J. Mycopathol, Res, ***49(2)*** : *329-332 (2011)*

Antimicrobial activity of *Pleurotus djamor*

SANDEEP ACHARYA[1] AND AJAY KRISHNA SAHA[2]
[1]*Plant Physiology and Biochemistry Laboratory, Department of Botany, Tripura University, Suryamaninagar,*
[2]*Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University, Suryamaninagar, West Tripura-799130, India.*

Received : 25.05.2011 Accepted : 30.08.2011 Published :24.10.2011

In vitro antimicrobial activity of the ethanolic extract of wild edible mushroom *Pleurotus djamor* (Fr.) Boedijn was examined in Potato Dextrose Agar (PDA) and Nutrient Agar (NA) media following the filter paper disc diffusion method. The microorganism studied was a plant pathogenic forms of *Ralstonia solanacearum* (Smith.) Yabuuchi *et al.* (bacteria) and *Magnaporthe grisea* (T.T.Hebert) M.E. Barr (fungi). The paper disc study was performed against a standard tetracycline (10 µg/disc for bacteria) and greseofulvin (10 µg/disc for fungi). The 50% ethanolic crude extracts prepared from the wild edible mushroom *Pleurotus djamor* showed better inhibition compared to aqueous extract (control) and Tetracycline and Greseofulvin (as standard antibiotics). The *Ralstonia solanacearum* (diameter of growth inhibition zone, 18.3 mm) was found to be most vulnerable towards the extract of juvenile stage of the mushroom in case of Nutrient Agar (NA) medium. The extract of juvenile stage of the mushroom *Pleurotus djamor* showed its best effect towards the plant pathogenic fungus *Magnaporthe grisea* (diameter of growth inhibition zone, 22.4 mm) used in case of Potato Dextrose Agar (PDA) medium.

Key words: *Pleurotus djamor* (Fr.) Boedijn., antifungal and antibacterial effect

INTRODUCTION

There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Nair and Chanda, 2007). This situation provides the impetus to the search for new antimicrobial substances from various sources like medicinal plants and mushrooms (Cordell, 2000). Now-a-days, there is a renewed interest in traditional medicine and increasing demand for more drugs from fungal source.

Macrofungi have long been used as a valuable food source and as traditional medicines around the world since ancient times (Sagakami *et al.*, 1991; Wasser and Weis, 1999). 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. A number of medicinal mushrooms are rich sources of natural antibiotics, where the cell wall glucans are well known for their immunomodulatory properties and many of the externalized secondary metabolites combat bacteria, fungi and viruses (Suzuki *et al.*,1990; Gao *et al.*, 2005; Lindequist *et al.*, 2005) and also have been used extensively in traditional medicine for curing various human ailments (Jagadish *et al.*, 2008; Jagadish *et al.*, 2009). Extract of *Pleurotus djamor* has been reported to inhibit proliferation of hepatoma cells and breast cancer cells *in vitro* (Wu *et al.*, 2010). Viewing this encouraging and successful reports, it is assumed that pan tropical white variety of *Pleurotus djamor* may possess some antimicrobial mechanism against

[1]E-mail: phytosandeep@yahoo.com;
[2]E-mail: aks.58@rediffmail.com

plant pathogens. As such, a screening test is conducted *in vitro* for antimicrobial activity of *P. djamor.*

MATERIALS AND METHODS

Collection and preparation of mushroom extracts

Wild mushrooms of two stages (juvenile and mature) were collected at the end of the rainy season from northern tropical moist deciduous forests of Tripura (20°51'-24°32' N latitude and 90°10'-92°21' E longitude) during July-August, 2010. Mushrooms were identified up to species level, using morphological and anatomical characteristics. Mushrooms samples were sent to Mycological Research Laboratory of ICAR, Lembucherra, for identification and were identified as *Pleurotus djamor* (Fr.) Boedijn. (white ecological variety). In order to minimize variability between individuals from the same species, all basidiocarps from the same species were homogenized according to the modified method of Alvarez-Parrilla *et al.* (2007). Two different stages of basidiocarp were used for experimental purpose and expressed in the following terms: juvenile (one day old) and mature (two days old). The method of Alade and Irobi (1993) with little modification (Santhi *et al.,* 2006) was adopted for preparation of mushroom extracts in 50% ethanol. For preparation of mushroom extracts, basidiocarps of each stages of same age (10 g) were collected in fresh condition and kept on ice 4-6 hrs for transportation to the laboratory and washed with tap water followed by distilled water. Then they were cut, weighed and frozen at -10°C for 1 day, lyophilized for 48 hrs (Labconco Freeze dry/shell freeze system), milled and stored at -10°C. The basidiocarps were ground separately in a mechanical grinder and coarse powder was prepared. An amount of 1 g of mushroom powder was extracted with 3 ml of 50% ethanol for 10 minutes using a mortar and pestle. The extracts thus obtained were subjected to low speed centrifugation (5000 rpm for 10 min) and the clear supernatants were collected and the extracts were used for paper disc diffusion method. Approximately, 10 µl per ml of crude 50% ethanol extract was impregnated in each of the paper disc.

Preparation of bacterial pathogen

The pure cultures of *Ralstonia solanacearum* were prepared from infected brinjal (*Solanum melongena* L. var. Bejo Sheetal, a local variety). The said culture was identified by IMTECH, Chandigarh. The antibacterial activities of the pure mushroom extracts were evaluated using nutrient agar medium in Petriplates. After solidification, pure isolates of *Ralstonia solanacearum* was inoculated in the plate using pour plate technique (Khan and Siddiqui, 2007). The *in vitro* antibacterial properties of pure mushroom extracts were tested by disc diffusion method (Nene and Thapliyal,1979). In this method, the medium (20 ml) inoculated with suspension of bacteria was poured into sterilized Petri dishes and left to gel at room temperature. Whatman No. 4 filter paper discs (6 mm diameter) was soaked in 10 µl 50% ethanol extract for use. The filter paper discs were placed equidistantly on inoculated medium and diffusion of solution was allowed to occur for 30 minutes at room temperature. Plates were incubated at 25 ± 2°C for 24 hrs. Ten plates were employed per treatment and the average zone of inhibition was recorded. Tetracycline (having 10 µg/disc) was used as standard for reference purpose and tested against the test organism.

Preparation of fungal pathogen

Again, antifungal activity of crude dried mushroom extracts in 50% ethanol was studied by disc diffusion method using Potato Dextrose Agar (PDA) medium (Nene and Thapliyal,1979). The pure culture of the fungal pathogen was obtained from Indian Microbial Type Culture Collection (IMTECH), Chandigarh. Potato Dextrose Agar (PDA) medium was sterilized at 121°C (for 15 min.) in autoclave. Pure isolates of *Magnaporthe grisea* was inoculated in the plate using pour plate technique (Khan and Siddiqui, 2007). About 20 ml PDA medium was poured into ten separate sterilized Petriplate in triplicates. After solidification, fungal isolates were inoculated on PDA medium by streak plate method (Vishunavat and Kolte, 2005) under aseptic condition. Whatman No. 4 filter paper discs (6 mm diameter) were soaked in 10 µl 50% ethanol extract for use. The filter paper discs were placed equidistantly on inoculated medium and diffusion of solution was allowed to occur for 30 min at room temperature. Plates were incubated at 25 ± 2°C. Ten plates were employed per treatment and the average zone of inhibition of mycelial growth after 3 days of incubation was recorded for each treatment. Greseofulvin (having 10 µg/disc) was used as standard for ref-

erence purpose and tested against the test organisms.

Antimicrobial Activity

The *in vitro* antimicrobial activity of mushroom extracts was evaluated by disc diffusion (DD) method and tried for at least three times to confirm accuracy (Tables 1 and 3). An amount of 10 µg/per ml extract was poured over each disc (diameter 6 mm) by micropipette. The bacterial ooze was spread by cotton swab (earlier autoclaved) moistened with bacterial solution and the fungal mycelia was spread by fine inoculation needle (earlier autoclaved) by streaking. The extracts were reconstituted in DMSO and applied on Whatman No. 4 filter paper. Whatman paper No.4 discs (6 mm diameter) were prepared using special quality absorbent paper of 1 mm thickness. The discs were soaked in standard amount (10 µg /ml) of crude mushroom extract or antibiotic agent. Clear discs (without any knot, spot or stain) were selected for impregnation of crude extracts (Nene and Thapliyal, 1979).

Statistical analysis

Values were presented as the mean ± SEM. For standard error of the mean (SEM), the obtained results (data) were statistically analyzed according to Steel and Torrie (1980). ANOVA analyses were performed in order to determine differences between various stages of mushrooms at 5% significant level, using the commercial software SPSS 13.0 (SPSS Inc. Headquarters Chicago, Illinois, USA).

RESULTS AND DISCUSSION

In the present investigation, the wild edible mushroom *Pleurotus djamor* (Fr.) Boedijn showed considerable inhibitory effect against both bacterial and fungal pathogens. Against *Ralstonia solanacearum,* the juvenile mushroom stage showed better inhibition than the mature stage as compared against Tetracycline as standard antibiotics. Similarly, against *Magnaporthe grisea,* the juvenile mushroom stage showed higher inhibitory effect compared to the mature stage and Greseofulvin as a standard antibiotics. The juvenile mushroom stage showed better inhibition than the mature stage due to presence of polyphenolics, flavonoids etc. and towards maturity, phenols and flavonoids were used to regenerate other secondary metabolites and metabolic derivatives like organic acids (Murcia *et al.*, 2002; Yang *et al.*, 2002; Cheung *et al.*, 2003; Song *et al.*, 2003). Different types of antibacterial and antifungal compounds were being extracted from mushrooms against plant and human pathogenic microorganisms. Cieniecka-Roslonkiewicz *et al.* (2007) reported antimicrobial activity of *Cantharellus cibarius* against certain microbes, bacteria, and fungi. *Cordyceps* spp. were found active against plant pathogens viz., *Bipolaris maydis*, *Mycosphaerella arachidicola*, and *Rhizoctonia solani* (Wong *et al.*, 2010).

Statistical analyses considering the antibacterial effect and antifungal effect of mushroom extract with that of two stages of wild edible mushroom *P. djamor* in each experimental set (one-way ANOVA) revealed that calculated value of F (112.241) for two stages of wild edible mushroom *P. djamor* in 50 % ethanol extract was much higher than tabulated value of F (3.107) and calculated value of F (132.453) for two stages of wild edible mushroom *P. djamor* in 50 % ethanol extract was much higher than tabulated value of F (3.107) respectively and hence it can be concluded that antibacterial and antifungal effect of mushroom between two stages of wild edible mushroom *P. djamor* was found significant with their fungicidal properties. The results from this study suggest that the extracts of *P. djamor* had strong inhibitory effect against *R. solanacearum* and *M. grisea* under *in vitro* condition.

Table 1. Antibacterial effect of stipe connecting pileus part of *Pleurotus djamor* against *Ralstonia solanacearum*

Mushroom sample	Zone of Inhibition (mm)	
	Disk (10 µg)	Tetracycline (10 µg)
Juvenile	18.32 ± 0.14	24.53 ± 0.89
Mature	17.30 ± 0.08	24.12 ± 0.75

**All the readings are based on replicates ± SEM; F value 112.24 (F value 0.05-3.10)

Table 2. Antifungal effect of stipe connecting pileus part of *Pleurotus djamor* against *Magnaporthe grasea*

Mushroom sample	Zone of Inhibition (mm)	
	Disk (10 µg)	Tetracycline (10 µg)
Juvenile	22.47 ± 0.02	24.10 ± 0.14
Mature	19.65 ± 0.05	24.25 ± 0.21

*All the readings are based on replicates ± SEM. F value 132.45 (F value 0.05-3.10)

REFERENCES

Alade, P.I., and Irobi, O.N.1993.Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiama*. *J. Ethnopharmacol.* **39**:171-174.

Alvarez-Parrilla E., de la Rosa L. A., Martínez N. R and González Aguilar G. A. 2007. Total phenols and antioxidant activity of commercial and wild mushrooms from Chihuahua, Mexico, *Ciencia y Tecnol. Aliment.* **5(5)**, 329-334.

Cheung L.M, Cheung P.C.K and Ooi V.E.C. 2003. Antioxidant activity and total phenolics of edible mushroom extracts, *Food Chem*. **81**, 249-255.

Cieniecka-Roslonkiewicz, A., Sas, A., Przybysz, E., Morytz, B., Syguda, A., and Pernak, J. 2007. Ionic liquids for the production of insecticidal and microbicidal extracts of the fungus *Cantharellus cibarius*. *Chemistry & Biodiversity,* **4**: 2218–24.

Cordell, G.A. 2000. Biodiversity and drug discovery symbiotic relationship. *Phytochem.,* **55**: 463-480.

Gao, Y., Tang, W., Gao, H., Chan, E., Lan, J., Li, X., and Zhou, S. 2005. Antimicrobial activity of the medicinal mushroom *Genoderma*. *Food Rev. Int.,* **21**: 211-229.

Jagadish, L.K., Shenbhagaraman, R., Venkatakrishnan, V., and Kaviyarasan, V. 2008. Studies on the phytochemical, antioxidant and antimicrobial properties of three indigenous *Pleurotus* species. *J. Mol. Biol. Biotechnol.,* **1**: 20-29.

Jagadish, L.K., Krishnan, V.V., Shenbhagaraman, R., and Kaviyarasan, V. 2009. Comparative study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J. E. Lange) Imbach before and after boiling. *Afr. J. Biotechnol.,* **8**(4): 654-661.

Khan, M., and Siddiqui,M. 2007. Antimicrobial activity of piper fruits, *Nat. Prod. Rad.* **6**(2):111-113.

Lindequist, U., Niedermeyer, T.H.J., and Julich, W.D. 2005. The pharmacological potential of mushrooms. *Evi. Compl. and Alt. Medicine,* **2**(3): 285-299.

Murcia, M.A., Martinez-Tome, M., Jimenez, A.M., Vera, A.M., Honrubia, M. and Parras, P.J. 2002. Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing. *Food Prot.,* **65**, 1614-1622.

Nair, R., and Chanda, S.V. 2007. Antibacterial activities of some medicinal plants of Western Region of India. *Turk. J. Biol.,* **31**: 231-236.

Nene,Y.L., and Thapliyal, P.N. 1979. In: *Fungicides in plant diseases control.* Oxford and IBH publishing Co., New Delhi, pp.415-416.

Sagakami, H., Aohi, T., Simpson, A., and Tanuma, S. 1991. Induction of immuno-potentiation activity by a protein-bound polysaccharide PSK. *Anticancer Res.,* **11**: 993-1000.

Santhi, R., Alagesaboopathi and Rajasekara Pandian, M. 2006. Antibacterial activity of *Andrographis lineata* Nees and *Andrographis echioides* Nees of the Shevaroy Hills of Salem district,Tamil Nadu. *Ad.Plant Sci.* **19**(2), 371-375.

Song, Y.S., Kim, S.H., Sa, J.H., Jin, C., Lim, C.J. and Park, E.H. 2003. Antiangiogenic, antioxidant and xanthine oxidase inhibition activities of the mushroom *Phellinus linteus*. *J. Ethnopharmacol.,* **88**, 113-116.

Steel, R.G.D., and Torrie, T.H. 1980. In: *Principles and procedures of statistics.* McGraw- Hill Book Co. Inc., New York.

Suzuki, H., Iiyama, K., Yoshida, O., Yamazaki, S., Yamamoto, N., and Toda, S. 1990. Structural characterization of immunoactive and antiviral water-solubilized lignin in an extract of the culture medium of *Lentinus edodes* mycelia (LEM). *Agric. Biol. Chem.,* **54**(2): 479-487.

Vishunavat, K., and Kolte, S.J. 2005. In: *Essentials of Phytopathological Techniques,* Kalyani Publishers, New Delhi, India.

Wasser, S.P., and Weis, A.L. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: Current perspectives (Review). *Int.J. Med. Mushoom,* **1**: 31-62.

Wong, J. H., Ng, T. B., Wang, H., Sze, S.C.W., Zhang, K.Y., Li, Q., and Lu, X. 2010. Cordymin, an antifungal peptide from the medicinal fungus *Cordyceps militaris*. *Phytomedicine,* **18**(5): 387.

Wu, X., Zheng, S., Cui, L., Wang, H., and Ng, T.B. 2010. Isolation and characterization of a novel ribonuclease from the pink oyster mushroom *Pleurotus djamor*. *J. Gen. Appl. Microbiol.,* **56**(3): 231–239.

Yang J.H., Lin H.C and Mau J.L 2002. Antioxidant properties of several commercial mushrooms, *Food Chem*. **77**, 229-235.