Research Article

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ISOLATION PURIFICATION AND IDENTIFICATION OF CONSTITUENTS OF *BORRERIA HISPIDA (Linn)*

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ABSTRACT

This study reports the isolation and characterization of a new chemical constituents extracted from the leaves of *Borreria Hispida* Linn. The isolation of the organic compounds was done using simple chromatographic technique. The method of isolation is simple, cost effective and efficient. Eight compounds were isolated for the first time from the air dried leaves of Borreria hispida (*Linn).* Four sterol compounds, one unsaturated terpenoidal ketone, one flovone, one Saponin, and one Terpene compound were isolated. These compounds were isolated and identified by physical and chemical methods; melting point, Rf values, IR and H^1 NMR spectroscopy. *Borreria Hispida* Linn has a long history of use in the Ayurvedic and Unani systems of medicine. It contains a number of phytoconstituents viz. alkaloids, phytosterols, glycosides, amino acids, proteins, phenolic acids, enzymes, vitamins, sugars, minerals, flavonoids, gums & mucilage, terpenoids etc [1-6]. The plant is used for the treatment of a number of diseases [1, 7-8]. The present study deals with isolation purification and identification of constituents from the leaves of ***Borreria hispida Linn***.

Key word: Leaf extract of B*orreria hispida,* isolation of constituents

INTRODUCTION:

Borreria hispida is belongs to the family Rubiaceae. It is widely distributed in throughout India, up to 900m in hills and on all dry lands as a weed. The seed of *Borreria hispida* is used as PPAR-alpha gene expression, antioxidant redox status, protein metabolism in STZ diabetic rats. Potential role of *Borreria hispida* in ameliorating cardiovascular risk factor. [9]*Borreria hispida* seed flavonoid-rich fraction possesses free radical scavenging and antioxidant activity both *in vitro* and *invivo*. [10] *Borreria hispida Linn* has been in use in the Indian system of medicine.

Various part of the plant are useful in the treatment of antifertility, Appetite, Bleeding in child birth, body ache, eye discan, Gum trouble, Scabies and skin disease, Stomach compliance, Ulcers, Wounds, head ache and tooth ache[6][7]Also the rural people of Kanyakumari District (Tamil Nadu, India) use the fresh juice or the paste form of whole plant for tooth ache and various skin diseases.

MATERIALS AND METHOD:

Collection and Identification of Plant Material:

The whole plant of *Borreria hispida* (Linn), were collected from Midalam, Kanyakumari District of Tamil Nadu, India.Taxonomic identification was done by Dr. D. Stephen, Lecturer, Dept. of. Botany, The American College. The leaves of *Borreria hispida Linn* were collected in the month of July and dried in the shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts:

About 1 kg of above dry powdered materials was successively extracted with petroleum ether (40-600C) by continuous hot percolation method in Soxhlet apparatus [11]. The extraction was continued for 72 hours. The petroleum ether extract was filtered and concentrated to dry mass by using vacuum distillation. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The dark green residue was obtained. (14 gms)

The marc left after petroleum ether extract was taken and subsequently extracted with chloroform for 72 hours.The chloroform extract was then filtered and concentrated to a dry mass. A dark green residue was obtained. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained (4 gms)

Column Chromatography Of Petroleum Ether And Chloroform Extracts:

The combined extracts of petroleum ether and chloroform were chromatographed over about 200 gms of silica gel (100 – 200 mesh) using petroleum ether, benzene, chloroform, ethyl acetate, acetone and their mixtures in various proportions in the order of their increasing polarities.

The column was packed by using the suspension of silica gel in petroleum ether. Each 50 ml of the eluate was collected and concentrated.

The obtained fractions were tested for the presence of various constituents and nature of the compound. The fractions 46 to 74 and 84 – 90 were showed three different sports, indicating the presence of three different compounds. Hence these fractions were rechromatographed over silica gel (100 – 200 mesh). Table 2 and 3.

Preparation of Thin Layer Chromatography Plates:

About 30 gms of silicagel G was weighed and it was shaken with 100 ml of water to form a homogenous suspension. The suspension poured into a thin layer chromatography applicator which was adjusted to 0.25 mm thickness.

The plates were kept in the hot air oven at 110^{o}C for 1 hour to activate the silica gel G. They were then stored in a dry atmosphere and used whenever required.

By using the capillary tube, the eluate were spotted on the TLC plates around 2 cm above its bottom and subjected to chromatogram with different solvent system. The compound moved according to their affinity towards different solvent system. The plates after developed in each solvent system were observed under UV lamp. The different spots developed in each solvent system were identified by means of different spraying system and also by placing in iodine chamber. The results have been recorded as shown in the Table1.

Table 1-Data Showing Column Chromatography of Combined Petroleum Ether and Chloroform Extract:

Fraction	Eluates	Nature of residue	TLC
1-8	Petroleum ether-100	Colorless	Nil
9-14	Petroleum ether: Benzene 95:5	Light Yellow	Single spot
15-20	Petroleum ether: Benzene 80:20	Light Yellow	Single spot
21-27	Petroleum ether: Benzene 70:30	Yellow	Single spot
28-39	Petroleum ether: Benzene 50:50	Reddish Yellow	Single spot
40-45	Benzene: Chloroform 90:10	Orange	Single spot
46-58	Benzene: Chloroform 80:20	Reddish Orange	Three spots
59-64	Benzene: Chloroform 50:50	Reddish Orange	Three spots
65-74	Chloroform-100	Orange	Three spots
75-77	Chloroform: Ethyl acetate 90:10	Yellowish Orange	Single spot
78-80	Chloroform: Ethyl acetate 80:20	Yellowish Orange	Single spot
81-83	Chloroform: Ethyl acetate 70:30	Yellowish Orange	Single spot
84-86	Chloroform: Ethyl acetate 50:50	Dark green	Three spots
87-90	Ethyl acetate- 100	Dark green	Three spots

Re-Chromatography of Fractions 84-90: Chloroform-Ethyl acetate fractions from 84 to 90 were mixed and rechromatographed over silicagel (100- 200 mesh). **Table 2:**

Fraction	Eluates	Nature of residue	TLC
1-12	Petroleum ether -100	Colorless	Nil
13-15	Petroleum ether: Benzene 50:50	Colorless	Nil
16-18	Benzene-100	Colorless	Nil
19-24	Chloroform-100	Yellow	Single spot
25-33	Chloroform: Ethyl acetate 10:90	Orange	Single spot
34-42	Ethyl acetate-100	Green	Single spot
43-45	Ethyl acetate: Acetone 10:90	Dark Green	Single spot
46-54	Acetone- 100	Light Green	Single spot

Re-Chromatography of Fractions 46-74: Benzene - Chloroform fractions from 46 to 74 were mixed and rechromatographed over silicagel (100- 200 mesh).**Table 3**

Fraction	Eluates	Nature of residue	TLC
1-3	Hexane	Oily Residue	Single spot
4-8	Petroleum ether 100	Orange Viscous	Two spot
9-16	Petroleum ether: Chloroform 80:20	Brown solid	Single spot
17-20	Chloroform 100	Yellow viscous	Single spot
21-24	Chloroform: Ethyl acetate 80:20	Yellow viscous	Single spot

The fractions 4 to 8 also showed two sports on TLC, indicating the presence of two different compounds. These fractions were mixed together and dried to become a residue. The residue was treated with water. The water soluble portion did not show any spot on TLC. The water insoluble fraction was treated with ethyl alcohol then filtered and separated into ethyl alcohol soluble and insoluble fraction.

The ethyl alcohol soluble portion was treated with equal volume of distilled water and ethanol mixture (1:1) and then treated with ethyl acetate in separating funnel, which separated into organic layer and the aqueous layers. The process was repeated for at least three times to ensure complete extraction. The aqueous layer and organic layer have been separated. Both fractions were distilled to get the residue. The compounds C and D are obtained from the organic and aqueous layer respectively.

The fraction 75 to 83 were showed same R_f value , hence they were mixed together and evaporated to get the residue. The quantity of the compound obtained is too less. So we could not process the compound.

IR Spectra:

The IR spectrum for Compound 'A' , Compound 'G' and Compound 'H' were taken on Perkin Elmer model IR spectrophotometer using neat. The IR spectrum for compound 'B' , Compound 'C', Compound 'D', Compound 'E' and Compound 'F' were taken on Perkin Elmer model IR spectrophotometer using KBr.

NMR Spectra:

NMR for all the Compounds was taken on av 300, ^{1}H NMR (CDCI,), 300 MHz, TMS as standard.

RESULTS AND DISCUSSION:

Characterization of compound A:

Fractions 1 to 39 (Table 1) were showed positive results for **Sterols** and showed same R_f value on TLC. These fractions were mixed together and the reddish orange power (103 mg), (Compound A) was obtained. The compound A was soluble in chloroform and benzene, melting point 135-140°C, R_f value 0.6808

Table -5:

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
3482	O-H stretching		0.07934 - 1.97443	Due to protons of CH_3, CH_2 and CH of cyclic compounds, Polycyclic compounds
3357	O-H stretching			
3289	O-H stretching			
2927	SP3 C-H stretching			
1604	C=C double bond stretching			
146 1	C-H bending			
1373	C-H bending			
1101	C-O stretching of alcohol		5.1205	Due to protons of alkenes nature
973	C-H bending (Olefinic)			
871	C-H bending			

Characterization of compound B:

Fractions 40 to 45 (Table 3) were showed positive results for **Sterols** and showed same R_f value on TLC. These fractions were mixed together and the reddish orange viscous (300 mg) residue (Compound B) was obtained. The Compound B was soluble in chloroform and benzene, melting point 190 - 195°C, R_f value 0.7142

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
2923	C-H Stretching		0.6 – 2.3	May be due to protons of CH_3, CH_2 and CH
2850	C-H Stretching			May be due to steroidal ring protons.
1737	C=O Stretching			
1461	C-H Bending			
1376	C-H Bending		5 – 5.5	May be due to unsaturation.

Characterization of compound C:

Compound C, white crystalline powder (135 mg) has been obtained from the Fractions 4-8, (Table 2) by extraction process. Compound C showed positive results for **Sterols** and was soluble in chloroform and benzene, melting point 125 - 130°C, R_f value 0.66

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
3428	O-H Stretching		0.678 – 2.3	Groups of peaks represents cyclic protons of steroid ring
2937	SP^3 C-H Stretching			
1461	C-H Bending of SP^3		5.345 – 5.593	Indicates unsaturation
1373	C-H Bending			
1054	C-O Stretching		7.264 – 7.610	May be due to unsaturated lactone.

Characterization of compound D:

Compound D, viscous white residue (35 mg) has been obtained from the Fractions 4-8(Table 2) by extraction process. Compound D showed positive results for **Steroidal Saponins** and was soluble in chloroform and benzene, melting point 225°C, R_f value 0.822

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
3407	O-H Stretching		0.838 – 2.0	Due to protons of steroid ring.
2923	C-H Stretching			
2848	C-H Stretching		4.6 – 4.82	Due to methane protons attached with ester.
1737	C=O Stretching			
1656	C=O Stretching		5.334 – 5.684	May be due to unsaturation.
1459	C-H Bending			
1376	C-H Bending		7.387 – 7.61	May be due to unsaturated lactone.
1159	C-O Stretching			
1072	C-O Stretching			

Characterization of compound E:

Fractions 1 to 39 (Table 3) were showed positive results for **Terpenes** and showed same R_f value on TLC. These fractions were mixed together and the brown viscous residue (103 mg), (Compound E) was obtained. The compound E was soluble in chloroform and benzene, melting point 89-92°C, R_f value 0.8095

Table- 5:

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
			0.0706 – 0.8760	Due to alicyclic protons
3447	O-H Stretching			
2917	SP^3 C-H Stretching		1.1657 – 1.2279	Due to CH_3 protons
2849	C-H Stretching			
1700	C=O Stretching		2.0051-52.1109	CH_2 proton attached to C=O group
1696	C=O Stretching			
1096	C-O Stretching		5.1030 – 5.1250	May be due to unsaturated compound

Characterization of compound F:

Fractions 17 to 20 (Table 3) were showed positive results for **Sterols** and showed same R_f value on TLC. These fractions were mixed together and the yellow viscous residue (40 mg), (Compound F) was obtained. The compound F was soluble in chloroform and benzene, melting point 150°C, R_f value 0.7826

Table 6

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
3521	O-H Stretching		0.8326 – 1.143	May be due to methyl (CH_3) protons.
2927	C-H Stretching			
1644	May be C=N Stretching		1.253 – 1.479	Protons of CH_3, CH_2 and CH of fused rings of polycyclic compounds.
1463	C-H Bending			
1367	C-H Bending (saturated)			
1105	C-O Stretching		1.501 – 1.523	May be due to methane(CH) protons
968	C-H Bending			
873	C-H Bending		5.125	May be due to olefinic protons of alkenes nature
840	C-H Bending			
642	C-H Bending			

Characterization of compound G:

Fractions 34 to 42 (Table 3) were showed positive results for **Unsaturated Terpenoidal Ketone** and showed same R_f value on TLC. These fractions were mixed together and the dark green viscous residue (1.2 gms), (Compound G) was obtained. Melting point 128°C, R_f value 0.7380

Table -7:

IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
3513	O-H Stretching		0.693 – 2.368	Due to CH_3, CH_2 and CH protons of fused rings of a alicyclic compounds.
2927	SP^3 C-H Stretching			
2856	C-H Stretching			
1708	C=O Stretching			
1602	C=O Stretching		5.126 – 5.411	Cyclic unsaturated ketones.
1461	C-H Bending			
1378	C-H Bending			
1307	C-H Bending			

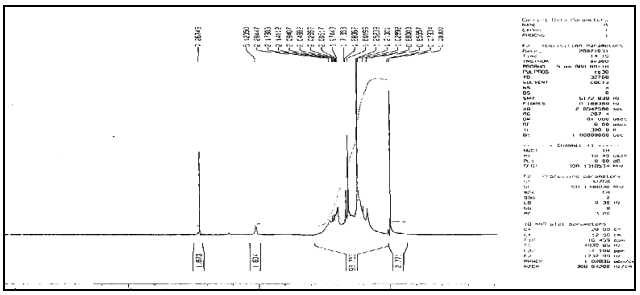
Characterization of compound H:

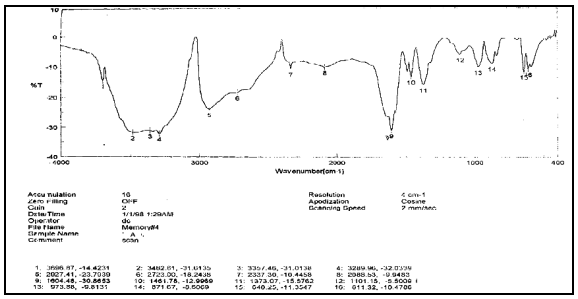
Fractions 43 to 54 (Table 3) were showed positive results for **Flavones** and showed same R_f value on TLC. These fractions were mixed together and the dark green viscous residue (30 mgs), (Compound H) was obtained. The compound H was soluble in chloroform and benzene, melting point 105°C, R_f value 0.8723

Table- 5:

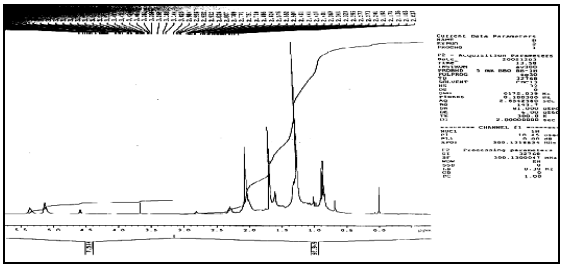
IR SPECTRAL DATA			NMR SPECTRAL DATA	
Frequency cm^{-1}	**Groups assigned**		**Signals(δ) values ppm**	**Groups assigned**
2925	SP^3 C-H Stretching		0.9765 – 1.0076	Due to CH_3, protons
2850	C-H Stretching		1.252	Due to CH_2, protons
1716	C=O Stretching		1.6776	Due to CH, protons
1602	C=C Stretching		2.0368 – 2.2881	Methylene group attached to carbonyls like CH_2-C=O
1376	C-H Bending			
983	C-H Bending (Olefinic)		5.35	Due to ethylenic proton

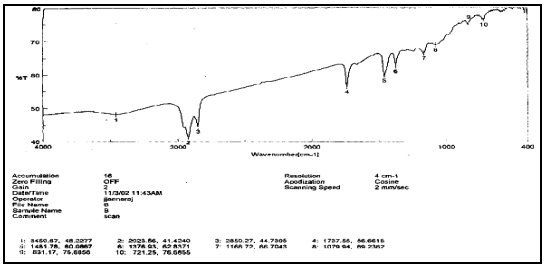
Spectra for Compound A:



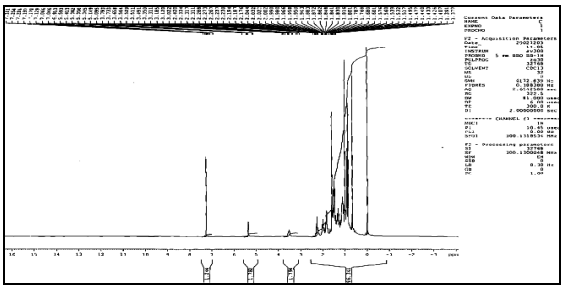


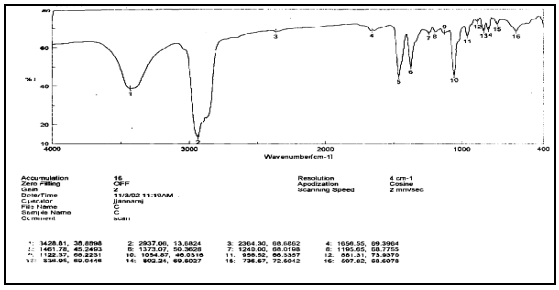
Spectra for Compound B:



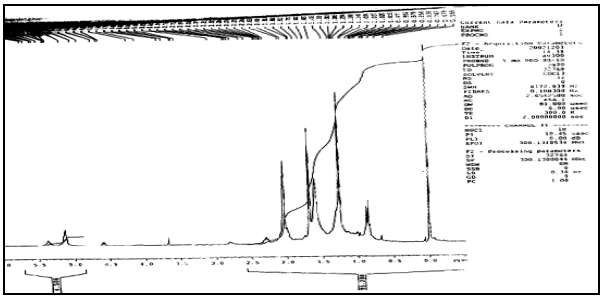


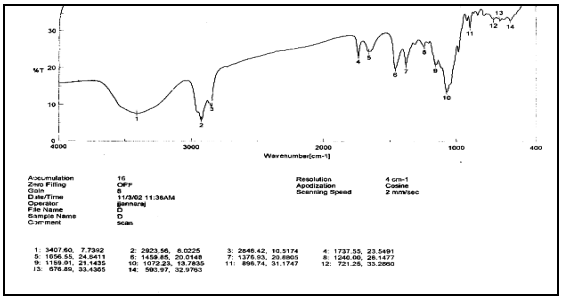
Spectra for Compound C:



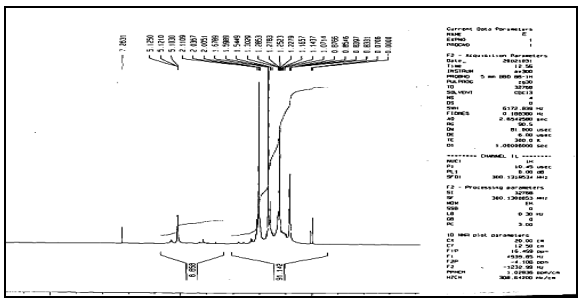


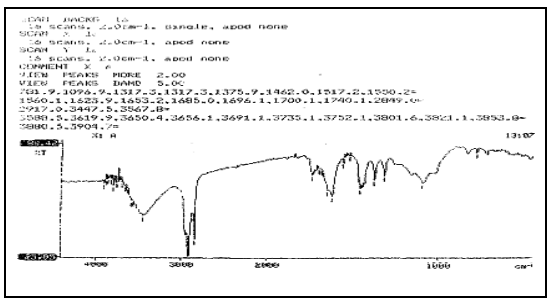
Spectra for Compound D





Spectra for Compound E





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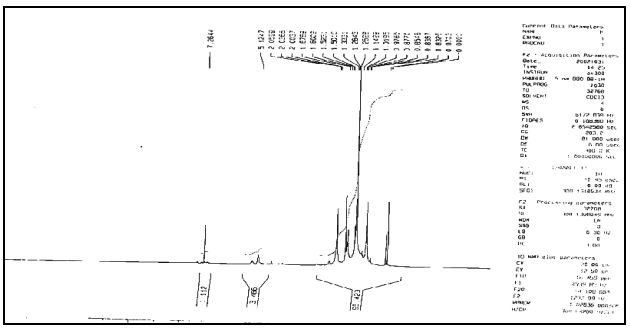
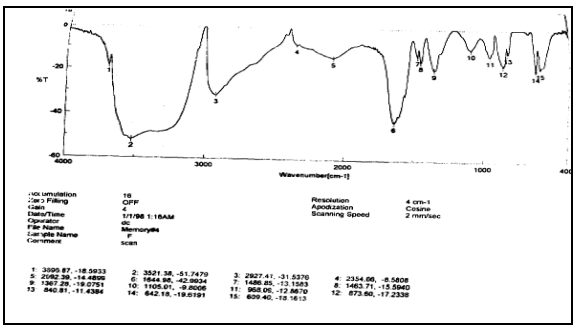
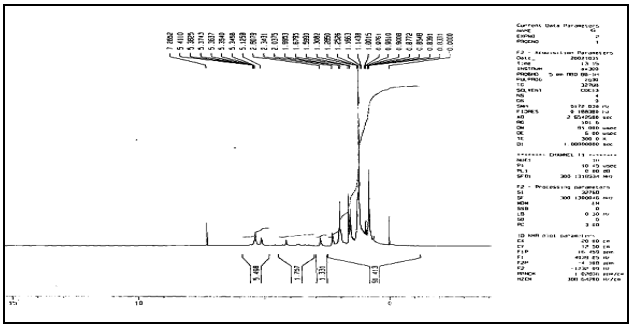
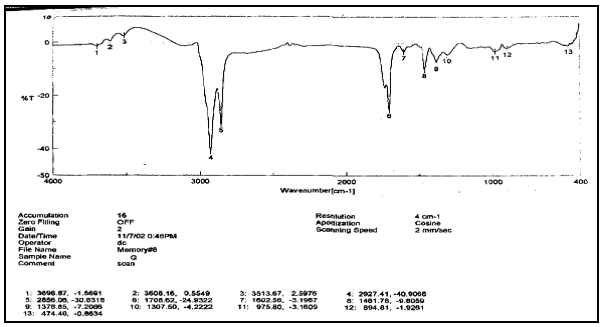


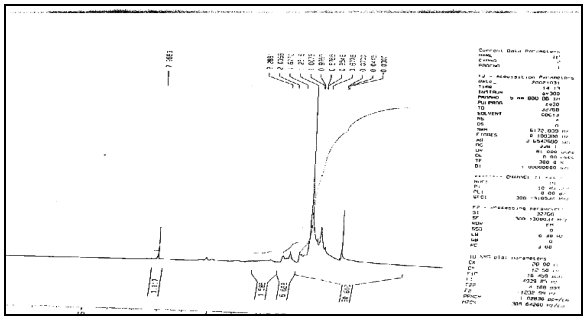


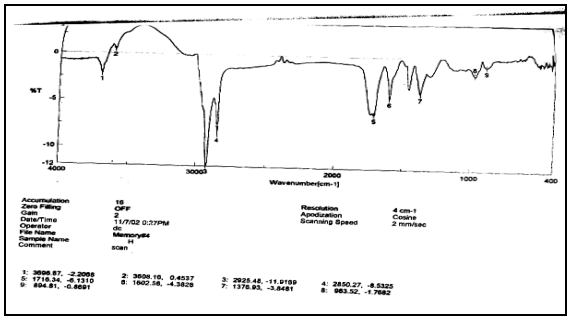
Spectra for Compound G:





Spectra for Compound H:





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