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ORIGINAL ARTICLE

In Vitro Antioxidant and Anticancer Effect of *Ganoderma Lucidum* from Wood

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ABSTRACT
Ganoderma lucidum is a fungus that has a wide range of medicinal applications. The findings demonstrate that polysaccharides and triterpenes extracted from Ganoderma lucidum have bioactive activities, and that it contains 10.2 percent mineral. The entire mushroom is edible, and the fruiting body, in particular, is high in protein. According to studies, 15 kDa protein and lectins extracted from the mushroom have immuno-potentiating and antifungal activities, whereas mycelium includes protein LZ-8, which suppresses the immune system. Anti-tumour action has been shown in long-chain fatty acids extracted from spores. Carbohydrates and crude fibre are also abundant in dried mushrooms. Treatments used to treat cancer, such as cisplatin and cyclophosphamide, can have a variety of adverse effects, including renal failure and cancer cell resistance to chemotherapeutic drugs. Owing to Patients with these negative effects were unable to continue their medication and were cured of their sickness. Ganoderma lucidum has the ability to cure cancer, which aids in the reduction of adverse effects. The studies show that Ganoderma lucidum has anti-proliferative, pro-apoptotic, and anti-migratory effects on cancer cells. As a result, combination therapy will assist patients in finishing their medications and saving lives by improving immunity. The present study focuses on the antibacterial, antifungal, and antioxidant properties of Ganoderma lucidum and also elucidates the presence of bioactive compounds.
Keywords: *Ganoderma lucidum , Anti-cancer, Anti-oxidant, Bioactive Compounds, chronic diseases.*

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INTRODUCTION

In nature, *Ganoderma lucidum* is a black fungus with a lustrous look and a woody feel. The Latin word lucidus means "bright" or "brilliant." In different parts of the world, *Ganoderma lucidum* is known by different names. In China, it is known as lingzhi (21), while in Japan, it is known as mannentake or reishi (20). *Ganoderma lucidum*, one of the edible mushrooms, has a great therapeutic potential; it has been used to heal ailments and as a medication to lengthen human life since ancient times. It is now commercially accessible in powder, meal supplement, and tea form (18). *Ganoderma lucidum* has been shown to aid with blood glucose management, hyperglycaemia, chronic bronchitis, asthma, cancer, and HIV (15) (20), immune development, and bactericidal action (15)(20). Hepatoprotection is also provided. It also inhibits anti-atherosclerotic, anti-inflammatory, anti-diabetic, anti-oxidative, anti-aging, anti-fungal, anti-viral, anti-coagulant (20) and immunity-boosting agents (20) (2) Because of its excellent medicinal potential, it is in high demand all over the world. To address this need, an artificial approach employing low-cost resources like as grain, sawdust, wood locks (19), and cock leftovers is advised. Artificial production of *Ganoderma lucidum* is especially challenging since each species requires a different environment to thrive.(3)
The fungus *Ganoderma lucidum* is a member of the genus *Ganoderma* (8), the family Ganodermataceae, the Order Polyporaes, and the Class Agaricomycetes (14). Major and minor nutrients are found in *Ganoderm*

lucidum. The components present in *Ganoderm lucidum* listed in Table 1. And it also contains micronutrients like iron, magnesium, manganese, zinc, copper, and calcium. (20). Polysaccharide present in the *Ganoderma lucidum* (GL – PS) is a heteropolymer that has xylose, mannose, galactose, and fructose, 1-3, 1-4, and 1-6 linked β and α – D or L substitutions (18). It helps to treat cancer and liver diseases (Gao et al., 2005), and also has anti-inflammatory, hypoglycaemic, anti-cancer, anti-tumerogenic, and immune-stimulating effects. (20). Triterpenoid is also present in this mushroom and possesses antimicrobial activity. Various countries like China, Korea, Japan, & USA have done detailed research and proved that polysaccharide and triterpenoid also help to treat hypertension, diabetes, and cancer. (10), fatigue syndrome, hepatitis, prostate cancer, and HIV-I. (15) (21).

Water soluble peptididoglycans derived from *Ganoderma lucidum* show antiviral action. They're also employed to modulate the immune system.(Yugendhar Parepalli, 2020). Other than the bioactive chemicals stated above, more than 300 bioactive compounds have been extracted from *Ganoderma*, and its therapeutic capabilities have been investigated and proven. (21). It also contains bactericidal enzymes such as lysozyme and acid proteases.

Drugs like cisplatin and cyclophosphamide are routinely used to treat cancer patients, however they have adverse effects include renal failure and induce malignant cells to acquire resistance. (19). To address this, research has been conducted to find phytomedicines that can be used to treat cancer. According to Shing et al., when patients are given bioactive components of *Ganoderma lucidum* as a medicine for six months, the mitogen-induced lymphoproliferative responses improve. (17). Bioactive substances extracted from basidiocarp and spores have been shown in studies to boost the immune systems of immuno-compromised youngsters with malignant malignancies. According to Chuanwei Jiao,2020 et al.,(7) the spore oil of *Ganoderma lucidum* causes breast cancer cells to apoptosis. (12). Triterpanes have been shown to inhibit the proliferation of malignant cells in Lewis-tumour-bearing animals (19). In mice with hepatoma, GLPS inhibits cancer cell proliferation (11.). The existence of bioactive components in Ganoderma lucidum, as well as its antibacterial and antioxidant activities, are demonstrated in this study.

MATERIAL AND METHODS

SAMPLE COLLECTION

A vacuum evaporator was used to dry the *Ganoderma lucidum* mushroom that was taken from the forest. (Fig – 1)

EXTRACTION OF METHENOL

Methanol was employed as an extraction solvent.

The extract (Fig – 3) was made from the dried powder of *Ganoderma lucidum* using the soxhlation process (1). (Fig-2)

Fig.1: *Ganoderma lucidum*

Fig. 2: Ganoderma solvent Extraction using Soxhlation method

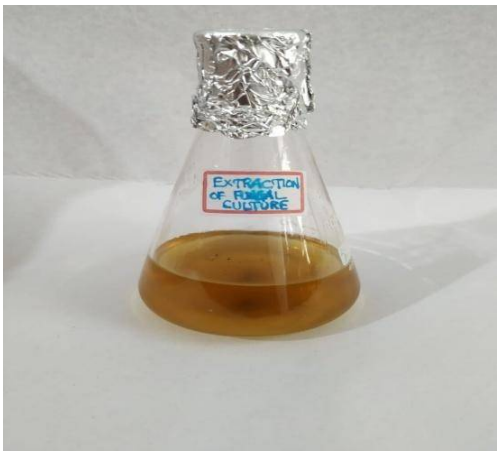


Fig. 3 showing: Solvent extraction

ANALYSIS OF BIOACTIVE COMPOUNDS

The presence of alkaloids was determined using the Wagner's test, which involved adding 4-5 drops of Wagner's reagent to a few millilitres of extract. To test for the presence of phenol, 2ml of extract was combined with a 10% ferric chloride solution. 1 ml of extract and a few drops of Fehling's reagent were added to a boiling water bath for 10 minutes to detect the presence of reducing sugar. The presence of saponins was determined using the foam test technique, which involved mixing 2 mL of extract with 6 mL of sterile distilled water and aggressively shaking to see if foam formed. The presence of flavonoids was determined by treating the extract with a 10% ferric chloride solution. To determine the presence of polysterols, Salkowski's test was done by mixing 2 mL chloroform with a few drops of acetic anhydride in 1 mL extract. The ninhydrin test was used to determine the amino acid by mixing the ninhydrin solution with a few ml of extract. The presence of steroids was determined by diluting 1 ml of extract with 2 ml chloroform and 0.2 ml concentrated sulfuric acid. The presence of tannin was determined by adding a few drops of weak ferric chloride solution to the extract. A few cc of concentrated sulphuric acid was added to the extract to evaluate the glycoside.

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Pure cultures of *E. coli, Staphylococcus aureus, Pseudomonas, Kelbsiella,* and *Proteus* were inoculated into nutritional broth and incubated at 37 degrees Celsius for 24 hours. *Candida albicans* and *Candida tropicalis* were inoculated into SDB broth and incubated for 48 hours at 35 degrees Celsius. The well diffusion technique was used to assess microbial activity. (4). The inoculum was evenly swabbed and Muller Hinton agar plates were made. On agar, wells were cut and the extract was applied in concentrations of 25µl, 50 µl, 75 µl, and 100 µl to each well. The plates with the bacterial culture injected were incubated for 24 hours at 37 degrees Celsius. The fungal culture plates were incubated for 48 hours at 35 degrees Celsius.

ANTIOXIDANT EFFECTIVENESS

Using the approach described by Braca et al.,(6). 2,2-diphenyl-1-picrylhydrazyl was used to assess the free radical scavenging activity. At varied concentrations of 5, 10, 15, 20, and 25 µl, 0.004 % of DPPH 3ml solution was added to the methanolic extract. The solution was well mixed and allowed to rest for 30 minutes at room temperature. A spectrophotometer was used to test the solution's absorbance at 517nm after it had been incubated.

The antioxidant ability of the extract was evaluated using the procedure described by Pulido et al.,(16). At 37°C, 900l of freshly produced FRAP reagent was stored. (5). 90 µl of distilled water and various quantities of methanol extract (5, 10, 15, 20, and 25 µl) were added to the FARP reagent and incubated at 37°C for 30 minutes. The measurements were calculated using a spectrophotometer at 593nm after the incubation period.

To the 5 µl, 10 µl, 15 µl, 20 µl, and 25 µl of extract 400 µl of 100 mM sodium nitropruside and 100 µl of PBS (pH 7.4) were added, accordingly. The mixture was well mixed and incubated for 2.5 hours at 25°C. After incubation, 0.5 ml Giess reagent was added and allowed to sit for 30 minutes at room temperature. The Absorbance values were then taken using a spectrophotometer set to 540 nm.

Radical scavenging activity, antioxidant capacity of the extract, and nitric oxide scavenging activity were calculated using the formula:

$$Scavenging\ activity = {}^{Control\ -\ Sample}/_{Control} \times 100$$

***IN VITRO* ANTICANCER ACTIVITY:**

Human liver cancer cells (Hp G2) were bought from the National Centre for Cell Science (NCCS), Pune, and cultured in Eagles' medium at 37°C with 5% CO_2, 95% oxygen, and 100% relative humidity. The progress of the cells was monitored twice a week. The cell line was treated with EDTA after forming a monolayer of cells to get a single cell suspension. A heamocytometer was used to count viable cells, and the cell solution was diluted with 5% FBS. A 96-well plate was used, with 100 µl of cell suspension put to each well and incubated at 37°C for 24 hours with 5% CO_2, 95% oxygen, and 100% relative humidity for cell adhesion to the well walls. After 48 hours of incubation, a variable concentration of extract was added to the well-containing media and incubated at 37°C, 5% CO_2, 95% oxygen, and 100% relative humidity.

MTT ASSESSMENT

The cells were then treated with 15 µl of MTT in phosphate saline buffer and incubated at 37°C for 4 hours after incubation. Formazan crystals were removed from the well plate after incubation by removing the surplus medium and dissolving them in 100 µl of DMSO. The absorbance was measured at 570nm using a microplate reader. The proportion of viable cells was computed as follows:

$$Percentage\ of\ Cell\ Viability\ = {}^{Test}/_{Control} \times 100$$

$$Cell0inhibition = {}^{sample\ absorbance}/_{control\ absorbance} \times\ 100$$

RESULT

BIO ACTIVE COMPOUND ANALYSIS

The extracts were subjected to a qualitative phytochemical analysis using the techniques listed below. The presence of alkaloids is confirmed by the production of a reddish-brown precipitate. The presence of phenol is confirmed by the production of a deep blue or black colour. The emergence of the blue colour was discovered to be caused by the presence of reducing sugars. A continuous foam appears after a few seconds, showing the presence of saponins. The presence of flavonoids is indicated by the production of a green or blue colour. The presence of phytosterols is indicated by the production of a blue green colour. The presence of amino acids and proteins causes a purple or violet colour to appear. The presence of steroids is indicated by the production of a red-colored precipitate. The creation of a dark green or blue tint confirms the presence of tannin. The presence of glycoside is shown by the production of a red colour. The results were tabulated below(Table no:2)

ANTIBACTERIAL ACTIVITY:

Antimicrobial activity and minimum inhibitory concentrations of extracts against bacteria such as *E. coli (Fig-7), Staphylococcus aureus (Fig – 5), Bacillus,(Fig-4) Pseudomonas (Fig-6), Candida tropicalis (Fig -8)*, and *Candida albicans (Fig- 9)* were determined using the agar well diffusion technique. The extracts have antibacterial properties against the microorganisms that were tested. (Table – 4)

DPPH Assay:

By measuring the absorbance at 518 nm, the decolourization of DPPH was measured. Instead of plant extract 1 mL of ascorbic acid, the appropriate vehicle was used as a control. By comparing the absorbance values of the control and experimental tubes, the Percentage of suppression of DPPH radicals by the extract/compound was measured.(Table -5) (Fig-10)

FRAP Assay:

The antioxidant concentration has the same ferric-TPTZ reducing capacity as 1 mmol/l FeSO4.7H2O. The concentration of antioxidant that causes an increase in absorbance in the FRAP test equal to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution obtained using the related regression equation was estimated as EC1. (Table -6)

NITRIC OXIDE (NO) SCAVENGING ASSAY:

The antioxidant activity of plant extract was assessed using nitric oxide scavenging activity at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 litres. Finally, at 540 nm, absorbance is measured. The formula was used to compute the % inhibition of each reaction, which was done in triplicates. (Table -7) (Fig – 11)

MTT ASSAY:

While increasing the concentration of the extract the cell viability has been reduced. That shows that the action of extract on cancer cells. The component present in the mushroom inhibit the growth and multiplication of the cancer cells. The Figure-12 showing the HpG2 Cell viability after treating with DMSO at different concentrations

Fig 4. Showing antibacterial activity against *Bacillus*

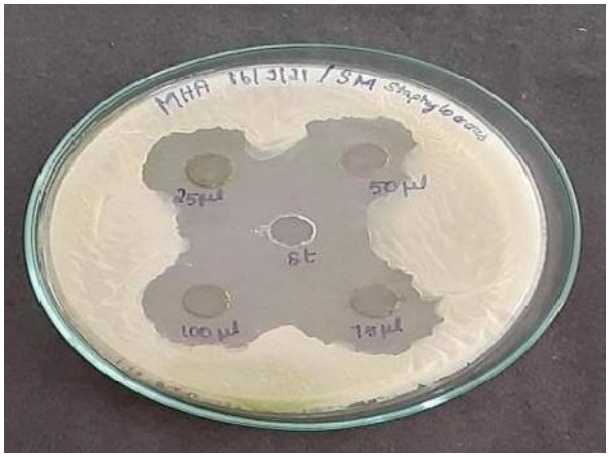


Fig 5. Showing antibacterial activity against *Staphylococcus aureus*

Fig 6 . Showing antibacterial activity against *Pseudomonas*

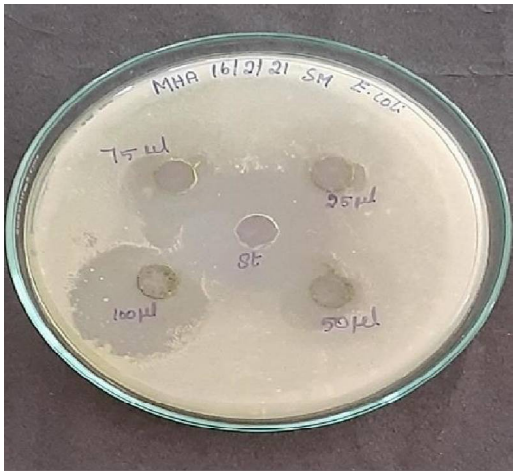


Fig . 7 Showing antibacterial activity against *Escherichia coli*

Fig. 8 **Showing antibacterial activity against** *Candida tropicalis*

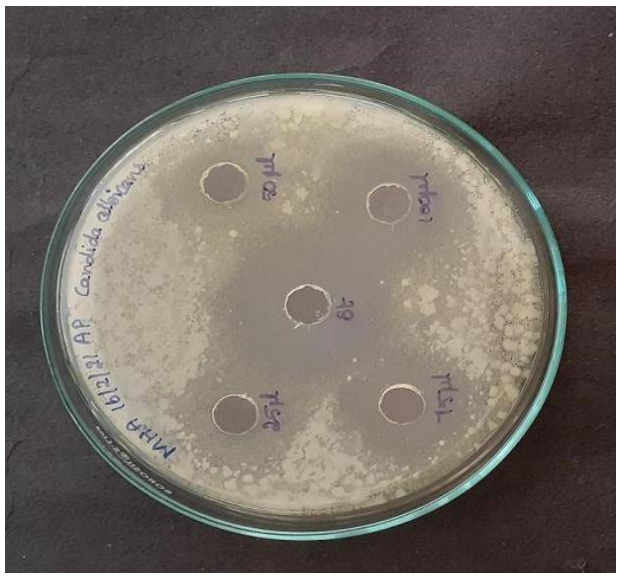


Fig: 9 **Showing antibacterial activity against** Candida albicans

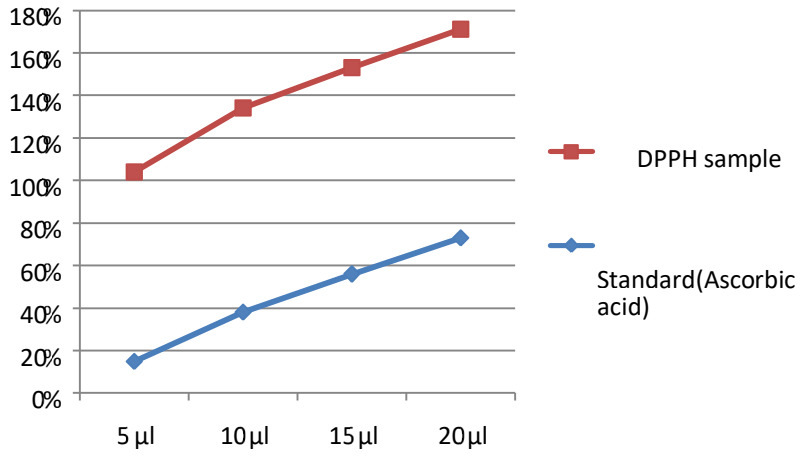


Fig showing: Graphical representation of DPPH assay

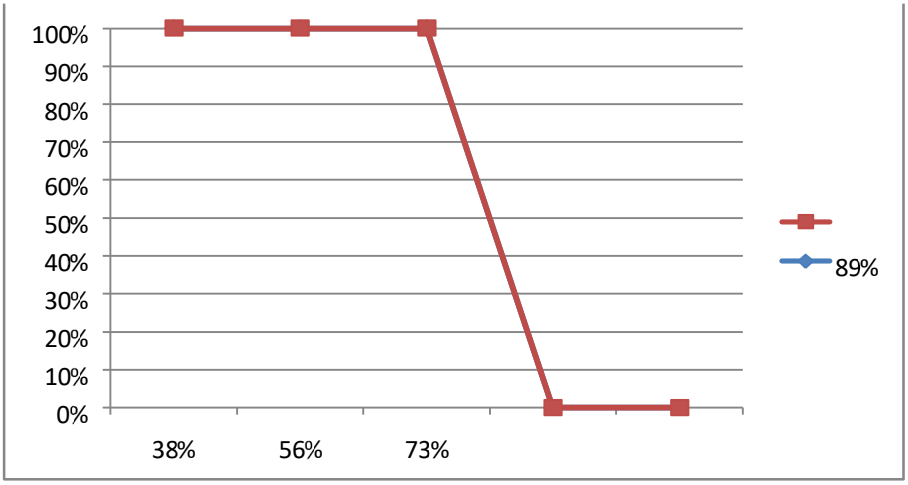
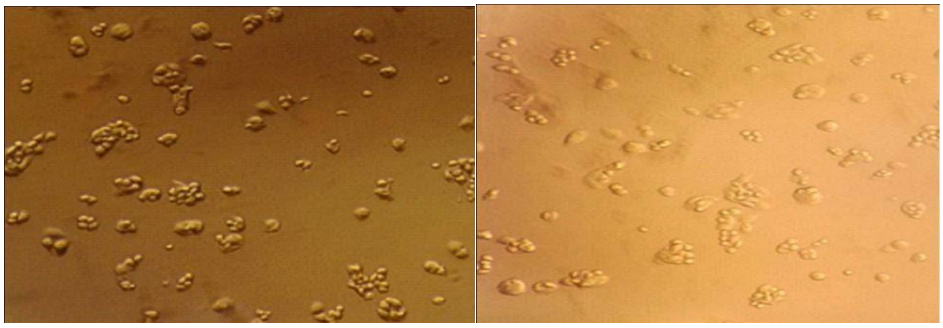
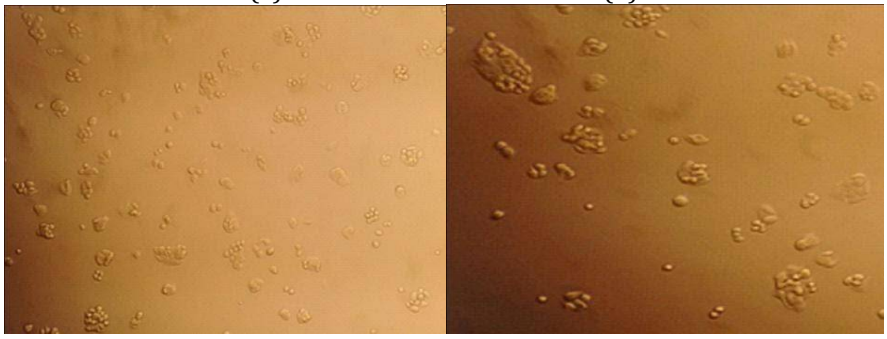


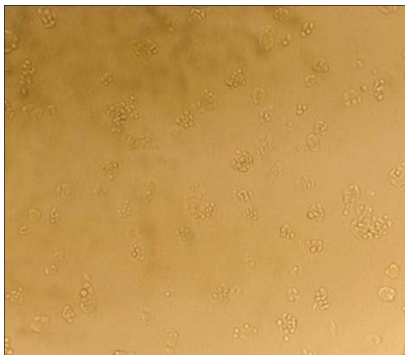
Fig : Graphical representations of nitric oxide scavenging assay



(a) (b)



(c) (d)



(e)

Fig (a) 18.75 µg ,(b)37.5 µg , (c)75 µg (d)150 µg (e)300 µg

Table – 1 List and percentage of components present in *Ganoderm lucidum*

Components present in *Ganoderma lucidum*	**%**
Ash	1.8%
Carbohydrate	26-28%
Crude Fat	3-5%
Crude Fibre	59%
Crude Protein	7-8%
Polysaccharides	1.1-5.8 %
Triterpenes	7.8%

Table - 2. Tabulation of bio active compound analysis:

BIO ACTIVE COMPOUND	**APPEARANCE**	**RESULTS**
Alkaloids	Reddish brown	+
Flavanoids	Green	+
Steroids	Red colour Precipitate	+
Ancinoacids & proteins	Present	+
Phytosteroids	Bluish green	+
Tannin	Blue colour	_
Reducing sugar	Blue colour	+
Phenol	Deep blue colour	+
Saponins	Present foam	+

*"+" =Present: "-" = Absent

Table – 3 Spectrophotometric reading at 593nm for FRAP Assay was taken and calculated

concentration	**FRAP Standard**	**FRAP Sample**
5 µl	33 %	92%
10 µl	44 %	94%
15 µl	52 %	95%
20 µl	71 %	96%
25 µl	83 %	99%

Table – 4 For nitric oxide scavenging activity Spectrometric readings were taken at 540nm and calculated

Concentration	**Standard (Ascorbic acid)**	**Sample**
5 µl	30 %	53%
10 µl	46 %	86%
15 µl	58 %	94%
20 µl	73 %	96%
25 µl	81 %	97%

Table - 4 Showing the Absorbance at 570nm in spectrophotometric analysis in MTT Assay

Concentration	**18.75 µg**	**37.5 µg**	**75 µg**	**150 µg**	**300 µg**	**Cont** ABS
	0.065	0.151	0.244	0.367	0.456	0.781
	0.066	0.151	0.245	0.362	0.457	0.785
	0.067	0.152	0.242	0.363	0.457	0.782
Avg	**0.066**	**0.151333**	**0.243667**	**0.364**	**0.456666667**	**0.782667**

DISCUSSION AND CONCLUSIONS

Cancer has become a major public health concern across the world. The entire globe requires cancer prevention through natural alternatives. G. lucidum pharmacologically active substances such as GA-T, GA-DM, GA-T, GA-H, GA-F, GA-, and GA-Me operate as natural alternatives to advanced and conventional combo treatments in the fight against cancer development. There are numerous facts in favour of compounds derived from *G. lucidum* demonstrating anticancer properties via various mechanisms, including cytotoxic effects on cancerous cells, activation of the host immune response, suppression of angiogenesis, induction of metabolizing enzymes phase II, induction of cell differentiation, and inhibition of uPA and uPA receptor expression in cancer cells. Furthermore, many triterpenes and polysaccharides, as well as their anticancer processes, remain unknown. For cancer prevention and therapy, facts about the mechanisms involved and biologically active elements should be investigated further.

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