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Ultra Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UPLC-Q-TOF-MS)-based metabolomic analysis of mycelial biomass of three *Ganoderma* isolates from the Lower Volta River Basin of Ghana

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ABSTRACT

In this work, we sought to determine the differences and/or similarities in the metabolite composition of the mycelial biomass of three ganoderma isolates (Ganoderma LVRB-1, Ganoderma LVRB-9 and Ganoderma LVRB-17) from the Lower Volta River Basin of Ghana. The cultured mycelial mass of the three isolates were subjected to DNA sequencing. BLASTn searches of the internal transcribed spacer. (ITS) sequences of the isolates were conducted in the GenBank and the data obtained subjected to ITS phylogenetic analysis. Thereafter, extracts of the cultured mycelial biomass of the three isolates were subjected to untargeted ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS)-based metabolomic analysis. A cursory examination of the total ion chromatograms of the isolates gave evidence of the differential levels of the metabolites present. Further analysis of the metabolomic data using multivariate analysis better captured these marked differences in terms of the presence and/or levels of the metabolites. Finally, four lanostane triterpenoids, namely ganoderic acid C6, ganoderenic acid A, Ganoderenic acid D and ganoderic acid G, together with two annotated compounds (ganoderic acids K and AM1) were detected in the mycelia biomass of the three ganoderma isolates from the Lower Volta River Basin of Ghana. The results provide the first ever metabolomic data on the chemical constituents of the mycelial biomass of ganoderma isolates from the Lower Volta River Basin of Ghana.

1. Introduction

Mushrooms have been consumed by humans because of their nutritional and pharmaceutical properties since time immemorial. The genus *Ganoderma* under the division of *Basidiomycota*, for example, holds an important place in food and traditional medical systems of China, Japan, Korea [1] and some African countries. In Namibia, for example, ganoderma mushrooms are reported to have been used in relieving stress when sniffed as ash mixed with tobacco, calming of nerves when put in water, used as a drink and healing of cold and flu symptoms when its smoke is inhaled and applied to infected skin to treat the wounds on children's heads [2]. In most Asian countries, *Ganoderma* popularly called "herb of spiritual potency," is regarded as a symbol of success, well-being, divine power and longevity. Taoist priests in China reportedly were adding ganoderma mushrooms to a special magic potion to attain a state of higher consciousness [3]. The State Pharmacopoeia of the People's Republic of China [4] stated ganoderma mushrooms can be used to ease the mind and this may partly explain why this biomedical fungus have been used in some ritual and cultural practices.

Several bioactive compounds have been isolated from the mycelia, fruiting body and spores of ganoderma mushrooms. These bioactive compounds of ganoderma mushrooms include triterpenoids, steroids, polysaccharides, proteins, amino acids, alkaloids, nucleosides, and nucleotides [1,5]. Extracts and preparations from

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this ancient biomedical fungus have been reported to have pharmacological activities such as anticancer, immunomodulation, anti-inflammation, antioxidation and hepatoprotection [6]. Ganoderma polysaccharides, for example, are known to exert their pharmacological activities primarily through enhanced mitogenicity and activation of some immune cells; leading to increase in the production of interferon (IFN)-γ and tumor necrosis factor (TNF-α) [7,8], enhanced cytotoxicity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells activity [8]. Ganoderma triterpenes (GTs), on the other hand, have been shown to suppress the inflammatory response in lipopolysaccharide (LPS) activated murine macrophages. GTs have been reported to suppress the secretion of inflammatory cytokines (TNF-α and IL-6), inflammatory mediator, nitric oxide (NO) and prostaglandin E2 (PGE2) from LPS-stimulated murine RAW264.7 cells. Mechanistically, GTs mediate their anti-inflammatory activity through inhibition of the transcription factor, NF-κB [9,10]. *Ganoderma* has been identified as one of the herbal anti-inflammatory agents that can suppress the secretion of inflammatory cytokines and prevent the development of diseases associated with chronic inflammation [11]. Owing to the wide range of biological activities, the commercialization of ganoderma products is increasing worldwide and are in high demands in many health shops in Asian, Western and European [12] and now in most West African countries including Ghana.

Through a mycological survey and molecular phylogenetic study, three novel ganoderma isolates have been identified from the Lower Volta River Basin of Ghana, West Africa. These isolates, however, have not been characterized for their metabolite composition and possible identification of their major bioactive secondary metabolites. There is, therefore, a need to study the metabolome of ganoderma isolates from the Lower Volta River Basin of Ghana to provide insight into their biopharmaceutical potentials and possible clinical applications. The goal of this study was to study the metabolome of mycelial biomass, (a combination of myceliaa, primordia and extracellular compounds) of three novel ganoderma isolates from the Lower Volta River Basin of Ghana to unlock their nutraceutical and biopharmaceutical potentials.

2. Materials and methods

2.1. Chemicals and reagents

Former acid (HPLC grade), methanol (HPLC grade) and acetonitrile (LC-MS grade) were purchased from Sigma–Aldrich, St. Louis, MO (USA). Ganoderenic acid A (CAS No. 100665-40-5), ganoderic acid G (CAS No. 98665-22-6), ganoderenic acid D (CAS No. 100665-43-8), ganoderic acid C6 (CAS No. 105742-76-5) were bought from Yuanye biotechnological Ltd (China). Purified water was obtained from Milli-Q water system (Millipore, USA). Aantibiotics malt extract agar (AMEA) was bought from Fungi Perfecti, LLC (USA).

2.1.1. Ganoderma tissue isolation

Freshly collected fruiting bodies of Ganoderma LVRB-1, Ganoderma LVRB-9 and Ganoderma LVRB-17 collected from the Lower Volta River Basin of Ghana were surface sterilized with 70% alcohol, cut with sterilized scalpel longitudinally and a small piece of tissue fragments was taken aseptically from the inner core of the fruiting body. The isolated tissue fragment was placed on antibiotic malt extract agar (AMEA) prepared following the manufacturer's instructions (Fungi Perfecti, LLC (USA) in a petri plate and incubated in the dark at 28 °C for 10 days. The resulting mycelium of each ganoderma isolate was transferred to malt extract agar (MEA), consisting of 2% w/v malt extract, 1.5.0% w/v agar without antibiotic and cultured for another 10 days to obtain pure mycelium of the fungal culture.

2.1.2. Production of mycelia biomass

A semi-solid medium of MEA was prepared in a petri plate and a small piece of a 10-day cultured pure mycelium of the isolate was placed in agar medium of the petri plate. This inoculated plate was incubated in the dark at 28 °C, 90% of relative humidity of air until primordial heads started forming. The mycelia biomass, a combination of mycelium, primordia and extracellular compounds, was harvested by gently scrapping from the surface of the agar plate for UPLC-Q-TOF-MS analysis.

2.1.3. Molecular identification and phylogeny of ganoderma isolates

Three fruiting bodies resembling the genus *Ganoderma* from the Lower Volta River Basin were subjected to DNA sequencing to facilitate their identification. BLASTn searches of the ITS sequences of the three isolates in comparison with the reference sequences at the GenBank of National Centre of Biotechnology Information (NCBI) were initially made to confirm that the fungal isolates were species of *Ganoderma* and the data matrices generated subjected to ITS phylogenetic analysis by Bayesian Inference (BI) approach [13] to establish their phylogenetic positions.

2.1.4. Preparation of mycelial biomass for UPLC-Q-TOF-MS analysis

A 0.02 g quantity of fresh mycelial biomass was weighed and cold macerated with 500 µL methanol (containing 0.1% v/v formic acid) at 4 °C for 48 h and ultrasonicated (100 Hz) for at 25 °C for 3 min. Each sample was then centrifuged using Eppendorf 5430 R at 9838 x g for 5 min at 4 °C and the supernatant filtered through 0.22 µm pore size sintered glass filter. The quality control sample consisted of a mixture of equal volumes of all samples (100 µL of each). A 5 µL aliquot of each mycelium biomass was injected for UPLC-Q-TOF-MS analysis.

2.1.5. UPLC-Q-TOF-MS analysis

Chromatographic separations were performed with an Agilent 1290 series (Agilent Corp., Santa Clara, CA, USA) HPLC system equipped with a binary pump, micro degasser, an autosampler and a temperature-controlled column compartment. Chromatographic separations were done on an ACQUITY UPLC HSST3 ODS column (1.8 µm, 2.1 mm × 100 mm; Waters, Ireland). The mobile phase consisted of two solvents, A and B. Mobile phase A was 0.1% formic acid water and B was acetonitrile. This mobile phase system was run in a gradient elution as follows: 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 oven temperature was set to 40 °C and the injection volume was 5 µL. Flow rate was 0.4 mL/min. Before each injection, the column was equilibrated for 5 min with 25% of phase B. The samples were injected randomly. The QC sample was initially injected three times to equilibrate the column prior to the injections of the samples and injected after every injection of the samples. Solutions of all reference compounds were subjected to same analytical conditions.

Separated components were detected with Agilent 6545 A Q-TOF mass spectrometer (Agilent Corp., Santa Clara, CA, USA) equipped with an ESI interface. For MS/MS analysis, the following operating parameters were used: drying N_2 gas flow rate, 11 L/min; temperature, 350 °C; nebulizer, 35 psig; capillary, 3000 V; skimmer, 65 V; OCT RFV, 750 V, fragmentor 175 V. An auto MS/MS was achieved in the negative ion mode in *m/z* range of 60–1000. At the scan rate of 2.0 spectra/sec using fixed collision energies (10.00, 20.00, 40 eV) MS/MS data were acquired with isolation width MS/MS medium (~4 amu).

2.1.6. Data processing

The raw LC-MS spectral data were initially transformed to the ".mzdata" format using the Agilent DA-reprocessor software (version B.06.00, Agilent Technologies). Therein, the threshold of 5000 counts was set for the peak heights. The data obtained were the run by

XCMS package (http://metlin.scripps.edu/download/) on the R platform to pretreat the data which included peak discrimination, filtering and alignment. The 80% rule was applied in the treatment of missing values. Finally, prior to multivariate analysis, the pretreated data were normalized by the relative peak abundance. The resultant data were then analyzed using R software (R 3.6.1) to construct the partial least squares-discriminate analysis (PLS-DA) and heatmap using the R packages 'vegan' and 'pheatmap' respectively. The hierarchical clustering dendrogram was constructed using the distance measure option "euclidean" and clustering algorithm, "ward" using same R software (R 3.6.1). The differences between the samples in terms of metabolite levels were captured by these three multivariate algorithms.

2.1.7. Identification of metabolites

Chemical composition of the mycelial biomass was determined on the basis of the mass spectral data (fragmentation patterns) of each metabolite with reference to relevant published literature [14–16] and reference compounds (ganoderic acid C6, ganoderenic acid A, ganoderenic acid D, and ganoderic acid G).

3. Results

3.1. Molecular identification and phylogeny of ganoderma isolates

The results of the ITS BLASTn search (Table 1) revealed that first isolate designated Ganoderma LVRB-1 had a high level of DNA sequence similarity (99.47%) with *G. enigmaticum* whereas the second the isolate labelled Ganoderma LVRB-9 had a high level of sequence similarity (98.72%) with *G. weberianum*. The third isolate designated *Ganoderma* LVRB-17 similarly had a high level of sequence similarity (99.48%) with *G. resinaceum*. Bayesian analysis of the ITS sequences revealed that Ganoderma LVRB-1 belongs to the species *G. enigmaticum* whereas Ganoderma LVRB-17 belongs to *G. resinaceum*. The third isolate designated, Ganoderma LVRB-9, however, matched with *G. weberianum* (99.24%) and *G. sichuanese* (98.73%) (Fig. 1) in the ITS BLASTn search.

3.2. Mycelial biomass metabolomic differences

To systematically compare the similarities and differences in terms of the chemical components of Ganoderma LVRB-1, Ganoderma LVRB-9 and Ganoderma LVRB-17, UPLC-Q-TOF-MS-based metabolomics approach was employed. This approach is based on the premise that identical ganoderma isolates would produce similar mass spectral features in terms of the presence and abundance of the components, whereas those of different isolates would generate different mass spectra features. The representative total ion chromatograms (TIC) of the mycelial biomass of the three ganoderma isolates are shown in Fig. 2**.**

A cursory examination of the chromatograms reveals that the mycelial biomass of the three ganoderma isolates are unambiguously different from each other. The subtle differences between them were more significantly captured in the metabolomics analyses. Partial least squares discriminate analysis (PLS-DA), a commonly used classification method for modeling the discrimination between species based on the levels of their metabolites, was employed to analyze the LC-MS data and the score plot is given in Fig. 3A. As shown, the clusters of the mycelial biomass of the ganoderma isolates are well separated in the PLS-DA plot. The clusters of Ganoderma LVRB-17 and Ganoderma LVRB-9 are located in the upper left and lower left quadrants of the PLS-DA plot, respectively, while clusters of Ganoderma LVRB-1 are located in middle of the right upper and lower quadrants of the PLS-DA plot, indicating explicit difference among the three ganoderma isolates. Also, the metabolome differences between the cultured biomass of the ganoderma isolates were captured in a heatmap (Fig. 3B). As illustrated, the ganoderma samples G1 = LVRB-1, G1.1 = LVRB-1.1, G9 = LVRB-9, G9.9 = LVRB-9.9 and G17 = LVRB-17, G17.1 = LVRB-17.1, exhibited marked variations in their secondary metabolite composition. Finally, the similarities and differences in the metabolomes of the various ganoderma isolates were captured in the hierarchical clustering dendrogram (Fig. 3C).

3.3. Identification triterpenoids

We sought to identify as much as possible the presence of triterpenoids in the cultured mycelial biomass of the three ganoderma isolates. In order to confirm or otherwise the identities of the triterpenoids, 4 compounds were identified based on the retention times and fragmentation patterns of their reference compounds but two other two triterpenoids were detected in the present study annotated with reference to relevant published literature. The structures of these compounds are illustrated in Fig. 4. Similar to previous studies, extracts of the ganoderma isolates were analyzed in the negative ion mode using electrospray ionization. This mode was found to be more sensitive and appropriate for the triterpenoids. A common pattern of the fragmentation pertained to losses of H_2O and CO_2 and

Table 1
ITS sequence of isolate (A) Ganoderma LVRB-1, (B) Ganoderma LVRB-9 and (C) Ganoderma LVRB-17 from the Lower Volta River Basin of Ghana.

Species ID	DNA sequence	Sequence similarity (%) showing species of top matching candidate
Ganoderma LVRB-1	GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTGCAACCTCTTTGCGGAGTTTGTAGGCTTGGACTTGGAGGGCTTGTCGGCCTTTAACGGTCGGCTCCTCTTAAATGCATTAGCTTGATTCCTTGCRGATCGGCTGTCGGTGTGATAAAATGTCTACGCCGTGACCGTGAAGCGTTTGGATGAGCTTCCAACCGTCTTGSTTCAAAGACAACTTTTTATGACCTCTGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA	*Ganoderma enigmaticum* voucher Ghana1a/ 93839899.49%
Ganoderma LVRB-9	CATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACGCCCTGCTCATCCACTCTACACCTGTGCACTTGCTGTGGGTTTCAAACGTCGTAAAGCGAGTCTCTTTACCGAGCTTGTAGAGCGGCGTCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATTAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCACCGATGAAGAACGCAGCGAAATGCGATAAAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCTGTTTGAGTGTCATGAAATCTTCAACTTACAGACCTTTGCA	*Ganoderma weberianum* strain CBS 12858199.24% and *Ganoderma sichuanese* 98.73%
Ganoderma LVRB-17	GTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATCGAGTTTTGACTGGGTTGTAGCTGGCCTTCCGAGGCATGTGCACACCCTGCTCATCCACTCTACACCTGTGCACTTACTGTGGGTTCCAGACGTTGTGAAGCGGGCTCTTTACGGAGCTTGTAAAGCGGCGTGCCTGTGCCTGCGTTTATCACAAACTCTATAAAGTATTAGAATGTGTATTGCGATGTAACGCATCTATATACAACTTTCAGCAACGGCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAG	*Ganoderma resinaceum* isolate F-299.48%

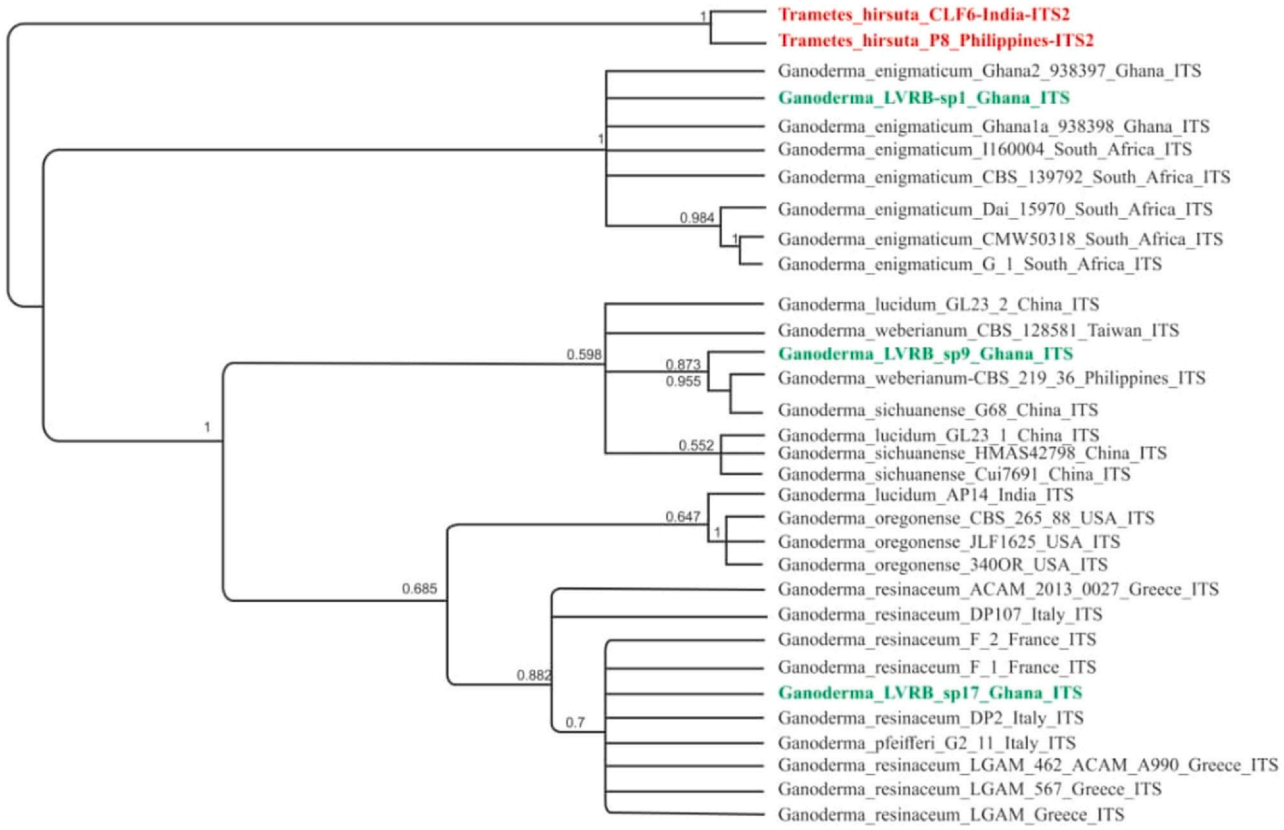

Fig. 1. Bayesian posterior probability (BPP) tree showing the phylogenetic position of *Ganoderma* collections from the Lower Volta River Basin of Ghana in comparison with reference ITS rDNA sequences at the GenBank of National Centre of Biotechnology Institute (NCBI). *Trametes hirsuta* CLF6 and *Trametes hirsuta* P8 were used as outgroups. Numbers at the branch nodes represent BPP values.

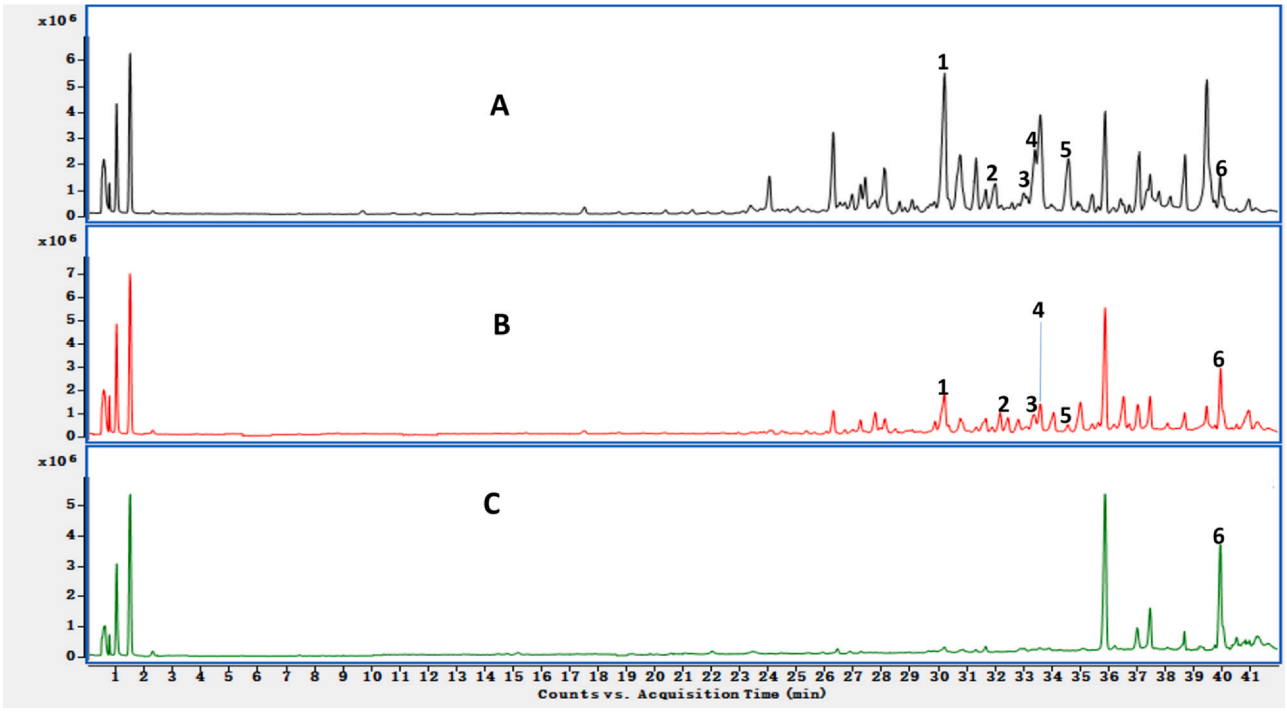

Fig. 2. Representative total ion chromagrams (TIC) of cultured mycelial biomass of three species of *Ganoderma*. A = *Ganoderma enigmaticum* (LVRB-1); B = *Ganoderma* LVRB-9; C = *Ganoderma resinaceum* (LVRB-17).

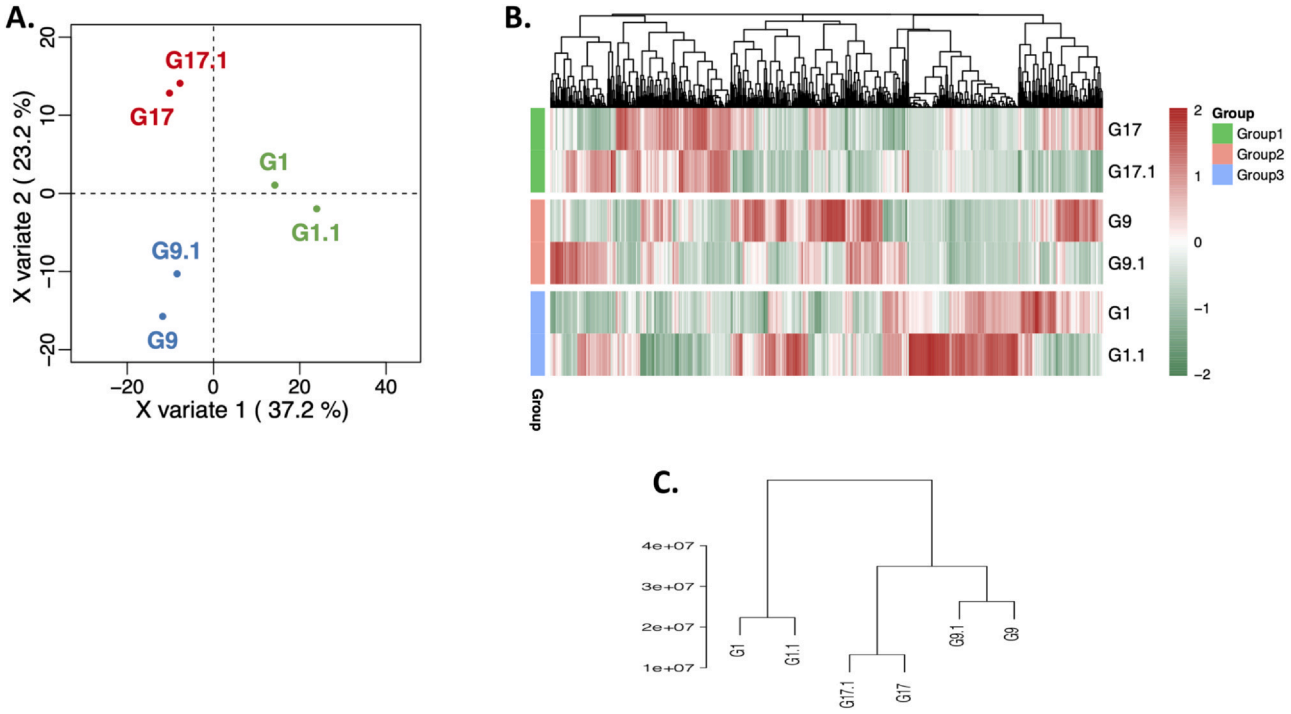

Fig. 3. Presentation of the metabolomic differences between the species of *Ganoderma* analyzed. (A) PLS-DA score plot showing the discrimination the metabolome of the various *Ganoderma* species [R^2Y(cum) = 0.962; Q2(cum) = 0.710]. (B). Heatmap representation of the metabolite differences between three Ganoderma species analyzed. (C). Hierarchical clustering dendrogram of all samples analyzed. G1 = LVRB-1, G1.1 = LVRB-1.1 mean two different samples of *Ganoderma* LVRB-1 strains; G9 = LVRB-9, G9.1 = LVRB-9.1 represent two samples of strain of *Ganoderma* LVRB-9 strain; G17 = LVRB-17, G17.1 = LVRB-17.1 are two samples of *Ganoderma* LVRB-17 strains.

rearrangement of fragments. The maximum mass error recorded for the molecular ions of all the compounds was ± 3.3258 ppm which meets the requirement for qualitative analysis. Details of the identification are described here and summarized in Table 2.

Compound **1** which was detected at 30.752 min, was identified as **ganoderic acid C6** based on the product ion, $[M - H]^-$ 529.2810 and the corresponding fragment ions of *m/z* values 511.2707, 496.2180, 467.2801, 437.2318, 303.1602, 273.1458, 209.3146. The fragment ion of *m/z* value 511.2707 is the product of a loss of water molecule by the product ion, $[M - H - H_2O]^-$. The rest correspond to losses of molecules of H_2O and CO_2. For instance, *m/z* value 496.2180 corresponds to losses of 2 H_2O and loss of 3H atoms ($[M - H - 2H_2O - 3H]^-$); *m/z* 467.2801 represents $[M - H - CO_2 - H_2O]^-$ etc. (Supplementary Fig. S1). This pattern of fragmentation was confirmed using reference compound.

The product ion, $[M - H]^-$ 571.2932 of compound **2** yielded fragment ions of *m/z* values 553.3533, 529.3535, 511.3429. *m/z* value of 553.3533 is the result of the loss of a H_2O molecule by the product ion, thus, $[M - H - H_2O]^-$. Sequential losses of a HCOOH molecule and a H_2O molecule from the product ion respectively account for *m/*

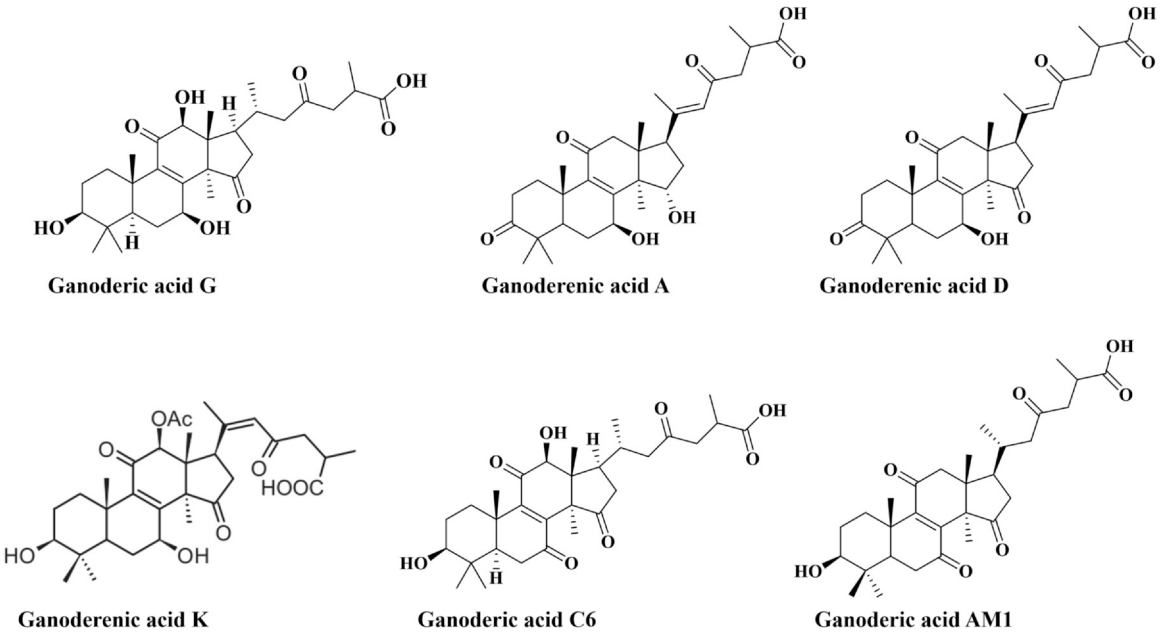

Fig. 4. Chemical structures of triterpenoids identified in all three *Ganoderma* species.

Table 2
Details of secondary metabolites (triterpenoids) identified in *Ganoderma* species.

No.	RT (min)	Formula	Cal. *m/z* $[M-H]^-$	Det. *m/z* $[M-H]^-$	Δ ppm	MS/MS fragmentation	Identity	Ganoderma species		
								A	B	C
1	30.752	$C_{30}H_{42}O_8$	529.2807	529.2810	0.5668	511.2707, 496.2180, 467.2801, 437.2318, 303.1602, 273.1458, 209.3146	Ganoderic acid G6[a]	Present	Present	Absent
2	32.232	$C_{32}H_{44}O_9$	571.2913	571.2932	3.3258	553.3533, 529.3535, 511.3429	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, 451.2858	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, 416.2314, 374.1863, 329.1749, 301.1811	Ganoderenic acid D[a]	Present	Present	Absent
5	34.596	$C_{30}H_{42}O_7$	513.2858	513.2860	0.3896	495.2752, 465.2677, 381.2426, 301.1822, 211.0972, 193.0873, 167.0714, 123.0814	Ganoderenic acid A[a]	Present	Present	Absent
6	40.392	$C_{30}H_{44}O_8$	531.2963	531.2964	0.1882	513.2858, 469.2956, 301.1820, 265.1443, 203.1444	Ganoderic acid G[a]	Present	Present	Present

A = Ganoderma LVRB-1; B = Ganoderma LVRB-17; C = Ganoderma LVRB-9.
[a] Denotes compounds confirmed with reference compounds.

z values 529.3535, 511.3429. Specifically, *m/z* 529.3535 represents $[M - H - HCOOH]^-$, while *m/z* 511.3429 is $[M - H - HCOOH - H_2O]^-$. Based on the observed pattern of fragmentation with reference to the work of Hennicke et al. [11], compound 2 was annotated as **ganoderenic acid K** (Supplementary Fig. S2).

Compound **3** which was annotated as **ganoderic acid AM1** (Supplementary Fig. S3) at a retention time of 33.592 min presented with the product ion, $[M - H]^-$ 513.2856 and fragment ions, 495.2752, 469.2963, 451.2858. *m/z* 495.2752 corresponds to $[M - H - H_2O]^-$ while $[M - H - CO_2]^-$ represents *m/z* 469.2963 and *m/z* 451.2858 corresponds to $[M - H - CO_2 - H_2O]^-$.

Compounds 4–6 were identified on the basis of the fragmentation patterns and retention times of their reference compounds. Compound 4 was identified as **ganoderenic acid D** (product ion, $[M - H]^-$ 511.2701). The fragment ions represent the following fragmentation pattern: *m/z* 493.2597 $[M - H - H_2O]^-$; *m/z* 449.2700 $[M - H - CO_2 - H_2O]^-$ etc (Supplementary Fig. S4).

The product ion of compound **5** was $[M - H]^-$ 513.2860 with fragments of the following *m/z* values 495.2752, 465.2677, 381.2426, 301.1822, 211.0972, 193.0873, 167.0714, 123.0814. Similar to the other triterpenic acids, the pattern of fragmentation of this compound which was confirmed to be **ganoderenic acid A** basically involved the losses of CO_2 and H_2O molecules. For instance, the following represent the characteristic patterns of fragmentation for these fragment ions: *m/z* 495.2752 is $[M - H - H_2O]^-$; *m/z* 381.2426 is $[M - H - 3CO_2]^-$; *m/z* 301.1822 is $[M - H - 4CO_2 - 2H_2O]^-$ etc. (Supplementary Fig. S5).

Finally, compound **6** which was detected at 40.392 min, was identified as **ganoderic acid G** based on the product ion, $[M - H]^-$ 531.2964 and corresponding fragment ions of *m/z* values 513.2858, 469.2956, 301.1820, 265.1443, 203.1444. The fragmentation pattern of these ions is summarized as follows: *m/z* 513.2858 $[M - H - H_2O]^-$; *m/z* 469.2956 $[M - H - CO_2 - H_2O]^-$; *m/z* 301.1820 $[M - H - 4CO_2 - 3H_2O]^-$; *m/z* 265.1443 $[M - H - 4CO_2 - 5H_2O]^-$; *m/z* 203.1444 $[M - H - 5CO_2 - 6H_2O]^-$ (Supplementary Fig. S6).

The results of this metabolomic study provides further evidence that the mycelial biomass of the three isolates differ in their metabolite compositions. All the 4 compounds positively identified were present in both Ganoderma LVRB-1 and Ganoderma LVRB-17 and but only Ganoderic acid G was detected in Ganoderm*a* LVRB-9 (Table 2). As described earlier, the amounts of metabolites vary significantly among the three ganodema isolates. Even though the other three triterpenoids were not found in the mycelia biomass of Ganoderma LVRB-9, the level of Ganoderic acid G in this particular isolate was more abundant as compared to the other two ganoderma isolates.

4. Discussion

The medical fungus ganoderma is receiving a special attention in the nutriceutical and cosmetic industries because of its rich macronutrients and bioactive compounds. Pharmacokinetics studies revealed ganoderic acids are the major bioactive components of the medicinal mushroom ganoderma with structures closely related to lanostane tetracyclic triterpenoids [17]. Metabolomics is one of the most powerful tools for identification of fungal species through exhaustive profiling of their metabolites. In this study, a combination of ultra performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS), ultra performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) and multivariate statistical analysis was employed to systematically profile the secondary metabolites of the mycelial biomass of three ganoderma isolates collected from the Lower Volta River Basin of Ghana to help unlock their nutraceutical, cosmeceutical and biopharmaceutical applications.

The results of the total ion chromatogram (TIC) showed variation in the metabolite profile of the mycelial biomass of the three

ganoderma isolates. The PLS-DA score plot has also revealed that the mycelial biomass of the three ganoderma samples unambiguously separated into three distinct clusters based on their mass spectra. This finding is also consistent with our DNA phylogenetic tree, which suggested the three isolates from the Lower Volta Basin of Ghana belongs to three different species namely *G. enigmaticum*, *G. resinaceum* and *G. weberanium-sichuanese* complex. The variation in the metabolite profile of the three isolates has also been further clarified in the heatmap.

The results further revealed that by combining the UPLC-Q-TOF-MS and UPLC-MS/MS, lanostane tetracyclic triterpenoids could be detected from the mycelial biomass of the three ganoderma isolates. [18] in a similar study, detected lanostane tetracyclic triterpenoids as the main metabolites from the primordial stage of *G. lingzhi*, another polypore fungus belonging to the division *Basidiomycota* by LC-MS analysis [18]. In this study where secondary metabolites in *G. lingzhi* were profiled by means of LC-IT-TOF-MS data, in combination with multivariate analysis, triterpenoids including ganoderenic acid C, ganoderic acid C2, ganoderenic acid A, ganoderic acid K, ganoderic acid H, ganoderic acid A, and ganoderenic acid D were found, together with some other unidentified metabolites at the primordial stage [18]. In another study, ganoderic acid A, ganoderenic acid A, ganoderic acid B, ganoderic acid H, ganoderic acid C2, ganoderenic acid D, ganoderic acid D, ganoderenic acid G, ganoderic acid Y, kaemferol, genistein and ergosterol were identified from the *G. lucidiun* extract by HPLC, EI-MS and NMR [19].

In this current study, 4 triterpenoids were identified from the mycelial biomass of the three ganoderma isolates from the Lower Volta River Basin of Ghana. The identified triterpenoids include ganoderic acid C6, ganoderenic acid A, ganoderenic acid D, and ganoderic acid G. Besides the above four triterpenoids, ganoderenic acid K and ganoderenic acid AM1 were annotated from Ganoderma LVRB-1 and Ganoderma LVRB-17 collected from the Lower Volta River Basin of Ghana. Intriguingly, ganoderic acid G was the only triterpenoid successfully identified in Ganoderma LVRB-9 although several other unknown metabolites were present in relatively higher quantities.

The identified triterpenoids in this study have been reported to be associated with a number of interesting biological activities. Ganoderic acid C6 and ganoderic acid G, for example, have been shown to exhibit antinociception using the acetic acid-induced writhing method [20] whereas ganoderenic acid D was reported have to cytotoxic effect against cervical (HeLa), colon (CaCo-2), and liver (HepG2) cancer cells [21].

It is known that most fatal mushroom poisonings are caused by species belonging to the genus *Amanita* because of the presence of α-, β- and γ-amanitin, which inhibit RNA polymerase II and thereby blocks the synthesis of proteins, leading to cell death. Wu et al. showed that ganoderic acid G from *G. lucidu*m has hepatoprotective effects on liver injury induced by α-amanitin (α-AMA) in mice, thereby reducing mortality rates [22]. The finding suggest that Ganoderma LVRB-1, Ganoderma LVRB-9 and Ganoderma LVRB-17 collected from the Lower Volta River Basin of Ghana may possess hepatoprotective effects on acute liver injury induced by α-AMA because of the presence of ganoderic acid G. Thus collectively, *Ganoderma* LVRB-1, *Ganoderma* LVRB-9 and *Ganoderma* LVRB-17 may possess anticancer, hepatoprotective and anti-nociceptive effects due to the presence of their bioactive components. Since the triterpenoids in the three ganoderma isolates have been reported to have various biological activities, specific condition products can be prepared from their mycelial biomass, which comprises of mycelium and primordia and extracellular compounds.

Angiotensin-converting enzyme (ACE) is a zinc metallopeptidase that plays a vital role in the regulation of vascular tone. ACE functions by converting the inactive peptide angiotensin I into active angiotensin II, which increases blood pressure by its vasoconstrictive effect and promotes sodium and water retention in the body [23]. Ganoderenic acid A identified in Ganoderma LVRB-11 and Ganoderma LVRB-1, and ganoderic acid G found in all the three isolates have been shown to inhibit hydrolyzing activity of angiotensin-converting enzyme (ACE) [24]. This finding is very interesting because current evidence indicates that angiotensin-converting enzyme inhibitors (ACEI) reduce mortality in cardiovascular disease and the progression of chronic kidney disease. This probably may explain why angiotensin-converting enzyme inhibitors (ACEI) are considered as the cornerstone for the treatment of heart failure and hypertension [25]. It is also known that elevated plasminogen activator inhibitor-1(PAI-1) levels are associated with increased cardiovascular risk. ACEI have been shown to lower PAI-1 levels and increase the release of tissue plasminogen activators (tPA) through elevated bradykinin and thereby prevents the formation of blood clot [26]. In a multicenter retrospective study, in-hospital use of ACEI or angiotensin II receptor blockers (ARB) was associated with lower risk of all-cause mortality due to COVID-19 compared with either nonuse of ACEI/ARB or use of a different class of antihypertensive agent among patients with hypertension [27]. Since ganoderenic acids A and G have been demonstrated to inhibit hydrolyzing activity of angiotensin-converting enzyme their detection in these two ganoderma isolates from the Lower Volta River may be useful in the treatment of heart failure and hypertension regardless of COVID-19 and therefore worthy of further investigation. In another interesting study, ganoderenic acid A exhibited aldose reductase inhibitory activity [28]; thereby reducing secondary complications induced by diabetes, specifically in tissues in which glucose uptake is not insulin-dependent. In our current study, ganoderenic acid A was detected in the mycelia biomass of *Ganoderma* LVRB −1 and *Ganoderma* LVRB-17. The combination of ACE inhibitory and aldose reductase inhibitory activity of ganoderenic acid A, suggest *Ganoderma* LVRB-1 and Ganoderma LVRB-17 from the Lower Volta River Basin of Ghana may be useful as antihypertensive and human aldose reductase inhibitory agent for treating hypertension and diabetic polyneuropathy, a common complication of diabetes mellitus that causes pain and sensory and motor deficits in the arms and legs [29].

5. Conclusion and study limitation

In conclusion, the outcome of this study revealed marked differences in the chemical composition of the three ganoderma isolates as clearly captured by the heatmap, and PLS-DA score plot analyses. A total of 4 lanostane triterpenoids, namely ganoderic acid C6, ganoderenic acid D, ganoderenic acid A and ganoderic acid G were found in Ganoderma LVRB-1 and Ganoderma LVRB-17, together with ganoderenic acid K and ganoderenic acid AM1 as annotated compound, which their identity need to be confirmed with pure reference compounds. In the current study, although ganoderic acid G was the only lanostane triterpenoids successfully detected in the isolate designated Ganoderma LVRB-9; which belongs to the species *Ganoderma weberianum-sichuanese* complex, several other unknown metabolites were present together with ganoderic acid G. This study provides the first ever metabolomic data on the chemical constituents of the mycelial biomass of these three ganoderma isolates from the Lower Volta River Basin of Ghana.

The main limitation of this study stems from the fact that most of the secondary metabolites particularly the abundant ones could not be identified due to the unavailability of reference compounds. In future studies, with the aid of reference compounds we would broaden the scope of the compounds to seek for and identify in these species of ganoderma. Other studies including bioassay-guided isolations of compounds and bioactivity studies would be conducted.

CRediT authorship contribution statement

GA, RNA, AKA conceived and designed the study. RNA, AKA, JCH supervised the study. GA, RNA, MAG, AQ performed the various experimental work. GA, AQ, MAG wrote a draft of the manuscript which was cross-checked RNA, AKA and JCH. RNA received funding for the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2021.114355.

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