AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Comparative Study of Extracts of *Ganoderma Lucidum* for Anthelmintic and Antibacterial Activity

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ABSTRACT

The love affair of Asian's with Ling zhi, the Chinese name for *Ganoderma lucidum* and related species can be traced back over two thousand years. *Ganoderma lucidum* has been used for a broad spectrum of health benefits from preventive measures and maintenance of health to the regulation or treatment of chronic as well as acute life threatening illness. The present study was done to compare the Aqueous and methanolic extract of *Ganoderma lucidum* for anthelmintic activity and to compare the methanolic and chloroform-acetone extract of *Ganoderma lucidum* for antibacterial activity. Aqueous extract of mushroom did not show any result for anthelmintic activity, while the methanolic extract (60 mg/ml) was equally effective as standard (Albendazole 20 mg/ml). For antibacterial activity both the methanolic (350 mg/ml) and chloroform-acetone (350 mg/ml) extracts showed effective results, but the results of choloroform-acetone extract were more effective than methanolic extract and standard (Gentamycin 40 mg/ml).

Key words: anthelmintic, antibacterial, methanolic extract, aqueous extract.

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Received 09 October 2012, Accepted 15 October 2012

Please cite this article in press as: Jasrotia N *et al.*, Comparative Study of Extracts of *Ganoderma Lucidum* for Anthelmintic and Antibacterial Activity.American Journal of PharmTech Research 2012.

INTRODUCTION

Mushrooms have long been considered to have medicinal value[1]. Of the 14000 to 15000 species of mushrooms in the world[2], around 700 have known medicinal properties. However, it has been estimated that there are about 1800 species of mushrooms that have potential medicinal attributes[1]. *Ganoderma lucidum* (Leyss. ex Fr.) Karst. (G. lucidum: Lingzhi in Chinese, Reishi in Japanese) has been used for a long time in China to prevent and treat various human diseases. *Ganoderma lucidum* has been under modern pharmacological and clinical research in the recent 30 years, and it has been reported to be effective in modulating immune functions, inhibiting tumor growth[3]. Chemical constituents in Ganoderma spp. are diverse such as triterpenes, sterols, alkaloids, polysaccharides, proteins, peptides, amino acids, nucleotides, lectins, vitamins and fatty acids[4]. It is widely used as a crude drug in the treatment of a variety of ailments such as like chronic hepatitis, nephritis, hepatopathy, neurasthenia, arthritis, bronchitis, asthma, gastric ulcer and insomnia[5].

Helminth infections are most widely found in those human beings particularly who are in low poverty level & does not maintain hygienic condition[6]. Worm diseases cause serious morbidity & affect population in endemc areas[7]. Anthelmintics or antihelminthics are drugs that expel helminth parasitic worms (helminths) from the body, either by stunning or killing them. However these worms have shown the development of resistance to some broad spectrum anthelmintics (benzimidazoles, levamisole, avermectins) and also some narrow spectrum wormers such as the salicylanilides (closantel)[8].

Bacteria are the smallest, unicellular organisms, equipped with all the machinery for growth and self-replication at the expense of foodstuffs. Only a few species of bacteria cause disease, often due to the cell's ability to produce specific poisons, toxins or aggresins[9]. Antibacterials are the substances produced by microorganisms, which selectively suppress the growth of or kill other microorganisms at very low concentrations. This definition excludes other natural substances which also inhibit micro-organisms but are produced by higher forms (e.g. antibodies) or even those produced by microbes but are needed in higher concentrations. Antibacterial drugs are the greatest contribution of the 20th century to therapeutics[10]. 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[11].

This present study was done to compare the activity of methanolic and aqueous extract of *Ganoderma lucidum* for anthelminitc activity and methanolic and chloroform-acetone extract for

antibacterial activity.

MATERIALS AND METHODS

Plant collection:

Dried fruiting bodies of *Ganoderma lucidum* were collected from Directorate on Mushroom Research, Solan. The fruiting bodies of the mushroom were grinded into coarse powder with the help of mechanical grinder.

Preparation of extract:

The powdered mushroom (300gm) was packed in three soxhlet extractors for three different extracts and the soxhlet assembly was then labelled. Methanol was used as a solvent for extraction of mushroom in one assembly and chloroform-acetone (1:1 v/v) was used as a solvent for extraction of mushroom in another assembly and water was used in third assembly. Extraction in all the three solvents was done for 72 hours.

Determination of anthelmintic activity:

Adult earthworms *Pheretima posthuma* were used for testing anthelmintic activity of *Ganoderma lucidum*. These worms were purchased from V.D.S Chambaghat, Solan. These worms were washed with normal saline to remove the faecal matter attached to them. The average size of earthworm was 5-7 cm. The earthworms resembled the intestinal roundworm parasites of human beings both anatomically and physiologically and hence were used to study the anthelmintic activity[12].

For determining anthelmintic activity, procedure of Lakshmanan Baskar, et al was followed but with minor modifications The worms were divided into respective groups containing six earthworms in each group. All the prototypes (methanolic and aqueous extract) were dissolved in minimum quantity of 2% v/v tween80 and the volume was adjusted to 25 ml with normal saline for making the concentration of 20, 40, 60 mg/ml. All the prototypes (methanolic and aqueous extract) and the standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 25 ml of respective formulations as follows, vehicle (2% v/v tween80 in normal saline), Albendazole (20 mg/ml) and prototypes (20, 40, 60 mg/ml). Six worms of about the same size per petridish were used. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. All the determinations were done in duplication.

Determination of antibacterial activity:

Antibacterial activity of mushroom extract was carried out by the filter paper disc diffusion technique, as per the method of Wani IA, with little modification[13].300 ml of Mueller Hinton Agar medium was prepared. The bacterial strain *Staphylococcus aureus* (ATCC-29737) was obtained from Kwality Pharmaceuticals, Amritsar. Mc Farland Standard was prepared as per the method mentioned by Wani IA and was compared with the bacterial culture[13].

After preparation of the Muller Hinton agar plate, the methanolic and chloroform-acetone extracts were prepared in a concentration of 50, 150, 250, 350 mg/ml. Finally the discs containing the standard antibiotic and the extracts of *Ganoderma lucidum* were prepared.

The discs containing methanolic extracts (50, 150, 250, 350 mg/ml) were placed with the help of sterile foreceps on one inoculated petridish. The discs containing chloroform-acetone extracts (50, 150, 250, 350 mg/ml) were placed with the help of sterile foreceps on the second inoculated petridish. Third petridish was taken as control with disc (not containing any solution) and in fourth petridish the disc containing standard antibiotic solution (Gentamycin, 40 mg/ml) was placed.

RESULTS AND DISCUSSIONS:

Anthelmintic activity:

The aqueous extract of *Ganoderma lucidum* did not showed anthelmintic activity against any of the worm but the methanolic extract showed the activity. From table 1 it was concluded that higher concentration of methanolic extract produced paralytic effect much earlier and the time of death was also reduced for the worms.

Table1: Anthelmintic activity of methanolic extract of *Ganoderma lucidum*

Groups	Concentration	Paralysis time	Death time
1	Control	Nil	Nil
2	20 mg/ml	54.66±0.3	131.83±0.8
3	40 mg/ml	18.66±0.6	30.5±0.4
4	60 mg/ml	11.33±0.7	23.83±0.2
5	Albendazole (20 mg/ml)	7.68±0.11	32±1.1

All determinations were done in duplication and results are expressed as Mean ± SEM.

P value was <0.01

In the present study it was revealed that aqueous extract of *Ganoderma lucidum* did not exhibited anthelmintic activity against the worms. Methanolic extract showed anthelmintic activity in dose-dependent manner. The minimum time of paralysis and death was recorded against the worms at 60 mg/ml of methanolic extract of *Ganoderma lucidum*. The anthelmintic results of the

methanolic extract were compared with reference standard, which was Albendazole (20 mg/ml). It was observed that the anthelmintic effect of methanolic extract was more or less comparable with the standard drug used. (Table 1)

Turbidity test:

The broth was more turbid than the standard, so sterile saline was added until it was comparable to the standard.

Antibacterial activity:

Many antimicrobial compounds such as terpenes, lectins, polysaccharides etc, act on the bacterial cytoplasmic membrane[14,15]. Antibacterial activity of methanolic and chloroform-acetone extract showed effective zone of inhibition against *Staphylococcus aureus* (ATCC-29737). The maximum zone of inhibition for chloroform-acetone extract was found to be 25 mm at 350 mg/ml and the maximum zone of inhibition for methanolic extract was found to be 18 mm at 350 mg/ml. The results were compared with the reference standard Gentamycin (40 mg/ml). It was revealed that chloroform-acetone extract showed a zone of inhibition which was more effective than the reference standard. The zone of inhibition of methanolic extract was not much effective as compared to chloroform-acetone extract but near the value of zone of inhibition of Gentamycin. Thus, it was observed that the antibacterial activity of choloform-acetone extract was much more effective as compared to methanolic extract and Gentamycin (Table 2). The results of antibacterial activity of methanolic extract of *Ganoderma lucidum* were in accordance with the results given by Sridhar Sekaran, et al[16].

Table 2: Antibacterial activity of extracts of *Ganoderma lucidum*

S.No	Concentration (mg/ml)	Zone of inhibition of Chloroform-acetone extract	Zone of inhibition of Methanolic extract
1	50	9mm	5mm
2	150	14mm	11mm
3	250	21mm	14mm
4	350	25mm	18mm

Standard (Gentamycin) 40 mg/ml showed 22 mm of zone of inhibition

CONCLUSION

Ganoderma lucidum is one of the most famous traditional Chinese medicinal herbs. Due to the wide variety of its biological activities, such as antitumor, immunomodulatory, cardiovascular, respiratory, antihepatotoxic and antinociceptive effect, the mushroom attracts international attention as a valuable herb[17,18]. Ganoderic acid, triterpenes and polysaccharides are the major compounds of the mushroom with significant pharmacological activities[19]. In this work it was

concluded that aqueous extract of *Ganoderma lucidum* did not show anthelmintic activity against any worm but the methanolic extract of the mushroom was equally effective as compared to Albendazole. Both the methanolic and chloroform-acetone extracts of *Ganoderma lucidum* showed zone of antibacterial inhibition but the zone of inhibition of cholorform-acetone extract was much more effective than Gentamycin and methanolic extract. With the presence of approximately 400 different bioactive compounds, *Ganoderma lucidum* is a blessing for mankind. It is evident from the present study that mushroom extracts from *Ganoderma lucidum* could be employed to combat bacterial and helmintic infections.

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