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In vitro Studies on Antibacterial Activity of *Pleurotus sajor caju*

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Antibacterial activity of acetone and methanolic extracts of *Pleurotus sajor caju* was determined *In vitro* against two pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus)* following agar well diffusion method using different concentrations (25, 50, 75 and 100%). Acetone and methanolic extracts showed potential antibacterial activity against selected strains. Methanolic extract showed maximum inhibitory effect against the growth of *S.aureus* and acetone extract against *E.coli*. The mushroom used in this study was found to have antimicrobial effects at a variety of degrees against microorganisms tested. There is need of further studies to isolate and characterize the antibacterial moieties in this fungus for practical disease control measures.

Key words: *Pleurotus sajor caju*, antibacterial, *in-vitro, Staphylococcus aureus, Escherichia coli.*

Edible mushrooms are a valuable source of biologically active compounds[1]. The use of mushrooms with potential therapeutic properties raises global interest from the scientific and clinical community based on two main reasons. First, mushrooms demonstrate their efficacy against numerous diseases and metabolic disturbances. Second the medicinal use of edible mushrooms extracts seems to be a more natural, less expensive approach and in general involves minimal unwanted side effects[2]. Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times[3,4]. Wild and cultivated mushrooms contain a huge diversity of biomolecules with nutritional[5] and/or medicinal properties[6]. Both fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity and their compounds could be isolated from many mushrooms species and could be of benefit for human. Oyster mushrooms are a good source of dietary fiber and other valuable nutrients[7]. This mushroom is credited to the third largest macrofungus cultivated for food and industrial purposes worldwide. Oyster mushrooms were found to contain antimicrobial and antioxidant potentials[8]. Thus, the present study focused on evaluation of antibacterial activities of acetone and methanolic extracts of *Pleurotus sajor caju* using agar well diffusion method against two clinical isolates *Escherichia coli* and *Staphylococcus aureus*.

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MATERIALS AND METHODS

Materials used in the present study were fruiting bodies and pure cultures of *Pleurotus sajor caju*. The fruiting bodies of the mushroom were procured from DMR, Solan (H.P.) and two bacterial pathogens (*Escherichia coli* and *Staphylococcus aureus*) were procured from IGMC, Department of Microbiology, Shimla.

Methodology

Isolation of pure culture of *Pleurotus sajor caju*: The cultures were raised from the stipe and stroma portion of healthy, sun-dried and fresh specimens. The specimens were first washed with distilled water and then the tissue from the stipe and stroma portion were cut with the help of a sterilized blade. The bits of tissue (2-3 mm) were taken up with a sterilized forceps and dipped in 0.1% mercuric chloride solution for 5-10 seconds.

Now the tissue was placed on filter paper to remove the excess moisture. The small bits of *Pleurotus* tissues were then transferred aseptically into the petriplates containing potato-dextrose agar (PDA) medium with the help of a sterilized forceps. These were then incubated at 25°C for at least 8-10 days and observed regularly for appearance of culture. The actively growing mycelial colonies were sub cultured to obtain pure cultures.

Preparation of crude mushroom extract

Acetone and methanolic extracts of experimental fruiting bodies were prepared following Indian Pharmacopoeia[9]. The fresh fruit bodies were dried in shade conditions and the dried material (50 g) as pulverized in a blender to get coarse powder and soaked separately in 300 ml of acetone and methanol in Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator[10,11]. The extracts were collected and stock solution of conc. 10 mg/ml was prepared.

Screening of extract of *Pleurotus sajor caju* for antibacterial activity

Screening of mushroom extracts (acetone and methanolic) of *Pleurotus sajor caju* was done using agar well diffusion method. Nutrient agar medium (Beef extract 1 g, yeast extract 2g, sodium chloride 1g, peptone 5g, agar 20g, distilled water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours. A 100 µl of bacterial suspension was spread on each nutrient agar plates. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each petriplate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared extracts of *Pleurotus sajor caju*. The control well containing the pure solvent. The plates were incubated at 37 ± 2°C for 24 hours in incubation chamber.

The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all three replicates and the average values were tabulated. Percentage inhibition of growth of bacterial microorganisms was calculated after subtracting control from the values of inhibition diameter using control as standard[12].

$$\text{Percentage of growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Control = average diameter of bacterial colony in control.

Test = average diameter of bacterial colony in treatment sets[13].

RESULTS AND DISCUSSION

Morphological and mycelial characters

The fruiting bodies of *Pleurotus sajor caju* were white or pale white in colour. Spores are round having spore print pale yellow (Plate 1A, C). *Pleurotus sajor caju* (Fr.)Sing. was having longitudinally radial hyphae aerial initially, creamish white, becoming densely matted and cottony in texture (Plate 1B).

Antibacterial activity of *Pleurotus sajor caju* against *S. aureus* and *E. coli*

The acetone and methanolic extracts of *Pleurotus* were screened against *S. aureus* and *E. coli*. Stock solution was prepared by making a concentration of 10 mg/ml, other concentrations were prepared by serial dilution of stock solution. Acetone and methanolic extract of *Pleurotus* showed considerable growth inhibition against two test bacteria in different concentrations (25% to 100%). Acetone extract of *P. sajor caju* showed maximum inhibition of 16.11% and 20.33% at 100% concentration against *S. aureus* and *E. coli* respectively (Table 1 Plate 2 B, D). Methanolic extract exhibited maximum inhibition of 18.88% against *S. aureus* and 22.22% against *E. coli* at 100% concentration. (Table 2 and Plate 2 A, C). It is evident from the results that acetone and methanolic extracts of *P. sajor caju* showed maximum percent inhibition against *E. coli.*

The results of the present study are in agreement with the work of the earlier workers[14, 15] who have also reported strong antibacterial activity of methanolic extract of *G. lucidum* against gram negative (*E. coli*) and comparatively less activity against gram-positive (*S. aureus*) bacteria. Similar trend in antibacterial activity of methanolic extract of *Lactarius delicious*[16], *Sparassis crispa*[17], *Morchella esculenta*[18] and *Ganoderma lucidum*[19]

have been reported against *S. aureus* and *E.coli.* Ramesh and Patter[20] have reported that extract of *Clavaria vermicularis* and *Marasmium oreades* offered more inhibition to gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) as compared to grampositive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Filipa *et al.*,[21] have reported that methanolic extract of *Cordyceps militaris* possesses antioxidant, antibacterial, antifungal and antiproliferative properties. Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide[22]. Mushroom based products either from the mycelia or fruit bodies are consumed in the form of capsules, tablets or extracts[23].

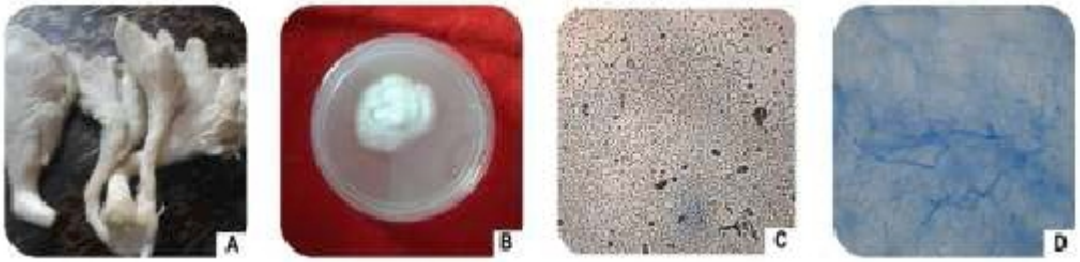

Plate 1.
A) Fruiting bodies of *P. sajor caju;* B) Pure culture of *P. sajor caju;* C) Basidiospores of *P. sajor caju*

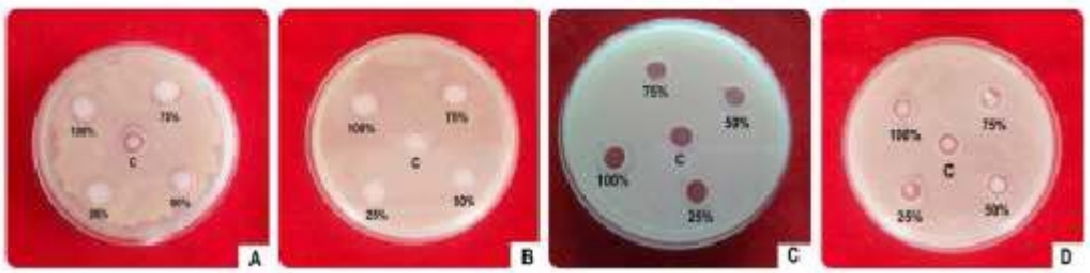

Plate 2.
(A) Inhibition in the growth of *S.aureus* at different concentrations of Methanolic extract
(B) Acetone extract of *P. sajor caju*
(C) Inhibition in the growth of *E.coli* at different concentrations of Methanolicextract
(D) Acetone extracts of *P. sajor caju*

Table 1. Percentage Inhibition of Growth of *S. aureus* and *E. coli* At Different Concentrations of Acetone Extract Of *P. sajor caju*

S.No.	Concentration of acetone extract of *P. sajor caju* (in %)	Percentage inhibition of growth of test bacteria	
		S.aureus	*E.coli*
1	Control	00	00
2	25	11.11±0.47	13.33±0.33
3	50	12.22±0.36	14.66±0.29
4	75	14.66±0.26	16.67±0.22
5	100	16.11±0.11	20.33±0.33

Table 2. Percentage Inhibition of Growth of *S. aureus* and *E. coli* at Different Concentrations of Methanolic Extract of *P. sajor caju*

S.No.	Concentration of methanolic extract of *P. sajor caju* (in %)	Percentage inhibition of growth of test bacteria	
		S.aureus	*E.coli*
1	Control	0	0
2	25	12.22±0.31	13.88±0.22
3	50	15.55±0.59	16.00±0.33
4	75	17.22±0.44	17.77±0.08
5	100	18.88±0.47	20.22±0.37

CONCLUSION

Mushroom taken as food has the advantage of being devoid of dose related adverse effect seen with antibiotics. Resistance to antibiotics due to its frequent and inappropriate use pose a threat to treatment of bacterial infections mostly in under developed countries and in many developed countries of the world as well. Therefore mushroom with antibacterial properties have received considerable attention in recent years. Search for new antimicrobial agents has gained considerable importance, and mushrooms for their antibacterial activity may be considered for their easy availability and cheapness. So, there is a need for further studies to isolate and characterize the bioactive compounds present in *P. sajor caju* .

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