African Journal of Biotechnology Vol. 10(42), pp. 8440-8447, 8 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2144
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Secondary metabolites of oil palm isolates of *Ganoderma zonatum* Murill. from Cameroon and their cytotoxicity against five human tumour cell lines

Tonjock R. Kinge and Afui M. Mih*

Department of Plant and Animal Sciences, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon.

Accepted 12 May, 2011

Three lanostane-type triterpenoids [lanosta-7,9(11),24-trien-3-one 15,26-dihydroxy, lanosta-7,9(11),24-trien-26-oic,3-hydroxy and ganoderic acid y], four steroids[(22E,24R)-ergosta-7,22-dien-3β,5α.6β-triol, 5α,8α-epidiory (22E,24R-ergosta-6,22-dien-3β-ol, ergosta-5,7,22-trien-3β-ol,7 (ergosterol) and ergosta-7,22-dien-3β-ol,6] and a benzene derivative (dimethyl phthalate) were isolated from ethyl acetate crude extract of *Ganoderma zonatum* Murill. of oil palm from Cameroon. Their structures were elucidated by nuclear magnetic resonance (NMR), electron impact ionization mass spectrum experiments (EI-MS) and by comparing with the data reported in literature. The highly oxygenated lanostane triterpenoid - ganoderic acid y- showed moderate cytotoxicity against two human tumour cell lines, SMMC-7721 (liver cancer) and A549 (lung cancer) with IC_{50} values of 33.5 and 29.9 µM, respectively and no activity on HL-60, MCF-7 and SW480, while lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy and lanosta-7,9(11),24-trien-26-oic,3-hydroxy, showed no activity. The three lanostane triterpeniods had the same molecular formula, molecular weight, behaviour on the TLC plates, with the same retention factor value of 3 (when petroleum ether-acetone was used in the ratio of 3:1), but with slightly different structures. These compounds have not been reported occurring together from any other species of *Ganoderma.* Their simultaneous occurrence might thus, serve as a diagnostic chemotaxonomic character for *G. zonatum*, The ^{1}H NMR data is provided for the first time for lanosta-7,9(11),24-trien-26-oic,3-hydroxy.

Key words: Secondary metabolites, *Ganoderma zonatum*, chemotaxonomic character, cytotoxicity.

INTRODUCTION

Ganoderma species belong to the division Basidiomycota, class Homobasidiomycetes, order Aphyllophorales and family Polyporaceae (Alexopoulos et al., 1996; Wasser and Weis, 1999a). *Ganoderma* species are not listed among the group of edible mushrooms because the fruiting bodies are always thick, corky and tough and do not have the fleshy texture characteristics of true edible fungi (Jong and Birmingham, 1991). Although species of *Ganoderma* cannot be eaten directly, they have attracted great attention all over the world because of their wide range of pharmacological values (Yoon et al., 1994; Wasser and Weis, 1999b). The bioactive compounds from them have been implicated for their high antioxidant, immune-regulatory and hypoglycemic activities. They have been known for their antihydrogenic, antitumor, antihepatotoxic, antinocieptic, immunodulatory, cardio-vascular, antibacterial and antiviral values (Chang and Buswell, 1996; Chang and Mshigeni, 2001).

Basidiomycetes, also called macromycetes, are among the many diverse organisms and are a major source of biologically active natural products (Liu, 2004). They provide a rich variety of active secondary metabolites (Turner, 1971). The number of different mushroom species on earth is estimated at 140000, of which only about 10% are known and from those, approximately 700 species are known to possess significant pharma-cological properties (Lull et al., 2005; Chang, 1996). Medicinal basidiomycetes represent an unlimited source of phytochemicals such as primary and secondary metabolites (Roja and Rao, 1998). Quite a wide range of substances from higher basidiomycetes belonging to

*Corresponding author. E-mail: afuimih@yahoo.com. Tel: 237 74 62 53 39 or 237 75 53 11 02. Fax: 237 33 32 22 72.

different classes of chemical compounds have been described and their biological properties evaluated (Lorenzen and Anke, 1998; Mizuno, 1999).

Over the past three decades, scientists all over the world have isolated more than 150 triterpenes and 50 pharmacologically active polysaccharides from different species of *Ganoderma* (Kim and Kim, 2002; Lin and Chou, 1984; Jong and Birmingham, 1992). Some of those bioactive compounds obtained from different basidiomycetes are a variety of terpenes, sesquiterpenes, steroids and polysaccharides (alone or forming complexes with proteins, like PSK), among others (Lindequist et al., 2005). Most of their main pharmacological properties like their antitumoral activity have been attributed to their ability to modulate the immune system. Most of them are immunostimulants, but others are immunosuppressive.

Basidiomycetes mushrooms have been used in folk medicine throughout the world since ancient times (Arisawa et al., 1986; Wasser, 1999; Stamets, 2000). Medicinal mush-rooms useful against cancer are known in China, Russia, Japan, Korea, as well as the USA and Canada, but less known in most African countries. Members of the genus *Ganoderma* have also been used in traditional medicine for centuries in, Japan and Korea. In 1997, the worldwide production of *Ganoderma* was approximately 4,300 tons, of which China contributed 3,000 tons (Chiu et al., 2000). Traditionally in China, *Ganoderma* is highly regarded as a herbal treatment and is claimed to alleviate or cure virtually all diseases.

The medicinal value of the mushrooms is largely a function of their secondary metabolites (Hajjaj et al., 2005). A large number of mushroom-derived compounds, both cellular components and secondary metabolites, have been shown to affect the immune system and could be used to treat a variety of disease states. Compounds that appear to enhance or potentiate host resistance are being sought for the treatment of cancer, immunodeficiency diseases or generalized immune suppression after drug treatment (Hajjaj et al., 2005). More than 140 highly oxygenated lanostane-type triterpenoids have been isolated from the fruiting bodies, mycelia and spores of *Ganoderma lucidium*, some of them exhibiting a very broad spectrum of biological activities and pharmacological functions. Some of them have been shown to inhibit histamine release from rats mast cells (Komoda et al., 1989), angiotensin-converting enzyme and hypocholesterol activities (Hajjaj et al., 2005).

Ganoderic acids and other triterpenoids have received considerable attention due to their conspicuous pharmacological activities. Despite the valuable dietary and therapeutic benefits of *G. lucidum* and related species, phytochemical evaluation of the active components have been performed predominantly in China, Korea, Japan and the United states (Paterson, 2006).

Herbalists usually consider *Ganoderma* as natural regulator, suppressing the immune system if it is overactive and boosting it if it is underactive such that traditional healers among the Yoruba people of south-western Nigeria have used species of *Ganoderma* in the treatment of skin disorder, high blood pressure and intestinal disorder (Gao and Yang, 1991). They usually regard *G. lucidum* as immune booster especially when combined with other medicinal ingredients.

In Cameroon and other countries such as Malaysia and Indonesia, species of *Ganoderma* are highly prevalent in oil palm plantations, where they are considered pathogenic (Turner, 1981; Utomo et al., 2005) and several control measures have been considered which are aimed at killing or reducing the basidiocarp development. Ethnomycological studies in Cameroon revealed that a species of *Ganoderma* identified as *G. lucidium* (W.Curt.Fr.) P. Karst is used in the treatment of skin infections, boils, abscesses, tumours and also as a component in other medicinal preparations (Yongabi et al., 2004). There is however, paucity of information on the chemical constituents of species of *Ganoderma* and other macro-fungi in general in Cameroon as is the case with other Sub-Sahara African countries. The aim of this study was to identify the chemical constituents of the Cameroonian oil palm isolate of *Ganoderma zonatum* and subsequently, to screen some of the pure compounds against cancer cell lines *in vitro* as a rationale for the conservation of this medicinal fungus.

MATERIALS AND METHODS

General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV spectra were measured in Shimadzu UV-2401 PC spectrophotometer. IR spectra were obtained on a Tensor 27 with KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. FAB-MS were recorded with a VG Autospec-3000 spectrometer. EI-MS were recorded with a VG Autospec-3000 spectrometer. ESI-MS and HRESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer. Preparative HPLC was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 µm, 9.4 × 150 mm) column. Preparative MPLC was performed on Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605 and manager C-615. Silica gel (200 to 300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), RP-18 gel (40 to 75 µm, Fuji Silysia Chemical Ltd., Aichi, Japan) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized under visible and UV light (254 and 365 nm). Spots were also visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. All solvents used were of analytical reagent grade.

Collection and identification of fungus

Ganoderma species were collected from naturally infected oil palm plants of the PAMOL Plantations Ltd, Lobe, South West Region, Cameroon and were identified using gross morphology and

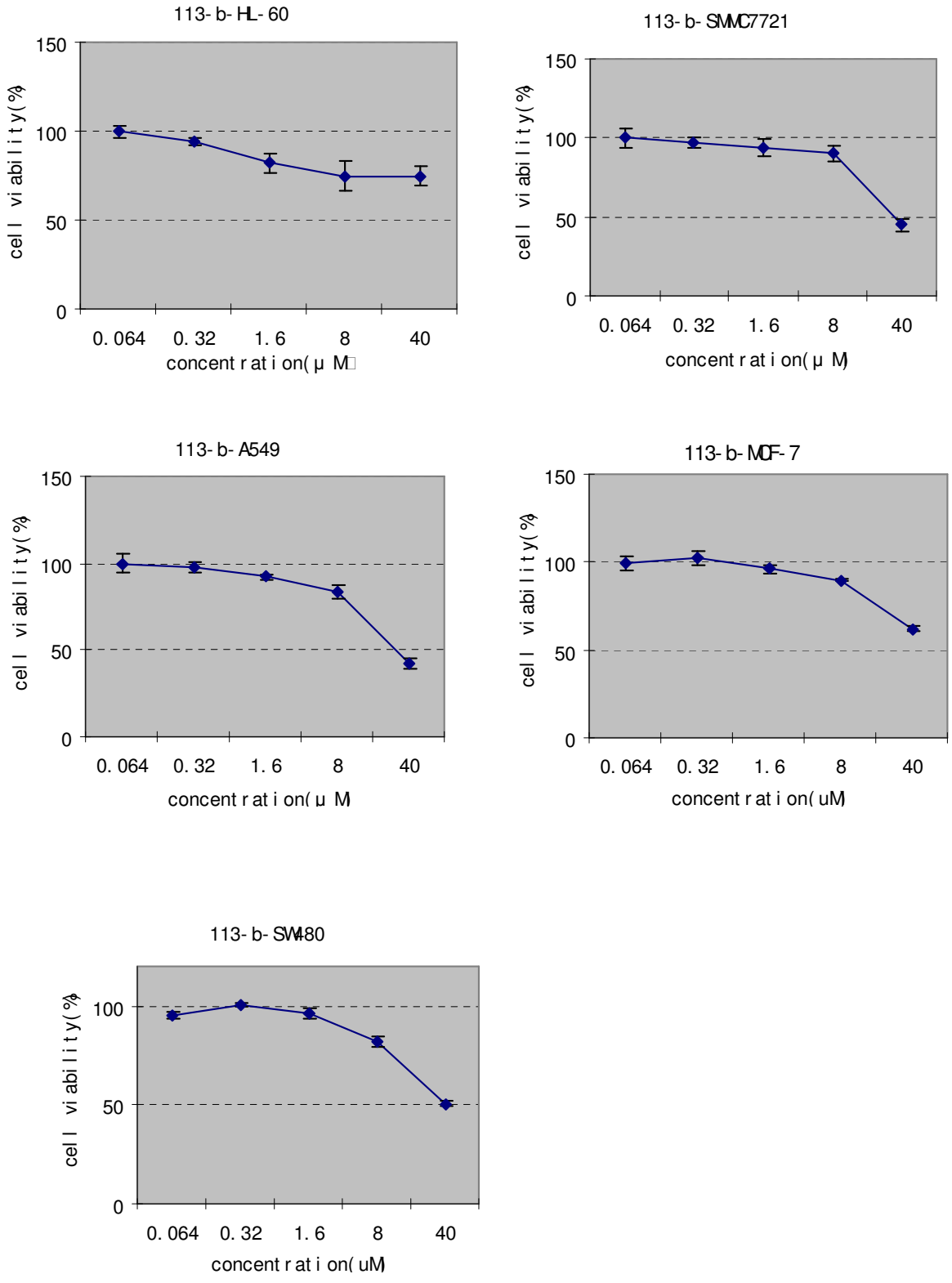


Figure 1. Percentage cell viability of five tumor cell lines against different concentrations (μM) of ganoderic acid Y.

microscopy (Ryvarden, 1984). The gross morphology is exemplified in Figure 1.

Basidiospores inclusive ornamentation measured 11 to 14 μm x 8 to 6 μm, while measurements exclusive of ornamentation were (8) 9 to 11 (12) x 7 to 5 μm. The microscopic investigation revealed that the species was *G. zonatum* (Figure 2). Duplicate voucher

Figure 2. Photograph of *Ganoderma zonatum*.

specimens (No. HKAS58060 and UB-0083) have been deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences and in Specimen Collection of the University of Buea, Cameroon, respectively.

Extraction and isolation of secondary metabolites

Fruiting bodies of *G. zonatum* (1.4 kg) were oven dried at 70°C for 48 h. The dried samples were powdered and then extracted three times with 8 L of chloroform/methanol in 1:1 ratio (v/v) at room temperature. The combined extract was evaporated in a rotator evaporator and concentrated *in vacuo* and the residue was suspended in water and partitioned with petroleum ether, ethylacetate and n-hexane. The ethylacetate extract (240 g) was subjected to MPLC and then TLC and like fractions were combined to give three fractions.

Fraction 1 (27 g) was chromatographed on silica gel (petroleum ether- methanol, 50:1-1:2) to give three sub-fractions 1.1 to 1.3. Sub-fraction 1.2 (9 g) was chromatographed on silica gel (petroleum ether- acetone, 20:1-1:2) to give compound 1 (15 mg), a white powder and compound 4 (90 mg), also a white powder. Sub fraction 1.3 (12 g) was subjected to silica gel CC (petroleum ether-acetone, 15:1-1:2) to yield compound 6 (21 mg), a white powder, while Sub fraction 1.1 was discarded because the quantity was too small for purification and analysis.

Fraction 2 (32 g) was chromatographed on silica gel (petroleum ether- methanol, 30:1-1:2) to give four sub-fractions; 2.1 to 2.4. Sub-fraction 2.2 (8 g) was chromatographed on silica gel (petroleum ether- acetone, 10:1 and 1:2) to yield compound 2 (10.5 mg). Sub-fraction 2.3 (10 g) was chromatographed using petroleum ether- methanol solvent (30:1 and 1:2) to give compound 5 (20 mg).

Fraction 3 (20 g) was subjected to petroleum ether-methanol, 25:1-1:2 to give three sub-fractions 3.1 to 3.3. Sub fraction 3.1 (5 g) was chromatographed on silica gel (petroleum ether- MeOH, 30:1-1:2) to give four sub-fractions 3.1.1 to 3.1.4. Fraction 3.1.4 (200 mg) was subjected to silica gel CC (petroleum ether- EtOAc, 12:1-1:2) to yield six sub-fractions 3.1.4.1 to 3.1.4.6. Sub-fraction 3.1.4.4 (20 mg) was chromatographed on silica gel (petroleum ether-acetone, 6:1) to give compound 7 (12 mg). Sub-fraction 3.1.4.6 (10 mg) was chromatographed on sephadex LH-20 ($CHCl_3$:MeOH 1:1) to yield compound 3 (8 mg). Sub-faction 3.1.4.2 was further subjected to HPLC to give compound 8.

Cytotoxicity assay

The following human tumour cell lines were used: breast cancer MCF-7, hepatocellular carcinoma SMMC7721, human myeloid leukaemia HL-60, colonic cancer W480 and lung cancer A549. These are cell lines maintained at the Kunming Institute of Botany of the Chinese Academy of Sciences, Kunming, China. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% foetal bovine serum (Hyclone, USA) at 37°C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), purchased from sigma, USA. Briefly, 100 µl adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended

cells were seeded just before drug addition, both with initial density of 1 × 105 cells/ml in 100 µl medium. Each tumour cell line was exposed to the test compound at various concentrations (0.064, 0.32, 1.6, 8 and 40 µM) in triplicates for 48 h, with cisplatin and taxol (sigma, USA) as positive control. After the incubation, MTT (100 µg) was added to each well and the incubation continued for 4 h at 37°C. The cells were lysed with 100 µl 20% SDS-50% DMF after removal of 100 µl medium. The optical density of the lysate was measured at 595 nm in a 96-well microtitre plate reader (Bio-Rad 680, USA). The concentration of inhibitors that result in 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity.

RESULTS

Following purification of the various fractions by preparative TLC methods, the spectral analysis revealed the following data for the eight compounds:

^{1}H NMR and EI Data of 1–8 (in chloroform and methanol-d_6, $d_7\delta$ in ppm and J in Hz)

Lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy

454(M)+,6.77(1H,t),5.51(IH,t),5.38(1H,m),2.26(2H,m),2.14(2H,m),2.05(2H),1.81,1.29(3H,s),1.02(3H,s),0.98(3H,s)0.95(3H,d),0.91(3H,s),0.87(3H,s),0.62(3H,s).

Lanosta-7,9(11),24-trien-26-oic,3-hydroxy

454(M)+,6.81(1H,t),5.44(1H,t),5.28(1H,t),4.81(1H,brs),3.41,2.24,2.15(3H,s),0.98(3H,s),0.96(3H,s),0.90(3H,d),0.86(6H,s),0.54(3H,s).

Ganoderic acid y

454(M)+,6.74(1H,t),5.40(1H,t),5.25(1H,m),3.14,2.15,1.75(3H,s),0.90(3H,s),0.86(3H,d),0.80(6H,s),0.49(3H,s).

(22E,24R)-ergosta-7,22-dien-3β,5α.6β-triol,

EI-MS M/Z(%): 430 (M)+ (1),412(42),394(45), 365(8), 269(33),251(53),81(45),69(100).

5α,8α-epidiory-(22E,24R)-ergosta-6,22-dien-3β-ol

Colorless amorphorus powder; mp: 182-184oC; EI-MS m/z(%): 428((M)+,13),410((M-H20)+,5),396((M-02)+, 100),363(35),303(8), 251(20),152(30), 107(24), 95(35),81(43),69(65);IR(KBR);3525, 3309,2955,2812, 1650,1460,1380,1074,1043,968,858,275.

Ergosta-5,7,22-trien-3β-ol,7(ergosterol)

Colorless needle; mp: 164-165oC; [α]D 22 = -103o (*c* 0.1,CHCl3); UV ($CHCl_3$): λmax nm (log ε) 274 (4.01), 284 (4.07), 296 (3.84); EI-MS *m/z*: 396 [M]+ (76), 271 [M - C9H17]+ (80), 69 (100).

Ergosta-7,22-dien-3β-ol,6 (Stella sterol)

Colorless amorphorus powder; [α]D 22 = -6.8o (*c* 0.1,CHCl3); UV ($CHCl_3$): λmax nm (log ε) 239 (3.42); EI-MS *m/z*: 398 [M]+ (33), 355 [M - (CH3)2CH]+ (8), 271 (100), 255 [M - C9H17 - H2O]+ (56).

Dimethyl phthalate

149(M)+(400mHz;CD_3Cl) 3.90(6H,s),7.55(2H,dd),7.72(2H,dd).

The structures and molecular formulae of the eight pure compounds are shown on Table 1.

Cytotoxic assays against five human cancer cell lines with the highly oxygenated lanostane-type triterpenoids (lanosta-7,9(11),24-trien-3-one 15,26-dihydroxy, lanosta-7,9(11),24-trien-26-oic,3-hydroxy and ganoderic acid y) revealed that ganoderic acid Y had moderate anticancer activity against two human cancer cell lines SMMC-7721(liver cancer) and A549 (lung cancer) with IC_{50} values of 33.5 and 29.9 µM, respectively (Table 2). Different concentrations of ganoderic acid Y and their percentage viability on the different cell lines is shown Figure 1.

DISCUSSION

Eight pure compounds were isolated for the first time from *G. zonatum* collected from oil palm plantations in Cameroon. The eight compounds composed of three lanostane-type triterpenoids [lanosta-7,9(11),24-trien-3-one 15,26-dihydroxy, lanosta-7,9(11),24-trien-26-oic,3-hydroxy and ganoderic acid y], four steroids [(22E,24R)-ergosta-7,22-dien-3β,5α.6β-triol, 5α,8α-epidiory (22E,24R-ergosta-6,22-dien-3β-ol,Ergosta-5,7,22-trien-3β-ol,7(ergosterol), ergosta-7,22-dien-3β-ol,6] and a benzene derivative (dimethyl phthalate). This is the first report of these compounds from *G. zonatum* from Cameroon. Species of the genus *Ganoderma* are known to be prolific producers of lanostane type triterpenoids with over 100 compounds having been associated with members of the genus (Shim et al., 2004). Although all three lanostane-type triterpernoids found in this study have been reported separately from different species

Table 1. Structure, molecular formula and weight of eight secondary metabolites from a Cameroonian oil palm isolate of *G.zonatum* Murill.

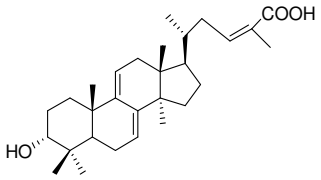
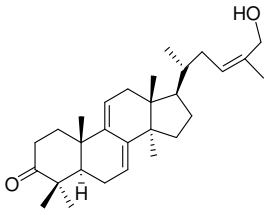
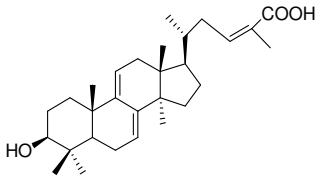
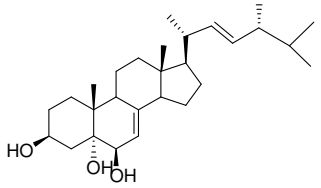
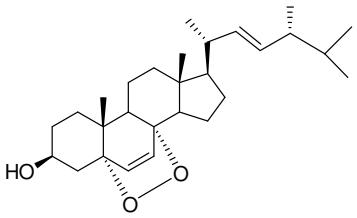
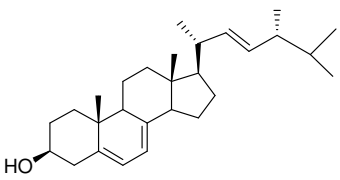
Compound	Structure, molecular formula and weight
Compound 1 Lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy	 **1** $C_{30}H_{46}O_3$ M=454
Compound 2 *Lanosta*-7,9(11),24-trien-26-oic,3-hydroxy	 **4.** $C_{30}H_{46}O_3$ M=454
Compound 3 Ganoderic acid Y(24E)-3-ol-5α-lanosta-7,9(110,24-trien-26-oic acid	 $C_{30}H_{46}O_3$ M=454
Compound 4 22E,24R)-ergosta-7,22-dien-3β,5α.6β-triol	 $C_{28}H_{46}O_3$ M= 430
Compound 5 5α,8α-epidiory-(22E,24R)-ergosta-6,22-dien-3β-ol	 C_{28} H_{44} O3 M=428
Compound 6 Ergosta-5,7,22-trien-3β-ol,7	 C_{28} H_{44} O M=396

Table 1. Contd.

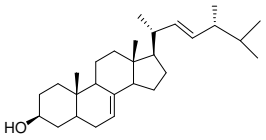
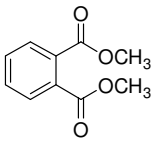
Compound 7 Ergosta-7,22-dien-3β-ol,6	 $C_{28}H_{46}O$ M=398
Compound 8 Dimethyl phthalate	 $C_{10}H_{10}O_4$ M=194

Table 2. Cytotoxicity of lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy, lanosta-7,9(11),24-trien-26-oic,3-hydroxy and ganoderic acid Y to five cancer cell lines.

Test compound	IC_{50} value (μM)*				
	HL-60 (leukaemia)	SMMC-7721 (liver cancer)	A-549 (lung cancer)	MCF-7 (breast cancer)	SW480 (colon cancer)
112-b[1]	>40	□40	□40	□40	□40
113-a[1]	□40	□40	□40	□40	□40
113-b[1]	□40	33.51	29.94	□40	□40
Cisplatin (DDP)	0.84	9.86	8.40	18.84	13.51
TAXOL	<0.008	<0.008	<0.008	<0.008	<0.008

*IC_{50} is the concentration that will allow for only 50% cell viability; [1]112-b, Lanosta-7,9(11),24-trien-3-one,15;26-dihydroxy; 113-a, lanosta-7,9(11),24-trien-26-oic,3-hydroxy; 113-b, ganoderic acid Y; Cisplatin (DDP) and TAXOL are the controls.

of the fungus, their simultaneous occurrence in *G. zonatum* is reported here for the first time. This simultaneous occurrence could be used as a diagnostic feature for the species and could serve as a chemo-taxonomic character.

The anti-tumour effects of *Ganoderma* are apparently mediated by numerous biologically active compounds such as polysaccharides, triterpenes and immuno-modulatory proteins (Silva et al., 2002). Due to differences in climate, composition of extracts and culturing methods, individual species of *Ganoderma* may differ in their bioactivity (Chen et al., 2004).

For the active compound ganoderic acid Y, the percentage cell viability of liver and lung cancer cell lines was less than 50% at 40 μM concentration, while for HL-60 (leukaemia), MCF-7 (breast cancer) and SW480 (colon cancer) it was greater or equal to 50% at the same concentration. The reasons for these differences are unclear and there is need for further investigation on the underlying mechanism of the action of ganoderic acid Y on liver and lung cancer cell lines.

Ganoderic acid Y (113-b), an oxygenated lanostane type triterpenoid, has been shown to have biological activity. For example, Haijaji et al. (2005) showed that it inhibits cholesterol biosynthesis. Also, Berger et al. (2004) found that organic fractions of *G. lucidum* containing oxygenated lanosterol derivatives inhibited cholesterol synthesis in T9A4 hepatocytes. Toth et al. (1983) also isolated ganoderic acid Y from *Ganoderma lucidum* and found that it showed cytotoxic activity *in vitro* on hepatoma cells, while Huie and Di (2004) showed that ganoderic acid Y from the fruiting body of *G. lucidum* possess antihypertensive properties and ACE (Angiotensin-converting enzymes). In this study, it was found to have moderate antiproliferative activity against two cancer cell lines; liver and lung cancer cell lines, respectively. This affirms the claims of the use of this fungus for the treatment of tumours by traditional medical practitioners in Cameroon (Yongabi et al., 2004). This shows that ganoderic acid Y (113-b) has pharmaceutical potentials and *G. zonatum* could serve as a source of this compound.

Conclusion

Based on the literature available to us, this is the first report on the isolation, structural elucidation and

bioactivity of compounds from the fruiting bodies of *G. zonatum*. It serves as a baseline study for further research on this medicinal mushroom species. The three highly oxygenated lanostane triterpenoids isolated from *G. zonatum,* can be used as a taxonomic marker for this species since they possess the same molecular weight, molecular formula and retention factor value, but with slightly different structures. Our recent surveys revealed that *G. zonatum*, a member of the *G. lucidum* complex, was among the most prevalent species on oil palm in south western Cameroon. These could serve as a source of secondary metabolites. There is need for further research *in vivo* to ascertain the efficacy of ganoderic acid Y on lung (A-549) and liver (SMMC-7721) cancer cell lines, since they are natural products. There is also the need for the research on different species of *Ganoderma* and other macro fungi from Cameroon and Africa for their secondary metabolites and subsequently, their bioactivity against different cell lines.

ACKNOWLEDGEMENTS

The first author was financed by TWAS-CAS Post-Graduate 2008 fellowship award. We also thank Prof. Lui Ji-Kai for providing the laboratory facilities in Kunming Institute of Botany, CAS. The field work was financed by the University of Buea Research Grant No. 2008/A24 to the second author.

REFERENCES

Alexopoulos CJ, Mims CW, Blackwell M (1996). Introductory Mycology (4th edition). New York John Wiley. p. 869

Arisawa M, Fujita M, Fukumura S, Hayashi T, Morita N (1986). Three new Lanostanoids from *Ganoderma lucidium.* J. Natural Products (Lloydia) . 49: 621-625.

Berger A, Rein D, Kratky E, Monnard I, Hajjaj H, Meirim I, Piguet-Welsch C, Hauser J, Mace K, Niederberger P (2004). Cholesterol-lowering properties of *Ganoderma lucidum* in vitro, ex vivo, and in hamsters and minipigs. Lipids Health Dis. pp. 1-12.

Chang R (1996). Functional Properties of Edible Mushrooms. Nutr. Rev. 54: 91-93.

Chang ST, Buswell JA (1996). Mushroom nutriceutical. World J. Microbiol. Biotechnol..12(5): 473-476.

Chang ST, Mshigeni KE (2001). Mushroom and Human Health: their growth significance as petentdietary supplement, University of Namibia, Windhoek, p. 79.

Chen HS, Tsai YF, Lin S, Lin CC, Khoo KH, Lin CH, Wong CH (2004). Studies on the immune-modulating and anti-tumor activities of *Ganoderma lucidum*(Reishi) polysaccharides. Bioorganic Med. Chem., 12: 5595-5601.

Chiu SW, Wang ZM, Leung TM, Moore D (2000). Nutritional value of *Ganoderma* extract and Assesment of its genotoxicity and anti-genotoxicity using comet assays of mouse lymphocytes. Food Chem. Toxicol., 38: 173-178.

Gao B, Yang G (1991). Effect of *Ganoderma applanatum* polysaccharides on cellularand humoral immunity in normal and sarcoma 180 transplanted mice. Phytopathol. Res., 5: 134-138.

Hajjaj H, Mace C, Roberts M, Niederberger P, Fay LB (2005). Effect of 26-oxygenosterols from *Ganoderma lucidium* and their activity as cholesterol synthesis inhibitors. Applied and Environ. Microbiol., 71(7): 3653-3658.

Huie CW, Di X (2004).Chromatographic and electrophoretic methods for Lingzhi pharmacologically active components. J. chromatogr., 812: 241-257.

Jong SC, Birmingham JM (1991). Medicinal benefit of the mushroom *Ganoderma*. Adv. Appl. Microbiol., 37: 104-132.

Jong SC, Birmingham JM (1992). Medicinal benefits of the mushroom *Ganoderma*. Adv. Appl. Microbiol., 34: 183-262.

Kim HW, Kim BK (2002).Recent advances on biologically active triterpenoids of *Ganoderma lucidum* In: *Ganoderma* Lin ZB (Ed), Beijing Medical university press, Beijing, Genet. Chem. Pharmacol. Ther. pp. 10-19.

Komoda Y, Shimizu M, Sonoda Y, Sato Y (1989). Ganoderic acid and derivatives as cholesterol synthesis inhibitors. Chem. Pharm. Bull., 37: 531-533.

Lin JY, Chou TB (1984). Isolation and characterization of lecitin from edible mushroom,*Volvariella volvacea.* J. Biol. Chem., 96: 135-140.

Lindequist U, Niedermeyer T, Jülich W (2005). The pharmacological potential of mushrooms. E-CAM, 2(3): 285-299.

Liu J (2004). N-containing Compounds of Macromycetes. Chem. Rev., 105(7): 2723-2744.

Lorenzen K, Anke Y (1998). Basidiomycetes as a source for new bioactive natural *Ganoderma lucidum* extract alone and in combination with some antibiotics. Arch. Pharm. Res., 17: 438-442.

Lull C, Wichers H, Savelkoul H (2005). Antiinflamatory and Immunomodulating Properties of Fungal Metabolites. Mediators of Inflammation. pp. 63-80.

Mizuno T (1999). The extraction and development of antitumor-active polysaccharides from medicinal mushrooms in Japan (Review). Int. J. Med. Mushrooms, 1: 9-30.

Paterson RRM (2006). *Ganoderma*-A therapeutic fungal biofactory. Phytochemistry, 67: 1985-2001.

Roja G, Rao PS (1998). Biotechnology investigation in medicinal plants for the product ion of secondary metabolites In: Role of Biotechnology in medicinal and Aromatic plants (Irfan AA and Atiya K. editors) Hong Kong, 1: p. 201.

Shim SH, Ryu J, Kim JS, Kang SS, Xu Y, Jung SH, Lee YS, Lee S, Shin KH (2004). New lanostane- type triterpenoids from *Garnoderma applanatum*. J. Nat. Prod. 67: 1110-1113.

Silva D, Labarrere C, Slivova V, Sedlak M, Lloyad Jr. FP, HO WWY (2002). *Ganoderma lucidum* suppresses motility of highly invasive breast and prostate cancer cells. Biochem. Biophys. Res. Commun. 298: 603-612.

Stamets P (2000). Mushrooms, civilization and history. In Growing Gourmet and Medicinal Mushrooms. Ten Speed Press. USA, pp. 1-4.

Toth JO, Luu B, Ourisson G (1983). Ganoderic acid T and Z: Cytotoxic triterpenes from *Ganoderma lucidum* (Polyporaceae). Tetrahedron Lett., 24: 1081-1084.

Turner PD (1981). Oil Palm Diseases and Disorders. Kuala Lumpur: Oxford University Press, p. 281.

Turner WB (1971). Fungi, their cultivation and their secondary metabolism. In Fungal Metabolites. Academic Press England, pp. 11-23.

Utomo C, Werner S, Niepold F, Deising HB (2005). Identification of *Ganoderma*, the causal agent of basal stem rot disease in oil palm, using a molecular method. Mycopathologia, 159: 159-170

Wasser SP, Weis AL (1999b). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives (Review). Int. J. Med. Mushroom, 1: 31-62.

Wasser SP, Weis AL(1999a). General description of the most important medicinal higher basidiomycetes mushrooms. Int. J. Med. Mushroom, 1: 351-370.

Wasser SP, Weis AL (1999). Therapeutic Effects of substances occurring in Higher Basidiomycetes Mushrooms: a Modern Perspective. Crit. Rev. Immunol., 19: 65-96.

Yongabi K, Agho M, Carrera MD (2004). Ethnomycological studies of wild mushrooms in Cameroon, Central Africa. Mycologia Applicada Int., pp. 34-36.

Yoon SY, EO SK, Kim YS, Lee CK, Han SS (1994). Antimicrobial activity of *Ganoderma* extract alone and in combination with some antibiotics. Arch. Pharmacol. Res., 17(6): 438-442.