RESEARCH ARTICLE

ANTIBACTERIAL EFFICACY OF *PLEUROTUS OSTREATUS* AGAINST HUMAN PATHOGENIC GRAM NEGATIVE BACTERIA

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DOI: https://doi.org/10.5281/zenodo.17001737

Abstract:

Pleurotus ostreatus is an edible mushroom that also has high medicinal values. Because of the rising threat of resistant bacterial pathogens, there is an immediate requirement to discover novel antimicrobial compounds especially from natural sources that can effectively eradicate infections. In this study, *P. ostreatus* was tested for its ability to inhibit the growth of three human pathogenic bacterial strains. The antibacterial activity of mushroom ethyl acetate extract was evaluated against three human pathogenic Gram negative bacteria such as *Klebsiella pneumoniae* (MTCC 7407), *Proteus vulgaris* (MTCC 7299) and *Salmonella enterica* ser. *typhi* (MTCC 8767) by agar disc diffusion assay and broth dilution assay. The results showed that *P. ostreatus* ethyl acetate extract has significant antibacterial activity against all the three bacteria tested, which indicates that *P. ostreatus* could be a potential source for novel antibacterial agents.

Keyword: *Pleurotus ostreatus,* Antibacterial Activity, Gram Negative Bacteria, Agar Disc Diffusion Assay, Broth Dilution Assay.

Introduction:

Pleurotus are white-rot fungi, most species growing on hardwood trees, although some also decay conifer wood. *Pleurotus* belongs to class Agaricomycetes and family Pleurotaceae. *Pleurotus ostreatus* is one of the 40 species under the genus *Pleurotus* commonly referred to as 'oyster mushrooms' (Bawadekji *et al*., 2017; Paul *et al*., 2017). Mushroom species are medicinally valuable due to their antimicrobial, antiviral, antioxidant, anti-inflammatory, anti-diabetic and anti-cancer activities (Bhambri *et al.,* 2022).

Infectious diseases account for a high proportion of health problems in most of the developing countries. Indiscriminate use of commercial antimicrobial drugs has led to the development of resistance to the existing antibiotics by the microorganisms. The spread of such drug resistant pathogens

has renewed the interest in traditional medicine (Llor & Bjerrum, 2014). Recently, in addition to medicinal plants, research on drugs derived from fungi such as mushrooms has gained interest (Bhambri *et al.,* 2022; Cohen et al., 2002).

The survey of literature revealed that *Pleurotus ostreatus* has many biological activities (Bawadekji *et al*., 2017; Paul *et al*., 2017; Vamanu, 2012; Waktola & Temesgen, 2020), but the work done related to antibacterial activity is comparatively less. Owing to this the present investigation was carried out. The objective of this work was to evaluate the antibacterial potential of the mushroom *P. ostreatus* with the following steps: Collection and storage of mushroom; Extraction of bioactive agents from mushroom using ethyl acetate and Evaluation of antimicrobial activity of the mushroom extracts against human pathogenic bacteria by agar well diffusion technique and broth dilution method.

Materials and Methods:

Collection of Sample

The samples (*Pleurotus ostreatus*) grown on fallen tree trunk were collected from rural region. The mushrooms were dried in sunlight and pulverised.

Ethyl Acetate Extract Preparation

The extraction was done by swirling about 5.0 g of powdered mushroom with 100 ml of ethyl acetate in a separatory funnel at room temperature for about 2-3 hours and filtering through Whattman No.1 filter paper. The residue obtained was then extracted twice with another 50 ml of solvent each time. The total extract was then evaporated to dryness at 37°C (in incubator) and redissolved in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml and stored at -20°C for further use. In the next step, from the working standard solution concentrations of 100 µg/ml, 250 µg/ml, 500 µg/ml and 750 µg/ml were prepared in sterile distilled water and screened. Later 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml and 4.0 mg/ml were prepared and tested. Control solution was prepared by mixing DMSO and water at 1:4 concentration.

Study of Antibacterial Activity by Agar Well Diffusion Method

The agar well diffusion method was used to screen the antibacterial activity of ethyl acetate extract of *P. ostreatus* against three human pathogenic Gram negative bacteria such as *Klebsiella pneumoniae* (MTCC 7407), *Proteus vulgaris* (MTCC 7299) and *Salmonella enterica* ser. *typhi* (MTCC 8767). 20 ml of nutrient agar (NA) medium was poured into 90 mm sterile Petri plates. The Petri plates were labelled properly with the name of the organism to be inoculated. After cooling, NA plates were inoculated with appropriate overnight broth culture of the bacteria with the help of sterile cotton swabs 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 on the plates by using standard sterile cork borer. Using micropipette about 100 µl of different concentrations of extract were transferred to respective wells and about 100 µl of control solution was transferred to the central well as control. Then the plates were incubated at 37°C for about 24 to 48 hours. At the end of incubation, plates were observed for zone of inhibition and the inhibition zones formed around the wells were measured and recorded. Triplicates were maintained for each bacterium.

Study of Antibacterial Activity by Broth Dilution Method

The antibacterial activity was also determined using broth dilution method. Two-fold serial dilutions of *P. ostreatus* ethyl acetate extract were prepared in sterile distilled water by initially mixing 500 µl of extract with 500 µl of distilled water and then diluting 500 µl of previous dilution with 500 µl of distilled water to have the next diltution. 4.0 mg/ml, 2.0 mg/ml, 1.0 mg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml dilutions were prepared and used. 100 µl of each dilution was transferred into a sterile effendorf tube of 1.5 ml volume separately and properly labelled. 500 µl of sterile nutrient broth was added to each of the tube and inoculated with 100 µl of bacterial inoculum. A control tube was maintained with all the components except mushroom extract. Following 24-hour incubation at 37ºC, bacterial growth inhibition was determined by measuring the optical density (OD) at 600 nm using a spectrophotometer. The growth inhibition in each of the tube was determined by the formula:

Percentage of Inhibition = (OD of Control – OD of Test) / (OD of Control) X 100

Results:

Agar Well Diffusion Method

At the concentrations of 100 µg/ml, 250 µg/ml, 500 µg/ml and 750 µg/ml, the ethyl acetate extract of *Pleurotus ostreatus* showed no zone of inhibition indicating its inactivity at these concentrations against tested bacteria. At the concentrations of 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml and 4.0 mg/ml, the ethyl acetate extract of *Pleurotus ostreatus* showed very good antibacterial activity against all the three bacteria screened such as *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella enterica* ser. *typhi* which was evident by the inhibition zones formed around the wells (Figure 1, Table 1). Comparatively, best activity was against *Klebsiella pneumoniae.*

Broth dilution method

At the concentrations of 1.0 mg/ml, 2.0 mg/ml and 4.0 mg/ml the ethyl acetate extract of *P. ostreatus* showed complete growth inhibition of all the three bacteria screened such as *Klebsiella pneumoniae*, *Proteus vulgaris* and *Salmonella enterica* ser. *typhi* which was evident by the OD values. However, at the concentrations of 500 µg/ml, 250 µg/ml and 125 µg/ml good growth of *P. ostreatus* was observed that was almost similar to the control, which was evident by the OD values. This clearly indicated the inactivity of the mushroom extract at these concentrations against the tested bacteria. This result was similar to that observed in agar dilution method. The percent growth inhibition of bacteria at different concentrations of *P. ostreatus* ethyl acetate extract is presented in figure 2.

Table 1: Antibacterial activity of *Pleurotus ostreatus* ethyl acetate extract by agar well diffusion method (after 24 h incubation)

Concentration of extract	Zone of inhibition (in mm)		
	Klebsiella pneumoniae	***Proteus vulgaris***	***Salmonella enterica* ser. *typhi***
1.0 mg/ml	27.6± 0.28	27.6± 0.28	24.0 ±0.32
2.0 mg/ml	30.0± 0.38	28.8± 0.34	27.6± 0.28
3.0 mg/ml	31.2± 0.46	31.2± 0.46	30.0± 0.38
4.0 mg/ml	32.4 ± 0.34	31.2 ±0.46	31.2± 0.46

Values are means of triplicate ± standard deviation

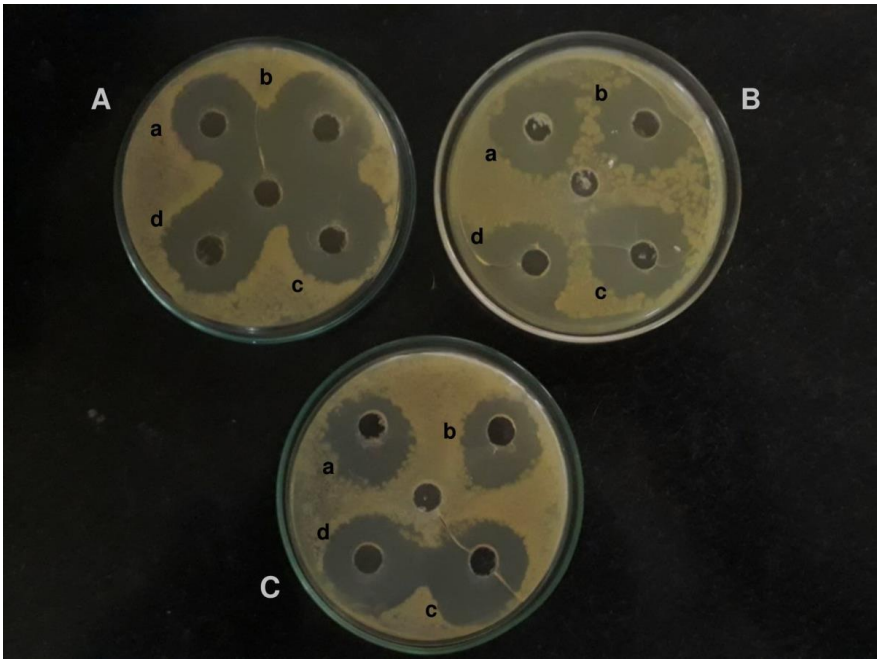

Figure 1: Antibacterial activity of ethyl acetate extract of *Pleurotus ostreatus* by agar well diffusion method (after 24 h incubation)

[**A** - *Klebsiella pneumoniae* (MTCC 7407), **B** - *Proteus vulgaris* (MTCC 7299) and **C** - *Salmonella enterica* ser. *typhi* (MTCC 8767) showing zones of inhibition against ethyl acetate extract of *Pleurotus ostreatus* at the concentrations of 1.0 mg/ml (a), 2.0 mg/ml (b), 3.0 mg/ml (c) and 4.0 mg/ml (d); Control solution (centre well)]

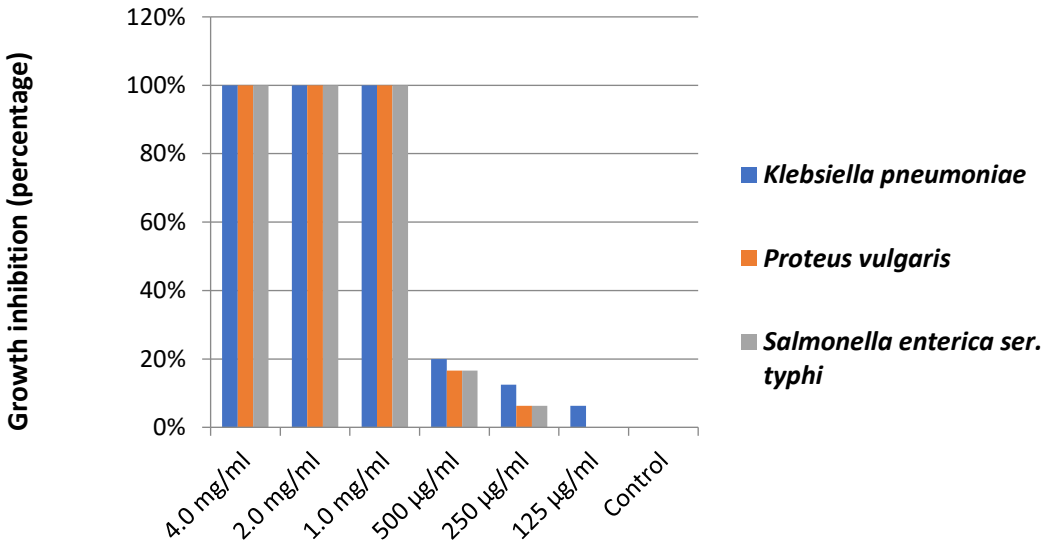

Figure 2: Antibacterial activity of *Pleurotus ostreatus* ethyl acetate extract by broth dilution method (after 24 h incubation)

Discussion:

The expanding bacterial resistance to presently available antibiotics has become a growing concern worldwide and this increasing bacterial resistance is prompting research on the novel antimicrobial agents from natural sources (Llor & Bjerrum, 2014). Oyster mushrooms (*Pleurotus*) are

edible and nutritious, and possess important bio-active compounds (Cohen et al., 2002; Paul et al., 2017). Significant antimicrobial effects of *P. ostreatus* have been reported by many scientists (Bawadekji *et al*., 2017; Paul *et al*., 2017; Vamanu, 2012; Waktola & Temesgen, 2020). The results of present study showed antibacterial efficacy of the crude ethyl acetate extract of this macro-fungus. The extract was found to be very effective at the concentrations of 1.0 mg/ml, 2.0 mg/ml, 3.0 mg/ml and 4.0 mg/ml against all the three bacteria tested such as *Klebsiella pneumoniae* (MTCC 7407), *Proteus vulgaris* (MTCC 7299) and *Salmonella enterica* ser. *typhi* (MTCC 8767) which suggests that *P. ostreatus* has significant antibacterial effects. Our findings are in accordance with other researchers, however the solvents and organisms studied may be different (Bawadekji *et al*., 2017; Vamanu, 2012).

Conclusion:

The results of present study showed promising evidence for the antibacterial potential of ethyl acetate extracts of *Pleurotus ostreatus.* This mushroom can be further studied to discover bioactive natural compounds that may help in the development of new drugs of potent antibacterial activity.

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