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HPTLC fingerprinting of phenolics profile of three *Curcuma* species

Mangesh Dagawal and Prabha Bhogaonkar

Abstract
Genus *Curcuma* L. (Zingiberaceae) comprising of 120 species is distributed throughout South and South-East Asia, with few species extending to China, Austrilia and South Pacific. Four species of *Curcuma* are reported from Melghat. Of these *C. longa* L. is cultivated, while *C. inodora* Blatt., *C. pseudomontana* J. Graham and *C. decipiens* Dalzell are wild. *C. decipiens* being rare coluld not be collected. *Curcuma inodora* Blatt. known as 'Jangali Halad' is a common herb of Melghat at higher elevations. In Melghat area populations of *C. inodora* are found to show many distinct variations in aerial as well as underground characters. Twelve distinct variants of *C. inodora* and single accession each of *C. pseudomontana* and *C. longa* were collected. HPTLC profile of phenolics showed significantly different banding pattern and Rf values. One of the phenolic compound at 0.75 Rf value is most common chemical compound in all samples except *C. longa*. Thus *C. longa* stands distinct. *C. pseudomontana* clubs with entire *C. inodora*. however, spot at Rf *0.65* is found only in *C. pseudomontana* thus making it distinct from *C. inodora* accessions. HPTLC screening of phenolics reflects distinctness as well as relatedness of the species. Phenolic profile can be used for the standardization of three *Curcuma* species studied here.

Keywords: *Curcuma inodora* Blatt, *Curcuma pseudomontana* J. Graham, *Curcuma longa* L., Melghat forest, HPTLC, Phenolics profile

1. Introduction
Genus *Curcuma* L. (Zingiberaceae) comprising of 120 species and is distributed throughout South and South-East Asia, with a few species extending to China, Austrilia and the South Pacific; 40 species being recorded from india. Four species of *Curcuma* are reported from Melghat [1, 2]. Of these *C. longa* L. is cultivated while *C. inodora* Blatt., *C. pseudomontana* J. Graham and *C. decipiens* Dalzell are wild. *C. decipiens* being rare could not be collected.
C. inodora is widely distributed throughout Maharashtra and is very common and abundant in Melghat. It is commonly called 'Jangli halad' and used in traditional medicine by locals. Fresh rhizome paste is applied over cuts, as strong antiseptic. The smoke of dried rhizome is used to hypnotise the person, some use it in Tantrik, Vashikarana and Mayajal Kriyas [3]. Paste of root stock is applied in glandular diseases and piles [4-6], psychosomatic disorders and constipation [7, 8]. *C. pseudomontana* is used in traditional medicine to cure jaundice and diabetes [9], body swellings and to increase lactation [10]. Fresh tubers are eaten as blood purifier [11, 12]. The dried rhizome of *Curcuma longa* L. has been found to be a rich source of beneficial phenolic compounds known as the curcuminoids [13. 14]. It is used in several ways in Ayurveda and traditional medicine world over.
Most of the secondary metabolites and pigments are medicinally important. Phenolics probably constitute largest group of plant secondary metabolites. They are wide spread in nature, and to be found in most classes of natural compounds having aromatic molecules. They are important constituents of some medicinal plants and in the food industry they are utilized as colouring agents, flavouring, aromatizer and antioxidants. Simple phenolics like resorsinol are narcotic in action. Fluroglucinol derivatives of hydroquinone have taenicidal properties; p-hydroxybenzene also shows same activity. Phenolic compounds have wide range of biological properties such as anti-platelet aggregation property, anti-inflammatory potential, antioxidant, anti-tumoral and oestrogenic activity [14, 15]. Oxidative stress, the consequence of an imbalance of prooxidents and antioxidents in the organism is rapidly gaining recognition as key phenomenon in chronic diseases [16].
HPTLC based methods are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase. This reduces time and cost of analysis. HPTLC also facilitates repeated detection of chromatogram with same or different parameters. HPTLC analysis is performed for the development of characteristics fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug.

Srivastava *et al*. [17] studied HPTLC profile of *Curcuma aeuriginosa* Roxb.; they found curcumin to be present in traces only in the species. HPTLC fingerprint of a plant species can provide sufficient information about phenolics present and for identification, standardization and quality control of the medicinal plant.

2. Material and Methods

Curcuma pseudomontana J.Graham, *Curcuma longa* L. and twelve variants of *Curcuma inodora* Blatt. were collected from various locations in Melghat Forests for HPTLC screening of phenolics. Identification of *Curcuma* species was done by using standard floras [2, 18, 19, 20]. For HPTLC studies leaves were washed with distilled water, air dried, powdered and stored at room temperature for further analysis. HPTLC screening was done following Wagner [21].

2.1 Sample preparation

500 mg of each sample was extracted with 5 ml methanol by sonication for 30min. Then these solutions were filtered and filtrate used for chromatography.

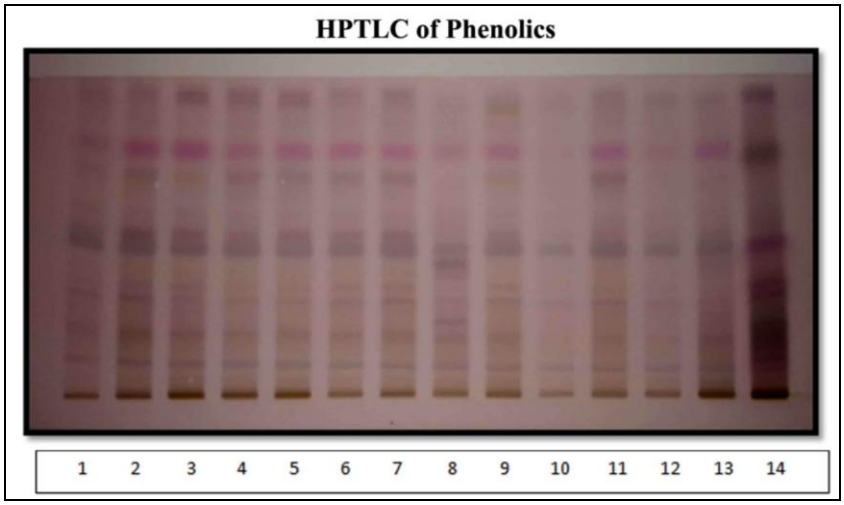
2.2 Chromatography

Chromatography was performed on Merck TLC plates precoated with silica gel 60 F_{254}. 10 µl sample extracts were loaded as band length 8.0 mm on TLC plate by CMAG linomat-5 sample applicator equipped with 100 µl syringe using Wincat's software. TLC plate was developed using mobile phase Toluene: Chloroform: Methanol (6:3:1 v/v/v). ASR was used for visualization. The sample loaded plate was kept in TLC developing chamber (after saturation with solvent vapor) and the plate was developed in the mobile phase. After removal of plate from chamber, plate was derivatised by using ASR reagent and kept in photo documentation chamber. The image was captured at UV 366 nm. Finally, the plate was fixed in scanner and Rf values were recorded.

3. Results and Discussion

HPTLC profile of methanolic extracts showed the presence of phenolics in chromatogram as well as in UV light after derivatization.

Banding pattern on TLC plate is shown in photoplate no. 1. Rf values and peak areas of each sample are represented in chromatograms. Spots at 0.75 Rf value is most common chemical compound in all samples except sample no. 14. Spot at 0.65 Rf is found only in sample no.13. Spots at 0.50 Rf values is most common chemical compounds in samples 1, 2, 3, 4, 5, 6, 7 and 9. Maximum number of peaks i.e. 9 is appear in sample 4, 5 and 9 while in sample 10 and 12 minimum number of peaks i.e. 5 are produced. Peak no. 5 in sample 2 shows highest concentration i.e. 55. 88%. Peak, Rf and area of the respective phenolics are given in Table no. 1 and chromatograms of each sample are presented in Fig no. 1 to 14. HPTLC profile of phenolics showed significantly different banding pattern and Rf values for all samples studied. One of the phenolic compound at 0.75 Rf value is most common chemical compound in all samples except *C. longa*. Thus *C. longa* stands distinct while *C. pseudomontana* clubs with *C. inodora*, however, spot at Rf 0.65 is found only in *C. pseudomontana* thus making it distinct from *C. inodora* accessions.



HPTLC of Phenolics image of 14 samples (Track 1 to 14) under wave length 366 nm after derivatsing with NP reagent

Table 1: HPTLC peak of 14 samples Phenolics with Rf values

Sample	Peak	Rf	Area	
			Area	%
Sample-01 (CI-01)	1	0.21	899.5	7.85
	2	0.39	255.3	2.33
	3	0.45	1295.3	11.31
	4	0.50	534.9	4.67
	5	0.57	464.4	4.05
	6	0.69	1356.2	11.84
	7	0.75	4512.7	39.39
	8	0.88	2138.3	18.66
Sample-2 (CI-02)	1	0.21	1564.3	7.81
	2	0.40	262.3	1.31
	3	0.50	4164.1	20.80
	4	0.55	706.5	3.53

	5	0.75	11070.3	55.88
	6	0.90	2257.0	11.27
Sample -3 (CI-03)	1	0.21	1482.2	6.80
	2	0.26	117.1	0.54
	3	0.46	1445.4	6.63
	4	0.50	2294.8	10.53
	5	0.56	570.6	2.62
	6	0.75	11635.4	53.39
	7	0.90	4246.7	19.49
Sample 4 (CI-04)	1	0.21	1647.9	6.40
	2	0.26	152.6	0.59
	3	0.39	251.5	0.98
	4	0.46	1873.1	7.26
	5	0.50	2619.7	10.18
	6	0.56	622.8	2.42
	7	0.69	4105.6	15.96
	8	0.75	9009.3	35.01
	9	0.90	5448.5	21.17
Sample 5 (CI-05)	1	0.21	2309.5	7.14
	2	0.27	315.5	0.98
	3	0.31	225.1	0.70
	4	0.40	338.6	1.05
	5	0.50	6298.0	19.48
	6	0.56	875.3	2.71
	7	0.69	4658.5	14.41
	8	0.75	10743.7	33.23
	9	0.89	6566.5	20.31
Sample 6 (CI-06)	1	0.22	2004.7	7.12
	2	0.27	235.3	0.84
	3	0.31	238.8	0.85
	4	0.40	330.8	1.17
	5	0.50	5663.3	20.11
	6	0.69	4088.4	14.52
	7	0.75	9776.1	34.71
	8	0.90	58.24	20.68
Sample-7 (CI-07)	1	0.22	2199.6	8.39
	2	0.27	264.1	1.01
	3	0.30	209.2	0.80
	4	0.39	293.7	1.12
	5	0.50	5639.4	21.51
	6	0.69	4582.1	17.48
	7	0.75	8177.4	31.20
	8	0.90	4847.3	18.49
Sample 8 (CI-08)	1	0.21	2150.1	10.31
	2	0.35	659.1	3.16
	3	0.40	1870.1	8.97
	4	0.46	2085.4	10.00
	5	0.51	3024.3	14.50
	6	0.64	1723.6	8.26
	7	0.75	4973.8	23.85
	8	0.85	4371.2	20.96
Sample 9 (CI-09)	1	0.21	1715.9	8.40
	2	0.31	167.0	0.82
	3	0.39	386.7	1.89
	4	0.45	2544.0	12.45
	5	0.50	1274.1	6.23
	6	0.56	322.7	1.58
	7	0.69	2027.9	9.92
	8	0.75	6762.4	33.09
	9	0.88	5234.9	25.62
Sample 10 (CI-10)	1	0.22	1086.8	7.89
	2	0.39	227.7	1.65
	3	0.46	2710.8	19.68
	4	0.75	6906.2	50.14
	5	0.90	2843.4	20.64
Sample 11 (CI-11)	1	0.21	1298.6	9.16
	2	0.31	184.6	1.30
	3	0.40	247.1	1.74
	4	0.46	3007.9	21.22

	5	0.69	2711.2	19.13
	6	0.75	4921.1	34.72
	7	0.90	1803.9	12.73
Sample 12 (CI-12)	1	0.22	813.9	8.26
	2	0.39	207.7	2.11
	3	0.46	2182.2	22.14
	4	0.75	4717.5	47.87
	5	0.90	1933.6	19.62
Sample 13 (CP-13)	1	0.21	1256.1	9.81
	2	0.30	158.0	1.23
	3	0.35	411.8	3.24
	4	0.40	860.7	6.72
	5	0.46	1885.9	14.73
	6	0.51	1100.1	8.59
	7	0.65	1127.4	8.80
	8	0.75	4410.9	34.44
	9	0.88	1591.9	12.43
Sample 14 (CL-14)	1	0.21	1182.7	12.38
	2	0.31	737.5	7.72
	3	0.46	1935.4	20.25
	4	0.59	80.6	0.84
	5	0.74	2483.5	25.99
	6	0.90	3136.4	32.82

HPTLC Chromatogram of Phenolics

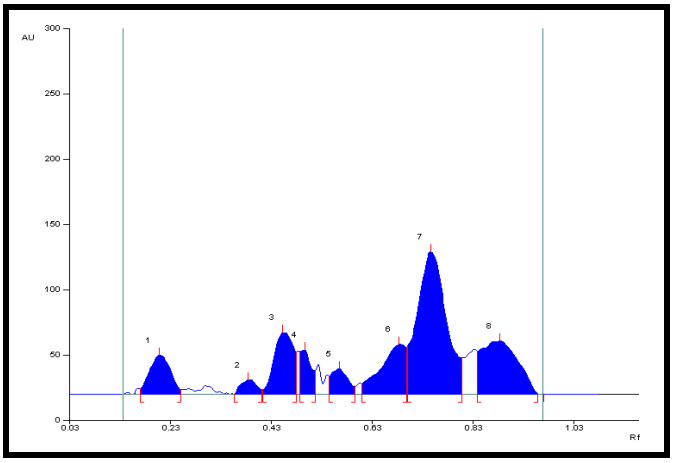


Fig 1: HPTLC Chromatogram of Phenolics Sample -1 (Variant- CI-01)

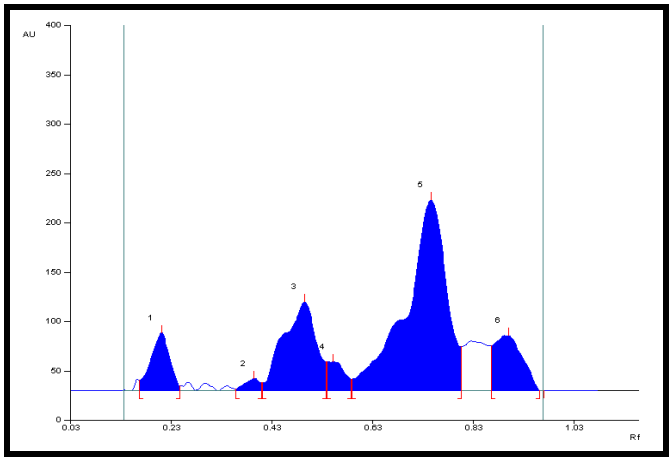


Fig 2: HPTLC Chromatogram of Phenolics Sample -2 (Variant- CI-02)

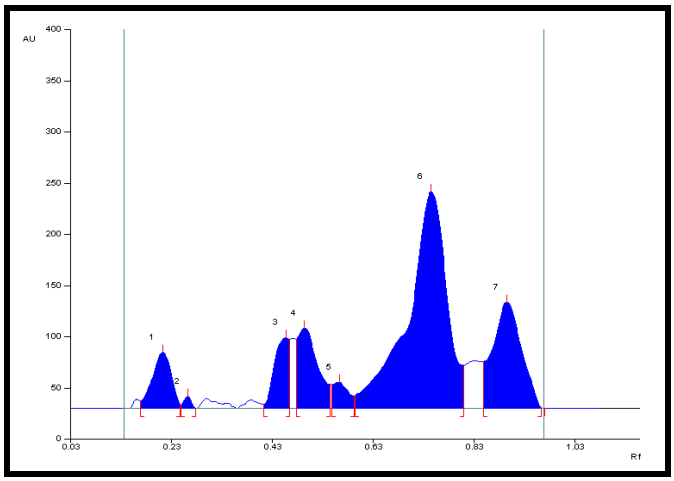


Fig 3: HPTLC Chromatogram of Phenolics Sample -3(Variant- CI-03)

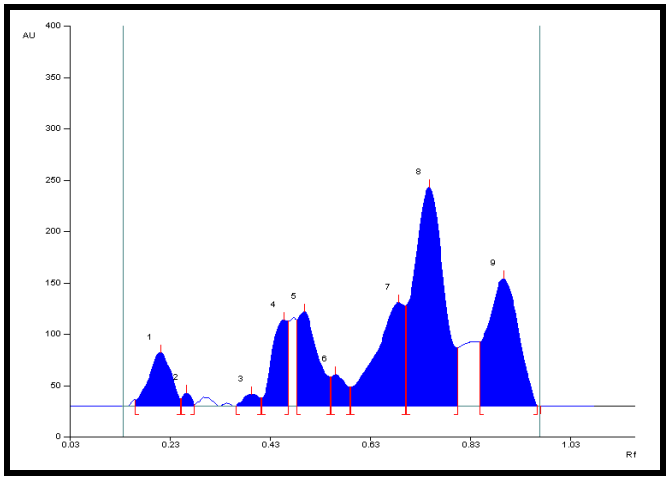


Fig 4: HPTLC Chromatogram of Phenolics Sample -4(Variant- CI-04)

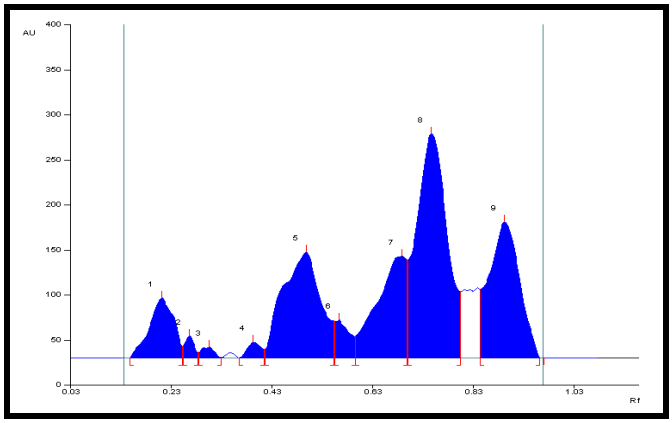


Fig 5: HPTLC Chromatogram of Phenolics Sample -5 (Variant- CI-05)

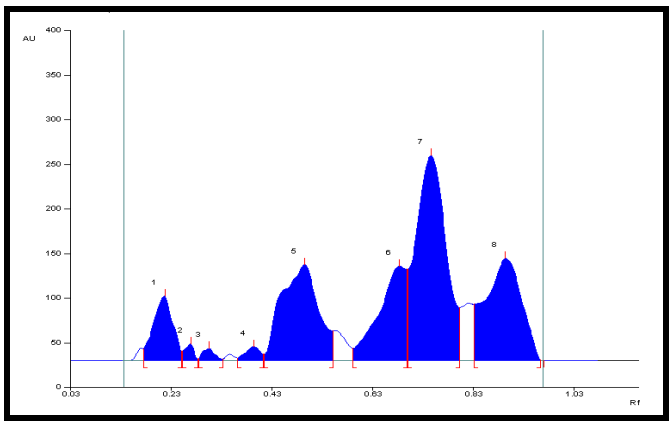


Fig 6: HPTLC Chromatogram of Phenolics Sample -6 (Variant- CI-06)

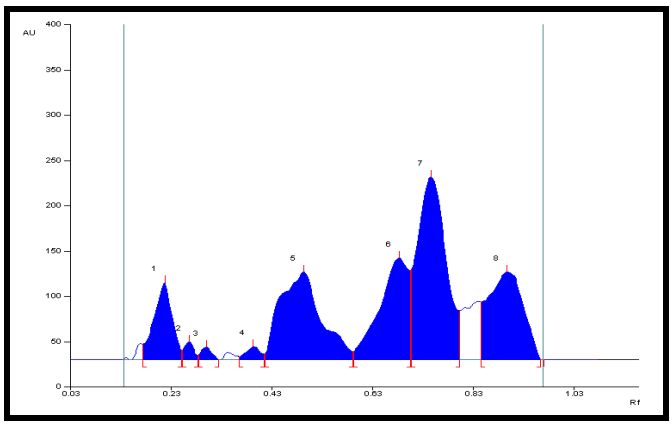


Fig 7: HPTLC Chromatogram of Phenolics Sample -7 (Variant- CI-07)

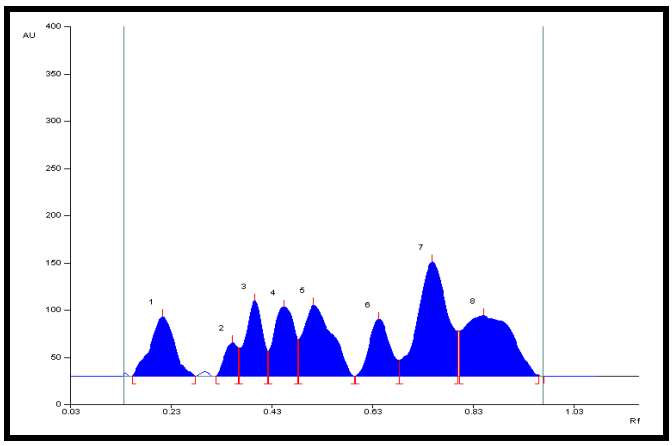


Fig 8: HPTLC Chromatogram of Phenolics Sample -8 (Variant- CI-08)

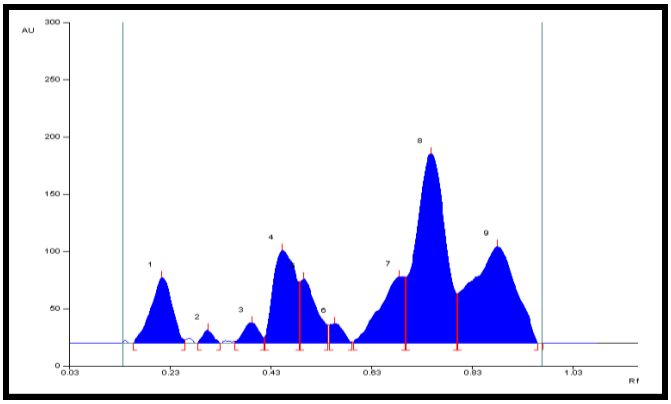


Fig 9: HPTLC Chromatogram of Phenolics Sample -9 (Variant- CI-09)

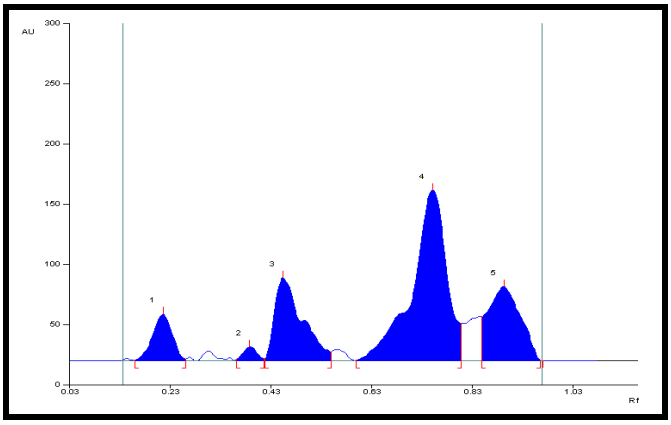


Fig 10: HPTLC Chromatogram of Phenolics Sample -10 (Variant- CI-10)

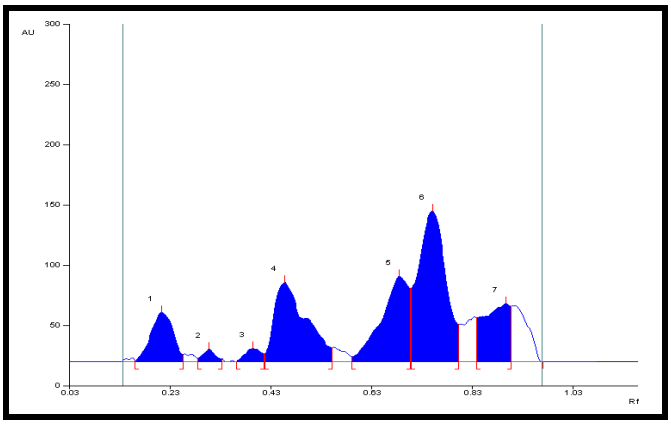


Fig 11: HPTLC Chromatogram of Phenolics Sample -11 (Variant- CI-11)

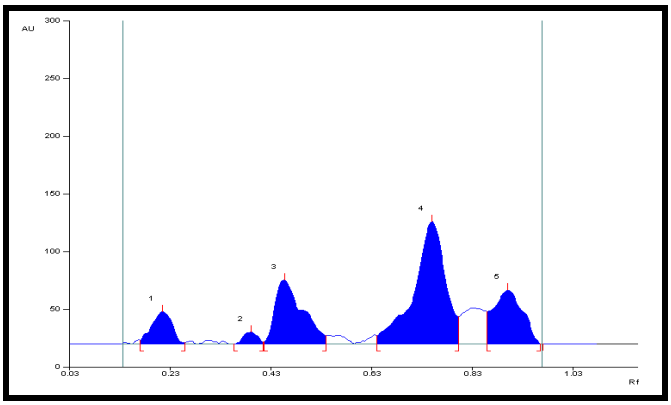


Fig 12: HPTLC Chromatogram of Phenolics Sample -12 (Variant- CI-12)

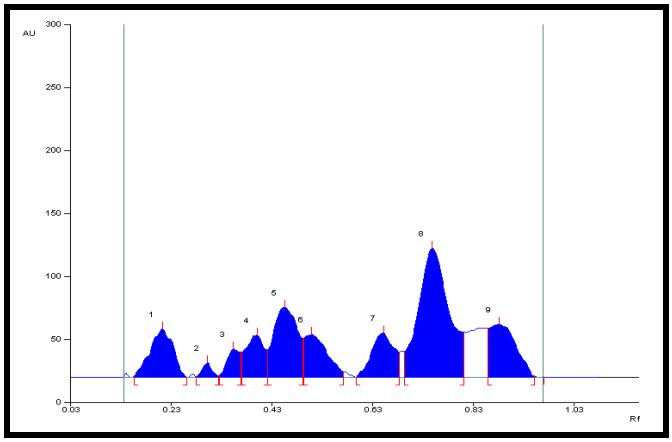


Fig 13: HPTLC Chromatogram of Phenolics Sample -13 (CP-13)

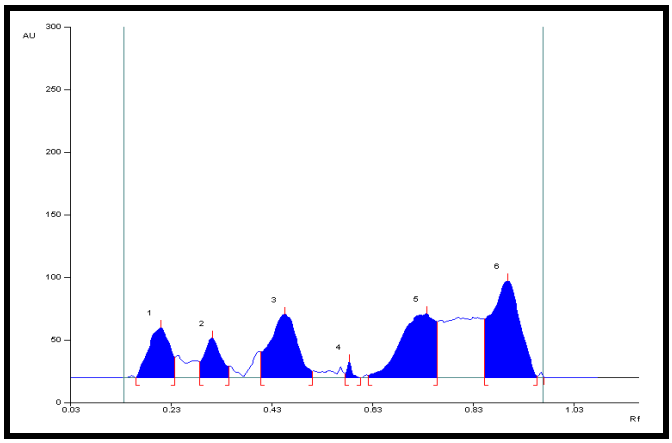


Fig 14: HPTLC Chromatogram of Phenolics Sample -14 (CL-14)

4. Conclusion

HPTLC profile of phenolics characterizes all the three species by virtue of presence or absence of a specific phenolic compound. Phenolic profile can be used for the standardization of three *Curcuma* species studied here.

5. Acknowledgment

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