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NEW SAPONINS FROM *ALBIZIA LEBBECK* (L) BENTH FLOWERS

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ABSTRACT: Two new oleanane triterpene type saponins named lebbeckanin I (1) and lebbeckanin II (2), in addition to a new isomer of methyl inositol, D-1-*O*-methyl-*cis*-inositol (3), were isolated from butanol fraction of *Albizia lebbeck* (L.) Benth flowers. Their structures were established on the basis of spectroscopic analysis including MS, ^{1}H & ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC & HMBC. The fraction containing mixture of compounds 1 and 2 exhibited significant cytotoxic activity against HePG-2 (Hepatocarcinoma), HEP-2 (Larynx carcinoma), HELA (Cervical carcinoma), MCF-7 (Breast carcinoma) and HCT-116 (Colon carcinoma) cell lines with LD_{50} values of 1.74, 3.42, 4.29, 0.65 and 0.74 µg respectively. In addition, the antimicrobial screening of the same fraction was evaluated and showed variable degrees of activity against all the tested organisms. It is notable that the same fraction showed antifungal activity against *Syncephalastrum racemosum* (RCBM 05922) stronger than that exhibited by Amphotericin B.

INTRODUCTION: *A. Lebbeck* (L.) Benth is a fast growing medium sized deciduous tree measuring 10-12 m in length, the tree is cultivated in Egypt. *A. lebbeck* is used in folk medicine to treat some inflammatory conditions as asthma, arthritis, burns allergic rhinitis, bronchitis and leprosy [1]. In addition, saponins of *A. lebbeck* have been claimed to be useful in treatment of Alzheimer`s and Parkinson`s diseases, moreover *A. lebbeck* extracts showed several biological activities [2], while flavonoids exhibit antioxidants properties [3]. As well as *A. lebbeck* extract exhibits potent hepatoprotective activity along with various pharmacological activities such as CNS, cardiotonic, lipid-lowering, antioxidant [4] and hypoglycemic activities [5].

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Biologically active triterpenoid saponins were isolated also from different Albizia species [6-15]. *A. lebbeck* growing worldwide revealed many biological and phytochemical interests. However, the species growing in Egypt has not received attention. The present study was undertaken for isolation of two new saponins named lebbeckanin I (1) and lebbeckanin II (2) from butanol extract of the flowers and a new isomer of methyl inositol, D-1-*O*-methyl-*cis*-inositol (3); in addition to the evaluation of cytotoxic and antimicrobial activities of the fraction containing mixture of compounds 1 and 2.

Experimental section:

General experimental section:

Büchi rotatory evaporator was used for evaporation of solvent; Melting points were determined by using Digital, electro-thermal LTD (England) apparatus; GL-58 (λ_{max} 254 and 365 nm) UV lamp was used for TLC visualization UVP; Circulating hot air oven W.T-binder 7200 (Germany); Infrared spectral analysis were recorded in potassium

bromide disks on a Pye Unicam SP 3000 and IR spectrophotometer, Jasko, FT/IR-460 plus. Bruker Daltonics flex analysis; acetonitrile: H_2O (1:5) was used as a matrix for ESI-mass. ^1H & ^{13}C-NMR spectral analyses were obtained by: Bruker at 400 & 125 MHz, Bruker at 600 & 150 MHz. Chemical shifts were given in ppm with the TMS as internal standard.

Chromatographic Solvent Systems:
The following solvent systems were used in TLC development

I. Ethyl acetate: acetic acid: formic acid: H_2O; 10:1.1:1.1:2.7

II. Ethyl acetate: methanol: H_2O; 6: 2: 0.8

III. Chloroform : methanol : acetic acid : H_2O; 15 : 8 : 3 : 2

IV. Butanol : acetic acid : H_2O; 10 : 2 : 8

Plant material:
The flowers of *Albizia lebbeck* (L) Benth family Fabaceae, were collected on May 2010 from the vicinity of Benha governorate, Qalioubia, Egypt. The identification was kindly identified by Prof dr. Hussain Abdel Baset, Professor of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt. A voucher specimen is deposited in Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt. The flowers were shade dried and powdered.

Extraction and isolation:
Isolation of compounds 1-3 from butanol Fraction of *A. lebbeck* flowers:
The dried powdered *A. lebbeck* flowers (320 g) was extracted with 95% ethanol and the resulting ethanolic extract (52.4 g, 14.9%) was dissolved in mixture of methanol and water (1:9) then partitioned with petroleum ether, chloroform, ethyl acetate and butanol saturated with water to give 3.5 g (1.8%), 0.89 g (0.46%), 1.2 g (0.62%) and 17.5 g (9.1%) of petroleum ether, chloroform, ethyl acetate and butanol fractions respectively. The butanol soluble fraction of flowers (15.5 g) was subjected to silica gel column packed by wet method using ethyl acetate. The elution was started with ethyl acetate then the polarity increased gradually with methanol. The eluate was collected in 50 fractions, 250 ml each, concentrated under reduced pressure, monitored by TLC using solvent systems (I, II & III), visualization spray reagent (anisaldehyde/ sulphric acid and aniline phthalate) and similar fractions were combined together. For purification, fractions (38-40) and (44-46) were separately rechromatographed over Sephadex LH_{20} column (2 x 35 cm, 10 gm) and eluted with methanol. The resulting fractions were concentrated to afford 27 mg of compound 1 and 15 mg of compound 2 respectively. Fractions 9-20 revealed the presence of one major green spot using aniline phthalate as spray reagent. The fractions were combined and concentrated to afford a white residue; this residue was crystallized from methanol to afford 750 mg of compound 3.

Compound (1):
(27 mg) golden yellow powder (methanol) with mp 200-202ºC; R_f value 0.42 (solvent systems III); FTIR spectrum ($\nu_{max.}$, KBr) cm^{-1}: 3410 (OH), 2926 (C-H), 1643 (C=C) & 1441 (-CH_2). The ESIMS: *m/z* 1583 $[M+H]^+$, 1387 [M-arabinose-H_2O-$COCH_3$]$^+$, 1226 [1357- 161 (aminoglucose)]$^+$, 1095 [1226-132 (xylose)]$^+$ & 963 [1095- 132 (arabinose)]$^+$; EIMS: *m/z* 472 $[M]^+$. The ^1H &^{13}C NMR data (CD_3OD, 600, 150 MHz) data are listed in **Table 1**.

Compound (2): (15 mg) shiny creamy powder (methanol) with mp 233-235ºC; R_f value 0.36 (solvent system I); FTIR spectrum (ν_{max}, KBr) cm^{-1}: 3415 (OH), 2925 (C-H), 1646 (C=C) and 1431 (-CH_2); The ESIMS: *m/z* 1761 $[M+H]^+$, 1599 [M-glu]$^+$, 1583[M-glu-OH]$^+$, 1387 [1583- arabinose-H_2O-$COCH_3$]$^+$, 1226 [1387-161 (aminoglucose)]$^+$, 1094 [1226- 132 (xylose)]$^+$ & 962 [1094-132 (arabinose)]$^+$; ^1H &^{13}C NMR data (CD_3OD, 600, 150 MHz) data are listed in **Table 2.**

Compound (3): (750 mg) white crystals (methanol) with mp 184-186ºC; R_f value 0.50 (solvent system I); FTIR spectrum ($\nu_{max.}$ KBr) cm^{-1}: 3405 and 3320 (OH), 2950 (C-H), 1453 (- CH_2), 1342 (- CH_3) and 1279 (- C-O); The EIMS *m/z*: 164 (M^+ - OCH_3, 3.90), 149 (6.24), 128 (8.88), 122 (12.52), 106 (11.73), 85 (62.49), 83(100.00) and 44

(30.55); ^{1}H & ^{13}C-NMR data (DMSO-d_6, 400, 125 MHz) data are listed in **Table 3**.

Acid hydrolysis of compounds 1 and 2: Compounds **1** (10 mg) and **2** (5 mg) were separately dissolved in 9 ml 2 N HCL and 9 ml CH_3OH then heated at 100ºC for 6 hrs. The mixture was left to cool, diluted with water and extracted with CH_2Cl_2. TLC for CH_2Cl_2 using solvent system (III), visualizing agent anisaldhyde sulphuric acid and heating 110ºC produced violet spot with R_f value 0.42 (compound 1) indicates triterpene or sterol skeleton. The aqueous layer was neutralized with $BaCO_3$ and filtered; the filtrate was concentrated under reduced pressure and investigated by paper chromatography against authentic sugars. The paper was developed using solvent system (IV) and aniline phthalate as revealing agent followed by heating.

Biological activities:

Cytotoxic activity: The cytotoxic activity of the fraction containing mixture of compounds 1 and 2 against HePG-2 (Hepatocarcinoma), HCT-11 6 (Colon carcinoma), HEP-2 (Larynx carcinoma), HELA (Cervical carcinoma) and MCF-7 (Breast carcinoma) cell lines was carried out using MTT assay according to method reported by Mosmann[16] and the results were reported in **Table 4**.

Antimicrobial activity:

Microbial strains: The antimicrobial activity of a fraction containing mixture of compounds 1 and 2 was tested against fungi; *Asperagillus fumigatus* (RCMB O2568), *Syncephalastrum racemosum* RCMB 05922, *Geotricum candidum* (RCMB 05097) and *Candia albicans* (RCMB 05036). The same fraction was also tested against Gram –ve bacteria *Pseudomonus aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010052) and Gram +ve bacteria including *Streptococcus pneumonia* (RCMB 010010) and *Bacillis subtilis* (RCMB 010067). All these microorganisms were obtained from the Regional Centre for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

The Method: Cup-plate method [17] was used to detect the preliminary antimicrobial activity of a fraction containing mixture of compounds 1 and 2. Amphotricin B, Ampicillin and Gentamycin were used as antibacterial and antifungal standards and the zones of inhibition were measured (mm) and recorded in **Table 5**.

RESULTS AND DISCUSSION:

Characterization of the isolated compounds:

Compound 1: The IR spectrum of compound 1 showed absorption bands at 3410, 2926 and 1643 cm^{-1}. Acid hydrolysis of compound 1 gave an aglycon (sterol or triterpene, R_f value 0.42, solvent system III) and glucose, xylose, rhamnose, arabinose and glucosamine as sugar moieties which were identified with authentic samples, The positive ESI-MS spectrum showed a molecular ion peak at *m/z* 1583 [M^+ + H] which is in a good agreement with the molecular formula $C_{74}H_{120}N_2O_{34}$. The EI-MS of aglycon showed M^+ at 472. The ^{1}H NMR data shown in Table 1 demonstrated the presence of seven singlet signals at δ_H 1.06 (s), 0.87 (s), 0.87 (s), 1.25 (s), 1.37 (s), 0.96 (s) and 1.06 (s), corresponding to CH_3-23, CH_3-24, CH_3-25, CH_3-26, CH_3-27, CH_3-29 and CH_3-30 respectively and connected via HSQC to carbon signals at δ_C 28.5 (q), 17.0 (q), 16.1 (q), 17.1 (q), 27.3 (q), 33.3 (q) and 24.6 (q).

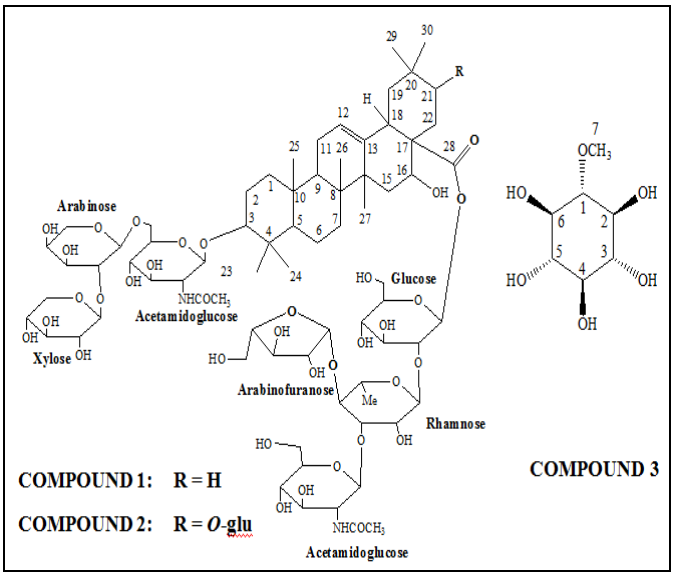


FIG.1: CHEMICAL STRUCTURES OF COMPOUNDS 1-3

The dihydroxylation pattern was evidenced from the ^{1}H NMR data which showed two multiplet signals at δ_H 3.25 (1H, m) and 3.35 (1H, m) for H-3 and H-16 respectively which showed direct coupling to carbons at δ_C 90.1(d) and 73.1 (d) in the HSQC experiment. The other ^{1}H NMR signal

at δ_H 5.32 (1H, br t) in addition to the ^{13}C NMR signals at δ_C 123.6 (C-12, d) and 144.7 (C-13, s) proved the presence of oleanane type triterpene nucleus carrying a double bond at (Δ^{12})[1 3 18], also the comparison of other NMR signals presented in table (1) with literature data [7] confirmed the presence of echinocystic acid. The presence of anomeric protons at δ_H 4.48 (1H, d, *J*=7.0 Hz), 4.52 (1H, d, *J*= 4.0 Hz) and 4.49 (1H, br d) which connected directly via HSQC to carbons at δ_C 104.8 (d), 103.3 (d) and 106.5 (d) respectively with the other carbons and proton signals presented in table (1) and through the By comparison with literature[7] suggested the presence of 2-deoxy-2-acetamidoglucose, L–arabinose and D-xylose as part of sugar moieties in the compound.

The presence of 2-deoxy-2-acetamidoglucose moiety was confirmed from the presence of carbonyl group at δ_C 173.4 with one methyl signals at δ_C 23.1 (q) and δ_H 1.95 (3H, s) and typical amide carbon at δ_C 57.8. In addition to the HMBC correlations (**Fig. 2**) of the methyl signal at δ_H 1.95 (3H, s) and H-2 at δ_H 3.65 (1H, m) of the 2-deoxy-2-acetamidoglucose with C=O at δ_C 173.4. The sugar sequence was determined from the ESI – mass fragmentation which showed fragment at *m/z* 1095 [1227-132 ($C_5H_8O_4$)], fragment at *m/z* 963 [1095-132 ($C_5H_8O_4$)] disclosing the sugar sequence may be xyl. (1→2) - ara - (1 → 6) -2-deoxy-2-acetamidoglucose-O-. ^{13}C NMR revealed C-2 position of arabinose and C-6 position of acetamidoglucose were shifted to 80.2 (d) and 69.5 (t) respectively which confirmed the above sugars sequence.

In addition to comparison with literature data [7]. Unfortunately the HMBC correlations were very few especially for sugars linkages, but the connection of these sugars with C-3 was very clear and confirmed from the downfield shift of C-3 signal at δ_C 90.1 instead of δ_C 78.1 of free OH group at position 3[18]. By careful inspection of ^1H NMR and ^{13}C NMR data it was noticed the presence of other four anomeric protons at δ_H 5.38 (1H, d, *J*=7.0 Hz), 5.30 (1H, br), 5.32 (1H, d, *J*=7.0 Hz) and 4.44 (1H, br) connected via HSQC to carbon signals at δ_C 95.3 (d), 101.4 (d), 111.1 (d) and 105.3 (d) respectively which with other NMR signals suggested the presence of glucose, arabinofuranose, rhamnose and 2-deoxy-2 acetamidoglucose as the second part of sugar moieties. Comparison of NMR data of these sugars with literatures [7, 9] we concluded that the linkages of these sugars with each other is branched chain of *α*-L-arabinofuranose, *α*-L-rhamnopyranose, 2-deoxy-2-acetamidoglucopyranose and *β*-D-glucospyranose and be in the same pattern reported by literature [7, 9] except the presence of terminal 2-deoxy-2-acetamidoglucose in compound 1 instead of glucose, the presence of 2-deoxy-2-acetamidoglucose was identified from the typical amide carbon at δ_C 56.9, with C=O at δ_C 173.3 and CH_3 at δ_C 23.1.

The branched sugars chain was suggested from the mass fragmentation pattern of compound 1 which showed the base peak at *m/z* 1227 [M^+-2-deoxy-2-acetamidoglucose-arabinose-H_2O]. ^{13}C NMR data of compound 1 revealed C-2 position of glucose in addition to C- 3 and C-4 of rhamnose were shifted to δ_C 80.1, 81.3 and 79.3 respectively which confirmed the connection of C-1 of glucose with the aglycon and C-2 with rhamnose which subsequently connected at their C-3 and C-4 with arabinofuranose and 2-deoxy-2-acetamidoglucoe respectively.

The position of the second sugar moieties at C-28 was confirmed also from the up field shift of C-28 at δ_C 177.3 instead of 180.1 for the free carboxylic group [18]. The presence of non occupied hydroxyl group at C-16 was confirmed from the mass fragment, at *m/z* 1227 which showed the loss of one molecule of water from the free OH at C-16 this confirmed also the position of the second sugar moieties at C-28. From the above data compound **1** was unambiguously identified as 3-*O*-*β*-D-xylopyranosyl-(1→2) - *α* – L - arabinopyranosyl-(1→6)- 2-deoxy-2-acetamido-*β*-D-glucopyranosyl-echinocystic acid -28-O-*β*-D-2-deoxy-2-acetamido-*β*-D-glucopyranosyl-(1→3)-[*α*-L-arabinofuranosyl-(1→4)]-*α*-L-rhamnopyranosyl-(1→2) – *β* - D-glucopyranosyl ester named as Lebbekanin **I** (**1**). This is the first repot about the isolation of compound **1** from the nature.

Compound 2: The positive ESI-MS spectrum showed a molecular ion peak at *m/z* 1761 [M+ H] $^+$ corresponding to the molecular formula $C_{80}H_{132}N_2O_{40}$. Investigation of ^{13}C & ^1H NMR data

of compound **2**, (**Table 2**), proved that compound **2** was also triterpenoid saponin and their data were identical to those of compound **1** except the presence of extra ^{1}H & ^{13}C NMR signals corresponding to one glucose moiety and one hydroxyl group **Table 2**. The downfield shift of C-21 and H-21 in compound **2** to δ_C 89.5 and δ_H 3.45 respectively instead of δ_C 36.4 and δ_H 1.80, 1.90 in compound **1** indicating that this carbon is hydroxyl-glucosidated one.

The above suggestion of presence of an extra glucose and OH group in compound **2** was established also from the ESI-MS spectrum of compound **2** which shows a molecular ion peak at *m/z* 1761 $[M+H]^+$ with 178 mass unit difference from that of compound **1**. Furthermore the mass fragmentation of compound **2** showed the first loss of glucose followed by loss of hydroxyl group through the fragments at *m/z* 1599 $[M-162\ (glu)]^+$ and fragment at *m/z* 1583 $[M-162\ (glu)-H_2O-H]^+$ reaching to the molecular weight of compound **1**. The third loss in mass spectrum was the loss of arabinose and one molecule of H_2O plus acetate group which is very clear in the fragment with 100% intensity at *m/z* 1387 [1583-($C_5H_8O_4$)-H_2O-$COCH_3$], another mass fragment at *m/z* 1226 $[1387\text{-aminoglu}–H]^+$ was detected. The fragment at *m/z* 962 showed the loss of two pentoses 962 [1226-arabinose-xylose].

The position of the extra glucose was established to be at C-21 from the downfield shift of C-21 to be at δ_C 89.5 instead of δ_C 36.4 in ^{13}C NMR spectrum. From the above data, compound **2** was identified as 3 - *O*- *β* – D - xylopyranosyl - (1→2) – *α* – L - arabinopyranosyl-(1→6)-2-deoxy-2- acetamido-*β*-D-glucopyranosyl-21-O - *β* – D - glucopyranosyl acacic acid -28-O-*β*-D-2-deoxy-2-acetamido-*β*-D-glucopyranosyl-(1→3) - [*α* - L - arabinofuranosyl-(1→4)]-*α*-L-rhamnopyranosyl - (1→2) – *β* - D-glucopyranosyl ester named as Lebbekanin **II**. This is the first isolation of Lebbekanin **II** from the nature.

TABLE 1: ^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND 1 IN CD_3OD

No	δ_C	δ_H	No	δ_C	δ_H
Aglycon					
1	37.9 t	1.80, 1.90 m	4	67.3 d	3.25 m
2	27.1 t	1.52, 1.54 m	5	64.3 t	3.60, 3.62 m
3	90.1 d	3.25 t	**xyl (1-- 2) ara**		
4	39.8 s	-	1	106.5 d	4.49 d (7.0 Hz)
5	55.0 d	0.78 m	2	75.7 d	3.75 m
6	19.3 t	1.38, 1.60 m	3	77.6 d	3.35 m
7	33.4 t	1.30, 1.58 m	4	70.6 d	3.72 m
8	40.0 s	-	5	67.2 t	3.88, 3.97 m
9	47.3 d	1.65 m	**sugars (C-28)**		
10	37.9 s	-	**Glu**		
11	23.1 t	1.85, 1.90 m	1	95.3 d	5.38 d (7.0 Hz)
12	123.6 d	5.32 br t	2	80.1 d	3.54 m
13	144.7 s	-	3	77.6 d	3.49 m
14	40.8 s	-	4	71.1 d	3.71 m
15	30.7 t	1.25 m	5	79.3 d	3.45 m
16	73.1 d	3.35 br t	6	62.5 t	3.65, 3.85 m
17	48.4 s	-	Rha (1-- 6) glu		
18	47.7 d	2.25 t (7.0 Hz)	1	101.4 d	5.30 br d
19	42.0 t	1.70 br d	2	69.1 d	3.52 m
20	31.3 s	-	3	81.3 d	3.72 m
21	36.4 t	1.80, 1.90 m	4	79.3 d	3.48 m
22	32.2 t	1.80, 1.90 m	5	68.5 d	3.40 m
23	28.5 q	1.06 s	6	17.7 q	1.36 d (7.0 Hz)
24	17.0 q	0.87 s	**ara (1-- 4) rha**		
25	16.1 q	0.87 s	1	111.1 d	5.32 br d
26	17.1 q	1.25 s	2	82.5 d	3.62 m
27	27.3 q	1.37 s	3	78.1 d	3.48 m
28	177.2 s	-	4	83.5 d	3.75 m
29	33.3 q	0.96 s	5	64.2 t	3.58, 3.62 m
30	24.6 q	1.06 s	**glu-2-NHAc**		
			(1 -- 3) rha		
sugars (C-3)			1	105.3 d	4.44 br d
glu-2-NHAc			2	56.9 d	3.65 m
1	104.8 d	4.48 d (7.0 Hz)	3	77.0 d	3.35 m

2	57.8 d	3.65 m	4	71.1 d	3.51 m
3	75.7 d	4.0 m	5	78.2 d	3.53 m
4	72.3 d	3.6 m	6	62.1 t	3.71, 3.78 m
5	76.7 d	3.44 m	C=O	173.3 s	-
6	69.5 t	4.05, 3.71 m	$COCH_3$	23.1 q	1.95 s
C=O	173.4 s				
$COCH_3$	23.1 q	1.95 s			
ara (1-6) glu					
1	103.3 d	4.52 d (4.0 Hz)			
2	80.2 d	3.55 m			
3	74.7 d	3.54 m			

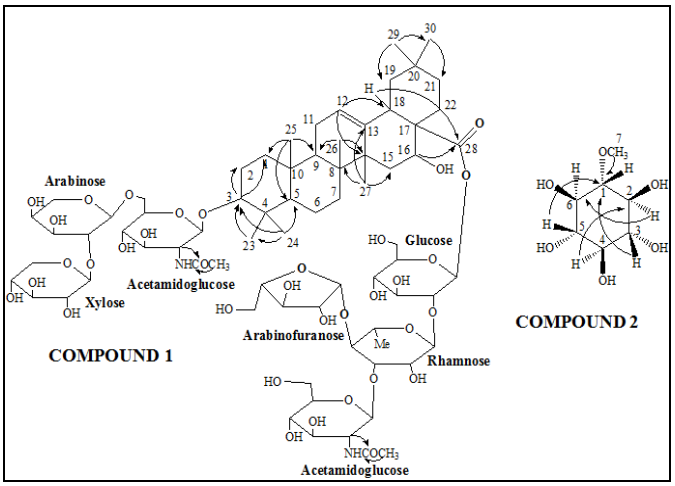


FIG. 2: IMPORTANT HMBC CORRELATIONS OF COMPOUNDS 1 AND 3

Compound 3: (**Fig. 1)** was obtained as white crystals, melting at 184-186ºC. The IR spectrum showed the absorption bands at 3405 & 3320 (OH), 2950 (H-C) and 1279 (C-O) cm^{-1}. The EI-MS showed the $[M^+ - OCH_3]^+$ peak at *m/z* 164 which is consistent with a molecular formula $C_7H_{14}O_6$. The broad band ^{13}C NMR and DEPT spectra of compound **3** showed six methine and one methyl carbon atoms. All the carbon signals showed downfield shifts due to their attachment to oxygen atom. The ^1H NMR spectrum showed methoxyl protons as singlet at δ_H 3.44 (3H, s) and six oxymethine protons in the range of δ_H 2.98 to 3.62. Since the molecular formula showed one double bond equivalent therefore compound 3 must be monocyclic.

Inositols have nine theoretically possible isomers among which seven are named as myo-, D-chiro, L-chiro-, neo-, muco-, scyllo- and allo-forms have previously been reported. Theoretically, 20 isomeric mono-*O*-methyl ethers of inositol are possible [19]. These isomers have distinct ^1H NMR spectra and characteristic coupling constant. In the 20 possible isomers of mono-*O*-methylated inositols *muco*-, *allo*- and *cis*-isomers have three axial and equatorial oriented hydroxyl or methoxyl groups. However, in *muco* form the three axial oriented substituents are adjacent to each other. On the other hand, in *allo* form the axial two oriented substituents are adjacent to each while the third axial substituent is next to the adjacent position i.e. 1, 2-4-disposition. Lastly, *cis* form has alternate axial and equatorial substituents.

In ^1H NMR spectrum of compound 3, there were clearly three axial and three equatorially oriented protons and methoxyl group. The most upfield oxymethine protons at δ_H 3.00 could be assigned to the axially oriented proton vicinal to the methoxyl group. The signal was observed a broad triplet (*J* = 9.2 Hz). The large coupling constant allowed us to assigned axial orientation to the oxymethine protons at C-2 and C-6, respectively. The H-6 signal observed as doublet of triplet at δ_H 3.34 (*J* = 4.4 Hz) and H-2 as broad singlet at δ_H 3.50. HSQC showed connectivity of C-3 with proton at δ_H 3.50 (d, *J*= 8.0 Hz), C-4 with proton at δ_H 3.62 (brs) and C-5 with proton at δ_H 3.48 (d, *J*= 8.0 Hz). From the relative coupling constant, it was evident that compound **3** has *cis*-inositol configuration. This could further be confirmed by ^1H-^1H COSY & HMBC correlations between all the oxymethine protons and carbons.

On the basis of these cumulative evidences, the structure of compound **3** could be assigned as 1-*O*-methyl- inositol. D-pinitol (D-3-*O*-methyl-chiro-inositol) was reported to be isolated before from genus Albizia [20] and (D-1-*O*-methyl-*cis*- inositol) is a new isomer and it is the first report about the isolation of this compound from Albizia species.

Results of Biological Activities:

Cytotoxic activity: The results of cytotoxic activity of a fraction containing mixture of

compounds **1** and **2** obtained from the butanol extract of *A. lebbeck* flowers against HePG-2 (Hepatocarcinoma), HEP-2 (Larynx carcinoma), HELA (Cervical carcinoma), MCF-7 (Breast carcinoma) and HCT-11 6 (Colon carcinoma) cell lines were reported in **Table 4**. It showed that the sample exhibited cytotoxic activity against all tested cell lines with LD_{50} values of 1.74, 3.42, 4.29, 0.65 and 0.74 µg respectively which is stronger than that reported for the bark extract of *A. lebbeck* [21, 22].

TABLE 2: ^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND 2 IN CD_3OD

No	δ_C	δ_H	No	δ_C	δ_H
Aglycon					
1	37.9 t	1.80, 1.90 m	4	67.2 d	3.25 m
2	27.1 t	1.52, 1.54 m	5	65.5 t	3.50, 3.82 m
3	90.5 d	3.18 br t	**xyl (1-- 2) ara**		
4	39.8 s	-	1	106.7 d	4.49 br d
5	55.0 d		2	75.7 d	3.75 m
6	19.1 t	1.38, 1.60 m	3	77.6 d	3.35 m
7	33.4 t	1.30, 1.58 m	4	70.3 d	3.72 m
8	40.0 s	-	5	67.5 t	3.25, 3.97 m
9	47.8 d	1.65 m	**sugars (C-28)**		
10	37.8 s	-	**Glu**		
11	23.1 t	1.85, 1.90 m	1	95.3 d	5.38 d (6.3 Hz)
12	123.7 d	5.32 br t	2	80.2 d	3.54 m
13	144.7 s	-	3	79.3 d	3.49 m
14	40.8 s	-	4	71.1 d	3.71 m
15	31.3 t	1.25	5	77.6 d	3.45 m
16	73.1 d	3.35 br t	6	63.3.4 t	3.73, 4.40 m
17	48.1 s	-	**Rha (1-- 6) glu**		
18	47.7 d	2.25 m	1	101.3 d	5.30 br d
19	42.1 t	1.70 m	2	77.1 d	3.52 m
20	31.3 s	-	3	81.8 d	3.72 m
21	89.5 d	3.45 br t	4	80.1 d	3.75 m
22	32.1 t	1.80, 1.90 m	5	68.9 d	3.73 m
23	28.6 q	1.29 s	6	17.8 q	0.95 d (7.0 Hz)
24	17.1 q	1.13 s	**Ara (1-- 3) rha**		
25	16.1 q	0.87 s	1	111.1 d	5.32 d (3.9 Hz)
26	17.8 q	1.25 s	2	82.5 d	3.62 m
27	27.4 q	1.37 s	3	78.1 d	3.48 m
28	176.9 s	-	4	84.2 d	3.75 m
29	33.3 q	0.87 s	5	66.2 t	3.58, 3.62 m
30	24.5 q	0.96 s	**glu-2-NHAc (1 -- 4) rha**		
sugars (C-3) glu-2-NHAc			1	106.5 d	4.44 br d
			2	56.9 d	3.65 m
1	104.8 d	4.48 d (7.0 Hz)	3	77.0 d	3.35 m
2	57.8 d	3.65 m	4	71.0 d	3.51 m
3	75.7 d	4.00 m	5	78.2 d	3.53 m
4	72.3 d	3.60 m	6	69.6 t	3.71, 3.78 m
5	76.9 d	3.44 m	C=O	173.3 s	-
6	69.5 t	4.40, 3.75 m	$COCH_3$	23.1 q	1.95 s
C=O	173.4 s	-	**sugar at C-21 glu**		
$COCH_3$	23.1 q	1.95 s	1	101.5	4.56 d (7.2 H)
ara (1-6) glu			2	75.5	3.27 m
1	103.3	4.52 d (4.0 Hz)	3	78	3.92 m
2	80.2	3.55 m	4	72.5	3.61 m
3	74.7	3.54 m	5	78.1	3.37 m
			6	63.0	3.74 , 3.60.m

TABLE 3: ^{1}H AND ^{13}C NMR SPECTRAL DATA OF COMPOUND 3 IN DMSO-D_6

No.	δ_H (*J*= Hz)	δ_C	No.	δ_H (*J*= Hz)	δ_C
1	2.98-3.02 (1H, t, *J*=9.2 Hz)	84.24	7	3.44 (3H, brs)	60.12
2	3.48-3.50 (1H, d, *J*=8.0 Hz)	71.43	C_2-OH	4.51 (d, *J*=9.8 Hz)	
3	3.50-3.52 (1H, d, *J*=8.0 Hz)	70.52	C_3-OH	4.63 (d, *J*= 1.6 Hz)	
4	3.62 (1H, br s)	72.42	C_4-OH	4.35 (d, *J*= 5.6 Hz)	
5	3.62 (1H, br s)	72.86	C_5-OH	4.72 (d, *J*= 1.6 Hz)	
6	3.33-3.34 (1H, dt, *J*= 4.4 Hz)	73.04	C_6-OH	4.52 (d, *J*= 4.4 Hz)	

TABLE 4: RESULTS OF CYTOTOXIC ACTIVITY AND LD_{50} IN µG OF FRACTION CONTAINING MIXTURE OF COMPOUNDS 1 AND 2 OBTAINED FROM THE BUTANOL FRACTION OF *A.LEBBECK* FLOWERS AGAINST DIFFERENT CELL LINES

Sample conc. (in µg)	% Viability for sample against different cell lines				
	MCF-7	HepG-2 cell	HCT-116	HEP-2	HELA cell
50	8.54	12.62	10.37	15.86	14.19
25	11.68	17.45	14.29	26.14	23.72
12.5	15.93	25.94	17.85	34.52	31.45
6.25	20.36	32.81	21.43	42.91	45.16
3.125	27.66	39.65	30.72	50.73	52.87
1.56	36.94	51.38	38.21	61.66	60.93
0.78	47.06	63.26	49.03	72.09	69.74
0.39	55.92	70.91	58.49	79.25	81.38
0	100	100	100	100	100
LD_{50} in µg	0.65	1.74	0.74	3.42	4.29

Antimicrobial activity:

The fraction containing mixture of compounds **1** and **2** exhibited (as shown in **Table 5**) strong or moderate activity against all tested microorganisms except *Candida albican* it showed no activity. It is notable that it exhibited antifungal activity against *Syncephalastrum racemosum* stronger than that of Amphotericin B, nearly the same effect of Gentamicin against *Escherichia coli* and stronger than that reported for the leaf extract of *A. lebbeck*[23].

TABLE 5: RESULTS OF ANTIMICROBIAL ACTIVITY OF FRACTION CONTAINING MIXTURE OF COMPOUNDS 1 AND 2 OBTAINED FROM THE BUTANOL FRACTION OF *A.LEBBECK* FLOWERS

Tested organisms	Fraction containing mixture of compounds 1 and 2	Standard
Fungi		**Amphotericin B**
Asperagillus fumigatus (RCMB O2568)	21.3 ± 0.72	23.7 ± 0.1
Syncephalastrum racemosum (RCMB 05922)	21.9 ± 0.63	19.7 ± 0.2
Geotricum candidum (RCMB 05097)	22.3 ± 0.58	28.7 ± 0.2
Candia albicans (RCMB 05036)	NA	25.4 ± 0.1
Gram Positive bacteria		**Ampicillin**
Streptococcus pneumonia (RCMB 010010)	22.2 ± 1.2	23.8 ± 0.2
Bacillis subtilis (RCMB 010067)	24.3± 0.72	32.4 ± 0.3
Gram Negative bacteria		**Gentamycin**
Pseudomonus aeruuginosa (RCMB 010043)	16.3 ± 0.58	17.3 ± 0.1
Escherichia coli (RCMB 010052)	19.8 ± 0.72	19.9 ± 0.3

NA= no activity, Conc. of standards and sample (500 µg/ml)

CONCLUSION: The phytochemical study of butanol fraction of *Albizia lebbeck* (L.) Benth flowers was carried out and led to isolation and characterization of three new compounds, lebbeckanin I (1), lebbeckanin II (2), and a new isomer of methyl inositol, D-1-*O*-methyl-*cis*-inositol (3), by using different spectroscopic methods. Furthermore, the biological activity of fraction containing mixture of compounds 1 and 2 exhibited strong cytotoxic and antimicrobial activity, therefore it could be considered as a promising source for new cytotoxic and antimicrobial drugs.

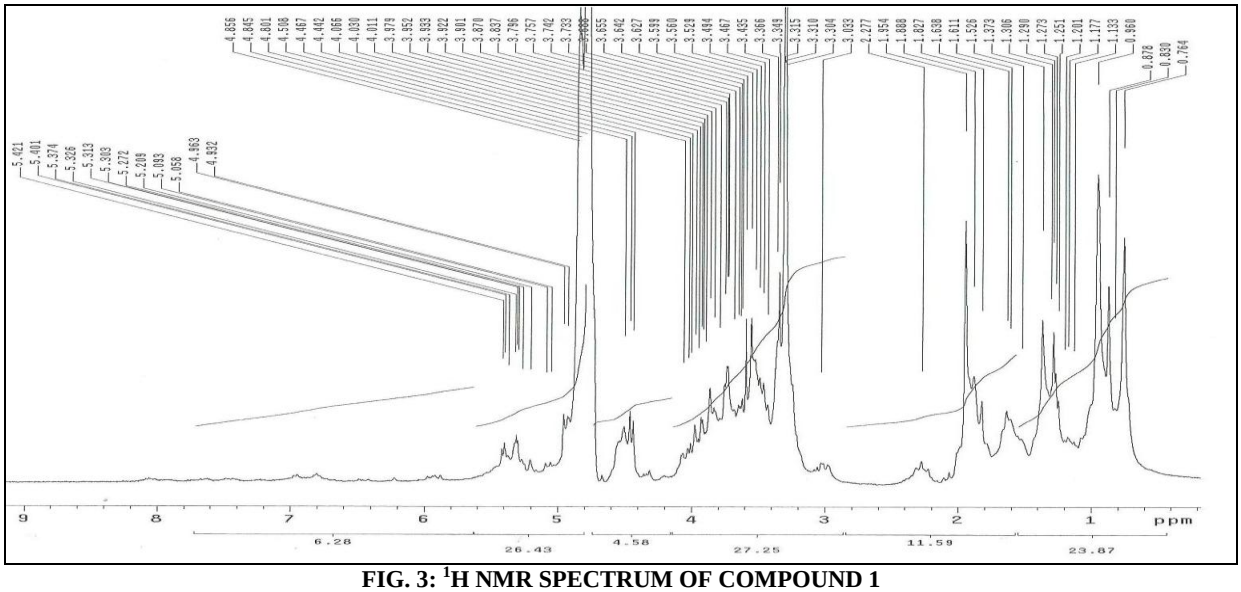


FIG. 3: ^{1}H NMR SPECTRUM OF COMPOUND 1

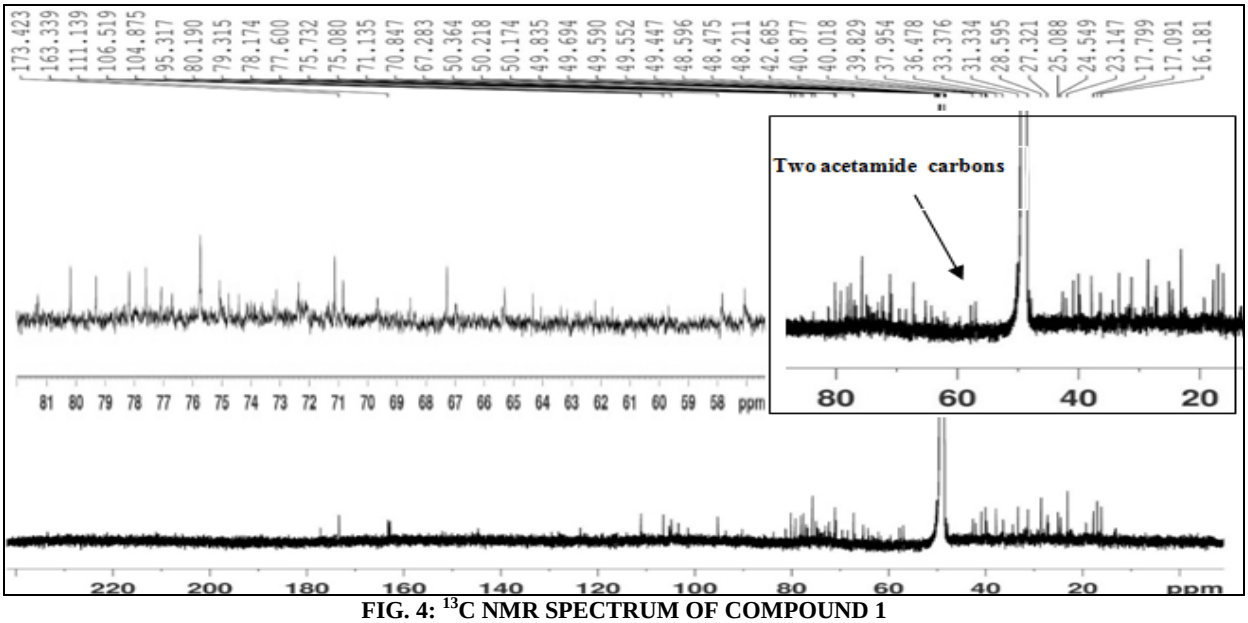


FIG. 4: ^{13}C NMR SPECTRUM OF COMPOUND 1

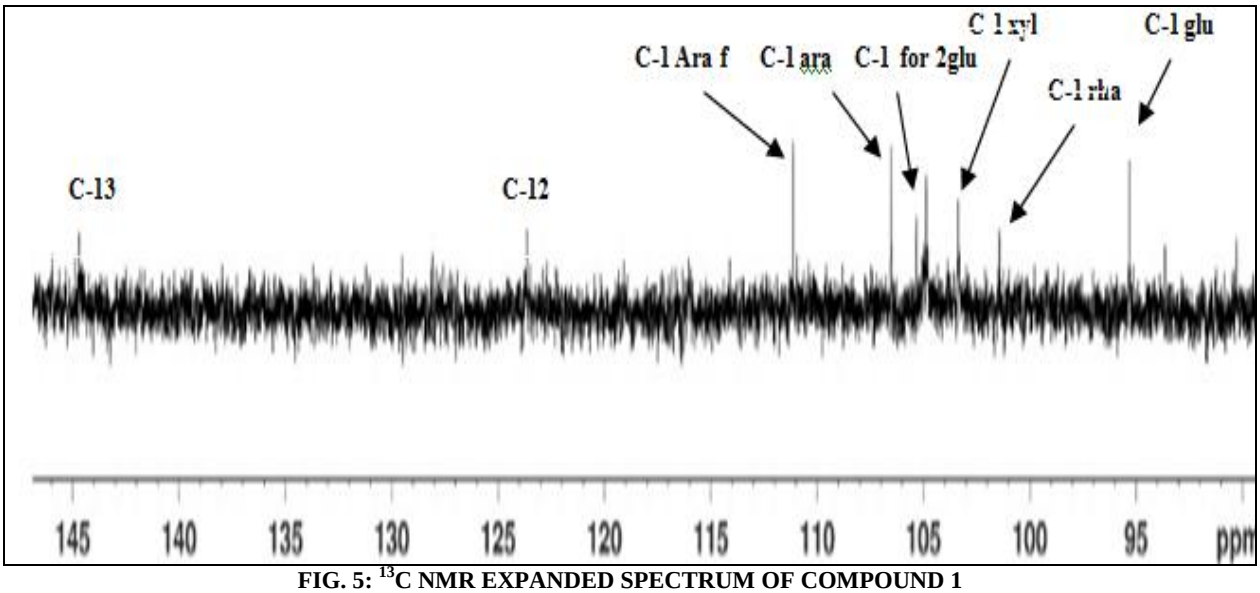


FIG. 5: ^{13}C NMR EXPANDED SPECTRUM OF COMPOUND 1

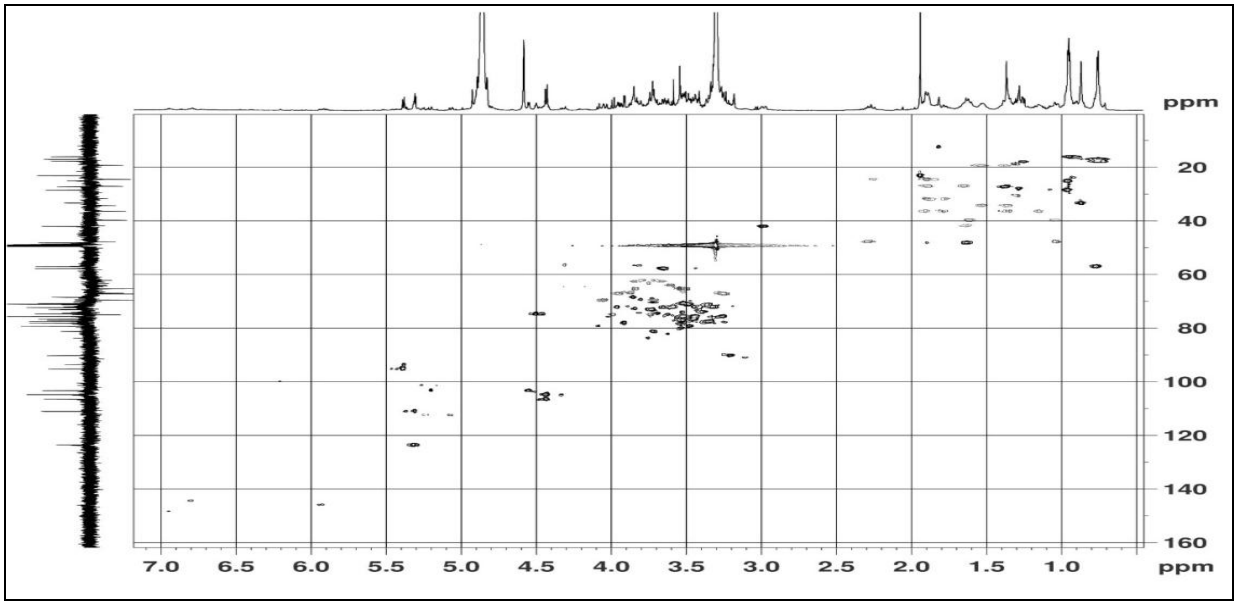


FIG. 6: HMQC SPECTRUM OF COMPOUND 1

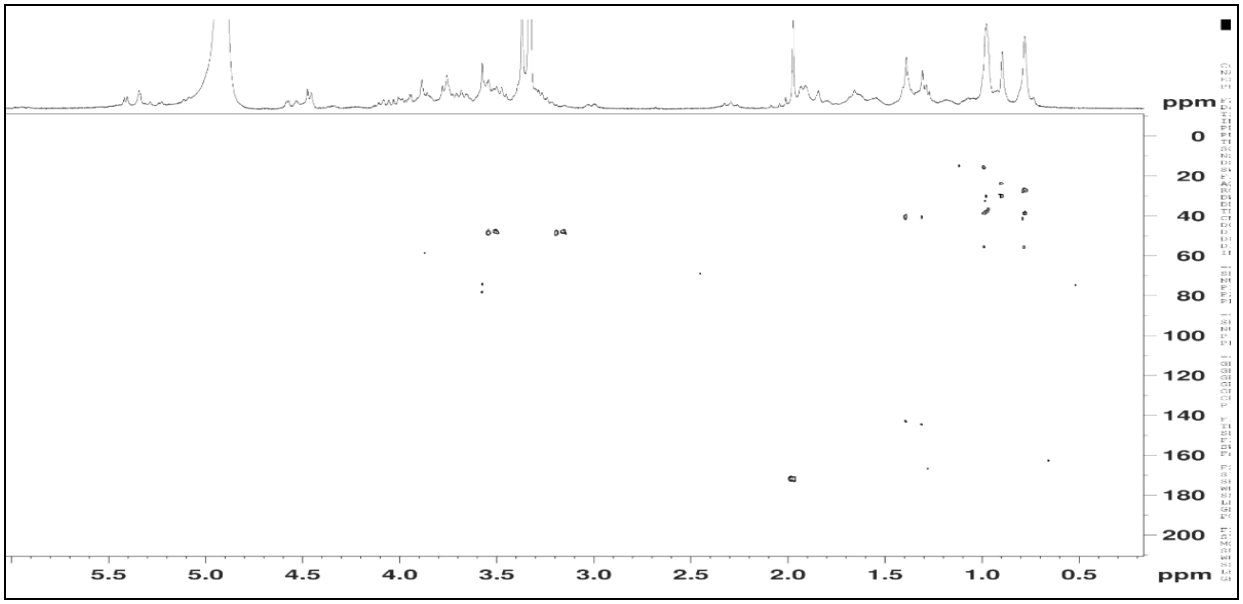
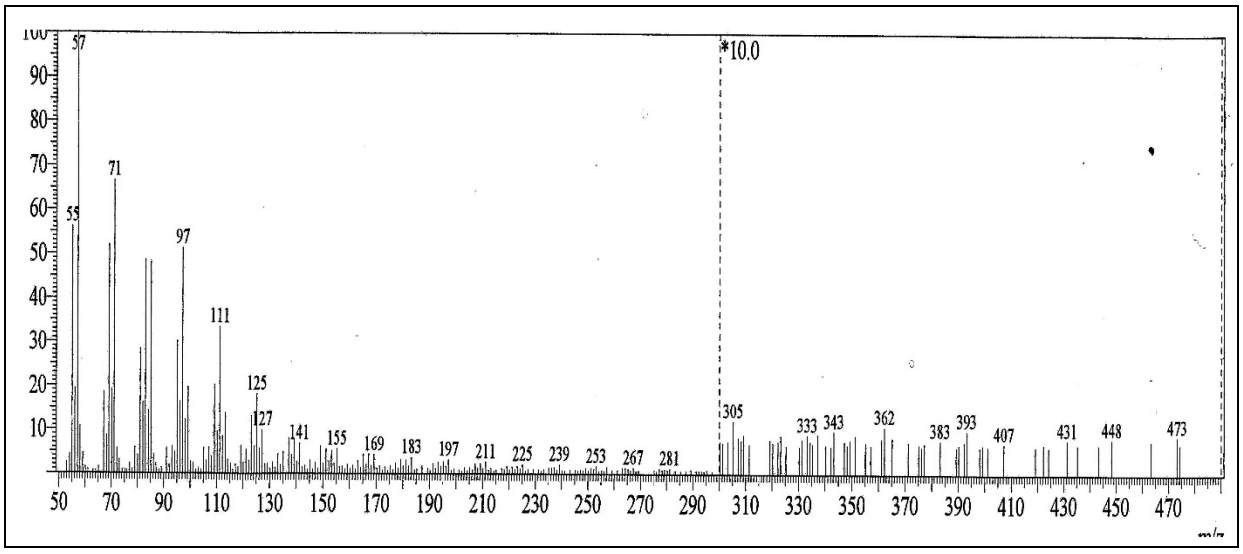


FIG. 7: HMBC SPECTRUM OF COMPOUND 1



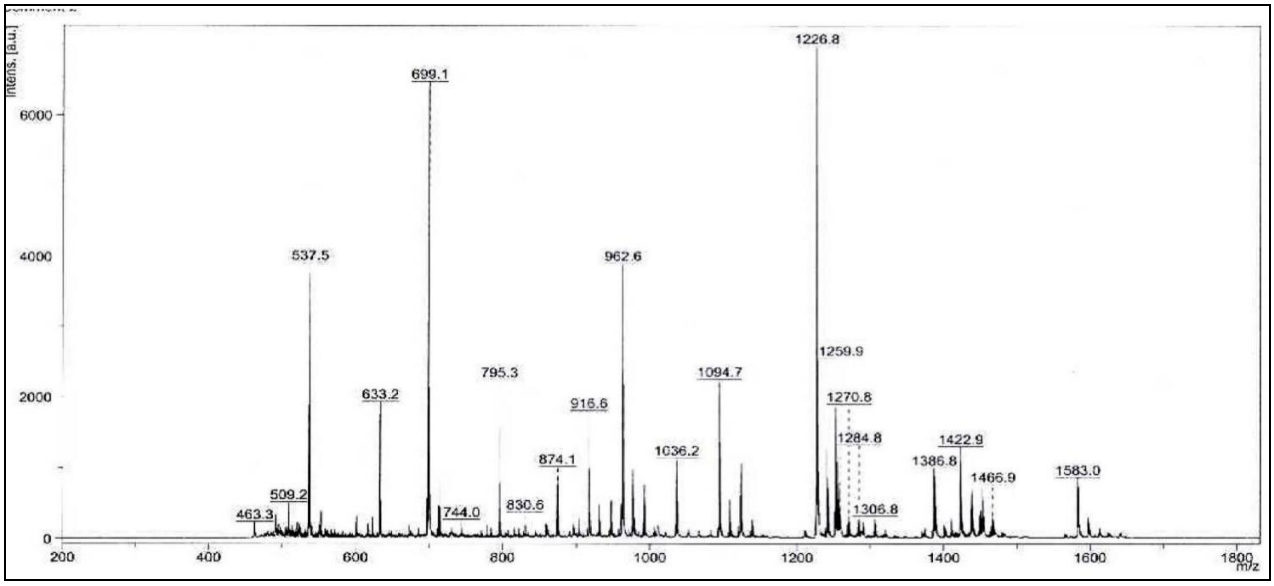


FIG. 8: EIMS (A) AND ESI MS (B) SPECTRA OF COMPOUND 1

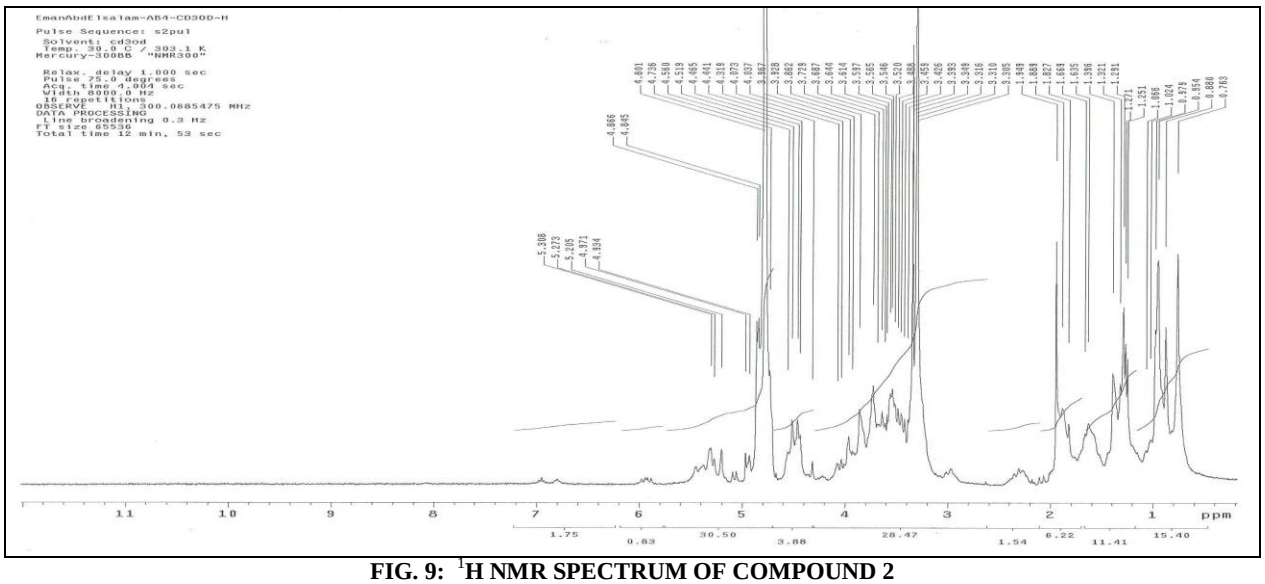


FIG. 9: ^{1}H NMR SPECTRUM OF COMPOUND 2

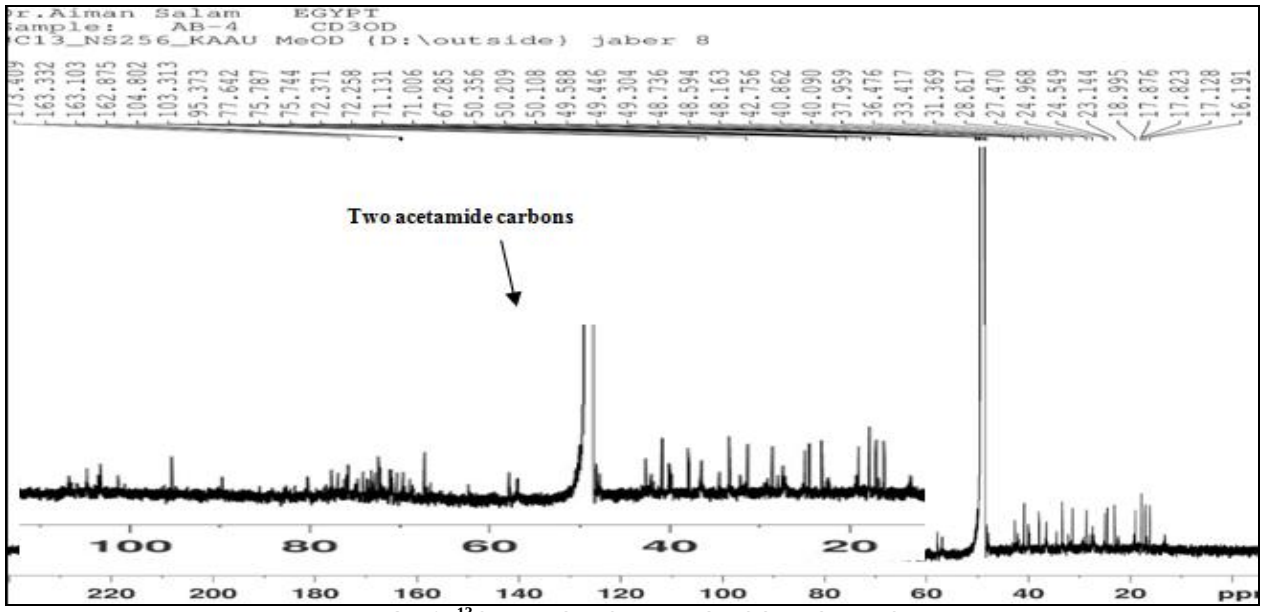


FIG. 10: ^{13}C NMR SPECTRUM OF COMPOUND 2

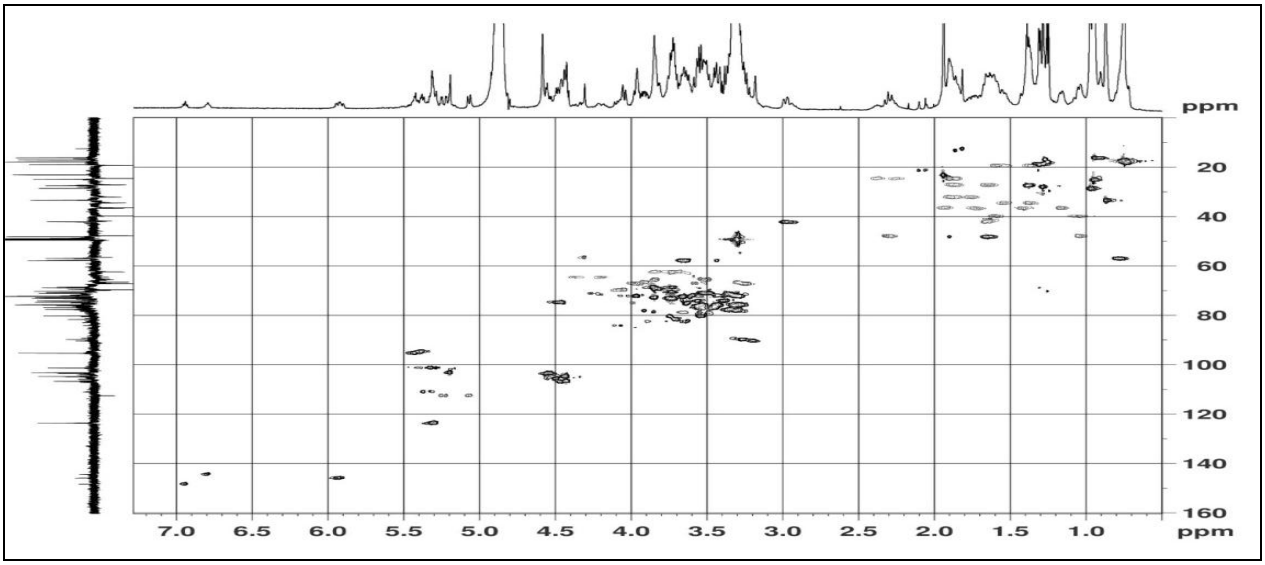


FIG. 11: HSQC SPECTRUM OF COMPOUND 2

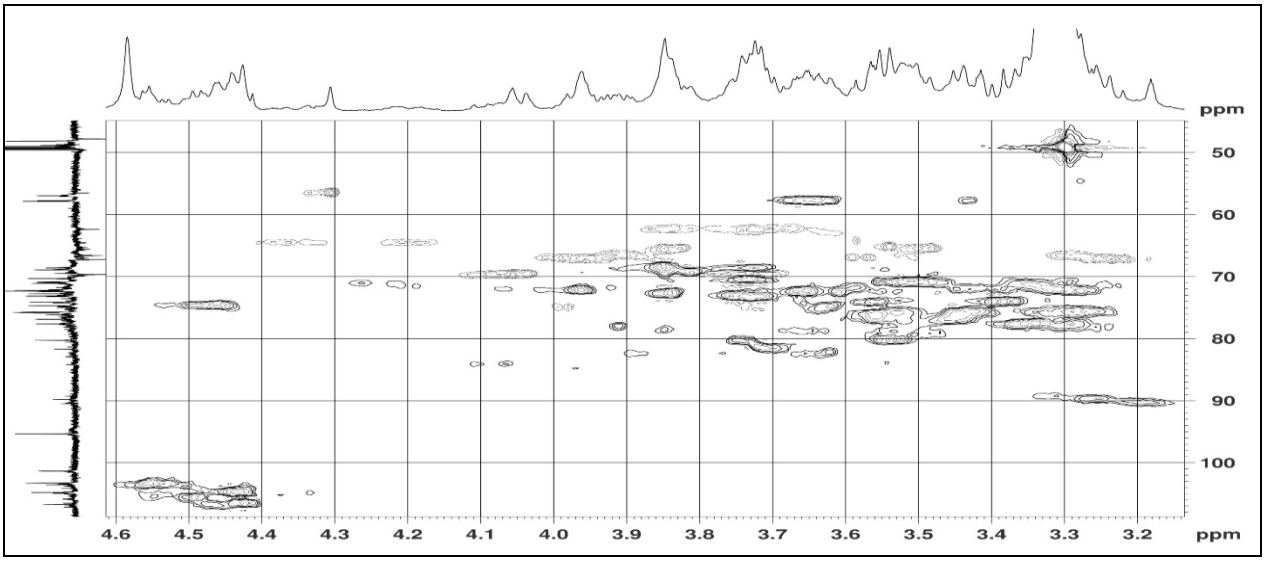


FIG. 12: HSQC SPECTRUM OF COMPOUND 2

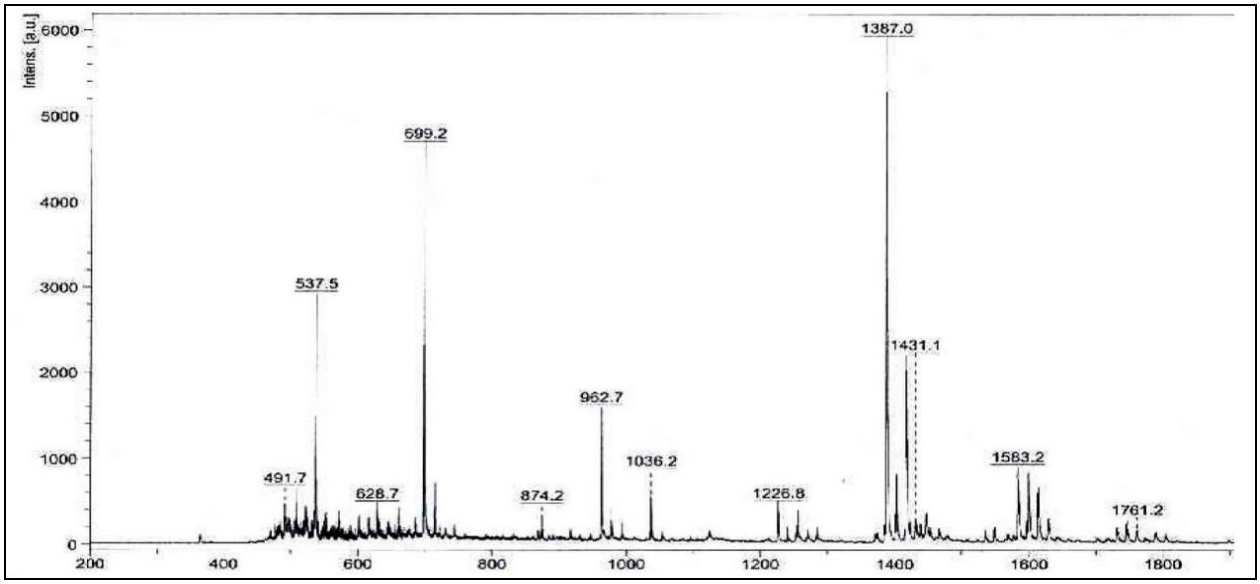


FIG. 13: ESI MS SPECTRUM OF COMPOUND 2

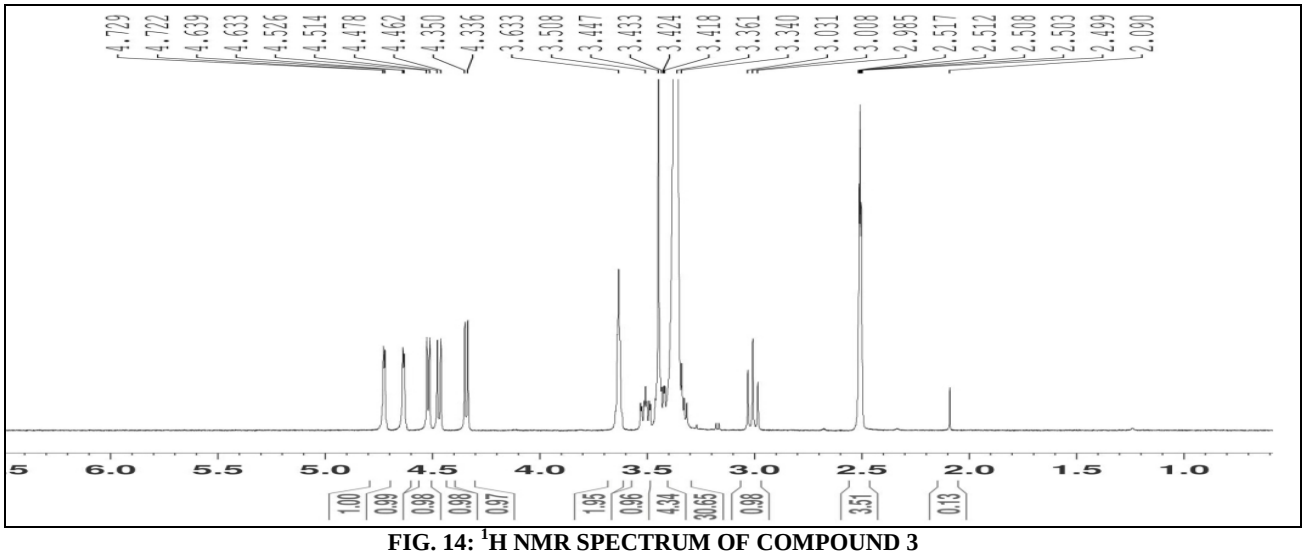


FIG. 14: ^{1}H NMR SPECTRUM OF COMPOUND 3

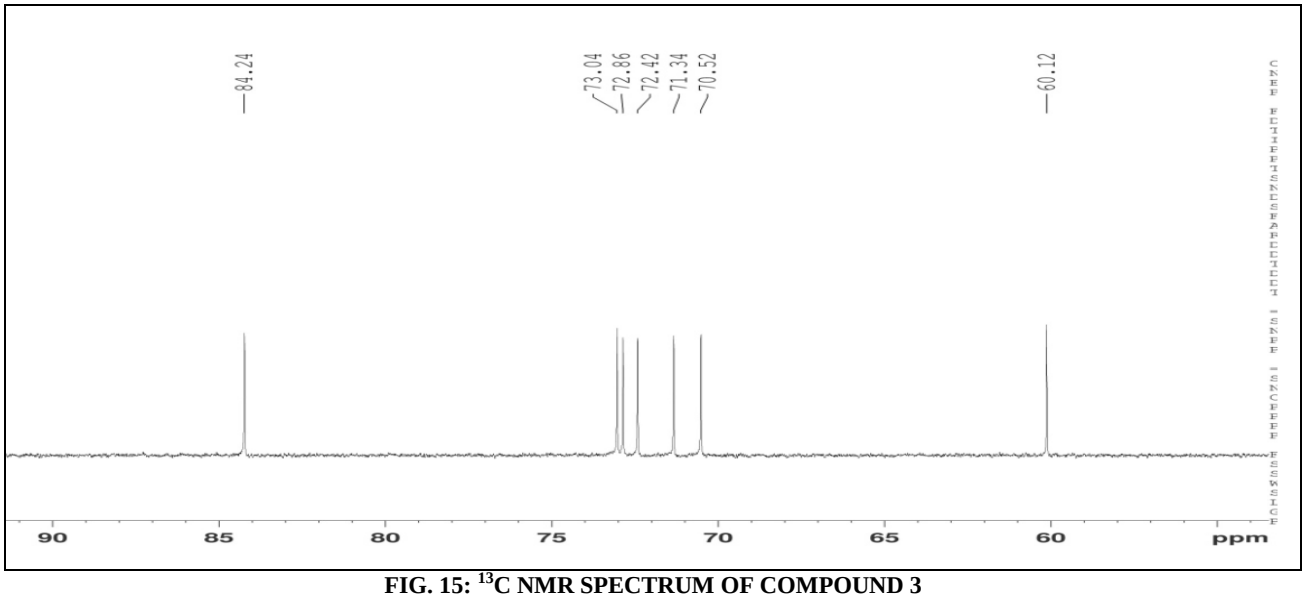


FIG. 15: ^{13}C NMR SPECTRUM OF COMPOUND 3

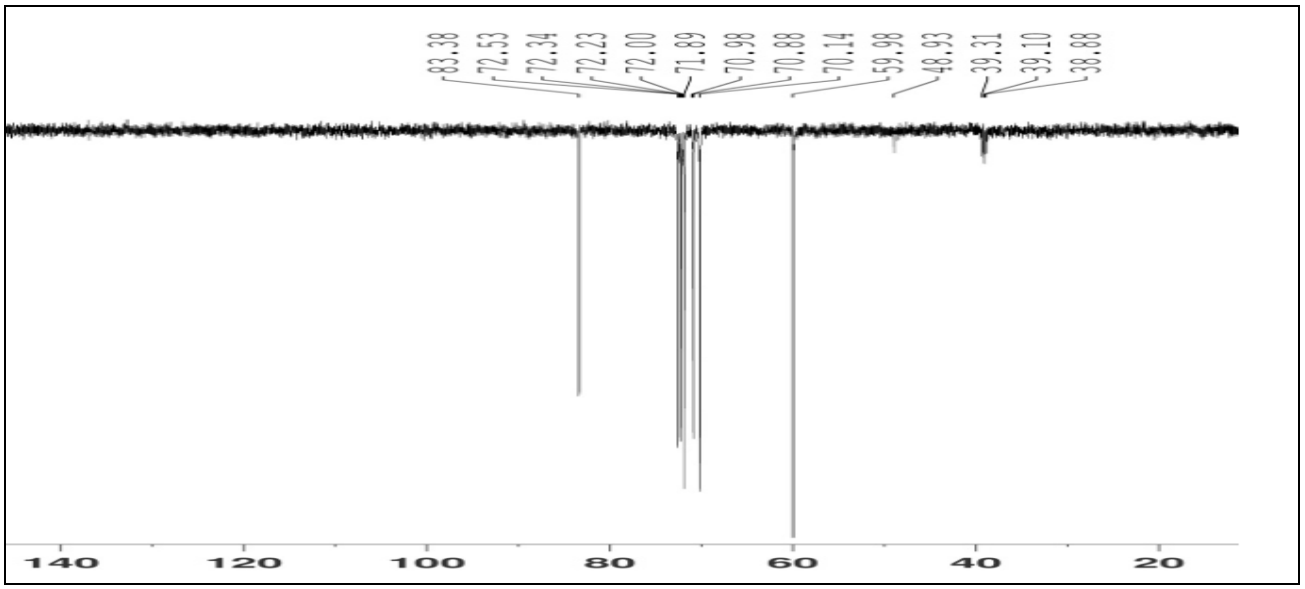


FIG. 16: DEPT SPECTRUM OF COMPOUND 3

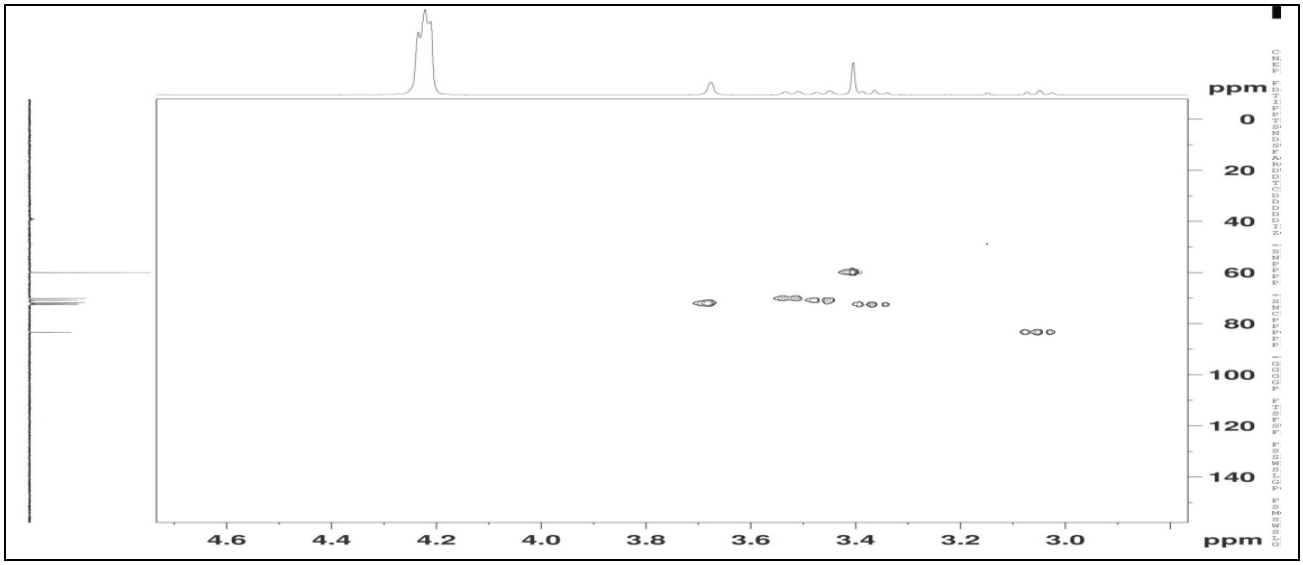


FIG.17: HSQC SPECTRUM OF COMPOUND 3

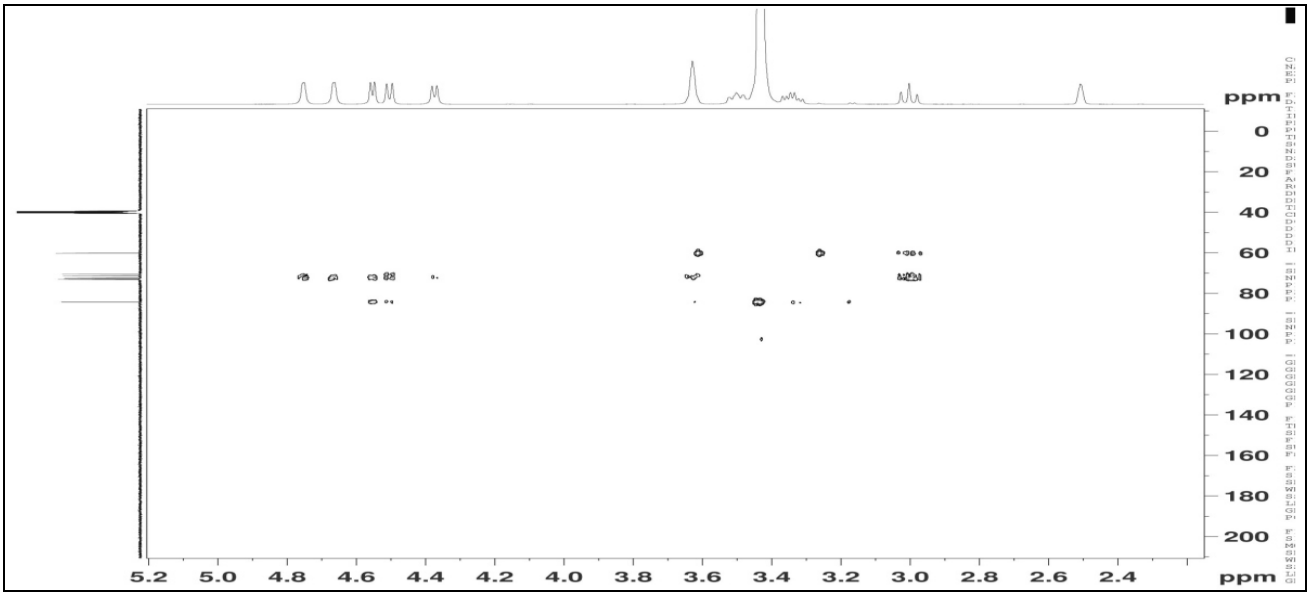


FIG. 18: HMBC SPECTRUM OF COMPOUND 3

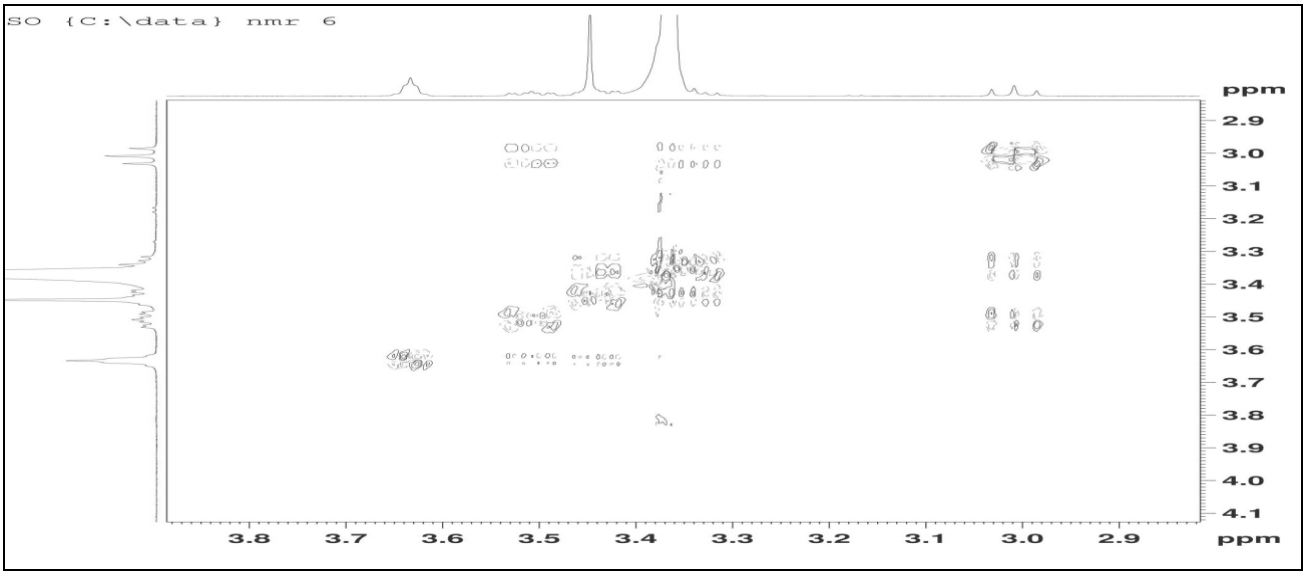


FIG. 19: COSY SPECTRUM OF COMPOUND 3

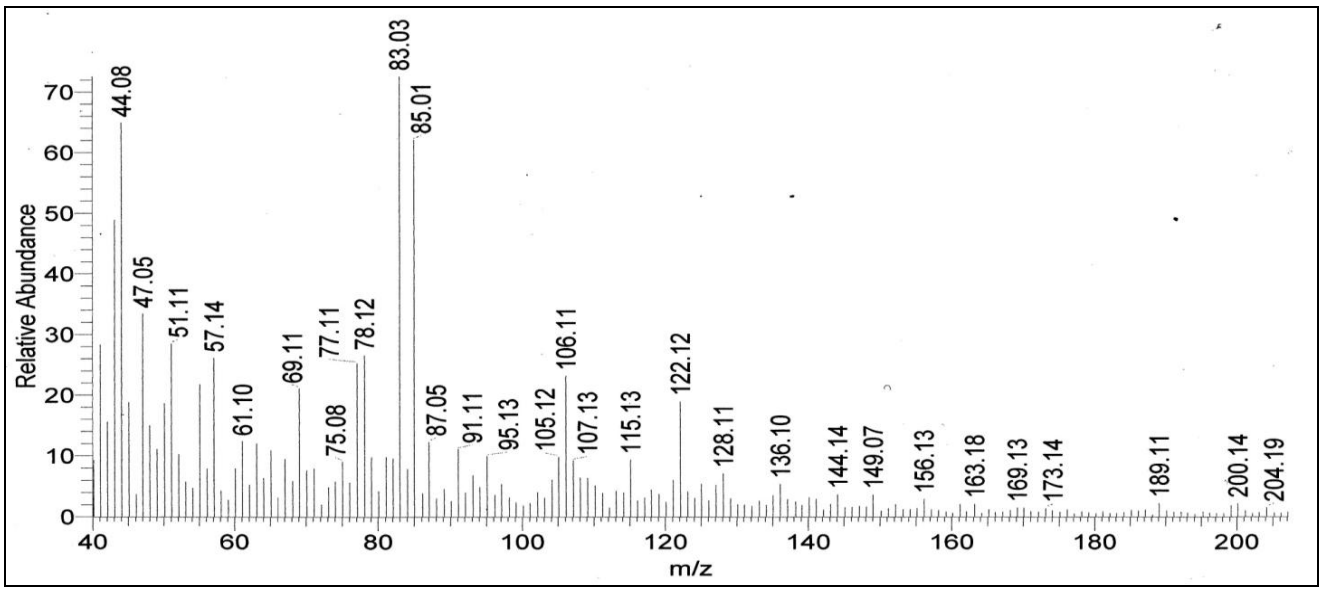


FIG. 20: EI MASS SPECTRUM OF COMPOUND 3

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CONFLICTS OF INTERREST: None.

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