكتريا *S. aureus* حيث بلغت نسبة التثبيط 22.5 مم و للسلالة *P. aeruginosa* 20.3 مم على التوالي . وكذلك للفطر *P. florida* كانت 21.8 و 20.7 مم على التتابع اما مستخلص وسط الفطر *P. sapidus* كان 20.3 و 20.1 مم. بينما اظهرت المستخلصات الاخرى تثبيطاً متفاوت لجنسي البكتريا في حين سجل المستخلص المائي البارد ادنى تثبيط بينما سجل الفطر *P. ostreatus* تثبيطا (12.2 و 14.7) مم للبكتريا على التوالي , وقد سجل الفطر *P. florida* (10.2 و 10.6) مم على التوالي, و الفطر *P. sapidus* (9.2 و 9.4) مم على التوالي. تشير هذه النتائج إلى أن المستخلص الميثانولي لوسط *P. ostreatus* أظهر أقوى نشاط مضاد للميكروبات، مما يسلط الضوء على إمكانية استخدامه كعامل مضاد للميكروبات طبيعي.

1-Introduction:

Pleurotus fungi is one of edible mushrooms that belong to Phylum Basidiomycota, Class Basidiomycetes, Subclass Holobasidiomycetidae, Order Agaricales, Family Tricholomatacae Genus *Pleurotus* [1]. Containing more than 40 commercial species include *P. eryngii, P. ostreatus, P. djmor, P. citrinoplieatus, P. tuberregium, P. plumonarius, P. nebrodensis, P. florida, P. sabidus,* and *P. cystidiosus* , etc. [2]. *Pleurotus* species have several biological impacts due to the presence of essential bioactive compounds, i.e., phenol, flavonoids, terpene, terpenoid, polysaccharides, and several other secondary metabolites that act as antimicrobial activities [3]. Therefore, they are characterized by low calories and fat while rich in proteins, vitamins, minerals, and chitin [3,4]. Oyster mushrooms are often ranked third after White Button mushrooms and Shiitake mushrooms for their health benefits beyond the traditional nutrients they contain. They are grown in tropical climates across the globe. Due to the widespread cultivation of Oyster mushrooms, a massive amount of Spent Mushroom Substrate (SMS) has been discarded [5]. Spent Mushroom Substrate mentions the main by-product residual waste after mushrooms have been harvested, After harvesting edible parts of the mushroom [6, 7]. The major components of SMS are lignocellulose materials, such as wood chips, sawdust, wheat, cotton, maize, rye or rice straw, corncobs, straw, sawdust, animal compost, and other organic materials and inorganic substances such as gypsum and limestone [8,9]. In addition to the mushroom mycelium that has broken down a significant portion of that substrate during the growing process, SMS is made by manuring organic substrate that is discarded after complete mushroom production [10]. Approximately 5,00,000 metric tons of SMS is generated annually, as solid waste in India raises worry about their management and disposal [11]. [12] referred in his study that (SMS) received global attention with the advantages including recycling resources, making it eco-friendly, low-cost, and highly efficient.

The SMS contains mushroom mycelia, which have high levels of bioactive compounds, including polysaccharides, polypeptides/proteins, and phenolics [13]. The characterization of bioactive compounds in *P. ostreatus* using a UV-visible spectrophotometer has been studied in detail [14]. UV spectrophotometry can be used to estimate the antibacterial activity of mushrooms by detecting phenolic compounds, which are directly related to antibacterial properties. Phytochemical and spectrophotometric analyses of *Cedrela serrata* methanolic leaf extract showed that the extracts of *C. serrata* contained a variety of phytochemicals, including alkaloids, carbohydrates (monosaccharides and disaccharides), flavonoids, steroids

(phytosterols), saponins, tannins, phlobatannins, terpenoids, cardiac glycosides, and anthraquinones [15]. A phytochemical screening and UV spectroscopic analysis of *Ganoderma lucidum*, identified key bioactive compounds such as triterpenoid content, while *Cordyceps* species were rich in polysaccharides and alkaloids [16]. According to [17], medicinal mushrooms like *Ganoderma lucidum* (reishi), *Cordyceps sinensis*, and *Pleurotus ostreatus* contain bioactive compounds that can be detected and quantified using UV spectrophotometry. While the results of the biochemical analysis of the medicinal mushrooms *P. ostreatus* and *A. bisporus* using a spectrophotometer showed that the extract of *P. ostreatus* had a higher phenolic content and was richer in different amino acids and polysaccharides than that of *A. bisporus*. Additionally, the study demonstrated that, in contrast to *P.ostreatus*, *A. bisporus* has a higher lipid content [18].

These bioactive compounds have been investigated in the antibacterial activity of *Pleurotus ostreatus* extract tested against *S. aureus* and *P. aeruginosa,* and found the petroleum ether, methanol, and acetone extracts showed different degrees of inhibition against pathogenic bacteria, inhibition zone between 10 -19 mm, where the ethanolic extract of *P. ostreatus* affected against pathogenic bacteria with inhibition zones of *S. aureus* was 29.6 mm and *P. aeruginosa* 24.4 mm [19]. It is possible to use the spent substrate of aqueous extracts from spent substrates of *P.ostreatus* as antibacterial activity against *S. epidermidis*, *B. subtilis,* and *E. coli*; the largest zone of inhibition against *S. epidermidis* was 40.00 mm [20].

SMS can possibly be utilized for bioremediation, as it is therapeutically active against pathogens. SMS was obtained from two Oyster mushroom species *P. ostreatus* and *P. djamor* extract, against *E. coli, S. aureus*, and *Xanthomonas*. The MIC was recorded for *P. djamor*, that is, 11.66 mm, 8.80 mm, and 9.04 mm, compared to *P. ostreatus*, which showed its highest MIC as 9.30 mm, 9.18 mm, and 9.28 mm for *E. coli, S. aureus,* and *Xanthomonas*, respectively [21].

2- Materials and Methods:

2-1. Source of Spent Mushroom substrate (SMS).

Spent Mushroom substrate obtained from three species of Oyster mushroom (*Pleurotus ostreatus, P. florida,* and *P.sapidus*) from the Iraqi Ministry of Agricultural/ Plant Protection Directorate/National Centre of Organic Farming. The species were cultivated individually on a substrate (Wheat straw with Corncobs and Wheat brain supplemented with Cowpea).

2-2 Source of pathogenic bacteria

Pathogenic bacterial strains Gram-positive (*Staphylococcus aureus),* and Gram-negative (*Pseudomonas aeruginosa*) were obtained from the laboratories of Al-Kadhimiya Teaching Hospital. The bacteria were activated on nutrient agar (NA) plates and allowed to grow for a duration of 18 to 24 h at a temperature of 37°C.

2-3 Preparation SMS Extracts

2-3-1 Drying and Grinding of SMS.

Spent mushroom species of *P. ostreatus , P. florida* and *P. sapidus* were dried in an oven at 40°C until weight stability. Then SMS is crushed well using grindery to obtain a fine powder that is easy to use in the laboratory for extraction experiments [22].

2-3-2 Alcohol Extract

Fifty grams of fine-dried powder SMS was taken and dissolved in 1000 ml of ethanol 85% and methanol 85% individually. The maceration process was carried out at room temperature, and then the shaker device was used at 150 rpm for 3 days. The mixtures were filtered through

the Whatman No.2 filter paper. Each solvent extract was combined and evaporated by a rotary to 10 ml semiliquid [23]. Extracts were kept in the dark at 4°C until use.

2-3-3 Aqueous Extraction

Fifty grams of fine dried SMS powder was used to prepare the aqueous extract (Hot water and cold water), were soaked in 500ml of sterile distal water, then shaken in a shaker device at 150 rpm for 2 hours and kept in a refrigerator at 4ºC for 48 hours. Then, it was sterilized by filtration using a Whatman disk filter of 0.2 µm caliber. These sterile, cold, and hot extracts were used to screen for antibacterial activities [24, 25].

2-4 Antibacterial Assay

The antibacterial activity of the methanol, ethanol, and aqueous extract of mushrooms was determined by the agar-well diffusion method to determine the minimal inhibitory concentration (MIC) [26], with slight modifications to suit the conditions of this experiment. Briefly, an overnight culture of each microbial isolate was prepared with nutrient broth to turbidity that was equivalent to 0.5 McFarland (1.5×10^8 cfu/ml). In order to determine the antimicrobial efficacy of the fractions. Bacteria were spread over the surface of the solidified agar (Mueller Hinton agar). The methanol extracts were dissolved in distilled water to a final concentration of 10 mg/ml and filter sterilized through a 0.45-µm membrane filter. Small wells (6 mm in diameter) were made in the agar plates using a sterile cork borer. 50–100 microliters of the extract of each mushroom species was loaded into the different wells. All the preloaded plates with the respective extract and test organism were incubated at 37°C, for 24 hours. After the incubation period, inhibition zones were measured from the edge of the zone to the edge of the well using millimeter calipers [27].

2-5 Statistical Analysis

All statistical analyses in this study were carried out using variance analysis (SAS software version 9.4). All data were calculated from at least 3 replicates, and the averages of the coefficients were compared using the least significant difference (LSD) test at the 0.05 probability level.

3- Results

3-1 Test isolates

An antibiotic susceptibility test was performed, and the results showed that *P. aeruginosa* isolates have high resistance against a wide range of antibiotics such as Cefotaxime, Tetracyclin, Imipenem, Meropenem, Amikacin, Piperacillin\Tazobactam, and Tobramycin, so it was considered a multidrug-resistant isolate.

The other isolate was *S. aureus*, which showed resistance to several groups of antibiotics such as Cefoxtin, Penicillin, Oxacillin, Meropenem, Mecillinam, Erythromycin, Cefitriaxone, and Cefotaxime, so it is considered multidrug-resistant [28, 29].

3-2 Antibacterial activity of SMS Pleurotus ostreatus extracts against Pathogenic bacteria.

The results showed a positive effect of SMS *P. ostreatus* extracts on pathogenic bacteria *S. aureus* and *P. aeruginosa* at two concentrations of 50 and 100 µg/mL **(**Table 1**)**. The obtained results indicate that *S. aureus* is significantly sensitive to extracts; the general mean recorded was 18.9 mm. Methanol extract had the highest impact on antibacterial activity against both bacteria. The inhibition zone was 18.7 mm, followed by ethanol extract at 14.4 mm (Figure 1).

The results of interaction between extract and bacteria species showed that methanol extract gave the best inhibition zone against *S. aureus* and *P. aeruginosa* were 22.5 and 20.3 mm, respectively, at high concentration (100 µg/mL), while 16.4 and 15.7 mm, respectively, at low

concentration (50 µg/mL). Ethanolic extract was more sensitive against *P. aeruginosa* at a concentration of 100 µg/mL, which exhibited an inhibition zone of 19.9 mm.

Hot aqueous extract of *P. ostreatus* significantly inhibited the growth of *P. aeruginosa* and *S. aureus* with an inhibition zone of 18.6 and 15.9 mm, respectively, at high concentration (100 µg/mL), and 12.6 and 9.9 mm respectively at low concentration. The cold aqueous extracts had the lowest antibacterial activity against *P. aeruginosa* and *S. aureus,* reaching 14.7 and 12.2 mm, respectively, at high concentration, and 7.6 and 6.6mm, respectively, at low concentration. The hot aqueous of SMS extract showed a higher effect than cold water at both concentrations, especially in bacteria *P. aeruginosa,* and achieved the highest inhibition zone of 18.6 mm.

Table 1: Effect SMS of *P. ostreatus* extract against pathogenic bacteria

extracts	Concentration per well (µg/mL)	Inhibition zone (mm)		Mean	L.S.D at 0.05
		Pseudomonas aeruginosa	*Staphylococcus aureus*		
Methanol	50	15.7	16.4	A 18.7± 2.20	0.452
	100	20.3	22.5		
Ethanol	50	10.9	9.9	B 14.4 ± 1.07	
	100	19.9	16.9		
Hot aqueous	50	9.9	12.6	B 14.3 ± 1.38	
	100	18.6	15.9		
Cold aqueous	50	7.6	6.6	C 10.3 ± 1.12	
	100	14.7	12.2		
mean		A 14.7 ± 1.89	B 18.9 ± 2.19		
Lsd$_{0.05}$	3.19				
Mean of the interaction of extracts vs bacteria species vs concentration (EBC)					
L.S.D at 0.05	2.04				

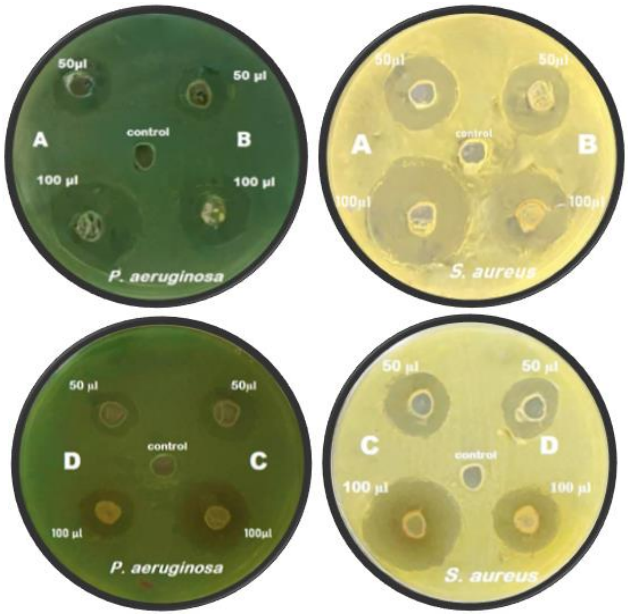


Figure 1: Effect SMS of *P. ostreatus* extracts against pathogenic bacteria.
Extract: **A** (methanol extract), **B** (ethanol extract), **C** (Hot aqueous water extract), and **D** (cold aqueous water extract)

3-3 Antibacterial activity of SMS P. florida extracts against Pathogenic bacteria.

The SMS *P. florida* were investigated to evaluate their antimicrobial activity against pathogenic bacteria, including *S. aureus* and *P. aeruginosa* using agar-well diffusion methods. Evaluation of antimicrobial activity was recorded in Table 2. The obtained results indicate that *S. aureus* is significantly sensitive to extracts. The general mean of inhibition zone was recorded at 12.9 mm. Methanol extract had the highest impact on antibacterial activity against both bacteria; the inhibition zone was 18.9 mm, followed by ethanol extract at 13.0 mm, as shown in Figure 2.

The interaction between extract and bacteria species showed that methanol extract gave the best inhibition zone against *S. aureus* and *P. aeruginosa* at 21.8 and 20.7 mm, respectively, at high concentration (100 µg/mL), and 16.6 and 16.4 mm, respectively at low concentration (50 µg/mL). Ethanolic showed an inhibition zone against *P. aeruginosa* at a concentration of 100 µg/mL, which was 18.9 mm.

Hot aqueous extract of *P.florida* significantly inhibited the growth of *S. aureus* and *P. aeruginosa* with an inhibition zone of 14.6 and 12.0 mm, respectively, at high concentration 100 µg/mL, and 10.6 and 6.5 mm at low concentration. The cold aqueous extracts had the lowest antibacterial activity against *P. aeruginosa* and *S. aureus* reaching 10.6 and 10.2 mm, respectively, at high concentration, and 6.2 and 5.5 mm respectively, at low concentration. The hot aqueous of SMS extract showed a higher effect than cold water at both concentrations, especially in bacteria *S. aureus,* and achieved the highest inhibition zone of 14.6 mm.

Table 2: Effect SMS of *P. florida* extract against human pathogenic bacteria.

extracts	Concentration per well (µg/mL)	Inhibition zone (mm)		Mean	L.S.D at 0.05
		Pseudomonas aeruginosa	***Staphylococcus aureus***		
Methanol	50	16.6	16.4	A 18.9 ± 2.31	0.756
	100	20.7	21.8		
Ethanol	50	8.5	9.2	B 13.0 ± 1.40	
	100	18.9	15.2		
Hot aqueous	50	6.5	10.6	C 10.9 ± 1.02	
	100	12.0	14.6		
Cold aqueous	50	6.2	5.5	D 8.13 ± 1.00	
	100	10.6	10.2		
mean		A 12.5 ± 1.07	A 12.9 ± 1.68		
$Lsd_{0.05}$	1.53				
Mean of interaction of extracts vs Bacteria species vs concentration (EBC)					
L.S.D at 0.05	1.47				

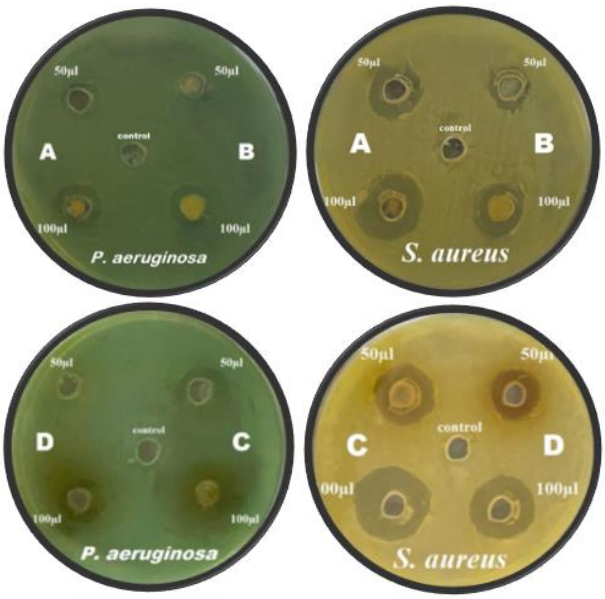


Figure 2: Effect SMS of *P. florida* extracts against pathogenic bacteria.
Extract: **A** (methanol extract), **B** (ethanol extract), **C** (Hot aqueous water extract) and **D** for (cold aqueous water extract)

3-4 Inhibitory effect of P. sapidus extracts against some human pathogenic bacteria.

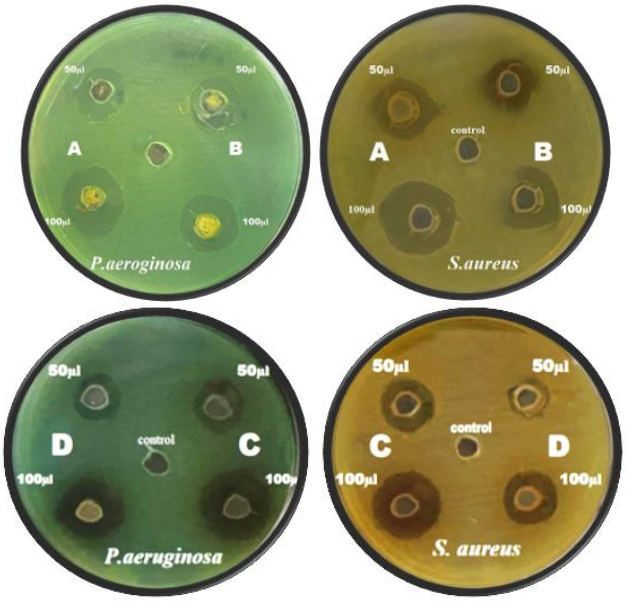
The results of SMS *P. sapidus* showed that extracts have a positive effect on pathogenic bacteria *S. aureus* and *P. aeruginosa* at two concentrations of 50 and 100 µg/mL, as demonstrated in Table 3**.** The highest inhibition zone was obtained from *P. aeruginosa,* which recorded a general mean of 11.59 mm. The highest inhibitory activity was determined against bacteria, which recorded 17.40 mm for methanol extract and 11.98 mm for ethanol extract.

The results of the interaction between extract and bacteria species showed that methanol extract gave the best inhibition zone against *S. aureus* and *P. aeruginosa* were 20.3 and 20.1 mm, respectively, at high concentration (100 µg/mL), and 16.5 and 12.7 mm, respectively at low concentration (50 µg/mL). Ethanolic extract was more sensitive against *P. aeruginosa* at a concentration of 100 µg/mL (18.5 mm).

Hot aqueous extract of *P. sapidus* significantly inhibited the growth of *S. aureus* and *P. aeruginosa* 12.8 and 10.2 mm, respectively, at high concentration (100 µg/mL), and 8.9 and 6.6 mm, respectively, at low concentration. The weakest inhibitory activity was determined against *P. aeruginosa* and *S. aureus* for cold aqueous extract with inhibition zones of 9.4 and 9.2 mm, respectively, at high concentration, and 5.6 and 4.2 mm, respectively, at low concentration. The hot aqueous of SMS extract showed a higher effect than cold water at both concentrations, especially in bacteria *P. aeruginosa,* and achieved the highest inhibition zone of 12.8 mm.

Table 3: Effect SMS of *P. sapidus* extract against human pathogenic bacteria.

extracts	Concentration per well (µl)	Inhibition zone (mm)		Mean	L.S.D at 0.05
		Pseudomonas aeruginosa	*Staphylococcus aureus*		
Methanol	50	12.7	16.5	A 17.40 ± 2.78	0.202
	100	20.1	20.3		
Ethanol	50	9.6	7.2	B 11.98 ± 1.69	
	100	18.5	12.6		
Hot aqueous	50	6.6	8.9	C 9.63 ± 1.78	
	100	10.2	12.8		
Cold aqueous	50	5.6	4.2	D 7.10 ± 1.56	
	100	9.4	9.2		
mean		A 11.59 ± 1.93	A 11.46 ± 1.42		
Lsd$_{0.05}$	1.143				
Mean of the interaction of extracts vs bacteria species vs concentration (EBC)					
L.S.D at 0.05	0.862				



Figures 3: Effect SMS of *P. sapidus* extracts against pathogenic bacteria.
Extract: **A** (methanol extract), **B** (ethanol extract), **C** (Hot aqueous water extract), **D** (cold aqueous water extract)

Discussion

SMS extracts have a strong inhibiting effect linked with phenolic compounds and other beneficial or therapeutic health effects in addition to the prevention of some skin-borne diseases. The SMS extracts could be used as a rich source of antibacterial in pharmaceutical-type products [30]. The methanolic extracts were characterized by the highest bacterial inhibition zones because CH_3OH is a highly polar solvent with a hydroxyl group (-OH) that makes it polar [31]. It may dissolve a variety of organic and inorganic compounds, such as sugars and organic acids in the cell wall of bacteria, and there are active materials in SMS, such as phenolic compounds found in the mycelium of mushrooms [32]. Ethanol is less polar

because it has only one hydroxyl group with an additional methyl group. Cold and hot water was used for aqueous extracts because it was looking for an extract that contained a wide range of compounds and biological activity. Hot water may be the best option because it helps extract a wider range of compounds, including proteins, polysaccharides, and other compounds that require higher temperatures to dissolve. Hot extracts often contain more biologically active compounds, such as polysaccharides (chitin), which may have stronger health or medical benefits [33]. While cold water extracts may sometimes be less effective in terms of biological or medical activities due to the lack of diversity in the compounds being extracted, such as sugars, some acids, some compounds with weak biological activity, and some sensitive compounds, they can be preserved and require a slower process [34]. The difference in the inhibitory effect of (hot and cold) aqueous extract may be attributed to the production of secondary metabolites from the shikimic acid and cinnamic acid pathways during lignocellulosic degradation by *P. ostreatus*, which may have antibacterial activity [35]. In conclusion, *P. ostreatus* contains many different bioactive compounds with diverse biological activities.

Conclusion and impact of the study:

Considering the inhibition zone, antibacterial examination revealed that the methanol extracts originating from *P. ostreatus* possess inherent capacity as antibacterial agents targeting specific pathogenic bacteria. As a result, these findings justify the need for further investigation into their potential applications in medicine.

REFERENCES

[1] K. Hosaka, K.-O. Nam, and T. Kasuya, "DNA barcoding of *Pleurotus cyatheae*, an 'extinct' mushroom species from Japan," *Bulletin of the National Museum of Nature and Science, Series B, Botany*, vol. 50, no. 2, pp. 41-49, 2024.

[2] R. Pandey, V. S. Pandey, and V. N. Pandey, "Nutraceutical metabolites, value addition and industrial products for developing entrepreneurship through edible fleshy fungi," in *Entrepreneurship with Microorganisms*, Academic Press, 2024, pp. 293-328.

[3] P. Devi, J. Islam, P. Narzary, D. Sharma, and F. Sultana, "Bioactive compounds, nutraceutical values and its application in food product development of oyster mushroom," *Journal of Future Foods*, pp. 335-342, 2024. [Online]. Available: http://creativecommons.org/licenses/by-nc-nd/4.0/.

[4] M. E. Effiong, C. P. Umeokwochi, I. S. Afolabi, and S. N. Chinedu, "Assessing the nutritional quality of *Pleurotus ostreatus* (oyster mushroom)," *Frontiers in Nutrition*, vol. 10, p. 1279208, 2024.

[5] A. Bhatia, Dr. N. Neeraj, R. Jarial, K. Jarial, and P. Bhatia, "Oyster Mushrooms: Versatile Mushrooms with Potential Health Benefits," *Zenodo*, vol. 3, pp. 2128-2133, 2023. doi: 10.5281/zenodo.8290368.

[6] M. Dedousi, E. M. Melanouri, D. Karayannis, E. I. Kaminarides, and P. Diamantopoulou, "Utilization of spent substrates and waste products of mushroom cultivation to produce new crops of *Pleurotus ostreatus*, *Pleurotus eryngii*, and *Agaricus bisporus*," *Carbon Resources Conversion*, vol. 7, no. 2, p. 100196, 2024.

[7] R. W. Mwangi, M. Mustafa, N. Kappel, L. Csambalik, and A. Szabó, "Practical applications of spent mushroom compost in cultivation and disease control of selected vegetable species," *Journal of Material Cycles and Waste Management*, pp. 1-16, 2024.

[8] B. Shingha Barua, A. Nigaki, and R. Kataoka, "A new recycling method through mushroom cultivation using food waste: Optimization of mushroom bed medium using food waste and agricultural use of spent mushroom substrates," *Recycling*, vol. 9, no. 4, p. 58, 2024.

[9] C. Martin, G. I. Zervakis, S. Xiong, G. Koutrotsios, and K. O. Strætkvern, "Spent substrate from mushroom cultivation: exploitation potential toward various applications and value-added products," *Bioengineered*, vol. 14, no. 1, pp. 2252138, Dec. 2023. doi: 10.1080/21655979.2023.2252138. PMID: 37670430; PMCID: PMC10484051.

[10] S. M. Stewart-Wade, "Efficacy of organic amendments used in containerized plant production: Part 1 – Compost-based amendments," *Scientia Horticulturae*, vol. 266, Art. no. 108856, 2020.
[11] M. H. Shanta, I. A. Choudhury, and S. Salman, "Municipal solid waste management: Identification and analysis of technology selection criteria using Fuzzy Delphi and Fuzzy DEMATEL technique," *Heliyon*, vol. 10, no. 1, 2024.
[12] S. K. Sahithya, T. Mouli, A. Biswas, and M. S. T. Mercy Scorlet, "Remediation potential of mushrooms and their spent substrate against environmental contaminants: An overview," *Biocatalysis and Agricultural Biotechnology*, vol. 42, Art. no. 102323, 2022.
[13] M. Fogarasi, S. A. Nemeș, A. Fărcaș, C. Socaciu, C. A. Semeniuc, M. I. Socaciu, and S. Socaci, "Bioactive secondary metabolites in mushrooms: A focus on polyphenols, their health benefits and applications," *Food Bioscience*, vol. 62, p. 105166, 2024, doi: 10.1016/j.fbio.2024.105166.
[14] L. D. Johnson, "Characterization of bioactive compounds in *Pleurotus ostreatus* using UV-Visible Spectrophotometry," *Mycological Research*, vol. 32, pp. 140-148, 2018.
[15] F. Perveen and S. Zaib, "Phytochemical and spectrophotometric analyses of the Hill Toon, *Cedrela serrata* Royle methanolic leaves extract and its fractions," *International Journal of Agricultural Innovations and Research*, vol. 1, no. 5, pp. xx-xx, 2013. [Online]. Available: https://www.ijaer.org.
[16] C. Pascale, R. Sirbu, F. Busuricu, and E. Cadar, "Spectral analysis and phytochemical screening of *Ganoderma lucidum* mushroom extracts," *European Journal of Natural Sciences and Medicine*, vol. 7, no. 1, pp. 44-53, 2024.
[17] A. C. Soria and C. Sánchez, "Medicinal Mushrooms: The Role of Bioactive Compounds in Human Health," *Journal of Natural Products*, vol. 81, no. 5, pp. 1257-1269, 2018.
[18] S. Dilfy, M. Hanawi, A. Al-Bideri, and A. Al-Attabi, "Determination of chemical composition of cultivated mushrooms in Iraq with spectrophotometrically and high performance liquid chromatographic," *Journal of Green Engineering*, vol. 10, pp. 6200–6216, 2020.
[19] H. Ahmed, M. Youssef, and T. Magraby, "Antimicrobial and antioxidant activities of *Pleurotus ostreatus* as edible oyster mushroom in Egypt," *Journal of Environmental Studies*, vol. 28, pp. 24–34, 2022. [Online]. Available: https://doi.org/10.21608/jesj.2022.164389.1031.
[20] M. A. Martinez, D. O. Ramírez, S. S. Simental, N. R. Perez, M. M. Mayo, and Z. Zepeda-Bastida, "Antibacterial activity of spent substrate of mushroom *Pleurotus ostreatus* enriched with herbs," *Journal of Agricultural Science*, vol. 7, no. 11, Art. no. 225, 2015.
[21] M. Afsar, A. Zia, M. B. U. Salam, M. N. Ahmad, A. A. Khan, T. ul Haq, T. Aziz, and A. F. Alasmari, "A multifaceted analysis of spent mushroom substrate of selected oyster mushrooms for enzymatic activity, proximate composition, and antimicrobial activity," *Italian Journal of Food Science*, vol. 36, no. 1, pp. 165–174, 2024.
[22] T. Elsakhawy and W. Tawfik, "Evaluation of spent mushroom substrate extract as biofertilizer for growth improvement of rice (*Oryza sativa*)," *Egyptian Journal of Soil Science*, vol. 10, no. 21608/ejss.2019.18835.1320, 2019.
[23] P. Saini, *Studies on cultivation of Pleurotus fossulatus (Cooke) Sacc. on locally available substrates*, Ph.D. dissertation, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, 2024.
[24] X. Zhang, Y. Bai, Y. Wang, C. Wang, J. Fu, L. Gao, ..., and Y. Deng, "Anticancer properties of different solvent extracts of *Cucumis melo* L. seeds and whole fruit and their metabolite profiling using HPLC and GC-MS," *BioMed Research International*, vol. 2020, no. 1, Art. no. 5282949, 2020.
[25] V. Mishra, S. Tomar, P. Yadav, S. Vishwakarma, and M. P. Singh, "Elemental analysis, phytochemical screening and evaluation of antioxidant, antibacterial and anticancer activity of *Pleurotus ostreatus* through in vitro and in silico approaches," *Metabolites*, vol. 12, no. 9, Art. no. 821, 2022. [Online]. Available: https://doi.org/10.3390/metabo12090821.
[26] G. Gashaw, A. Fassil, and F. Redi, "Evaluation of the antibacterial activity of *Pleurotus* spp. cultivated on different agricultural wastes in Chiro, Ethiopia," *International Journal of Microbiology*, vol. 2020, no. 1, Art. no. 9312489, 2020.
[27] D. Ma, Z. Zhou, G. Wang, C. Xu, L. Cao, F. Dong, ... and Y. Li, "Efficient control of Fusarium head blight and reduction of deoxynivalenol accumulation by a novel nanopartner-based strategy," *Environmental Science & Technology*, 2024.

[28] CLSI, *M100: Performance Standards for Antimicrobial Susceptibility Testing*, 33rd ed., Clinical and Laboratory Standards Institute, 2023.

[29] A. M. Al-Dahbi and H. J. Al-Mathkhury, "Distribution of Methicillin Resistant *Staphylococcus aureus* in Iraqi Patients and Healthcare Workers," *Iraqi Journal of Science*, vol. 54, no. 2, pp. 293-300, 2013.

[30] S. Rubab, S. Bahadur, U. Hanif, A. I. Durrani, A. Sadiqa, S. Shafique, U. Zafar, M. Shuaib, Z. Urooj, M. M. Nizamani, and S. Iqbal, "Phytochemical and antimicrobial investigation of methanolic extract/fraction of *Ocimum basilicum* L.," *Biocatalysis and Agricultural Biotechnology*, vol. 31, p. 101894, 2021.

[31] S. Iram, K. S. Ahmad, I. Shaheen, A. M. Aljuwayid, and G. A. Ashraf, "Synthesis and analysis of *Euphorbia cognata* Boiss-assisted organic–inorganic complex: Photosynthesis and stabilization of CoO nanoparticles," *Applied Biochemistry and Biotechnology*, pp. 1-15, 2024.

[32] N. Saetang, T. Rattanapot, N. Manmai, D. Amornlerdpison, R. Ramaraj, and Y. Unpaprom, "Effect of hot water extraction process on schizophyllan from split gill mushroom," *Biomass Conversion and Biorefinery*, vol. 14, no. 1, pp. 1017-1026, 2024.

[33] M. J. Ng, N. Y. Goh, C. S. Tan, M. F. M. Razif, H. Y. Y. Yap, B. H. Kong, and S. Y. Fung, "Comparative cytotoxicity evaluation of heat-assisted vs cold water extractions of six medicinal fungi against breast and lung cancer cells," *Food Technology and Biotechnology*, vol. 62, no. 2, pp. 254-263, 2024.

[34] S. A. Norrel and K. E. Messley, *Microbiology Laboratory Manual Principles and Applications*, Upper Saddle River, NJ, USA: Prentice Hall, 1997.

[35] A. J. Mattoo and S. Nonzom, "Endophytes in lignin valorization: A novel approach," *Frontiers in Bioengineering and Biotechnology*, vol. 10, p. 895414, Jul. 19, 2022, doi: 10.3389/fbioe.2022.895414.