JSR Publications

Available Online

J. Sci. Res. **17** (1), 305-315 (2025)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Evaluation of the Antimicrobial Potential of *Pleurotus ostreatus* Extracts

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Received 25 May 2024, accepted in final revised form 22 September 2024

Abstract

Antimicrobial activity of *Pleurotus ostreatus* was studied *in vitro* using its aqueous and methanolic extracts, against nine human pathogenic bacteria (MTCC cultures) such as *Bacillus subtilis*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae* - Gram positive bacteria; *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella enterica* ser. *typhi* and *Shigella flexneri* - Gram negative bacteria. Antifungal potential was screened against phytopathogenic fungi like *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp. and *Helminthosporium* sp. The *P. ostreatus* aqueous extract failed to show growth inhibitory effect against any of the human pathogenic bacteria or phytopathogenic fungi studied. The *P. ostreatus* methanolic extract exhibited significant inhibition of all the bacteria tested at higher concentrations of 2000, 3000, 4000 and 5000 ppm. But, even *P. ostreatus* methanolic extract could not inhibit the growth of phytopathogenic fungi studied, at any of the concentrations screened. *P. ostreatus* methanolic extract was found to have phytochemicals such as alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. The results of present studies reveal the antibacterial potential of *Pleurotus ostreatus*, which could be further evaluated to obtain potent antibacterial bioactive principles for new drug development.

Keywords: *Pleurotus ostreatus*; Aqueous extract; Methanolic extract; Antimicrobial activity.

doi: https://dx.doi.org/10.3329/jsr.v17i1.74102 J. Sci. Res. **17** (1), 305-315 (2025)

1. Introduction

Mushrooms are macro fungi that have distinct fleshy and edible fruiting bodies [1]. Mushrooms are extensively consumed as nutritious and delicious foods [2], while some mushrooms are also used as traditional medicine [3]. Mushrooms serve as a functional food that can offer both nutrients as well as health benefits.

The genus *Pleurotus* belongs to the class Agaricomycetes, the order Agaricales and the family Pleurotaceae (Fig. 1). *Pleurotus fungi* are found in both tropical and temperate forests, and they don't require complex controlled environmental conditions for growth. They naturally occur on decomposing logs or sometimes on dried trunks [4].

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Pleurotus ostreatus
Class : Agaricomycetes
Order : Agaricales
Family : Pleurotaceae
Genus : *Pleurotus*
Species : *P. ostreatus*
Common name : Oyster mushroom
Part used : Fruiting bodies

Fig. 1. *Pleurotus ostreatus*

There are about 40 species, including *Pleurotus ostreatus*, under the genus *Pleurotus*, which are commonly called 'oyster mushrooms'. Commercially, the oyster mushroom stands third in the worldwide mushroom production [5]. *P. ostreatus* has been reported to have multiple biological activities. It is reported to reduce cholesterol levels as it contains lovastatin, a form of cholesterol lowering statin [6]. The fungal carpophore is a good source of lignin and phenol degrading enzymes [7].

It has been used in the mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons [8,9]. The fruiting bodies and also the mycelia of *Pleurotus ostreatus* have numerous therapeutic properties such as anti-inflammatory, immune-stimulating and immunomodulating, anticancer activity, ribonuclease activity, etc. [10].

In most of the developing countries, majority of health problems are due to infectious diseases. Many synthetic antimicrobial drugs are available commercially, but their indiscriminate use has resulted in the development of multi-drug resistant microbial strains. The spread of these drug resistant pathogens has made it very difficult to successfully treat many of the diseases caused by microorganisms [11,12]. This has led to an increase in the interest for traditional medicines that use natural substances to control as well as treat a variety of infections and diseases, creating a demand for medicines from natural sources, particularly medicinal plants. Recently, fungi have attracted the interest of researchers as a source of novel antimicrobial drugs [13], especially mushrooms [3,5]. Many mushrooms are a potential source for numerous biologically active substances with therapeutic value [14,15].

Many researchers have reported various biological activities of *Pleurotus ostreatus* [10,16,17], but the work done regarding the antimicrobial activity is relatively less. Two of the studies reporting the antimicrobial activity of *P. ostreatus*, have used lab-cultured *P. ostreatus* for extraction and only three bacterial cultures examined in the present study have been used, and the strains used were also different. Screening was carried out using the disc diffusion method. Evaluation against phytopathogenic fungi and also phytochemical screening of the extract were not reported in these studies [18,19]. Phytochemical screening

of *P. ostreatus* has been reported [20,21], but the phytochemical components of a particular species can differ based on the geographic area [22]. In the present study, the antimicrobial activity of extracts from naturally grown *P. ostreatus* has been evaluated against both human pathogenic bacteria (MTCC strains) and phytopathogenic fungi by the agar well diffusion method, in addition to the photochemical screening of one of the extracts.

2. Experimental

2.1. *Materials*

All the chemicals used in this study are of analytical-grade and used directly without any additional purification. Nutrient Agar (NA) was acquired from Himedia, India and methanol was acquired from Merck, India and throughout the investigations distilled water was utilized wherever required. All the reactions were conducted in an ambient condition as mentioned in standard procedures.

2.2. *Collection and storage of* Pleurotus ostreatus *sample*

The *Pleurotus ostreatus* grown on fallen tree trunk were collected from Hallibylu estate, Halekote village, Mudigere taluk, Chikkamagaluru, Karnataka. The precise identification of the mushroom as *Pleurotus ostreatus* was done by the help of S. Lokesh, DOS in Biotechnology, University of Mysore, Mysuru. The mushrooms were dried in sunlight, powdered and packed in an air tight container and then stored at ambient condition for further studies (Figs. 2. and 3).

Fig. 2. Mushroom dried under sunlight.

Fig. 3. Pulverized mushroom.

2.3. *Preparation of* P. ostreatus *aqueous extract*

10 g of powdered mushroom was added to 100 mL of sterile water and boiled for about 10 - 15 min. The mixture was cooled to room temperature (RT). Then the mixture was filtered through double layer muslin cloth and the residue was discarded. The filtrate obtained was

again filtered using Whattman No. 1 filter paper. The extract was transferred into a clean conical flask and stored at -20 °C until further use.

For experiment, 25 %, 50 %, 75 % and 100 % concentrations of aqueous extract were prepared in different eppendorf tubes by dissolving the appropriate volumes of extract with sterile distilled water and the tubes were labeled accordingly.

2.4. *Preparation of* P. ostreatus *methanolic extract*

The extraction was done by swirling about 5.0 g of mushroom powder with 100 mL of methanol in a separatory funnel at room temperature (RT) for about 2-3 h, the solution was decanted and filtered using Whattman No. 1 filter paper. The solid fraction was re-extracted twice with fresh 50 mL of methanol respectively. All the three extract solutions were mixed and then evaporated to dryness at 37 °C (in incubator). The solid residue obtained was weighed and found to be 160 mg, which was re-dissolved in methanol to have a solution of 160 mg/mL concentration and stored at -20 °C until further use.

From this methanolic extract stock solution (160 mg/mL i.e., 1,60,000 ppm) the working standard solution of 20,000 ppm was prepared. Initially, from the working standard solution, four concentrations (250 ppm, 500 ppm, 750 ppm and 1000 ppm) were prepared. Later, four higher concentrations were prepared (2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm) from the working standard solution. The dilutions were made using sterile distilled water.

2.5. *Growth and maintenance of test microorganisms for antimicrobial studies*

All the test bacteria (Table 1) were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. About 10 test tubes containing peptone broth were sterilized and each tube was labeled properly with the organism name to be inoculated. Then a loopful of test cultures (MTCC bacteria) was inoculated into the appropriately labeled test tube under aseptic conditions. The inoculated test tubes were incubated at 37 °C overnight.

Table 1. Bacterial strains used in the study.

Bacteria	Accession No.
Gram Positive Bacteria	
Bacillus subtilis	MTCC 441
Listeria monocytogenes	MTCC 839
Staphylococcus aureus	MTCC 3160
Streptococcus pneumoniae	MTCC 2672
Gram Negative Bacteria	
Enterobacter aerogenes	MTCC 7661
Escherichia coli	MTCC 1560
Klebsiella pneumoniae	MTCC 7407
Proteus vulgaris	MTCC 7299
Salmonella enterica ser. typhi	MTCC 8767
Shigella flexneri	MTCC 9543

All the test fungi such as *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., and *Helminthosporium* sp., were obtained from the pure cultures maintained at the Laboratory, Postgraduate Department of Microbiology, Maharani's Science College for Women, Mysuru, India. About four test tubes containing physiological saline were sterilized and tubes were properly labeled with the names of four different fungi. Then about 3-4 fungal discs were taken from the maintained pure cultures of fungi and were transferred into the tubes. These test tubes were shaken well to get spore suspensions.

2.6. *Antibacterial and Antifungal activity assay*

The agar well diffusion method was used to screen the antibacterial activity using Nutrient Agar (NA, Himedia) medium and using Potato Dextrose Agar (PDA, Himedia) medium for antifungal activity. The Petri plates were labelled properly with the name of the organism to be inoculated. 20 mL of sterilized NA and PDA media was poured into respective 90 mm sterile Petri plates. After cooling, NA plates were inoculated with appropriate overnight culture of the bacteria with the help of sterile cotton swabs and allowed to set for a while. The PDA plates were inoculated with 1.0 ml spore suspension of the fungus with the help of sterile spreader and allowed to set for a while. Then about 5 wells (one for control in center and other four at four sides for 4 different concentrations) were bored using standard sterile cork borer on the plates. 100 µL of different concentrations of extract were added to respective wells and 100 µL of sterile distilled water was transferred to the central well as control. Then the NA plates were incubated at 37 °C for about 24-48 h, whereas the PDA plates were kept undisturbed at RT for about 4 to 5 days for incubation. After incubation, plates were examined for zone of inhibition and the inhibition zones formed around the wells were measured and recorded. Triplicates were maintained for each bacterium and fungus.

2.7. *Phytochemical screening of* P. ostreatus *methanolic extract*

The required amount of methanolic extract was obtained following the procedure as mentioned earlier and subjected to phytochemical tests following standard procedures [23,24] to detect phytochemicals like alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids and anthroquinone.

3. Results and Discussion

3.1. *Antibacterial activity of* P. ostreatus *aqueous extract*

The *Pleurotus ostreatus* aqueous extract, in any concentration tested, did not show any antibacterial activity against different human pathogenic bacteria studied. None of the bacteria exhibited zone of inhibition for treatment with *P. ostreatus* aqueous extract.

3.2. *Antibacterial activity of* P. ostreatus *methanolic extract*

Antibacterial activity was also not observed with the 250, 500, 750 and 1000 ppm concentrations of *P. ostreatus* methanolic extracts (no inhibition zone observed) against any of the ten bacteria studied, similar to aqueous extract. However, the higher concentrations of methanolic extract of *P. ostreatus viz.,* 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm, tested showed significant but varied antibacterial activity against all the human pathogenic bacteria studied. The antibacterial activity of methanolic extracts of the mushroom at higher concentrations is mentioned in Table 2. At 2000, 3000, 4000 and 5000 ppm concentrations all the bacteria tested were inhibited at varying levels by the methanolic extracts of the mushroom, with few bacteria being completely inhibited (Fig. 4).

At higher concentrations of 2000, 3000, 4000 and 5000 ppm the *P. ostreatus* methanolic extract showed very good antibacterial activity against both Gram positive as well as Gram negative bacteria, thereby exhibiting a broad spectrum of activity. Comparatively, *Proteus vulgaris* was highly susceptible followed by *Listeria monocytogenes*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*, while *Bacillus subtilis* was least susceptible or more resistant followed by *Streptococcus pneumoniae* (Table 2; Fig. 4).

Table 2. Antibacterial activity of methanolic extract of *P. ostreatus.*

Bacteria	Zone of inhibition (in mm)				
	Control	2000 ppm	3000 ppm	4000 ppm	5000 ppm
Gram Positive Bacteria					
Bacillus subtilis (MTCC 441)	0	16.33 ± 0.49	17.5 ± 0.76	18.67 ± 0.67	19.33 ± 0.49
Listeria monocytogenes (MTCC 839)	0	38.5 ± 1.12	37.83 ± 0.75	40.83 ± 1.11	41.5 ± 0.89
Staphylococcus aureus (MTCC3160)	0	31.0 ± 0.82	32.17 ± 0.75	33.0 ± 0.89	34.33 ± 0.67
Streptococcus pneumoniae (MTCC 2672)	0	23.5 ± 0.89	24.33 ± 0.71	25.0 ± 0.73	25.0 ± 0.77
Gram Negative Bacteria					
Enterobacter aerogenes (MTCC 7661)	0	33.17 ± 0.83	36.33 ± 0.56	37.5 ± 0.76	39.33 ± 0.99
Escherichia coli (MTCC 1560)	0	17.17 ± 0.75	19.0 ± 0.77	21.83 ± 0.91	22.83 ± 0.48
Klebsiella pneumoniae (MTCC 7407)	0	35.5 ± 0.92	39.0 ± 0.63	40.5 ± 0.62	41.0 ± 1.13
Proteus vulgaris (MTCC 7299)	0	43.0 ± 0.52	44.83 ± 0.70	45.83 ± 0.60	46.0 ± 0.68
Salmonella enterica ser. typhi (MTCC 8767)	0	31.0 ± 0.77	32.0 ± 0.86	32.83 ± 0.70	34.5 ± 0.62
Shigella flexneri (MTCC 9543)	0	30.67 ± 0.71	31.83 ± 0.75	32.33 ± 0.49	34.5 ± 0.43

Values are means of two experiments, each with three replicates ± SE

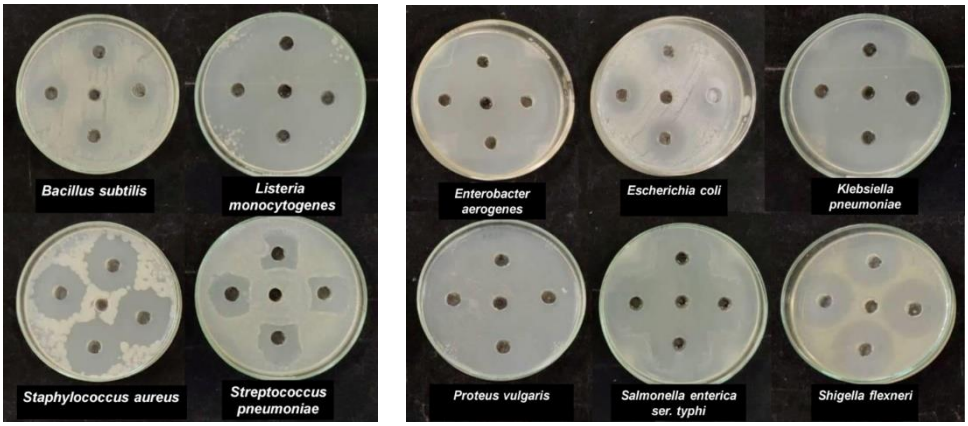

Fig. 4. Antibacterial activity of methanolic extract of *Pleurotus ostreatus* against MTCC Gram positive bacterial strains (A) and Gram negative bacterial strains (B) (showing zones of inhibition against different concentrations of methanolic extract; 2000, 3000, 4000 and 5000 ppm concentrations in clockwise direction from top well; central well - control).

3.3. *Antifungal activity of aqueous and methanolic extracts of* P. ostreatus

Both aqueous and methanolic extracts of *P. ostreatus* showed no inhibitory effect against different phytopathogenic fungi studied (*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., and *Helminthosporium* sp.), in any of the concentrations screened. The fungi tested did not exhibit zone of inhibition for treatment with either aqueous or methanolic extracts of the mushroom (Fig. 5).

3.4. *Phytochemical screening of* P. ostreatus *methanolic extract*

Results of different tests for screening phytochemicals in *P. ostreatus* methanolic extract showed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids in the extract (Table 3).

Presently, search for new antimicrobial agents from diverse sources of nature has become inevitable owing to the uncontrollable emergence and spread of multi drug resistant pathogenic microbes. The diseases caused by such pathogens are very difficult to cure [25]. Down the ages, extracts of many mushrooms have been identified to be good sources of natural drugs. They have been examined for their use as potential alternative therapeutic agents to treat many infections and diseases [26]. Medicines obtained from fungi can be used in Unani and Ayurvedic systems to treat many diseases or can be a source for the production of novel drugs [26].

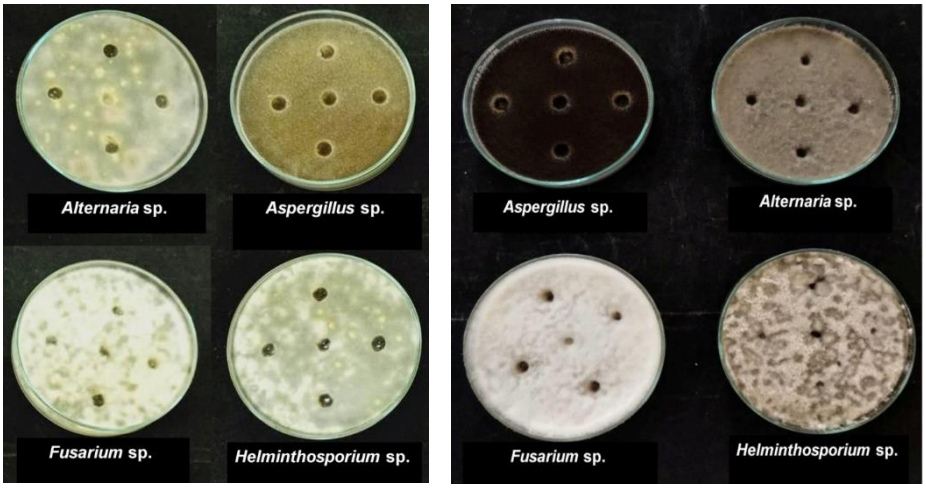

Fig. 5. Antifungal activity of aqueous extract of *Pleurotus ostreatus.* A: Fungal plates showing no zones of inhibition against different concentrations of aqueous extract (25, 50, 75 and 100 % concentrations in clockwise direction from top well; central well – control); B: Fungal plates showing no zones of inhibition against different concentrations of methanolic extract (2000, 3000, 4000 and 5000 ppm concentrations in clockwise direction from top well; central well – control).

Table 3. Phytochemicals of *P. ostreatus* methanolic extract.

Phytochemicals	+ Present /- Absent
Alkaloids	+
Flavonoids	+
Phenol	+
Saponins	+
Steroids	+
Tanins	+
Terpenoids	+
Anthroquinone	-

In the current investigation, *Pleurotus ostreatus* was evaluated for its antimicrobial potential using its aqueous and methanolic extracts *in vitro.* Screening was done by agar well diffusion method against a few human pathogenic bacteria such as *Bacillus subtilis* (MTCC 441), *Listeria monocytogenes* (MTCC 839), *Staphylococcus aureus* (MTCC 3160), *Streptococcus pneumoniae* (MTCC 2672) - Gram positive bacteria; *Escherichia coli* (MTCC 1560), *Enterobacter aerogenes* (MTCC 7661), *Klebsiella pneumoniae* (MTCC 7407), *Proteus vulgaris* (MTCC 7299), *Salmonella enterica* ser. typhi (MTCC 8767) and *Shigella flexneri* (MTCC 9543) - Gram negative bacteria; and phytopathogenic fungi (*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., and *Helminthosporium* sp.).

The *P. ostreatus* aqueous extract did not inhibit the growth of any human pathogenic bacteria or phytopathogenic fungi examined. None of the bacteria or fungi tested exhibited inhibition zone on treatment with *P. ostreatus* aqueous extract at 25 %, 50 %, 75 % and 100

% concentrations. The *P. ostreatus* methanol extract also failed to inhibit the growth of any of the bacteria tested at lower concentrations (250, 500, 750 and 1000 ppm), but was very effective showing significant inhibition of all the bacteria tested at 2000, 3000, 4000 and 5000 ppm concentrations. Maximum inhibition was observed against *Proteus vulgaris, Listeria monocytogenes, Klebsiella pneumoniae* and *Enterobacter aerogenes*. However, methanolic extract also failed, at any of the concentrations screened, to inhibit the growth of any of the plant pathogenic fungi examined.

Although in our studies water extract was ineffective against both bacteria and fungi, water extract of *P. ostreatus* was reported to significantly inhibit the growth of a few fungi like *Candida albicans, Cryptococcus humicola*, and *Trichosporon cutaneum*, and also bacteria such as *Staphylococcus aureus* and *Escherichia coli* [27]. The reason for this may be the heat labile nature of bioactive principles in *P. ostreatus* that were inactivated by heat treatment in our study. The methanolic extracts exhibited a broad spectrum of antibacterial activity being effective on both Gram positive as well as Gram negative bacteria, which is of notable significance. Similar results have been reported by many workers. Both the ethanol and methanol extracts of *P. ostreatus* were effective against both Gram-negative and Gram-positive bacteria [28]. Both petroleum ether and acetone extracts of *P. ostreatus* were effective against Gram positive bacterium - *Bacillus subtilis*, Gram negative bacterium - *Escherichia coli* and fungus - *Saccharomyces cerevisiae* [29]. Significant growth inhibition of *Escherichia coli* (87.5 %) and *Bacillus subtilis* (57.5 %) was caused by dehydrated mycelium extract [30]. Crude extract of *P. ostreatus* fruit-bodies inhibited the growth of *Pseudomonas aeruginosa, Staphylococcus aureus* and *C. albicans* [31]. Methanolic extracts of *P. ostreatus* exhibited very good antibacterial potential by effective growth inhibition of *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Streptococcus faecalis* in the disc diffusion assay [19].

The results of this study showed rich content of phytochemicals in the *P. ostreatus* methanolic extract, which is on par with the reports of other researchers [20,21]. The antimicrobial potential of the mushroom observed in this study could be credited to these phytochemical compounds. These phytochemicals with medicinal value could serve as important source for the development of novel chemotherapeutic drugs [24].

4. Conclusion

Worldwide, one of the most significant areas of traditional medicine is the natural medicine. The proper use of antimicrobial mushrooms as medicines and screening them for new drugs, essentially requires scientific study of many different mushrooms. Scientific study of mushroom extracts for their antimicrobial potential may result in the identification of new antimicrobial compounds. In the current investigations, antimicrobial activity of aqueous and methanol extracts of *P. ostreatus* was studied by agar well diffusion method and the results showed promising evidence for the antibacterial potential of methanol extract of *P. ostreatus*. Phytochemical screening showed the presence of multiple bioactive compounds in the extract, which may be the reason for antimicrobial potential observed. Thus,

Pleurotus ostreatus could be a potential source for bioactive natural compounds that might serve as novel drugs of effective antimicrobial activity.

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