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Antibacterial and antifungal activity of fruit bodies of *Phellinus* mushroom extract

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Abstract

The present study was carried out to evaluate the antibacterial and antifungal activity of methanol and aqueous extract of fruit bodies from *Phellinus* on selected five bacterial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans* and five fungal strains *Penicillium* sps., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus*. For antimicrobial test, well diffusion technique was used and the zone of inhibition of microorganisms was measured in mm. The fruit body of *Phellinus* showed potential antimicrobial activities against the selected strains and maximum inhibition zone 42mm was recorded from 200mg of aqueous extract of *Phellinus* fruit body against *Pseudomonas aeruginosa* and minimum (5mm) by the above pathogen at 50 mg of methanol extract. The methanolic extract showed the maximum antifungal activity 35mm inhibition zone was recorded from 200mg of extract against *Aspergillus flavus* and minimum 3mm by 50 mg of extract against *Penicillium* sp.

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Introduction

Mushrooms belong to a special group of macroscopic fungi. Macromycetes arranged in the phylum Basidiomycota and some of them in the Ascomycota are known as the higher fungi (Moradali *et al.*, 2007, Sicoli *et al.*, 2005). It is estimated the existence of about 140.000 different species of mushrooms in the planet, however, only about 10% is known. Half of them present nutritious properties. 2.000 species of mushrooms are safe and, approximately, 70 are known for presenting some pharmacological properties. Edible mushrooms are attractive because of their flavor, taste, and delicacy (Diyabalanage, 2008). Although many species of edible mushrooms exist in the nature, less than 20 species are used as food and only 8–10 species are regularly cultivated in significant extent.

Phellinus is a genus of fungi in the family Hymenochaetaceae. Many species cause white rot. Fruiting bodies, which are found growing on wood, are resupinate, sessile and perennial. The flesh is tough and woody or cork-like, and brown in color. Clamp connections are absent, and the skeletal hyphae are yellowish-brown (Ellis and Ellis, 1990).
Several compounds with important pharmaceutical properties have been isolated from these organisms. Substances that act as anti-aging, in longevity, modulating the immune system, having hypoglycemic activity and to inhibit tumor growth have been isolated from mushrooms, such as polysaccharides. Polysaccharides can interconnect several points forming a wide variety of branched or linear structures, for example, ß4 glucans (Ooi and Liu, 2000). Furthermore, other bioactive substances such as triterpenes, lipids and phenols have also been identified and characterized in mushrooms with medicinal properties (Maiti *et al.,* 2008). Mushroom contain vitamins A and C of ß-carotene and a great variety of secondary metabolites such as phenolics compounds, polyketides, terpenes, steroids and phenols, all have protective effects because of their antioxidant properties (Jayakumar *et al*., 2009; Soares *et al*., 2009).

In disc diffusion method, the discs are very expensive and their acquisition in developing countries is sometimes difficult. In an attempt to combat this, in 1997 Magaldi developed a modification of the disc diffusion method which she named the 'well diffusion' method (WD). The procedure is similar; the discs are supplemented with dilutions of the drug placed in wells which have been cut out in the agar. This allows the use and standardization of various concentrations of any drug for different fungal species. It has proven to be a cheap, simple and reliable method of antifungal drug susceptibility testing for *Candida* spp., and it produces results comparable with the disc diffusion test (Magaldi and Camero, 1997, Magaldi 2004)

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The aim of the present work is to evaluate the antibacterial and antifungal activity of *Phellinus* sp. extracts with the help of methanol and aqueous extract against to bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans*) and to the fungal species (*Penicillium* sps., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus*) were investigated.

Materials and methods

Collection of fungal fruit body

A mushroom species – *Phellinus* Quél. was used in this study and collected from NCC ground, Govt. Arts College, Thiruvannamalai. These Agaricomycetes were identified by Department of Mycology, Centre for Advanced Studies in Botany, University of Madras, Chennai - 25.

Preparation of crude extract

Various extracts of the experimental fruit body was prepared according to the methodology of Indian

Pharmacopoeia (Anonymous, 1966). The fresh fruit bodies were dried in shade conditions and the dried materials were pulverized in a blender to get coarse powder. The coarse powder material was used to Soxhlet extraction successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50°C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for antibacterial and antifungal activity.

Test organisms

The stored culture of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

Pathogenic fungal strains *Penicillium* sps., *Aspergillus fumigatous* , *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial studies

Bacterial Media (Muller Hindon Agar Media)

Thirty Six grams of Muller Hindon Agar Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method (Bauer *et al*., 1996). The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of 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 three different fixed directions in all 3 replicates and the average values were tabulated.

Results

The methanol and aqueous extract of the *Phellinus* fruit body were screened against five human pathogenic bacteria and five fungal pathogens to check antibacterial and antifungal activities by well diffusion method which showed valuable zone of inhibition. The specific zone of inhibition against various types of pathogenic bacteria and fungus was shown in table 1 and 2. Methanol and aqueous extract were effective against both bacteria and fungus, and aqueous extract was better than methanol extract against bacteria but in the case of fungal pathogens in voce versa.

The zone of inhibition against bacterial pathogens ranged between 42 – 19mm in aqueous extract and 17 – 5mm in methanolic extract. The maximum activity (42mm) was recorded from 200mg of aqueous extract of *Phellinus* against *Pseudomonas aeruginosa* followed by 34mm against *Salmonella typhi* and

minimum (19mm) against *Streptococcus mutans* at 50mg level whereas, the methanolic extract showed the maximum activity (17mm) was recorded from 200mg of fruit body extract against *Streptococcus mutans* followed by 16mm against *Staphylococcus aureus* and minimum (5mm) by 50mg of extract against *Pseudomonas aeruginosa*.

Table 1. Inhibition zone of methanol and aqueous extracts of fungal fruit bodies against bacterial pathogens.

Name of the pathogen	Zone of the Inhibition (mm)					
	Methanol extract (mg)			Aqueous extract (mg)		
	50	100	200	50	100	200
Escherichia coli	09 ± 1.4	12 ± 2.4	15 ± 2.4	24 ±1.4	28 ± 2.8	30 ± 3.7
Pseudomonas aeruginosa	05 ± 1.4	09 ± 1.4	14 ± 1.4	22 ± 2.8	23 ± 3.7	42 ± 2.4
Salmonella typhi	-	-	-	21 ± 1.4	24 ± 3.7	34 ± 2.8
Staphylococcus aureus	10 ± 2.4	12 ± 2.4	16 ± 2.8	24 ± 4.9	24 ± 3.7	29 ± 1.4
Streptococcus mutans	09 ± 2.4	11 ± 2.4	17 ± 2.8	19 ± 1.4	24 ± 3.7	30 ± 1.4

The zone of inhibition against fungal pathogens ranged between 20 - 5mm in aqueous extract and 35 – 3mm in methanolic extract. The maximum activity (20, 12mm) was recorded from 200mg of aqueous extract against *Aspergillus niger* and *Aspergillus flavus,* respectively and minimum (5mm) by *Aspergillus flavus* at 100 mg level whereas, the methanolic extract showed the maximum activity (35mm) was recorded from 200mg against *Aspergillus flavus* and minimum (3mm) by 50 mg against *Penicillium sp*. *Aspergillus fumigatous* was not respond for both methanol and aqueous extracts.

Discussion

Many pharmaceutical substances with potent and unique health-enhancing properties have been isolated from medicinal mushrooms and distributed worldwide (Cairney *et al*., 1999). Mushroom based products either from the mycelia or fruiting bodies are consumed in the form of capsules, tablets or extracts (Nitha *et al*., 2006).

In present study, the selected macrofungus showed antibacterial and antifungal activity in high level which was screened against the selected bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans* and the fungal species (*Penicillium* sp., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus*).

Table 2. Inhibition zone of Methanol and Aqueous extracts of fungal fruit bodies against fungal pathogens.

Name of the pathogen	Zone of the Inhibition (mm)					
	Methanol extract (mg)			Aqueous extract (mg)		
	50	100	200	50	100	200
Penicillium sp.	03 ± 1.4	09 ± 1.4	11 ± 3.7	-	-	-
Aspergillus fumigatous	-	-	-	-	-	-
Aspergillus niger	07 ± 2.8	14 ± 4.2	15 ± 2.4	11 ± 2.4	14 ± 4.9	20 ± 2.8
Aspergillus flavus	17 ± 2.8	18 ± 3.7	35 ± 2.4	-	05 ± 1.4	12 ± 2.4
Mucor indicus	09 ± 3.7	10 ± 2.4	14 ± 3.7	-	-	-

The methanol extract of *Phellinus merrillii* and *Phellinus swieteniae* showed marked activity against almost all strains of Acinetobacter *baumannii.* The diameter of zone of Inhibition (ZOI) of methanol extract of *P. merrillii* and *P. swieteniae* were 10.8 - 21.0 mm and 10.5 - 15.5 mm respectively. The ethyl acetate extracts of both *Phellinus* spp. showed moderate activity against all strains of *Acinetobacter*. The ZOI of ethyl acetate extract of *P. merrillii* and *P. swieteniae* were 10.6 - 16.7 and 11.2 - 22.2 mm, respectively. The MIC value of methanol extract of both species was found to be much higher as compared to ethyl acetate extract which suggests that methanol extracts of *Phellinus* spp. May not be effective for antibacterial activity against *Acinetobacter baumannii.* (Belsare, 2010). In our study the fruit body of *Phellinus* showed potential antimicrobial activities against the selected strains and the maximum activity (42mm) was recorded from 200mg of aqueous extract against *Pseudomonas aeruginosa* and minimum (5mm) by the above

pathogen at 50 mg of methanol extract whereas, the methanolic extract showed the maximum antifungal activity (35mm) was recorded from 200mg of extract against *Aspergillus flavus* and minimum (3mm) by 50 mg of extract against *Penicillium* sp.

The methanol extract shown the normal and minimum zone of inhibition against *Escherichia coli, Pseudomonas aeruginosa*, and maximum amount of inhibition was recorded against Steptrococcus *mutans,*. In other hand, against the fungal strain maximum zone was recorded against *Aspergillus flavus* and minimum zone against *Penicillum sp*. The antifungal activity depends upon the host defense system and drug reaction were factors looked into anti-microbial chemotheraphy but depression in host immune activity were ignored during antifungal drug screening (Gunji *et al*., 1983) .

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