Current Trends in Biotechnology and Pharmacy
Vol. 15 (4) 383 - 389, October 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.4.39



# *Ganoderma mbrekobenum*: A pharmacologically Important Mushroom Naturally Growing in Raisen, India

**Shailendra Singh Parihar, Sharda Sahu, Govind Gupta, Anil Prakash***

Department of Microbiology, Barkatullah University, Bhopal
*Corresponding author Email: dranilprakash98@gmail.com

**Abstract**

*Ganoderma* is well-known medicinal species of mushroom that has been used for many years in the treatment of different diseases which include migraine, headache, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, hypercholesterolemia, nephritis, dysmenorrhea, constipation, hepatitis, leukopenia and cardiovascular disorders. In present study, wild species *Ganoderma mbrekobenum* was collected from agroclimatic zone of Vindhya plateau (Raisen 23° 2′ 12″ N and 78° 5′ 3″ E), Madhya Pradesh, India in single and same host plant (lemon tree). On the basis of ITS Gene, BLASTN and phylogenetic analysis, the three collected samples were identified as *Ganoderma mbrekobenum* (MK940286), *G. mbrekobenum* (KY865253) and *G. mbrekobenum* (MK940290).

Further, in background of the therapeutic values of *Ganoderma*, estimation of bioactive compounds was performed by using LC-MS technique on hot water ethanolic extract of one of the identified species, *G. mbrekobenum* (MK940286) at IIT, Mumbai. This species has shown to be a source of important bioactive compounds such as Olmesartan Medoxomil, Benazeprilat, Isopropamide, Ganodermic acid A, Ramipri Glucuronide, Desmethyldoxepine, Oleamide, Phorbol myristate acetate and Cepharanthine which are in common medicinal use as a remedy for several chronic diseases. Collectively, the study indicates that the extracted compounds from *G. mbrekobenum* could be a promising molecular mortar for further exploration as an anti-tumour and anti- inflammatory agent.

**Key words**: *Ganoderma*, *G. mbrekobenum,* bioactive compounds, anti-tumour, anti- inflammatory.

## Introduction

Mushroom being the ultimate health food, proved by several recent experimental studies records its medicinal attributes. In the recent scenario, a variety of medicinal preparations of mushrooms in the form of tablets, capsules and extracts have been produced and marketed. *Ganoderma* is a white rot popular medicinal mushroom that has been used in traditional Chinese medicine for centuries. It has been consumed for its broad medicinal properties in various countries of Asia like China, Korea, Japan, Taiwan, Malaysia, and Vietnam for over centuries (Klupp et al. 2015) Practice of consumption of *Ganoderma* was frequent to increase the immunity and as antitumor, antimicrobial, anti-inflammatory, antioxidant and acetylcholinesterase inhibitory activity. *Ganoderma* is also a constituent of several cosmetics produced especially in China, Korea, USA and some other Asian and European countries, and in many places for skin lightening (Jiang 2015). In the contemporary world, it has approximated current global trade of nearly 2 billion dollars involving its considerably large trade share in India (Rai 2008). The global production of *Ganoderma* mushroom was about 4900–5000 tons in 2002, of which 3800 tons were produced in China. India contributes very least quantity of *Ganoderma* production and imports this from Malaysia and China.

The genus *Ganoderma*, belongs to Ganodermataceae family, is a group of higher macro fungi widely growing in tropical and temperate regions. *Ganoderma* mushroom is a natural gift to mankind, as countless merits are found in the content metabolic compounds. The bioactive metabolites of *Ganoderma* are used to cure several diseases such as cancer, diabetes, heart disease, liver ailments, kidney dysfunction and AIDS. About 300 species of *Ganoderma* have been described so far, but among them only fifty species have been studied and identified on the molecular basis, ITS sequences, and the other are synonymous of each other (Wachtel-Galor et al. 2011, Furtado 1965). However, in pace of time, the environmental factors have greatly influenced the genomic structure of *Ganoderma* and led to the development of the new species of *Ganoderma*. New species of *Ganoderma* are reported to possess certain new useful bioactive compounds (Hapuarachchi 2018).

A few years ago, *G. mbrekobenum* was discovered in Ghana (South Africa) and named on the Ghanaian word Twi, ‘mbrekoben’ denoted for reddish brown mushroom (Otto et al. 2015). *G. mbrekobenum*, looks similar to the *Ganoderma lucidum* and identified as separate entity as *G. mbrekobenum* on the basis of ITS and LSU gene studies (Otto et al. 2016). We have

collected the similar wild species from Raisen district of Madhya Pradesh India. In present study, morphological identification and molecular characterization were carried out in order to identify the species followed by detection of bioactive compounds.

## Materials and Methods

### *Sample collection and Processing*

Samples were collected from root and stem portions of the selective lemon tree grown in Vindhya plateau (Raisen 23° 2′ 12″ N and 78° 5′ 3″ E), Madhya Pradesh India in September 2016 and October 2017. The collected samples were brought to laboratory and debris were removed followed by processing for pure culture isolation. In the process, a fresh Ganoderma fruiting body was cut into small pieces and surface sterilized using 1% $HgCl_2$, then placed on the surface of malt extract agar plates and potato dextrose agar plates, with the help of sterilized forceps and incubated at 28ºC for 5 days. After observing the mycelial growth around the fruiting tissue, pure mycelium was transferred on fresh MEA and PDA plates.

### *Morphological Identification*

The samples were identified morphologically by following standard literature (Alexopoulos et al. 1995) based on fruiting body's colour, size, stem colour and size, spores and spore print.

### *Molecular characterization*

Isolation of mycelium DNA was done by following CTAB (Cetyl Trimethyl Ammonium Bromide) method. The mycelial mat grown on Potato Dextrose Broth (PDB) at 25ºC for 10 days was harvested on sterilized filter paper. The mycelium was frozen, dried using liquid nitrogen, and ground with the help of mortal and pistol to make it powder. The ground material was processed for DNA isolation according to modified CTAB method as described in an earlier study (Oyetayo and Yao 2010).

Isolated DNA was quantified using agarose gel electrophoresis and stored in deep freezer in TE buffer for future use. Further ITS gene of isolated DNA was amplified using PCR (polymerase chain Reaction) in thermal Cycler machine (BIORAD) by using ITS -1 and ITS-4 universal Primer, 10X PCR Buffer, 25mM of $MgCl_2$, 2mM dNTPs and Taq polymerase, MQ water and Isolated Genomic DNA. PCR reaction was carried under the following circumstances: 35 cycles having denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min. The amplified products were purified using gel purification kit (Promega, US) and separated in 1.2% agarose gel by gel electrophoresis method, gel was observed under the UV light in Gel Documented System (BIORAD) to obtain DNA band in gel with the help of transilluminator. The DNA band gel cube was cut and collected in the minicentrifuge tube. Extraction of the fragment DNA was carried out following the protocol as described in the Wizard SV Gel and PCR Clean-Up System Promega USA.

Isolated and amplified ITS genes were sent for gene sequencing to Xcelris-Ahmedabad and Bio innovations-Mumbai. The obtained sequences were submitted to NCBI GeneBank for accession number. The data were analysed using NCBI BLAST (Altschul et al. 1990) to match the best similarities with other related ITSs on database. The similar ITS sequences were obtained from NCBI GenBank and aligned using CLUSTAL W (Thompson et al. 1994). Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2018) inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura 1980). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed (Felsenstein 1985).

### *Extraction of bioactive compounds*

The mature fruiting body of *Ganoderma mbrekobenum* MK94029 were dried and ground to powder with the help of heavy-duty grinder. About 100 gm of powder was dissolved in 300 ml of distilled water and incubated at 80 ºC on water bath for 8 hours, thereafter, extract was filtered in a separate beaker and 200 ml of 95% ethanol was added to it and incubated at 4ºC in refrigerator for 5 days. After five days, precipitate was found settled in the bottom of the beaker and the extract was centrifuged at 3000 rpm for 10 min, and dried in rotary vacuum evaporator to obtain concentrated crude sample.

### *Estimation of bioactive compounds by HRLCMS*

The ethanolic extract of *Ganoderma mbrekobenum* MK94029 (10mg) was sent to Indian Institute of Technology (IIT) Bombay for the identification of produced bioactive compounds. It was performed by an Agilent LCMS TOF/Q TOQ Mass -spectrometer (MSQ-TOF-G6550A) binary pump (64220B). The chromatographic separation was performed using an hypersil gold 3micron 100 x 2.1 MM used as a stationary phase at 40°C as temperature. The mobile phase consisted of acetonitrile, water with formic Acid (90% ACN +10% H2O+ 0.1% FA) (solvent B) and water with formic Acid as solvent A (0.1% FA in water). The flow rate was kept 0.300 mL/min. The gradient elution started with 95% A/5% B 0-2 min,0%A 100% B -20 min, 0% A/100% B 20-25 min, 95% A/5% B 25-26 min,95%A/5%B26-30min. Photodiode array detector was set at 350 nm for acquiring chromatograms. The injection volume was 3.00 µL and peaks were monitored at 250 nm. Mass spectra data were recorded on an ionization mode for a mass range of m/z 163-1000. Other mass spectrometer conditions were as follows: nebulizing gas pressure: 35 psi; drying gas flow: 1L/min; drying gas temperature: 250 °C. Scan Source Parameters value, VCap-3500, Nozzle voltage-1000, Fragmentor-175, Skimmer-65 and

Current Trends in Biotechnology and Pharmacy
Vol. 15 (4) 383 - 389, October 2021, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2021.4.39

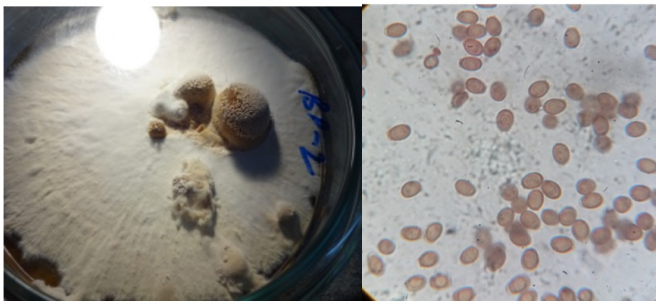

octopole RF peak-750.

## Results and Discussion

### *Morphological Identification*

The three collected Ganoderma samples were identified on the basis of macroscopic and microscopic studies. All the isolates were found to bear brownish, red colour (size-20cm-25cm) of fruiting body having red brown stem colour and spore print (Figure 1A) On pure culture characterization of *Ganoderma* isolates on MEA plate, cottony brownish white coloured culture appeared and after few days some fruiting body like structure was observed on culture plate (Figure 1B). The spores were ellipsoidal to oval thin walled reddish brown in colour (Figure 1C). Morphologically it seems very much similar to *Ganoderma lucidum* but Pegler and Yao (1996) noted that a wild species of *Ganoderma* isolated from different places were different from *G. lucidum* by molecular systematic studies.

A



B C

Figure 1: Collected sample of G. mbrekobenum fruiting body (A), mycelial growth of G. mbrekobenum on culture plate of MEA (B), microscopic structure of G. mbrekobenum spores (C).

### *Molecular identification and Phylogenetic tree analysis*

Molecular characterization is a useful and reliable step in the proper identification of pharmacologically important *Ganoderma species.* After DNA isolation and amplification of ITS 5.8s rDNA through PCR technique by using universal ITS-Gene (ITS-1/ITS-4) primers, 650 bp amplicon was obtained for all the three samples (Figure 2). The amplified product was sequenced and submitted to NCBI gene bank and received the accession no. MK940287, KY865253 and MK940290.

For phylogenetic analysis, total 23 representative homologous sequences of ITS rDNA above 97% similarity were selected for cluster analysis. The results of the ITS gene sequence obtained from NCBI GenBank BLAST search revealed that the percentage relationship of isolated *Ganoderma* species is 99% sequence identity with several other *Ganoderma mbrekobenum* species (KX000898; NR147647) and 98% identical with MH221092;1KM229613; KM229610; MK453307). None of the ITS sequences of *Ganoderma species* were 100% homologous with ITS of collected Ganoderma samples from NCBI GenBank (Figure 3).

*Ganoderma mbrekobenum* species sequences from Raisen region formed two different clades in the phylogenetic tree. *G. Mbrekobenum* MK940290 and *G. Mbrekobenum* MK940287 form a clade while *G. mbrekobenum* KY865253 form another clade. This indicates that these three isolated *G. mbrekobenum* species are not from the same ancestral stock with *Ganoderma species* sequences that are in NCBI GenBank. This species of *Ganoderma* was isolated first time from Ghana by Otto et al (2016) from roots and trunks of angiosperm trees in Ghana. These species were 99.22% similar. Similar isolate of *Ganoderma species* (KM229612) from *Ficus benghalensis* was found in Pune, Maharashtra (India) that showed 98.9% similarity with *G. mbrekobenum* KY865253 being the major strain of the species in our study. NCBI accession no. MK453307 of *G. mbrekobenum* from Guangzhou, China was found 98.80% similar with our major strain *G. mbrekobenum* KY865253.

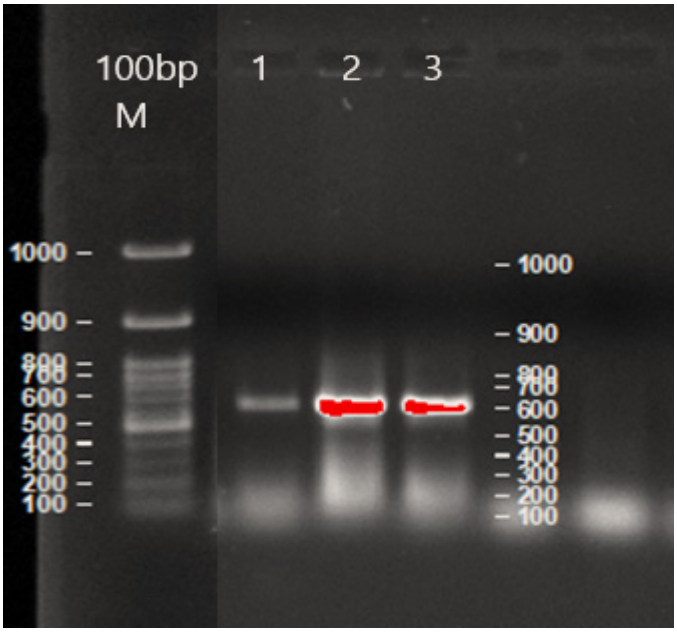



Figure 2: PCR Amplified ITS gene of three samples of *G. mbrekobenum.*

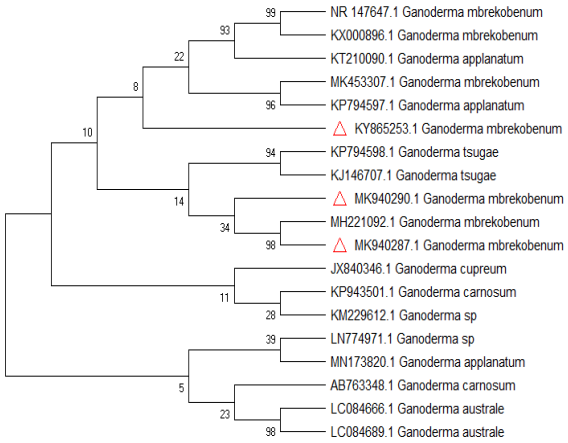



Figure 3: Phylogenetic relationships of collected and characterized *G. Mbrekobenum by* ITS sequences inferred by Maximum Likelihood method: bootstrap method with 1000 replicates analysis. Sequences used for this comparison were obtained under the following GenBank accession numbers: MK940287; KY865253; MK940290.

### *Estimation of Bioactive Compound*

The LC-MS study of *Ganoderma mbrekobenum* MK940287 extract indicated the presence of several bioactive compounds. A total of 67 compounds were identified with different retention time. The chromatogram or the mass spectra (Figure 4) was analysed by using the database of Indian Institute of Technology Bombay (IITB). On the basis of LC-MS data the compounds Ganoderic acid A, Ganoderic acid C1, Ganoderic acid S, Ganoderiol F, Ganodermanondiol were found in our samples were also shown to exist in other *Ganoderma species* (Ma et al. 2011)

but the others compound that were found in our sample such as Olmesartan Medoxomil, Benazeprilat, Isopropamide, Ramipri Glucuronide, Desmethyldoxepine, Oleamide, Phorbol myristate acetate and Cepharanthine were not reported previously in *Ganoderma*. The zoomed spectra of these compounds are shown in figure 5. These compounds play very important role in remedies of various disorders that involve blood pressure, kidney dysfunction, cardiac disorder, cancer diseases, immune defence mechanism, antineoplastic, diabetes etc. (Table1). Obodai et al (2017) have reported chemical characterization and antioxidant potential of twelve wild strains of *Ganoderma sp*. from Ghana. Sharma et al (2019) have also reported *G. lucidum* as rich source of polysaccharides, alkaloids, and ganoderic acid. They also proposed the cellular mechanisms to elucidate the mode of action as anticancer, antiviral, antioxidant and in treatment of liver and other diseases. But no study has yet been reported the extraction and characterization of bioactive compounds of *G. mbrekobenum*.

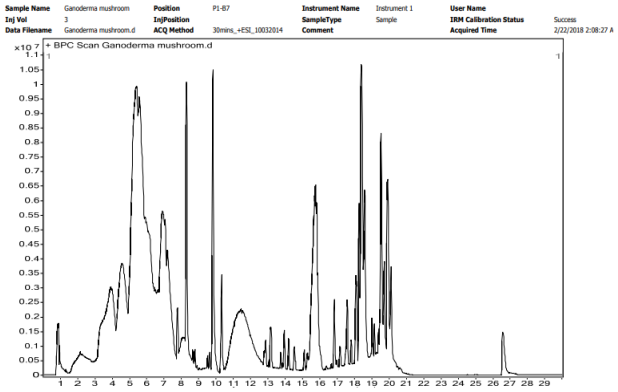
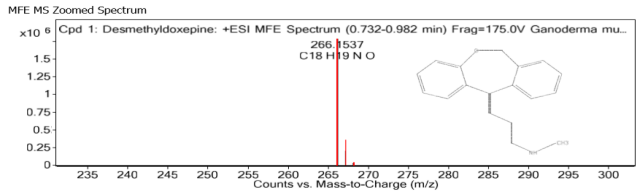
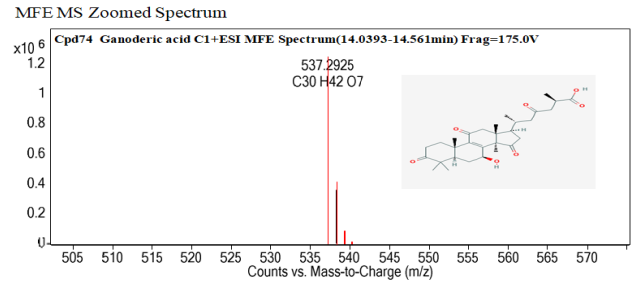



Figure 3: LC–MS chromatogram of *G. mbrekobenum* MK940286 extract for bioactive compounds.

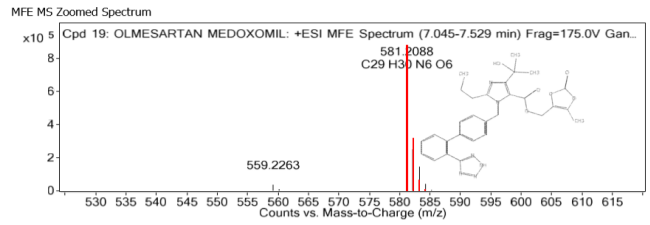


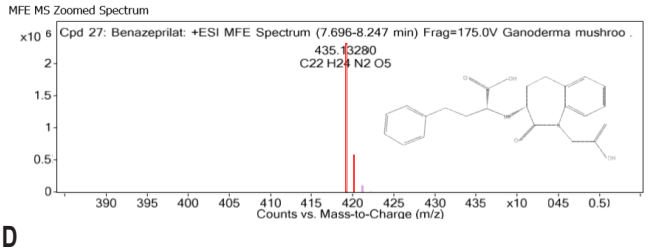

A

B

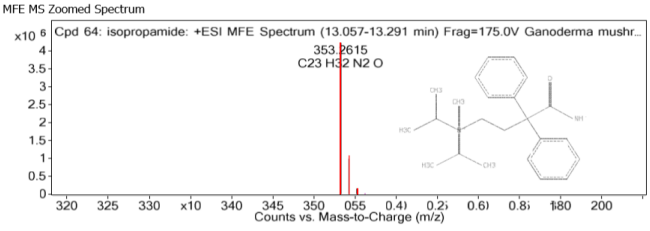



C




D



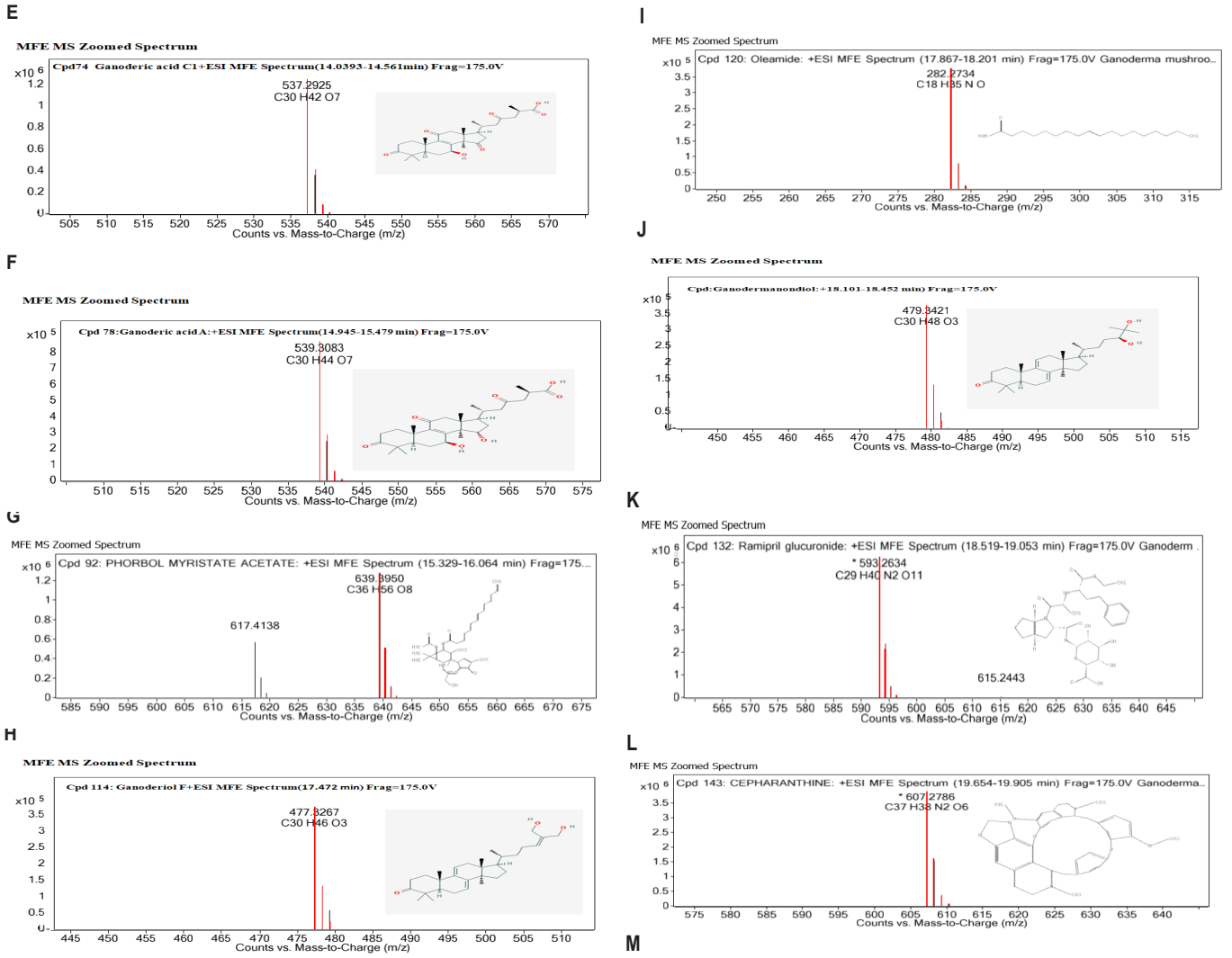



Figure 4: Zoomed spectrum and details of some important compounds, A- Desmethyldoxepine , B-Ganoderic acid S, C-Olmesartan Medoxomil, D- Benazepril, E- Isopropamide, F-Ganoderic acid C1,G-Ganoderic acid A,H- Phorbol myristate acetat, I- Ganoderiol F, J- Oleamide, K-Ganodermanondiol, L- Ramipril Glucuronide, M-Cepharanthine.

Table -1 Bioactive compounds of Ganoderma mbrekobenum and its biological activity

| S.No. | RT | M/Z | Bioactive Compound | Activity | Reference(s) |
|---|---|---|---|---|---|
| 1. | 0.86 | 266.15 | Desmethyldoxepine- | It's used in Treatment of mild depression, Antidepressive Agents, | Kirchheiner (2005) |
| 2. | 6.474 | 453.3342 | Ganoderic Acid S | It is compound showed, Immune Restoration Effects, and anticancer active. | Radwan et al. (2013) |
| 3 | 7.143 | 581.20 | Olmesartan Medoxomil | It's used as medicine in high blood pressure, heart failure diabetic and kidney related disease. | Brenner et al. (2001), Blankfield (2002), Bergmann et al. (2001), Puchler et al. (2001) |

**RT=** Retention Time, **M/Z =** Mass/ Charge

## Conclusion

The differences in environmental factors with pace of time result in the change in genetic makeup of *Ganoderma species* that can consecutively affect the health promoting properties of these wild Ganoderma. On the basis of ITS gene sequence, all three isolated samples were identified as a wild species of *Ganoderma mbrekobenum*. This is the first report for collection and

identification of wild *Ganoderma mbrekobenum* species collected from root and stem region of lemon tree agroclimatic zone of Vindhya plateau (Raisen 23° 2′ 12″ N and 78° 5′ 3″ E), Madhya Pradesh, India.

On the LCMS analysis of extracts of *G. mbrekobenum*, several pharmacologically important bioactive compounds were obtained. Some compound such as Olmesartan Medoxomil, Benazeprilat, Isopropamide, Ramipri Glucuronide, Desmethyldoxepine, Oleamide, Phorbol myristate acetate and Cepharanthine were not reported previously in *Ganoderma*. Most of these compounds are reported for the treatment of cardiovascular ailments, mainly high blood pressure, heart failure, renal disorders and also against diabetes. This important and untapped species of *Ganoderma* has great potential as antitumor and anti-inflammatory drugs. Further studies may unfold certain other characteristics features of this species which would be useful in treatment of many other health conditions.

**Acknowledgement**

Authors are thankful to the DBT Builder Programme of Barkatullah University, Bhopal, Madhya Pradesh, India and Department of Microbiology, Barkatullah University, Bhopal for providing financial support and necessary facilities.

**References**

1. Alexopoulos, C. J., Mims, C. W. and Blackwell, M. (1996). Introductory mycology (No. Ed. 4). John Wiley and Sons.

2. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. Journal of molecular biology, 215(3), 403-410.

3. Bakris, G. L., Williams, M., Dworkin, L., Elliott, W. J., Epstein, M., Toto, R., ... and Sowers, J. (2000). Preserving renal function in adults with hypertension and diabetes: a consensus approach. American journal of kidney diseases, 36(3), 646-661.

4. Balfour, J. A. and Goa, K. L. (1991). Benazepril. Drugs, 42(3), 511-539.

5. Bergmann K. V., Laeis P., Püchler K., Sudhop, T., Schwocho, L. R., & Gonzalez, L. (2001). Olmesartan medoxomil: influence of age, renal and hepatic function on the pharmacokinetics of olmesartan medoxomil. Journal of hypertension. Journal of hypertension. Supplement: official journal of the International Society of Hypertension, 19(1), S33-40.

6. Blankfield, R. P. (2002). Angiotensin-receptor blockers, type 2 diabetes, and renoprotection. N Engl j Med, 346(9), 705-707.

7. Boss, E. G. and Buchanan, G. C. (1957). Effect of isopropamide iodide on basal gastric secretion in the human. Gastroenterology, 33:730-6.

8. Brenner, B. M., Cooper, M. E., de Zeeuw, D., Keane, W. F., Mitch, W. E., Parving, H. H., Remuzzi, G., Snapinn, S. M., Zhang, Z. and Shahinfar S. (2001). Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy The New England Journal of Medicine, 345(12):861-869.

9. Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39(4), 783-791.

10. Frampton, J. E. and Peters, D. H. (1995). Ramipril. Drugs, 49(3), 440-466.

11. Furtado, J. S. (1965). *Ganoderma colossum* and the status of Tomophagus. Mycologia, 57(6), 979-984.

12. Go, A. S., Bauman, M. A., King, S. M. C., Fonarow, G. C., Lawrence, W., Williams, K. A. and Sanchez, E. (2014). An effective approach to high blood pressure control: a science advisory from the American Heart Association, the American College of Cardiology, and the Centers for Disease Control and Prevention. Journal of the American College of Cardiology, 63(12), 1230-1238.

13. Hapuarachchi, K. K., Elkhateeb, W. A., Karunarathna, S. C., Cheng, C. R., Bandara, A. R., Kakumyan, P. ... and Wen, T. C. (2018). Current status of global *Ganoderma* cultivation, products, industry and market. Mycosphere, 9(5), 1025–1052.

14. Huang, H., Hu, G., Wang, C., Xu, H., Chen, X. and Qian, A. (2014). Cepharanthine, an alkaloid from *Stephania cepharantha* Hayata, inhibits the inflammatory response in the RAW264. 7 cell and mouse models. Inflammation, 37(1), 235-246.

15. Huitrón-Reséndiz, S., Gombart, L., Cravatt, B. F. and Henriksen, S. J. (2001). Effect of oleamide on sleep and its relationship to blood pressure, body temperature, and locomotor activity in rats. Experimental neurology, 172(1), 235-243.

16. Jedinak, A., Thyagarajan-Sahu, A., Jiang, J. and Sliva, D. (2011). Ganodermanontriol, a lanostanoid triterpene from *Ganoderma lucidum*, suppresses growth of colon cancer cells through ss-catenin signaling. International journal of oncology, 38(3), 761-767.

17. Jiang, L. (2015). *Ganoderma lucidum* (Reishi mushroom): potential application as health supplement and cosmeceutical ingredient. Global

Journal for Research Analysis, 4, 124-125.

18. Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of molecular evolution, 16(2), 111-120.

19. Kirchheiner, J., Henckel, H. B., Franke, L., Meineke, I., Tzvetkov, M., Uebelhack, R. ... and Brockmöller, J. (2005). Impact of the CYP2D6 ultra-rapid metabolizer genotype on doxepin pharmacokinetics and serotonin in platelets. Pharmacogenetics and genomics, 15(8), 579-587.

20. Klupp, N. L., Chang, D., Hawke, F., Kiat, H., Cao, H., Grant, S. J. and Bensoussan, A. (2015). *Ganoderma lucidum* mushroom for the treatment of cardiovascular risk factors. Cochrane Database of Systematic Reviews, (2).

21. Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6), 1547-1549.

22. Liu, J., Tamura, S., Kurashiki, K., Shimizu, K., Noda, K., Konishi, F. ... and Kondo, R. (2009). Anti-Androgen Effects of Extracts and Compounds from *Ganoderma lucidum*. Chemistry & biodiversity, 6(2), 231-243.

23. Ma, B., Ren, W., Zhou, Y., Ma, J., Ruan, Y. and Wen, C. N. (2011). Triterpenoids from the spores of *Ganoderma lucidum*. North American journal of medical sciences, 3(11), 495.

24. Obodai, M., Narh Mensah, D. L., Fernandes, Â., Kortei, N. K., Dzomeku, M., Teegarden, M. and Ferreira, I. C. (2017). Chemical characterization and antioxidant potential of wild *Ganoderma* species from Ghana. Molecules, 22(2), 196.

25. Otto, E. C., Blanchette, R. A., Held, B. W., Barnes, C. W. and Obodai, M. (2016). *Ganoderma mbrekobenum*. Pers. Mol. Phylogeny Evol. Fungi, 36: 416–417.

26. Oyetayo, V.O. and Yao, Y. J. (2010). Identification of Wild *Ganoderma* species indigenous to Nigeria using ITS region of the DNA. Niger J Econ Soc Stud, 24(1):2140-2144.

27. Püchler, K., Laeis, P. and Stumpe, K. O. (2001). Blood pressure response, but not adverse event incidence, correlates with dose of angiotensin II antagonist. 19(Suppl. 1): S41-8.

28. Radwan, F. F., Perez, J. M. and Haque, A. (2011). Apoptotic and immune restoration effects of ganoderic acids define a new prospective for complementary treatment of cancer. Journal of clinical & cellular immunology, 004.

29. Rai, R. D. (2008). Cultivation of Reishi Mushroom (*Ganoderma lucidum*). Folder. National Research Centre for Mushroom (ICAR), Chambaghat, Solan.

30. Remuzzi, G., Macia, M. and Ruggenenti, P. (2006). Prevention and treatment of diabetic renal disease in type 2 diabetes: the BENEDICT study. Journal of the American Society of Nephrology, 17(4 suppl 2), S90-S97.

31. Seeherman, R. (1957). Isopropamide iodide: a long-acting anticholinergic. Delaware medical journal, 29(10), 265-269.

32. Sharma, C., Bhardwaj, N., Sharma, A., Tuli, H. S., Katyal, P., Beniwal, V. ... and Sharma, A. K. (2019). Bioactive metabolites of *Ganoderma lucidum*: Factors, mechanism and broad-spectrum therapeutic potential. Journal of Herbal Medicine, 100268.

33. Sibai B. M. (1996) Treatment of hypertension in pregnant women The New England Journal of Medicine. 335:257-65.

34. Strair, R. K., Schaar, D., Goodell, L., Aisner, J., Chin, K. V., Eid, J. and Rabson, A. B. (2002). Administration of a phorbol ester to patients with hematological malignancies: preliminary results from a phase I clinical trial of 12-O-tetradecanoylphorbol-13-acetate. Clinical cancer research, 8(8), 2512-2518.

35. Thompson, J. D., Higgins, D. G. and Gibson. T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res, 22:4673-4680.

36. Wachtel-Galor, S., Yuen, J., Buswell, J. A. and Benzie, I. F. (2011). *Ganoderma lucidum* (Lingzhi or Reishi). In Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. CRC Press/Taylor & Francis.

37. Zhang, Q., Zuo, F., Nakamura, N., Ma, C. M. and Hattori, M. (2009). Metabolism and pharmacokinetics in rats of ganoderiol F, a highly cytotoxic and antitumor triterpene from *Ganoderma lucidum*. Journal of natural medicines, 63(3), 304-310.