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GANODERMA ISOLATES FROM THE LOWER VOLTA RIVER BASIN OF GHANA: MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS, METABOLOMICS AND BIOLOGICAL ACTIVITY EVALUATION

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In Partial Fulfillment of the requirement for the Degree

of Doctor of Philosophy (PhD) in Biochemistry

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DECLARATION

I hereby declare that except for references to other people's work, this exercise is a result of my own research, and this thesis, either in whole, or in part has not been presented for another degree.

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DEDICATION

To my mother, Felicia Adzovi Bokor,

my father, Adzorlolo Awuku Adotey

And the entire Adotey Family

for

All your love and care

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ABBREVIATIONS

ACEI	angiotensin-converting enzyme inhibitors
ADP	Adenosine diphosphate
AMA	amanitin
AMEA	antibiotic malt extract agar
AP-1	activator protein-1
APBP	acidic protein bound polysaccharide
ARB	angiotensin receptor blockers
ARS	Apostles Revelation Society
Bax	Bcl-2-associated X
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
CM-GL	carboxymethylated *G. lucidum*
Covid-19	Coronavirus disease 2019
COX-2	cyclooxygenase-2
CREB	cyclic element-binding protein
D1	domain 1
D2	domain 2
D3	domain 3
DCs	dendritic cells
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
DWV	deformed wing virus
EI-MS	Electron Ionization-Mass Spectrometry
ELISA	enzyme-linked immunosorbent assay
EPS	exopolysaccharide
ESI	Electrospray Ionization
EV71	Enterovirus 71
FIP -LZ-8	fungal immunomodulatory proteins-ling-zhi-8
FIP-gap1	fungal immunomodulatory proteins *G. applanatum* 1
FIP-gap2	fungal immunomodulatory proteins *G. applanatum* 2
FIP-gsi,	fungal immunomodulatory proteins-*G. sinense*
FIP-gts	fungal immunomodulatory proteins-*G. tsugae*
FIPs	fungal immunomodulatory proteins
FIPs	fungal immunomodulatory proteins
G0	gap 0
GA-Me	ganoderic acid Me
G-CSF	granulocyte colony-stimulating factor
GLIS	*G. lucidum* immunomodulating substance
GLPS	*G. lucidum* polysaccharides
GLPss58	*G. lucidum* polysaccharides sulfated 58
GLTs	*G. lucidum* triterpenoids
GM-CSF	granulocyte-macrophage colony-stimulating factor
GMI	*G. microsporum*
GPS	*Ganoderma* polysaccharides
GSPS	*G. sinense* polysaccharide

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GTs	*Ganoderma* triterpenoids
Hep G2	human hepatocellular carcinoma
HL-60	human promyelocytic leukemia cells
HMG-CoA	β-Hydroxy β-methylglutaryl-CoA
HPBLs	human peripheral blood lymphocytes
HPLC	high performance liquid chromatography
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
IC50	half maximal inhibitory concentration
IFN-1	interferon-1
IFN-α	interferon-alpha
IFN-γ	interferon-gamma
IL-17A	interleukin-17A
IL-17F	interleukin-17F
IL-1β	interleukin-1 β
IL-2	interleukin-2
IL-22	interleukin-22
IL-23	interleukin-23
IL-6	interleukin-6
ITS	internal transcribed spacer
ITS	internal transcribed spacer 1
ITS1	internal transcribed spacer 3
ITS2	internal transcribed spacer 2
ITS4	internal transcribed spacer 4
kDa	kilodalton
LC-MS	Liquid chromatography–mass spectrometry
LPS	lipopolysaccharide
LSU	large subunit
LSV	Lake Sinai virus
LVRB-1	Lower Volta River Basin -1
LVRB-14	Lower Volta River Basin -14
LVRB-16	Lower Volta River Basin -16
LVRB-17	Lower Volta River Basin -17
LVRB-2	Lower Volta River Basin -2
LVRB-9	Lower Volta River Basin -9
LZ-8	ling-zhi-8
LZP-F3	ling-zhi polysaccharide fraction 3
m/z	mass-to-charge ratio
MAPK	mitogen-activated protein kinase
MCF-7	human breast adenocarcinoma
M-CSF	macrophage colony-stimulating factor
MDA-MB-231	human breast cancer cells
MDR	multidrug resistance
MEA	malt extract agar
MHC	histocompatibility complex
MMP-9	matrix metalloproteinase-9
MTT	3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide
NCBI	National Center for Biotechnology Information
NF-κB)	nuclear factor-κB

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NK	natural killer
nLSU	nuclear large subunit
NMR	Nuclear magnetic resonance
NO	nitric oxide
NOS	nitric oxide synthase
nSSU	nuclear small subunit
PAI-1	plasminogen activator inhibitor-1
PBS	phosphate buffered saline
PCR	Polymerase Chain Reactions
pDC	plasmacytoid dendritic cell
PGE2	prostaglandin E 2
PI3K	phosphoinositide 3-kinase
PLS-DA	partial least squares-discriminate analysis
PMA	phorbol-12-myristate-13-acetate
rDNA	ribosomal deoxyribonucleic acid
rLZ-8	recombinant ling-zhi-8
RPMI-1640	Roswell Park Memorial Institute-1640
S-180	Sarcoma 180
SeGLP-2B-1	Se-enriched *G. lucidum* polysaccharide-2B-1
S-GL	sulfated *G. lucidium*
SI	selectivity index
Th1	T helper 1
TIC	total ion chromatograms
TNF-α	tumor necrosis factor alpha
tPA	tissue plasminogen activators
UC	urothelial carcinoma
uPA	urokinase-plasminogen activator)
vRNA	viral RNA

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ABSTRACT

Ganoderma, a cosmopolitan genus of polypore mushroom, is known to have a number of interesting medicinal properties. The Lower Volta River Basin is reportedly rich in several species of polypore mushrooms resembling *Ganoderma*. Despite the medicinal importance, the *Ganoderma* mushroom isolates obtained from this river basin have not been well studied. In this present research study, sequence analysis of the internal transcribed spacer 2 (ITS 2), complete internal transcribed spacer (ITS) and the nuclear large subunit (nLSU) was used to identify collected *Ganoderma* from this riverine Lower Volta Basin. Ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS) was used to study the chemical constituents of the mycelial biomass of these *Ganoderma* mushrooms and the effect of their extracts and fractions on the human carcinoma cell line PC-3 and two human lymphoma cell lines; Jurkat, derived from a T cell leukemia and plasmacytoid dendritic cell (pDC) derived from acute leukemia evaluated using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The result of the sequence analysis revealed that the *Ganoderma* sample designated *Ganoderma* sample 2 belongs *G. mbrekobenum* species whereas three of the *Ganoderma* muhrooms belong to the species *G. enigmaticum*. The sequence analysis further demonstrated that *Ganoderma* sample coded *Ganoderma* sample 17 belongs to the species *G. resinaceum* whereas the sample designated *Ganoderma* sample 9 belongs to *G. weberianum-sichuanese* species complex. Thus, the native *Ganoderma* mushrooms collected in the present study belong to four mushroom species, namely *G. mbrekobenum*, *G. enigmaticum*, *G. resinaceum and G. weberianum-sichuanese* species complex. The current data on molecular identity and phylogeny of *Ganoderma* mushrooms from Ghana would be helpful in future studies relating to molecular evolution and medical implications of *Ganoderma* isolates from different regions of Ghana and other part of the world. The total ion chromatogram (TIC) data demonstrated an interesting metabolic profile difference, suggesting UPLC-Q-TOF-MS could be used to differentiate between Lower Volta River Basin *Ganoderma* isolates based on their mass spectra. The PLS-DA score plot of the mycelial biomass was separated into three distinct clusters, consistent with the phylogenetic analysis in the current study

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which showed that the *Ganoderma* mushrooms used in the current metabolomic study belong to three different species. UPLC-Q-TOF-MS analysis revealed the presence of six lanostane-triterpenoids in the mycelia biomas of three *Ganoderma* mushrooms. Ganoderenic acid A, Ganoderenic acid D, Ganoderic C6 and Ganoderic acid G were identified in the mycelia biomass by comparing their mass spectra with pure reference compounds. The remaining two (Ganoderenic acid K and Ganoderic acid AM1), due to absence of reference pure compounds, were annotated by comparing their mass spectra with *Ganoderma* lanostane triterpenoids previously reported in literature. The result of the biological activity evaluation showed the fraction **GL-C2** significantly ($\leq 0.05\%$) inhibited the proliferation and survival of the three cancer cell lines, PC-3, pDC and Jurkat with increasing concentrations and with IC_{50} values of 27.73±5.25, 21.31±2.40 and 17.09±0.86 µg/mL, respectively compared to Chang liver cells (CVCL_0238) with an IC_{50} value of 75.41±1.95 µg/mL. The study further demonstrated that the subfraction **GL-C2-C1** from **GL-C2** demonstrated a potent cytotoxic effect against PC-3 with IC_{50} value of 3.24± 0.10 µg/mL compared to curcumin with IC_{50} = 5.13± 0.86 µg/mL. This finding suggests that the subfraction **GL-C2-C1** could be an excellent candiadate for developing new treatment option for prostate cancer prevention or treatment. The results also revealed that the subfractions **GL-C2-C4** and **GL-C2-C5** potently inhibited the growth and survivl of pDC with IC_{50} values of 19.95±0.50 and 13.57±2.14 µg/mL, respectively, suggesting **GL-C2-C4** and **GL-C2-C5** may be useful in modulating the production of type I interferon (IFN-1) by suppressing the viability of pDCs and may thereby be useful in developing biopharmaceuticals for treating disorders associated with pDCs. Thus, the current findings demonstrated that specific mycelial fractions are selectively cytotoxic to the three human cancer cell lines suggesting their potential efficacy in the treatment of malignancies. Future study with other cancer cell lines, primary pDCs, T cells, B cell and macrophages as well as animal models is worthy of investigation. The isolation of the bioactive compounds in **GL-C2-C1** may lead to a novel bioactive compound that can be used in developing new treatment for prostate cancer whereas novel bioactive compounds from **GL-C2-C4** and **GL-C2-C5** may lead to a novel compound for developing new treatment for disorders associated with pDC.

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CHAPTER 1

INTRODUCTION

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1.1 *Ganoderma*: - The Ancient Biomedical Fungus

Medicinal mushrooms have been used for long because of their novel pharmaceutical attributes and have gained increasing attention in health research (Cheung *et al*., 2010; Zhang *et al*., 2016). Several medicinal mushroom species have been developed into dietary supplements for health maintenance or therapeutic "agents" for prevention or treatment of chronic disorders and neurodegenerative diseases (Zhang *et al*., 2016). In China, Japan and Korea, *Ganoderma* mushroom is one of such medicinal mushrooms that plays a major role in their traditionl medical system because of its health-promoting properties (Xu *et al*., 2011; Wachtel-Galor *et al*., 2011). In Namibia for example, *Ganoderma* mushrooms are burnt and the smoke is inhaled for relieving flu. This ancient biomedical fungus *Ganoderma* is regarded in some cultural practices as a symbol of 'longevity and immortality' (Wasser, 2005; Halpern, 2007; Lin, 2009). *Ganoderma* was, therefore, represented in different ancient Chinese art work most probably because of its medicinal and cultural importance (Wasser, 2005; Halpern, 2007). The inclusion in the Chinese pharmacopoeia dating several years back (Halpern, 2007; Chen *et al*., 2012) and recently in Herbal pharmacopoeia and therapeutic compendium of America (Upton, 2000) strongly highlighted the medicinal and economic importance of *Ganoderma* as a biomedical fungus. It has been reported that ancient Malaysian traditional healers cut *Ganoderma* mushrooms into pieces and worn round the neck of children in the form of strings for treating epilepsy (Tan, 2015). However, in modern traditional medicine *Ganoderma* mushrooms, have been cited for treating chronic fatigue syndrome, diabetes, hepatitis, lower cholesterol level, prevent formation of blood clot and tumor growth (Halpern, 2007).

1.2. *Ganoderma* Bioactive Metabolites

Modern biochemical studies revealed the mycelia, fruit bodies or spores of *Ganoderma* mushrooms are rich in diverse biologically active compounds, including polysaccharides, triterpenoids, proteins, steroids, sterols,

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nucleotides, fatty acids and vitamins. These biologically active compounds are reported to have a number of interesting biopharmaceutical properties (Radwan *et al*., 2011; Xu *et al*., 2011; Ahmad *et al*., 2018). *Ganoderma* is considered by many as a cell factory for producing pharmacologically active compounds. *Ganoderma* polysaccharides (GPS), for example, have been shown to activate a number of important immune cells in the body (Xu *et al*., 2011). The activated cells include T lymphocytes, macrophages and natural killer (NK) cells. Numerous authors reported that the activation of these cells by GPS leads to production of IL-6, IL-12 and IFN-γ in these cells. GPS are interestingly known to inhibit mast cells but activate lymphocytes and complement system (Min *et al*., 2001, Seo *et. al*., 2009).

Ganoderma triterpenoids (GTs), on the other hand, possess wide spectrum of biological activities. The most important biological activities of GTs include antitumour, antiviral, antihypertensive, antiangiogenic, immunomodulating, antihepatitis, antioxidant, anticomplement, and antimicrobial (Akihisa *et al*., 2007; Boh *et al*., 2007 and Xu *et al*., 2011). Dudhgaonkar *et al.* (2009) reported that GTs unlike GPS have been shown to remarkably suppressed the secretion of TNF-α, IL-6, NO and PGE(2)) from lipopolysaccharide (LPS)-stimulated murine RAW264.7 cells In another similar study, GLTs have been shown to suppress in RAW264.7 cells the expression of inducible nitric oxide synthase (NOS), the enzyme catalyzing the production of nitric oxide (NO) and cyclooxygenase 2 (COX-2), the enzyme catalyzing the synthesis of the proinflammatory mediators, prostaglandins (Bhardwaj *et al*., 2014). These authors observed that the antiinflammatory and antiproliferative actions of GLT on cells such as macrophages was mediated through the inhibition of NF-kappaB and activator protein-1 (AP-1) signaling pathways. Thus, while GPS activate secretion of inflammatory cytokines, GTs suppress the secretion of cytokines. The opposing role of GPS and GTs, with regard to secretion of inflammatory cytokines has, therefore, made *Ganoderma* mushroom an interesting biomedical fungus to study.

Numerous ergostane bioactive compounds with multifaceted biopharmacological activities have been isolated from *Ganoderma* mushrooms and they include antitumour (Chen *et al*., 2009; Cui *et al*., 2010), antiaging (Weng *et al*., 2010), antiinflammatory (Akihisa *et al*., 2007), anticomplement (Seo *et al*., 2009), and cholesterol lowering

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(Kim, 2010). B-sitosterol and its related glycosides is another important class of bioactive compounds found in *Ganoderma* mushrooms. B-sitosterol and its glycosides are known to have biological activity against breast, prostate, colon, lung, stomach and ovarian cancer cells (Bin *et al.*, 2015). Other interesting biological activities of β-sitosterol include mosquito larvicidal, trypanocidal, and neutralizing effect on viper and and cobra venom among others (Saeidnia *et al.*, 2014). Silva *et al.* (2003), in another interesting study, isolated two novel cerebrosides from some species of *Ganoderma* mushrooms and the isolated cerebrosides have been shown to have inhibitory effect against DNA polymerase, which may lead to cell death. In addition, several biologically active long chain fatty acids, including nonadecanoic, heptadecanoic and hexadecanoic acids with antitumour proliferation effect have been isolated from some species of *Ganoderma* mushrooms (Fukuzawa *et al.*, 2008; Gao *et al.*, 2012). Nucleosides and nucleobases are another interesting class of bioactive compounds isolated from some *Ganoderma* mushrooms (Gao *et al.*, 2007). Generally, nucleosides and nucleobases from different sources, including those from *Ganoderma* mushrooms, have been reported to modulate a number of human physiological processes (Jacobson *et al.*, 2002, Sánchez-Pozo *et al.*, 2002; Guo *et al.*, 2013), which include antiplatelet aggregation, antiarrhythmic and antiseizure effects (Schmidt *et al.*, 2000, Anfossi *et al.*, 2002 and Wang *et al.*, 2008). Similarly, proteins with remarkable immunomodulatory effects, for example, LZ-8, a 12 kDa protein, have been purified from the mycelium culture of *G. lucidum*Wu *et al.* (2008), indicating *G. lucidum*may be good candidate for the prevention or treatment of cancer and autoimmune diseases. Besides the above biologically active compounds, trace minerals, including selenium and germanium, known to have anticancer effect, have been reported from some *Ganoderma* mushroom species.

1.3. Health-Promoting Properties of *Ganoderma*

Sanodiya *et al.* (2009) and Ahmad *et al.* (2018) reported a wide range of striking therapeutic properties associated with *Ganoderma* mushrooms. These striking therapeutic properties include anticancer and mmunomodulatory

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among others (Ahmad *et al*., 2018) As a result of these striking medicinal properties mushrooms, belonging to the genus *Ganoderma*, are used in traditional medicine in many parts of the world (Radwan *et al*., 2011)

1.3.1. Anticancer and Immunomodulatory Activities of *Ganoderma*

Lin and others (2004) showed that polysaccharides from *Ganoderma* mushrooms can induce macrophages or T lymphocytes to secrete TNF-α and IFN-γ to suppress tumour cells growth by apoptosis. Although most of the immunomodulatory attributes of *Ganoderma* mushroom is credited to *Ganoderma* polysaccharides, recent studies have shown that methanol or ethanol soluble extracts, containing triterpenes from *Ganoderma*, can enhance expression of cellular immune activity and antigen processing and presentation (Radwan *et al*., 2011). For example, administration of ganoderic acid Me (GA-Me) has been shown to increase significantly NK cell activity and production of IL-2 and IFN-γ through upregulation of nuclear factor-κB (NF-κB) (Radwan *et al*., 2011). In another study, GA-Me has been shown to reverse the multidrug resistance (MDR) in colon cancer cells by inducing apoptosis through upregulation of p-p53, Bax, caspase-3, caspase-9 and downregulation of Bcl-2 (Jiang *et al.*, 2011). Similarly, ganoderic acid X, another lanostanoid triterpene, was shown to suppress topoisomerases and induce apoptosis in numerous tomour cell lines (Li *et al*., 2005). In a similar intriguing study, dried powder of *G. lucidum*, consisting of 13.5% polysaccharides and 6% triterpenes, was shown to suppres angiogenesis (Stanley *et al*., 2005). The finding suggested *G. lucidum* could inhibit prostate cancer and, therefore, may have use for reatment of angiogenesis-dependent prostate cancer (Stanley *et al*., 2005). These interesting anticancer and immune modulating activities may explain why *Ganoderma* polysaccharides and triterpenes are used as chemoimmunotherapeutic agents against some cancers.

1.4. *Ganoderma* and its Health Supplements

Health supplements derived from *Ganoderma* mushrooms come in diverse and various forms such as coffee, tea, capsules or tablets (Lai *et al*., 2004, Singh *et al*., 2013) and are formulated from intact fruiting bodies that processed

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into capsules or tablets (Wachtel-Galor *et al*., 2011). These health supplements have gained wide acceptance in developed countries such as China, Japan, USA (Paterson (2006) and several developing countries including Ghana although theirv effectiveness hass not be established in properly controlled clinical study. However, Wang *et al*. (2012) reported health supplements from *Ganoderma* are used for enhancing cellular immune response or treating diseases associated with immune disorders. Nevertheless, it takes a long time to produce *Ganoderma* mushroom fruiting bodies and the cultivation technique does not always guarantee production of standardized products. As a result, liquid or solid fermentation techniques have been introduced to produce *Ganoderma* health supplements to meet the increasing demand and ensure quality products throughout the year (Wachtel-Galor *et al*., 2011). In liquid or solid fermentation approach, *Ganoderma* health supplements are formulated from either (i) dried and powdered mycelia or (ii) pulverised mycelial and mushroom primordia combinations (Wachtel-Galor *et al*., 2011). Gano Ultra^{TM}, manufactured by Aloha Medicinals Inc, a U.S. biotechnology company, is one of such *Ganoderma* mycelial-based health supplements. This supplement, according to the manufacturer, is a combination product of the mycelium, primordia, fruiting body, spores, and extracellular compounds. (https://alohamedicinals.com, accessed 13 July 2019). However, other supplement manufacturing companies reportedly formulate their supplements from *Ganoderma* polysaccharides and triterpenoids extracted from the fruit bodies and mycelia and then tableted or encapsulated separately or integrated together in some proportions (Wachtel-Galor *et al*., 2011).

1.5. Identification of *Ganoderma* Species

In view of the growing interest in *Ganoderma* mushroom as health promoting biomedical fungus, it is very important to establish correctly the identity of the various species used in formulating health supplements. The key morphological features used traditionally in identification of *Ganoderma* mushroom species include (i) shape and size of basidiospores, (ii) inner core context color and consistency and (iii) microanatomy of fruit body pilear crust (Wachtel-Galor *et al*., 2011). However, these morphological features vary widely a result of differences in geographical locations, climatic conditions and natural genetic influences such as mutations and recombination of

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individual species. This has imposed serious limitation on *Ganoderma* identification (Hapuarachchi *et al*., 2018). Furthermore, fungal identification by traditional taxonomy is an experience-based science and most researchers in Africa lack expertise in this field. As a result, researchers have recommended nuclear based molecular approach as one of the most reliable methods for identifying fungi such as *Ganoderma* (Richter *et al.*, 2015; Welti *et al.*, 2015). The nuclear based molecular approach, which involves DNA sequence analysis, is one of the reliable practical methods for molecular identification of *Ganoderma* muhrooms. Recently, Ghana Food Research Institute collaborated with with University of Minnesota to establish the molecular identity of *Ganoderma* from Ghana using nuclear based molecular approach. This collaborative study led to naming of two *Ganoderma* species, *Ganoderma wiiroense* (Otto *et al*., 2015) from Sisala district of Northern Ghana and *Ganoderma mbrekobenum* from Brong Ahafo and Greater Accra Regions of Ghana (Otto *et al*., 2016). The Lower Volta River Bank (LVRB) of Ghana, undulating land covered with extensive water bodies and high vegetation, has a widespread distribution of polypore mushrooms resembling *Ganoderma* but no well-structured studies have been conducted on *Ganoderma* species from this part of Ghana. The extensive water bodies and high vegetation may explain why there is a widespread distribution of polypore mushrooms in the area.

1.6. Research Question

- What is the molecular identity of the *Ganoderma* mushrooms collected from the riverine Lower Volta Basin using sequence analysis of nuclear ribosomal regions (ITS2, ITS and LSU)?
- What are the major active components (lanostane triterpenoids) present in the mycelia biomass of these native *Ganoderma* mushrooms using LC-MS-MS metabolomic approach?
- How would the extracts and fractions of the mycelial biomass influence survival or vibility of human prostate carcinoma (PC-3), human T lymphoblastic leukemia (Jurkat) and human plasmacytoid dendritic cell (pDC) deived from acute leukemia, in comparison with Chang (normal) liver cell?

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1.7. Aims and Objectives

17.1. Aim of Study:

To study molecular identity, metabolomics and biological activity of polypore mushroom resembling *Ganoderma* collected in the present study from the Lower Volta River Basin

1.7.1.1 Specific Objectives:

The specific objectives of this study were to:

1.7.1.1.1 To establish molecular identity and phylogenetic position of *Ganoderma* mushrooms collected from LVRB by analyzing the nuclear ribosomal ITS2, ITS and LSU regions

1.7.1.1.2. To characterize the major active components (lanostane triterpenoids) present in the native *Ganoderma* mushroom from Lower Volta Basin by LC-MS-MS metabolomic analysis.

1.7.1.1.3 To elucidate the effect of extracts, fractions and subfractions of mycelial biomass on human prostatic tumor cell line (PC-3) and human lymphoma cell lines; Jurkat, derived from a T cell leukemia and plasmacytoid dendritic cell (pDC) derived from acute leukemia, in comparison with Chang liver cell (normal liver cell)

1.8. Justification for the Study

The study would help to establish the molecular identity and phylogenetic status of the collected *Ganoderma* mushrooms from the riverine Volta Basin of Ghana, which would be helpful in future studies relating to molecular evolution and medical implications of *Ganoderma* isolates from Ghana and other parts of the world. The UPLC-Q-TOF-MS-MS metabolomic study would not only help to clarify the differences and similarities in the metabolites present in the *Ganoderma* mushrooms from this area but would also provide insight into the major secondary metabolites (lanostane triterpenoids) produced by these local *Ganoderma* muhrooms. The evaluation

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of effect of extracts, fractions and subfractions of mycelial biomass on human carcinoma cell lines (PC-3) and two human lymphoma cell lines; Jurkat, derived from a T cell leukemia, and PMDC05, a plasmacytoid dendritic cell (pDC) derived from acute leukemia, may help to unlock biopharmaceutical potentials of native LVRB *Ganoderma* isolates

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CHAPTER 2

LITERATURE REVIEW

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2.1 *Ganoderma* and Historical Account

Li *et al*. (2014) described *Ganoderma* as a medicinally famous mushroom belonging to the family *Ganodermataceae*, order *Polyporales*, class *Agaricomycetes* and phylum *Basidiomycota* The name *gan* is a Latin word which mens "shiny" referring to the surface appearance and *derm*, which means "skin," denoting the glossy exterior and woody texture of this biomedical fungus (Wachtel-Galor *et al*., 2011). *Ganoderma* mushrooms has been classified into six main categories (red, black, yellow, white, blue and purple) based on the fruit body colour. However, at the macroscopic or morphological level, Zheng *et al*. (2007) reported *Ganoderma* mushrooms have been divided into two distinctive subgenera namely, *G. lucidum* complex and *G. applanatum* complex. While in Japan, the medicinal mushroom *Ganoderma* is called *Reishi*, which means 'divine mushroom' or *Mannetake*, meaning '10,000-year mushroom', in China, it is variously called by names, including *Ling Chu*, *Ling Chih* and *Ling Zhi*, denoting mushroom of immortality (Halpern, 2007). *Ganoderma* is known to have a reputation as a medicinal material and referred to as a "herb of spiritual potency," particularly in most Asian countries (Halpern, 2007). The consumption of this ancient medical fungus, prior to its artificial cultivation, was the preserve of only the rich and privileged in society (Wachtel-Galor *et al*., 2011). Currently, *Ganoderma* mushroom gained popularity as functional food supplement in China, Japan, USA (Lindequis *et al*., 2005) and now can be found in health shops in several other countries including Ghana. Although the medicinal attributes of *Ganoderma* is based largely on hearsay rather than hard facts, traditional uses as well as ritual and cultural practices, several modern biopharmacological studies, to some extent now, support many of the ancient therapeutic claims (Halpern, 2007, Wachtel-Galor *et al*., 2011, Radwan *et al*, 2015, Cheng *et al*., 2010, Jiang *et al*.2004).

Ganoderma has a long and memorable history. It has been reported that Taoist priests were the first to experience the medicinal effects of *Ganoderma* mushrooms. Halpern (2007) reported that Chinese Taoist priests were using *Ganoderma* to prepare special spiritual or magic food that would enable them to attain higher spiritual state. Professor Dr. Georges M. Halpern disclosed in his book "Healing Mushrooms" an interesting poem regarding the spiritual use of *Ganoderma* mushroom written by Wang Chung of the Han Dynasty as follows:

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“They dose themselves with the germ of gold and jade

And eat the finest fruit of the purple polypore fungus

By eating what is germinal, their bodies are lightened

And they are capable of spiritual transcendence”

The poem suggested the Chinese priests were using purple polypore fungus (purple *Ganoderma*) which might contain metabolites that were helping them to build energy, increase stamina and calm cells of the brain (Halpern, 2007). In the Chinese oldest *materia medica* called Herbal Classic, *Ganoderma* was assigned the first place in the superior grade ahead of ginseng, a popular Korean herbal drug. Halpern (2007) reported that *Ganoderma* muhrooms were attributed with numerous medicinal properties. These attributes include spleen tonifying, energy enhancing, strengthening of cardiac function, memory enhancing and anti-aging. The famous State Pharmacopoeia of China (2000) stated that *Ganoderma* mushrooms can relx the mind. This may explain why this medical fungus may be used to attain a higher state of consciousness during meditation.

2.2. *Ganoderma* Systematics and Phylogenetics

Richter *et al*. (2015) reported *Ganoderma* mushroom species in the world ranged from 250 to more than 400. The origin of *Ganoderma* is currently in doubt and several authors argued this biomedical fungus originates from the tropical regions, because they can survive under hot and humid conditions, before spreading to the temperate zones of the world (Pilotti *et al*., 2004; Jargalmaa *et al*., 2017). The reproductive structure of *Ganoderma*, which is the form that grows from a living or dead wood trunk which may influence the metabolites they are likely to produce, is characterized by a shiny pileus surface and a two-layered basidiospore wall with a truncated apex (Moncalvo, 2000). Although these structural features are used in traditional fungal systematics, they have very limited value for species identification.

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2.2.1. Traditional Systematics

Morphological features such as (i) basidiospore shape and size, (ii) context colour and consistency and (iii) microstructure of pilear crust seem to be more reliable for *Ganoderma* mushroom identification. However, it has been observed that a typical spore is similar for dozens of *Ganoderma* species. This has made accurate identification of *Ganoderma* based on basidiospore shape and size very difficult (Jargalmaa *et al*., 2017). Although colour and consistency of context and pilear crust microstructure are used by some researchers in *Ganoderma* mushroom identification, they are known to change with age and exposure to different environmental conditions, leading to variation in these macroscopic and microscopic features (Hong *et al*., 2001). As a result, identifying *Ganoderma* mushrooms based on macroscopic and microscopic features only is very difficult (Zheng *et al*., 2007). Although chlamydospore production and shape as well as optimum growth temperature are reported to be useful cultural characters for differentiating similar species, they have also been found to be limited in addressing phylogenetic relationships between taxa (Moncalvo 2000), thereby affecting the development of a natural classification system. As a result, the traditional taxonomic methods for identifying *Ganoderma* are not only confusing but inconclusive (Hong *et al*., 2002) and *Ganoderma* is said to be in a state of "taxonomic chaos" (Jargalmaa *et al*., 2017).

2.2.2. Molecular Systematics

Molecular systematics is now being considered as one of the most practical methods for identifying fungal species. This is because genetic composition of every fungus is not only unique but is also not affected by factors such as age and environmental conditions (Chan, 2003). Lee (2006) reported that molecular method for identifying fungal species has a number of advantages such as being simple, rapid, accurate and doe not require large amount of samples. Above all, the molecular method can be employed easily for *Ganoderma* mushroom samples in the powdered forms or drug formulation combintions (Lee, 2006). Although molecular systematic is a valuable tool

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for identifying fungal species, it does not directly reflect the pharmacological activity. Nevertheless, it can provide relevant information regarding pharmaceutical product quality necessary for public health protection.

Currently, a number of PCR based molecular markers are used in identifying various *Ganoderma* species. The most popular among these molecular markers include nuclear large subunit (Lee *et al*., 2006), nuclear small subunit (Latiffah *et al*., 2002), internal transcribed spacer (Moncalvo, 2000; Gottlieb *et al*., 2000; Latiffah *et al*., 2002) and some other specific genes (Zhou *et al*., 2008). Several authors including Kim and Lee, 2000; Park *et al*., 2000 and Chen *et al*., 2004 reported that the nLSU and nSSU genes are conserved at the genus or species levels. As a result, most researchers focussed rather on ITS for establishing the natural relationship of mushroom species such as *Ganoderma*. Hong *et al*. (2000) and Wesselink *et al*. (2002) interestingly established that the D1, D2 and D3 regions of the nLSU have enough divergency. These regions of the nLSU are, therefore, used in some studies for inferring phylogenetic relationships between fungal species Since the nLSU region has more diversity than the nSSU, it is used by some researchers alongside ITS (Hong *et al*., 2000; Wesselink *et al*., 2002) for establishing the natural relationship within *Ganoderma* species. Indeed, the ITS region has been shown to have greater sequence variation between closely related species and have a high rate of evolution for fungal species identification (Monard *et al*., 2013). Although the ITS has been used to characterize severl fungal species, it failed to reolve the molecular identity of some other fungal species (Gazis *et al*. 2011), therefore, calling for need for additional markers.

Recently, the internal transcribed spacer 2 (ITS2) region, for several reasons, has been proposed as one of the most suitable DNA barcodes for fungal species identification. Firstly, the ITS2 region is short and the sequences are easy to amplify with one pair of universal primers, secondly, the region has high inter-specific divergence and thirdly its identification accuracies have been shown to be very high (Chen *et al*., 2010). Although ITS2 has advantages over other nuclear genomic regions including the ITS, many researchers are not using this nuclear ribosoml region for identification of fungal species. This is partly because some past studies suggested that ITS1 and ITS display higher species diversity relative to ITS2 (Kress *et al*., 2005). Nevertheless, so far no universal

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primers for ITS1 and ITS have been developed for general taxonomic use, which has led to low DNA amplification, thus calling for need for specific PCR additives and conditions (Chase *et al*, 2007; Kress *et al*., 2007). Based on a recent available evidence and findings, some researchers indicated the ITS2 region could be used as a universal barcode for the identification, especially in closely related species (Müller *et al*., 2007 and Chen *et al*., 2010)

Although *Ganoderma lucidum* is the most cited *Ganoderma* species in scientific literature, current cumulative evidence suggests that many of the *Ganoderma* species have been cited wrongly (Moncalvo, 2005). In Ghana, most identified *Ganoderma* species lack supporting molecular data (Obodai *et al*., 2017); making phylogenetic position of *Ganoderma* isolates reported from Ghana previously published in research doubtful. This development calls for well-structured research into phylogenetic of *Ganoderma* from Ghana. This study would pave the way not only for monitoring but also managing diseases caused by *Ganoderma* mushrooms to cash crops such as cocoa, coffee, cashew in Ghana. This is achievable because the vegetative *Ganoderma* mycelia isolated, for example, from cash crops and the entire forest ecosystem could easily be identified using modern DNA molecular techniques.

Currently, molecular phylogenetic evidence available suggests Africa habours the highest diversity of *Ganoderma* mushrooms. However, many of these *Ganoderma* mushroom from Africa have not been reported in published research and the few reported *Ganoderma* mushroom species were identified based on morphological features (Moncalvo, 2005). Considering the difficulties associated with *Ganoderma* mushroom identification based on traditional taxonomic approach, the nuclear sequence-based method may be a more practical tool for identifying the poorly sampled different *Ganoderma* mushrooms from most African countries, including Ghana. In fact, this position is strongly supported by the current ease, low cost of PCR amplification and rapid expansion of molecular databases for *Ganoderma* at the GenBank.

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2.2.3. *Ganoderma* Phylogenetics

The systematics of the *Ganodermataceae* is known to have been carried out hundreds of years. *Ganodermataceae* family include *Ganoderma* with a laccate pileal surface and truncated basidiospores and *Amauroderma* with globose to ellipsoid basidiospores without a truncated apex (Sun *et al*., 2022). Although *Ganoderma* mushrooms can easily be recognized in the field based on the macro-morphological character of the sporocarp, a number of researchers recommended that species discrimination should be supported with molecular phylogenetic analysis in order to attain a more stable taxonomic identification. This may explain why, in recent times, numerous studies have been carried on phylogenetic relationships of *Ganoderma* mushrooms from different geographical regions of the world. Moncalvo *et al*. (1995), for example, sequenced the 25S ribosomal RNA gene and the internal transcribed spacers of *Ganoderma* species. The results of the phylogenetic analysis showed tnucleotide sequences of the internal transcribed spacers could discriminate between most *Ganoderma* species except *G. tsugae* group which was misnamed. Based on combined data of the D2 region of the 25S ribosomal RNA gene and the internal transcribed spacers, the subgenus *Elfvingia* was shown to be monophyletic. Furthermore, the results of the phylogenetic analysis of the D2 region alone supported *Amauroderma* as a sister taxon of *Ganoderma*. Moncalvo *et al*. (1995), therefore, concluded that D2 region could be suitable for *Ganodermataceae* systematics at higher taxonomic levels.

In another interesting development, the nuclear ribosomal DNA ITS sequences were used to study the phylogenetic relationships between 34 *Ganoderma* isolates cultivated in China and the results of the study revealed the 34 isolates clustered into five distinct groups, namely the subgenus *Elfvingia*, the sect. *Phaeonema*, and three groups within the sect. *Ganoderma*. It was also observed in the study that 85.7% of the *Ganoderma* isolates formed a single group within the sect. *Ganoderma* (Su *et al*., 2007) and the genetic diversity between the subgenus *Elfvingia* and the sects *Phaeonema* and *Ganoderma* was to distinctly clear. This observation made the authors to conclude that phylogenetic analysis is a more effective and useful approach not only for studying the taxonomy of *Ganoderma* but also for establishing phylogenetic relationships within the genus, compared to methods based on morphological analysis (Su *et al*., 2007).

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In a recent field trip, an interesting *Ganoderma* specimen characterized by perennial, sessile fruiting body, fuscous to black pileal surface with laccate crust, was collected from South Africa (Xing *et al*., 2016). To establish the phylogenetic relationships, the nuclear internal transcribed spacer regions (ITS) and the translation elongation factor 1-α gene (EF1-α) sequences were analyzed. The results of the phylogenetic analysis based on combined ITS and EF1-α sequences showed the collected specimen clustered with *G. enigmaticum,* but forming a distinct lineage and, therefore, proposed as a new *G. aridicola* species within *G. lucidum* complex (Xing *et al*., 2016). In a similar study, seven specimens of *Ganoderma* were collected from Yunnan Province of China and the results of phylogenetic analysis of the internal transcribed spacer (ITS), translation elongation factor 1-α (TEF1-α) and the second subunit of RNA polymerase II (RPB2) sequences showed five out of the seven collections clustered together with high bootstrap support, forming a clade sister to *G. shanxiense.* The remaining two other collected specimens clustered with *G. aridicola, G. bambusicola*, *G. casuarinicola, G. calidohilum*, *G. enigmaticum* and *G. thailandicum*, but formed distinct lineages and, therefore, proposed as new *Ganoderma* species, namely *G. dianzhongense* and *G. esculentum*.

In a more recent study, Gunnels *et al*. (2020) amplified and sequenced the nuclear ribosomal internal transcribed spacer regions (ITS) of *Ganoderma* mushrooms commonly used in developing food supplements. The results of phylogenetic analyses of this interesting study revealed the presence of *G. lingzhi* DNA in all seven herbal supplements. The authors concluded that ITS-based phylogenetic analysis is a successful and cost-effective method for DNA-based species authentication of fungal and plant species that are otherwise difficult to identify by morphological or biochemical methods (Gunnels *et al*., 2020). In an earlier study, Liao *et al*. (2015) studied *Ganoderma* containing crude drugs, mycelia, spores, and authentic extracts and spore oils using DNA barcoding and the results revealed that *G. lucidum* cultivated in China was different from those cultivated in Europe. The study also revealed that *G. lucidum* and *G. sinense* clustered into two clades that were separated from the other *Ganoderma* species, strongly supporting the hypothesis that *G. lucidum* species originating from Europe and East Asia are not the same species. By comparing the ITS2 sequences and RNA secondary structures, the fruiting bodies and commercial products of *G. lucidum* and *G. sinense* were successfully distinguished from those of other

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in this interesting study (Liao *et al*., 2015). The researchers concluded that DNA barcoding method is applicable to the authentication of commercial products containing *Ganoderma* species. In a more recent study, Zhang *et al* (2017) sequenced seven internal transcribed spacer (ITS) sequences of *Ganoderma lucidum* strains. Phylogenetic analysis of the ITS1 sequences differentiated the strains into three geographic groups while the ITS2 could only differentiate the strains into two groups. It was further observed that the secondary structures of the ITS1 sequences exhibited similar structures with a conserved central core and differed helices but the ITS2 sequences shared similar structures with the difference in helix 4. Thus, compared to ITS2 region, ITS1 region could differentiated *Ganoderma lucidum* into three geographic originations based on phylogenetic analysis and secondary structure prediction but it is not clear whether the ITS 1 would successfully delineate other *Ganoderma* mushroom strains at the intraspecific level.

Back in Africa, two *Ganoderma* species were collected from Ficus and Citrus trees from the North East Nile Delta, Egypt. To establish the taxonomic positions, phylogenetic analysis of the ribosomal 5.8S rRNA gene including the flanking internal transcribed spacers (ITS) was performed. The results of this study confirmed the status of the collected ganodeerma mushrooms as *G. resinaceum* EGM and *Ganoderma* sp EGDA (El-Fallal *et al*., 2015). In India, molecular taxonomy of *Ganoderma* was studied by analyzing the ITS rDNA sequences (Malarvizhi Kaliyaperumal, 2013) because the authors believed identification based on macro-microscopic features could lead to a large number of synonyms resulting in several taxonomic names. The authors found that the Indian isolate coded MYC1 as *Ganoderma cupreum* clustered with Malaysian and Australian 'cupreum'. This finding according to the author represented the first molecular evidence of *G. cupreum* from Asian origin. Kinge *et al*. (2012) studied the phylogenetic relationships among species of *Ganoderma* from Cameroon using molecular techniques. Analysis of the internal transcribed spacer and mitochondria small subunit of 28 isolates revealed the isolates belong to eight species, which only *G. ryvardense* was previously described from Cameroon while *G. cupreum* and *G. weberianum* are new records. The remaining five species did not match with any previously described species and have been designated as *Ganoderma* with different species affinities (Kinge *et al*., 2012). Recently, Du *et al*. (2023) performed a phylogenetic studies on the type materials of *G. sichuanense* (holotype, epitype, and topotype) and *G.*

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lingzhi (holotype) and found that *G. lucidum* is a name mistakenly applied to the widely cultivated *Ganoderma* species in China, that the scientific binomial for *Lingzhi* is *G. sichuanense* and *G. lingzhi* is the later synonym of *G. sichuanense*. In another novel phylogenetic studies on *Ganoderma*taceae using six gene loci including the internal transcribed spacer regions (ITS), the large subunit of nuclear ribosomal RNA gene (nLSU), the second largest subunit of RNA polymerase II gene (rpb2), the translation elongation factor 1-αgene (tef1), the small subunit mitochondrial rRNA gene (mtSSU) and the small subunit nuclear ribosomal RNA gene (nSSU), 14 genera, namely *Amauroderma, Amaurodermellus, Cristataspora, Foraminispora, Furtadoella, Ganoderma, Haddowia, Humphreya, Magoderna, NeoGanoderma, Sanguinoderma, SinoGanoderma, Tomophagus and Trachydermella* were confirmed (Sun *et al*., 2022). These authors recommended that a combined multi-gene dataset with ITS, nLSU, rpb2, tef1, rpb1 and tub is better for phylogenetic analyses of *Ganoderma*taceae since the internal transcribed spacer region (ITS) considered as the universal barcode of fungi may be limited in identifying complex groups or potential new species.

2.3. Bioactive Molecules of *Ganoderma*

Boh *et al*. (2007) reported that the main bioactive metabolites responsible for these pharmacological activities of *Ganoderma* mushrooms include triterpenoids, polysaccharides, steroids, proteins, fatty acids, amino acids, nucleosides and alkaloids.

2.3.1 *Ganoderma* Polysaccharides

Huie and Di (2004) and Wasser (2010) reported that over 200 polysaccharides have been isolated from *Ganoderma* mushrooms. It has been reported by several authors that the *Ganoderma* polysaccharides (**Figure 1**) belong to three main classes, namely β-D-glucans, heteropolysaccharides and glycoproteins (Wasser, 2011; Chang *et al*., 2012, Mizuno *et al*., 2013).

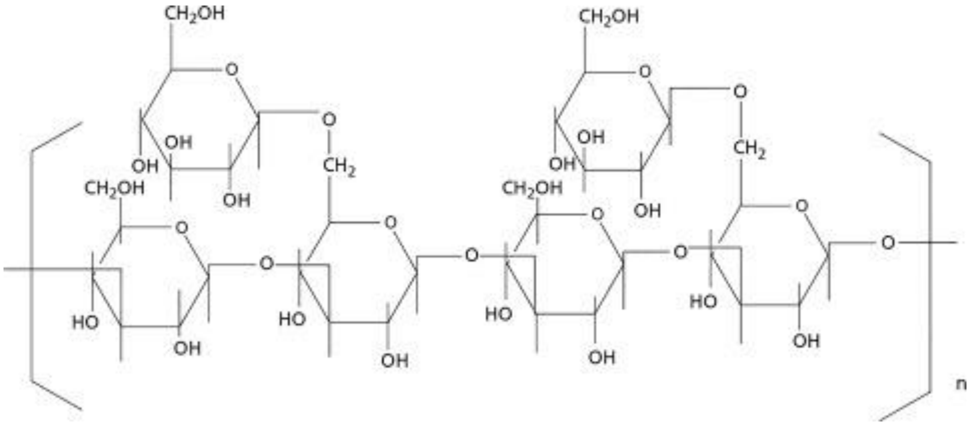

Figure 1. Typical *Ganoderma* polysaccharide structure (Zeng, *et al*., 2019)

2.3.1.1. *Ganoderma* β-D-glucans

Structurally, *Ganoderma* β-D-glucans contain β-(1→3) D-glucopyranosyl as main chain and glucosyl residue side chains at C-6 position of the main chain (Benkeblia, 2015). Several authors have documented that the degree of substitution of the backbone chain and the length of chain play a role in the biological activities of *Ganoderma* polysaccharides (Bao *et al*., 2002; Lin *et al*., 2005). Zhu *et al*. (2005), however, argued that biological activities of *Ganoderma* polysaccharides are rather dependent on the molecular mass, solubility in water and triple-helical structure formation. Other researchers, however, disagreed and insisted β -D-(1→3)-glucosidic linkage is the essential structural feature exclusively responsible for the biological activities of *Ganoderma* polysaccharides and this may explain why polysaccharides such as starch, consisting of α -D-(1→4)-glucosidic linkages so far, has no known biological activity. Although some authors reported helical structures are neither essential nor advantageous for biological activities, Bao *et al*. (2002) is of the view that triple-helical structure of β -D-1,3-linked glucans is favourable for T-lymphocyte proliferation.

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2.3.1.2. *Ganoderma* Heteropolysaccharides

Several heteropolysaccharides, containing different combinations of sugars, have been isolated from different species of *Ganoderma* mushrooms. Li *et al*. (2007), for example, isolated a polysaccharide, consisting of galactose, mannose, glucose, arabinose and rhamnose, from submerged mycelial culture of *Ganoderma lucidium*. Pana *et al*. (2012) isolated a neutral heteropolysaccharide, which consists of galactose, rhamnose and glucose in the molar ratio of 1.00:1.15:3.22. Wang *et al*. (2011) similarly isolated 5 water soluble heteropolysaccharides (GL-I to GL-V) from artificially cultured fruit body of the same mushroom species.

2.4. *Ganoderma* Proteins

2.4.1. *Ganoderma* Glycoproteins

In one interesting study, Wang *et al.* (2002) isolated a fucose-containing glycoprotein fraction which has the ability to stimulate proliferation of spleen cell and expression of cytokines, including IL-1, IL-2 and IFN-γ from water-soluble extract of *G. lucidum*. Wu and Wang (2009) in another study purified a water-soluble glycopeptide (PGY), consisting of two moieties of carbohydrate and peptide, from *G. lucidum* fruit bodies. The glycopeptide (PGY) has been shown to have low DPPH (1, 1diphenyl-2-picryl hydrazyl) radical-scavenging activity but exhibited strong superoxide radical-quenching effect, suggesting this glycopeptide may be a good of source of natural antioxidants.

2.4 2. *Ganoderma* Lectins

Thakur *et al*. (2007) isolated a 114 kDa hexametric lectin from the fruiting bodies of *G. lucidum*. Biochemical characterization revealed the lectin has neutral sugar (9.3%) by composition. This 114 kDa hexametric lectin has shown to have hemagglutinating activity against protease treated human erythrocytes. Girjal *et al* (2011), on the

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other hand, isolated a smaller (15 kDa) lectin, which has shown biological activity against a number of microorganisms.

2.4 3. *Ganoderma* Protease Inhibitors

Currently, there is a substantially great interest in protease inhibitors. Dunaevsky *et al*. (2013) reported protease inhibitors are becoming useful for treating diseases such as cancer, malaria, autoimmune and neurodegenerative diseases. El Zawawy *et al*. (2016) studied antiproteolytic effect of methanol extract of *G. lucidum* on *Pseudomonas aeruginosa*, host tissue damaging bacteria. The result revealed methanol extract of *G. lucidum* could serve as promising approach for treating skin burn infections caused by protease-producing extended spectrum β-lactamase and multidrug resistant *Pseudomonas aeruginosa* (ESβLMDRPA).

2.5. *Ganoderma* Triterpenes

The triterpenes derived from *Ganoderma* possess lanostane structure (Figure 2), consisting of 30 or 27 or 24 carbon atoms.

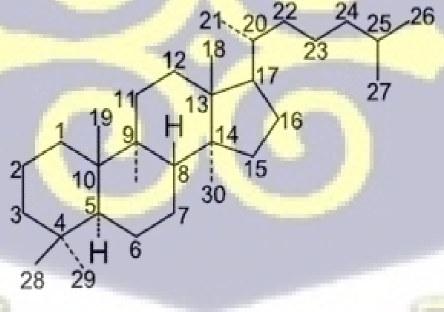

Figure 2. Typical lanostane structure (Xia et al., 2014)

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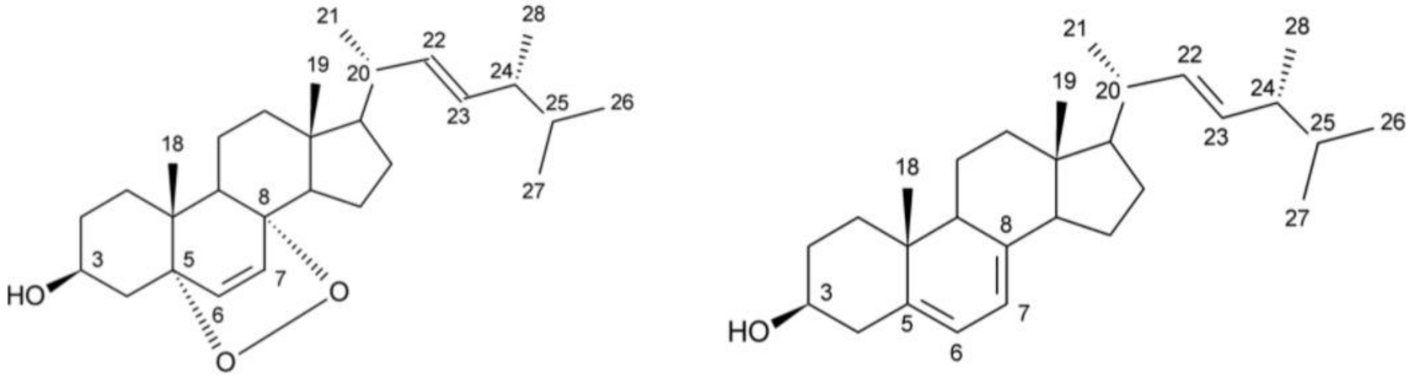
The majority of triterpenes derived from *Ganoderma* mushrooms possess a double bond at C-8 (9) on the ring, however, a double bond can also be found at C-7(8), C-9(11), C-20(22), C-22 (23) and C-24(25). In addition to a double bond, some of the triterpenes may have substituent groups at C-3, 7, 11, 12, 15, 22, 23, 24 and 25 positions of the parent nucleus (Xia *et al*., 2014). On the basis of position of double bond and substituent group, *Ganoderma* triterpenes can be classified into different structural types. These structural types include ganoderic acid, lucidenic acid, ganoderenic acids, ganoderiols, epoxyganoderiols and lucialdehydes among others.

Kubota *et al*. (1982) isolated the first two lanostane triterpenes, namely ganoderic acid B and ganoderic acid A. Nishitoba *et al*. (1984) two years after isolated lucidenic acids A, were B and C, possessing C27 carbon atoms, from cultured mycelium of *Ganoderma* mushrooms. One year later, another structurally similar group, ganoderenic acids A, B, C and D, were isolated *G. lucidum* Komoda *et al*., 1985). The same researchers isolated ganoderic acid E, F, G and lucidenic acid D2 from the same *Ganoderma* mushroom. Over time, several oxygenated compounds, including ganoderic acid C1 to O, P to T, U, W, X, Y, Z and lucidenic acids D1, D2, E1, E2, F, G to M, N, O, P, were isolated from the fruit bodies and cultured mycelia of *Ganoderma* mushrooms (Baby *et al*., 2015).

Several terpenes, including ganoderiol A, B, C, D, E, F, G, H, I and J as well as epoxyganoderiol A, B and C were isolated from *Ganoderma* mushrooms such as *G. lucidum*, *G. concinna*, *G. sinense*, *G. hainanense* and *G. amboinense* (Baby *et al*., 2015). Similarly, lucialdehydes A, B, C and lucidal D and E, possessing an aldehydic group in their side-chains, were isolated from *G. lucidum*, *G. pfeifferi* and *G. concinna* (Gao *et al*., 2002; Niedermeyer *et al*., 2005; Ma *et al*., 2012).

2.6. *Ganoderma* Steroids

A number of steroids have been isolated from *Ganoderma* mushrooms. Structurally, these *Ganoderma* steroids (**Figure 3**) have at least one double at C5–C6, C6–C7, and C7–C8, two double bonds at C5–C6/C7–C8, C4–C5/C7–C8, C6–C7/C9–C11, C7–C8/C16–C17 or three double bonds at C4–C5/C6–C7/C8–C14 in the ring systems. Although most of these *Ganoderma* steroids have C22–C23 double bonds in their side-chains, some have no side-chain double bonds (Baby *et al*., 2015).



(1) **(2)**

Figure 3. Examples of *Ganoderma* steroids: (1) ergosterol peroxide and (2) ergosterol (Seo *et al*., 2009)

The popular *Ganoderma* steroids include ergosterol, ergosterol peroxide and stella sterol (Seo *et al*., 2009) among several other steroidal compounds, including β-sitosterol (Joseph *et al*., 2011) and daucosterol (Lee *et al*., 2005).

2.7. *Ganoderma* Alkaloids

Zhao *et al*. (2015) isolated four novel polycyclic alkaloids (lucidimine A, B, C and D (**Figure 4)** from the methanol extract of *G. lucidum*fruit bodies.

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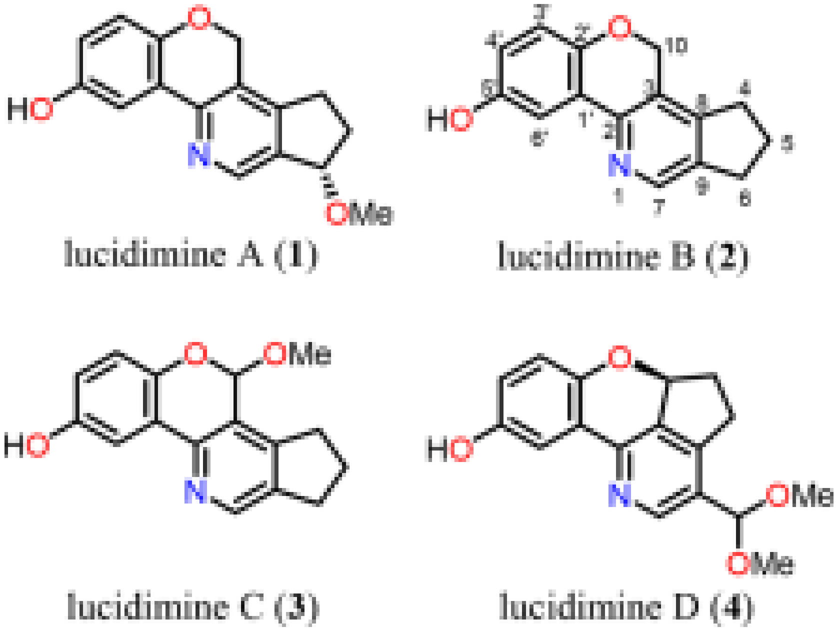

lucidimine A (1)

lucidimine B (2)

lucidimine C (3)

lucidimine D (4)

Figure 4. Structures of lucidimine A, B, C and D (Chen *et al*., 2018)

Huang *et al.* (2016) similarly isolated lucidimines A and B from *G. calidophilum*, but they were named as ganocalicines A and B.

2.8. *Ganoderma* Biopharmacological Activities

Numerous authors observed that a number of biopharmacological activities have been attributed to *Ganoderma* mushrooms. These include anticancer, immunomodulation, antiinflammatory, radioprotective, antiviral, antioxidative, cholesterol synthesis inhibitory, hypoglycemic, hepatoprotective, inhibition of lipid peroxidation/oxidative DNA damage, antimicrobial and anti-aging properties (Smith *et al*., 2002; Xia *et al*, 2014; Baby *et al.*, 2015; Kao *et al*., 2016)

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2.8.1. Anticancer Activities of *Ganoderma*

Normal cells are known to divide at a self regulated rate which controls their cell cycle. Nevertheless, when the controls fail, the cell cycle is deregulated and it causes abnormal cell reproduction, which eventually leads to cancer (Williams *et al*., 2012). Cancer has been recognized as a tremendous threat to human health in most nations of the world, including Ghana. As a result, several efforts have been directed to finding antitumour drugs with low toxicity but high efficacy. Several authors reported that *Ganoderma* species have inhibitory effect on a number of cancer cell lines (Silva, 2003; Xia *et al*., 2014). This may partly explain why *Ganoderma* mushrooms are used for treating cancers. The human cancer cell lines that *Ganoderma* mushrooms demonstrated biological activity against include prostate (Wang *et al*., 2015; Qu *et al*., 2017), breast (Rios-Fuller *et al*., 2018; Yang *et al*., 2018), leukemia (Müller *et al*., 2006, Calviño *et al*., 2010; Yang *et al*., 2016;), cervix (Liu *et al*., 2015), ovarian (Dai *et al*., 2014), colonic (Hong *et al*., 2004), bladder MTC-11 (Lu *et al*., 2004) and uroepithelial (Lu *et al*., 2004). Currently, the mechanism by which *Ganoderma* mushrooms exert anticancer activities is not completely understood. Wu *et al*. (2013), however, reported that anticancer activity of *Ganoderma* mushrooms is exerted, for exmples, through cell cycle arrest and programmed cell death among others. G. *lucidium*, for example, has been shown to induce cell cycle arrest in estrogen-independent breast cancer cells at G0/G1 phase (Jiang *et al*., 2006) and at G1 phase in lung cancer cells (Tang *et al*., 2006). Wachtel-Galor *et al* (2011) observed that *G. lucidum*ca used cell cycle arrest at G2 phase in human cancer cells such as bladder, prostate, and leukemia cells. In another study, Jiang *et al*. (2004) investigated the effects of *G. lucidum* extracts on apoptosis of human prostate cancer cells (PC-3). The results of this study revealed that the mushroom decreased the expression of NF-κB regulated B-cell lymphoma 2 (Bcl-2) and B-cell lymphoma-extra large (Bcl-xL), two important anti-apoptotic proteins that inhibit apoptosis and therefore play a critical role in cancer development and resistance to treatment. The result of this study further revealed that *G. lucidum* markedly unregulated expression of Bcl-2-associated X Protein (Bax), a proapoptotic protein, leading to the enhancement of the ratio of Bax/Bcl-2 and Bax/Bcl-xL, indicating *G. lucidum* hold promise as a natural anticancer source. Smina *et al*. (2017) reported that *Ganoderma* total triterpene can induce apoptosis

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in human breast adenocarcinoma (MCF-7) cells by down-regulating the levels of cyclin D1, Bcl-2, Bcl-xL but by up-regulating the levels of Bax and caspase-9.

In addition to the total triterpenes, some isolated triterpenes have shown significant cytotoxic effect against several carcinoma cell lines (Cheng *et al*., 2010). Radwan *et al* (2015), for example, reported that ganoderic acid A can induce apoptosis in lymphoma cells through caspase-3 and caspase-9. Ganoderic acid A has also been shown to enhance HLA-II mediated antigen presentation and $CD4^+$ T-cell recognition, indicating ganoderic acid A is a candidate for future drug design for the treatment of lymphoma. In another study, ganoderic acid DM demonstrated a number of interesting anticancer effects, including inhibition of cell proliferation, induction of DNA damage, cell cycle arrest at the G1 phase, and apoptosis in human breast cancer cells (Wu *et al*., 2012). Ganoderic acid DM, similarly, has been shown to improve $CD4^+$ T-cell recognition in melanoma cells (Hossain *et al.*, 2012). This indicates ganoderic acid DM has a chemo-immunotherapeutic potential for inducing a cross-talk between autophagy and apoptosis, as well as improving immune recognition for sustained melanoma tumor clearance (Hossain *et al.*, 2012). On the other hand, ganoderenic acid D has been shown to have cytotoxicity against human hepatocellular carcinoma (hep G2), colorectal adenocarcinoma (caco-2) and cervical adenocarcinoma cells growth (Ruan *et al*., 2014). Similarly. *Ganoderma*nontriol, a lanostanoid triterpene alcohol, has been shown to suppress the proliferation of human breast cancer cells MDA-MB-231 by expressing the regulatory protein CDC20, which is overexpressed in tumours compared to normal mammary epithelial cells from breast cancer patients (Jiang *et al*., 2011). *Ganoderma*nontriol has also been shown to inhibit breast cancer cell lines. The inhibition is chieved through urokinase-plasminogen activator (uPA suppression and expression of uPA receptor inhibitiob. This finding suggests *Ganoderma*nontriol as a natural agent has potential for breast cancer treatment (Jiang *et al*., 2011). In another recent investigation, a new triterpenoid (ethyl lucidenate A) obtained from *G. lucidum* ethyl acetate fraction has been shown to be cytotoxic human leukemia cells and Burkitt's lymphoma cells with IC_{50} values of 25.98 and 20.42 μg /mL, respectively. (Li *et al*., 2013). In one other interesting study, lucidenic acid B has been shown to suppress phorbol-12-myristate-13-acetate (PMA)-induced HepG2 cells by suppressing the matrix metalloproteinase (MMP)-9 activity in a dose-dependent manner at the transcriptional level. The study further

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revealed that lucidenic acid B inhibit nuclear factor-kappa B (NF-kB) and activator protein-1 (AP-1) DNA-binding effects of HepG2 liver cells, which lead to downreguling of Matrix metalloproteinase-9 (MMP-9) expression (Weng *et al*., 2008).

Besides triterpenes, *Ganoderma* polysaccharides have been shown to suppress tumorigenesis and tumor growth (Shang *et al*., 2011). The polysaccharide designated SeGLP-2B-1, a nutritionally available organic seleno-compound purified and characterized from *G. lucidum*, for example, has been shown to inhibit growth of the breast cancer cell line MCF-7 by disrupting the mitochondrial membrane potential, and increasing the activities of caspase-9, -3 and poly (ADP-ribose) polymerase (Shang *et al*., 2011). The combination of ling-zhi polysaccharide fraction 3 (LZP-F3) and arsenic trioxide have been shown to have a significant synergistic growth inhibition on human urothelial carcinoma (UC) cell and arsenic-resistant cell through proapoptotic pathway (Huang *et al*., 2010). Two water-soluble derivatives, sulfated and carboxymethylated *G. lucidum*polysaccharides, coded S-GL and CM-GL, have been demonstrated to inhibit proliferation of Sarcoma 180 (S-180) tumor cells with an IC_{50} value of 26 and 38 μg/mL, respectively. The *Ganoderma* polysaccharides S-GL and CM-GL have also been shown to inhibit the growth of S-180 solid tumors implanted in BALB/c mice, with low toxicity (Wang *et al*., 2009). The antitumor enhancement effects of S-GL and CM-GL indicate they could be developed into anticancer drugs.

2.8.2. Immunomodulatory Activities

The development of tumours is known to be supported by immune evasion. This is why natural bioactive compounds with immunomodulatory capabilities are critically needed against immune evasion by cancer cells. *Ganoderma* is reported by several researchers as one of the natural sources of immunomodulatory compounds. Lin *et al*. (2004) reported *G. lucidum* modulates the immune system through immune system enhancement. Liu *et al*. (2018) has demonstrated that administration of β-D-glucan in phosphate buffered saline (PBS) stimulates lymphocyte proliferation, promotes macrophages to form pseudopodia, and enhances the levels of inflammatory cytokines IL-6 and TNF-α. Structural analysis revealed that the β-D-glucan fractions with molecular weight higher

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than 1.82×10^6 g·mol^{-1} exhibit better activity in enhancing the release of inflammatory cytokines, suggesting the bioactivity of *Ganoderma* polysaccharides is largely influenced by molecular weight. Wang and others also investigated the effects of *G. atrum* polysaccharide (PSG-1) on dendritic cells (DCs) and found that PSG-1 induce activation and maturation of murine myeloid-derived DCs through mitogen-activated protein kinase (MAPK) pathways (Wang *et al*., 2017). In an earlier molecular mechanistic study, PSG-1 was found to induce TNF-α secretion through phosphoinositide 3-kinase (PI3K)/Akt, MAPK and NF-κB signaling pathways in RAW264.7 macrophage cells (Yu *et al*., 2012), thus providing a theoretical basis for the potential of PSG-1 as a novel immunomodulating agent.

Zhang *et al*. (2010) investigated the immunoactivity capacities of proteoglycan fraction (GLIS) of *Ganoderma* mushroom fruit body on spleen-derived B lymphocytes and bone marrow-derived macrophages. It was found in the study that GLIS exerted anticancer effect by increasing humoral and cellular immune responses. In an earlier study, GLIS treatment was found to enhance proliferation of bone marrow macrophages (BMMs), increase significantly nitric oxide (NO) production, induce cellular respiratory burst, and increase levels of interleukins (IL-1β), IL-6, IL-12p35, IL-12p40, IL-18, and TNF-α gene expression and levels of TNF-α, IL-1β, and IL-12 secretion.

Rubel *et al*. (2010) studied effect of *G. lucidum* supplemented diet, formulated from *Ganoderma* mycelium grown by solid-state culture on wheat grain, and found that the diet produced a significant decrease in T lymphocytes (CD3$^+$ and CD8$^+$) population in spleen cells for three months. Although, in the study, the IFN-γ concentration was significantly increased in both plasma and supernatant of the adherent peritoneal cell cultures from mice fed with this supplement, the adherent peritoneal cells showed a significant increase in IL-10 production, decrease in TNF-α production and decrease in nitric oxide production (Rubel *et al*., 2010). The study suggests that the *G, lucidum* mycelium supplemented diet used in the study did not only enhance immunity against cancer cells or pathogenic microorganisms, but also alleviated adverse effects associated with immune system dysfunction. Meng and his research team conducted a comparative tudy on immunomodulatory activity of polysaccharides from two official species of *Ganoderma:* namely *G. lucidum* and *G. sinense*. The finding revealed both GSPS and GLPS potently

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promote macrophage phagocytosis. The polysaccharides are also known to increase release of nitric oxide and cytokines such as IL-1α, IL-6, IL-1

Fungal immunomodulatory proteins, a special class of glycoproteins have been isolated from *Ganoderma* mushrooms. Four such glycoproteins, LZ-8 from *G. lucidum*, FIP-gts from *G. tsugae*, GMI from *G. microsporum* and FIP-gsi from *G. sinensis*, have been shown to have immunomodulatory and cancer prevention effects. LZ-8, the first FIPs isolated from *G. lucidium*, is regarded as a potential candidate for treating and preventing autoimmune diseases (Bao *et al*., 2018) while the second, FIP-gts, isolated from *G. tsugae*, have been shown to possess antitumor activity against lung and urothelial cancer cells (Bao *et al*., 2018). Hsin *et al*. (2016) investigated combination treatment of FIP-gts and chloroquine and found that chloroquine increased FIP-gts-induced cytotoxicity in parental and cisplatin-resistant urothelial cancer cell lines, showing the combination treatment may provide an intereting strategy for urothelial cancer treatment (Hsin *et al*., 2016).

Chen and others studied the antiinflammatory and neuroprotective effects of FIPs extracted from *G. microsporum* (GMI). These researchers found GMI reduced LPS/IFN-γ-induced inflammatory mediator production. Chen *et al*. (2018) reported antiinflammatory and neuroprotective effects of GMI was achieved through suppression of NO, TNF-α, IL-1β, and PGE2 production. The authors suggested GMI may have potential for treating neuroinflammation and neurodegenerative diseases such as Parkinson's, Alzheimer's and stroke. In a related study, Lu *et al*. (2018) demonstrated that GMI combined with chidamide induced apoptosis and suppressed distal tumor metastasis in melanoma cells, indicating the combination has potential as an immunotherapeutic adjuvant for metastatic melanoma.

2.8.3. Anti-Oxidative Activities

It is well known that reactive oxygen species or free radicals seriously harm cells in the body through oxidative processes. There is, therefore, a great interest in antioxidative molecules that could prevent or treat free radical

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and reactive oxygen species-mediated diseases. Rani *et al.* (2015) evaluated the free radical scavenging activity of aqueous and methanol extracts of *G. lucidum* fruiting bodies cultivated on bread fruit (*Artocarpus heterophyllus*). The study results revealed the methanol extract has stronger scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (DPPH) with IC_{50} value of 290 μg/ml and 2,2'-azino-bis (3-ethylbenzothiazoline- 6-sulphonic acid (ABTS), IC_{50} value of 580 μg/ml) compared to the substrate, whereas the aqueous extract had better scavenging activity for ferric reducing antioxidant power with IC_{50} value of 5 μg/ml. In a similar study, Wong *et al.* (2004) investigated the antioxidative effect of *G. lucidum* against ethanol-induced heart toxicity in the mouse model and found that *G. lucidum* exhibits a dose-dependent antioxidative effect on the mouse heart homogenate. These researchers attributed the observed antioxidative activity to the cardioprotective effect of *G. lucidum* and suggest the mushroom may be helpful in protecting the heart from superoxide induced damage (Wong *et al.* (2004). In another closely related study, Sun *et al.* (2004) studied antioxidant activity of *G. lucidum* peptide (GLP) using different oxidation systems and found that GLP has remarkable antioxidant activity in the rat liver tissue homogenates and mitochondrial membrane peroxidation systems and block hemolysis of rat red blood cells in a dose-dependent manner. On the basis of this result, Sun and his co-researchers suggested GLP could play an important role in the inhibition of lipid peroxidation in biological systems through its antioxidant and free radical scavenging activities (Sun *et al.*, 2004). Mahendran *et al.* (2012) studied the antioxidant capacity of crude exopolysaccharide (EPS) extracts of *Ganoderma lucidum* by hydrogen peroxide scavenging, 1-1-Diphemy1-2picryl-hydrazyl (DPPH) radical scavenging and ABTS (2,2 azino bis (3-etheylbenzothiazoline- 6-sulphonicacid) diammonium salt) assays and found that crude exopolysaccharides of the fruiting bodies of *G. lucidum* has potent antioxidant activity.

Park and Kim (2018) cultured *G. lucidum* mycelium on black bean (*Rhynchosia nulubilis*) to verify if the mycelium could be used as a functional health ingredient for formulation of dietary supplements. The biological activity of the mycelium ethanol extract was evaluated by DPPH and ABTS assays. The results revealed that *G. lucidum* mycelium cultivated on *Rhynchosia nulubilis* has significantly higher radical scavenging activity compared to only the ethanol extract from *Rhynchosia nulubilis* (Park and Kim, 2018). This finding suggests *G. lucidum* mycelium

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cultivated on *Rhynchosia nulubilis* can significantly enhance antioxidant activity better compared to raw *Rhynchosia nulubilis* which G. *lucidum* mycelium was not cultivated on.

In another study, the antioxidant activities of four polysaccharides obtained from fermented soybean curd residue by *G. lucidum* was investigated by hydroxyl radical, reducing power, DPPH free radical, chelating activity, ABTS radical-scavenging and SOD-like activity. The results showed four polysaccharides exhibit antioxidant activities in a concentration-dependent manner. Among the four polysaccharides, GLP-III and GLP-IV exhibited the higher scavenging effects on hydroxyl radicals, ABTS radical, DPPH free radical, and stronger reducing power and SOD-like activity than GLP-I and GLP-I. Shi *et al*. (2013), therefore, reported that GLP from *Ganoderma* fermented soybean could have applications in medical and food industries. Liu and others studied antioxidant activity of two low-molecular-weight polysaccharide, GLP(L)1 and GLP(L)2, from fruit body of *G. lucidum* and found that both GLP(L)1 and GLP(L)2 displayed antioxidant activities but GLP(L)1 was more effective in free radicals scavenging and Fe $(^{2+})$ chelating. These authors therefore concluded that these two low-molecular-weight polysaccharides may play an important role in the exploration of natural antioxidants in food industry and pharmaceuticals (Liu *et al*., 2010). Peng *et al*. (2016) in a phytochemical study isolated 8 aromatic terpenoids from fruiting bodies of *G. capense* and the isolated compounds showed potent the DPPH radical scavenging antioxidant activity with IC_{50} values ranging from 6.00±0.11 to 8.20±0.30μg/ml.

2.8.4. Anti-viral Activity

Several reports indicate *Ganoderma* produces different substances with demonstrable antiviral activity (Eo *et al.*, 2000). Recently, Stamets *et al.* (2018) demonstrated that cultured mycelium extracts of *G. resinaceum* reduce the levels of honey bee deformed wing virus (DWV) and Lake Sinai virus (LSV), in a dose-dependent manner, indicating honey bees may gain health benefits from this polypore mushroom because of its antimicrobial compounds. Enterovirus 71 (EV71), one of the main causative pathogens of hand, foot and mouth disease (HFMD), has emerged as a major concern among pediatric infectious diseases. Zhang *et al.* (2014) evaluated the

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antiviral activities of two *G. lucidum* triterpenoids (GLTs), lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy (GLTA) and ganoderic acid Y (GLTB), against Enterovirus 71 (EV71 and found *Ganoderma* triterpenoids prevent EV71 infection. In this interesting study, GLTA and GLTB also demonstrated the ability to significantly inhibit the replication of the viral RNA (vRNA) of EV71. The findings indicate that these two *G. lucidum* triterpenoids may be potential therapeutic agents to control and treat EV71 infection (Zhang *et al.*, 2014).

Niedermeyer *et al*. (2005) isolated three bioactive compounds (ganoderone A, lucialdehyde B and ergosta-7,22-dien-3beta-ol) from *G. pfeifferi* and reported that they have potent inhibitory activity against herpes simplex virus. In a similar study, Kim *et al*. (2000) showed that the antiviral activity of acidic protein bound polysaccharide (APBP) isolated from carpophores of *G. lucidum* in combination with IFN alpha showed more potent effect on herpes simplex virus than synergistic effects of APBP with IFN gamma, suggesting APBP may be good candidate for developing antiherpetic agent.

Several other substances isolated from *Ganoderma* mushrooms showed biological activity against influenza and HIV virus. El-Mekkawy *et al*. (1988), for example, reported that ganoderiol F and *Ganoderma*nontriol demonstrated activity against HIV while *Ganoderma*diol showed *in vitro* antiviral activity against influenza virus type A. Similarly, ganoderic acid GS-2, 20-hydroxylucidenic acid N, 20(21)-dehydrolucidenic acid N and ganoderiol F have been shown to have inhibitory properties against human immunodeficiency virus-1 protease (Sato *et al*., 2009).

2.8.5. Anti-inflammatory

Chen *et al*. (2019) investigated the anti-inflammatory activity of *G. lucidum* polysaccharides (GLPS) on carbon tetrachloride (CCl_4)-induced acute liver injury in mice and found GLPS have potential for the prevention and treatment of liver inflammation. In an earlier study, Chen *et al.* (2018) investigated the anti-inflammatory potential of fungal immunomodulatory protein extracted from *G. microsporum* (GMI) in an *in vitro* rodent model of primary

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neuron/glia cultures. The study results revealed that GMI has the ability to suppress NO, TNF-α, IL-1β, and PGE2 production, indicating GMI may have a potential towards the treatment of neuroinflammation, responsible for the pathogenesis of a number of neurodegenerative diseases. The anti-inflammatory activity evaluation of two new farnesyl phenolic compounds namely, ganoduriporols A and B from *G. duripora* showed the two compounds exhibit antiinflammatory effects in RAW 264.7 cells by inhibiting the production of TNF-α, IL-1β, IL-6 and PGE2 through the suppression of COX-2, MAPK and NF-κB signaling pathway in LPS-induced macrophage cells. These findings suggest the two new farnesyl phenolic compounds could serve as interesting antiinflammatory agents (Liu *et al*., 2018). In another development, Zhang *et al.* (2018) investigated the antiinflammatory properties of GLPss58, a sulfated polysaccharide from *G. lucidum* formed by chemical sulfation and found that GLPss58 inhibits L-selectin/sTyr-sLeX binding significantly, blocks binding of anti-l-selectin antibodies to L-selectin on the surface of human peripheral blood lymphocytes (HPBLs) and inhibit the secondary lymphoid tissue chemokine-induced chemotactic invasion of HPBLs. This study further revealed that GLPss58 has the ability to inhibit the complement system and cytokines mediated inflammation; suggesting GLPss58 is a favorable potential antiinflammatory agent (Zhang *et al*., 2018).

2.9. Principles and Theories of Metabolomics

2.9.1. What metabolomics?

Metabolomics refers to the systematic and comprehensive study of small molecules present in a biological system including cells, tissues, biological fluids and organisms at a specific point in time. Although the analysis of complete set of metabolites also known as metabolome has been present in biological research for decades, according to the *Patti et al*. (2012) the term “metabolomics” was only recently coined. As an emerging field of ‘omics’, metabolomics aims principally to compare the endogenous metabolites present in a biological system or a specific physiological state by applying a combination of analytical chemistry, bioinformatics, statistics and

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biochemistry; explaining why metabolomics is considered an interdisciplinary field of science (Bouhifd *et al*., 2013).

2.9.2. Metabolomics Analytical Tools

This interdisciplinary field of science is known to deal with quantitative measurement of thousands of metabolites with a wide range of chemical and physical properties usually from a small amount of biological materials. As a result, metabolomics requires highly analytical instruments. Over the past decades, numerous analytical instruments have been developed and applied for metabolomics analysis. Several authors reported that the two analytical instruments that meet the high requirements are nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Kim *et al*., 2011; Scalbert *et al*., 2009; Fuhrer *et al*., 2015). NMR, for example, is typically known as a fast and highly reproducible spectroscopic technique, which works by absorption and re-emission energy by atom nuclei as a result of variations in an external magnetic field (Bothwell and Griffin (2011). Alonso *et al*. (2015) explained that by applying magnetic field to a targeted atom nucleus, different types of spectral data are generated, which allows not only the quantification of the concentration of metabolites present but provides also information about its chemical structure. Mass spectrometry, on the other hand, is another sensitivity analytical technique, which acquires spectral data in the form of a mass-to-charge ratio (m/z) and a relative intensity of the measured compounds but the biological sample first needs to be ionized with a spectrometer to enable the resulting ionized compounds to generate different peak patterns, defining the fingerprint of the original molecule (Alonso *et al*., 2015). Several ionization and mass selection methods are currently available for MS spectrometry (El-Aneed *et al*., 2009). MS analysis is generally preceded by a separation step to reduce the high complexity of the biological sample and to allow analysis of different sets of metabolites at different times. Theodoridis *et al.* (2011) reported liquid and gas chromatography columns are the most commonly used separation techniques, which is based on the interaction of the different metabolites in the sample with the adsorbent materials inside the chromatographic column. This approach enables metabolites with different chemical properties to pass through

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the column at different amounts of time and the time that each metabolite requires, called retention time together with the m/z MS values is then used to generate the LC-MS or GC-MS spectral data.

2.9.3. Metabolomics Approaches and Application

In metabolomic studies, two high-throughput strategies, namely untargeted and targeted, are used. The untargeted, also known as top-down strategy is characterized by simultaneous measurement of a large number of metabolites from each sample, thereby avoids the need for a prior specific hypothesis on a particular set of metabolites. This results in analysis of global metabolomic profile of the sample and accounts for generation of large amounts of data that are not only characterized by its volume but also by its complexity. This explains why metabolomics requires high performance bioinformatic tools (Alonso *et al*., 2015). Considering its high-throughput and minimal sample requirements, untargeted metabolomics has wide applicability across a myriad of biological investigations and has been used to address a number of biomedical issues. An excellent example of biomedical applications in which untargeted metabolomics provided unique insight into is the identification of altered metabolic pathways in disease that represent novel drug targets, an evolving biomedical application referred to as “therapeutic metabolomics” (Rabinowitz *et al*., 2011). Another interesting example of this application is the discovery of increased levels of the metabolite 2-hydroxyglutarate in cancer cells with isocitrate dehydrogenase 1 mutations, which are a known common feature of a major subset of primary human brain cancers (Dang *et al*., 2009). Saghatelian *et al*. (2004) reported another interesting area in which untargeted metabolomics has been successfully applied is in characterizing gene and protein function. As highlighted by the above examples, untargeted metabolomics have implications for therapeutic screening and provides chemical insight across a broad area of mechanistic cell biology but its full potential to shape the understanding of global metabolism is yet to be realized.

Targeted metabolomics, on the other hand, is a hypothesis-driven method in which a specified list of metabolites is measured, typically focusing on one or more related pathways of interest (Dudley *et al*., 2010). This approach

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is reported to be effective for pharmacokinetic studies of drug metabolism as well as for measuring the influence of therapeutics or genetic modifications on a specific enzyme (Nicholson *et al*., 2002).

2.9.4. Statistical Analysis and Data Visualization

Since metabolomics experiments often generate large and complex data sets, a comprehensive evaluation of these outputs requires specialized data analysis such as cheminformatics, bioinformatics, and statistics (Al-Sulaiti *et al*., 2023). To successfully achieve this, data normalization is required to accurately quantify the features detected in the metabolomics analysis. By this appraoch, undesirable systematic biases and background signals will be minimized, resulting in a modified data set that highlights better the important metabolite differences (Considine *et al*., 2017).

Principal component analysis (PCA) is arguably the most widely used multivariate analysis method for metabolomic studies. The objective of PCA is to determine a linear transformation that preserves the variance in the original data as much as possible in the lower dimensionality output data (Jolliffe, 2002). Thus, PCA is used for an unsupervised evaluation to identify outliers and common sample clusters. As a result of the unsupervised nature of the PCA, its application only reveals group structure when within-group variation is sufficiently less than between-group variation. In view of this, supervised forms of discriminant analysis such as Partial Least Squares Discrimination Analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA) that rely on the class membership of each observation are commonly applied in metabolomics studies (Wold *et al*., 2001; [Barker *et al*., 2003). Although PCA, PLS-DA and OPLS-DA are often the methods used in multivariate analysis, they are by no means the only tools available of metabolomics studies. Hierarchical clustering analysis (HCA) and nearest-neighbor clustering may also be applied to multivariate spectral data to reveal differences between samples without supervision. In some other interesting studies, a new learning machine technique widely used in genome studies called support-vector machine (SVM) (Cortes and Vapnik, 1995) have been applied to human urine metabolomics NMR and was found to be superior PLS-DA in terms of both predictive accuracy and better predictive power compared to that of

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PLS-DA (Mahadevan *et al*., 2008). Artificial neural networks (ANN), another interesting pattern recognition method in metabolomics data mining, have also been used in combination with PCA for plant metabolic profiling and fingerprinting in order to method improve performance in manipulating metabolomics data Xia *et al*., 2007).

INTEGRI PROCEDAMUS

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CHAPTER 3

MATERIALS AND METHODS

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3.1. Chemicals and Reagents

Acetonitrile and formic acid were purchased from Sigma–Aldrich, MO (USA). Pure reference compounds of ganoderic acid C6, ganoderic acid G, ganoderenic acid A, ganoderenic acid D were obtained from China Yuanye Biotechnological Limited. Antibiotic malt extract agar (AMEA) and malt extract agar (MEA) without antibiotic were purchased from Fungi Perfecti (USA). The remaining items were purchased from the following organiztions: Ultra Clean Plant DNA Isolation Kit from MoBio Laboratories, California (USA), Gene Clean Spin kit from Qbiogene, Inc., California (USA), BigDye Terminator sequencing enzyme v.3.1 from Applied Biosystems, California (USA), PCR Master Mix from Promega Corp., Wisconsin (USA) and Montage PCR Centrifugal Filter Devices from Millipore Corp., MA (USA).

3.2. Molecular Identification and Phylogenetic Analysis

3.2.1. Origin, Collection and Sampling of Mushroom Fruit Bodies

Mushroom fruit bodies (basidiomata) resembling *Ganoderma* mushrooms were opportunistically collected from April to June, 2015 during a mycological surveys. Samples were collected from different locations (Agortigagorme, Azaglo Torkor, Kizito Campus, Lukunu and Degorme (**Figure** 2) within 2 km radius of Mepe in the North Tongu District of Ghana. The study site was approximately 110 km east of Accra, the capital of Ghana. The land is undulating and covered with rich vegetation and extensive water bodies. The collected basidiomata were carefully cleaned aseptically, transferred into paper bags and transported to Accra Technical University for fungal hyphae iissue isolation.

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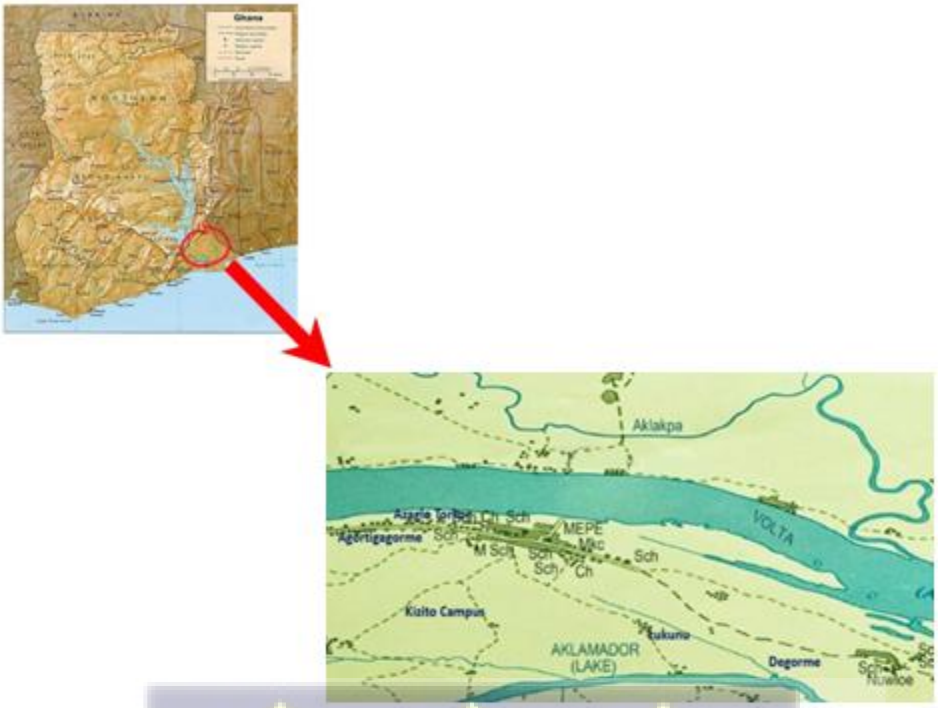

Figure 5. Locational map of sample collection site

3.2.2. Fungal Tissue Isolation

The collected fruit bodies were first surface cleaned, longitudinally cut with scalpel and pull apart to expose the inner core. A hyphae fragment piece taken from the inner core was placed on AMEA medium prepared in a petri dish per the manufacturer's instructions. The inoculated petri dish was cultured at 29-30°C for 14 days. The resultant pure mycelium was transferred to MEA prepared from 2 % w/v malt extract and 1.5 % w/v agar with no antibiotic and incubated for 14 days in the dark.

3.2.3. DNA Extraction

Genomic DNA extraction from pure *Ganoderma* mycelial culture was done by protocol of Aime and Mora (2005) with slight modification. Briefly, 2–4 mm of pure mycelium in a petri dish was carefully excised from actively growing zone of cultured fungal mycelium. Genomic DNA was extracted from this material with UltraClean Plant

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DNA Isolation Kit per the manufacturer's instructions and fungal pigments or polysaccharides co-extracted with the DNA smples were cleaned with the GeneClean Spin kit, according to the manufacturer's protocol (Qbiogene, Inc., Irvine, California).

3.2.4. Polymerase Chain Reactions (PCR)

ITS3 and ITS4 primers designed by White *et al*. (1990) were used for PCR amplification of the internal transcribed spacer 2 (ITS2) region for rapid identification of the collected *Ganoderma* mushroom samples. The amplification was performed per the protocol of Liao *et al*. (2015) with a slight modification using 25-µL reaction mixtures, containing 12.5 µL of 2 × PCR buffer, 1 µL of each PCR primer (2.5 µM), and 2 µL of DNA extract. The total volume was adjusted to 25 µL with sterile deionized water and PCR amplification was achieved with an initial denaturation step of 5 min at 94^{o} C; 35 cycles of 30 s at 94 C,45 s at 50^{o} C, and 1 min at 72^{o} C; and a final extension of 7 min at 72^{o} C.

ITS1-F and ITS4 primers designed by Gardes and Bruns (1993) were used to amplify the internal transcribed spacer (ITS) region. PCR amplification for ITS was performed by the protocol of Aime and Mora (2005) in 25 mL reaction volumes with 12.5 mL of PCR Master Mix (Promega Corp., Madison, Wisconsin), 1.25 mL each of 10 mM primers (upstream and downstream), and 10 mL of diluted (10- to 100-fold) DNA template. Cycle sequencing parameters consisted of a 2 min denaturation step at 94^{o} C, then 35 cycles at 94^{o} C for 39 s, 50^{o} C for 15 s and a final extension of 45s at 60^{o} C.

To further resolve the phylogenetic placement of the six isolates, the nLSU ribosomal region was amplified using the primer pairs LROR and LR6 according to the procedures outlined in Aime & Philips-Mora (2005). The PCR was performed in 25 µL reaction volumes same as described above for the ITS. LSU amplification was carried out under the same cycling program as for the ITS, except the cyclic extension step was increased to 4 mins All

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the PCR products were cleaned with Montage PCR Centrifugal Filter Devices (Millipore Corp., Billerica, Massachusetts).

3.2.5. Cycle Sequencing

Sequencing reactions were cleaned by ethanol precipitation and sequenced with BigDye Terminator sequencing enzyme v.3.1 (Applied Biosystems, Foster City, California) by following the method described by Aime and Mora (2005) in the reaction: 2 mL of diluted BigDye in a 1: 3 dilution of BigDye: dilution buffer (400 mM Tris pH8.0, 10 mM MgCl2); 0.3 mL of 10 mM primer; 10–20 ng of cleaned PCR template; and H2O to 5 mL total reaction volume. For ITS2 and ITS, the sequencing primers were the same as those used for PCR but for LSU, LR0R, LR3R, LR5, and LR16 (Moncalvo *et al*., 2000) were used as internal sequencing primers.

3.2.6. DNA Sequence Comparison

Preliminary moleculr identification of the collected *Ganoderma* mushrooms was done by Blastn search of the ITS2, ITS and nLSU sequences against sequences at GenBank of the National Center for Biotechnology Information (NCBI). DNA sequence of *Ganoderma* species sharing at least 98% nucleotide identity with those from *Ganoderma* mushroom isolates colleted in the present study were downloaded and used for the purpose of comparison and preliminary molecular identification.

3.2.7. Molecular Phylogenetic Analyses

Molecular phylogenetic trees were generated based on Bayesian inference (BI), a probabilistic method developed by Reverend Thomas Bayes based on Bayes' theorem. The sequence data set generated were aligned with Clustal X in Geneious Prime Version 2020.03 and refinements to the alignment executed manually. Bayesian inference

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(BI) analysis was conducted for the ITS2, ITS and nLSU using Mr. Bayes Geneious Prime Version 2020.03 with parameters set to 1,100 000 generations, two runs and four chains.

3.3. LC-MS Metabolomic Study of Cultured *Ganoderma* Mycelial Biomass

3.3.1. *Ganoderma* Samples for Metabolome Study

LC-MS-MS metabolomic analysis was used to characterize three of *Ganoderma* samples coded *Ganoderma* smple 1, *Ganoderma* smple 9 and *Ganoderma* smple 17. The three coded *Ganoderma* mushrooms were identified as *Ganoderma enigmaticum*, *Ganoderma weberianum-sichuanese* and *Ganoderma resinaceum* respectively in the molecular phylogenetic analysis in the present study. *Ganoderma* sample 2, *Ganoderma* sampe 14 and *Ganoderma* sample 16 were lost through microbial contamination and were excluded from the subsequent analysis.

3.3.2. Mycelia Biomass Production

Malt extrct agar (MEA) was prepared and 2 mm piece of fourteen (14) days cultured mycelium excised from pure actively growing zone of each ganoderm mushroom was inoculated in the prepared MEA. The inoculated MEA plate was cultured at 28-30ºC in the dark, 85-90% relative humidity. The mycelia biomass was harvested when pin heads started forming by scrapping from the agar surface for LC-MS analysis.

3.3.3. Sample Preparation for LC-MS Analysis

Mycelia biomass (0.02 mg) was macerated with 500 µL methanol containg 0.1% formic acid. The macerated sample was ultrasonicated for 48 h (100 Hz) at 25°C for 30 min, centrifuged for 5 min at 9838 x g at 4°C using Eppendorf 5430R. The supernatant filtered with sintered glass filter and of sample (5 µL) injected for LC-MS analysis. Equal volume of 100 µL of each mixed and used as quality control sample.

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3.3.4. LC-MS Analysis

The chromatographic separations were performed using Agilent 1290 series (Agilent Corp., Santa Clara, CA, USA) High Performance Liquid Chromatogrphy (HPLC) system. ODS 1.8 µm, 2.1 mm ×100 mm column from Waters, Ireland was used for the separations. The mobile phase A: 0.1 % formic acid in water and B: acetonitrile. The gradient elution was 25 % B at 0-2 min; 25-42 % of B at 2-20 min; 42-75% of B at 20-35 min; 75-95 % of B at 35-40 min; 95-25 % of B at 40-42 min. The flow rate for injection volume (5 µ) was 0.4 mL/min and the oven temperature was 40 °C. The column was equilibrated for 5 min using 25% of the mobile phase B before each injection. The column was equilibrated by injecteing three times quality control (QC) sample before and after each sample and reference compound injection. The separated components were detected with Q-TOF mass spectrometer from Agilent Corp., CA (USA) equipped with an electrospray ionization (ESI) interface. MS-MS was run in negative ion mode with m/z values ranging from 60-1000.

3.3.5. LC-MS Data Processing

LC-MS data obtained were changed to mzData format. R-Package XCMS was used to perform peak finding, filtering and alignment. PLS-DA and heatmap were performed with R software (R 3.6.1) in order to capture the differences between the mycelia biomass of three samples.

3.3.6. Identification of Metabolites

Chemical composition was determined by comparing fragmentation patterns and retention times of detected metabolites with reference pure compounds or reference to relevant published literature (Hennicke *et al*., 2016; Xin *et al*., 2018 and Yang *et al*., 2007).

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3.4. Evalution of Biological Activity

3.4.1. Cell Viability Inhibition

The effect of ethanol extract, fractions and subfractions of mycelial biomass of *Ganoderma* LVRB-9 on viability on the human carcinoma cell line PC-3 and two human lymphoma cell lines; Jurkat, derived from a T cell leukemia, and PMDC05, a plasmacytoid dendritic cell (pDC) derived from acute leukemia was test by MTT assay. TIC results from UPLC-Q-TOF-MS analysis showed that the the most prominent peaks are found *Ganoderma* LVRB-9. *Ganoderma* LVRB-9 was therefore selected for the biological activity evaluation.

3.4.2. Production of Mycelial Biomass

Malt extract agar solid medium was prepared in a microbox per the manufacturer's instruction (Combinessnv, Belgium) and a layer of sterile cellophane was carefully overlaid on the agar medium. A small piece of 10-days cultured mycelium of *Ganoderma* LVRB-9 was placed onto the agar medium overlaid with the layer of cellophane. The microbox was sealed and incubated at 28ºC and 90% of relative humidity of air until the fungal mycelial spread through the agar medium onto the overlaid cellophane. The fungal mycelial biomass was peeled from the cellophane layer 35 days later when the primordial heads started forming out of the mycelial biomass. The mycelial biomass was dried to <7% moisture at 50ºC with circulating air for 3 days and ground into fine powder using micropul model 3TH electrical mill (Aloha Medicinal, USA).

3.4.3. Mycelial Biomass Extraction

Three hundred (300) grams of *Ganoderma* LVRB-9 mycelial biomass was extracted three (3) times with 1.5L of absolute ethanol for six hours at 50ºC and filtered using a mixture of cotton and glass wool. The filtrates were

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combined and dried under vacuum with a Heidolph Rotary evaporator at 40ºC and 1 atmospheric pressure to obtain the crude ethanol extract (**GL-CO1**).

3.4.4. Column Chromatographic fractionation

Crude ethanol extract (**GL-CO1**) was subjected iilica gel column chromatography using differing mixtures of solvents of increasing polarity to obtained seven solvent fraction **GL-C1to C7.** **GL-C1** was eluted with 100 ml hexane/ethyl (90/10) mixture. **GL-C2** was eluted with 200 ml hexane/ethyl acetate (70/30) mixture while **GL-C3** was eluted with 400 ml hexane/ethyl acetate (40/60) mixture and **GL-C4** was eluted with 300 ml ethyl acetate/methanol (90/10) mixture. **GL-C5** was eluted with 200 ml ethyl acetate/methanol (50/50) mixture and **GL-C6** and **GL-C7**, were eluted with 300 ml methanol/ethyl acetate (80/20) and 200 ml of 100 % methanol respectively. The seven fractions concentrated under reduced pressure were transferred into 1.5 ml sample vial for bioactivity assays. **GL-C2** was found promising and, therefore, subjected refractiionation by silica gel column chromatography as described above to yield nine sub-fractions **GL-C2-C1** to **C9**. Further bioactivity analysis of **GL-C2-C1** to **C9**, revealed the **GL-C2-C1**, **GL-C2-C4** and **GL-C2-C5** were very interesting. The flow chart for the silica column chromatography purification of GL-ethanol extract is shown in Figure **6**.

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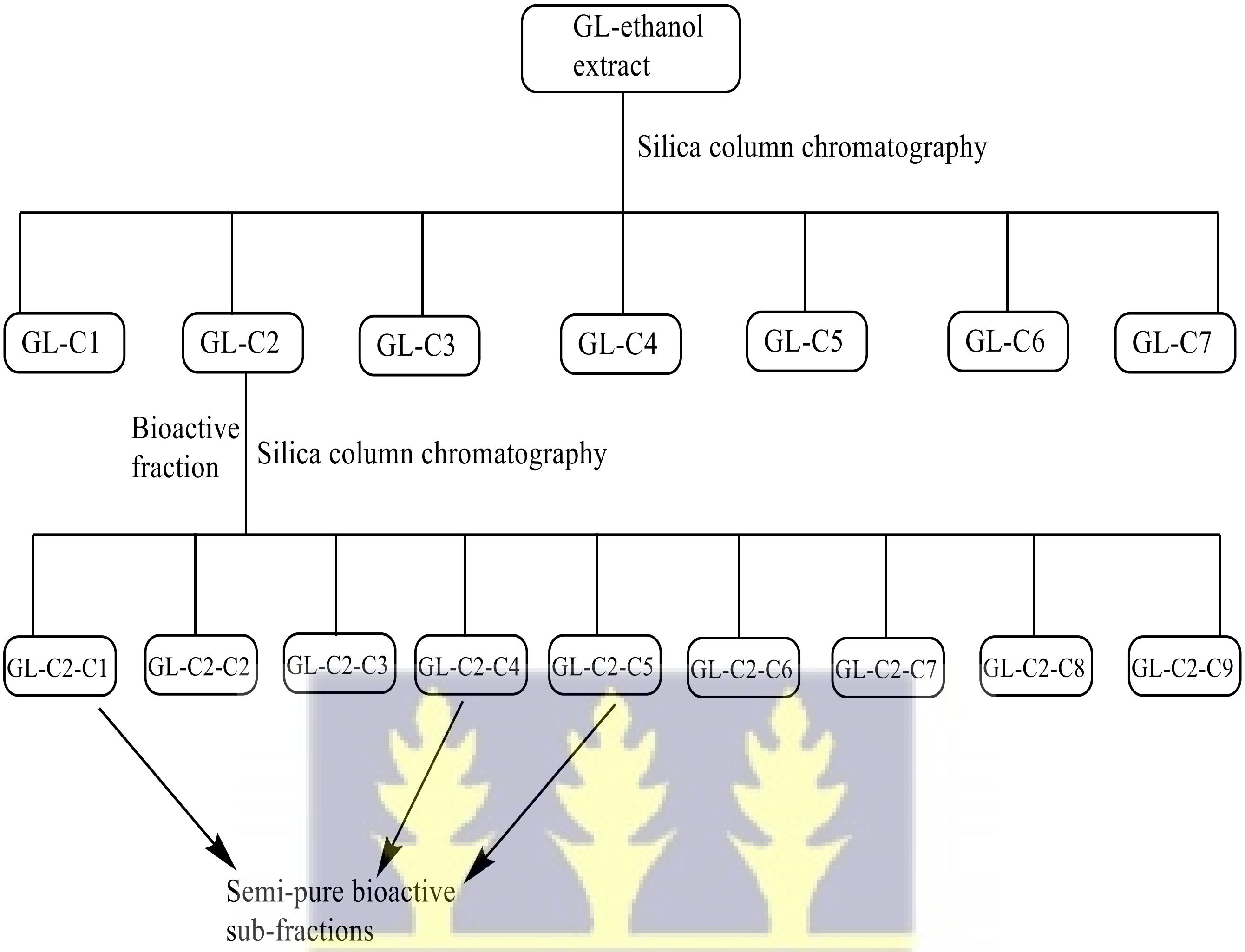

Figure 6. A flow chart of silica column chromatographic fractionation of *Ganoderma* LVRB-9ethanol extract.

3.4.5. In-vitro Cell Viability (MTT) Assay

3.4.5.1. Cell Cultures Used in the Study

Human prostatic tumor cell line (PC-3), T cell lymphoma line (Jurkat) and Chang liver cell (normal liver cell) used in this study were donated by Professor Itoh of Tokyo University of Japan. The specialized innate immune cell, plasmacytoid dendritic cell (pDC) was donated by Professor Vincent C. Lombardi, Department. The cells were

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maintained on RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 Ag/ml streptomycin in 25-cm^2 culture flasks at 37 ºC in humidified atmosphere with 5% CO_2.

3.4.5.2. Cell Viability (MTT) Assay

Cell viability of the crude ethanol extract, fractions and subfractions of the mycelial biomass was evaluated by 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, Jurkat, PC-3, pDC and Chang liver cells in their exponential growth stage were harvested and resuspended in fresh medium at a cell density of 1 x 10^5 cells/milliliter, 100 µL aliquots transferred into wells in 96-well plates and incubated for 24 hours in a CO_2 incubator with 5% CO_2 at 37 ºC. The 24-hour incubated cells were treated with various concentrations of either (i) crude mycelial biomass ethanol extract (**GL-C01**) (1000 µg/mL- 62.5 µg/mL) or (ii) fractions from the crude mycelial ethanol extract (**GL-C1** to **GL-C7**) (100 µg/mL-6.25 µg/mL) or (iii) subfractions of the crude mycelial biomass fraction (**GL-C2-C1** to **GL-C2-C9**) (100 µg/mL-6.25 µg/mL) or (iv) curcumin (2.3-0 36.80 µg/mL) as a positive control or media alone as negative control. The treated cells were incubated for 72 hours at 37 ºC with 5% CO_2 and 50 µl of PBS solution containing 1 mg/mL MTT was added to each well in the dark place, covered with aluminum foil. The media was removed and 100 µL of DMSO added to solubilize purple formazan crystals at room temperature in the dark and the optical density read at 570 nm. The experiment was performed in triplicate (n=3) to determine the percentage cell viability. The inhibitory concentration at fifty percent (IC_{50}) was determined and the selectivity index (SI), a ratio of the IC_{50} value of each sample in the normal cell line (Chang liver) to IC_{50} values of the other cells used in the study calculated.

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`CHAPTER 4

RESULTS

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4.1. Molecular Identification and Phylogenetic Analysis

4.1.1. Origin and Sampling of *Ganoderma* Isolates

Six fungal isolates resembling *Ganoderma* were opportunistically collected from different locations (Agortigagorme, Azaglo Torkor, Kizito Campus, Lukunu and Degorme) (**Figure 5**) during the rainy season of May–June 2015. Of the six collections, *Ganoderma* isolates LVRB-2 and LVRB-17 were found growing on dead *Acacia* trees (**Figure 7A and 7F**). Another two isolates coded *Ganoderma* LVRB-9 and *Ganoderma* LVRB-16 were found growing on *Mangifera indica* (**Figure 7C and 7E**). Out of the remaining two, one of the collections designated *Ganoderma* LVRB-1 was found growing on dead *Azadirachta indica* (**Figure 7A**), while the last collection designated *Ganoderma* LVRB-14 was found on *Baphia nitida* (**Figure 7D**). The upper hymenial surface of the fruiting bodies collected from *Mangifera indica* and *Acacia spp*. were reddish brown in colour (**Figure 7B, 7C, 7E and 7F**) while collections from *Azadirachta indica* and *Baphia nitida* were yellowish brown in colour (**Figure 7A, and 7D**). The *Ganoderma* sample LVRB-2 and *Ganoderma* sampleLVRB-17 from *Acacia* spp. showed single bracket growth pattern. The rest of the collections, however, exhibited storey growth pattern. As summarized, *Ganoderma* sample LVRB-14 and *Ganoderma* sample LVRB-16 were from Lukunu and one each was collected from Agortigagorme (Sample LVRB-1), Degorme (Sample LVRB-2), Kizito Campus (Sample LVRB-9) and Azaglo Torkor (Sample LVRB-17) (**Table 1**). The sample collection site has extensive water bodies (Volta River, Aklakpa River and Aklamador River) and undulating land is covered with luxuriant vegetation (**Figure 5**).

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Table 1. Collection details and morphological features of *Ganoderma* isolates

Sample code	Host tree species	Collection site	Collection Date	Color	Concentric zone growth	Growth pattern
LVRB-sp1	*Azadirachta indica*	Agortigagorme	2/5/2015	Yellowish brown	Fairly present	Storey
LVRB-sp2	*Acacia* spp.	Degorme	2/5/2015	Reddish brown	Absent	Single bracket
LVRB-sp9	*Mangifera indica*	Mepe Kizito	2/5/2015	Reddish Brown	Absent	Single bracket
LVRB-sp14	*Baphia nitida*	Lukunu	31/05/2015	Yellowish brown	Fairly present	Storey
LVRB-sp16	*Mangifera indica*	Lukunu	31/05/2015	Reddish Brown	Fairly present	Storey
LVRB-sp17	*Acacia spp.*	Azaglo Torkor	14/06/2015	Reddish Brown	Fairly present	Single bracket

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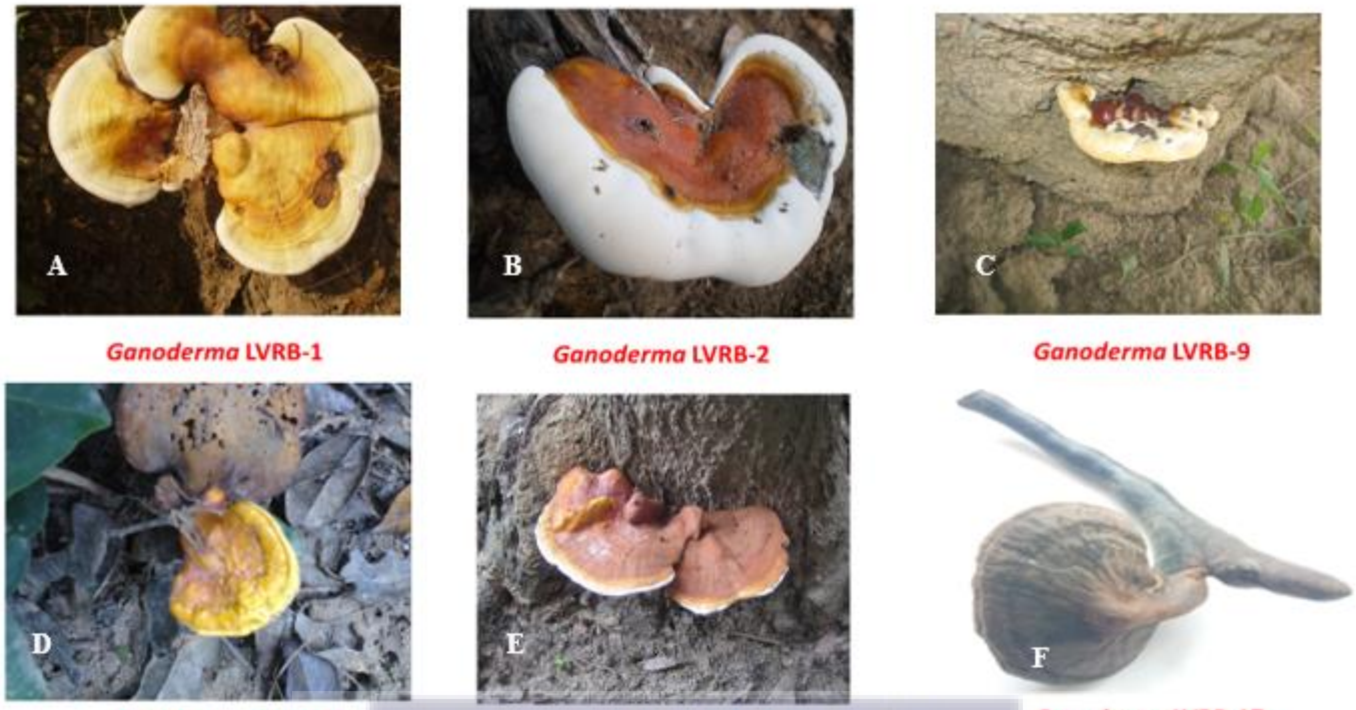

Ganoderma LVRB-1

Ganoderma LVRB-2

Ganoderma LVRB-9

Ganoderma LVRB-14

Ganoderma LVRB-16

Ganoderma LVRB-17

Figure 7. Collected *Ganoderma* specimen. **A**: *Ganoderma* isolate LVRB-1 growing on dead *Azadirachta indica* collected from Agortigagorme; **B**: *Ganoderma* isolate LVRB-2 growing on dead *Acacia* spp. collected from Degorme; **C**: *Ganoderma* isolate LVRB-9 growing at the base of *Mangifera indica* collected from Kizito Campus; **D**: *Ganoderma* isolate LVRB-14 growing on dead *Baphia nitida* collected from a farm in Lukunu; **E**: *Ganoderma* isolate LVRB-16 growing on at the base of *Mangifera indica* collected from Lukunu and **F**: *Ganoderma* isolate LVRB-17 collected from Azaglo Torkor.

4.1.2. Sequence Generation

Genomic DNA amplification and sequencing of the ITS2 region was successful for all the *Ganoderma* isolates (**Table 2**), except *Ganoderma* LVRB-9, which yielded poor-quality sequence data. The ITS2 sequence was also found not to be sufficient for resolving the phylogeny of *Ganoderma* isolates LVRB-2 and LVRB-17. As a result, the complete ITS region was sequenced for *Ganoderma* isolates LVRB-2, LVRB-17 and LVRB-9 (**Table 3**). The nLSU region was also amplified and sequenced for all the six *Ganoderma* mushrooms collected (**Table 4**).

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Table 2. ITS2 sequence matching results of *Ganoderma* isolatesLVRB-1, LVRB-2, LVRB-14, LVRB-16 and LVRB-17

Ganoderma isolates	DNA sequence	Highet matching sequence similarity of (%)
LVRB-1	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGT TTGAGTGTCATGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACT TGGAGGGCTTGTCGGCCTTTAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTT GCRGATCGGCTGTCGGTGTGATAAAATGTCTACGCCGTGACCGTGAAGCGTTTGGAT GAGCTTCcAACCGTCTTGsTTCAAAGACAACTTTttATGACCTCTGACCTCAAATCAGGT AGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma enigmaticum* voucher Ghana1a/938398 99.49
LVRB-2	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGT TTGAGTGTCATGAAATCTTCAACCTGCAAGCTTTTAATCGGTTTGTAGGCTTGGATTT GGAGGCTATTGTCGGCCTTTATCGGTCGGCTCCTCTTAAATGTATTAGCTTGGTTCCTT GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGCGTTTGGGCA AGCTTCTAACCGTCTCACTTTAGAGACAACCTTATGACCTCTGACCTCAAATCAGGTA GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma mbrekobenum* voucher UMN7-4 GHA 100.00
LVRB-14	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGT TTGAGTGTCATGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACT TGGAGGGCTTGTCGGCCWTTAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCT TGCGGATCGGCTGTCGGTGTGATAAAATGTCTACGCCGTGACCGTGAAGCGTTTGGAT GAGCTTCCAACCGTCTTGCTTCAAAGACAACTTTTTATGACCTCTGACCTCAAATCAG GTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma enigmaticum* voucher Ghana1a/938398 99.74
LVRB-16	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGT TTGAGTGTCATGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACT TGGAGGGCTTGTCGGCCWTTAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCT TGCGGATCGGCTGTCGGTGTGATAAAATGTCTACGCCGTGACCGTGAAGCGTTTGGAT GAGCTTCcAACCGTCTTGCTTcAAaGACAaCTTTttATGACCTCTGACCTCAAATCAGGTA GGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma enigmaticum* voucher Ghana1a/938398 99.74
LVRB-17	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTG AATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGT TTGAGTGTCATGAAATCTTCAACCTGCAAGCTTTTAATCGGTTTGTAGGCTTGGATTT GGAGGCTATTGTCGGCCTTTATCGGTCGGCTCCTCTTAAATGTATTAGCTTGGTTCCTT GCGGATCGGCTTGTCGGTGTGATAATGTCTACGCCGCGACCGTGAAGYGTTTGGGCA AGCTTCTAACCGTCTCACTTTAGAGCAACCTTATGACCTCTGACCTCAAATCAGGTAG GACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma mbrekobenum* voucher UMN7-4 99.48

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Table 3. ITS sequence matching results of *Ganoderma* isolate LVRB-2, LVRB-9 and LVRB-17

Ganoderma isolates	DNA sequence	Highet matching sequence similarity of (%)
LVRB-2	GTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAG TTTTGACTGGGTTGTAGCTGGCCTTACGAGGCATGTGCACGCCCTGCTCATCCGCT CTACACCTGTGCACTTACTGTGGGTTACAGACGGTGAAGCGGGCTTCTTACGGGG AGCTTGTGAAGCGTGTCTGTGCCTGCGTTTACCACAAACTCTTTAAAGTATTAGAA TGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTTGGCTCT CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCA GTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCAT	*Ganoderma mbrekobenum voucher UMN7-4 GHA* 99.49
LVRB-9	CATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTG CTCATCCACTCTACACCTGTGCACTTGCTGTGGGTTTCAAACGTCGTAAAGCGAGT CTCTTTACCGAGCTTGTAGAGCGGCGTCTGTGCCTGCGTTTATCACAAACTCTATA AAGTATTAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACG GATCTCTTGGCTCTCGCACCGATGAAGAACGCAGCGAAATGCGATAAAATGTGAA TTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATT CCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTACAGACCTTTGC	*Ganoderma weberianum* strain CBS 128581 98.72
LVRB-17	GTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAG TTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACACCCTGCTCATCCACT CTACACCTGTGCACTTACTGTGGGTTCCAGACGTTGTGAAGCGGGCTCTTTACGGA GCTTGTAAAGCGGCGTGCCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATTAG AATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGCTCTTGGCT CTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAG	*Ganoderma resinaceum* isolate F-2 99.48

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Table 4. nLSU sequence matching results of *Ganoderma* isolates LVRB-1, LVRB-2, LVRB-9, LVRB-14, LVRB-16 and LVRB-17

Ganoderma isolates	DNA sequence	Highet matching sequence similarity of (%)
LVRB-1	GAGCGGTCCAATCAAGCGACGGCTCGTTCTTACATATTTAAAGTTTGAGAATAGGTTAAGGTTGTTTCAACCCCAAGGCCTCTAATCATTCGCTTTACCACATAAATCTGATAATGAGTTTCTGCTATCCTGAGGGAAACTTCGGCAGGAACCAGCTACTAGATGGTTCGATTAGTCTTTCGCCCCTATACCCAAATTTGACGATCGATTTGCACGTCAGAATCGCTACGAGCCTCCACCAGAGTTTCCTCTGGCTTCACCCTATTCAGGCATAGTTCACCATCTTTCGGGTCCCAACATACATGCTCTACCGCGGATCCGTCAGAGAACGTCAGGTCCGGGCGTCGATGCCCCCCACGACAGGGGTCTCAACTTTCACTTTCATTACGCGCTCGGGTTTTCCACCCAAACACTCGCAGGTATGTTAGACTCCTTGGTCCGTGTTTCAAGACGGGTCGTTTAAAGCCATTATGCCAGCATCCTAAGCGCGAAAGTGGGATAAACCCCTGCCTTACGGCGCGCTGCGTTCCTCGATCCCAACCGCCGTATGCGACCAGAGTCTATAACACACCATAAGGTGCCACATTACTCCAGCCCTTTTCCGACGGTCAAAATCGATGCTGACCCGTCAATCCGGAAAGTGCACCAAGCAAAAAAGCAAGGCTGAGTTCCGGATGACGCGACTGACTTCAAGCGTTTCCCTTTCAGCAATTTCACGTACTGTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCCCTCACGGTACTTGTTCGCTATCGGTCTCTCGCCAATATTTAGCTTTAGATGGAATTCACCACCCATTTTGAGCTGCATTCCCAAACAACTCGACTCTTTGAGAGCGCATCACAAAGCACTGGTAGTCCGTGTCAAAGACGGGATTCTCACCCTCTATGACGCTCTGTTCCAAGAGACTTATACACGGTCCAGCGCGGAAAGCACTTCTCCAGACTACAACTCGGACGGCCAAAGACCGCCAGATTTTAAATTTGAGCTTTTCCCGCTTCACTCGCAGTTACTAGGGGAATC	*Ganoderma enigmaticum* CBS 139792 99.90
LVRB-2	TAAGCATATCAATAAGCGGAGGAAAAGAAACTAACAAGGATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTTGTCCGGAACTCAGCCTTGCTTTTGCTTGGTGCACTTTCCGGATGACGGGTCAGCATCGATTTTGACCGTCGGAAAAGGGCTAGAGTAATGTGGCACCTTCGGGTGTGTTATAGACTCTGGTCGCATACGGCGGTTGGGATCGAGGAACGCAGCGCGCCGTAAGGCAGGGGTTCTCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTAAACGACCCGTCT	*Ganoderma mbrekobenum* voucher UMN7-4 GHA 100.0
LVRB-9	ATCAAGCGACGGCTCGTTCTTACATATTTAAAGTTTGAGAATAGGTTAAGGTTGTTTCAACCCCAAGGCCTCTAATCATTCGCTTTACCACATAAATCTGATAATGAGTTTCTGCTATCCTGAGGGAAACTTCGGCAGGAACCAGCTACTAGATGGTTCGATTAGTCTTTCGCCCCTATACCCAAATTTGACGATCGATTTGCACGTCAGAATCGCTACGAGCCTCCACCAGAGTTTCCTCTGGCTTCACCCTATTCAGGCATAGTTCACCATCTTTCGGGTCCCAACATACATGCTCTACCGCGGATCCGTCAGAGAACGTCAGGTCCGGGCGTCGATGCTCCCCACGACAGGGATCTCAACTTTCACTTTCATTACGCGCTCGGGTTTACCACCCAAACACTCGCAGGTATGTTAGACTCCTTGGTCCGTGTTTCAAGACGGGTCGTTTAAAGCCATTATGCCAGCATCCTAAGCGCGAAAGTGGGCGAACCCCTGCCTTGCGGCGCGCTGCGTTCCTCGATCCCAACCGCCGTATGCGACCGGAGTCTATAACACACCCGGAGGTGCCACATTACTCCAGCCCTTTTCCGACGGTCAAAATCGATGCTGACCCGTCATCCGGAAAGTGCACCAAGCGAAAGCAAGGCTGAGTTCCGGACAACGCGACTGACTTCAAGCGTTTCCCTTTCAGCAATTTCACGTACTGTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCCCTCACGGTACTTGTTCGCTATCGGTCTCTCGCCAATATTTAGCTTTAGATGGAATTCACCACCCATTTTGAGCTGCATTCCCAAACAACTCGACTCTTTGAGAGCGCATCACAAAGCACTGGTAGTCCGTGTCAAAGACGGGATTCTCACCCTCTATGACGCTCTGTTCCAAGAGACTTATA	*Ganoderma pseudoferreum* strain CATAS-RRI-Gp-15 99.71

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	CACGGTCCAGCGCGGAAAGCACTTCTCCAGACTACAACTCGGACGGCCAAAGACCGCCAGATTTTAAATTTGAGCTTTTCCCGCTTCACTCGCAGTTACTAGGGGAATC	
LVRB-14	GATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCGTCCGGAACTCAGCCTTGCTTTTTTGCTTGGTGCACTTTCCGGATTGACGGGTCAGCATCGATTTTGACCGTCGGAAAAGGGCTGGAGTAATGTGGCACCTTATGGTGTGTTATAGACTCTGGTCGCATACGGCGGTTGGGATCGAGGAACGCAGCGCGCCGTAAGGCAGGGGTTTATCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTAAACGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATACCTGCGAGTGTTTGGGTGGAAAACCCGAGCGCGTAATGAAAGTGAAAGTTGAGACCCCTGTCGTGGGGGGCATCGACGCCCGGACCTGACGTTCTCTGACGGATCCGCGGTAGAGCATGTATGTTGGGA	*Ganoderma* enigmaticum voucher Ghana1a/938398 100.00
LVRB-16	GATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCGTCCGGAACTCAGCCTTGCTTTTTTGCTTGGTGCACTTTCCGGATTGACGGGTCAGCATCGATTTTGACCGTCGGAAAAGGGCTGGAGTAATGTGGCACCTTATGGTGTGTTATAGACTCTGGTCGCATACGGCGGTTGGGATCGAGGAACGCAGCGCGCCGTAAGGCAGGGGTTTATCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTAAACGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATACCTGCGAGTGTTTGGGTGGAAAACCCGAGCGCGTAATGAAAGTGAAAGTTGAGACCCCTGTCGTGGGGGGCATCGACGCCCGGACCTGACGTTCTCTGACGGATCCGCGGTAGAGCATGTATGTTGGGA	*Ganoderma enigmaticum* CBS 139792 99.71
LVRB-17	GATTCCCCTAGTAACTGCGAGTGAAGCGGGAAAAGCTCAAATTTAAAATCTGGCGGTCTTTGGCCGTCCGAGTTGTAGTCTGGAGAAGTGCTTTCCGCGCTGGACCGTGTATAAGTCTCTTGGAACAGAGCGTCATAGAGGGTGAGAATCCCGTCTTTGACACGGACTACCAGTGCTTTGTGATGCGCTCTCAAAGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTGAATTCCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGAACAAGTACCGTGAGGGAAAGATGAAAAGCACTTTGGAAAGAGAGTTAAACAGTACGTGAAATTGCTGAAAGGGAAACGCTTGAAGTCAGTCGCGTCGTCCGGAACTCAGCCTTGCTTTTTTGCTTGGTGCACTTTCCGGATTGACGGGTCAGCATCGATTTTGACCGTCGGAAAAGGGCTGGAGTAATGTGGCACCTTATGGTGTGTTATAGACTCTGGTCGCATACGGCGGTTGGGATCGAGGAACGCAGCGCGCCGTAAGGCAGGGGTTTATCCCACTTTCGCGCTTAGGATGCTGGCATAATGGCTTTAAACGACCCGTCTTGAAACACGGACCAAGGAGTCTAACATACCTGCGAGTGTTTGGGTGGAAAACCCGAGCGCGTAATGAAAGTGAAAGTTGAGACCCCTGTCGTGGGGGGCATCGACGCCCGGACCTGACGTTCTCTGACGGATCCGCGGTAGAGCATGTATGTTGGGA	*Ganoderma resinaceum* voucher LGAM 566 100.00

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4.1.3. DNA Sequence Comparisons by BLASTn

As indicated in **Table 2**, ITS2 BLASTn search revealed *Ganoderma* mushrooms LVRB-1, LVRB-14 and LVRB-16 displayed highest matching sequence similarity of 99.49%, 99.74% and 99.74% with *G. enigmaticum* (**Table 2**). Similarly, the isolates designated *Ganoderma* LVRB-2 and *Ganoderma* LVRB-17 showed the highest similarity of 100%, and 99.48 respectively with the species *G. mbrekobenum* (**Table 2***)*.

In the ITS BLASTn search (**Table 3**), *Ganoderma* LVRB-9 showed the highest similarity with *G. weberianum* (98.72%), whereas *Ganoderma* LVRB-2 displayed the highest similarity with *G. mbrekobenum* (99.47%). Interestingly, the isolate *Ganoderma* LVRB-17 showed the highest similarity with *G. resinaceum* (99.48%), contrary to the similarity with the species *G. mbrekobenum* observed earlier, suggesting *Ganoderma* LVRB-17 was misidentified in the ITS2 Blastn search.

BLASTn analysis of the nLSU sequence (**Table 4**) revealed high similarity with four different species of *Ganoderma*. *Ganoderma* LVRB-1 showed the highest sequence similarity with *G. enigmaticum* (99.90%) and the isolate designated *Ganoderma* LVRB-2 showed highest sequence similarity with *G. mbrekobenum* (100%). Ganoderrma LVRB-9 showed the highest similarity with *G. pseudoferreum* (99.71%) compared to G. *weberianum* 98.72 % in ITS analysis. *Ganoderma* LVRB-14 showed the highest similarity with *G. enigmaticum* (100.00%) and the isolate designated *Ganoderma* LVRB-16, showed the highest similarity with *G. enigmaticum* (99.71%). Finally, *Ganoderma* sample *LVRB*-17 showed the highest matching similarity with *G. resinaceum* (100%).

4.1.4. DNA Sequence and Data Sets for Phylogenetic Analysis

DNA sequences of *Ganoderma* species sharing at least 98% nucleotide identity with those from the Lower Volta River Basin of Ghana were downloaded and used for the purpose of comparison. The detailed information on the sequences, in this study is presented in **Table 5**.

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Table 3. ITS2, ITS and nLSU *Ganoderma* sequences from the Lower Volta River Basin of Ghana and GenBank accession numbers of other isolates used in this study.

Species	ID	Origin	GenBank Accession No.		
			ITS-2	ITS	LSU
G. oregonense	CBS 265.88	USA	JQ781875	JQ781875	
G. oregonense	340OR	USA	MG654189	MG654189	
G. oregonense	JLF1625	USA	MH277959	MH277959	
G. mbrekobenum	UMN7-4 GHA	Ghana	KX000898	KX000898	
G. mbrekobenum	MIN 850481	Ghana	NR_147647	NR_147647	
G. mbrekobenum	SSP:10	India	KY865253	KY865253	
G. mbrekobenum	SSPG5	India	MK940290		
G. mbrekobenum.	SSPG7 [a]	India	MK940289		
G. mbrekobenum	SSPG6 [a]	India	MK940286		
G. mbrekobenum	NGM	India	MH221092	MH221092	
Ganoderma sp.	LVRB17[a]	Ghana	This Study	This study	This study
G. resinaceum	GR-102	India	GU451247		
G. resinaceum	GR33	Egypt	KX428468		
G. resinaceum	GR-101	India	GU451246		
G. enigmaticum	Ghana1a/938398	Ghana	KR150678	KR150678	KR150679
G. enigmaticum	Ghana2/938397	Ghana	KR014265	KR014265	KR014266
G. enigmaticum	CBS 139792	South Africa	NR_132918		NG_058156
G. enigmaticum	Dai 15970	South Africa	KU572486	KU572486	
G. enigmaticum	Dai 15971	South Africa	KU572487	KU572487	
G. enigmaticum	I160004	South Africa	MK453308	MK453308	
Ganoderma sp.	LVRB1[a]	Ghana	This Study	This study	This study
Ganoderma sp.	LVRB16[a]	Ghana	This Study	This Study	This study
Ganoderma sp.	LVRB 9[a]	Ghana		This Study	This study
Trametes hirsuta	CLF6	India	MH091710	MH091710	
Trametes hirsuta	P8 s	Philippines	MF377416	MF377416	
G. weberianum	CBS 219_36	Philippines		MK603804	
G. weberianum	CBS 128581	Taiwan		MH864975	
G. weberianum	CBS 128581	Taiwan		MH864975	
G. lucidum	GL23 2	China		DQ424976	
G. sichuanense	G68	China		KX055552	
G. sichuanense	Cui7691	China		JQ781878	
G. sichuanense	HMAS42798	China		JQ781877	
G. lucidum	GL23 1	China		DQ424975	
G. resinaceum	ACAM 2013-0027	Greece		MG706235	
G. resinaceum	DP107	Italy		AM906064	

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G. lucidum	AP14	India	FJ463905	
G. resinaceum	LGAM 568	Greece	MG706255	
Ganoderma resinaceum	F-2	Italy	KJ509597	
Ganoderma resinaceum	F-1	France	JN588588	
Ganoderma resinaceum	DP2	Italy	AM906060	
Ganoderma pfeifferi	G2/11	Italy	AM269774	
Ganoderma resinaceum	LGAM 567	Greece	MG706254	MG706200
Ganoderma resinaceum	LGAM 462	Greece	MG706250	
G._enigmaticum	CMW50318	South Africa	MH571697	
G._enigmaticum	CBS 139792	South Africa	NR_132918	
G._enigmaticum	G-1	India	KU870313	
G. pseudoferreum	CATAS-RRI-Gp-21	China		KX454438
G. pseudoferreum	CATAS-RRI-Gp-18	China		KX454435
G. destructans	CBS 139793	South Africa		MH878652
G. destructans	CMW43671	South Africa		KR183861
G. austroafricanum	CBS 138724	South Africa		NG_064272
G. lingzhi	Dai 15799	China		KU220019
G. lingzhi	Dai 15798	China		KU220018
G. lucidum	Cui 9164	China		JN048793
G. lucidum	IUM01122	Korea		DQ208411
G. weberianum	CBS 128581	Taiwan		MH876427
G. weberianum	CBS 219.36	Philippines		MH867289
G. resinaceum	LGAM 566	Greece		MG706199
G. resinaceum	LGAM 486	Greece		MG706197
G. resinaceum	ACAM DD0863	Greece		MG706184
G. enigmaticum	CMW43669	South Africa		KR183859
Trametes hirsuta	CBS 282.73	Germany		MH872390
Trametes hirsuta_ _	*CBS 320.29*	*Canada*		MH866536

4.1.5. Phylogenetic Analysis

4.1.5.1. ITS2 Phylogenetic Analysis

Bayesian posterior probability (BPP) was conducted for all the successfully sequenced *Ganoderma* collections using MrBayes Geneious Prime Version 2020.03 and the resulting phylogenetic tree is presented in **Figure 8**. As shown in **Figure 8**, *Ganoderma* isolates LVRB-1, LVRB-14 and LVRB-16 formed a well-supported clade (BPP = 0.991) with *G. enigmaticum*, indicating they belong to the species *G. enigmaticum*. On the other hand,

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Ganoderma isolates LVRB-2 and LVRB-17 clustered with *G. mbrekobenum* with a very strong support (BPP = 1.00). The BPP analysis of ITS2 sequence suggests the *Ganoderma* collections belong to two distinct clades, G. *enigmaticum* and *G. mbrekobenum* (**Figure 8**).

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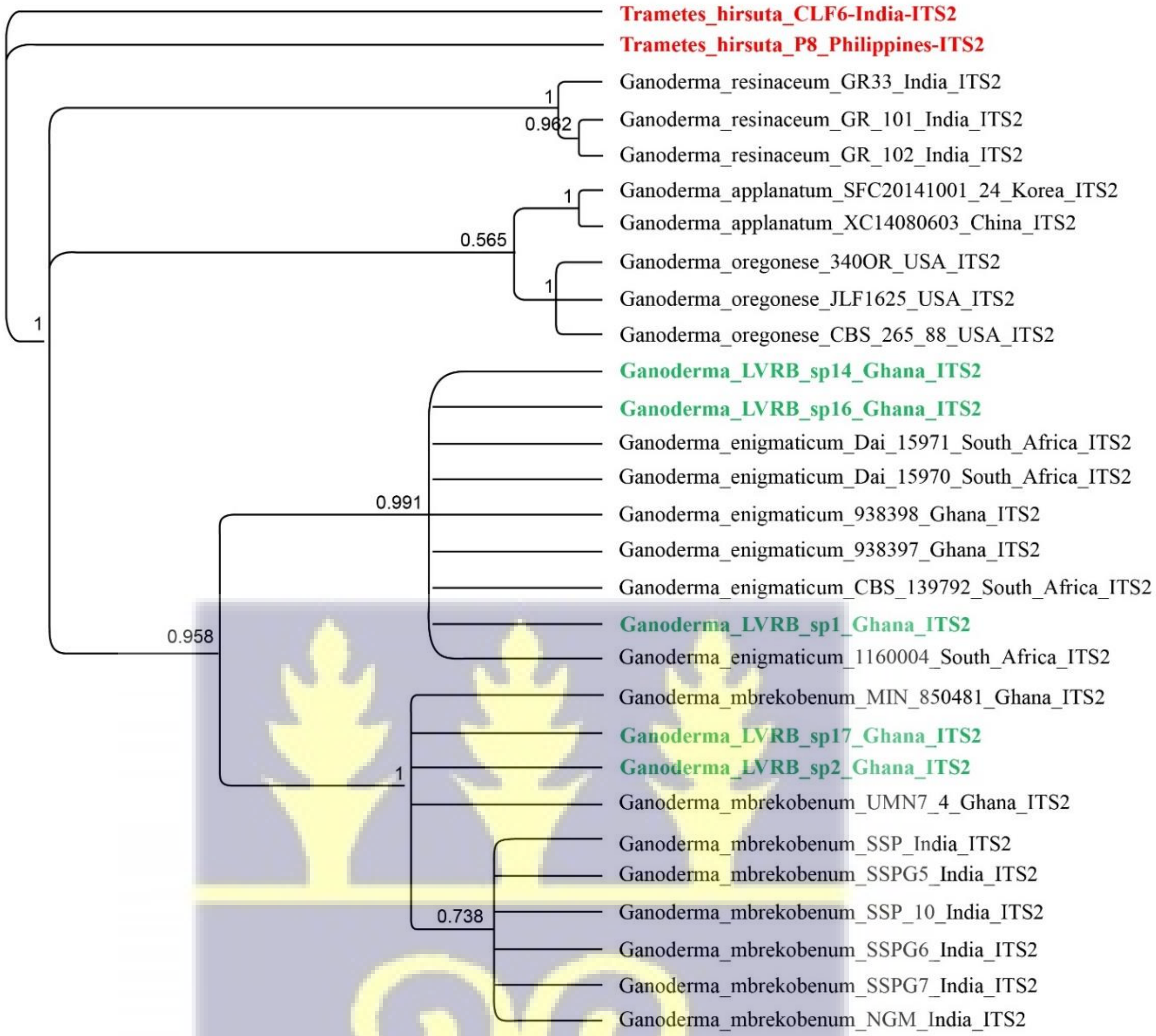

Figure 8. Bayesian tree showing position of collected *Ganoderma* compared to ITS2 sequences at GenBank. Branch node values represent Bayesin posterior probability (BPP). Tramete hirsuta from India and Phillipnes used as outgroups.

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4.1.5.1.2. ITS2 RNA Secondary Structure Analysis

The ITS2 sequences in the present study were subjected to RNA secondary structure analysis using ITS2 database (Keller *et al.*, 2009). As illustrated in **Figure 9**, *Ganoderma* iolates LVRB-1, LVRB-14 and LVRB-16, which formed a well-supported clade with *G. enigmaticum* in the ITS2 Bayesian Posterior Probability (BPP) analysis, displayed similar RNA secondary structures. *Ganoderma* isolates LVRB-2 and LVRB-7, which clustered with *G. mbrekobenum* exhibited similar ITS2 RNA secondary structures.

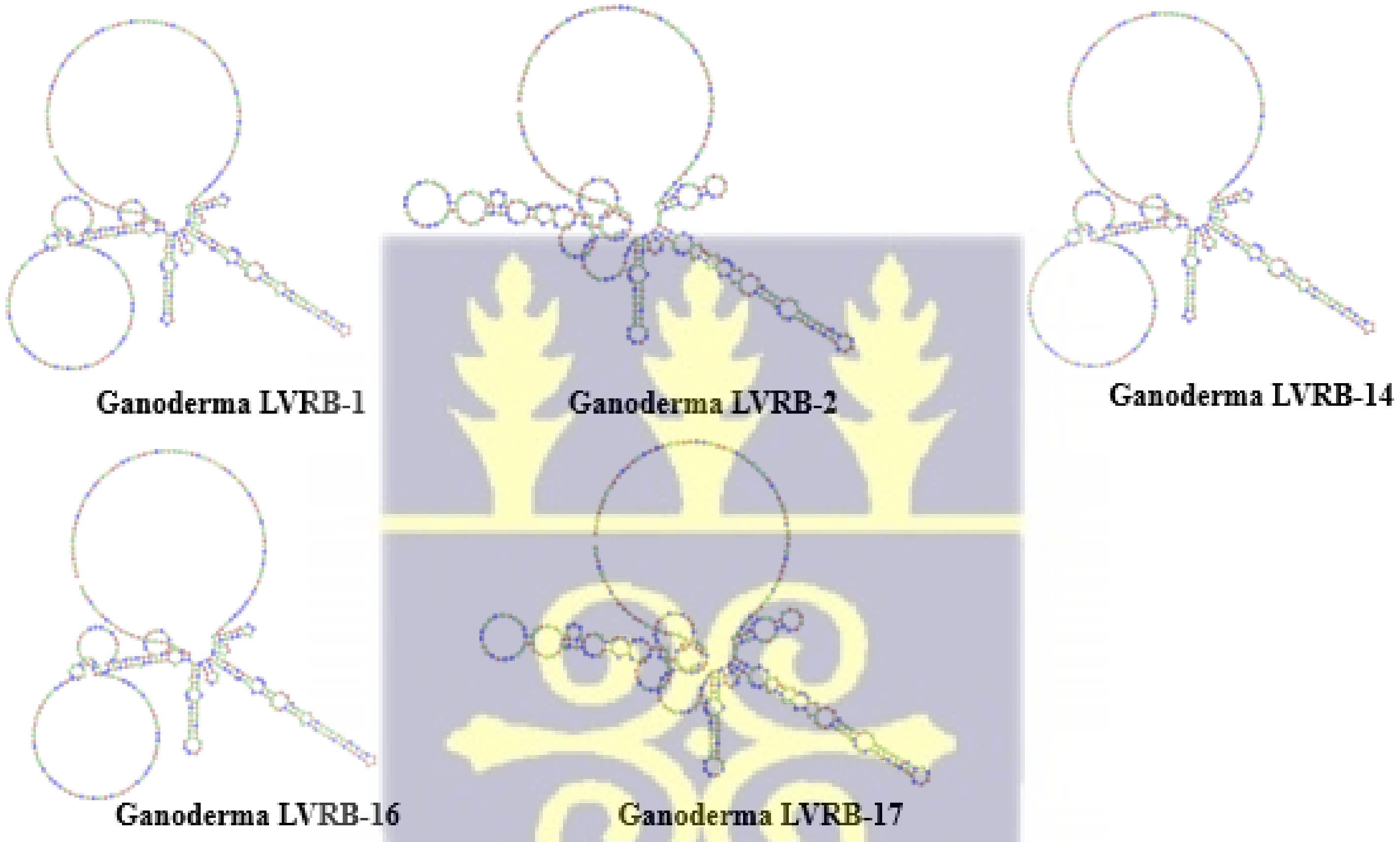

Figure 9. RNA secondary structures of ITS2 of *Ganoderma* isolates.

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However, a close morphological examination of *Ganoderma* samples LVRB-2 LVRB-17 suggest the two isolates are phenotypically different (**Figure 7A** and **B**); indicating the two isolates are not closely related. To address the above challenge, complete ITS of *Ganoderma* samples LVRB-2 and LVRB-17 together with *Ganoderma* sample

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LVRB-9, which yielded poor-quality ITS2 sequence data, were sequenced and analyzed to provide better insight into their molecular identity and phylogenetic placement**.**

4.1.5.2. ITS Phylogenetic Analysis

The results of Bayesian analysis of *Ganoderma* samples LVRB-2, LVRB-9 and LVRB-17 is presented in **Figure 10**. As shown (**Figure 10**), *Ganoderma* LVRB-17 clustered with *G. resinaceum* but with a low statistical support (BPP = 0.665). Although the statistical support for the clade was not very strong, the finding suggested *Ganoderma* LVRB-17 might have been misidentified in the ITS2 sequence analysis. This finding is not too much surprising because identification accuracies of ITS2 region were reported to be < 92.7% (Chen *et al*., 2010) and ITS is known to display higher species diversity relative to ITS2 (Kress *et al*., 2005. *Ganoderma* LVRB-9, however was found to be closely related to *G. weberianum* and *G. sichuanese* with a moderately higher statistical support (BPP = 0.774) (**Figure 10**). As clearly shown in **Figure 10**, *Ganoderma* LVRB-2 clustered with the species of *G. mbrekonenum* with a strong statistical support (BPP = 0.847), providing further evidence that the *Ganoderma* sample designated LVRB-2 belongs to the species *G. mbrekobenum*. The results of ITS Bayesian analysis suggested *Ganoderma* samples LVRB-2, LVRB-9 and LVRB-17 are separated phylogenetically from each into three clades namely, *Ganoderma mbrekobenum*, *Ganoderma weberianum*-sichuanese complex and *Ganoderma resinaceum*.

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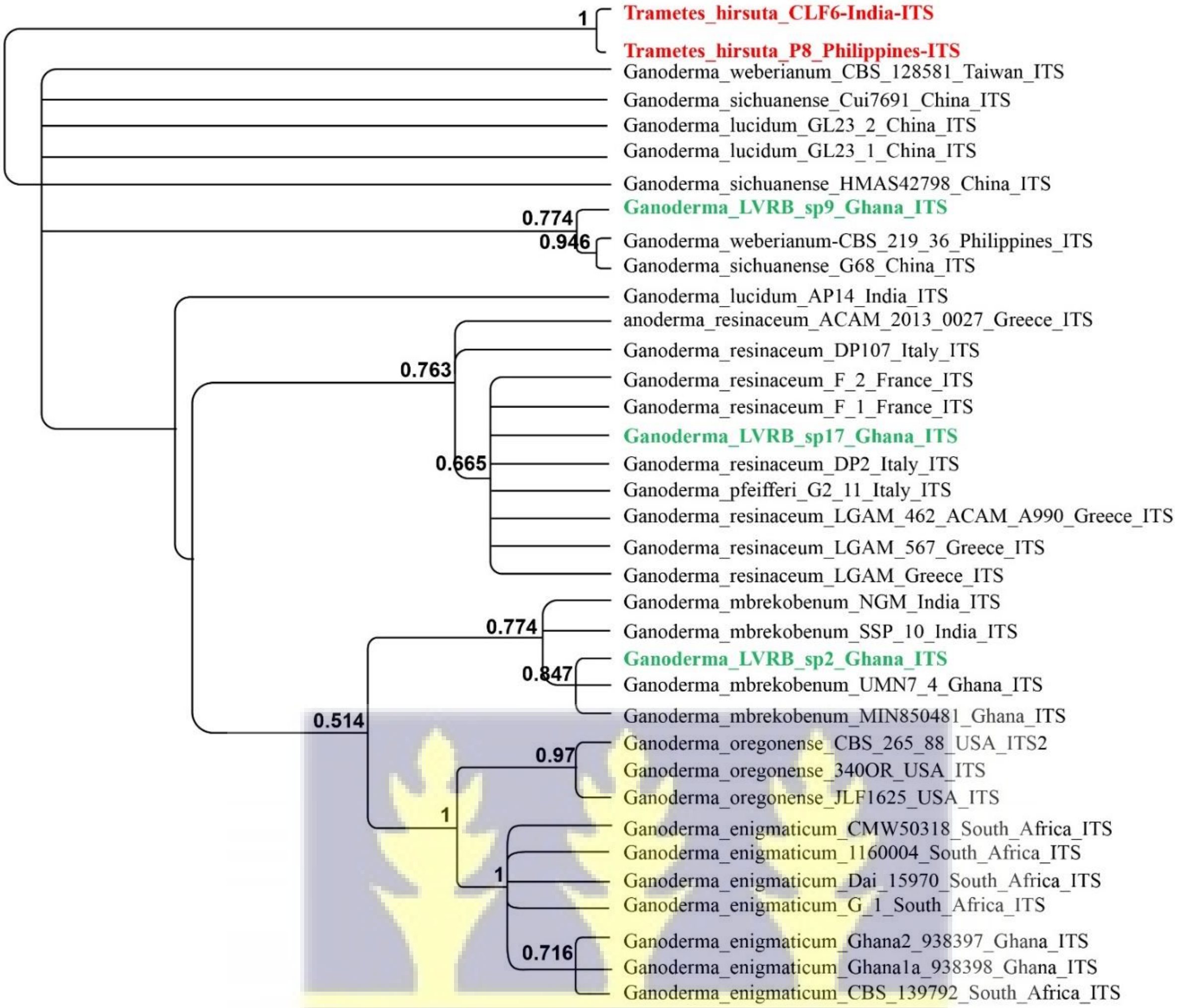

Figure 10. Bayesian phylogenetic tree showing position of collected *Ganoderma* compared to ITS sequence data at GenBank. Branch node values represent Bayesian poterior probability (BPP). Trametes hirsute from India and Philippines used as out groups.

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4.1.5.3. LSU Phylogenetic Analysis

The 28S (nLSU) was sequenced for all the *Ganoderma* mushrooms (n = 6) and the resultant phylogenetic tree is presented in **Figure 11**. As illustrated in **Figure 11**, Gnoderma sample LVRB-2 clustered with *G. mbrekobenum* with a moderately strong statistical (BPP = 0.887), thus, confirming that LVRB-2 belongs to the species *Ganoderma mbrekobenum*. *Ganoderma* sample LVRB-17 clustered with *G. resinaceum* with a very high statistical support (BPP = 0.922), which was consistent with the ITS the phylogenetic analysis further confirming this particular mushroom sample belongs to the species *G. resinaceum* (**Figure 10** and **11**).

The LSU phylogenetic tree generated from the Bayesian analysis further revealed *Ganoderma* samples LVRB-1 and LVRB-16 formed same clade with *G. enigmaticum* with a moderately strong support (BPP = 0.803); consistent with the ITS2 Bayesian analysis which indicated *Ganoderma* LVRB-1 and LVRB-16 both belong to *G. enigmaticum*. The *Ganoderma* sample coded LVRB-9, however, formed a sister clade with *Ganoderma* samples LVRB-1 and LVRB-16, both of which belong to the species *G. enigmaticum*, with a very strong support (BPP = 1.0). Although *Ganoderma* isolate LVRB-9 clustered with *G. enigmaticum* clade, it clustered with *G. weberianum* clade in the ITS analysis with a lower support (BPP = 0.774); revealing a conflict in the phylogeny signals between the LSU and ITS data sets (**Figure 8 and 11**). It has been reported that the ITS region have greater sequence variation between closely related species and a higher rate of evolution, is thus used as a DNA barcode for fungal identification (Kõljalg *et al.*, 2005; Monard *et al.*, 2013, Schoch *et al.*, 2012). In the current study *Ganoderma* isolate LVRB-9 clustered with *G. weberianum* and *G.sichuanese* based on the ITS BLASTn search and the ITS phylogenetic analysis and therefore tentatively identified as belonging *to Ganoderma weberianum-sichuanese* species complex. *Ganoderma* samples LVRB-14, similar to *Ganoderma* sample LVRB-1 and LVRB-16, clustered with *G. enigmaticum* with a moderate support (BPP =0.708), consistent with the observation made in the ITS2 analysis (**Figure 8**).

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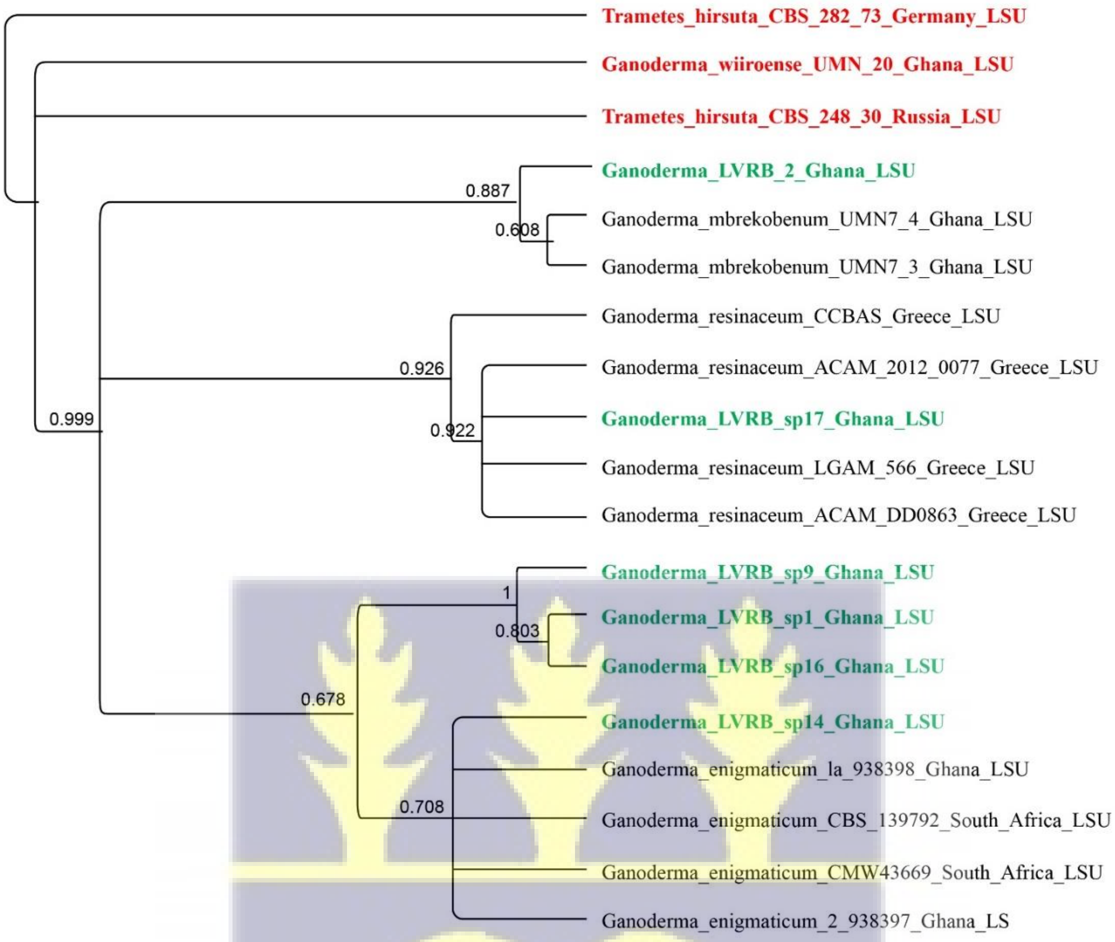

Figure 11. Bayesian phylogenetic tree showing position of collected *Ganoderma* samples compared to LSU sequence data of *Ganoderma* in GenBank. Branch node value represent Bayesian posterior probability (BPP) values. *Trametes hirsuta* from Germany and Russia used as outgroups.

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4.2. LC-MS Metabolomics Study

4.2.1. Metabolomic Comparison

The metabolite constituent of mycelia biomass of the three *Ganoderma* samples were analyzed by LC-MS-based metabolomics approach to provide insight into their metabolomic similarities and differences. LC-MS-based metabolomics approach was used on the assumption that similar *Ganoderma* mycelia biomass would display identical produce metabolites and show similar spectral features while different *Ganoderma* mycelia biomass would produce different metabolites and show different mass spectral features. Total ion chromatogram (TIC) of the three *Ganoderma* mycelia biomass are preented in **Figures 12 and 13**.

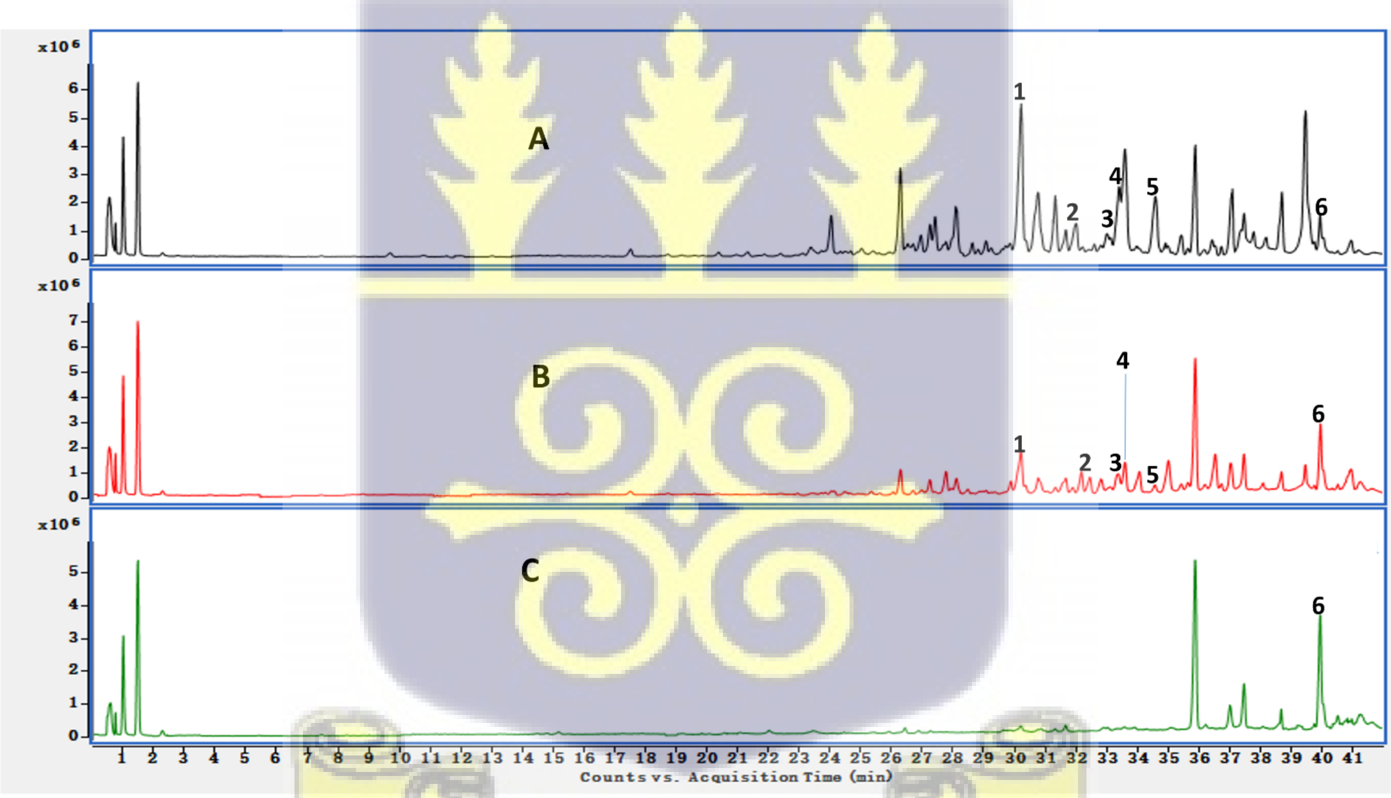

Figure 12. Total ion chromagram (TIC) of mycelia biomass. A: *Ganoderma* sample LVRB-1 (*G. enigmaticum*); B: *Ganoderma* sample LVRB-17 (*G. resinaceum*); C: *Ganoderma* sample LVRB-9 (*Ganoderma weberianum-sichuanese*)

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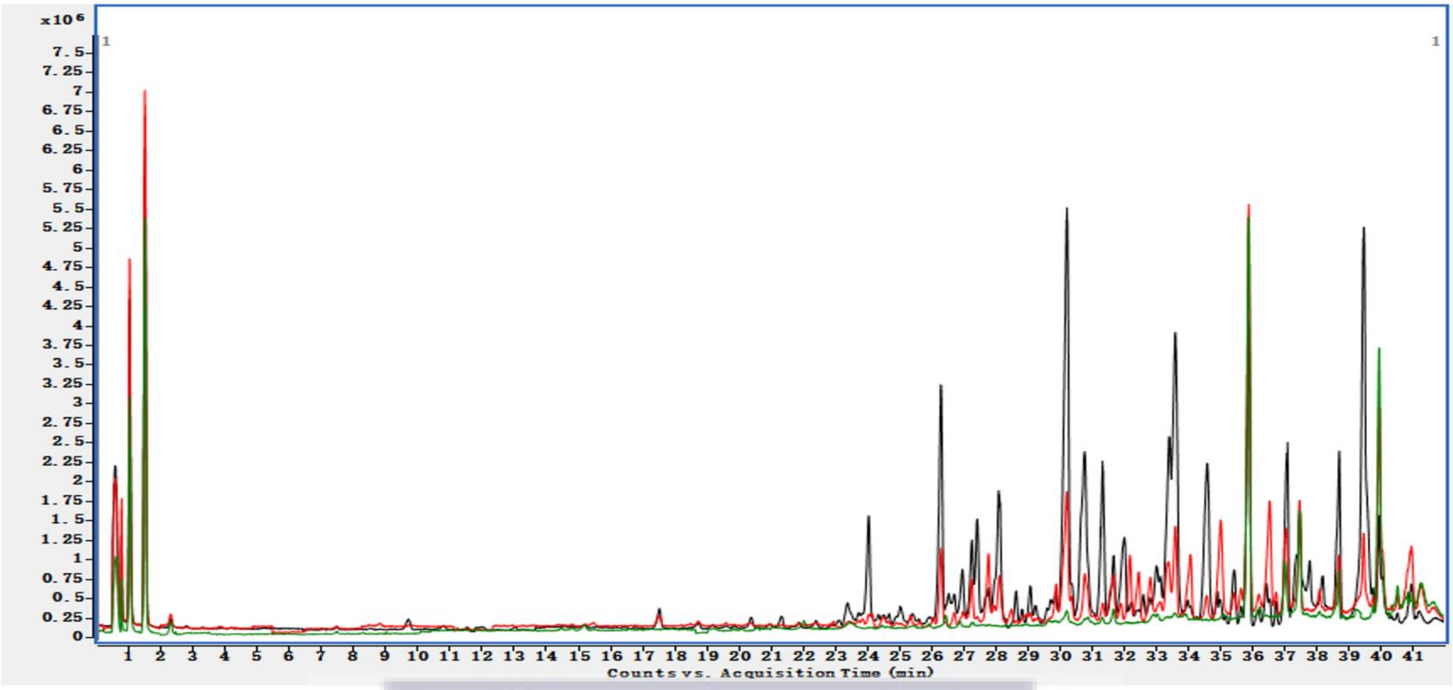

Figure 13. An overlaid TIC presentation of mycelial biomss analyzed by LC-MS. Color code is the same as in Figure 12.

Cursory examination of the chromatograms in **Figures 12** and **13** showed the *Ganoderma* mycelia biomass are different from each other. The PLS-DA score plot generated is shown in **Figure 14A.** As presented in **Figure 14**, the clusters of the three *Ganoderma* mycelia biomass were clearly separated. The mycelia biomass clusters for *Ganoderma* samples 17 and 9 were found in left upper and left lower quadrants but mycelia biomass clusters for *Ganoderma* sample 1 were located in right upper middle and lower quadrants of the PLS-DA plot, revealing explicit variation among the three *Ganoderma* isolates. These differences and similarities in three *Ganoderma* mycelia biomass were further captured in the heatmap representation in **Figure 14B**. As illustrated in **Figure 14 A** and **B**, the three *Ganoderma* mycelia biomass were markedly different in terms of metabolite composition.

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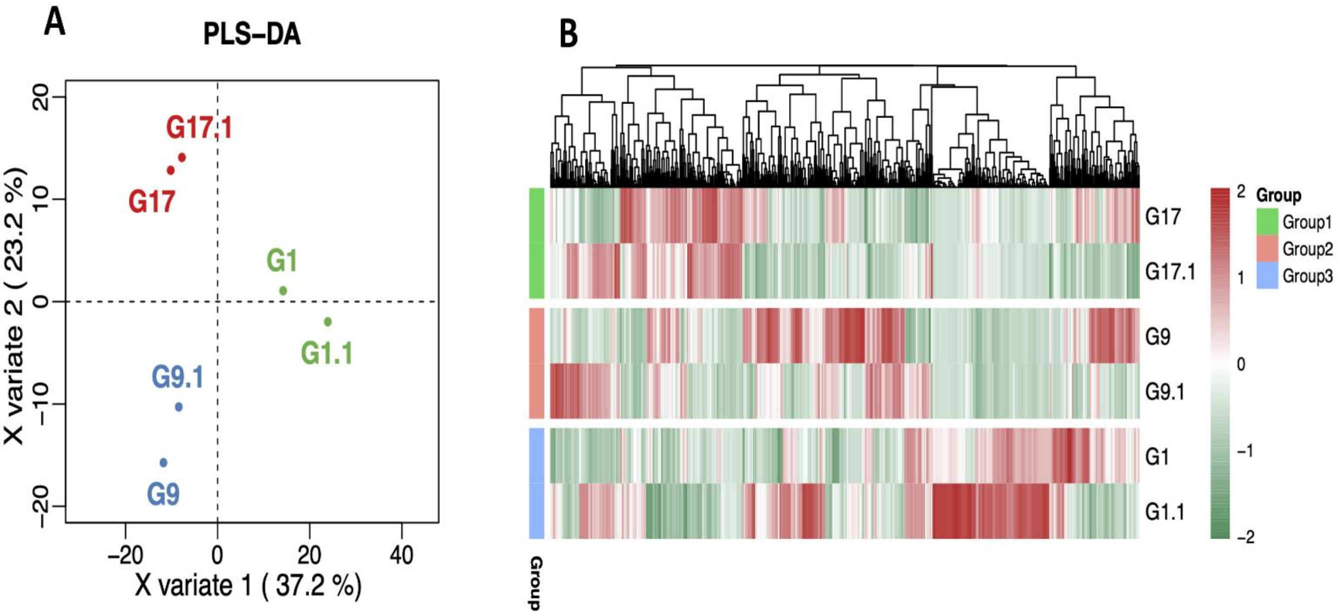

Figure 14. Metabolomic differences between mycelia biomas analyzed. **A:** PLS-DA score plot and **B**: Heatmap representation of sample. G1 and G1.1 correspond to two different *Ganoderma* mycelia biomass sample 1; G9 and G9.1 represent two *Ganoderma* mycelia sample 9; G17 and G17.1 are two different *Ganoderma* mycelia biomass sample 17.

4.2.2. Identification of Compounds

This aspect of the study aimed at identifying triterpenoids in the three *Ganoderma* mycelial biomass. Four tetracyclic triterpenoids were identified. The identification was made by comparing the retention time and fragmentation pattern with corresponding pure compounds. Two other lanostanoid compounds detected were tentatively identified due to lack of reference compounds. **Figure. 15** illustrates the chemical structure of the compounds detected.

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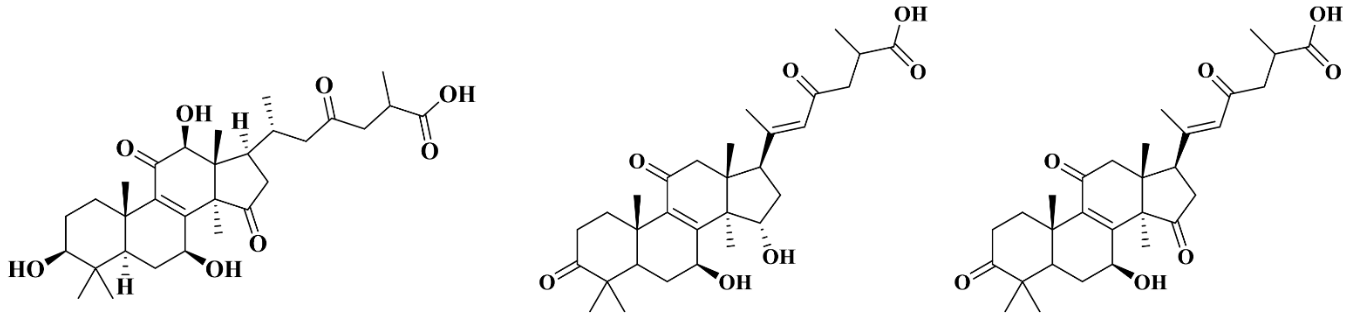
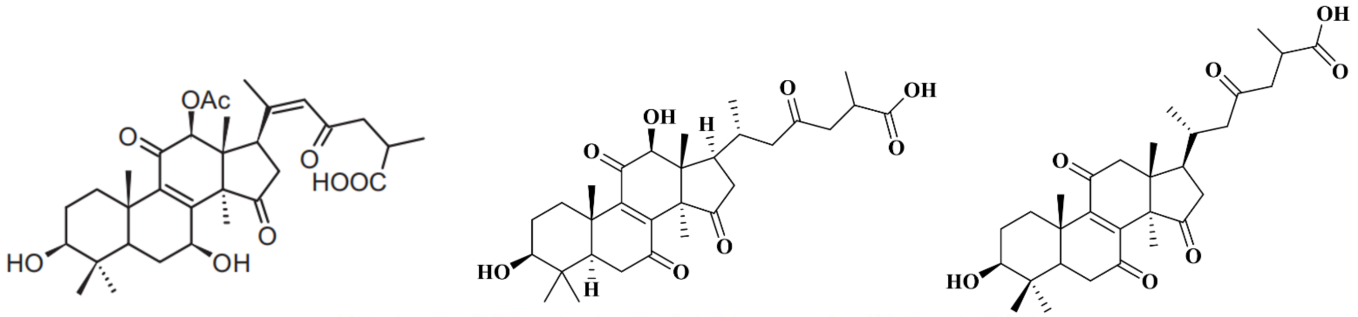


Ganoderic acid G **Ganoderenic acid A** **Ganoderenic acid D**



Ganoderenic acid K **Ganoderic acid C6** **Ganoderic acid AM1**

Figure 15. Structures of triterpenoids compounds in mycelial biomass

Similar to previous studies, *Ganoderma* mushroom extracts were analyzed in negative mode because it was found to be more sensitive and appropriate for detection of triterpenoids compounds. In the study, the *Ganoderma* mycelia samples were analysed by LC-MS in the negative mode, The results showed that the common fragmentation pattern pertains to loss of water (H_2O), carbon dioxide (CO_2) and fragment rearrarangement in some cases. The identification details are summarized in **Table 6**.

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Table 6. Details of triterpenoids identified.

No.	Rention Time in mins.	Formula	Calculted *m/z* [M-H]$^-$	Determined. *m/z* [M-H]$^-$	Δ part per minute (ppm)	MS-MS fragmentation	ID of compound	*Ganoderma* mycelia biomss		
								A	B	C
1	30.752	$C_{30}H_{42}O_8$	529.2807	529.2810	0.5668	511.2707, 496.2180, 67.2801, 437.2318, 303.1602, 73.1458, 209.3146	ganoderic acid C6*	Present	present	Absent
2	32.232	$C_{32}H_{44}O_9$	571.2913	571.2932	3.3258	553.3533, 529.3535, 11.3429	ganoderenic acid K	Present	present	Absent
3	33.592	$C_{30}H_{42}O_7$	513.2858	513.2856	0.3896	495.2752, 469.2963, 51.2858	ganoderic acid AM1	Present	present	Absent
4	33.607	$C_{30}H_{40}O_7$	511.2701	511.2701	0.0000	493.2597, 449.2700, 16.2314, 374.1863, 329.1749, 01.1811	ganoderenic acid D*	Present	present	Absent
5	34.596	$C_{30}H_{42}O_7$	513.2858	513.2860	0.3896	495.2752, 465.2677, 81.2426, 301.1822, 211.0972, 93.0873, 167.0714, 123.0814	ganoderenic acid A*	Present	present	Absent
6	40.392	$C_{30}H_{44}O_8$	531.2963	531.2964	0.1882	513.2858, 469.2956, 01.1820, 265.1443, 203.1444	ganoderic acid G*	Present	present	Aresent

A: *Ganoderma* sample 1 mycelia biomass; B: *Ganoderma* sample 17 mycelia biomass; C: *Ganoderma* sample 9 mycelia biomsss. * Shows confirmed compounds using reference compounds.

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Compoud 1 has product ion, [M—H]$^-$ 529.2810. The product ion was detected at 30.752 min. The fragment ions have *m/z* values ranging from 511.2707 to 209.3146 (**Table 6**). The fragmeent ion, which has *m/z* value of 511.2707, was formed by loss of one molecule of water by the product ion. Similarly, the fragment ion, which has *m/z* value of 496.2180 corresponds to loss of two water molecules ($2H_2O$) and loss of three hydrogen atom while *m/z* of 467.2801 represents [M—H—CO_2—H_2O]$^-$ (**Figure 16**). The fragmentation pattern was confirmed with pure reference compound (**Table 6**).

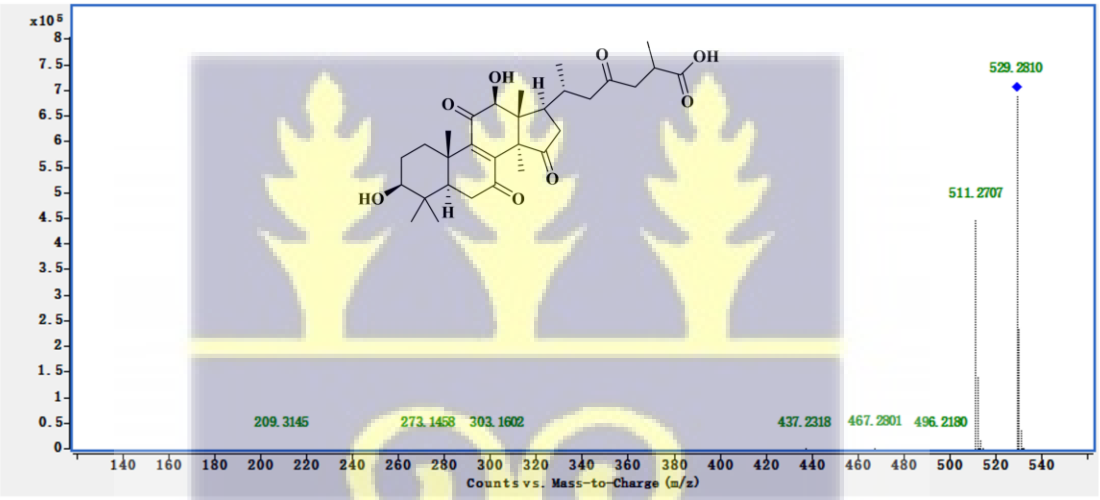

Figure 16. Electron ion chromatogram (EIC) for ganoderic acid C6.

Compound **2** yielded product ion, [M—H]$^-$ with m/z value of 571.2932. The fragment ions have *m/z* values ranging from 553.3533 to 511.3429 (**Table 6**). The fragement ion, which has *m/z* value of 553.3533 was formed by loss of H_2O molecule from the product ion [M—H—H_2O]$^-$. Subsequent loss of HCOOH and H_2O molecule accounted for the *m/z* values of 529.3535 and 511.3429. Specifically, the fragment ion

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which has *m/z* value of 529.3535 represents $[M-H-HCOOH]^-$, whereas *m/z* of 511.3429 represents $[M-H-HCOOH-H_2O]^-$. Based on the above obervations and comparison of fragmentation patterns and retention times to reference work of Hennicke *et al.* (2016) **Compound 2**, was tentatively identified as Ganoderenic acid K as illustrated in **Figure 17**.

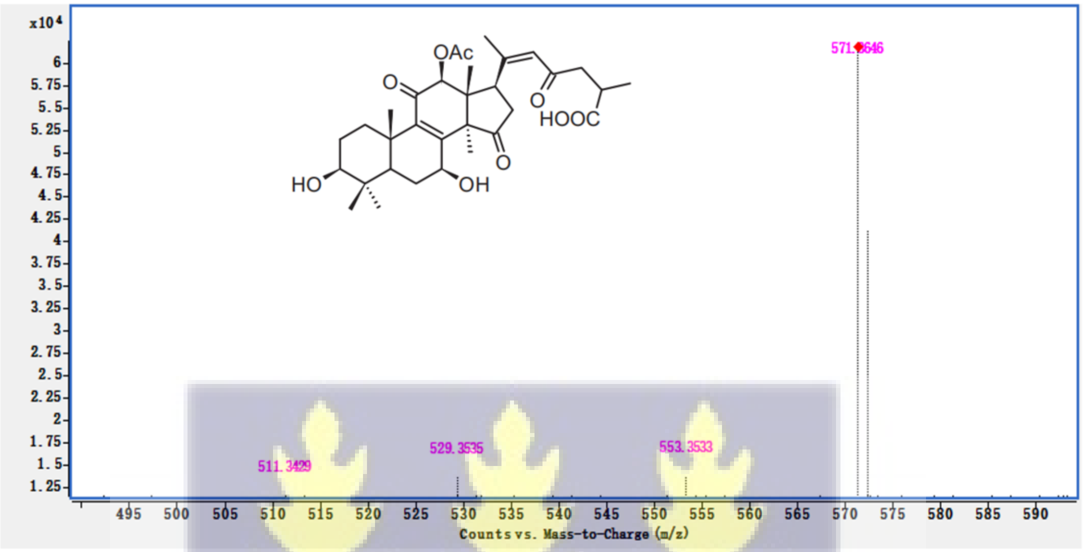

Figure 17. Electron ion chromatogram (EIC) for ganoderenic acid K.

Compound 3, which has product ion $[M-H]^-$ and m/z values of 513.2856 was detected at 33.592 min. It was tentatively identified as ganoderic acid AM1 (**Figure 18**). The m/z values of fragment ion range from 495.2752 to 451.2858 (**Table 6**). The fragment ion has m/z value of 495.2752 corresponds to $[M-H-H_2O]^-$, fragment ion with m/z of 469.2963 represents $[M-H-CO_2]^-$ while the fragment ion with m/z of sa451.2858 corresponds to $[M-H-CO_2-H_2O]^-$ (**Table 6).**

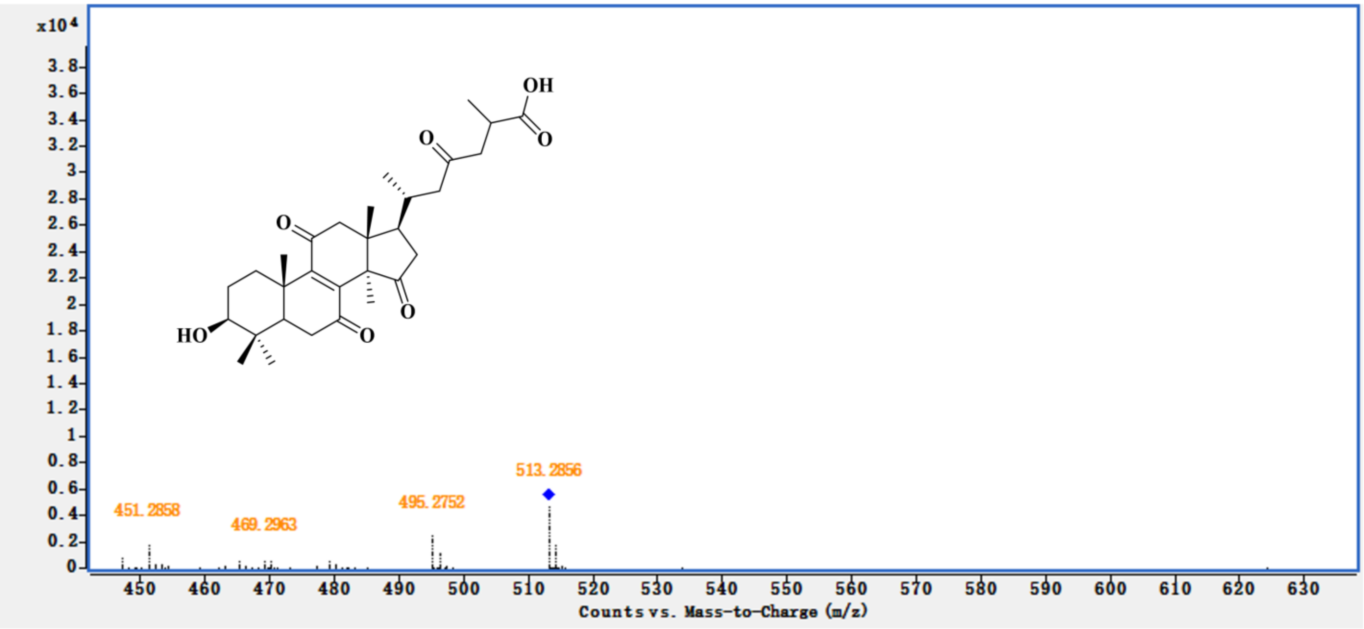

Figure 18. Electron ion chromatogram (EIC) for ganoderic acid AM1.

Compounds 4, 5 and **6**, unlike **Compounds 2 and 3** were identified by comparing the fragmentation patterns and retention times with reference compounds. **Compound 4**, which has product ion, $[M-H]^-$ and the m/z value of the product ion is 511.2701, was identified as ganoderenic acid D. The fragment ions with m/z value of 493.2597 correponds to $[M-H-H_2O]^-$ and the fragment ion with m/z of 449.2700 repressents $[M-H-CO_2-H_2O]^-$ (**Figure 19**).

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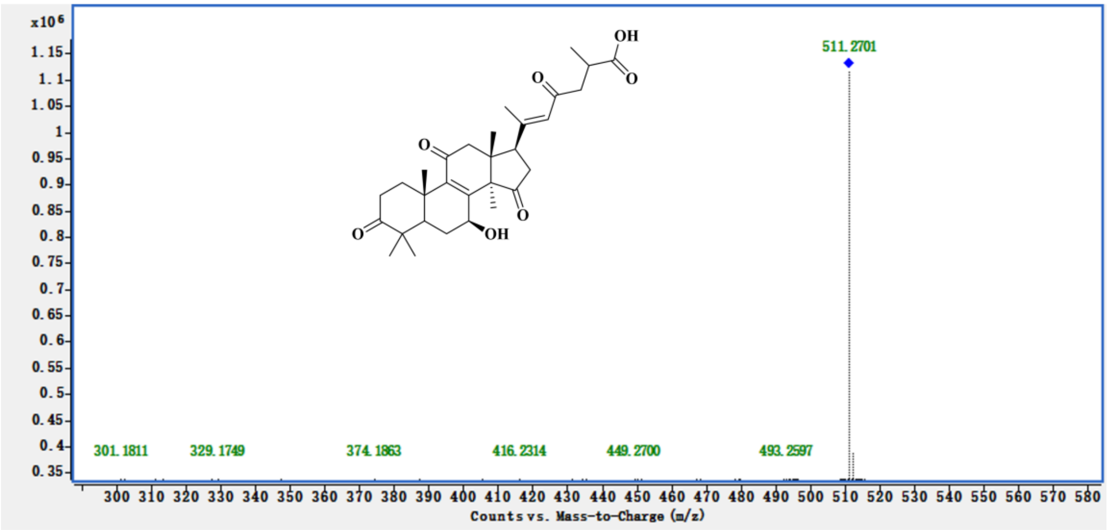

Figure 19. Electron ion chromatogram (EIC) for ganoderenic acid D.

Compound 5 has the product ion $[M-H]^-$ with m/z value of 513.2860. The fragments have m/z values ranging from 495.2752 to 123.0814 (**Table 6**). The fragment ions with m/z value of 495.2752 corresponds to $[M-H-H_2O]^-$ whereas the fragment ion with m/z value of 381.2426 represents $[M-H-3CO_2]^-$. Similarly, the fragment ion with m/z of 301.1822 corresponds to $[M-H-4CO_2-2H_2O]^-$ (**Figure 20**).

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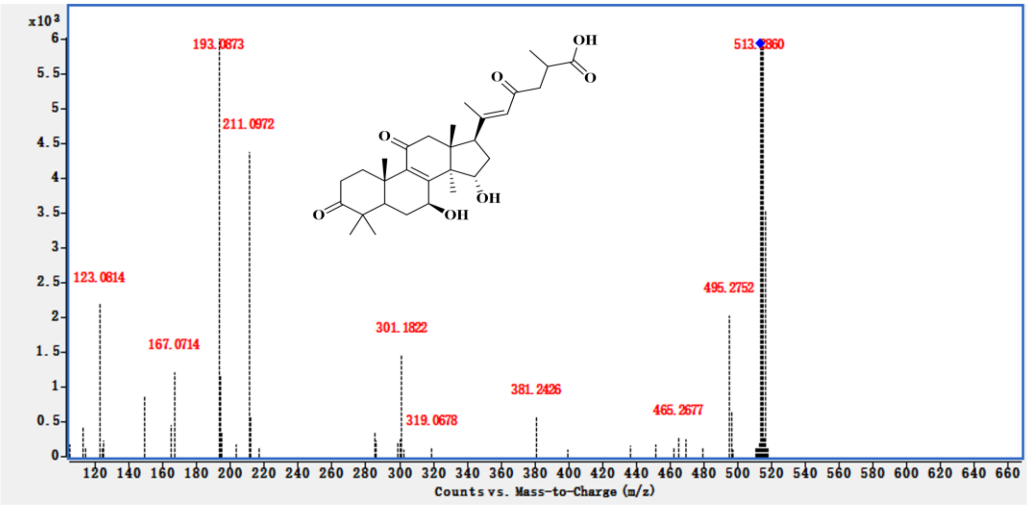

Figure 20. Electron ion chromatogram (EIC) for ganoderenic acid A.

Compound 6, which has the product ion $[M - H]^{-}$ with corresponding m/z value of 531.2964 was detected at 40.392 min and identified as ganoderic acid G. The fragment ions have m/z values of 513.2858, 469.2956, 301.1820, 265.1443 and 203.1444. The fragment ion with m/z value of 469.2956 represents $[M - H - CO_2 - H_2O]^{-}$ while the fragment ion with m/z value of 301.1820 corresponds to $[M - H - 4CO_2 - 3H_2O]^{-;}$. Similarly, the fragment ion with m/z value of 265.1443 represents $[M - H - 4CO2 - 5H_2O]^{-}$ and the one with m/z vlue of 203.1444 represents $[M - H - 5CO_2 - 6H2O]^{-}$ (**Figure 21**).

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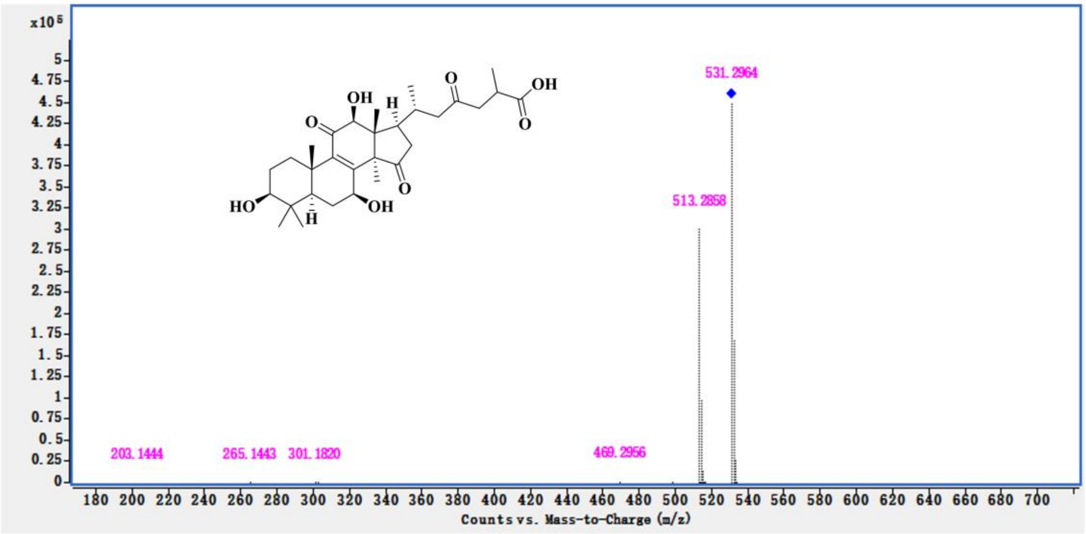

Figure 21. Electron ion chromatogram (EIC) for ganoderic acid G.

The six detected compounds were present in *Ganoderma* mycelia biomss of *Ganoderma* sample 1 and 17. Ganoderic acid G was the only triterpenoid successfully identified in *Ganoderma* sample 9 mycelia biomass (**Table 6**).

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4.3. Biological Activity Evaluation Studies

4.3.1. Cytotoxic Effect of *Ganoderma* LVRB-9 Mycelial Biomass

The cytotoxic effect of *Ganoderma* LVRB-9 mycelial biomass on human prostatic tumor cell line (PC-3), T cell lymphoma line (Jurkat) and plasmacytoid dendritic cell (pDC), in comparison with Chang liver cell (normal liver cell) was investigated by MTT assay. The cultured mycelial biomass of *Ganoderma* LVRB-9 was selected because it was found to contain more abundant metabolites compared to *Ganoderma* LVRB-1 and *Ganoderma* LVRB-17 based on our metabolomics analysis. The effect of different concentrations of the crude ethanol extract (**GL-CO1**) on PC-3, Jurkat and pDC, in comparison with Chang liver cell following 72 hours treatment is shown in **Figure 22**.

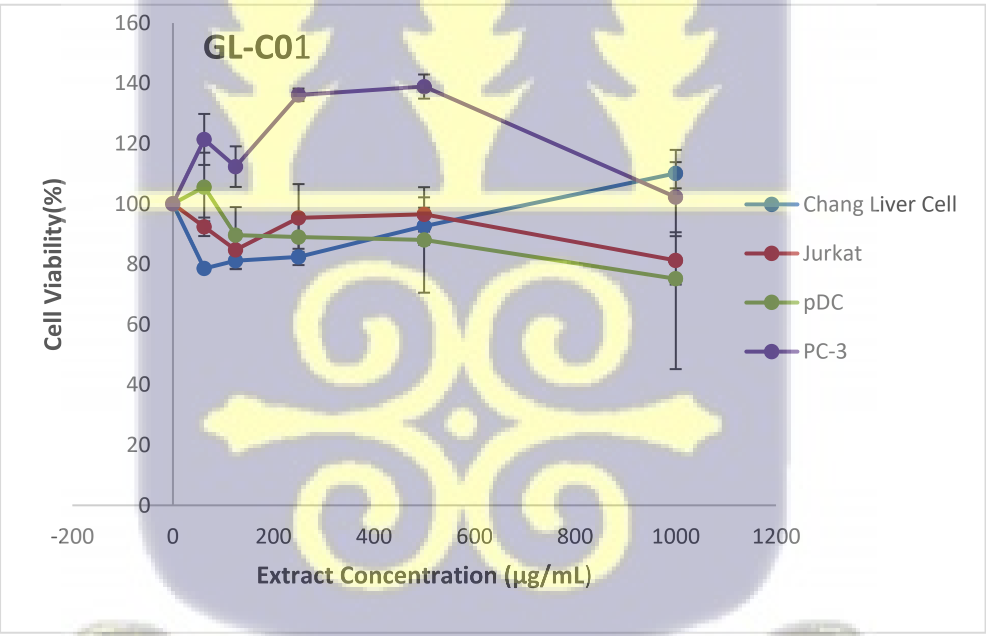

Figure 22. Mycelia biomass crude ethanol extract (**GL-CO1)** cytotoxic effect on PC-3, Jurkat, pDC and normal Chang liver cell lines evaluated by MTT assay.

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As shown in **Figure 22**, the crude ethanol extract (**GL-CO1**) exhibited no significant cytotoxic effect on the four cell lines at the concentration range of 0-500 µg/ml. However, a marginal cytotoxic effect on Jurkat and pDC was observed at the concentration range of 500-1000 µg/mL but the IC_{50} values were >1000 µg/ml (**Figure 22** and **Table 7**). Curcumin the positive control, on the other hand, markedly suppressed the growth and survival of the four tested cells in a concentration dependent manner (**Figure 23**). The highest cytotoxic effect of curcumin was against pDC with IC_{50} value of 1.21 ± 0.09 µg/mL and SI = 6.71, followed by Jurkat with IC_{50} value of 2.35 ± 0.38 µg/mL and SI = 3.45 and PC-3 with IC_{50} value of 4.29 ± 2.29 µg/mL and SI = 1.89 , respectively (**Figure 23** and **Table 7**). As described in (**Figure 23** and **Table 7**), curcumin demonstrated the lowest inhibitory effect against Chang liver (normal liver) cells with IC_{50} value of 8.12 ± 0.00µg/ml; indicating curcumin was selectively cytotoxic to pDC, Jurkat and PC-3 cells but non-toxic to Chang liver cell.

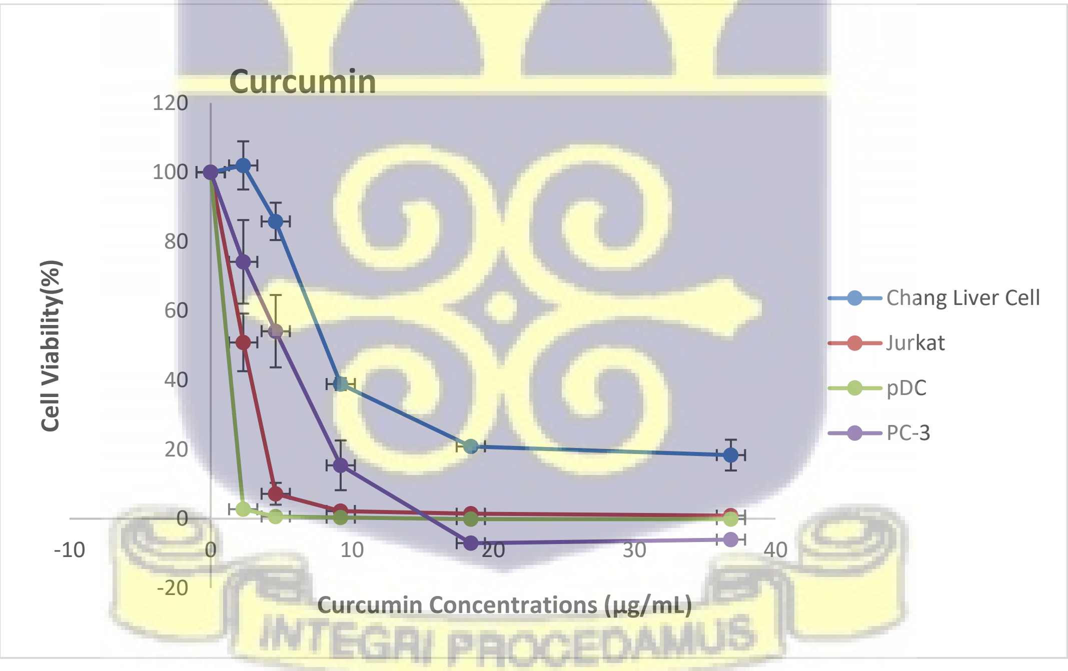

Figure 23. Curcumin cytotoxic effect on Jurkat, PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

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Table 7. Mean IC_{50} value and selectivitity index (SI) of fractions of ethanol extract of cultured *Ganoderma* LVRB-9 mycelial biomass on Jurkat, PC-3, pDC and Chang liver cell line.

	Solvent fractions of mycelial biomass of *Ganoderma* LVRB-9 ethanol extract (µg/mL)								Curcumin positive control
Cell line	GL-C01	GL-C1	GL-C2	GL-C3	GL-C4	GL-C5	GL-C6	GL-C7	(µg/mL)
Jurkat	>1000	0.00	17.09±0.86	48.82±5.08	>100	>100	>100	>100	2.35± 0.37
	*0.00	*0.00	*5.85	*2.05	*0.00	*0.00	*0.00	*0.00	* 3.45
PC-3	>1000	>100	27.73±5.25	>100	>100	>100	>100	>100	4.29±2.29
	*0.00	*0.00	*3.60	*0.00	*0.00	*0.00	*0.00	*0.00	*1.89
PMDC05	>1000	>100	21.31±2.40	35.69±9.65	>100	>100	>100	>100	1.21±0.09
	*0.00	*0.00	*4.69	*2.80	*0.00	*0.00	*0.00	*0.00	*6.71
Chang	>1000	>100	75.41±1.95	>100	>100	>100	>100	>100	8.12±0.01

Data are presented as Mean IC_{50} values and standard deviations by MTT assay from three independent experiments, performed in triplicate on Jurkat, PC-3, pDC, and Chang Liver cells. * Denotes selectivitity index (SI)

4.3.2. The cytotoxic Effect of Solvent Fractions

The cytotoxic effect of the solvent fractions (**GL-C1**, **GL-C2**, **GL-C3**, **GL-C4**, **GL-C5**, **GL-C6** and **GL-C7**) on PC-3, Jurkat, pDC and Chang liver cells; following 72 hours treatment at 37°C was also investigated. The results revealed that the fraction GL-C1 showed no cytotoxic effect on PC-3, pDC, Jurkat and Chang (**Figure 24** and **Table 7**).

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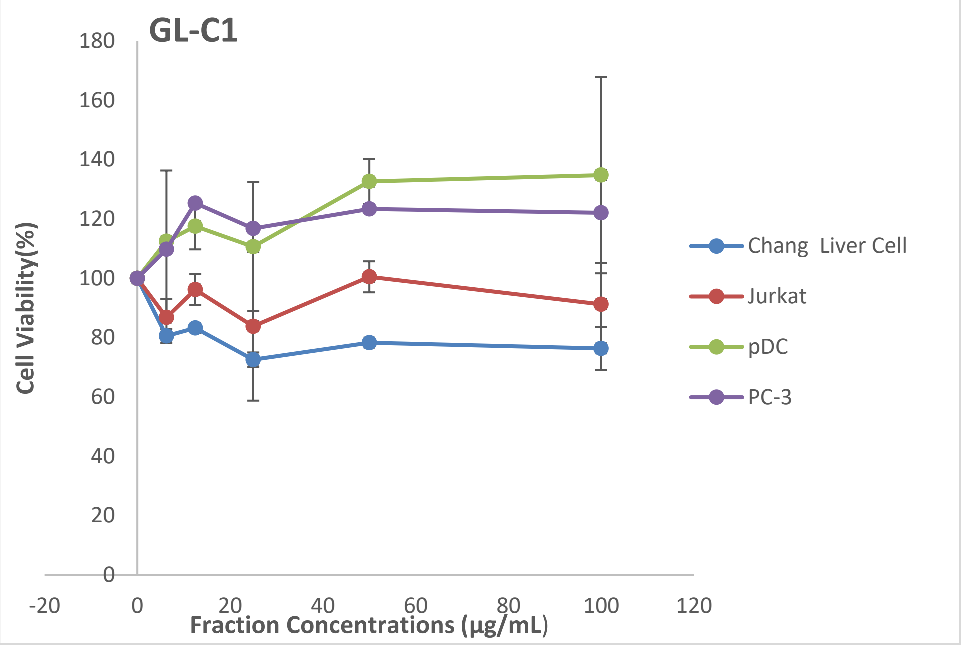

Figure 24. Mycelia biomass of *Ganoderma* LRVB-9 solvent fraction **GL-C1** cytotoxic effect on Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

Nevertheless, the fraction **GL-C2** (**Figure 25**) displayed a profound inhibitory effect on Jurkat, pDC and PC3 in a concentration dependent manner with IC_{50} value of 17.09±0.86 µg/mL and SI = 5.85), pDC with IC_{50} = 21.31±2.40 µg/mL and SI = 4.69 and PC-3 with IC_{50} value of 27.73±5.25 µg/mL and SI = 3.60 compared to Chang liver cell with IC_{50} of 75.41±1.95 µg/mL respectively (**Table 7**). Thus, the solvent fraction GL-C2, displayed the highest cy-totoxic effect against Jurkat followed followed by pDC and then PC-3.

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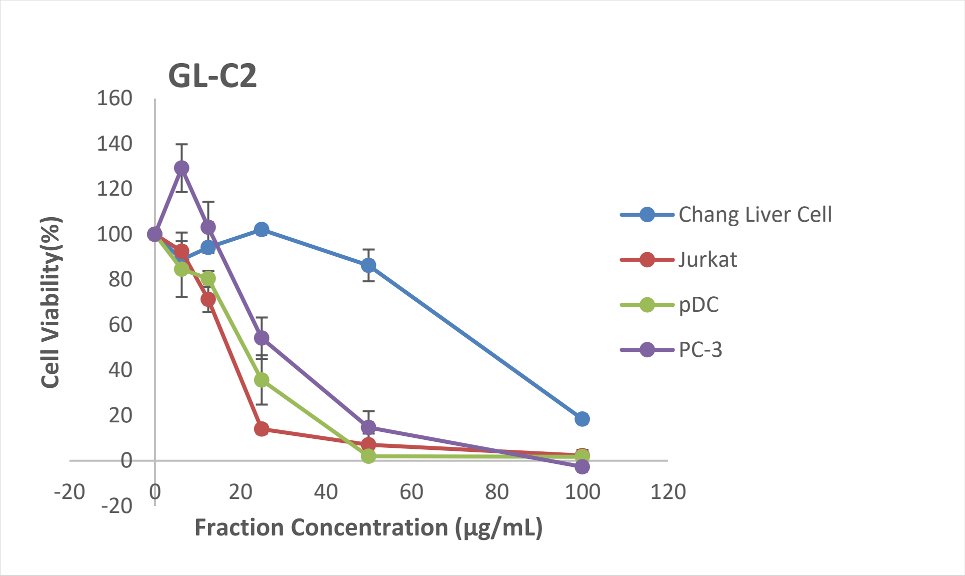

Figure 25. Mycelia biomass of *Ganoderma* LRVB-9 solvent fraction GL-C2 inhibitory effect on Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

Figure 26 illustrates the cytotoxic effect of the solvent fraction **GL-C3** on PC-3, Jurkat, pDC and Chang liver cells. As shown in **Figure 26**, **GL-C3** showed no significant cytotoxic effect against PC-3 and Chang liver cells but displayed a moderately strong cytotoxic effect against pDc with IC_{50} value of 35.69±9.65 µg/ml and SI = 2.80 and Jurkat with IC_{50} value of 48.82±5.08 µg/mL and SI = 2.05. Thus, compared to **GL-C2**, the cytotoxic effect of **GL-C3** was generally lower than that of **GL-C2 (Figure 26 and Table 7)**.

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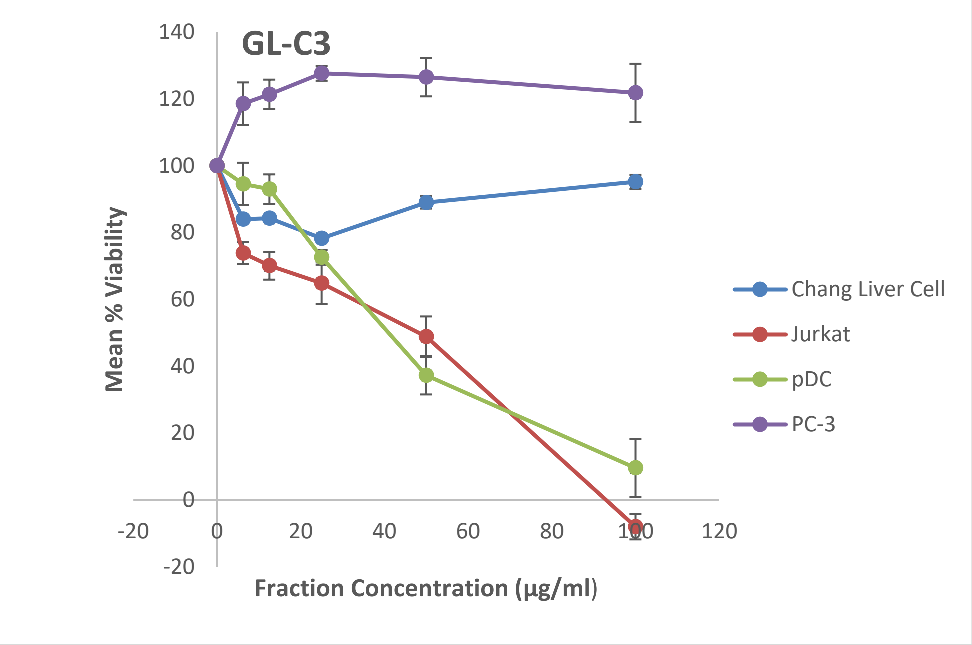

Figure 26. Mycelia biomass of *Ganoderma* LRVB-9 solvent fraction GL-C3 inhibitory effect on viability of Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

The inhibitory effect of **GL-C4** on viability of PC-3, Jurkat, pDC and Chang liver cells was also investigated using MTT assay (**Figure 27**). As illustrated, the solvent fraction **GL-C4**, unlike **GL-C1**, **GL-C2** and **GL-C3** failed to show inhibitory effect on the viability of all the four cell lines tested.

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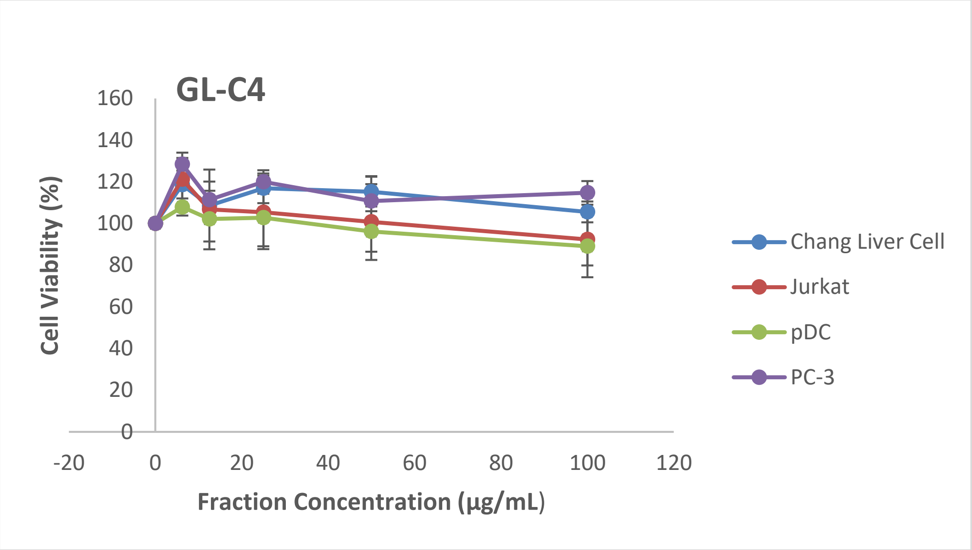

Figure 27. Mycelia biomass of *Ganoderma* LRVB-9 solvent fractions GL-C4 inhibitory effect on Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

The inhibitory effects of **GL-C5**, **GL-C6** and **GL-C7** similar to **GL-C4** showed no inhibitory effect on all the cell lines tested.

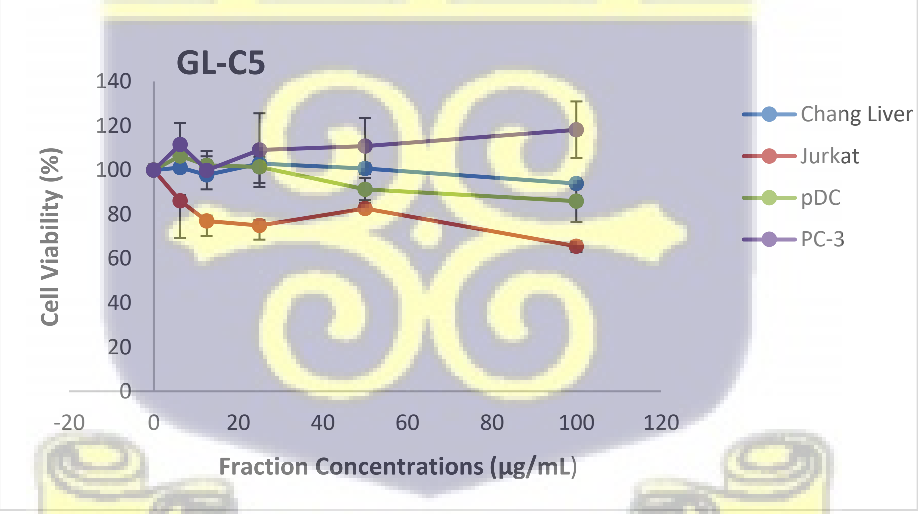

Figure 28. Mycelia biomass of *Ganoderma* LRVB-9 solvent fractions GL-C5 inhibitory effect on Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

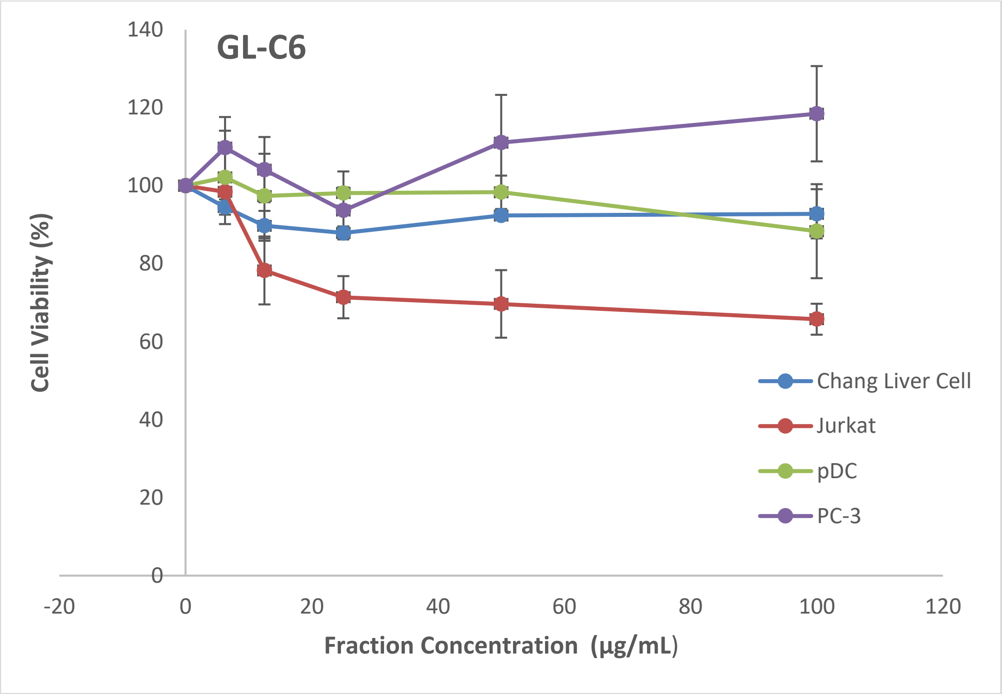

Figure 29. Cultured mycelia biomass of *Ganoderma* LRVB-9 solvent fractions GL-C6 inhibitory effect on Jurkat, PC-3, pDC and Chang liver cell lines evaluated by MTT assay.

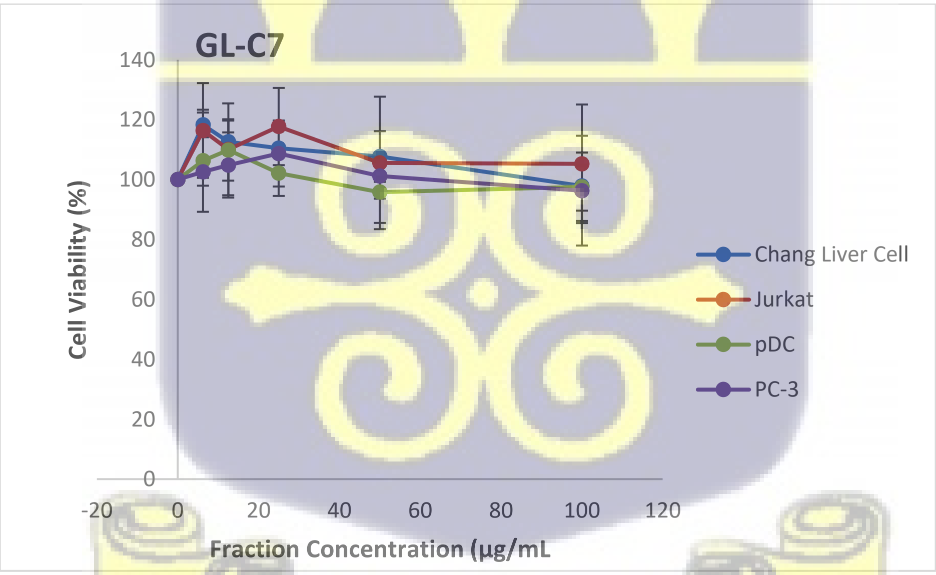

Figure 30. Mycelial biomass of *Ganoderma* LRVB-9 solvent fractions GL-C7 inhibitory effect on Jurkat, PC-3, pDC and Chang liver cell line evaluated by MTT assay.

In summary, besides solvent fraction **GL-C1**, **GL-C2** and **GL-C3**, the remaining (**GL-C4**, **GL-C5**, **GL-C6** and **GL-C7**) (**Figure 27, 28, 29 and 30**) showed no inhibitory effect on the four cells tested.

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Considering the comparatively low cytotoxic effect of **GL-C2** against Chang liver cells but markedly profound cytotoxic effect against Jurkat, pDC, and PC-3, it was further fractionated (**Figure 6**) and the cytotoxic effect of the corresponding subfractions tested against the four cell lines.

4.3.3. Cytotoxic effect of GL-C2 subfractions

The cytotoxic effect of mycelial biomass solvent subfractions (**GL-C2-C1, GL-C2-C2, GL-C2-C3, GL-C2-C4, GL-C2-C5, GL-C2-C6, GL-C2-C7, GL-C2-C8,** and **GL-C2-C9**) was tested against PC-3, Jurkat, pDC and Chang liver cells after a 72-hour treatment at various concentrations by MTT assay. The results revealed the subfraction **GL-C2-C1** showed no substantial cytotoxic effect on pDC, Jurkat, and Chang liver cells. The subfraction **GL-C2-C1**, however, demonstrated a potent cytotoxic effect against PC-3 with IC_{50} value of 3.24± 0.10 µg/mL and SI = 30.86 compared to curcumin with IC_{50} value of 5.13± 0.86 µg/mL and SI = 1.30 (**Figure 31 and Table 8**).

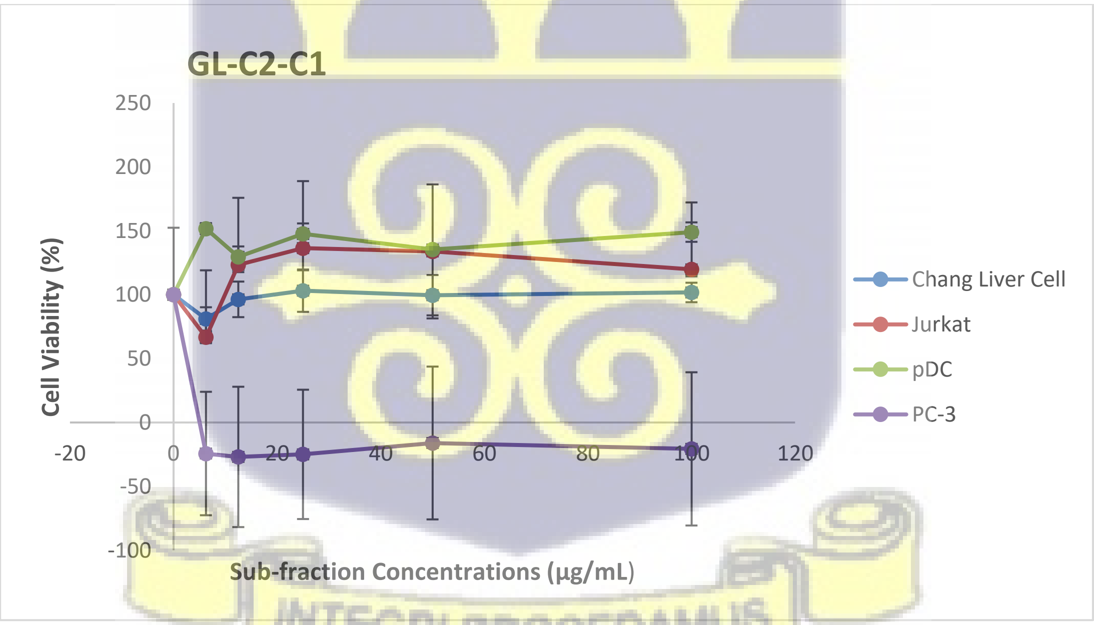

Figure 31. Mycelia biomass of *Ganoderma* LVRB-9 sub-fractions GL-C2-C1 cytotoxic effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

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The subfraction **GL-C2-C2**, unlike **GL-C2-C1**, exhibited poor cytotoxic effects against all the four cell lines tested (**Figure 32 and Table 8**). The subfraction **GL-C2-C3** (**Figure 33 and Table 8**), similar to **GL-C2-C2**, demonstrated a low cytotoxic effect against PC-3, Jurkat, and Chang liver cells but a mildly cytotoxic against pDc with IC_{50} value of 81.11±4.98 μg/mL and SI = 1.23 (**Figure 33 and Table 8**).

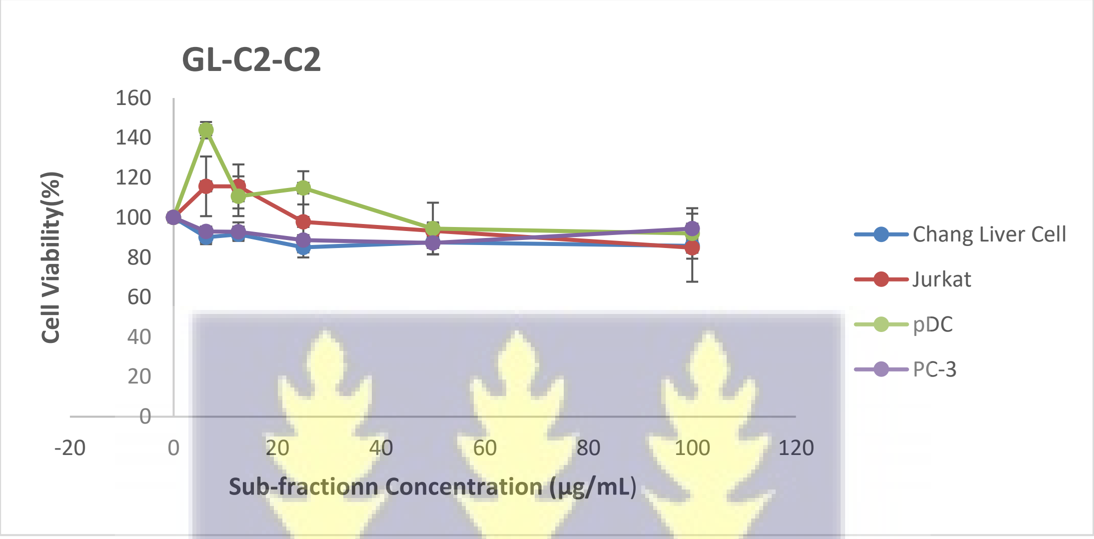

Figure 32. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C2 cytotoxic effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

The subfraction **GL-C2-C3**, similar to **GL-C2-C2**, demonstrated a low cytotoxic effect against PC-3, Jurkat, and Chang liver cells but a mildly cytotoxic against pDc with IC_{50} value of 81.11±4.98 μg/mL and SI = 1.23 (**Figure 33 and Table 8**).

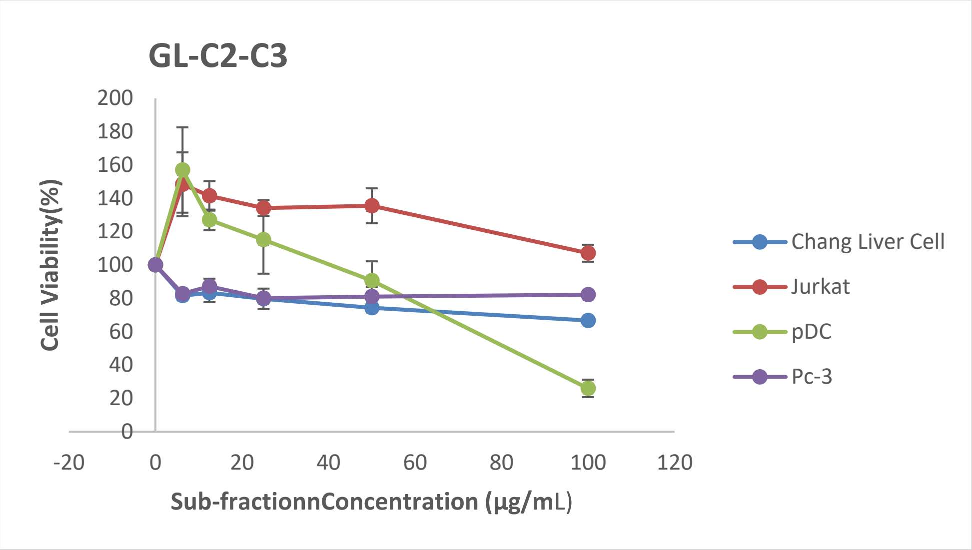

Figure 33. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C3 cytotoxic effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

On the other hand, the assessment of the cytotoxic effect against **GL-C2-C4** revealed the subfraction displayed cytotoxic activity against PC-3, Jurkat, and pDC but not Chang liver cell. As shown in **Figure 34** as well as **Table 8**, the subfraction **GL-C2-C4** exhibited the highest cytotoxic effect against pDC with IC_{50} value of 19.95± 0.50 µg/mL with SI = 5.01, followed by Jurkat with IC_{50} value of 48.14± 1.07 µg/ml and SI = 2.07 and then PC-3 with IC_{50} = 77.39± 2.79 µg/mL and SI = 1.29.

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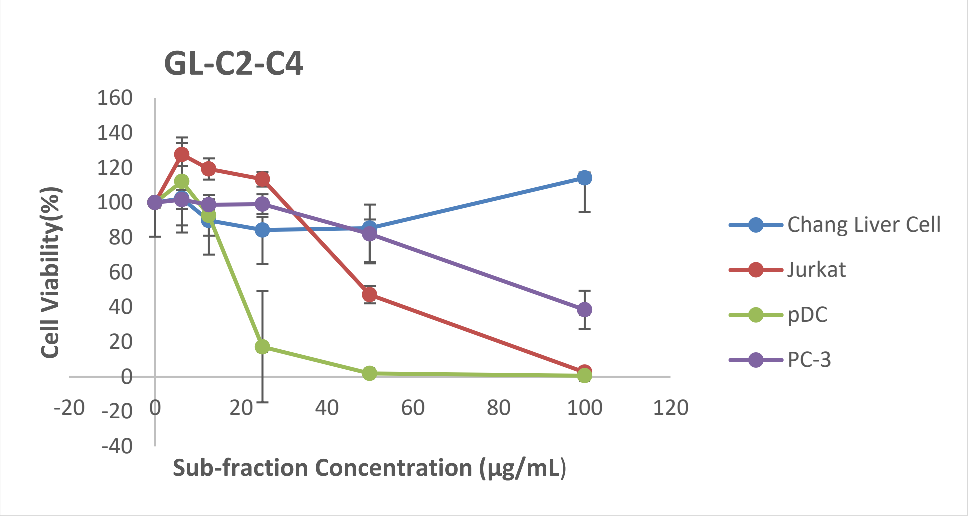

Figure 34. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C4 cytotoxic effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

As illustrated in **Figure 35** and **Table 8**, the subfraction **GL-C2-C5** in a manner similar to GL-C2-C4, demonstrated a strong cytotoxic effect against pDC with IC_{50} value of 13.57 ± 2.14 µg/ml and SI = 7.37 but a moderate cytotoxic effect against Jurkat with IC_{50} value of 52.83 ± 2.85 µg/mL and SI = 1.89. As shown, the subfraction GL-C2-C5 had no significant cy-totoxic influence on PC-3 and Chang liver cells at the concentration <100 µg/mL.

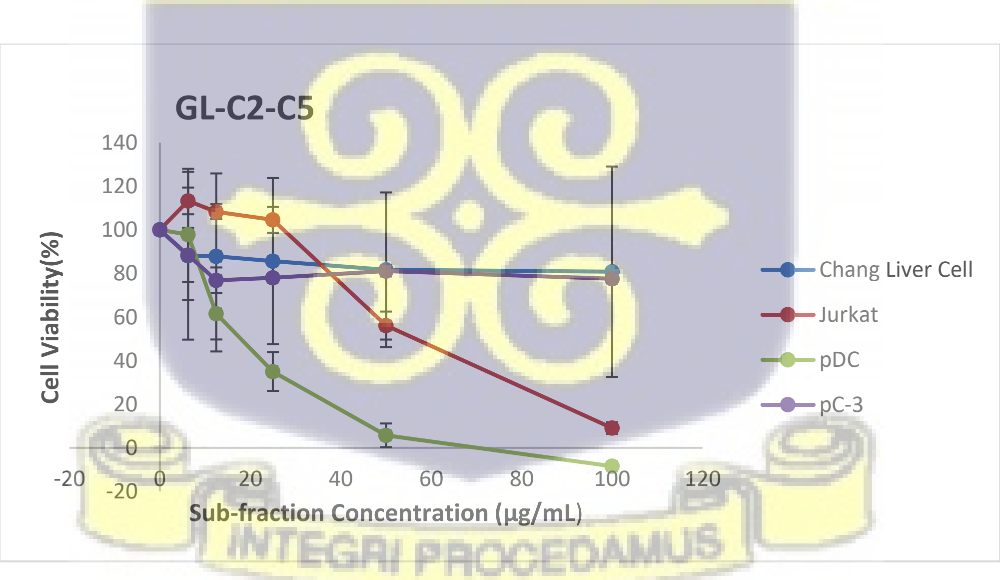

Figure 35. Mycelia biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C5 cytotoxic effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by mitochondrial activity using the MTT assay

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The subfraction **GL-C2-C6** displayed no cytotoxic effect on Chang liver cells) but a moderate cytotoxic effect against pDC with IC_{50} value of 52.83± 2.85 µg/mL and SI = 1.94 (**Figure 36** and **Table 8)**.

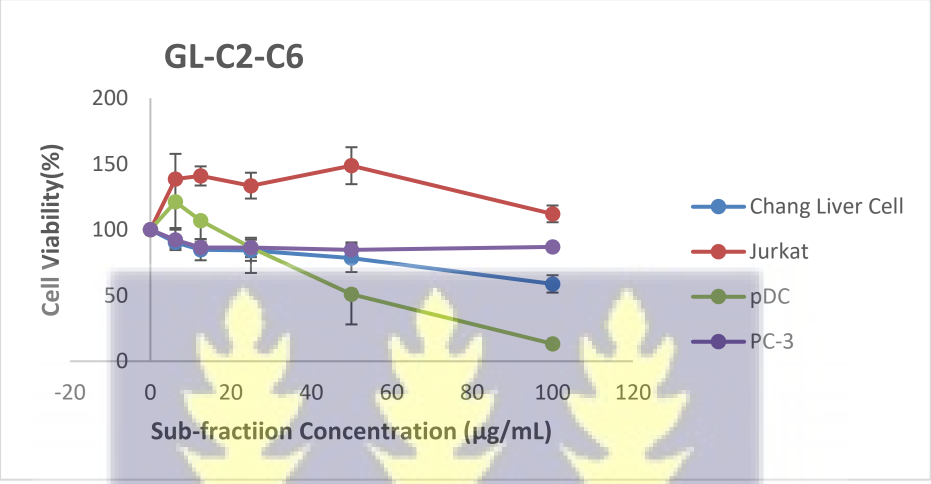

Figure 36. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C6 inhibitory effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

The subfraction **GL-C2-C7** like **GL-C2-C6** showed no cytotoxic effect against Chang liver cells, Jurkat and PC-3 but a mild cytotoxic effect against pDC, IC_{50} = 88.80±4.03 µg/mL and SI =1.12 (**Figure 37** and **Table 8**).

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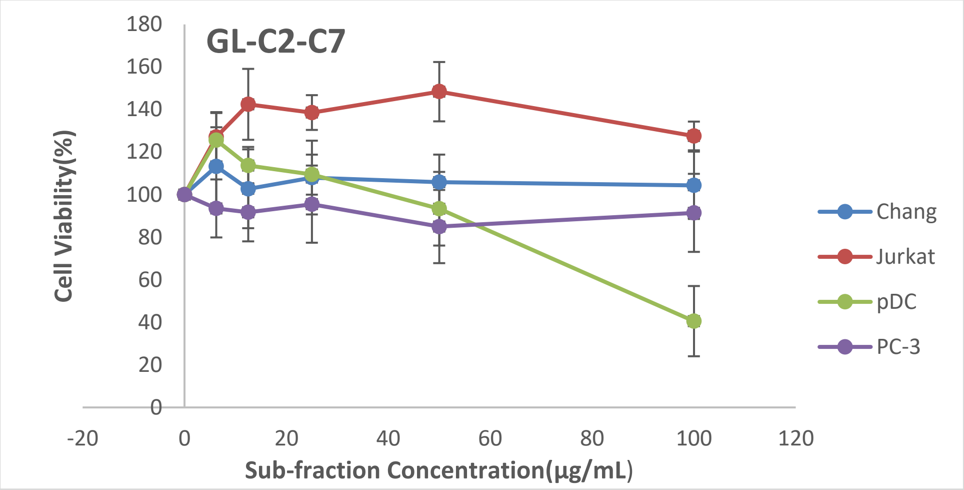

Figure 37. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C7 inhibitory effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

The subfraction **GL-C2-C8** (**Figure 38** and **Table 8**), similarly to **GL-C2-C2**, displayed no inhibitory effect on the proliferation of all the cell lines tested in this study.

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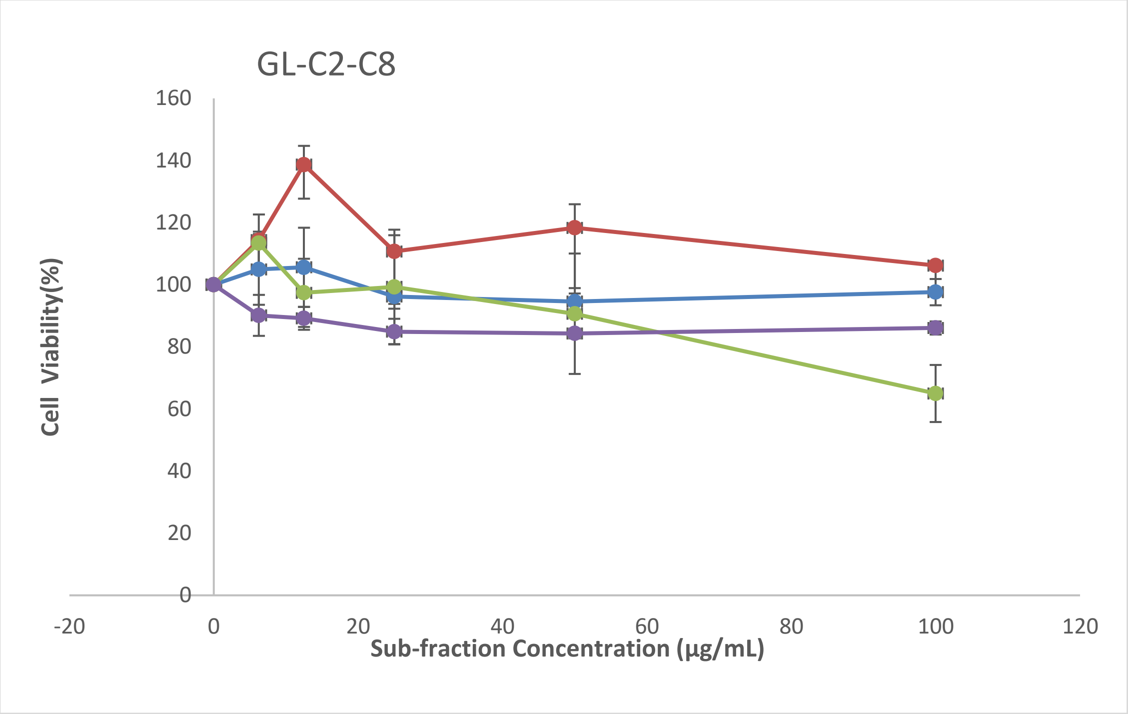

Figure 38. Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C8 inhibitory effect on Jurkat and PC-3, pDC and normal Chang liver cell lines evaluated by MTT assay.

The last subfraction **GL-C2-C9** displayed no inhibitory effect on the viability of PC-3 and Jurkat and Chang liver cell but mildly suppressed the viability of pDC. The last subfraction GL-C2-C9 displayed no cytotoxic effect against PC-3 and Jurkat and Chang liver cells but mildly suppressed the viability of pDC with IC50 of 46.18±1.21µg/mL and SI = 2.16 (**Figure 39** and **Table 6**).

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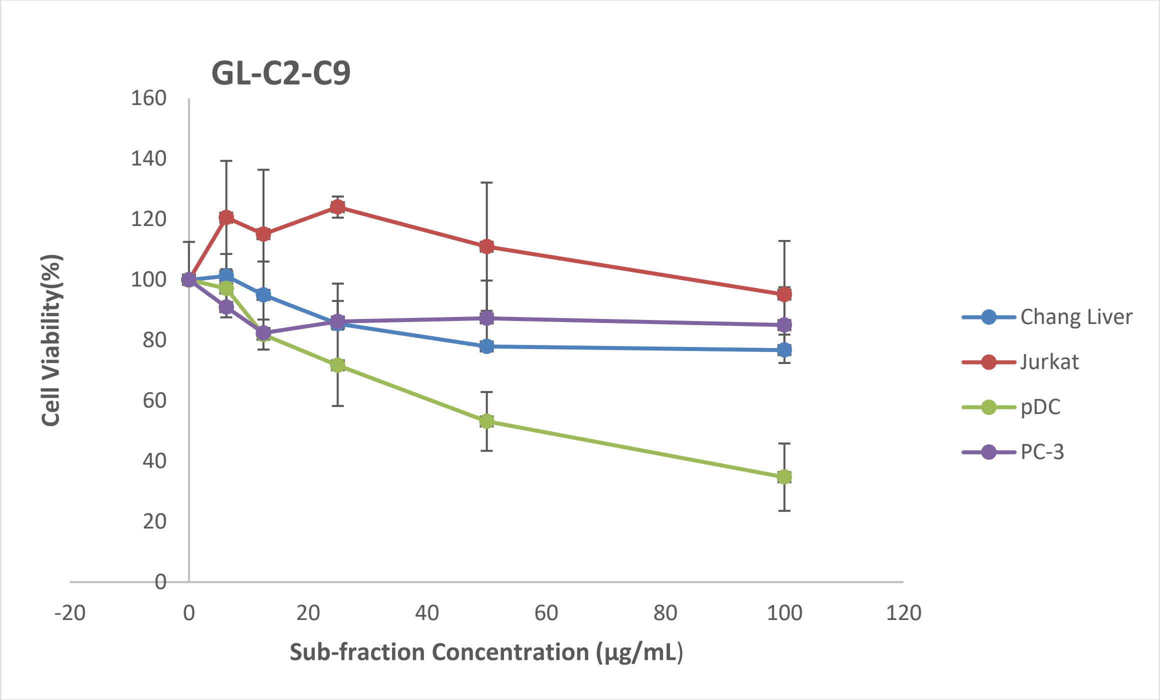

Figure 39 Mycelial biomass of *Ganoderma* LVRB-9 sub-fraction GL-C2-C8 inhibitory effect on Jurkat and PC-3, pDC and normal Chang liver cell line evaluated by MTT assay.

Table 8. Mean IC50 values and selectivity index (SI) of sub-fractions of ethanol extract of cultured *Ganoderma* LVRB-9 mycelial biomass on Jurkat, PC-3, and Chang liver cell lines

	Sub-fractions of *Ganoderma* LVRB-9 mycelial biomass ethanol extract (µg/ml)									Curcumin Control
Cell line	GL-C2-C1	GL-C2-C2	GL-C2-C3	GL-C2-C4	GL-C2-C5	GL-C2-C6	GL-C2-C7	GL-C2-C8	GL-C2-C9	(µg/mL)
Jurkat	>100 *0.00	>100 *0.00	>100 *0.00	48.14± 1.07 *2.07	52.83± 2.85 *1.89	>100 *0.00	>100 *0.00	>100 *0.00	>100 *0.00	3.78±0.52 *1.76
P-C-3	3.24± 0.10 *30.86	>100 *0.00	>100 *0.00	77.39± 2.79 *1.29	>100 *0.00	>100 *0.00	>100 *0.00	>100 *0.00	>100 *0.00	5.13± 0.86 *1.30
PMDC05	>100 *0.00	>100 *0.00	81.11±4.98 *1.23	19.95± 0.50 *5.01	13.57± 2.14 *7.37	51.39±2.38 *1.94	88.80±4.03 *1.12	>100 *0.00	46.18±1.21 *2.16	3.30± 0.07 *2.01
Chang	>100	>100	>100	>100	>100	>100	>100	>100	>100	6.65 ± 0.54

Data are presented as IC_{50} values and standard deviations by MTT assay from three independent experiments, performed in triplicate on two tumor cell lines (Jurkat and PC-3) and two other cell lines (plasmacytoid dendritic cell (pDC) and normal Chang liver cell. .* Denotes selectivitity index (SI)

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The subfraction **GL-C2-C8**, similar to the subfraction **GL-C2-C2,** failed to suppress the viability of the four cell lines tested. As shown in Table 6, it was only the subfractions **GL-C2-C-4** and **GL-C2-C5** that demonstrated inhibitory effect against Jurkat with IC_{50} value 48.14± 1.07 µg/mL and IC_{50} = 52.83± 2.85 µg/mL respectively. Apart from these two subfractions, the remaining seven out of the nine subfractions displayed no inhibitory effects on Jurkat cell. Similarly, as summarized in **Table 6**, it was only subfractions **GL-C2-C1** and **GL-C2-C4** that displayed inhibitory effect against the viability of PC-3 with IC_{50} = 3.24± 0.10 µg/ml and IC_{50} = 77.39 ± 2.79 µg/ml, respectively. As shown the highest cytotoxic effect on PC-3 was displayed by the subfraction **GL-C2-C1**. The IC_{50} value of **GL-C2-C1** (= 3.24± 0.10 µg/ml) was almost half lower than curcumin, IC_{50} = 5.13± 0.86 µg/ml, indicating **GL-C2-C1** has the ability to inhibit the viability of PC3 far more than curcumin. It is interesting to note that the IC_{50} value of **GL-C2-C1** for Chang liver cell was >100 but the IC_{50} value of curcumin against this normal liver cell was 8.12 µg/ml. This finding suggests the subfraction **GL-C2-C1** was selectively 12 times less toxic to the normal Chang liver cell compared to curcumin, the positive control. Considering the markedly profound cytotoxic effect of the subfractions **GL-C2-C1** against PC-3 and the strong cytotoxic effect of **GL-C2-C4** and **GL-C2-C5** on pDC and their low cytotoxic effects on normal Chang liver cell, it will be interesting to identify the bioactive compounds responsible for the growth inhibitory effects observed in this study.

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CHAPTER 5

DISCUSSION

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Medicinal mushrooms have been recognized as remarkable therapeutic agents by humans for very long because of their health and nutritional benefits. Several recent studies have shown that medicinal mushrooms promote immune functions, enhance cardiac health, reduce risks of cancer, help balance blood sugar levels, protect against pathogenic organisms, combat allergies, reduce inflammation and support detoxification process of the body (Smith *et al.,* 2002; Halpern, 2007; Sanodiya *et al.,* 2009; Xia *et al*, 2014; Baby *et al.*, 2015 and Kao *et al.*, 2016). *Ganoderma*, a polypore mushroom, which belongs to the family *Basidiomycota*, is an example of such prominent medicinal mushrooms regarded as a disease treating panacea (Wachtel-Galor *et al.,* 2011). Boh *et al*. (2007) reported *Ganoderma* mushrooms play a key role in modern traditional medicine in China, Japan and Korea and in some African countries (Kadhila-Muandingi & Chimwamurombe, 2012) and considered, therefore, as a reservoir of potential innovative drugs. Despite the medicinal and nutritional importance of the *Ganoderma* mushrooms, knowledge pertaining to the molecular identity, metabolome, anticancer and biological activity of the species from Ghana is limited. This study was aimed at establishing the molecular identity, through phylogenetic analysis, elucidating the major secondary metabolites (lanostane-type triterpenoids) and evaluating the cytotoxic activity of *Ganoderma* mushrooms collected from the riverine Lower Volta Basin.

Previously, molecular phylogenetic analysis of the internal transcribed spacer 2 (ITS2) (Grajales *et al.* 2007; Han *et al*., 2013; Wang *et al.,* 2014; Liao, *et al*., 2015), complete internal transcribed spacer (ITS) (Zing *et al*., 2018; Luangharn *et al*., 2019; Gunnels *et al*., 2020) and nuclear large subunit (nLSU) (Luangharn *et al.*, 2019) of the nuclear ribosomal regions were used to identify various *Ganoderma* mushrooms from different geographical regions of the world.

Recently, Otto *et al*. (2016) described G. *mbrekobenum*, a wood-rotting fungus originally from two regions of Ghana (Brong Ahafo and Greater Accra) using ITS and LSU and genes and named G. *mbrekobenum* after the Ghanaian Twi word ‘mbrekoben’, which translates to reddish brown mushroom

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(Otto *et al*., 2016). A few years later, *Ganoderma mbrekobenum* was dentified in Nigeria (Ofodile *et al*., 2022) and Raisen district of Madhya Pradesh India India from lemon tree (Parihar *et al*., 2021) on the basis of ITS BLASTN search and phylogenetic analysis. In the present study, molecular phylogenetic analysis of *Ganoderma* mushrooms collected from the Lower Volta River Basin, West Africa by ITS2, ITS and nLSU showed that the isolates designated *Ganoderma* LVRB-2, collected from Degorme on a dead Acacia trunk, belongs to the species G. *mbrekobenum.* Besides these two regions, this wood-rotting fungus has not been reported from any other part of Ghana, making this current isolation from the Volta River Basin a very important observation on the occurrence of *G. mbrekonenum* in Ghana. Since G. mbrekobenum from Ghana is not well studied, there is the need to study comprehensively Ghanaian *G. mbrekobenum* species to help reveal the potential utilization in comparison with *Ganoderma* mushrooms from other geographical regions of the world.

Coetzee *et al.* (2015) studies *Ganoderma* species, including new taxa associated with root rot of the iconic Jacaranda mimosifolia in Pretoria, South Africa. Phylogenetic trees generated using DNA sequences obtained from the ITS and LSU regions of the ribosomal RNA operon.in this study confirmed the discovery of *G. enigmaticum* as a new species of *Ganoderma* sampled from Ceratonia siliqua tree. A few years ago, *G. enigmaticum* was reported in Nigeria in using internal transcribed spacer sequences (ITS1 and ITS4) (Ofodile *et al*., 2022) Molecular phylogenetic analysis of the ITS2 and nLSU nuclear ribosomal regions in this present study revealed *Ganoderma* samples 1, 14 and 16 belong to *G. enigmaticum*, which was first described from Pretoria Province, South Africa (Coetzee *et al.*, 2015). The second discovery was made in Sissala West District of Upper West (Otto *et al*., 2016) and the third from Nigeria (Ofodile *et al*., 2022). This current isolation of *G. enigmaticum* from the Lower Volta River Basin is a very useful information and represents the second new record of *G. enigmaticum* in Ghana. Han *et al.* (2013) compared the ITS and ITS2 regions and found that ITS2 was more suitable for fungal species identification. The researchers attributed the superiority of ITS2 over ITS to its short length and high

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PCR amplification efficiency. In other study, Liao *et al*. (2015) reported that *Ganoderma* species could be distinguished from each other by comparing the ITS2 sequences and RNA secondary structures.

Ganoderma resinaceum is reported in Asian traditional pharmacopoeia to be used for immunoregulation, hyperglycemia and liver disease (Chen *et al*., 2017) and in Nigeria, West Africa (Oyetayo *et al*., 2011). Náplavová *et al*. (2020) recently studied fungal specimens from Slovakia (Central Europe), morphologically identified as *G. resinaceum*. The internal transcribed spacer (ITS) regions, partial translation elongation factor (tef1-α) region and partial 25S large subunit ribosomal RNA gene (25S LSU rRNA), all based on based on analysis of DNA sequences, as well as matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF MS), new method to determine relatedness of microorganisms based on analysis of protein profiles, were used to evaluate the genetic variability of *G. resinaceum*. The molecular data in the current study reveal new unrecognized diversity in the European *G. resinaceum*, thus suggesting much higher variability within the whole *G. resinaceum* group (Hong *et al*., 2004). Phylogeny based on tef1-α and LSU gene sequence comparisons indicates that the sequences of genotype A could be more closely related to the *G. sessile* sequences from isolates from USA than to the sequences of genotype B. However, no such grouping between *G. resinaceum* and *G. sessile* sequences was observed for ITS sequences and further studies are necessary to understand the true diversity within *G. resinaceum* group (Náplavová *et al*., 2020). Previously, El-Fallal *et al*. (2015) confirmed the status of *Ganoderma* mushroom collected the North East Nile Delta, Egypt as G. *resinaceum* by analyzing the ribosomal 5.8S rRNA gene and the flanking internal transcribed spacers (ITS). In the current study, ITS and nLSU molecular phylogenetic analysis revealed that Ganoderm sample LVRB-17 clusterd with G. *resinaceum* species. The isolation of *G. resinaceum* in the present study represents the first molecular evidence of the occurrence of *G. resinaceum* in Ghana. The results of the ITS2 phylogenetic and RNA secondary structure analyses showed *Ganoderma* LVRB-2 and *Ganoderma* LVRB-17 belong to the same species, suggesting ITS2 was not able to resolve the identity of *Ganoderma* LVRB-17 in the current study. This is contrary to the report of Han *et al*. (2013) and Liao *et al*. (2015) that ITS2 is suitable for

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identification of *Ganoderma* species. Recently, Badotti *et al.* (2017) performed a large-scale analysis of Basidiomycota sequences to determine the performance of the complete ITS region and sub-regions (ITS1 and ITS2) and found that neither the complete ITS nor the sub-regions were useful in identifying 11 of the 113 Basidiomycota genera. As a result, it has been suggested that nLSU should be used alongside ITS for identifying *Ganoderma* species (Hong *et al*., 2000; Wesselink *et al*., 2002). However, other researchers argued strongly that ITS is the universal barcode for identifying fungi species.

Ganoderma sichuanense, a medicinal mushroom originally described from China has been widely used as traditional medicine in Asia because of its potential nutritional and therapeutic values. Thawthong *et al*. (2017) collected 8 specimens of *Ganoderma* species from Thailand and analysis of ITS sequence data confirm that the collections from Thailand belong to *G. sichuanense*. The authors reported this is the first discovery of the species in Thailand and recommended that the study of more collections of this species is needed to help estimate better the variability of this taxon. Phylogenetic studies on the genus *Ganoderma* revealed that the correct name for widely cultivated *Ganoderma* species in China is *G. sichuanense*, and *G. lingzhi* is a later synonym (Zhou *et al*., 2023) although other earlier authors rejected this position and indicated that the name *G. lingzhi* was based on the unwarranted ITS sequence claimed to be of the holotype of G. sichuanense (Yao *et al*., 2020). As a result, some authors recently proposed that G. *lucidum* from China be renamed as *G. sichuanense* or *G. lingzhi*. In another interesting, related study, phylogenetic analysis using the internal transcribed spacer region rDNA sequences of the *Ganoderma* species showed that all Korean 'G. *lucidum*' strains clustered with *G. sichuanense* and *G. lingzhi* from China but strains from Europe and North American, considered as true G. *lucidum*, clustered in a clearly different group. Based on these results, the authors proposed that the Korean cultivated strains of *'G. lucidum'* should be renamed as *G. lingzhi* (Known *et al*., 2016).

Recently, airborne transmission of COVID-19 has drawn immense attention to bioaerosols. As a result, knowledge of the microbial communities in the major hospitals dealing with COVID patients is needed to enable precautionary measures to be taken prevent hospital-mediated outbreak and better assess

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occupational exposure of the healthcare workers (Habibi *et al*., 2022). Recently, the baseline of the bacterial and fungal population of two major hospitals dealing with COVID patients in Kuwait was studied through targeted amplicon sequencing and the fungi recorded in the indoor air of the hospitals include *Ganoderma scichuanese* along with several other pathogens (Variovorax (9.44%), Parvibaculum (8.27%), Pseudonocardia (8.04%), Taonella (5.74%), Arthrospira (4.58%), Comamonas (3.84%), Methylibium (3.13%), Sphingobium (4.46%), Zoogloea (2.20%), and Sphingopyxis (2.56%). The findings, therefore, highlight the need for regular surveillance of indoor hospital air. Kinge *et al*. (2012) reported *G. weberianum* as new record from Cameroon based on analysis of the ITS nuclear ribosomal regions. In the present study, molecular phylogenetic analysis of ITS nuclear ribosomal region demonstrated that *Ganoderma* LVRB-9, collected from St. Kizito campus, clustered with both G. *sichuanes*e and *G. weberianum*, indicating this particular *Ganoderma* mushroom sample belongs to the species *G. weberianum-sicuanese*. The isolation of *G. weberianum-sichuanese* in the present study is very important since it represents the first molecular evidence of the occurrence of *G. weberianum-sichuanese* species complex in Ghana. Recently, the mitochondria small subunit (mtSSU) and β-tubulin genes were used to distinguish fungal isolates which belong to the genera *Ganoderma*taceae. Malarvizhi (2014), for example, reported the β-tubulin gene could provide more robust phylogenetic information for separating fungal species than nLSU and ITS. In this current study, mtSSU and β-tubulin were not employed in molecular phylogenetic analysis of the *Ganoderma* samples because of logistic constraints. The two multilocus genes could be explored in a future study to further confirm the identity *Ganoderma* sample LVRB-9.

In summary, the molecular and phylogenetic analysis in the present study revealed that the *Ganoderma* muhrooms collected in the study belong to four known species, namely *Ganoderma mbrekobenum*, *Ganoderma enigmaticum*, *Ganoderma. weberianum-sichuanese* and *Ganoderma resinaceum*. The current study has provided important information that will be useful for future studies regarding the molecular evolution, biomedical implications and phytopathogenic significance of ganodema isolates in Ghana.

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Since *Ganoderma* mushrooms are being taken orally in powder, extract, or modern pill form, there is the need know the composition of the compounds ingested and how they work. There is also the need to evaluate how this medically important fungus response to various forms of stress in its natural environment. Liquid chromatography–mass spectrometry (LC–MS)-based metabolomics has been used widely not only to characterize and standardize compounds in *Ganoderma* mushrooms but also to rapidly differentiate the species (Zhao *et al*., 2018; Satria *et al*., 2019). Meng *et al*. (2022), for example, combined transcriptomics and nontargeted metabolomics analysis to evaluate the effect of exogenous ethylene on the production of endogenous ethylene and ganoderic acid in *G. lucidum*. The transcriptomics and nontargeted metabolomics data revealed that genes involved in the tricarboxylic acid (TCA) cycle, polyamine metabolic pathway, acetyl-CoA carboxylase (ACC) pathway, and triterpenoid metabolism were up-regulated, but the metabolic intermediates involved in these metabolic pathways were down-regulated. This clearly indicates that ethylene can potentially accelerate normal glucose metabolism by increasing the number of intermediates available for downstream biological processes such as polyamine metabolism, ethylene synthesis pathway, and ganoderic acid biosynthesis. In a similar study, Jiang *et al*. (2019) employed integrated proteomics and metabolomics analysis to evaluate the respobse of methyl jasmonate, plant hormone, on ganoderic acid biosynthesis. The results of this study revealed that methyl jasmonate treatment lead to metabolic rearrangement that inhibit the normal glucose metabolism, energy supply, and protein synthesis of cells but promoted production secondary metabolites, including ganoderic acids. This study confirmed ganoderic acid biosynthesis promoting effect and, therefore, provided the basis for further investigation of the molecular mechanisms of methyl jasmonate signal response and ganoderic acid biosynthesis. Liu *et al*. (2023), on the other hand, studied the effects of fermentation on the metabolites of *Ganoderma* under different pineapple leaf residue treatments and the composition of the compounds produced identified using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Multivariate analyses (principal component analysis (PCA), orthogonal least squares

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discriminant analysis (OPLS-DA), and volcano plots (VP)) of the spectra data revealed that the metabolites exhibit significant differences ($p < 0.05$) and were well clustered under various pineapple leaf residue treatments. It was observed that amino acids such as histidine and lysine were upregulated, whereas tyrosine, valine, L-alanine, and L-asparagine were downregulated.

The isolation and characterization of individual lanostane triterpernoids in *Ganoderma* is known to be laborious, time-consuming steps, and requires commercial reference standards which are not readily available in most laboratories. To address this challenge, Qi *et al*. (2012) developed a rapid and confirmatory method to identify ganoderic acids in *Ganoderma* mushrooms. This rapid and confirmatory method is easily performed by running LC–MS analysis on the tests samples and then comparing the retention times and mass spectra of the test sample with the reference standards of the 14 ganoderic acids published in their work. This method has been adopted by some laboratories and extraction factories to examine the quality and quantity of the triterpene composition in incoming mushrooms, crude extracts, finished goods, and competitor products (Qi *et al.*, 2012). Although this method could be a useful tool for researchers in future studies, its use is limited to the identification of the 14 compounds reported in their study. This development, therefore, calls for other identification methods. In this study, LC-MS metabolomic approach combined multivariate statistical analysis was used to to characterize and differentiate between the metabolites present in mycelia biomass of three *Ganoderma* samples collected from various locations along the bank of the Lower Volta River. The generated LC-MS mass spectral of the chemical constituents of the mycelia biomass served as a fingerprint for characterization and differentiation of the three *Ganoderma* mushrooms. Total ion chromatogram (TIC) revealed markedly significant diffrences in the metabolite profile of the three *Ganoderma* mycelia biomass. The result further revealed that LC-MS could be used to differentiate between the three *Ganoderma* mushrooms based on their mass spectra data. The mycelia biomass of three *Ganoderma* mushrooms was further characterized by PLS-DA technique. The resultant score scatter plot showed that the three *Ganoderma* mycelia biomass were separated into three clear clusters; confirming that they could be could be

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distinguished from each other based on the PLS-DA score scatter plot analysis of their spectral data This finding showed positive support to the current molecular phylogenetic study, which indicated that the three *Ganoderma* mycelia biomass belong to three different species, which are *Ganoderma enigmaticum*, *Ganoderma resinaceum* and *Ganoderma weberanium-sichuanese* species. Heat map analysis in the current study clarified further significantly the differences between the three *Ganoderma* mycelia biomass in terms of their metabolite constituents, suggesting they may have different biological activities.

Previous research revealed LC-MS could be used to characterize the metabolite consituents during the various developmental stages of *Ganoderma* species. Satria *et al.* (2019) studied metabolites in *G. lingzhi* at different developmental stages using ion trap-time-of-flight MS coupled with liquid chromatography (LC-IT-TOF-MS). The results revealed lanostane triterpenoids were the main metabolite constituents at the primordial stage. The lanostane triterpenoids detected in the study were Ganoderic acid A, Ganoderic acid C2, Ganoderenic acid C, Ganoderic acid K, Ganoderenic acid A, ganoderic acid H, and Ganoderenic acid D and several other metabolites that could not be identified. It was observed that the level of the lanostane triterpenoids decreased as the mushroom matured, making the researchers in the study to conclude that *Ganoderma* matured muhroom fruit bodies are not always best for top quality *Ganoderma* muhroom product development. Chen *et al.* (2012) previously observed that triterpenoid content of *G. lucidum*was extremely low in cultured aerial mycelia but was markedly increased in the primordia and reduced during the fruiting body formation. The above observations suggest that harvesting time of *Ganoderma* mushroom may depend on the intended purpose of the specific product to be developed. Zhao *et al*. (2018) in an interesting HPLC, EI-MS and NMR-based metabolomic study identified, ganoderenic acid A, ganoderenic acid D, ganoderenic acid G, ganoderic acid A, ganoderic acid B, ganoderic acid C2, , ganoderic acid D, ganoderic acid H, ganoderic acid Y, kaemferol, genistein and ergosterol were identified from *G. lucidiun*.

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Correct identification of cultivation sources is important for proper quality assurance but nost often, dependent on subjective morphological examinations. This calls for the need to develop efficient ways for discriminating the cultivation sources of *Ganoderma* mushrooms. Xxx collected *Ganoderma* samples from Korea and China and subjected them to NMR-based metabolomics and multivariate statistical analysis. The principal component analysis in this study showed some overlaps but the orthogonal projections to latent structure discriminant analysis (OPLS-DA) provided clear distinction between samples from the two countries, indicating NMR-based approach could contribute to addressing the important aspect of quality control process of *Ganoderma* mushrooms and cane easily be applied to other herbal medical products (Wen *et al*., 2010)

Mushroom mycelia biomass refers to a combination of mycelia and young fruiting bodies called primordia before the mushroom blooms. Biochemically, mushroom mycelial biomass contains nutrients and bioactive compounds such as β-glucans, secondary metabolites and functional enzymes (Barros *et al*., 2016). The mycelial extracts from a Thai wild mushroom identified as *Ganoderma* australe were analysed using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) and the presence of lovastatin and tentative compounds including p-coumaric, nicotinamide, gamma-aminobutyric acid, choline, nucleosides, amino acids, and saccharides were reavealed (Wongkhieo *et al*., 2023). The presence of lovastatin indicates Thailand's wild G. austral has the potential use as a functional food to prevent or alleviate hypercholesterolemia. In this current study, a total of four lanostanoid triterpenes were identified in cultured mycelia biomass of three *Ganoderma* mushrooms from Ghana by comparing their retention times and fragmentation patterns with pure reference compounds. They include ganoderenic acid A, ganoderenic acid D, ganoderic acid C6, and ganoderic acid G were identified. Besides the four triterpenoids, ganoderic acid AM1 and ganoderenic acid K were annotated as candidate chemical structures by comparing their rention time nd fragmentation pattern with metabolites reported in previously published research. Although several other unidentified metabolites with relatively high peaks were detected in *Ganoderma* sample LVRB-9, ganoderic acid G was the only compound suceesfully

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identified in this particular sample, making in *Ganoderma* sample LVRB-9 and interesting *Ganoderma* mushroom to study further.

The lanostanoid compounds present in the three *Ganoderma* mycelia biomass have been reported to be associated with several intriguing biological activities. Koyama *et al.* (1997), for example, reported that Ganoderic acid C6 has antinociceptive (pain-relieving) effect. This observation is interesting because most current pain-relieving drugs are known to have potential side effects, suggesting that the development of *Ganoderma*-based medication from in *Ganoderma* samples LVRB-1 and LVRB-17 for pain management may be of beneficial interest. This may be particular true if *Ganoderma* samples LVRB-1 and LVRB-17 display high therapeutic efficacy but with fewer or no side effects so that they can serve as a replacement for conventional analgesics such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs). Ganoderenic acid D, on the other hand, has been shown to have cytotoxic effect against human cervical, colon, and liver cancers (Ruan *et al.*, 2014). These observations indicated that *Ganoderma* samples LVRB-1 and LVRB-7 could be developed into new drugs for treating cervical, colon and liver cancers. Ganoderenic acid K, which was an annotated compound was found in two of the *Ganoderma* mycelia is known to have inhibitory activity against HMG-CoA reductase, which is a rate-limiting enzyme in hepatic cholesterol synthesis (Chen *et al.*, 2017). Yue *et al.* (2010) reported Ganoderic acid AM1, the second annotated compound in the current study is known to have cytotoxic activity against human cervical carcinoma cells. In another study, Ganoderic acid AM1, has been reported to have hepatoprotective activity (Liu *et al.*, 2014).

The genus *Amanita* is a well-known poisonous mushroom. The fatal poisoning of this mushroom is caused by α-, β- and γ-amanitin, which have inhibitory activity against RNA polymerase II. This inhibitory action blocks proteins synthesis cauing cell death. Wu *et al*. (2018) mentioned that ganoderic acid G posess hepatoprotective effects against liver injury caused by α-amanitin (α-AMA) mice, thereby reducing mortality rates, suggesting the three *Ganoderma* mycelia biomass may have protective effects

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against liver injury. The above observations suggest the three *Ganoderma* mycelia biomass may have anticancer, hepatoprotective, antihyperglycemic, antihyperlipidemic and anti-nociceptive effects because of their bioactive chemical constituents.

Angiotensin-converting enzyme (ACE) functions by converting the inactive peptide angiotensin I into active angiotensin II, increasing blood pressure through vasoconstriction. The enzymatic action of ACE also promotes sodium and water retention in the body (Al Shukor *et al*., 2013). Ganoderic acid G identified in the three *Ganoderma* mycelia biomas is known to inhibi ACE (Hai-Bang *et al*., 2015). This observation is intriguing because available evidence suggests enzyme inhibitors of ACE could reduce cardiovascular mortality and chronic kidney disease progression. This observation could explain the reason why enzyme inhibitors of ACE are being considered as a hallmark for heart failure and hypertension treatment of (Sanchis-Gomar *et al*., 2020). Elevated plasminogen activator inhibitor-1(PAI-1) levels are known to be implicated in cardiovascular diseases. Henry *et al* (2020) reported that inhibitors of ACE suppress PAI-1 level and release tissue plasminogen activators (tPA); elevate bradykinin levels and thereby prevent blood clot formation. In a clinicl study, use of ARB or ACEI was associated with lower risk of all-cause of mortality, including COVID-19 compared to without ACEI/ARB or other class of antihypertensive agent among patients with hypertension (Zhang *et al*., 2020). Since ganoderic acid G and ganoderenic acid A have been demonstrated to have inhibitory effect on angiotensin-converting enzyme (Hai-Bang *et al*., 2015), the detection in these native Lower Volta Basin *Ganoderma* mushrooms suggests that they would be useful in the treatment of hypertension nd heart failure regardless of COVID-19 and therefore worthy of investigation. Fatmawati *et al*. (2010) demonstrated that ganoderic acid C2 and ganoderenic acid A isolated the fruiting body of *Ganoderma lucidum*potently inhibit human aldose reductase in vitro and attributed inhibitory activity to the free carboxyl group because their methyl ester elicits much lower inhibitory activity. The combination of aldose reductase and ACE inhibitory activities of ganoderenic acid A suggests *Ganoderma* samples LVRB-1 and LVRB-17 may be useful as antihypertensive agent (Chalk *et al*., 2007). The above observations suggest that the three Lower Volta

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Basin *Ganoderma* mushrooms may be suitable candidates for the development of functional health supplements. Wang *et al*. (2015) demonstrated that *Ganoderma lucidum* triterpenoids (GLT) at different doses and for different time periods suppress prostate cancer cell growth by inducing growth arrest and apoptosis, suggesting triterpenoids of *Ganoderma* mushrooms could be used as a potential therapeutic drug for prostate cancer. Although the triterpenoids identified in the present study have not been reported in literature, the total triterpenoid extracts may synergistically induce cell growth and apotoptosis against human prostate cancer.

LC-MS metabolomic analysis demonstrated that the three *Ganoderma* mycelia biomas differ markedly from each other in terms of their chemical constituents as shown in the TIC, heatmap and PLS-DA score plot. The results have provided the first time ever metabolomic data on the metabolites of the mycelia biomass Lower Volta *Ganoderma* mushrooms. The main limitation of the current study is that most of secondary metabolites could not be identified because of lack of pure reference compounds. This is especially true for *Ganoderma* sample LVRB-9 which contained most the unidentified metabolites with high masss spectral peaks.

Recently, Geng *et al*. (2020) reported a broad range of bioactivities for *Ganoderma* mushrooms. The reported bioactivities include antioxidation, anti-inflammation, anti-liver disorders, antitumor growth, and metastasis. In the present study, the cytotoxic effect of extracts and fractions of mycelial biomass of *Ganoderma* LVRB-9, *Ganoderma weberianum-sichuanese* species complex isolated from the Lower Volta River Basin of Gha-na, on PC-3, Jurkat, and pDC was investigated in comparison with Chang liver cells. Curcumin, a bioactive compound from the rhizomatous herbaceous plant Curcuma longa, was used as the positive control because of its ability to inhibit the viability of many cancer cell lines, including pancreatic cancer, breast cancer, colorectal cancer, and skin cancer (Kang *et al.*, 2016). All the four cell lines (PC3, pDCs, Jurkat and Chang liver) were significantly ($p< 0.05$) inhibited by curcumin with IC_{50} values of 4.29±2.29, 1.21±0.09, 2.35±0.37 and 8.12±0.01µg/mL, respectively. Interestingly, the viability

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of pDCs was affected highest by curcumin, the positive control. Since curcumin demonstrated potent cell viability inhibitory effect on all the four cell lines, the effect of the fractions from the mycelia biomass of *Ganoderma* sample LVRB-9 was investigated. The results revealed that fraction **GL-C2** decreased the viability of pDC with increasing concentrations and with an IC_{50} value of 21.31±2.40 µg/mL. This finding suggests that **GL-C2** may be potential agent for modulating the viability and biological activities of pDCs and may be useful in treating disorders associated with pDC such as persistent virus infection (for example, HIV infection), autoimmune disease (for exmple, systemic lupus erythematosus), and cancer (for exmple, pDC-derived leukemia) as well as disorders associated with tissue accumulation of pDC (Su Lishan, 2015). The results of the present study also demonstrated that **GL-C2** significantly ($p< 0.05$) affected the viability of Jurkat and PC-3 with IC_{50} values of 17.09±0.86 and 27.73±5.25 µg/mL, respectively. The suppressive activity of GL-C2 on the viability of Jurkat is in agreement with the work of Zhang *et al*. (2005) and Gill *et al*. (2008), which demonstrated that extracts from *G. lucidum*suppressed the cell viability of Jurkat in both time and concentration-dependent manner.

As a result of the suppressive activity on the viability of PC-3, pDC, and Jurkat, the fraction **GL-C2** was further fractionated and the subfractions were tested. The results of the current study demonstrated that the viability of PC-3 was strongly suppressed by subfraction **GL-C2-C1** with an IC_{50} value of 3.24± 0.10 µg/mL compared to that of curcumin with an IC50 value of 5.13± 0.86 µg/mL. This finding suggests that **GL-C2-C1** may have a stronger anticancer activity against PC-3 compared to curcumin and could be useful in developing *Ganoderma* mushroom-based products for treating human prostate carcinoma. This current finding is consistent with the work of Jiang *et al*. (2004) which demonstrated that G.*lucidum*extract suppressed the viability of PC-3 and induced apoptosis in human prostate cancer (PC-3) cells. In another similar study, four human prostate can-cer cell lines (LNCaP, 22Rv1, PC-3, and DU-145) were treated with *G. lucidum triterpenoids* (GLT) and the results showed that GLT suppresses prostate cancer cell growth by inducing growth arrest and apoptosis (Wang *et al*., 2015). Since lanostane triterpenoids, such as Ganoderic acid G, were detected in the mycelial biomass of *Ganoderma* LVRB-9 in this present study,

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it suggests that the bioactive compounds present in **GL-C2-C1** may be similar to GLT and may explain **GL-C2-C1** growth suppressing effect of against PC-3 in the current study.

It has been documented that pDCs play a pivotal role in the pathogenesis of a number of disease conditions, which include persistent virus infection, for example HIV infection, autoimmune disease, for example systemic lupus erythematosus, and cancer for example pDC-derived leukemia as well as disorders associated with tissue accumulation of pDC (Su Lishan, 2015). It is therefore of great importance to search for biological molecules that can modulate the viability of pDCs (Hirai *et al*., 2011). The results of the present study demonstrated that subfraction **GL-C2-C4** decreases the vi-ability of pDC with increasing concentrations and with an IC_{50} value of 19.95± 0.50 µg/ml. On the other hand, subfraction **GL-C2-C5** suppressed the viability of pDC with a better IC_{50} value of 13.57± 2.14 µg/mL, indicating that **GL-C2-C5** has stronger suppressive activity on the viability of pDC than **GL-C2-C4**.

Several authors reported that pDCs infiltrate the inflamed lymphoid tissues, nasal allergic mucosa, rheumatoid arthritis synovium and skin lesions in systemic lupus erythematosus and psoriasis (Jahnsen *et al*., 2000; Rönnblom *et al*., 2003; Cavanagh *et al*., 2005) and, therefore, play an important role in the pathogenesis of these autoimmune diseases. Since pDC is the highest IFN-α and β secreting cell in the body, the remarkable suppressive activity **GL-C2-C4** and **GL-C2-C5** suggest these two subfractions may be helpful in suppressing the ability of pDCs to produce IFN-α and β., indicating that **GL-C2-C4** and **GL-C2-C5** may be instrumental in treatment of the above mentioned autoimmune or inflammatory disorders. In another study, Boichuk *et al.* (2015) reported that gut-associated pDCs in HIV patients have poor or low IFN-producing capacity; resulting thereby in increased expression of cytotoxic and proapoptotic granzyme (GZMB), which play a contributing role in gut-inflammatory disorders. Future investigation on the suppressive activity of **GL-C2-C4** and **GL-C2-C5** in controlling such pDC mediated gut inflammatory disorders in HIV patients will be very interesting.

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In conclusion, this current study demonstrated that the mycelial biomass subfraction **GL-C2-C1** possesses anticancer activity against PC-3 carcinoma cell line and could therefore be useful in developing a prostate cancer medication. Another notable finding in this study is that the subfractions **GL-C2-C4** and **GL-C2-C5** potently inhibited the viability of pDC, the highest IFN-α and β secreting cell in the body. The results have therefore provided an interesting basis for the development of **GL-C2-C4** and **GL-C2-C5** as therapeutic agents to selectively kill dysfunctional pDCs in order to restore immune function and prevent cancer cell growth. **GL-C2-C4** and **GL-C2-C5** may be good candidates for developing biopharmaceuticals for treating pDCs associated disorders where immune activation and cytokine production is over elevated. The isolation and characterization of the biologically active components responsible for the biological activity of **GL-C2-C1** in a future study could serve as a natural anticancer treatment for the prostate while the bioactive compounds from **GL-C2-C4** and **GL-C2-C5** could help explain the basis of their use in treating pDC associated disorders.

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CHAPTER 6

SIGNIFICANCE, LIMITATION, SUGGESTION AND CONCLUSION

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6.1 Significance

The results of our molecular phylogenetic study are not only consistent with earlier findings in which the occurrence of *G. mbrekobenum* and *G. enigmaticum* was reported but also provide the first ever molecular evidence of the occurrence of *G. resinaceum* and *G. weberianum sichuanese* species complex in Ghana. The current molecular phylogenetic study has provided important information that would be useful for future studies regarding the molecular evolution, biomedical implications and phytopathogenic significance of ganodema isolates in Ghana. The results of current study have also provide the first ever metabolomic data on the chemical constituents (ganoderic acid C6, ganoderenic acid A, ganoderenic acid D and ganoderic acid G, together with two annotated compounds, ganoderic acids K and AM1) of the mycelial biomass of three *Ganoderma* isolates from the Lower Volta River Basin of Ghana, thus providing insight into their biopharmaceutical potentials and unlocking their potential nutraceutical and biopharmaceutical applications. The cytotoxic study findings demonstrated that specific mycelial fractions of *Ganoderma* weberianum-sichuanese species complex are selectively cytotoxic to the three human cancer cell lines (PC-3, Jurkat and pDC) suggesting their potential efficacy in the treatment of malignancies and pDC related disorders.

6.2 Limitations

The first main limitation of the current study is the sample size is small and it limited to only the Lower Volta River Basin of Ghana. The second main limitation stems from the fact that the most of the secondary metabolites particularly the abundant ones could not be identified due to the unavailability of reference compound and the third limitation is that the biologically active components responsible for the observed biological activity couldn't be isolated and characterized due to logistic challenges.

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6.3 Suggestions

Since the current study is limited to only the Lower Volta River Basin of Ghanam, there is the need to collect *Ganoderma* mushrooms from different parts of Ghana and study the phylogenetic status to help understand not only diversities but also potential utilization in comparison with *Ganoderma* mushrooms from other geographical regions of the world. There is also the need to identify o the annotated compounds in the current liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS)-based metabolomic analysis with the aid of reference compounds. There is also the need to isolate and characterize the compounds responsible for cytotoxic activity of subfractions **GL-C2-C1**, **GL-C2-C4** and **GL-C2-C4**. Studies on other cancer cell lines as well as animal models are worthy of investigation. Similarly, studies on antimalarial, antiviral and immunomodulatory activiy among others in future are recommended.

6.4 Conclusion

In conclusion, current finding represents the first ever molecular evidence of occurrence is G. *resinaceum* and G. weberianum-sichuanese species complex in Ghana. The results provide the first ever metabolomic data on lanostane triterpenoids (Ganoderic acid G, Ganoderenic acid A, Ganoderenic acid D, Ganoderic acid C6, Ganoderenic acid K and Ganoderenic acid AM1) of the mycelial biomass of *Ganoderma* mushrooms from Ghana. The fraction **GL-C2-C1** may be useful candidate for developing a new treatment option for human prostate cancer whereas subfractions **GL-C2-C4** and **GL-C2-C4** would be useful treating pDC related disoders.

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REFERENCES

University of Ghana http://ugspace.ug.edu.gh

Ahmad M. F. (2018). *Ganoderma lucidum*: Persuasive biologically active constituents and their health endorsement. Biomedicine & pharmacotherapy Biomedecine & pharmacotherapie, 107, 507–519.

Aime, M. C., & Phillips-Mora, W. (2005). The causal agents of witches' broom and frosty pod rot of cacao (chocolate, *Theobroma cacao*) form a new lineage of Marasmiaceae. Mycologia, 97(5), 1012–1022.

Akihisa, T., Nakamura, Y., Tagata, M., Tokuda, H., Yasukawa, K., Uchiyama, E., Suzuki, T., & Kimura, Y. (2007). Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus *Ganoderma lucidum*. Chemistry & biodiversity, 4(2), 224–231.

Al Shukor, N., Van Camp, J., Gonzales, G. B., Staljanssens, D., Struijs, K., Zotti, M. J., Raes, K., & Smagghe, G. (2013). Angiotensin-converting enzyme inhibitory effects by plant phenolic compounds: a study of structure activity relationships. Journal of agricultural and food chemistry, 61(48), 11832–11839.

Aloha Medicinals. Medicinal Mushroom Products. (2019). https://alohamedicinals.com (accessed 13 July

Alonso, A., Marsal, S., & Julià, A. (2015). Analytical methods in untargeted metabolomics: state of the art in 2015. Frontiers in bioengineering and biotechnology, 3, 23.

Al-Sulaiti, H., Almaliti, J., Naman, C. B., Al Thani, A. A., & Yassine, H. M. (2023). Metabolomics Approaches for the Diagnosis, Treatment, and Better Disease Management of Viral Infections. Metabolites, 13(8), 948.

Anfossi, G., Russo, I., Massucco, P., Mattiello, L., Cavalot, F., Balbo, A., & Trovati, M. (2002). Adenosine increases human platelet levels of cGMP through nitric oxide: possible role in its antiaggregating effect. Thrombosis research, 105(1), 71–78

Baby, S., Johnson, A. J., & Govindan, B. (2015). Secondary metabolites from *Ganoderma*. Phytochemistry, 114, 66–101.

Badotti, F., de Oliveira, F. S., Garcia, C. F., Vaz, A. B., Fonseca, P. L., Nahum, L. A., Oliveira, G., & Góes-Neto, A. (2017). Effectiveness of ITS and sub-regions as DNA barcode markers for the identification of Basidiomycota (Fungi). BMC microbiology, 17(1), 42.

University of Ghana http://ugspace.ug.edu.gh

Bao, D. P., Bai, R., Gao, Y. N., Wu, Y. Y., & Wang, Y. (2018). Computational Insights into the Molecular Mechanism of the High Immunomodulatory Activity of LZ-8 Protein Isolated from the *Lingzhi* or Reishi Medicinal Mushroom *Ganoderma lucidum* (Agaricomycetes). International journal of medicinal mushrooms, 20(6), 537–548.

Bao, X. F., Wang, X. S., Dong, Q., Fang, J. N., & Li, X. Y. (2002). Structural features of immunologically active polysaccharides from *Ganoderma lucidum*. Phytochemistry, 59(2), 175–181.

Bao, X., Fang, J., & Li, X. (2001). Structural characterization and immunomodulating activity of a complex glucan from spores of *Ganoderma lucidum*. Bioscience, biotechnology, and biochemistry, 65(11), 2384–2391.

Barker, M., & Rayens, W. (2003). Partial least squares for discrimination. Journal of Chemometrics: A Journal of the Chemometrics Society, 17(3), 166-173.

Barros, A., B., Bell, V., Ferrão, J., Calabrese, V., Fernandes, T. H (2016). Mushroom Biomass: Some Clinical Implications of β-Glucans and Enzymes. Curr Res Nutr Food Sci 2016;4(Special Issue Conference October 2016.

Benkeblia, N. (2015). *Ganoderma lucidum* Polysaccharides and Terpenoids: Profile and Health Benefits. Journal of Food Nutrition and Dietetics. 1(1): 101

Bhardwaj, N., Katyal, P., & Sharma, A. K. (2014). Suppression of inflammatory and allergic responses by pharmacologically potent fungus *Ganoderma lucidum*. Recent patents on inflammation & allergy drug discovery, 8(2), 104–117.

Bin Sayeed, M. S., & Ameen, S. S. (2015). Beta-Sitosterol: A Promising but Orphan Nutraceutical to Fight Against Cancer. Nutrition and cancer, 67(8), 1214–1220.

Boh B, Berovic M, Zhang J, Zhi-Bin L (2007) *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev 13:265–301.

Boichuk, S. V., Khaiboullina, S. F., Ramazanov, B. R., Khasanova, G. R., Ivanovskaya, K. A., Nizamutdinov, E. Z., Sharafutdinov, M. R., Martynova, E. V., DeMeirleir, K. L., Hulstaert, J.,

University of Ghana http://ugspace.ug.edu.gh

Anokhin, V. A., Rizvanov, A. A., & Lombardi, V. C. (2015). Gut-Associated Plasmacytoid Dendritic Cells Display an Immature Phenotype and Upregulated Granzyme B in Subjects with HIV/AIDS. Frontiers in immunology, 6, 485.

Bouhifd, M., Hartung, T., Hogberg, H. T., Kleensang, A., & Zhao, L. (2013). Review: toxicometabolomics. Journal of applied toxicology: JAT, 33(12), 1365–1383.

Calviño, E., Manjón, J. L., Sancho, P., Tejedor, M. C., Herráez, A., & Diez, J. C. (2010). *Ganoderma lucidum* induced apoptosis in NB4 human leukemia cells: involvement of Akt and Erk. Journal of ethnopharmacology, 128(1), 71–78.

Cavanagh, L. L., Boyce, A., Smith, L., Padmanabha, J., Filgueira, L., Pietschmann, P., & Thomas, R. (2005). Rheumatoid arthritis synovium contains plasmacytoid dendritic cells. Arthritis research & therapy, 7(2), R230–R240.

Chalk C, Benstead TJ, Moore F. (2007) Aldose reductase inhibitors for the treatment of diabetic polyneuropathy. Cochrane Database of Systematic Reviews 2007, Issue 4. Art. No.: CD004572.

Chan K (2003). Some aspects of toxic contaminants in herbal medicine. Chemosphere 52: 1361-1371.

Chang, S. T., & Wasser, S. P. (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. International journal of medicinal mushrooms, 14(2), 95–134.

Chase, M., Cowan, R., Hollingsworth, P., Van den Berg, C., Madriñán, S., Petersen, G., Wilkinson, M. (2007). A Proposal for a Standardised Protocol to Barcode All Land Plants. Taxon, 56(2), 295-299.

Chen, B., Tian, J., Zhang, J., Wang, K., Liu, L., Yang, B., Bao, L., & Liu, H. (2017). Triterpenes and meroterpenes from *Ganoderma lucidum* with inhibitory activity against HMGs reductase, aldose reductase and α-glucosidase. Fitoterapia, 120, 6–16.

Chen, H. S., Tsai, Y. F., Lin, S., Lin, C. C., Khoo, K. H., Lin, C. H., & Wong, C. H. (2004). Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. Bioorganic & medicinal chemistry, 12(21), 5595–5601.

University of Ghana http://ugspace.ug.edu.gh

Chen, S., Xu, J., Liu, C., Zhu, Y., Nelson, D. R., Zhou, S., Li, C., Wang, L., Guo, X., Sun, Y., Luo, H., Li, Y., Song, J., Henrissat, B., Levasseur, A., Qian, J., Li, J., Luo, X., Shi, L., He, L., … Sun, C. (2012). Genome sequence of the model medicinal mushroom *Ganoderma lucidum*. Nature communications, 3, 913.

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. PloS one, 5(1).

Chen, W. Y., Chang, C. Y., Li, J. R., Wang, J. D., Wu, C. C., Kuan, Y. H., Liao, S. L., Wang, W. Y., & Chen, C. J. (2018). Anti-inflammatory and Neuroprotective Effects of Fungal Immunomodulatory Protein Involving Microglial Inhibition. International journal of molecular sciences, 19(11), 3678.

Chen, X. Q., Chen, L. X., Zhao, J., Tang, Y. P., & Li, S. P. (2017). Nortriterpenoids from the Fruiting Bodies of the Mushroom *Ganoderma resinaceum*. Molecules (Basel, Switzerland), 22(7), 1073.

Chen, X., Hu, Z. P., Yang, X. X., Huang, M., Gao, Y., Tang, W., Chan, S. Y., Dai, X., Ye, J., Ho, P. C., Duan, W., Yang, H. Y., Zhu, Y. Z., & Zhou, S. F. (2006). Monitoring of immune responses to a herbal immuno-modulator in patients with advanced colorectal cancer. International immunopharmacology, 6(3), 499–508.

Chen, Y. K., Kuo, Y. H., Chiang, B. H., Lo, J. M., & Sheen, L. Y. (2009). Cytotoxic activities of 9,11-dehydroergosterol peroxide and ergosterol peroxide from the fermentation mycelia of *Ganoderma lucidum* cultivated in the medium containing leguminous plants on Hep 3B cells. Journal of agricultural and food chemistry, 57(13), 5713–5719.

Chen, Y. S., Chen, Q. Z., Wang, Z. J., & Hua, C. (2019). Anti-Inflammatory and Hepatoprotective Effects of *Ganoderma lucidum* Polysaccharides against Carbon Tetrachloride-Induced Liver Injury in Kunming Mice. Pharmacology, 103(3-4), 143–150.

Chen, Y., & Lan, P. (2018). Total Syntheses and Biological Evaluation of the *Ganoderma lucidum* Alkaloids Lucidimines B and C. ACS omega, 3(3), 3471–3481.

University of Ghana http://ugspace.ug.edu.gh

Chen, Y., Zhu, S. B., Xie, M. Y., Nie, S. P., Liu, W., Li, C., Gong, X. F., & Wang, Y. X. (2008). Quality control and original discrimination of *Ganoderma lucidum* based on high-performance liquid chromatographic fingerprints and combined chemometrics methods. Analytica chimica acta, 623(2), 146–156.

Cheng, C. R., Ding, J., Yang, Y., Liang, X. Y., Guo, D. A., Yang, M., & Guan, S. H. (2016). Pharmacokinetic Studies of Ganoderic Acids from the *Lingzhi* or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes), by LC-MS/MS. International journal of medicinal mushrooms, 18(5), 405–412.

Cheng, C. R., Yue, Q. X., Wu, Z. Y., Song, X. Y., Tao, S. J., Wu, X. H., Xu, P. P., Liu, X., Guan, S. H., & Guo, D. A. (2010). Cytotoxic triterpenoids from *Ganoderma lucidum*. Phytochemistry, 71(13), 1579–1585.

Cheung, P. C. K. (2010). The nutritional and health benefits of mushrooms. Nutrition Bulletin 35(4):292 - 299

Chinese Pharmacopoeia Commission, Chinese Pharmacopoeia of the People's Republic of China, vol. 1, China Medical Science and Technology Press, Bejing, 2010, pp. 198-199.

Coetzee, M. P., Marincowitz, S., Muthelo, V. G., & Wingfield, M. J. (2015). *Ganoderma* species, including new taxa associated with root rot of the iconic Jacaranda mimosifolia in Pretoria, South Africa. IMA fungus, 6(1), 249–256.

Considine, E. C., Thomas, G., Boulesteix, A. L., Khashan, A. S., & Kenny, L. C. (2017). Critical review of reporting of the data analysis step in metabolomics. Metabolomics: Official journal of the Metabolomic Society, 14(1), 7.

Cortes, C., & Vapnik, V. (1995). Support-vector networks. Machine learning, 20, 273-297.

Cui, Y. J., Guan, S. H., Feng, L. X., Song, X. Y., Ma, C., Cheng, C. R., Wang, W. B., Wu, W. Y., Yue, Q. X., Liu, X., & Guo, D. A. (2010). Cytotoxicity of 9,11-dehydroergosterol peroxide isolated from

University of Ghana http://ugspace.ug.edu.gh

Ganoderma lucidum and its target-related proteins. Natural product communications, 5(8), 1183–1186.

Dai, S., Liu, J., Sun, X., & Wang, N. (2014). *Ganoderma lucidum* inhibits proliferation of human ovarian cancer cells by suppressing VEGF expression and up-regulating the expression of connexin 43. BMC complementary and alternative medicine, 14, 434.

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Ward, P. S., Yen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., & Su, S. M. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature, 462(7274), 739–744.

Du, Z., Li, Y., Wang, X. C., Wang, K., & Yao, Y. J. (2023). Re-Examination of the Holotype of *Ganoderma* sichuanense (*Ganoderma*taceae, Polyporales) and a Clarification of the Identity of Chinese Cultivated *Lingzhi*. Journal of fungi (Basel, Switzerland), 9(3), 323.

Dudhgaonkar, S., Thyagarajan, A., & Sliva, D. (2009). Suppression of the inflammatory response by triterpenes isolated from the mushroom *Ganoderma lucidum*. International immunopharmacology, 9(11), 1272–1280

Dudley, E., Yousef, M., Wang, Y., & Griffiths, W. J. (2010). Targeted metabolomics and mass spectrometry. Advances in protein chemistry and structural biology, 80, 45–83.

El Zawawy, N. A., & Ali, S. S. (2016). Anti-proteolytic activity of *Ganoderma lucidum* methanol extract against Pseudomonas aeruginosa. Journal of infection in developing countries, 10(9), 1020–1024.

El-Fallal, A. A., El-Sayed A. K., A., El-Esseily, S. R. (2015). First record of two *Ganoderma* species from North East Nile Delta-Egypt. Mycosphere 6(3), 248–259,

el-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T., & Otake, T. (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. Phytochemistry, 49(6), 1651–1657

University of Ghana http://ugspace.ug.edu.gh

Eo, S. K., Kim, Y. S., Lee, C. K., & Han, S. S. (2000). Possible mode of antiviral activity of acidic protein bound polysaccharide isolated from *Ganoderma lucidum* on herpes simplex viruses. Journal of ethnopharmacology, 72(3), 475–481.

Fatmawati, S., Shimizu, K., & Kondo, R. (2010). Inhibition of aldose reductase in vitro by constituents of *Ganoderma lucidum*. Planta medica, 76(15), 1691–1693.

Fernández-García, M., Rojo, D., Rey-Stolle, F., García, A., & Barbas, C. (2018). Metabolomic-Based Methods in Diagnosis and Monitoring Infection Progression. Experientia supplementum (2012), 109, 283–315.

Fuhrer, T., & Zamboni, N. (2015). High-throughput discovery metabolomics. Current opinion in biotechnology, 31, 73–78.

Fukuzawa, M., Yamaguchi, R., Hide, I., Chen, Z., Hirai, Y., Sugimoto, A., Yasuhara, T., & Nakata, Y. (2008). Possible involvement of long chain fatty acids in the spores of *Ganoderma lucidum* (Reishi Houshi) to its anti-tumor activity. Biological & pharmaceutical bulletin, 31(10), 1933–1937.

Gao, J. L., Leung, K. S., Wang, Y. T., Lai, C. M., Li, S. P., Hu, L. F., Lu, G. H., Jiang, Z. H., & Yu, Z. L. (2007). Qualitative and quantitative analyses of nucleosides and nucleobases in *Ganoderma* spp. by HPLC-DAD-MS. Journal of pharmaceutical and biomedical analysis, 44(3), 807–811.

Gao, P., Hirano, T., Chen, Z., Yasuhara, T., Nakata, Y., & Sugimoto, A. (2012). Isolation and identification of C-19 fatty acids with anti-tumor activity from the spores of *Ganoderma lucidum* (reishi mushroom). Fitoterapia, 83(3), 490–499.

Gao, Y.; Zhou, Sh.; Chen, G.; Dai, X.; Ye, J. (2002). A phase I/II study of a *Ganoderma lucidum* (Curt.:Fr.) P. Karst. extract (ganopoly) in patients with advanced cancer. Int. J. Med. Mushrooms. 4(3): 207–214.

Gardes. Bruns T.D. (1993). ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts Mol. Ecol., 2 (1993), pp. 113-118

University of Ghana http://ugspace.ug.edu.gh

Gazis, R., Rehner, S., & Chaverri, P. (2011). Species delimitation in fungal endophyte diversity studies and its implications in ecological and biogeographic inferences. Molecular ecology, 20(14), 3001–3013.

Geng, X., Zhong, D., Su, L., Lin, Z., & Yang, B. (2020). Preventive and therapeutic effect of *Ganoderma lucidum* on kidney injuries and diseases. Advances in pharmacology (San Diego, Calif.), 87, 257–276.

Gill, S. K., & Rieder, M. J. (2008). Toxicity of a traditional Chinese medicine, *Ganoderma lucidum*, in children with cancer. The Canadian journal of clinical pharmacology = Journal canadien de pharmacologie clinique, 15(2)

Girjal, U.V., Neelagund, S., & Krishnappa, M. (2011). *Ganoderma lucidum*: a source for novel bioactive lectin. Protein and peptide letters, 18(11), 1150–1157.

Gottlieb, A.M., Ferrer, E., Wright, J.E. (2000). rDNA analyses as an aid to the taxonomy of species of *Ganoderma*. Mycol. Res. 104: 1033-1045.

Grajales, A., Aguilar, C., & Sánchez, J. A. (2007). Phylogenetic reconstruction using secondary structures of Internal Transcribed Spacer 2 (ITS2, rDNA): finding the molecular and morphological gap in Caribbean gorgonian corals. BMC evolutionary biology, 7, 90.

Gunnels T, Creswell M, McFerrin J, Whittall JB (2020). The ITS region provides a reliable DNA barcode for identifying reishi/*lingzhi* (*Ganoderma*) from herbal supplements. PLoS ONE 15(11): e0236774.

Guo, S., Duan, J. A., Qian, D., Wang, H., Tang, Y., Qian, Y., Wu, D., Su, S., & Shang, E. (2013). Hydrophilic interaction ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry for determination of nucleotides, nucleosides and nucleobases in Ziziphus plants. Journal of chromatography. A, 1301, 147–155.

Gupta, S., Afaq, F., & Mukhtar, H. (2002). Involvement of nuclear factor-kappa B, Bax and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Oncogene, 21(23), 3727–3738.

University of Ghana http://ugspace.ug.edu.gh

Hai-Bang T., Shimizu K. (2015) Structure–activity relationship and inhibition pattern of reishi-derived (*Ganoderma lingzhi*) triterpenoids against angiotensin-converting enzyme. Phytochem. Lett. 2015; 12:243–247.

Halpern, G.M. (2007). Healing Mushrooms. Garden City Park (NY): Square One Publishers.

Han, J., Zhu, Y., Chen, X., Liao, B., Yao, H., Song, J., Chen, S., & Meng, F. (2013). The short ITS2 sequence serves as an efficient taxonomic sequence tag in comparison with the full-length ITS. BioMed research international, 2013, 741476.

Hapuarachchi, K. K., Karunarathna, S.C., Raspé,O., De Silva, K.H.,W.,L., Thawthong, A., Wu, X.L., Kakumyan, P., Hyde, K.D., and Wen, T.C. (2018). High diversity of *Ganoderma* and Amauroderma (*Ganoderma*taceae, Polyporales) in Hainan Island, China. Mycosphere 9(5), 931–982.

Hapuarachchi, K.K., Wen, T.C., Deng, C.Y., Kang, J.C., Hyde. K.D. (2015). Mycosphere Essays 1: Taxonomic confusion in the *Ganoderma lucidum* species complex. Mycosphere 6(5), 542–559,

He, J., Luo, Z. L., Tang, S. M., Li, Y. J., Li, S. H., & Su, H. Y. (2021). Phylogenetic analyses and morphological characters reveal two new species of *Ganoderma* from Yunnan province, China. MycoKeys, 84, 141–162.

Hennicke, F., Cheikh-Ali, Z., Liebisch, T., Maciá-Vicente, J. G., Bode, H. B., & Piepenbring, M. (2016). Distinguishing commercially grown *Ganoderma lucidum* from *Ganoderma lingzhi* from Europe and East Asia on the basis of morphology, molecular phylogeny, and triterpenic acid profiles. Phytochemistry, 127, 29–37.

Henry, B. M., Vikse, J., Benoit, S., Favaloro, E. J., & Lippi, G. (2020). Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: A novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. Clinica chimica acta; international journal of clinical chemistry, 507, 167–173. Advance online publication.

Hirai, M., Kadowaki, N., Kitawaki, T., Fujita, H., Takaori-Kondo, A., Fukui, R., Miyake, K., Maeda, T., Kamihira, S., Miyachi, Y., & Uchiyama, T. (2011). Bortezomib suppresses function and survival of

University of Ghana http://ugspace.ug.edu.gh

plasmacytoid dendritic cells by targeting intracellular trafficking of Toll-like receptors and endoplasmic reticulum homeostasis. Blood, 117(2), 500–509.

Hong, K. J., Dunn, D. M., Shen, C. L., & Pence, B. C. (2004). Effects of *Ganoderma lucidum* on apoptotic and anti-inflammatory function in HT-29 human colonic carcinoma cells. Phytotherapy research PTR, 18(9), 768–770.

Hong, S. G., & Jung, H. S. (2004). Phylogenetic analysis of *Ganoderma* based on nearly complete mitochondrial small-subunit ribosomal DNA sequences. Mycologia, 96(4), 742-755.

Hong, S. G., Jeong, W., & Jung, H. S. (2002). Amplification of mitochondrial small subunit ribosomal DNA of polypores and its potential for phylogenetic analysis. Mycologia, 94(5), 823–833.

Hong, S.G., Chun, J., Nam, J.S... Park, Y.D,. Bae. K.S. (2000). Phylogenetic analysis of genus Sporobolomyces based on partial sequences of 26S rDNA. Journal of Microbiology and Biotechnology. 10, 363-366.

Hossain, A., Radwan, F. F., Doonan, B. P., God, J. M., Zhang, L., Bell, P. D., & Haque, A. (2012). A possible crosstalk between autophagy and apoptosis in generating an immune response in melanoma. Apoptosis: an international journal on programmed cell death, 17(10), 1066–1078.

Hsin, I. L., Wang, S. C., Li, J. R., Ciou, T. C., Wu, C. H., Wu, H. M., & Ko, J. L. (2016). Immunomodulatory proteins FIP-gts and chloroquine induce caspase-independent cell death via autophagy for resensitizing cisplatin-resistant urothelial cancer cells. Phytomedicine: international journal of phytotherapy and phytopharmacology, 23(13), 1566–1573.

Huang, C. Y., Chen, J. Y., Wu, J. E., Pu, Y. S., Liu, G. Y., Pan, M. H., Huang, Y. T., Huang, A. M., Hwang, C. C., Chung, S. J., & Hour, T. C. (2010). Ling-Zhi polysaccharides potentiate cytotoxic effects of anticancer drugs against drug-resistant urothelial carcinoma cells. Journal of agricultural and food chemistry, 58(15), 8798–8805.

University of Ghana http://ugspace.ug.edu.gh

Huang, M., Mei, X., & Zhang, S. (2011). Mechanism of nitric oxide production in macrophages treated with medicinal mushroom extracts (review). International journal of medicinal mushrooms, 13(1), 1–6.

Huang, S. Q., Li, J. W., Li, Y. Q., & Wang, Z. (2011). Purification and structural characterization of a new water-soluble neutral polysaccharide GLP-F1-1 from *Ganoderma lucidum*. International journal of biological macromolecules, 48(1), 165–169.

Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England), 17(8), 754–755.

Huie, C. W., & Di, X. (2004). Chromatographic and electrophoretic methods for *Lingzhi* pharmacologically active components. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences, 812(1-2), 241–257.

Huiping, H., Yuanchao, L., Xiaowei, L., Xiangmin, L., Weipeng, M., Yizhen, X., Zhi, Z., Qingping, W. (2021). Artificial Cultivation Anti-tumor Activity of *Ganoderma* mbrekobenum. Sains Malaysiana 50(3) (2021): 723-733

Jacobson, K. A., Jarvis, M. F., & Williams, M. (2002). Purine and pyrimidine (P2) receptors as drug targets. Journal of medicinal chemistry, 45(19), 4057–4093.

Jahnsen, F. L., Lund-Johansen, F., Dunne, J. F., Farkas, L., Haye, R., & Brandtzaeg, P. (2000). Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. Journal of immunology (Baltimore, Md. 1950), 165(7), 4062–4068.

Jargalmaa, S., Eimes, J. A., Park, M. S., Park, J. Y., Oh, S. Y., & Lim, Y. W. (2017). Taxonomic evaluation of selected *Ganoderma* species and database sequence validation. PeerJ, 5, e3596.

Jiang, A. L., Liu, Y. N., Liu, R., Ren, A., Ma, H. Y., Shu, L. B., Shi, L., Zhu, J., & Zhao, M. W. (2019). Integrated Proteomics and Metabolomics Analysis Provides Insights into Ganoderic Acid Biosynthesis in Response to Methyl Jasmonate in *Ganoderma lucidum*. International journal of molecular sciences, 20(24), 6116

University of Ghana http://ugspace.ug.edu.gh

Jiang, J., Slivova, V., & Sliva, D. (2006). *Ganoderma lucidum* inhibits proliferation of human breast cancer cells by down-regulation of estrogen receptor and NF-kappaB signaling. International journal of oncology, 29(3), 695–703.

Jiang, J., Slivova, V., Valachovicova, T., Harvey, K., & Sliva, D. (2004). *Ganoderma lucidum* inhibits proliferation and induces apoptosis in human prostate cancer cells PC-3. International journal of oncology, 24(5), 1093–1099.

Jiang, Z., Jin, T., Gao, F., Liu, J., Zhong, J., Zhao, H. (2011). Effects of Ganoderic acid Me on inhibiting multidrug resistance and inducing apoptosis in multidrug resistant colon cancer cells. Process Biochemistry, 46: 1307-1314.

Jolliffe, I. T. (2002). Principal component analysis for special types of data. In: Principal Component Analysis. Springer Series in Statistics. Springer, New York, NY. (pp. 338-372).

Joseph, S., Janardhanan, K.K., George, V., & Baby, S. (2011). A new epoxidic ganoderic acid and other phytoconstituents from *Ganoderma lucidum*. Letters. 4, 386–388.

Kadhila-Muandingi, P.N., Chimwamurombe, P.M. (2012). Uses of *Ganoderma* and other mushrooms as medicine in Oshana and Ohangwena regions of Northern Namibia. Journal of Research in Agriculture, 1(2): 146-151.

Kang, D., Li, B., Luo, L., Jiang, W., Lu, Q., Rong, M., & Lai, R. (2016). Curcumin shows excellent therapeutic effect on psoriasis in mouse model. Biochimie, 123, 73–80.

Keller, A., Schleicher, T., Schultz, J., Müller, T., Dandekar, T., & Wolf, M. (2009). 5.8S-28S rRNA interaction and HMM-based ITS2 annotation. Gene, 430(1-2), 50–57.

Keypour, S., Rafati, H., Riahi, H., Mirzajani, F., Moradalic, M. F. (2010). Qualitative analysis of ganoderic acids in *Ganoderma lucidum* from Iran and China by RP-HPLC and electrospray ionisation mass spectrometry (ESI-MS). Food Chemistry, 119(4):1704-1708.

Kim, H. K., Choi, Y. H., & Verpoorte, R. (2011). NMR-based plant metabolomics: where do we stand, where do we go? Trends in biotechnology, 29(6), 267–275.

University of Ghana http://ugspace.ug.edu.gh

Kim, K.S. and Lee, Y.S. (2000). Rapid accurate species-specific detection of Phytophthora infestans through analysis of ITS regions in its rDNA. Journal of Microbiology and Biotechnology,10,652-655.

Kim, S., D. (2010). Isolation and structure determination of a cholesterol esterase inhibitor from *Ganoderma lucidum*. Journal of Microbiology and Biotechnology, 20(11):1521-3.

Kinge, T. R., Mih, A. M., Coetzee M. P. A. (2012). Phylogenetic relationships among species of *Ganoderma* (*Ganoderma*taceae, Basidiomycota) from Cameroon. Australian Journal of Botany 60(6) 526-538

Ko, H. H., Hung, C. F., Wang, J. P., & Lin, C. N. (2008). Antiinflammatory triterpenoids and steroids from *Ganoderma lucidum* and G. tsugae. Phytochemistry, 69(1), 234–239.

Kõljalg, U., Larsson, K. H., Abarenkov, K., Nilsson, R. H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F., Tedersoo, L., Vrålstad, T., & Ursing, B. M. (2005). UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. The New phytologist, 166(3), 1063–1068.

Komoda Y. (1985). Iyo Kizai Kenkyujo hokoku. Reports of the Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 19, 45–54.

Koyama, K., Imaizumi, T., Akiba, M., Kinoshita, K., Takahashi, K., Suzuki, A., Yano, S., Horie, S., Watanabe, K., & Naoi, Y. (1997). Antinociceptive components of *Ganoderma lucidum*. Planta medica, 63(3), 224–227.

Kress, W. J., & Erickson, D. L. (2007). A two-locus global DNA barcode for land plants: the coding rbcL gene complements the non-coding trnH-psbA spacer region. PloS one, 2(6), e508.

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., & Janzen, D. H. (2005). Use of DNA barcodes to identify flowering plants. Proceedings of the National Academy of Sciences of the United States of America, 102(23), 8369–8374.

University of Ghana http://ugspace.ug.edu.gh

Kubota, T.; Asaka, Y.; Miura, I.; Mori, H. (1982). Structure of Ganoderic acid A and B, two new lanostane type bitter triterpenes from *Ganoderma lucidum* (FR.) Karst. Helvetica Chimica Acta. 65: 611-619.

Kwon, O. C., Park, Y. J., Kim, H. I., Kong, W. S., Cho, J. H., & Lee, C. S. (2016). Taxonomic Position and Species Identity of the Cultivated Yeongji '*Ganoderma lucidum*' in Korea. Mycobiology, 44(1), 1–6.

Lalani, S., & Poh, C. L. (2020). Flavonoids as Antiviral Agents for Enterovirus A71 (EV-A71). Viruses, 12(2), 184.

Latiffah Z, Harikrishna K, Tan SG, Tan SH *et al*. (2002). Restriction analysis and sequencing of the ITS regions and 5.8S gene of rDNA of *Ganoderma* isolates from infected oil palm and coconut stumps in Malaysia. *Ann. Appl. Biol*. 141: 133-142.

Lee, J. S., Lim, M. O., Cho, K. Y., Cho, J. H., Chang, S. Y., & Nam, D. H. (2006). Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. Journal of microbiology (Seoul, Korea), 44(1), 29–34.

Lee, S. Y., Kim, J. S., Lee, S., & Kang, S. S. (2011). Polyoxygenated ergostane-type sterols from the liquid culture of *Ganoderma* applanatum. Natural product research, 25(14), 1304–1311.

Lee, S., Shim, S. H., Kim, J. S., Shin, K. H., & Kang, S. S. (2005). Aldose reductase inhibitors from the fruiting bodies of *Ganoderma* applanatum. Biological & pharmaceutical bulletin, 28(6), 1103–1105.

Li, C. H., Chen, P. Y., Chang, U. M., Kan, L. S., Fang, W. H., Tsai, K. S., & Lin, S. B. (2005). Ganoderic acid X, a lanostanoid triterpene, inhibits topoisomerases and induces apoptosis of cancer cells. Life sciences, 77(3), 252–265.

Li, G., Cheng, M., Nunoya, J., Cheng, L., Guo, H., Yu, H., Liu, Y. J., Su, L., & Zhang, L. (2014). Plasmacytoid dendritic cells suppress HIV-1 replication but contribute to HIV-1 induced immunopathogenesis in humanized mice. PLoS pathogens, 10(7), e1004291.

University of Ghana http://ugspace.ug.edu.gh

Li, J. R., Cheng, C. L., Yang, W. J., Yang, C. R., Ou, Y. C., Wu, M. J., & Ko, J. L. (2014). FIP-gts potentiate autophagic cell death against cisplatin-resistant urothelial cancer cells. Anticancer research, 34(6), 2973–2983.

Li, J., Wu, B., Xu, J., & Liu, C. (2014). Genome-wide identification and characterization of long intergenic non-coding RNAs in *Ganoderma lucidum*. PloS one, 9(6), e99442.

Li, N., Hu, Y. L., He, C. X., Hu, C. J., Zhou, J., Tang, G. P., & Gao, J. Q. (2010). Preparation, characterisation and anti-tumour activity of *Ganoderma lucidum* polysaccharide nanoparticles. The Journal of pharmacy and pharmacology, 62(1), 139–144.

Li, P., Deng, Y. P., Wei, X. X., & Xu, J. H. (2013). Triterpenoids from *Ganoderma lucidum* and their cytotoxic activities. Natural product research, 27(1), 17–22.

Li, W. D., Zhang, B. D., Wei, R., Liu, J. H., & Lin, Z. B. (2008). Reversal effect of *Ganoderma lucidum* polysaccharide on multidrug resistance in K562/ADM cell line. Acta pharmacologica Sinica, 29(5), 620–627.

Li, Y.F., Fang, L., & Zhang, K. (2007). Structure and bioactivities of a galactose rich extracellular polysaccharide from submergedly cultured *Ganoderma lucidum*. Carbohydrate Polymers 68(2):323-328

Liao, B., Chen, X., Han, J., Dan, Y., Wang, L., Jiao, W., Song, J., & Chen, S. (2015). Identification of commercial *Ganoderma* (*Lingzhi*) species by ITS2 sequences. Chinese medicine, 10, 22.

Lin, S. B., Li, C. H., Lee, S. S., & Kan, L. S. (2003). Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. Life sciences, 72(21), 2381–2390.

Lin, Y. L., Liang, Y. C., Lee, S. S., & Chiang, B. L. (2005). Polysaccharide purified from *Ganoderma lucidum* induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappaB and p38 mitogen-activated protein kinase pathways. Journal of leukocyte biology, 78(2), 533–543.

University of Ghana http://ugspace.ug.edu.gh

Lin, Z. B., & Zhang, H. N. (2004). Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta pharmacologica Sinica, 25(11), 1387–1395.

Lin, Z.B. (2009). *Lingzhi*: from mystery to science. Peking University Medical Press, Beijing 1–162.

Lindequist, U., Niedermeyer, T. H., & Jülich, W. D. (2005). The pharmacological potential of mushrooms. Evidence-based complementary and alternative medicine: eCAM, 2(3), 285–299..

Liu, J. Q., Lian, C. L., Hu, T. Y., Wang, C. F., Xu, Y., Xiao, L., Liu, Z. Q., Qiu, S. Q., & Cheng, B. H. (2018). Two new farnesyl phenolic compounds with anti-inflammatory activities from *Ganoderma duripora*. Food chemistry, 263, 155–162.

Liu, L. Y., Chen, H., Liu, C., Wang, H. Q., Kang, J., Li, Y., & Chen, R. Y. (2014). Triterpenoids of *Ganoderma theaecolum* and their hepatoprotective activities. Fitoterapia, 98, 254–259.

Liu, R. M., Li, Y. B., Liang, X. F., Liu, H. Z., Xiao, J. H., & Zhong, J. J. (2015). Structurally related ganoderic acids induce apoptosis in human cervical cancer HeLa cells: Involvement of oxidative stress and antioxidant protective system. Chemico-biological interactions, 240, 134–144.

Liu, W., Wang, H., Pang, X., Yao, W., & Gao, X. (2010). Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. International journal of biological macromolecules, 46(4), 451–457.

Liu, Y., Qian, Y., Wang, C., He, Y., Zhu, C., Chen, G., Lin, L., & Chen, Y. (2023). Study of the Metabolite Changes in *Ganoderma lucidum* under Pineapple Leaf Residue Stress via LC-MS/MS Coupled with a Non-Targeted Metabolomics Approach. Metabolites, 13(4), 487.

Liu, Y., Tang, Q., Zhang, J., Xia, Y., Yang, Y., Wu, D., Fan, H., & Cui, S. W. (2018). Triple helix conformation of β-d-glucan from *Ganoderma lucidum* and effect of molecular weight on its immunostimulatory activity. International journal of biological macromolecules, 114, 1064–1070.

Liu, Y., Zhang, J., Tang, Q., Yang, Y., Guo, Q., Wang, Q., Wu, D., & Cui, S. W. (2014). Physicochemical characterization of a high molecular weight bioactive β-D-glucan from the fruiting bodies of *Ganoderma lucidum*. Carbohydrate polymers, 101, 968–974.

University of Ghana http://ugspace.ug.edu.gh

Lu, Q. Y., Jin, Y. S., Zhang, Q., Zhang, Z., Heber, D., Go, V. L., Li, F. P., & Rao, J. Y. (2004). *Ganoderma lucidum* extracts inhibit growth and induce actin polymerization in bladder cancer cells in vitro. Cancer letters, 216(1), 9–20.

Luangharn, T., Karunarathna, S. C., Mortimer, P. E., Hyde, K. D., & Xu, J. (2019). Additions to the knowledge of *Ganoderma* in Thailand: *Ganoderma casuarinicola*, a new record; and *Ganoderma thailandicum* sp. nov. MycoKeys, 59, 47–65.

Ma, K., Ren, J., Han, J., Bao, L., Li, L., Yao, Y., Sun, C., Zhou, B., & Liu, H. (2014). Ganoboninketals A-C, Antiplasmodial 3,4-seco-27-Norlanostane Triterpenes from *Ganoderma boninense* Pat. Journal of natural products, 77(8), 1847–1852.

Mahadevan, S., Shah, S. L., Marrie, T. J., & Slupsky, C. M. (2008). Analysis of metabolomic data using support vector machines. Analytical chemistry, 80(19), 7562–7570.

Mahendran, S., Anandapandian, K., T., K., Shankar, T, Chellaram, C., and Vijayabaska P. (2012). Antioxidant Properties of *Ganoderma Lucidum* Crude Exopolysaccharide. Indian Journal of Innovations and Developments. Vol. 1 No. S 8.

Malarvizhi, K. (2014). Phylogenetic relationships of *Ganoderma* species based on mitochondrial and nuclear dna sequences from tamil nadu, india. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8) 2014

Meng, L. Z., Xie, J., Lv, G. P., Hu, D. J., Zhao, J., Duan, J. A., & Li, S. P. (2014). A comparative study on immunomodulatory activity of polysaccharides from two official species of *Ganoderma* (*Lingzhi*). Nutrition and cancer, 66(7), 1124–1131.

Meng, L., Zhou, R., Lin, J., Wang, Q., Wang, P., Wang, W., Wang, L., & Li, Z. (2022). Integrated Transcriptomics and Nontargeted Metabolomics Analysis Reveal Key Metabolic Pathways in *Ganoderma lucidum* in Response to Ethylene. Journal of fungi (Basel, Switzerland), 8(5), 456

Min, B. S., Gao, J. J., Hattori, M., Lee, H. K., & Kim, Y. H. (2001). Anticomplement activity of terpenoids from the spores of *Ganoderma lucidum*. Planta medica, 67(9), 811–814.

University of Ghana http://ugspace.ug.edu.gh

Mizuno, M., & Nishitani, Y. (2013). Immunomodulating compounds in Basidiomycetes. Journal of clinical biochemistry and nutrition, 52(3), 202–207.

Monard, C., Gantner, S., & Stenlid, J. (2013). Utilizing ITS1 and ITS2 to study environmental fungal diversity using pyrosequencing. FEMS microbiology ecology, 84(1), 165–175.

Moncalvo J. M. (2000) Systematics of *Ganoderma*. In: *Ganoderma* Diseases of Perennial Crops. Wallingford, UK: CAB International; 2000. pp. 23–45.

Moncalvo, J, M (2005). Molecular Systematics of *Ganoderma*: What Is Reishi? International Journal of Medicinal Mushrooms, 7(2005)353-354.

Moncalvo, J., M., Wang, H., H., Hseu, R. (1995) Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences, Mycologia, 87:2, 223-238,

Müller, C. I., Kumagai, T., O'Kelly, J., Seeram, N. P., Heber, D., & Koeffler, H. P. (2006). *Ganoderma lucidum* causes apoptosis in leukemia, lymphoma and multiple myeloma cells. Leukemia research, 30(7), 841–848.

Müller, T., Philippi, N., Dandekar, T., Schultz, J., & Wolf, M. (2007). Distinguishing species. RNA (New York, N.Y.), 13(9), 1469–1472.

Náplavová, K., Beck, T., Pristaš, P., Gáperová, S., Šebesta, M., Piknová, M., Gáper, J. (2020) Molecular Data Reveal Unrecognized Diversity in the European *Ganoderma resinaceum*. Forests 2020, 11, 850.

Nicholson, J. K., Connelly, J., Lindon, J. C., & Holmes, E. (2002). Metabonomics: a platform for studying drug toxicity and gene function. Nature reviews. Drug discovery, 1(2), 153–161.

Niedermeyer, T. H., Lindequist, U., Mentel, R., Gördes, D., Schmidt, E., Thurow, K., & Lalk, M. (2005). Antiviral Terpenoid Constituents of *Ganoderma pfeifferi*. Journal of natural products, 68(12), 1728–1731.

Nishitoba, T., H. Sato, T. Kasai, H. Kawagishi, and S. Sakamura. (1984). New bitter C27 and C30 terpenoids

University of Ghana http://ugspace.ug.edu.gh

Obodai, M., Mensah, D. L., Fernandes, Â., Kortei, N. K., Dzomeku, M., Teegarden, M., Schwartz, S. J., Barros, L., Prempeh, J., Takli, R. K., & Ferreira, I. C. (2017). Chemical Characterization and Antioxidant Potential of Wild *Ganoderma* Species from Ghana. Molecules (Basel, Switzerland), 22(2), 196.

Ofodile, L. N., Isikhuemhen, O. S., Anike, F. N., & Adekunle, A. A. (2022). The Domestication and Cultivation of *Ganoderma* (Agaricomycetes) Medicinal Mushroom Species from Nigeria. International journal of medicinal mushrooms, 24(6), 69–78.

Otto, E. C., R. A. Blanchette, C. W. Barnes and B. W. Held (2015). *Ganoderma wiiroense*, a new species of *Ganoderma* from Ghana. Persoonia (Fungal Planet Description Sheets) 35:316-317.

Otto, E.C., Blanchette, R. A., Barnes, C.W. and Held, B.W. (2015). Diversity of *Ganoderma* in Ghana. https://www.ncbi.nlm.nih.gov/nuccore/KR150679.1(accessed on 29 May 2020).

Otto, E.C.; Blanchette, R.A.; Held, B.W.; Barnes, C.W.; Obodai, M. (2016). Fungal planet 449 *Ganoderma mbrekobenum*. Pers. Mol. Phylogeny Evol. Fungi 2016, 36, 416–417.

Oyetayo, O. V. (2011). Medicinal uses of mushrooms in Nigeria: towards full and sustainable exploitation. African Journal of Traditional, Complementary and Alternative Medicines, 8(3).

Pana, D., Wang, L., Chen, C., Teng, B., Wang, C., Xu, Z., Hu, B., & Zhou, P. (2012). Structure characterization of a novel neutral polysaccharide isolated from *Ganoderma lucidum* fruiting bodies. Food chemistry, 135(3), 1097–1103.

Parihar, S. S., Sahu, S., Gupta, G., Prakash, A. (2021). *Ganoderma mbrekobenum*: A pharmacologically Important Mushroom Naturally Growing in Raisen, India. Current Trends in Biotechnology and Pharmacy Vol. 15 (4) 383 – 389.

Park, H.G., Go, H.G., Kim, S.H., and Park. W. M. (2004). Molecular identification of Asian isolates of medicinal mushroom *Hericum* erinaceum by phylogenetic analysis of nuclear ITS rDNA. Journal of Microbiology and Biotechnology, 14, 816-821.

University of Ghana http://ugspace.ug.edu.gh

Park, M. H., & Kim, M. (2018). Antioxidant and Anti-Inflammatory Activity and Cytotoxicity of Ethanol Extracts from *Rhynchosia nulubilis* Cultivated with *Ganoderma lucidum* Mycelium. Preventive nutrition and food science, 23(4), 326–334.

Paterson R. R. (2006). *Ganoderma* - a therapeutic fungal biofactory. Phytochemistry, 67(18), 1985–2001.

Patti, G. J., Yanes, O., & Siuzdak, G. (2012). Innovation: Metabolomics: the apogee of the omics trilogy. Nature reviews. Molecular cell biology, 13(4), 263–269.

Peng, X., Li, L., Wang, X., Zhu, G., Li, Z., & Qiu, M. (2016). Antioxidant farnesylated hydroquinones from *Ganoderma capense*. Fitoterapia, 111, 18–23.

Pilotti, C. A., Sanderson, F. R., Aitken, E. A., & Armstrong, W. (2004). Morphological variation and host range of two *Ganoderma* species from Papua New Guinea. Mycopathologia, 158(2), 251–265.

Qi, Y., Zhao, L., & Sun, H. H. (2012). Development of a rapid and confirmatory method to identify ganoderic acids in *Ganoderma* mushrooms. Frontiers in pharmacology, 3, 85

Qu, L., Li, S., Zhuo, Y., Chen, J., Qin, X., & Guo, G. (2017). Anticancer effect of triterpenes from *Ganoderma lucidum* in human prostate cancer cells. Oncology letters, 14(6), 7467–7472.

Rabinowitz, J. D., Purdy, J. G., Vastag, L., Shenk, T., & Koyuncu, E. (2011). Metabolomics in drug target discovery. Cold Spring Harbor symposia on quantitative biology, 76, 235–246.

Radwan, F. F., Hossain, A., God, J. M., Leaphart, N., Elvington, M., Nagarkatti, M., Tomlinson, S., & Haque, A. (2015). Reduction of myeloid-derived suppressor cells and lymphoma growth by a natural triterpenoid. Journal of cellular biochemistry, 116(1), 102–114.

Radwan, F. F., Perez, J. M., & 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, S3, 4.

Rani, P., Lal, M. R., Maheshwari, U., & Krishnan, S. (2015). Antioxidant Potential of *Lingzhi* or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) Cultivated on Artocarpus

University of Ghana http://ugspace.ug.edu.gh

heterophyllus Sawdust Substrate in India. International journal of medicinal mushrooms, 17(12), 1171–1177.

Richter, C., Wittstein, K., Kirk, P., Stadler, M. (2015). An assessment of the taxonomy and chemotaxonomy of *Ganoderma*. Fungal Diversity 71, 1–15.

Rios-Fuller, T. J., Ortiz-Soto, G., Lacourt-Ventura, M., Maldonado-Martinez, G., Cubano, L. A., Schneider, R. J., & Martinez-Montemayor, M. M. (2018). *Ganoderma lucidum* extract (GLE) impairs breast cancer stem cells by targeting the STAT3 pathway. Oncotarget, 9(89), 35907–35921.

Rönnblom, L., & Alm, G. V. (2003). Systemic lupus erythematosus and the type I interferon system. Arthritis research & therapy, 5(2), 68–75.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic biology, 61(3), 539–542.

Ruan, W., Lim, A. H., Huang, L. G., & Popovich, D. G. (2014). Extraction optimisation and isolation of triterpenoids from *Ganoderma lucidum* and their effect on human carcinoma cell growth. Natural product research, 28(24), 2264–2272.

Ruan, W., Lim, A. H., Huang, L. G., & Popovich, D. G. (2014). Extraction optimisation and isolation of triterpenoids from *Ganoderma lucidum* and their effect on human carcinoma cell growth. Natural product research, 28(24), 2264–2272.

Rubel, R., Dalla Santa, H. S., Bonatto, S. J., Bello, S., Fernandes, L. C., Di Bernardi, R., Gern, J., Santos, C. A., & Soccol, C. R. (2010). Medicinal mushroom *Ganoderma lucidum* (Leyss: Fr) Karst. triggers immunomodulatory effects and reduces nitric oxide synthesis in mice. Journal of medicinal food, 13(1), 142–148.

Ryvarden, L., Johansen, I. A (1980). Preliminary polypore flora of East Africa. Oslo: Fungiflora. 636 p.

Saeidnia Soodabeh, Manayi Azadeh, Gohari Ahmad R, Abdollahi Mohammad. The Story of Beta-sitosterol- A Review. European Journal of Medicinal Plants, 4(5): 590-609.

University of Ghana http://ugspace.ug.edu.gh

Saghatelian, A., Trauger, S. A., Want, E. J., Hawkins, E. G., Siuzdak, G., & Cravatt, B. F. (2004). Assignment of endogenous substrates to enzymes by global metabolite profiling. Biochemistry, 43(45), 14332–14339.

Sánchez-Pozo, A., & Gil, A. (2002). Nucleotides as semi essential nutritional components. The British journal of nutrition, 87 Suppl 1, S135–S137.

Sanchis-Gomar, F., Lavie, C. J., Perez-Quilis, C., Henry, B. M., & Lippi, G. (2020). Angiotensin-Converting Enzyme 2 and Antihypertensives (Angiotensin Receptor Blockers and Angiotensin-Converting Enzyme Inhibitors) in Coronavirus Disease 2019. Mayo Clinic proceedings, . Advance online publication.

Sanodiya, B. S., Thakur, G. S., Baghel, R. K., Prasad, G. B., & Bisen, P. S. (2009). *Ganoderma lucidum*: a potent pharmacological macrofungus. Current pharmaceutical biotechnology, 10(8), 717–742.

Sato, N., Zhang, Q., Ma, C. M., & Hattori, M. (2009). Anti-human immunodeficiency virus-1 protease activity of new lanostane-type triterpenoids from *Ganoderma sinense*. Chemical & pharmaceutical bulletin, 57(10), 1076–1080.

Sauce, D., Larsen, M., Fastenackels, S., Pauchard, M., Ait-Mohand, H., Schneider, L., Guihot, A., Boufassa, F., Zaunders, J., Iguertsira, M., Bailey, M., Gorochov, G., Duvivier, C., Carcelain, G., Kelleher, A. D., Simon, A., Meyer, L., Costagliola, D., Deeks, S. G., Lambotte, O., … Appay, V. (2011). HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. Blood, 117(19), 5142–5151.

Scalbert, A., Brennan, L., Fiehn, O., Hankemeier, T., Kristal, B. S., van Ommen, B., Pujos-Guillot, E., Verheij, E., Wishart, D., & Wopereis, S. (2009). Mass-spectrometry-based metabolomics: limitations and recommendations for future progress with particular focus on nutrition research. Metabolomics: Official journal of the Metabolomic Society, 5(4), 435–458.

University of Ghana http://ugspace.ug.edu.gh

Schmidt, A. P., Lara, D. R., de Faria Maraschin, J., da Silveira Perla, A., & Onofre Souza, D. (2000). Guanosine and GMP prevent seizures induced by quinolinic acid in mice. Brain research, 864(1), 40–43.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Fungal Barcoding Consortium, & Fungal Barcoding Consortium Author List (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America, 109(16), 6241–6246.

Seo, H. W., Hung, T. M., Na, M., Jung, H. J., Kim, J. C., Choi, J. S., Kim, J. H., Lee, H. K., Lee, I., Bae, K., Hattori, M., & Min, B. S. (2009). Steroids and triterpenes from the fruit bodies of *Ganoderma lucidum* and their anti-complement activity. Archives of pharmacal research, 32(11), 1573–1579.

Shang, D., Li, Y., Wang, C., Wang, X., Yu, Z., & Fu, X. (2011). A novel polysaccharide from Se-enriched *Ganoderma lucidum* induces apoptosis of human breast cancer cells. Oncology reports, 25(1), 267–272.

Shi, M., Zhang, Z., & Yang, Y. (2013). Antioxidant and immunoregulatory activity of *Ganoderma lucidum* polysaccharide (GLP). Carbohydrate polymers, 95(1), 200–206.

Singh, S., K., Doshi, A., Pancholy, A., Pathak, R. (2013) Biodiversity in wood–decay macro–fungi associated with declining arid zone trees of India as revealed by nuclear rDNA analysis. European Journal of Plant Pathology, 1–10.

Sliva D. (2003). *Ganoderma lucidum* (Reishi) in cancer treatment. Integrative cancer therapies, 2(4), 358–364.

Smina, T. P., Nitha, B., Devasagayam, T. P., & Janardhanan, K. K. (2017). *Ganoderma lucidum* total triterpenes induce apoptosis in MCF-7 cells and attenuate DMBA induced mammary and skin carcinomas in experimental animals. Mutation research, 813, 45–51.

University of Ghana http://ugspace.ug.edu.gh

Smith, J.; Rowan, N.; Sullivan, R. (2002). Medicinal Mushrooms. Their Therapeutic Properties and Current Medical Usage with Special Emphasis on Cancer Treatment; Special Report Commissioned by Cancer Research UK; The University of Strathclyde in Glasgow, 2002; 256.

Stamets, P. E., Naeger, N. L., Evans, J. D., Han, J. O., Hopkins, B. K., Lopez, D., Moershel, H. M., Nally, R., Sumerlin, D., Taylor, A. W., Carris, L. M., & Sheppard, W. S. (2018). Extracts of Polypore Mushroom Mycelia Reduce Viruses in Honey Bees. Scientific reports, 8(1), 13936.

Stanley, G., Harvey, K., Slivova, V., Jiang, J., & Sliva, D. (2005). *Ganoderma lucidum* suppresses angiogenesis through the inhibition of secretion of VEGF and TGF-beta1 from prostate cancer cells. Biochemical and biophysical research communications, 330(1), 46–52.

Su Lishan (2015). Depletion of plasmacytoid dendritic cells. International Application Patent Cooperation Treaty (PCT). International Publication Number: W O 2015/095143 A1. https://patentimages.storage.googleapis.com/07/d1/15/56e82177057236/AU2014365838A1.pdf Accessed June 5, 2021

Su, C. L., Tang, C. H., Zhang, J. S., Chen, M. J., & Pan, Y. J. (2007). Acta microbiologica Sinica, 47(1), 11–16.

Sun, J., He, H., & Xie, B. J. (2004). Novel antioxidant peptides from fermented mushroom *Ganoderma lucidum*. Journal of agricultural and food chemistry, 52(21), 6646–6652.

Sun, Y. F., Xing, J. H., He, X. L., Wu, D. M., Song, C. G., Liu, S., Vlasák, J., Gates, G., Gibertoni, T. B., & Cui, B. K. (2022). Species diversity, systematic revision and molecular phylogeny of *Ganodermat*aceae (Polyporales, Basidiomycota) with an emphasis on Chinese collections. Studies in mycology, 101, 287–415.

Tan, W. C., Kuppusamy, U. R., Phan, C. W., Tan, Y. S., Raman, J., Anuar, A. M., & Sabaratnam, V. (2015). *Ganoderma* neo-japonicum Imazeki revisited: Domestication study and antioxidant properties of its basidiocarps and mycelia. Scientific reports, 5, 12515.

University of Ghana http://ugspace.ug.edu.gh

Tang, W., Liu, J. W., Zhao, W. M., Wei, D. Z., & Zhong, J. J. (2006). Ganoderic acid T from *Ganoderma lucidum* mycelia induces mitochondria mediated apoptosis in lung cancer cells. Life sciences, 80(3), 205–211.

Upton, R, (2000). American Herbal Pharmacopeia and Therapeutic Compendium: Reishi Mushroom, *Ganoderma lucidum*. Standards of Analysis, Quality Control, and Therapeutics. U.S.A. Canada: Santa Cruz; 200

Vaughan, D. E., Rouleau, J. L., Ridker, P. M., Arnold, J. M., Menapace, F. J., & Pfeffer, M. A. (1997). Effects of ramipril on plasma fibrinolytic balance in patients with acute anterior myocardial infarction. HEART Study Investigators. Circulation, 96(2), 442–447.

Wachtel-Galor, S., Yuen, J., Buswell , J.A., Benzie, I.F.F. (2011). *Ganoderma lucidum* (*Lingzhi* or Reishi): A Medicinal Mushroom. In: Benzie, I.F.F., Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 9.

Wang, D. M., Wu, S. H., & Yao, Y. J. (2014). Clarification of the concept of *Ganoderma orbiforme* with high morphological plasticity. PloS one, 9(5), e98733.

Wang, H., Yu, Q., Nie, S. P., Xiang, Q. D., Zhao, M. M., Liu, S. Y., Xie, M. Y., & Wang, S. Q. (2017). Polysaccharide purified from *Ganoderma atrum* induced activation and maturation of murine myeloid-derived dendritic cells. Food and chemical toxicology an international journal published for the British Industrial Biological Research Association, 108(Pt B), 478–485.

Wang, J. G., Ma, Z. C., Zhang, L. N., Fang, Y. P., Jiang, F. T., & Phillips, G. O. (2011). Structure and chain conformation of water-soluble heteropolysaccharides from *Ganoderma lucidum*. Carbohydrate Polymers, 86(2), 844–851.

Wang, J., Huang, Z. G., Cao, H., Wang, Y. T., Hui, P., Hoo, C., & Li, S. P. (2008). Screening of anti-platelet aggregation agents from Panax notoginseng using human platelet extraction and HPLC-DAD-ESI-MS/MS. Journal of separation science, 31(6-7), 1173–1180.

University of Ghana http://ugspace.ug.edu.gh

Wang, J., Zhang, L., Yu, Y., & Cheung, P. C. (2009). Enhancement of antitumor activities in sulfated and carboxymethylated polysaccharides of *Ganoderma lucidum*. Journal of agricultural and food chemistry, 57(22), 10565–10572.

Wang, P. Y., Zhu, X. L., & Lin, Z. B. (2012). Antitumor and Immunomodulatory Effects of Polysaccharides from Broken-Spore of *Ganoderma lucidum*. Frontiers in pharmacology, 3, 135.

Wang, T., Xie, Z. P., Huang, Z. S., Li, H., Wei, A. Y., Di, J. M., Xiao, H. J., Zhang, Z. G., Cai, L. H., Tao, X., Qi, T., Chen, D. L., & Chen, J. (2015). Total triterpenoids from *Ganoderma Lucidum* suppresses prostate cancer cell growth by inducing growth arrest and apoptosis. Journal of Huazhong University of Science and Technology. Medical sciences, 35(5), 736–741.

Wang, X. C., Xi, R. J., Li, Y., Wang, D. M., & Yao, Y. J. (2012). The species identity of the widely cultivated *Ganoderma*, 'G. *lucidum*' (Ling-zhi), in China. PloS one, 7(7), e40857.

Wang, Y. Y., Khoo, K. H., Chen, S. T., Lin, C. C., Wong, C. H., & Lin, C. H. (2002). Studies on the immuno-modulating and antitumor activities of *Ganoderma lucidum* (Reishi) polysaccharides: functional and proteomic analyses of a fucose-containing glycoprotein fraction responsible for the activities. Bioorganic & medicinal chemistry, 10(4), 1057–1062.

Wasser S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied microbiology and biotechnology, 60(3), 258–274.

Wasser S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied microbiology and biotechnology, 89(5), 1323–1332.

Wasser, S. P (2005). Reishi or Ling Zhi (*Ganoderma lucidum*). In: Coates, P.M., Blackman, M.R., Cragg, G.M., Levine, M., Moss, J., White, J.D. (eds) Encyclopedia of dietary supplements. Marcel Dekker, New York 603–622

Wasser, S.P. (2010). Medicinal mushroom science, history, current status, future trends, and unsolved problems. International Journal of Medicinal Mushrooms 12(1):1-16.

University of Ghana http://ugspace.ug.edu.gh

Welti, S., Moreau, P.A., Decock, C., Danel, C., Duhal, N., Favel, A., Courtecuisse, R. (2015). Oxygenated lanostane-type triterpenes profiling in laccate *Ganoderma* chemotaxonomy. Mycological Progress 14(7), 45.

Wen, H., Kang, S., Song, Y., Song, Y., Sung, S. H., & Park, S. (2010). Differentiation of cultivation sources of *Ganoderma lucidum* by NMR-based metabolomics approach. Phytochemical analysis: PCA, 21(1), 73–79.

Weng, C. J., Chau, C. F., Hsieh, Y. S., Yang, S. F., & Yen, G. C. (2008). Lucidenic acid inhibits PMA-induced invasion of human hepatoma cells through inactivating MAPK/ERK signal transduction pathway and reducing binding activities of NF-kappaB and AP-1. Carcinogenesis, 29(1), 147–156.

Weng, Y., Xiang, L., Matsuura, A., Zhang, Y., Huang, Q., & Qi, J. (2010). *Ganoderma*sides A and B, two novel anti-aging ergosterols from spores of a medicinal mushroom *Ganoderma lucidum* on yeast via UTH1 gene. Bioorganic & medicinal chemistry, 18(3), 999–1002.

Wesselink, J. J., De La Iglesia, B., James, S. A., Dicks, J. L., Roberts, I. N., & Rayward-Smith, V. J. (2002). Determining a unique defining DNA sequence for yeast species using hashing techniques. Bioinformatics (Oxford, England), 18(7), 1004–1010.

White, T.J. Bruns T., Lee, S., Taylor J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics M. Innis, D. Gelfand, J. Sninsky, T. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press, Orlando, Florida (1990), pp. 315-322

Williams, G.H., Stoeber, K. (2012). The cell cycle and cancer. J Pathol. 2012;226(2):352-364.

Wold, S., Sjöström, M., & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. Chemometrics and intelligent laboratory systems, 58(2), 109-130.

Wong, K. L., Chao, H. H., Chan, P., Chang, L. P., & Liu, C. F. (2004). Antioxidant activity of *Ganoderma lucidum* in acute ethanol-induced heart toxicity. Phytotherapy research: PTR, 18(12), 1024–1026.

University of Ghana http://ugspace.ug.edu.gh

Wongkhieo, S., Tangmesupphaisan, W., Siriwaseree, J., Aramsirirujiwet, Y., Wiriyajitsomboon, P., Kaewgrajang, T., Pumloifa, S., Paemanee, A., Kuaprasert, B., Choowongkomon, K., Chester, A. H., & Swainson, N. M. (2023). In vitro cholesterol lowering activity of *Ganoderma* australe mycelia based on mass spectrometry, synchrotron Fourier-transform infrared analysis and liver-spheroid bioactivity. Scientific reports, 13(1), 13619.

Wu, G. S., Guo, J. J., Bao, J. L., Li, X. W., Chen, X. P., Lu, J. J., & Wang, Y. T. (2013). Anti-cancer properties of triterpenoids isolated from *Ganoderma lucidum* - a review. Expert opinion on investigational drugs, 22(8), 981–992.

Wu, G. S., Lu, J. J., Guo, J. J., Li, Y. B., Tan, W., Dang, Y. Y., Zhong, Z. F., Xu, Z. T., Chen, X. P., & Wang, Y. T. (2012). Ganoderic acid DM, a natural triterpenoid, induces DNA damage, G1 cell cycle arrest and apoptosis in human breast cancer cells. Fitoterapia, 83(2), 408–414. .

Wu, H., Tang, S., Huang, Z., Zhou, Q., Zhang, P., & Chen, Z. (2016). Hepatoprotective Effects and Mechanisms of Action of Triterpenoids from *Lingzhi* or Reishi Medicinal Mushroom *Ganoderma lucidum* (Agaricomycetes) on α-Amanitin-Induced Liver Injury in Mice. International journal of medicinal mushrooms, 18(9), 841–850

Wu, Y., & Wang, D. (2009). A new class of natural glycopeptides with sugar moiety-dependent antioxidant activities derived from *Ganoderma lucidum* fruiting bodies. Journal of proteome research, 8(2), 436–442.

Xia, J. M., Wu, X. J., & Yuan, Y. J. (2007). Integration of wavelet transform with PCA and ANN for metabolomics data mining. Metabolomics, 3, 531-537

Xia, Q., Zhang, H., Sun, X., Zhao, H., Wu, L., Zhu, D., Yang, G., Shao, Y., Zhang, X., Mao, X., Zhang, L., & She, G. (2014). A comprehensive review of the structure elucidation and biological activity of triterpenoids from *Ganoderma* spp. Molecules (Basel, Switzerland), 19(11), 17478–17535.

Xin, H., Fang, L., Xie, J., Qi, W., Niu, Y., Yang, F., Cai, D., Zhang, Y., & Wen, Z. (2018). Identification and Quantification of Triterpenoids in *Lingzhi* or Reishi Medicinal Mushroom, *Ganoderma lucidum*

University of Ghana http://ugspace.ug.edu.gh

(Agaricomycetes), with HPLC-MS/MS Methods. International journal of medicinal mushrooms, 20(10), 919–934.

Xing, J. H., Song, J., Decock, C., Cui, K. (2016). Morphological characters and phylogenetic analysis reveal a new species within the *Ganoderma lucidum* complex from South Africa. Phytotaxa 266 (2): 115–124.

Xing, J. H., Sun, Y. F., Han, Y. L., Cui, B. K., & Dai, Y. C. (2018). Morphological and molecular identification of two new *Ganoderma* species on Casuarina equisetifolia from China. MycoKeys, (34), 93–108.

Xu, J. W., Zhao, W., & Zhong, J. J. (2010). Biotechnological production and application of ganoderic acids. Applied microbiology and biotechnology, 87(2), 457–466.

Xu, Z., Chen, X., Zhong, Z., Chen, L., & Wang, Y. (2011). *Ganoderma lucidum* polysaccharides: immunomodulation and potential anti-tumor activities. The American journal of Chinese medicine, 39(1), 15–27.

Yang, G., Yang, L., Zhuang, Y., Qian, X., & Shen, Y. (2016). *Ganoderma lucidum* polysaccharide exerts anti-tumor activity via MAPK pathways in HL-60 acute leukemia cells. Journal of receptor and signal transduction research, 36(1), 6–13.

Yang, M., Wang, X., Guan, S., Xia, J., Sun, J., Guo, H., & Guo, D. A. (2007). Analysis of triterpenoids in *Ganoderma lucidum* using liquid chromatography coupled with electrospray ionization mass spectrometry. Journal of the American Society for Mass Spectrometry, 18(5), 927–939.

Yang, Y., Zhou, H., Liu, W., Wu, J., Yue, X., Wang, J., Quan, L., Liu, H., Guo, L., Wang, Z., Lian, X., & Zhang, Q. (2018). Ganoderic acid A exerts antitumor activity against MDA-MB-231 human breast cancer cells by inhibiting the Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway. Oncology letters, 16(5), 6515–6521. .

University of Ghana http://ugspace.ug.edu.gh

Yao, Y. J., Li, Y., Du, Z., Wang, K., Wang, X. C., Kirk, P. M., & Spooner, B. M. (2020). On the Typification of *Ganoderma* sichuanense (Agaricomycetes)-the Widely Cultivated *Lingzhi* Medicinal Mushroom. International journal of medicinal mushrooms, 22(1), 45–54.

Yue, Q. X., Song, X. Y., Ma, C., Feng, L. X., Guan, S. H., Wu, W. Y., Yang, M., Jiang, B. H., Liu, X., Cui, Y. J., & Guo, D. A. (2010). Effects of triterpenes from *Ganoderma lucidum* on protein expression profile of HeLa cells. Phytomedicine: international journal of phytotherapy and phytopharmacology, 17(8-9), 606–613

Yuen, J. W., & Gohel, M. D. (2005). Anticancer effects of *Ganoderma lucidum*: a review of scientific evidence. Nutrition and cancer, 53(1), 11–17.

Zeng, P., Chen, Y., Zhang, L., & Xing, M. (2019). Ganoderma lucidum polysaccharide used for treating physical frailty in China. Progress in molecular biology and translational science, 163, 179–219.

Zhang X, Xu Z, Pei H, Chen Z, Tan X, Hu J, *et al*. (2017) Intraspecific Variation and Phylogenetic Relationships Are Revealed by ITS1 Secondary Structure Analysis and Single-Nucleotide Polymorphism in *Ganoderma lucidum*. PLoS ONE 12(1): e0169042.

Zhang, J. J., Li, Y., Zhou, T., Xu, D. P., Zhang, P., Li, S., & Li, H. B. (2016). Bioactivities and Health Benefits of Mushrooms Mainly from China. Molecules (Basel, Switzerland), 21(7), 938.

Zhang, J., Tang, Q., Zhou, C., Jia, W., Da Silva, L., Nguyen, L. D., Reutter, W., & Fan, H. (2010). GLIS, a bioactive proteoglycan fraction from *Ganoderma lucidum*, displays anti-tumour activity by increasing both humoral and cellular immune response. Life sciences, 87(19-22), 628–637.

Zhang, K., Liu, Y., Zhao, X., Tang, Q., Dernedde, J., Zhang, J., & Fan, H. (2018). Anti-inflammatory properties of GLPss58, a sulfated polysaccharide from *Ganoderma lucidum*. International journal of biological macromolecules, 107(Pt A), 486–493.

Zhang, P., Zhu, L., Cai, J., Lei, F., Qin, J. J., Xie, J., Liu, Y. M., Zhao, Y. C., Huang, X., Lin, L., Xia, M., Chen, M. M., Cheng, X., Zhang, X., Guo, D., Peng, Y., Ji, Y. X., Chen, J., She, Z. G., Wang, Y., … Li, H. (2020). Association of Inpatient Use of Angiotensin-Converting Enzyme Inhibitors and

University of Ghana http://ugspace.ug.edu.gh

Angiotensin II Receptor Blockers with Mortality Among Patients With Hypertension Hospitalized With COVID-19. Circulation research, 126(12), 1671–1681.

Zhang, W., Tao, J., Yang, X., Yang, Z., Zhang, L., Liu, H., Wu, K., & Wu, J. (2014). Antiviral effects of two *Ganoderma lucidum* triterpenoids against enterovirus 71 infection. Biochemical and biophysical research communications, 449(3), 307–312.

Zhao, R. L., & He, Y. M. (2018). Network pharmacology analysis of the anti-cancer pharmacological mechanisms of *Ganoderma lucidum* extract with experimental support using Hepa1-6-bearing C57 BL/6 mice. Journal of ethnopharmacology, 210, 287–295.

Zhao, X. R., Huo, X. K., Dong, P. P., Wang, C., Huang, S. S., Zhang, B. J., Zhang, H. L., Deng, S., Liu, K. X., & Ma, X. C. (2015). Inhibitory Effects of Highly Oxygenated Lanostane Derivatives from the Fungus *Ganoderma lucidum* on P-Glycoprotein and α-Glucosidase. Journal of natural products, 78(8), 1868–1876.

Zhu, X. L., & Lin, Z. B. (2005). Effects of *Ganoderma lucidum* polysaccharides on proliferation and cytotoxicity of cytokine-induced killer cells. Acta pharmacologica Sinica, 26(9), 1130–1137.

Zhu, X. L., Chen, A. F., & Lin, Z. B. (2007). *Ganoderma lucidum* polysaccharides enhance the function of immunological effector cells in immunosuppressed mice. Journal of ethnopharmacology, 111(2), 219–226.

Zjawiony J. K. (2004). Biologically active compounds from Aphyllophorales (polypore) fungi. Journal of natural products, 67(2), 300–310.

Zou, W., Machelon, V., Coulomb-L'Hermin, A., Borvak, J., Nome, F., Isaeva, T., Wei, S., Krzysiek, R., Durand-Gasselin, I., Gordon, A., Pustilnik, T., Curiel, D. T., Galanaud, P., Capron, F., Emilie, D., & Curiel, T. J. (2001). Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. Nature medicine, 7(12), 1339–1346.