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Chromatographic And Spectral Analysis Of Sesbania Grandiflora L.Bark

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ABSTRACT
Current research is being conducted to investigate the chromatographic analysis and appearance of the Sesbania grandiflora plant. L tools. Soxhlet is used for the extraction of organic solvent. Water and methanol are used as solvents. Plant quality analysis was performed using standard chemical methods. The results show that the presence of alkaloids, carbohydrates, phenolic compound, tannin, flavonoids and saponins was found in plant extracts. An investigation was under way to determine if there were any chemical compounds in the Sesbania grandiflora. L by analysis of HPTLC, UV, IR and NMR.

Keywords:- Phytochemical study, Sesbania grandiflora (L.) bark, spectroscopy, chromatography etc.

1. INTRODUCTION

The *Sesbania grandiflora* belonging to the family Fabaceae better known as *'sesbania'*, is widely used as a traditional Indian medicine. *S. grandiflora* has common names for Agati, Corkwood Tree and West Indian Pea, hummingbird tree (or red wisteria). In India, it is known as vaka or basna. Traditionally *Sesbania gradiflora* is used alone or with other medicinal plants to treat various ailments. It is a small tree believed to have originated in India or Southeast Asia and grows mainly in tropical and subtropical regions of the world. Originally from Asian countries such as India, Malaysia, Indonesia and the Philippines where it is often seen growing in costs between rice fields, roadblocks and vegetable gardens. This plant contains Grandifloral, arginine, cystine, histidine, isolucine, phenylalanine, tryptophan, valine, threonine, alanine, aspargine, aspartic acid and saponin which produces oleanolic acid, galactose, rhamnose and glucuronic acid and contains and flavonol glycoside, kaemp. The root-bark of the variety of red flowers helps in the rejuvenated state of vata and arthralgia. The bark separates, cools, becomes bitter, crumbly, anthelmintic and febrifuge. Squeezed bark is used externally to treat scabies. Bark juice is good for dyspepsia, diarrhea and gastralgia. [(Kirthikar and Basu, 1998), (Chatterjee, 1992), (Rastogi, 1960)]. Based on the above therapeutic features of the Sesbania grandiflora, in this study, we performed chromatographic analysis and appearance of plant bark scales.

2. MATERIALS AND METHODS

2.1. Plant Material

The plant material Sesbania grandiflora. (Fabaceae) bark is collected in the local area of Dhule district, MS, India. The planting material was clean and dry. It was also identified and validated by the Department of Agriculture, Shri Shivaji Vidya Prasarak Sanths's Late K. Dr. P. R. Ghogarey Science College, Dhule (Maharashtra) is a Voucher Specimen No. 110.

2.2. Preparation of the Extract

Dry bark is mechanically reduced to rough powder and then supported and stored in a sturdy air container at room temperature. The extraction method was based on the presence of active drug components, using a variety of solutions ranging from non-polar to polar. Dry powder was extracted in a sequence of methanol and refined water using a soxhlation method. The discharge was centered on the stand by simply removing the solvent at low temperatures using a rotating evaporator. The discharge was stored in an airtight container.

2.3. Chromatographic Separation

[(Wagner, 2007), (Egon, 2007), (Rangari, 2012)]

2.3.1 Thin layer chromatography:

Methanolic extract were evaluated by thin layer chromatography to identify the presence of number of phytoconstituent present in extract using specific solvent system and detecting reagents, which was found to give proper separation.

2.3.2 High performance thin layer chromatography (HPTLC)

The well-developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical chromatographic techniques. thin layer chromatography and high performance thin layer chromatography are methods commonly applied for the identification, assay and the testing of purity, stability, dissolution or content uniformity of raw materials and formulated products.

REAGENTS AND OTHER MATERIALS

Standard Quercetin, toluene, ethyl acetate [all reagents of analytical grade, [E-Merck] and silica gel 60 F 254 precoated thin layer chromatography aluminum plates [E-Merck]. Ammonia vapour and iodine crystal was used as a spraying reagent.

APPARATUS

Spotting device: Linomat V Automatic Sample Spotter; CAMAG (Muttenz, Switzerland)

Syringe: 100µL Hamilton (Bonadzu, Switzerland)

Thin layer chromatography (TLC) Chamber: Glass with trough chamber (20×10 ×4 cm) (CAMAG)

Densitometer: Thin layer chromatography scanner 3 linked to Win Cats Software (CAMAG)

High performance thin layer chromatography plates:

Identification and determination of the drug was performed on (10 cm × 10 cm. layer thickness 0.2

mm, E-Merck, Darmstadt, Germany) aluminum backed silica gel 60 F_{254} TLC plates, pre-washed with methanol.

Selection and optimization of mobile phase

Toluene: Ethyl acetate: Methanol, the mobile phase consisting of (5:4:1) (*v/v*) gave good resolution, sharp and symmetrical peak with R_f value for. Ammonia solution and Iodine crystal was used as a spraying reagent.

Selection of detection wavelength

For detection after derivatization of plate with Anisaldehyde- H_2SO_4, 5 min heated at 105^0C and scanned densitometrically at 370nm.

PREPARATION OF STANDARD AND SAMPLE SOLUTIONS

1) Preparation of sample solutions.

Sample solution was prepared by dissolving 10 mg of Methanolic extracts of plants *Sesbania grandiflora* in Methanol and making up the volume to 10 ml.

2) Standard solution of Quercetin

The quercetin stock solution was prepared by dissolving 5 mg and 1 mg of well-balanced quercetin in methanol and creating a volume up to 10 ml with methanol to obtain a final concentration of 500 µg / ml and 100 µg / ml.

2.4 Column Chromatography

Column chromatography is one of the most useful methods of separation and purification of solids and liquids. Column chromatography is another solid liquid process in which both phases are solid (vertical) and liquid (Phase).

Isolation of phytocontituent from Solvent ether soluble fraction by column chromatography

Isolation of phycontsituent was carried out on Solvent ether soluble fraction of methanolic extract of *Sesbania grandiflora*, as the particular fraction revealed compound.

Absorbent: Silica gel 60-120.

Column dimension: Length -45cm, Diameter-2.2 cm.

Packed adsorbent length: 26cm

Elution Rate: 5-6 drops/min

Volume of elute collected : 20ml

Elution type: Isocratic elution

Column packing:-

Sample preparation:

20ml of solvent ether soluble fraction of methanolic extract was mixed well with 20 gm silica gel and at 45°C dried in vaccum oven. The adsorbed material transferred to the column.

PREPRETION OF COLUMN:

150 gm of silica gel for column chromatography was activated in hot air oven at 110°C for 1hr. The adsorbed bed was developed in mobile phase which was initially packed with glass wool. The glass wool is trimmed at the bottom of the column. The activated silica slurry made was made in Toluene: Ethyl acetate: Methanol (5: 4: 1) and applied to the column in small portions by keeping the buttocks open with a small tap after each installation to ensure uniform packaging. Then fractions collected with eluted mobile phase. Fractions collected were further concentrated. Each fraction was evaluated by thin layer chromatography to detect the number of phytoconstituent present in it.

Thin layer chromatography of isolated fraction after column chromatography:

Stationary phase : Silica gel G

Mobile phase ; Toluene: Ethyl acetate: Methanol

Proportion : 5: 4: 1

Visualizing agent : Ammonia solution and Iodine crystal

All fractions showed one spot on thin layer chromatography plates. Hence all fractions are collected.

2.5 Characterization of isolated compound:

The characterization of isolated compound was carried out through evaluation of physical properties, Chemical tests, Melting point.

2.5.1 Physical properties:

The Purified crystals of isolated compound observed for color, shape.

2.5.2 Chemical tests:

The isolated compound observed for compound observed for chemical tests.

2.5.3 Melting point.

The purified isolated compound observed for melting point.

2.6 Spectroscopic Analysis

2.6.1 Ultraviolet Spectrophotometer (U.V. Spectrum):

The solution of parts per million (ppm) was prepared. Isolated fraction compound and shimadzu 1800 UV Spectrophotometer with 1cm matched quartz cells was used to obtained U.V Spectrum of isolated fraction compound.

2.6.2 Fourier transform infrared spectroscopy (FTIR):

FTIR has proven to be available tool for the characterization and identification of compound functional

groups (chemical bonds) present in an unknown mixture of plant extract

2.6.3 ^{1}H- Nuclear magnetic resonance (NMR):

^{1}H-NMR spectra has been important tool for identification of purity and characterization of isolated compound.

2.6.4 Mass spectrum:

A mass spectrum has been also important tool for identification of purity and characterization of isolated compound.

3. RESULTS

3.1 Chromatographic Studies

3.1.1 Thin layer chromatography of Metabolic extract of Bark of *Sesbania grandiflora*

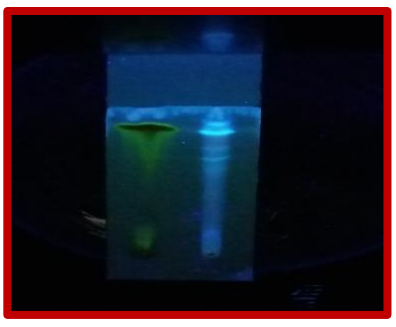
Silica gel G used as Stationary phase, Toluene: Ethyl acetate: methanol (5:4:1) as mobile phase and detected by ammonia solution and iodine crystal.

A B

Fig 3.1 Under UV cabinet without spraying reagent

A:-Spot of Standard Quercetin

B: -Spot of Methanolic extract of plant *Sesbania grandiflora*



A B

Fig 3.2 TLC with Ammonia solution

A B

Fig 3.3 TLC with Iodine crystal

A:-Spot of Standard Quercetin

B: - Spot of Methanolic extract of plant *Sesbania grandiflora*

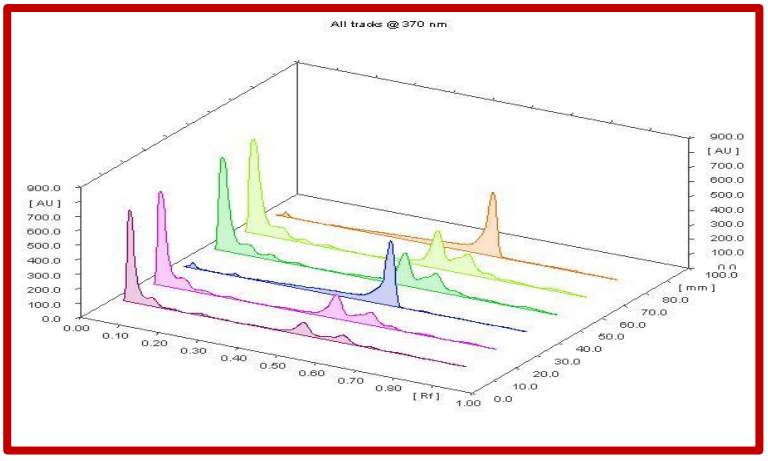


Figure 3.4 3D images of all tracks of Standard Quercetin and extract at 370 nm.visualized by Ammonia solution and Iodine crystal.

3.1.2 High performance thin layer chromatography (HPTLC)

Stationary phase : Silica gel G pre coated plate.

Mobile phase : Toluene: Ethyl acetate: Methanol

Proportion : 5:4: 1

Detection : Ammonia solution and Iodine crystal

Solvent front : 8cm

No of tracks **:** 6

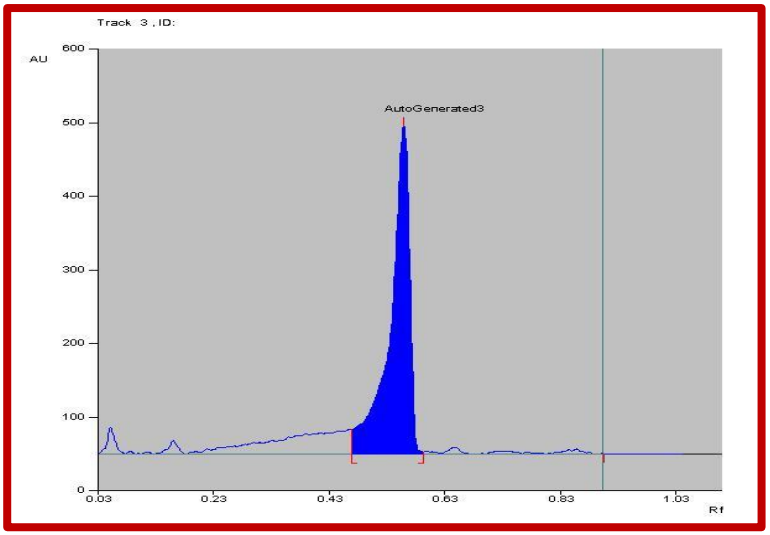


Figure 3.5 HPTLC Chromatogram for Methanol extract of bark of plant *Sesbania grandiflora*

With Toluene: Ethyl acetate: Methanol (5:4:1) as mobile phase Scanned at 370 nm.

Table 3.1 Linearity of Methanol extract of bark of plant *Sesbania grandiflora* L.

Sr No.	Peak area	Rf Value
1	2074	0.59
2	3644	0.58
3	12772	0.57
4	4958	0.59
5	5583	0.58
6	13365	0.59

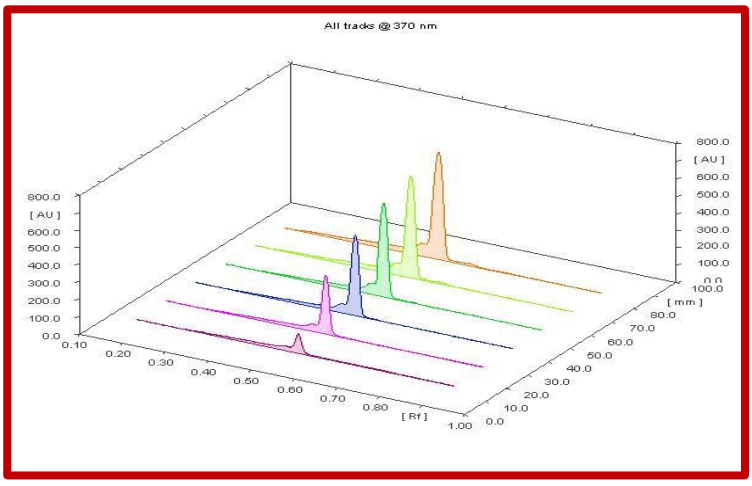


Figure. 3.6 3D spectra of Standard Quercetin in linearity curve

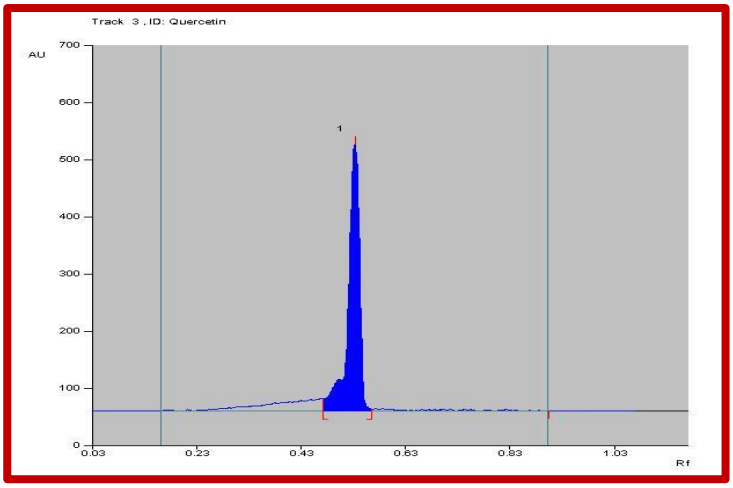


Figure 3.7 HPTLC Chromatogram of Standard Quercetin

With mobile phase Toluene: Ethyl acetate: Methanol (5:4:1) Scanned at 370nm.

Table 3.2 Linearity of Quercetin.

Sr No. N	Concentration (ng/µl)	Peak area
1	500	1566
2	1000	4842
3	1500	8300
4	2000	10331
5	2500	12061
6	3000	14224

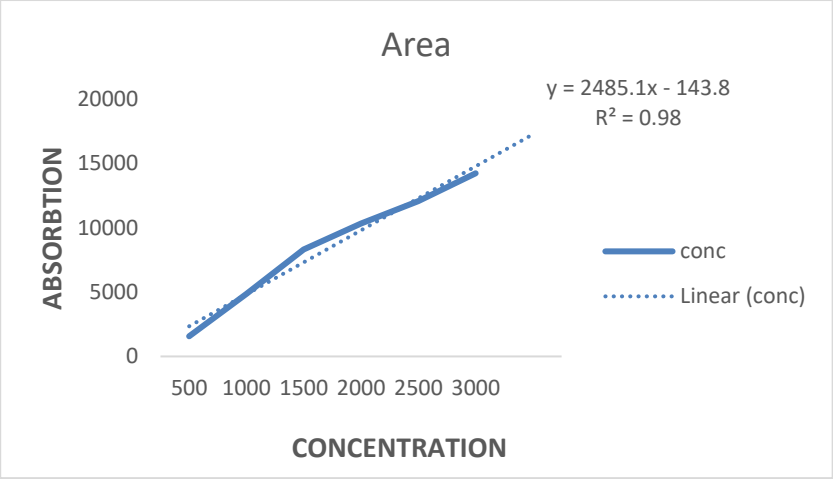


Figure 3.8 Calibration Curve for Standard Quercetin.

Y= 2485.1X + 143.8

Coefficient of correlation = 0.98; Slope =2485.1

3.2 Column chromatography

Table 3.3 Result of Column chromatography of plant *Sesbania grandiflora*

Sr. No.	Fractions	No of spots	Color	Rf value
1	1-4	No spot	-	-
2	5-8	No spot	-	-
3	9-14	Single spot	Yellowish green	0.59
4	15-20	Single spot	Yellowish green	0.59

Table 3.4 Chemical test of isolated compound

Sr. No.	Chemical test	Absorbance	Infernce
1	Sodium hydrate	Decolorise	Flavonoid may be present

Table 3.5 Yield of isolated compound

Sr. No.	Isolated compounds	Yield from column
1	Compound Q	1mg

Table 3.6 Parameters of isolated compounds

Sr. No.	Parameters	Isolatedcompounds
1	Physical state	Solid crystalline
2	Color	Yellowish green
3	Solubility	Methanol, ethanol, water
4	Meltingpoint	312-314 °C

3.3 Spectral Analysis

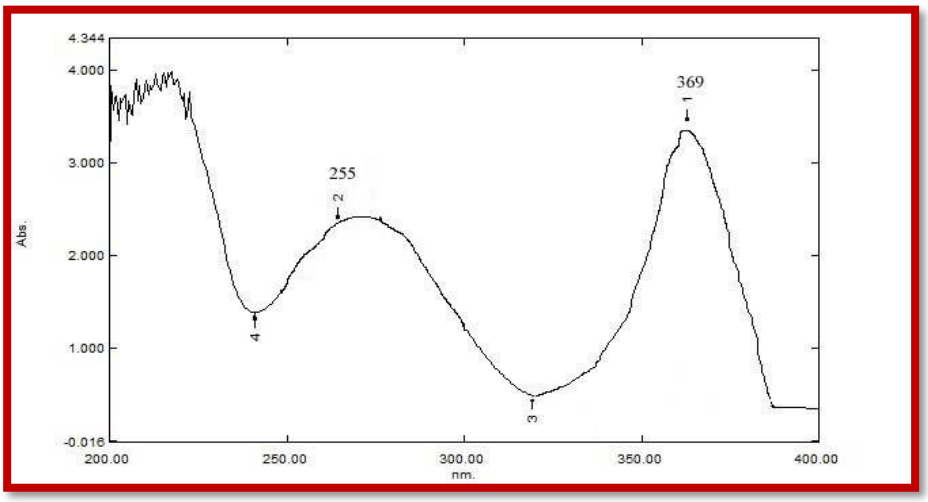


Figure 3.9 UV spectra of isolated compound-Q

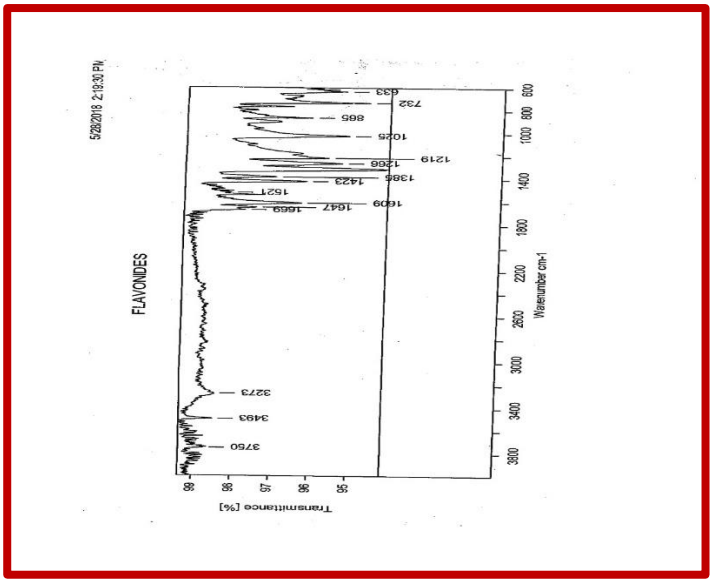


Figure 3.10 FT-IR spectra of isolated compound-Q

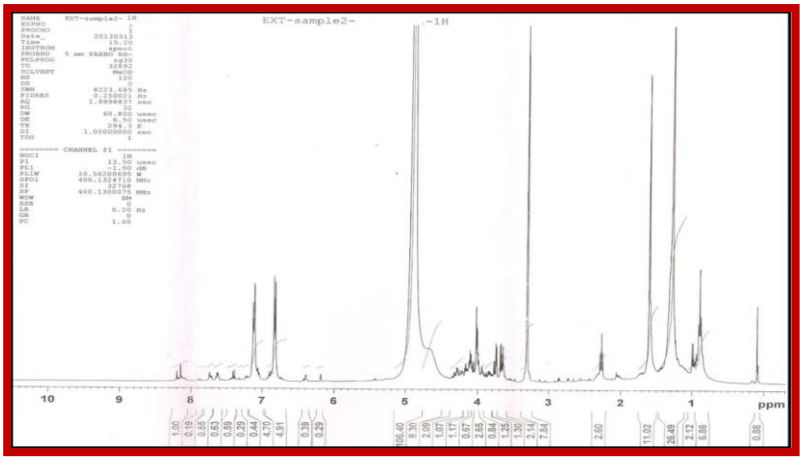


Figure 3.11 NMR spectra of isolated compound-Q

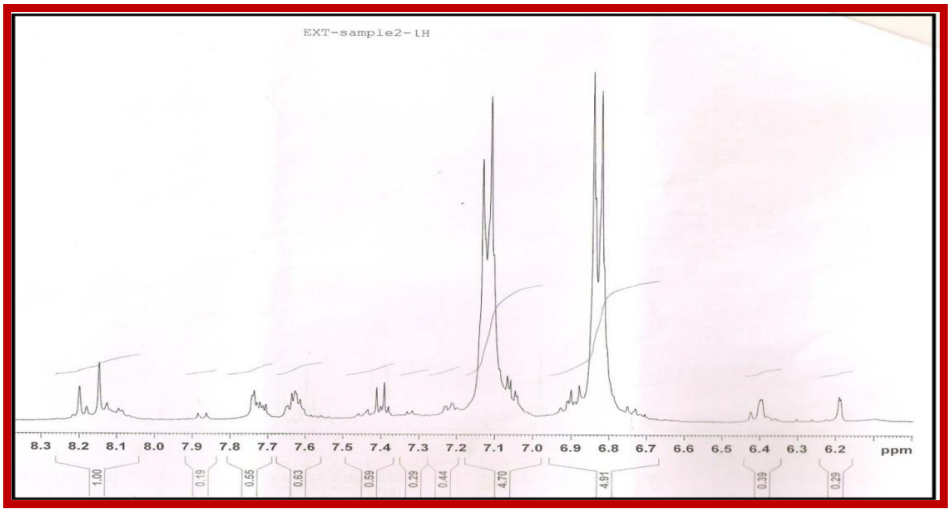


Figure 3.12 NMR spectra of isolated compound-Q

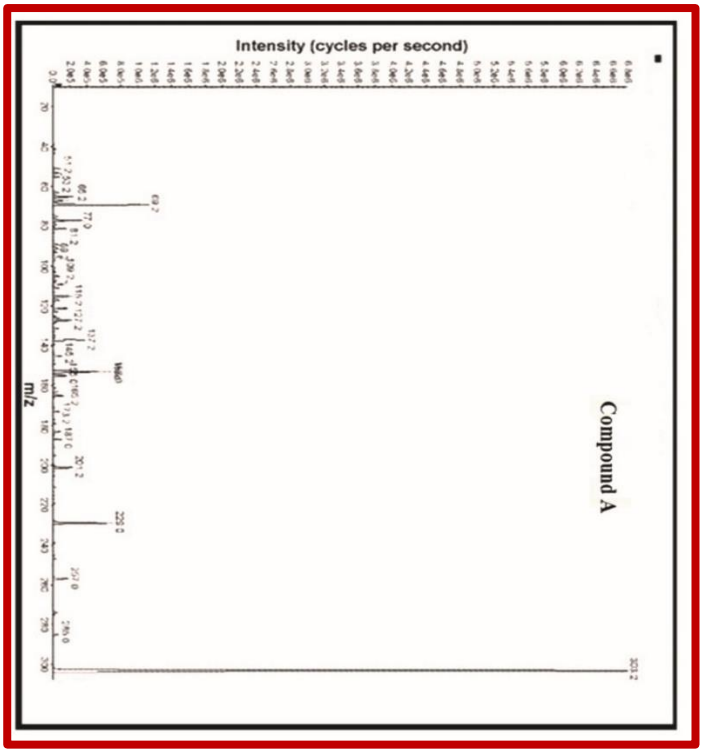


Figure 3.13 MASS spectra of isolated compound-Q

Peak	v(F1) [ppm]	v(F1) [Hz]	Intensity [rel]
1	8.2158	3287.3881	0.02
2	8.1985	3280.4659	0.07
3	8.1798	3272.9834	0.03
4	8.1465	3259.6591	0.11
5	8.1254	3251.2164	0.04
6	8.0951	3239.0924	0.03
7	8.0848	3234.9711	0.03
8	7.8848	3154.9451	0.02
9	7.8626	3146.0622	0.02
10	7.7372	3095.8859	0.06
11	7.7302	3093.0850	0.04
12	7.7224	3089.9640	0.04
13	7.7143	3086.7229	0.03
14	7.7060	3083.4018	0.04
15	7.6465	3059.5941	0.04
16	7.6360	3055.3927	0.06
17	7.6281	3052.2317	0.06
18	7.6139	3046.5499	0.05
19	7.4348	2974.8866	0.03
20	7.4117	2965.6436	0.07
21	7.4007	2961.2421	0.04
22	7.3915	2957.5610	0.08
23	7.3807	2953.2395	0.03
24	7.3310	2933.3531	0.03
25	7.3182	2928.2314	0.03
26	7.2286	2892.3798	0.04
27	7.2137	2886.4178	0.04
28	7.2004	2881.0961	0.03
29	7.1331	2854.1674	0.49
30	7.1116	2845.5646	0.60
31	7.0665	2827.5187	0.09
32	7.0584	2824.2776	0.08
33	7.0461	2819.3560	0.06
34	7.0408	2817.2354	0.06
35	6.9267	2771.5805	0.03
36	6.9078	2764.0181	0.05
37	6.9003	2761.0171	0.07
38	6.8892	2756.5756	0.04
39	6.8790	2752.4943	0.07
40	6.8427	2737.9696	0.65
41	6.8374	2735.8489	0.42
42	6.8207	2729.1667	0.61
43	6.7512	2701.3577	0.04
44	6.7296	2692.7149	0.03
45	6.4252	2570.9153	0.03
46	6.3990	2560.4319	0.05
47	6.3949	2558.7914	0.05
48	6.1914	2477.3649	0.06
49	6.1869	2475.5643	0.05
50	4.9062	1963.1178	15.00
51	4.4335	1773.9764	0.04
52	4.4178	1767.6943	0.04
53	4.3561	1743.0063	0.05
54	4.3460	1738.9650	0.05
55	4.3277	1731.6426	0.07
56	4.3168	1727.2812	0.07
57	4.3059	1722.9198	0.06
58	4.2898	1716.4777	0.10
59	4.2785	1711.9562	0.10
60	4.2638	1706.0743	0.06
61	4.2566	1703.1934	0.06
62	4.2501	1700.5925	0.08
63	4.2243	1690.2692	0.08

Figure 3.14 MASS spectra of isolated compound-Q

Table 3.7 Results of plant *Sesbania grandiflora.*

Spectra	Characteristics
U.V	Two peak with λ max at 369nm
FT-IR	Peaks at following wave number are observed Wave number(cm^{-1}) 3037.02 C-H Stretching 1699.34 C=O Stretching 3354.32 O-H Stretching 1606.76 C=C Stretching 1291.39 O-H bending 1119.71 C-O-C Stretching
^{1}H NMR	Peaks at following delta values are observed. Delta value 7.73 (1H, H-6, S) 4.90 (1H, H-3, H-4, S) 6.87 (1H, H-3) 6.394 (1H, H-7, S) 6.186 (1H, H-5, S) 7.613-7.737 (Multiplets, all remaining protons Of aromatic rings
Mass	Base peak at 303.2

4. DISCUSSION AND CONCLUSION

In case of phytochemical investigation the Methanolic extract were introduced for chromatography separation by using TLC, HPTLC chromatography for separation of important phytochemical present in Methanolic extract which shows potent pharmacological activity. The Methanol extract were subjected for Thin Layer Chromatography and observe numbers of spots compared with standard Quercetin. The methanolic extract was subjected to TLC with the mobile phase toluene: ethyl acetate: methanol with (5:4:1) ratio. Visualizing agent were used is UV cabinet, Ammonia vapors, iodine crystals. The methanolic extract were shows few phytoconstituents at R_F value 0.59. The R_f value for standard Quercetin (0.53).

In case of natural product analysis, High Performance Thin layer Chromatography (HPTLC) is more widely used than other chromatographic methods. In the present work, an attempt has been made to develop and validate new, fast, precise, accurate, and robust HPTLC method for concurrent quantification of Quercetin. Results were obtained indicate the reliability of the proposed densitometric method. To obtain the desired Rf value, minimum resolution, different solvent systems containing various ratios of toluene, dichloromethane, n-hexane, ethanol, methanol, water, ethyl acetate, and acetone were tried. Finally, the solvent system composed of toluene: ethyl acetate: methanol (5:4:1) was selected for obtaining well separated peaks. The wavelength used for detection and quantification

was 370 nm. The Rf value for standard Quercetin was found to be 0.54.

Column chromatography was used for separation of active phytoconstituent present in methanolic extract by using mobile phase toluene: ethyl acetate: methanol (5:4:1).

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