Eco. Env. & Cons. 28 (February Suppl. Issue) : 2022; pp. (S399-S405)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i02s.063

Extraction of Bioactive Compounds in *Ganoderma lucidium* from Himachal Pradesh

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(Received 15 July, 2021; Accepted 10 August, 2021)

ABSTRACT

Ganoderma lucidium from the region of Himachal Pradesh was taken for the study to identify the bioactive compounds. Two extraction methods *viz.,* water and methanol extract were used for extracting the compounds. From the results, when comparing with water extract of fruiting body of *Ganoderma lucidium* the methanol extract has high number of phenolic and triterpenoid compounds. Elemental analysis revealed that calcium, sodium phosphorous, potassium was in high concentration. While potassium, magnesium, copper, iron, zinc, manganese were found to be in moderate level. TLC spotted with chromatographic fractions showed pink spots when sprayed with anisaldehyde–sulphuric acid reagent which indicate the presence of triterpenes. In HPLC chromatogram of methanolic extract of *Ganoderma lucidium* eight compounds were detected and these compounds were extracted at retention time 4.87, 21.67, 26.2, 29.1, 30.09, 36, 38.2, 39 respectively. From GC-MS chromatograms, 13 compounds were identified mostly these compounds were saturated and unsaturated fatty acid. These compounds were analysed for pharmacological study.

Keywords: *Ganoderma, GCMS, HPLC, Phytochemicals, Triterpenes*

Introduction

Phytochemicals are abundant, locally renewable, user-friendly and environmentally safe, and attracts low capital. Mushroom contains a vast number of compounds among which are polysaccharides and triterpenes. Terpenes or terpenoids are the compounds responsible for the medicinal, culinary and fragrance nature. In Chinese term, aqueous extract of *Ganoderma Ganoderma lucidium* are called as Lingzhi. The name represents a combination of spiritual potency and essence of immortality and is regarded as the "Herb of spiritual potency'' symbolizing success, well-being, divine power, and longevity. For over 2000 years, Lingzhi has been recognized as a medicinal mushroom, and in ancient scripts its powerful effects have been documented. The fungus is cosmopolitan with interwoven hypha that forms a lamellaless mycelium employed in the treatment of various medical conditions ranging from cancer, tumour, wound healing, hypotension, microbial infections and inflammatory condition. About 80% of the world's population need the herbal medicines to serve the health especially for millions of people in the vast rural areas of developing countries. Medicinal plants as a primary health care modality more than 65% of the global population used. *Ganoderma lucidium* was investigated for its antibacterial effect on Gram positive bacteria and Gram-negative bacteria. The extracts derived from

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the mushroom contain bacteriolytic enzymes which has an interesting aspect of its performance as antimicrobial effect. Its major compound with significant pharmacological activities are ganoderic acid, triterpenes (Zhao *et al*., 2015).

It is interesting that during the last three decades more than 150 triterpenes have been isolated and are known to be unique compounds in this mushroom. *Ganoderma lucidium* products with different triterpenes likely to result in different pharmacological activities. A new class of compound with nutritional and medicinal features extractable from the fruiting bodies of mushroom have been referred to as" mushroom nutraceutical'. *Ganoderma lucidium* is rich in mushroom nutraceutical components with potential therapeutic values (Sasikumar *et al.,* 2010).

Chemical compounds and nutrients play important role in nutrition, maintaining good health and physiological functions of the body (Cohen *et al*., 2014). For vaccinations they promote growth performance and humoral immune response (Ogbe *et al*., 2008). Copper (Cu), zinc (Zn), chromium (Cr), cadmium (Cd), manganese (Mn), iron (Fe), nickel (Ni), and lead (Pb) (Ogbe *et al*, 2012) are trace elements found in most mushrooms. For metabolic reactions, firm bone formation, transmission of nerve impulses, and regulation of water and salt balance minerals in the diet are essential.

Ganoderma lucidum and its pharmaceutically active compounds are responsible for the major medicinal properties. The main aim of the work is to detect phytochemical, elemental analysis and compound present in *Ganoderma lucidium* fruiting body powder.

Materials and Methods

Ganoderma lucidium fruiting body powder 1 kg was collected from Daxen Agritech India Pvt LTD, Himachal Pradesh. The dried sample was stored in clean polythene bags labeled properly and stored in refrigerator at 4°C until analysis.

Preparation of Aqueous Extract

The aqueous extract of fruiting body of *Ganoderma* were prepared by transfer of one gram(1g) of the fruiting bodies powder to 50 ml capacity of sterile wide-mouthed screw-capped bottles. 10 ml of sterile de-ionized distilled water was added to the powdered samples which were allowed to soak for 24 hours at room temperature and was heated for 2 hour at 100 °C. The mixtures were then centrifuged at 2000 rpm for 10 minutes at 4 °C. The supernatants were filtered through a sterile funnel containing sterile Whatman filter paper (No.1) and then filter sterilized using 5ml sterile syringe with 0.2 membrane filter. (Pooja *et al.,* 2004).

Solvent extraction

Air dried powder of *Ganoderma lucidium* fruiting body powder was extracted by using soxhlet apparatus. 10 g of fruiting powder was taken in a paper cone and placed in soxhlet apparatus (Dulger *et al.,* 2004).

Determination of total triterpenoids

The total triterpenoids determination was performed according to the colorimetric method of Lei Wei *et al* (2015). At 100°C in a water bath, the test samples or ursolic acid standards in tubes were evaporated to dryness. 0.4 ml of 5% vanillin/glacial acetic acid (w/v) and 1.0 ml of perchloric acid solution were added to each tube successively. The tubes were placed in a water bath for the reaction at 60 °C for 15 min, 5.0 ml of glacial acetic acid added to the cooled samples and the absorbance of the sample was measured at 548 nm.

Determination of Total Flavanoid Content

The modified aluminium chloride colorimetric method (Barros *et al*. 2007) was followed to determine flavonoid content. 500 µl distilled water, sodium nitrite, NaNO2 (5%, 30 µl) and Ganoderma extract (100 µl, 10 mg/ml) was mixed. Then the mixture was allowed to stand for 5 minutes. Aluminium chloride solution, $AlCl_3.H_2O$ (10%, 60 µl) was added to the mixture and left for 6 minutes. Sodium hydroxide, NaOH (1M, 200 µl) mixed with 110µl distilled water were added to the solution. At 510nm absorbance of the solution was measured. Based on standard curve of rutin (0.2-1.0 mg/ml) with the linear equation y = 0.0101x + 0.2238, where R2 = 0.9563 the concentration of total flavonoids content was calculated. The extracts results were expressed as ìg of rutin equivalent (RE/µg) per gram.

Elemental analysis

1g of crude powder of *Ganoderma lucidium* fruiting body was ashed in an oven 650 °C for 5-6 hours in the porcelain crucible. The ash was dissolved in HNO_3 solution and digested with HCL and H_2O_2, cooled, centrifuged and diluted up to 10 ml with de-

ionized water. (Muhammed *et al*., 2011). Then it was analyzed for elements using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS Z-5000).

Thin Layer Chromatography

Methanolic extracts and aqueous extracts of the samples were separated on silica gel thin layer aluminium plates of 15x5 cm with 2mm thickness. With a pencil, a thin mark was made at the bottom of the plate to apply the sample spots. Extracts were spotted manually using capillary tube. Allow sufficient time for the development of spots by spraying anisaldehyde–sulphuric acid reagent. Then remove the plates and allow them to dry (Ebenezer *et al*., 2017). Colour of the spots were noted and retention factor (Rf) values were calculated by using the following formula

$$Rf = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

High performance liquid chromatography

The high-performance liquid chromatography was performed for *Ganoderma lucidium*. The sample was made to run for 10 min and the volume of sample taken for analysis is 10 µl and the analysis was carried at 254 nm. Methanol was used as the solvent system for the study. After the analysis, peaks were obtained (Gao, 2004).

GC-MS Determination of bioactive components

Phytochemical investigation of methanolic extract was performed using GC-MS equipment (Thermo Scientific Co.). Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II.GC-MS system experimental conditions were as follows: DB35-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25ìm. At 1.0 ml/min flow rate of mobile phase (carrier gas: He) was set. Temperature programme (oven temperature) was 70°C raised to 260 °C at 6 °C/min in the gas chromatography part, and injection volume was 1ìl. Samples dissolved in methanol were run fully at a range of 50-650 m/z and by using Wiley Spectral library search programme the results were compared (Olukayode *et al.,* 2016)

Retention indices (RI) of the compounds were determined by comparing the retention times of a series and identification of each component was confirmed by comparison of its retention index with data from the literature. The spectrum of the unknown components was compared with the spectrum of known components which was stored in the NIST library. The molecular weight, name, chemical structure and molecular formula of the components of the test materials were ascertained. The peak in GCMS of methanol extract of *Ganoderma lucidium* showed the presence of the secondary phytochemical compounds like phenolic and fatty acids and its esters.

Results and Discussion

The total triterpenoid content in methanol and water extract was 6.2 ± 5.1 and 4.8 ± 0.2 respectively. Methanol extract had highest number of triterpenoids which was given in Table 1. The total flavanoid content in methanol extract was found to be high (159.21 ± 3.26) than in water extract (99.50 ± 2.14) Table 2. Elemental composition of crude extract of *Ganoderma lucidium* was summarized in Table 3. Elements present in crude extract were calcium (344.8 Mmg/kg), magnesium (9 mg/kg), potassium (31.1 mg/kg) copper (0.799 mg/kg), iron (46.2 mg/kg), zinc (14 mg/kg), manganese (0.99 mg/kg), phosphorous (184.3 mg/kg), sodium (187.7 mg/kg). Among those, calcium, sodium and phosphorous were in high concentration. While potassium, magnesium, copper, iron, zinc, manganese were found to be in moderate concentration. The results are in line with the findings of Falandysz *et al*. (2013). Sodium(Na), magnesium (Mg), phosphorous (P), potassium (K), and calcium (Ca), the essential micronutrients like zinc(Zn), iron (Fe), manganese (Mn) and copper (Cu) and some hazardous

Table 1. Total triterpenoids content

Sample	Total triterpenoid (mg urosolic acid /g)
Aqueous extract	4.8 ± 0.2
Methanol extract	6.2 ± 5.1

Table 2. Total Flavanoid Content

Sample	Total Flavonoid content (mg ruting/ g of extract)
Aqueous extract	99.50 ± 2.14
Methanol extract	159.21 ± 3.26

non-essential nutrient like lead(Pb), mercury(Hg) were present in edible mushroom. From the elemental analysis of the *G. lucidium* powder appreciable concentration of various elements was revealed. Manganese complex helps in blood clotting. For the maintenance of fluid balance, sodium and potassium are required, while potassium and calcium are important in stimulating action potential across nerve endings. For heme formation and to enhance oxygen carrying capacity of red blood cells iron is highly required physiologically. For protein synthesis, normal body development and recovery from illnesses, zinc is an important requirement (Muhammad *et al.*, 2011), it is also a necessary part of DNA, for cell division and synthesis hence its importance in wound healing (Ko *et al.*, 2008).

From qualitative analysis of thin layer chromatography, the spot visualized in two lanes in methane extract of *Ganoderma lucidium* L1 Rf = 0.54, and L2 Rf = 0.86 and in water extract Rf=0.6 (Figure 1). TLC spotted with chromatographic fractions showed pink spots when sprayed with anisaldehyde–sulphuric acid reagent which indicate the presence of triterpenes.

In HPLC chromatogram of methanolic extract of *Ganoderma lucidium* eight compounds *viz.*, Ganoderic acid E, Ganodermanontriol, Ganoderic acid K, Lucidadiol, Ganoderic acid H, Ganoderic acid B, Ganoderic acid D, Ganoderic acid A were extracted at retention time 4.87, 21.67, 26.2, 29.1, 30.09, 36, 38.2, 39 respectively (Figure 2) (Table 4). Compounds analysed by chromatography agrees with Dr. Duke's Phytochemical and Ethnobotanical

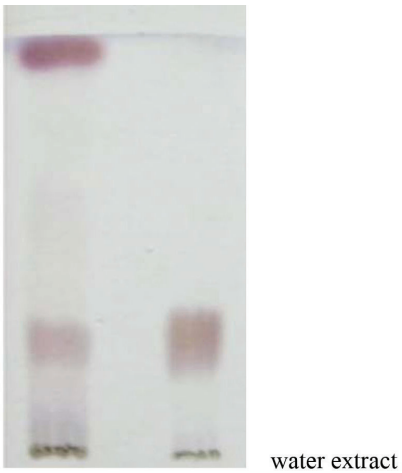

Fig. 1. Thin Layer Chromatography

Table 3. Elemental analysis of crude extract of *Ganoderma lucidium*

Element	Oxidant gas pressure Flow rate(kPa)	Content (mg/kg)	WHO standards (/g)
Calcium	160	344.8	3600-80000
Magnesium	160	9	100-200
Potassium	160	31.1	10-100
Copper	160	0.799	100-300
Iron	160	46.2	50-5000
Zinc	160	14.9	150-20000
Manganese	160	0.99	100-20000
Lead	160	0.081	5-30
Phosphorous	160	184.3	5-300
Sodium	160	187.7	400-500

Table 4. List of compound in methanolic extract of *Ganoderma lucidium* body powder detected by HPLC

Compound	Molecular formula	Retention Time/min	Molecular weight g/mol
Ganoderic acid E	C30H40O7	4.87	612.8
Ganodermanontriol	C30H48O7	21.67	472.7
Ganoderic acid k	C32H46O9	26.2	574.7
Lucidadiol	C30H48O3	29.1	456.7
Ganoderic acid H	C32H44O9	30.09	572.7
Ganoderic acid B	C30H44O7	36	516.7
Ganoderic acid D	C30H42O7	38.2	514.6
Ganoderic acid A	C30H44O7	39	516.7

Databases National Agricultural Library).

The GC-MS chromatograms of methanolic extract showed that most of the extracted compounds were saturated and unsaturated fatty acids. Compounds such as butanoic acid, butyl ester, propanoic acid, 2-methyl-dodecyl ester, 17-pentatriacontene, cyclo octacosane, tricosyl trifluoroacetate, hexacosyl heptafluorobutyrate, 5-eicosene, 3-eicosene, (E), 1-nonadecene, 1-docosene were extracted at retention time 1.5, 3.9, 4.3,5.9, 7.2, 8.1, 11.5, 12.8, 21.2,23.2, 26.8, 28.1, 29.2, respectively (Table 5) (Fig. 3). In this study, some compounds agree with the finding of Olukayode *et al.* (2016).

Conclusion

In conclusion the compounds identified by phytochemical, elemental analysis, TLC, HPLC, GC-MS in the methanol extract of *Ganoderma lucidium* fruiting body was discussed for pharmacological study which showed that they can be used as potential drug targets for various ailments.

Conflict of interest

The authors have no conflicts of interest to declare.

All co-authors have seen and agree with the contents of the manuscript and there is no financial in-

Table 5. List of compounds in methanolic extract of Ganoderma lucidium fraction detected by GC-MS

S. No.	Retention Time	Name of the compound	Molecular Formula	Peak Area %	Class
1	1.5	Propanoic acid,2-methyl-3-hydroxy-2,4,4-trimethypentyl ester	C12H24O3	2.81	Saturated Fatty acid
2	3.9	Propanoic acid, 2-methyl-dodecyl ester	C14H28O	0.56	Saturated fatty acid
3	4.3	17-Pentatriacontene	C37H74	1.45	Saturated fatty acid
4	5.9	Cyclo octacosane	C28H56	0.70	Saturated fatty acid
5	7.2	Tricosyl trifluoroacetate	C25H47F	0.52	Saturated fatty acid
6	8.1	Hexacosyl hepatafluorobutyrate	C30H53F7O2	3.42	Saturated fatty acid
7	11.5	5-Eicosene, (E)	C20H40	3.84	Unsaturated fatty acid
8	12.8	Cetene	C16H32	18.55	Unsaturated fatty acid
9	21.2	1-Octadecene	C18H36	5.5	Unsaturated fatty acid
10	23.2	3-Eicosene, (E)-	C20H40	3.40	Unsaturated fatty acid
11	26.8	1-Nonadecene	C19H38	3.30	Unsaturated fatty acid
12	28.1	1-Docosene	C22H44	55.20	Unsaturated fatty acid
13	29.9	Butanoic acid, butyl ester	C8H16O2	0.67	Saturated fatty acid

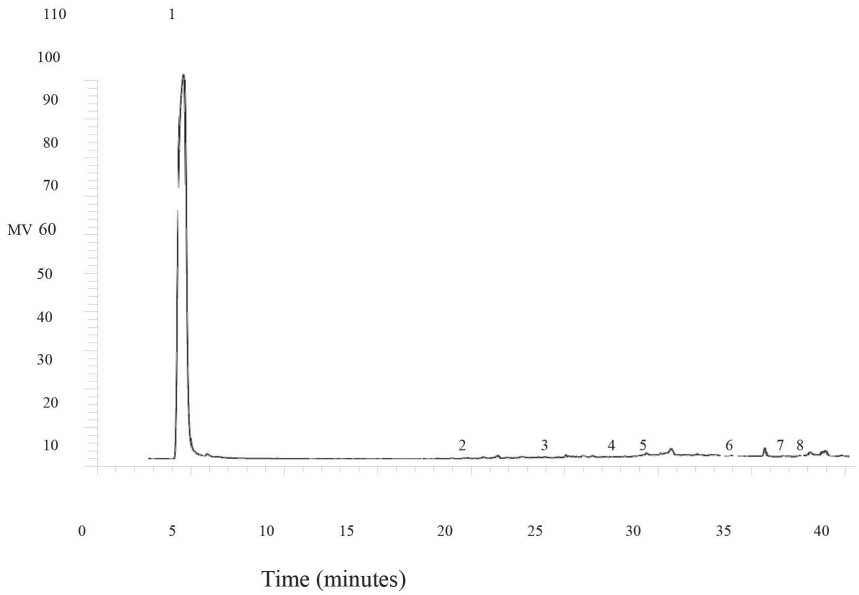

Fig. 2. HPLC Chromogram of Methanolic extract of *Ganoderma lucidium*

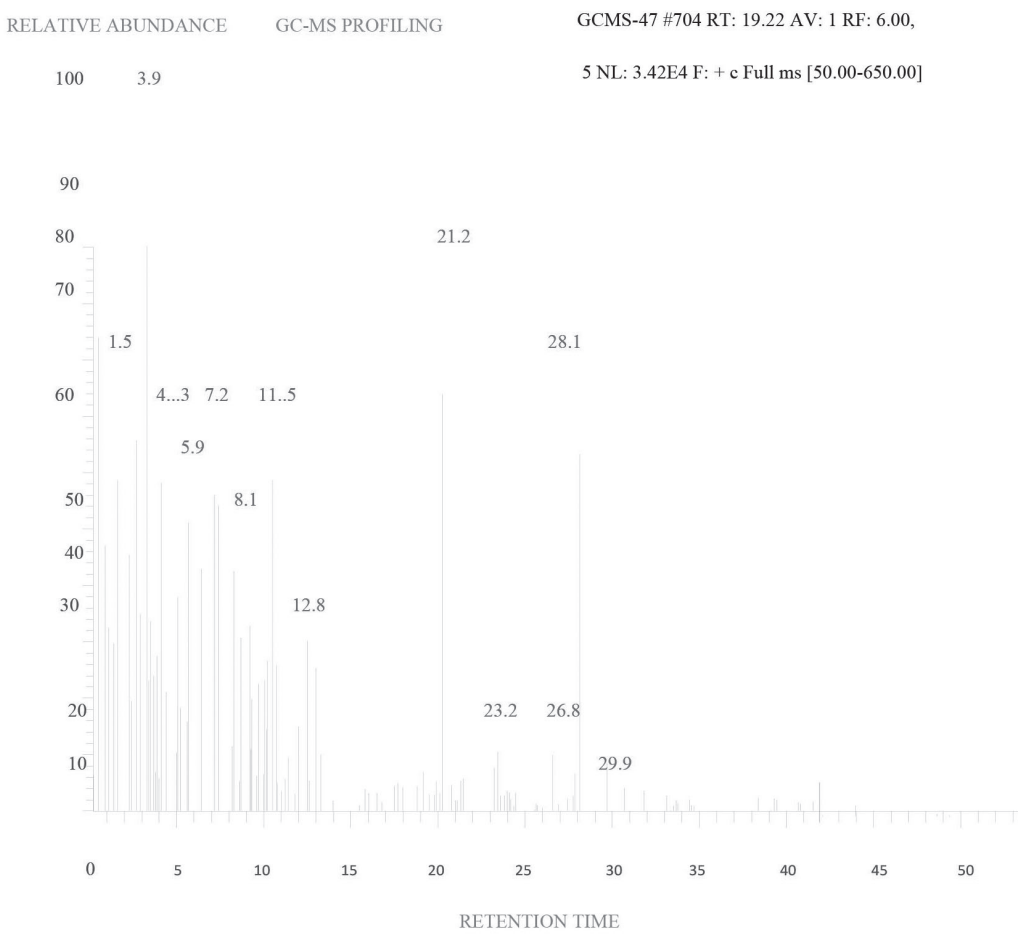

Fig. 3. Gc-Ms chromogram of methanolic extract of *Ganoderma lucidium* fruiting body

terest to report. We certify that the submission is original work and is not under review at any other publication.

Data availability statement

This is to bring to your kind notice that all the data pertaining to this research article have been included in the manuscript. No other data to be shared

Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Acknowledgement

The authors are thankful to The South Indian Textile Research Association, Coimbatore, and also thankful to Daxen Agritech Pvt Lmt Himachal Pradesh for providing fruiting body powder of *Ganoderma lucidium*.

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