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HPTLC FINGERPRINTING OF FLAVONOIDS PROFILE OF THREE *CURCUMA* SPECIES

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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* 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 flavonoid showed significantly different banding pattern and Rf values. Peak at 0.88 Rf value is most common chemical compound present in sample 2, 3, 7, 8, 9, 10 and 12 i.e. in 50 % samples. *C. longa* is distinct from others showing peaks at Rf 0.38, 0.62 and 0.71 not produced by rest 13 accessions. HPTLC screening of flavonoid reflects distinctness as well as relatedness of the species. Flavonoid 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; Flavonoid profile.

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 (Dhore and Joshi 1988 and, Bhogaonkar and Devarkar 1999). 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 (Devarkar, 2001). Paste of root stock is applied in glandular diseases and piles (Shah and Gopal, 1982, Mudaliar *et al*, 1987 and, Bhogaonkar and Kadam, 2005), psychosomatic disorders and constipation **(**Rommand-Monnier, 2009 and Jagtap, 2009). *C. pseudomontana* is used in traditional medicine to cure jaundice and diabetes (Panal *et al*., 2012), body swellings and to increase lactation (Ramarao *et al*., 2000). Fresh tubers are eaten as blood purifier (Acharya *et al*., 2012).

Most of the secondary metabolites and pigments are medicinally important. Flavonoids occur both in free state and as glycosides. They are the largest group of naturally occurring phenols. About 8000 flavonoids are known. (Babu and Liu 2009). Flavonoids are known to exhibit several biological activities such as antioxidants, anti-inflamatory, anti-allergic, anti-carcinogenic, antiviral, hepatoprotective, antithrombotic, antibacterial and antifungal (Gabor, 1979; Harborne, 1998; Middleton, 1984; Hertog *et al.*1993; Miller, 1996; Jaggi and Kapoor, 1999; Andreson *et al.* 2000; Narayana *et al.*2001).

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.

HPTLC fingerprint of a plant species can provide sufficient information about flavonoids present and for identification, standardization and quality control of the medicinal plant.

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 flavonoids. Identification of *Curcuma* species was done by using standard floras (Bhogaonkar and Devarkar, 1999, Cooke,1967, Dhore, 1986 and, Yadav and Sardesai, 2002). 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 (1996).

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.

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 plates and developed using mobile phase Ethyl Acetate: Glacial Acetic Acid: Formic Acid : water (10:0.5:0.5:1 v/v/v/v)). NP reagent (1gm of 2 –Aminoethyl diphenyl borate + 200 ml of methanol) 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 dipped in NP reagent and the image was captured at UV 366 nm. Finally, the plate was fixed in scanner and Rf values were recorded.

RESULTS AND DISCUSSION

HPTLC profile of methanolic extracts showed the presence of flavonoids 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.

Sample 1 shows only single spot and peak at Rf 0.84 and maximum number of peaks i.e. 8 peaks are present in sample 3 and 5. Peak at 0.88 Rf value is most common chemical compound present in seven samples 2, 3, 7, 8, 9, 10 and 12. All 14 samples tested are

significantly different. The area of peak reflects the concentration of the compound present. Sample no. 1 has only one flavonoid hence the percentage is 100%. However, the other samples show presence of more than one compound; here sample 6 peak no. 5 shows maximum concentration i.e. 48.79%.

The peak, Rf and area of the respective flavonoid is given in Table 1 and chromatograms of each sample are presented in Fig no. 1-14.

HPTLC profile of flavonoid showed significantly different banding pattern and Rf values for all samples studied. Peak at 0.88 Rf value is most common chemical compound present in sample 2, 3, 7, 8, 9, 10 and 12 i.e. in 50% samples. Flavonoid profile thus can be used to distinguish not only different species but also different variants. *C. longa* is distinct from others showing peaks at Rf 0.38, 0.62 and 0.71 not produced by rest 13 accessions. Thus flavonoid profile can serve as reliable tool to identify the variants as well as species.

Table No 1: HPTLC peak of 14 samples flavonoides with Rf values.

Sample	Peak	Rf	Area	
			Area	%
Sample-01 (CI-1)	1	0.84	1512.5	100
Sample-2 (CI-02)	1	0.60	677.5	3.82
	2	0.69	2887.5	18.29
	3	0.75	4884.1	27.56
	4	0.83	2918.2	16.47
	5	0.88	6356.0	35.86
Sample -3 (CI-03)	1	0.27	785.1	3.62
	2	0.37	287.1	1.32
	3	0.60	1070	4.93
	4	0.69	3528.4	16.25
	5	0.74	3040.9	14.01
	6	0.79	2070.0	9.53
	7	0.83	2443.7	11.26
	8	0.88	8484.6	39.08
Sample 4 (CI-04)	1	0.28	479.2	2.62
	2	0.61	928.6	5.08
	3	0.70	2943.1	16.09
	4	0.74	2004.0	10.96
	5	0.84	4402.2	24.06
	6	0.89	4015.1	21.95
	7	0.90	3521.1	19.25
Sample 5 (CI-05)	1	0.27	554.6	2.03
	2	0.37	772.4	2.83
	3	0.61	1670.1	6.11

	4	0.65	1663.8	6.09
	5	0.70	2629.4	9.62
	6	0.74	2305.8	8.44
	7	0.79	2275.1	8.32
	8	0.89	15460.0	56.57
Sample 6 (CI-06)	1	0.61	1243.4	4.26
	2	0.70	4313.4	14.79
	3	0.75	5788.5	19.85
	4	0.80	3470.4	11.90
	5	0.89	14230.8	48.79
	6	1.01	120.9	0.41
Sample-7 (CI-07)	1	0.61	1128.5	3.28
	2	0.70	4180.1	12.15
	3	0.75	6603.4	19.19
	4	0.79	3311.6	9.62
	5	0.84	5551.3	16.13
	6	0.88	13408.0	38.96
	7	1.00	228.6	0.66
Sample 8 (CI-08)	1	0.58	649.7	3.26
	2	0.70	4562.8	22.90
	3	0.74	2901.1	14.56
	4	0.78	2025.0	10.16
	5	0.83	3028.8	15.20
	6	0.88	6678.3	33.51
	7	1.01	80.4	0.40
Sample 9 (CI-09)	1	0.70	4140.4	16.63
	2	0.74	2509.2	10.08
	3	0.83	11o31.8	44.31
	4	0.88	7216.9	28.99
Sample 10 (CI-10)	1	0.70	2416.5	18.65
	2	0.73	1207.6	9.32
	3	0.78	1059.8	8.18
	4	0.83	3253.5	25.12
	5	0.88	5016.4	38.73
Sample 11 (CI-11)	1	0.63	709.2	8.07
	2	0.69	1905.1	21.69
	3	0.83	3971.6	45.21
	4	0.87	2006.4	22.84
	5	0.97	192.1	2.19
Sample 12 (CI-12)	1	0.69	1352.2	32.92
	2	0.83	1576.2	38.37
	3	0.88	1179.5	28.71
Sample 13 (CP-13)	1	0.31	114.4	1.58
	2	0.36	243.8	3.37
	3	0.68	1845.8	25.51
	4	0.75	1267.5	17.52
	5	0.87	2544.6	35.16
	6	0.92	1220.4	16.86

Sample 14 (CL-14)	1	0.27	588.6	9.21
	2	0.38	496.1	7.16
	3	0.62	509.3	7.97
	4	0.71	1710.1	26.75
	5	0.75	1043.4	16.32
	6	0.90	2045.7	32.00

Photoplate 1.

HPTLC of Flavonoid

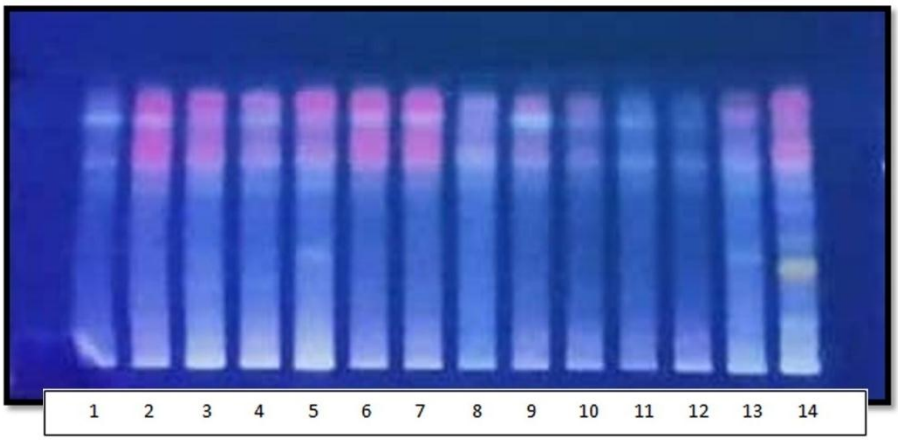

Fig: HPTLC of Flavonid image of 14 samples (Track 1 to 14) under wavelength 366 nm after derivatising with NP reagent

HPTLC Chromatogram of Flavonoids:

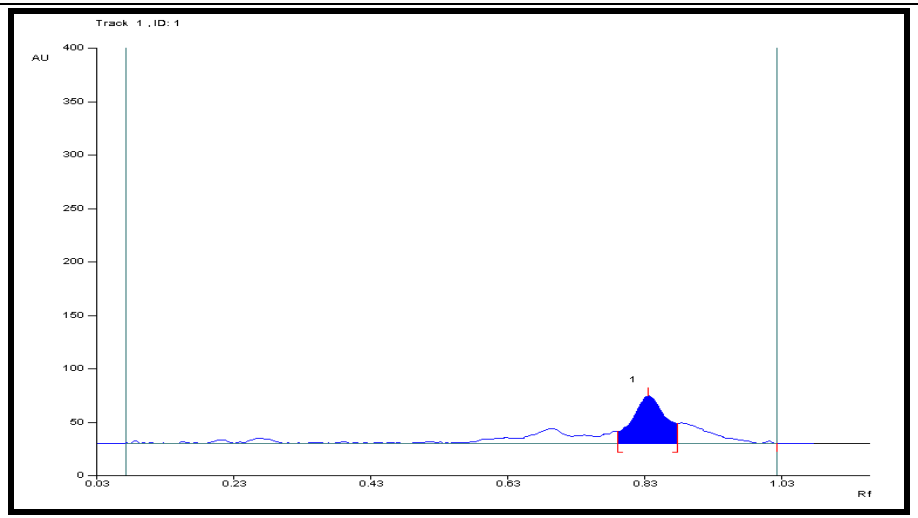

Fig 1: HPTLC Chromatogram of Flavoniod Sample -1 (Variant- CI-01)

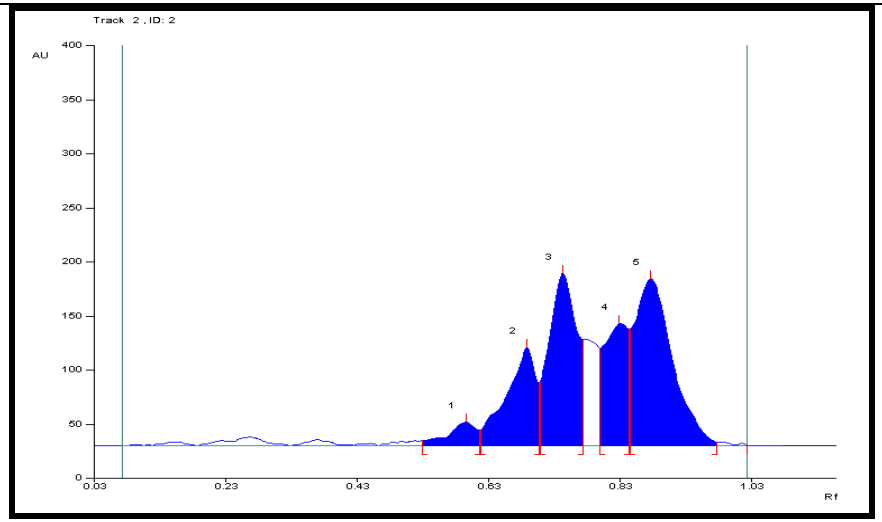

Fig 2: HPTLC Chromatogram of Flavoniod Sample -2 (Variant- CI-02)

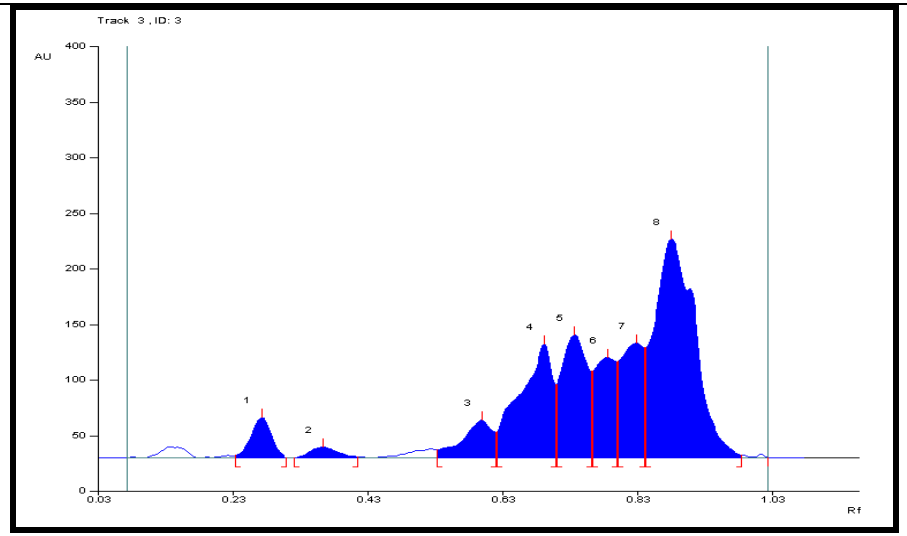

Fig 3: HPTLC Chromatogram of Flavoniod Sample -3(Variant- CI-03)

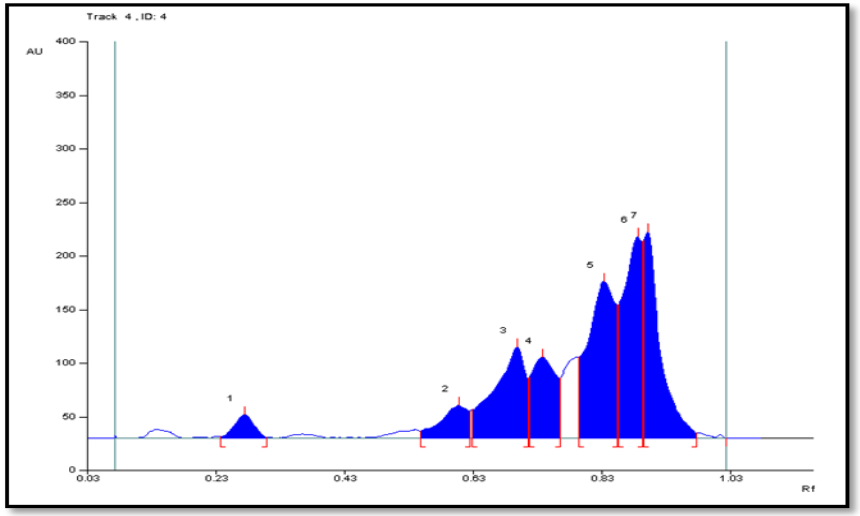

Fig 4: HPTLC Chromatogram of Flavoniod Sample -4(Variant- CI-04)

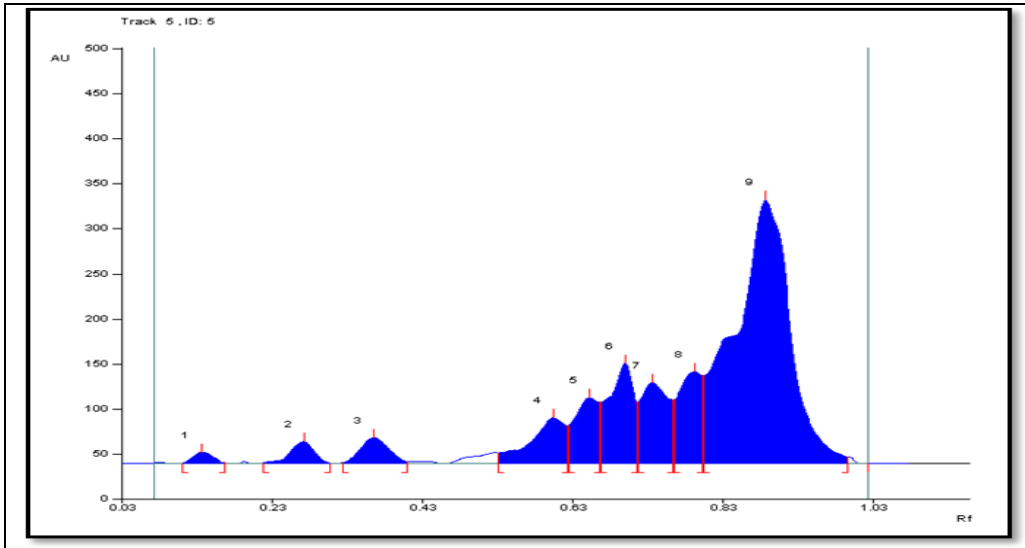

Fig 5: HPTLC Chromatogram of Flavoniod Sample -5 (Variant- CI-05)

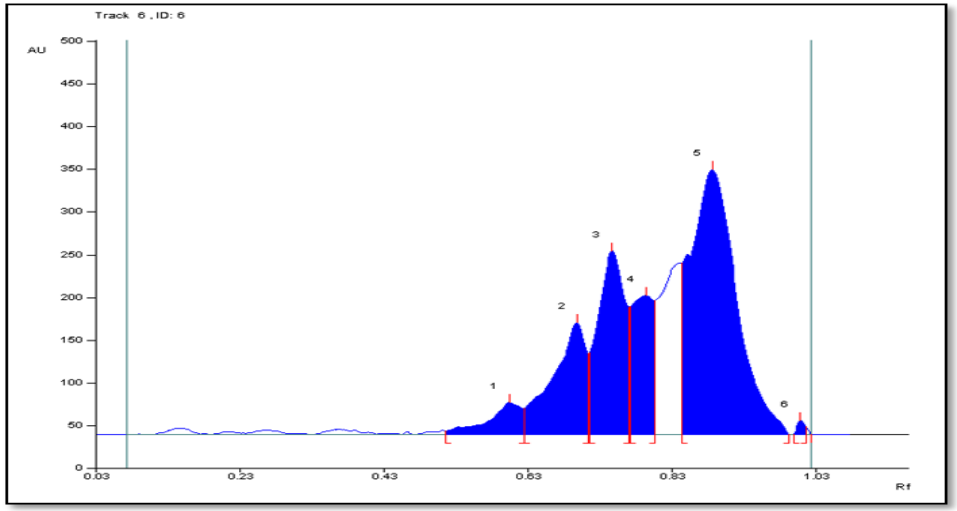

Fig 6: HPTLC Chromatogram of Flavoniod Sample -6 (Variant- CI-06)

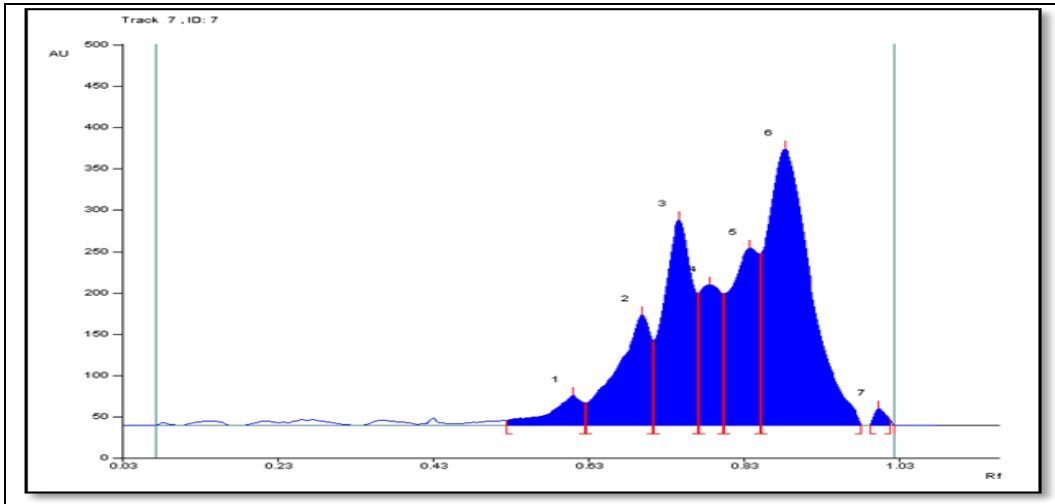

Fig 7: HPTLC Chromatogram of Flavoniod Sample -7 (Variant- CI-07)

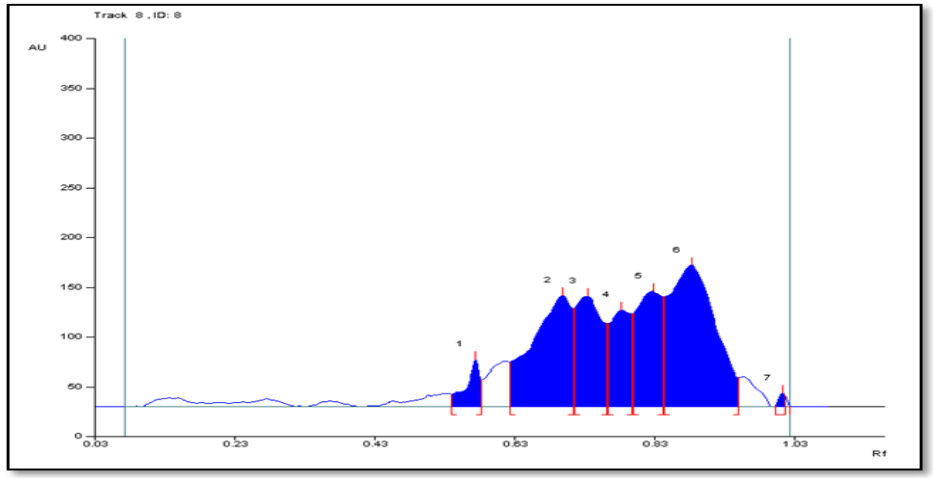

Fig 8: HPTLC Chromatogram of Flavoniod Sample -8 (Variant- CI-08)

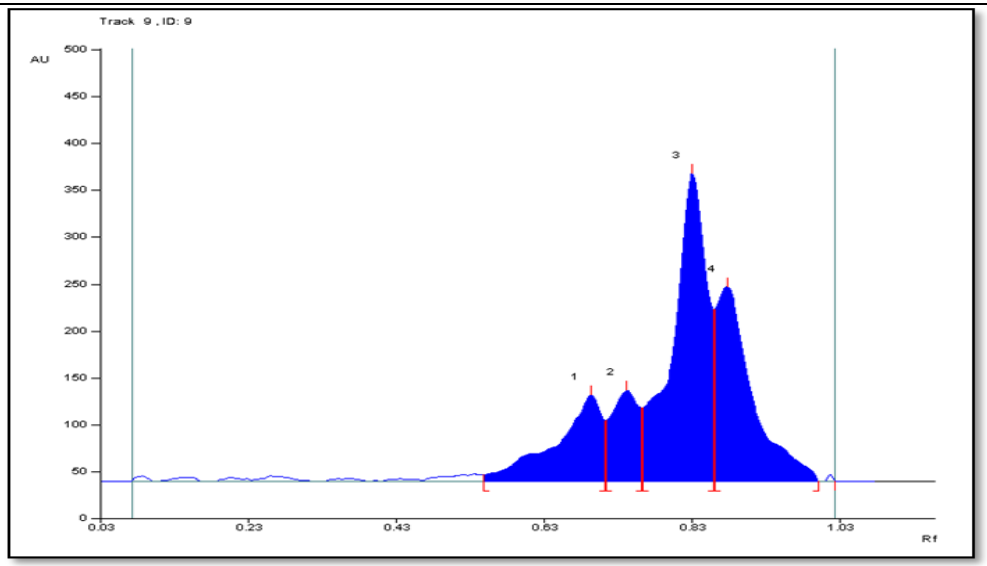

Fig 9: HPTLC Chromatogram of Flavoniod Sample -9 (Variant- CI-09)

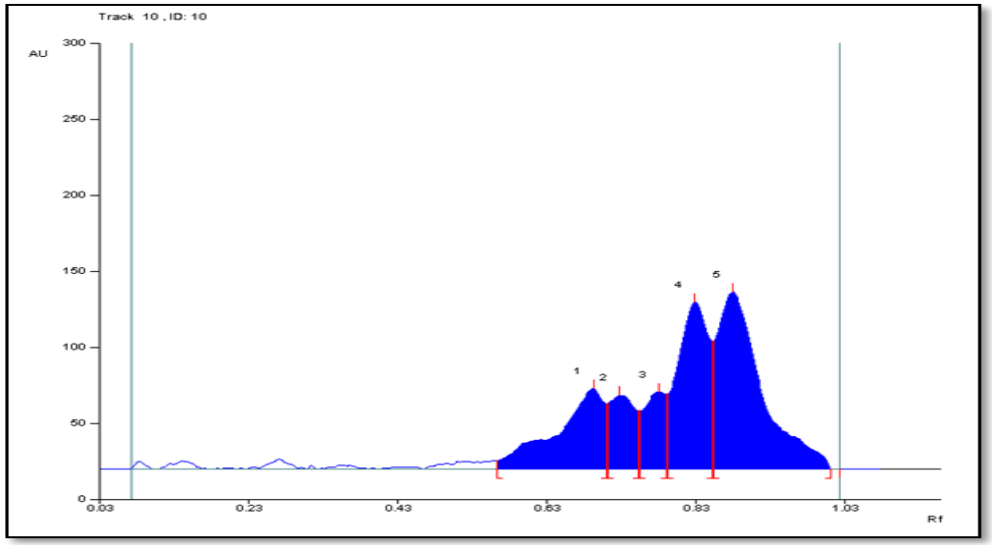

Fig 10: HPTLC Chromatogram of Flavoniod Sample -10 (Variant- CI-10)

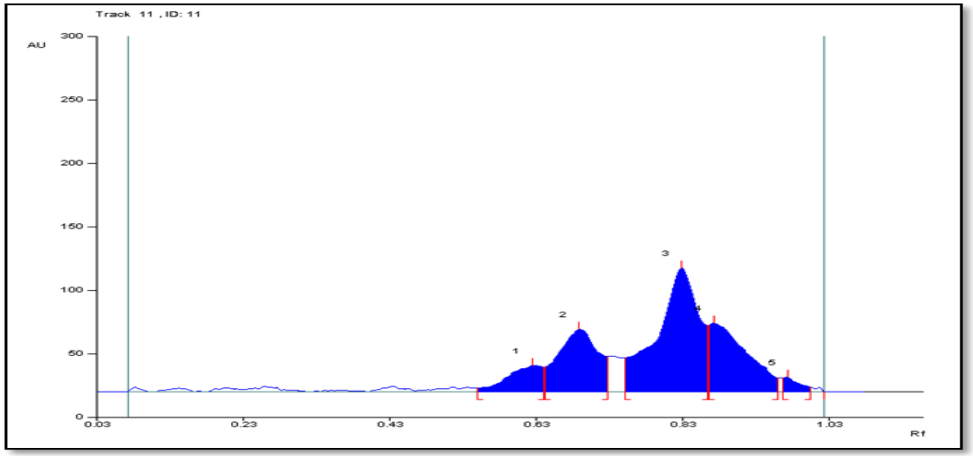

Fig 11: HPTLC Chromatogram of Flavoniod Sample -11 (Variant- CI-11)

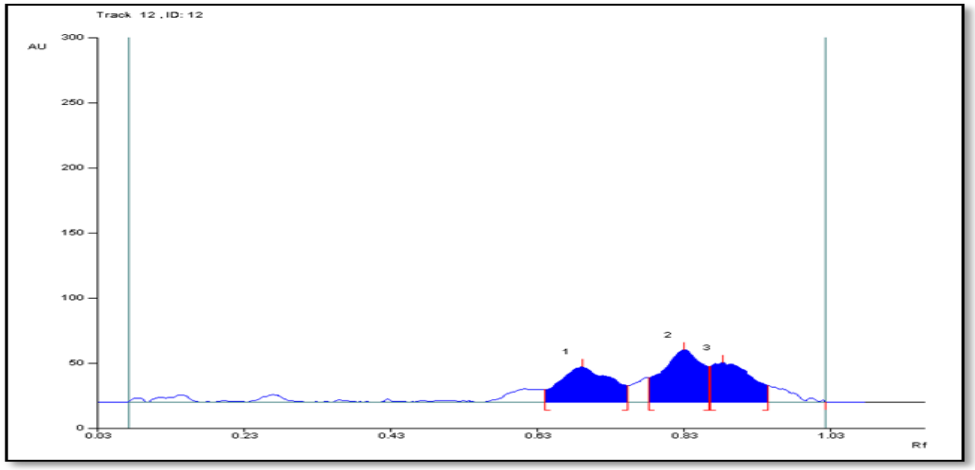

Fig 12: HPTLC Chromatogram of Flavoniod Sample -12 (Variant- CI-12)

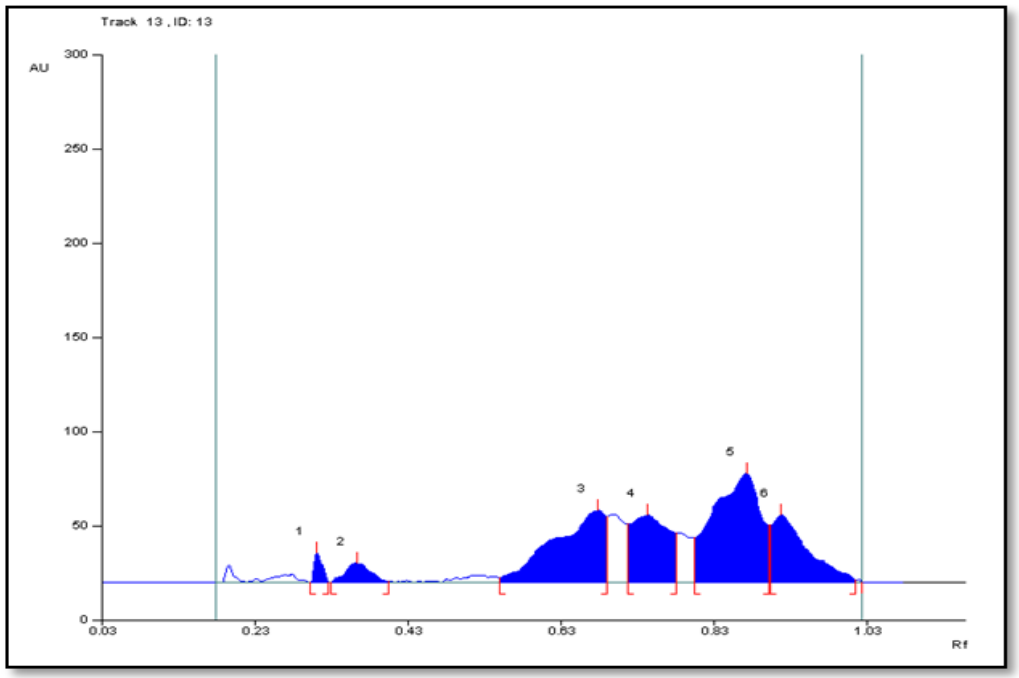

Fig 13: HPTLC Chromatogram of Flavoniod Sample -13 (CP-13)

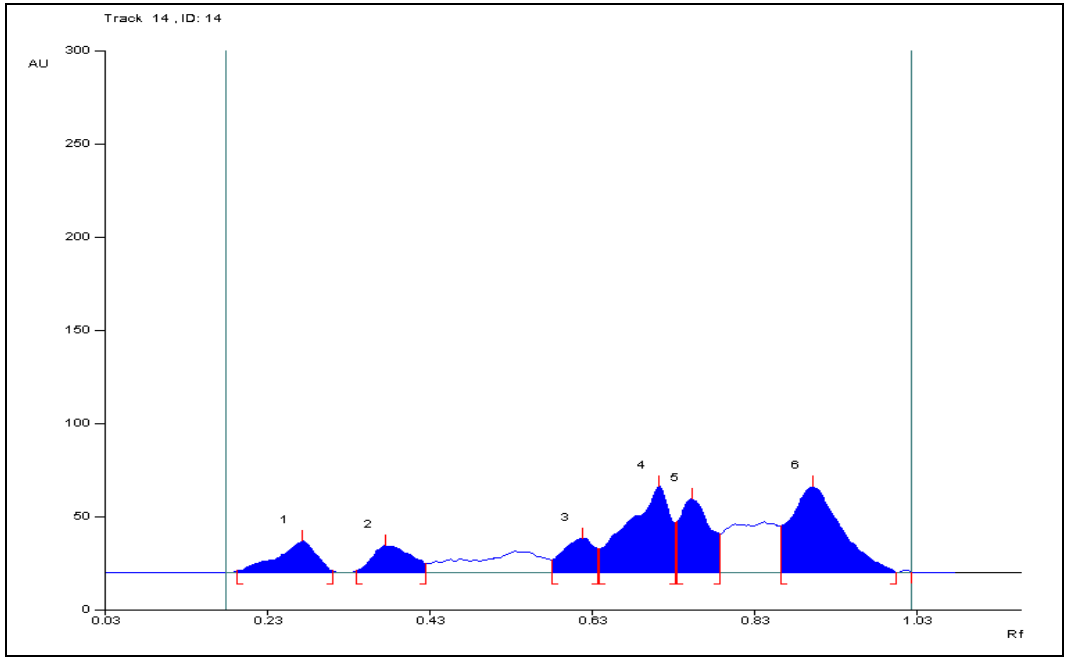

Fig 14: HPTLC Chromatogram of Flavoniod Sample -14 (CL-14)

CONCLUSION

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

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