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Research Article

EVALUATION OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *GANODERMA LUCIDUM* EXTRACTS AGAINST HUMAN PATHOGENIC BACTERIA

ANITA KAMRA*, A. B. BHATT

*Dept of Botany and Microbiology, HNB Garhwal Central University, Srinagar 246174 Uttarakhand, India. Email: kamra.anita@gmail.com

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ABSTRACT

The aim of the present study was to investigate the antimicrobial and antioxidant properties of *Ganoderma lucidum* fruit bodies grown under tropical habitat. The aqueous and organic solvents (Hexane, dichloromethane, ethyl acetate, and methanol) extracts from the fruit bodies of *Ganoderma lucidum* were tested against *Bacillus subtilis*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Streptococcus mutans*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhimurium and Pseudomonas aeruginosa* by agar diffusion method. The susceptibility patterns of test bacteria against the crude extracts were determined at two different concentrations 0.5mg/100µl and 1.0mg/100µl respectively. Among these, the methanol and aqueous extracts were found to possess more potent inhibitory effects at 0.5mg/ml and rest of three extracts showed negligible effect. The result revealed that most susceptible micro organism was *Proteus vulgaris* (23.66 ± 0.57mm zone of inhibition in methanol extract) followed by *Pseudomonas aeruginosa* (23.13 ±0.49mm zone of inhibition in aqueous extract). *Listeria monocytogenes* exhibited no susceptibility to aqueous extract. The minimum inhibitory concentration was found to be 31.25µg/ml for all tested strain except *Streptococcus mutans* (62.50µg/ml). We present antioxidant power of various extracts of *Ganoderma lucidum* by FRAP assay. The antioxidant value was found to be highest in the order of dichloromethane followed by aqueous, methanol, ethyl acetate and hexane extract. Preliminary phytochemical analysis of methanol and aqueous extract revealed the presence of phenols, flavonoids and ascorbic acid. The positive results of screening of *Ganoderma lucidum* fruit bodies for antibacterial activity forms a primary platform for further phytochemical studies and development of new drugs for therapy of urinary tract infections.

Keywords: *Ganoderma lucidum*, Antimicrobial, Antioxidant, Minimum inhibitory concentration.

INTRODUCTION

In the last three decades the search for new therapeutic bioactive compounds that can serve as antioxidant and antimicrobial agents had increased tremendously due to multiple drug resistance in human pathogenic microorganisms. Oxidative Damage in human cells or tissues is mainly caused by uncontrolled production of oxygen derived free radicals. Free radicals are dangerous substances produced in the body along with toxins and waste which are formed during the normal metabolic process of the body. Environmental agents like toxicity of lead, pesticides, ionizing radiations, alcohol, cigarette smoking, burning of organic matter, and excessive use of certain drugs, UV radiations and automobile pollution also initiate free radical generation leads different complication in body[1]. The infection caused by bacteria, viruses and fungus also produces reactive oxygen species in the body[2]. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules (DNA lipid and proteins) are damaged. Although there are several enzyme systems within the body that scavenge free radicals like superoxide dismutase, catalase, and glutathione peroxidase and glutathione reductase[3]. Currently available synthetic antioxidants example Butylated hydroxyl toluene (BHT), Butylated hydroxyl anisome (BHA) and Gallic acid etc. has been suspected to cause negative health effects. Hence their application has been restricted and there is a trend to substitute them with naturally occurring antioxidants. The multiple drug resistance in the human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases[4]. The researchers found that low levels of antibiotics may not be enough to kill the bacteria but they stress them. That stress causes them to produce free radicals. These free radicals damage the bacterial DNA and might cause mutation leading to the development of resistance[5].

Ganoderma is polypore, non edible mushroom that is soft (when fresh), corky and flat with a conspicuous red varnished, kidney-shaped cap and depending on the specimen age, white to brown pores underneath[6]. It lacks gills on its underside and releases its spores through the fine pores, leading to its morphological classification as a Polypore. *Ganoderma* species belong to the division Basidiomycota, class Homobasidiomycetes or Aphyllophorales, family Polyporaceae[7]. *Ganoderma lucidum* (Fr.) P. Karst (Lingzhi) is a popular medicinal mushroom and have many biologically active components like triterpenoids, polysaccharides, ganoderic acids and so on, giving it, its antimicrobial, antioxidant, antiviral and anticancer properties[8]. Several compounds with important pharmaceutical properties such as Polysaccharides forming a wide variety of branched or linear structures known as ß4glucans and have been isolated from medicinal mushrooms, act as anti aging agents, and provide longetivity, modulating the immune system and hypoglycaemic activity[9]. Furthermore other bioactive substances such as triterpenes, lipids and phenols have also been identified and characterized in mushrooms with medicinal properties[10]. Mushroom contain vitamins A and C or ß carotenes and a great variety of secondary metabolites such as phenolics compounds, polyketides, terpenes, steroids and phenols, all have protective effects because of their antioxidant properties[11,12]. This situation forced scientist for discovering new antibacterials and antioxidants from the division Basidiomycota.

The aim of present work was to carry out *invitro* experiments to screen antibacterial potential of different extracts of tropically grown red *Ganoderma lucidum*. Not much literature is available with regard to their antioxidant and antimicrobial activities of fruit body of *Ganoderma lucidum* for treatment of urinary tract infections and other infections in human body. The present study reveals its biopharmaceutical properties.

MATERIAL AND METHODS

Chemicals, Media, Micro organisms

All chemicals used were of analytical grade. The *Ganoderma lucidum* (fruit bodies) were procured from National Research centre for Mushroom (NRCM) Solan, INDIA. Bacterial strains were procured from IMTECH Chandigarh. Muller Hinton Agar was purchased from Himedia, Mumbai. The microorganism used for investigations are: *Bacillus subtilis* MTCC-121, *Enterococcus faecalis* MTCC-439, *Listeria monocytogenes* MTCC-839, *Streptococcus mutans* MTCC-497, *Klebsiella pneumoniae* MTCC-432, *Proteus vulgaris* MTCC-1771, *and Salmonella typhimurium MTCC-98 Pseudomonas aeruginosa* MTCC-1036.

Preparation of crude extracts

In the present study, the fruit bodies were grounded to a fine powder with the help of pestle and mortar. Five grams of mushroom powder was taken in a thimble and subjected to soxhlet extraction

for 8 hours using 250 ml each of the following solvents: hexane, dichloromethane, ethyl acetate, methanol and water. All solvent extracted fractions were dried in rotary vaccum evaporator to remove the traces of solvents and to obtain residues. The extracts were stored at 4˚C in air tight containers for further investigations[13]. The yield percentage (w/w) of red *Ganoderma lucidum* in different solvents is shown in Table 1.

Table 1: Represents the Yield % of red *Ganoderma lucidum* in different Extracts

S. No	Solvent used	Yield %
1.	Hexane	2.4
2.	Dichloromethane	2.2
3.	Ethyl acetate	1.0
4.	Methanol	5.2
5.	Water	12.0

Preliminary screening of phytoconstituents:

Preliminary qualitative phytochemicals analysis of all the extracts was carried out by employing standard conventional procedures[14, 15, 16].

Screening of Antibacterial activity

Antibacterial activity of mushroom extracts was carried out by modified agar well diffusion method. The test bacterial strains were transferred into a tube containing 5 ml of nutrient broth and incubated at 35 ± 2˚C. To standardize the inoculum density for susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 Mcfarland turbidity standard was used[17]. The inoculum size of the test strain was 1×10^8 to 2×10^8 cfu/ml. 0.02ml inoculum of known turbidity was applied on the dried surface of prepared Muller Hinton Agar plate. The inoculated plates were left for 15-20 minutes at room temperature. For antibacterial screening hexane, dichloromethane, ethyl acetate, methanol and water extracts were dissolved in DMSO to a final concentration of 0.5mg/100µl and 1mg/100µl. The wells were bored with 9mm borer in seeded agar and 0.1ml volume of each extract reconstituted in DMSO was transferred into wells. After holding the plates at room temperature for some time to allow diffusion of the extract into the agar, the plates were incubated at 37^{0}C for 24h. After 24 hours each plate was examined for zone of inhibition. As reference antibiotic Gentamycin sulphate (0.5mg/100µl and 1mg/100µl) was used. The tests were performed in triplicates and final values were expressed as mean ± standard deviation.

Minimum Inhibitory concentration

Minimum inhibitory concentration (MIC) is defined as the lowest concentration which results in maintenance or reduction of inoculum viability over a period of 24 hours[18]. DMSO as a negative and Gentamycin as positive control were prepared in the mictrotiter well plates with Muller Hinton Broth as a diluent. The plates were incubated at 37˚C. The least concentration of extract or standard drug (Gentamycin) showing no visible growth after 24 hours was taken as MIC.

FRAP method for Antioxidant power

A modified method was adopted for the FRAP assay[19]. The stock solution included 300mM acetate buffer, pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40mM HCl and 20 mM $FeCl_3.6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5ml TPTZ and 2.5ml $FeCl_3.6H_2O$. In the FRAP assay, reductants (antioxidant) of extract reduces Fe^{+3}/2, 4, 6-tripyidyl-s-triazine (TPTZ) complex present in stoichiometric excess to blue colored Fe^{2+} form. The stock solutions of various extract of concentration ($1 \times 10^2 - 10 \times 10^2$ g/l) were prepared. The tubes were incubated at 37˚C for 15 minutes. Reading of the colored product (Ferrous tripyridyltriazine complex) was taken at 593nm in spectrophotometer. Aqueous solution of known Fe (II) concentration was used for calibration (in a range of 100-1000 µmol/l). Zero was set with blank (FRAP reagent + distilled water). Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

RESULTS AND DISCUSSION

The yield % of all extracts of mushroom fruit bodies are presented in Table 1. The aqueous extract yield was relatively higher as compared to other extracts. Preliminary phytochemicals screening *Ganoderma lucidum* revealed that extracts contain phenols, flavonoids and ascorbic acid. Our findings are in accordance with other investigators who have also reported the presence of these components in the member of family Ganodermataceae[20].

Results of antibacterial activity of different extracts of concentration 0.5 mg/100µl and 1.0 mg/100µl of *Ganoderma lucidum* were determined by Agar Well diffusion method (Table 2,3). In terms of zone of inhibition the aqueous and methanol extracts (0.5mg/100µl) revealed prominent effects and the rest of three extracts showed negligible effects. It was apparent from the Table: 2 methanol extract of the *Ganoderma lucidum* possessed strong antimicrobial activity against *Proteus vulgaris* (23.66 ± 0.57mm) closely followed by *Enterococcus faecalis* (20.30 ± 0.30mm). It showed moderate effect against *Salmonella typhimurium* (16.06 ± 0.40mm), *Pseudomonas aeruginosa* (13.96 ± 0.25mm) and *Listeria monocytogenes* (12.26 ± 0.55mm) at the same concentration. But it was highly reduced in case of *Streptococcus mutans* (10.00 ± 0.60mm), *Bacillus subtilis* (9.00 ± 0.40mm) and *Klebsiella pneumoniae* (8.20 ± 0.20mm).

Table 2: Antimicrobial activity of methanol extract of *Ganoderma lucidum*

S. No	Micro-Organisms	*Zone of Inhibition Methanol	
		0.5mg/100µl	1.0mg/100µl
1.	*Bacillus subtilis*	9.0±0.4	10.16±0.20
2.	*Enterococcus faecalis*	20.3±0.30	22.26±0.30
3.	*Listeria monocytogenes*	12.26±0.55	12.33±0.32
4.	*Streptococcus mutans*	10.0±0.60	12.23±0.32
5.	*Klebsiella pneumoniae*	8.2±0.20	9.00±0.20
6.	*Proteus vulgaris*	23.66±0.57	23.90±1.058
7.	*Salmonella typhimurium*	16.06±0.40	18.06±0.60
8.	*Pseudomonas aeruginosa*	13.96±0.25	17.40±0.45

Antimicrobial activity = Average zone of inhibition ± SD

Aqueous extract of *Ganoderma lucidum* fruit body was equally inhibitory against all the tested strains except *Listeria monocytogenes* (Table:3). *Listeria monocytogenes* exhibited no susceptibility to the extract (0.5mg/100µl). Maximum inhibition halos were observed against *Pseudomonas aeruginosa* (23.13 ± 0.49mm) which were closely followed by *Proteus vulgaris* (22.00 ± 0.50mm) and *Enterococcus faecalis* (21.93 ± 0.30mm). Aqueous extract reveals moderate ZOI against *Salmonella typhimurium* (17.00 ± 0.32mm), *Klebsiella pneumoniae* (15.16 ± 0.66mm) and *Streptococcus mutans* (13.33 ± 0.23mm). The lowest antagonistic effect of aqueous extract was observed with *Bacillus subtilis* (13.1 ± 0.26mm). The hexane, dichloromethane and ethyl acetate were found to be ineffective against all the strains showing negligible ZOI.

Table 3: Antimicrobial activity of aqueous extract of *Ganoderma lucidum*

S. No	Micro-Organisms	Zone of Inhibition (mm) Water	
		0.5mg/100µl	1.0mg/100µl
1.	*Bacillus subtilis*	13.1±0.26	14.76±0.208
2.	*Enterococcus faecalis*	21.93±0.30	22.43±0.49
3.	*Listeria monocytogenes*	-	32.50±0.78
4.	*Streptococcus mutans*	13.33±0.23	14.56±0.51
5.	*Klebsiella pneumoniae*	15.16±0.66	15.40±1.216
6.	*Proteus vulgaris*	22.00±0.50	28.46±0.75
7.	*Salmonella typhimurium*	17.0±0.32	17.80±0.264
8.	*Pseudomonas aeruginosa*	23.13±0.49	11.76±0.68

Antimicrobial activity = Average zone of inhibition ± SD

From this study it was clearly observed that the *Ganoderma lucidum* fruit bodies grown in tropical habitat demonstrated high level of antimicrobial activities against the urinary tract infection causing microbes which also causes various other infections. In the present study, the aqueous extract exhibited less antibacterial activity than methanol extract. These results are consistent with already reported literature, methanol is a better extracting solvent than water and most active components are generally water insoluble hence it is expected that low polarity organic solvents yields more active extract[21, 22].The antimicrobial activity showed by *Ganoderma lucidum* is due to the presence of lectins, terpenes, polysaccharides in their fruit bodies and the solubility of those components in the extracts used for the present investigation[23, 24].

Table 4: MIC of methanol and aqueous extract of red *Ganoderma lucidum*

S. No	Micro-Organisms	Minimum Inhibitory Concentration (µg/ml)	
		Methanol	Water
1.	*Bacillus subtilis*	31.25	31.25
2	*Enterococcus faecalis*	31.25	31.25
3.	*Listeria monocytogenes*	31.25	-
4.	*Streptococcus mutans*	62.50	62.50
5.	*Klebsiella pneumoniae*	31.25	31.25
6.	*Proteus vulgaris*	31.25	31.25
7.	*Salmonella typhimurium*	31.25	31.25
8.	*Pseudomonas aeruginosa*	31.25	31.25

All the values in the table were represented as average ± SD of three experiments

The MIC assay leads to the determination of effective concentration of the extract required to manage the bacterial pathogenecity caused by the microbes in reference. Therefore MIC studies of aqueous and methanol extracts were carried out (Table: 4). The MIC was found to be 31.25µg/ml for all the microbes except *for Streptococcus mutans* which had the MIC value to be 62.50µg/ml (Table 4). Minimum inhibitory concentration reported in the present study is lower in comparison to MIC already reported in the literature[25]. The lower is the MIC the more sensitive and promising the extract[26].

Assessment of Antioxidant power

Oxidative stress occurs when there is an excess of reactive oxygen species or decrease in antioxidant levels that leads to tissue damage by physical, chemical and physiological factors that causes different type of human diseases including aging, infertility, immunodepression, tumors, gastrointestinal disorders, renal disorders (urinary tract infection). Antioxidants are the substances capable to mop up free radicals and prevent them from causing diseases. Antioxidant activity by this method was evaluated on the basis of antioxidant content where ferric to ferrous ion reduction at low pH causes a ferrous-tripyridyl-triazine at 593 nm. All the extracts of *Ganoderma lucidum* at different concentrations exhibited strong antioxidant activity. All values increased with the increasing concentrations (1×10^2- 10×10^2 g/l) of every extract (Fig 1). Reducing ability of dichloromethane extract (1.70 - 60.80 µM/L) was observed highest among all the extracts (Fig 1) followed by aqueous (7.30 - 52.60 µM/L), methanol (5.10 - 40.90 µM/L), ethyl acetate (1.30 - 27.50 µM/L) and hexane (0.70 - 24.80 µM/L) respectively. The results of preliminary phytochemical investigation showed the presence of flavonoids and phenolic compounds which corresponds to the strong antioxidant activity shown by the extracts. Hence the observed *invitro* antioxidant activity may be because of these phytoconstituents, which needs further investigation to isolate the purified compounds.

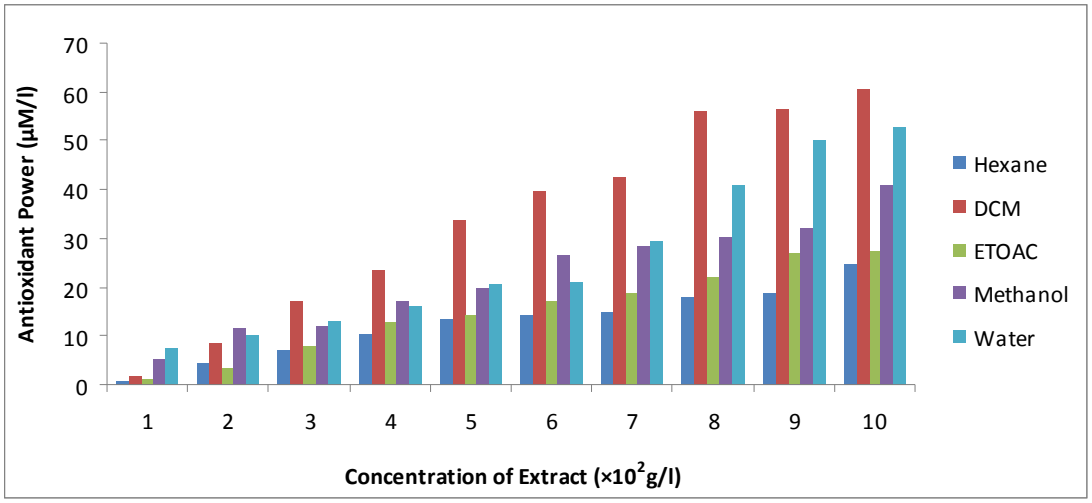

Fig. 1: Antioxidant power (µM/L) of different extract of red *Ganoderma lucidum*

CONCLUSION

The results from the present study supported the usage of *Ganoderma lucidum* fruit body as an ideal bio-pharmaceutics and suggested that the methanol and aqueous extract exert strong antimicrobial activity. All the extracts in this study exhibited potent antioxidant activity. This might be due to presence of rich phytochemical constituents such as phenols, flavonoids and ascorbic acid. The results of preliminary phytochemical analysis are in agreement with the reports of other workers. Further work is therefore under progress to identify the bioactive principles and elucidate their mechanism of action to scavenge the free radicals. This study is strongly suggestive that *Ganoderma lucidum* can be used as antibacterial agent in the development of new drug for the therapy of urinary tract infections which is caused by bacterial pathogenesis and harmful activity of excess free radicals in humans.

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