

Development of HPTLC Fingerprinting for Different Extract of *G. Asiatica* Linn. Leaf and Fruit.

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Abstract

HPTLC Chromatogram of *G. asiatica* Linn. leaf, petroleum ether, chloroform, ethanol and aqueous extract, had shown the presence of 9, 5, 6 and 3 numbers of compounds respectively. There may be two compounds in all the extracts having similar R_f value 0.19 - 0.22 and R_f value 0.34 - 0.39. While HPTLC Chromatogram of *G. asiatica* Linn. fruits, petroleum ether, chloroform, ethanol, methanol and aqueous extract, had shown the presence of 11, 11, 10, 6 and 7 numbers of compounds respectively. There may be two compounds in all the extracts having similar R_f value 0.36 - 0.38 and R_f value 0.85 - 0.87.

Introduction:

Synonyms: *Grewia subinaequalis* DC

Biological Source¹: Drug consists of dried whole plant of *Grewia asiatica* Linn. belonging to family Tiliaceae.

Part used: bark, fruits, leaves²



Plant of *Grewia asiatica* Linn.

Vernacular names³

Sanskrit : Dharmana, Parusha

Bengali : Shakri, Phalsa

English : Phalsa

Gujrati : Phalsa

Hindi : Phalsa

Malayalam : Sataschi

Marathi : Daman, Damni, Karavarani

Tamil : Tadachit, Sadachi, Una, Tarra
Telugu : Phutiki, Charachi, Ettatada, Nulijana
Punjabi : Phalna, Pharua

Description^{2,3}

A shrub or small tree, young parts stellately pubescent.

- **Bark:** Rough and gray.
- **Leaves:** Leaves are 7-17/6-12 cm, ovate or suborbicular, acute or subacuminate or cuspidate, sharply and often coarsely doubly serrate, subglabrous above, hairy-tomentose beneath, rounded or only slightly cordate at the base 5-6-7 nerved; petioles 6-12 mm long, thickened at the top; stipules nearly as long as the petioles, linear, lanceolate.
- **Flower buds:** Flower-buds broadly cylindrical or clavate. Peduncles axillary, usually many, long, slender, far exceeding the petioles and often 3-4 times as long, sometimes 4 cm long.
- **Flowers:** Flowers large. Bracts beneath the pedicels lanceolate. Sepals about 10 cm. long, linear oblong, acute, stellately pubescent or tomentose. Petals yellow, oblong or ovate-oblong, jagged or entire, about 6 mm. long, not bifid, gland with a wide fleshy margin, pubescent towards the edges. Gonophore long. Stigma with 4 short, rounded lobes; style much thickened above.
- **Fruit:** Fruit red, globose, 6-8 mm. diameter; pyrenes 1-2, always 1- celled only.

Habitat: Drier woodlands and on most soils as well as drier vine thickets and coastal regions".

Materials and Method:^[4,5,6,7]

Collection of Plant Material

Aerial parts of *Grewia asiatica* Linn. herbs growing in natural habitat in Rampura, Panchmahal, Gujarat, India, were collected in June, 2018.

Extraction and Phytochemical Investigations

The powder of *G. asiatica* Linn. leaves and fruits were successively extracted with Petroleum Ether, Chloroform, Methanol by soxhlet apparatus & Maceration with Chloroform water.

HPTLC Fingerprinting Of *G. Asiatica* Linn. Fruit & Leaf Extract

HPTLC is the most simple separation technique available today which gives better precision and accuracy with extreme flexibility for various steps (stationary phase, mobile phase, development technique and detection). The HPTLC was carried out using a Hamilton 100 µl HPTLC syringe, Camag Linomat V automatic spotting device, Camag twin trough chamber, Camag TLC Scanner-3, WINCAT integration software, aluminium sheet precoated with Silica Gel 60F254 (Merck), 0.2 mm thickness. HPTLC finger printing technique is useful to identify and to check the purity of raw herbal extracts as well as finished product. Hence forth it is very useful tool in standardizing process of raw herbal extracts and finished products.

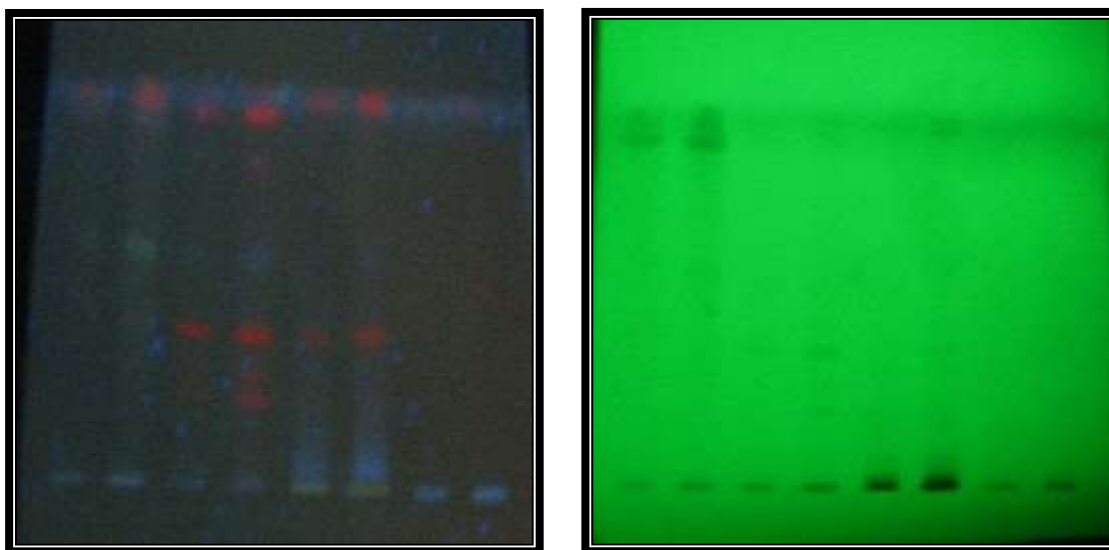
Steps involved in HPTLC analysis

- **Selection of plate and adsorbent:** Precoated aluminium plates with Silica Gel 60F254 (E. Merck, India) of 10 x 10 cm and 0.2 mm thickness, were used for the detection. The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography.
- **Sample solution:** Accurately weighed 100 mg of petroleum ether, chloroform, ethanol and aqueous extract of leaf and petroleum ether, chloroform, ethanol, methanol and aqueous extract of fruit of *G. asiatica* Linn. were taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume was made up to the mark with methanol. This solution was further used for HPTLC finger-printing.

- **Application of sample:** Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of *G. asiatica* Linn. extracts solution viz. 2,4,6,8 µl.
- **Development:** The plate was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2 °C, relative humidity 40%). The development distance was 8 cm. Subsequently scanning was done.
- **Detection:** The plate was scanned at UV 366 nm and 254 nm using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of each compound which were separated on plate and data of peak area of each band was recorded.

HPTLC Fingerprinting Of Various Extract Of *Grewia Asiatica* Linn. Leaf And Fruit

HPTLC fingerprinting of *G. asiatica* Linn. leaf extracts



UV 366

UV 254

Fig. 1: HPTLC fingerprinting of *G. asiatica* Linn. leaf extracts

[Track 1: 2 µg/ml Pet. ether extract of GA leaf; Track 2: 4 µg/ml Pet. ether extract of GA leaf; Track 3: 2 µg/ml Chloroform extract of GA leaf; Track 4: 4 µg/ml ; Chloroform extract of GA leaf; Track 5: 2 µg/ml Alcoholic extract of GA leaf; Track 6: 4 µg/ml Alcoholic extract of GA leaf; Track 7: 2 µg/ml Aqueous extract of GA leaf; Track 8: 4 µg/ml Aqueous extract of GA leaf]

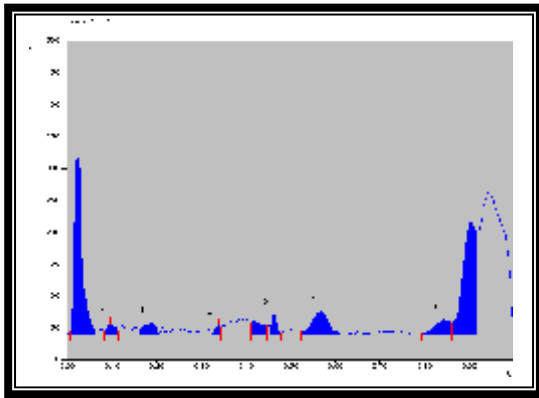


Fig. 2 HPTLC fingerprinting of Petroleum ether extract of *G. asiatica* Linn. leaf.

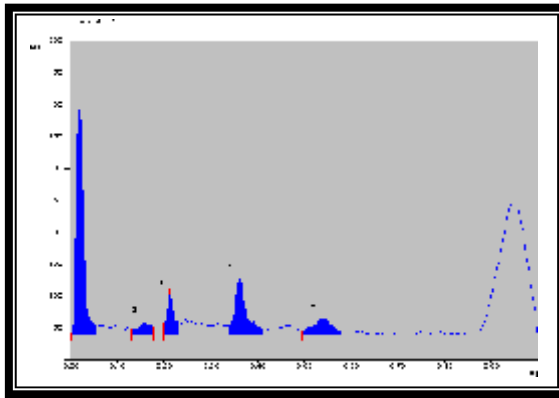


Fig. 3 HPTLC fingerprinting of Chloroform extract of *G. asiatica* Linn. leaf

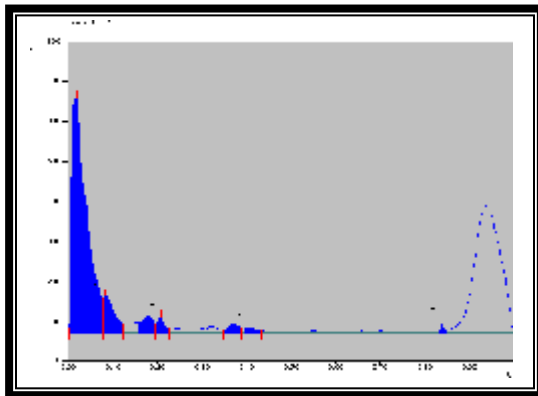


Fig. 4 HPTLC fingerprinting of alcoholic extract of *G. asiatica* Linn. leaf

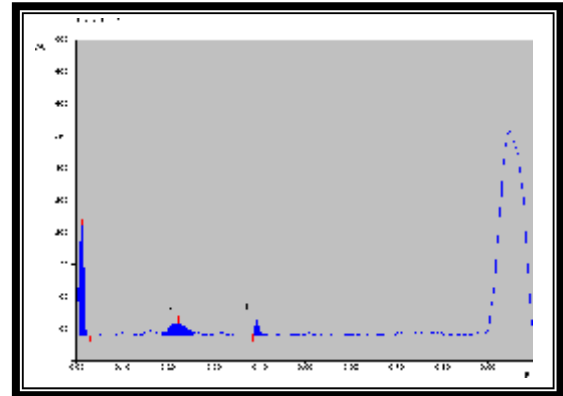


Fig. 5 HPTLC fingerprinting of aqueous extract of *G. asiatica* Linn. leaf

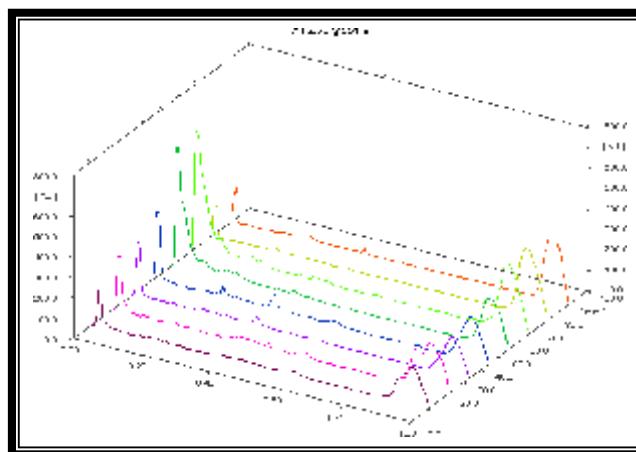


Fig. 6 3D Fingerprinting of *G. asiatica* Linn. leaf extracts

Table: 1 Rf and AUC in HPTLC of various extract of GA leaf

Peak	Pet. Ether ext.		Chloroform ext.		Alcohol ext.		Aqueous ext.	
	Rf	AUC	Rf	AUC	Rf	AUC	Rf	AUC
1	0.03	4036.8	0.02	2974.6	0.02	13098.8	0.01	1380.4
2	0.10	254.8	0.12	167.6	0.09	149.6	0.20	603.5
3	0.19	409.6	0.21	395.0	0.17	940.0	0.39	183.9
4	0.34	142.6	0.37	1036.9	0.19	607.3		
5	0.42	472.6	0.55	331.8	0.21	689.9		
6	0.46	281.7			0.36	462.1		
7	0.57	1074.5						
8	0.84	669.9						
9	0.90	4327.0						

The result suggested that HPTLC Chromatogram of *G. asiatica* Linn. leaf, petroleum ether, chloroform, ethanol and aqueous extract, had shown the presence of 9, 5, 6 and 3 numbers of compounds respectively. There may be two compound in all the extracts having similar Rf value. Compound one was in pet. Ether extract at Rf 0.19, in chloroform extract at Rf 0.21, in alcohol extract at Rf 0.19 and in aqueous extract at Rf 0.22. Compound second was in pet. Ether extract at Rf 0.34, in chloroform extract at Rf 0.37, in alcohol extract at Rf 0.37 and in aqueous extract at Rf 0.39.

HPTLC fingerprinting of *G. asiatica* Linn. fruit extracts

For the HPTLC fingerprinting of *G. asiatica* Linn., here we put two different concentration of petroleum ether extract, chloroform extract, ethanol extract, methanol extract and aqueous extract respectively on HPTLC plate. Here we use chloroform : methanol (7 : 3) as mobile phase for separation of compounds.

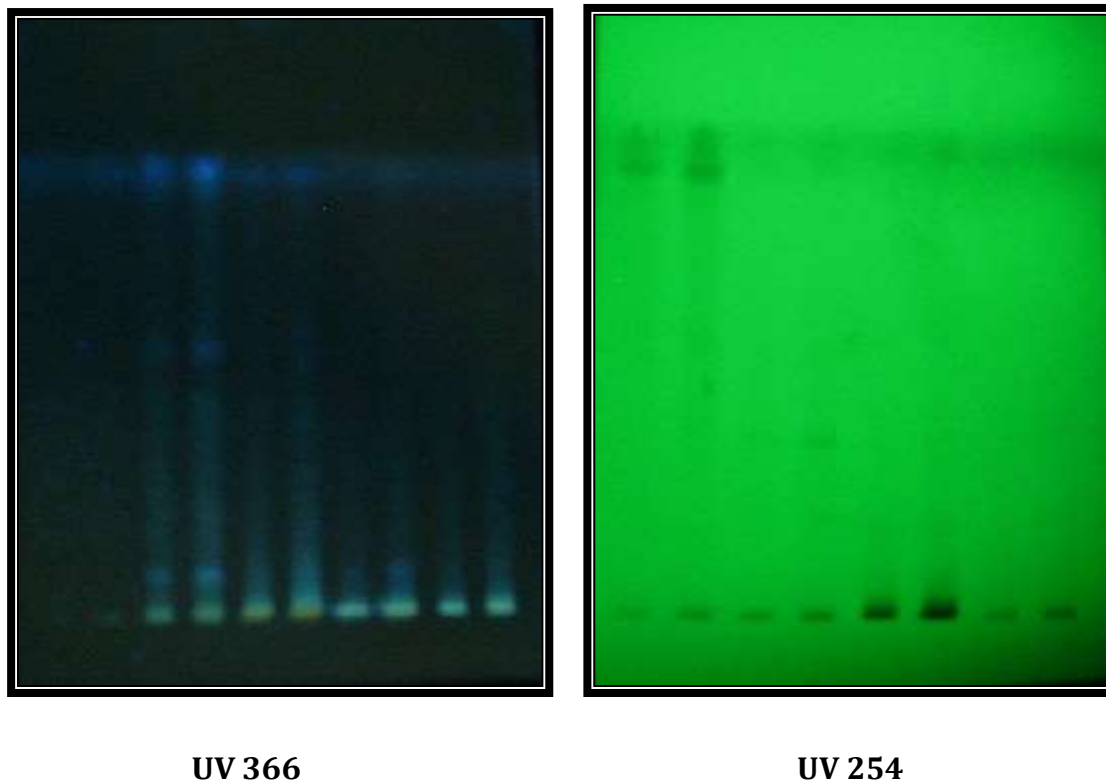


Fig 7 HPTLC fingerprinting of *G. asiatica* Linn. fruit extracts

[**Track 1:** 2 µg/ml Petroleum ether extract of GA fruit; **Track 2:** 4 µg/ml Petroleum ether extract of GA fruit; **Track 3:** 2 µg/ml Chloroform extract of GA fruit; **Track 4:** 4 µg/ml Chloroform extract of GA fruit; **Track 5:** 2 µg/ml Alcoholic extract of GA fruit ; **Track 6:** 4 µg/ml Alcoholic extract of GA fruit; **Track 7:** 2 µg/ml Methanolic extract of GA fruit; **Track 8:** 4 µg/ml Methanolic extract of GA fruit; **Track 9:** 2 µg/ml Aqueous extract of GA fruit; **Track 10:** 4 µg/ml Aqueous extract of GA fruit]

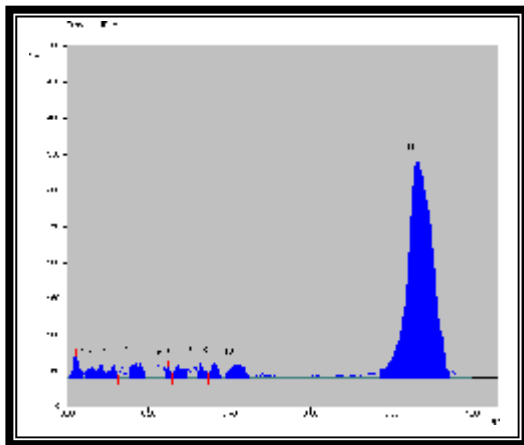


Fig. 8 HPTLC fingerprinting of ether extract of GA fruit

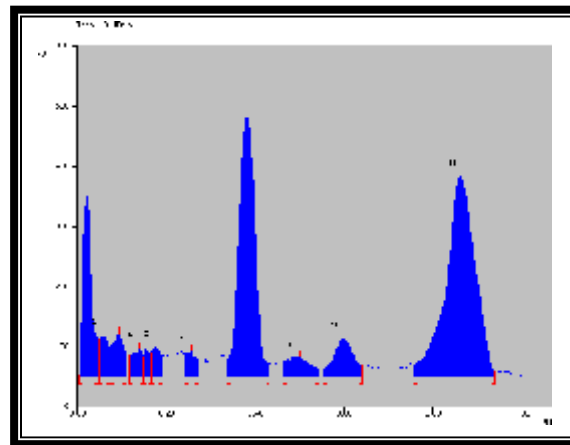


Fig. 9 HPTLC fingerprinting of Pet. chloroform extract of GA fruit

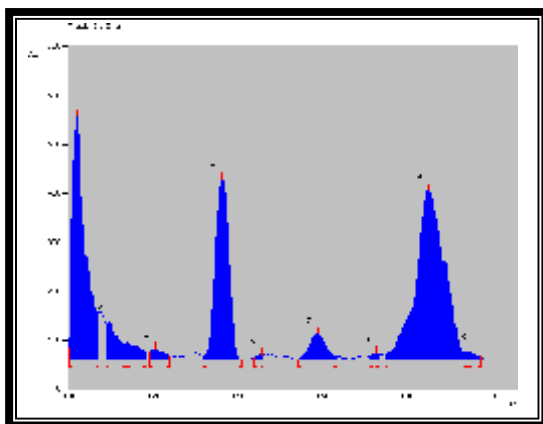


Fig. 10 HPTLC fingerprinting of extract of GA fruit

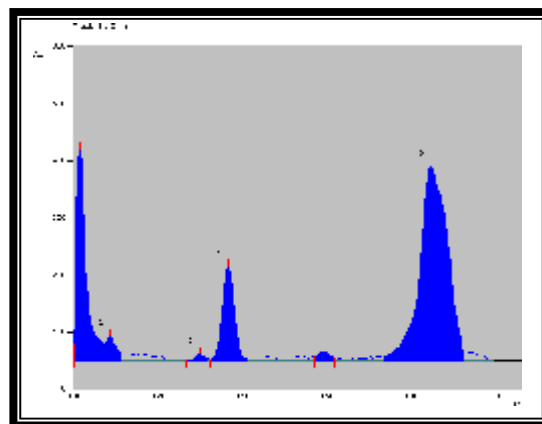


Fig. 11 HPTLC fingerprinting of ethanol methanolic extract of GA fruit

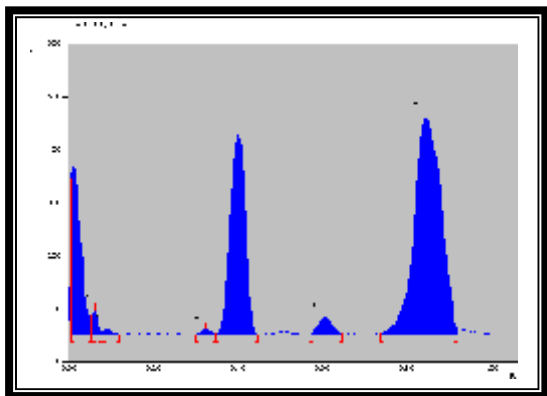


Fig. 12 HPTLC fingerprinting of aqueous extract of GA fruit

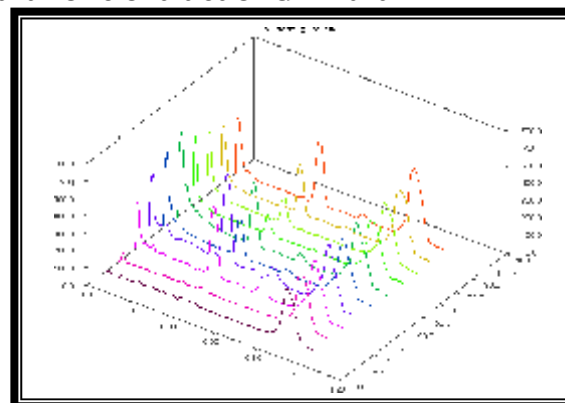


Fig.13 3D Diagram of fingerprinting of GA fruit extracts

Table. 2 Rf and AUC in HPTLC of various extract of GA fruit

Peak	Pet. Ether ext.		Chloroform ext.		Alcohol ext.		Methanol ext.		Aqueous ext.	
	Rf	AUC	Rf	AUC	Rf	AUC	Rf	AUC	Rf	AUC
1	0.02	399.7	0.02	5068.3	0.02	13416.3	0.01	7791.3	0.01	6228.6
2	0.06	245.3	0.06	1151.2	0.11	18961.3	0.09	3946.5	0.06	555.2
3	0.08	208.1	0.09	1578.3	0.14	954.6	0.3	189.6	0.09	208.7
4	0.11	233.9	0.14	887.6	0.21	580.3	0.37	4153.2	0.36	227.5
5	0.17	458.7	0.15	520.6	0.36	10428.2	0.59	389.9	0.40	11992.1
6	0.25	158.4	0.18	853.2	0.46	140.7	0.85	19664	0.61	1027.9
7	0.27	335.7	0.26	821.1	0.59	1802.6			0.85	22936.2
8	0.33	240.4	0.38	13609.0	0.73	193.6				
9	0.36	303.5	0.50	1533.7	0.85	20347.7				
10	0.42	562.0	0.60	2427.0	0.95	257.0				
11	0.87	16068.6	0.86	18916.0						

The result suggested that HPTLC Chromatogram of *G. asiatica* Linn. fruits, petroleum ether, chloroform, ethanol, methanol and aqueous extract, had shown the presence of 11, 11, 10, 6 and 7 numbers of compounds respectively. There may be two compound in all the extracts having similar Rf value. Compound one was in pet. Ether extract at Rf 0.36, in chloroform extract at Rf 0.38, in alcohol extract at Rf 0.36, in methanol extract at Rf 0.37 and in aqueous extract at Rf 0.36. Compound second was in pet. Ether extract at Rf 0.87, in chloroform extract at Rf 0.86, in alcohol extract at Rf 0.85, in methanol extract at Rf 0.85 and in aqueous extract at Rf 0.85.

References:

1. Nadkarani KM, "Indian Materia Medica with ayurvedic, unani tibbi, siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & Indexes", Popular Prakashan Pvt. Ltd., Mumbai 1997; 1:593.
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