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ANTIMICROBIAL ACTIVITY OF *PLEUROTUS SAJOR*-CAJU (GREY OYSTER MUSHROOM) AGAINST *PSEUDOMONAS AERUGINOSA*

Dr. Dande Swapna Sree, Lecturer, Department of Botany,

Silver Jubilee Government College, Cluster University, Kurnool, Andhra Pradesh, India

Abstract: Mushrooms are macroscopic fungi known for their nutritional and therapeutic importance. *Pleurotus sajor-caju* (grey oyster mushroom) is considered as one of the edible fungus meant for oyster shaped fruiting bodies and hence considered as grey oyster mushroom. This fleshy edible fungus is rich in nutrients like carbohydrates, proteins, fats, minerals, and multivitamins. The present research article investigated the antimicrobial effect of *Pleurotus sajor-caju* extracts on the bacterial strain *Pseudomonas aeruginosa.* Antimicrobial activity was tested with two different extracts of Pleurotus by using the disc-diffusion method. The two Both aqueous and methanolic powdered extracts exhibited antibacterial activity against tested bacterial strains. Comparatively, the Methanol extract revealed higher effects over aqueous extracts among the bacterial strain tested thus stating that *Pleurotus sajor-caju* extracts are one of the promising sources of novel antimicrobial agents.

Keywords: *Pleurotus sajor-caju*, *Pseudomonas aeruginosa*, Antibacterial activity

Introduction: Mushrooms are macroscopic fungi used from ancient civilizations to modern society due to their high nutritional values and therapeutic importance. *Pleurotus sajor-caju*, also called Grey oyster mushroom, is a macroscopic, aerobic fleshy edible fungus belonging to the family Pleurotaceae. The Pleurotus genus comprises various species of cultivated mushroom, with significant pharmacological properties and high nutritional value and are among the most consumed in the world (Rathore *et al*., 2020). Studies have reported *Pleurotus sajor-caju* associated with high nutritional value, bioactive compounds and therapeutic properties like anti-viral, anti-bacterial, anti-fungal, anti-parasitic, anti-hypertensive, anti-inflammatory, and anti-diabetic (Heleno *et al*., 2015; Kim *et al*., 2010). The antimicrobial activity is due to the ability of the mushroom compounds to disrupt the bacteria cellular membranes causing cell lysis (Finimundy *et al.*, 2018; Kandasamy *et al.,* 2019)

Materials and Methods:

1. **Collection of Mushrooms**: Pleurotus mushrooms were raised using Paddy straw as substrate. They were harvested and cleaned with tap water, cut into little bits, air-dried in room temperature and were ground into fine powder. The powder is used for antimicrobial activity.
2. **Aqueous extraction of Mushrooms**: To three hundred grams of fine powder of mushroom, 1000 ml of distilled water was added and subjected to boiling in a conical flask for 20 minutes using a water bath. This was cooled to room temperature and the supernatant obtained was subjected to centrifugation at 5400 x gravity for 10 minutes. This was filtered through Whatman No 1 filter paper and subjected to freeze drying. This extract was desiccated at 4°C in a refrigerator.
3. **Methanol extraction of Mushrooms**: Each three hundred grams of mushroom powder was soaked in one Liter of methanol and left standing for two days. The extracts were then filtered, and the filtrate was concentrated by a rotary evaporator separately. The concentrate was then stored in air-tight containers and refrigerated.
4. **Preparation of Inoculum**: The stock cultures were maintained on slants of nutrient agar. The bacterial culture used for this experiment was made by picking a loopful of cells out of the stock culture and placing in test tubes that contained Mueller–Hinton broth (MHB). This was reactivated by incubating them overnight at 37°C. Cultures were diluted with fresh MHB and compared with McFarland standard to achieve values corresponding to 2×10^6 colony-forming unit for bacteria (Al-Salt, 2012).
5. **Evaluation of extract for antimicrobial activities**: Aqueous and methanol extracts of the mushroom were used to evaluate their antibacterial activity using disc-diffusion method as described in Mbwambo et al., 2007. The bacterial strains selected to test include *Pseudomonas aeruginosa*. The purity of the bacteria was tested by culturing on nutrient agar and were maintained on nutrient agar slants. Whatman No.1 filter papers were made into 5mm diameter discs and sterilised. These sterilized discs were soaked with mushroom extracts at concentrations of 25, 50, 100, and 200 mg/ml. The discs which were soaked in dimethylsulfoxide represented the negative controls (Valadbeigi et al., 2014). The bacteria were inoculated in nutrient broth and incubated at 30°C for 24 hours . Sterilized Petri dishes were inoculated with 0.01 ml of the above culture media (10^5-10^6 bacteria per ml). To this Mueller–Hinton agar was added and subjected to swirling for homogenous distribution of the culture. The sterile filter paper discs injected with the mushroom extracts were placed on the solid medium by pressing gently. Ciprofloxacin 0.2 mg/ml (Mulatu, 2020) was used as the standard drug to test bacterial strains. The Petri dishes which were treated were kept at 4°C for 1-2 hours and thereafter they were incubated at 35°C for 18–24 hours. The zones of inhibition which had been formed on the media were measured with a transparent ruler in Triplicates were maintained and statistical analysis of the date was carried out.
6. **Statistical Data Analysis**: Raw data was recorded in a data book and then exported to STATA statistical software version 14.2 for analysis. Descriptive statistics were expressed as mean ± standard deviation. One-way analysis of variance was used to determine the statistical difference among

different treatment groups followed by Bonferroni post hoc test for comparison of means of different treatment groups. The level of significance was set at 95% ($p \leq 0.05$).

Results: Both aqueous and methanolic extracts revealed antibacterial activities at different concentrations tested against P. aeruginosa. The antibacterial effect of the ciprofloxacin was significantly higher compared to that of Methanol extract at all the concentrations tested against P. aeruginosa. The zones of inhibition of Methanol extract at the concentration of 200 mg/ml were statistically higher than those of 25, 50 and 100 mg/ml against P. aeruginosa (Table 1; $p < 0.05$). Further, the effect of the negative control was comparable to the effect of methanol extract at all tested concentration against P. aeruginosa. Also, the aqueous extract at higher concentrations revealed antibacterial activity against P. aeruginosa. The concentrations of aqueous extract that never showed the antibacterial effect had zones with a diameter of 5 mm. The zones of inhibition of ciprofloxacin, were significantly higher compared to those of aqueous extract at all the tested concentrations against P. aeruginosa. The antibacterial effect of the aqueous extract at the concentrations of 50, 100, and 200 mg/ml was not significantly different against P. aeruginosa. However, the aqueous extract at concentrations of 25 mg/ml never showed antibacterial effect and was comparable to the negative control (Table 1).

The increased antimicrobial inhibition zone of methanol extracts over aqueous extracts was observed which might be due to the extraction of active lipophilic constituents in methanol than in aqueous extracts. The methanol extracts of Pleurotus sajor-caju performed better than aqueous extracts. As most of the antimicrobial active components being non-polar and saturated organic molecules and for active lipophilic constituents that do not extract into water, methanol extraction may provide more consistent antimicrobial activity compared to those extracted in water. Water being more polar than methanol presented smaller microbial growth inhibition zones than the methanol extracts. As Negative control is used to compare the result to a new experiment against an already known result and involves testing the experiment with known result and that result will have no effect on the experiment. In this experiment different concentrations exhibited comparatively less inhibition zones than negative control proving that mushroom extract possesses antimicrobial effect against the bacteria. The results were compared to ciprofloxacin as a chemical inhibitor against P. aeruginosa.

Table 1: Antibacterial activity of Aqueous and methanolic extracts of mushroom against *Pseudomonas aeruginosa*

Treatment	**Methanol extract**	**Aqueous extract**
200 mg/ml	8.33 ± 0.58	6.53 ± 0.29
100 mg/ml	7.33 ± 0.58	6.33 ± 0.29
50 mg/ml	6.67 ± 0.58	6.17 ± 0.29
25 mg/ml	5.37 ± 0.58	5.00 ± 0.00
Negative control	5.00 ± 0.00	5.00 ± 0.00
Ciprofloxacin	19.33 ± 58	19.33 ± 58

Conclusions: The present study concluded that the methanol, and aqueous extracts of *Pleurotus sajor-caju* revealed potent antibacterial effect against Gram-negative *Pseudomonas aeruginosa* bacteria. The methanol and aqueous extracts showed significant antimicrobial activity against *Pseudomonas aeruginosa* and hence may be used as antibacterial agent either solely or in Integrated Disease Management.

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