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Bio-assay guided isolation of bioactive molecules from chloroform extract of fruits of *Lantana camara* (L.)

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Abstract
Current progress in drug discovery research and search for new novel molecules have intensified the efforts for exploring bioactive lead molecule from herbal and traditional system of medicine around all parts of world. The present resaerch highlights about bioassay guided isolation of active phytochemical from bioactive chloroform crude extract of fruits of *Lantana camara* (L.). Column chromatography separation of chloroform extract leads to the isolation of LC-1 to LC-3 active molecules. The structure of active lead molecules was confirmed with help of IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, DEPT-95 spectral techniques. Further antimicrobial assay studies were carried out by using *Pseudomonas aeruginosa, Salmonella thyphimurium, Staphylococcus aureus* and *Escherichia coli* by disc diffusion method found that LC-2 & LC-3 is promisingly active molecule.

Keywords: *Lantana camara* (L.), column chromatography, spectral technique, antimicrobial activity

Introduction
The potential role of medicinal plants for betterment of human life is since from ancient time, as they represent a formidable reservoir of potentially useful bioactive leads for new medicines [1, 2]. This plant-based, traditional medicine system continue to play an essential role in health care [3], with about 80% of the worlds inhabitants relying mainly on traditional medicines for their primary health care [4, 5]. *Lantana camara* (L.), Family: Verbenaceae has been important coniferous plant in ayurvedic and indigenous medicinal systems [6, 7], it is an evergreen plant is widely distributed in all parts of the world and well known as medicinal plant[8] since from ancient times people used for curing fever, cold, cough and other body ailments and they were supported by scientific data's [9]. In last few decades, scientist and researchers around the globe have elaborately studied the chemical composition of whole plant of L. camara as well as biological pharmacological activities [10, 11]. In Ethiopia medicinal plant play major supplementary roles to the limited modern health care available. The rich traditional folklore knowledge of the people led to the application of plants for supplement, medicine and other uses. Ethiopian plants have shown very effective medicinal value for ailments of human and domestic animals thus medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care in the country [12, 13].
The vernacular name of *L. camara* (L.) in Ethiopia is 'Yewof-qolo' (in Amharic) and 'Midhandubara' (in Afan Oromo) found all over Ethiopia as major weed in agricultural areas mostly on fertile sandy and light clay soil. Fruits are edible, the fresh and ripe one is eaten by children in dry area. In northern region south Gonder and southern region wonago district it is the most important traditional medicine in treating diarrhea [14, 15]. When eaten in excess of tolerable amounts it is also perceived as poisonous while edible by children and currently by adults [16].

Phytochemical constituents of *L. camara* (L.): Based on the literature survey of *L.camara* (L.), gave insight about previously isolated compounds as triterpenoids, flavonoids, iridoids, phenyl propanoids, glycosides and verbascoside [17, 18]. Phytochemical screening revealed that leaf [19], stem [20, 21] and root [22] contains tannin, catachin, saponin, steroids, alkaloids, phenol, anthroquinone, protein, alkaloids, glycosides and reducing sugar, mainly responsible for diverse biological activities [23-25] and include different types of the essential oil been reported [26-28]. The toxic nature of unripe fruit of *L.camara* (L.) species is due to a series of pentacyclic triterpenes, of which lantadenes A and B [29-31].

Materials and Methods

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker NMR spectrometer. The chemical shifts were given in ppm (δ) and were referenced relative to $CDCl_3$ (δ 7.28 and 76-77 ppm for ^{1}H and ^{13}C-NMR respectively) and the chemical shifts were expressed in (ppm) values with TMS as an internal reference. The IR spectra were recorded on a Bruker FT-IR spectrometer. Structural elucidation was done by comparing the values with the reported data in the literature.

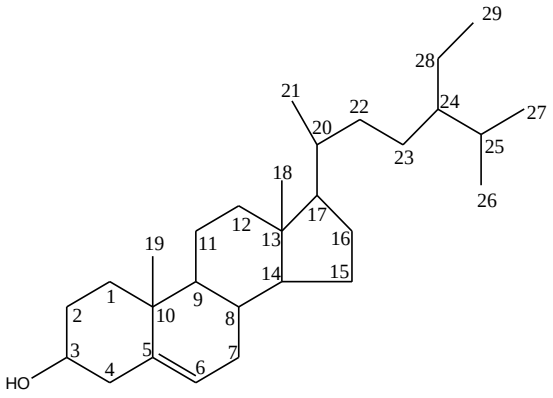
Isolation and characterization of compounds

Dried and powdered fruit material of *Lantana camara* (L.) was subjected to sequential solvent extraction with petroleum ether, chloroform, acetone, and methanol by using maceration technique and antibacterial studies was carried out by using *invitro* method. We encouraged from the previous results [32], bioactive chloroform extract of fruits of *L.camara* (L.) was subjected to column chromatography to isolate the active lead molecule. A glass column was packed with 100 g activated silica gel (60-120 mesh) slurry dissolved in petroleum ether and the crude extract was adsorbed on to dry silica gel. Then the solvent was allowed to evaporate, and the dry sample was applied into the column that was already packed with silica gel. Ideal solvent system for elution of the column was determined from TLC analysis of the extracts in various combinations of petroleum ether, chloroform, ethyl acetate, acetone and methanol, among them petroleum ether: ethyl acetate show spots with clear separation. The column was eluted with 100% petroleum ether and polarity slowly increased using the ratio of ethyl acetate. The developed spots on TLC plates were visualized under UV light at 254 and 365 nm and then by exposure to iodine vapor. The fractions that showed the same TLC development profiles (color and R_f) were combined and concentrated to dryness under reduced pressure using rotary evaporator. The isolated bioactive pure compounds were then characterized by the various spectral techniques namely, IR, ^{1}HNMR, ^{13}CNMR, DEPT-135 as well as comparison of these data with data in literature. All spectroscopic analysis were carried out at Department of chemistry, Addis Ababa University.

Literature survey also revealed that β-sitosterol has been isolated from previously the different parts such as leaves and stems of other plants [33, 34], in addition to this a wide variety of plants and foods such as milk, butter, and meats are found to be the main sources of natural di-(2-ethylhexyl) phthalate and other phthalates [35, 36]. The structure of the compounds (LC-1, LC-2 and LC-3) those were isolated from chloroform extract of *Lantana camara* were discussed below.

Table 1: ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of LC-1 with reported data of [β-sitosterol] [37] *Data from Behnam M. 2014

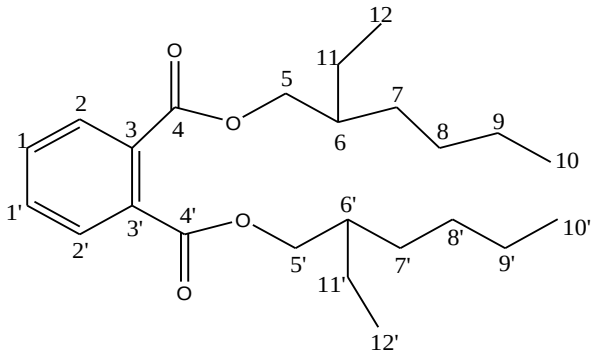
Position	^{13}C-NMR data of LC-1	Reported data of β-sitosterol*	DEPT-135 of LC-1	^{1}H-NMR data of LC-1	Reported ^{1}H-NMR data of β-sitosterol* (important values)	Nature of carbon
1	37.25	37.3	37.25			CH_2
2	31.53	31.7	31.59			CH_2
3	71.87	71.8	71.87	3.55	3.53	CH
4	42.23	42.2	42.22			CH_2
5	140.72	140.8				C
6	121.76	121.7	121.77	3.56	5.36	CH
7	31.60	31.8	31.59			CH_2
8	31.92	31.9	31.93			CH
9	50.13	50.1	50.13			CH
10	36.52	36.5				CH_2
11	21.10	21.1	21.09			CH_2
12	39.78	39.8	39.77			CH_2
13	42.34	42.3				C
14	56.77	56.9	56.77			CH
15	24.32	24.4	24.32			CH_2
16	28.27	28.2	28.27			CH_2
17	56.07	56.1	56.05			CH
18	11.88	11.9	11.88	0.69	0.69	CH_3
19	19.41	19.4	19.42	1.02	1.03	CH_3
20	36.16	36.2	36.16			CH
21	18.80	18.8	18.8	0.93	0.93	CH_3
22	33.95	34.0	33.87			CH_2
23	26.09	26.1	26.05			CH_2
24	45.85	45.9	45.83			CH
25	29.71	29.1	29.72			CH
26	19.84	19.8	19.85	0.82	0.83	CH_3
27	19.04	19.0	19.04	0.84	0.85	CH_3
28	23.08	23.1	23.07			CH_2
29	12.00	12.1	12.00	0.86	0.87	CH_3



LC-1: β-sitosterol

Table 2: ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of LC-2 [di-(2-ethylhexyl) phthalate] [38] *Data from Fadipe AL, 2014

Position	^{13}C-NMR data of LC-4	Reported data of di-(2-ethylhexyl) phthalate *	DEPT-135 of LC-4	DEPT-135 of di-(2-ethylhexyl) phthalate*	^{1}H-NMR data of LC-4	Reported ^{1}H-NMR data of di-(2- ethylhexyl) phthalate *	Nature of carbon
1,1’	130.88	130.87	130.87	130.88	7.56	7.55-7.57	CH
2,2’	128.81	128.80	128.81	128.80	7.72-7.73	7.70-7.74	CH
3,3’	132.48	132.47	-	-	-	-	
4,4’	167.76	167.75	-	-	-	-	
5,5’	68.18	68.16	68.17	68.16	4.22-4.25	4.20-4.28	CH_2
6,6’	38.77	38.74	38.76	38.74	1.69-1.71	1.63-1.76	CH
7,7’	30.39	31.43	30.38	30.37	1.34	1.30-1.35	CH_2
8,8’	28.95	29.70	28.95	28.93	1.28	1.29	CH_2
9,9’	23.01	22.98	23.00	22.98	1.28	1.29	CH_2
10,10’	14.06	14.04	14.06	14.04	0.86	0.89-1.00	CH_3
11,11’	23.78	23.75	23.77	23.75	1.43	1.40-1.47	CH_2
12,12’	10.98	10.95	10.98	10.96	0.94	0.89-1.00	CH_3

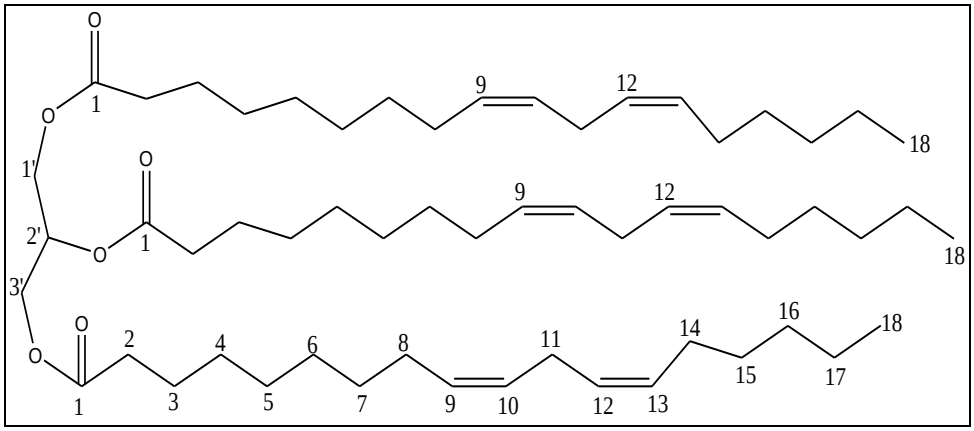


LC-2: di-(2-ethylhexyl) phthalate

Table 3: ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of LC-3 with reported data [39-40] of [trilinolein] *Data from Fierro 2012 and Ragasa *etal* 2013

Position	^{13}C-NMR data of LC-5	Reported data of trilinolein *	DEPT-135 of LC-5	^{1}H-NMR data of LC-5	Reported ^{1}H-NMR data of trilinolein *	Nature of carbon
1’a 1’b	62.13 62.13	62.10 62.10	62.13 62.13	4.31 4.10	4.28 4.12	CH_2
2’	68.92	68.88	68.92	5.36	5.32	CH
3’a 3’b	62.13 62.13	62.10 62.10	62.13 62.13	4.31 4.10	4.28 4.12	CH_2
1	172.87, 173.29	172.84, 173.26	-	-	-	C
2	34.08	34.05	34.07	2.38	2.31	CH_2
3	24.70	24.83	24.69	1.65	1.35	CH_2
4	29.08	29.08	29.08	1.28	1.25	CH_2
5	29.26	29.27	29.26	1.28	1.25	CH_2
6	29.09	29.11	29.08	1.28	1.25	CH_2
7	29.72	29.62	29.69	1.32	1.30	CH_2
8	29.16	29.19	29.16	2.06	2.03	CH_2
9	130.02	130.01	130.02	5.38	5.33	CH
10	128.08	128.06	128.08	5.38	5.33	CH
11	25.65	25.63	25.65	2.79	2.75	CH_2

12	127.91	127.90	127.91	5.38	5.33	CH
13	130.22	130.22	130.22	5.38	5.33	CH
14	27.23	27.19	27.22	2.06	2.03	CH_2
15	29.38	29.36	29.38	1.32	1.30	CH_2
16	31.95	31.52	31.95	1.28	1.25	CH_2
17	22.71	22.57	22.71	1.28	1.25	CH_2
18	14.12	14.07	14.12	0.90	0.87	CH_3



LC-3: trilinolein

Results

Evaluation of antibacterial activities of the isolated compounds

The antimicrobial activities of three isolated compounds (LC-1, LC-2, and LC-3) have been investigated with four bacterial species by disc diffusion method, *Pseudomonas aeruginosa*, *Salmonella thyphimurium*, *Staphylococcus aureus* and *Escherichia coli*, activity of the compounds were expressed as growth inhibition zones (in mm), given in (Table-4). The result revealed that LC-2: [di-(2-ethylhexyl) phthalate] showed maximum antibacterial activity against *P.aeruginosa* 26mm, *S. thyphimurium* 24mm, *S. aureus* 22mm and *E.coli* 20mm. The results of this study were in agreement with literature values, with inhibition in the range of 13-24 mm [41, 42].

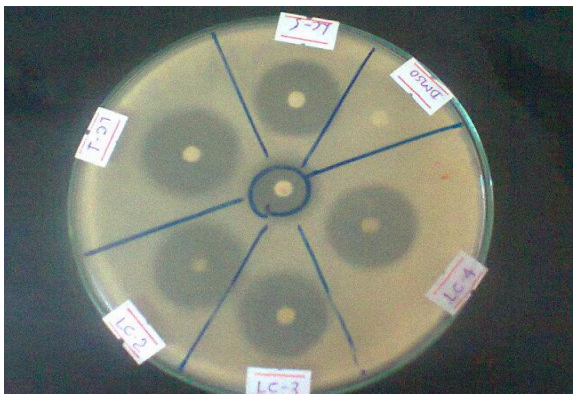
Similarly, LC-3: (trilinolien) showed antibacterial activity against *P. aeruginosa* 21 mm, 20mm against *E.coli*, 19mm against *S. aureus*, and 11mm against *S. thyphimurium*. LC-1 (β-sitosterol) displayed inhibitory activity 24mm against *P. aeruginosa*, 19mm against *S.aureus*,17mm against *S.thyphimurium*, and 14mm against *E.coli*. Literature reported value of β-sitoterol shown the inhibition zone 14mm (*E. coli*), 13 mm (*S. aureus*), and 11 mm (*P. aeruginosa)* [43].

Among the three isolated compounds LC-3 showed least activity with the tested four bacteria strains that means 19mm against *P. aeruginosa*, 18mm against *S. aureus*, 15mm against *S. thyphimurium* and 14mm against *E. coli*. The overall results of this study provide evidence that *L. camara* (L.) fruits extract as well as the isolated compounds exhibit antibacterial activity for both Gram negative and Gram positive pathogens.

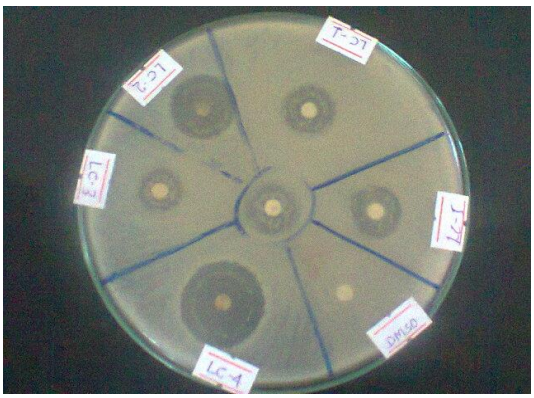
Table 4: Antibacterial activity of isolated compounds in disc diffusion methods

Bacterial strain	Diameter of zone in mm					
	LC-1	LC-2	LC-3	Gentamicine	DMSO*	Acetone*
Escherichia coli	14	20	20	22	NI	NI
Staphylococcus aureus	19	22	19	23	NI	NI
Salmonella thyphimurium	17	21	18	24	NI	NI
Pseudomonas aeruginosa	24	23	21	25	NI	NI

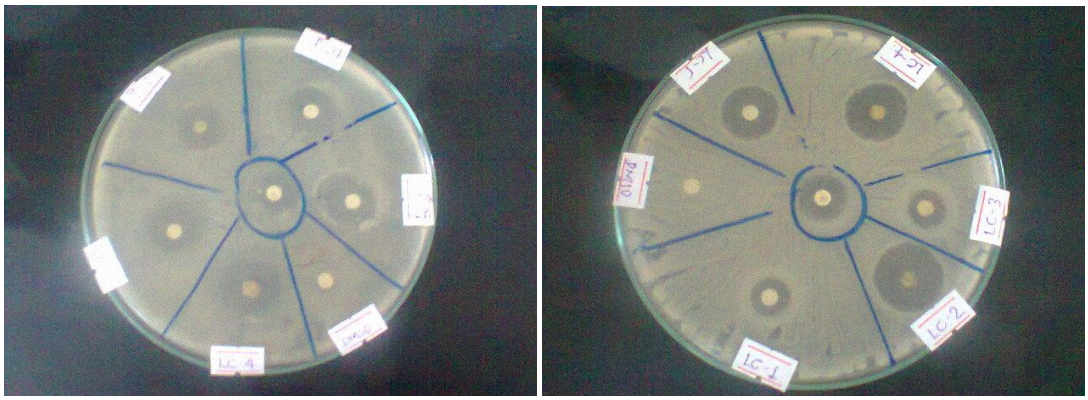
Note, Acetone and DMSO* NI = No Inhibition



Pseudomonas aeruginosa



Staphylococcus aureus



Salmonella thyphimurium *Escherichia coli*

Fig 1: Antimicrobial activities of LC-1, LC-2, LC-3 at different solvents

Discussion

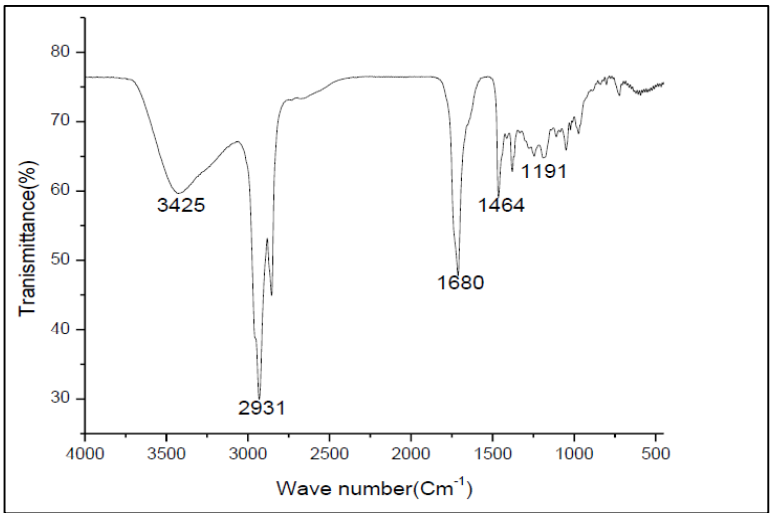
At present traditional herbal based medicine gains importance and there is an increasing demand to herbal drugs in comparison with existing system of medicine. Plants contain plethora of complex functional moieties with varied pharmacological activities. Many valuable, potent bioactive lead molecules used for treating dreadful diseases which have been isolated from plant kingdom. *L. camara* (L.) considered as weed but used as effective folk medicine in many parts of the world. The results of current study showed promising antibacterial activity against the tested bacteria, among these chloroform crude extract were found to possess a more potent inhibitory effect when compared to petroleum ether, ethyl acetate and methanol extract. The current study established the therapeutic potential of *L. camara* (L.) in modern medicines and a possible candidate for the drug discovery.

Declaration of conflict of interest

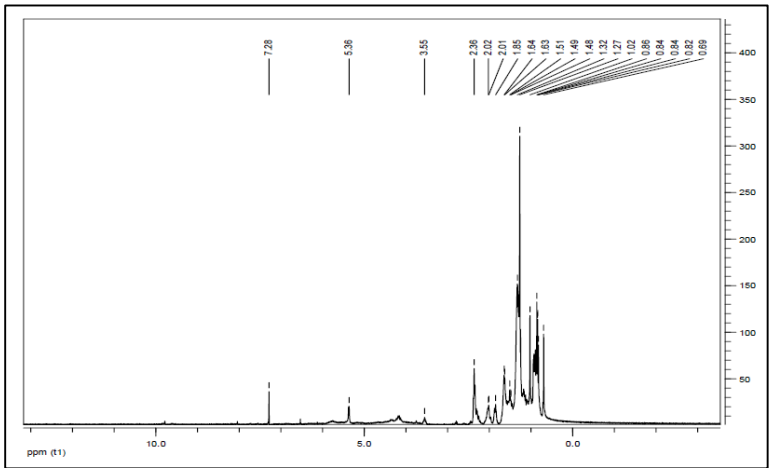
The authors declare that there is no conflict of interest.

Appendixes

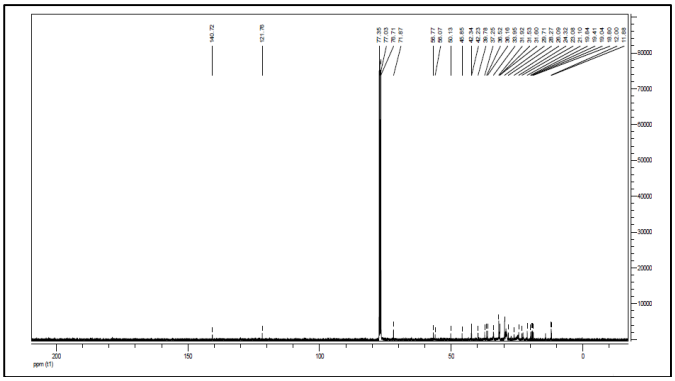
Appendix-1 IR spectrum of LC-1



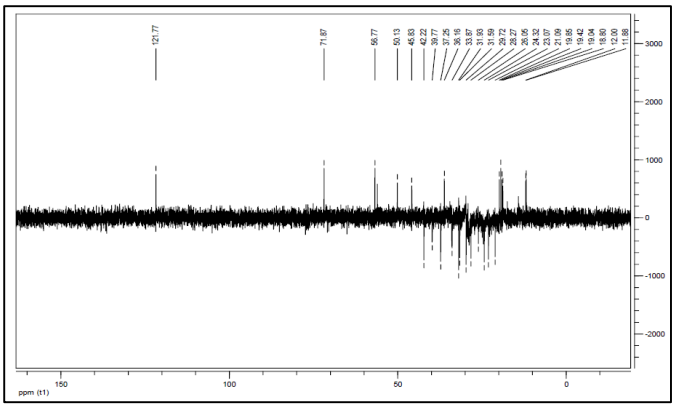
^{1}H-NMR spectrum of LC-1



Appendix-2 13C-NMR spectrum of LC-1

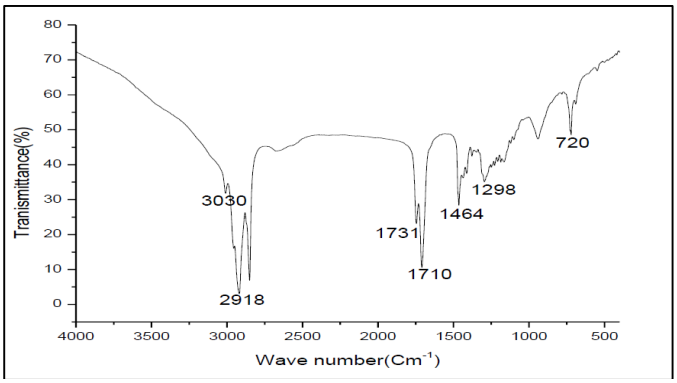


DEPT-135 of LC-1

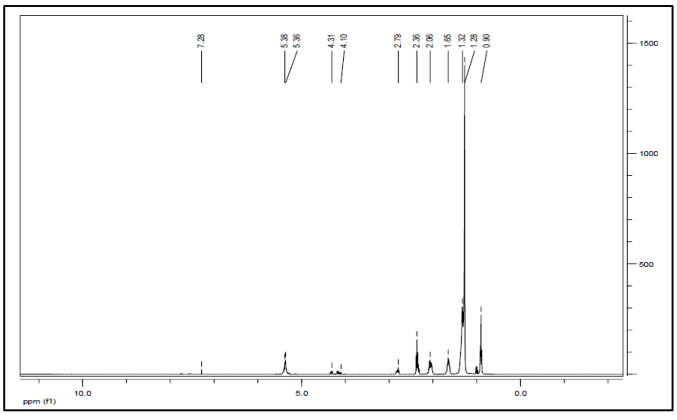


Appendixes

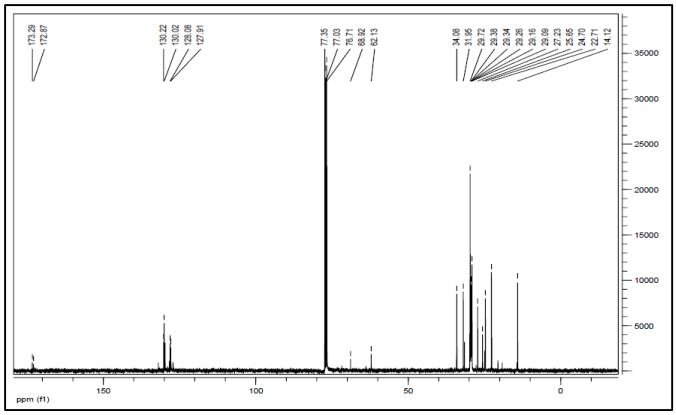
Appendix-5 IR spectrum of LC-3



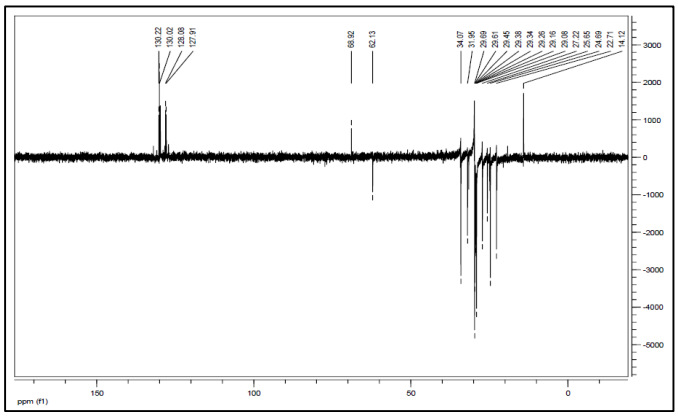
[1]H-NMR spectrum of LC-3



Appendix-6 13C-NMR spectrum of LC-3



DEPT-135 of LC-3



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