ANALYSIS OF THE ANTIBACTERIAL ACTIVITY OF FIVE MUSHROOMS ON CLINICAL ISOLATES AND COMPARISON OF THE EFFICACY OF THEIR VARIOUS EXTRACTS

DIVYA IYER, SANTHINI.S.NAIR

Abstract: The need for less toxic, more potent and non anti - infective antibiotics, as well as the evolving resistance of microorganisms are some of the medical areas that have posed a challenge to therapeutics since 1990s. These factors have the combined effect of injecting a sense of urgency into the search for new bioactive compounds. Drug resistance is more frequently encountered in hospital – acquired pathogens. The basidiomycetes (mushrooms) are valuable as gene pool sources, which have not yet been the subject of extensive screening for any possible antifungal and antibacterial activity. Medicinal mushroom research has focused on discovery of compounds that can modulate positively or negatively the biological response of immune cells.

In this study, the antimicrobial activity of various solvent extracts of 5 Mushrooms viz .*Agaricus bisporus, Ganoderma lucidum, Pleurotus sajorcaju, Auricularia auricular* and a local mushroom from Gondia, Nagpur were assessed *in vitro* for their ability to inhibit clinical pathogens. Clinical samples of stool, urine, pus and sputum from patients were screened for to isolate the pathogens. Total of 11 species of bacteria, 9 Gram negatives: 2 pathogenic strains of *Pseudomonas*, 1 strain each of *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Proteus vulgaris, Proteus mirabilis* and 2 Gram positives: *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from the clinical samples were used for the study. The solvents used were ethanol, chloroform and petroleumether. Chloroform extract exhibited maximum antibacterial activity.*Auricularia auricular was* most potent and capable of inhibiting the growth of 9 of the 11 isolates.

Keywords: Mushroom,*Agaricus bisporus, Ganoderma lucidum, Pleurotus sajorcaju, Auricularia auricula*, antibacterial activity, clinical isolates.

Introduction: For centuries, people have enjoyed mushrooms for their flavor, texture and mystique. When used as medicine, mushrooms are made into soup or tea, or taken as a tonic or elixir. Studies conducted over the past 30 years mostly in Asia have provided data suggesting that mushrooms or substances extracted from mushrooms may aid in the treatment of certain types of cancer, boost the immune system and reduce the risk of coronary heart disease. Mushrooms contain a wide variety of bioactive molecules including terpenoids, steroids, phenols, nucleotides and their derivates, glycoproteins, and polysaccharides. Mushrooms also accumulate a variety of secondary metabolites which have been found to have potential antibacterial property. Research has shown that mushroom metabolites tested on some bacteria exhibited inhibitory responses against gram positive and gram negative organisms [1]-[2]

Ganoderma lucidum, a mushroom, is one of the

most famous traditional Chinese medicinal herbs. One interesting aspect of its performance is antimicrobial effect due to the extracts derived from this mushroom which contain bacteriolytic enzyme, lysozyme and acid protease (Fig no 1) [3]-[4]-[5].

Auricularia auricular widely known as Jew's ear is widely used in China for food and medicine. It contains polysaccharides that have been used as immune toxins, anticoagulants and to lower cholesterol (Fig no. 1). [6]

Figure 1: Fruiting bodies of the Mushrooms used in the study.

(1) Local mushroom from Gondia, (2) *Auricularia auricula*, (3)*Ganoderma lucidum*, (4) *Agaricus bisporus*, (5) *Pleurotus sajorcaju*

Pleurotus sajorcaju, the common oyster mushroom has been studied for its role in numerous diseases with its anti-cancer activity, immunomodulating effects, and antiviral, antibiotic and anti-inflammatory activities (Fig no 1)[7].

Agaricus bisporus, white button mushrooms promotes innate immunity against tumors and viruses through the enhancement of a key component, natural killer (NK) cell activity (Fig no 1). [7]

Aim of this study is to screen five mushrooms namely *Agaricus bisporus, Ganoderma lucidum, Pleurotus sajorcaju*, *Auricularia auricula* and a local mushroom from Gondia, Nagpur for their antimicrobial activity against human pathogens isolated from clinical specimens and screening of such compounds effective against common bacterial infections and also due to drug resistant bacteria.

Material and Methods:

Test Microorganisms:

Clinical isolates isolated from patients suffering from varied common infections like UTI, Diarrhea, skin infections and burns. Samples were collected from at Dr. Dhiren Shah's Diagnostic and Research Centre, Ghatkopar.

The samples were screened and following isolates were isolated nine Gram negative bacteria (*Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa* isolated from urine, *Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B* from stool sample, *Pseudomonas spps, Klebsiella pneumonia* isolated from sputum and 2 gram positive bacteria viz *Staphylococcus aureus* isolated from pus and *Streptococcus pyogenes* was isolated from sputum.

Mushroom samples *G.lucidum, P.sajorcaju, A.auricular* were obtained from Aarya Biotech, Ahmedabad. *A.bisporus* was obtained from a local store. The local mushroom from Gondia was collected from Ruchi Oyster Mushroom, Gondia.

Media, Standards & Reagents:

Chloroform, Ethanol, Petroleum ether was of analytical grade purity from Merck. All culture media, reagents and stains were obtained from HiMedia Laboratories.

Extracts preparation:

50 grams of sunlight dried fruiting body masses of the mushrooms were obtained. They were ground to a fine powder using a mortar and pestle. The extraction of the mushroom was carried out using three solvents [ethanol, chloroform, petroleum ether]. All the extracts used were crude extracts.[8]-[9]

Ethanol / Chloroform extract:

For ethanol and chloroform extract, 10 grams of

the dried mushroom powder was soaked in 100ml of absolute alcohol and chloroform inside 250 ml flasks respectively. They were covered with aluminum foil to prevent evaporation and allowed to stand for 7 days for extraction. The mixture was then filtered using Whatman filter paper no. 1. This filtrate was stored at 4°C in screwed capped bottles for further use. The concentration of the extract was 10mg / ml. [10]

Petroleum ether extract:

10g of the extraction solvent petroleum ether was added to a 250 ml round bottom flask and Soxhlet extraction was carried out in a hot mantle for a period of 4 hrs. After the extraction was complete, this filtrate was stored at 4°C in screwed capped bottles for further use. The concentration of the extract was 10mg / ml.[5]-[9]

Antibacterial activity:

Antibacterial activity of mushroom extract was carried out by the Agar cup diffusion technique. To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard was used. 0.1 ml of test organism was added to 20 ml molten & cooled Sterile Mueller – Hinton Agar butt, mixed and poured into assay plates .Agar cups of 8 mm diameter are punched out with a sterile cork borer. Plugs are removed and disposed into Petri plate containing phenol.3-4 drops of the control solvent in added to 1 well and the testing solution to the remaining wells. The plates were then incubated in the refrigerator at 4°C for 2 hours to allow the extracts to diffuse through the medium. Then the plates were incubated at 37°C for 24 hours. Growth inhibition was measured as diameters of inhibitory zones and compared with the control. [5] [8][11]

Results and Discussion:

Result of the antibacterial activity of different extracts of concentration (10 g/ml) for *G.lucidum, P.sajorcaju, A.auricula, A.bisporus and of the local mushroom from Gondia* determined by agar cup diffusion method are shown in the Table 1.

It is apparent from Table 1 that, *A. biosporus* extracts were different in their antimicrobial effectiveness depending on the extract used. The results show that petroleum ether is not a very good solvent for extracting *A. bisporus* because they could not be compared with the effectiveness of chloroform. The ethanol extract was comparatively effective against 4 out of the 11 strains tested. The inhibitory zone of 22mm was demonstrated against *Ps. aeruginosa* for the ethanolic extract. While chloroform and petroleum ether extract was tested against *Ps aeruginosa* and the zone of inhibition was 13mm and 10mm respectively.

However the chloroform extract was found to be the most effective as it inhibited 9 of the 11 cultures tested. It was ineffective against *P. mirabilis* and *S. paratyphi.*

G. lucidum extracts were also different in their antimicrobial effectiveness depending on the extract used. (Table No 1) The results showed that petroleum ether is not a very good solvent for extracting *G. lucidum.* The ethanolic extract was also ineffective as it exhibited activity against only 4 out of the 11 strains tested. *S. typhi* was inhibited by ethanolic extract and showed an inhibitory zone of 22mm. The chloroform extract was found to be the most effective as it inhibited 8 of the 11 cultures tested. However it was ineffective against *E. coli, K. pneumonia* and *S. paratyphi A*. Table no 1 depicts the antimicrobial effectiveness of the various extracts of *A. auricula*. The results show that petroleum ether was not very good with its effectiveness. However, showed good results against *K. pneumoniae, P. aeruginosa* and *S. aureus.* The ethanolic extract was not much effective as it could inhibit only 4 out of the 11 strains tested. Here again the chloroform extract

was found to be the most effective. It could inhibit 9 of the 11 cultures and showed inhibitory zone of 24mm against *Ps. aeruginosa.* The ethanolic extract of *P. sajorcaju* was effective against only 3 out of the 11 strains tested. While the chloroform extract was found to be the most effective as it inhibited 6 of the 11 cultures tested. It was effective against S. *typhi* and *S paratyphi A with* inhibitory zone of 16mm (Table 1).

The petroleum ether extract of the local mushroom was effective against only 3 cultures namely *P. mirabilis, Pseudomonas spps* and *S. aureus*. The ethanol extract was comparatively effective as it inhibited 8 out of the 11 strains tested. The chloroform extract was found to be as effective as the chloroform extract as it inhibited 8 of the 11 cultures tested.

From the results obtained, it could be concluded that chloroform was the best solvent for extracting antimicrobial substances from these mushrooms.

Table 1: Antibacterial activity of different organic solvents of *Auricularia auricula, Agaricus bisporus* , *Ganoderma lucidum,* Local Mushroom from Gondia and Pleurotus sajorcaju (Zone of inhibition in mm)

TEST BACTERIA	*Auricularia auricula* (10mg/ml)			*Agaricus bisporus* (10mg/ml)			*Ganoderma lucidum* (10mg/ml)			*Local Mushroom* (10mg/ml)			*Pleurotus sajorcaju* (10mg/ml)		
	E	C	PE	E	C	PE	E	C	PE	E	C	PE	E	C	PE
Escherichia coli	-	17	-	-	18	-	13	13	-	14	14	-	11	13	-
Klebsiellapneumoniae	-	13	12	-	12	14	10	10	-	10	12	-	12	10	-
Proteus vulgaris	11	12	-	16	13	-	13	13	-	12	12	-	13	10	-
Proteus mirabilis	-	15	-	17	11	-	11	13	-	15	-	11	10	12	11
Ps aeruginosa	14	24	13	22	13	10	12	16	-	14	14	-	12	14	-
Pseudomonas spps.	11	14	-	16	15	-	13	13	-	13	15	12	12	13	11
Salmonella typhi	12	14	-	14	12	-	22	17	-	10	12	-	10	16	-
Salmonella paratyphi A	11	23	-	10	16	-	10	13	-	21	15	-	10	16	-
Salmonella paratyphi B	12	-	-	10	12	-	10	12	-	15	10	-	10	13	-
Staphylococcus aureus	13	-	12	-	13	12	13	11	-	15	13	14	10	-	12
Streptococcus pyogenes	-	13	-	12	12	-	10	10	-	11	12	-	10	10	-

Key: E –Ethanol, C-Chloroform, PE-Petroleum ether

Further analysis of the antibacterial spectrum of the chloroform extract on individual isolates was carried out in order to determine the most effective mushroom extract for each of them.

Conclusion: Mushrooms have an established history for use in traditional oriental therapies. In recent years, multiple drug resistance in human pathogenic microorganism has forced scientists to search for new therapeutic alternatives. This study demonstrates that

mushrooms, similar to plant and other natural compounds have a great potential as a prolific resource for drugs.

In conclusion, the extracts of all the mushrooms used in this study inhibited some medically important microorganisms. This suggests that they are potential sources of new antimicrobial agents. Chloroform extract was the most effective whereas Petroleum ether the least. *A. auricula, the black mushroom* proved to be the most potent with a wide antibacterial spectrum. It inhibited 9 of the 11 isolates. The order of antibacterial potential of these mushrooms is as follows: *A. auricular*>*A. bisporus*>*G. lucidum* > *Local mushroom from Gondia*> *P. sajorcaju.*

Acknowledgement: Authors thank the Department of Microbiology, VES College of Arts, Science & Commerce, Chembur, Mumbai for their technical & financial support. Special gratitude to Prof. Ms. Rita Ramsinghani, Dept of Chemistry for supporting the extraction activities and Mr. Mahindra Thakur for his extensive guidance on Mushrooms.

References:

1. Chang ST, Buswell JA: Mushroom nutriceuticals. World Journal of Microbiology & Biotechnology, 12(5):473-476, 1996.
2. Cowan MM: Plant products as antimicrobial agents. Clinical Microbiology Review, 12(4):564-582, 1999.
3. Gao Y, Zhou S, Huang M, Xu A: Antibacterial and antiviral value of the genus Ganoderma P. Karst. Species (Aphyllophoromycetideae) : A review. International Journal of Medicinal Mushrooms, 5(3): 235-246, 2003
4. Keypour S, Riahi H, Moradali MF, Rafati H: Investigation of the antibacterial activity of a chloroform extract of Ling Zhi or Reishi Medicinal Mushroom, Ganodermalucidum (W.Curt.: Fr) P. Karst. (Aphyllophoromycetideae) from Iran. International Journal of Medicinal Mushrooms,10(4): 345-349, 2008.
5. Sheena N, Ajith TA, Mathew A, Janardhanan KK: Antibacterial activity of three macrofungi,Ganoderma lucidum, Navesporusfloccosa and Phellinus rimosus occurring in South India. Pharmaceutical Biology, 41(8): 564-567, 2003.
6. Chang ST, Mshigeni KE: Mushroom and Human health: their growing significance as petent dietary supplements. University of Namibia, Windhoek, Namibia. pp: 79, 2001
7. D.H. Tambekar, T.P. Sonar, M.V. Khodke and B.S. Khante, The Novel Antibacterials from Two Edible Mushrooms: Agaricusbisporus and Pleurotussajorcaju, International Journal of Pharmacology 2 (5): 584 – 587, 2006.
8. Jonathan S. Gbolagade and Ishola O. Fasidi Antimicrobial Activities of Some Selected Nigerian Mushrooms, African Journal of Biomedical Research, Vol. 8 (2005); 8 3- 87. Klaus A, Miomir N: Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceedings of National Science, MaticaSrpska Novi Sad, 113: 219-226, 2007
9. Klaus A, Miomir N: Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceedings of National Science, MaticaSrpska Novi Sad, 113: 219-226, 2007
10. Dulger B, Gonuz A: Antimicrobial activity of certain plants used in Turkish traditional medicine. Asian Journal of Plant Science, 3: 104-107, 2004
11. K. ShresthaVaidya, S. Thapa, A. Shrestha, K. Shrestha Antibacterial Activity of the Wild Mushrooms against Human Pathogens Nepal Journal of Science and Technology Vol. 7, 2006.

***

Divya Iyer/ Santhini.S.Nair/Department of Microbiology/V.E.S College of Arts/ Science& College/ Chembur/ University of Mumbai/ santhinisnair@gmail.com