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ResearchArticle

HPTLC Fingerprint Profile of Extracts from Leaf, Bark and Flower of *Tecomella undulata* (Seem) in Different Solvents

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ABSTRACT

Tecomella undulata (Seem), family Bignoniaceae, is an important medicinal plant found in western region of India and traditionally used in indigenous system of medicine. An attempt has been made to give a complete HPTLC profile for leaf, bark and flower of *Tecomella undulata* (Seem). Methanolic, chloroform and acetone extracts were prepared using sonication. The mobile phase of Toluene: Ethyl acetate: Formic acid was used in different ratio for good separation. The fingerprint showed better resolution after derivatisation in methanolic sulphuric acid reagent. The fingerprint has revealed a distinct and repetitive pattern of bands. Acetone extract of leaf and bark shows 14 different Rf values whereas flower shows maximum separation in chloroform. HPTLC profile of the plant will be helpful for crude drug identification, serve to check the adulteration and also act as biomarker for this plant in the Pharmaceutical industry.

Keywords: HPTLC, *Tecomella undulata* (Seem), Standardization.

INTRODUCTION

Tecomella undulata (Seem) is a very important medicinal plant commonly found in Rajasthan, India. It is commonly known as Rohida (Hindi), Rohitaka (Sanskrit) and Ammora (English). Due to its presence in arid and semi-arid regions of Thar Desert, it is also known as Desert teak. Locally it is popularly known and traded by the name of Marwar teak or Desert teak. *Tecomella undulata* (Seem) is famous for its quality timber; use of its leaves, flowers and pods as fodder; and its secondary metabolites having therapeutic properties. It was observed that the traditional medicinal practitioners use a total of seven Bignoniaceae family species, including *Tecomella undulata* (Seem), for treatment of ailments like cancer, snake bite, skin disorders, gastrointestinal disorders, respiratory tract disorders, gynecological disorders, hepatic disorders, epilepsy, cholera, pain, urinary problems, malaria, heart problems, and sexually transmitted diseases[1]. Its therapeutic uses are also mentioned in Ayurveda[2,3]. Pharmacological activities having phytochemicals like flavonol[4], triterpenoids[5] phytopsterols[4], napthoquinone[6,7] iridoid glucoside[8] etc are already been identified. It is used in the treatment of several diseases like hepatitis syphilis, gonorrhea, conjunctivitis[9]. It also acts as a blood purifier, have antioxidants[10], anti-inflammatory[11], antibacterial and analgesic activities[12,13]. Mixtures of medicinal plants are prescribed by the traditional healers for diseases ranging from common colds to malaria, arthritis, ulcers, etc. and they show positive effects in treatment of deadly diseases like AIDS and cancer[14]. *Tecomella undulata* (Seem) leaves shows presence of compounds like oleanolic acid, ursolic acid and betulinic acid with strong HIV prohibition potential. Octadimethyl succinate derivatives of oleanolic and betulinic acid, reported from *Tecomella undulata* (Seem) is 24 times more active than azidothymidine (AZT), an antiretroviral medication used to prevent and treat HIV/AIDS[15]. *Tecomella undulata* (Seem) is a good source of minerals which are required for structural and functional integrity of the living cells and organisms. Arial plant parts is a good source of mineral to treat number of diseases that are mainly caused due to the deficiency of those minerals and hence is utilized in Ayurvedic system to cure disease[16]. Nowadays well developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical techniques including chromatographic ones. In the present scenario, TLC/HPTLC is very important, essential and viable tool for qualitative and quantitative analysis of herbal products. High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. HPTLC based method is being explored as an important tool in routine drug analysis. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. In HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analysis. Hence, an attempt has been made to give a complete

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Table 1: HPTLC profile of different Plant parts of *Tecomella undulata* (Seem) along with Solvent system, detection system and R_f values.

Sr. No.	Name of extract	Solvent system	Detected at wavelength	R_f values
1	Leaf Methanolic Extract	Tol: Etac : F.A. (7 : 4 : 1)	254nm BD	0.09,0.12,0.17,0.21,0.26,0.35,0.44,0.52,0.64,0.71
2	Leaf Chloroform Extract	Tol: Etac : F.A. (7 : 4 : 1)	254nm BD	0.09,0.12,0.17,0.22,0.26,0.28,0.32,0.36,0.47,0.59,0.63,0.67,0.76
3	Leaf Acetone Extract	Tol: Etac : F.A. (7 : 4 : 1)	254nm BD	0.09,0.12,0.18,0.22,0.26,0.29,0.33,0.36,0.45,0.54,0.59,0.68,0.73,0.78
4	Bark Methanolic Extract	Tol: Etac : F.A (5 : 5 : 1)	254nm BD	0.38,0.50,0.59
5	Bark Chloroform Extract	Tol: Etac : F.A. (5 : 5 : 1)	254nm BD	0.13, 0.34, 0.51, 0.61, 0.68
6	Bark Acetone Extract	Tol: Etac : F.A. (5 : 5 : 1)	254nm BD	0.12, 0.23, 0.36 0.48, 0.53, 0.61, 0.67, 0.70
7	Flower Methanolic Extract	Tol: Etac : F.A. (6 : 4 : 1)	254nm BD	0.07, 0.14, 0.18, 0.29, 0.39, 0.45, 0.55, 0.61, 0.71
8	Flower Chloroform Extract	Tol: Etac : F.A. (6 : 4 : 1)	254nm BD	0.17,0.29,0.34,0.40,0.45,0.54,0.61,0.68
9	Flower Acetone Extract	Tol: Etac : F.A. (6 : 4 : 1)	254nm BD	0.08,0.19,0.39,0.49,0.58,0.64,0.72
10	Leaf Methanolic Extract	Tol: Etac : F.A. (7 : 4 : 1)	540 nm AD	0.10,0.18,0.22,0.27,0.34,0.37,0.44,0.47,0.61,0.64,0.68, 0.76
11	Leaf Chloroform Extract	Tol: Etac : F.A. (7 : 4 : 1)	540 nm AD	0.10,0.13, 0.18, 0.22, 0.26, 0.33, 0.36, 0.39, 0.47, 0.64,0.68
12	Leaf Acetone Extract	Tol: Etac : F.A. (7 : 4 : 1)	540 nm AD	0.10, 0.19, 0.22, 0.28, 0.34, 0.37, 0.46, 0.61, 0.70
13	Bark Methanolic Extract	Tol: Etac : F.A. (5 : 5 : 1)	540 nm AD	0.40, 0.48, 0.53, 0.60, 0.65
14	Bark Chloroform Extract	Tol: Etac : F.A. (5 : 5 : 1)	540 nm AD	0.09, 0.16,0.37, 0.42, 0.53, 0.62, 0.69
15	Bark Acetone Extract	Tol: Etac : F.A. (5 : 5 : 1)	540 nm AD	0.19, 0.38, 0.43, 0.55,0.64,0.72
16	Flower Methanolic Extract	Tol: Etac : F.A. (6 : 4 : 1)	540 nm AD	0.06,0.12,0.21,0.28,0.33,0.37,0.43,0.47, 0.52,0.57,0.73
17	Flower Chloroform Extract	Tol: Etac : F.A. (6 : 4 : 1)	540 nm AD	0.05,0.12,0.24,0.29,0.33,0.37,0.41, 0.47,0.51, 0.57, 0.66,0.72
18	Flower Acetone Extract	Tol: Etac : F.A. (6 : 4 : 1)	540 nm AD	0.06,0.15,0.21,0.31,0.35,0.39,0.46,0.54,0.60,0.70, 0.76

HPTLC profile for leaf, bark and flower of *Tecomella undulata* (Seem). It can further lead to provide a beneficial information for plant based medicament and standardization of herbal products.

MATERIAL AND METHODS

Collection of plant material

Fresh plant parts were collected from Nagaur, Rajasthan, India in the month of December –January. The plant was identified and authenticated at Blatter's Herbarium, St. Xavier's College, Mumbai. (Accession No 22800).

Preparation and Extraction of Plant Material

The leaf, bark and flower were subjected for preliminary phytochemical studies and HPTLC finger print studies. The leaf, bark and flower of *Tecomella undulata* (Seem) were separated, washed thoroughly in distilled water and cut into small pieces. They were shade dried at room temperature. Dried pieces were then uniformly grinded separately using mechanical grinder to make fine powder. The powdered form of plant leaf, bark and flower were stored for future use. The powdered material is then used for preliminary phytochemical studies and HPTLC fingerprinting.

HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Wagner[18], Harborne[19], and Eike Reich[20].

Sample Preparation

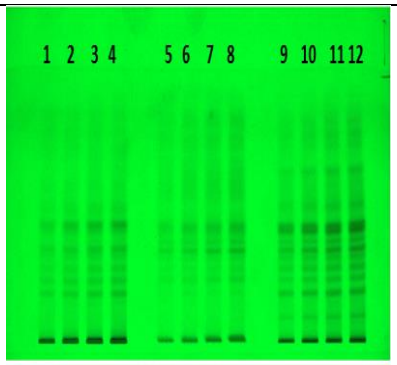
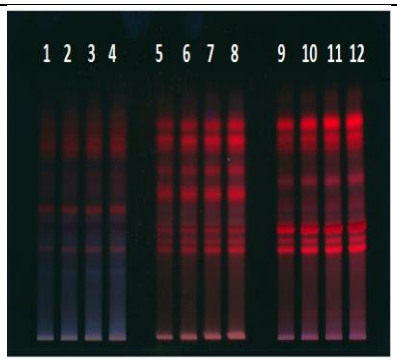
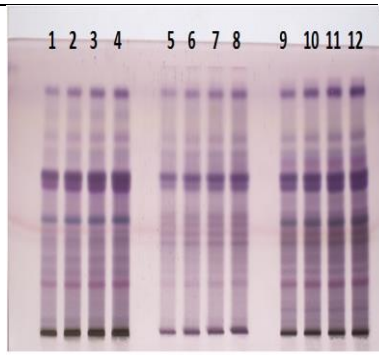
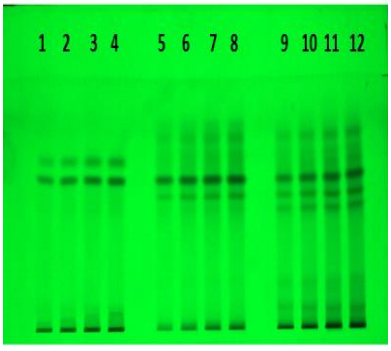
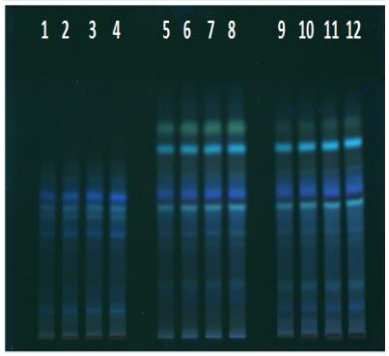
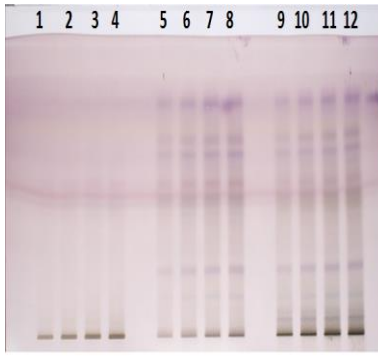
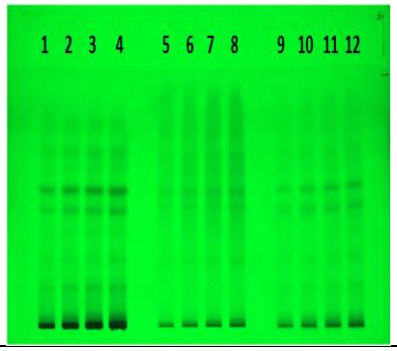
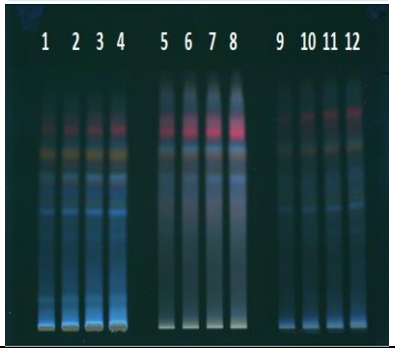
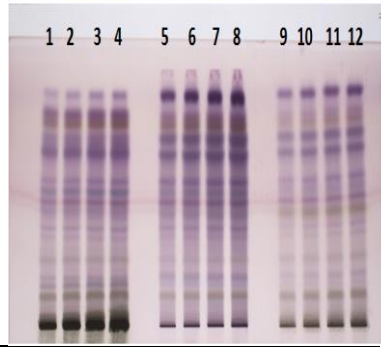
Air dried plant powder was extracted with Methanol, chloroform and acetone. This extracts were evaporated to reduce the volume.

Developing Solvent System

Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The desired aim was achieved using different compositions of mobile phase as it is giving in table no 1[18,20]

Sample Application

Table 2: HPTLC Plates at different wavelength (254, 366 and A.D. 540 nm) of methanolic (lane 1-4), chloroform (lane 5-8) and acetone (lane 9-12) extracts of *Tecomella undulata* (Seem).

Wavelength	254 nm	366 nm	540 nm after dervatization
LEAF			
BARK			
FLOWER			

Chromatograph was performed on 20x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F254 ((E. Merck Ltd, Darmstadt, Germany) stored in desiccators. Methanol, chloroform and acetone, each one of them used four Different aliquots (4, 6 8, and 10 µl) of for leaf, bark and flower extracts was applied on 8 mm wide band by Hamilton micro syringe (Switzerland), with the nitrogen flow providing a delivery speed of 150 nl/s. The syringe was mounted on a Linomat V applicator attached to CAMAG HPTLC system and was programmed through WIN CATS software. Spotting was performed at 25±2ºC ascending development of the plate with elution distance of 80 mm (distance to the lower edge was 10 mm).

Development of Chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 20 x 10 cm saturated with solvent vapors for 20 - 25 minutes. The linear ascending development was carried out and 20 ml of mobile phase was used per chromatography run.

Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with methanolic sulphuric acid reagent as spray reagent and dried at 100ºC on CAMAG plate heater for 3 - 5 min.

Photo documentation

The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 nm, 366 nm and visible light. The R_f values and finger print data were recorded by WIN CATS software.

Densitometric scanning

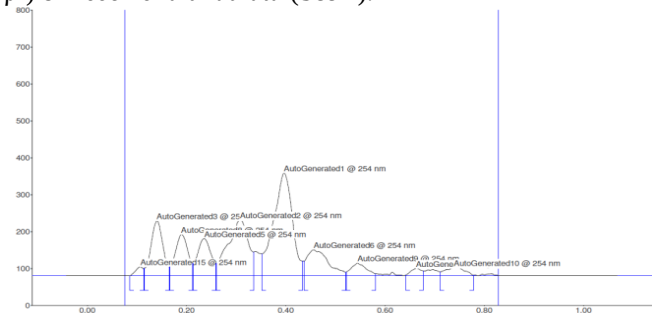
Scanning was taken place at 540 nm. The plate was fixed in scanner stage and scanning was done at 540 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

RESULTS AND DISCUSSION

Many compositions of mobile phase for HPTLC analysis were tested in order to obtain high resolution and

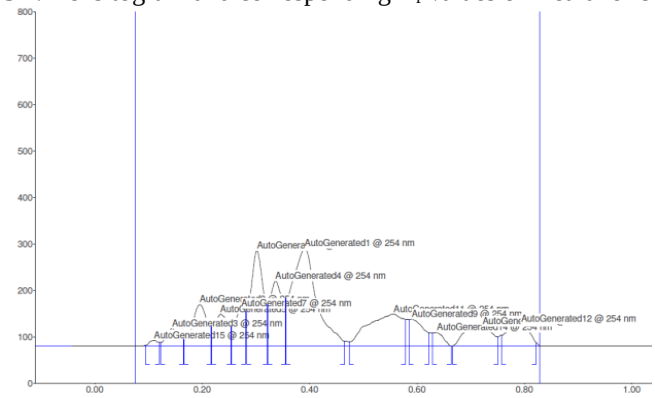
reproducible peaks. The mobile phases for different extracts of the plant has been used is given in table no 1. Different ratios were use for different extracts as it is given in tabular form. Simultaneously solvent system, Rf values

Table 3: Densitogram and corresponding R_f values of methanolic, chloroform and acetone extracts at 254 of Leaf (10 µl) of *Tecomella undulata* (Seem).



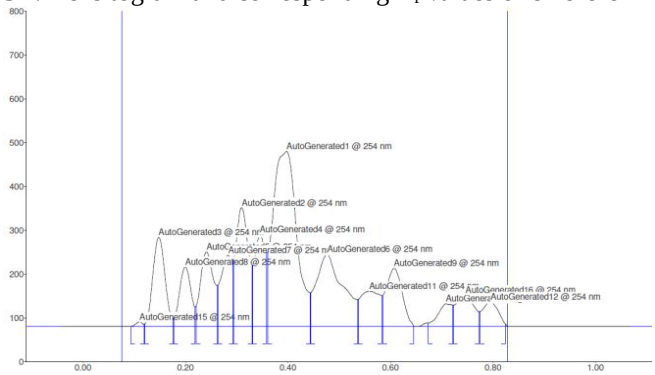
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.0	0.11	24.6	2.55	0.11	20.4	309.7	1.16
2	0.12	20.7	0.14	148.8	15.44	0.17	24.1	2977.7	11.16
3	0.17	24.6	0.19	111.9	11.61	0.21	33.9	2385.9	8.94
4	0.21	34.3	0.24	100.9	10.46	0.26	34.0	2306.6	8.65
5	0.26	34.2	0.31	151.5	15.72	0.34	65.1	5228.5	19.60
6	0.35	59.6	0.40	278.7	28.91	0.43	39.9	8885.6	33.31
7	0.44	39.8	0.46	70.4	7.30	0.52	9.9	2582.8	9.68
8	0.52	10.5	0.55	34.6	3.59	0.58	6.4	888.2	3.33
9	0.64	2.1	0.66	20.2	2.09	0.68	13.9	359.4	1.35
10	0.71	11.2	0.74	22.4	2.32	0.78	1.8	749.3	2.81

3A. Densitogram and corresponding R_f values of methanolic leaf extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	1.9	0.11	12.6	1.16	0.12	7.6	168.5	0.57
2	0.12	7.9	0.14	37.7	3.46	0.17	14.5	751.1	2.54
3	0.17	14.8	0.20	89.6	8.21	0.22	41.2	1993.9	6.73
4	0.22	41.4	0.23	68.7	6.29	0.25	42.0	1506.3	5.08
5	0.26	42.1	0.27	80.4	7.36	0.28	75.3	1317.3	4.45
6	0.28	75.7	0.30	205.6	18.82	0.32	88.8	4024.8	13.59
7	0.32	90.0	0.34	140.3	12.84	0.36	102.8	2791.7	9.42
8	0.36	103.6	0.39	210.8	19.30	0.47	10.5	8151.0	27.52
9	0.47	9.1	0.56	68.9	6.31	0.58	57.2	3796.8	12.82
10	0.59	56.9	0.59	57.8	5.29	0.62	28.5	1293.0	4.36
11	0.63	28.5	0.64	29.9	2.73	0.67	0.0	475.0	1.60
12	0.67	0.4	0.72	42.0	3.85	0.75	20.2	1751.3	5.91
13	0.76	23.8	0.80	47.8	4.38	0.82	8.2	1603.1	5.41

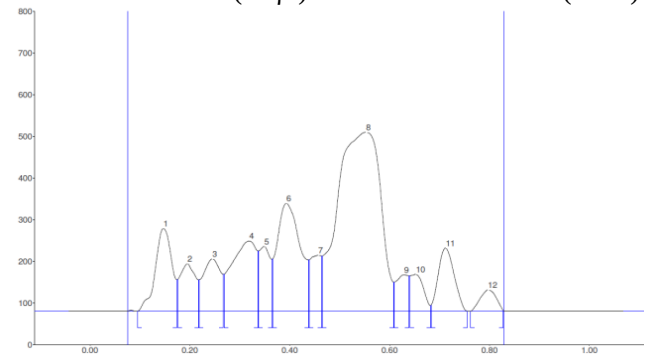
3B. Densitogram and corresponding R_f values of chloroform leaf extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.2	0.11	10.8	0.51	0.12	5.8	121.7	0.22
2	0.12	6.3	0.15	204.2	9.60	0.18	18.7	4048.0	7.18
3	0.18	19.4	0.20	136.4	6.41	0.22	44.8	2586.8	4.59
4	0.22	45.6	0.24	170.5	8.01	0.26	93.8	3685.3	6.54
5	0.26	95.0	0.28	163.0	7.67	0.29	150.8	3024.6	5.36
6	0.29	153.4	0.31	271.5	12.77	0.33	138.3	5623.1	9.97
7	0.33	138.5	0.35	210.2	9.89	0.36	169.7	3796.3	6.73
8	0.36	171.5	0.40	399.9	18.80	0.44	77.4	15552.4	27.58
9	0.45	77.6	0.48	165.0	7.76	0.54	62.3	7171.7	12.72
10	0.54	63.0	0.56	80.9	3.80	0.58	70.4	2557.2	4.53
11	0.59	71.4	0.61	133.7	6.28	0.65	1.2	3496.1	6.20
12	0.68	7.9	0.71	52.2	2.45	0.72	49.1	1212.1	2.15
13	0.73	49.5	0.75	72.2	3.40	0.77	34.6	2078.6	3.69
14	0.78	34.8	0.80	56.3	2.65	0.83	6.1	1436.5	2.55

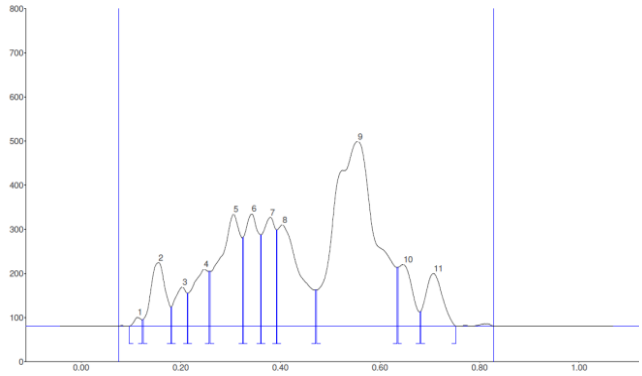
3C. Densitogram and corresponding Rf values of acetone leaf extract of *Tecomella undulata* (Seem) at 254nm

Table 4: Densitogram and corresponding Rf values of methanolic, chloroform and acetone extracts at 540 nm after dervatization of Leaf (10 µl) of *Tecomella undulata* (Seem).



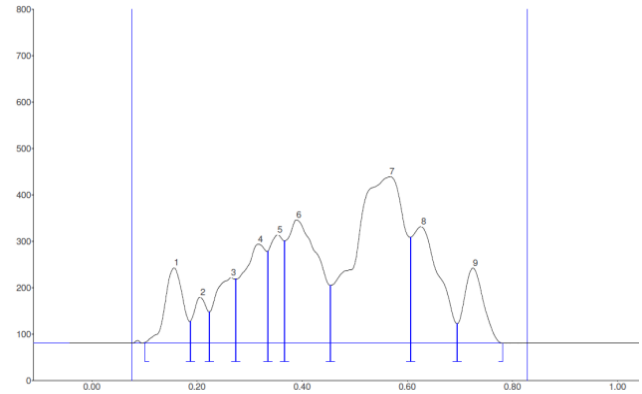
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	0.2	0.15	198.6	10.09	0.17	75.7	5522.2	7.39
2	0.18	76.5	0.20	114.3	5.81	0.22	75.4	2947.9	3.95
3	0.22	75.7	0.25	126.2	6.41	0.27	89.0	3738.0	5.00
4	0.27	89.3	0.32	169.1	8.59	0.34	144.7	6938.4	9.29
5	0.34	144.8	0.35	155.8	7.91	0.37	125.0	2880.9	3.86
6	0.37	125.7	0.39	259.3	13.17	0.44	123.5	10064.3	13.47
7	0.44	123.9	0.46	135.0	6.86	0.47	132.6	2498.1	3.34
8	0.47	132.8	0.55	429.8	21.84	0.61	70.0	30900.6	41.36
9	0.61	70.0	0.63	87.9	4.47	0.64	85.0	1797.8	2.41
10	0.64	85.1	0.65	88.6	4.50	0.68	13.7	1982.3	2.65
11	0.68	14.8	0.71	151.7	7.71	0.76	0.3	4054.4	5.43
12	0.76	0.2	0.80	52.0	2.64	0.83	2.3	1388.8	1.86

4A. Densitogram and corresponding Rf values of methanolic leaf extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	0.1	0.11	20.6	1.01	0.12	15.1	249.5	0.34
2	0.13	15.8	0.16	144.4	7.05	0.18	43.7	3520.1	4.78
3	0.18	44.1	0.20	88.8	4.33	0.21	75.2	1755.0	2.38
4	0.22	75.3	0.25	129.5	6.32	0.26	124.4	3341.5	4.53
5	0.26	124.5	0.31	254.3	12.42	0.32	200.8	8987.3	12.20
6	0.33	201.8	0.34	254.9	12.44	0.36	207.5	5949.1	8.07
7	0.36	207.6	0.38	247.0	12.06	0.39	218.7	5278.9	7.16
8	0.39	218.7	0.41	230.1	11.23	0.47	81.9	8658.7	11.75
9	0.47	81.9	0.56	418.7	20.44	0.64	133.6	29333.1	39.81
10	0.64	133.9	0.65	140.0	6.83	0.68	32.7	3272.0	4.44
11	0.68	33.9	0.71	119.9	5.85	0.75	0.4	3342.7	4.54

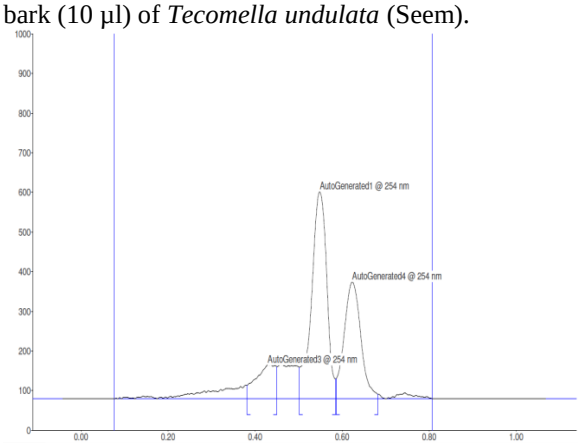
4B. Densitogram and corresponding Rf values of chloroform leaf extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.10	2.1	0.16	162.9	8.62	0.19	46.7	4673.1	5.72
2	0.19	48.4	0.21	99.6	5.27	0.22	67.5	2079.6	2.54
3	0.22	67.8	0.27	141.6	7.49	0.27	138.3	4303.8	5.26
4	0.28	138.4	0.32	213.8	11.31	0.33	197.9	7934.9	9.71
5	0.34	199.0	0.35	234.0	12.38	0.37	220.7	5114.9	6.26
6	0.37	220.8	0.39	266.1	14.07	0.45	125.0	13361.1	16.34
7	0.46	125.1	0.57	358.9	18.98	0.61	228.5	28404.0	34.74
8	0.61	228.8	0.63	251.5	13.30	0.70	42.4	10882.1	13.31
9	0.70	42.5	0.73	162.3	8.58	0.78	0.1	5002.1	6.12

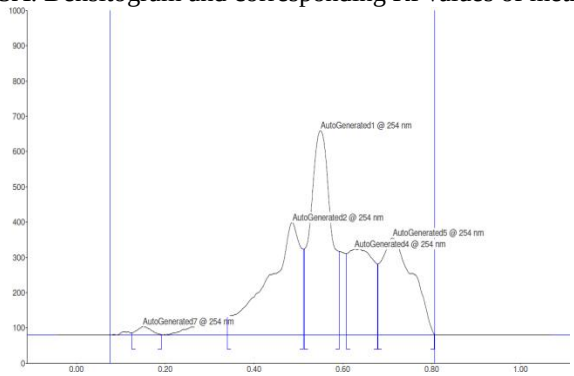
4C. Densitogram and corresponding Rf values of acetone leaf extract of *Tecomella undulata* (Seem) at 540nm

Table 5: Densitogram and corresponding R_f values of methanolic, chloroform and acetone extracts at 254 nm of bark (10 µl) of *Tecomella undulata* (Seem).



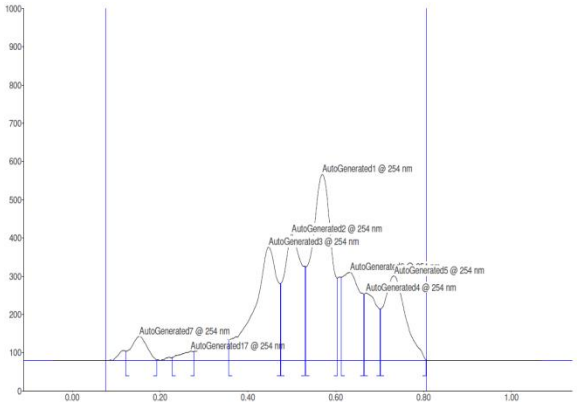
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.38	33.0	0.43	84.9	9.43	0.45	80.6	3276.2	11.68
2	0.50	78.9	0.55	521.4	57.89	0.59	49.5	15335.0	54.65
3	0.59	50.5	0.63	294.3	32.68	0.68	11.4	9450.0	33.68

5A. Densitogram and corresponding Rf values of methanolic bark extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.13	5.8	0.15	23.9	1.66	0.19	1.6	629.7	0.89
2	0.34	48.9	0.49	318.5	22.12	0.51	243.7	19703.1	27.77
3	0.51	244.0	0.55	578.8	40.21	0.59	237.6	22530.4	31.76
4	0.61	230.6	0.63	243.2	16.90	0.68	200.7	11800.4	16.63
5	0.68	201.1	0.71	275.1	19.11	0.81	2.6	16279.0	22.95

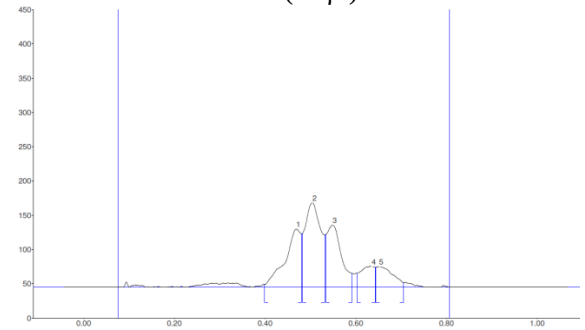
5B. Densitogram and corresponding Rf values of methanolic bark extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.12	24.2	0.15	62.6	3.42	0.19	0.9	1840.7	2.78
2	0.23	7.1	0.27	24.5	1.34	0.28	23.3	629.2	0.95
3	0.36	53.3	0.45	296.3	16.22	0.47	200.6	13285.8	20.07
4	0.48	201.4	0.50	330.1	18.07	0.53	245.9	10878.6	16.43
5	0.53	244.5	0.57	486.0	26.60	0.61	215.6	18426.7	27.83
6	0.61	217.4	0.63	230.2	12.60	0.67	173.8	7912.5	11.95
7	0.67	173.9	0.67	176.6	9.67	0.70	134.0	4308.4	6.51
8	0.70	134.6	0.73	220.7	12.08	0.81	2.1	8923.2	13.48

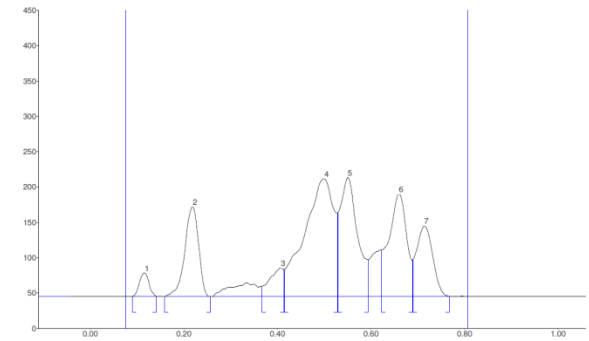
5C. Densitogram and corresponding Rf values of methanolic bark extract of *Tecomella undulata* (Seem) at 254nm

Table 6: Densitogram and corresponding R_f values of methanolic, chloroform and acetone extracts at 540 nm after dervatization of bark (10 µl) of *Tecomella undulata* (Seem).



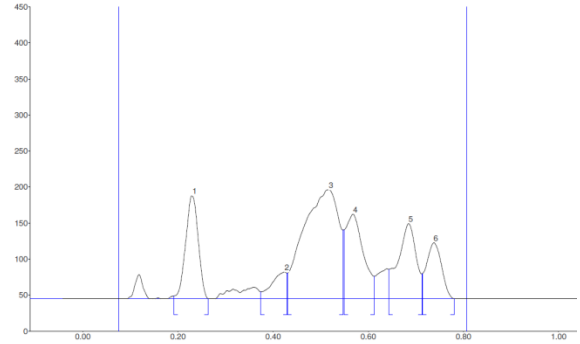
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.40	4.3	0.47	85.0	23.57	0.48	78.0	2527.2	24.24
2	0.48	78.6	0.51	123.5	34.26	0.53	77.0	3648.6	35.00
3	0.53	77.0	0.55	91.1	25.26	0.59	20.6	2483.5	23.83
4	0.60	20.6	0.64	30.5	8.46	0.64	29.5	817.7	7.84
5	0.65	29.6	0.65	30.4	8.44	0.71	6.8	947.0	9.08

6A. Densitogram and corresponding Rf values of methanolic bark extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.09	0.2	0.12	33.6	4.30	0.14	0.3	574.4	2.17
2	0.16	0.0	0.22	127.1	16.26	0.26	0.0	3138.0	11.87
3	0.37	15.0	0.41	40.6	5.20	0.42	38.3	1018.8	3.85
4	0.42	38.5	0.50	166.6	21.31	0.53	118.1	8761.2	33.15
5	0.53	118.7	0.55	168.4	21.54	0.60	52.2	5315.8	20.11
6	0.62	65.6	0.66	145.2	18.57	0.69	51.8	4721.4	17.86
7	0.69	52.8	0.72	100.3	12.83	0.77	0.6	2903.3	10.98

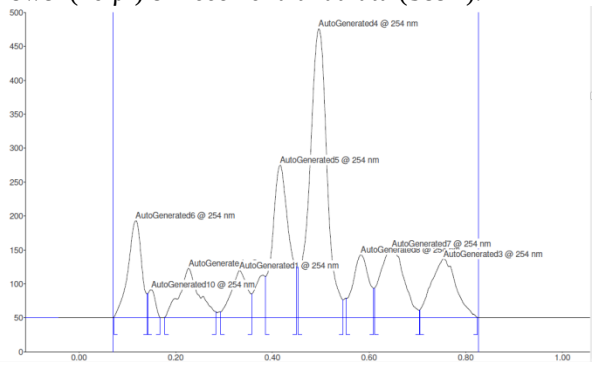
6B. Densitogram and corresponding Rf values of chloroform bark extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.19	3.7	0.23	143.1	22.71	0.26	0.1	3160.8	14.02
2	0.38	9.4	0.43	36.9	5.85	0.43	35.8	990.9	4.40
3	0.43	35.9	0.52	150.9	23.95	0.55	95.2	9319.5	41.35
4	0.55	95.4	0.57	117.4	18.63	0.61	31.2	3718.4	16.50
5	0.64	40.9	0.69	104.3	16.55	0.71	34.5	3288.3	14.59
6	0.72	35.0	0.74	77.6	12.31	0.78	0.1	2060.3	9.14

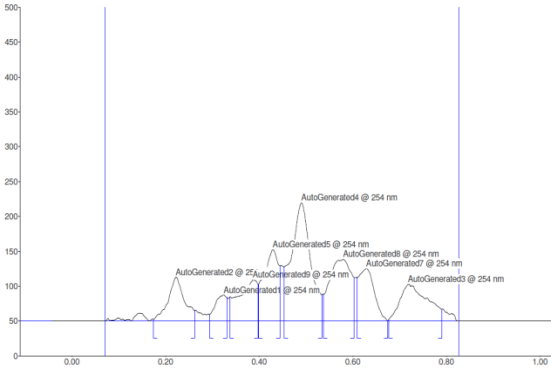
6C. Densitogram and corresponding Rf values of acetone bark extract of *Tecomella undulata* (Seem) at 540nm

Table 7: Densitogram and corresponding R_f values of methanolic, chloroform and acetone extracts at 254 nm of flower (10 µl) of *Tecomella undulata* (Seem).



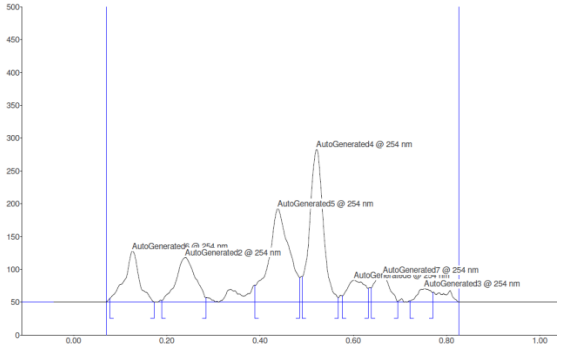
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.07	0.6	0.12	143.6	11.37	0.14	35.4	3405.4	8.87
2	0.14	36.2	0.15	41.5	3.29	0.17	0.0	502.0	1.31
3	0.18	1.0	0.23	73.1	5.79	0.28	9.4	2517.1	6.55
4	0.29	9.2	0.33	69.9	5.54	0.36	35.3	2008.5	5.23
5	0.39	61.6	0.42	225.4	17.86	0.45	75.9	6488.9	16.90
6	0.45	75.2	0.50	426.0	33.75	0.55	26.7	12917.8	33.64
7	0.55	28.8	0.58	93.1	7.38	0.61	43.6	2597.4	6.76
8	0.61	44.1	0.65	102.5	8.12	0.71	11.3	4223.3	11.00
9	0.71	11.8	0.76	86.9	6.89	0.83	0.8	3744.2	9.75

7A. Densitogram and corresponding Rf values of methanolic flower extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.17	2.6	0.22	63.1	9.72	0.26	15.3	1820.1	8.58
2	0.29	9.5	0.32	37.4	5.76	0.33	33.7	764.1	3.60
3	0.34	34.4	0.39	59.4	9.15	0.40	52.5	1932.6	9.11
4	0.40	53.0	0.43	102.8	15.83	0.45	79.3	2710.5	12.78
5	0.45	77.9	0.49	170.0	26.18	0.53	37.8	5890.1	27.77
6	0.54	38.8	0.58	88.7	13.66	0.60	62.2	3444.5	16.24
7	0.61	62.8	0.63	75.3	11.60	0.68	0.7	2088.2	9.84
8	0.68	1.5	0.72	52.7	8.11	0.79	17.0	2563.5	12.08

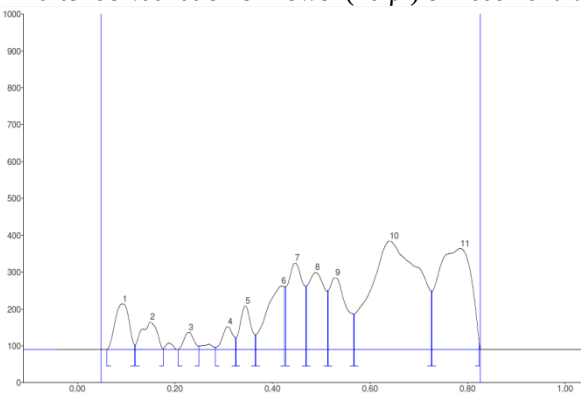
7B. Densitogram and corresponding Rf values of chloroform flower extract of *Tecomella undulata* (Seem) at 254nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.08	5.6	0.13	77.9	12.58	0.17	0.6	2069.5	11.50
2	0.19	2.7	0.24	68.5	11.05	0.28	7.0	2351.4	13.07
3	0.39	25.9	0.44	142.9	23.08	0.49	37.5	5220.3	29.02
4	0.49	39.0	0.52	233.3	37.66	0.57	6.8	5660.1	31.46
5	0.58	10.0	0.60	33.9	5.47	0.63	21.0	1059.7	5.89
6	0.64	22.2	0.67	42.0	6.78	0.70	0.9	1121.2	6.23
7	0.72	2.0	0.75	20.9	3.37	0.77	15.2	507.5	2.82

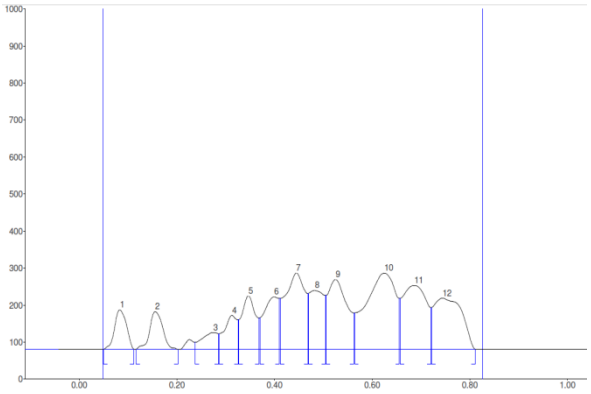
7C. Densitogram and corresponding Rf values of acetone flower extract of *Tecomella undulata* (Seem) at 254nm

Table 8: Densitogram and corresponding R_f values of methanolic, chloroform and acetone extracts at 540 nm after dervatization of flower (10 µl) of *Tecomella undulata* (Seem).



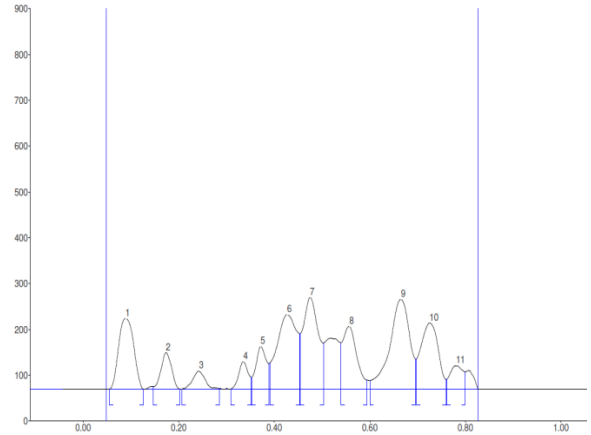
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	0.7	0.09	124.3	6.86	0.12	12.0	2954.0	4.10
2	0.12	12.4	0.15	75.8	4.18	0.18	2.4	1944.3	2.70
3	0.21	0.1	0.23	47.6	2.63	0.25	9.6	789.4	1.10
4	0.28	6.0	0.31	62.4	3.45	0.32	32.4	1125.2	1.56
5	0.33	32.6	0.35	118.8	6.56	0.37	40.4	2190.1	3.04
6	0.37	40.4	0.42	172.7	9.53	0.43	170.5	5209.1	7.24
7	0.43	170.5	0.45	235.1	12.98	0.47	172.1	6237.9	8.67
8	0.47	172.5	0.49	209.3	11.55	0.51	159.0	6006.4	8.35
9	0.52	159.9	0.53	195.8	10.81	0.57	96.6	5819.4	8.09
10	0.57	96.8	0.64	295.1	16.29	0.73	158.1	24266.8	33.72
11	0.73	158.3	0.79	274.6	15.16	0.83	14.4	15417.5	21.43

8A. Densitogram and corresponding Rf values of methanolic flower extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	0.4	0.08	107.3	6.27	0.11	0.4	2181.5	3.85
2	0.12	0.6	0.16	102.5	5.99	0.20	0.1	2303.0	4.07
3	0.24	19.7	0.28	46.2	2.70	0.29	42.3	1281.7	2.26
4	0.29	42.5	0.31	92.4	5.40	0.33	80.5	2030.3	3.59
5	0.33	80.8	0.35	145.0	8.47	0.37	85.5	3433.1	6.06
6	0.37	85.9	0.40	142.2	8.31	0.41	138.4	3673.4	6.49
7	0.41	138.4	0.45	207.2	12.11	0.47	151.7	7124.1	12.58
8	0.47	152.2	0.48	159.9	9.34	0.51	146.1	4018.8	7.10
9	0.51	146.3	0.53	189.3	11.06	0.56	99.0	6245.4	11.03
10	0.57	99.2	0.63	206.7	12.08	0.66	138.2	10466.6	18.49
11	0.66	138.3	0.69	173.3	10.13	0.72	113.2	7166.7	12.66
12	0.72	113.6	0.75	139.2	8.14	0.81	0.7	6688.9	11.82

8B. Densitogram and corresponding Rf values of chloroform flower extract of *Tecomella undulata* (Seem) at 540nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.06	0.0	0.09	154.4	11.71	0.13	0.2	4041.9	11.22
2	0.15	4.9	0.17	79.7	6.04	0.20	0.1	1454.7	4.04
3	0.21	0.2	0.24	39.2	2.98	0.29	0.6	844.8	2.34
4	0.31	0.3	0.34	60.3	4.58	0.35	26.1	994.4	2.76
5	0.35	26.4	0.37	93.4	7.08	0.39	55.6	1741.5	4.83
6	0.39	56.6	0.43	162.7	12.35	0.45	121.2	5638.7	15.65
7	0.46	121.3	0.48	200.3	15.20	0.50	100.5	5405.5	15.00
8	0.54	101.3	0.56	136.9	10.38	0.60	19.7	3454.6	9.59
9	0.60	18.8	0.67	195.7	14.84	0.70	65.5	6856.4	19.03
10	0.70	65.6	0.73	144.4	10.95	0.76	21.0	4408.8	12.24
11	0.76	21.3	0.78	51.2	3.88	0.80	37.6	1192.5	3.31

8C. Densitogram and corresponding Rf values of acetone flower extract of *Tecomella undulata* (Seem) at 540nm

and detection at different wavelengths is also given in this table no.1. However, in this present study we used four different volumes (4, 6, 8 & 10 µl) of leaf, bark and flower methanolic, chloroform and acetone extracts but 10 µl volume got maximum peaks that is why here we were considering 10 µl's Rf values for analysis. Table no. 2 is showing picture of plate at 254, 366 and 540 nm and Table no. 3-8 is showing chromatograms and related Rf- values of respective extract at 254 nm and after dervatization 540 nm. Leaf methanol, chloroform and acetone extract are showing 10, 13 and 14 Rf values respectively at 254 nm and12, 11& 9 peaks at 540 after dervatization respectively. For bark methanol, chloroform and acetone extract are showing 3, 5 & 8 and 14 Rf values respectively at 254 nm and 5, 7 & 6 peaks at 540 after dervatization respectively. And finally flower methanol, chloroform and acetone extract are showing 9, 8 & 7 and Rf values respectively at 254 nm and 11, 12 & 11 peaks at 540 after dervatization respectively. Results are showing different solvents for better extraction for different Arial part at different wavelength (254 and 540 A.D.). For leaf Acetone and methanol is good solvent as compare to chloroform. Bark is showing maximum peaks in Acetone and chloroform as compare to methanol. And Flower methanol and chloroform as compare to acetone. Maximum bands we got in leaf extracts at 254 nm.

CONCLUSION

This Study gives a complete HPTLC profile of Leaf, bark and flower of *Tecomella undulata* (Seem). In future this would be helpful for determining the quality of the crude drug. Further studies like component isolation, define structure and identification can be done by using sophisticated techniques. HPTLC analysis revealed a better separation of individual secondary metabolites. The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. These plant derived bioactive compounds in addition of being developed directly as drugs can also serve as prototype drug molecules known as "Lead Compounds" and as pharmacological probes to help better understand biochemical and physiological mechanisms.

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