September 2015

IJESR Volume 3, Issue 9 ISSN: 2347-6532

Aushada-Trends in translational Research

A STUDY ON THE ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF THE MEDICINAL MUSHROOM *GANODERMA LUCIDUM*

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ABSTRACT

Drug resistance is one of the most serious global threats to the treatment of infectious diseases. Currently, there is a growing interest in using natural antifungal compounds derived from alternative sources such as medicinal mushrooms for the treatment of these opportunistic infections. Different species of mushroom have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer. *Ganoderma lucidum*, commonly known as "Reishi," is recognized as a powerful medicinal fungus. The *Ganoderma lucidum* solvent extracts were analysed for determining the anticandidal and antimold activity. The antifungal efficacy was analysed by the standard agar disc diffusion method followed by the determination of Minimum Inhibitory Concentration. Ethanol extracts of *Ganoderma lucidum* showed maximum antifungal activity against most of the strains tested. It is apparent from the present study that mushroom extracts derived from *Ganoderma lucidum* could be employed to fight against several diseases caused by pathogenic fungal microorganisms. The present results have offered organic extracts of *Ganoderma lucidum* as a new and safe antifungal agent.

Keywords: drug resistance, medicinal mushroom, antifungal agent

Introduction

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial medicine that was initially effective for the treatment of diseases caused by it. Resistant organisms (include bacteria, viruses and some parasites) are able to withstand attack by antimicrobial medicines, such as antibiotics, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist and may spread to others. Antimicrobial resistance (AMR) is a consequence of the use, particularly the misuse, of antimicrobial medicines and develops when a microorganism mutates or acquires a resistance gene (Barros L. *et al.*, 2007)

Mushroom is a macro fungus with a distinctive fruiting body that is large enough to be seen by the naked eyes. It includes both edible and non edible species. Medicinal values associated with mushrooms have been reported (Bhosle S.R *et al.*, 2010). Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer (Chang S.T and Buswell J.A, 1996). Although the advances brought by technology has made life easier to people, many are still looking for better organic alternatives that are proven to be more effective in their most natural form, like *Ganoderma lucidum*, a fungus known by its many names like “Reishi,” “Ling Zhi,” and “Mannentake,” for hundreds or even thousands of years, is recognized as powerful medicinal fungi (Chang S.T and Buswell J.A, 2003).

The present study was planned to determine the antifungal efficacy of *Ganoderma lucidum* against fungal molds and *Candida albicans* strains using various crude extracts of the medicinal mushroom, *Ganoderma lucidum.*

Materials & Methods

Collection of the medicinal mushroom:

The medicinal mushroom powder was authenticated and obtained from Camillotek India Private Limited, Chennai.

Preparation of crude extracts (Klaus A. and Miomir N.,2007)

The crude extracts were prepared by weighing 20 grams of the powdered material which was then mixed with 100 ml of the solvent, incubated in a shaker at 37ºC for 4 hours at 250 rpm. The supernatant was filtered and the filtrate was then dried in air at room temperature. The solvents used were water, petroleum ether, ethanol, methanol, chloroform, acetone, ethyl acetate, hexane and dichloromethane. . The residue obtained after drying was dissolved in the appropriate solvent and stored at 4ºC for further use. Sterility testing of the extracts was done by inoculating into Nutrient agar and Saboraud's Dextrose Agar and observing for the presence/absence of bacterial/fungal growth. The extracts were then used for antifungal activity evaluation.

Fungal cultures used:

The test organisms used for screening were *Candida albicans* strain 1, *Candida albicans MTCC 3017, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium spps., Alternaria alternata, Mucor and Rhizopus.* All the strains were laboratory isolates of the Department of Microbiology, JBAS College for Women, Chennai.

ANTIFUNGAL PRELIMINARY SCREENING ASSAY (Bauer *et al.*, 1996; Navarro *et al.*, 1996)

Disc [5mm] prepared from whatmann No: 1 filter paper was sterilized and impregnated with 20µl of various crude solvent extracts [Conc : 100mg/ml].. Respective solvents without crude mushroom extracts were used as the negative control. Sabouraud's Dextrose agar with chloramphenicol & gentamicin was the media used for the assay. One laboratory strain of *Candida albicans* and one standard strain of *Candida albicans MTCC 3017* were used. A 24 hours old culture of *Candida albicans* was taken and a loopful of organism mixed with sterile saline. For preparation of inoculum for fungal molds, a 3 day old culture was suspended in sterile saline. The absorbance of the prepared culture was read at 530nm and adjusted with sterile saline to match that of (4) MacFarland standard. A lawn culture of the organism was made on Sabouraud's Dextrose agar and allowed to dry for 30 minutes. Sterile filter paper discs with the corresponding solvent extracts were placed on the plate in a way such that each disc is separated from one another by 20 mm. The plates were incubated at 37ºC for 24 hours for *Candida albicans* and 48-72 hours for fungal molds. The zone of inhibition seen around the disc was observed and the zone diameter measured in mm and recorded.

MICROBROTH DILUTION ASSAY FOR MIC DETERMINATION (Pattnaik et al., 1996)

In this dilution method, samples being tested are mixed with a suitable medium that has previously been inoculated with the test organism. After incubation, growth of the microorganism may be determined by direct visual or trubidometric methods. After incubation, the end point of the test is taken as the highest dilution, which will just prevent perceptible growth of the test organism (MIC Value).The broth dilution assay was performed on a microtitre plate. Doubling dilutions of the selected crude extracts were prepared in Saboraud's Dextrose broth with the first one considered as neat. Fungal and candidal cultures of 10^6 cfu/ml dilution were prepared with McFarland standard (4) and 10 ul were added to each well of the microtitre plate and mixed well. The microtitre plates were incubated at 37°C for 24 hours for *Candida albicans* and at room temperature for 48 hours for fungal molds. A loopful of the broth were streaked on to SDA plates. Plates were incubated at the same temperature as mentioned. The growth /no growth pattern of the organism corresponded to the MIC/MBC of the crude mushroom extracts.

Results & Discussion

The resistance of pathogenic fungi including *Candida albicans* and non-*Candida albicans* species isolated from patients against antifungal agents has increased. Systemic mycoses may involve any part of the body, and a lot of species formerly considered as non pathogenic are recognised as opportunistic pathogens which cause deep seated mycoses. These pathogens include fungi belonging to the genera of *Aspergillus, Rhizopus, Mucor, Alternaria and Fusarium*. Based on the toxicity and low potency, combined with the increasing side effects of these drugs, novel fungal therapies with fewer side effects on humans are urgently required for effective management of these fungal infections. These negative health trends call for a renewed interest on the strategies for treatment and prevention (Rekha Sharanappa and Vidyasagar, 2013).Many *Ganoderma* species have been studied for different therapeutic properties as antitumor and antiviral agents but far less investigation have been carried out on their antifungal potential (Dunham, 2009).

In the present study, the extracts of the medicinal mushroom, *Ganoderma lucidum* was screened against *Candida albicans* and fungal molds, the results of which are represented in

Table I. In the preliminary screening disc diffusion assay, the acetone and ethanol extracts of *Ganoderma lucidum* showed maximum inhibition zone followed by the methanol, chloroform, dichloromethane and petroleum ether extracts. Hexane, ethyl acetate and aqueous extracts of *Ganoderma lucidum* showed the least inhibition zones.

TABLE 1: **Preliminary Screening: Antifungal Efficacy of *Ganoderma lucidum***

Method of Assay: *1.Agar disc diffusion assay* , Temperature of incubation: *37˚C, 25ºC*

ORGANISM	ZONE OF INHIBITION (mm)								
	SOLVENTS EMPLOYED								
	Chloroform	E.acetate	D.water	P.ether	Ethanol	Dichloromethane	Acetone	Hexane	Methanol
1.Candida albicans MTCC3017	-	9	7	11	**18**	13	**18**	-	15
2. Candida albicans	-	11	-	10	**17**	12	11	-	15
2.Aspergillus fumigates	5	8	-	-	10	**17**	9	9	8
3.Aspergillus flavus	13	-	-	-	10	9	**13**	12	10
4.Aspergillus niger	8	7	-	5	11	-	**13**	-	12
5. Rhizopus spp.	11	8	-	3	11	13	**17**	5	8
6. Mucor spp.	13	-	-	3	**15**	-	**19**	-	10
7. Penicillium spp.	11	10	-	-	12	10	**17**	9	13
8. Alternaria alternate	21	-	-	-	15	13	**24**	-	17

In the microbroth dilution assay, which is represented in Table II, only those extracts which showed maximum activity for particular fungi were analysed. In the determination of

minimum bactericidal concentration of the solvent extracts of *Ganoderma lucidum*, most of the extracts showed an MBC value of 12.5mg/ml and 6.25mg/ml. Only the acetone extract showed an MBC of 3.125mg/ml for *Alternaria alternata.*

TABLE 2: **Determination of MIC/MBC of various crude extract solvents of *Ganoderma lucidum***

		Concentration of the extracts in mg/ml						
ORGANISMS TESTED	**CRUDE EXTRACTS**	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**	**1.56**
1.Candida albicans MTCC 3017	Ethanol	-	-	-	-	+	+	+
	Acetone	-	-	-	-	-	+	+
2. Candida albicans	Ethanol	-	-	-	-	+	+	+
3. Aspergillus fumigates	Dichloromethane	-	-	-	-	+	+	+
4. Aspergillus flavus	Acetone	-	-	-	+	+	+	+
5. Aspergillus niger	Acetone	-	-	-	-	+	+	+
6. Penicillium spp.	Acetone	-	-	-	-	+	+	+
7. Mucor	Acetone	-	-	-	-	-	+	+
8. Rhizopus spp.	Acetone	-	-	-	-	+	+	+
9. Alternaria alternate	Acetone	-	-	-	-	-	-	+

- denotes no growth; +denotes growth

Antifungal proteins obtained from different medicinal mushrooms are known to possess high potentiality but there are very few studies in this area of research. Studies have demonstrated the existence of a 15K-Da antifungal protein, designated ganodermin isolated from *Ganoderma lucidum*. Ganodermin inhibited the mycelia growth of *Botrytis cinerea, Fusarium oxysporum and Physalospora piricola.* (Hexiang Wang and T.B.Ng, 2006). In the

study by Jaya Singh *et al*., 2014, *Ganoderma lucidum* acetone extracts demonstrated maximum activity against 5 strains of fungi such as *Aspergillus niger* , *Curvularia lunata*, *Fusarium oxysporum* ,*Alternaria alternata* and *Drashelaria* spp. at a concentration of 1000mg/ml which is similar to our studies. Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts. In the present study the aqueous extract exhibited least antifungal activity than the organic extracts.

Identification of a novel antifungal agent derived from medicinal mushroom is significant, since majority of natural products are non toxic to human and environment.

CONCLUSION

Fungal pathogens include the plant pathogenic fungi which cause extensive losses to agriculture and human pathogens which can cause severe life-threatening infections. Currently there are only a few effective antifungal agents to combat these fungal pathogens. Despite extensive research being done in the development of new therapeutic strategies, there are only few successful drug candidates. *G. lucidum* , native to Asia, is a well known medicinal mushroom that has more pharmaceutical value than nutritional value. It has a huge repertoire of bioactive compounds that can be harnessed to develop a candidate antifungal agent. To conclude, acetone and ethanol crude solvent extracts of *Ganoderma lucidum* can be used as a potential antifungal agent either alone or in combination with other synthetic antifungals.

REFERENCES:

1. Barros L., Calhelha R.C., Vaz J.A. , Ferreira I. C. F. R., Baptista P. and Estevinho L. M., 2007, Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts, European Food Research and Technology, 225 ,pp 151-6.
2. Bauer, H.W., Kirby, W.M.M., Slerris, J.C. andTruck, M., 1996. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology, 45, pp 493-496.

3. Bhosle S. R., Bapat G., Vaidya Jitendra G.,Garad Sandhya A. and Sonawane Hiralal B.,2010. Antimicrobial activity of terpenoid extracts from Ganoderma samples. International Journal of Pharmacy and Life Science, 1(4) ,pp 234-240.
4. Chang ST, Buswell JA, 1996, Mushroom nutriceuticals. World journal of Microbiology & Biotechnology,2(5),pp 473-476.
5. Chang, S.T. and J.A. Buswell, 2003, Medicinal mushrooms—a prominent source of nutriceuticals for the 21st century. Current Topics in Nutraceutical Research, 1, pp 257-280.
6. Dunham M., 2009. Potential of Fungi Used in Traditional Chinese Medicine: II *Ganoderma.* http://www.alternative2cancer.com/docs/potential.pdf
7. Hexiang W., and T.B.Ng, Ganodermin, an antifungal protein from fruiting bodies of the medicinal mushroom Ganoderma lucidum, 2006, Peptides 27, pp 27-30.
8. Jaya Singh, Saurabh Gupta, Sonam Malviya, and Bharti Ahrwar,2014, *In-vitro* Evaluation of Antimicrobial Activity of *Ganoderma lucidum*, International Journal of Advanced Research ,Volume 2, Issue 6,pp 460-466.
9. Klaus A, Miomir N, 2007, Influence of the extracts isolated from *Ganoderma lucidum* mushroom on some microorganisms. Proceedings of National Science, Matica Srpska Novi Sad.,113,pp 219-226.
10. Marjorie Murphy Cowan, 1999, Plant products as Antimicrobial agents. Clinical Microbiology Reviews, Oct.12 (4), pp564-582
11. Navarro V, Villarreal M.L, Rojas G and Lozoya X, 1996, Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of Ethanopharmacology, 53,pp 143-147
12. Pattnaik. S , Subramanyam V.R and Kole. C. Antibacterial and antifungal activity of ten essential oils *in vitro*, 1996, Microbios , 86(349), pp 237-46
13. Rekha Sharanappa and Vidyasagar G.M, 2013, Anti-Candida activity of Medicinal Plants.A Review, International Journal of Pharmacy and Pharmaceutical Sciences; 5(4),pp 9-16