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Chrysin Quantification from Leaf Samples of Dolichandrone Falcata

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ABSTRACT

The chrysin quantitative from leaf samples were analysed using HPTLC. All the samples were active on TLC plate and revealed the presence of chrysin. The maximum amount of chrysin was obtained from in vivo leaf powder (0.60 µg) than in vitro leaf powder.

Keywords- Chrysin, Dolichandrone falcata, leaves. HPTLC

I. INTRODUCTION

Dolichandrone falcata contains secondary metabolite from the flavonoids group [7]. The detection of these flavonoids from in vitro grown plants was carried out [13]. Thin Layer Chromatography (TLC) has been in use in analysis of flavonoids from 1960. Markham's technique in identification of flavonoid is also useful [5]. The chromatographic techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) were well-known, although GC and HPLC are now a days considered to be the leading techniques [1], [10]. The modern high-performance TLC (HPTLC) is an efficient instrumental technique and optimized quantitative HPTLC using a densitometric evaluation can produce results that are analogous to those obtained with GC and HPLC.

II. METHODS AND MATERIAL

Procurement of plant material and extraction procedure:

Leaves were collected from Botanical Garden and *in vitro* grown cultures of *Dolichandrone falcata* maintained at Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad; Maharashtra, India. The powder of leaf after defatting with petroleum ether was dried and the dried fractions were stored in airtight containers until use.

Chemicals and Reagents:

Pure sample of chrysin was purchased from Sigma-Aldrich Chemical Co. Spectrochem, petroleum ether anisaldehyde, Sulphuric acid and HPLC grade methanol. All different organic solvents and chemicals used for extraction under study were of analytical grade (A.R. grade) obtained from S.D. Chem. Pvt. Ltd., Mumbai, India.

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HPTLC quantification in test samples:

Sample 1, 2, 3, 4, 5, 6 – methanolic extracts of leaf 7 and 8 – Chrysin standard as a reference compound

Test solution preparation:

The methanol extracts of D. falcata was weighed in an electronic balance and dissolved in methanol and kept for ultra-sonication at 100 rpm for 25 minutes. This solution was used as test solution for HPTLC analysis.

Mobile phase

Chloroform (18.6): Methanol (0.4): Formic Acid (0.2).

Sample application:

20µl of test solution and 2µl of standard solution was loaded as 8mm band length in the 20 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT -5 instrument.

Spot development:

The sample's loaded plate was kept in TLC developing chamber and the plate was developed with respective mobile phase.

Photo-documentation:

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG TLC Scanner) and captured the images at White light, UV 254nm and UV366nm.

Derivatization:

The developed plate was sprayed with anisaldehyde - Sulphuric acid reagent dried at 100°C in hot air oven. The plate was photo-documented in white light and UV 366 nm mode using photo-documentation (CAMAG visualiser) chamber.

Scanning:

After derivatization, the plate was fixed in scanner stage and scanning was done at UV 366 nm. The peak table, peak display and peak densitogram was noted.

III. RESULTS AND DISCUSSION

Table I: Application of sample on TLC plate at Linomat-5

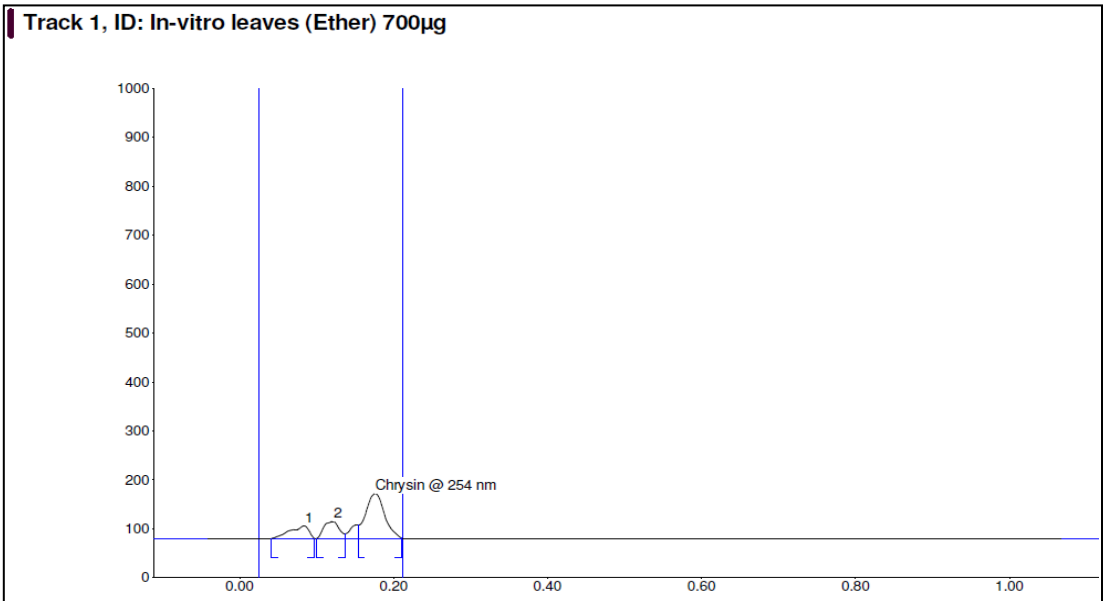
No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
1	20.0 mm	20.0 µl	1	*In-vitro* leaves (Ether) 700µg	Yes
2	32.3 mm	20.0 µl	2	*In-vivo* leaves (Ether) 100µg	Yes
3	44.6 mm	20.0 µl	3	*In-vivo* green leaves 825µg	Yes
5	69.2 mm	10.0 µl	5	*In-vitro* leaves 500µg	Yes
6	81.5 mm	10.0 µl	6	*In-vivo* leaves 500µg	Yes
7	106.1 mm	2.0 µl	8	Chrysin 2µg	Yes

Table II: Quantitative HPTLC analysis of chrysin from various leaf samples.

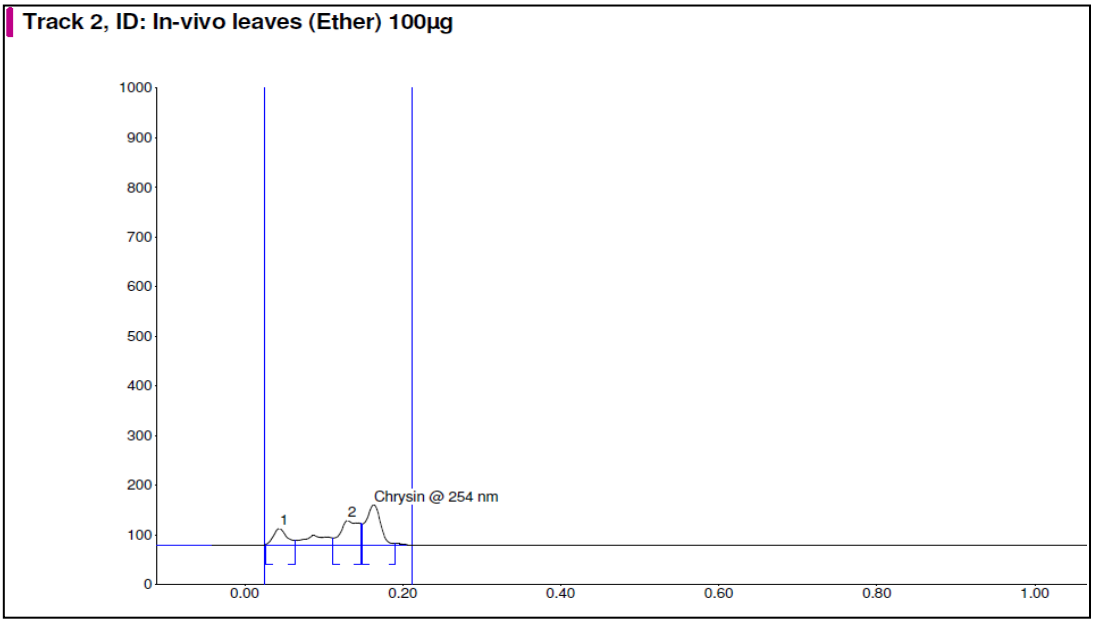
Sr. No.	Sample	Applied quantity	Area	Chrysin in µg
1	*In vitro* leaves powder (EE)	20.0 µl	1935.6	0.33
2	*In vivo* leaves powder (EE)	20.0 µl	1256.4	0.22
3	*In vivo* green leaves powder (EE)	20.0 µl	2626.9	0.45
4	*In vivo* leaves powder (WEE)	10.0 µl	3493.9	0.60
5	*In vitro* leaves powder (WEE)	10.0 µl	2043.6	0.35
6	Standard Chrysin powder	2.0 µl	11580.7	2.0

EE- Ether Extracted,

WEE- Without Ether Extracted.

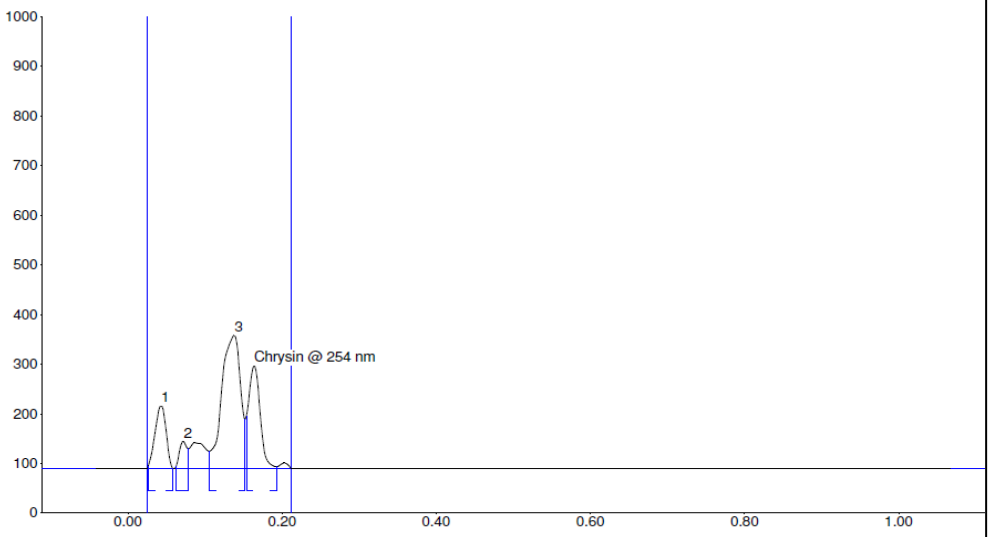


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	0.4	0.08	26.4	17.13	0.10	0.9	550.7	17.87	unknown *
2	0.10	0.1	0.12	35.5	23.05	0.14	9.9	595.2	19.31	unknown *
3	0.15	27.9	0.18	92.2	59.82	0.21	1.1	1935.6	62.81	Chrysin



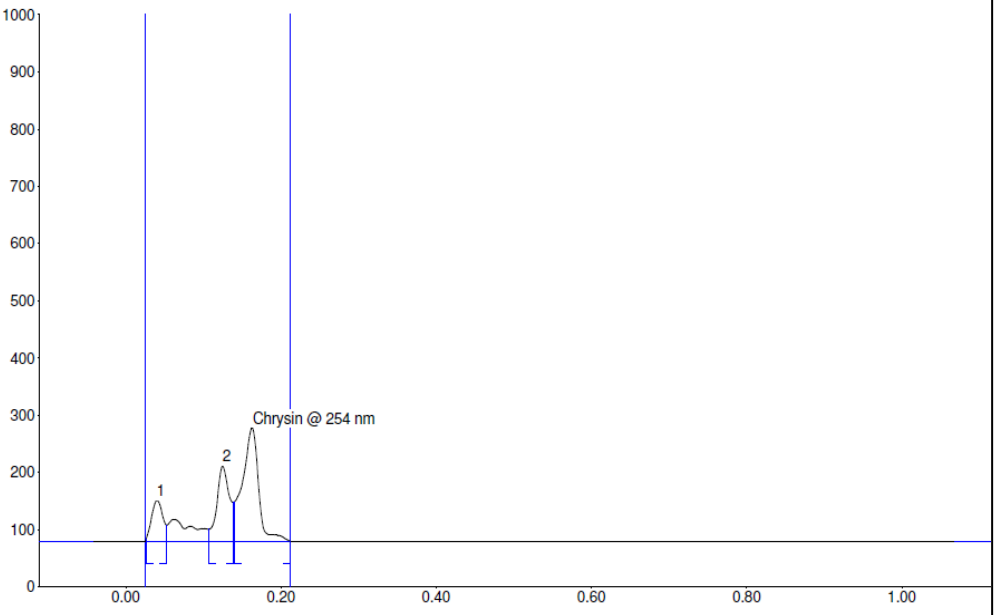
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	0.6	0.04	33.2	20.24	0.06	9.0	468.9	17.63	unknown *
2	0.11	13.8	0.13	49.7	30.31	0.15	42.8	934.1	35.12	unknown *
3	0.15	42.2	0.16	81.1	49.46	0.19	2.9	1256.4	47.24	Chrysin

Track 3, ID: In-vivo green leaves 825µg

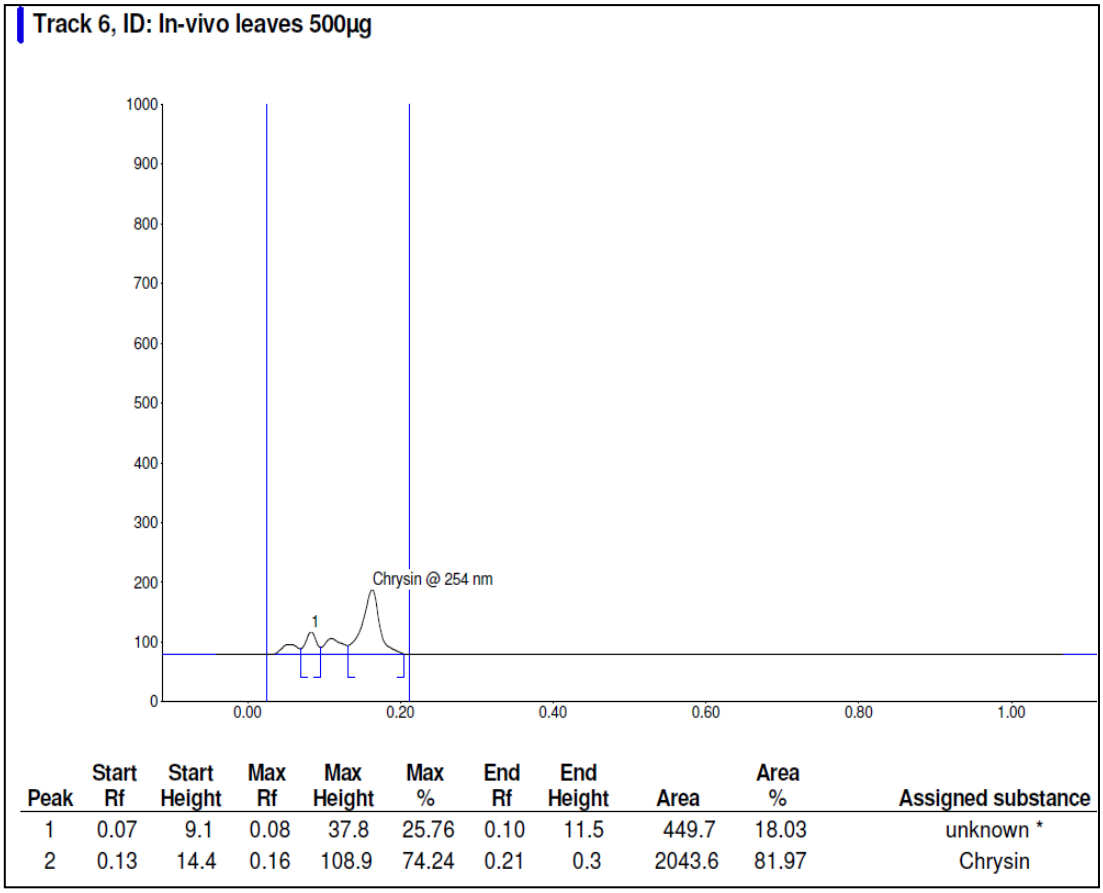


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	4.9	0.04	127.3	19.31	0.06	0.4	1516.9	14.91	unknown *
2	0.06	3.2	0.07	55.4	8.41	0.08	40.1	487.8	4.80	unknown *
3	0.11	34.8	0.14	268.8	40.78	0.15	99.9	5541.1	54.47	unknown *
4	0.15	105.8	0.16	207.7	31.50	0.19	4.2	2626.9	25.82	Chrysin

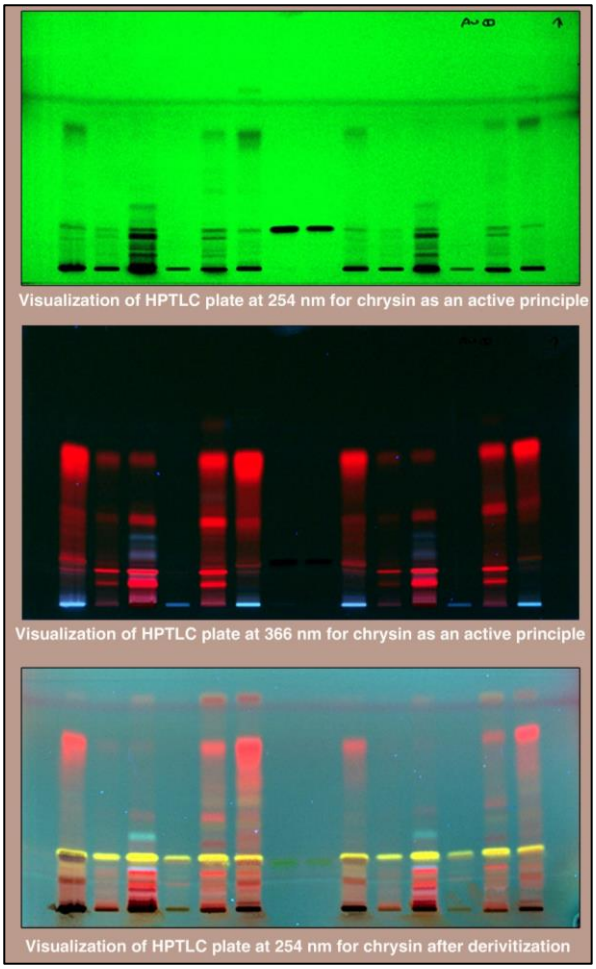
Track 5, ID: In-vitro leaves 500µg



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	4.2	0.04	71.6	17.83	0.05	28.2	874.4	14.17	unknown *
2	0.11	21.0	0.13	131.8	32.84	0.14	67.8	1803.7	29.22	unknown *
3	0.14	68.9	0.16	198.0	49.33	0.21	0.0	3493.9	56.61	Chrysin



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	9.1	0.08	37.8	25.76	0.10	11.5	449.7	18.03	unknown *
2	0.13	14.4	0.16	108.9	74.24	0.21	0.3	2043.6	81.97	Chrysin



All the samples were active on TLC plate and ran within 15 minutes as shown in Table-I. HPTLC analysis of various samples of D. falcata revealed the presence of chrysin, except in callus. However, variations in the quantity of chrysin in samples were observed. The data in Table-II can indicate the variation in chrysin among the samples. The maximum amount of chrysin was obtained from in vivo leaf powder (without ether extracted) (0.60 µg), followed by the ether extracted in vivo green leaf powder (0.45 µg), without ether extracted in vitro leaf powder (0.35 µg), and the ether extracted in vitro leaf powder (0.33 µg). The minimum amount of chrysin was obtained from in vivo leaf powder (Ether extracted) (0.22 µg).

In the present investigation, the method developed for HPTLC fingerprinting provided a quick quantitative analysis for the methanolic extract of various leaf powders of D. falcata. The conditions used have led a good separation of the peaks which could be identified in the chromatogram for chrysin.

IV. DISCUSSION

Flavonoids have been described as health-promoting, disease preventing dietary supplements and have activity as cancer preventive agents [6]. Chrysin (5, 7-Dihydroxyflavone), extensively distributed in plants, has been reported to have many biological activities, including antioxidant, antibacterial, anticancer, anti-inflammatory, anti-allergenic, and anxiolytic activities ([4], [9], [3], [2], [8], [11] and [12]).

In the present investigation, the method developed for HPTLC fingerprinting provided a quick quantitative analysis for the methanolic extract of various leaf powders of D. falcata. The conditions used have led a good separation of the peaks which could be identified in the chromatogram for chrysin.

V. CONCLUSIONS

The good separation of the peaks identified in the chromatogram for chrysin. The method developed for HPTLC fingerprinting provided a quick quantitative analysis for the methanolic extract of various leaf powders of D. falcata.

VI. ACKNOWLEDGEMENT

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