Asian Journal of Applied Chemistry Research

5(1): 48-57, 2020; Article no.AJACR.54802
ISSN: 2582-0273

Isolation, Characterization and Antibacterial Activity of Ergosta-5, 7, 22-triene-3β, 14α – diol (22Z) from Kenyan *Ganoderma lucidum*

Ebrahim Sande[1], Danstone Lilechi Baraza[1*], Selline Ooko[1] and Peter Kuloba Nyongesa[2]

[1]Department of Chemistry, Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya.
[2]Department of Biology, Masinde Muliro University of Science and Technology, P.O. Box 190-50100, Kakamega, Kenya.

Authors' contributions

This work was carried out in collaboration among all authors. Author ES designed the study, did experiments, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DLB and SO did experiments, wrote the protocol, managed the analyses of data, literature surveys and wrote the manuscript. Author PKN did the bioassay experiments and analyses, managed the literature searches and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2020/v5i130127
Editor(s):
(1) Dr. Angélica Machi Lazarin, State University of Maringá, Brazil.
Reviewers:
(1) Ishtiaq Hussain, Quaid-I-Azam University, Islamabad, Pakistan.
(2) Beatrice Ifesan, Nigeria.
(3) Maciej Masłyk, The John Paul II Catholic University of Lublin, Poland.
Complete Peer review History: http://www.sdiarticle4.com/review-history/54802

Original Research Article

Received 28 December 2019
Accepted 05 March 2020
Published 28 March 2020

ABSTRACT

Aims: To determine the chemical composition and antibacterial activity of Kenyan *Ganoderma lucidum*.
Study Design: Structural determination of the isolated compound was done using spectral evidences and in comparison with literature. The antibacterial properties of the compound was done using disc diffusion method.
Place and Duration of Study: Department of Pure and Applied Chemistry, Masinde Muliro University of Science and Technology, between January and November, 2019.
Methodology: Sequential extraction of dried samples of Kenyan *G. lucidum* were done using solvents hexane, ethyl acetate and methanol. Chromatographic separation of hexane extract of

**Corresponding author: Email: dbaraza@mmust.ac.ke, bdanstone1970@yahoo.com;*

Ganoderma lucidum was done using spectroscopic data. The compound was assayed against *Escherichia coli*, *Klebsiella pneumoniae*, Methicillin–Resistant *Staphylococcus aureus* (*MRSA*), *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. Standard antibiotic namely; ampicillin was used as the control. Disc diffusion method was used and zones of inhibition, after respective incubation periods, were used to quantify antibacterial activity.

Results: From hexane extract of *Ganoderma lucidum,* Ergosta-5, 7, 22-triene-3β, 14α – diol (22Z) was isolated. Ethylacetate and methanol extracts produced a mixture of complex compounds. Ergosta-5,7,22-triene-3β,14α-diol (22Z) exhibited significant activity against Methicillin-Resistance *Staphylococcus aureus* (MRSA) (p=0.022) and *Streptococcus pyogenes* (p = 0.05). The most sensitive microbe was *Streptococcus pyogenes*.

Conclusion: One major compound, Ergosta-5, 7, 22-triene-3β, 14α – diol (22Z) was isolated, characterized and antibacterial activity determined.

Keywords: Ganoderma lucidum; Ergosta-5, 7, 22-triene-3β, 14α – diol (22Z); antibacterial.

ABBREVIATIONS

^{13}C-NMR	– carbon 13 Nuclear Magnetic Resonance
^{1}H-NMR	– Proton Nuclear Magnetic resonance
IR	– Infra-red Spectroscopy
R_f	– Retention factor
G. lucidum	– Ganoderma lucidum
E. coli	– Escherichia coli
MRSA	– Methicillin Resistant Staphylococcus aureus
K. pneumoniae	– Klebsiella pneumoniae
P. aeruginosa	– Pseudomonas aeruginosa
S. pyogenes	– Streptococcus pyogenes

1. INTRODUCTION

A mushroom is a hypogenous or epigenous macro fungus with a fruiting body which can be observed and plucked by hand [1]. Mushrooms produce mycochemicals and nutraceuticals which are sources of useful drugs [1]. Isolates from mushrooms are biodegradable, less toxic to the environment, readily available and cheap as opposed to synthetic drugs [2]. *Ganoderma lucidum* is one of the major mushrooms that are known to have medicinal value and are traditionally used in treatment of various ailments [3,4]. Investigations on immune boosting properties have been documented. For example, In Nigeria and China, herbalists use *Ganoderma lucidum* to boost the immune system [5,6]. In other parts of the world, *Ganoderma* species are used in treatment of skin disorder, high blood pressure and intestinal disorder [7]. The traditional uses of these mushrooms has enabled scientists to search for more effective drugs from these species to prevent microbial resistance. Thus, increased resistance to current synthetic drugs by microbes makes it vital to search for new bioactive compounds that could inhibit these strains [8].

Previous investigations from *Ganoderma* species have yielded mainly ergosterol derivatives, steroids and other triterpene derivatives which were responsible for various biological activities [9,10,11]. Ergosterol and its derivatives have been reported to be responsible for biological activity such as cytotoxicity against HeLa cells, rheumatoid arthritis and immune promoting properties [12,13,14]. Very few studies have been done on the isolation of compounds and determination of antibacterial properties from the Kenyan *Ganoderma lucidum*. This therefore prompted us to isolate, characterize and determine the antibacterial activity of Ergosta-5,7,22-triene-3β,14α-diol (22Z), which was the major component in the above mushroom species.

2. MATERIALS AND METHODS

2.1 Collection of Mushroom Material and Identification

The cultivated species of *Ganoderma lucidum* was obtained from a mushroom farm in Kakamega County in Kenya. The county lies at an altitude of 1500-1600 m above sea level and is 358 km west of Nairobi, the capital city of Kenya. The mushroom species were identified by a staff at the East African Herbarium-national museums of Kenya where a voucher specimen was deposited and identification number EAHNMK 261 assigned to the mushroom.

2.2 Extraction and Isolation of Compounds from *Ganoderma lucidum*

2.2.1 Extraction

Samples of fresh mushroom material were collected, cleaned, air dried for 7 days, ground

into fine powder using an electric grinder and stored under dry conditions ready for analysis. One kilogram (kg) of dried powdered *G. lucidum* mushroom was soaked in 3 liters of hexane for two days and concentrated using a rotary evaporator. This resulted into about 6.0 g of hexane crude extract.

2.2.2 Isolation and Identification

The Hexane extract (6.0 g) was fractionated by use of column chromatography with silica gel as stationary phase. The column was eluted using hexane/ethyl acetate of increasing polarity. The fractions were collected in volumes of 10ml. Sixty eight fractions were collected but the dominant compound was obtained at 30% ethyl acetate in hexane.. All the fractions were then concentrated at room temperature. Fractions 39-43 resulted into impure colourless, needle like crystals. 32 mg of combined impure fractions (39 to 43) was collected. Recrystalisation of these fractions in hexane yielded pure needle like colorless crystals (26 mg), which were identified using combined spectroscopic methods (^{1}HNMR, ^{13}C-NMR and IR spectra) and in comparison with literature sources.

2.3 Bioactivity Tests of Ergosta-5,7,22-triene-3β,4α-diol-(22Z) from *Ganoderma lucidum*

2.3.1 Culture of test micro-organisms

The selected test micro-organisms [(*Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes* and Methicillin-Resistant *Staphylococcus aureus* (MRSA)] were resuscitated by culturing them on Mueller Hinton Agar (Himedia) and incubated at 37°C for 18 hours. The micro-organisms were later transferred to Mueller Hinton Broth (Himedia) and incubated at 37°C for 6 hours for a uniform and young culture for the antibacterial tests.

2.3.2 Antibacterial tests

Mueller Hinton agar media was prepared according to the manufacturer`s instructions. The media was then sterilized by autoclaving at 121°C and 15psi pressure applied for 15 minutes. Using a 6mm diameter cork borer, wells were made through the agar in the petri dishes (agar well diffusion method) [15]. The concentrations of 100 mg/ml in Dimethylsulphoxide (DMSO) of the isolated compound and 10 mg/ml of the control (Ampicillin) were prepared. Only 100 microlitre aliquot was dispensed into each well using micropipettes and left at 4°C for 4 hours for the sample to completely diffuse into the media. The samples were run in replicates of three. The micro-organisms (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Streptococcus pyogenes* and Methicillin-Resistant *Staphylococcus aureus,* (MRSA) were then uniformly spread on each plate and incubated at 37°C for 24 hours. Zones of bacterial growth inhibition were observed and the diameter of each measured in millimeters to assess the antibacterial activity. The values obtained were used to determine the antibacterial activity by comparing the test and standard antimicrobial (Ampicillin).

3. RESULTS AND DISCUSSION

3.1 Spectral Analysis Ergosta-5,7,22-triene-3β,14α-diol (22Z)(1)

From hexane extract of dried mushroom of *Ganoderma lucidum*, a compound with white needle-like crystals was isolated with a m.p of 178-180° and R_f = 0.6. This compound was identified as Ergosta-5, 7, 22-triene-3β, 14α – diol (22Z), with molecular formula $C_{28}H_{45}O_2$ by combined spectroscopic analysis (^{1}H-NMR,^{13}C-NMR and HMBC).

Both ^{1}H and ^{13}C-NMR spectra and in comparison with observed data of similar structures [11,16,17,18] showed that most peaks were consistent with Ergosta-5,7,22-triene-3β,14α-diol (22Z).

^{1}H-NMR spectrum for compound 1 revealed four olefinic protons (δ_H 5.570, d, *J* = 2.5Hz, 1H; 5.581, d, *J*=2.5Hz, 1H; 5.138, dd, *J* = 2.5, 5.5Hz, 1H; 5.205, dd, *J* = 5.0, 5.5Hz). Doublet of doublets at δ_H 5.20 and δ_H 5.21 were assigned to protons at C -22 and C- 23 which is similar to splitting pattern in sterols [10,15]. Two hydroxyl (-OH) protons with signals (3.598, 1H, m) and [3.489, 1H (s)] were observed. The doublet signal at 3.958 ppm was assigned to carbon 3 of the sterol moiety. The proton with the signal 3.489 ppm (a singlet) appeared at carbon -14. This situation was in agreement with the one reported by Nao-Yun et al. [18] which was recently isolated as a sterol from the mushroom, *Algicolous Gibberella zeae* strain. The protons at the double bond between C-22 double bond was deduced to be cis from the^{1}H – ^{1}H coupling

constant (*J* = 10Hz. According to previous investigations [17], coupling constant for cis molecule occurs in the range of 6 – 10Hz, while trans-isomers have been reported with *J-values* between 11 – 18Hz. ^{1}H-NMR spectrum of compound 1 indicated a coupling constant of 5.5Hz that was in consistent with a cis Isomer. Therefore, it was evident from the information given that the molecule would most likely be Ergosta-5, 7, 22-trien-3β, 14α-diol (22Z) **(1).**

^{13}Carbon NMR spectra indicated the presence of 28 carbons as evident for steroid molecules [7], including six methyl groups (12.06, 16.27, 17.59, 19.63, 19.94 and 21.53), seven methylenes (21.1, 22.95, 28.19, 31.96, 38.36, 39.07 and 40.76), ten methane carbons (33.08, 40.48, 42.82, 46.23, 55.71, 70.46, 116.9, 119.6, 131.91, and 135.61) and five quaternary carbons (37.07, 42.81, 71.07, 139.8, and 141.37) by Miguel et al. [11], Baraza et al. [16].

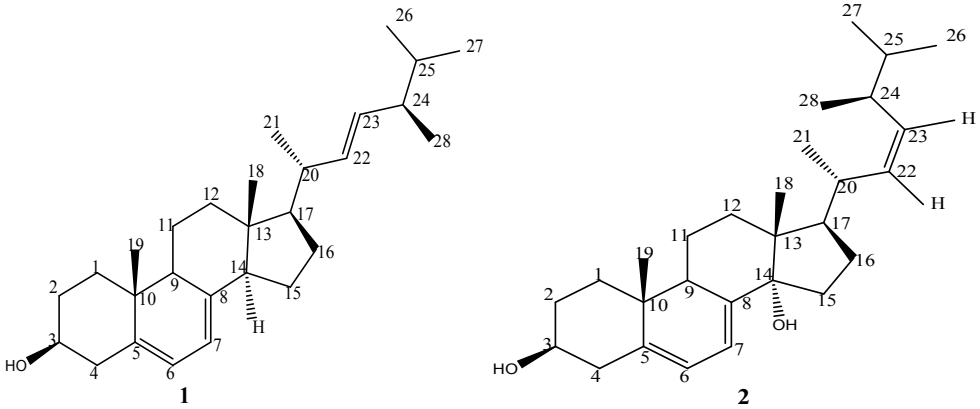
Further confirmation of Compound **1** was done using infra-red spectroscopic analysis and prominent strong absorption at 3300 cm^{-1} for hydroxyl group (O-H peak), 3176.5 cm^{-1} (weak) for vinyl =C-H stretch, 2954.7 cm^{-1} (strong) for methyl (CH_3) stretch, 2869.9 cm^{-1} (weak) for methylene (-CH_2) stretch, 2657.7 cm^{-1} (weak) -C-H (methine) sp C-H stretch, 1596.9 cm^{-1} (sharp) for cyclic double bond, (-C=C-), 1226.6 cm^{-1} (strong) for C-O stretching vibration. The above IR spectrum was consistent with reported data as described by Pavia et al. [17]. Compound 1 compared well with the reported data for compound **(2)** with only different at the coupling constant values (*J* values) at C-22 and C-23 (*J* value of 15.6Hz) [11,16,17].

Table 1. ^{1}H and ^{13}C NMR chemical shifts (δ, ppm) for Ergosta-5,7,22-triene-3β,14α-diol (22Z) (1) and Ergosta-5,7,22-triene-3β-ol-(22E)(2)

	^{13}C-NMR (CD_2Cl_2) (500MHz)	^{1}H-NMR (CD_2Cl_2) (500MHz)	^{13}C-NMR	^{1}H-NMR
	Compound 1		**Compound 2 [11]**	
1	38.36	1.844(2H, t)	38.4	1.86
2	31.96	2.312 (2H,m)	32.0	2.44
3	70.46	3.598 (1H,m)	70.4	3.58
4	40.76	1.28, 2.28 (2H, d)	40.8	1.90, 2.06
5	139.8		139.8	
6	119.58	5.570 (1H, d, J = 2.5Hz)	119.6	5.60
7	116.27	5.581 (1H, d, J =2.5Hz)	116.3	5.45
8	141.37		141.3	
9	46.23	1.582 (1H, m)	46.2	1.61
10	37.07		37.0	
11	21.10	1.595 (2H, m)	21.1	1.50
12	39.07	1.907 (2H, t, J = 3.0Hz)	39.1	1.90
13	42.81		42.8	
14	71.07	3.489 (s)	54.6	1.85
15	22.95	1.503,1.831 (2H, m)	23.0	1.50,1.86
16	28.19	1.309,1.745 (2H, m)	28.3	1.31,1.74
17	55.71	1.254 (1H, m)	55.7	1.26
18	12.06	0.631 (3H, s)	12.0	0.79
19	17.59	0.797 (3H, s)	17.6	0.64
20	40.48	1.874 (1H,m)	40.4	1.87
21	21.53	1.031 (3H, d, J = 4Hz)	21.1	1.05
22	135.61	5.138 (1H, dd, J = 2.5, 5.5Hz	135.6	5.20 J=15.6Hz)
23	131.91	5.205 (1H, dd, J = 5.0, 6.5Hz	132.0	5.21 J=15.6Hz
24	42.82	2.043 (1H, m)	42.8	2.05
25	33.08	1.450 (1H, m)	33.1	1.47
26	19.63	0.830 (3H, d, J = 6.5Hz)	19.6	0.84
27	19.94	0.880 (3H, d, J = 6.0Hz)	19.9	0.82
28	16.27	0.920 (3H, d, J = 4.5Hz)	16.2	0.92

Table 2. Infra-red spectrum for Ergosta-5,7,22-triene-3β,14α-diol (22Z)

Functional group	Signal
Hydroxyl (-OH)	3300cm^{-1} (strong)
Vinyl (=C-H)	3176.5cm^{-1} (weak)
Methyl (-CH_3) (sp^3 stretch)	2954.7cm^{-1} (strong)
Methylene (-CH_2) (sp^2 stretch)	2869.9cm^{-1} (weak)
Methine (-C-H) (sp stretch)	2657.7cm^{-1}) (weak)
Cyclic double bond (-C=C-)	1596.9cm^{-1} (sharp)
Carbonyl stretch (-C-O)	1226.6cm^{-1} (strong)



Biological assays of Ergosta-5,7,22-triene-3β,14α-diol (22Z) (1) from Kenyan *Ganoderma lucidum*

Table 3. Antibacterial activity of Ergosta-5,7,22-triene-3β,14α-diol (22Z) from *G. lucidum* and control

Micro-organism	Ampicillin	Ergosta-5,7,22-triene-3β,14α-diol (22Z)	*P*-value
Escherichia coli	NI	NI	
Pseudomonas aeruginosa	NI	NI	
Klebsiella pneumoniae	NI	NI	
MRSA	31±0.3	10.3±0.3	P=0.022
Streptococcus pyogenes	40.3±0.3	10.3±0.3	P=0.05

Key: NI: No Inhibition

Ergosta-5,7,22-triene-3β,14α-diol (22Z) (compound **1**) and the control Ampicillin were subjected to antibacterial tests using well diffusion methods. Compound **1** was active against MRSA (10.3 mm) and *S. pyogenes* (10.3 mm) as shown in Table 3. Both the isolated compound and the control did not cause inhibition of *P. aeruginosa*, *E. coli* and *K. pneumoniae*. These results show that concentration of the bioactive compound may have been low or the compound might have been inactive against the tested microbes. The findings in this study compared favourably with the results in a research by Nwachukwu et al. [19], Gebreselema Gebreyohannes et al. [20], Alves et al. [21] in which E. *coli*, was found to be resistant against all the control antibiotics used while *E. coli, K. pneumoniae*, and *P. aeruginosa* were resistant against the extracts (50 mg/ml) and the control antibiotic ampicillin [22].This results therefore show that the compound does not possess antimicrobial activity against *P. aeruginosa, K. pneumoniae and E. coli* at the given concentration. Further bioactivity studies with higher concentration may reveal the actual concentration that the compound may inhibit against *P. aeruginosa, K. pneumoniae and E. coli.* Other researchers have also revealed that ergosterol from *G. lucidum* has been used by traditional healers in the treatment of malaria [23] and has also proved to be toxic against brine shrimp larvae [16].

4. CONCLUSION

A sterol, Ergosta-5,7,22-triene-3β,14α-diol (22Z) was isolated from hexane extract of Kenyan *Ganoderma lucidum*. It was found to be active against *MRSA* and *Streptococcus pyogenes*.

5. RECOMMENDATIONS

There is need to support cultivation of mushrooms for large scale extraction of useful compounds. These compounds would form a basis for useful drugs to prevent anti-microbial resistance.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Matthias (University of Postdalm, Germany) for running spectral data for the isolated compounds. Our appreciation goes to Mr. Peter Nyongesa, Chief technician, microbiology department of Masinde Muliro University for his help, advice and guidance in antibacterial investigation. We also thank the herbarium staff at the East African Herbarium - National Museums of Kenya, botany department in Nairobi for identifying *Ganoderma lucidum* mushroom.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shu-ting C, Miles PG. Mushrooms: Cultivation, nutritional value, medicinal effect and environmental impact. 2nd Ed. CRC Press, London; 2004.
2. Pauw DBE. Is there need for new antifungal agents? Clin. Microbiol. Infec. 2000;6:23-28.
3. Soji F, Kehinde TA, Adedamola AO. Biopharmaceutical Assessment of Active Components of *Deadaleopsis confragosa* and *Ganoderma lucidum.* Open Journal of Medical Microbiology. 2013;3:135-138.
4. Vinay GU, Shivayogeeswar N, Madappa K. *Ganoderma lucidum*: A source for novel bioactive lectin. Protein and Peptide Letters. 2011;18:1150-1157.
5. Gao B, Yang G. Effect of *Ganoderma applanatum* polysaccharides on cellular humoral immunity in normal and sarcoma 180 transplanted mice. Phytopathological Research. 1991;5:134-138.
6. Jonathan SG. Vegetative growth requirements and antimicrobial activities of some higher fungi from Nigeria. Ph.D thesis, University of Ibadan, Ibadan, Nigeria. 2002;267.
7. Akpaja EO, Okhuoya JA, Ehwerheferere BA. Ethnomycology and indigenous uses of mushrooms among the Bini-speaking people of Nigeria: A case study of Aihuobabekun community near Benin City. Nigeria. International Journal of Medicinal Mushrooms. 2005;7:373-374.
8. World Health Organization. Antimicrobial resistance: Global Report on Surveillance. 2014;257.
9. Donadio S, Carrano L, Brandi L, Serina S, Soffientini A, Raimondi E, et al. Targets and assays for discovering novel antibacterial agents. Journal of Biotechnology. 2002;99:175-185.
10. Ely SR, Naaik, CG. Antibacterial activity of marine organisms collected from the coast of South East India. Journal of Explore Biology and Ecology. 2004;309: 121-127.
11. Miguel M, Alviez T, Cami S, Geraldine M, Jose B, Jose V. Ergosterol from the mushroom *Laetiporus* sp.; Isolation and chemical characterization. Bolivian Journal of Chemistry. 2015;32(4):90-94.
12. Wasser SP, Weiss AL. Medicinal properties of substances occuring in higher basidiomycetes mushrooms: Current perspectives (Review). Int. J. Med. Mushr. 1999;1:31-62.
13. Bok JW, Lermer L, Chilton J, Klingeman HG, Towers GHN. Antitumor sterols from the mycelia of *Cordyceps sinensis*. Phytochemistry. 1999;51:891-898.
14. Nam KS, Jo YS, Kim YH, Hyun JW, Kim HW. Cytotoxic activities of acetoxyscirpenediol and ergosterol peroxide from *Paecilomyces tenuipes*. Life Sci. 2001;69:229-237.
15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antibacterial activity: A review. Journal of Pharmaceutical Analysis. 2015;11(5).
16. Baraza LD, Joseph CC, Moshi MJ, Nkunya MHH. Chemical constituents and biological activity of three Tanzanian wild mushroom species. Tanz. J. Sci. 2007;33.
17. Pavia DL, Lampman, GM, Kriz, GS. Introduction to spectroscopy: A guide for

students of organic chemistry. Philadelphia: Harcourt College Publishers; 1996.

18. Nao-Yun Ji, Xiang-Hong L, Xiao-Zhen T, Feng-Ping M. A new pyrrolidine derivative and steroids from an algicolous *Gibberella zeae* strain. Natural Product Communications. 2011;6(9):1243-1246.
19. Nwachukwu NC, Iheukwumere I, Ekeleme E. Antibacterial effects of the mushroom *Termitomyces letestui* on *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. Academic Journal of Science. 2017; 07(02).
Available: http://universitypublications.net
[ISSN: 2165-6282]
20. Gebreselema Gebreyohannes, Andrew Nyerere, Christine Bii, Desta Berhe Sbhatu. Determination of antimicrobial activity of extracts of Indigenous Wild Mushrooms against pathogenic organisms; Evidence-Based Complementary and Alternative Medicine. 2019;2019:7.
21. Alves MJ, Ferreira ICFR, Martins A, Pintado M. Antimicrobial activity of wild mushroom extracts against clinical isolates resistant to different antibiotics. J Appl Microbiol. 2012;113(2):466-75.
22. Asogwa FC, Okoye COB, Ugwu Okechukwu PC, Edwin Nzubechukwu, Alum Esther U, Egwu Chinedu O. Phytochemistry and antimicrobial assay of *Jatropha curcas* extracts on some clinically isolated bacteria - A comparative analysis. European Journal of Applied Sciences. 2015;7(1):12-16.
23. Garcia J, Morin C. Complete structural assignments of an Ergosterol Derivative from *Entandrophragma utile*. Journal of Natural Products. 1991;54(1):136-42.

APPENDIX

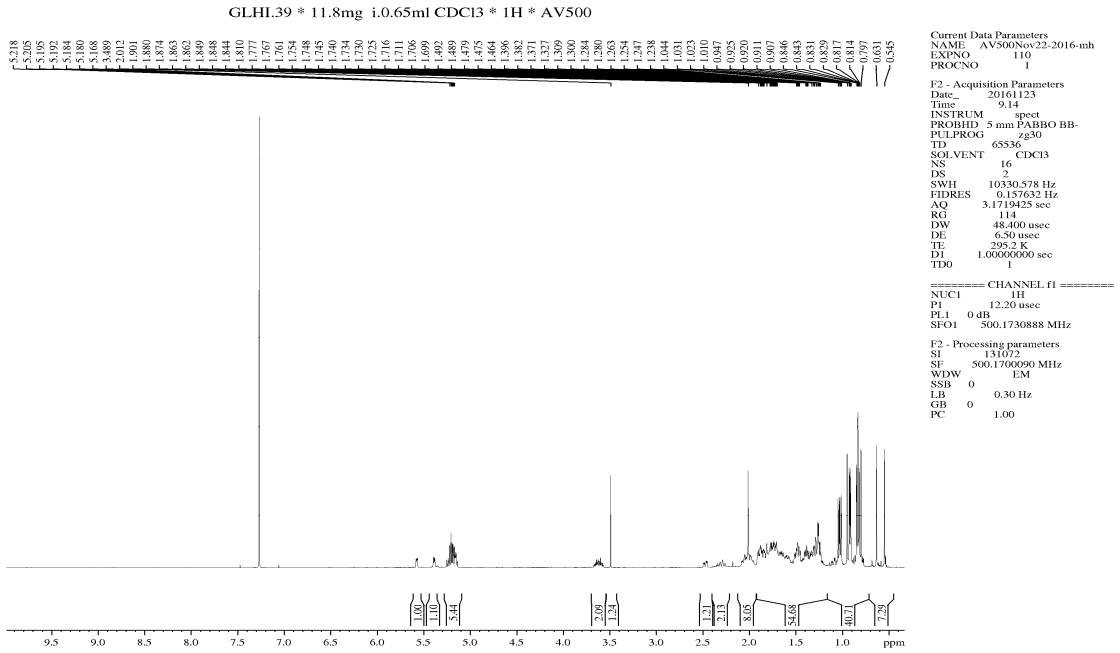


Fig. A1. ^{1}H-NMR for Ergosta-5,7,22-triene-3β,14α-ol (22Z) (1)

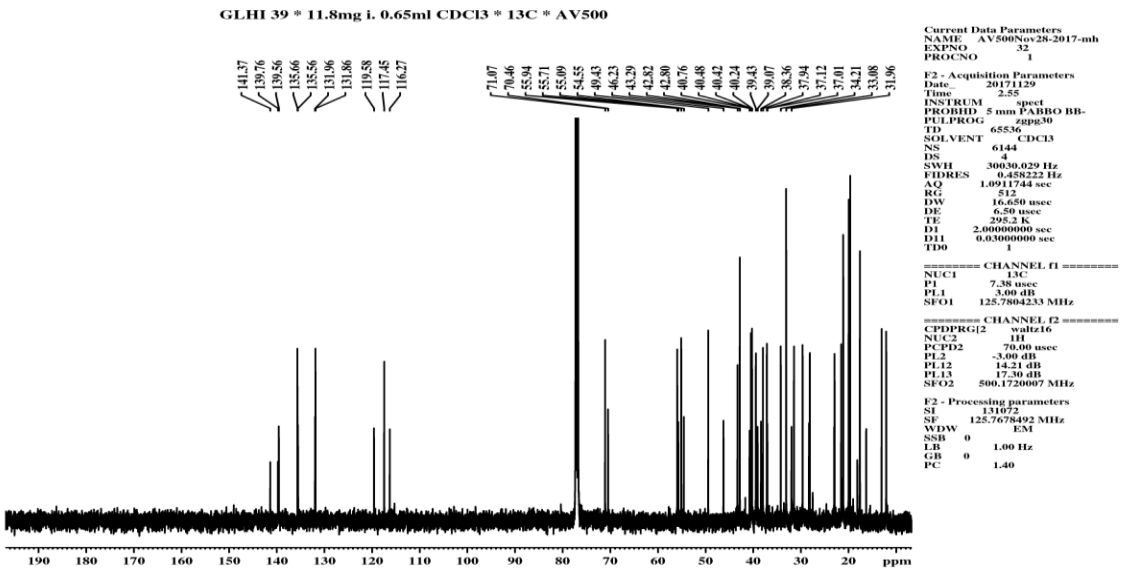


Fig. A2. ^{13}C-NMR spectrum for Ergosta-5,7,22-triene-3β,14α-ol (22Z) (1)

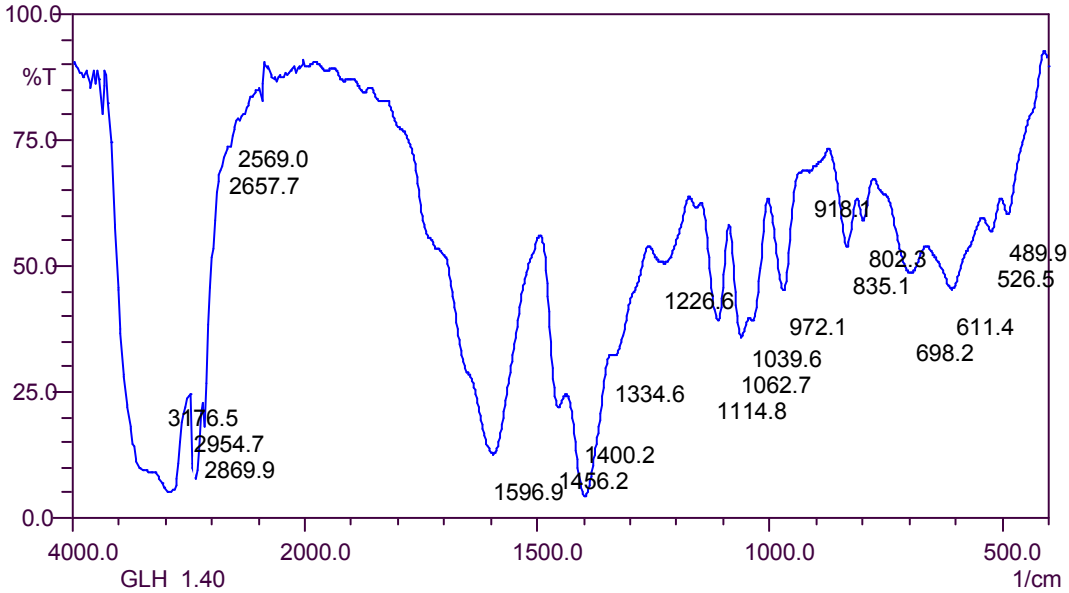


Fig. A3. Infra-red spectra for Ergosta,5,7,22-triene-3β,14α-diol (22Z)

Fig. A4. *Ganoderma lucidum* from Kakamega, Kenya

Fig. A5. *S. aureus*. Number 1 is compound (Ergosta-5,7,22-triene-3β,14α-ol (22Z) (10mm). Numbers 2, 3 and 4 are impure compounds from EtOAc and MeOH extracts of *G. lucidum*

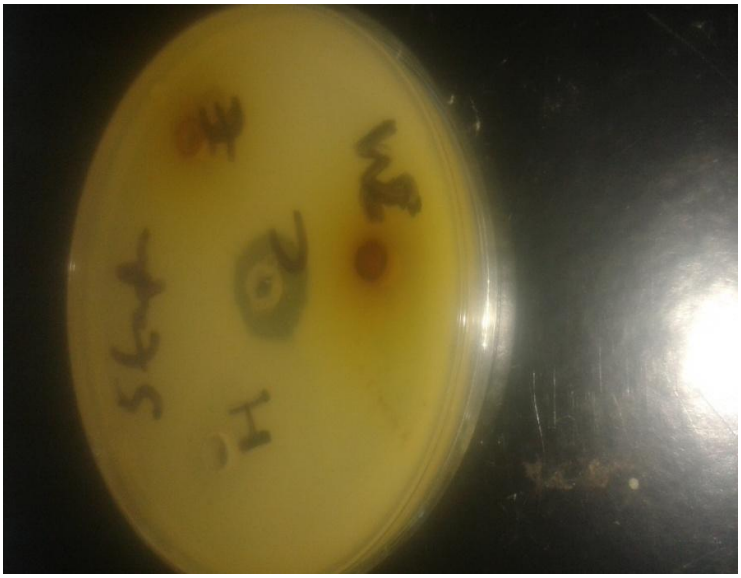


Fig. A6. *S. pyogenes*. At the centre of the petri dish is the positive control (Ampicillin) (31mm), the next three are crude extracts of hexane, ethylacetate and methanol of *Ganoderma lucidum*

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/54802