Antimicrobial activity of isolated Stigmast-5-en-3β-ol (β-Sitosterol) from Honeybee Propolis from North-Western, Nigeria.

Jephthah O. Odiba[a*], Aliyu M. Musa[a], Halimatu S. Hassan[a], Sani M. Yahaya[a] and Ephraim I. Okolo[b]

[a]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.
[b]Department of Chemistry, Kogi State University, Anyigba, Nigeria.
Email: jefexy@gmail.com

Abstract

The new reported exploration into the chemical profiling of constituents of honey bee propolis from North Western, Nigeria led to the isolation of a sterol; β-Sitosterol from the n-hexane-soluble fraction of the ethanol extract of the propolis. The structures of these compounds were characterised by comparing their spectral data including 1D and 2D NMR with those reported in the literature. The isolated compound was active against 6 out of 12 tested microorganisms including; *Staphylococcus aureus, Bacillus subtilis, Streptococci pyrogene, Klebsiella pneumonia Shigella dysentariae* and *Candida krusei.* The zones of inhibition ranged between 26mm to 34mm against the test microorganisms. The MIC and MBC value was as low as 12.5-25µg/mL and 25-100µg/mL respectively against test bacteria's while the MFC value was 50µg/mL against *Candida krusei*. This is a new report of the isolation of this compound from the propolis around this region.

Keywords: Propolis, Sterol, Antimicrobial activity, 2D NMR

Introduction

Antimicrobial agents from natural products have been one of the world reliable sources of drug discovery in order to treat infectious diseases. It has been estimated that 25% of prescription drugs and 11% of drugs considered essential by the World Health Organization (WHO) are derived from natural products and a large number of these synthetic drugs are obtained from precursor compounds originating from plants and natural products (Rates, 2001). Hence, secondary metabolites such as steroid, flavonoids, alkaloids, terpenes, anthocyanin and tannins plays important role as a potential lead for novel medications and also, due to the overwhelming rate of bacterial resistance to medication there is a continuous need in search of novel and potent antimicrobial agents.

Propolis (bee glue) which is a waxy resinous natural produce of honey producing bee *Apis mellifera* Linn, has been reported to compose of 50% resin (admixture of flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds (Ashraf, 2009), and the chemical constituents of each propolis varies quantitatively and qualitatively from different subcontinent and locality (Ashraf, 2009; Bankova, Castro, & Marcucci, 2000). This is associated with the different types of vegetation as well as medicinal plants found around that region it was collected.

Similarly, propolis have been shown to possess diverse biological activities such as anti-tumour (Feng et al., 2009), antimicrobial (Ashraf, 2009), antioxidant, anti- inflammatory (allergy, rhinitis and asthma), antimicrobial action, anti-ulcer activity, anti-viral (Jose & Vassya, 2010; Viuda-Martos, Ruiz, Fern, & Perez, 2008) and is being used in Nigeria traditionally as an emollient in treatment of measles, ringworm, chickenpox disease as well as healing potential for septic wounds (Abel & Banjo, 2012; Adewumi & Ogunjinmi, 2011). Therefore, it is of noteworthy that diverse melliferous plants grow around the Northern guinea savannah (North West) of Nigeria which could account for the medicinal properties of that propolis collected from this region (Abel & Banjo, 2012). Therefore as a result of the therapeutic potential of this natural product this has steered research into the chemical profiling of North- Western Nigeria honey bee propolis in order to provide a scientific backing of its ethno medicinal uses.

2.0 Materials and Methods

2.2 Collection of Honey bee propolis

Honey bee propolis was collected from Zango Kataf Local Government Area of Kaduna State, Nigeria, from Honeybee cultivators, propolis was pressed to remove stored honey from it, sizes reduce with scissors and stored for use

2.3 Extraction and Fractionation of Propolis

The raw honey bee propolis (2500g) was extracted with 4 Litres of 95% ethanol (Chia-Chi, Ming-Hua, Hwei-Mei, & Jing-Chuan, 2002) using cold maceration method for 7days. The extract was filtered using Whatman

No. 1 filter paper and concentrated in vacuo to yield a brown semi solid residue (350g) referred to as honeybee propolis ethanol extract (CR). The ethanol extract (300g) was suspended in distilled water and partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol to obtain n-hexane fraction 18.5g (HH), chloroform fraction 4.6g (CC), ethyl acetate fraction 7.1g (EE), n-butanol fraction 27.8g (BB), and the residual aqueous fraction 230.7g (AF) respectively.

2.4 Chromatography Separation

The n-hexane soluble sub fraction of the ethanol extract of propolis was subjected to thin layer chromatography using silica gel as stationary phase and n-hexane: ethyl acetate (9:1) and n-hexane: ethyl acetate (8:2) as mobile phase. The chromatograms when developed yielded eight and four spots respectively after being sprayed with 10% sulphuric acid and also showed zones for steroidal nucleus with Salkowski detecting reagent. Column chromatography of HH (7g) was conducted using silica gel (Mesh 60-120 µm) that was packed using wet packing method. The column was eluted continuously using hexane and ethyl acetate by gradient elution technique. Thin Layer Chromatography (T.L.C) was used to monitor the eluates. A total of 95 eluates, 100ml each were collected. Similar fractions were pooled together to give eight major fractions (H1 to H8). Eluates H5 was further subjected to small column chromatography to yield three major fractions H5-1, H5-2 and H5-3 respectively. Further purification of fraction H5-2 led to the isolation of a white crystalline powder (10mg) coded as compound J1 which yielded a single homogenous spot when subjected to T.L.C using solvent systems n-hexane: ethyl acetate (9:1, 8:2) and a melting point of (136-137^{0}C) using thermal melting point apparatus. J1 was further subjected to Infra-Red (IR) Spectroscopy, Proton Nuclear Magnetic Resonance Spectroscopy (1HNMR), ^{13}CNMR and 2D NMR analysis to ascertain the chemical structure.

2.5 Antimicrobial Assay

The antimicrobial activity of the isolated compound J1 was determined according to the described agar diffusion method (Agbagwa & Okolo, 2012). Sparfloxacin, Ciprofloxacin and fluconazole at 5µg/mL were used as the standard antimicrobial agent. Clinical isolates; *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Corynebacterium ulcerans*, *Bacillus subtillis*, *Shigella dysentariae*, *Proteus mirabilis*, *Candida albicans*, *Candida krusei* and *Candida tropicalis* were used as the test organisms which were obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital Zaria, Nigeria (ABUTH).

The inoculum were prepared using muller hinton broth and incubating for 24 hours at 37^{0}C for bacteria and while fungi, saboraud dextrose broth was used and incubated for 48 hours at 25^{0}C and standardised. Stock solution 0.1mg/mL of compound J1 was prepared using Dimethyl Sulfoxide (DMSO), and 0.1ml of the stock was introduced separately into each 6mm diameter well (cups) bored in a solidify Muller Hinton agar plates already seeded with 0.1ml of standard inoculum and incubated according to standard specification of the organism. The antimicrobial activities were expressed as the diameter to the nearest millimetres of zone of inhibition by the compound J1. The test where perform in triplicates with controls.

2.6 Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined on the organisms that were sensitive to the compound J1, and was carried out using broth dilution method (Agbagwa & Okolo, 2012; Vollekova, Kostalova, & Sochorova, 2001). Two fold serial dilutions of variable concentrations of the compound J1 (100-62.5µg/mL) were prepared and the test organisms (0.1 mL) were inoculated into each tube containing the test compound and broth. The tubes were incubated at 37ºC for 24 hours for bacteria and 48 hours at 25ºC for fungi. The lowest concentration of the extract showing no visible growth (turbidity) of the test organism was considered to be the MIC.

2.7 Minimum bactericidal and fungicidal concentration (MBC/MFC)

The contents of the MIC tubes in the serial dilution were sub cultured onto appropriately labelled Mueller Hinton and Saboraud dextrose agar plates, and incubated at 37ºC for 24 hours and 25^{0}C for 48 hours for bacteria and fungi respectively, then they were observed for colony growth. The lowest concentration of the sub culture with no growth was considered as the MBC and MFC (Damintoti, Mamoudou, Jacques, & Alfred, 2005; Vollekova et al., 2001).

3.0 Result and Discussion

The IR absorption spectrum showed a broad band absorption peaks at 3390.9cm^{-1}(O-H stretching) and moderate intense band at 2935.7cm^{-1} (aliphatic C-H stretching), weak intense band at 1450.5cm^{-1} (C=C band), 1377.2cm^{-1} (C-H bending) and 1051.2cm^{-1} (C-O stretching band) (Pateh et al., 2008).

The 1H NMR spectrum (400MHZ, $CDCl_3$) of J1 revealed the presence of six methyl protons at δ 0.74, 0.87, 0.88, 0.89, 0.93 and 1.06 (3H each), this proton signal between (0.7-1.8) are attributed to resonance of overlapping methyl and methylene protons, a characteristic frame work of steroids (Yun-song, Jin-Hua, Hong-Bin, & Liang, 2006). Multiplets of two protons at δ 2.30 were observed and the H-3 proton at δ 3.50 is an

indication of a carbinol proton in steroids or triterpenes nucleus, while a doublet of one proton at δ 5.39 representing an olefinic protons at H-6. All these resonances are similar to that of β- sitosterol compound reported (Hamada, Soumia, Catherine, & Mohammed, 2012).

The result of ^{13}C-NMR and DEPT of J1 showed 29 C- signals. Six methyl groups at [(δc: 11.9 (C-18), 19.8 (C-19), 18.3 (C-21), 21.1 (C-26), 19.4 (C-27), and 11.9 (C-29)], an olefinic carbons appeared at δc: 140.8 (C-5) and 121.7 (C-6), and a secondary hydroxyl bearing carbon at [(δc: 71.8 (C-3)], in addition to eleven methylene, nine methine and three quaternary carbons. The de-shielded signal at δc 71.8 was due to C-3 with a hydroxyl group attached to it. These carbon-13 resonances are also in agreement with reported β-sitosterol compound (Hamada et al., 2012; Pateh et al., 2008).

The COSY spectrum of J1 showed some cross peak between the methylene proton at (δH 1.90, H-1), correlation with methylene proton at (δH 1.30, H-2). The carbinol proton of (δH 3.50, H-3), showed cross peak correlation with methylene protons at (δH 1.30, H-2) and (δH 2.30, H-4). The singlet of olefinic proton of (δH 5.39, H-6), showed cross peaks correlation with methylene proton at (δH 1.51, H-7). The methylene proton of (δH 1.51, H-7) showed correlation with methine proton at (δH 1.88, H-8). The methylene proton of (δH 1.28, H-11) showed cross peaks correlation with methylene protons at (δH 2.06, H-12).

The NEOSY spectra of J1, the methyl proton of (δH 0.74, H-18) showed space coupling with methylene proton at (δH 2.06, H-12). While methyl proton of (δH 1.06, H-19) showed space coupling with methylene proton at (δH 1.90, H-1).

The HMBC spectra of J1, the methylene proton of (δH: 1.90, H-1) showed J^2 correlation with methylene carbon signal at (δc 35.9, C-2), and J^3 correlation with methine carbon signal at (δc:71.8, C-3), quaternary carbon signal at (δc:140.8, C-5) and methyl carbon signal at (δc:11.9, C-19). The methylene proton of (δH 2.30, H-4) showed long range J^3 correlation with methylene carbon signal at (δc: 35.9, C-2), and quaternary carbon signal at (δc 140.8, C-5), and J^2 correlation with methine carbon at (δc:71.8, C-3) and (δc:121.7, C-6). The methine proton at (δH 5.39, H-6) showed correlation with methylene carbon signal at (δc: 42.3, C-4), (δc: 33.9, C-7) and J^3 correlation with methine carbon signal at (δc 36.1, C-8). Also methylene proton at (δH 1.51, C-7) showed J^3 correlation with methine carbon signal at (δc 50.1, C-9) and (δc 56.8, C-14). The methine proton at (δH 1.98, H-8) showed J^2 correlation with methine carbon signal at (δc 50.1, C-9) and J^3 correlation with quaternary carbon signal at (δc 42.4, C-13). The methine proton at (δH 1.04, H-14) showed J^3 correlation with methylene carbon signal at (δc 33.7, C-16) and with methine carbon signal at (δc 56.1, C-17). The methylene proton at (δH 2.0, H-20) showed J^2 correlation with methyl carbon signal at (δc 18.3, C-21) and J^3 correlation with quaternary carbon signal at (δc 42.4, C-13), while the methyl proton at (δH 0.88, H-26) showed J^3 correlation with methine carbon signal at (δc 31.9, C-25) and methyl carbon signal at (δc 19.4, C-27).

Confirmation of the structure of J1 was accomplished by 2D NMR experiments (COSY, NOESY, HSQC and HMBC). Based on the result of 1D and 2D NMR and comparing the data obtained with that in the literature (Hamada et al., 2012; Pateh et al., 2008), J1 was suggested to be β-Sitosterol as shown in Figure 3

Table 1: Comparative ^{1}H and ^{13}C-NMR Chemical Shifts of Compound J1 in $CDCl_3$, 400 MHz with a known compound.

Position carbon	**δ_c (ppm)***	**δ_c (ppm)**	**δ_H (ppm)***	**δ_H(ppm)**
1	37.2	37.3	1.90	1.90
2	31.6	35.9	1.56	1.30
3	71.8	71.8	3.58	3.50
4	42.3	42.3	2.30	2.30
5	140.7	140.8	-	-
6	121.7	121.7	5.40	5.39
7	31.9	33.9	1.50	1.51
8	31.8	36.1	2.30	1.98
9	50.1	50.1	0.98	0.98
10	36.5	39.8	-	-
11	21.1	29.7	1.50	1.28
12	39.7	42.3	2.06	2.06
13	42.3	42.3	-	-
14	56.7	56.8	1.04	1.04
15	24.3	29.2	1.11	1.28

16	28.2	33.7	1.30	1.30
17	56.0	56.1	1.16	1.05
18	11.8	11.9	0.74	0.74
19	19.4	19.8	1.06	1.06
20	36.1	38.8	1.40	2.00
21	18.8	18.3	0.93	0.93
22	33.9	36.5	1.07	1.07
23	26.0	31.7	1.23	1.22
24	45.8	45.8	0.97	0.97
25	29.1	31.9	1.71	1.74
26	19.8	21.1	0.88	0.88
27	19.0	19.4	0.87	0.87
28	23.0	23.1	1.31	1.31
29	12.0	11.9	0.89	0.89

Data from Hamada *et al.*, (2012)*****

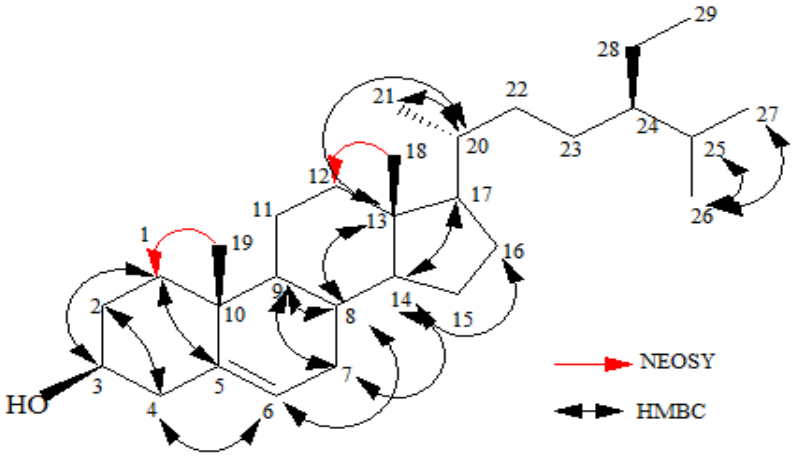


Fig 2 NEOSY and HMBC of isolated β-Sitosterol

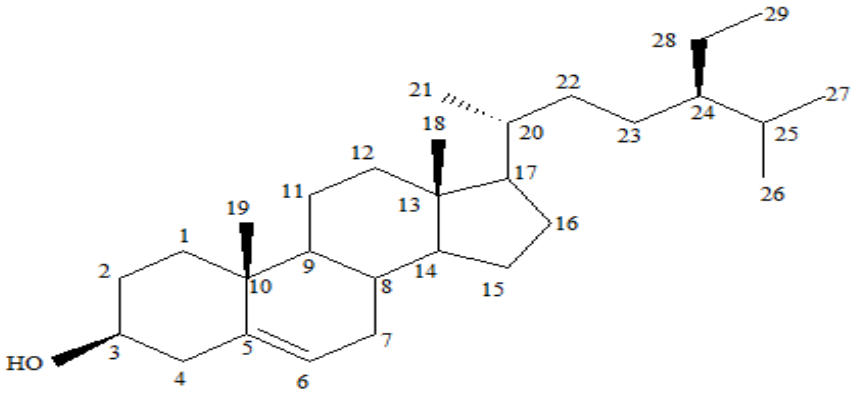


Fig 3: Stigmast-5-en-3β-ol (β-Sitosterol)

3.1 Antimicrobial Assay Result

The antimicrobial actiivity tests results were expressed in terms of MIC, MFC, MBC, and diameter of zones of inhibition of the test organism. The attained results are shown in Tables 2 - 3. The results from the sensitivity test showed that compound J1 had remarkable activity against some tested microorganisms with inhibition zones ranging 26mm to 34mm. The isolated compound J1 was active against *S. aureus, B. subtilis, S. pyogenes, K. pneumoniae, S. dysenteria and C. krusei* with the exception of *C. ulcerans, E. coli, P. mirabilis, P.*

aeruginosa, C. albicans and C. tropicalis The MIC values ranging from 12.5-25µg/mL and MBC values ranging from 25-100µg/mL, while MFC value of J1 was 50µg/mL.

Table 2: Zones of Inhibition of J1 and Standard Drug against Test Organism

	Zone of Inhibition (mm)			
Test organism	**J1**	**SPX**	**CPO**	**FLZ**
S. aureus	27	37	32	-
B. subtilis	34	42	37	-
S. pyogenes	30	40	38	-
C. ulcerans	0.0	34	36	-
E. coli	0.0	32	30	-
K. pneumoniae	26	47	32	-
P. aeruginosa	0.0	30	0.0	-
S. dysenteria	28	35	37	-
P. mirabilis	0.0	30	32	-
C. albicans	0.0	-	-	37
C. krusei	27	-	-	40
C. tropicalis	0.0	-	-	35

Key: SPX – sparfloxacin CPO- ciprofloxacin FLZ- fluconazole

Table 3: MIC and MBC/MFC of compound J1 (µgmL^{-1})

	MIC (µg/mL^{-1})	**MBC/MFC (µg/mL^{-1})**
Test organism	**J1**	**J1**
S. aureus	25.0	50.0
B. subtilis	12.5	25.0
S. pyogenes	12.5	50.0
K. pneumoniae	25.0	100.0
S. dysenteria	12.5	50.0
C. krusei	25.0	50.0

Similarly, the isolated β-Sitosterol were observed to have an appreciable and slightly lower zones of inhibition (26mm to 34mm) compare to standard drugs (Sparfloxacin Ciprofloxacin and Fluconazole). In addition, the lower MIC value of the isolated compound is a characteristics of high potent antimicrobial activity; in relationship with the fact that compounds possessing MIC value lower than 100µg/mL are good antimicrobial

agents (Tang et al., 2003). Hence, these antimicrobial potency of β-Sitosterol could be associated with its mechanism of action which could be liken to membrane disruptions of the susceptible test organism (Cowan, 1999). The steroid exhibit a broad spectrum of activity with more pronounce effect on gram positive organism compare to gram negative organism and slight activity on fungi cells (Table 2). However, illness such as; wound infections, pneumonia and sepsis caused by pathogenic *S. aureus, S. pyrogenes* and *B. subtilis* (Centers for Disease Control and Prevention, 2014), urinary tract infection and dysentary cause by pathogenic *K. pneumoniae* and *S. dysenteria* (National Iinstitute of Allergy and Infectious Disease, 2012) as well as, candida infection cause by *C. krusei* (Rajendra, Kura, Arvind, & Panthaki, 2010) shows susceptibilty to the steriods, hence indicating their usefulness in the treatment of such disease. However, their biological activities show that they are in accordance to β-Sitosterol isolated from some reported medicinal plants (Sen, Poonan, Kshitiz, Sanjay, & Tejovathi, 2012; Sileshi, Legesse, Yinebab, Diriba, & Tadesse, 2012).

Therefore, β-Sitosterol which is a known secondary metabolite presence in oil producing parts of natural products; fruits, plants, seeds exhibit several biological activities alongside with the aforementioned properties therefore making propolis a potenciate source of bioactive compounds, however, a robust clinical trials and pharmacological screening are need to established it therapeutic effectiveness.

Conclusion

The study reaveled a new investigation into the chemical profiling of n-hexane soluble sub fraction of ethanol extract of honey bee propolis from North Western Nigeria. Similarly, this led to the isolation of β-Sitosterol. The compound possesses good antimicrobial activity justifying the scientific backing of the propolis use traditional in Nigeria and as also a potential source for bioactive compounds to treat infectious disease. Further chemical profiling should be carried out on other soluble sub-fractions of the propolis in order to ascertain more biological activity.

Acknowledgement

We wish to acknowledge Mr Dilip Jagjivan, of the school of chemistry and physics, University of KwaZulu-Natal Westville campus, Durban, South Africa, for the technical assistance with 1D and 2D NMR.

Reference

[1] Abel, A. A., & Banjo, A. D. (2012). Apitherapy in Southern Nigeria: An assessment of therapeutic potentials of some honeybee products. Journal of Pharmaceutical and Biomedical Sciences, 2(2), 9-15.
[2] Adewumi, A. A., & Ogunjinmi, A. A. (2011). The healing potential of honey and propolis lotion on septic wounds. Asian Pecific Journal of Tropical Biomedical Science, S55-S57.
[3] Agbagwa, O. E., & Okolo, I. (2012). Antibacterial activity of honey and propolis marketed in Nigeria using well-in-agar and disc diffusion method. Internationalk Research Journal of Microbiology, 3(3), 101-105.
[4] Ashraf, A. E. (2009). New prenilated compound from Egyptian propolis with antimicrobial activity. Revista Lantinoamericana e Quimica, 37(1), 85-90.
[5] Bankova, V., Castro, S. L., & Marcucci, M. C. (2000). Propolis: Recent advances in chemistry and plant origin. Apidologie, 31, 3-15.
[6] Centers for Disease Control and Prevention. (2014). Disease/pathogen associated with antimicrobial resistance. Retrived 30, October 2014 From: http://www.cdc.gov/drugresistance/diseasesconnectedar.html.
[7] Chia-Chi, C., Ming-Hua, Y., Hwei-Mei, W., & Jing-Chuan, C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10(3), 178-182.
[8] Cowan, M. M. (1999). Plants products as antimicrobial agents. Clinical Microbiology Review, 12, 564-582.
[9] Damintoti, K., Mamoudou, H. D., Jacques, S., & Alfred, S. T. (2005). Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. Africa Journal of Biotechnology, 4(8), 823-828.
[10] Feng, L., Suresh, A., Hongyan, Z., Yasuhiro, T., Hiroyasu, E., & Shigetoshi, K. (2009). Chemical constituents of propolis from Myanmar and their preferential cytotoxic against a human pancreatic cancer cell lines. Journal of Natural Product, 72, 1283-1287.
[11] Hamada, H., Soumia, M., Catherine, L., & Mohammed, B. (2012). Chemical constituents of Centaurea omphalotricha Coss and Durieu ex Bath and Trab. Records of Natural Products, 6(3), 292-295.
[12] Jose, M. S., & Vassya, B. (2010). Propolis: Is there a potential for development of new drugs. Journal of Ethnopharmacology, 133, 253-260.
[13] National Iinstitute of Allergy and Infectious Disease. (2012). Antimicrobial (Drug)Resistance. Retrieved October 30, 2014 From: http://www.niaid.nih.gov/topics/antimicrobialresistance/examples/gramnegative/Pages/default.aspx.
[14] Pateh, U. U., Haruna, A. K., Garba, M., Iliya, I., Sule, I. M., Abubakar, M. S., & Ambi, A. A. (2008). Isolation of stigmasterol, beta-sitosterol and 2-Hydroxyhexadecanoic acid methyl ester from Rhizome of Stylochiton lancifolius Pyer and Kotchy (Araceae). Nigeria Journal of Pharmaceutical Sciences, 8(1), 19-25.
[15] Rajendra, J. K., Kura, M. M., Arvind, G. V., & Panthaki, M. H. (2010). Candida tropicalis: its prevalence, pathogenecity and increasing resistance to fluconazole. Journal of Medical Microbiology, 59(8), 873-880.
[16] Rates, S. M. (2001). Plants as source of drugs. Toxicon, 39, 603-613.
[17] Sen, A., Poonan, D., Kshitiz, K. S., Sanjay, S., & Tejovathi, G. (2012). Analysis of IR, NMR and antimicrobial activity of Beta sitosterol isolated from Momordica charantia. Science Secure Journal of Biotechnology, 1(1), 9-13.
[18] Sileshi, W., Legesse, A., Yinebab, T., Diriba, M., & Tadesse, B. (2012). Evaluation of antibacterial activities of compounds isolated from Sida rhombifolia Linn. (Malvaceae. Natural Products Chemistry and Research, 1(1), 1-8.
[19] Tang, T., Bremner, P., Kortenkemp, A., Schlage, C., Gray, A. I., Gibbons, S., & Heinrich, M. (2003). Biflavonoids with cytotoxic and antibacterial activity from Ochna macrocalyx. Plan Med., 69, 247-253.
[20] Viuda-Martos, M., Ruiz, N. Y., Fern, A. L. J., & Perez, A. J. A. (2008). Functional properties of honey bee propolis and royal jelly. Journal of Food Science, 73(9), R117-124.
[21] Vollekova, A., Kostalova, D., & Sochorova, R. (2001). Isoquinoline alkaloids from Mahonia aquifolium stem bark are active against Melssezia species. Folia Microbiol, 46, 107-111.

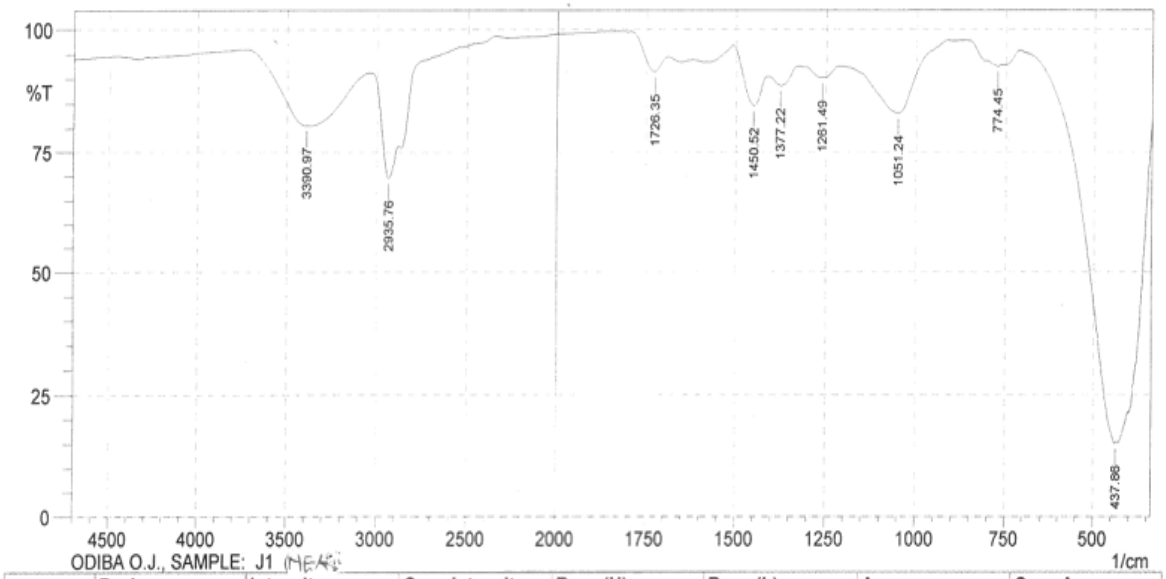
[22] Yun-song, W., Jin-Hua, Y. S., Hong-Bin, Z., & Liang, L. (2006). New cytotoxic steriod from Stachyrus himalaicus Var himalaicus. Molecules, 11, 536-542.

Appendix

SHIMADZU

FTIR ANALYSIS RESULT NARICT,ZARIA

FTIR- 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER



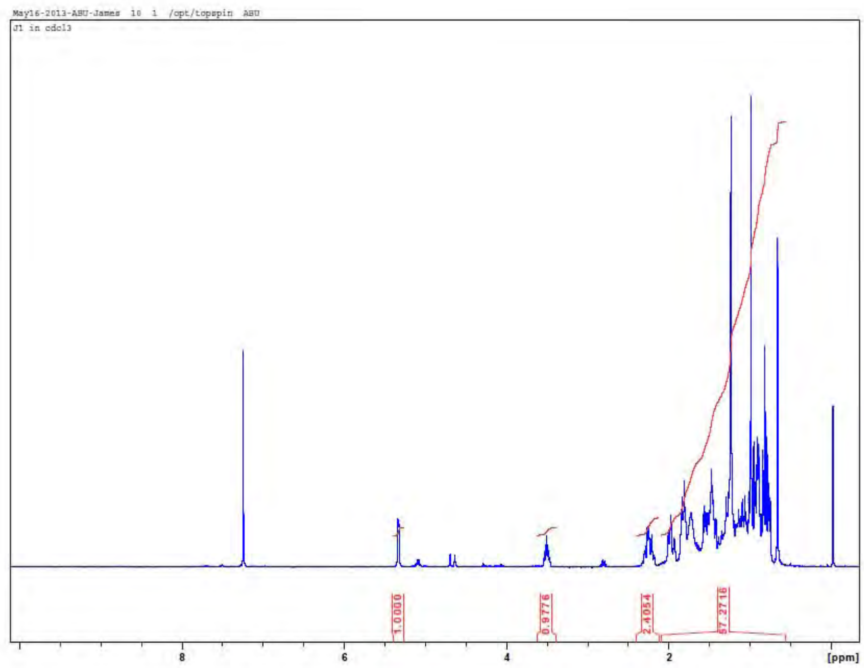
	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	437.86	15.147	0.197	440.75	434	5.507	0.014
2	774.45	92.648	0.471	804.34	759.01	1.434	0.058
3	1051.24	83.06	12.439	1216.16	915.25	13.687	7.28
4	1261.49	90.189	2.36	1307.78	1216.16	3.674	0.592
5	1377.22	88.703	2.697	1410.98	1329	3.721	0.587
6	1450.52	84.623	8.45	1509.35	1410.98	4.836	2.025
7	1726.35	91.519	0.226	1729.24	1689.7	1.246	-0.008
8	2935.76	69.58	11.471	3042.81	2884.64	16.078	3.582
9	3390.97	80.585	0.198	3673.55	3387.11	17.113	0.602

Comment;
ODIBA O.J., SAMPLE: J1 (NEAT)

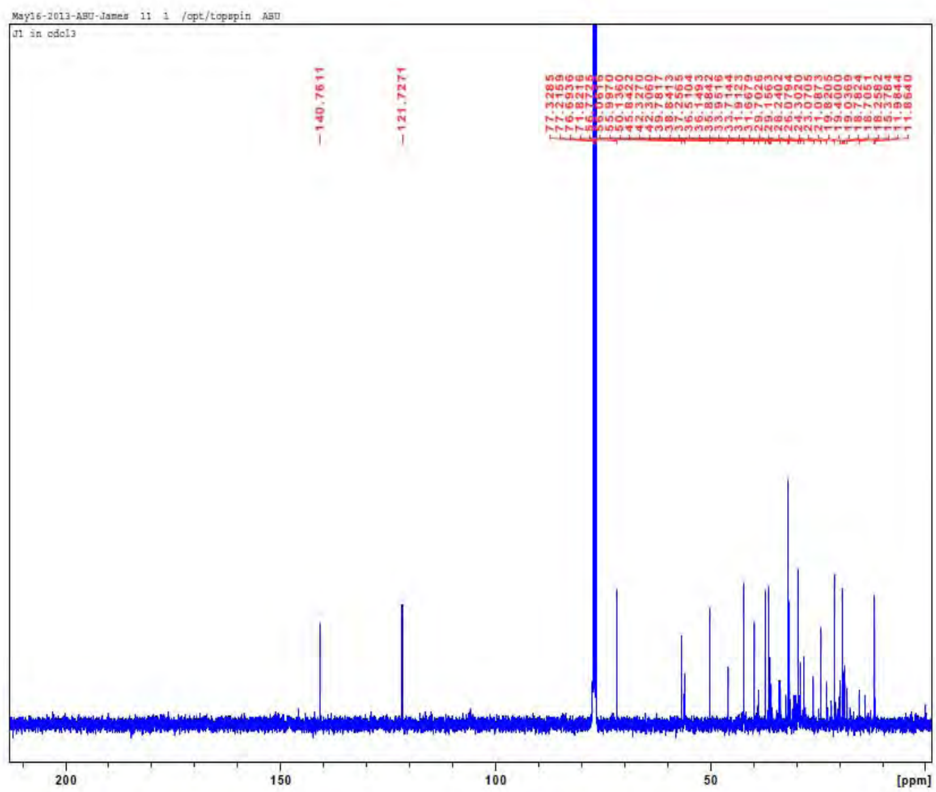
User; Administrator

No. of Scans;
Date/Time; 5/13/2013 11:41:02 AM
Resolution;

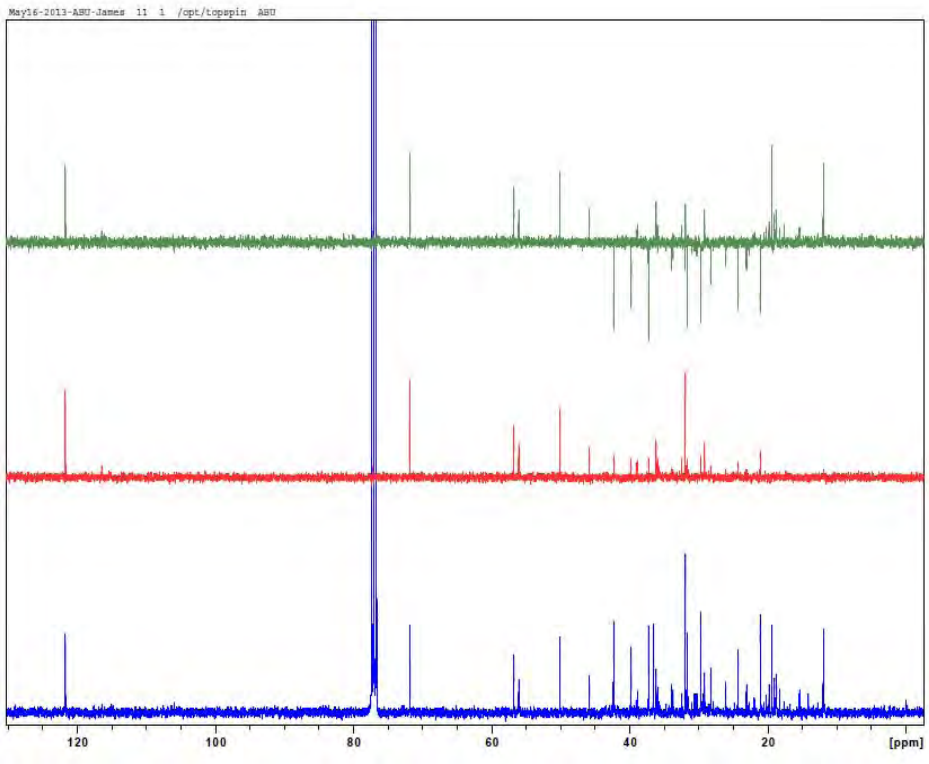
IR spectrum of J1



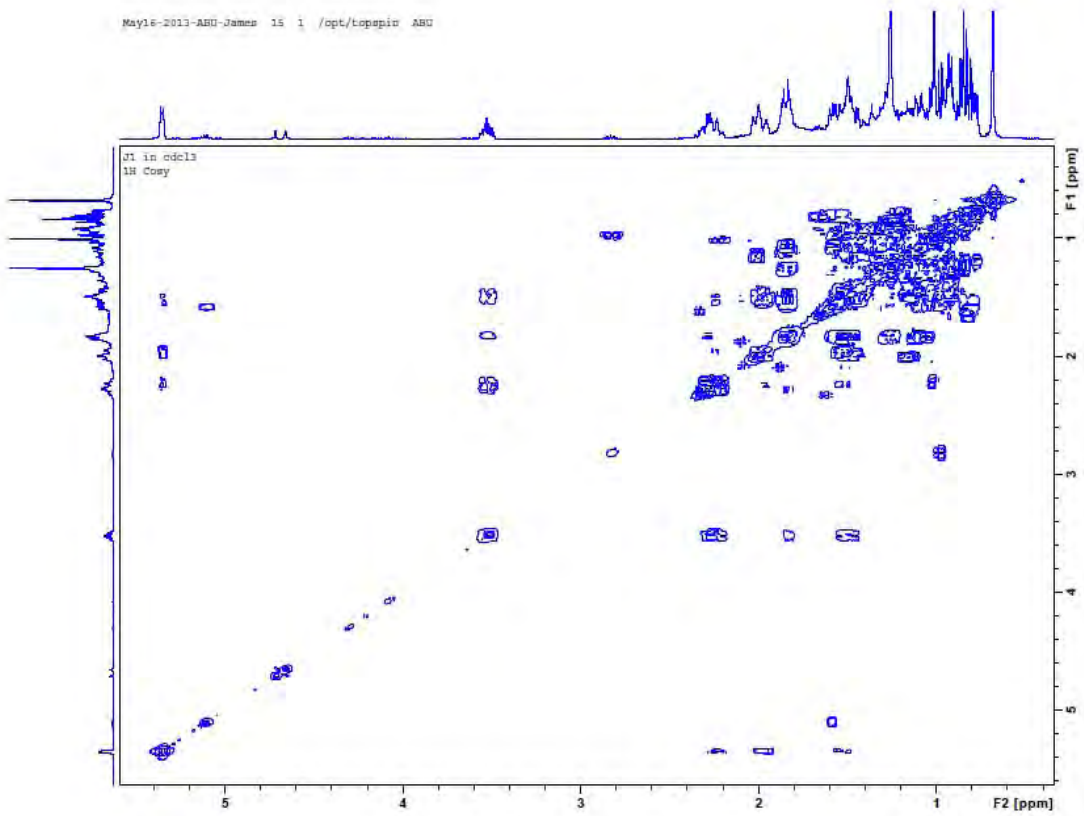
[1]H NMR Spectrum of J1 in $CDCl_3$



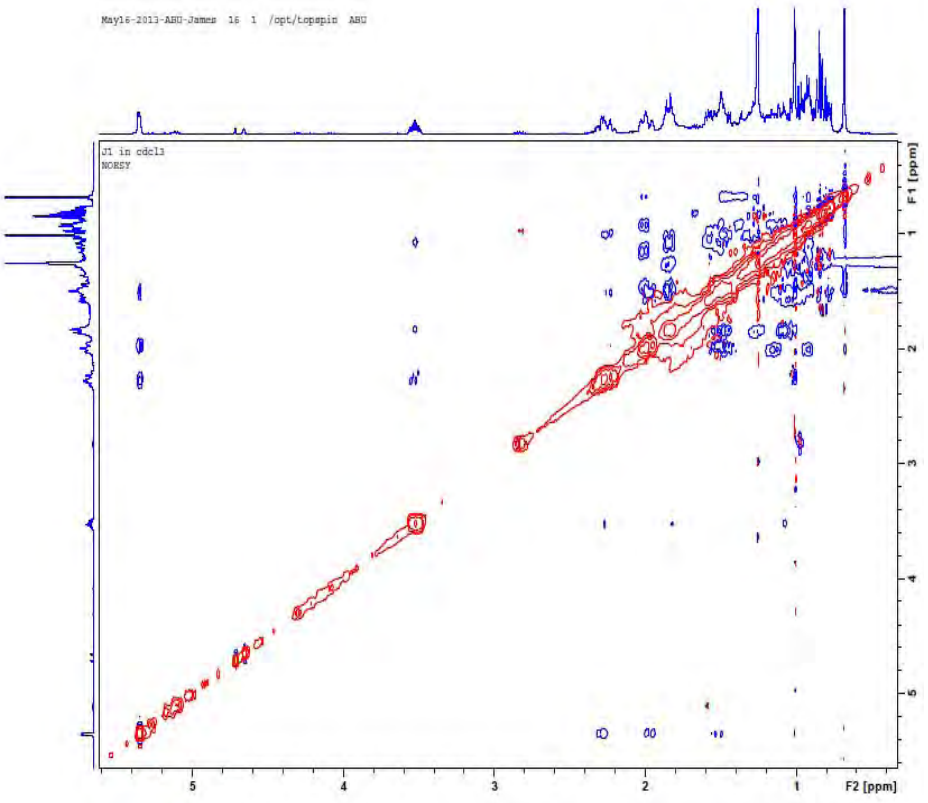
[13]C NMR Spectrum of J1 in $CDCl_3$



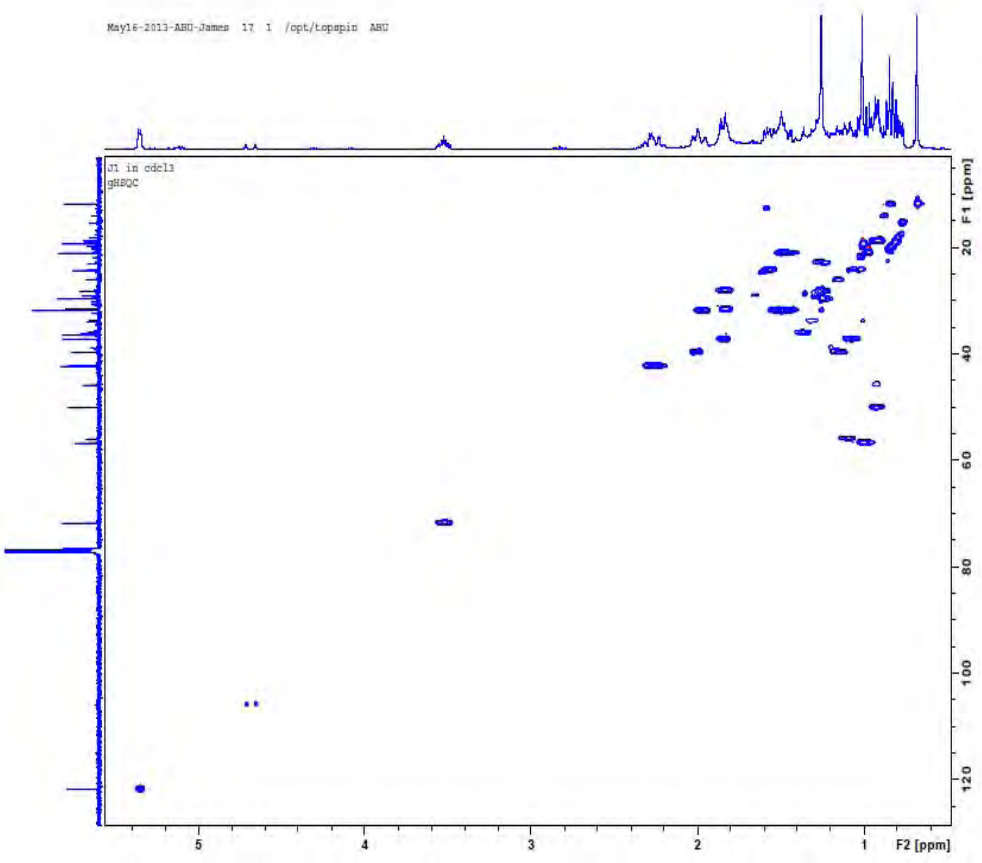
DEPT Spectrum of J1 in $CDCl_3$



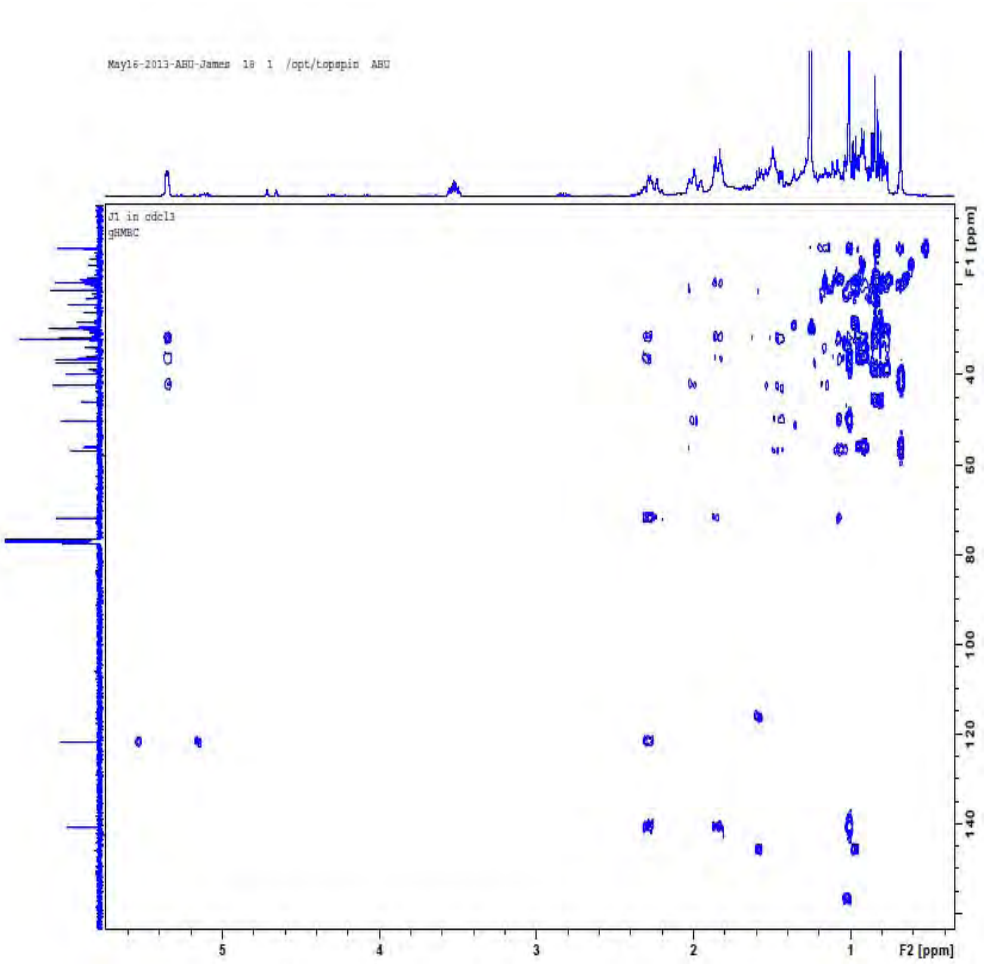
^{1}H-^{1}H COSY Spectrum of J1 in $CDCl_3$



NOESY Spectrum of J1 in $CDCl_3$



HSQC Spectrum of J1 in $CDCl_3$



HMBC Spectrum of J1 in $CDCl_3$