

Antibacterial Activity Of *Ganoderma lucidum* Extracts Against Mdr Pathogens

R. Radhika*

Department of Microbiology, M. R. Government Arts College, Mannargudi

* Corresponding author

ABSTRACT

Ganoderma lucidum provides multiple benefits to human in terms of nutrient and medicine. Antibacterial potentials of this medicinal mushroom were assessed by making use of well diffusion and drug dilution methods on MDR bacterial isolates. Results revealed that all five extracts produced effective zone of inhibition with best by methanol extract against *Escherichia coli* and *Pseudomonas aeruginosa* (19.3 ± 0.4 mm respectively) and also with 266 ± 23.6 MBC. *Ganoderma lucidum* extracts could be considered as an effective antibacterial agent.

Key word: Antibacterial, *Ganoderma lucidum*, fruiting body, disc diffusion

INTRODUCTION

Mushroom fungi are known for its natural bioactive compounds (Ho et al., 2007). Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately, the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. The antimicrobial activity of various polysaccharides from medicinal mushrooms is being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. They succeeded in the isolation and identification of Pleuromutilin (Kavanagh et al., 1950), a diterpene that is especially useful for the treatment of *Mycoplasma* infections in animals (Brizuela et al., 1998) and served for the development of the first commercial antibiotic of basidiomycete origin. With the development of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics (Maziero et al., 1999, Suay et al., 2000). In fact, several compounds that inhibit the growth of a large spectrum of saprophytic and phytopathogenic fungi were isolated from basidiomycetes (Anke, 1997). Despite their potential and enormous diversity in tropical ecosystems (Hawksworth, 1991), few studies aiming at the discovery of bioactive compounds from basidiomycetes are directed to edible mushrooms (Ishikawa et al., 2001, Paccola et al., 2001, Oliveira et al., 2002) or common, easily recognized species (Smânia et al., 1999). This work was carried out to evaluate the antibacterial activity of aqueous, hexane, chloroform, methanol and ethanol extract of fruit bodies from *Ganoderma lucidum* on selected Multidrug resistant five bacterial Species.

MATERIALS AND METHODS

Collection of *Ganoderma lucidum*

Ganoderma lucidum was collected as wild from the paddy fields of Thiruvavur (Dt), Tamil Nadu and identified and authenticated in the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The selected strains were multiplied on potato dextrose agar (PDA) petriplates and slant culture was also maintained for further analysis.

Processing and Extraction

Ganoderma lucidum is dried completely, powdered using mechanical grinder and extracted using water and ethanol.

Assessment of Antibacterial activity

Test Organisms

Escherichia coli, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* were isolated from clinical cases and identified by making use of microscopic methods, cultivation of selective cum differential agar and biochemical tests.

Antibiotic sensitivity assay

All isolates were subjected into antibiotic sensitivity test according to Bauer *et al.*, (1966). The susceptibility of isolates were examined by a disc diffusion assay. Antibiotics like Gentamycin (Gen) - 30µg/disc, Ciprofloxacin (CF) - 30 µg/disc, Ampicillin (A) -10 µg/disc, Erythromycin (E) - 15 µg/disc, Co-trimoxazole (Co) - 30 µg/disc, Cephalosporins (CE) - 10 µg/disc, Novobiocin (NV) - 05 µg/disc, Cefpodoxime (CPD) - 30 µg/disc, Tetracycline (T) - 30 µg/disc, Ceftriaxone (CEF) - 30 µg/disc.

Preparation for Mushroom Extract

Ten grams mushroom powder was mixed with 50 ml of water, hexane, chloroform, methanol and ethanol separately in a beaker and it was placed on a shaker for 24 hrs. The aqueous solutions were filtered through Whatman (No.1) filter paper and then it was placed on the rotary evaporator vacuum, for 15 minutes at 37°C. Then the residue was dissolved with 10 ml of dimethyl sulfoxide and stored at 40°C for further analysis.

Antibacterial activity-Well diffusion method

A 24 hr culture of bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) was prepared prior to the methods of Well diffusion assay. It was inoculated in sterile Brain Heart Infusion broth with the turbidity 1.5×10^8 CFU/mL. A volume of the inoculum was swabbed on Mueller- Hinton agar plate using a sterile cotton swab within 15 minutes after standardization. The well was prepared in the medium using sterile 5mm cork borer. The prepared extracts were loaded in different concentration of 50µl in each well separately. Later the plates were incubated at 37°C for 24 hours. After 16-24 hours, the zone of inhibition was measured in mm. 30 µg Tetracycline was used as the positive control while 1ml DMSO were used as negative controls (Balouiri *et al.*, 2015).

Results

Antibacterial activity of various solvent extracts of *Ganoderma lucidum* were done with well diffusion method against MDR strains of five different bacterial isolates. Antibiotic susceptibility patterns of the test isolates were assessed against standard antimicrobials before performing antibacterial activity of the mushroom extracts. It was determined that all the isolates were completely resistant to Gentamycin (GEN), Ampicillin (A), Cephalosporin (cef) and Novobiocin (NV); all were susceptible to Ciprofloxacin (CF), Cefpodoxime (CPD) and Ceftriaxone (Table 1) and hence were multidrug resistant strains (MDR). Tetracycline susceptibility was noted only with *E. coli*, and *Salmonella only*, which could cause gastrointestinal infections. Similarly, tetracycline only inhibited *Escherichia coli* and *Salmonella typhimurium*. As multidrug resistance was confirmed with all the test isolates, the clinical significance of the five test pathogens was very obvious.

Table 1
Antibiotic resistance pattern of the test isolates

S. No	Antibiotics and its concentration	Clinical isolates				
		<i>Escherichia coli</i>	<i>Salmonella</i> sp.,	<i>Pseudomonas</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>
1	Gentamycin	R	R	R	R	R
2	Ciprofloxacin	S	S	S	S	S
3	Ampicillin	R	R	R	R	R
4	Erythromycin	S	S	S	S	S
5	Co-trimoxazole	S	R	S	R	R
6	Cephalosporin	R	R	R	R	R
7	Novobiocin	R	R	R	R	R
8	Cefpodoxime	S	S	S	S	S
9	Tetracycline	S	S	R	R	R
10	Ceftriaxone	S	S	S	S	S

The maximum antibacterial activity of *G.lucidum* was noted with Methanolic extract with highest zone of inhibition against *Pseudomonas aeruginosa* (19.5 ± 2.6 mm) followed by *Escherichia coli* (19.3 ± 0.4), *Salmonella typhimurium* (17.6 ± 1.2), *Streptococcus pyogenes* (16.8 ± 0.9) and *Streptococcus pyogenes* (16.4 ± 1.3) (Table 2). Ethanol extract also produced good antibacterial activity against all the test organisms which was varied from 14.7 to 18.3mm zone of inhibition. Lowest zone of inhibition was produced by chloroform extract against *Pseudomonas aeruginosa* (09.8 ± 2.7 mm)

The MBC values of the different extracts against the tested bacteria are represented in terms of microgram per ml concentration (Table 3). *Escherichia coli* was effectively killed by methanol extract at 266 ± 23.5 mg/ml concentration. It was followed by *Salmonella typhimurium* (266 ± 62.3) mg/ml concentrations. Highest concentration of extracts required to kill bacteria were 733 ± 62.3 mg/ml against *Escherichia coli*. This study also indicated almost all the extracts inhibited the growth of all tested bacteria. The extracts of *Ganoderma lucidum* could be considered as a bactericidal agent.

Table 2
Antibacterial nature of *Ganoderma lucidum* extracts on different bacterial isolates

S. No	Test Organism	Extract and zone of inhibition in mm				
		Water	Hexane	Chloroform	Ethanol	Methanol
01	<i>Escherichia coli</i>	13.8 ± 2.0	13.0 ± 3.2	12.7 ± 2.3	17.6 ± 3.5	19.3 ± 0.4
02	<i>Salmonella typhimurium</i>	16.8 ± 0.9	13.3 ± 1.6	14.3 ± 1.7	18.3 ± 2.6	17.6 ± 1.2
03	<i>Pseudomonas aeruginosa</i>	11.9 ± 0.7	14.6 ± 0.7	09.8 ± 2.7	15.2 ± 1.8	19.3 ± 0.4
04	<i>Staphylococcus aureus</i>	18.6 ± 1.6	14.8 ± 1.0	11.6 ± 1.3	17.2 ± 0.6	16.4 ± 1.3
05	<i>Streptococcus pyogenes</i>	16.7 ± 1.5	10.7 ± 2.6	14.7 ± 3.2	14.7 ± 1.5	16.8 ± 0.9
06	Negative Control	Nil	Nil	Nil	Nil	Nil

Table 3
Minimum Bactericidal of *Ganoderma lucidum* extracts on different bacterial isolates

S. No	Test Organism	MBC mg/ml				
		Water	Hexane	Chloroform	Ethanol	Methanol
01	<i>Escherichia coli</i>	350±40.8	733±62.3	600±40.8	416±47.1	266±23.5
02	<i>Salmonella typhimurium</i>	433±23.5	733±84.9	633±23.5	466±62.3	266±62.3
03	<i>Pseudomonas aeruginosa</i>	400±40.8	650±40.8	566±23.5	483±62.3	383±124.7
04	<i>Staphylococcus aureus</i>	433±47.1	666±23.5	583±62.3	583±62.3	383±154.5
05	<i>Streptococcus pyogenes</i>	616±23.5	600±40.8	533±62.3	666±23.5	366±62.3

DISCUSSIONS

Fungi, with an estimated 1.5 million species (Hawksworth, 2001), are the source of approximately one quarter of known natural bioactive compounds (Ho et al., 2007). Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Consequently, the antimicrobial activity of various anti-tumour polysaccharides from medicinal mushrooms is being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilising the body's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Polysaccharide Krestin has been shown to induce potent antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes* and *Candida* (Sakagami et al., 1991). Brizuela et al., (1998) succeeded in the isolation and identification of Pleuromutilin, a diterpene that is especially useful for the treatment of mycoplasma infections in animals and served for the development of the first commercial antibiotic of basidiomycete origin. Moreover, interest in the metabolites produced by basidiomycetes declined as streptomycetes were considered to be a more prolific and easier to manipulate source of antibiotics (Anke, 1989). However, over 6000 metabolites were already identified from these imperfect fungi, making it more and more difficult to isolate novel bioactive metabolites from them. With the development of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics (Anke, 1989, Maziero et al., 1999, Suay et al., 2000). In fact, several compounds that inhibit the growth of a large spectrum of saprophytic and phytopathogenic fungi were isolated from basidiomycetes. Furthermore, these organisms are able to inhibit the development of bacteria, actinomycetes and other fungi from their microhabitat, indicating that the antimicrobial substances produced by them have important ecological implications (Sidorova and Velikanov, 2000). Despite their potential and enormous diversity in tropical ecosystems (Hawksworth, 1991), few studies aiming at the discovery of bioactive compounds from basidiomycetes are directed to edible mushrooms (Ishikawa et al., 2001, Paccola et al., 2001, Oliveira et al., 2002) or common, easily recognized species (Smânia et al., 1999). The antimicrobial activity of aqueous, methanol, hexane, and ethyl acetate extracts from edible wild and cultivated mushrooms against nine food borne pathogenic bacterial strains was screened with a disk diffusion assay. Methanol, ethyl acetate, and aqueous extracts accounted for 92.8% of the positive assays, whereas the hexane extracts accounted for only

7.2%. Gram-positive bacteria were more sensitive than gram-negative bacteria to fungal extracts, and *C. perfringens* was the most sensitive microorganism. Aqueous extracts from *Clitocybe geotropa* and *Lentinula edodes* had the highest antimicrobial activity against all the bacterial strains tested (Venturini et al., 2008). This study also confirms antibacterial efficiency of *Ganoderma lucidum* extracts on MDR isolates. This study also incurred insights from the reports of Lakshmi Priya and Srinivasan, (2013); Kumiko et al., (2015); Mustafa et al., (2017) and Gebreselema et al., (2019). They also stated antimicrobial potentials of mushroom fungi against different microbial species.

REFERENCES

1. Anke, T., 1989. Basidiomycetes: a source for new bioactive secondary metabolites. *In*: Bioactive metabolites from microorganisms. *Progress in Industrial Microbiology*, 27: 51-57
2. Brizuela, M.A., Garcíam, L., Pérez, L., and Mansur, M., 1998. Basidiomicetos: nueva fuente de metabolites secundários. *Rev. Iberoam Micol.*, 15: 69-74.
3. Chelladurai, G., and Uma, V., 2017. Screening for bioactive potential of edible mushroom extracts against tooth decay bacteria. *World Journal of Pharmaceutical Research*. 6(8):
4. Hawksworth, D.L., 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycological Research*, 95: 641–655.
5. Hawksworth, D.L., 2001. Mushrooms: The extent of the unexplored potential. *Int. J. Med. Mushrooms.*, 3: 333- 337.
6. Ho, Y.W., Yeung, J.S., Chiu, P.K., Tang, W.M., Lin, Z.B., Man, R.Y., and Lau, C.S., 2007. *Ganoderma lucidum* polysaccharide peptide reduced the production of proinflammatory cytokines in activated rheumatoid synovial fibroblast. *Mol. Cell Biochem.*, 301: 173-179.
7. Ishikawa, N.K., Kasuya, M.C.M., and Vanetti, M.C.D., 2001. Antibacterial activity of *Lentinula edodes* grown in liquid medium. *Brazilian Journal of Microbiology*, 32: 206-210.
8. Kavanagh, F., Hervey, A., and Robibins, W.J., 1950. Antibiotic substances from basidiomycetes. VI. *Agrocybe dura*. *Proc. Natl. Acad. Sci., USA*. 36: 102 -106.
9. Khajuria, R., and Batra, P. 2014. Supplementation of nitrogen source in wheat straw for improving cellulolytic potential of *Ganoderma lucidum*. *International Journal of Pharma. and Bio Science*. 5: 90–99.
10. Kumiko Oka, Atsushi Ishihara, Nanase Sakaguchi, Shigeki Nishino, Roxana, Y. Parada, Akira Nakagiri, and Hiroshi Otani, 2015. Antifungal Activity of Volatile Compounds Produced by an Edible Mushroom *Hypsizygus marmoreus* against Phytopathogenic Fungi. *Journal of Phytopathology*, 163(11-12): 987-996.
11. Lakshmi Priya, J., and Srinivasan, V., 2013. Studies on the antibacterial activities of mushroom. *nt. J. Curr. Microbiol. App. Sci.*, 2(6): 184-189.
12. Maafi, R.I., Omar, M., Moyen Uddin, P.K., Md. Rubel, and Kamrunnahar, M., 2015. Phytochemical and antibacterial activity screening of three edible mushrooms, *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinus edodes* accessible in Bangladesh. *American Journal of Biomedicine and Life Science*, 3(2): 31-35.
13. Malhotra, G.K., 2010. Histological, molecular and functional subtypes of breast cancers. *Cancer Biol. Ther.*, 10(10): 955-960.
14. Matuszewska, A., Jaszek, M., Stefaniuk, D., Ciszewski, T., and Matuszewski, Ł., 2018. Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus *Cerrena unicolor*. *PLoS ONE*, 13(6): e0197044.

15. Maziero, R., Cavazzoni, V., and Bononi, V.L.R., 1999. Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. *Rev Microbiol.*, 30: 77-84.
16. Muhammad Ezzudin, R., Rabeta, M.S., Shaida Fariza, S., Leong Ooi, K., and Nuru Shafiqah, H., 2019. Primary and secondary antioxidant activities of nine edible mushrooms species. *Food Research*, 3 (1): 14–20.
17. Najeeb Khatian, and Muhammad Aslam, 2019. Effect of *Ganoderma lucidum* on memory and learning in mice. *Clinical Phytoscience*, 5: 4.
18. Oliveira, J.M., Jordão, B.Q., Ribeiro, L.R., Eira, A.F., and Mantovani, M.S., 2002. Antigenotoxic effect of aqueous extracts of sun mushroom (*Agaricus blazei* Murill lineage 99/26) in mammalian cells *in vitro*. *Food Chem Toxicol.*, 40: 1775-1780.
19. Ooi, V.E.C., and Liu, F., 2000. Immunomodulation and anti-cancer activity of polysaccharideprotein complexes. *Curr. Med. Chem.*, 7: 715-728.
20. Paccola, A.S., Maki, C.S., Nobrega, G.M.A., and Meirelles, L.D.P., 2001. Antagonistic effect of edible mushrooms extract on *Candida albicans* growth. *Braz. J. Microbiol.*, 32: 176-178.
21. Rakrudee Sarnthima, Saranyu Khammaung, and Piyawan Sa-ard, 2017. Culture broth of *Ganoderma lucidum* exhibited antioxidant, antibacterial and α -amylase inhibitory activities. *J. Food Sci. Technol.*, 54(11): 3724–3730.
22. Ramalingam Balakumar, Elumalai Sivaprakasam, Devendiran Kavitha, Sekaran Sridhar, and Jebamalai Suresh Kumar, 2011. Antibacterial and antifungal activity of fruit bodies of *Phellinus* mushroom extract. *International Journal of Biosciences*, 3(1): 72-77.
23. Romi Singh. 2017. A Review on Different Benefits of Mushroom. *IOSR Journal of Pharmacy and Biological Sciences* (IOSR-JPBS), 12(1): 107-111.
24. Sakagami, H., Aoki, T., Simpson, A., and Tanuma, S.I., 1991. Induction of immunopotentiating activity by protein-bound polysaccharide, PSK (Review). *Anticancer Res.*, 11: 993-1000.
25. Shon, Y.H., and Nam, K.S., 2002. Cancer chemoprevention inhibitory effect of soybeans fermented with basidiomycetes on 7,12-dimethylbenz[a] anthracene/12-Otetradecanoylphorbol- 13-acetate-induced mouse skin carcinogenesis. *Biotechnol. Lett.*, 24: 1005–1010.
26. Sidorova, I.I., and Velikanov, L.L., 2000. Bioactive substances of agaricoid basidiomycetes and their possible role in regulation of myco- and microbiota structure in soils of forest ecosystems. I. Antibiotic activity of water extracts from basidioms of several dominant agaricoid basidiomycetes. *Mikol Fitopatol.*, 34: 11-17.
27. Smania, A., Monache, F.D., Smania, E.F.A., and Cuneo, R.S., 1999. Antibacterial activity of steroidal compounds isolated from *Ganoderma applanatum* (Pers.) Pat. (Aphyllorphomycetideae) fruit body. *Int. J. Med. Mushrooms*, 1: 325–330.
28. Suay, I., Arenal, F., Asensio, F.J., Basilio, A., Cabello, M.A., Díez, M.T. , García, J.B. , Val, A.G., Gorrochategui, J., Hernández, P., Peláez, F., and Vicente, M.F., 2000. Screening of basidiomycetes for antimicrobial activities. *Antonie Van Leeuwenhoek.*, 78: 129 - 139.
29. Venturini, M.E., Rivera, C.S., and Gonzalez, C., 2008. Blanco antimicrobial Activity of Extracts of Edible Wild and Cultivated Mushrooms against Foodborne Bacterial Strains. *Journal of Food Protection*, 71(8): 1701–1706.
30. Vishwakarma, P., Singh, P., and Tripathi, N.N., 2017. In-vitro antioxidant activity and nutritional value of four wild oyster mushroom collected from North-Eastern Part of Uttar Pradesh. *Mycosphere*, 8(4): 592–602.
- 31.