KAVAKA 60(2): 28-37 (2024) DOI: 10.36460/Kavaka/60/2/2024/28-37

Production and Purification of Bioactive Compounds from *Ganoderma lucidum* Medicinal Mushroom

Gomathi S.*[1], Ambikapathy V.[1], Thajuddin N.[2], And Panneerselvam A.[1]

[1]*PG and Research Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur (Dt), Tamil Nadu, India (Affiliated to Bharathidasan University, Tiruchirappalli).*

[2]*Crescent Global Outreach Mission (CGOM), B.S. Abdur Rahman Crescent Institute of Science & Technology, (Deemed to be University), Vandalur, Chennai - 600 048, India.*

**Corresponding Author Email: mathigo10@gmail.com*

(Submitted on March 23, 2024; Accepted on June 2, 2024)

ABSTRACT

The *Ganoderma lucidum* extracts were active against the microbial pathogen. But the level of inhibition varied significantly. The ethanol and methanol extract exhibited inhibitory zone against bacteria and fungi. The potential mycelial extracts of *G. lucidum* to be employed for combating several pathogenic diseases. It also showed that fractionation of this mushroom extract can alter the presence or absence of a bioactive compounds and this can affect some of the claimed effects of the mushroom in disease conditions. Hence the need to find out the toxicity and anti-microbial activity of both the crude and organic solvent fraction of this extract. This is concluded the study that the polysaccharides extracts of medicinal mushrooms of *G. lucidum* showed bioactive compounds and antimicrobial properties. Polysaccharides extract may be good sources for the development of antioxidant food additives. It focused on the superiority of mushroom *G. lucidum* on different substrates and biotechnological applications of selected potential strains.

Keywords: *Ganoderma lucidum*, Bioactive compounds, Antimicrobial properties, Medicinal mushrooms, Organic solvent

INTRODUCTION

The ‘mushroom’ is a macrofungus with distinctive fruiting bodies or large context fungi with stalks and caps. Mushrooms have been part of the fungal diversity for around 300 million years. Prehistoric humans probably used mushrooms collected in the wild as food and possibly for medicinal purposes. It represents world’s one of the greatest untapped resources of nutritious foods which can substantiate the sufferings from malnutrition partly, owing to its richness in minerals, vitamins, fibers and essential amino acids (Chadha and Sharma, 1995). Additionally, mushrooms can be produced artificially in the controlled cropping rooms in large quantities within a short time and provide more protein per unit area than other crops (Gupta, 1986).

The medicinal mushroom Shiitake (*Lentinula edodes*) has been cultivated for at least 1000 years (Bhosle *et al*., 2010) and it’s been used for medicinal effect for 2000 years. Similarly *G. lucidum*, which is known as Ling zhi, in Chinese it means mushroom of 10000 years, revealing its ancient use.

Mushroom as Nutrition and Medicine

The number of mushrooms on earth is estimated to be 140,000 out of which approximately 14,000 have been identified (Wasser, 2002). These mushrooms have been used by many civilizations around the globe for various purposes like food, medicine, rituals, worshiping god etc. However fear of mushroom poisoning also exists to great extent throughout many cultures at different parts of this earth and in few places it achieves phobic extremes e.g., UK, Ireland, North America in west, while few countries in east like India. In contrast there are mushroom loving cultures throughout Asia, many parts of Europe, Russia, Poland, Italy, France and Germany.

Ganoderma lucidum

Systematic position

Class	:	Basidiomycetes
Order	:	Polyporales
Family	:	Ganodermataceae

Genus : *Ganoderma*

Species : *G. lucidum*

Ganoderma lucidum (Curt: Fr.) P.Karst. is a species of Basidiomycetes which belongs to Ganodermataceae of Aphyllophorales (Yang *et al.,* 2000). Its fruiting body is called Lingzhi in China and Reishi in Japan. For thousands of years, this fungus has been regarded as a traditional Chinese medicine (TCM) or a folk medicine for its medicinal properties. *G. lucidum* has been used for promotion of vitality, longevity (Lu *et al*., 2004) prevention and treatment of various human diseases in China and other Asian countries. It is used for the treatment of asthma, diabetes, altitude sickness, cardiovascular disease, AIDS and cancer (Lin, 2001; Wasser and Weis, 1999). *G. lucidum* appears to be very safe since oral administration of the extracts does not display any toxicity, and its merits have been investigated as a potential prophylactic agent for human health (Kim and Kim, 1999).

MATERIALS AND METHODS

Sample collection

Fruit bodies of *G. lucidum* were collected from various places of Orathanadu, Thanjavur district in Tamil Nadu, India. Salient features such as colour, size, shape, texture were observed for each strain. The study of the basidiomyetes was made on macro (size, colour, number pores/mm, length of tubes) and microscopic characters (somatic and reproductive structures). Colours are according to Munsell (1975) and Herbaria abbreviations following Holmgren *et al*., (1990). The collected fruit bodies were carefully stored in polythene bags and transported to the laboratory for mycological examination.

Microscopical observation

Morphological observations were mainly followed in the methods of Wang *et al.,* (2006). Lactophenol cotton blue staining was used as the mounting the strain. Microscopic characters were observed using a light microscope. For microscopic observation dermis elements were carefully examined and measured in thin sections perpendicular to the Pileus surface.

Extract preparation (Anonymous 1955)

Various extracts of the experimental samples were prepared according to the methodology of Indian pharmacopoeia. The chemical and physical state of the mushroom powder makes it difficult to dissolve in distilled water. Hence the mushroom materials were soaked in distilled water for 24 hrs. One gram of powder was dissolved separately in 10 ml of solvents such as aqueous, benzene, ethanol and methanol in cleaned screw cap bottle for 24 hrs. The dissolved extracts from the bottles were transferred to centrifugal tubes and centrifuged at 300 rpm for 10 min. The centrifuged extracts (supernatant) were again re-centrifuged and filtered with millipore filter. The filtered solvents with dissolved chemicals were concentrated and stored separately in refrigerated at 4 °C.

Antimicrobial Activity (Stokes and Ridgway, 1980)

To determine the antimicrobial activities of *G. lucidum* with some solvents of aqueous, benzene, ethanol and methanol extract was carried out by using agar well diffusion method.

Test organisms

The culture of microbes such as *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, K. pneumoniae* and fungal strains of *Aspergillus fumigatus, A. terreus, A. ochraceus, Penicillium* sp. and *Trichoderma viride* were purchased from Indian Biotrack Institute, Thanjavur.

Antibacterial studies

Nutrient agar medium was sterilized in an autoclave at 15 lbs pressure for 15 minutes. The sterilized media were poured into petridishes. Then the solidified plates were used for the antibacterial studies.

Antifungal studies

200 grams of potato slices were boiled with 1000 ml of distilled water. The potato infusion was used as water source of media preparation. Twenty grams of dextrose was mixed with potato infusion. Eighteen grams of agar was added as a solidifying agent. These constituents were mixed and autoclaved. Then the solidified plates were used for antifungal activity.

Well Diffusion Method (Bauer *et al.* 1996)

Antibacterial and antifungal activity of *G. lucidum* extract was tested using well diffusion method. The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 5 mm cork borer. 100 µl extracts were poured into each well by using sterile dropping pipette. The plates were incubated at 37±2 °C for 24 hours for bacterial activity and 48 hours for fungal activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. From the results of mean value were measured zone of inhibition.

Gas Chromatography-Mass Spectrometry (Kumaravel *et al*., 2010)

Gas Chromatography-Mass Spectrometry analyses were performed using a Hewlett Packard Gas Chromatography (Model, 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230 °C respectively. A fused silica capillary column HP-Innowax (30° MX 0.25 mm, film thickness 0.25 µm) was used. The oven temperature was maintained at 50°C for 5 min holding time and the temperature was raised, from 50-230°C at a rate of 2° C/min. Helium was the carrier gas at a flow rate of 28 cm/sec. One millilitre of extract mixed with methanol (80%) at a split ratio of 1:30 was injected (24). GC-MS analysis was carried out on a Agilent Technologies Network Mass Spectrometer (Model, 5973) coupled to HP gas chromatography (Model, 6890) equipped with NBS 75 K Library Software data. The capillary column and GC conditions were as described above. Helium was the carrier gas, with a flow rate of 22 cm/s. Mass spectra were recorded at 70 cV/200°C. The scanning rate of 1 scan/sec and the run time was 90 min. Compound identification was accomplished by comparing the GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices (RI) and by comparison to reference compounds.

RESULTS

Sample collection

Morphological characters such as color, size, shape, thickness, and texture were included for each species besides a synoptic comparison between all species considered and the distribution pattern of species recorded from the state of Tamil Nadu (**Plate 1**)

Antimicrobial activity

G. lucidum extracts with four solvents like aqueous, benzene, ethanol and methanol were tested against five fungal species and five bacterial species viz., *Bacillus cereus, E. coli, K. pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus A. fumigatus, A. ochraceus, A. terreus, Penicillium* sp. and *Trichoderma viride* were analyzed. (**Table 1** and **2**; **Plate 2** and **3**).

Aqueous extract

The aqueous extract of fruiting bodies of *G. lucidum* to act antimicrobial activity against *Bacillus cereus, E. coli, K. pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus, A. fumigatus, A. ochraceus, A. terreus, Penicillium* sp. and *Trichoderma viride* was 5, 7, 8, 7, 6, 7, 6, 2, 6, and 7 mm zone of inhibition was measured respectively.

Benzene extract

In benzene extract of fruiting bodies of *G. lucidum* act against some fungal and bacterial pathogen. In highest zone of inhibition determined in bacteria of *K. pneumoniae* (10 mm) and fungus *A. ochraceous* as 13 mm (fungi) and *T. viride* as 12 mm followed by *Penicillum* sp. (10 mm), *B. cereus, P. aeruginosa* and *A. fumigatus* (8 mm), *S. aureus* (7 mm), *E. coli* (6 mm) and *A. terreus* (5 mm) recorded respectively.

Ethanol extract

The ethanol extract of *G. lucidum* have high activity against all fungal and bacterial pathogen when compared to other solvents. The maximum zone was measured in *A. ochraceus* (20 mm), *T. viride* (19 mm), *Penicillium* sp. (18 mm), *A. terreus* (17 mm) and *A. fumigatus* (12 mm) were recorded correspondingly. At the same time the bacteria *K. penuminiae* (13 mm), *B. cereus* and *S. aureus* (12 mm), *E. coli* and *P. aeruginosa* (9 mm) recorded respectively.

Plate 1: Morphological view of *Ganoderma lucidum*

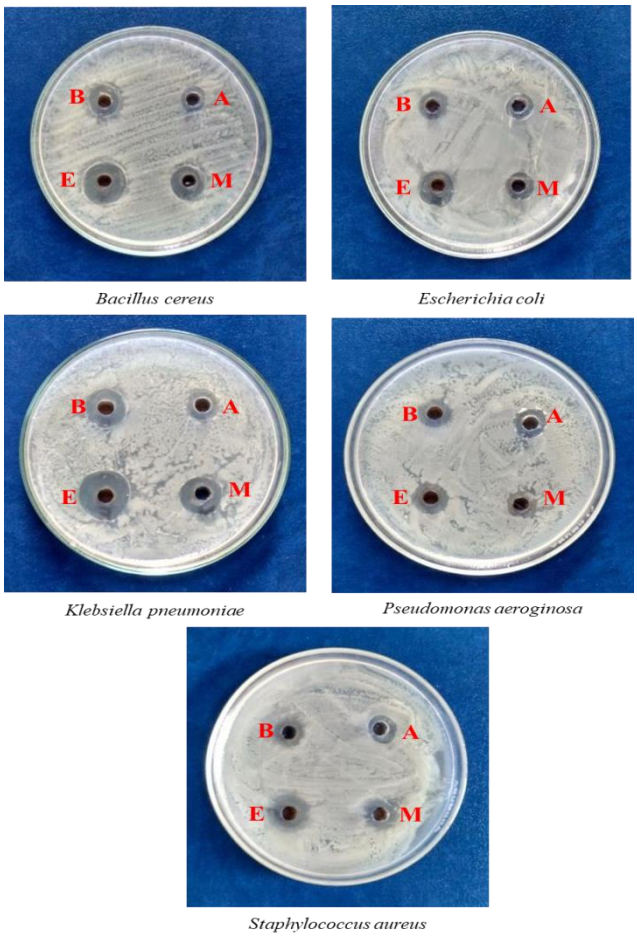

Plate 2: Studies on the effect of *Ganoderma lucidum* against clinical bacteria

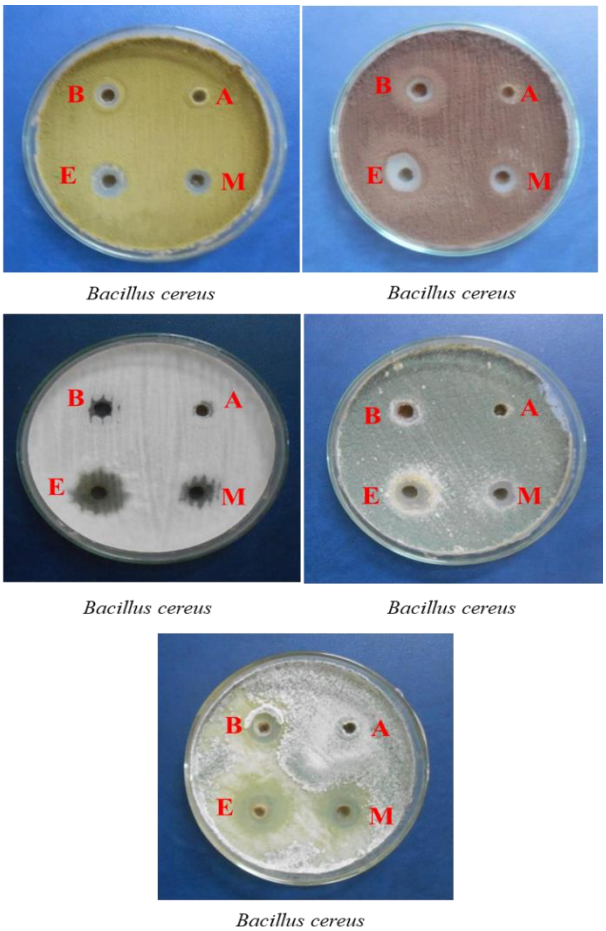

Plate 3: Studies on the effect of *Ganoderma lucidum* against clinical fungi

Table 1: Antibacterial activity of *Ganoderma lucidum* with different solvents extract

Name of the bacteria	Zone of inhibition (mm)			
	Aqueous	**Benzene**	**Ethanol**	**Methanol**
Bacillus cereus	5	8	12	9
E. coli	7	6	9	6
K. pneumoniae	8	10	13	10
Pseudomonas aeruoginosa	7	8	9	6
Staphylococcus aureus	6	7	12	7

Table 2: Antifungal activity of *Ganoderma lucidum* with different solvents extract

Name of the fungi	Zone of inhibition (mm)			
	Aqueous	**Benzene**	**Ethanol**	**Methanol**
Aspergillus fumigatus	7	8	12	9
Aspergillus chraceous	6	13	20	15
A.terreus	2	5	17	11
Penicillium sp.	6	10	18	8
Trichoderma viride	7	12	19	17

Methanol extracts

The methanol extracts was observed in the zone of inhibition of bacteria as *Bacillus cereus, E. coli, K. pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* of 9, 6, 10, 6, and 7 mm diameter and the fungal species of *A. fumigatus, A. ochraceus, A. terreus, Penicillium* sp. and *Trichoderma viride* as 9, 15, 11, 8, and 17 mm zone were recorded (**Table 1** and **2**).

Bioactive compound separated in *G. lucidum* by Gas Chromatography-Mass Spectrometry

Bioactive compound analysis of *G. lucidum* through GC-MS revealed the presence of fourteen components representing fourteen prominent peaks. The peaks with retention time of 10.60 min representing à-D-Glucopyranoside, O-à-D-glucopyranosyl- (1.fwdarw.3)-á-D-fructofuranosyl, 11.13 min corresponds to Dodecanoic acid, 2-(acetyloxyl)-1-[(acetyloxy)methyl]ethyl ester, 12.12 min corresponds to Ethaneperoxoic acid, 1-cyano-1-(2-(2-phenyl-1,3-dioxolan-2-yl)ethyl)pentyl ester, 12.29 min corresponds to N-Hexadecanoic acid, 13.52 min retention time corresponds to Cyclopropaneacetic acid, 2-hexyl-, 14.38 min corresponds to Myristoleic acid, 17 min retention time corresponds to Z-8-Methyl-9 tetradecanoic acid, 19.36 min corresponds to 1,2-Benzenediccarboxylic acid, mono(2-ethylhexyl) ester, 23.04 min corresponds to Erucic acid, 26.36 min corresponds to 9-Hexadecenoic acid, 27.75 min retention time corresponds to 9-Octadecenoic acid (Z)- 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester, 28.98 min corresponds to 7-Hexadecenoic acid, methyl ester, (Z)-, 29.43 min corresponds to 12-Methyl-E,E-2,13- octadecadien-1-ol, 30.06 min retention time corresponds to 9,12,15 Octadecatrienoic acid, 2,3- bis(acetyloxy)propyl ester, (Z,Z,Z) (**Table 3** and **Figure 1**).

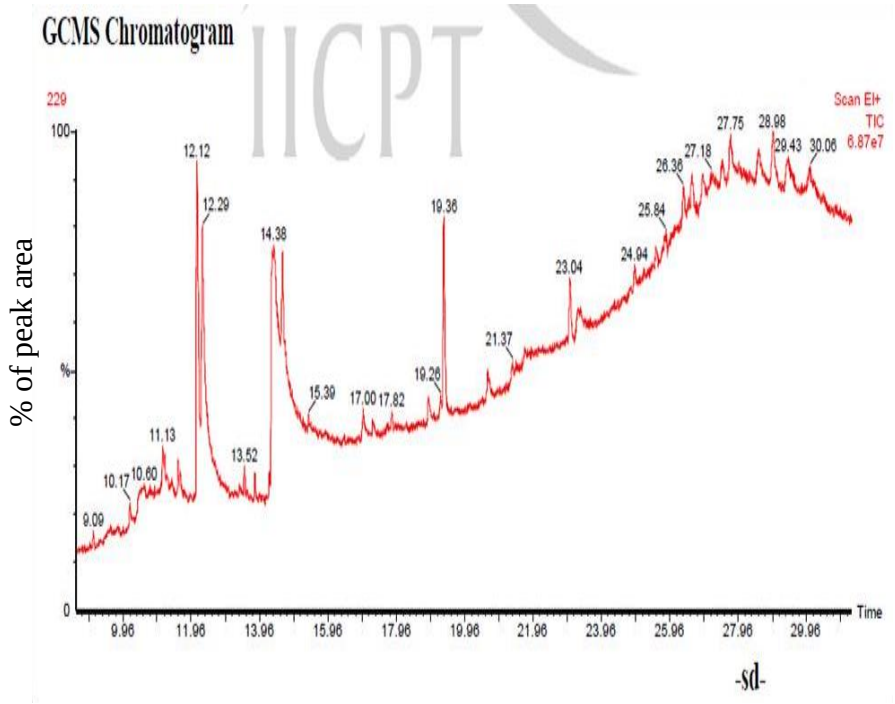

Figure 1: Bioactive compounds of *G. lucidum* by GC-MS

Table 3: Bioactive compounds of *G. lucidum* by GC-MS

S. No	Retention Time (min)	Name of the compounds	Molecular Formula	Molecular weight (KDa)	Peak area (%)
1.	10.60	à-D-Glucopyranoside,O-à-D-glucopyranosyl- (1.fwdarw.3)-á-D-fructofuranosyl	C18 H32O16	504	2.34
2.	11.13	Dodecanoic acid, 2-(acetyloxyl)-1- [(acetyloxy)methyl]ethyl ester	C19 H34 O6	338	1.24
3.	12.12	Ethaneperoxoic acid, 1-cyano-1-(2-(2-phenyl-1,3-dioxolan-2-yl)ethyl)pentyl ester	C19H35 NO5	347	4.62
4.	12.29	N-Hexadecanoic acid	C16H32 O2	256	9.47
5.	13.52	Cyclopropaneacetic acid, 2-hexyl-	C11H20O2	184	0.21
6	14.38	Myristoleic acid	C14H26O2	226	10.60
7.	17	Z-8-Methyl-9-tetradecanoic acid	C15H28O2	240	0.55

S. No	Retention Time (min)	Name of the compounds	Molecular Formula	Molecular weight (KDa)	Peak area (%)
8.	19.36	1,2-Benzenediccarboxylic acid, mono(2-ethylhexyl) ester	C16H22O4	278	2.30
9.	23.04	Erucic acid	C22H4O2	338	4.17
10.	26.36	9-Hexadecenoic acid	C16H30O2	254	12.93
11.	27.75	9-Octadecenoic acid (Z)- 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	C25H44O6	440	21.02
12.	28.98	7-Hexadecenoic acid, methyl ester, (Z)-	C7H32O2	268	13.60
13.	29.43	12-Methyl-E,E-2,13-octadecadien-1-ol	C19H36O	280	12.89
14.	30.06	9,12,15-Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z)-	C25H40O6	436	4.06

DISCUSSION

The *Ganoderma* species produce several secondary metabolites and bioactive compounds like polysaccharides, phenols, and triterpenes, which are largely responsible for their therapeutic properties. Throughout this review, several extracts obtained from *Ganoderma* species have been studied to delve into their therapeutic characteristics and mechanisms. Such properties like immunomodulation, antiaging, antimicrobial, and anticancer activities have been demonstrated by several *Ganoderma* species and are supported by a large body of evidence. Although its phytochemicals play a vital role in its therapeutic properties, identifying the therapeutic potentials of fungal-secreted metabolites for human health-promoting benefits is a challenging task. Identification of novel compounds with distinct chemical scaffolds and their mechanism of action could help suppress the spread of rising pathogens. Thus, this review provides an updated and comprehensive overview of the bioactive components in different *Ganoderma* species and the underlying physiological mechanisms.

In this study, the bioactive chemical has therapeutic use in cardiac failure, when administered; it acts by increasing intracellular calcium concentration, thus increasing cardiac output through increase in the force of contraction. The moderate presence of this bioactive chemical in this finding can suggest the cardio tonic activity of the mushroom extracts.

Ganoderma has long been used for the management of incessant infectious conditions such diabetic foot ulcers, pneumonia, and chronic hepatitis. While there is little information on *Ganoderma* antiviral and antibacterial properties in humans, preliminary (*in vitro and in vivo*) research show that the plant possesses a wide range of these properties. Furthermore, gram- positive and gram-negative bacteria are inhibited *in vitro* by antibacterial components found in *Ganoderma* species. The outcomes of preclinical (*in vitro*) and clinical investigations on the antibacterial and antifungal properties of *Ganoderma* species are brought to light in this review (Asha Arora 2023)

The microorganisms including bacteria and fungi were chosen for screening of the antimicrobial activity produced by *G. mbrekobenum* strain EGDA. The bioactive compounds were extracted from aqueous, petroleum ether, chloroform, ethyl acetate, and methanol extracts. The higher antibacterial activity produced by methanol extract was against *Bacillus subtilis* and *B. cereus* (14.13 ± 0.12 mm,

13.03 ± 0.12 mm, respectively). Water fraction showed antibacterial effect against most of the test bacterial strains. The highest antifungal activity produced by methanol extract was against *Fusarium oxysporum* I and *F. oxysporum* f. sp. lycopersici (16.37 ± 0.03 mm 15.67 ± 0.19 mm, respectively). Gas chromatography/mass spectrometry analysis of the separated fractions revealed the identification of 46 compounds.(Nour El Dein, Mahmoud *et al*., 2023)

In the present study two solvents extract of *Ganoderma lucidum* fruit bodies were act against in some fungal and bacterial pathogen. All of the fungi and bacteria viz., *Bacillus cereus, E.coli, K.pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus A.fumigatus, A.ochraceus, A.terreus, Penicillium* sp. and *Trichoderma viride* have a sensitive and maximum zone of inhibition were recorded.

The results of the present study agreement with the work of the earlier workers (Nasim and Ali, 2011; Kamra and Bhatt, 2012), who have also reported strong antibacterial activity of methanolic extract of *G. lucidum* against gram negative bacteria (*E. coli*) and comparatively less activity against gram-positive (*S. aureus*) bacteria. Similar trend in antibacterial activity of methanolic extract of *Lactarius delicious* (Sagar and Thakur, 2012a), *Sparassis crispa* (Sagar and Tandon, 2012b), *Morchella esculenta* (Sagar and Kumari, 2012c) and *Ganoderma lucidum* (Sagar and Kumari, 2012d) have been reported against *S. aureus* and *E.coli.* Ramesh and Patter (2010) have reported that extract of *Clavaria vermicularis* and *Marasmium oreades* offered more inhibition against gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) as compared to gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*).

Ganoderma lucidum is a forest fungus with a woody texture that has been used since ancient times to enhance health and prevent and treat many diseases due to its wide range of secondary metabolites with abundant medicinal properties. So far, many cellular mechanisms have been proposed to explain how the active metabolites function and the health and hygienic properties of this valuable medicinal fungus, including anti-cancer, anti-viral, anti-bacterial, enhanced cellular immunity, antioxidant and many more. This article reviews the scientific findings of scientists on the antimicrobial properties of the medicinal fungus *G. lucidum*.(Mohammad Hadi Rezghi Jahromi and Milad Mozafari 2021)

Ganoderma lucidum methanolic extract (GLME) has attracted tremendous attention due to its exceptional antimicrobial and anticancer properties that can be delicately tuned by controlling the initial extraction's content and concentration. In the present investigation the characterization, antimicrobial, and cytotoxic performance of GLME as a potential multi-functional therapeutic agent is focussed. Accordingly, FTIR, XRD, FESEM, EDX, and HPLC analyses were employed to assess the samples, followed by disc diffusion and microdilution broth methods to test its antibacterial effects against four Gram-positive and Gram-negative bacterial strains, viz., *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The results showed that the antibacterial property of this product against *E. coli* bacteria was higher than streptomycin, so the zone of inhibition was observed as 44 ± 0.09 mm and 30 ± 0.11 mm, respectively. These data provide valuable insights into the therapeutic usage of GLME for treating breast and blood cancers. This work is motivated by research studies looking for pharmacological products to address chronic and acute diseases, where further resources and studies are required to explore such products' adverse effects and toxicity (Mousavi *et al*., 2023).

In the present study, the peaks with retention time of 10.60min representing O-à-D- glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl, 11.13min corresponds to Dodecanoic acid, 2- (acetyloxyl)-1-[(acetyloxy)methyl]ethyl ester, 12.12min corresponds to Ethaneperoxoic acid, 1- cyano-1-(2-(2-phenyl-1,3-dioxolan-2-yl)ethyl)pentyl ester, 12.29 min corresponds to N- Hexadecanoic acid, 13.52min retention time corresponds to Cyclopropaneacetic acid, 2-hexyl-, 14.38min corresponds to Myristoleic acid, 17min retention time corresponds to Z-8-Methyl-9 tetradecanoic acid, 19.36min corresponds to 1,2-Benzenediccarboxylic acid, mono(2-ethylhexyl) ester, 23.04min corresponds to Erucic acid, 26.36min corresponds to 9-Hexadecenoic acid, 27.75min retention time corresponds to 9-Octadecenoic acid (Z)- 2-(acetyloxy)-1-

[(acetyloxy)methyl]ethyl ester, 28.98min corresponds to 7-Hexadecenoic acid, methyl ester, (Z)-, 29.43min corresponds to 12-Methyl-E,E-2,13-octadecadien-1-ol, 30.06min retention time corresponds to 9,12,15 Octadecatrienoic acid, 2,3-bis(acetyloxy)propyl ester, (Z,Z,Z).

Hatira Taskın *et al*., (2013) reported that fresh sample of *G.lucidum* extract was performed by Gas Chromatography (GC/MS). Volatile aroma compounds, Alcohols, aldehydes, acids, phenol, L-Alanine, d-Alanine, 3- Methyl, 2-Butanamine, 2-Propanamine were determined. 1-Octen-3-ol (Alcohol) and 3-methyl butanal (Aldehyde) were identified as major aroma compounds.

REFERENCES

Asha, A., 2023. *In-vitro* Antibacterial Activity of some *Ganoderma* Species: A Review. *Qeios,* 1-18; doi: 10.32388/65JGU9.

Bauer, A.W., Kirby, M.D.K., Sherris, J.C., *et al.*, 1996. Antibiotic susceptibility testing by standard single disc diffusion method. *American Journal of Clinical Pathology,* **45**:493-486.

Blundell R., Camilleri E., Baral B., *et al*., 2023.The Phytochemistry of *Ganoderma* Species and their Medicinal Potentials. *The American journal of Chinese medicine*; doi: 10.1142/S0192415X23500404.

Chadha, K.L. and Sharma, S.R. 1995. Mushroom Bio-technology - Advances in Horticulture. vol. 13. Malhotra Publishing House, New Delhi.

Deng, P., Linqiang, W., Congheng, C., *et al*., 2012. Structure characterization of a novel neutral polysaccharide isolated from *Ganoderma lucidum* fruiting bodies. *Food Chemistry;* **135:**1097–1103.

Gao, Y. and Zhou, S. 2003. Cancer prevention and treatment by *Ganoderma*, a mushroom with medicinal properties, *Food Reviews International*, **19**:275-325.

Gupta, R.S., 1986. Mushroom cultivation, Indian Horticulture. 31(1):1.

Hatira, T., Ebru, K., Ozgun, C., *et al*., 2013. Determination of Volatile Aroma Compounds of *Ganoderma Lucidum* by Gas Chromatography Mass Spectrometry (HS-GC/MS)**.** *Afr. J. Tradit. Complement Altern. Med*.**, 10(2):** 353-355**.**

Holmgren, P.K, Holmgren, N.H., Barnett, L.C. 1990. Index Herbariorum. Pt. 1. The Herbaria of the World. International Association for Plant taxonomy. New York: New York Botanical Garden, pp.693.

Kamra, A. and Bhatt A.B. 2012. Evaluation of antimicrobial and antioxidant activity of *Ganoderma lucidum* extracts against human pathogenic bacteria. *International Journal of Pharmacy and Pharmaceutical Sciences;* **4**:359 - 362.

Kim, K.C. and Kim, I.G. 1999. *Ganoderma lucidum* extract protects DNA from strand breakage caused by hydroxyl radical and UV irradiation. *International Journal of Molecular Medicine*, **4**:273-277.

Kumaravel, S., Praveen Kumar P., and Vasuki, P. 2010. GC-MS Study on microbial degradation of lindane. *International Journal of Applied Chemistry,* **6(3):**363-366.

Lin, Z.B. 2001. Pharmacological functions of *Ganoderma lucidum*. *In:* Modern Research of *Ganoderma lucidum* (Ed. Lin, Z. B.). *Beijing: Beijing Medical University Press.*

Lu, Q.Y., Jin, Y.S., Zhang, Q., *et al*., 2004. *Ganoderma lucidum* extracts inhibit growth and induce action polymerization in bladder cancer cells *in vitro*. *Cancer Letters*, **216**:9-20.

Mohammad, H.R.J. and Milad, M. 2021. *Ganoderma lucidum* and Antimicrobial Activity. *Ethno Pharm Product*, **2(2)**:35-41.

Mousavi, S.M., Hashemi, S.A., and Gholami, A. 2023. *Ganoderma lucidum* methanolic extract as a potent phytoconstituent: characterization, *in-vitro* antimicrobial and cytotoxic activity. *Scientific Reports,* **13**:17326; doi: 10.1038/s41598-023-44135-1.

Munsell, L. 1975. Munsell soil color charts. U.S. Department Agriculture, Hand.18. Soil Survey Manual, New York.

Nasim, G. and Ali, M. 2011. Estimation of antimicrobial potential of *Ganoderma lucidum*

(Leyss. Ex. Fr.) Karst. extracts. *Pakistan Journal of Botany*, **43**:183-189.

Naveen kumar, C., Srikumar, R., Swathi, S., *et al.*, 2018. Phytochemical analysis and antifungal activity of *Ganoderma lucidum*. *Indian Journal of Public Health Research & Development*, **9(12)**:130; doi: 10.5958/0976-5506.2018.01820.X .

Nour, El. Dein. and Mahmoud. 2023. Antimicrobial Activities of *Ganoderma mbrekobenum* (Agaricomycetes) EGDA from Egypt. *International Journal of Medicinal Mushrooms*, **25(9)**:31-41*;* doi: 10.1615/ *Int J MedMushrooms*.2023049502.

Wasser, S.P. 2002. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, **60**:258-274.

Wasser, S.P. and Weis, A.L. 1999. Therapeutic effects of substance occurring in higher Basidiomyetes mushrooms:Modern perspective. Critical Reviews in Immunology, **19**:65-69.

Whitaker R.H., 1969. Whittaker's five kingdom. *Science*, **163**:150-160.