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Phytochemical Investigation and Fingerprinting of *Gymnosporia spinosa* Leaves Using Sophisticated Chromatographic Techniques

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Abstract : *Gymnosporia spinosa* leaves are often consumed by Indians for treatment of liver diseases.They also possess analgesic, anti-inflammatory and anticancer activity, along with uses in gastrointestinal, tooth and eye disorders. The present work focuses on developing an HPTLC fingerprint of*G. Spinosa*l eaves and detecting phytochemical constitution of the same using GC-MS. Methanolic extract of the leaves was prepared by maceration. This extract was used to develop a suitable mobile phase for fingerprinting. After mobile phase development involving several pilot TLC, the mobile phase showing distinct spots in TLC was found to be Chloroform: Methanol (9:1). The extract was further subjected to HPTLC fingerprinting where R_f and Area Under Curve were calculated. The extract was also analyzed using GC-MS. HPTLC fingerprinting of the methanolic extract showed five peaks at 254nm and eight peaks at 366nm, whereas GC-MS study structurally identified five phytoconstituents. This work provides simple techniques for the Pharma industry which can be utilized for standardization, quality control and detection of adulteration of *Gymnosporia spinosa* leaf formulations.
Keywords : Celastraceae, GC-MS, *Gymnosporia montana*, HPTLC, *Maytenus emarginata*, *Maytenus senegulensis*, Quality control.

Introduction

*Gymnosporia spinosa*syn. *Gymnosporia montana*, *Maytenus emarginata*, *Maytenus senegulensis* (Family – Celastraceae) leaves are found commonly across India and are known as Vyaghrapaadi (Sanskrit) and Vikdo (Gujarati)[1,2].They are said to possess potent hepatoprotective activity along with anti-inflammatory, anticancer and analgesic potential. They are also used in gastro-intestinal, teeth and eye-related disorders[1-7].The present work focuses on developing an HPTLC fingerprint of *G. spinosa* leaves as well as its phytochemical analysis using GC-MS (Fig. 1).

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Figure 1. *Gymnosporia spinosa* leaves

Experimental[8-22]

Crude drug material

G. spinosa leaves were collected from medicinal garden of RK University, in July 2015 and authenticated by Dr. Vivek Vegda, Botanist, School of Science, RK University.

Extraction

50g dry powder of *G. spinosa* leaves was macerated with 200ml methanol for 24h at room temperature. Methanolic extract was filtered and evaporated on water bath at 60°C to obtain the dried extract.

Mobile phase development

Pilot TLC were developed for methanol extract by preparing various mobile phases using various solvents like toluene, chloroform, n-butanol, ethyl acetate, methanol and distilled water. After observing the pilot results, further TLC were developed by adding ammonia & ethyl acetate for removal of tailing.

HPTLC

HPTLC fingerprinting of methanolic extract was performed in Dept. of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, using the mobile phase Chloroform: Methanol (9:1), as it gave most appropriate TLC fingerprint, under the following conditions…

Stationary phase: Silica gel 60 F 254 (E. Merck KGaA)
Sample application: CAMAG Linomat 5
Detection: CAMAG TLC Scanner 3
Lamp: D2 & W
Measurement type: Remission
Measurement mode: Absorption
Optical filter: Second order
Data filtering: Savitsky-Golay 7

Four tracks of same extract at different concentrations were run for the HPTLC fingerprinting and scanned under UV 254nm and UV 366nm.

Mobile phase development

Pilot TLC were developed for methanol extract by preparing various mobile phases using various solvents like toluene, chloroform, n-butanol, ethyl acetate, methanol and distilled water. After observing the pilot results, further TLC were developed by adding ammonia & ethyl acetate for removal of tailing.

GC-MS analysis

The methanolic extract was analyzed for its phytochemical constitution using GC-MS (Agilent) at Bioresearch & Characterization Centre, RK University, Rajkot, Gujarat.

Results and Discussion

Five peaks were detected at 254nm (Table 1, Fig. 2, 3) and eight peaks were detected at 366nm (Table 2, Fig. 4, 5) upon HPTLC of methanolic extract of *G. spinosa*leavesusing mobile phase Chloroform: Methanol (9:1).

Table 1. R_f & Area Under Curve of HPTLC of methanol extract at 254nm

Peak	Max R_f	Area	Area %
1	0.02	19810.1	80.07
2	0.16	450.0	1.82
3	0.47	301.7	1.22
4	0.59	3699.6	14.95
5	0.79	480.0	1.94

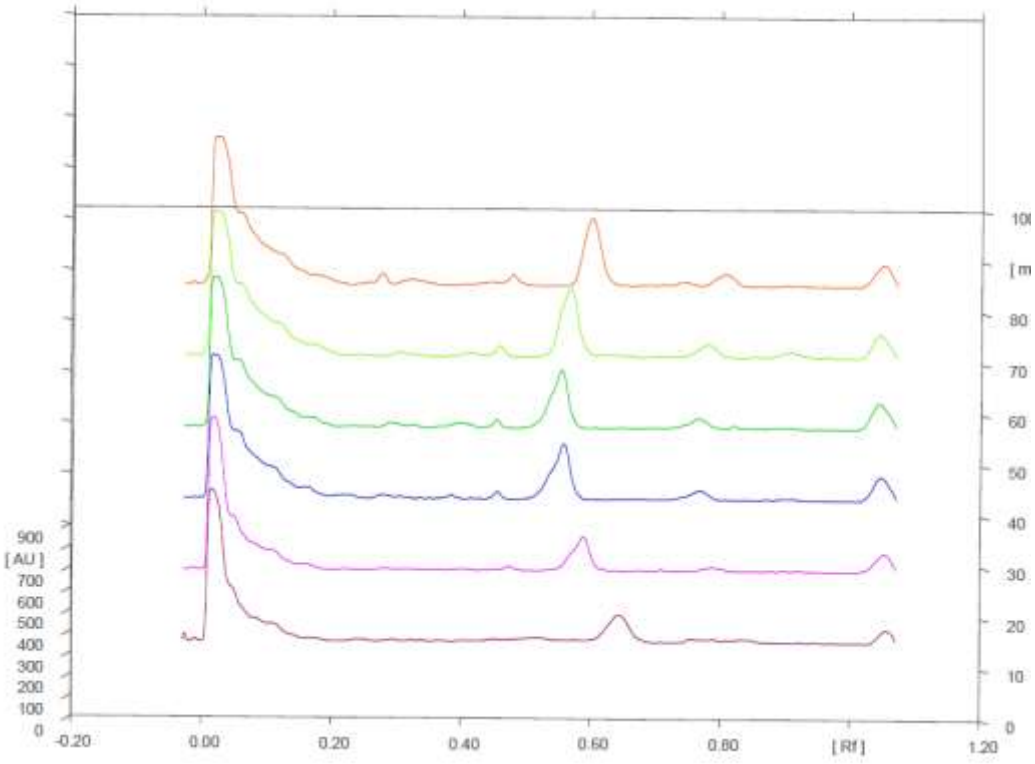

Figure 2: HPTLC 2Ddensitometric superimposable chromatogram of methanol extract at 254nm (chloroform: methanol 9:1)

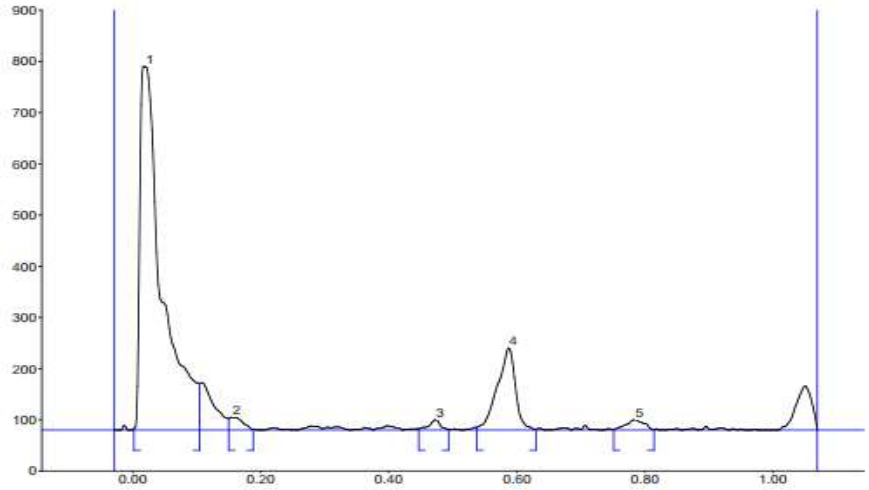

Figure 3: HPTLC chromatogram of methanol extract at 254nm(chloroform: methanol 9:1)

Table 2. R_f & Area Under Curve of HPTLC of methanol extract at 366nm

Peak	Max Rf	Area	Area %
1	0.02	14955.6	45.40
2	0.12	180.7	0.55
3	0.28	1614.1	4.90
4	0.40	762.3	2.31
5	0.48	397.4	1.21
6	0.59	12777.3	38.76
7	0.64	146.3	0.44
8	0.79	2123.6	6.44

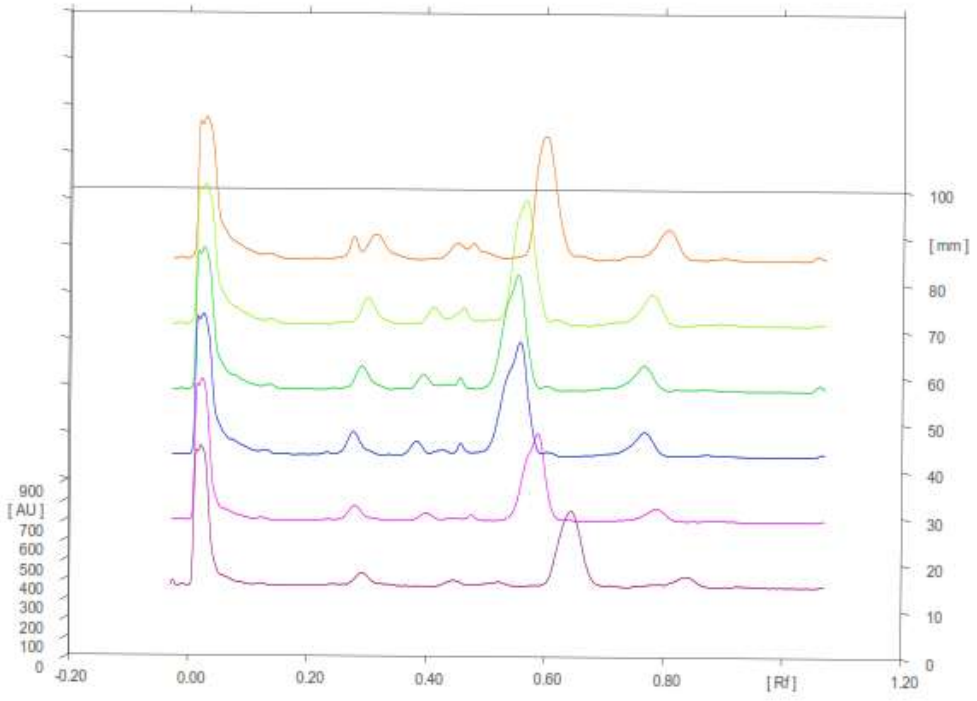

Figure 4: HPTLC 2D Densitometric superimposable chromatogram of methanol extract at 366nm (chloroform: methanol 9:1)

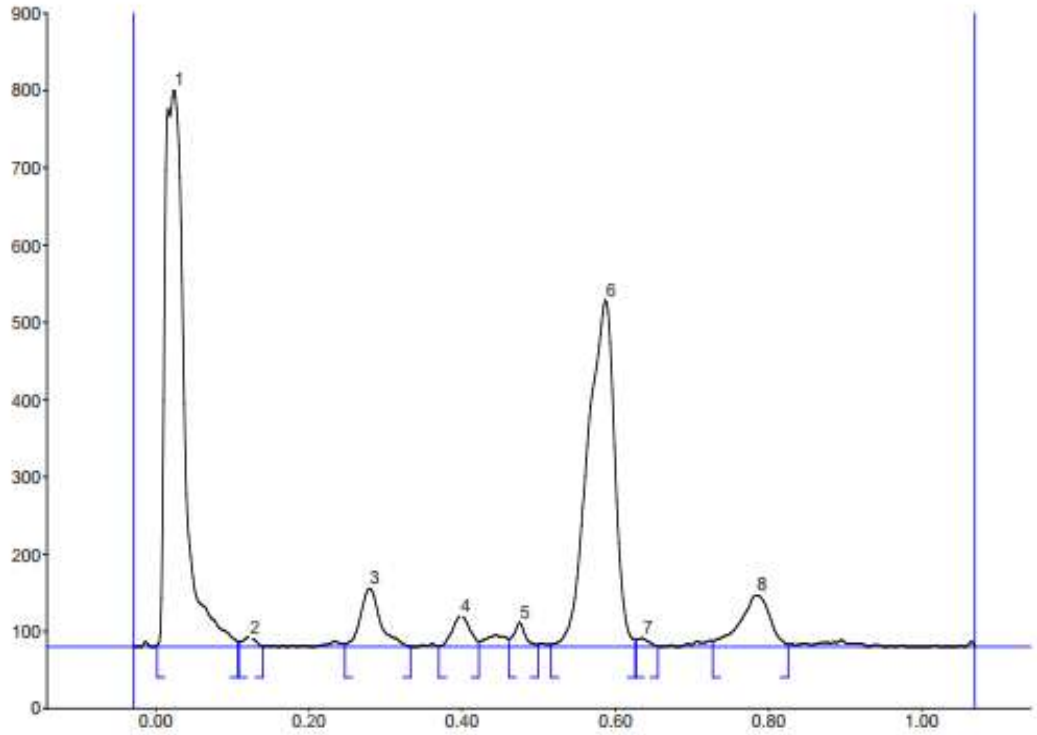

Figure 5: HPTLC chromatogram of methanol extract at 366nm (chloroform: methanol 9:1)

In the GC-MS analysis of the methanolic extract, five phytoconstituents were structurally identified: α-Amyrin, β-Amyrin, Dihydrotachysterol, Friedelan-3-one and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol.

Table 3. GC-MS data of Methanolic extract

Phytoconstituent	**Retention Time**	**Height**	**Area**	**Area%**
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	28.889	167922.37	483720.93	3.37
β-Amyrin	50.266	56339.8	334991.38	2.33
α-Amyrin	52.354	1532763.94	14355648.5	100
Friedelan-3-one	53.487	203985.95	2908056.61	20.26
Dihydrotachysterol	56.583	128282.82	675732.38	4.71

Table 4. m/z vs. Abundance of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

m/z	**Abund**
55.1	6147.76
57.1	5765.22
67.1	4178.82
68.1	4681.53
69.1	4921.87
71.1	8142.45
81.1	6827.58
82.1	5043.07
95.1	4342.79
123.1	5249.11

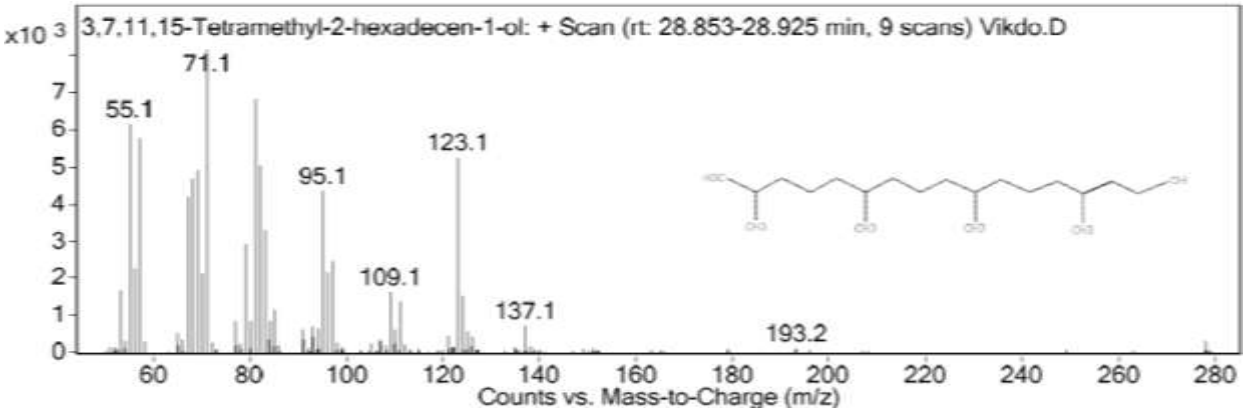

Figure6: Counts vs. m/z of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

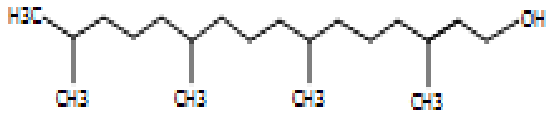

Figure 7: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol

Table 5. m/z vs. Abundance of β-Amyrin

m/z	Abund
55.1	19477.63
69.1	20212.53
91.1	16839.43
93.1	16558.16
95.1	19722.32
105.1	18786.46
119.1	19105.78
189.2	19905.93
203.2	60169.76
218.2	93116.83

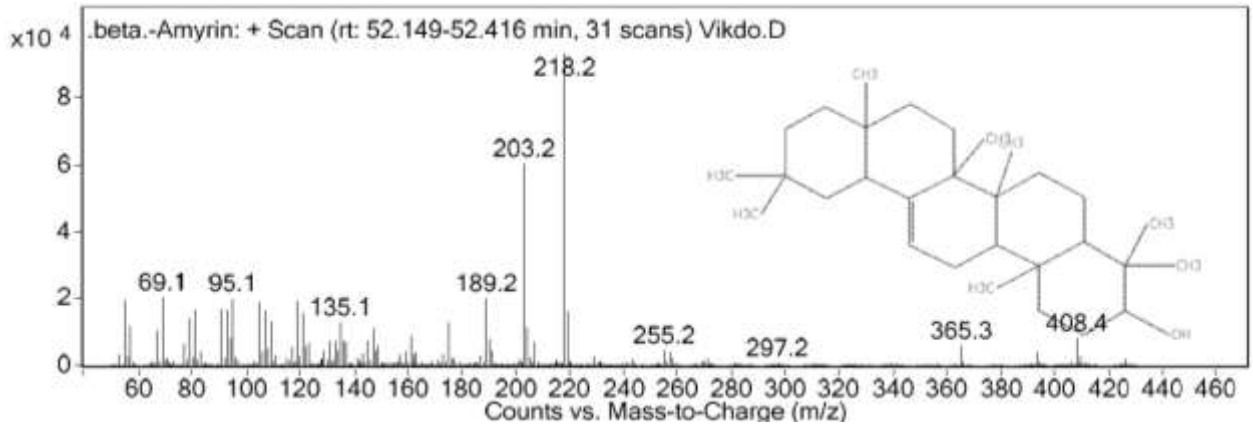

Figure 8: Counts vs. m/z of β-Amyrin

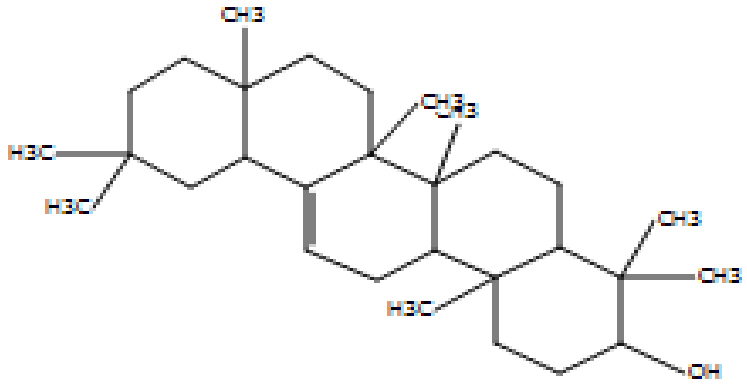

Figure 9: β-Amyrin

Table 6. m/z vs. Abundance of α-Amyrin

m/z	Abund
55.1	3418.71
67.1	2755.08
81.1	3328.15
91.1	2913.87
93.1	3659.66
107.1	3660.96
109.1	2796.04
121.1	3521.02
189.2	3833.97
218.2	5058.13

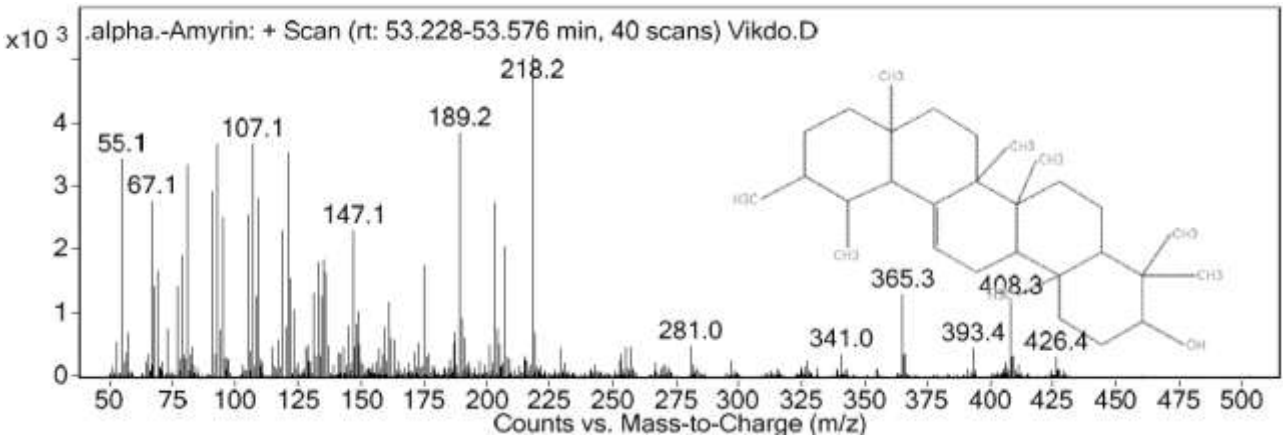

Figure 10: Counts vs. m/z of α-Amyrin

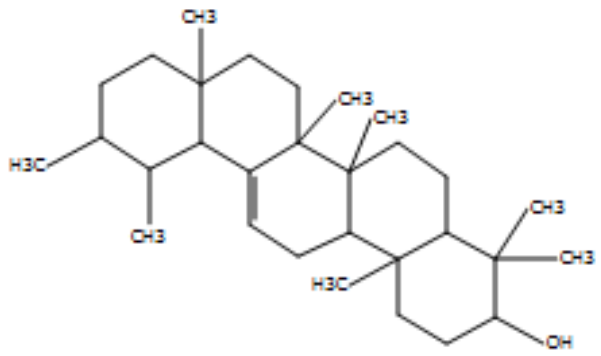

Figure 11: α-Amyrin

Table 7. m/z vs. Abundance of Friedelan-3-one

m/z	Abund
67.1	1901.8
69.1	2694.46
73.1	1742.33
81.1	2522.14
95.1	2167.32
109.1	2347.58
121.1	1591.11
123.1	2212.21
207	2665.56
281	1570.87

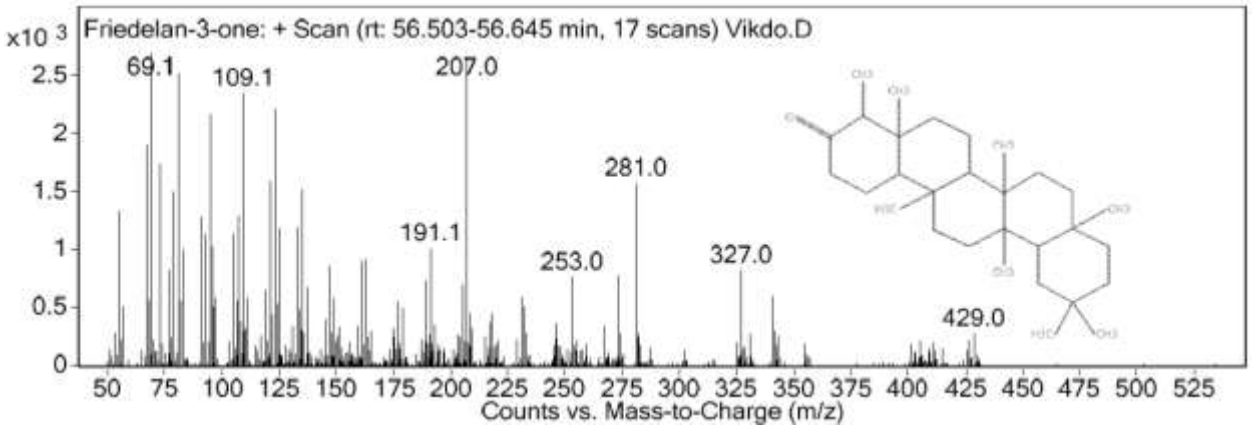

Figure 12: Counts vs. m/z of Friedelan-3-one

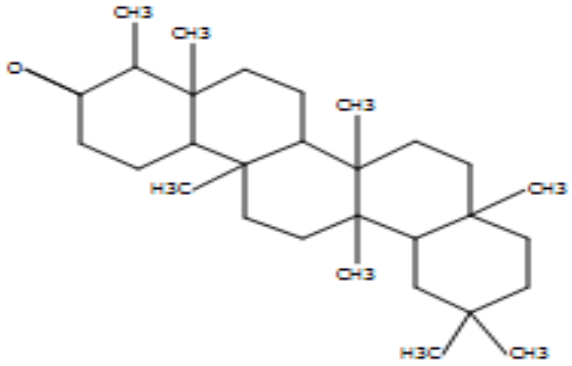

Figure 13: Friedelan-3-one

Table 8. m/z vs. Abundance of Dihydrotachysterol

m/z	Abund
55.1	808.44
57.1	497.3
73	764.15
91.1	502.23
95.1	583.3
105	493.22
107.1	665.55
147.1	501.76
207	549.23
381.3	501.62

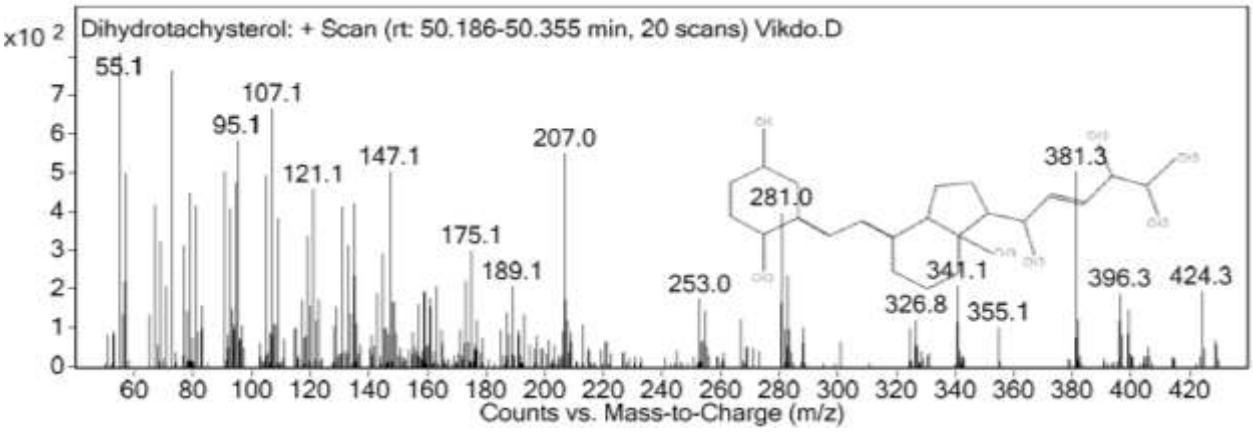

Figure 14: Counts vs. m/z of Dihydrotachysterol

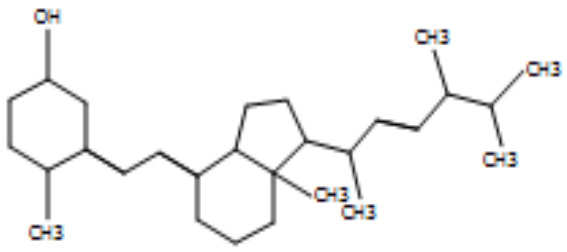

Figure 15: Dihydrotachysterol

The present work can be helpful to the herbal industry as an important quality control and standardization parameter of *G. spinosa* leaves, since they have shown tremendous potential in various diseases, specifically against liver disorders like jaundice, wherein they are popularly utilized[4-6].This work can be specifically useful for authentication of raw material of the leaves and in detection of its adulteration, which will ultimately benefit the people who consume *G. spinosa* leaf formulations.

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