

Antimicrobial Activity of *Lyophyllum decastes* an Edible Wild Mushroom

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Abstract: The antibacterial activity of various solvent extracts of *Lyophyllum decastes* was tested against 7 species of bacteria: *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Micrococcus luteus*. The methanol extract exhibited maximum activity. Gram positive *Bacillus subtilis* and *Micrococcus luteus* was most susceptible than Gram negative *Klebsiella pneumoniae* and *Salmonella typhi*. The solvent extracts of *Lyophyllum decastes* was also tested against 5 species of fungi: *Candida albicans*, *Cryptococcus*, *Aspergillus ocraceus*, *Curvularia* and *Alternaria*. The acetone extract exhibited maximum activity. *Curvularia* was most susceptible than other fungi and *Cryptococcus* was found to be resistant.

Key words: Antibacterial activity · Antifungal activity · Medicinal mushroom

INTRODUCTION

Mushrooms are used as food stuffs containing both nutritive and medicinal values. They are also known to prevent diseases such as hypertension, hypercholesterolemia and cancer [1]. It has been known that macro fungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity [2]. Modern clinical practice in Japan, china, Korea and other Asian countries continues to rely on mushroom derived preparations. The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and has found variable therapeutic activity such as anticarcinogenic, anti-inflammatory, immunosuppressor and antibiotic [3]. It was believed that mushrooms need antibacterial compounds to survive in their natural environment. Antimicrobial activities of many polypores and agarics from different countries were screened from both basidiocarps and their mycelia in liquid cultures. Nearly 75% of polypore fungi that have been tested show strong antimicrobial activity [4]. *Ganoderma lucidum* and other *Ganoderma* species were reported to have polysaccharides or triterpenoids and used against more often in combination with chemotherapeutic agents have been used to treat various bacterial diseases and against Herpes simplex virus, Hepatitis B virus, HIV and Epstein-Barr virus *in vitro* or in animal models. They inhibit both Gram-positive and

Gram-negative bacteria *in vitro* and hence tried to use in combination with chemotherapeutic agents to treat various bacterial diseases. Methyl australate, derivative from *G.lucidum* was used against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Salmonella typhi*

[5, 6]. Various solvents such as water, ethyl alcohol, methyl alcohol, acetone etc. were employed for extraction of active compounds. Similarly mushrooms such as species of *Cantherellus*, *Lentinus*, *Russula*, *Agaricus*, *Pleurotus* etc. showed some antimicrobial property [7- 9]. It is now focused that the mushrooms are vital sources of medicinal compounds that may be used to cure different disorders and prevent pathogenic microorganisms.

Lyophyllum decastes is an edible mushroom, well known in American and European countries. Basidiocarps growth in dense (and often huge) clusters on the ground, usually in areas where the ground has been disturbed (roadbeds, paths, landscaping areas, etc.) but, sometimes in woods. Pileus white, yellowish to greyish brown; stipe centric, solid; gills white to cream at age. Spores are inamyloid, smooth, more or less round. Gill faces and edges lack cystidia. An antimicrobial activity of *Lyophyllum decastes* in the literature is scanty. Hence, the antimicrobial potential of the different solvent extracts of *Lyophyllum decastes* on bacteria and fungi has been evaluated in the present work.

MATERIALS AND METHODS

The basidiocarps of *Lyophyllum decastes* were collected Rajajinagar, located in the Bangalore July. Macroscopic and microscopic characters were used to identify the fungus.

Preparations of Solvent Extract Form the Basidiocarps of the Mushroom: In the present study, the mushroom material was made into fine powder. Ten grams of mushroom powder was subjected to extraction using 100 ml of various solvents at room temperature for 24 hrs and filtered through Whatman No. 4 filter paper. The extracts were recovered by filtration and kept at 40°C in a rotary vacuum evaporator [9]. The residue was collected and store at 4°C for further use.

Microorganisms Tested

Bacterial Cultures: *Escherichia coli* (ATTC 25992), *Bacillus subtilis* (ATTC 6633), *Klebsiella pneumoniae* (ATTC 27736), *Pseudomonas aeruginosa* (clinical isolate), *Staphylococcus aureus* (ATTC 12598), *Salmonella typhi* (M 733); *Micrococcus luteus* (clinical isolate) were used

Fungal cultures: *Candida albicans*, *Cryptococcus sp*, *Aspergillus ocraceous*, *Curvularia sp* and *Alternaria sp* (clinical isolates) were used.

Screening of Antibacterial and Antifungal Activity of Mushroom Extract:

The antimicrobial activities of the extracts were determined by the Kirby-Bauer agar diffusion method according to NCCLS standards [10, 11]. All the bacterial cultures were incubated at 37°C for 24 hours by inoculation into Nutrient broth. The fungal

cultures were incubated at room temperature for 48 hours in Potato Dextrose Agar. Nutrient agar and Potato Dextrose Agar of about 20 ml were poured into each sterilized Petri plates aseptically and allowed to solidify. With the help of sterilized cork borer a well of 6mm diameter was bored at the centre of the media in the plate. The bacterial and fungal suspension was swabbed on the solidified media using sterilised swabs in the respective plates.

The mushroom extract was dissolved in Dimethylsulfoxide and the suspension was sterilized by filtration through a membrane filter [12]. The mushroom extract of about 100 microliters was filled into the wells of the agar plates. The bacterial plates were incubated at 37°C for 24 to 48 hours. The fungal plates were incubated at 28°C for 3-4 days. Inhibitory activity of DMSO was also tested. Nystatin was used as the reference disc for fungi. And Penicillin and Tetracycline was used as the reference disc for bacteria. After the period of incubation the zone of inhibition was measured, tabulated and compared with reference discs.

RESULTS

The antimicrobial effect of different solvent extracts of *Lyophyllum decastes* was tested against seven bacterial isolates and five fungal isolates. Among the organic solvent extracts methanol extract showed more effective inhibitory activity against bacteria. Gram positive *Bacillus subtilis* and *Micrococcus luteus* were susceptible than Gram negative *Klebsiella pneumoniae*, *Salmonella typhi*. *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed very less activity (Table 1).

Table 1: Antibacterial activity of different organic solvents of *Lyophyllum decastes* (Zone of inhibition in mm)

Sl.	Test organism	Acetone extract	Ethanol extract	Methanol extract	Penicillium	Tetracycline
01	<i>B.Subtilis</i>	-	10	12	12	17
02	<i>E.coli</i>	11	-	-	-	11
03	<i>S.aureus</i>	09	-	09	25	16
04	<i>K.pneumoniae</i>	13	07	12	-	08
05	<i>Styphi</i>	-	07	16	-	17
06	<i>P.aeruginosa</i>	-	-	-	-	08
07	<i>M.luteus</i>	12	-	12	33	20

Table 2: Antifungal activity of different organic solvents of *Lyophyllum decastes* (zone of inhibition in mm)

SINo.	Test organism	Acetone extract	Ethanol extract	Methanol extract	Nystatine
01	<i>Candida albicans</i>	-	07	-	18
02	<i>Cryptococcus sp</i>	-	-	-	22
03	<i>A.ocraceous</i>	10	07	-	18
04	<i>Curvularia sp</i>	25	-	-	14
05	<i>Alternaria sp</i>	10	-	-	12

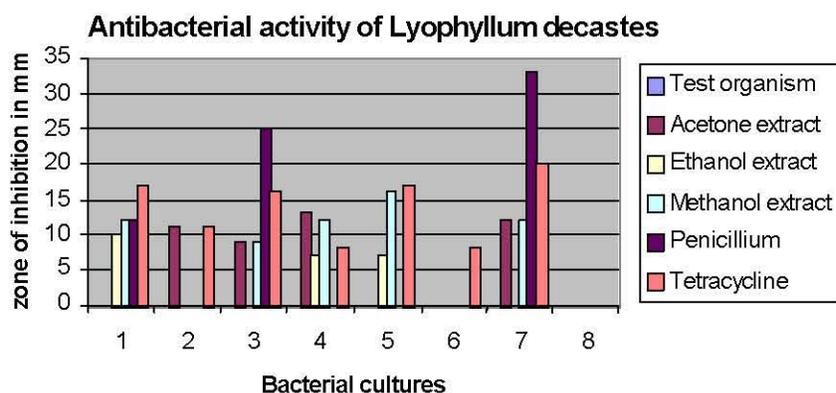


Fig. 1: Antibacterial activity of *Lyophyllum decastes*.

The acetone extract showed maximum activity against fungal cultures. *Curvularia* was found to be more susceptible than *Aspergillus ocraceous* and *Alternaria*. *Candida* and *Cryptococcus* was found to be resistant to acetone extract. However *Candida* was found to be little sensitive to ethanol extract. The reference antibiotic discs are more active than the organic solvent extracts of *Lyophyllum decastes* (Table 2).

DISCUSSION

Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc. act on the bacterial cytoplasmic membrane [13]. In our present work, the medicinal mushroom *Lyophyllum decastes* has been investigated for its antimicrobial activity. Among the three different extracts Methanol extract exhibited maximum antibacterial activity against Gram positive bacteria such as *Bacillus subtilis* and *Micrococcus luteus* and Gram negative bacterial such as *Klebsiella pneumoniae* and *Salmonella typhi*. To our knowledge, no investigation has been performed for comparing antibacterial activity of *Lyophyllum decastes*.

Most of the mushroom antifungal peptides and proteins reported to date resemble, in their broad spectrum of activity against different fungal species. It is note worthy that some antifungal proteins have a specificity of action against only certain fungal species [14]. The synergic action of an antifungal protein and ribosome inactivating protein lyophyllin from the mushroom *Lyophyllum shimeji* has been reported [15]. In our present investigation the acetone extract of the mushroom showed susceptibility to *Curvularia*, *Aspergillus ocraceous* and *Alternaria*. To our knowledge, no investigation has been performed for comparing antibacterial and antifungal activity of *Lyophyllum decastes*.

In conclusion, our research suggests that the *Lyophyllum decastes* collected from Bangalore, India has high potential it would be used as natural antimicrobial agent. Due to toxicological concerns associated with the usage of synthetic substances in food and increasing awareness about natural foods, there has been increased interest in the use of natural substances as food preservatives and antioxidants [16]. Hence further studies on isolation and identification of the active compounds may provide a better source for developing new therapeutic agents especially to *Klebsiella* and *Curvularia* related infection. Hence this property of the mushroom can be exploited in food and pharmaceuticals industries.

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