Biochemical and Antimicrobial Activities of $AgNO_3$ Treated Ethanolic Extracts of G*anoderma lucidum*

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Abstract- **In recent years, varieties of mushrooms have been identified and the number of mushrooms being cultivated for medicinal purposes. Fresh *Ganoderma lucidum* fruiting bodies were collected from Belgahana, Bilaspur District, Chhattishgarh during August and September 2020 and were tested for biochemical and antimicrobial activities from ENT screened microbes. Protein, Carbohydrate, Tannins and Terpenoids are present in ethanol, methanol, water of crude and silver nitrate treated extracts. The crude extract of *Ganoderma lucidum* and $AgNO_3$ from ethanol, methanol, and aqueous was revealed good antimicrobial activity against test organisms. Ciprofloxacin has used for standard was shown good zone of inhibition (20 to 28mm). The Ethanol extract of $AgNO_3$ treated with of *Ganoderma lucidum* has shown effective inhibition zone against all the organisms. Methanol extract of crude and $AgNO_3$ of *Ganoderma lucidum* shown inhibitory effect against test organisms (*candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa and staphylococcus aureus*). Ethanolic extract of $AgNO_3$ treated *Ganoderma lucidum* has shown good inhibitory effect against ENT infected microbial species while in crude extract it shown low inhibition of zone.**

Keywords: Biochemical screening, antimicrobial activity, *Ganoderma lucidum*

1.INTRODUCTION

In recent years, varieties of mushrooms have been identified and the number of mushrooms being cultivated for medicinal purposes (Smith et al., 2002; Chang and Wasser, 2012). *Ganoderma lucidum* (Family - Polyporaceae), is a medicinal mushroom, commonly known as Reishi, this mushroom is a valuable herb due to its biological activities such as, immuno - modulatory, cardiovascular, respiratory, anti-tumor, antiviral, antihepatotoxic effect (Ahmed et al., 2019). Extracts of mycelia of *Ganoderma* species showed an antibiotic effect against organisms such as *Pseudomonas syringae* and *Bacillus subtilis* (Basnet et al., 2017). *Ganoderma* contains a variety of bioactive compounds including a range of proteins, triterpenoids, and other lipids, polysaccharides and nucleotides (Kamble et al., 2011).

Bioproducts of *Ganoderma lucidum* have multi beneficial effects for human welfare. These are widely used as traditional medicinal ingredients for the treatment of various health problems. . It's major compounds with significant pharmacological activities are, triterpenes, ganoderic acid and polysaccharides (Wang et al., 2020). A new class of compounds with medicinal and nutritional features extractable from either the fruiting bodies or the mycelium of mushrooms have been referred to as "mushroom nutraceuticals" (Quereshi et al., 2010).

Mushroom contain vitamins A and C or ß carotenes and a wide variety of secondary metabolites such as phenolics compounds, terpenes, phenols, and steroids, all have protective effects because of their antioxidant properties (Valverde et al., 2015). The present work is to carry out experiments to screen antibacterial potential of different extracts of *Ganoderma lucidum*.. Present study is strongly suggestive that *Ganoderma lucidum* can be used as antimicrobial agent in the development of new drug for the treatment of various infections caused by bacterial pathogenesis and harmful activity of excess free radicals in humans (Kamra and Bhatt 2012).

2.MATERIALS AND METHODS

1.1 Collection and extraction of *Ganoderma lucidum*
Fresh *Ganoderma lucidum* (Figure 1) were collected from Belgahana, Bilaspur District, Chhattishgarh during August and September 2020. The *Ganoderma lucidum* were washed systematically and dried out in sunlight. The

mycelia of *Ganoderma lucidum* were grinded to powder and were used for the extraction.

Figure 1: *G. lucidum* samples in the present work

1.2 Mycelial Extracts

Dried mycelia were extracted in different solvent such as ethanol , methanol, water separately using hot extraction method, twenty gram of *G. lucidum* powder was dissolved in 200 ml of 70% ethanol and kept the Soxhlet apparatus for the hot extraction process soxhlet apparatus. The extract was then stored in a refrigerated condition for further use.

1.3 Synthesis of $AgNO_3$ treated extracts from *G.lucidum* extracts

1 ml of ethanol extract of *G. lucidum* was added to the 1 mM silver nitrate solution and kept the solution in sunlight for 5 to 6 hrs and finally, the reddish-brown colour change was observed. 1 ml of ethanol extract of *G. lucidum* was added to the 1 mM silver nitrate solution. The same procedure was carried out for the remaining four different volumes of *G. lucidum* extracts and silver nitrate solution

1.4 Preliminary screening

Various extracts were used for preliminary screening for biochemical such as protein, carbohydrates, tannins, amino acids, phlobatanins, terpenoids, steroids, phenols.

Test of proteins To 2 ml of the extract, 2 ml of Biuret reagents is to be added. An appearance of violet color ring indicates the presence of protein.

Test for Carbohydrates

To 2 ml of extract add 2 drops of Molisch's reagent and mix the solution. Nearly 2 ml of Conc. H_2SO_4isto be added drop by drop from the sides of test tube. A reddish violet color ring appearance at the junction of two layers indicates the presence of carbohydrates.

Test for Amino acids

To 2 ml of the extract, 2 ml of Ninhydrin reagent is to be added and keep the solution in hot water bath for 15 minutes. The formation of purple color indicates the presences of aminoacids in the sample.

Test for Tannins

To 5 ml of extract, few drops of 1% of lead acetate are to be added. A yellow colored precipitate formed in the test tube shows the presence of tannins.

Test for Phlobatinins

To 2 ml of extract add 1% aqueous HCl and boiled for few minutes. A red precipitate formed and deposited in the test tube is an evidence for the presence of phlobatinins.

Test for Terpenoids

To 2 ml of extract add 2 ml of Chloroform and 3 ml of Conc. H_2SO_4. Formationof a monolayer of reddish brown coloration of an interface shows a positive result for the terpenoids.

Test Microorganism

Some microorganism screened from Ear, Nose, Throat infected samples used in current research work are *Candida albicans, Enterococcus faecalis , Pseudomonas aeruginosa and Stathylococcus aureus* were used as the test organisms . A loopful of these culture were inoculated onto nutrient agar broth and separately incubated at 37^0C for 24 hr.

3.ANTIMICROBIAL ACTIVITY USING ZONE METHOD

Antimicrobial activity has been conducted based on agar well diffusion method. Freshly prepared Mueller Hinton broth has been prepared and inoculated with selected broth cultures. The broth was inoculated into growth medium. The Mueller Hinton agar growth medium has prepared in the petri-plates wells of 8mm size were made in the growth media with sterile borer. About extract were added to the wells of growth media. Petri-plates were incubated at 37^0 C for 24 hr. The zone of inhibition was measured for

each extract after incubation. Standard ciprofloxacin solution was used as a standard.

4.RESULTS AND DISCUSSIONS

The present investigation reported that ethanol, methanol, and aqueous extracts of *Ganoderma lucidum* contain protein positive in crude extract. $AgNO_3$ extract of *Ganoderma lucidum* indicated moderately positive in methanol extract. Carbohydrate indicated positive in ethanol, and AgNO3 extracts, but highly positive in methanol extract, and moderately present in aqueous extract. Tannins showed moderately positive in methanol and aqueous extract in AgNO3. Amino acids and phlobatinis absent in all extract. Terpenoids are strongly present in $AgNO_3$ of methanol extract and moderately present in $AgNO_3$ of aqueous extract. Protein, Carbohydrate, Tannins and Terpenoids are present in all the selected extracts.

Table 1: Biochemical screening of Ganoderma *lucidum* extracts

Name of the compound	Ethanol extract		Methanol extract		H2O extract	
	Crude	AgNO3 extract	Crude	AgNO3 extract	Crude	AgNO3 extract
Protein	+	+	+	++	+	+
Carbohydrate	+	+	+++	+	++	+
Tannins	+	+	+	++	+	++
Amino acids	-	-	-	-	-	-
Phlobatinins	-	-	-	-	-	-
Terpenoids	+	+	+	+++	+	++

Note: +++ is strongly positive (Color with more intensity); ++ is moderately positive (color intensity medium); + is weekly positive (color intensity low); - is negative

The crude extract of *Ganoderma lucidum* and $AgNO_3$ from ethanol, methanol, and aqueous was revealed good antimicrobial activity against test organisms. Ciprofloxacin has used for standard was shown good zone of inhibition (20 to 28 mm). The ethanol extract of $AgNO_3$ of *Ganoderma lucidum* has shown effective inhibition zone against all selected organisms. Methanol extracts of crude and $AgNO_3$ shown inhibitory effect against test organisms like *candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa and staphylococcus aureus*. Ethanolic extract of $AgNO_3$ shown good inhibitory effect against ENT infected microbial species while in crude extract it shown low inhibition of zone.

Table 2: Measurement of zone of inhibition of $AgNO_3$ and crude extracts of *Ganoderma lucidum*.

Name of microorganims	$AgNO_3$			Crude			Blank	Standard	$AgNO_3$
	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol			
Candida albicans	10	14	13	0	10	10	0	20	10
Enterococcus faecalis	9	13	12	0	0	0	0	30	0
Pseudomonas aeruginosa	10	11	11	0	0	9	0	19	0
Stathylococcus aureus	10	12	11	9	9	9	0	28	0

The antimicrobial studies conducted with four extract viz, Ethanol , Methanol, Aqueous and $AgNO_3$ of *Ganoderma lucidum* showed wide variation with respect to their effect, from present study it was proved that the ethanol extract with AgNO3 was very efficient to control all the four test organisms. The ethanol extract with $AgNO_3$ has maximum antimicrobial activity against test organisms, however the methanol extract with $AgNO_3$ indicated inhibitory effect for all test organisms, and however in crude extract it indicated inhibitory effect in test organisms except *Enterococcus faecalis*. In aqueous extract with $AgNO_3$ indicated zone inhibition of all test organisms while in crude extract it indicated inhibition against *Staphylococcus aureus*. Present study showed that the ethanol extract possesses more potential as an antimicrobial agent.

Ganoderma lucidum are good sources for the treatment of numerous diseases and in many countries, these natural resources were used in their

treatment system from ancient time (Islam et al., 2018). Recently, these become a principal target of investigation for searching a novel biologically active compound to develop many natural antibiotics that have been used for different infectious diseases (Godzieba et al ., 2020). Cytotoxic effects of *Ganoderma lucidum* extracts represent the pharmacological and medicinal activities and it is evaluated by a widely used, easy, and cheapest.

5.CONCLUSION

Protein, Carbohydrate, Tannins and Terpenoids are present in ethanol, methanol, water of crude and silver nitrate treated extracts. The present study showed that the ethanol extract possesses more potential as an antimicrobial agent. Further purification, *in vitro* and *in silico* studies has to be conducted for better understanding of usage of *Ganoderma lucidum* in the treatment in ENT species.

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