Supporting Information

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Isolation of a novel indigoferamide-A from seeds of *Indigofera heterantha* Wall

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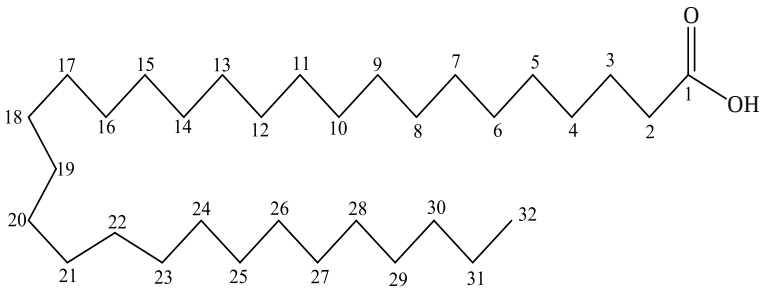
Table-1: ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectral data of **1** in MeOD

C. No.	**^{1}H NMR δ_H (J in Hz)**	**^{13}C NMR δ_C**	**Multiplicity**	**HMBC**
1	3.74, (d, J =5)	62.0	CH_2	C-3
2	4.10, m	52.9	CH	C-1’
3	3.55, m	76.0	CH	--
4	3.52, m	73.2	CH	C-2, C-5
5	1.28, m	33.8	CH_2	--
6-7	1.28, m	27.2	CH_2	--
8-13	1.28, m	30.5	CH_2	--
14	1.28, m	33.7	CH_2	--
15	1.28, m	33.1	CH_2	--
16	5.43, m	131.6	CH	C-15
17	5.45, m	131.6	CH	--
18	1.28, m	32.7	CH_2	C-17
19	1.28, m	23.8	CH_2	--
20	0.89, (t, J = 7)	14.5	CH_3	C-18
1′	--	176.9	-C-	--
2′	4.02, m	72.9	CH	C-1’, C 3’
3′	1.61, m	35.8	CH_2	--
4′, 5′	1.28, m	30.5	CH_2	--
6′, 20’	1.28, m	30.5	CH_2	--
21’	1.28, m	33.8	CH_2	--
22’	1.28, m	23.8	CH_2	--
23’	0.89, (t, J =7)	14.5	CH_3	--
NH	8.5, s	--	--	--



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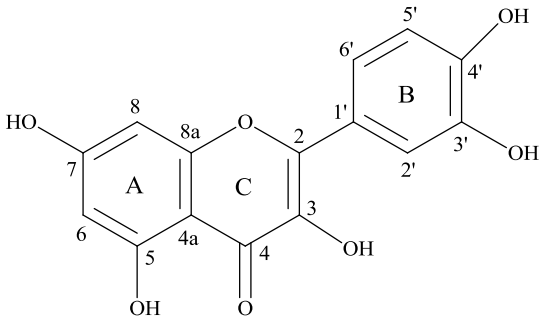

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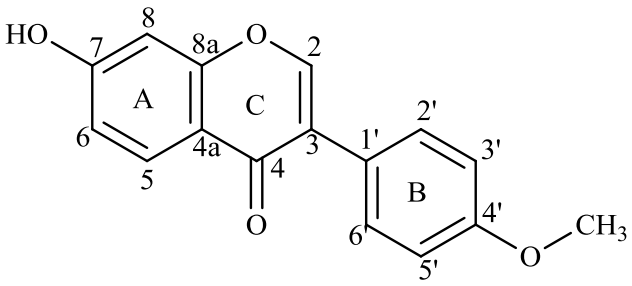

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Figure 4: The chemical structures of compounds **1/2/3/4**

5.1.1. Bacterial culture

Five diffèrent concentrations (1%, 2%, 3%, 4% and 5%) of both the bacteria i.e. *B. subtillis* and *E.* coli were prepared by adding 60, 120, 180, 240 and 300 μl from the stock solution to the sterile test tube containing 6 ml of the LB media.

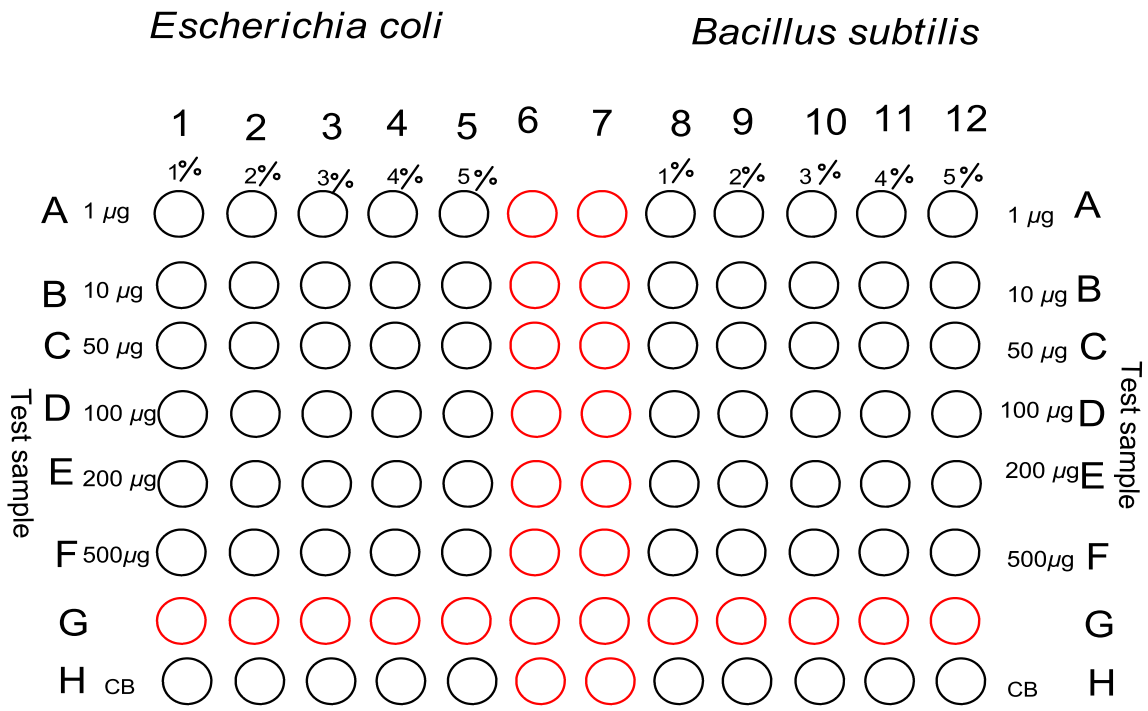
5.1.2. Test sample

Six concentrations (1, 10, 50, 100, 200 and 500 μg) of the sample were prepared in Dimethyl Sulfoxide (DMSO) and tested against all concentrations of the bacterial strains.

5.1.3. Preparation of samples for analysis

The plates consisted of 96 wells were used for the antibacterial testing in spectra max 190 (spectrophotometer). There are 12 longitudinal and 8 vertical wells in the plate marked by

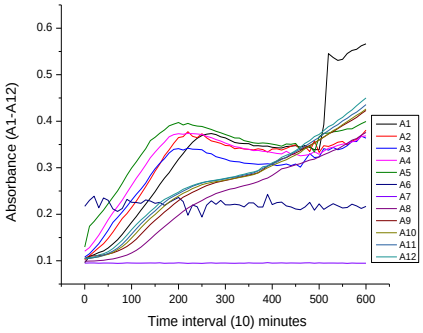
number from left to right i.e. (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) and alphabets from top to bottom i.e. (A, B, C, D, E, F, G and H). 200 μl of the 1% *E. coli* was poured to 1st vertical column with seven wells, 200 μl of 2% *E.coli* to 2nd vertical column with seven wells, 3% to 3rd, 4% to 4th and 5% to 5th vertical column while 6th and 7th vertical column were remained blank. Same procedure was applied for the *B. subtillis* started from 8th to 12th vertical column and each well were filled with 200 μl of the media containing experimental organism. Whereas one of the horizontal row G were remained blank. After filling the various concentrations of the experimental organisms the sample were added to each well in the following manner. 1 μg/ml of the test sample was added to 10 wells from A1 to A5 against (*E. coli*) and from A8 to A12 against (*B. subtillis*), while the same procedure was applied for the other concentrations such as 10 μg/ml to row B, 50 μg/ml to row C, 100 μg/ml to row D, 200 μg/ml to row E, 500 μg/ml to row F. The row G was remained blank. The 2 μl/ml of the carbanicillin standard was added to row H. After preparation of the samples for analysis it was immediately loaded to the spectra max 190 for analysis, the temperature was adjusted at 37 ^{0}C, absorbance were adjusted at 600 nm and the experiment were allowed for 10 hours with readings interval of 10 minutes. Then the results were recorded after 10 hours and were then compared with the standard antibiotic.



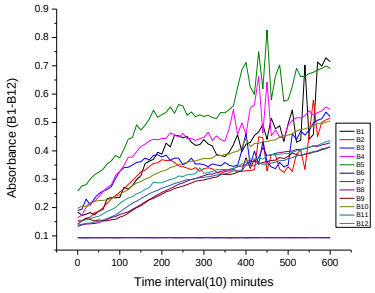
6. Antibacterial activity of new Indigoferamide-A

The indigoferamide-A (**1**) isolated from the seeds of *I. heterantha* was assayed for its anti-bacterial activity. The bacterial growth was high at low concentration and was decreased with the increase of concentrations. In graph (A) 1μg/ml of the test sample was used against *E. coli and B. subtillis* which showed high bacterial growth, while the bacterial growth decreases with the increase of concentration of test sample from 10 μg/ml in graph (B), 50 μg/ml in graph (C), 100 μg/ml in graph (D), 200 μg/ml in graph (E) and 500 μg/ml in graph (F)

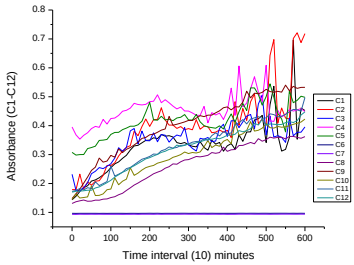
respectively. The bacterial growth inhibition is maximum at 500 μg/ml concentration. The data was obtained as absorbance vs time and were then plotted in graphical form.



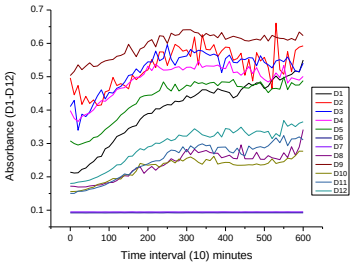
A (1μg/ml)



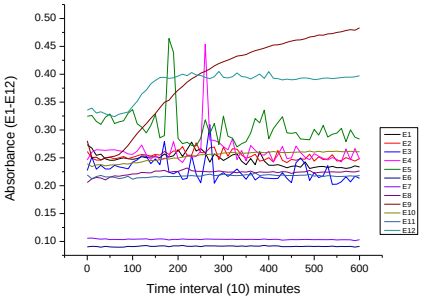
B (10 μg/ml)



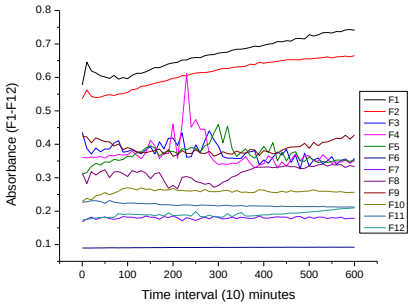
C (50 μg/ml)



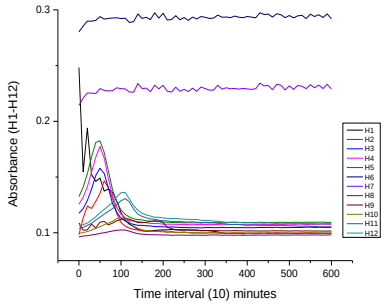
D (100 μg/ml)



E (200 μg/ml)



F (500 μg/ml)



H (Standard 100 μg/ml)

Figure-3: Graphs (**A-H**); anti-bacterial activity of inidigoferamide-A (**1**)

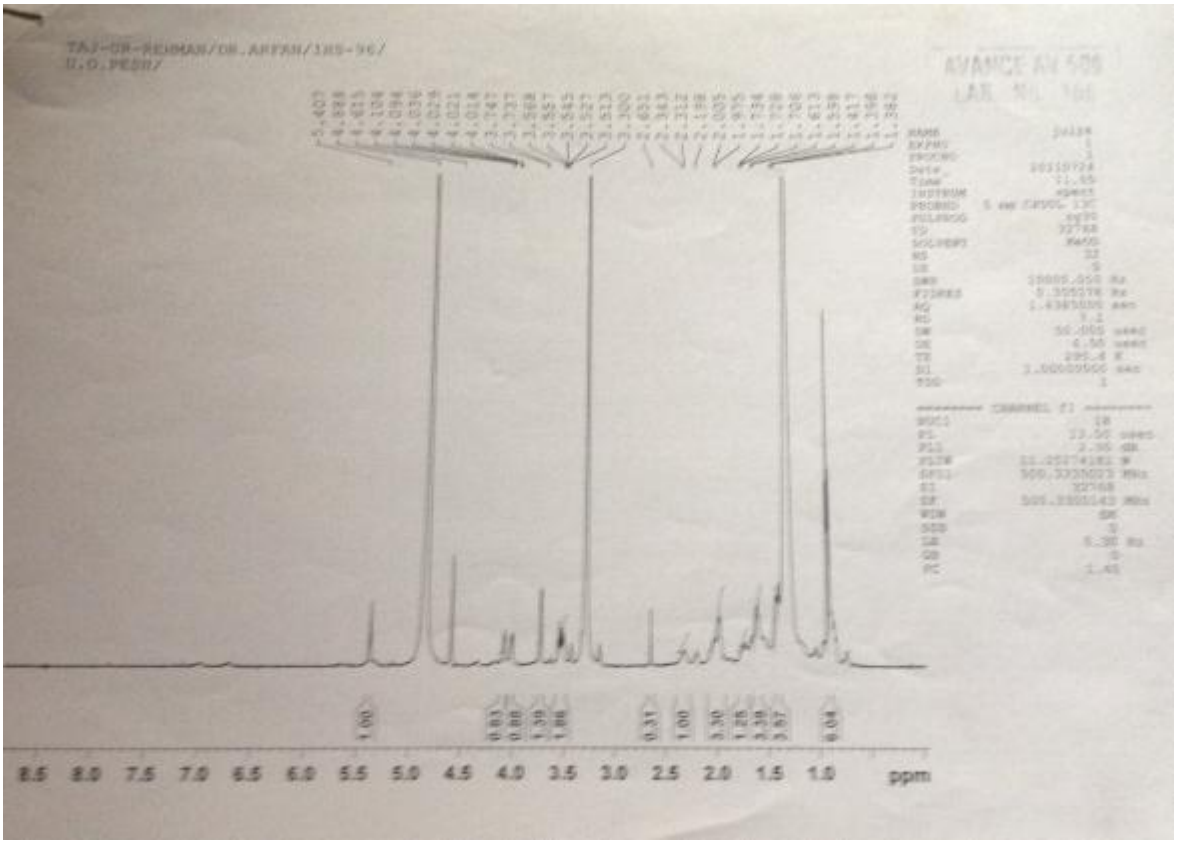
From the results of graphs (A), (B), (C), (D), (E) and (F) (**Figure-3**) of the test sample concluded that the growth of bacteria is higher at low concentration as compaired to standard anti-biotic carbanicillin graph H, while bacterial growth inhibition at higher concentration graphs (F) is comparable to that of standard carbanicillin. These results proved that the new isolated indigoferamide-A can be used as a potent antibacterial agent.

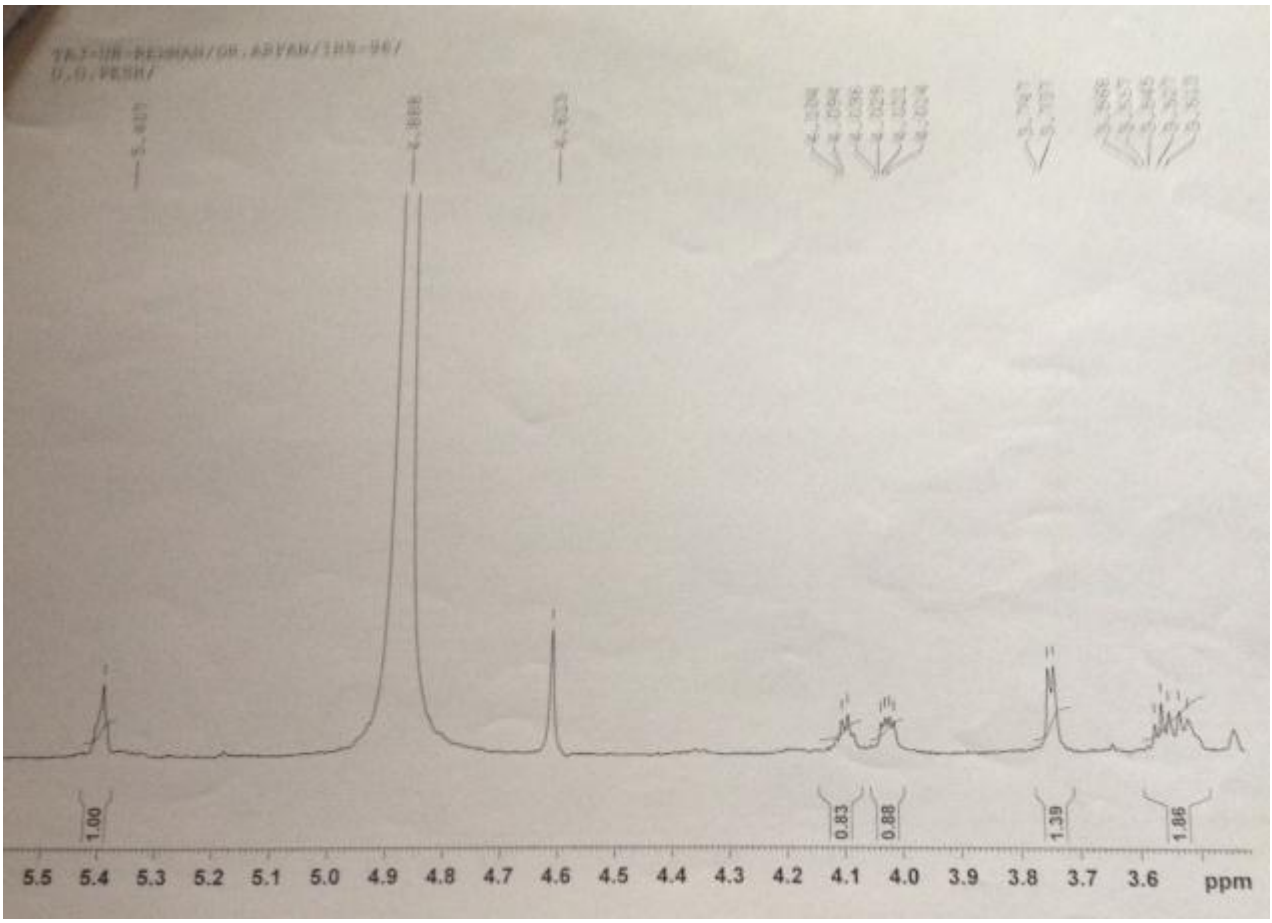
Supporting information:

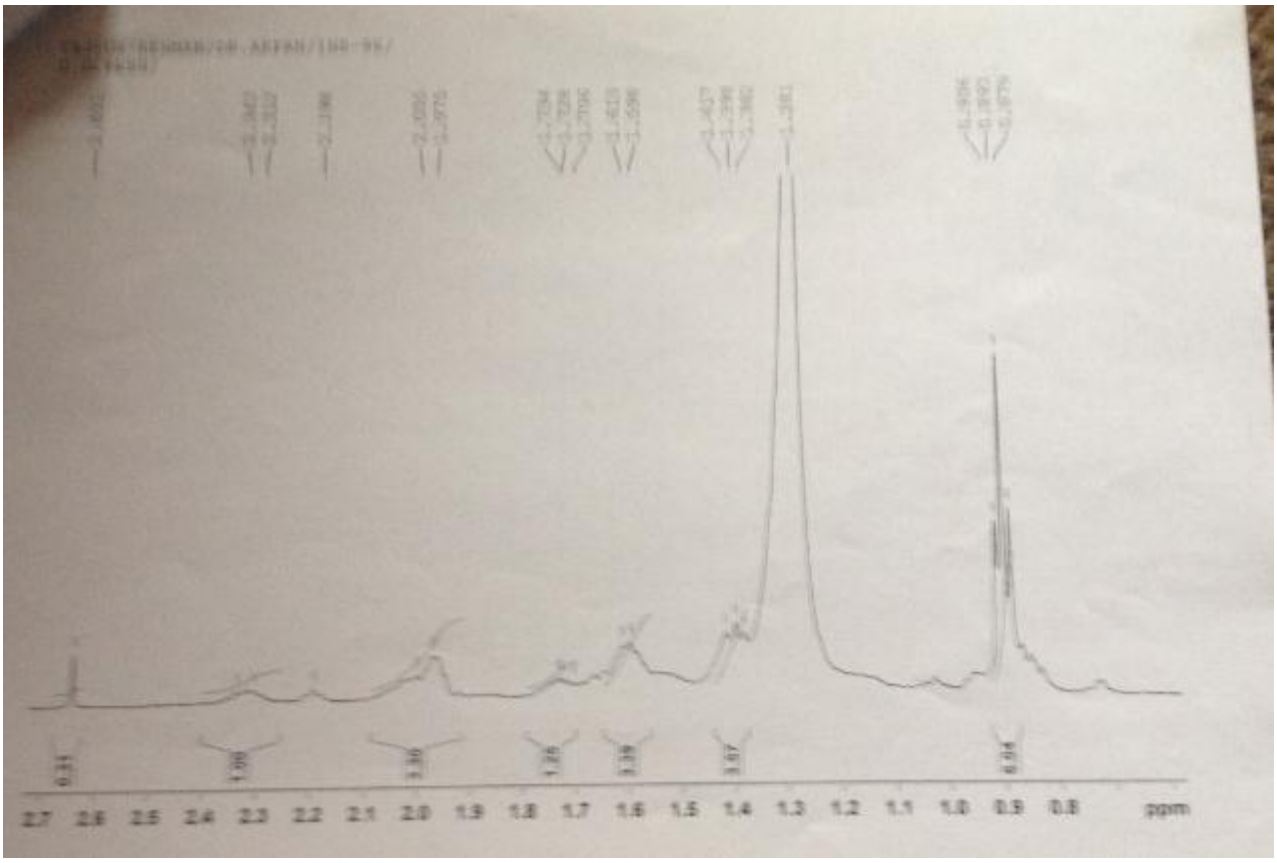
The supporting information according to reviewers, for plate 96 wells and graphs (A-H) figures are explained as follow:

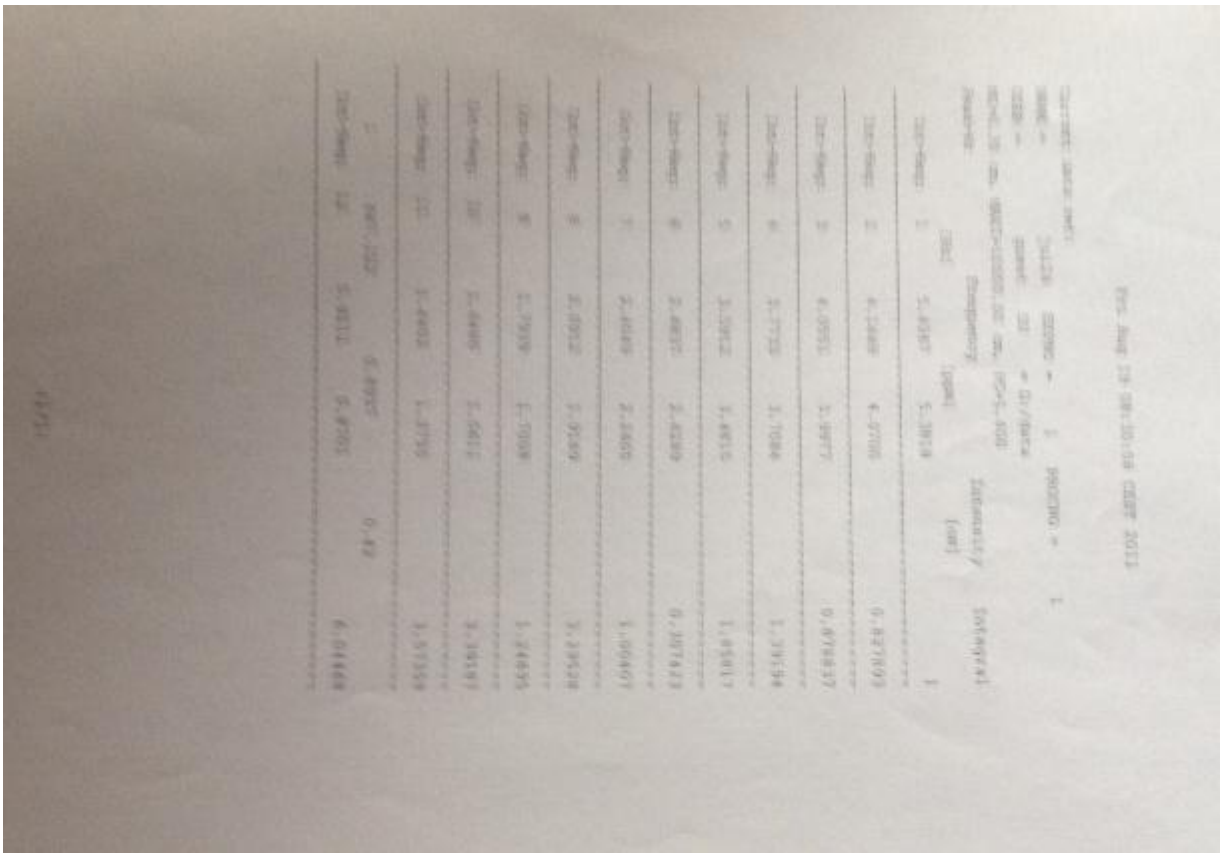
All these activities were carried out on spectra max 190 spectrophotometer. In this instrument a plate consist of 96 wells are used. Before starting the experiment the plate is prepared according to the protocol given in this paper. After this the plate is put in the instrument and the experiment is started. The data obtained from this experiment is then plotted in the form of graphs (A-H) via software known as origin.

[1]H-NMR Spectra of novel indigoferamide-A

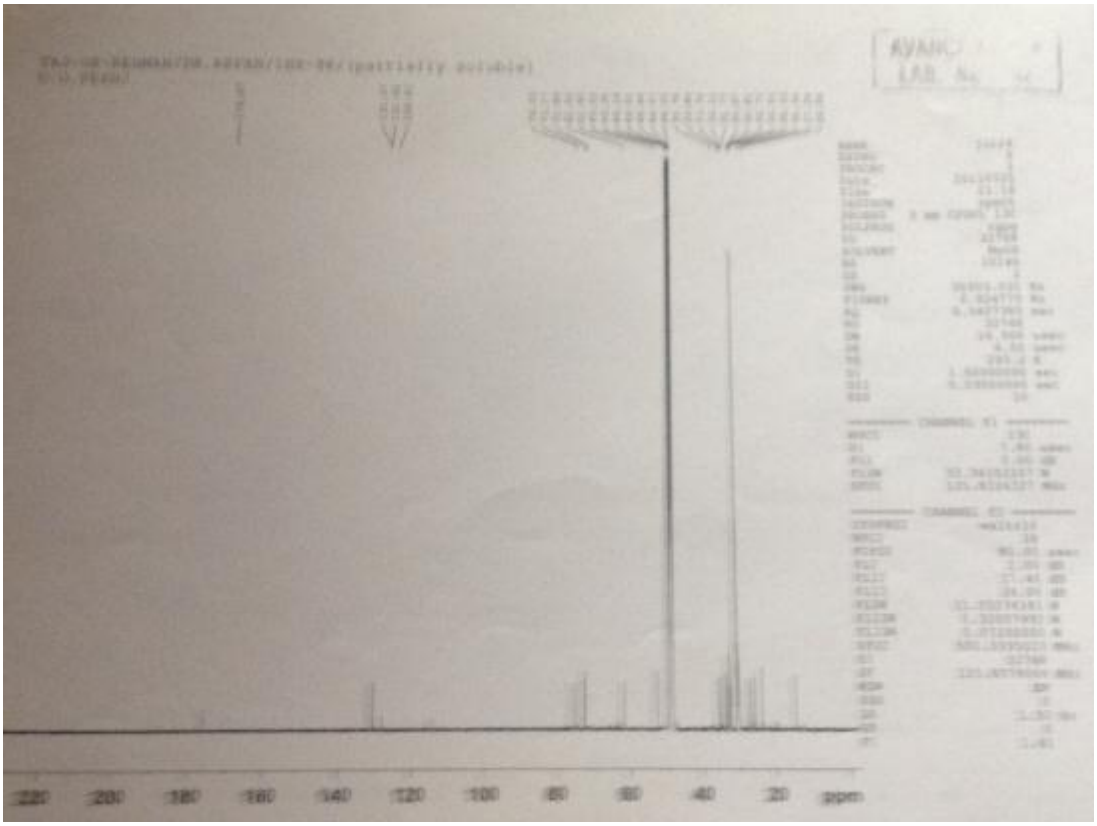


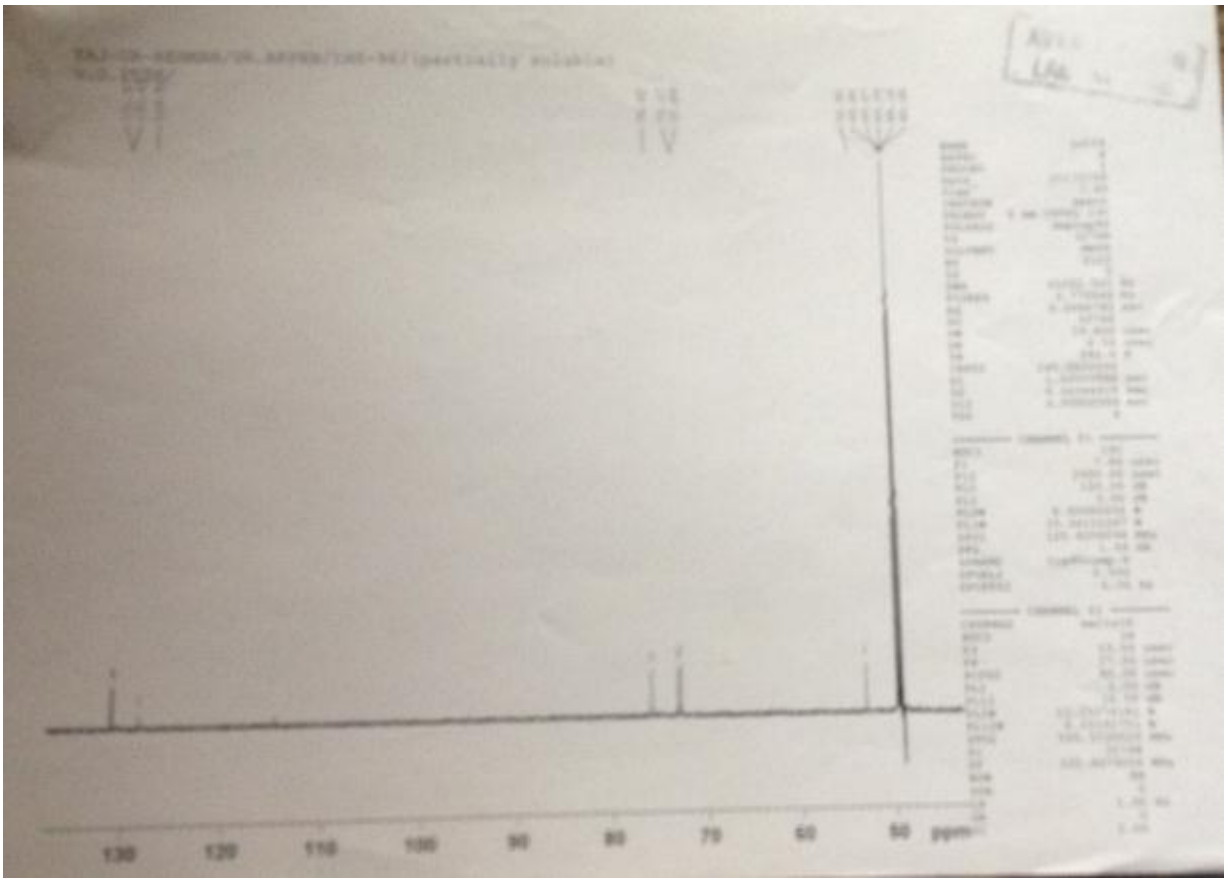


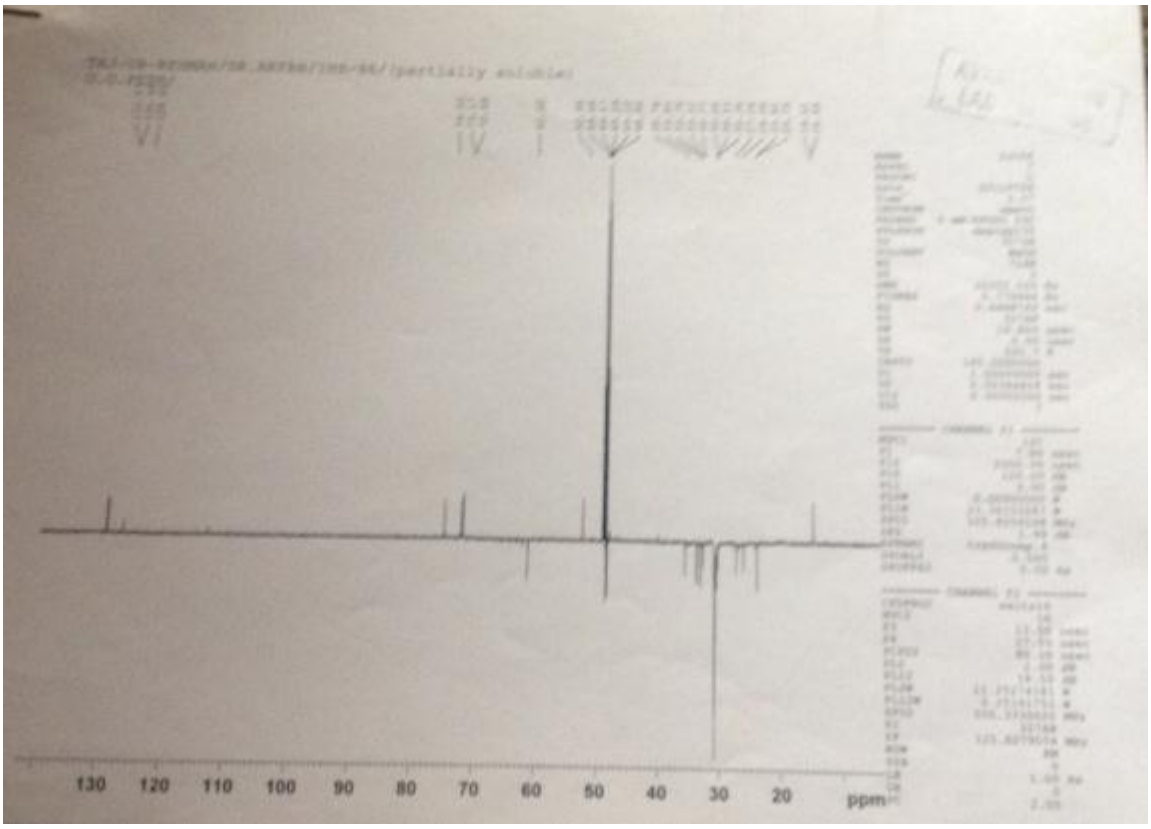

0.31
1.00
1.36
1.25
1.39
3.67
6.84
2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 ppm



^{13}C-NMR Spectra of novel indigoferamide-A



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90
80
70
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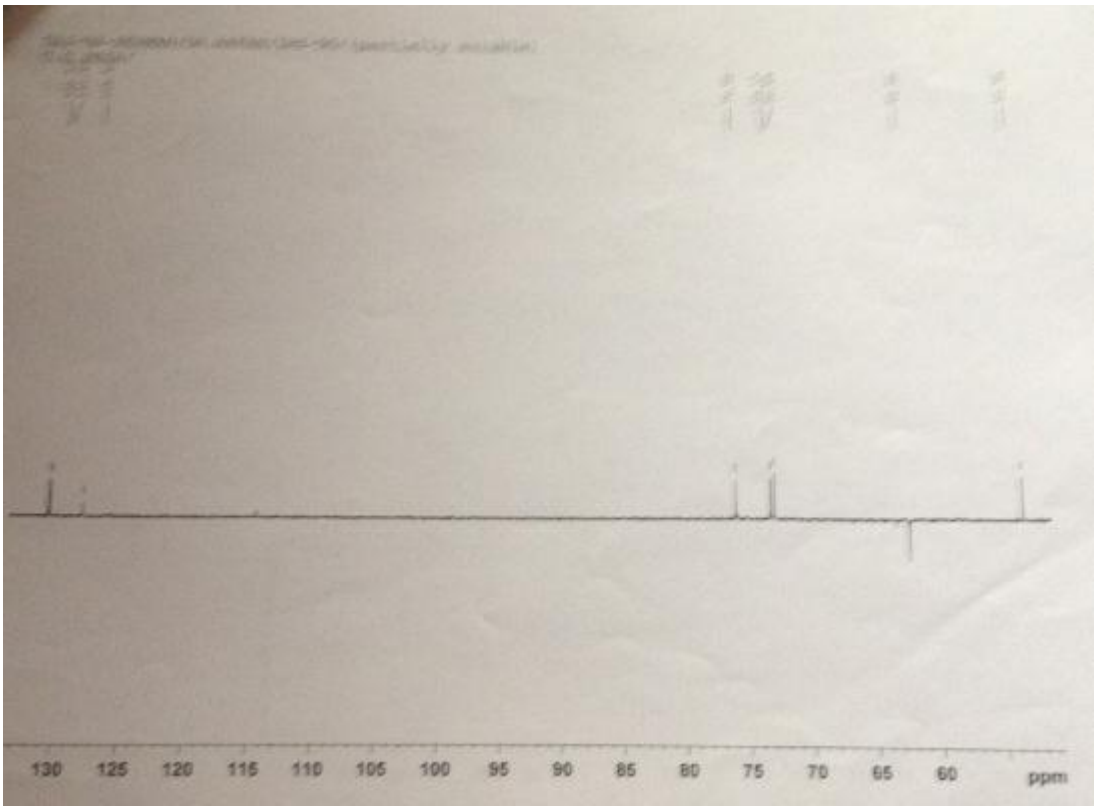
50
40
30
20
ppm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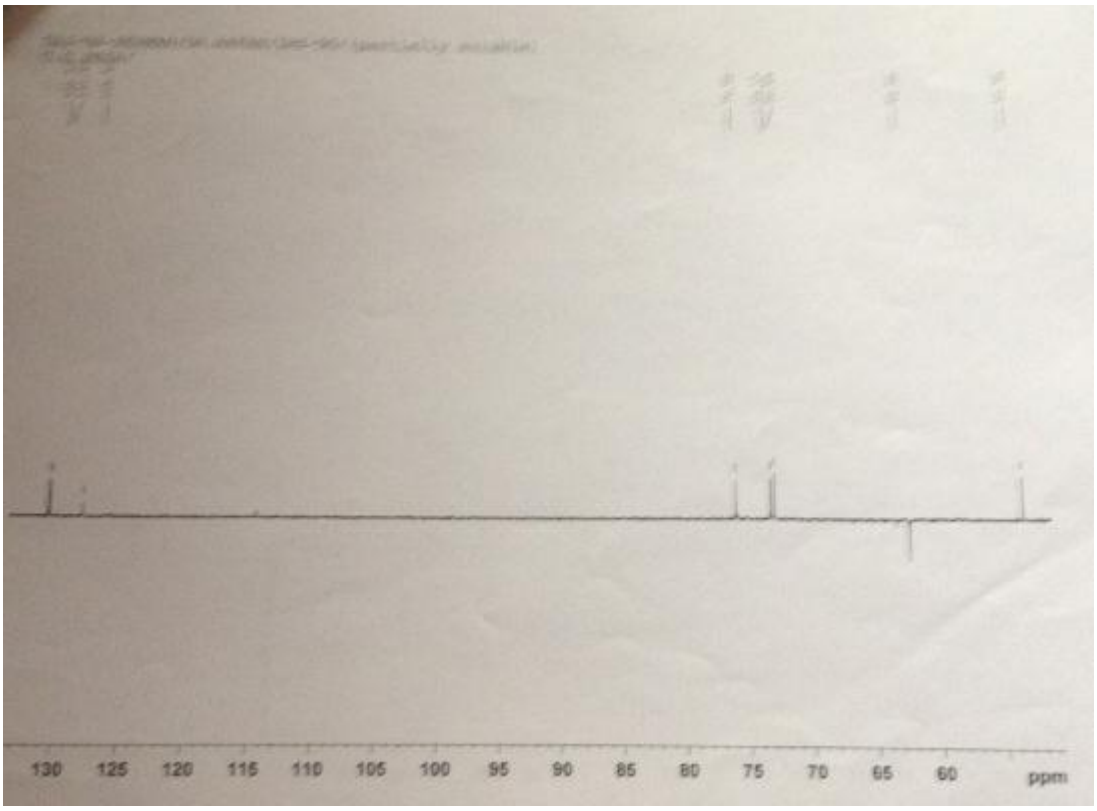
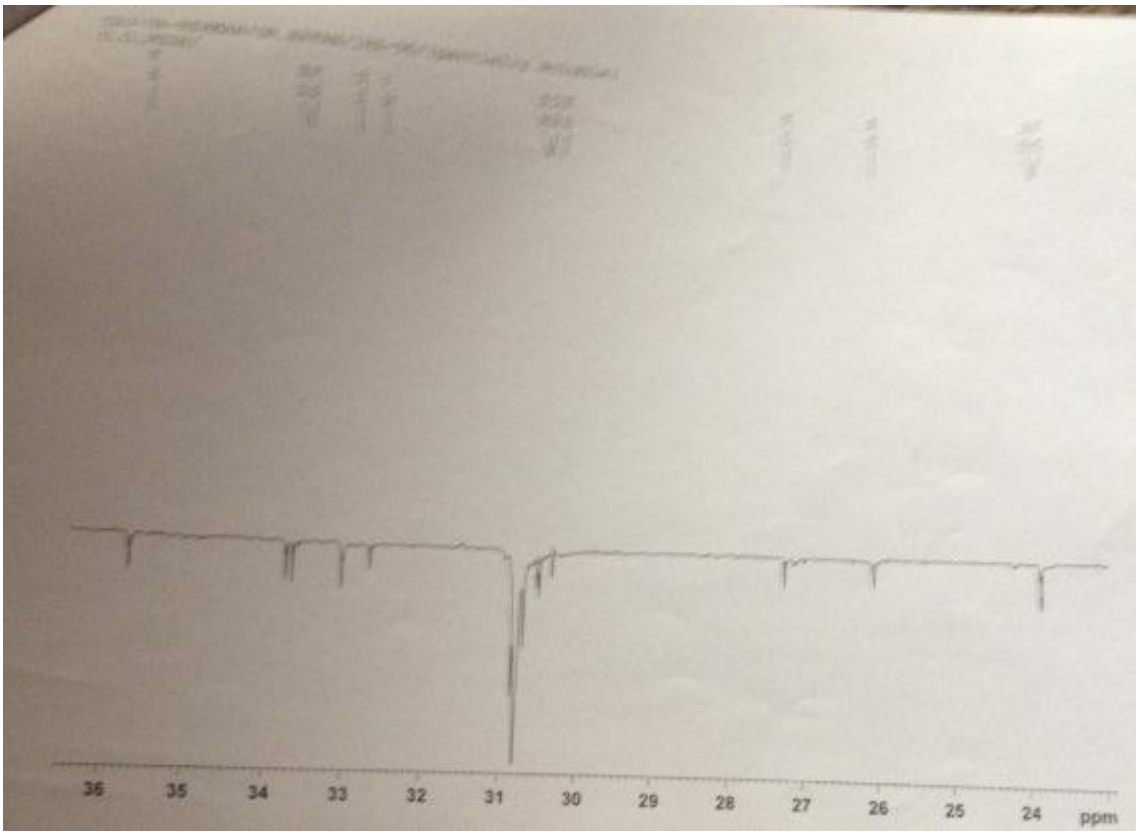
ppm


130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 ppm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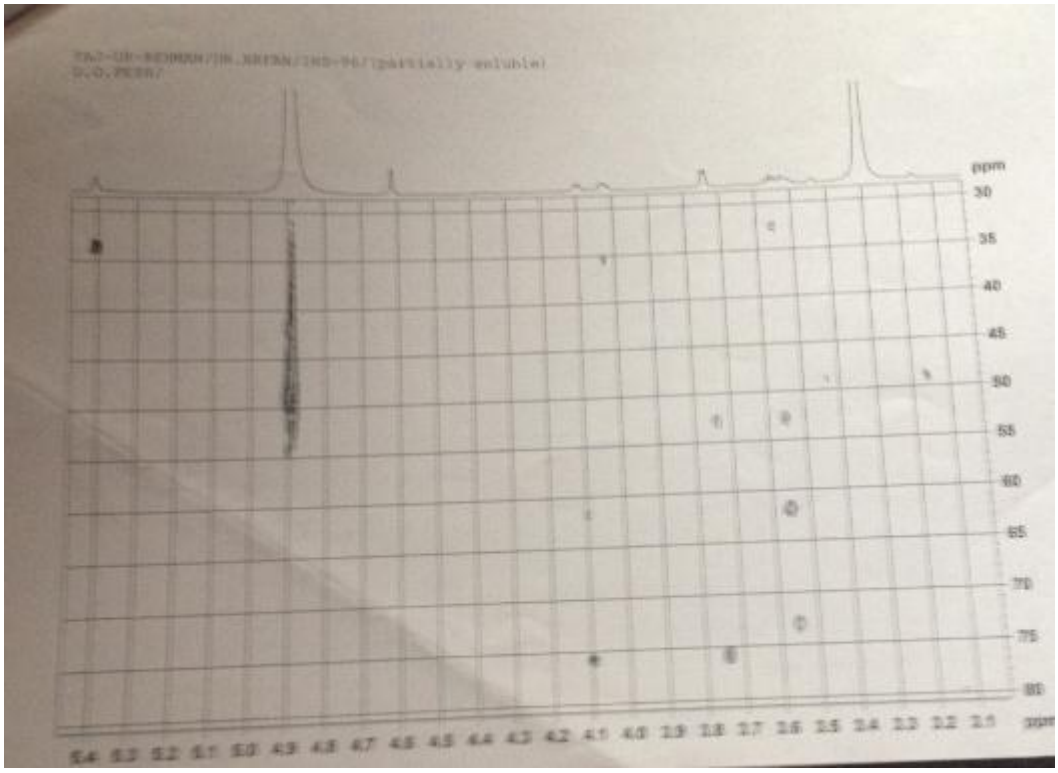
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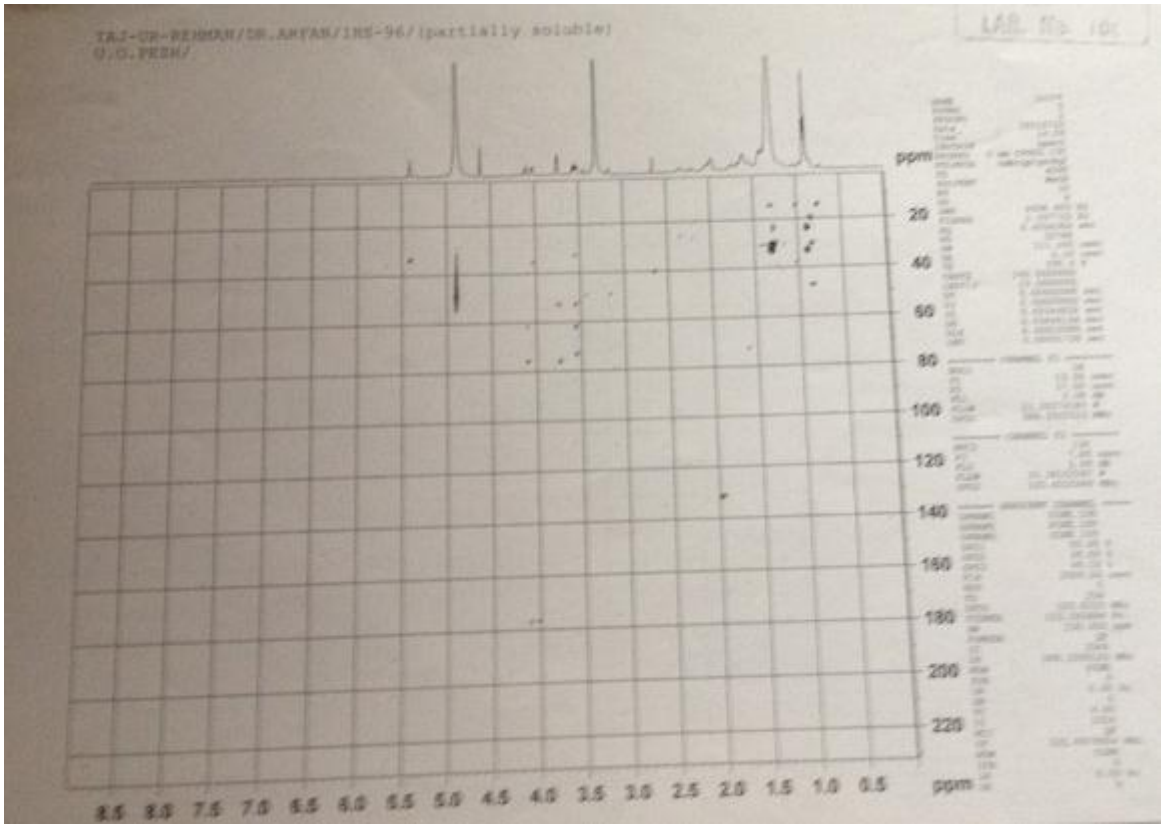
40
45
50
55
60
65
70
75
80
ppm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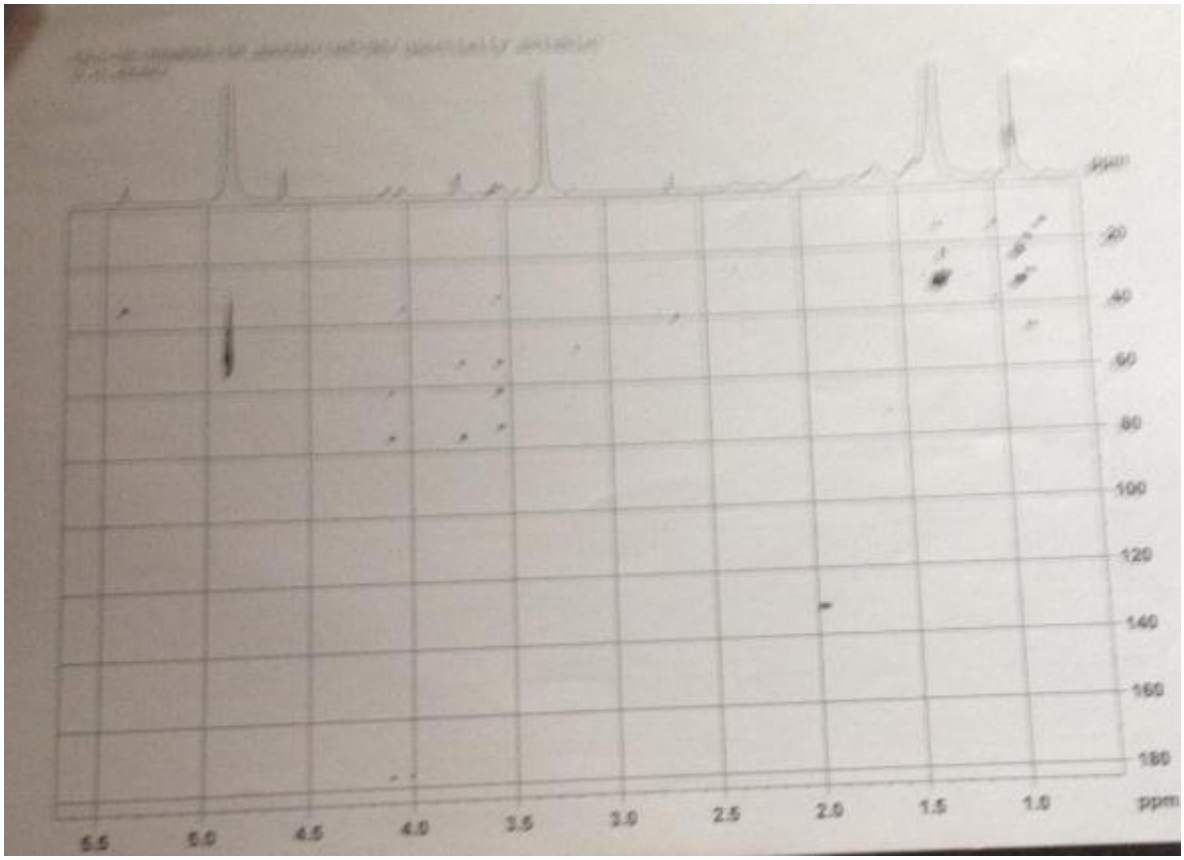
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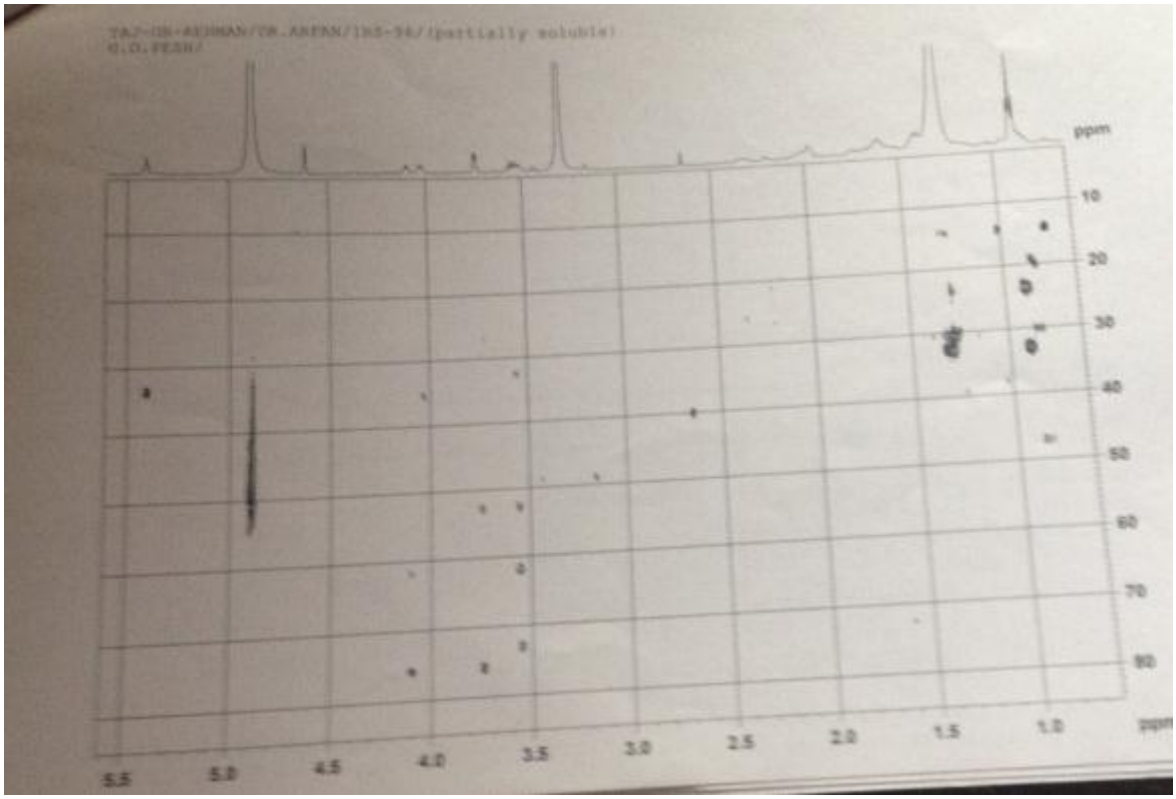
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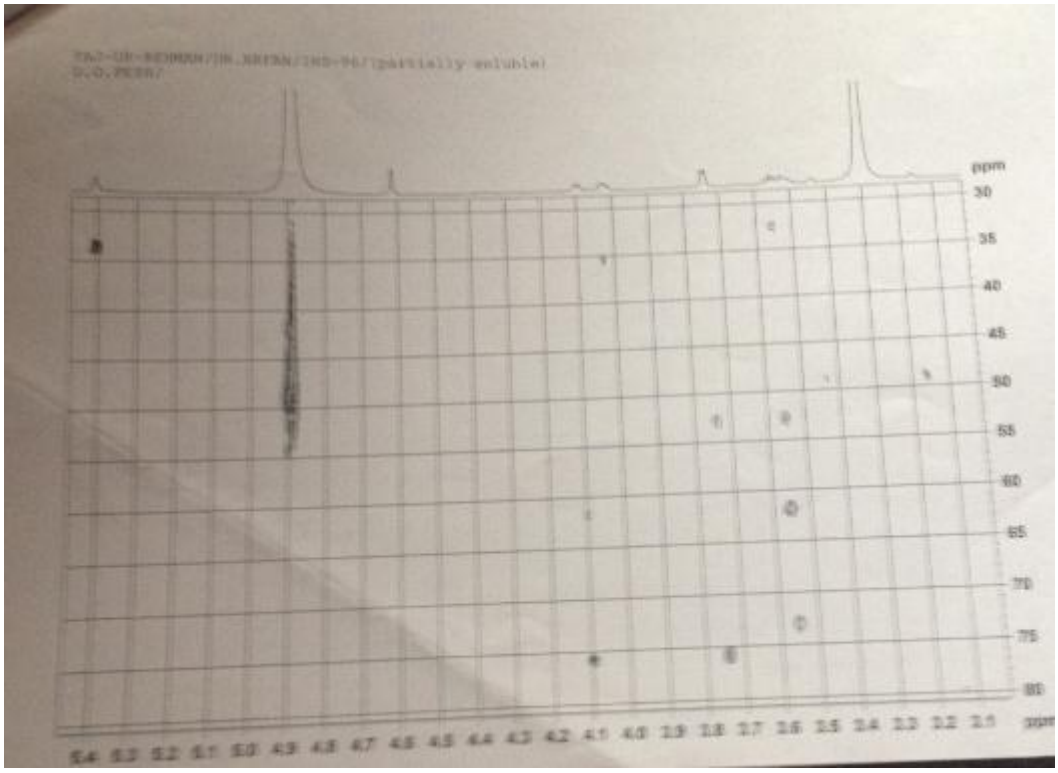
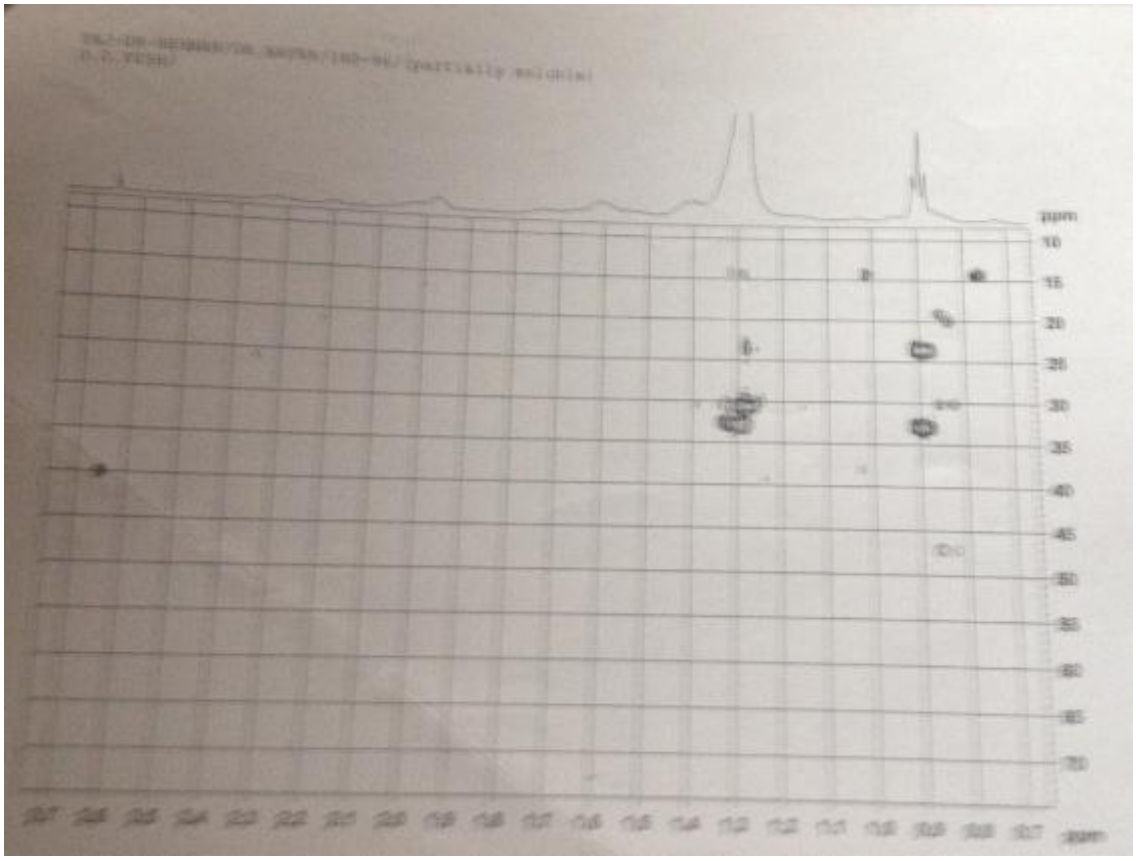
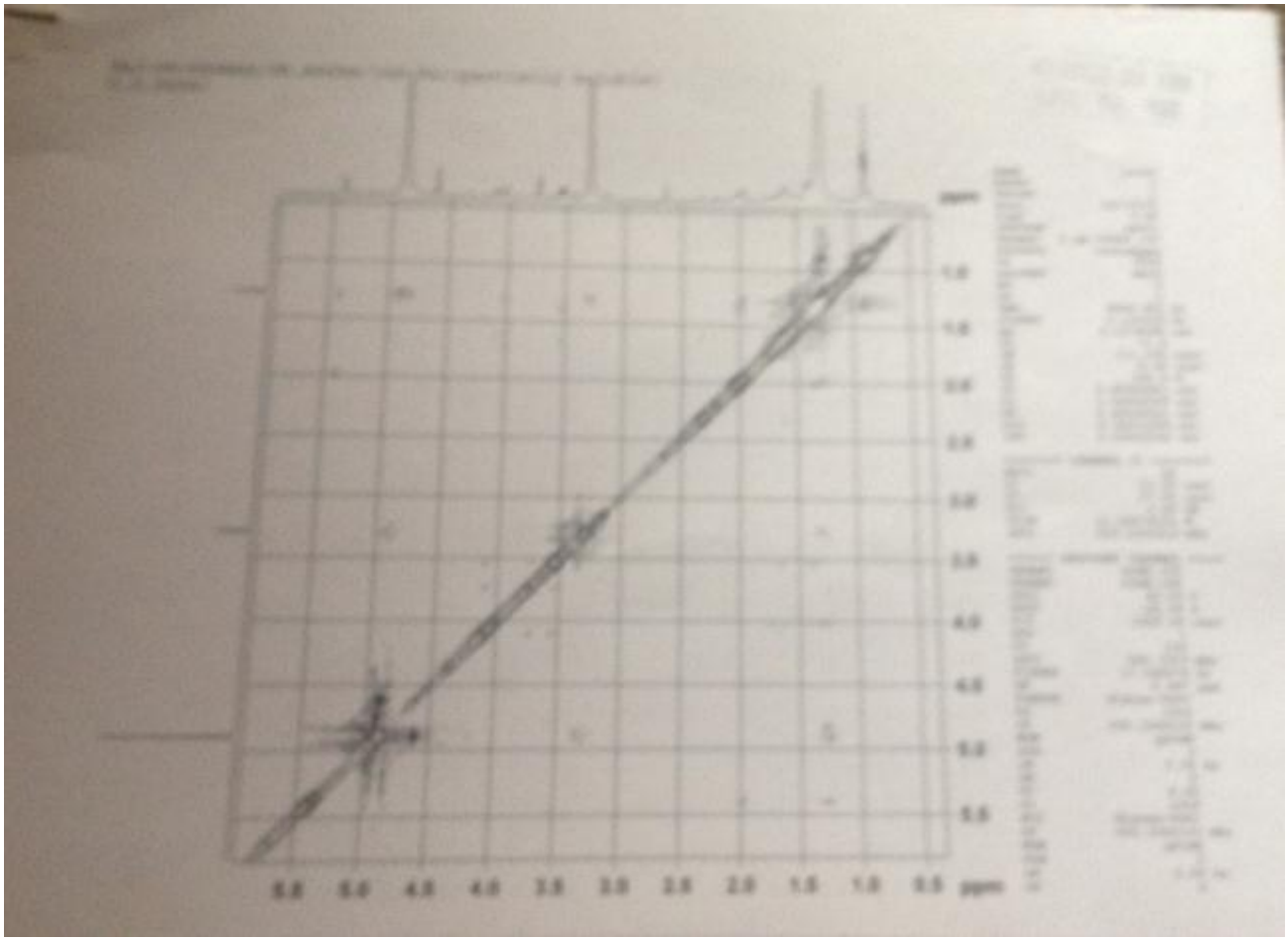
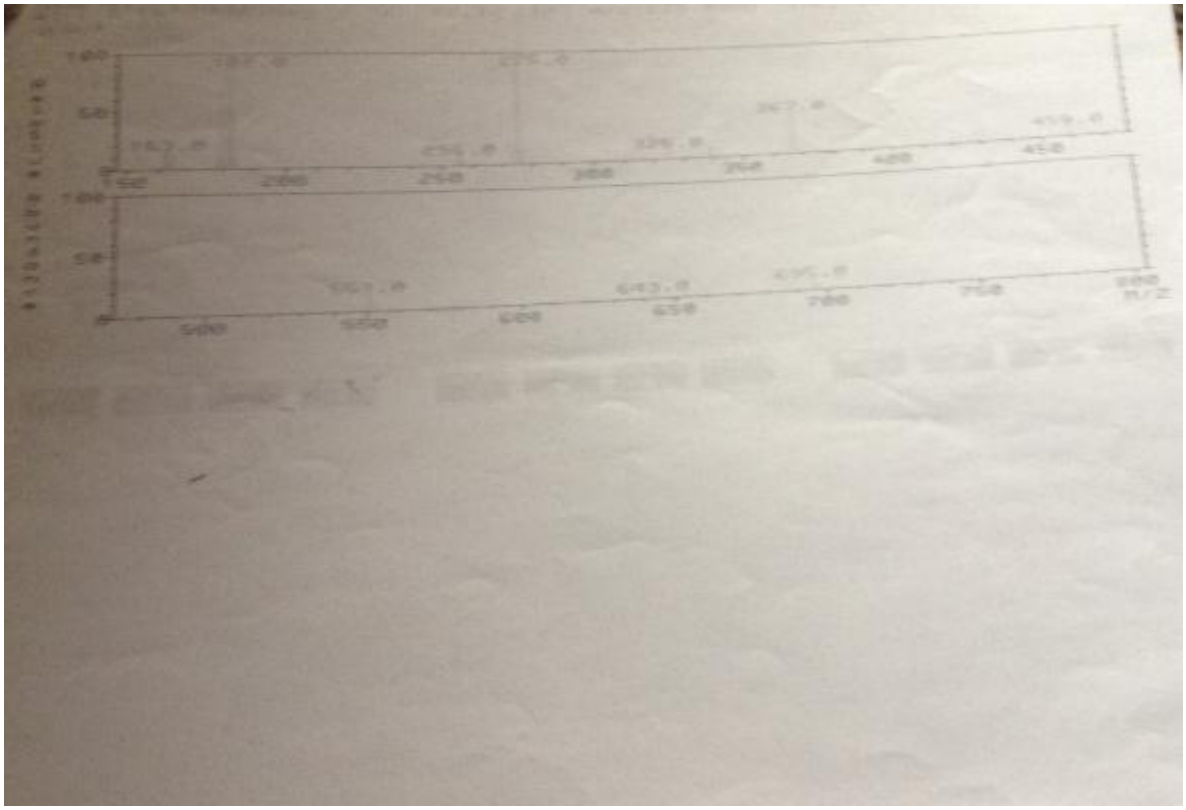






FAB of novel indigoferamide-A



MS Spectra of novel indigoferamide-A