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Original Article

Detection and quantification of flavonoids and phenolic acids in leaves of mussaenda *glabrata* (hook.f.) Hutch. by HPTLC technique

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ABSTRACT

Mussaenda glabrata (Hook.f.) Hutch. ex Gamble (Rubiaceae) is traditionally used as diuretic, antiasthmatic, antimicrobial and anti- inflammatory. In this prospective study to evaluate the chromatogram detection of chloroform and ethanol extract of *Mussaenda glabrata* leaf with standard flavonoid markers such as Rutin, Quercetin and Gallic acid by HPTLC techniques. HPTLC Chromatogram was developed in both the extracts by using Toluene-Ethyl acetate-Formic acid-Methanol (3: 6: 1.6: 0.4) as mobile phase. The identity of the bands of compounds 3-7 in both the extracts of *Mussaenda glabrata* were estimated by their UV absorption spectra with the standard marker rutin, quercetin, and gallic acid at 254 nm. By comparing with the Rf value of standard antioxidants markers. Quercetin was present in both extracts but ethanol extract contains nearly (0.56 %) when comparing with chloroform extracts, ethanol extract contain comparatively more rutin (0.09%) and gallic acid (0.32). Thus the present study provided a scientific validation for the traditional claims of *Mussaenda glabrata* revealed the presence flavonoids quercetin, rutin and gallic acid in extracts of Mussaenda *glabrata*.

Keywords: *Mussaenda glabrata*, HPTLC,Rutin, Quercetin,Gallic acid

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INTRODUCTION

The therapeutic efficiency of the drugs depends greatly on the use of proper and genuine raw materials. Because of this, the guarantee of safety, quality and subsequent efficacy of the medicinal plants and herbal products have now become a major and explanation area under discussion, so the standardization of plant materials is compulsory[1] The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing[2,3] High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs due to its simplicity, high sensitivity, accuracy and less expensive.[4]. *Mussaenda glabrata* is a shrub about 90 cm tall with spreading habit native to India, Nepal, Sri Lanka, Vietnam, Malaysia and Indonesia. In India, it is found in tropical Himalayas, Bihar, Bengal, Assam and Kerala [5]. Darsan Menon [6] investigated *invitro* antioxidant and anti-inflammatory activities of the methanolic extract of *Mussaenda glabrata* roots. Vidyalakshmi [7] evaluated the free radical scavenging activity in the flowers of *Mussaenda glabrata*. Sambrekar Sudhir [8] investigated the protective activity of alcoholic and aqueous extract of *Mussaenda frondosa* Linn. in Isoniazid (INH) induced hepatotoxicity in wistar rats. Patil Suhas [9] evaluated the antibacterial and wound healing activity of ethanolic extract of the leaves of *Mussaenda frondosa* Linn. The wound healing activity of *Mussaenda frondosa* was evaluated by using different types of wound healing models such as incision wound, excision wound and dead space wound. So the endeavour of the present job is to screen by HPTLC finger print analysis of chloroform and ethanol extracts of Mussaenda *glabrata* leaf.

MATERIALS AND METHOD

Collection and preparation of extract: The leaves of the plant *Mussaenda glabrata* was collected from Palakkad District of kerala and authenticated from the Botanical survey of India (BSI), Southern circle, Coimbatore, Tamil Nadu. The authentication certificate number is No. BSI/SRC/5/23/2015/Tech/2550. Soon after collection of the aerial parts, the leaves were cleaned and shade dried. After drying, these leaves were crushed to a coarse powder, stored in air tight plastic container for further use. The extraction is done by using Soxhlet apparatus. The coarse powders of the leaves were first extracted with petroleum ether. Obtained defatted material is again extracted with chloroform and ethanol. After extraction, the *Mussaenda glabrata* chloroform extract (MGCE) and *Mussaenda glabrata* ethanol extract (MGEE) were evaporated by using rotary evaporator and dried at room temperature to give a viscous mass. The obtained crude extracts were weighed and stored at 4^0C for the further analysis.

Chemicals and Instruments: Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. Rutin, gallic acid, and quercetin were procured from Sigma Chemical Company Inc., USA. TLC was carried out using Merck aluminium sheet coated with silica gel GF_{254} (0.2 mm).

Equipment: A Camag HPTLC system comprising of Linomat 5 applicator and Camag TLC scanner and single pan balance of Shimadzu model was used for weighing the samples.

Sample application: MGCE and MGEE (5,10µl each) and 5µl of standard solution were loaded as 6mm band length in the 10 x 10 Silica gel $60F_{254}$ HPTLC plate using Hamilton micro liter syringe with CAMAG LINOMAT 5 instrument.

Spot development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (standards) and the plate was developed in the respective mobile phase up to 80mm.

Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was Photo documented the images at UV 254nm.

Scanning: The dried plate was observed under UV light at 254nm and 366nm and photo documentation was done. Densitometric scanning was performed on Camag TLC scanner 3 in the absorbance mode at 254nm. The percentage of active constituents present in MGCE and MGEE was compared with that of standard markers.

RESULTS

The following different solvent compositions were tried for monitoring the elution of components in both the extracts. Ethyl Acetate: Methanol: Water Toluene (100:15.5:13.5:2), Toluene: ethyl acetate (93:7) Ethyl acetate: glacial acetic acid: formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Chloroform (6:4:0.3).Ethyl acetate: methanol: water (100:15.5:13.5), Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4). Totally 6 mobile phase were trailed for better elution of formulations. Of which Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) were given better elution for both the extracts to screen in one

plate. The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometry analysis was performed at 254 nm in absorbance mode. The elution of both the extracts were carried out in mobile phase of toluene: ethyl acetate: formic acid: methanol (3:6:1.6: 0.4) and in this mobile phase elution was good results were tabulated by considering every Rf value as one ingredients of extracts whether it may be pharmacologically active or inert but for screening the number of principle in the extracts can be considered as one of the principle in it. Therefore the obtained Rf value were compared with Rf value of the standards and well known free radical scavengers rutin, quercetin and gallic acid in chloroform and ethanol extracts (Fig:1 and Table :1). For identifying these free radical scavengers' rutin, quercetin and gallic acid, we used UV light at 254 nm. Separation of chloroform extract in chromatogram (5 µl) was not good since the extracts move along with solvent front but it shows three components at three fourth distance of the developed plate among one is equal to Rf value of quercetin and in (10 µl) the chloroform extract show traces of rutin (Fig: 2 and 3). Partition of ethanol extract (5 µl and 10 µl) chromatogram (Fig: 4 and 5) show the presence of rutin, gallic acid and quercetin. Fig: 6, 7 and 8 represents the chromatogram of standard marker rutin, gallic acid and quercetin respectively.

DISCUSSION

By comparing with the Rf value of standard antioxidants markers in both extracts rutin, quercetin and gallic acid (Table 1). Quercetin was present in both extracts but ethanol extract contains nearly (0.56 %) when comparing with chloroform extracts, ethanol extract contain comparatively more rutin (0.09%). *Mussaenda glabrata* plant was reported to have anti-inflammatory, hepato protective and wound healing activity by various extracts[6,7,8].By fingerprint analysis and screening for antioxidants markers in both extracts of *Mussaenda glabrata*, the pharmacological action may be due to presence of above detected flavonoids and phenolic acids and many scientific literature also support these markers as therapeutic component for various diseases. This was supplementary confirmed by Kandaswami[10] and Duthie [11] reported that rutin scavenges free radicals. Middleton reveled that rutin suppresses cellular immunity [12] as well as anti-inflammatory [13].Quercetin has been reported to inhibit the allergic and inflammatory responses of the immune system [14] by modulating several aspects of cell function relevant to inflammatory arthritis. At the molecular level, quercetin is known to inhibit nuclear factor kappa B (NF-kB), a central transcription factor in inflammatory and proliferative diseases [15]. Based on detection of antioxidants markers in both extracts from the leaves of *Mussaenda Glabrata* (Hook.f.) Hutch**.** by high performance thin layer chromatography we conclude that the pharmacological action may be due to presence of rutin, quercetin and gallic acids.

CONCLUSION

It can be concluded that rutin, quercetin and gallic acid were simultaneously detected in both the extracts from the leaves of *Mussaenda glabrata* (Hook.f.) Hutch by high performance thin layer chromatography. Hence we conclude that the pharmacological action may be due to presence of rutin, quercetin and gallic acids.
The developed HPTLC method may be adopted for routine detection of rutin, quercetin and gallic acid in plant extracts by simultaneous detection.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest regarding this publication.

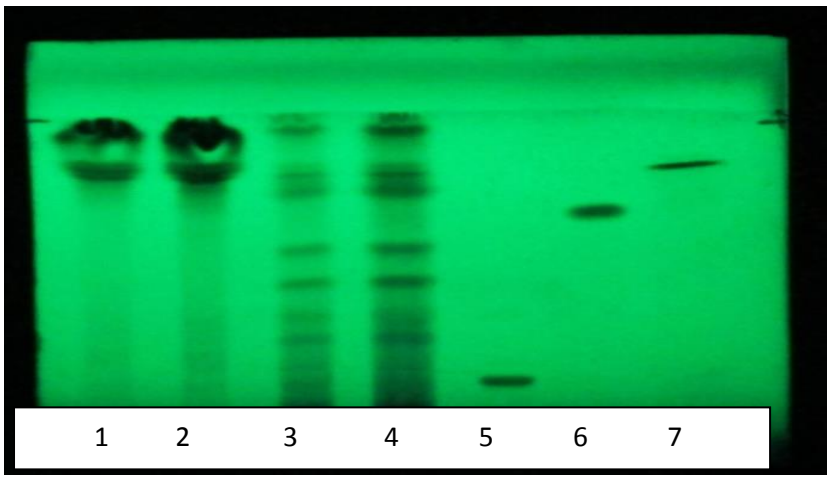
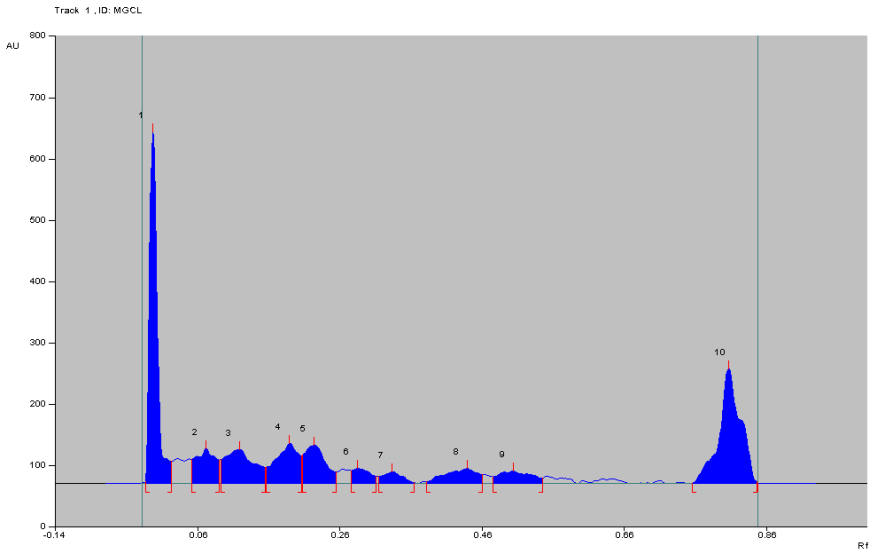


Fig. 1: TLC Profile Chloroform and Ethanol Extracts of Mussaenda *glabrata* at 254 nm.

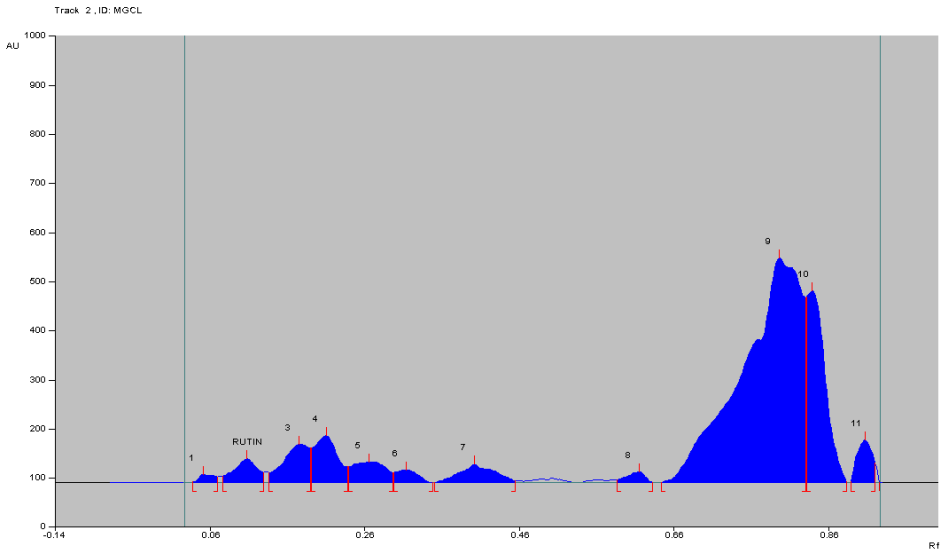
1. Chloroform extract (5 µl) 2. Chloroform extract (10 µl) 3.Ethanol extract (5 µl)
4. Ethanol extract (10 µl) 5.Standard marker Rutin 6. Standard marker Gallic acid
7. Standard marker Quercetin.



winCATS Planar Chromatography Manager

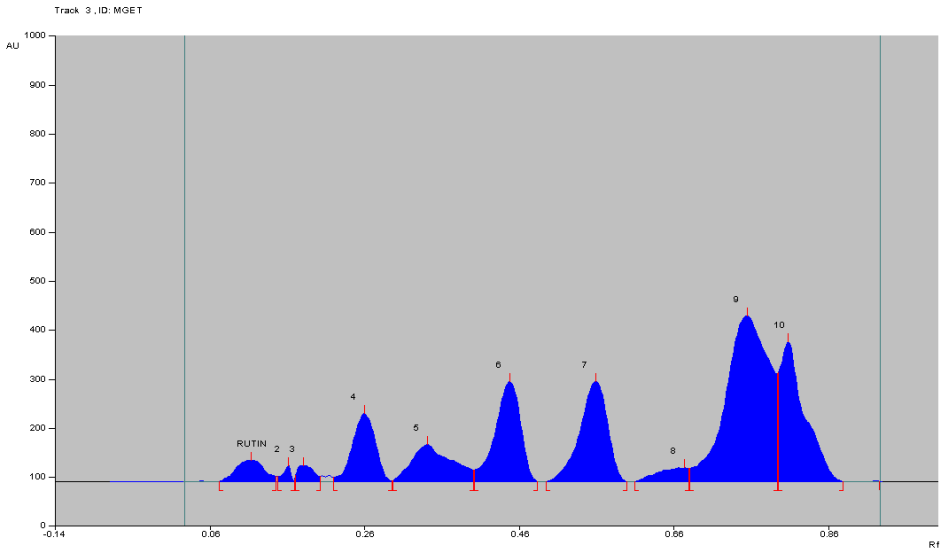
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	3.1	-0.01	572.4	52.60	0.02	35.5	5171.6	27.92	unknown *
2	0.05	39.0	0.07	56.6	5.20	0.09	38.2	1260.5	6.80	unknown *
3	0.09	38.5	0.12	55.2	5.07	0.15	26.2	1856.4	10.02	unknown *
4	0.15	26.4	0.19	65.6	6.03	0.20	45.2	1681.4	9.08	unknown *
5	0.21	45.9	0.22	62.9	5.78	0.25	18.4	1489.7	8.04	unknown *
6	0.27	20.8	0.28	24.4	2.25	0.31	12.2	494.1	2.67	unknown *
7	0.31	11.7	0.33	19.2	1.76	0.36	0.8	419.1	2.26	unknown *
8	0.38	2.6	0.44	24.8	2.28	0.46	14.2	882.0	4.76	unknown *
9	0.47	10.8	0.50	19.9	1.83	0.54	8.1	750.0	4.05	unknown *
10	0.75	0.2	0.80	187.3	17.21	0.84	2.5	4520.8	24.40	unknown *

Fig. 2: Chromatogram of *Mussaenda glabrata* chloroform extract 5 µl



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	1.3	0.05	16.9	1.30	0.07	11.6	266.1	0.57	unknown *
2	0.07	13.4	0.11	48.1	3.70	0.13	20.6	1168.3	2.51	RUTIN
3	0.13	20.6	0.17	77.7	5.98	0.19	70.8	2054.6	4.41	unknown *
4	0.19	70.8	0.21	95.4	7.34	0.24	31.8	2349.4	5.04	unknown *
5	0.24	31.9	0.26	42.5	3.27	0.29	20.0	1466.0	3.15	unknown *
6	0.30	20.3	0.31	25.4	1.96	0.35	0.1	579.7	1.24	unknown *
7	0.35	0.0	0.40	37.1	2.86	0.45	4.1	1342.9	2.88	unknown *
8	0.58	5.7	0.61	22.3	1.72	0.63	0.5	418.4	0.90	unknown *
9	0.64	0.2	0.79	457.7	35.23	0.83	376.9	28094.7	60.30	unknown *
10	0.83	378.4	0.84	389.8	30.00	0.88	2.1	7469.1	16.03	unknown *
11	0.89	2.1	0.91	86.4	6.65	0.92	40.3	1385.6	2.97	unknown *

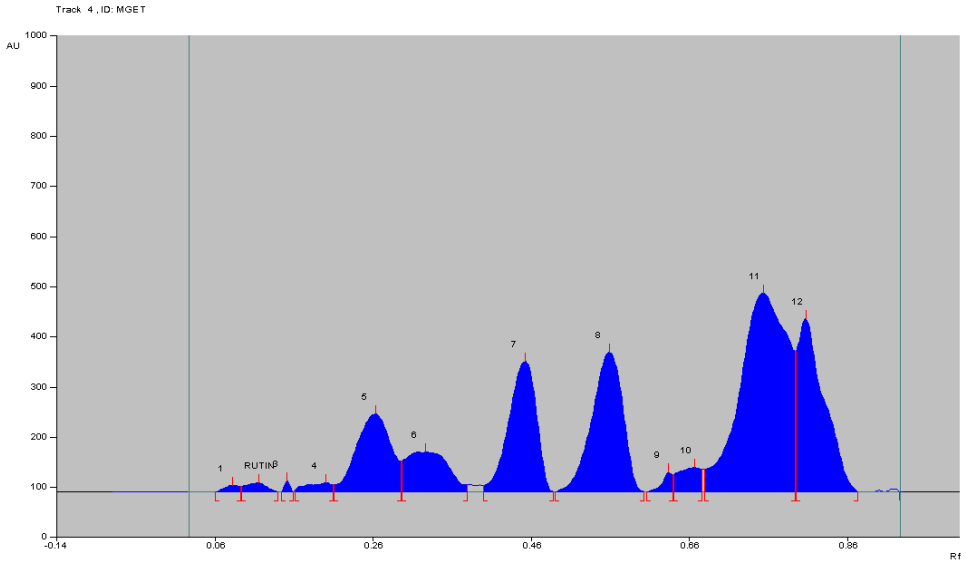
Fig. 3: Chromatogram of *Mussaenda glabrata* chloroform extract 10 µl



winCATS Planar Chromatography Manager

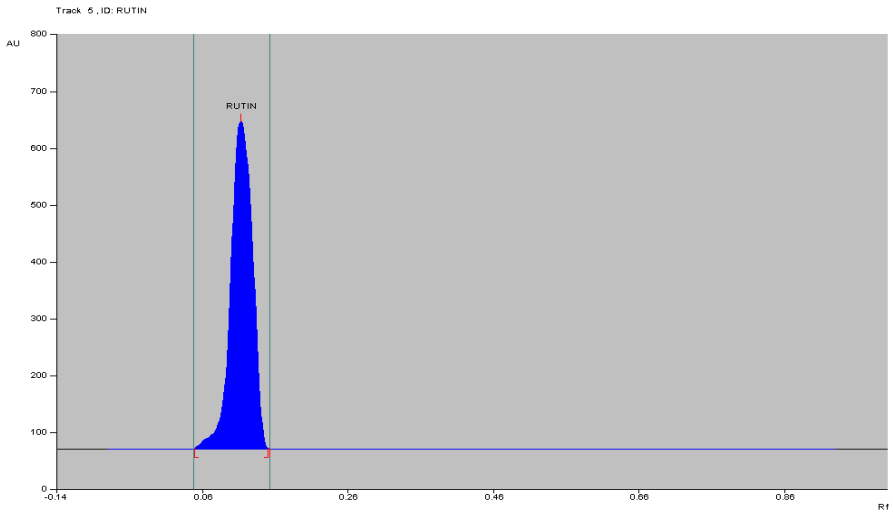
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.3	0.11	44.1	3.18	0.14	10.9	1316.9	3.02	RUTIN
2	0.15	11.1	0.16	33.0	2.38	0.17	3.6	261.8	0.60	unknown *
3	0.17	12.3	0.18	33.2	2.40	0.20	11.5	571.3	1.31	unknown *
4	0.22	8.5	0.26	138.6	10.00	0.29	1.8	3299.9	7.57	unknown *
5	0.29	2.1	0.34	76.0	5.48	0.40	23.8	3143.6	7.22	unknown *
6	0.40	24.2	0.45	204.1	14.73	0.48	0.6	5414.8	12.43	unknown *
7	0.49	0.2	0.56	205.2	14.80	0.60	0.1	5925.2	13.60	unknown *
8	0.61	0.2	0.67	28.3	2.04	0.68	27.1	915.7	2.10	unknown *
9	0.68	27.2	0.75	338.3	24.40	0.79	220.5	15279.4	35.07	unknown *
10	0.79	222.5	0.81	285.3	20.59	0.88	0.1	7440.3	17.08	unknown *

Fig. 4: Chromatogram of *Mussaenda glabrata* ethanol extract 5 µl



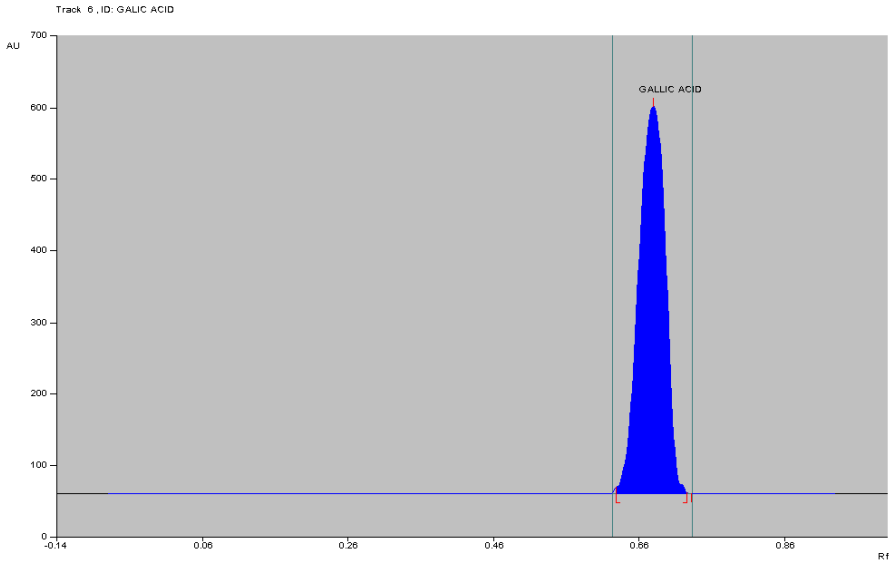
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.06	0.0	0.08	13.1	0.78	0.09	11.0	194.9	0.34	unknown *
2	0.09	11.2	0.11	18.0	1.07	0.14	0.1	372.9	0.66	RUTIN
3	0.14	0.5	0.15	21.4	1.28	0.16	0.0	119.4	0.21	unknown *
4	0.16	1.6	0.20	18.3	1.09	0.21	14.0	473.2	0.84	unknown *
5	0.21	14.2	0.26	154.8	9.26	0.29	61.6	5467.3	9.65	unknown *
6	0.29	62.0	0.32	79.0	4.72	0.38	13.7	3490.6	6.16	unknown *
7	0.40	13.2	0.45	260.2	15.56	0.49	0.0	7406.6	13.07	unknown *
8	0.49	0.1	0.56	278.4	16.64	0.60	0.2	8816.0	15.56	unknown *
9	0.60	0.1	0.63	39.4	2.35	0.64	34.5	405.2	0.72	unknown *
10	0.64	35.1	0.66	48.4	2.89	0.67	45.5	1140.4	2.01	unknown *
11	0.68	45.9	0.75	396.2	23.69	0.79	280.1	19314.3	34.09	unknown *
12	0.79	281.2	0.81	345.5	20.66	0.87	0.6	9459.2	16.69	unknown *

Fig. 5: Chromatogram of *Mussaenda glabrata* ethanol extract 10 µl



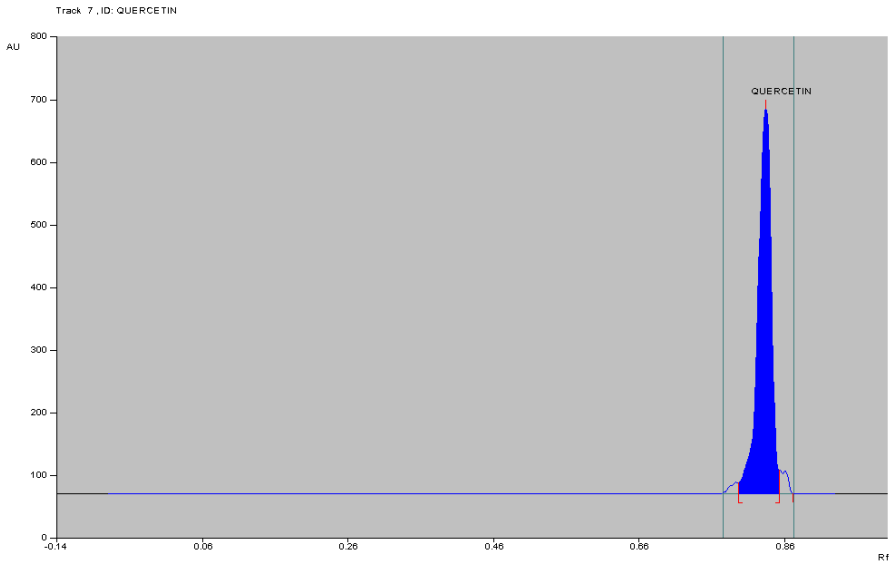
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	1.7	0.11	573.1	100.00	0.15	0.1	13836.0	100.00	RUTIN

Fig. 6: Chromatogram of rutin 5 µl



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.63	9.9	0.68	537.9	100.00	0.72	7.4	16028.6	100.00	GALLIC ACID

Fig. 7: Chromatogram of gallic acid 5 µl



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.79	1.9	0.83	593.4	100.00	0.85	13.5	8397.3	100.00	QUERCETIN

Fig. 8: Chromatogram of quercetin 5 µl

Table 1: Rf values of Chloroform Ethanol extracts and standard markers rutin, querctin and gallic acid

Track Number/Name of the formulation	Amount of Sample applied in µl	Total Number of compunds in extracts	Rf Value of the marker in extracts	Name of the marker present in extracts.	Area of Standard Marker	Area of marker in sample	Amount of marker present	% of marker in Extracts
Track-1-Chloroform extract	5 µl	10	0.81	quercetin		4520.8	2.69 µg	0.54%
Track-2 Chloroform extract	10 µl	11	0.11	Rutin	--------	1168.3	0.42 µg	0.04%
			0.84	quercetin	--------	7469.1	4.45 µg	0.45%
Track-3 Ethanol extact	5 µl	10	0.11	Rutin,	--------	1316.9	0.48 µg	0.09%
			0.67	gallic acid	--------	915.7	0.25 µg	0.05%
			0.81	quercetin	--------	7440.3	4.43 µg	0.88%
Track-4 Ethanol extact	10 µl	12	0.11	Rutin,	--------	372.9	0.13 µg	0.01%
			0.66	gallic acid	--------	1140.4	0.32 µg	0.32%
			0.81	quercetin	-------	9459.2	5.63 µg	0.56%
Track-5 Rutin standard	5 µl	1	0.11	Rutin	14899.5	--------	5 µg	------
Track-6 Gallic acid standard	5 µl	1	0.68	Gallic acid	18840.5	--------	5µg	------
Track-7 Quercetin standard	5 µl	1	0.83	Querctin	11249.8	--------	5 µg	--------

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