International Journal of Applied Research 2016; 2(5): 73-76

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(5): 73-76
www.allresearchjournal.com
Received: 01-03-2016
Accepted: 02-04-2016

Kalpna Kumari
Department of Biosciences,
HPU Shimla- 171005,
Himachal Pradesh, India.

Anand Sagar
Department of Biosciences,
HPU Shimla- 171005,
Himachal Pradesh, India.

Correspondence
Kalpna Kumari
Department of Biosciences,
HPU Shimla- 171005,
Himachal Pradesh, India.

Evaluation of antibacterial activity of methanolic and acetone extract of *Ganoderma lucidum* (Curt.) P. Karst.

Kalpna Kumari, Anand Sagar

Abstract
Medicinal mushrooms are widely used as traditional medicinal ingredients for the treatment of different diseases and related health problems. *Ganoderma lucidum* provides bioactive compounds that claim to possess antibacterial activity. The fruiting bodies of *Ganoderma lucidum* were collected from various places in Bilaspur district of Himachal Pradesh, India. The aim of this research is to know antibacterial activity of methanolic and acetone extracts of *Ganoderma lucidum*. Antibacterial activity of *Ganoderma lucidum* was done against four human pathogenic bacteria i.e. *Yersinia pestis*, *Pseudomonas aeruginosa, Listeria monocytogenes* and *Bacillus cereus* using agar well diffusion assay. The result showed that the extracts had antibacterial activity and methanolic extract had higher antibacterial activity than acetone extract.

Keywords: *Ganoderma lucidum*, bioactive compounds, antibacterial activity, agar well diffusion

1. Introduction
Ganoderma lucidum like any other fungi grow wild on living or dead/dying wood log of hardwood and many times on dead roots. Mostly *Ganoderma lucidum* is found at the base of living hardwoods or occasionally on the stumps or roots of a wide range of deciduous hosts. The medicinal mushroom *Ganoderma lucidum* (Curt.) P. Karst. called "Lingzhi" in China, has been used in traditional medicines in many Asian countries. The fruiting bodies and mycelium of *G. lucidum* contain immunomodulating polysaccharides, some of which inhibit the growth of several cancer cells [1]. In chemical structure the polysaccharides are 1, 3-β- D-glucans that contain a large number of D-glucose molecules linked by glycoside bonds and which are highly branched [2]. The activities of *Ganoderma lucidum* are mainly because of polysaccharides and/or triterpenoids of the fungus. Some of the triterpenoids showed antioxidant, anticancer [3], antimicrobial activities [4]. *Ganoderma lucidum* have effects on angiogenesis reduction of benign prostatic hyperplasia, antibacterial and antiviral effects on lipid metabolism, antidiabetic activity, vitality and performance enhancement, antioxidant effects and beneficial cosmetic effects on the skin [5]. The aim of this research is to know antibacterial activity of methanolic and acetone extracts of *Ganoderma lucidum* against *Yersinia pestis*, *Pseudomonas aeruginosa, Listeria monocytogenes and Bacillus cereus*. Fruiting bodies of *Ganoderma lucidum* were extracted by following the method of Jonathan and Fasidi[6] using 90% methanol and acetone respectively.

2. Material and Methods
Materials used to check antibacterial activity of the mushroom were, fruiting body of *Ganoderma lucidum* and four bacterial pathogens i.e. *Yersinia pestis, Pseudomonas aeruginosa, Listeria monocytogenes* and *Bacillus cereus.*

2.1 Procurement of test bacteria
Pure cultures of pathogenic bacteria (*Yersinia pestis, Pseudomonas aeruginosa, Listeria monocytogenes* and *Bacillus cereus*) were procured from Department of Biotechnology, H.P.U., Shimla (H.P.) India. Cultures were maintained on nutrient agar medium.

2.2 Maintenance and preservation of pure culture of test bacteria

Pure cultures of each of the bacterial species (*Yersinia pestis*, *Pseudomonas aeruginosa, Listeria monocytogenes and Bacillus cereus*) were maintained on nutrient agar medium and were preserved in refrigerator. Sub culturing was done at regular intervals in order to maintain the cultures. Each bacterial species was transferred from parent source to maintain and preserve the cultures.

2.3 Preparation of *Ganoderma lucidum* extract

Fruiting bodies of *Ganoderma lucidum* were dried under aseptic conditions. Dried mushrooms were pulverized in a blender and 50g each of the powdered samples were soaked separately in 300ml of 95% methanol and acetone until the complete exhaustion in an Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 7 days for extraction. The extracts were filtered through Whatman filter paper no.1 and were evaporated and dried using rotary evaporator at 40 ^{0}C [6]. The extracts were collected and stock solution of concentration 10mg/ml was prepared.

2.4 Antibacterial screening with methanolic and acetone extracts of *Ganoderma lucidum* against *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus*

Screening of methanolic and acetone extract of *Ganoderma lucidum* was done by using agar well diffusion method. Nutrient Agar Medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled water 1000ml) was used throughout the investigation for the growth of microorganisms. The medium was autoclaved at 121.6 ^{0}C for 30 minutes and poured into petri-plates. A 100 µl of bacterial suspension was spread on nutrient agar plates. Agar wells of 8mm diameter were prepared with the help of sterilized stainless steel cork borer. One well was prepared in each agar plate. The well in each plate was loaded with 25%, 50%, 75% and 100% concentration prepared separately by dissolving requisite amount of methanol and acetone in the extract. The plates containing bacterial colonies were incubated at 30 ^{0}C for 24 hours in incubation chamber. All the tests were repeated in triplicates. Diameter of bacterial colonies of treatment and control sets were measured in mutually perpendicular direction on second day. Percentage inhibition of bacterial microorganisms was calculated after subtracting the value of control from the value of extracts using control as standard [7].

$$\text{Percentage} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Control = average diameter of bacterial colony in control
Test = average diameter of bacterial colony in treatment sets [8].

3. Results and discussion

Methanolic and acetone extracts of *Ganoderma lucidum* were seen to show gradual inhibition in the growth of test bacteria. There was a regular increase in inhibition zone diameter of *Y. pestis P. aeruginosa, L. monocytogenes* and *B. cereus,* when their cultures were subjected to increasing concentration of extracts of *Ganoderma lucidum* (Table-1 and 2, Fig. A and B) respectively. Stock solution of conc. 10mg/ml was considered as 100% and other concentrations were prepared by serial dilution of stock solution. Inhibition zone diameter was 24.11 ± 0.23 mm at 25% conc., 26.47 ± 0.27 mm at 50% conc., 29.41 ± 0.21 mm at 75% conc. and 33.05 ± 2.05 mm at 100% conc. of methanolic extract in case of *Y. pestis* respectively. In case of *P. aeruginosa* inhibition zone diameter was 19.15 ± 0.19 mm at 25% concentration, 20.45 ± 0.27 mm at 50% concentration, 22.33 ±0.33 mm at 75% concentration and 25.11 ±1.75 mm at 100% concentration of methanolic extract of *G. lucidum*. In case of *L. monocytogenes* inhibition zone diameter was 15.41 ± 1.70 mm at 25% concentration, 16.94 ± 0.79 mm at 50% concentration, 18.88 ± 0.62 mm at 75% concentration and 20.75 ± 0.33 mm at 100% concentration of methanolic extract of *G. lucidum*. Similar trend of increase in inhibition zone diameter of *Bacillus cereus* was observed when its culture was subjected to the methanolic extract of *Ganoderma lucidum* i.e. 14.35 ±0.18 mm at 25% conc., 15.00 ± 0.11 mm at 50% conc., and 17.26 ± 0.66 mm at 75% conc. and 19.05 ± 0.18 mm at 100% concentration. Plates kept as control did not show growth inhibition zone against test bacteria.

Similar trend of growth inhibition was noticed in case of *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus* with increasing concentration of acetone extract of *Ganoderma lucidum.* It is evident that acetone extract of *Ganoderma lucidum* showed inhibition of around 16.21 ± 0.03 mm at 25% conc., 19.95 ± 0.05 mm at 50% conc., 21.55 ± 0.08 mm at 75% conc. and 24.50 ± 0.04 mm at 100% conc. respectively in case of *Y. pestis.* In case of *P. aeruginosa* inhibition zone diameter was 15.59 ± 0.05 mm at 25% concentration, 16.85 ± 0.11 mm at 50% concentration, 18.22 ± 1.11 mm at 75% concentration and 20.32 ± 0.79 mm at 100% concentration of acetone extract of *G. lucidum*. In case of *L. monocytogenes* inhibition zone diameter was 13.95 ± 0.90 mm at 25% concentration, 15.25 ± 0.22 mm at 50% concentration, 16.99 ± 1.33 mm at 75% concentration and 19.05 ± 1.11 mm at 100% concentration of acetone extract of *G. lucidum*. Similar trend of increase in percentage inhibition of growth was observed in case of *B. cereus.* when the concentration of acetone extract of *Ganoderma lucidum* was gradually increased (i.e. 10.25 ±1.35 mm at 25% conc., 12.19 ± 0.77 mm at 50% conc., and 14.68 ± 0.94 mm at 75% conc. and 16.09 ± 0.49 mm at 100% concentration).

Thus it is evident that the methanolic extract of *Ganoderma lucidum* exhibit higher antibacterial activity as compared to acetone extract of the fungus against the test bacteria. Maximum inhibition was observed in case of *Yersinia pestis* (Gram-negative), followed by *Pseudomonas aeruginosa* (Gram-negative), *Listeria monocytogenes* (Gram-positive) and minimum inhibition was observed in case of *Bacillus cereus* (Gram-positive). Antibacterial activity of different extracts of *Ganoderma lucidum* against various microorganisms have been investigated [9-11]. They reported that the methanolic extract of *G. lucidum* possessed maximum antibacterial activity as compared to other organic and aqueous extracts.

Table 1: Inhibition zone diameter of growth of *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus* at different concentrations of methanolic extract of *Ganoderma lucidum*

Concentration of methanolic extract of *G. lucidum* (%)	Inhibition Zone diameter (In mm ± S.D.)			
	Y. pestis	***P. aeruginosa***	***Listeria monocytogenes***	***Bacillus cereus***
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
25	24.11±0.23	19.15±0.19	15.41±1.70	14.35±0.18
50	26.47±0.27	20.45±0.27	16.94±0.79	15.00±0.11
75	29.41±0.21	22.33±0.33	18.88±0.62	17.26±0.66
100	33.05±2.05	25.11±1.75	20.75±0.33	19.05±0.18

Table 2: Inhibition zone diameter of growth of *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus* at different concentrations of acetone extract of *Ganoderma lucidum*

Concentration of acetone extract of *G. lucidum* (%)	Diameter of inhibition zone (mm) ± SD			
	Y. pestis	***P. aeruginosa***	***L. monocytogenes***	***B. cereus***
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
25	16.21±0.03	15.59±0.05	13.95±0.90	10.25±1.35
50	19.95±0.05	16.85±0.11	15.25±0.22	12.19±0.77
75	21.55±0.08	18.22±1.11	16.99±1.33	14.68±0.94
100	24.50±0.04	20.32±0.79	19.05±1.11	16.09±0.49

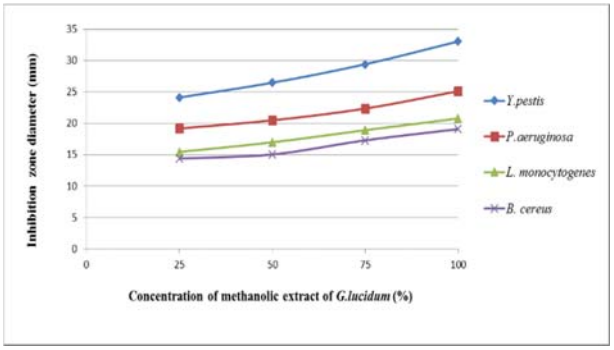

Fig A: Antibacterial activity of *G. lucidum* (methanolic extract) against *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus.*

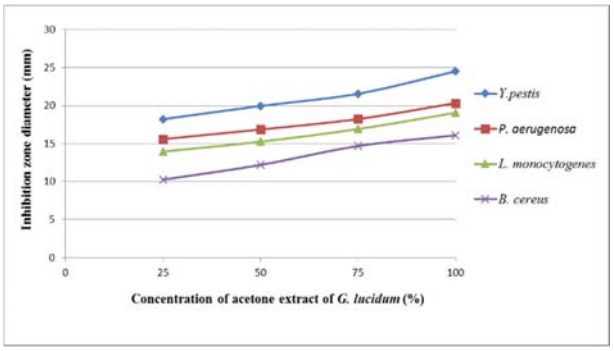

Fig B: Antibacterial activity of *G. lucidum* (acetone extract) against *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus.*

4. Conclusion

Antibacterial activity of methanolic and acetone extract of *G. lucidum* against *Y. pestis, P. aeruginosa, L. monocytogenes* and *B. cereus* was investigated in this study. It is evident that the methanolic extract of *Ganoderma lucidum* exhibit higher antibacterial activity as compared to acetone extract of *G. lucidum* against the test bacteria. Maximum inhibition was observed in case of *Yersinia pestis* (Gram negative), followed by *Pseudomonas aeruginosa* (Gram negative), *Listeria monocytogenes* (Gram positive) and minimum inhibition was observed in case of *Bacillus cereus* (Gram positive). Hence it is concluded that *Ganoderma lucidum* possess antibacterial activity against human pathogenic bacteria.

5. Acknowledgments

Thanks are due to the UGC, New Delhi for providing financial assistance in the form of BSR fellowship and Chairperson, Department of Biosciences, H.P. University, Shimla for providing laboratory facilities.

6. Conflict of interest

The authors declare that there is no conflict of interest.

7. References

1. Sone Y, Okuda R, Wada N, Kishida E, Misaki A. Structure and antitumor activities of the polysaccharide isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. Agricultural and Biological Chemistry. 1985; 49:2641-2653.
2. Wagner R, Mitchell DA, Sassaki GL. Lopes de Almeida Amazonas MA Links between morphology and physiology of *Ganoderma lucidum* in submerged culture for the production of exopolysaccharide. Journal of Biotechnology. 2004; 114:153-164.
3. Lin SB, Li CH, Lee SS, Kan LS. Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. Life Sciences. 2003; 72:2381-2390.
4. Prasad Y. Wesely WEG Antibacterial Activity of The bio-Multidrug (*Ganoderma lucidum*) on Multidrug resistant *Staphylococcus aureus* (MRSA). Advanced Biotechnology. 2008, 9-16.
5. Boh B, Berovic M, Zhang J, Zhin-Bin L. *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnology Annual Review. 2007; 13:265-301.
6. Jonathan SG, Fasidi IO. Antibacterial activities of Nigerian edible macro fungi- *Lycoperdon pusillum* (Bat. Ex) and *Lycoperdon giganteus* (Pers.). African Journal of Biomedical Research. 2003; 6:85-90.
7. Hemashenpagam N, Selvaraj T Antimicrobial potentials of different extracts of *Solanum Xanthocarpum* Chard and Wendt. Plant Archives. 2010; 1:387-390.
8. Kannan P, Ramadevi SR, Hopper W. Antibacterial activity of *Terminalia chebula* fruit extract. African Journal of Microbiology Research. 2009; 3:180-184.

9. Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. People's journal of Scientific Research. 2010; 3:9-13.
10. Djide MN, Sartini RL, Hasyim N. Antibacterial activity of various extracts from the fruiting bodies of *Ganoderma lucidum* growing at samanea saman (jacq.) Merr) trunk. International Journal of Scientific and Technology Research. 2014; 3:15-16.
11. Sagar A, Kumari K. Study on ultrastructure and antibacterial activity of *Ganoderma lucidum* (Curt.) P. Karst. Indian Journal of Mushrooms. 2012; 30(2):5-9.