Chemical Constituents and Antibacterial Evaluation of *Deinbollia pinnata* (Schum and Thonn) Sapindaceae.

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ABSTRACT

Roots of *Deinbollia pinnata* are investigated for phytochemicals and antibacterial activities. Preliminary phytochemical screening of the *n*-hexane, ethyl acetate, and methanol extracts of *D. pinnata* revealed presence of terpenoids, steroids, phenols, flavonoids, saponins, and cardiac glycosides. Chromatographic separation of the n-hexane and EtOAc extracts afforded two known compounds: 3,3',4',5,7-pentahydroxyl flavonoid (quercetin, 1) and stigmasteryl-3β-ol-5,22-diene (stigmasterol, 2).

Structures of isolated compounds were established on the basis of infrared, ^{1}H-, ^{13}C-NMR spectroscopy and comparison with spectral of related compounds. The extracts and isolated compounds demonstrated *in-vitro* antibacterial activities against four multidrug resistant Gram-positive bacterial strains: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus albus, Staphylococcus epidermidis* and three Gram-negative bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa,* and *Klebsiella pneumonia* using disc diffusion method for the assay. The diameter zone of inhibition ranges from 5 mm – 20 mm. In each case, the standard drugs, *gentamycin*, *ceftriaxone* and *ofloxacin* are more active than the extracts and isolated compounds.

(Key words: *Deinbollia pinnata*, chemical constituents, antibacterial activity, isolated compounds)

INTRODUCTION

Herbal plants in various forms are known to contain variety of chemical constituents (metabolites) that are therapeutic or serve as precursor for chemo-pharmaceutical applications (WHO, 1996). Herbal plants and their preparations have been reported for antimicrobial, antimalarial, anti-inflammatory, antidiabetic, anthelmintic, antiparasitic, anti-obesity, anticancer, and antiviral activities (Nimri *et al*., 1999). Resistance to antimicrobial agents has become an increasingly important and global problem of people presented for bacterial infections (Alexandria, 2004).

Sapindaceae (soapberry family) is a family of flowering plants with about 136 genera and 2000 species occurring from temperate to tropical regions throughout the world (Burkhill, 2000). Members of this family are widely reported for pharmacological, antioxidant, antidiabetic, and anti-inflammatory activities (Sofidiya *et al*., 2007; Simpson *et al*., 2010; Veeramani *et al*., 2010; Muthukumran *et al*., 2011). Ethnobotanical reports indicated that plants in Sapindaceae are used for treating ulcer, boils, pain, dermatological problems, wound healing, diarrhea and dysentery (Burkhill, 2000; Sofidiya *et al*, 2007; Agboola *et al,* 2012).

The genus *Deinbollia* consists of 59 species, including *D. accuminata, D. angustifolia, D. borbonica, D. boinensis, D. calaophylla, D. crassipes D. cuneifolia, D. dasybotrys, D. overadii, D. fanshawei, D. fulvotomentella, D. gossweileri, D. grandifolia, D. hierniana, D, insignis,D. laurenti, D. pinnata, D. laurifolia*, *D. longiacumainata, D. macarantha, D. macrocarpa,*

D. macroura, and *D. mexima* (Temitope and Oluwatoyin, 2012).

Roots and leaves of *D. pinnata* are used in folforic medicine as remedy for febrifuge, analgesic, bronchiasis intercostals, intestinal pains, jaundice, cough, asthma, and infections (Margret *et al.,* 2011; Agboola *et al.,* 2012, Basile *et al*., 2005)). Despite numerous pharmacological potentials of *D*. *pinnata*, no compound has so far been isolated from the plant, and scientific reports on its phytochemical and antibacterial activities are scarce in the literature. In furtherance of our search for useful phytochemicals from Nigerian forest, we investigated phytochemical and antibacterial activities of *D*. *pinnata*. Herein, we report for the first time, isolation of quercetin, β-sitosterol and antibacterial activities of the extracts and isolated compounds from the root extracts of *D*. *pinnata*

MATERIALS AND METHOD

Experimental

Melting points (mp) were determined on Gallenkamp apparatus and are uncorrected. Column chromatography was carried out using open column packed with silica gel (60, 60-200 mesh, ASTM (Merck, Darmstadt, Germany).

Aluminium sheets pre-coated with silica gel 60 PF_{254} (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and visualized under UV light. The infra-red (FT-IR) spectra were run in KBr pellets, in a Fourier transform infra-red spectrophotometer (FTIR) and absorption expressed in cm^{-1}. The IR was recorded on a Perkin Elmer, Model 2000, USA. The Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) spectra were taken in deuterochloroform ($CDCl_3$) and deuterodimethylsulphoxide (DMSO) in Fourier transform mode in a Shimadzu NMR Spectrophotometer. 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR, chemical shift (δ) are recorded in ppm, with tetramethylsilane (TMS) as internal reference. The coupling constant, *J* values are recorded in Hertz (Hz).

Plant Collection and Authentication

The root of *D. pinnata* used in this study was collected from the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State, Nigeria in 2013by Mr. Michael, a forester in the herbarium section of the institute. The plant material was authenticated at FRIN through comparison with the voucher specimen in the herbarium under the accession number FHI 3251.

Extraction of Plant Material

The roots of *D. pinnata* were air-dried at ambient temperature in the Organic Research Laboratory and crushed by means of automated grinding machine at Lafenwa, Abeokuta, Ogun State, Nigeria. The pulverized powdered material (2.9 kg) was exhaustively and successively extracted in an aspirator bottle by percolation with refluxing n-hexane (68^{o} C) for 24 h. The extract was concentrated at reduced pressure to yield a light brown oily solid (19.4 g).

Analytical TLC plates using pre-coated silica gel (Merck, Kieselgel 60$PF_{254+360}$) was carried out on the roots n-hexane extract of *D. pinnata* to reveal number of components in the extract. The plate was developed in n-hexane and ethyl acetate mixture, (3:1, vol: vol) and visualized under iodine vapor. Thereafter, the n-hexane residue (marc) was re-extracted by percolation with EtOAc for 36 h and concentrated at reduced pressure to yield dark brown solid (11.2 g). The marc was bathed with methanol by percolation and concentrated by distillation to produce dark green solid (42.0 g). The n-hexane and EtOAc extracts were spotted on analytical TLC (silica gel, Merck, Kieselgel60$PF_{254+360}$) plates developed in n-hexane and EtOAc mixture (3:1, vol: vol) and (2:1, vol: vol), respectively.

Fractionation of Root *n*-Hexane and EtOAc Extracts of *D. pinnata* by Column Chromatography

10 g of root *n*-hexane extract of *D. pinnata* was chromatographed in a silica gel (Kieselgel 60-200 mesh) packed column, and eluted from the column using varying polarity of solvents, starting with 100% *n*-hexane, 5% EtOAc in *n*-hexane, up to 100% EtOAc. 100 mL fraction was collected each time and a total of 205 fractions were obtained. Fractions were bulked into 10 subfractions on the basis of their similar TLC profile. Fractions 90-93, eluted with 10% EtOAc, was purified in *n*-hexane and crystallised in a mixture of *n*-hexane: EtOAc (3:1, vol: vol), to

yield pure white feathery powder (Compound 1, 150 mg).

8.0 g of root EtOAc extract of *D. pinnata* was chromatographed in a silica gel (Kieselgel 60-200 mesh) packed column and eluted progressively with gradient of EtOAc in n-hexane. The eluents are monitored using analytical thin layer chromatograph, on a pre-coated silica gel (Kieselgel $60PF_{254+360}$) plates. 100 mL of each fraction was collected at a time and 418 fractions were collected altogether. Each fraction was concentrated by distillation and stored in dried labeled sample bottles for further analysis.

Fractions were bulked into 18 sub fractions, on the basis of similarity in their TLC profile. Sub fraction 1 – 15, eluted with 100 % *n*-hexane, yielded yellow semi- solid (180 mg). This fraction was purified by washing severally in *n*-hexane, warmed on water bath and filtered repeatedly to yield pure yellow solid (compound 1, 50 mg). Fractions 41 – 48, eluted with 5% EtOAc in n-hexane contains oily dark green semi solid (180 mg). Sub-fraction 41-48 was purified by washing it in *n*-hexane and crystallized using MeOH, to yield pure yellow solid (compound 2, 100 mg).

Phytochemical Screening of Extracts of *D. pinnata*

Extracts of *D*. *pinnata* are subjected to preliminary phytochemical screening to evaluate the phytochemicals present. The procedure of Harbone (1984) was adopted to test for phytochemicals in the extracts.

Collection and Isolation of Tested Strains

Human clinical isolates were collected from Microbiology and Parasitological Laboratory, Federal Medical Centre (FMC), Idi-Aba, Abeokuta, Ogun State, Nigeria. Seven clinical isolates consisting of three Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*), four Gram-positive bacteria (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Staphylococcus albus, and Staphylococcus epidermidis)* were obtained from samples of patients who had pneumonia and urinary tract infections. After collection of all the isolates, were labeled, sub cultured and the isolates were maintained on nutrient agar slants at 4°C.

Antimicrobial Sensitivity Testing

Antimicrobial sensitivity test of each isolate was carried out as described by the Kirby – Bauer disc diffusion method (Bauer *et al*., 1986) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2007). Turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution. The standardized bacterial suspension was swabbed and inoculated on nutrient agar (Lab M Laboratories, U.K) using sterile cotton swabs and left to dry for 10 min, before dispensing the anti-microbial discs were placed onto the surface of the inoculated agar plate.

Three commercially prepared antibiotics discs, *gentamycin* (10 µg/mL), *ceftriaxone* (30 µg/mL) and *ofloxacin* (5 µg/mL) by (Abtek Biological Ltd, UK) were used to determine the drug sensitivity and resistance pattern of bacteria. The plates were inverted and placed in an incubator at 37^{o} C within 15 min after the discs were applied. After 16 -18 h of incubation, each plate was examined for the determination of the diameter zone of inhibition of the antibiotics. The anti-microbial activities of discs were determined by measuring the zone of inhibition expressed in millimeter (mm).

Antibacterial Assay of n-Hexane, EtOAc and MeOH Extracts of *D. pinnata* and Isolated Compounds

Antibacterial activity of the *n*-hexane, ethyl acetate, and methanol extracts of *D*. *pinnata* and isolated compounds were carried out using disc diffusion method (Bauer *et al.,* 1986). The suspension of tested bacteria (25 µL) was spread on nutrient agar plates. The discs (6 mm) were impregnated with 10 µL of each extract (20, 40, 60, 80 and 100 mg/mL), air-dried and placed on the prepared agar plates. The turbidity of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution.

Negative controls were prepared with extracting solvents (*n*-hexane, ethyl acetate and methanol). Antibiotics drugs *gentamycin* (10 µg/mL), *ceftriaxone* (30 µg/mL) and *ofloxacin* (5 µg/mL) (Abtek Biological Ltd., UK) were used as positive controls to compare the sensitivity of bacterial strain. The plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by

measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

RESULTS AND DISCUSSION

Results of preliminary phytochemical screening of the root *n*-hexane, EtOAc and MeOH extracts indicated flavonoids, tannins, saponnins, phlobatannins, steroids, and cardiac glycosides. Alkaloids, anthraquinones and reducing sugar are absent in the extracts (Table 1).

Presence of flavonoids, steroids and tannins have been reported for antibacterial properties in plants (Udochukwu, *et al*., 2015; Gabriele, 2010; Sofidiya *et al.,* 2007). Reference drug, G*entamycin* was active on five tested strains: *Staphylococcus albus*, *Staphylococcus aureus*, *Klebsiella pneumonia* (Figure 1), *Escherichia coli* and *Streptococcus pneumoniae* with diameter zones of inhibitions (DMZI) of 25 mm, 16 mm, 19 mm, 20 mm and 15 mm, respectively (Plates 3, 4, 5 and 6, Table 2). It exhibits no activity on *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. *Ceftriaxone* was active on *Staphylococcus albus*, *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Streptococcus pneumoniae* with DMZI values of 13 mm, 23 mm, 33 mm, and 22 mm, respectively.

No activity was recorded on *Escherichia coli* and *Pseudomonas aeruginosa*. *Ofloxacin* was active on the three tested strains *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* with DMZI values of 29 mm, 30 mm and 31 mm (Plates 4, 5 and 6, Table 2) but revealed no activity on *Staphylococcus albus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Table 2). *Ceftriaxone* showed a broad spectrum activity on *Streptococcus pneumoniae* at DMZI 22 mm, G*entamycin* has DMZ1 of 15 mm and *Ofloxacin* exhibited no activity on *Streptococcus pneumonia*, a bacteria responsible for cough (Siemieniuk *et al.,* 2011). *Pseudomonas aeruginosa* exhibited resistance to the antibiotic drugs: *gentamycin, ceftriaxone and ofloxacin* (Table 2).

Hexane extract (DPH) and EtOAc extract (DPE) of *D*. *pinnata* are active on *Streptococcus pneumoniae* (DMZI = 17 mm and 8 mm respectively, Table 2), it exhibited no activity on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Staphylococcus albus, and Staphylococcus epidermidis*.

Table 1: Preliminary phytochemical screening of root extracts of *D. pinnata.*

Phytochemicals	DPH	DPE	DPM
Saponnins	+	+	+
Tannins	+	+	+
Phlobatannins	+	+	+
Cardiac glycosides			
Legal test	+	+	+
Kedde test	+	+	+
STEROIDS			
Lieberman's test	+	+	+
Salwoski test			
FLAVONOIDS			
Shinoda test	+	+	+
Lead acetate test	+	+	+
Free Flavonoids	-	+	+
Phenol (Ferric chloride test)	+	+	+
Alkaloids	-	-	-
Reducing sugar	-	-	-

KEY: DPH = *D. pinnata* hexane extract, DPE = *D. pinnata* EtOAc extract and DPM = *D. pinnata* MeOH extract, + = present, - = Absent

Table 2: Antibacterial Activities of DPH, DPE and DPM and Reference Drugs.

Extracts / Reference drugs	Diameter of zones inhibition (DMZI) (mm)					*S.p*	*P.a*
	S. a	*S.au*	*K.p*	*E.c*	*S.e*		
Extracts							
DPH	-	-	-	-	-	17±1.155^{b}	-
DPE	-	-	-	-	-	8±1.155^{b}	-
DPM	-	6±0.577^{b}	10±1.155^{c}	6±0.577^{b}	5±0.577^{b}	16±1.155^{d}	-
Extracts (100mg/mL)							
DPH	-	5±0.577^{b}	-	-	-	15±1.155^{b}	-
DPE	-	-	-	-	-	7±1.155^{b}	4±0.577^{b}
DPM	-	5±0.577^{b}	-	-	-	14±1.155^{d}	5±1.155^{d}
Gentamycin	-	25±0.577^{c}	16±1.155^{bc}	19±2.309^{bc}	20±2.887^{c}	-	15±1.732^{b}
Ceftriaxone	-	13±1.155^{b}	23±1.155^{d}	33±2.309^{b}	-	22±1.732^{c}	-
Ofloxacin	-	-	29±2.906^{cd}	30±2.333^{d}	31±2.333^{d}	-	-
n - Hexane	-	-	-	-	-	-	-
EtOAc	-	-	-	-	-	-	-
MeOH	-	-	-	-	-	-	-

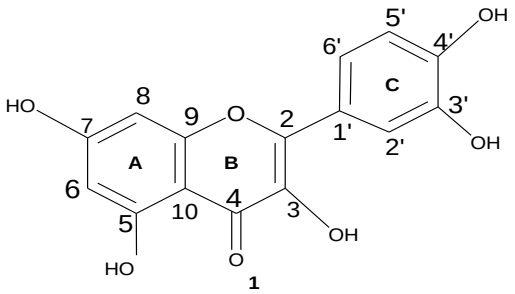
n = 3, X ±SEM. Means along the same column with different superscripts are significantly different at P< 0.05. *Staphylococcus albus* (*S. a*), *Staphylococcus aureus* (*S.au), Klebsiella pneumoniae* (*K.p*), *Escherichia coli* (*E.c*), *Staphylococcus epidermidis* (*S.e*) , *Streptococcus pneumoniae* (*S.p*) , *Pseudomonas aeruginosa* (*P.a*). *Gentamycin* (GEN), *Ceftriaxone* (CTR) and *Ofloxacin* (OFL). *D. pinnata* n-Hexane extract (DPH), *D. pinnata* ethyl acetate (DPE) and *D. pinnata* Methanol (DPM)

The MeOH extract DPM showed higher activity on the*Streptococcus pneumoniae* (16 mm), *Staphylococcus aureus* (6 mm)*, Escherichia coli* (6 mm)*, Klebsiella pneumoniae* (10 mm), and *Staphylococcus epidermidis* (5 mm) (Table 2). Highest DMZI was exhibited at 100 mg/mL by DPH on *Streptococcus pneumoniae* (DMZI = 15 mm). DPM and DPE gave DMZI of 14 mm and 4 mm on *Streptococcus pneumoniae,* respectively, at 100 mg/ mL (Table 4). *Streptococcus pneumoniae* has been reported as bacteria responsible for cough (Siemieniuk *et al.,* 2011). In this vein, it can be established that the folkloric use of *D. pinnata* in the treatment of cough is hereby supported with experimental evidence.

Characterization of Compounds 1-2

Compound 1 (Quercetin): Fractions 41-48 from the EtOAc extract, eluted with 5% EtOAc in n-hexane afforded quercetin after recrystallization as pure powdery yellow solid (50 mg), mp (314-315^{o}C), Lit mp 315 ^{o}C, R_f 0.21 on analytical TLC (Merck, Kieselgel 60$PF_{254+360}$), developed in n-hexane: EtOAc (3:1, vol:vol), visualized under iodine vapour in iodine tank as dark – brownish stains.

Infra-red (IR) spectrum of **1** exhibits characteristic stretching vibrations at 1728.5cm-^{1}, suggesting ketone and a weak peak at 1461.5 cm^{-1} indicates C=C stretch of aromatic moiety. Stretching vibration at 1374 cm^{-1} suggests C-O stretch connected to unsaturated moiety. A weak peak at 3400cm^{-1} suggests phenolic O-H stretch. A comparison of ^{1}H- and ^{13}C- NMR spectrum of **1** with reported work on quercetin showed close similarity (Table 3) (Addae-Mensah and Achenback, 1985; Addae-Mensah and Munense, 1989). Hence, compound **1** is confirmed as quercetin.



1

Compound 2 (Stigmasterol): Compound **2** was purified as feathery white solid (100 mg), mp 216°C, Lit mp 215 ^{o}C, TLC

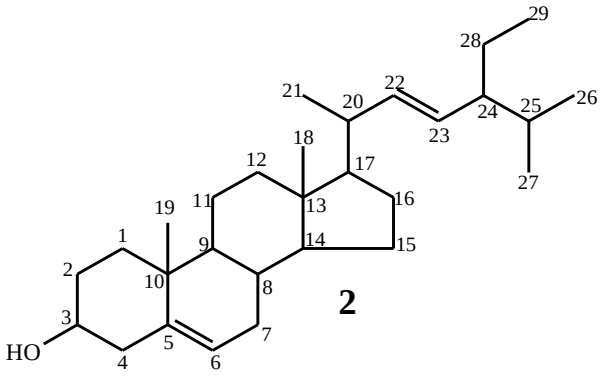
Table 3: Comparison of the ^{1}H- NMR and ^{13}C- NMR of Compound 1 and Quercetin.

C	Quercetin (Addae-Mensah and Achenback, 1985)		BMD 2	
	δ_H (100 MHz) DMSO	δ_C (500 MHz) DMSO	δ_C (125 MHz) DMSO	δ_H (500 MHz) DMSO
2	-	160.30	161.30	-
3	-	129.68	129.68	-
4	-	179.2	-	-
5	-	156.1	155.48	-
6	6.19	113.5	113.12	6.21 *d,* 2.0
7	-	164.8	-	-
8	6.13	102.4	102.15	6.18 *d,* 2.0
9	-	157.3	-	-
10	-	104.9	-	-
1'	7.81	121.8	-	-
2'	-	114.0	113.09	7.94 *d,* 2.0
3'	-	145.3	-	-
4'	-	149.1	-	-
5'	7.48	130.1	112.70	7.53 *d,* 8.5
6'	6.82	114.2	113.35	6.79 *dd,* 8.5

^{1}H and ^{13}C NMR spectral were acquired in DMSO at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shift values are shown in δ scale (ppm) with coupling constants (*J* in Hz) in parentheses, *d*-doublet and *dd*- doublet of doublet.

(Kieselgel 60$PF_{254+360}$), $R_{f\ =}$ 0.9, developed in n-hexane: EtOAc (2:1, vol: vol), visualized in iodine vapor on analytical TLC. Infrared spectrum (KBr disc) of BMD 3 displayed characteristics intense broad absorption band at 3432.1 cm^{-1}, suggestive of –OH stretch of alcohol (hydrogen bonded), a weak band at 1184.8cm^{-1} and 737.4cm^{-1} are connected with presence of hydroxyl group in **2** (Jain and Bari, 2010).

A medium band at 1053.8 cm^{-1} is assigned for C-O stretch of alcohol. A medium intense band at 1636.21cm^{-1} is indicative of vinylic C=C stretching vibration of alkene, along with out-of-plane C-H vibrations of unsaturated moiety at 879.06 cm^{-1} (Edilu *et al.,* 2015). A sharp band at 2923.59 cm^{-1} suggests C-H stretch of alkane and few C-H bending vibration of $–CH_3$ and $–CH_2$ appearing at 1462.94 (C-H bending of $–CH_2$), and 1377.27 cm^{-1} (C-H bending of $–CH_3$). Compound 2 was confirmed as stigmasteryl -5,22-dien-3-β-ol (Stigmasterol, **2**), on the basis of its spectral properties (^{1}H- and ^{13}C-nmr) and comparison with related work of stigmasterol in literature (Table 4, Edilu *et al.,* 2015; Jain and Bari, 2010).



2

Antimicrobial evaluation of compounds **1** and **2** (Table 5) demonstrated higher antibacterial activities (DMZI of 15 mm and 12 mm respectively) against S*treptococcus pneumoniae*, reported as causative agent for cough (Siemieniuk *et al.,* 2011).

These finding validates the use of *D. pinnata* roots in traditional medicine for the treatment of cough and bronchial asthma. Hence, *D. pinnata* may provide a drug lead for pharmaceutical preparations.

Table 4: Comparison of ^{1}H- and ^{13}C-NMR of Compound 2 and that of Stigmasterol.

C	Compound 2		STIGMASTEROL (Edilu *et al.*, 2015)		DEPT
	δ_C	δ_H	δ_C	δ_H	
1	37.23		37.3		CH_2
2	31.65		31.6		CH_2
3	71.79	3.53 (1H, *tdd*)	71.8	3.54	CH
4	42.29		42.3		CH_2
5	140.74	5.35	140.8		C
6	121.71		121.7	5.3	CH
7	31.87		31.9		CH_2
8	31.9		31.9		CH
9	51.22		51.2		CH
10	36.48		36.5		C
11	21.06		21.1		CH_2
12	39.74		39.7		CH_2
13	42.29		42.3		C
14	56.85		57.9		CH
15	24.35		24.4		CH_2
16	28.23		28.4		CH_2
17	56.03		56.1		CH
18	11.84	0.68	11.0	0.68	CH_3
19	21.20	1.02	21.2	1.02	CH_3
20	40.49		40.5		CH
21	21.20	1.04	21.2	1.01	CH_3
22	138.31	5.137	138.3	5.01	CH
23	129.24	5.01	129.3	5.0	CH
24	51.22		51.2		CH
25	31.87		31.9		CH_3
26	19.01	0.84	19.0	0.86	CH_3
27	21.20	0.69	21.2	0.76	CH_3
28	25.39		25.4		CH_2
29	12.24	0.79	12.1	0.78	CH_3

^{1}H and ^{13}C NMR spectral were acquired in DMSO at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shift values are shown in δ scale (ppm) with coupling constants (*J* in Hz) in parentheses, *d*-doublet and *dd*- doublet of doublet

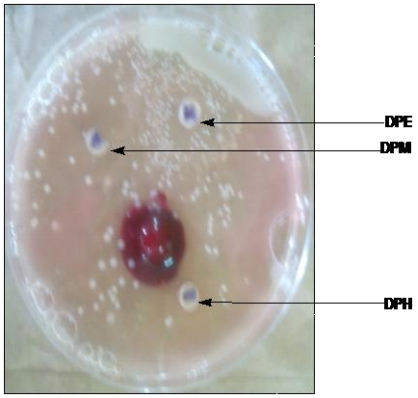
Table 5: Antibacterial Activities of Compounds 1 and 2.

Compound	Diameter of zones inhibition (DMZI) (mm)						
	S. a	*S.Au*	*K.p.*	*E.c.*	*S.e.*	*S.p.*	*P.a*
1	--	13	-	18	-	20	-
2		10	-	16	-	15	-

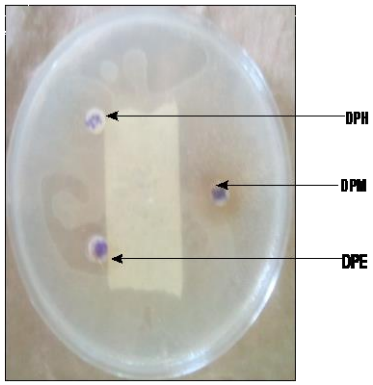
CONCLUSION

The results of antibacterial assay on extracts of *D. pinnata* and compounds isolated from *n*-hexane and EtOAc extracts suggests inhibitory actions of the plant on important bacterial. This lends scientific credence and justification for ethnobotanical information for the use of *D. pinnata* in traditional medicine. Hence, *D. pinnata* may provide a drug lead for pharmaceutical preparations.

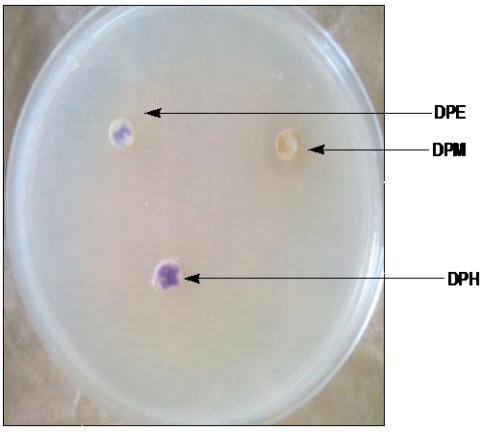
PLATES:



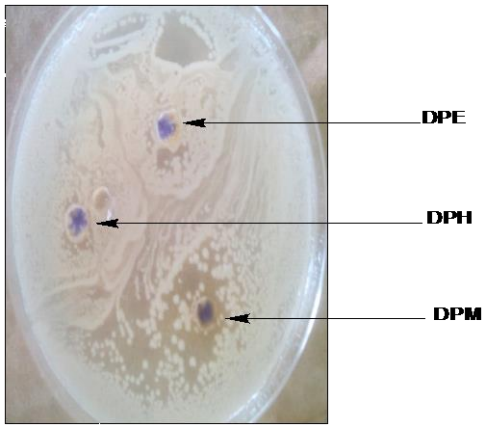
Antibacterial activities of DPH, DPE and DPM on *Streptococcus pneumonia*



Antibacterial activities of DPH, DPE and DPM on *S. aerus*

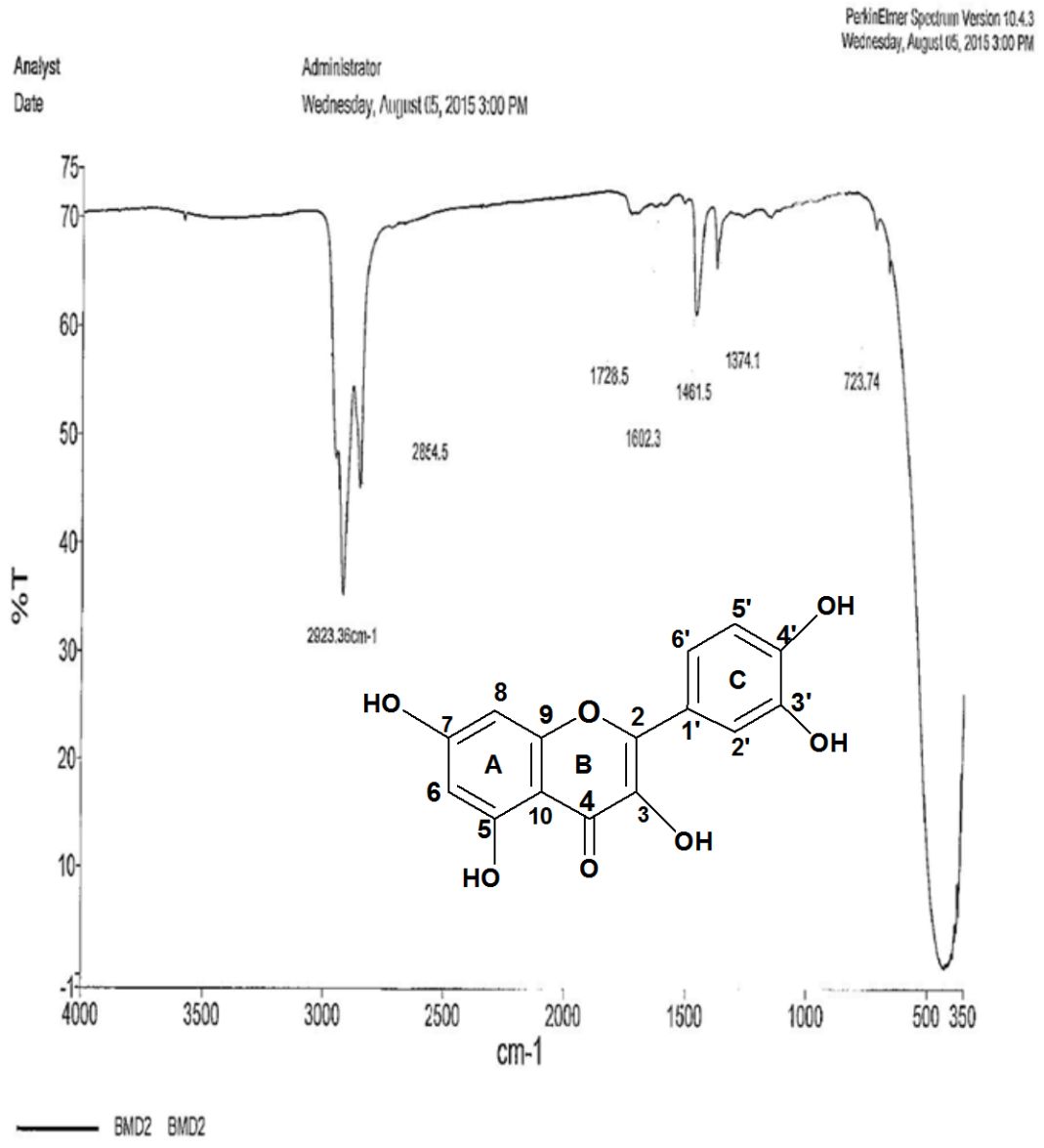


Antibacterial activities of DPH, DPE And DPM on *E. Coli*.

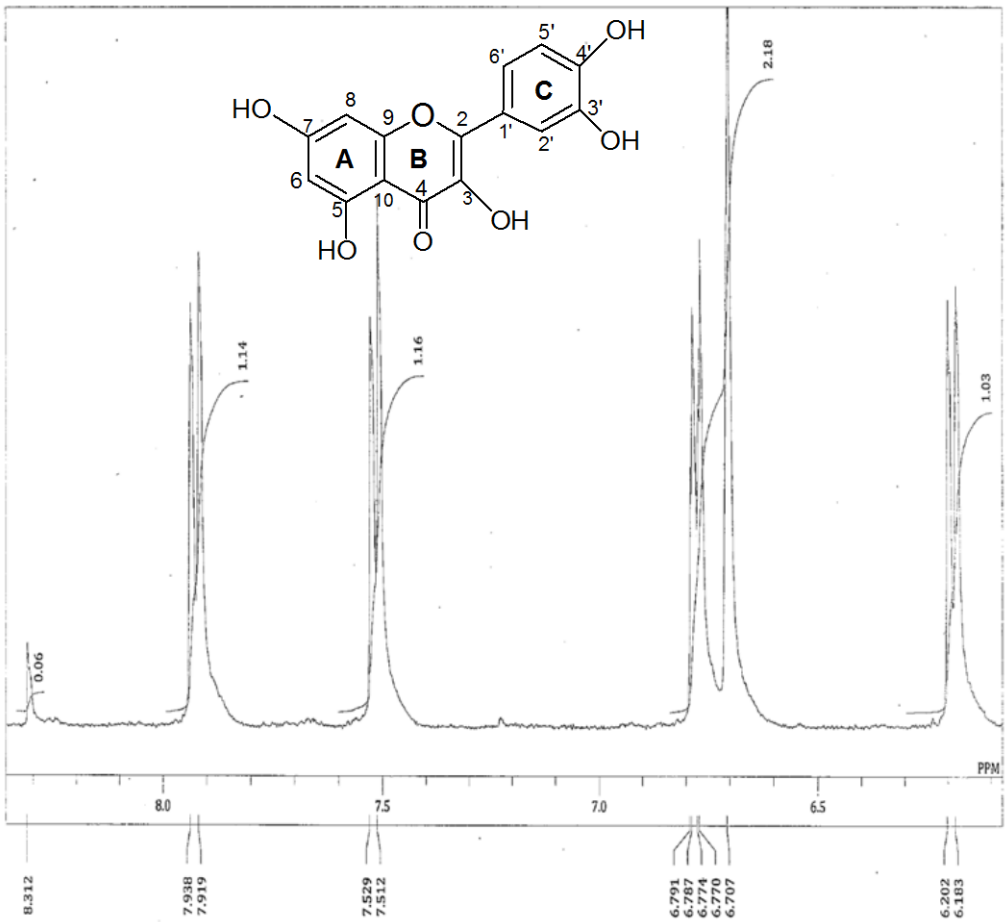


Antibacterial activities of DPH, DPE and DPM on *Klebsiella*. P*pneumonia*e

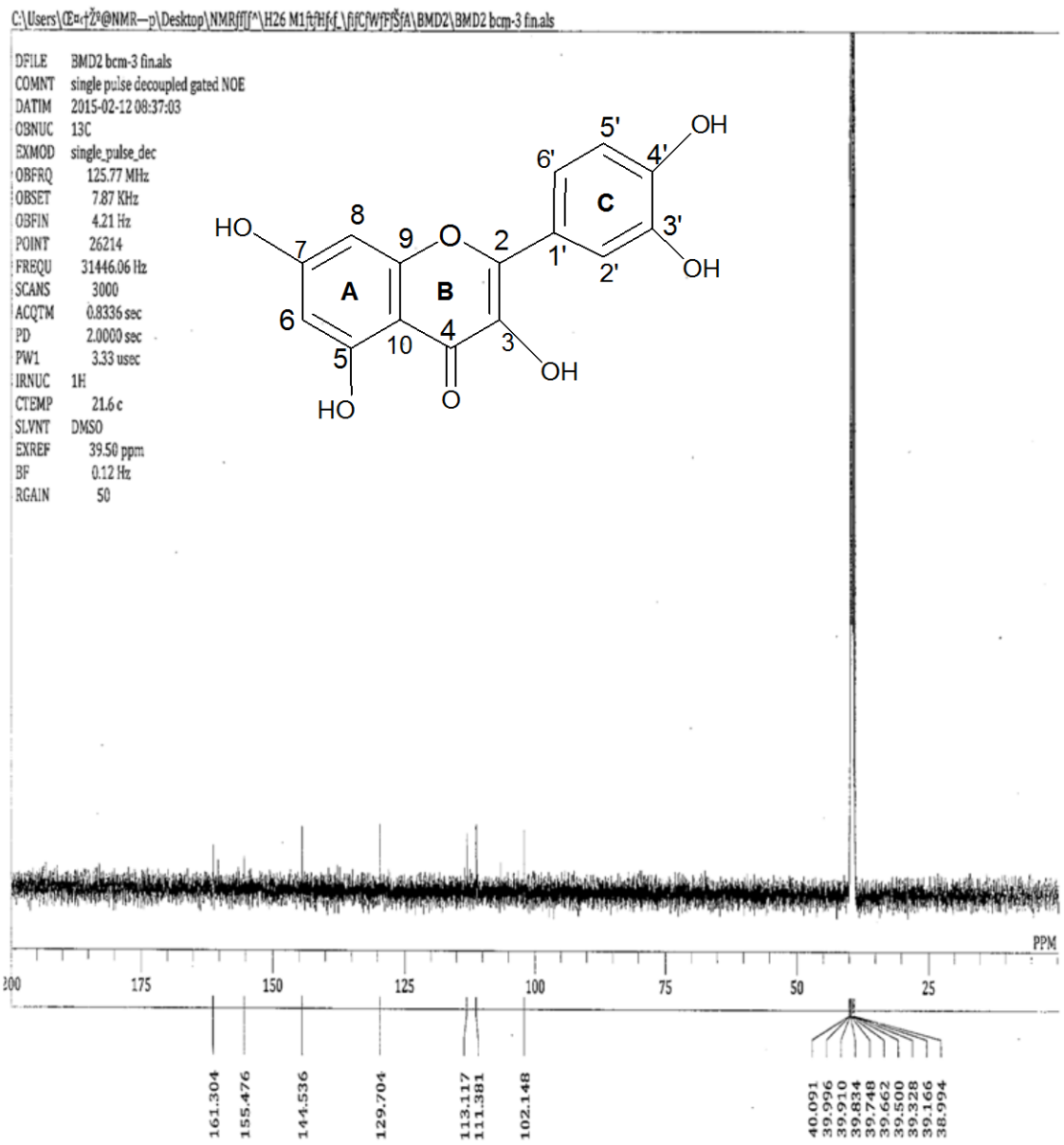
FT-IR of Compound 1:



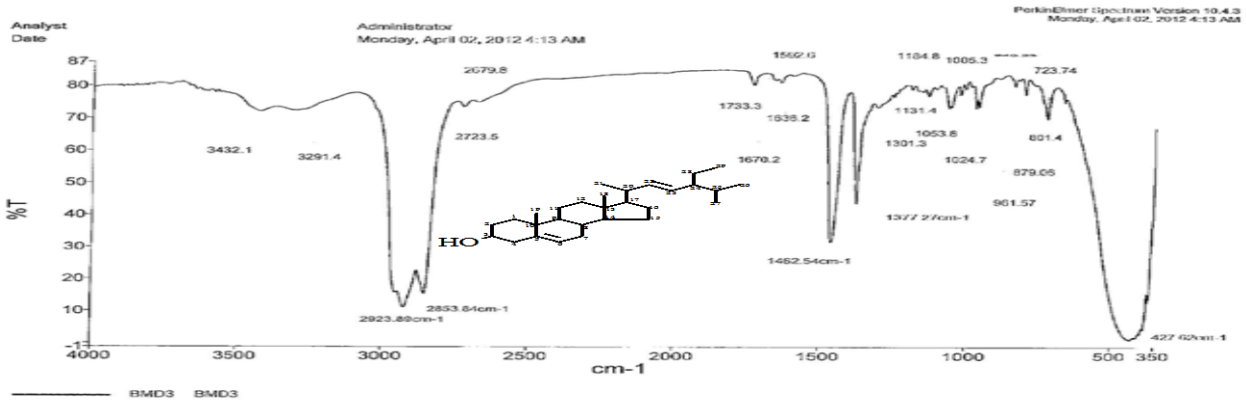
Expanded ^{1}H-NMR Spectrum of Compound 1:



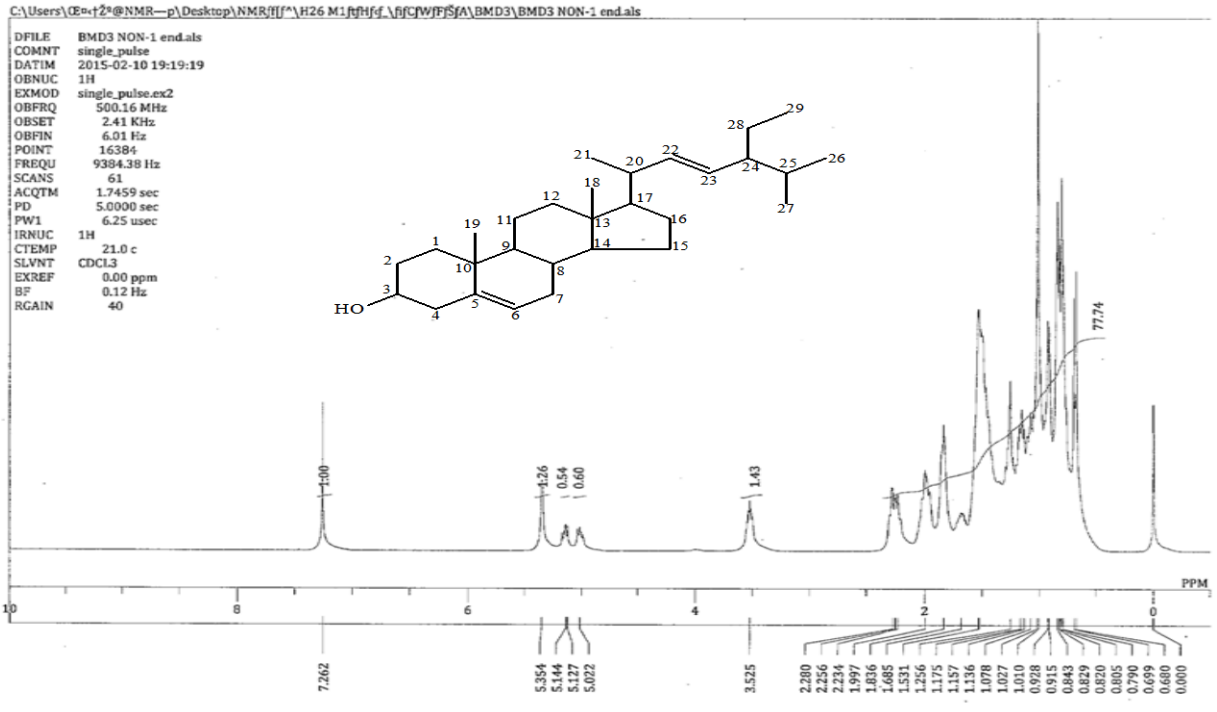
^{13}C-NMR Spectrum of Compound 1:



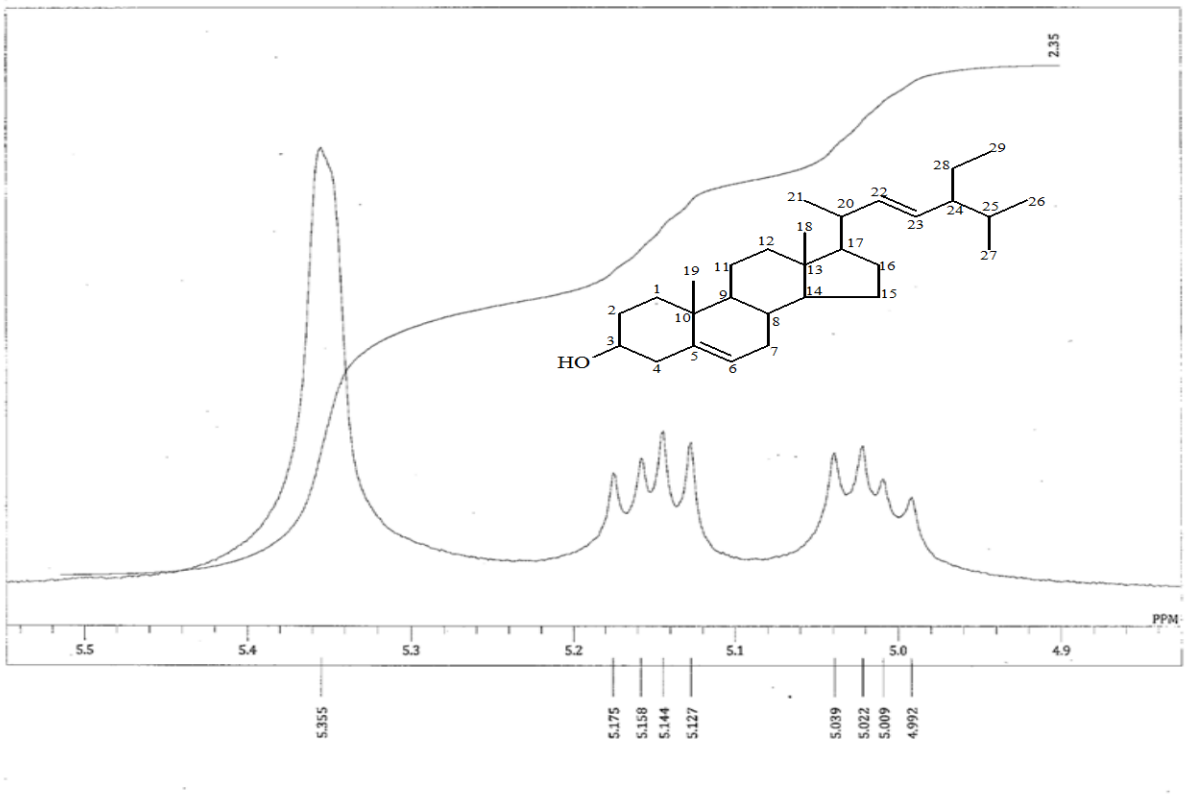
FT-IR of Compound 2:



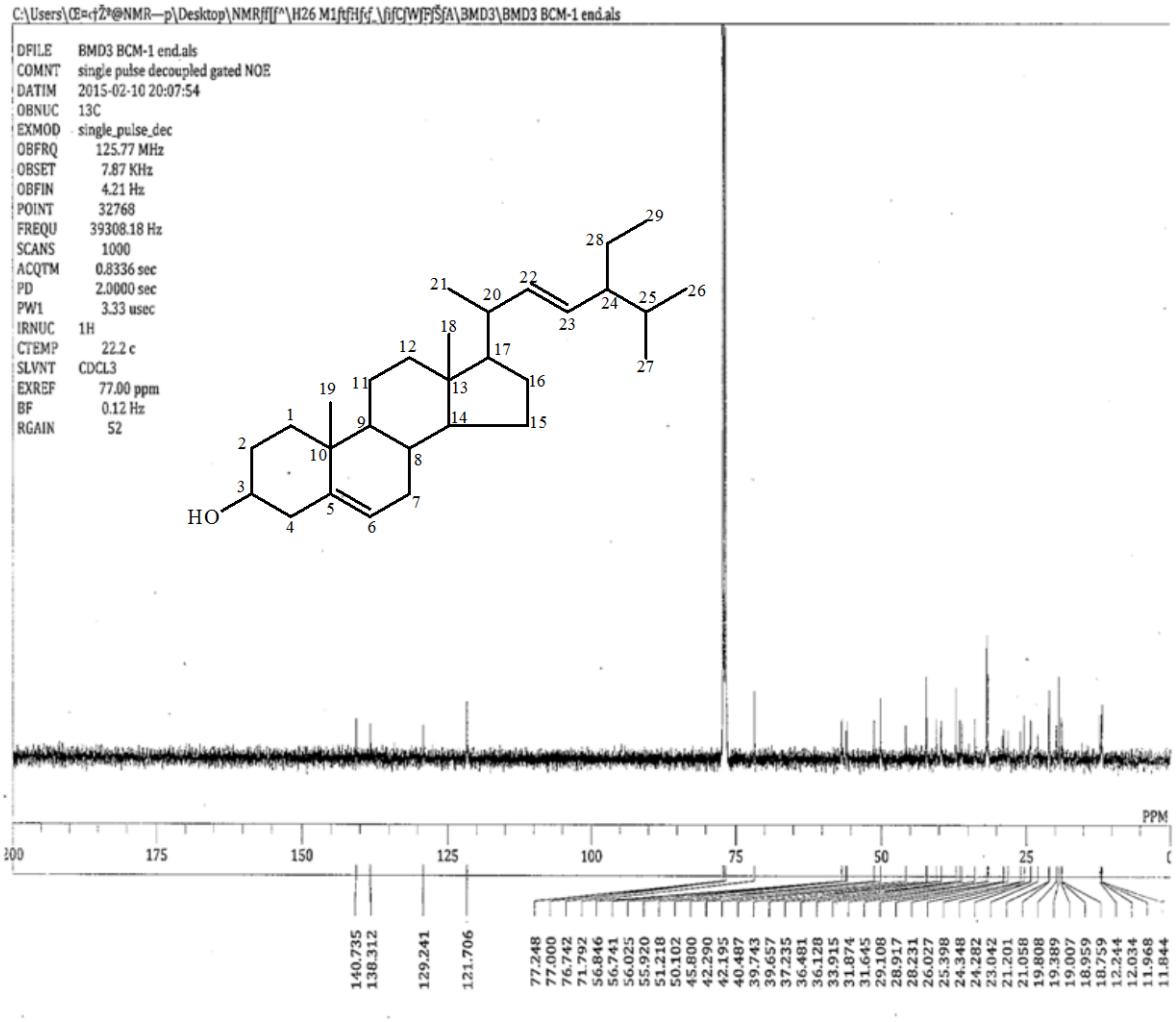
^{1}H-NMR of Compound 2:



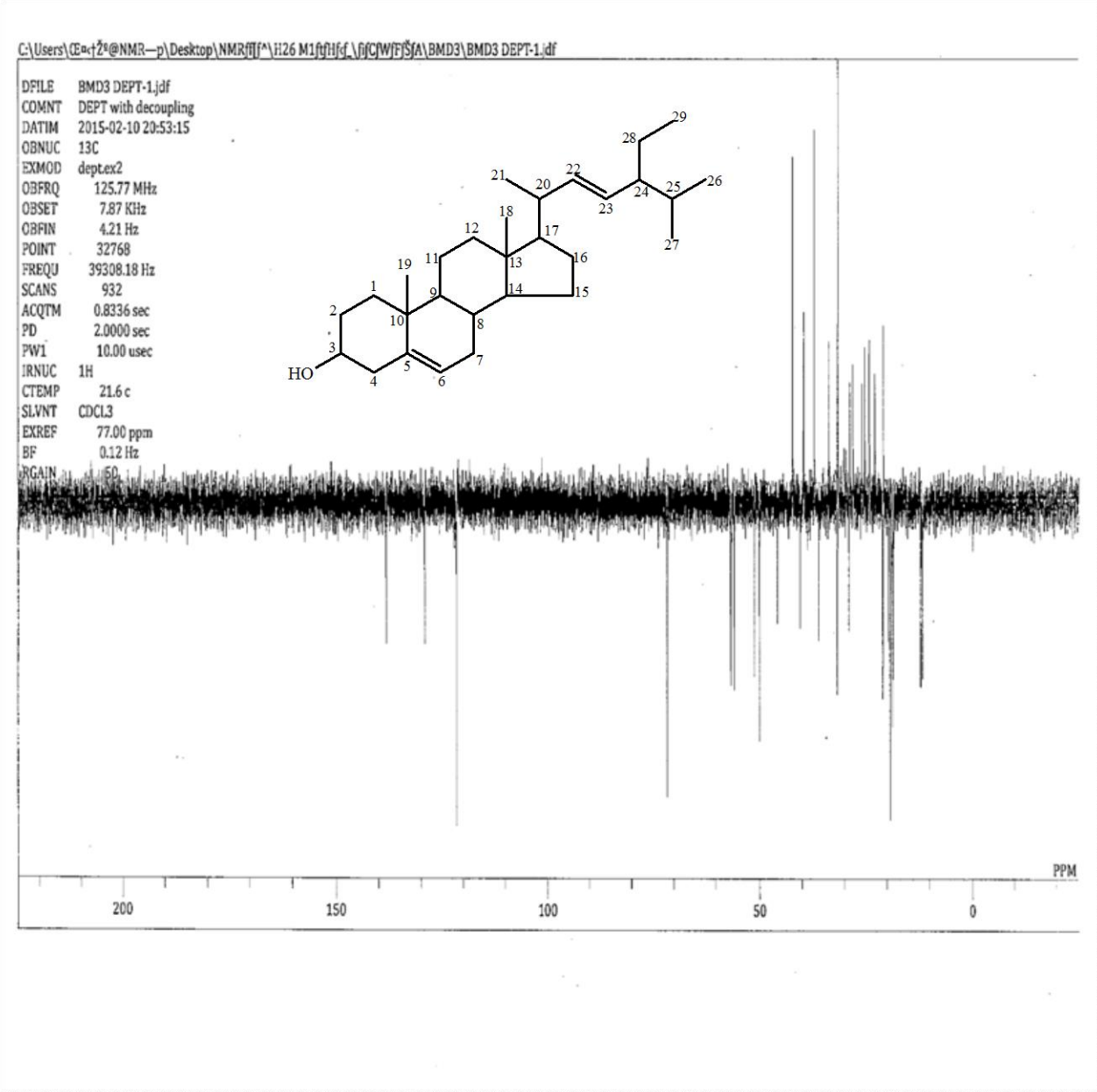
Expanded ^{1}H-NMR of Compound 2:



^{13}C-NMR of Compound 2:



C-13 DEPT Spectrum of Compound 2



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