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ANTIBACTERIAL AND ANTIFUNGAL POTENTIAL OF FRUIT BODY EXTRACTS FROM *DALDINIA CONCENTRICA* (BOLTON) CESATI & DE NOTARIS

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ABSTRACT

Aqueous and methanol extracts of fruit bodies of *Daldinia concentrica* were tested against five pathogenic fungi like *Penicillium* sps., *Aspergillus fumigatous*, *Aspergillus niger*, *Aspergillus flavus* and *Mucour indicus* and also five bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus mutans* . The antibacterial and antifungal activities of the above extracts were determined by well diffusion assay. Nearly both the extracts were found effective against these bacteria and fungi. The aqueous extract showed higher zone of inhibition than the methanol extract tested. The extracts exhibited antibacterial activities with zone of inhibition ranging from 14 - 54 mm and 5 - 23 mm for aqueous and methanol extracts whereas, antifungal activities zone of inhibition ranging from 9 - 25 mm and 7 - 18 mm, for aqueous and methanol extracts, respectively. The organisms were more sensitive to the aqueous extract of the fungal fruit bodies than that of methanol extract.

INTRODUCTION: Since the discovery of penicillin, a potent antibiotic produced by *Penicillium notatum* [1], a new area in natural product research has started. Fungi were noticed as a source of chemically new compounds with various biological activities. The isolation from soil was a common method to get fungal isolates. However, fungal strains from terrestrial sources yielded often already described secondary metabolites. Thus, fungi from new origins were needed. The marine environment offers a wide array of potential fungal sources including sediment, sand, driftwood, mangrove wood, sea water, algae, sponges and other invertebrates [2].

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 [3]. Many worldwide cultures, especially in the Orient, recognize that extracts from some edible and non-edible mushrooms are known for their potential health benefits. In China, the dietary supplements and nutraceuticals made from mushroom extracts are used, along with various combinations of other herbal preparations [4, 5].

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, ß glucans [6].

Furthermore, other bioactive substances such as triterpenes, lipids and phenols have also been identified and characterized in mushrooms with medicinal properties [7].

Many antibiotics in clinical use were isolated/ developed from fungi or the order Actinomycetales. Although production of important antibiotics such as penicillin, cephalosporin, and griseofulvin by fungi is well known, the occurrence of antibiotics in mushrooms is less well documented for discovery of new antibiotics with different structural types. It has been known since Greek and Roman antiquity that macro fungi are used as food and medicine and thus these may be a source of new and useful bioactive compounds [8].

Mushrooms are currently available in Taiwan, including *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. Ethanolic extracts, usually were more effective than hot water in antioxidant activity. However, for analyses of scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, hot water extracts were more effective in reducing power, scavenging ability on hydroxyl radicals, and chelating ability on ferrous ions [9].

MATERIALS AND METHODS:

Collection Fruit Bodies: A mushroom species - *Daldinia concentrica* (Bolton) Cesati & De Notaris was used in this study and collected from NCC ground, Govt. Arts College, Thiruvannamalai. This Xylariaceae member was identified by Department of Mycology, Centre for Advanced Studies in Botany, University of Madras, Chennai - 25, India.

Preparation of Crude Extract: Various extracts of the experimental fruit body was prepared according to the methodology of Indian Pharmacopoeia [10]. 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's extraction successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{o}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 *Mucour indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies:

Bacterial Media (Muller Hindon Media): Thirty Six grams of Muller Hindon 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. 20 g of dextrose was mixed with potato infusion. 20 g 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 [11]. 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^{o}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 results presented in **Table 1 and 2** showed the effect of aqueous and methanol fruit bodies extracts of *Daldinia concentrica* against the five test bacteria and fungi.

TABLE 1: INHIBITION ZONE OF METHANOL AND AQUEOUS EXTRACTS OF *DALDINIA CONCENTRICA* FRUIT BODIES AGAINST BACTERIAL PATHOGENS

Name of the pathogen	Zone of the Inhibition (mm)					
	Methanol extracts (mg)			Aqueous extract (mg)		
	50	100	200	50	100	200
Escherichia coli	11 ± 2.4	14 ± 3.7	16 ± 2.8	14 ± 5.1	23 ± 2.8	32 ± 2.8
Pseudomonas aeruginosa	14 ± 2.8	16 ± 2.4	23 ± 3.7	23 ± 2.4	23 ± 1.4	29 ± 4.9
Salmonella typhi	-	-	-		15 ± 3.7	22 ± 2.8
Staphylococcus aureus	08 ± 3.7	11 ± 1.4	15 ± 6.2	21 ± 2.4	28 ± 3.7	54 ± 4.9
Streptococcus mutans	05 ± 1.4	07 ± 2.8	11 ± 1.4	23 ± 3.7	24 ± 4.9	29 ± 3.7

TABLE 2: INHIBITION ZONE OF METHANOL AND AQUEOUS EXTRACTS OF *DALDINIA CONCENTRICA* FRUIT BODIES AGAINST FUNGAL PATHOGENS

Name of the pathogen	Zone of the Inhibition (mm)					
	Methanol extracts (mg)			Aqueous extract (mg)		
	50	100	200	50	100	200
Penicillium sp.	09 ± 1.4	10 ± 2.8	14 ± 5.1	10 ± 3.7	13 ± 3.7	25 ± 5.1
Aspergillus fumigatous	-		-	09 ± 2.8	14 ± 4.2	16 ± 1.4
Aspergillus niger	-	11 ± 2.4	13 ± 2.8	-	11 ± 1.4	15 ± 2.8
Aspergillus flavus	08 ± 2.8	11 ± 1.4	18 ± 3.7	17 ± 2.8	23 ± 3.7	25 ± 2.8
Mucor indicus	-	07 ± 2.4	16 ± 2.4	-	07 ± 1.4	15 ± 2.8

Antibacterial activity of aqueous fruit bodies extracts of *Daldinia concentrica* were showed effective zone of inhibition as compared methanol extract against the test bacterial pathogens. The maximum antibacterial activity of aqueous extract of whole fruit bodies of *Daldinia concentrica* was found 54 mm at 200 mg against *Staphylococcus aureus* and minimum 14 mm at 50 mg level against *Escherichia coli* whereas, in methanol extract showed maximum 23mm of inhibition zone establish at 200 mg of extract against *Pseudomonas aeruginosa* and the minimum inhibition zone (5 mm) was recorded at 50 mg level against *Streptococcus mutans*. There was no activity in *Salmonella typhi* against the methanol extract.

The significant antifungal activity of aqueous extract was found 25 mm at 200 mg against *Penicillium sp.* and *Aspergillus flavus* and less significant activity was found 9 mm at 50 mg against *Aspergillus fumigatous* but in the case of methanol extract showed maximum inhibition zone was produced 18 mm at 200 mg of extract against *Aspergillus flavus* and minimum activity (8 mm) was recorded from 50 mg of methanol extract of *D. concentrica* against *Aspergillus flavus*. *Aspergillus fumigatous* was not respond for methanol extracts.

DISCUSSION: The antibacterial and antifungal activities of extracts of fungal fruit body tested were recordable with pathogenic bacteria i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans* as well as, the pathogenic fungus such as *Penicillium* sps., *A. fumigatous*, *A. niger*, *A. flavus* and *M. indicus.* Both the bacterial and fungal pathogens were inhibited by the fruiting body extracts. Al-Fatimi *et al.,* (2006) used a paper disc method and stated that the fungus *Podaxis pistillaris* was found to exhibit a strong antibacterial activity against several Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Micrococcus flavus*, *Bacillus subtilis*, *Proteus mirabilis*, *Serratia marcescens* and *Escherichia coli*. Gbolagade and Fasidi (2005) found in their study that *Auricularia polytricha, Corilopsis occidentalis, Daldinia concentrica, Daedalea elegans* and *Tricholoma lobayensis* exhibited various degrees of antagonistic effects against the *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Microsporum boulardii.*

In the present study, the antibacterial and antifungal activities of the aqueous and methanol extracts were determined by well diffusion assay. The aqueous extract showed the highest and lowest zone of inhibition against *Staphylococcus aureus* was 54 mm at 200 mg concentrations, But in methanol extract, zone of inhibition of *Pseudomonas aeruginosa* was found to be the highest (23 mm) at 200 mg concentrations.

An antimicrobial activity of the extracts of *Cantharellus cibarius* Fr. against various bacteria and filamentous fungi including *F. oxysporum*, found that all the extracts showed more antifungal activities than antibacterial activities [14]. But in our study, we found that both the extracts showed more antibacterial activities than antifungal activities. It can therefore be suggested that crude extracts contain potential antimicrobial and antifungal compounds and the obtained results may also be useful for evaluating substances of interest. Further investigations to isolate and characterize the potential antimicrobial and antifungal compounds are underway.

REFERENCES:

1. Fleming A. On the antibacterial action of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. British Journal of Experimental Pathology 1929: 10**:** 226-236.
2. Jensen PR and Fenical W. Marine microorganisms and drug discovery: current status and future potential. In: Drugs from the Sea. (ed. Fusetani, N.) Basel, Karger**,** 2000**:** 6-29.
3. Moradali MF, Mostafavi H, Ghods, S, Hedjaroude GA. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macro fungi). International Immuno pharmacology 2007: 7: 701–724.
4. Barros L, Falcao S, Baptista P, Freire C, Vilas-Boas M, Ferreira ICFR. Antioxidant activity of *Agaricus* sp. Mushroom by chemical, biochemical and electrochemical assays. Food Chemistry 2008: 111: 61-65.
5. Carbonero ER, Gracher AHP, Smiderle FR, Rosado FR, Sassaki GL, Gorin PAJ, Iacomini MA. β-glucan from the fruit bodies of edible mushrooms *Pleurotus eryngii* and *Pleurotus ostreatoroseus*. Carbohydrate Polymers 2006: 66: 252–257.
6. Ooi VE and Liu F. Immunomodulation and anticancer activity of polysaccharide-protein complexes. Current Medical Chemistry 2000: 7: 715–729.
7. Maiti S, Bhutia SK, Mallick SK, Niyati Khadgi AK and Matti TK. Antiproliferative and immunostimulatory protein fraction from edible mushrooms. Environmental Toxicology and Pharmacology 2008: 26: 187–191.
8. Anke T. Basidiomycetes: A source of new bioactive secondary metabolites. Progress in Industrial Microbiology 1989: 27: 51-66.
9. Tsai SY, TsaI HL and Mau JL. Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea*, and *Boletus edulis*. LWT – Food Science and Technology 2007: 40, 1392–1402.
10. Anonymous. Indian Pharmacopoeia, 2nd ed. Government of India, NewDelhi, 1966: 23.
11. Bauer HW, Kirby WMM, Slerris JC and Truck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 1996: 45: 493-496.
12. Al-Fatimi MAA, Julich WD, Jansen R and Lindequist U. Bioactive components of the traditionally used mushroom Podaxis pistillaris. eCAM. 2006: 3: 87-92.
13. Gbolagade JS, Fasidi IO. Antimicrobial activities of some selected Nigerian mushrooms. African Journal of Biomedical Research 2005: 8: 83-87.
14. Dulger B, Gonuz A and Gucin F. Antimicrobial activity of the macrofungus *Cantharellus cibarius*. Pakisthan journal of biological science 2004: 7(9): 1535-1539.

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