"The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.- Science& Technology is now dominates almost every field of our activities.In summary, The faculties (Science stream) of GEMS Arts & Science college have made an excellent attempt to bring about this book Homo Scientia", covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts."

Dr. Balagopalan Unni

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HOMO SCIENTIA

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Dr. K Gopalakrishnan,
Smitha Pramod V

GEMS

HOMO SCIENTIA

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Brief Biography

Dr. B.G.Unni, (Balagopalan Unni) Ph.D
(Allahabad central University)
FRES (London), FIANSc , FISAgBc, FICCE

Former Chief Scientist and Area Coordinator (Biotechnology & Biological Sciences) DADD and Fulbright Fellow retired from CSIR service in 2015 after 38 years of research career at CSIR North East Institute of Science & Technology Jorhat Assam. Appointed at Assam down town University as Director-Research in March 2015 and continued up to June 2019 and then re-designated as Adviser Research in August 2019). Back in Kerala, Dr.Unni is appointed as Director Academic & Research at GEMS College of Arts & Science affiliated to University of Calicut from August 2019. Both the positions are on honorary basis to strengthen the institutions in research areas. He did his BSc Biology (1972-74, Ewing Christian College, Alld University), MSc in Biochemistry(1974-76)(Second Rank) and Ph.D in Biochemistry from Allahabad University(1976-80) and PDF in Molecular Biology from Texas A&M University, USA(1988-91). Dr. Unni is specialized in Biochemistry, Molecular Biology, and Biotechnology and well established in his area of research and completed more than 40 years of research in both basic and applied fields of research. Dr.Unni got more than 130 research papers, 190 abstracts, 35 papers in proceedings, 7 patents,1 technology.18 chapters in books, edited 3 books and 29 students

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received PhD degrees under his guidance and supervision. Dr. Unni had completed more than 20 projects sponsored by Commonwealth Science Council, London, Ministry of Non conventional Energy Sources, Department of Non conventional Energy Sources Govt of India, North Eastern Council Govt of India, Department of Science &Technology, Department of Biotechnology, Central Silk Board,GB Pant Institute of Himalayan Environment and Development, CSIR and DRDO, Ministry of Defense, Govt of India during his scientific tenure at CSIR NEIST. Dr Unni received- Fulbright Travel Award/ Fellowship (USA) Dr. B.M. Das Memorial Science award, Hebrew University Award , H.R. Cama Memorial Travel Award,COSTED Travel Award,. DAAD- fellowship-Germany, Well Mark International Scholarship (USA) & Technology award in life sciences by CSIR,Govt of India . Best Fulbright Alumni Chapter Leader-South Asia Selected by the United States Education Foundation In India (USIEF), New Delhi .Nominated to represent India at the International Fulbright Scholars meet at Marrakech, Morocco- Nominated by United States Education Foundation In India, New Delhi . Dr. Unni is in the editorial board of more than eight indexed journal in the country .Dr.Unni was nominated to various state and central committees such as High power committee for development of sericulture activities Muga, Eri,Tassar and Mulberry in Assam nominated by Governor of Assam, .Expert in the area of non mulberry sericulture, Ministry of Textiles, Advisory Board, Post graduate Biotechnology programme, Academic Council, Assam Agricultural University, Research Council, Central Silk Board, Ministry of Textiles , DBT's Nominee for Biosafety Committee ,Vice President SBC (India) Indian Institute of Science Bangalore, Vice President Indian Academy of Neuro-sciences, Member Fulbright Academy of Science & Technology, USA, Board of studies- Botany Nagaland University and Biotechnology Saugar University Madhya Pradesh., Fellow, Indian Academy of Neurosciences & Indian Society of Agricultural Biochemists, Fellow Royal Entomological Society, London UK and Scientific

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Advisor International Foundation of Science, Sweden, Member, Board of Studies Raiganj University (2017----), Member Research Review committee Tea Board of India (2016-2019), Member Advisory Committee Cancer Research Advisory Board, North East Cancer Hospital & Research Institute (2017--) President, Tea Improvement Consortium, Ltd, Tocklai Assam (2018-2020) .

Dr.Unni visited USA, Germany, Israel, Jordan, France, Morocco ,UK, Thailand ,Jordan, Singapore , China and UAE under various exchange program.

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Preface

I am very happy to learn that, the GEMS Arts & Science College is bringing out a series of books written by the faculty in this academic year. The college is occupying a very important position among the colleges in Kerala, the same way the college is having unique standing in both academic and research fronts too. This is because of the excellent management, faculties and the best performances of the students.. I have full confident that in the course of time, and with the sincere commitment and dedication of the faculties , students and with management , the college will attain high level perfection and excellence and became a model college in the state of Kerala

This book entitled " Homo Scientia" had comprehensive research topics in various aspects in the topics of cyber security, biotechnology, microbiology and geology.A brief description about the cybersecurity, the protection of computer set up such as hardware, software data from several threats have been described in the chapter The best practices for deploying and managing IPS network security tools have been explored. The integration of intrusion prevention system (IPS) solutions, adherence to security policies, regular updates, monitoring and the implementation of incident response procedures are considered to be the essential components of a comprehensive network security framework. The risk management in cyber security, various cyber-attack kinds, malware, and some strategies to tackle these attacks are also explained by the authors. A comprehensive overview of the evolution of computer graphics, exploring the advancements in hardware, software, algorithms, and techniques that have propelled the field from its early pixel-based beginnings to the current state of realism etc also described. Optical character recognition has been extensively investigated in the past few years, and has been proven that high recognition rates can be achieved in specific

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application scenarios using some standard and well-studied methods such as neural network, support vector machine (SVM), etc. The possibility of learning an appropriate set of features for designing optical character recognition (OCR) has been investigated

Biotechnology is an interdisciplinary science using modern technologies to construct biological processes in research, agriculture, formulation of pharmaceutical products and other related fields. The better understanding of advances in plant genetic resources, genome modifications, omics technologies to generate new solutions for food security under changing environmental scenarios etc have been discussed in this chapter. The increasing demand for food had a great impact on the agriculture sector to address the various challenges associated with crop productivity. The tremendous advancement in plant research helps in understanding plant biology for sustainable food security, functional ecosystems, crop improvement and human health. One of the sustainable farming techniques is the use of fertilizer at nano level. Nanomaterials that enhance plant nutrition could be considered as an alternative to the conventional chemical fertilizers. one chapter covered the importance of nano fertilizer to enhance metabolic processes in plants and reviewed the concerns in developing nanotechnological methods in the future. Metabolomics has now emerged as a powerful tool for the comprehensive analysis of metabolites within biological systems. One of the chapters provides a review on metabolomics, encompassing its methodologies, applications, potential impact on personalized medicine ,and discusses further the need for advancements in analytical technologies. The antifungal activity of mangroves, particularly Rhizophora species are one of the main sources for fungicidal compounds due to the presence of high concentration of phenols. The antifungal activity of Rhizophora species has been elucidated, and could be further utilized as biocontrol agents for fungal disease in agricultural crops. One of the chapters discussed the species identification and its impact on economical and ecological level in the species like Nutmeg, one of the important medicinal plants that had a greater attention ,however, it was very difficult to differentiate the sexual identity

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in the seedling stages. But the protein content screening among the studied plantlets had differentiated the sexes in the species as explained by the author.

AI (Artificial Intelligence) or machine intelligence enables farmers to enhance the quality and ensure a quick go-to market strategy for crops, and adoption of these algorithms to improve food industries. Artificial intelligence (AI) has also the potential to revolutionize education, from personalized learning to assessment and grading. Additionally, AI-powered tools can provide greater accessibility to students with disabilities, while also enabling more engaging and interactive content. AI continues to develop and become more prevalent in education, towards responsible and equitable implementation. However the negative and positive part of the AI may also be looked into.

The chapters related to microbiological aspects have also been incorporated in this book . Carbapenem-resistant A. baumannii (CRAb), bacteria that cause multi-infections in humans and resistant to multiple drugs too. The study attempted to isolate and characterize the bacterial species from the clinical specimens using biochemical techniques. The enzyme, carbapenemase produced by the bacteria was isolated and determined by different assays. Another study identified the antibacterial, antioxidant and anticancer activities of Ganoderma lucidum by various chromatographic techniques. Anticancer activity was also assessed on HeLa cell lines using MTT assay and DPPH assay. In one of the chapters,the author discussed L-asparaginase, one of the widely exploited enzymes for the treatment of acute lymphoblastic leukemia (ALL). Also attempted to isolate and characterize the enzyme from soil samples collected from different locations at Kerala. The study indicated that soils can provide a rich source for L-asparaginase which has got ample application in pharmaceutical industries.

The studies on various geological aspects with respect to different geographical areas in Kerala soil has been included in the book. The vertical geochemical variation and elemental mobility of the lateritic terrain in the Makkaraparamba of Malappuram District, Kerala has been very well investigated. Under extremely oxidizing and leaching conditions, laterite

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soil transformed into a variety of rocks and further developed into stable secondary product in the existing humid tropical and subtropical environments. The hydrogeological conditions in Kumbala- Kaliyar river basin, Kasaragod district, Kerala was assessed by means of Vertical Electrical Sounding (VES). The digital spatial data output of the present study would be much helpful for planning and management of surface and sub-surface water resources of Kasaragod River basin in which the Kasaragod township is centrally located

The contributed chapters in the book written by the faculties of science stream in the light of the recent thinking and developments in the field of science and education.Science& Technology is now dominates almost every field of our activities. In summary , The faculties (Science stream) of GEMS Arts & Science college have made a n excellent attempt to bring about this book Homo Scientia".covering almost all the important areas from biological sciences to artificial intelligence. Every article has its own merits in both academic and research fronts..I record my grateful appreciation and thanks to the contributors of this book for their untiring efforts.

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ANTICANCER AND ANTIBACTERIAL ACTIVITIES OF GANODERMA LUCIDUM

Shana Parveen TT
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PG Department of Microbiology

ABSTRACT

Ganoderma lucidum like any other fungi grow wild on living or dead or dying wood log of hardwood and on dead roots. The fruiting bodies and mycelium of *G. lucidum* contain immunomodulating polysaccharides, some of which inhibit the growth of several cancer cells . The activities of *Ganoderma lucidum* are mainly because of polysaccharides and/or triterpenoids of the fungus. The anticancer and antibacterial activity of methanolic and acetone extracts of *Ganoderma lucidum* was studied . The anticancer activity on HeLa cell lines was checked by MTT Assay and DPPH assay was carried out to check the antioxidant properties. The phytochemical screening and compound identification of *Ganoderma lucidum* was done by LCMS Analysis. *Ganoderma lucidum* exhibits antioxidant, anticancer and antimicrobial activities. Therefore, *G. lucidum* is an interesting plant extract that has been widely used in alternative medicine and proven to have numerous implications to be used as a potential anticancer drug.

INTRODUCTION

Ganoderma lucidum commonly referred to as Lingzhi, is a fungus which has been widely used through the centuries for the general promotion of health and longevity in Asian countries.

It has been known to have numerous pharmacological effects including immuno-modulating, anti-inflammatory, anti-cancer, anti-diabetic, anti-oxidative and radical-scavenging, and anti-aging effects.

Ganoderma lucidum

G. lucidum contains a wide range of bioactive compounds associated with the promotion of good health. These bioactive compounds can be extracted via water-based or ethanol-based extraction methods. The extraction process is very important for consumption of *G. lucidum* as the fungus is tough and indigestible by humans. There are many ways to consume *G. lucidum;* one method of consumption is to submerge the fungus in alcoholic beverages for a prolonged period of time. Since alcoholic beverages contain both water and ethanol components, this method can potentially extract the active ingredients of *G. lucidum* associated with both solvents.16 Various extracts of *G. lucidum* have been shown to have anticancer effects in both in vivo (mouse model) and in vitro (cancer cell lines) studies. These include cytotoxic,antimetastatic, and immune-modulating effects. however, still much research needs to be done to quantify it for personalized medicine, especially in treating specific cancers such as prostate cancer .

The medicinal fungus *Ganoderma lucidum* has been used in traditional Chinese medicine for millennia to improve health

and promote longevity. The idea of using *G. lucidum* for cancer treatment is based on numerous laboratory and preclinical studies with cancer and immune cells as well as animal models demonstrating various biological activities in vitro and in vivo. For example, *G. lucidum* possesses cytotoxic, cytostatic, antimetastatic, anti-inflammatory, and immunomodulating activities

Most mushrooms are 90% water by weight. For *G. lucidum*, the remaining 10% consists of 26–28% carbohydrate, 3–5% crude fat, 59% crude fibre, and 7–8% crude protein . In addition, *G. lucidum* contains a wide variety of bioactive constituents such as terpenoids, steroids, phenols, glycoproteins, and polysaccharides . Numerous authors have shown that triterpenes and polysaccharides are the major physiologically active components of *G. lucidum*.

Triterpenes are one of the possible pharmaceutically active compounds contributing to the medicinal activities of *G. lucidum* . Triterpenes are a subtype of Terpene, a class of naturally occurring compounds, composed of one or more isoprene units. Terpenes are widely distributed throughout the plant world. Many subtypes of Terpenes have been found to have anti-inflammatory, anti-tumourigenic, and hypolipidemic activity. Triterpenes contain six isoprene units. The isoprenes may form linear chains or fold-up and form a ring-like structure. Ganoderic acid is a sub-type of triterpenes with four cyclic and two linear isoprenes. There are over 140 species of triterpenes and triterpenoids identified in *G. lucidum*.

Polysaccharides are long chain sugar molecules joined together by glycosidic bonds. Various types of polysaccharides, with molecular weights ranging from 4 × 105 to 1 × 106 Da have been identified in *G. lucidum;* mostly in the fruiting body and mycelia, and a few have been found in the spores.

Structural analysis shows that polysaccharides of *G. lucidum* are all heteropolymers . Glucose forms the major share of the sugar molecules; with xylose, mannose, galactose, and fucose in different conformations. It is hypothesised that the

polysaccharides extracted from different parts of *G. lucidum* induce different immune responses with varying immune potencies . The different branching conformation and solubility characteristics affects the anti-tumourigenic properties of these polysaccharides . β-D-glucans consisting of (1-3)-, (1-4)- and (1-6)-β-D linkages are known to have a stronger anti-tumour potency and better absorption than other polysaccharides in *G. lucidum*. Together with the high concentration of high molecular-weight polysaccharides, the mushroom also consists of a matrix of the polysaccharide chitin, which is largely indigestible and is partly responsible for the physical hardness of the mushroom.

METHODOLOGY

The mushroom *Ganoderma lucidum* was collected from IRTC Mundoor. The mushroom was washed thoroughly and was dried in shade and grounded to fine powder using mortar and pestle.

Extraction of bioactive compounds from *Ganoderma lucidum*

Alcoholic extraction

The dried powder of *Ganoderma lucidum* was subjected to alcoholoic extraction by using solvents –ethyl acetate ,methanol and petroleum ether. The powdered mushroom along with each solvents kept in a magnetic stirrer at 300rpm for 30 minutes.the extracts were recovered by filtration using Whatman No. filter paper.

Water extraction

The dried powder of *Ganoderma lucidum* was subjected to water extraction by using hot water. The powdered muhroom soaked in hot water and kept in a magnetic stirrer at 300rpm for 30 minutes.the extracts were recovered by filtration using Whatman No. filter paper.

Anti-oxidant activity of *Ganoderma lucidum*

DPPH Assay

The Ether acetate and water extract were subjected for DPPH assay.

- Antiradical activity was measured by a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample.
- A stock solution of DPPH (1.3 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9.
- Decrease in the absorbance in the presence of sample extract and standard at different concentrations was noted after 30 Minutes.
- EC50 was calculated from% inhibition. A blank reading was taken using methanol instead of sample extract .
- Absorbance at 517 nm is determined after 30 min. using UV-visible Spectrometer(Systronic double beam- UV-2201), and IC50 (Inhibitory concentration to scavenge 50% free radicals) is also determined. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity.
- IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.

The capability to scavenge the DPPH radical was calculated using the following equation.

Percentage Inhibiton = C-T/A X 100

Where C = Absorbance of DPPH alone

T = Absorbance of DPPH along with different concentrations of extracts.

IC50 was calculated from equation of line obtained by plotting a graph of concentration versus % inhibition

Anticancer Activity Of *Ganoderma lucidum*

MTT Assay (HeLa Cell Lines)

1. For adherent cells, remove the medium and replace it with 100 µL of fresh culture medium. For non-adherent cells,

centrifuge the microplate, pellet the cells, carefully remove as much medium as possible and replace it with 100 µL of fresh medium.

2. Add 10 µL of the 12 mM MTT stock solution (prepared in step 1.1) to each well. Include a

negative control of 10 µL of the MTT stock solution added to 100 µL of medium alone.

3. Incubate at 37°C for 4 hours. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 hours.

4. Add 100 µL of the SDS-HCl solution (prepared in step 1.2) to each well and mix thoroughly using the pipette.

5. Incubate the microplate at 37°C for 4– hours in a humidified chamber. Longer incubations will decrease the sensitivity of the assay.

6. Mix each sample again using a pipette and read absorbance at 570 nm.

Antimicrobial Activity Of *Ganoderma Lucidum*

The ethyl acetate and water extracts were used for the study of antimicrobial activity of *Ganoderma lucidum*. The antimicrobial assay was performed and MIC was calculated by broth dilution method. 60 sterile test tubes were set up and labelled with test organism and concentration of sample extract. 2ml of sterile nutrient broth was inoculated with 0.05 ml overnight culture of each test organisms in each test tubes. Varying concentrations of the sample extracts were added to specifically labelled tubes. The tubes were incubated at 370C for 24 hours. Positive controls were put up without any sample extract.

After the incubation,the MIC values were determined by checking the turbidity in a spectrophotometer. In the tubes tested , the tube that showed no growth with minimum concentration of the sample extract was considered as the MIC value.

Compound Identification

LCMS analysis

10 µl of the filtered sample was injected to the manual injector using a Microsyringe (1-20µl, Shimadzu). The mobile phase used was water:methanol (50:50) in an isocratic mode. The column used was RP-C- 18 (phenomenex). The separated compounds were then ionized using APCI Method and using the split mode (50:50). The flaw rate was maintained to 2 ml/ mn with a temperature of 25=2 0C. The class VP integration software were used for the data analysis.

Preliminary Phytochemical Screening Of The Mushroom

The methanolic extract was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

Molisch's test:

Dissolved small quantity of 300mg alcoholic extract of *Ganoderma lucidum* in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test:

Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.:

Test for flavanoids

Shinoda test:

To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

Lead acetate test:

To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

Braemer's test:

To 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid

Liebermann-Burchardt test:

To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids.

Hager's test:

The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.

Wagner's test:

The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids. Tests for Glycosides

Tests for Glycosides

Legal's test:

Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

Foam test:

1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.A1cm layer of foam formation indicates the presence of Saponins

Test for Amino acids

Ninhydrin test:

Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the

presence of amino acids.

Test for fixed oils

Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

RESULTS AND DISCUSSIONS

The preliminary phytochemical screening of *Ganoderma lucidum* revealed that the extracts contain carbohydrates, flavanoids, terpenes, alkaloids, glycosides ,and aminoacids. It does not contain any tannins or saponins. The antioxidant activity was determined by DPPH assay.The IC 50 of ethyl acetate extract is 3.36 mg/ml and IC 50 of water extract is 13.4mg/ml. The ethyl acetate extract of *Ganoderma lucidum* shows more antioxidant activity than the water extract. The anticancer activity was determined by MTT assay. The LD 50 of ethyl acetate extract is 178microgram/ml and LD 50 of water extract is 213microgram/ml. The ethyl acetate extract of Ganoderma lucidum shows more anticancer activity than the water extract.

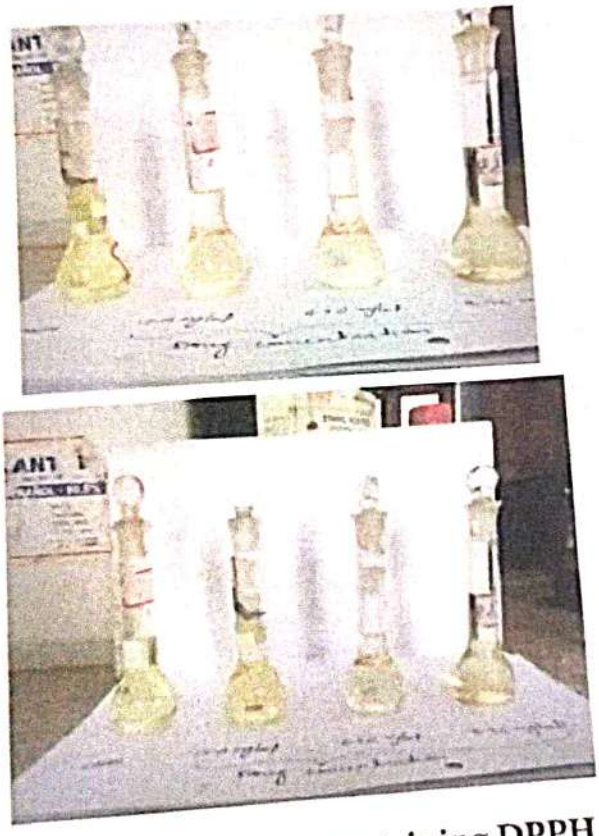
Sample concentration (microgram/ml	%viability	
	Water extract	Alcoholic extract
Control	96.58%	96.58%
50	82.05%	55.26%
100	69.25%	62.54%
150	62.32%	56.23%
200	52.02%	45.23%
250	42.02%	42.25%

Anticancer activities of *Ganoderma lucidum*-MTT Assay (HeLa Cell Lines)

LD 50(alcoholic extract)=178 microgram /ml

LD 50(water extract)=213 microgram /ml

The antibacterial activity of alcoholic and water extract of *Ganoderma lucidum* on *Escheritia coli, Klebsiella,Pseudomonas, Staphylococcus* and *Candida* was studied and their growth was inhibited by both the two extracts. The result of the antibacterial activity showed that the alcoholic extract of *G. lucidum* showed the strongest antimicrobial activity among than water exract against the tested microorganisms. This indicates that the active principle which inhibits the growth of susceptible bacteria may dissolve better in methanol than in other solvents.



Alcoholic and water extract containing DPPH reagent