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(RESEARCH ARTICLE)

Stigma -5, 22, -Diene-3-O-β-D-Glucopyranoside: A new antimicrobial glycoside from *Tetrapleura tetraptera*

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Abstract

Indigenous medical practices have been the subject of much attention in the literature of various disciplines to date but the specificity, mode of action and clinical efficacy of most traditional plants have not been established in a manner consistent with standards of modern pharmacognosy and pharmacology. In order to find antimicrobial lead compounds and to provide scientific validation for the use of *Tetrapleura tetraptera* (Schum.& Thonn.) in traditional medicine . Extensive chromatographic separation of stem bark and leaf extracts led to the isolation of three distinct compounds. Anti-microbial spectrum of these obtained compounds revealed that stigma-5, 22, - diene-3-O-β-D-glucopyranoside showed activity against *Staphylococcus aureus* (32 mm), *Streptococcus pyrogenes* (30 mm) and *Candida tropicalis* (30 mm). It is therefore recommended for further work for a possible development of new drugs.

Keywords: Antimicrobial; Zone of inhibition; Isolates; *Tetrapleura tetraptera*; Glucopyranoside.

1. Introduction

Traditional medicine practices have remained a module of health care system of many societies in spite of the accessibility of well-established alternatives [1]. It is our socio-economic and socio-cultural legacy as well as the earliest choice healthcare treatment for at least 80% of Africans who suffer from high fever and common ailments [2]. This is due to the fact that traditional medicine is the most inexpensive and available system of healthcare [3]. Therefore, many people in the developing countries still rely on traditional healing practices and medicinal plants for their vital healthcare needs [4]. In industrialized countries, adaptations of traditional medicine are termed “Complementary” or “Alternative” Medicine.

The World Health Organization (WHO) estimates that in several African countries traditional birth attendants assist in the majority of births [5]. In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicine at home [6]. The World Health Organization (WHO) encourages the inclusion of herbal medicine of proven safety and efficacy in the healthcare programmes of developing countries [7]. The extent of sensitization and mobilization by the WHO has encouraged some African countries to initiate serious development on traditional medicine [8]. Over the past years, plants have become an indispensable source of food and medicine. To a large extent, most people depend greatly on medicinal plants as an important source of remedy and treatment for most casual and life-threatening diseases. As a result, there is a growing demand all over the world for these medicinal plants. Aside from tackling diseases, people are resorting more to these medicinal plants as a means of reducing the use of chemical (orthodox) medicines that could potentially be detrimental to human health. Interestingly, most of these plants are used in our everyday food preparations as herbs, spices, seasonings and preservatives. But the truth is that we often consume most of these essential medicinal plants in the form of spices

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without even acknowledging what our bodies gain from them. Medicinal plants have contributed significantly as starting points for the development of modern drugs, as evidenced by taxol in cancer and artemisinin in malaria [9].This may be attributed to their chemical diversity, biomedical specificity, possession of a greater number of chiral centres than in synthetic or combinatorial libraries, and evolutionary pressures to create biologically active compounds by interactions with different proteins and biological targets [10]. Plants therefore, represent potential sources of new drugs acting through novel mechanisms in the search for new and more potent bioactive agents that target a variety of ailments including tuberculosis. The medicinal properties of plants have not been sufficiently harnessed. The difficulty encountered with alternative medicine has been that of reliable documentation of known traditional herbal medicine since uses vary from tribe to tribe.

Indigenous medical practices have been the subject of much attention in the literature of various disciplines to date but the specificity, mode of action and clinical efficacy of most traditional plants have not been established in a manner consistent with standards of modern pharmacognosy and pharmacology. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are flavonoids, steroids, saponins, tannins, alkaloids and phenolic compounds [9]. Many of these indigenous plants are used as spices and food plants. Therefore, with the increased demand for organic materials that serve as food additives, constituents of functional foods, nutraceuticals and prevention of plant diseases, it is imperative to examine the applicability and benefits of indigenous plant materials to mankind using modern analytical methods [11]. In Nigeria, therapeutic information for a lot of such plants is based on folklore and one of such plants suggested by folklore for the treatment of different pathogens that trigger malaria infections is *Tetrapleura tetraptera*, (Figure 1) commonly called “Aidan tree” in South-west region of Nigeria [12]. As part of our investigation on some medicinal plants in Nigeria, the anti-microbial activity displayed by isolated compounds from *T. tetraptera* is being reported for the first time from this plant.

Figure 1 Picture of *Tetrapleura tetraptera* leaves and fruits.

2. Material and methods

2.1. General experimental procedures

The infrared (IR) spectra were obtained on a Perkin Elmer Spectrum 100 Fourier transform infrared spectrophotometer (FT-IR) equipped with universal attenuated total reflectance (ATR) sampling accessory (Perkin Elmer, Waltham, USA). ^{1}H, ^{13}C and 2D nuclear magnetic resonance (NMR) spectra were obtained using deuterated dimethyl sulfoxide (DMSO) or chloroform ($CDCl_3$) at room temperature on a Bruker Avance^{III} 400 spectrometer (Bruker, Rheinstetten, Germany). High resolution mass spectra (HRMS) were obtained on a Waters Micromass LCT Premier Time of Flight-Mass Spectromerty instrument (Waters, Massachusetts, USA). Column chromatography was done using Merck silica gel 60 (0.040-0.063 mm) and Merck 20 cm × 20 cm silica gel 60 F_{254} aluminium sheets were used for thin-layer chromatography (TLC). The TLC plates were viewed under ultraviolet (UV) (254 and 366 nm) lamp before further visualization using a stain made of 10% sulfuric acid in methanol (MeOH) solution followed by heating. Organic solvents of analytical grade and other chemicals used were purchased from Merck (Darmstadt, Germany) or Sigma (St. Louis, USA) chemical companies.

2.2. Plant Material

The leaves and stem bark of *Tetrapleura tetraptera* (Figure 1) were collected in fresh condition from Owo region of Ondo state, Nigeria. Taxonomical identification was done at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria and herbarium number (FHI110372) was deposited at the Institute.

2.3. Extraction of plant material

Each ground plant sample (1000 g) was exhaustively extracted using Soxhlet extraction method with 95 % ethanol as the solvent [13]. The extracts were concentrated at 40˚C using a rotary evaporator and later air-dried to give dried crude extracts.

2.4. Fractionation of crude extracts

The crude residues of stem bark and leaves were fractionated into acidic, basic, polar neutral and non-polar neutral using a modified bio-assay guided method [14]. A flowchart of the intricate steps is depicted below (Figure 1). The extracts obtained were coded A (Crude Extract Stem Bark), B (Acidic Fraction Stem Bark), C (Basic Fraction Stem Bark), D (Non-Polar Fraction Stem Bark), E (Polar Fraction Stem Bark), F (Crude Extract Leaves), G (Acidic Fraction Leaves), H (Basic Fraction Leaves), I (Non-Polar Fraction Leaves), J (Polar Fraction Leaves).

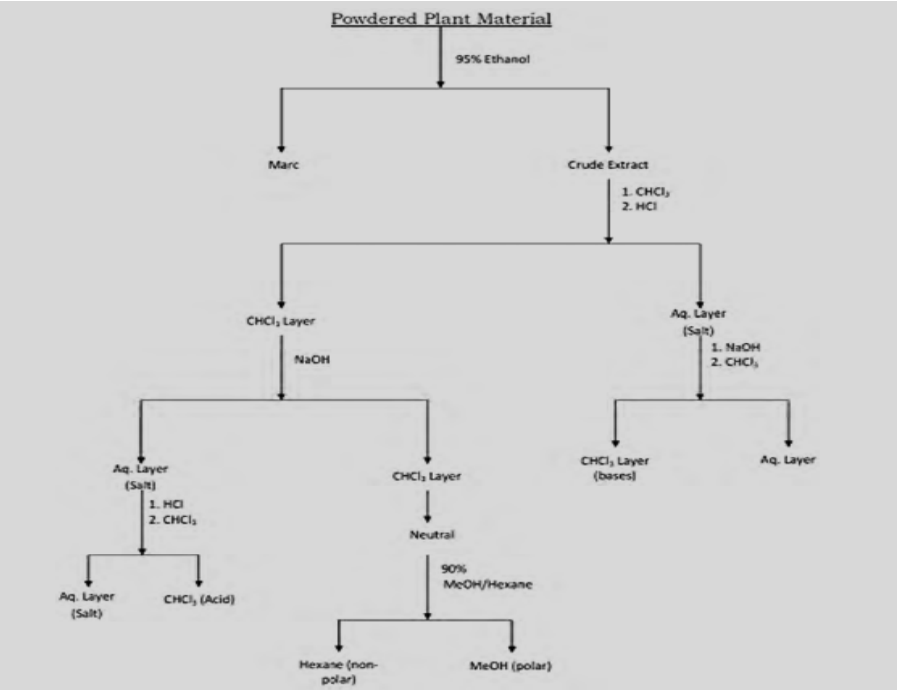


Figure 2 Flowchart of fractionation protocol

2.5. Chromatographic Separation of fractions

The column was packed with fine silica gel (Kieselgel 60), a column having 40 cm length and 3 cm in diameter was packed with 150 g of silica gel up to a height of 23 cm under reduced pressure. The column was washed with n-hexane to facilitate compact packing. The neutral polar fraction (13.0 g) of the stem bark loaded onto a silica gel column using wet method and eluted with hexane : ethyl acetate: methanol (100:0-100:10 v/v) gradient to give 68 fractions of 100 ml each. Fractions with similar TLC profiles were combined. Fractions 19-20 were combined and purified in hexane to give compound A, a white needle-like crystal (18 mg) and fractions 52-53 were filtered out from 100 % ethyl acetate to obtain a white solid crystal compound B (19 mg). Fractions 59-60 were also purified using 100 % ethyl acetate and filtered to give a white cotton-wool like solid compound C (42 mg).The neutral non-polar fraction (15 g) of the leaf was subjected to column chromatography using hexane: ethyl acetate: methanol (100:0-100:20, v/v) gradient to give 60 fractions of 100 ml. Fractions 29-31 were combined to give a dark green amorphous solid compound D (15 mg); and neutral polar fraction of the leaf (20.0 g) was also subjected to column chromatography using the same solvent system, 52 fractions of 100 ml were collected. Fractions 8-9 were washed with acetone, dried and to give a shiny white compound E (19 mg).

2.6 Antimicrobial Screening

All the media were purchased from Sigma-Aldrich and were prepared in accordance with the manufacturer's instructions. The clinical bacterial and fungal isolates were obtained from Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria. The identities of all isolates used were confirmed using

standard biochemical test [26]. Agar diffusion method adopted from EUCAST [27] was employed. The minimum inhibitory concentration (MIC) was determined on the test isolates and was done by broth dilution method [28]. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Muller Hinton agar medium for estimation of MBC [28]. The MBC / MFC values were the plates with lowest concentrations of the isolates without colony growth.

3. Results

3.1. Spectroscopic characterization

Chromatographic separation of neutral polar stem bark and non- polar leaf fractions gave five compounds which were subjected to different spectroscopic analyses to elucidate the structures of the isolated compounds.

Compound (A): White needle-like crystal with melting point between 162- 168 ^{0}C, TLC R_f 0.55 (Hex-EtOAc, 7:3). IR (KBr) $\sqrt{}_{max}$ cm^{-1}: 3336, 2916, 2848, 1638, 1461, 1367, 1052, 1023, 970, and 888. ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 5.33 (1H, t, H-6), 5.15 (1H, m, H-22), 5.02 (1H, m, H-23), 3.53 (1H, m, H-3), 0.81 (3H, s, H-18), 0.88 (3H, s, H-19), 1.08 (3H, d, J= 3.50 Hz, H-21), 0.83 (3H, t, H-29), 0.85 (3H, d, J=2.50 Hz, H-26), 0.92 (3H, d, J=7.05 Hz, H-27). ^{13}CNMR ($CDCl_3$) δ: 140.75 (s, C-5), 121.70 (d, C-6), 138.31 (d, C-22), 129.28 (d, C-23), 71.80 (d, C-3), 12.25 (q, C-29), 19.40 (q, C-19), 21.22 (q, C-26).

Compound (B): White solid crystal with melting point between 264-266 ^{0}C, TLC R_f 0.55 (DCM-MeOH, 8.5: 1.5). IR (KBr) $\sqrt{}_{max}$ cm^{-1}:3383, 2931, 2866, 1666, 1458, 1366, 1254, 1166, 1050, 1019,826. ^{1}HNMR [$(CD_3)_2SO$), 400 MHz] δ: 5.32 (br, s ,H-6), 5.31 (m, H-22), 5.17 (m, H-23), 1.22 (br, s), 4.22 (1H,d, J= 7.62 Hz, H$^{1'}$), 3.01 (1H,m,H$^{2'}$), 3.45 (1H,m, H$^{3'}$), 3.12 (1H, m, H$^{4'}$), 3.04 (1H, m, H$^{5'}$), 3.62 (d, J=10.50, H$^{6a'}$), 3.37 (1H,s,H$^{6b'}$). ^{13}CNMR [$(CD_3)_2SO$] δ: 76.91 (d, C-3), 121.13 (d, C-6), 140.42 (s, C-5), 137.99 (d, C-22), 128.79 (d, C-23), 100.75 (d, C-1'), 70.05 (d, C-2'), 76.31 (d, C-3'), 73.42 (d, C-4'), 76.69 (d, C-5'), 61.04 (t, C-6').

Compound (C): White cotton-wool like compound with melting point between 273-275 ^{0}C, TLC R_f 0.69 (DCM-MeOH, 8.5:1.5). IR (KBr) $\sqrt{}_{max}$ cm^{-1}: 3297, 2941, 1686, 1631, 1569, 1266, 1175. ^{1}HNMR [$(CD_3)_2SO$), 400 MHz] δ: 0.64, 0.70, 0.85, 0.86, 0.88, 1. 08, 1.30 (Me × 7), 5.15 (1H, m, olefinic) , 7.7 (d ,N-H signal) and 4.91 (1H, d, anomeric proton). ^{13}CNMR [$(CD_3)_2SO$] δ: 178.52 (COOH), 168.66 (NAc), 143.78 (C-13), 121.46 (C-12) and 87.99 (C-3). The carbohydrate carbon atoms appeared at 103.50 (C-1'), 55.80 (C-2'), 76.63 (C-3'), 73.97 (C-4'), 70.69 (C-5') and 61.13 (C-6').

Compound (D): a dark green amorphous solid, TLC R_f 0.27 (Hex-EtoAC, 7:3). IR (KBr) $\sqrt{}_{max}$ cm^{-1} : 3384, 2918, 2850, 1733, 1694, 1619, 1498, 1453, 1302, 1196, 1161, 1060.^{1}HNMR ($CDCl_3$, 400 MHz) δ: 9.47 (s), 8.55 (s), 8.0 (m), 3.40 (s), 3.24 (s).

Compound (E): a shiny white powder with melting point between 70-73 ^{0}C, TLC R_f 0.46 (Hex: EtOAc, 8:2). IR (KBr) $\sqrt{}_{max}$ cm^{-1}: 3314, 2916, 2848, 1710, 1462, 1171, 1062, 889, 718. ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 3.63 (2H, t, br, H-1), 1.56 (2H, m, H-2), 1.23 (42H, s, br, H3-H23), 0.87 (3H, t, H-24). ^{13}CNMR (δ): 63.12 (t, C-1), 32.82 (t, C-2), 32.82-22.69 (t, C3-C23), 14.11 (q, C-24).

Table 1. Microbial sensitivity and resistance summary of obtained compounds 2, 3, 5

Test Organism	A (2)	B(3)	E (5)	Ciprofloxacin	Fluconazole	Fulcin
Staphylococus aureus	S	S	S	S	R	R
Streptococus pneumonia	S	S	S	R	R	R
Streptococus pyogenes	R	S	S	S	R	R
Klebsiella pneumonia	R	R	S	S	R	R
Corynebacterium ulcerans	S	S	R	S	R	R
Candida albicans	S	S	S	R	S	R
Candida krusei	S	S	R	R	S	R
Candida tropicalis	R	S	R	R	S	S
Aspergillus fumigates	R	R	S	R	R	S

KEY: S=Sensitive; R= Resistance; 2, 3 = Stem Bark Polar neutral fraction; 5 = Non Polar neutral fraction Leaf

Table 2. Zone of inhibition of the Compounds 2, 3, 5 against the test Microorganisms

Test Organism	(2)A 0.2µg/ml	(3)B 0.2µg/ml	(5)E 0.2µg/ml	Ciprofloxacin 10µg/ml	Fluconazole 10µg/ml	Fulcin 10µg/ml
Staphylococus aureus	27	32	30	35	0	0
Streptococus pneumoniae	30	30	29	0	0	0
Streptococus pyogenes	0	30	25	30	0	0
Klebsiella pneumoniae	0	0	32	29	0	0
Corynebacterium ulcerans	28	28	0	30	0	0
Candida albicans	31	29	27	0	35	0
Candida krusei	28	26	0	0	32	0
Candida tropicalis	0	30	0	0	34	29
Aspergillus fumigates	0	0	24	0	0	30

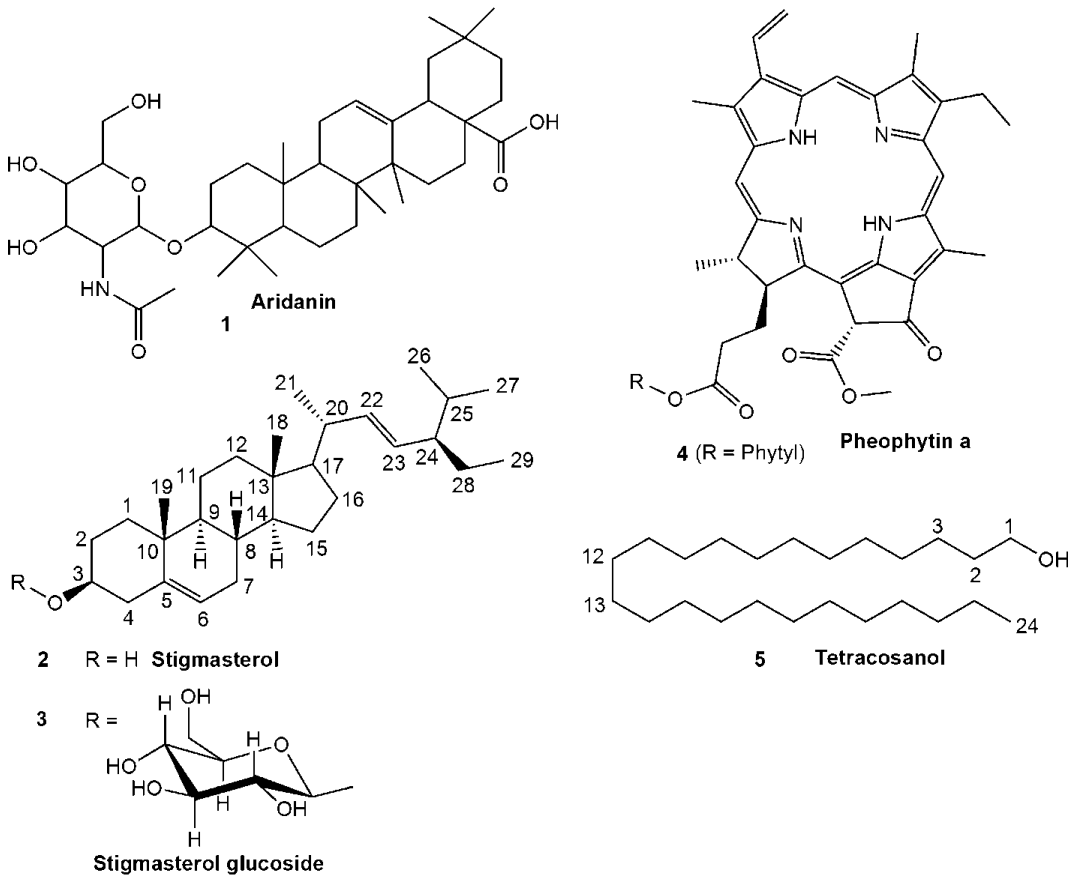
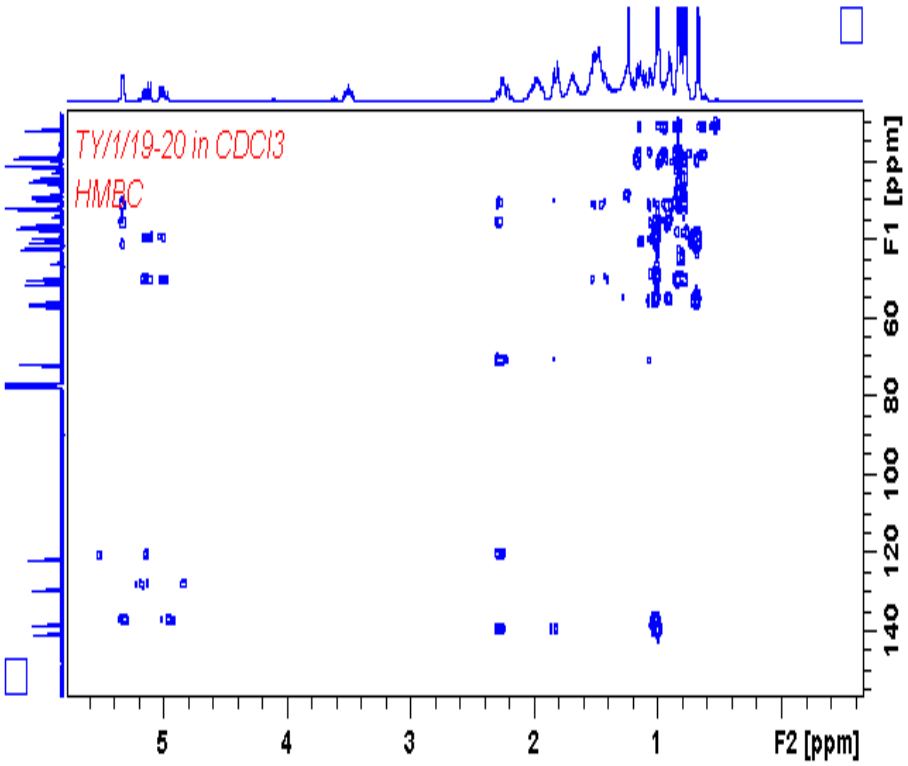
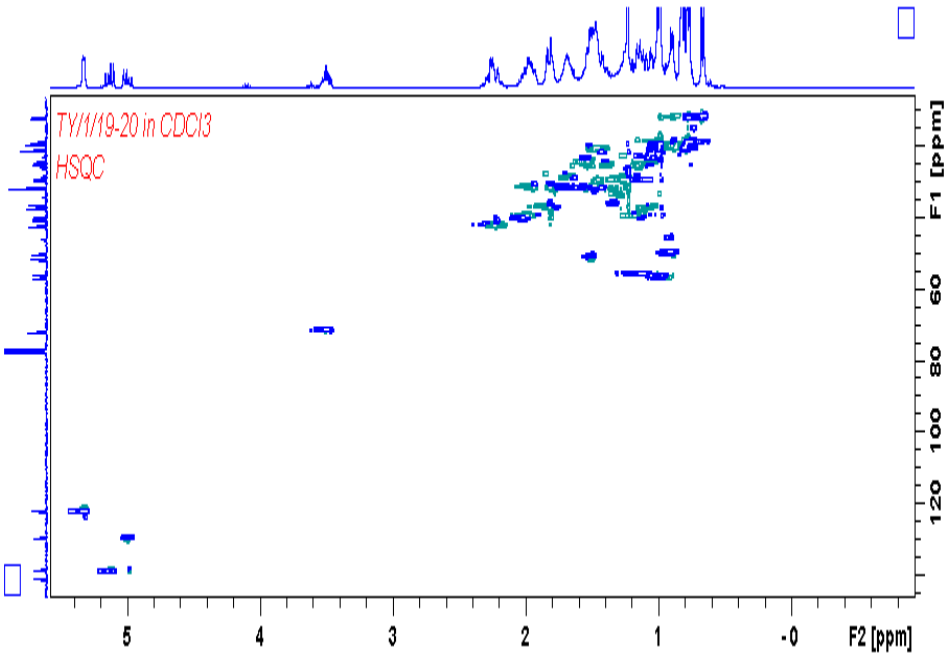


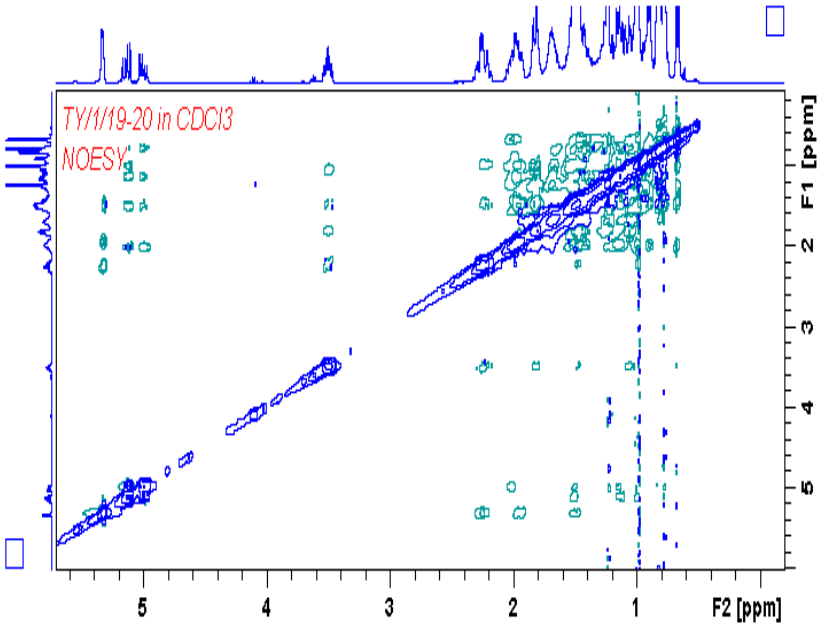
Figure 3 Chemical structures of compounds isolated from *Tetrapleura tetraptera*



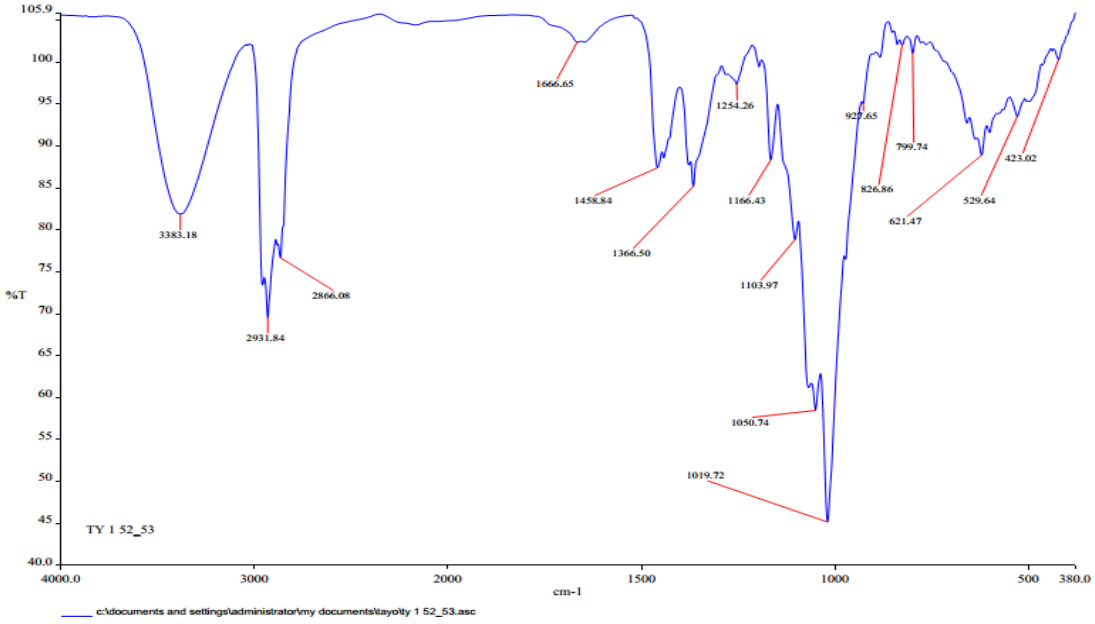
HMBC for Compound 2



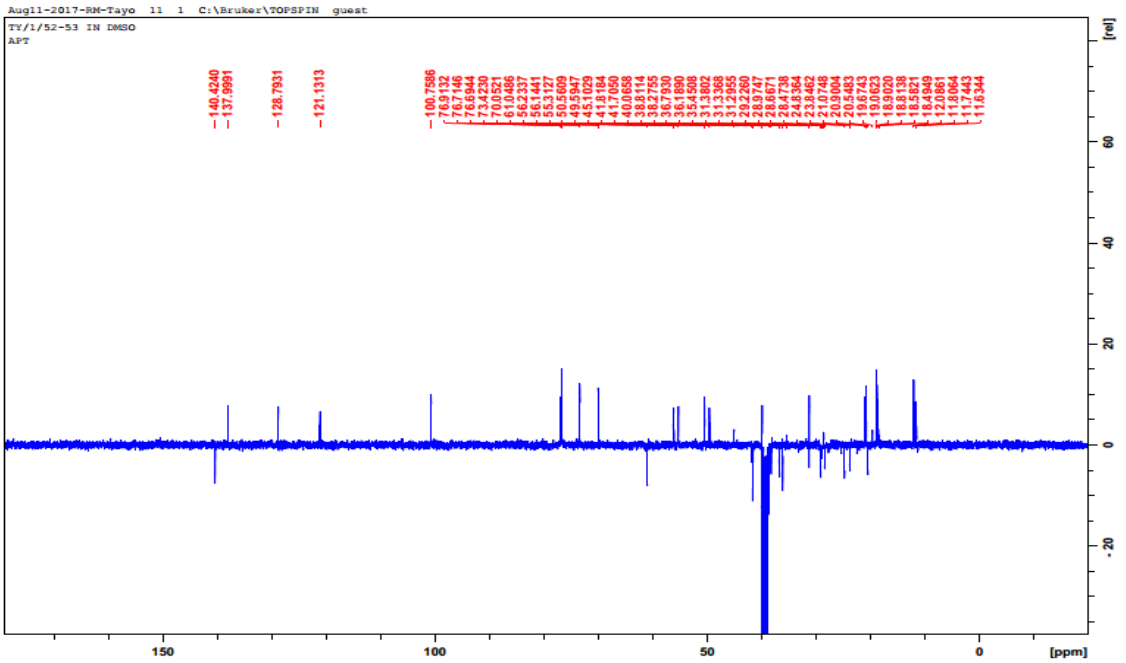
HSQC for Compound 2



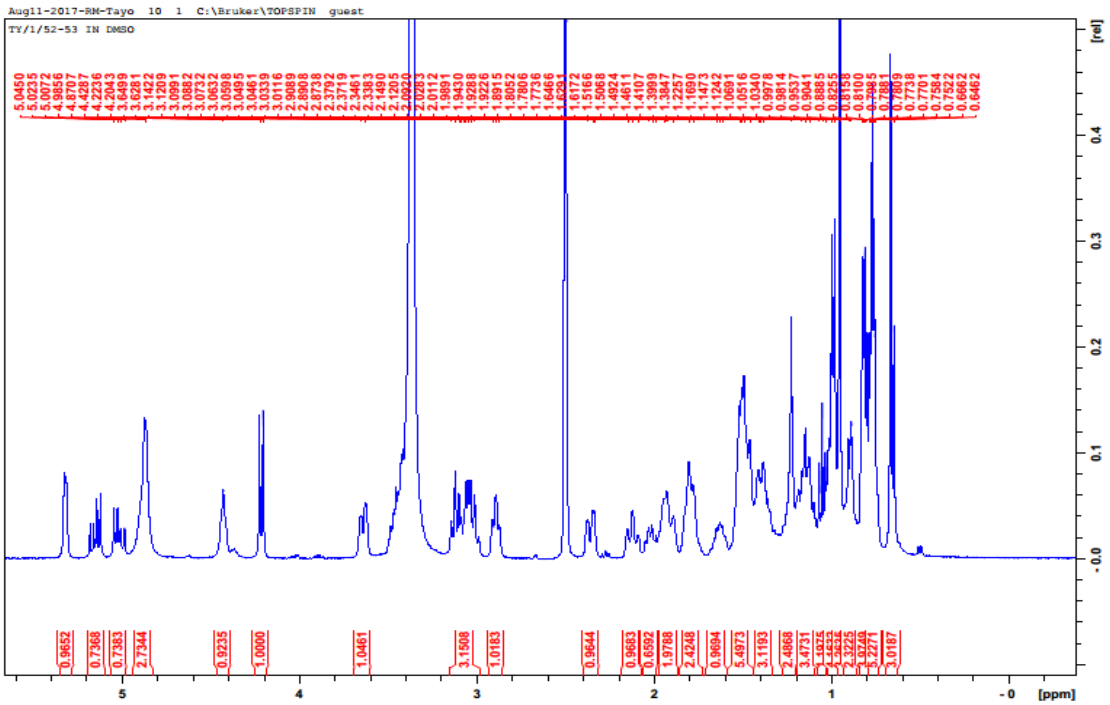
NOESY for Compound 2



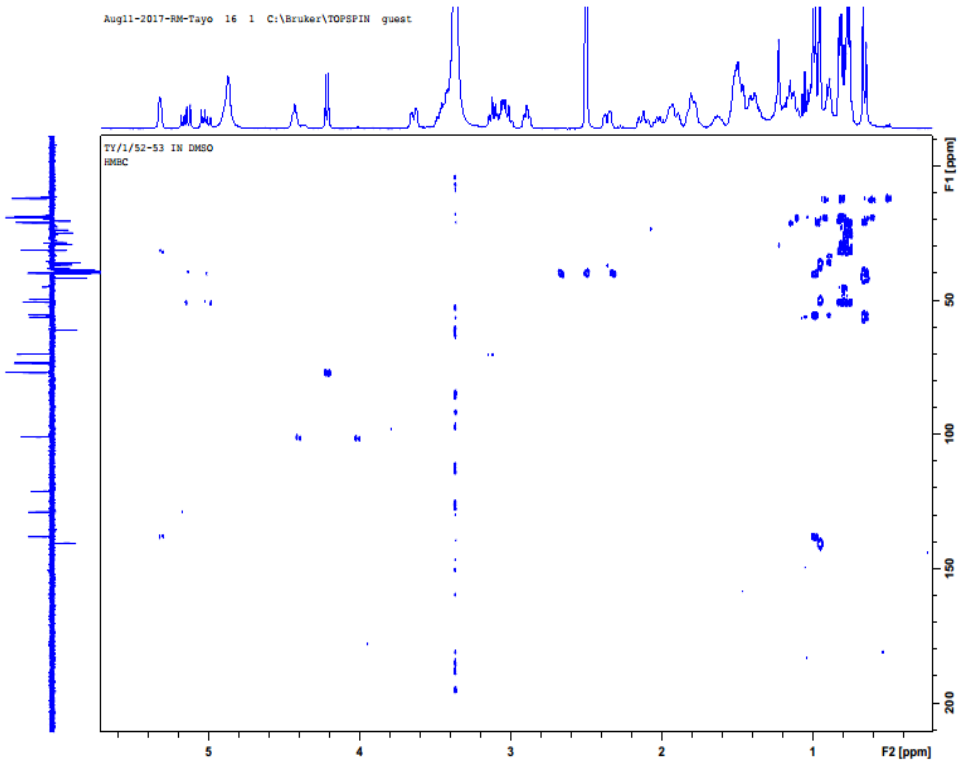
FT-IR Chromatogram of compound 3



^{13}C NMR Compound 3



^{1}H NMR Compound 3



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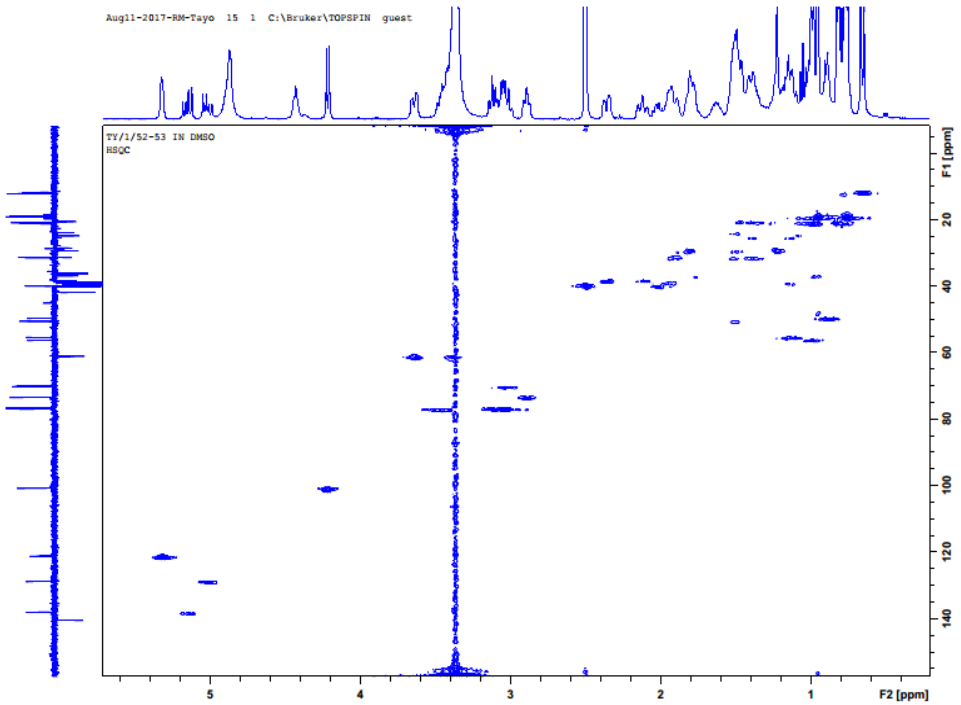

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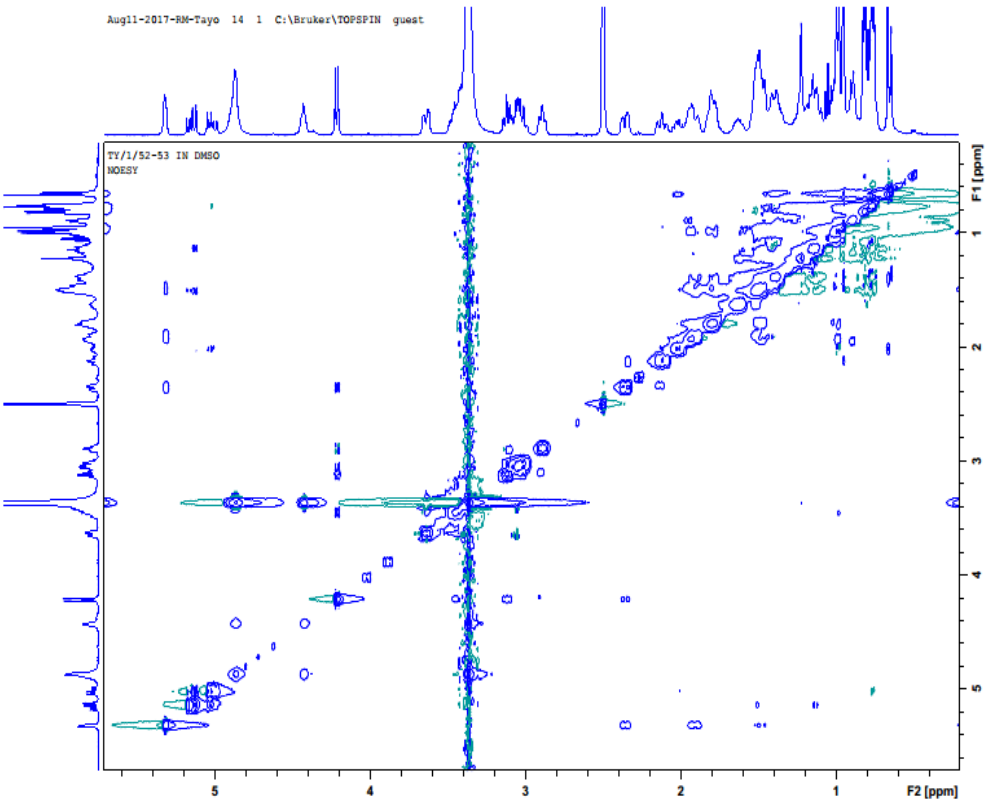

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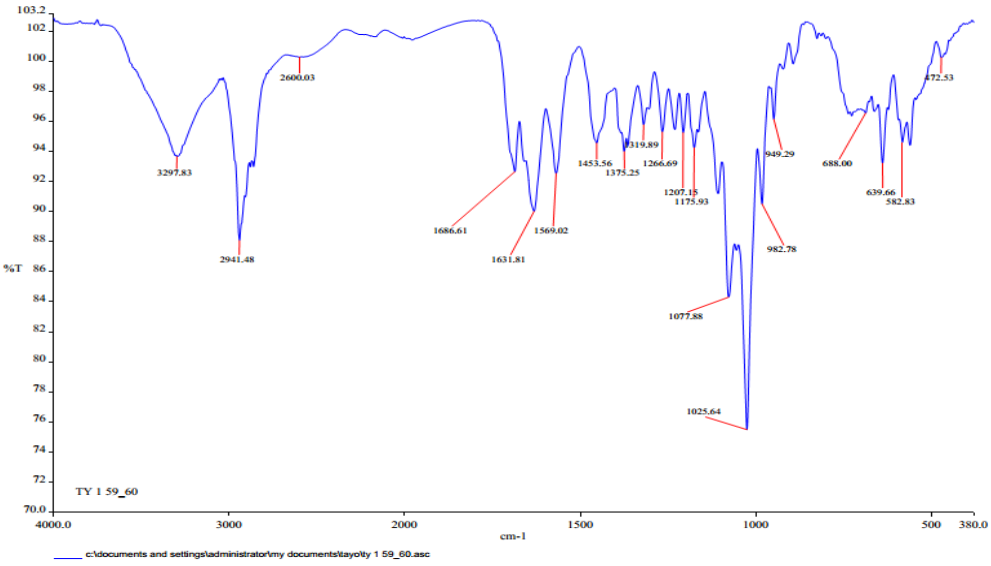
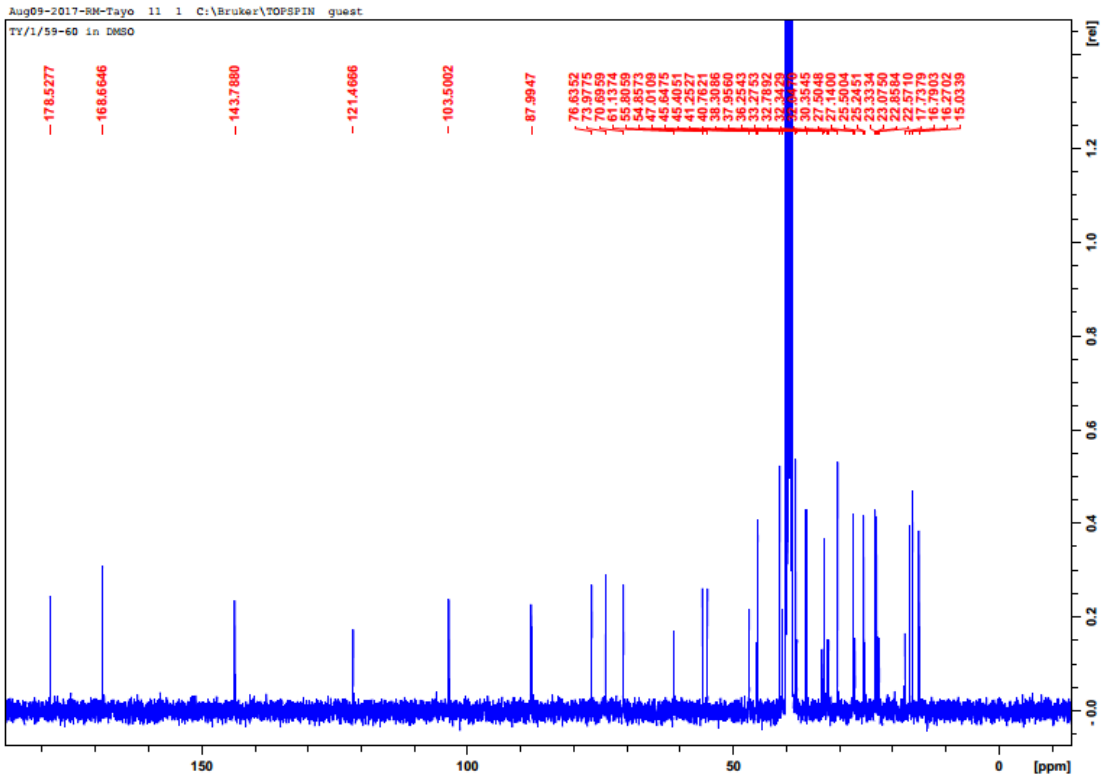
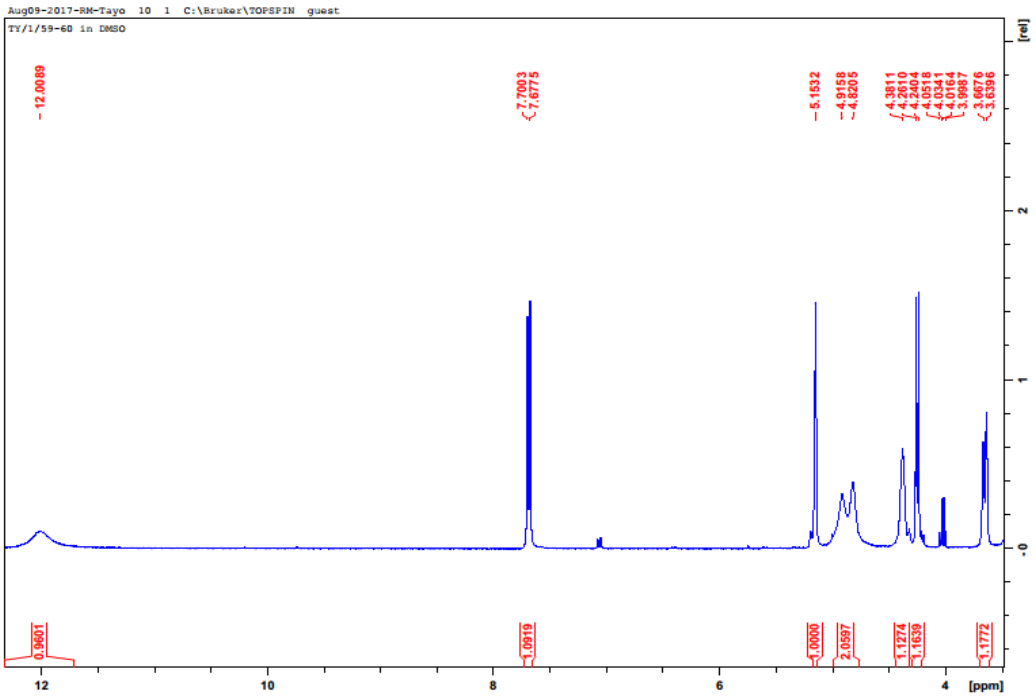


FT-IR Chromatogram of compound 1



^{13}C NMR Compound 1



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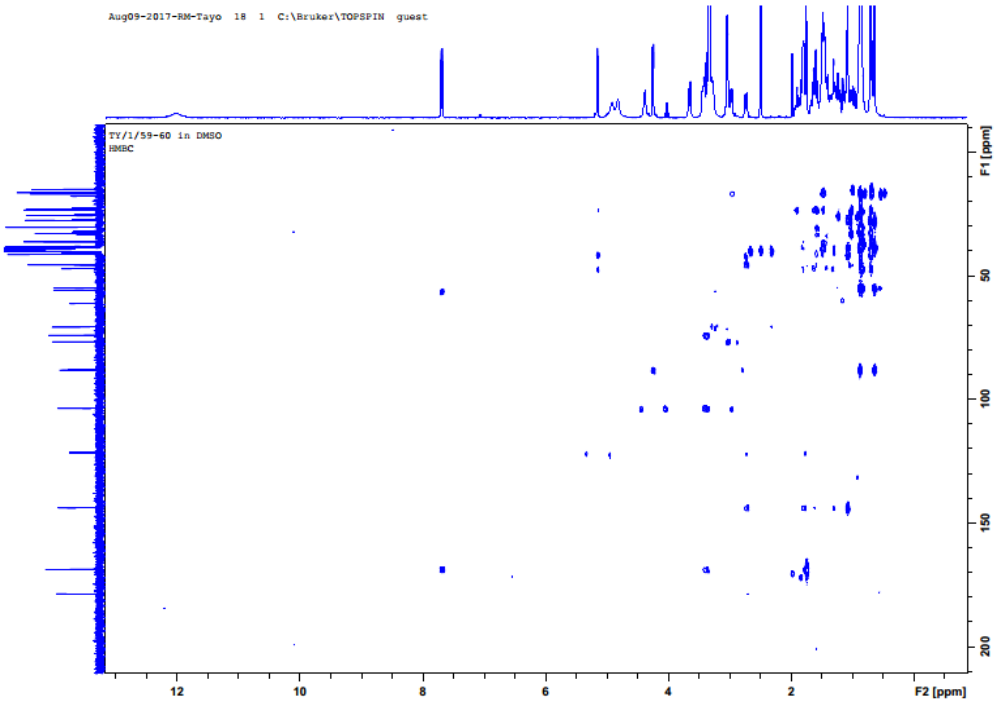

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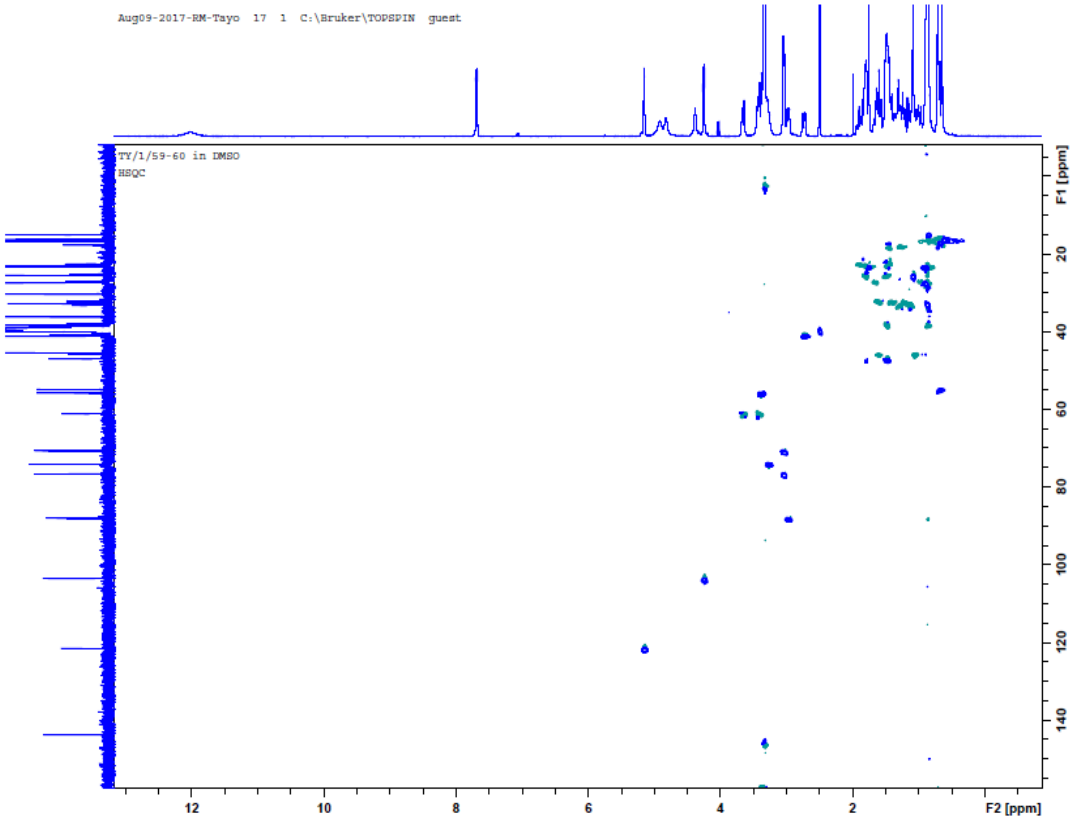

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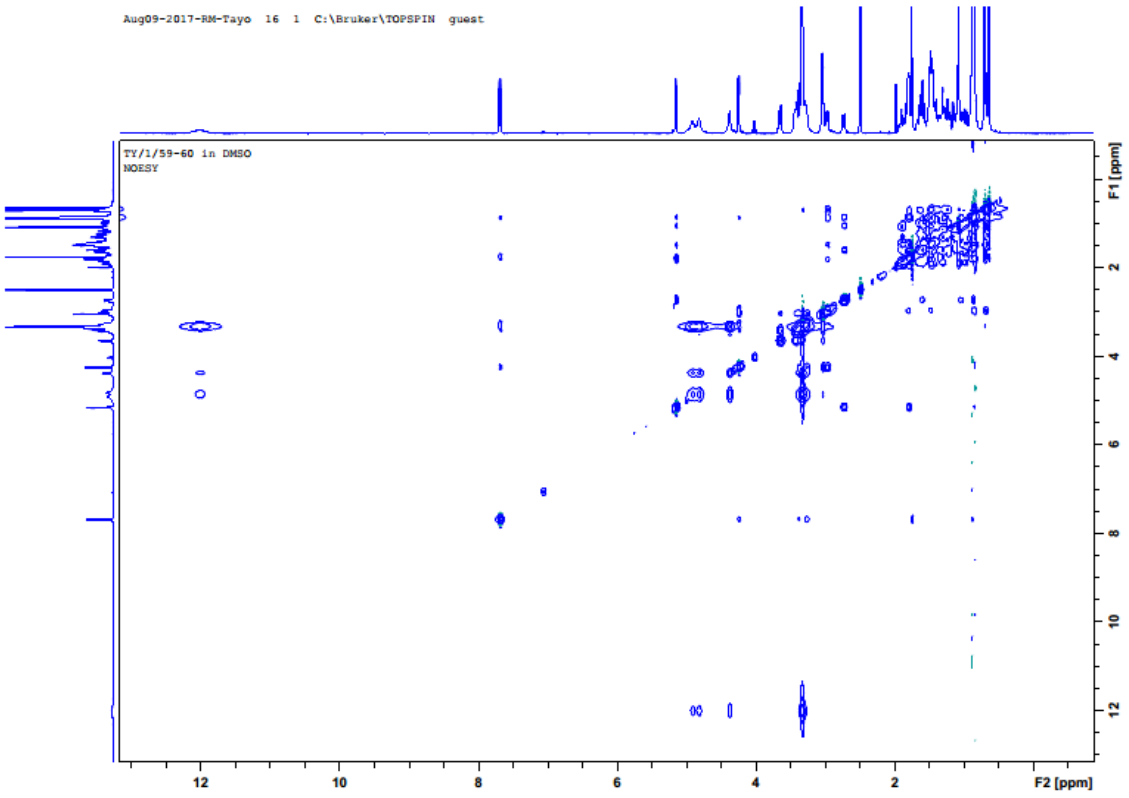

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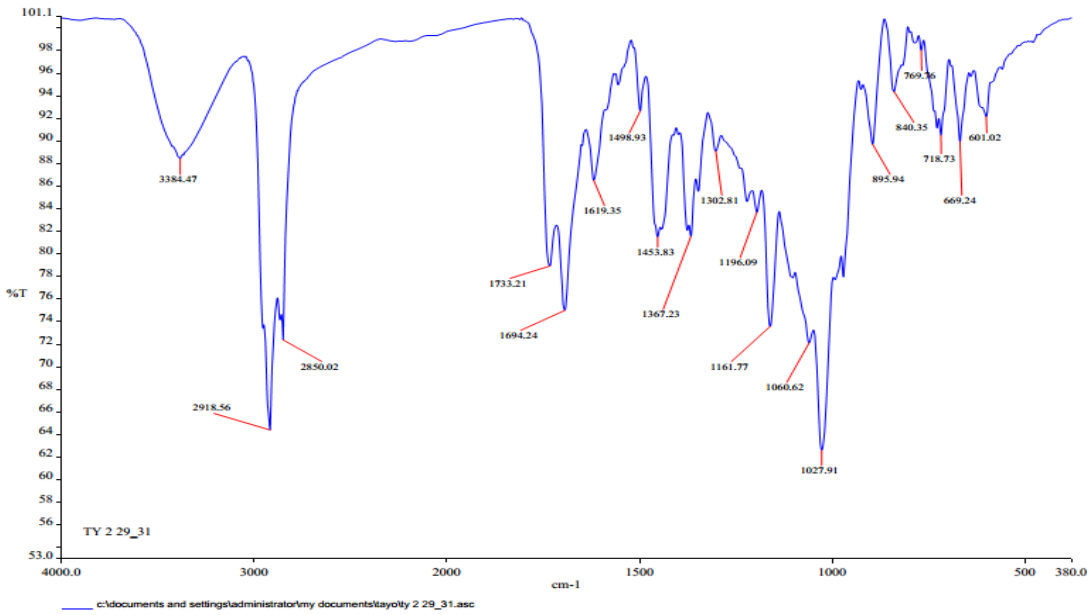
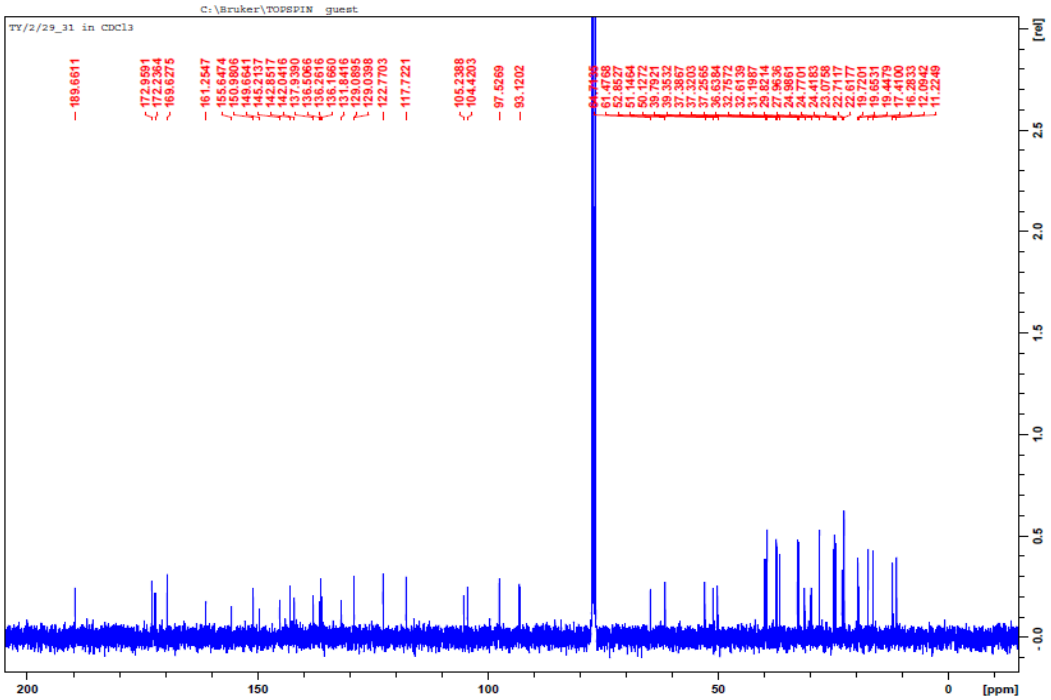


FT-IR Chromatogram for Compound 4



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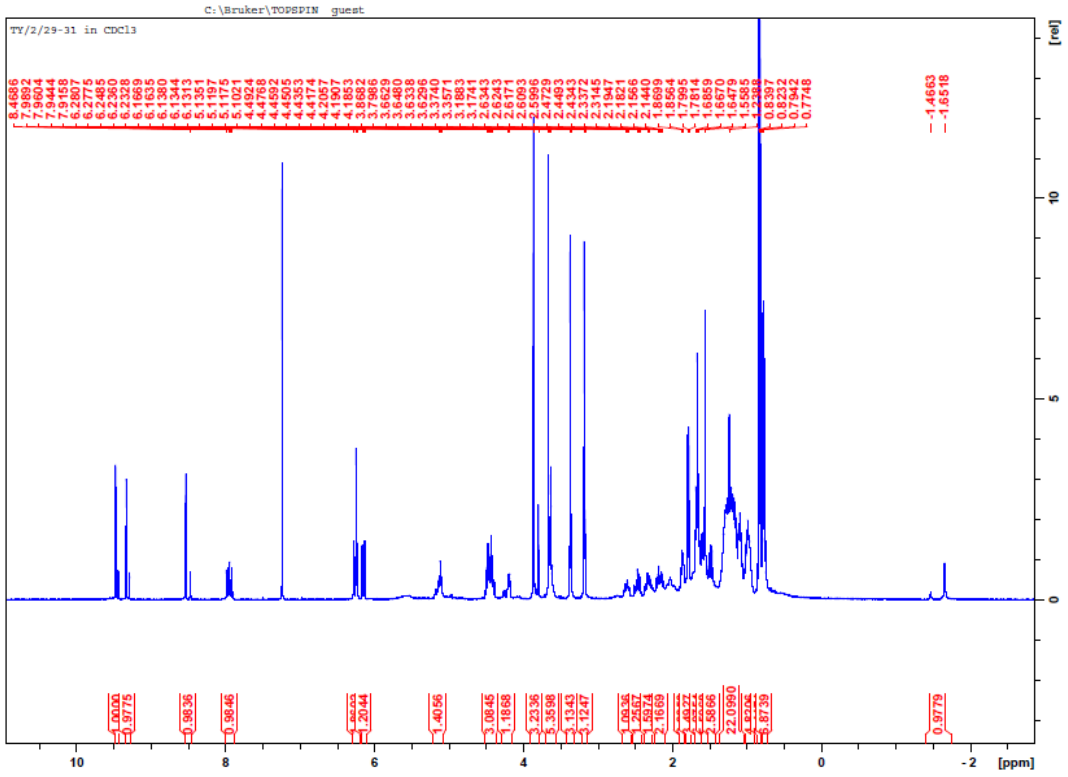

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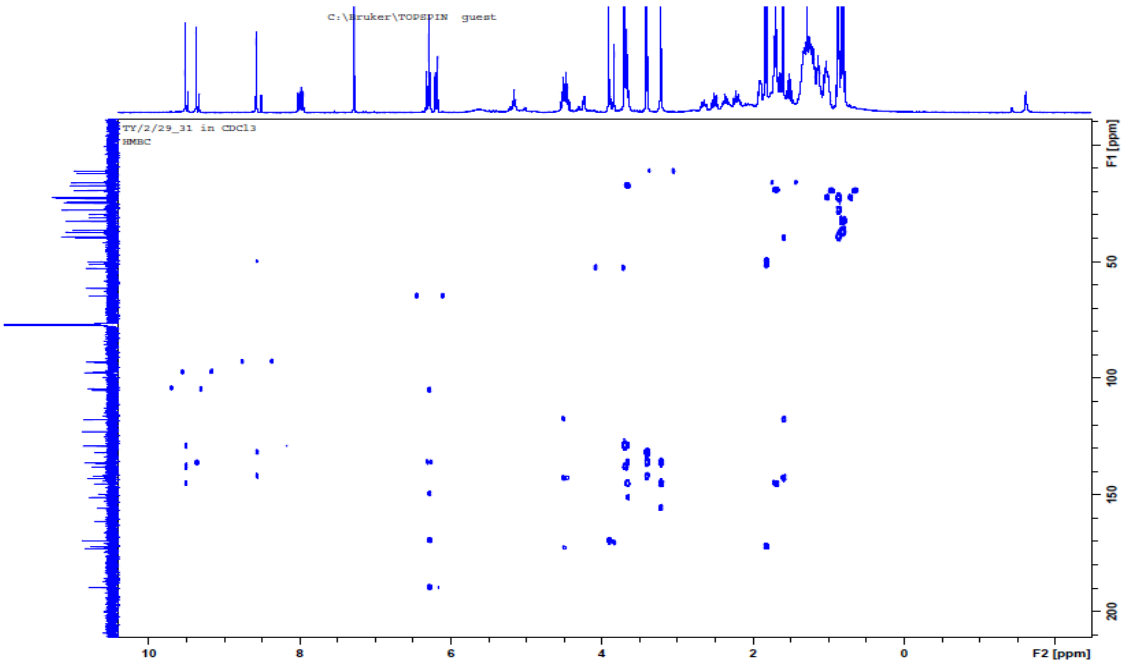

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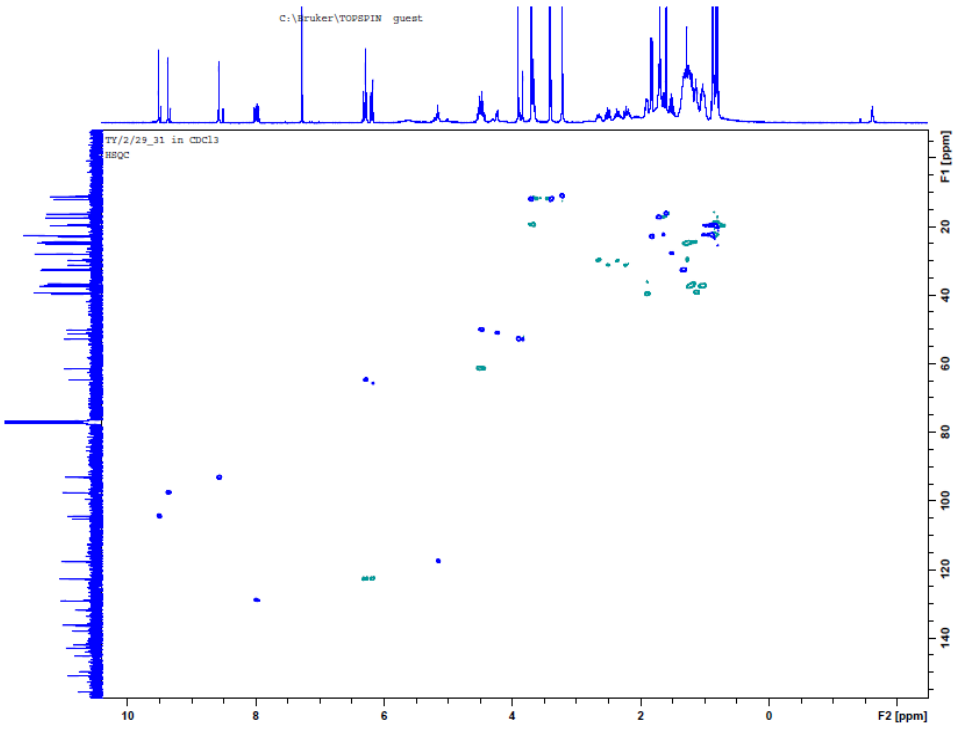

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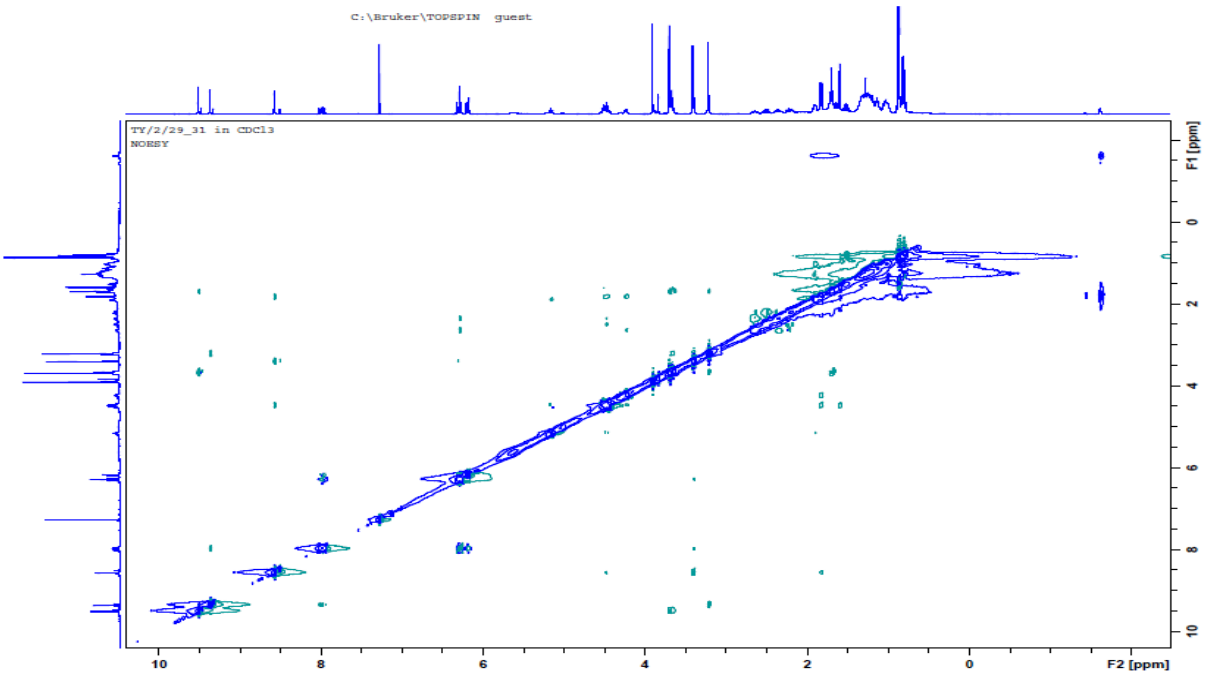

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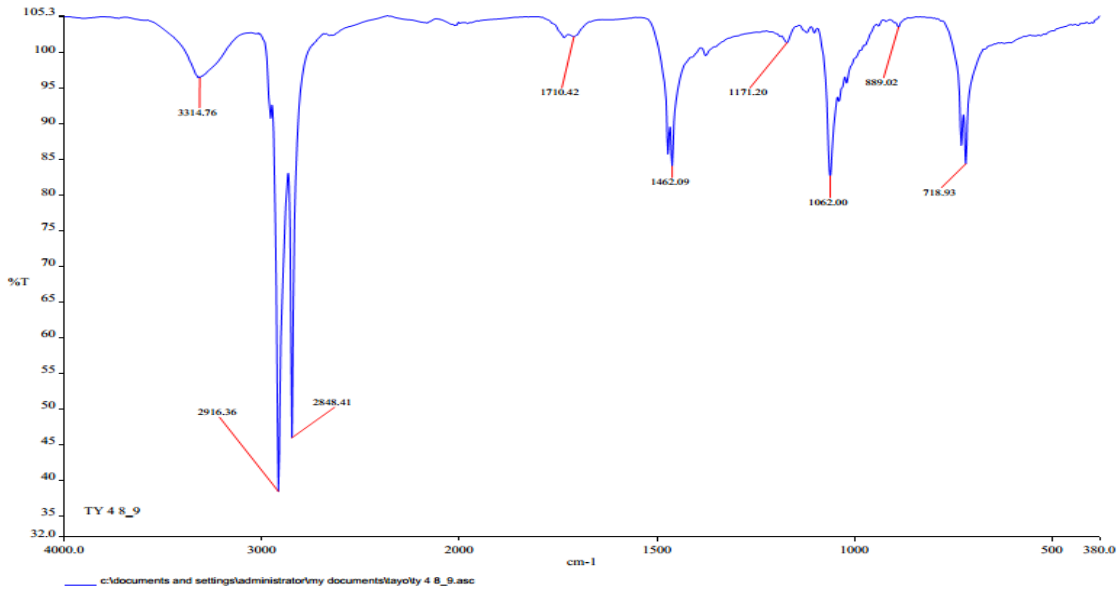
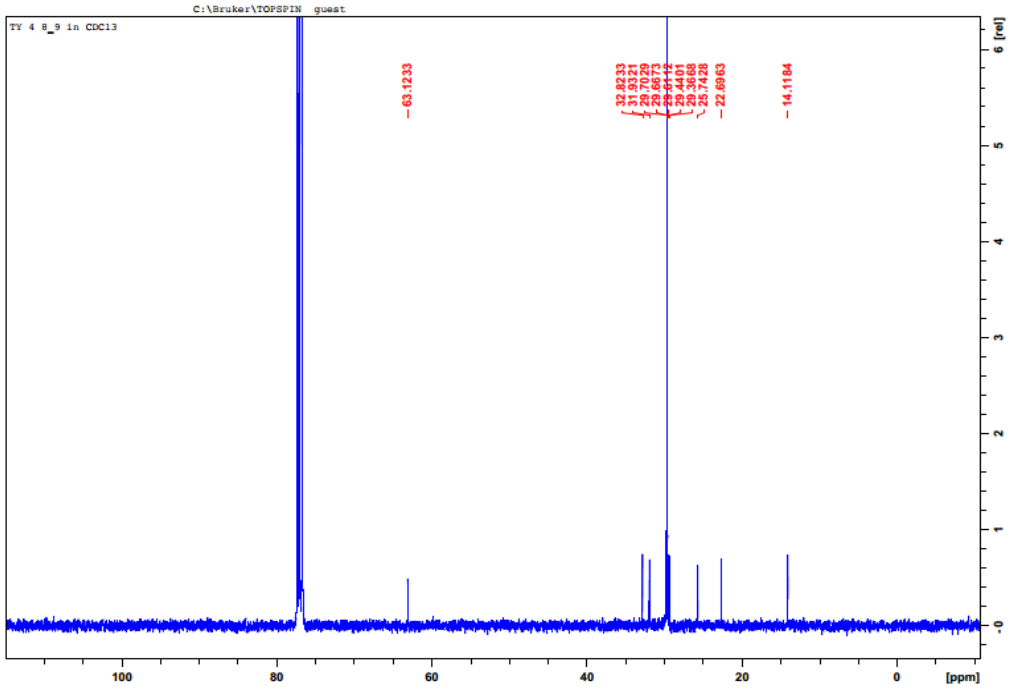
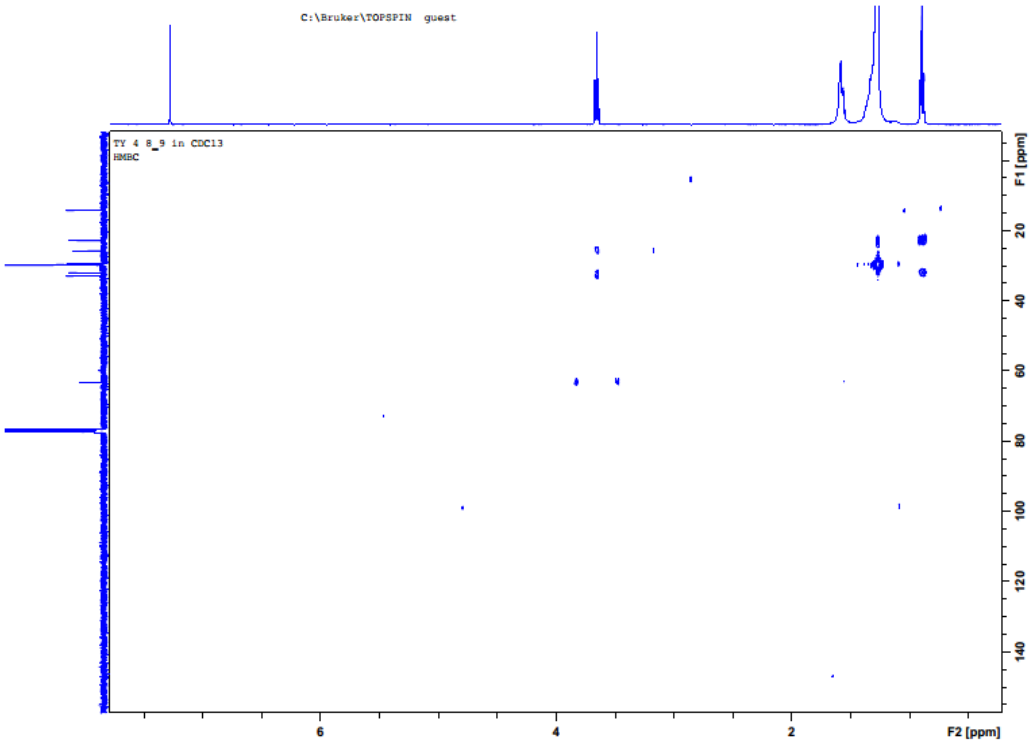


FT-IR Chromatogram of compound 5

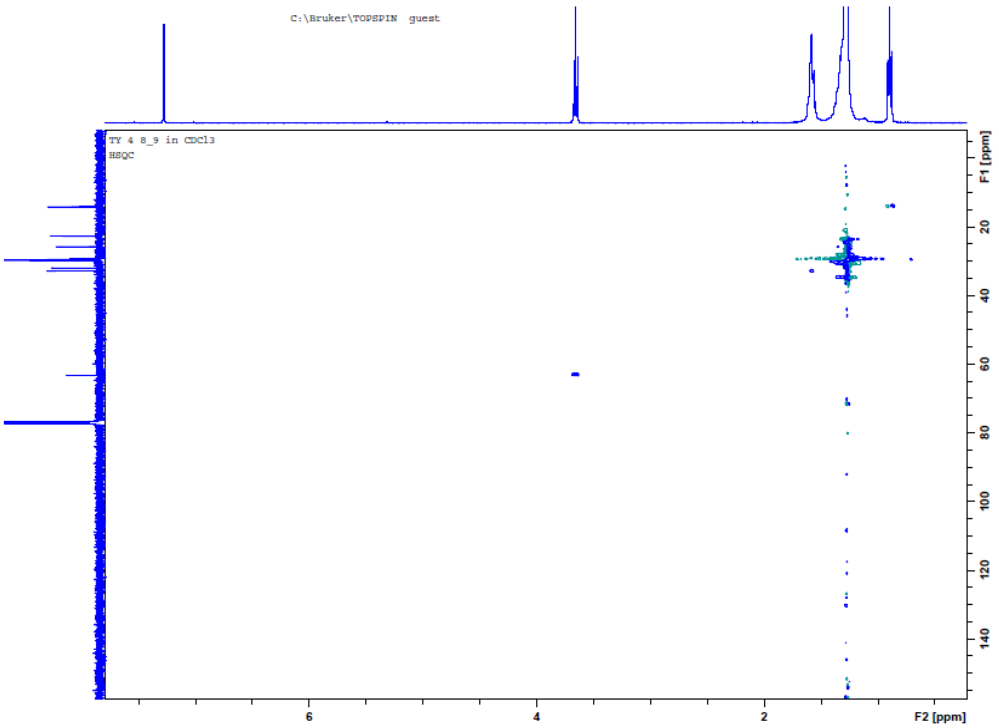


^{13}C NMR Compound 5

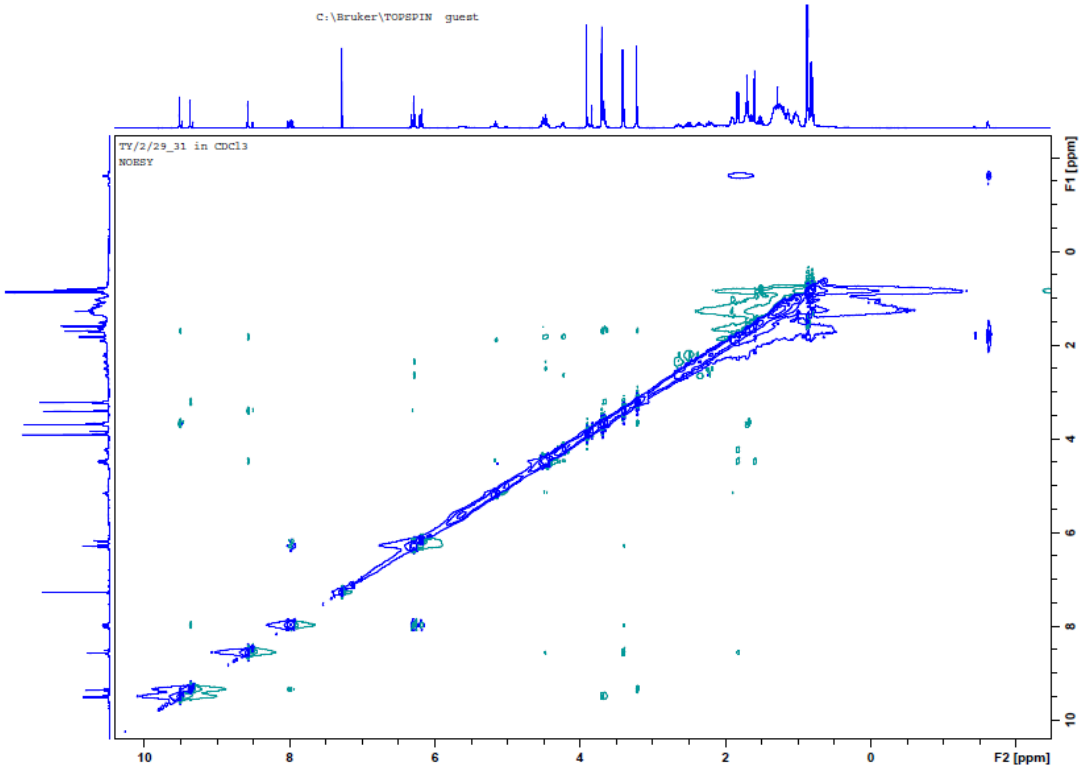
^{1}H NMR Compound 5



HMBC for Compound 5



HSQC for Compound 5



NOESY for Compound 5

4. Discussion

The five compounds (1-5) were isolated from the stem bark and leaves of *Tetrapleura tetraptera.* The compounds belonging to a sterol, a saponin, mono-N- acetyl glycoside, a straight chain fatty alcohol and a natural pigment were identified based on their ^{1}H and ^{13}C-NMR spectra and 2D NMR spectra which were compared to values in literature. This is the first time these compounds were isolated from *T. tetraptera* stem bark (except a mono- N- acetyl glycoside) and leaf though they are known compounds.

The compound A is a white needle-like crystal with melting point 162-168 ^{0}C which gave positive Salkowski and Lieberman-Burchard test for steroid (Figures 3-9). The FTIR (Fourier Transform Infra-red) spectrum exhibits characteristic absorption band at 3336 cm^{-1} which is the O-H stretching. Absorptions at 2918 and 2848 cm^{-1} are due to aliphatic C-H stretching. The absorptions at 1638 and 1052 cm^{-1} are as a result of C=C and C-O stretching. These assignments with reported values were all in agreements with other reported studies [15]; [16]. The ^{1}H spectrum showed the presence of six methyl groups of a stigmastane carbon skeleton, identical to stigmasterol which appeared at δ_H 0.81, 0.83, 0.85, 0.88, 1.08 and 1.65 ppm. The de-shielding effect of protons at δ_H 3.53 ppm (1H, m, H-3) indicated, they were bonded to oxygenated tertiary carbon. The ^{13}C spectrum displayed 29 carbons signals including six methyls. The correlation of carbons to their respective hydrogens was found by using HSQC (Heteronuclear Single Quantum Correlation) spectrum and the assignments were supported by H-C correlation based on HMBC (Heteronuclear Multiple Bond Correlation) spectrum. Signals at 140.75, 121.70 ppm are for C-5 and C-6 double bond while 71.80 ppm for C-3 hydroxyl group, 138.31 ppm for C-22 and 129.28 for C-23 respectively. Therefore C-5, C-6, C-22 and C-23 are alkene carbons. If we compare DEPT (Distortionless Enhancement by Polarization Transfer) 90 and 135 experiments, we confirmed that this compound was having six methyl, nine methylene, eleven methine and three quaternary carbon groups. Similarity of the above spectral data (Figures 3-8) to those of published data [17];[18] suggested that it is stigmasterol, 2.

Compound B gave a positive Liebermann- Burchard test and IR spectrum exhibited strong bands at 3383 and 1050 cm^{-1} characteristic of a glycoside (Figure 10). The calculated m/z suggested to be 574.42 but due to the loss of glucose from the molecular ion peak, gave a fragment at m/z 396. The $^{1}HNMR$ spectrum of B revealed the signals of two tertiary methyl at δ 0.64 (CH_3-18) and 1.03 (CH_3-19), four secondary methyls at δ 0.98 (CH_3-21), 0.88 (CH_3-26), 0.81 (CH_3-27) and 0.83 (CH_3-29), one trisubstituted olefinic proton at δ 5.32 (H-6), two di-substituted olefinic protons at δ 5.31 (H-

22) , 5.17 (H-23) and anomeric proton at δ 4.22. The ^{13}CNMR spectrum showed the existence of 35 carbon atoms in the molecule. An anomeric signal at δ 100.76 indicated the presence of a single monosaccharide moiety. The degree of protonation of each carbon atom was determined by DEPT experiments. The ^{13}C DEPT NMR spectrum showed the presence of six methyls, 10 methylene and 16 methine carbon atoms. The four methine resonances at δ 70.05, 76.71, 73.42 and 76.69, one methylene resonance at δ 61.04 were due to C-2', C-3', C-4', C-5' and C-6' respectively of the β-D-glucopyranoside. The chemical shift of the anomeric carbon at 100.76 (C-1') as well as the other sugar carbon chemical shifts were in agreement with α- configuration and with a pyranose form [19]. The olefinic resonances at δ 121.13, 137.99, and 128.79 corresponded to the C-6, C-22 and C-23 methine carbons and a signal at 140.42 corresponded to the C-5 quaternary carbon of the sterol moiety. The COSY and relayed COSY experiments showed that the anomeric doublet at δ 4.22 belonged to one glucose residue. In the COSY spectra all coupling constants were large and conformed to the equatorial proton of one β -D-glucopyranose residue. If one has a pure stereoisomer, the presence of one peak as a doublet between δ 4.0 and 4.6 with J around 8 Hz will point to β- isomer. While a doublet between δ 4.6 and 5.2 (comparatively de-shielded) with smaller J around 4 Hz, the presence of α- isomer will be indicated. The HMBC showed correlation between H-1' and C-3 (H-1'→C-3) and based on the above spectral data (Figures 9-14) which were in complete concurrence with the literature [8]; [20] this compound was identified as stigmast -5, 22-diene-3-O-β-D-glucopyranoside, 3.

Compound C is a white cotton-wool like powder with melting point between 273-275^{0}C and treatment of the compound with 2% H_2SO_4 in ethanol for 6hrs gave oleanolic acid and acetyl glucose. Its molecular formula, $C_{38}H_{60}NO_8$ was established by NMR (Nuclear Magnetic Resonance) spectroscopy and HRMS (High Resolution Mass Spectroscopy) which gave an exact mass of 658.4312 (calculated mass 658.4319), an $[M+1]^+$ at m/z 660 for $C_{38}H_{61}NO_8$ corresponding to 9.5 Double Bond Equivalent (DBE) as in the appendices. ^{1}HNMR and ^{13}C NMR signals at 168.73 clearly showed the presence of an acetyl group. The FTIR bands at 1631 and 1175 cm^{-1} revealed the carbonyl for amide and C-N stretch of amine in –NH-$COCH_3$.The HSQC revealed that carbohydrate residue was linked with oleanolic acid through the hydroxyl at C-3. The results of the ^{13}C NMR spectrum showed that the acetamido group can only be on C-2'. This assignment agrees with published data for similar structures [21]; [22]; [4]. Based on the above spectral data (Figures 15-20), the structure of the compound was established as 3-*O*-[β-D-glucopranosyl-2'-acetamido-2'-deoxy]-oleanolic acid, 1.

Compound D appears to be a natural pigment called pheophytin a and was obtained from the non- polar fraction of the leaf of *T. tetraptera*. Pheophytins are the degraded products of chlorophylls. During metabolite extraction the chlorophylls may lose their magnesium ions and become pheophytins. The signals of part of phytal fragments (-O-CH_2-CH=C (CH_3)-) of chlorophylls and other parts of pheophytins probably appeared at δ 9.47, 9.33, 8.537.98, 3.37 and 3.18 [23]. The ratio of chlorophylls a and b was determined as 3:1 by integration of singlet signals at δ 9.37 and 9.55. Other minor signals in the range of δ 11-7 may have appeared due to oxidized products of chlorophylls. The presence of two signals in the up field region (δ -1.46 and -1.65) of the spectrum of leaves was characteristic of N-H group of the porphyrins (Figures 21-26) [23]. The identification of this compound as pheophytin a, 4 was achieved by comparing the ^{1}H-NMR, ^{13} C-NMR, HMBC, HSQC, COSY and NOESY spectroscopic data with those in literature [5]; [24].

Compound E was obtained as a white powder (19 mg) and FTIR spectrum showed strong band of –OH (alkanol) at 3314 cm^{-1}. The ^{1}H-NMR spectrum had four sets of proton signals at δ 3.63, 1.56, 1.23 and 0.87 ppm corresponding to protons at C-1, C-2, C-3- C-23 and C-24 respectively. The ^{13} C-NMR spectrum had characteristic signals for a fatty acid derivative at δ 63.12 (CH_2), 32.82 (CH_2), 31.92 (21 CH_2) and 14.11 (CH_3) corresponding to an oxygenated methylene (C-1), a methlene (C-2), a methylenic side chain (C-3 to C-23) and a methyl (C-24) groups, respectively. The spectroscopic data (Figures 27-32) are in agreement with those reported in the literature [25] for n- tetraeicosanol, 5. Details of all the spectral characterization of isolated compounds (A-E) are attached as supplementary data.

The antimicrobial efficiency of selected isolates was investigated under the same parameters as the crude and bioassay guided fractions (Tables 1, 2). The results revealed that isolate with designation 52-53(B) was the most active against *Staphylococcus aureus* (32 mm), equally as active as isolate 19-20(A) against *Streptococcus pneumonia* (30 mm) and *Corynebacterium ulcerans* (28 mm), most active against *Streptococcus pyrogenes* (30 mm), less active (29 mm, 26 mm) than isolate 19-20(A) against *Candida albicans* and *Candida krusei* respectively (31 mm, 28 mm). Both isolates 52-53(B) and 19-20(A) represent pure compounds obtainable from the polar neutral fraction of the stem bark. Isolate 52-53(B) competed particularly favourably with 10 (µg/ml) of Ciprofloxacin against *Streptococcus pyrogenes.*

5. Conclusion

Isolation of three new active compounds and a natural pigment were compared with that of literature and confirmed to be stigmasterol, Stimast -5, 22- diene-3-O-β-D-glucopyranoside, n-tetraeicosanol and Pheophytin which are known compounds that have very good medicinal potentials. More compounds should be isolated from other bioassay guided

fractions, subjected to biological and pharmacological studies to investigate the medicinal potential of *Tetrapleura tetraptera*. Also, derivatives of the isolated compounds should be synthesized to improve on its activity.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declared no conflict of interest.

References

[1] Balunas M. J., and Kinghorn A. D., Drug discovery from medicinal plants. *Life science Journal,* 2005; 78:431-441.

[2] Chin Y. W., Balunas M. J., Chai H. B., and Kinghorn A. D., Drug discovery from natural sources, *AAPS Journal,* 2006; 8:239-253.

[3] Sobolev A. P., Brosio E., Gianferri R and Segre A. L., Metabolic Profile of Lettuce leaves by High field NMR Spectra. *Magnetic Resonance in Chemistry*, 2005; 43: 625-638.

[4] Amos S., Kolarole E., Akali P., Wambebe C., and Gamaniel K., Behavioural effects of the aqueous extracts of *Guiera senegalensis* in mice and rats. *Phytomedicine*, 2001; 8:356-361.

[5] Balunas M. J and Kinghorn A. D., Drug discovery from medicinal plants. *Life science Journal,* 2005; 78:431-441.

[6] Wolfender J. L., HPLC in natural products analysis. The detection issue. *Planta Medica,* 2009; 79:719-734.

[7] Adebayo A. S., Gbadomosi I. A., and Adewunmi C. O., 2000 Formulation of antimicrobial dried powdered herbs in soap basis. In C. O. Adewunmi and S.K. Adesina, eds. Phyto Medicines in Malaria and Sexually Transmitted Disease, Challenges for the New Millennium. Obafemi Awolowo University, Ile-Ife. 97.

[8] Alam M. S., Chopra N., Ali M., Niwa M., Oleanen and stigmasterol derivatives from *Ambroma augusta. Phytochemistry*, 1996; 41:1197-1200.

[9] Elujoba A. A., Odeleye O. M., and Ogunyemi C. M., Traditional medicine development from medical and dental healthcare delivery system in Africa. *Africa Journal of Traditional, Complementary and Alternative medicine*, 2005; 2:46-61.

[10] Wang S. Y., Tseng C. P., Tsai K. C., Lin C. F., Wen C. Y., Tsay H. S., Sakamoto N., Tseng C. H., Cheng J. C., Bioactivity-guided screening identifies pheophytin as a potent anti- hepatitis C virus compound from *lonicera hypoglauca Miq. Biochemical and Biophysical Research communication*, 2009; 385:230-235.

[11] Abdel R. S., Abd-Ellatif S., Deraz S., and Khalil A., Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *African Journal of Biotechnology,* 2011; 10:10733-10743.

[12] Okwute S. K., Olajide O. O., Etuk-Udo G., Orishadipe A. T., Phytochemical screening, *In-vitro* antimicrobial activity and Antioxidant characteristics of *Tetrapleura tetraptera* extracts. *European Journal of Medicinal Plants*, 2016; 17:1-10.

[13] Oloye O. I., Chemical profile of unripe pulp of *Carica papaya*. *Pakistan Journal of Nutrition,* 2005; 4:379-381.

[14] Okwute S. K., and Rufai Y., Antimicrobial and antioxidant potentials and chemical constituents of the leaf extract of the Nigerian *Piliostigma thonningii* (Caesalpiniaceae) Schum. *European Journal of Medicinal Plants*, 2015; 7: 137-145.

[15] Muhit M. A., Tareq A. M., Apu A. S., Basak D., and Islam M. S., Isolation and identification of compounds from the leaf extract of *Dillenia indica Linn, Bangladesh Pharmaceutical Journal,* 2010; 13:49-53.

[16] Enwere N. J., Foods of Plant Origin. Afro-orbis publication Ltd, University of Nsukka, 1998; 194-199.

[17] Ahmed Y., Rahman S., Akhtar P., Islam F., Rahman M., and Yaakob Z. Isolation of steroids from N-hexane extract of the leaves of *Saurauia roxburghii. International Food Research Journal,* 2013; 20:2939-2943.

[18] Luhata L. P., and Munkombuse N. M., Isolation and characterization of stigmasterol and β-sitosterol from *Odontonema strictum (Acanthaceae). Journal of Innovations in Pharmaceuticals and Biological Sciences,* 2015; 2: 88-95.

[19] Jares E. A., Tettamanzi M. C., and Pomilio A. B., Sitosterol 3-O-β-D-glucuronopyranoside from *Senecio bonariensis. Phytochemistry,* 1990; 29: 340- 341.

[20] Nasser W., Beres S. B., Olsen R. J., Dean M. A., Rice K. A., Long S. W., Kristinsson K. G., Gottfredsson M., Vuopio J., Raisamen K., Cangant D. A., Low D. E., Van-Beneden C. A., Hoffmann S., and Musser J. M., Evolutionary Pathway to increase virulence and Epidemic group: A *Streptococcus* disease derived from 3,615 genome sequences. *Proceedings of the National Academy of Sciences of the United States of America (PNAS)*, 2014; 111:1768-1776.

[21] Otsuka H., Akiyama T., Kawai K., Shibata S., Inoue O., Ogihara Y., (1973). *Phytochemistry*, 17: 1349.

[22] Adesina S. K., and Reisch J., A triterpenoid glycoside from *Tetrapleura tetraptera* fruit. *Phytochemistry,* 1985; 24: 3003-3006.

[23] Sobolev A. P., Brosio E., Gianferri R and Segre A. L., Metabolic Profile of Lettuce leaves by High field NMR Spectra. *Magnetic Resonance in Chemistry*, 2005; 43:625- 638.

[24] Wang S. Y., Tseng C. P., Tsai K. C., Lin C. F., Wen C. Y., Tsay H. S., Sakamoto N., Tseng C. H., Cheng J. C., Bioactivity-guided screening identifies pheophytin as a potent anti- hepatitis C virus compound from *lonicera hypoglauca Miq. Biochemical and Biophysical Research communication*, 2009; 385:230-235.

[25] Makhafola T. J., Elgorashi E. E., Mcgaw L. J., Awouafack M. D., Verschaeve L., and Eloff J. N., Isolation and characterization of the compounds responsible for the anti- mutagenic activity of *Combretum microphyllum* (Combretaceae) leaf extracts. *Complementary and Alternative Medicine,* 2017; 17:446 - 448.

[26] Cheesbrough M., Reaction isolates on tropical diseases: The effects. Cambridge University Press, London, 2002; 2 : 76-100.

[27] European Committee on Antimicrobial Susceptibility Testing [EUCAST]., 2012; version 2:12.

[28] Suffredini I. B, Sander H. S, Goncalves A.G, Reis A. O, Gales A. C, Varella A. D, Younes R. N., Screening of anti-bacterial extracts from plants native to Brazilian Amazon Rain forest and Atlantic forest., *Brazilian Journal of Medical and Biological Research*., 2004; 37: 379-389.