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CHROMATOGRAPHIC AND SPECTRAL STUDIES OF ACALYPHA INDICA

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ABSRACT

The prent study was used investgate the different techniques like TLC, HPTC used to finding the Rf values. Rf value of TLC is 0.49 and in HPTC of Rf value 0.49, scanned at 490nm and confirmed as stigma sterol. from the HPTLC study solvent ratio is optimized. the optimized solvent ratio used to isolate the stigma sterol through column chromatography. such isolated compound is analysed by spectral studies like HPLC, FTIR, MASS, NMR. from the HPLC study we know the percentage purity of the compound and FTIR sudy used confirm the functional grops of isolated compound, mass can be used to confirm the molecular weight of isolated compound, NMR can be used to determine the number of carbon atoms, hydrogens and various methyl, ethyl, hydroxyl grops of isolated compound.

KEYWORDS: TLC plates, HPTLC, HPLC, FTIR, MASS, NMR Spectroscopy.

INTRODUCTION

Chromatography is the separation of two or more compounds or ions by the distribution between two phases, one which is moving and the other which is stationary. These two phases can be solid-liquid, liquid-liquid or gas-liquid. The term chromatography means to write in color in greek and was introduced by the Russian botanist “Michel tswett” described his result by saying that the solvents according to the adsorption sequences are resolved in to variously colored zones such a preparation is termed as chromatogram and the corresponding method is the chromatographic method.[1,2] When column of stationary phase is used, technique is called as column chromatography. Based on the nature of stationary phase, that is whether it is solid, it is called as column adsorption chromatography.[3] Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of

absorption or emission of a solid or gas. an FTIR spectrophotometer simultaneously collects high spectral resolution data over a wide spectral range.[5] The technique of high permance liquid cromatography is so called because of its improved permance when compared to classical column chromatography.[6] It is also called as high pressure liquid chromatography. Mass spectra are also called as positive ion spectra or line spectra. Un like other kinds of spectroscopy, we do not use any electromagnetic radiation for excitation. We use electron bombardment to convert a neutral molecule to a positive charged one.[7,8] When protoan is studied, then it is called as proton magnetic resonance. When other nuclei like C^{13}, F^{19C}, CL^{35} etc is studied, then it is called as NMR. Generally in practice, th study of hydrogen itself is called as NMR spectra.[9,10,11]

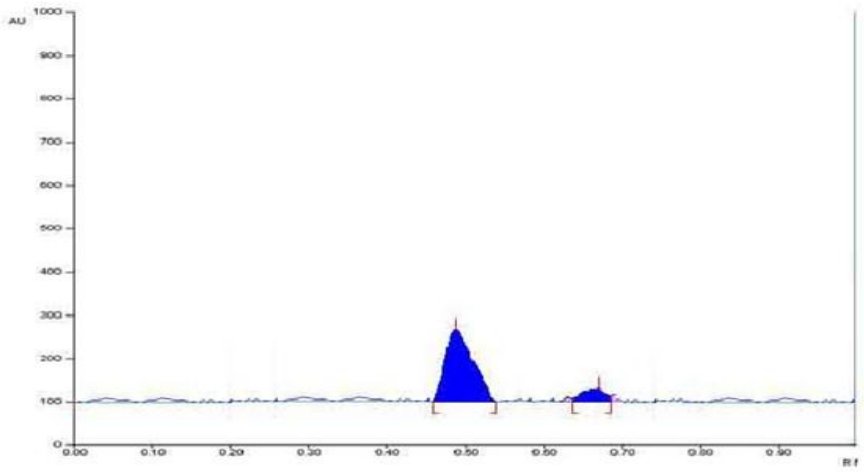
METHOD[3]

Packing the Column for Chromatography:

1. Take a cylindrical glass column and plug in a small piece of cotton at the bottom.
2. Mount the column on the stand.
3. 5 Lit mobile phase is prepared as per the optimization done by the HPTLC (i.e Chloroform: Ethanol (9.5: 0.5).
4. Sonnicate the mobile phase for 15 mins so as to remove the dissolved solvents from mobile phase.
5. Activation of silica gel is done at 130o C to 300o C for a 15-30 mins**.**
6. Take 200g of activated silica gel (for column chromatography 60-120 mesh) in a beaker.
7. Pour 500 ml of mobile phase into the beaker with constant stirring using a glass rod to make
8. slurry of the silica.
9. Initially pour mobile phase in to column and collect part of it from the bottom.
10. Pour the slurry into the column and column is tapped with rubber tubing to make perfect
11. packing without void in the packed column.
12. Washed sand was placed on top of the column packing so as to prevent disturbances in column packing. Then selected methanol exrtract of sample is placed on the top of the column then collect venis then analyzed by various spectral studies.

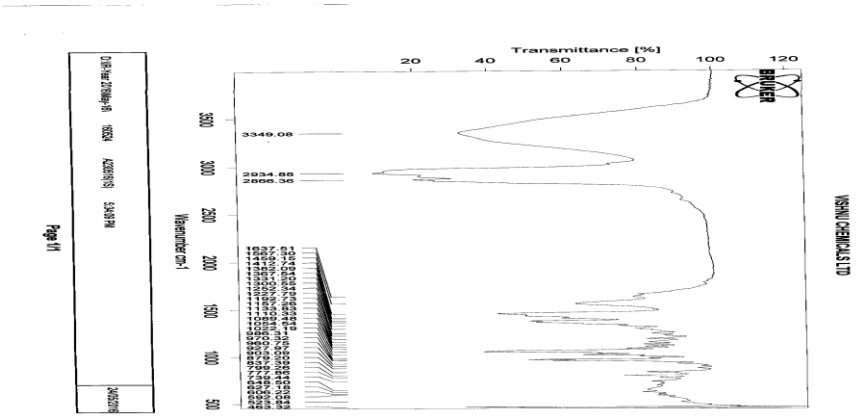
In chromatography study we can identify the Rf value of isolated compound stigmasterol. after that again isolated compound is analyzed by various spectral studies like TLC, HPTLC,

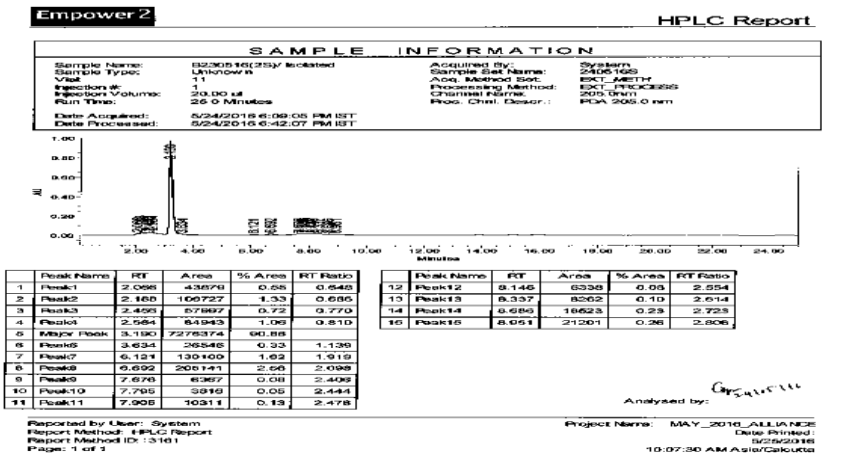
HPLC, FTIR, MASS, NMR spectral studies used to confirm the purity, functional group analysis, mass determination and confirmation of carbon numbers and proton numbers etc.



HPTLC chromatogram

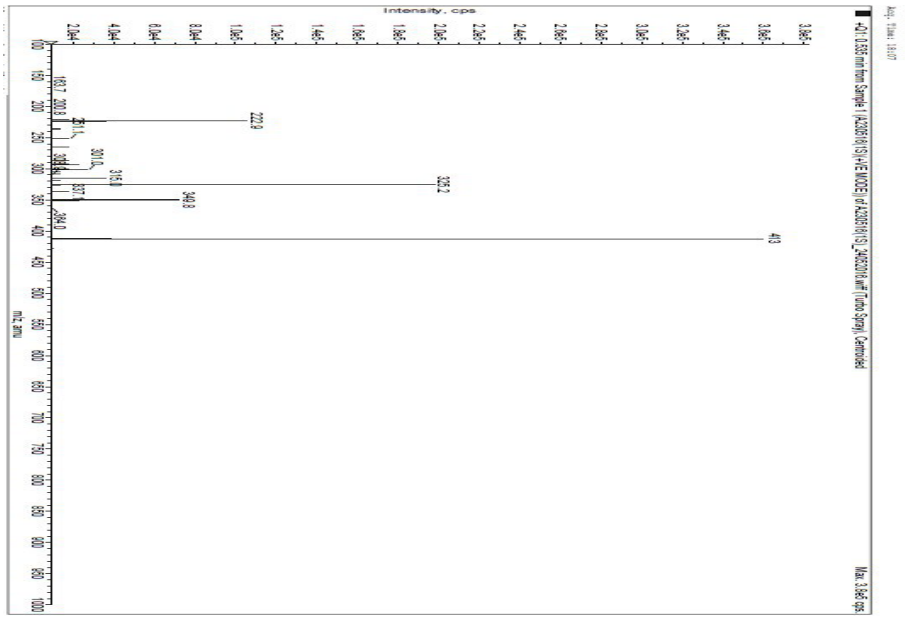
Chromatograph obtained at Scan of plate at 490 nm, Rf -0.49





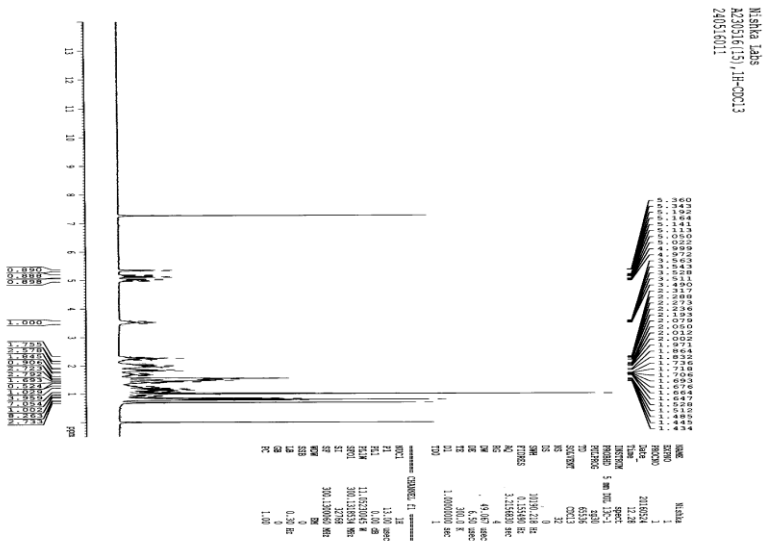
	Peak Name	RT	Area	% Area	RT Ratio
1	Peak1	2.066	43579	0.55	0.548
2	Peak2	2.165	106727	1.33	0.686
3	Peak3	2.455	57897	0.72	0.770
4	Peak4	2.584	84943	1.06	0.810
5	Major Peak	3.190	7276374	90.88	
6	Peak5	3.634	26546	0.33	1.139
7	Peak7	6.121	130160	1.62	1.919
8	Peak8	6.692	206141	2.56	2.098
9	Peak9	7.676	6367	0.08	2.405
10	Peak10	7.795	3816	0.05	2.444
11	Peak11	7.905	10311	0.13	2.478

	Peak Name	RT	Area	% Area	RT Ratio
12	Peak12	8.146	8338	0.05	2.554
13	Peak13	8.337	8262	0.10	2.614
14	Peak14	8.686	18623	0.23	2.723
15	Peak15	8.951	21201	0.26	2.806



HIGHEST BASE PEAK M+1=412

411+1=412



RESULTS AND DISCUSSION

from the HPTLC study wecan confirm the isolated compound stigma sterol. in case of spectral studies from interpretation of data we know the isolate compound may be stigma sterol.

The **FTIR** spectra of the **Isolated Compound (A230516)** show characteristic absorption bands at 3349 (-OH *Str*), 2934-2867(-CH *Str*, aliphatic), 1637(C=C *Str*), 1457(-CH- *Str*), 1054 & 881(Cycloalkane) groups respectively.

The molecular ion peak in their mass spectra was 413 (M+1), 411 (M-1) peaks identified respectively. Based on the above spectral data including IR, MASS, 1H NMR data and 13C NMR data is similar with respect standard values of stigmasterol. So the **Isolated Compound (A230516)** may be **STIGMASTEROL** as per the spectral data.

Interpretation of Spectral Data

The structures of the **Isolated Compound (A230516)** compounds were characterized as on the basis of satisfactory analytical and spectral data including, 1H NMR data and 13C NMR data.

The **1H-NMR** spectra of **Isolated Compound (A230516)** show signals in the range **1H NMR.**

(CDCL*3* δ ppm): 3.56(1H at C3), 5.36(1H at –C=CH, C6), 1.89-1.97(3H at -CH3,C18), 0.78(3H at-CH3,C19), 1.43-1.97(3H at -CH3,C21), 5.02(1H at –HC=C,C22), 5.36(1H at -C=CH,C23), 0.80(3H at -CH3,C26), 0.94(3H at -CH3,C27), 1.03(3H at -CH3,C29), 4.1(1H, -OH), 1.12-1.73(Cycloprotons) respectively.

13C-NMR (CDCL*3* δ ppm): 140.9 (C5), 138.4 (C20), 129.4 (C21), 121.8 (C6), 71.9 (C3), 12-57 (Cyclocarbons) respectively.

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