



Antimicrobial Activity of Whole Fruiting Bodies of *Trametes hirsuta* (Wulf. Fr.) Pil. Against Some Common Pathogenic Bacteria and Fungus

E. Sivaprakasam, D. Kavitha, R. Balakumar, S. Sridhar*, J. Suresh Kumar

Department of Botany, Govt. Arts College, Thiruvannamalai – 606 603, Tamil Nadu, India

ABSTRACT

Aqueous and methanolic extracts of fruit bodies of *Trametes hirsuta* were tested against five pathogenic fungi like *Penicillium* spp., *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* and *Mucor indicus* and five bacterial stains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus mutans*. The antibacterial and antifungal activities of all these extracts were demonstrated by well diffusion assay. Nearly all the extracts were found effective against these bacterial and fungal strains. The minimum inhibitory concentration of the various extract range from 200 mg to 50 mg on tested bacteria and fungi. The maximum antibacterial activity of aqueous extract of whole fruit bodies of *Trametes hirsuta* was found 33 mm at 200 mg against *Staphylococcus aureus* than that of methanol extract. The significant antifungal activity of aqueous extract was found 46 mm at 200 mg against *Aspergillus flavus* than that of methanol extract. The antimicrobial activity was showed at concentration dependent.

Keywords: *Trametes hirsuta*, fruit bodies, antibacterial, antifungal, well diffusion.

INTRODUCTION

Basidiomycetes could be a good source of new antibacterials and antifungals. [1-2] Already, a number of antibacterial compounds have been isolated from Basidiomycete fungi, including Illudin [3], Collybial [4], Pleurotin [5], Drosophilin A [6] and Frustulosin. [7]

Trametes hirsuta (Wulf. Fr.) Pil. (Polyporaceae) is a species of Basidiomycota which belongs to Polyporaceae of Polyporales. *Trametes* means "one who is thin"; *hirsuta* means "hairy with rather coarse, erect or ascending hairs." Occurrence on wood substrate Saprobiic; solitary or clustered, often overlapping, mostly on dead deciduous wood; year-round. Caps 1.5-10 cm wide, 1.5-6 cm long, and up to 2 cm thick. Densely hairy; grayish to yellowish or brownish, often with brownish margin; zonate or not; usually concentrically grooved.

Mushrooms and polypores are rich source of natural antibiotics. The cell wall glucans are well known for their immunomodulatory properties, and the secondary metabolites are active against bacteria [8] and viruses. [9] Exudates from mushroom mycelia are active against protozoa such as the malaria parasite *Plasmodium falciparum*. [10] Since humans and fungi share common microbial antagonists such as *Escherichia coli*,

Staphylococcus aureus and *Pseudomonas aeruginosa*, humans can benefit from the natural defense strategies of fungi to produce antimicrobials. [11] The general hypothesis increasingly substantiated is that polypores provide a protective immunological shield against a variety of infectious diseases. [12] A novel compounds derived from fungi of genus *Verticillium*, especially the fungus *Verticillium balanoides* useful for inhibiting activity of enzyme protein kinase C. Since the activation of protein kinase C has been implicated in several human disease processes, including cancer tumors, inflammation, and reperfusion injury, inhibition of protein kinase C should be of great therapeutic value in treating these conditions. [13]

Macro fungi that have been implicated of having curative effect against diseases such as high blood pressure, pneumonia, urinary tract infection, intestinal disorder by Nigerian herbalists include *Ganoderma lucidum*, *Fomes lignosus*, *Daldinia concentrica*, *Termitomyces* species, *Pleurotus* species, *Lycoperdon* species *Polyporus* species, *Calvatia cyathiformis* and *Psathyrella atroumbonate*. [14]

The aim of the present work is to evaluate the antibacterial and antifungal activity of *Trametes hirsuta*. Extracts with the help of Methanol and Aqueous against to bacterial and to the fungal species were investigated.

MATERIALS AND METHODS

Collection Fruit Bodies

A mushroom species-*Trametes hirsuta* (Wulf. Fr.) Pil. was used in this study and collected from NCC ground, Govt.

*Corresponding author: Dr. S. Sridhar, Asst. Professor, Department of Botany, Govt. Arts College, Thiruvannamalai - 606 603, Tamil Nadu, India; Tel.: +91-9443105935; E-mail: sekarsridhar@rediffmail.com

Arts College, Thiruvannamalai. These Basidiomycota 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. [15] 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 fumigatus*, *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 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. 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. [16] 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.

RESULT AND DISCUSSION

The first antimicrobial agent (antibiotic) to be produced was Penicillin, and it was discovered through the sheer serendipity of Alexander Fleming in 1928. This was derived from the ascomycetous fungus *Penicillium notatum*. The antibiotic was put into mass production and large scale therapeutic use because of the scale up work subsequently carried out by Howard Florey and Ernst Chain in the 1940s, and this work was supported by the necessity to cure wounded soldiers of infections during the II world war. [17]

In present investigation was to evaluate the antimicrobial activity of methanol and aqueous extract of whole fruit bodies of *Trametes hirsuta* by well diffusion against five bacterial and five fungal species. 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. The aqueous extract was better than methanol extract against bacteria as well as fungal pathogens. The maximum antibacterial activity of aqueous extract of whole fruit bodies of *Trametes hirsuta* was found 33 mm at 200 mg against *Staphylococcus aureus* and minimum 5mm at 50 mg level against *Escherichia coli* whereas, in methanol extract showed maximum 21mm of inhibition zone establish at 200 mg of extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the minimum inhibition zone (6mm) was recorded at 50 mg level against *Salmonella typhi*. The significant antifungal activity of aqueous extract was found 46 mm at 200 mg against *Aspergillus flavus* and less significant activity was found 11 mm at 100 mg against *Aspergillus fumigatus* but in the case of methanol extract showed maximum inhibition zone was produced 24mm at 200 mg of extract against *Aspergillus flavus* and minimum activity (7mm) was recorded from 50 mg of methanol extract of *T. hirsuta* against *Aspergillus niger* and *Mucor indicus*.

As on today, the important antibiotics derived from fungi, other than Penicillin, are: Cephalosporin from *Cephalosporium* spp., Griseofulvin from *Penicillium griseofulvum*, Lentinan from *Lentinus* sp., and Schizophyllan from *Schizophyllum commune*. Penicillin and Cephalosporin are antibacterial antibiotics acting against Gram-positive bacteria, whereas, Griseofulvin is an antifungal antibiotic useful in treating dermatophyte infections. Lentinan is active against *Mycobacterium tuberculosis*, *Listeria* sp., and Herpes Simplex Virus-1 (HSV-1). Schizophyllan is both antibacterial and antifungal in activity. It is useful in controlling *Candida albicans* and *Staphylococcus aureus*. Mushrooms and polypores are rich source of natural antibiotics. The cell wall glucans are well known for their immunomodulatory properties, and the secondary metabolites are active against bacteria. [8] It is also necessary for successful separation, purification and characterization of biologically active compounds using chromatographic and spectroscopic techniques for the synthesis novel antibiotics.

Table 1: Inhibition zone of Methanol and Aqueous extracts of *Trametes hirsuta* fruit bodies against bacterial pathogens

S. No	Name of the pathogen	Zone of the Inhibition (mm)					
		Methanol extract (mg)			Aqueous extract (mg)		
		50	100	200	50	100	200
1.	<i>Escherichia coli</i>	10 ± 1.4	12 ± 2.4	19 ± 3.7	05 ± 1.4	20 ± 2.4	24 ± 1.4
2.	<i>Pseudomonas aeruginosa</i>	13 ± 3.7	15 ± 2.4	21 ± 1.4	19 ± 1.4	22 ± 2.4	25 ± 3.7
3.	<i>Salmonella typhi</i>	06 ± 1.4	11 ± 2.4	19 ± 3.7	14 ± 5.1	19 ± 5.1	24 ± 4.9
4.	<i>Staphylococcus aureus</i>	07 ± 2.8	10 ± 2.8	21 ± 2.4	19 ± 3.7	22 ± 2.8	33 ± 3.7
5.	<i>Streptococcus mutans</i>	09 ± 2.8	13 ± 2.8	15 ± 3.7	12 ± 2.8	16 ± 1.4	19 ± 5.1

Table 2: Inhibition zone of Methanol and Aqueous extracts of *Trametes hirsuta* fruit bodies against fungal pathogens

S. No	Name of the pathogen	Zone of the Inhibition (mm)					
		Methanol extract (mg)			Aqueous extract (mg)		
		50	100	200	50	100	200
1.	<i>Penicillium sp.</i>	16 ± 2.8	17 ± 1.4	19 ± 3.7	14 ± 5.1	24 ± 3.7	27 ± 2.8
2.	<i>Aspergillus fumigatus</i>	09 ± 3.7	09 ± 2.4	14 ± 5.1	-	11 ± 2.4	13 ± 3.7
3.	<i>Aspergillus niger</i>	07 ± 1.4	09 ± 4.2	15 ± 2.8	-	12 ± 2.8	21 ± 1.4
4.	<i>Aspergillus flavus</i>	11 ± 2.4	13 ± 2.8	24 ± 3.7	20 ± 3.7	38 ± 2.8	46 ± 2.8
5.	<i>Mucor indicus</i>	07 ± 2.8	15 ± 5.1	17 ± 2.4	-	05 ± 1.4	18 ± 1.4

REFERENCES

- Lorenzen K, Anke T. Basidiomycetes as a source for new bioactive natural products. *Curr Org Chem.* 1998; 2: 329-364.
- Luo DQ, Wang F, Bian XY, Liu JK. Rufuslactone, a new antifungal sesquiterpene from the fruiting bodies of the basidiomycete *Lactarius rufus*. *J Antibiot.* 2005; 58: 456-459.
- McMorris TC, Anchel M. Fungal metabolites. The structures of the novel sesquiterpenoids illudin S and M. *J Am Chem Soc.* 1965; 87: 1594-1600.
- Simon B, Anke T, Anders U, Neuhaus M, Hansske F. Collybia, a new antibiotic sesquiterpenoid from *Collybia confluens* (Basidiomycetes). *Z Naturforsch C.* 1995; 50: 173-180.
- William JR, Frederick K, Annette H. Antibiotic substances from basidiomycetes: I. *Pleurotus griseus*. *Proceedings of the National Academy Sciences USA,* 1947, 33: 171-176.
- Marjorie A. Identification of drosophilin A as p-methoxytetra-chlorophenol. *J Am Chem Soc.* 1952; 74: 2943.
- Jordan KZ. Biologically active compounds from Aphylophorales (Polypore) fungi. *J Nat Prod.* 2004; 67: 300-310.
- Kupra J, Anke T, Oberwinkler G, Schramm G, Steglich W. Antibiotics From basidiomycetes VII. *Crinipellis stripitaria*(Fr.) Pat. *Journal of Antibiotics* 1979; 32: 130-135.
- Brandt CR, Piriano F. Mushroom antivirals. *Recent Research Developments for Antimicrobial Agents and Chemotherapy* 2000; 4: 11-26.
- Isaka M, Tantichareon M, Kongsaree P, Thebtaranonth Y. Structures of cordypyridones A-D, antimalarial N-hydroxy- and N-methoxy-2-pyridones from the insect pathogenic fungus *Cordyceps nipponica*. *Journal of Organic Chemistry* 2001; 6: 4803-4808.
- Hardman A, Limbird L, Gilman A. *The Pharmacological Basis of Therapeutics.* Edn 10, McGraw Hill, New York. 2001
- Mizuno T, Saito H, Nishitoba T, Kawagishi H. Antitumor active substances from mushrooms. *Food Reviews International* 1995; 11: 23-61.
- Damjan J, Samo K, Maja J, Katja S, Borut S. Antibacterial activity in higher fungi (mushrooms) and endophytic fungi from Slovenia. *Pharmaceutical Biology* 2007; 45(9): 700-706.
- Jonathan SG. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph.D. Thesis University of Ibadan. Ibadan, Nigeria. 2002
- Anonymous 1966. *Indian Pharmacopoeia*, 2nd ed. Government of India, New Delhi, 23.
- Bauer HW, Kirby WMM, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* 1996; 45, 493-496.
- Abraham EP. The beta-lactam antibiotics. *Scientific American* 1981; 244, 76-86.