ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

HPTLC FINGERPRINT PROFILE OF STEMS OF *TINOSPORA CORDIFOLIA* (WILD) MIERS AND ROOTS OF *HEMIDESMUS INDICUS* (L.) R.BR WITH THEIR POLYHERBAL MIXTURE

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Received on: 02-05-2019 *Accepted on: 30-06-2020*

Abstract

The present work emphasized on developing a simple HPTLC fingerprint profile of ethanolic extracts of *Hemidesmus indicus* roots and stems of *Tinospora cordifolia* as well as their combine mixture for separation of active compounds and their qualitative study Ethanolic extracts of the stem and roots parts were prepared by Soxhlet extraction. CAMAG HPTLC system with the help of aluminum thin layer chromatography (TLC) plate precoated with silica gel 60 F 245 Linomat applicator, TLC scanner 3 and win CATS software were used, ethanolic extracts of different samples at different concentrations were developed in suitable solvent system Toluene: ethyl acetate: formic acid (10:3:1) and scanned by densitometric scanner in UV light reflectance- absorbance mode regulated by wins cats software. The plate was further placed in photo-documentation chamber (CAMAG REPROSTAR3) and images were captured at UV 366nm and 254nm, it was further subjected to HPTLC fingerprinting where Rf value, Area Under Curve and percentage area were calculated. HPTLC profile displayed several peaks with distinguished Rf values.

Keywords: *Tinospora cordifolia* (Wild) Miers, *Hemisdesmus indicus* (L.)R.Br, HPTLC, Retention factor (Rf).

Introduction

"Hemidesmus" is derived from Latin word "Hemidesmos" which means 'half bond'. It is so named in allusion to sub connate filaments at their base – joint pods and connected stamens. Word "indicus" stands for 'of India'. Hemidesmus indicus belongs to family Asclepiadaceae which is derived from word "Askleplos" – means 'God of Medicine[1] It is a diffusely twining shrub with several slender laticiferous

branches with purple brownish bark This plant is found throughout India growing under mesophytic to semi dry conditions in the plains and up to an altitude of 600 m . It is quite common in open scrub jungles, hedges, uncultivated soil etc. It is found in India, Sri Lanka, Pakistan, Iran, Bangladesh and Moluccas[2-5.] '*Hemidesmus indicus'* was previously placed under the family "Asclepiadaceae" but currently because of its pollinial features transisted to Periplocaceae [6]. Plant is also called "Anantmool" in hindi means "eternal roots" as the roots are spread largely in a long way under the ground. roots have sweet fragrance similar as camphor known as "karpoori" [7] Different parts of the plant especially root contain various compounds such as 2-hydroxy 4-methoxy benzaldehyde, 4-hydroxy 3methoxy benzaldehyde, hemidesmine, hemidine, hemidesine, rutin [8-10] In Ayurveda, its roots are identified to treat ailments such as skin diseases, arthritis, rheumatism, epilepsy, nervous diseases, tonsillitis, liver disease,syphilis, stomach disorders and peptic ulcers. Syrup prepared from the roots is used as a flavoring agent and in the preparation of a sherbet and wine which possess cooling properties.[11]

Tinospora cordifolia (Wild).Miers ex Hook.F & Thoms (Family; Minispermaceae) commonly known as "Amrita" or "Guduchi" is an essential herbs for "Indian system of Medicine" and has been used in Ayurvedic formulation for the treatment of various ailments throughout the decades[12] *Tinospora cordifolia* is widely used in Ayurvedic system for its general tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antipyretic, anti-arthritic, anti-leprotic, anti-allergic and anti-diabetic properties.[13] The stem is bitter, stomachic, diuretic, stimulates bile secretions and purifies the blood and cures jaundice. The extract. of the stem is useful in skin problems and also prescribed in combination with other drugs as an antidote for snakebite and scorpion.[14] The stems of the plant possesses various phytoconstituents such as Alkaloids (Berberine, Choline, Tembetarine, Magnoflorine, Tinosporin, Palmetine)[15-17], Glycosides (18-norclerodane glucoside, Furanoid diterpene glucoside, Tinocordiside, Tinocordifolioside, Cordioside, Cordifolioside)[18-20], Lactones Clerodane deravatives, Tinosporon, Tinosporides, Jateorine, Columbin Steroids B-Sitostrerol, Sitosterol,20βHydroxy ecodysone, MakisteroneA, Giloinsterol Sesquiterpenoid Tinocordifolin and Diterpenoids Furanolactone[21-23]

Based on the previous literature reviews these two plants has a crucial role in treatment of several diseases. The therapeutic activity of these herbs is due to presence of major chemical constituents. Since we know that

HPTLC method is most reliable and being accurate can used as an effective characteristic tool for authentication and identification of components of the plants.so this work was aimed for separation of biologically active compounds in individual extracts of these plants as well as their combine mixture of extracts in order to enhance for their role in possible mechanism of action for treatment in various ailments.

Material and Methods

1) Collection and authentication of plant materials

Stems of the *Tinospora cordifolia* (Wild.) Miers from family menispermaceae were collected from botanical garden of Utthan near Jhalwa Prayagraj (U.P) Further identification was conducted by Dr. G.P Sinha, Scientist-E and Head of Office in Botanical Survey of India (BSI) Prayagraj (U.P). Specimen was deposited in the Herbaruim of BSI for future reference, Accession number - (98883).

The Roots of *Hemidesmus indicus*(L.)R.Br. were purchased from authorized drug store and authenticated by Scientist & Head Dr. A.K.S Rawat, Pharmacognosy & Ethano-pharmacology Division at CSIR-National Botanical Research Institute (NBRI), Lucknow, Uttar pradesh (India). A voucher specimen (NBRI/CIF/536/2017) has been deposited in the Herbarium for future reference, Specification: (NBRI-SOP-202.)

2) Preparation of extracts

Roots of *Hemidesmus indicus* (L.) R.Br has been dried and powdered with the help of mixer and grinder then made ready for soxhlet extraction.

The stem of *Tinospora cordifolia* (Wild)Miers were taken and chopped off into several pieces about 15-20 cm, rinsed off with distilled water , air-dried then finely powdered and used further for extraction by Soxhlet apparatus. Soxhlet extraction was done in each drug with 250 ml of ethanol.

.**3) Sample preparation**

25 mg of ethanolic extracts of stem of *Tinospora cordifolia* (EESTC) and roots of *Hemidesmus indicus*(EERHI) was dissolved in 5ml ethanol separately and their polyherbal mixture were also dissolved (PHC-1:1) for 48 hours. These extracts were stored in air tight bottle.

4) HPTLC Analysis

The studies were carried out with the help of following method Wagner[24] and Sethi [25]

a) Chromatographic technique

The chromatogram was developed on 10×20cm aluminum thin layer chromatography (TLC) plate precoated with silica gel 60 F 245 stored in a desiccator. The sample application was done by Camag Linomat 5 applicator using inert gas as a spray gas at dosage speed of 150nl/s, at a predosage volume 0.2µl using 100µl syringe size at band length 8mm. Different samples were applied with the help of Linomat applicator attached to Camag HPTLC system which was programmed through win CATS software (Version 1.3.0) at 254nm and 366nm using Deuterium light source.[26]

Table.1 Sample Application using -CAMAG Linomat 5

Applied volume		3.0µl	5.0µl	10.0µl
Applied position		15.0mm	50.0mm	85.0mm
Samples	1.	EERHI	EERHI	EERHI
	2.	EESTC	EESTC	EESTC
	3.	PHC(EERHI+EESTC)	PHC(EERHI+EESTC)	PHC(EERHI+EESTC)

b) Development chamber

The plate was developed in an ascending manner with a solvent system consisting of Toluene: Ethyl acetate: Formic acid [10:3:1 (v/v)] with volume of 50 ml in a development twin trough glass tank (20×10cm) pre-saturated with the mobile phase for each extracts. Solvent front position was found to be 80mm After development, the plate was dried at 60^{o}C in an oven for 5 minutes

c) Detection

Plate was scanned using CAMAG TLC Scanner 3 operated in reflectance–absorbance mode equipped by Win CATS software. The slit dimensions were 6 x 0.30 mm and the scanning speed was 20 mm/s. The source of radiation used was deuterium lamp emitting continuous UV spectra at 254 nm, data resolution 100 μm/step and filter factor (Savitsky-golay7).

The plate was kept in photo-documentation chamber (CAMAG REPROSTAR3) and captured the images at UV 366nm and 254nm. The peak numbers with its height and area, peak display and peak densitogram were identified. Rf value and Fingerprint data were also recorded.

Result and Discussion

A. Table 2. HPTLC-EERHI (R. No-201806270123)

No.	Applied position	Applied volume	Active
Track 1.	15.0mm	3.0µl	yes
Track 2.	50.0mm	5.0µl	yes
Track 3.	85.0mm	10.0µl	yes

The HPTLC fingerprint Profile of ethanolic extracts of *H.indicus* is shown in fig 2,3 and 4 where at a concentration of 3µl ,5µl and 10µl were applied respectively in track 1,2 and 3, there were 3 spots observed in 5µl and 10µl. i.e., at 5µl Rf values were 0.25,0.39 and 0.46 and at 10µl Rf values were obtained 0.24,0.38 and 0.45 indicating the presence of 3 different components at two different concentration of samples. Out of 3 spots, the spots with Rf values 0.39 and 0.38 of conc. 5µl and 10µl were found to be more distinguished as the percentage area was higher with 50.63 and 41.34 respectively. The remaining components were found to be less in quantity as the percentage area for the remaining spots were less. 2 spots were seen at a concentration of 3µl it means Rf values were obtained 0.41 and 0.47. The components with higher percentage area in 3 µl sample were found to be 56.45 with Rf value 0.41.

Win CATS Planar Chromatography Manager

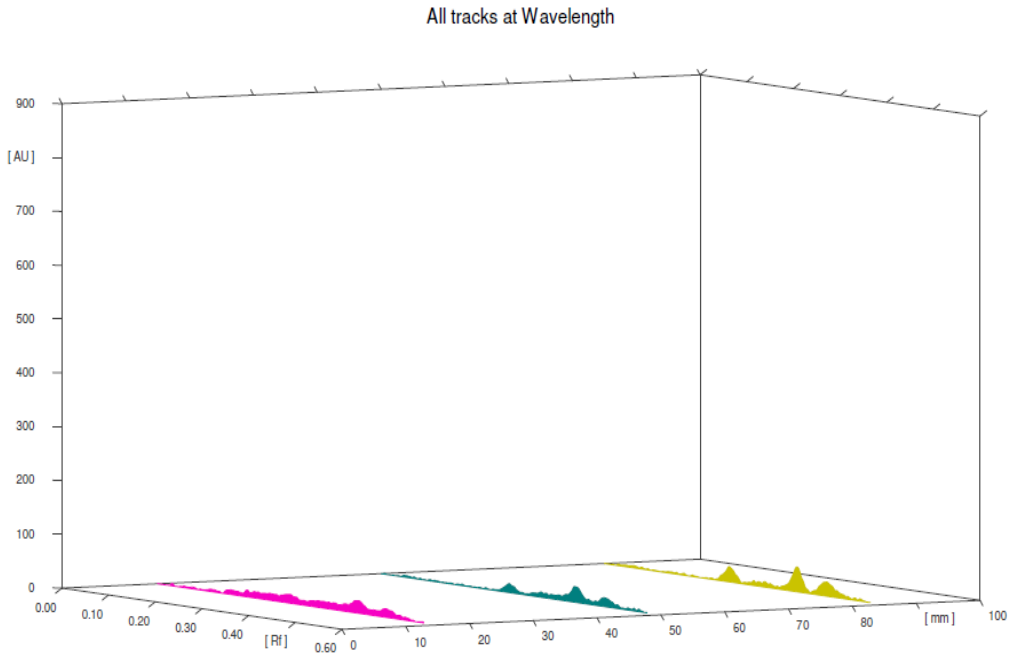

Fig 1: HPTLC fingerprint profile of all the tracks at 254nm of ethanolic extracts of roots of *Hemidesmus indicus* (L.) R. Br

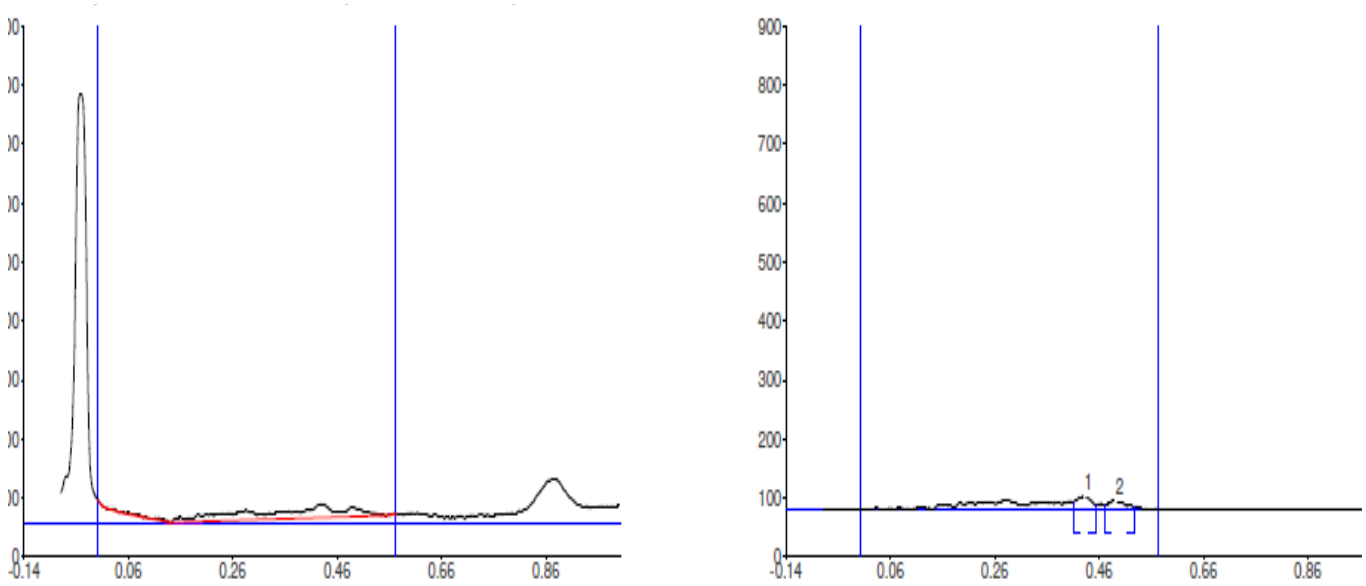

Fig 2. Track 1- HPTLC chromatogram of ethanolic extracts of roots of *Hemidesmus indicus* (L)R.br

Table-3. Track 1-Rf value of the chromatogram of EERHI.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.41	13.3	0.43	23.7	60.15	0.45	9.6	554.0	56.45
2.	0.47	9.9	0.49	15.7	39.85	0.53	4.3	427.4	43.55

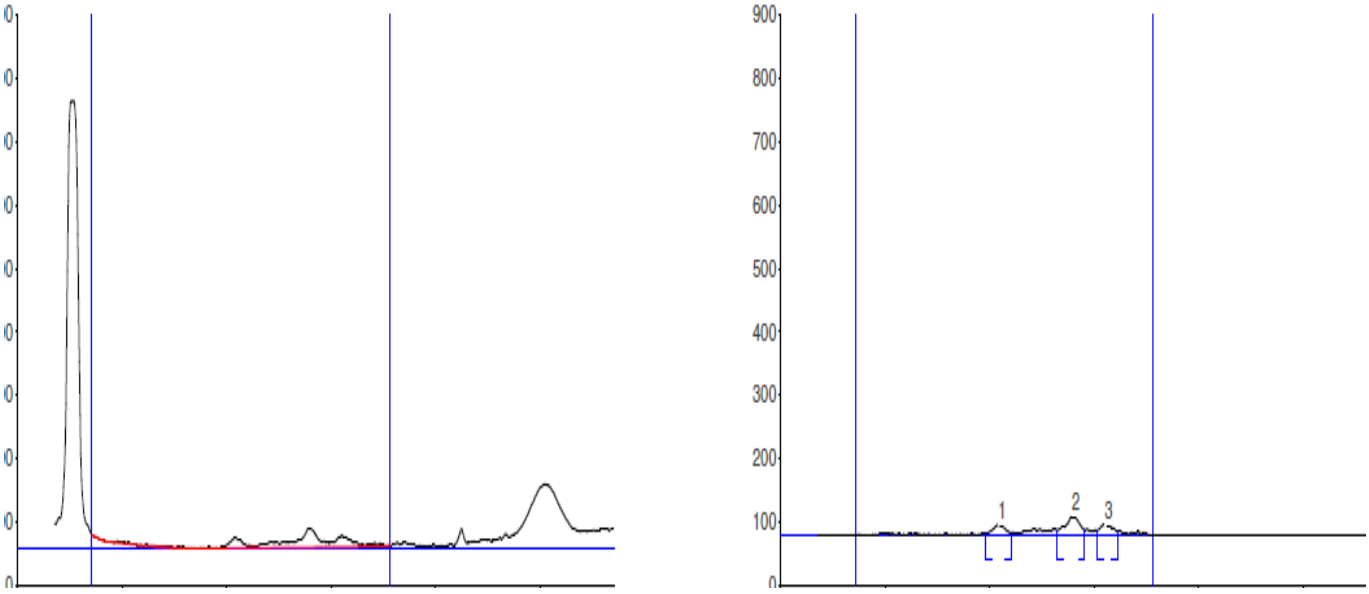

Fig 3. Track 2- HPTLC chromatogram of ethanolic extracts of roots of *Hemidesmus indicus* (L)R.br

Table-4. Track-2 Rf value of the chromatogram of EERHI.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.25	1.7	0.27	16.1	26.38	0.30	3.2	303.3	23.67
2.	0.39	7.2	0.42	28.8	47.02	0.44	7.4	648.7	50.63
3.	0.46	6.8	0.48	16.3	26.60	0.50	5.3	329.3	25.70

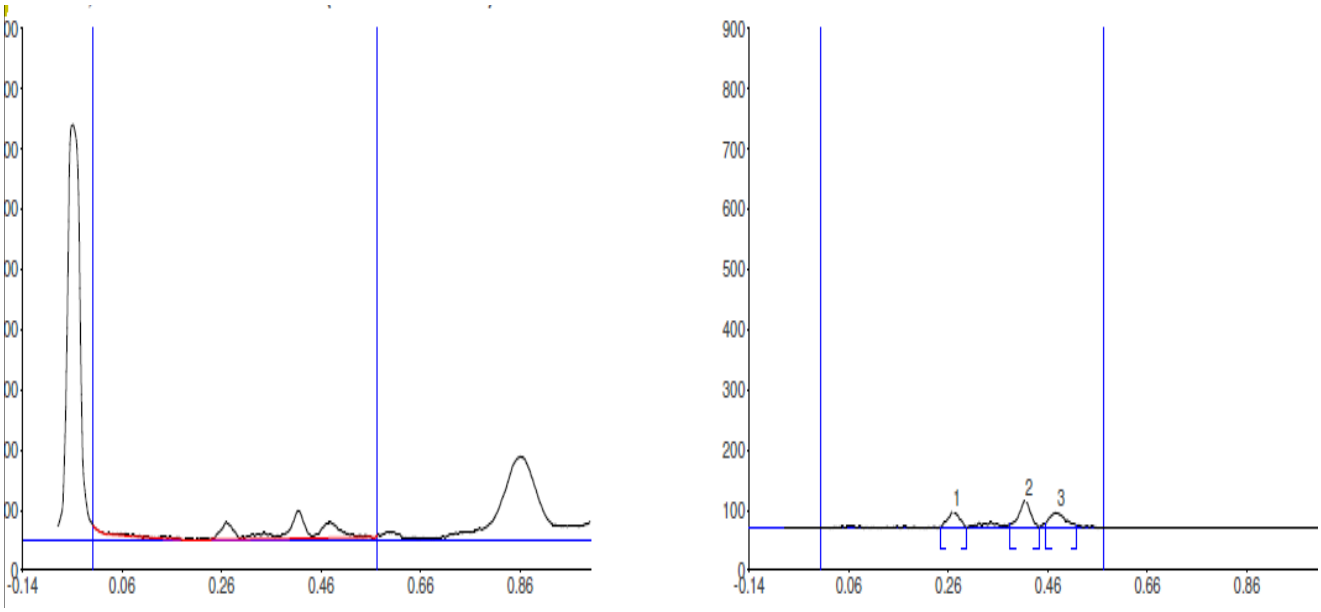

Fig 4. Track 3- HPTLC chromatogram of ethanolic extracts of roots of *Hemidesmus indicus* (L)R.br

Table-5. Track -3 Rf value of the chromatogram of EERHI.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.24	2.3	0.27	28.3	28.12	0.30	1.5	558.8	25.52
2.	0.38	6.6	0.41	46.1	45.82	0.44	2.3	905.3	41.34
3.	0.45	7.1	0.48	26.2	26.06	0.52	4.7	725.7	33.14

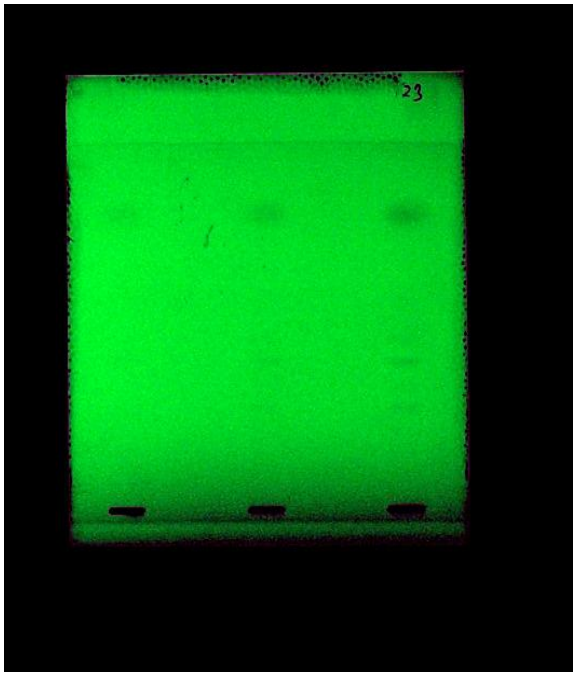

Fig.5 U.V-254 nm

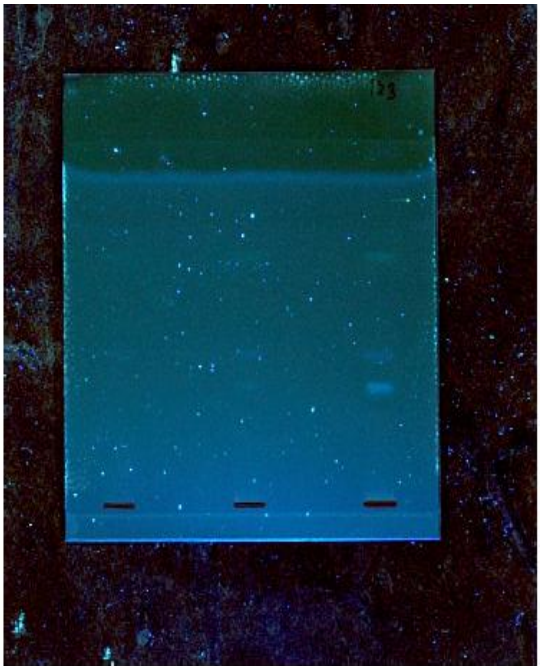

Fig.6 U.V-366nm

B. Table-6. HPTLC-EESTC (R. No-201806270122).

No.	**Applied position**	**Applied volume**	**Active**
Track 1.	15.0mm	3.0µl	Yes
Track 2.	50.0mm	5.0µl	Yes
Track 3.	85.0mm	10.0µl	Yes

The HPTLC fingerprint Profile of ethanolic extracts of *Tinospora.cordifolia* is shown in fig 8,9 and 10 where at a concentration of 3µl ,5µl and 10µl were applied respectively in track 1,2 and 3, there were 5 spots observed in 3µl. and 6 spots were observed in 5µl and10µl.At 3µl, Rf values were 0.02,0.07,0.20,0.34 and 0.43. Out of 5 spots with Rf values 0.20 and 0.34 having higher percentage area were found i.e., 33.35 and 28.04 respectively. At 5µl, Rf values were obtained 0.01,0.08,0.18,0.25,0.32 and 0.39 and at 10µl, Rf values were obtained 0.01,0.08,0.20,0.26,0.32 and 0.40 indicating the presence of 6 different components at two different concentration of samples. Out of 6 spots in both cases, the spots with Rf values 0.18, 0.20 and 0.32 of conc. 5µl and 10µl were found to be more prominent and distinguished as the percentage area was higher with 44 ,37.07 and 20.74 respectively. The remaining components were found to be less in quantity as the percentage area of few spots were less than 7. Rf values with 0.01,0.08 and 0.32 were found to be common in both concentrations indicating the similarity of a constituent.

Win CATS Planar Chromatography Manager

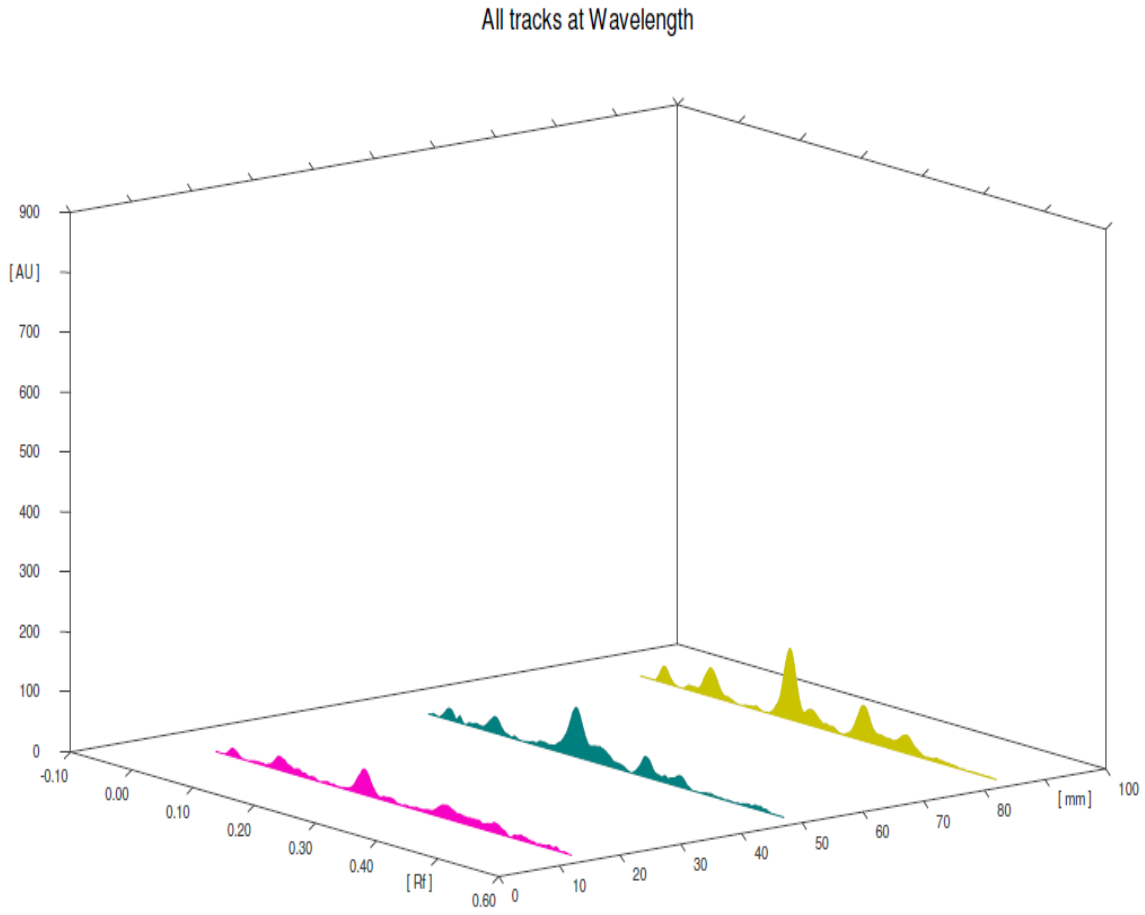

Fig 7: HPTLC fingerprint profile of all the tracks at 254nm of ethanolic extracts of stems of *Tinospora cordifolia* (Wild) Miers.

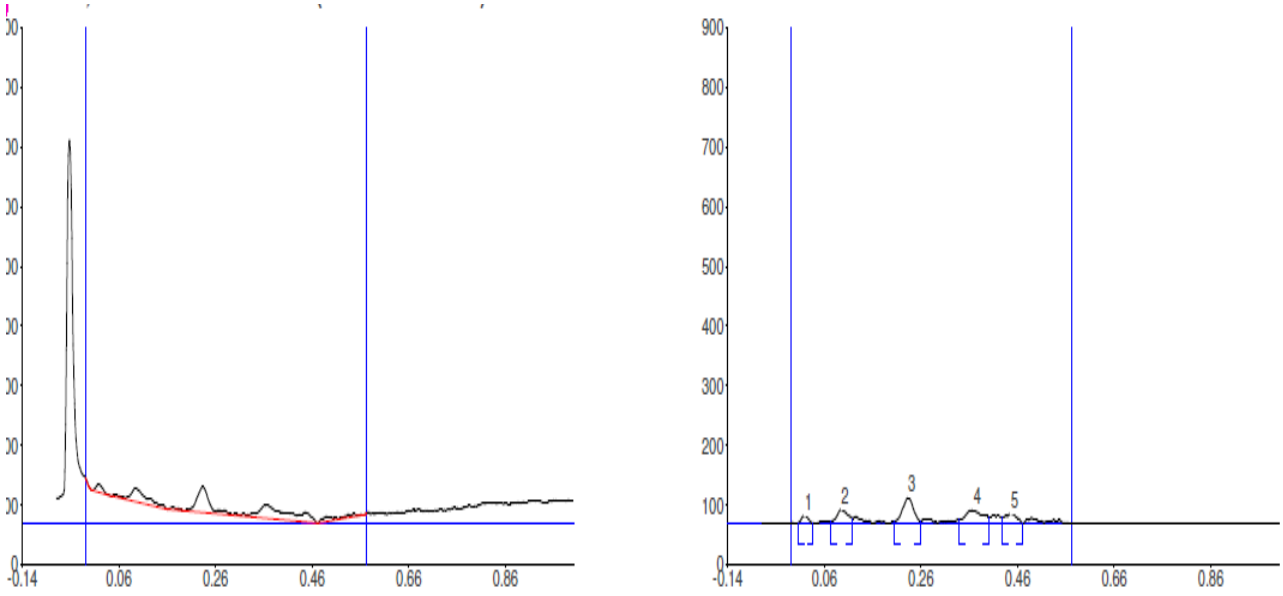

Fig 8. Track 1- HPTLC chromatogram of ethanolic extracts of stems of *Tinospora cordifolia* (Wild) Miers.

Table-7. Track 1- Rf value of the chromatogram of EESTC.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.00	0.2	0.02	13.3	11.22	0.03	0.8	149.4	6.27
2.	0.07	1.8	0.09	23.3	19.72	0.12	8.4	438.4	18.39
3.	0.20	2.7	0.23	42.8	36.14	0.26	3.8	795.2	33.35
4.	0.34	5.9	0.37	22.3	18.82	0.40	11.7	668.7	28.05
5.	0.43	9.3	0.44	16.7	14.09	0.47	0.0	332.6	13.95

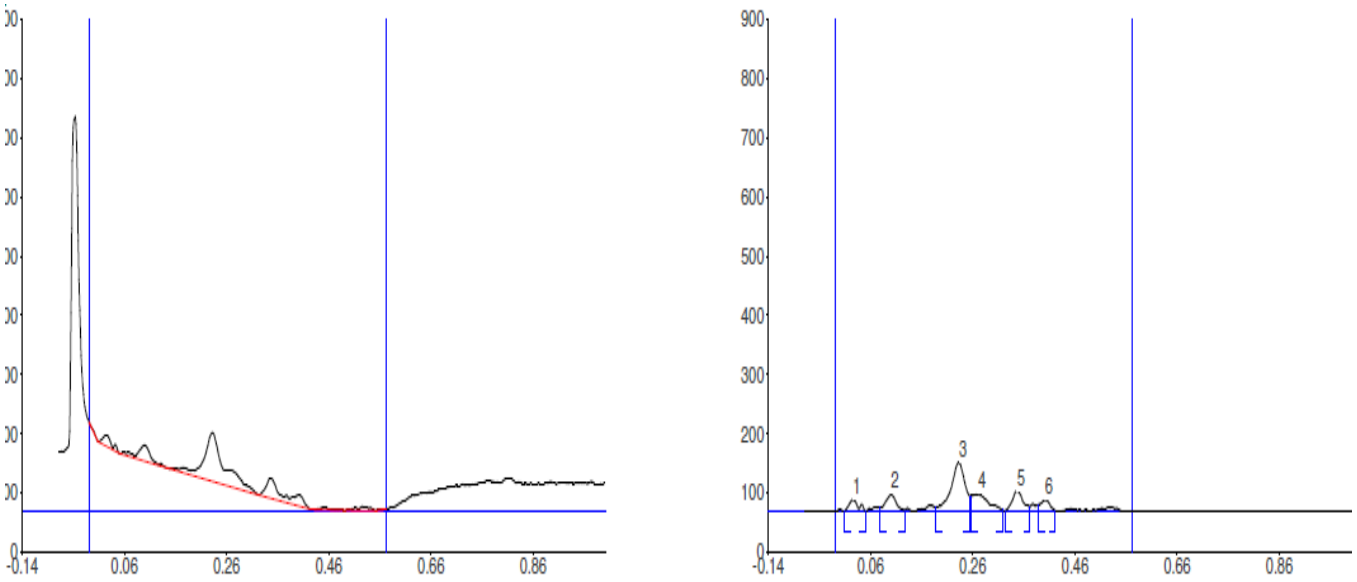

Fig 9. Track 2- HPTLC chromatogram of ethanolic extracts of stems of *Tinospora cordifolia* (Wild) Miers.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.01	0.3	0.02	19.0	9.06	0.05	0.1	276.0	6.57
2.	0.08	5.3	0.10	27.7	13.20	0.12	1.2	461.6	10.98
3.	0.18	6.9	0.23	82.0	39.06	0.25	24.8	1849.7	44.00
4.	0.25	24.9	0.27	28.5	13.58	0.32	1.9	754.0	17.94
5.	0.32	0.1	0.34	33.8	16.08	0.37	7.8	585.3	13.92
6.	0.39	11.3	0.40	18.9	9.01	0.42	0.3	277.2	6.59

Table-8. Track-2 Rf value of the chromatogram of EESTC.

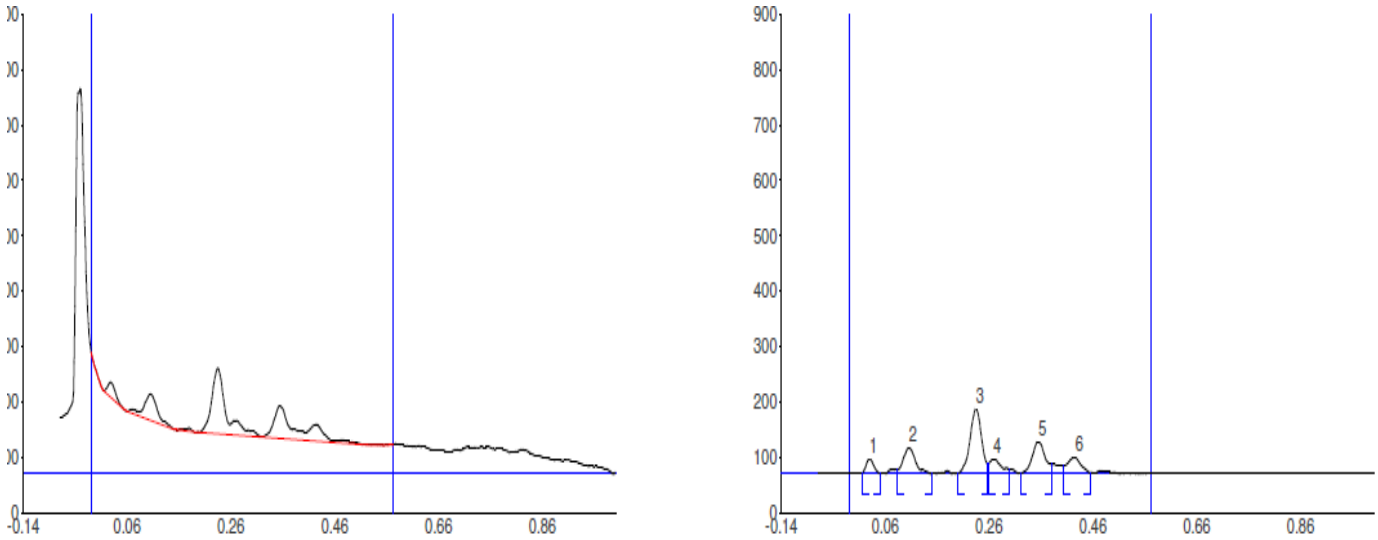

Fig 10. Track 3- HPTLC chromatogram of ethanolic extracts of stems of *Tinospora cordifolia* (Wild) Miers.

Table-9. Track-3 Rf value of the chromatogram of EESTC.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.01	0.5	0.03	27.7	8.94	0.05	0.7	335.3	5.77
2.	0.08	7.4	0.10	47.5	15.34	0.15	0.0	931.3	16.02
3.	0.20	0.8	0.23	118.7	38.33	0.26	18.8	2155.8	37.07
4.	0.26	19.2	0.27	26.9	8.70	0.30	8.1	512.0	8.81
5.	0.32	1.1	0.35	58.6	18.93	0.38	17.8	1206.0	20.74
6.	0.40	14.8	0.42	30.2	9.77	0.45	2.8	674.4	11.60

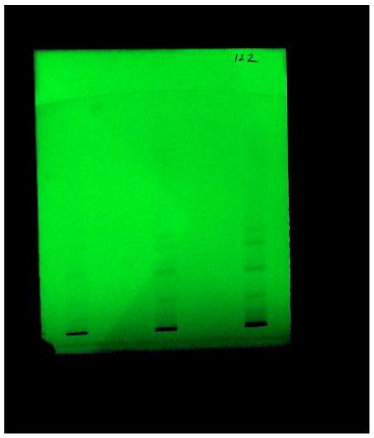

Fig.11 U.V- 254 nm

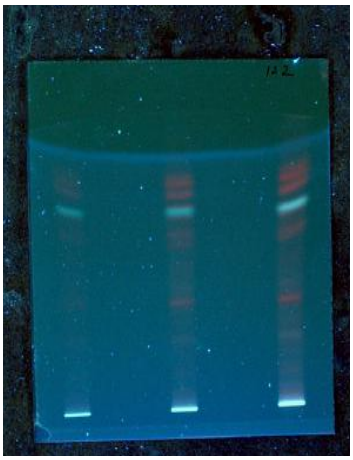

Fig.12 U.V- 366nm

C. Table 10. HPTLC-PHC(EERHI+EESTC) (R. No-201806270121).

No.	Applied position	Applied volume	Active
Track 1.	15.0mm	3.0µl	Yes
Track 2.	50.0mm	5.0µl	Yes
Track 3.	85.0mm	10.0µl	Yes

The HPTLC fingerprint profile of polyherbal combine extracts is shown in fig 14,15 and 16 where at a concentration of 3µl ,5µl and 10µl were applied respectively in track 1,2 and 3, there were 2 spots observed in 3µl and 7 spots as well as peaks were observed at a concentration of 5µl and 10µl i.e., At 3µl, Rf values were 0.20 and 0.40. At 5µl, Rf values were obtained 0.00,0.07,0.13,0.19,0.30,0.37 and 0.44. At 10µl, Rf values were obtained 0.06,0.12,0.17,0.23,0.29,0.37 and 0.43 indicating the presence of 7 different component at two different concentration of samples. Out of 7 spots, the spots with Rf values 0.37 and 0.17 of conc. 5µl and 10µl were found to be more prominent as the percentage area was larger with 25.77 and 25.24 respectively. The remaining components were found to be less in quantity as the percentage area for remaining spots were less. The spots with Rf values 0.37 has been found common in both concentration 5µl and 10µl indicating the similarity of a component.

Win CATS Planar Chromatography Manager

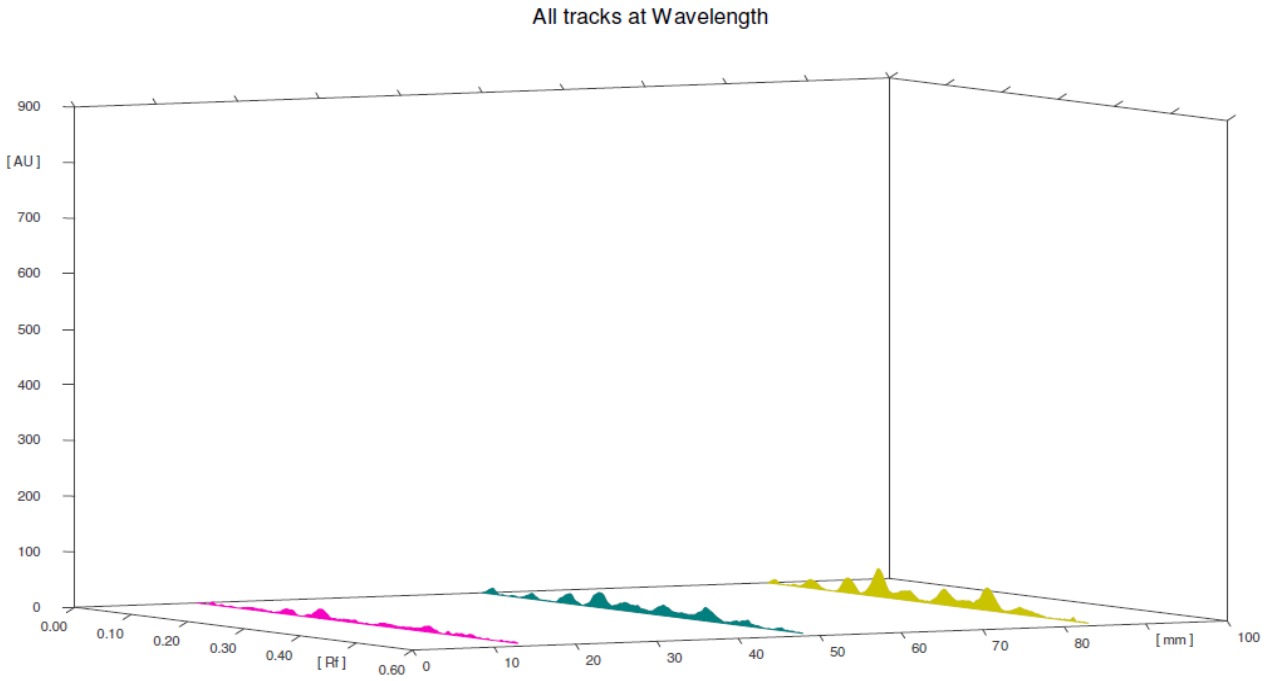

Fig 13: HPTLC fingerprint profile of all the tracks at 254nm of polyherbal extracts of both plants.

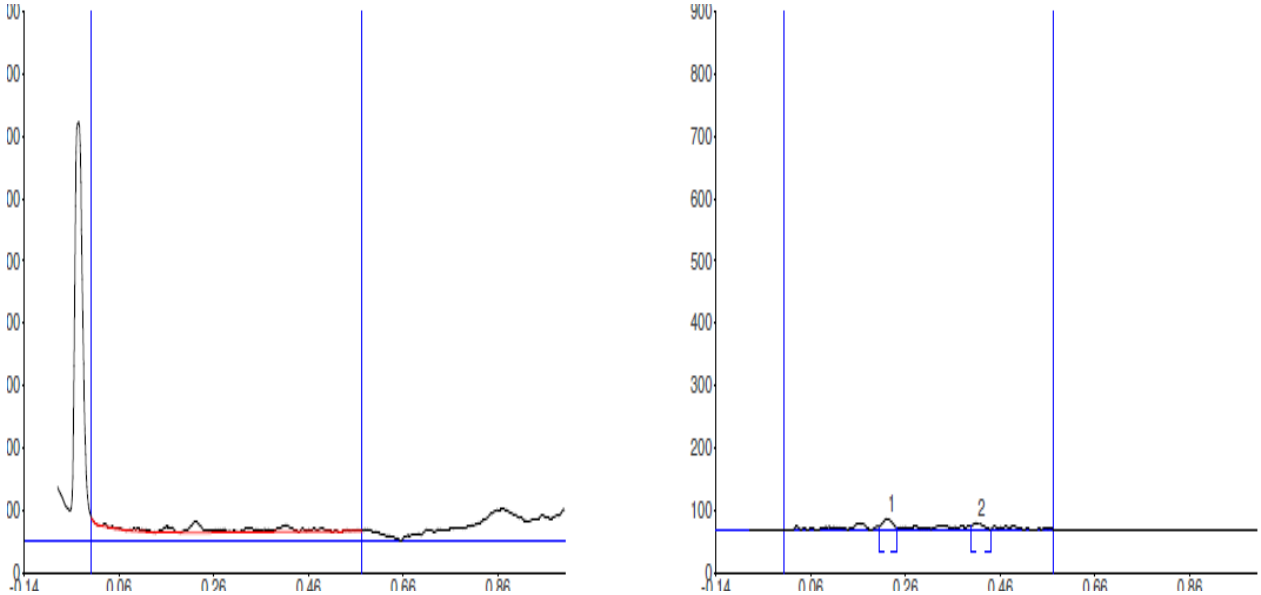

Fig 14. Track 1- HPTLC chromatogram of polyherbal extracts of above two plants.

Table-11. Track-1 Rf value of the chromatogram of PHC.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.20	4.3	0.22	17.1	60.82	0.24	3.0	294.6	60.81
2.	0.40	5.4	0.41	11.0	39.18	0.44	0.3	189.8	39.19

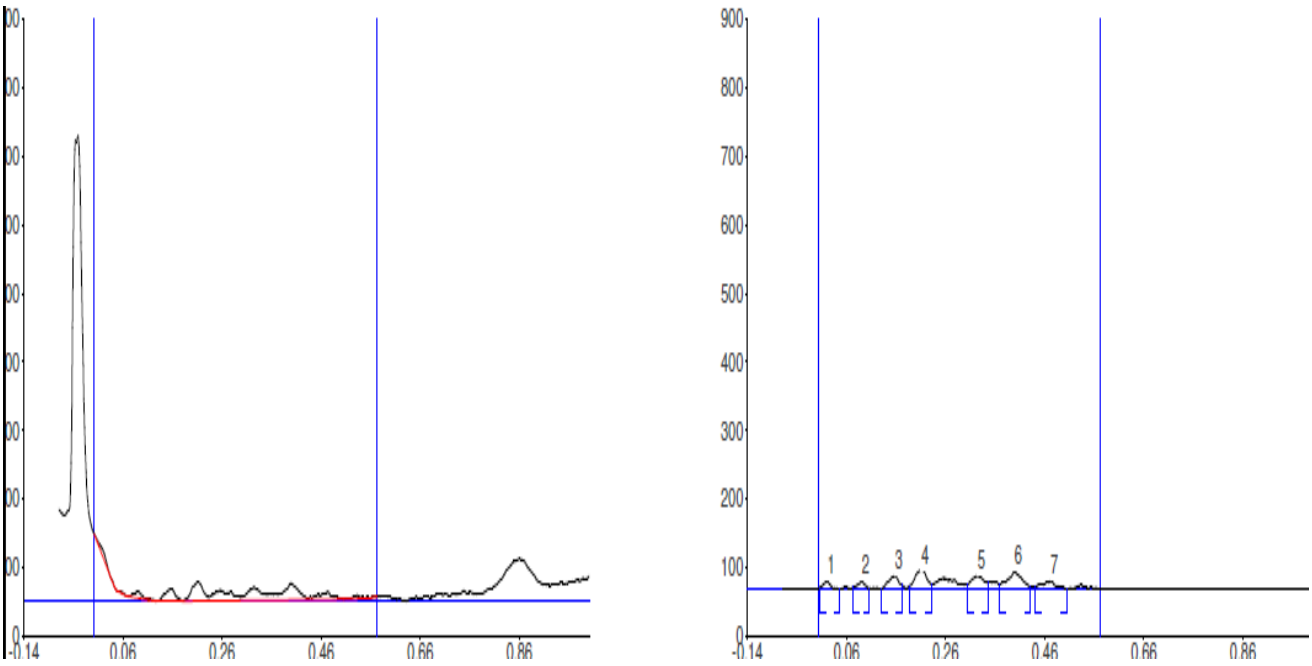

Fig 15. Track 2- HPTLC chromatogram of polyherbal extracts of above two plants.

Table-12. Track-2 Rf value of the chromatogram of PHC.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.00	1.1	0.02	10.4	8.63	0.04	0.0	116.8	5.12
2.	0.07	1.5	0.09	10.4	8.60	0.10	0.8	121.7	5.34
3.	0.13	0.2	0.16	18.3	15.23	0.17	4.8	299.7	13.15
4.	0.19	3.9	0.21	27.0	22.42	0.23	5.7	522.5	22.93
5.	0.30	6.0	0.32	19.0	15.76	0.34	8.4	393.8	17.28
6.	0.37	7.6	0.40	24.3	20.21	0.43	2.5	587.2	25.77
7.	0.44	3.0	0.47	11.0	9.15	0.50	0.2	236.9	10.40

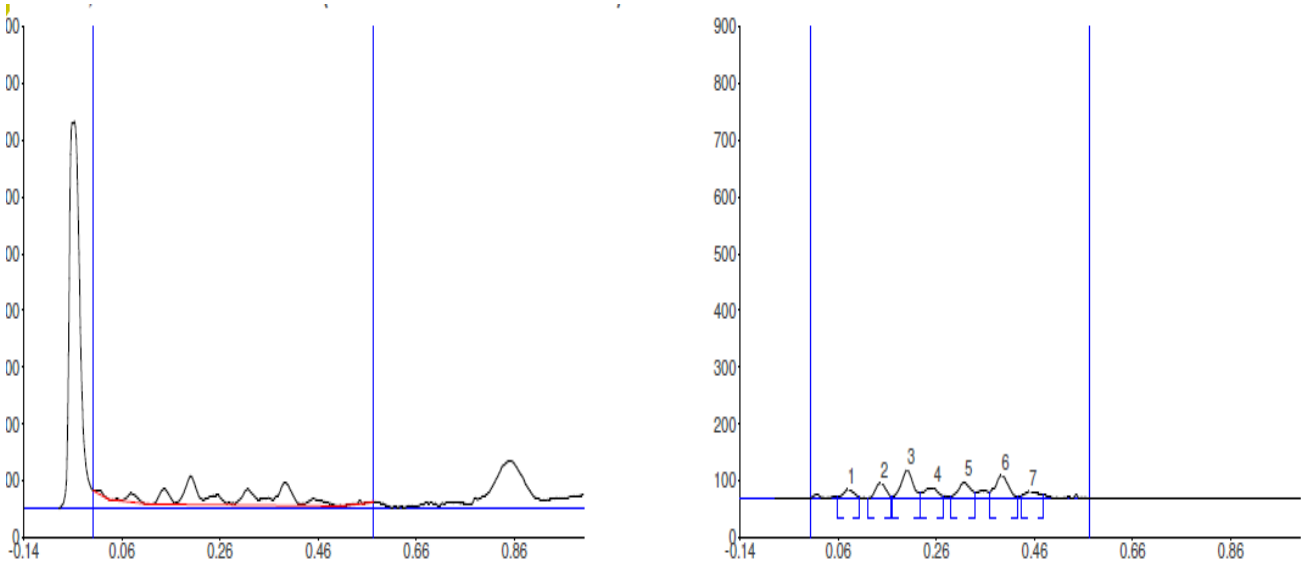

Fig-16. Track 3- HPTLC chromatogram of polyherbal extracts of above two plants.

Table-13. Track-3 Rf value of the chromatogram of PHC.

	Start		Max			End		Area	
Peak	**Rf**	**H**	**Rf**	**H**	**%**	**Rf**	**H**	**Area**	**%**
1.	0.06	2.1	0.08	15.9	8.28	0.10	2.0	264.6	6.99
2.	0.12	0.1	0.14	26.5	13.86	0.17	2.4	452.9	11.96
3.	0.17	2.6	0.20	50.1	26.15	0.22	8.8	955.7	25.24
4.	0.23	9.0	0.25	17.4	9.10	0.27	3.1	402.1	10.62
5.	0.29	2.4	0.32	28.1	14.69	0.34	10.0	565.9	14.95
6.	0.37	9.9	0.39	40.3	21.06	0.42	2.5	863.1	22.80
7.	0.43	4.2	0.45	13.1	6.86	0.48	5.1	281.5	7.44

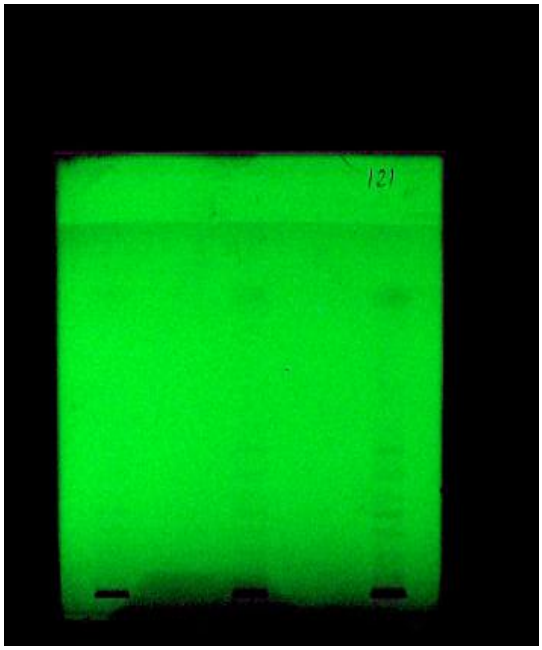

Fig-17 U.V-254nm

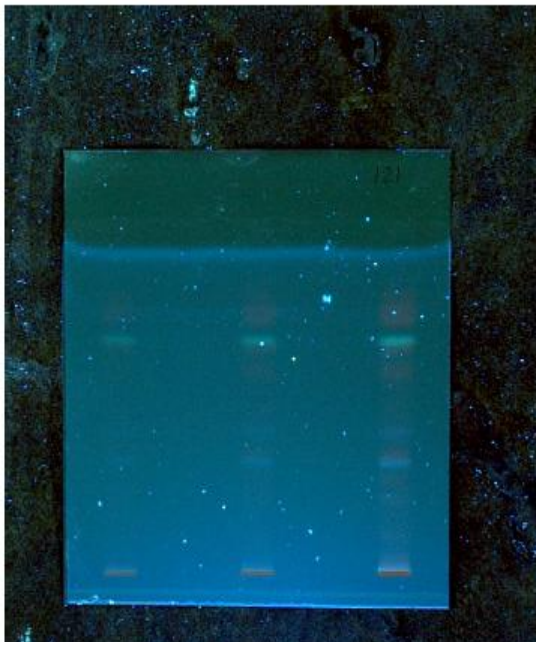

Fig-18 U.V-366nm

Conclusion

The present study revealed about the HPTLC one of the most effective, reliable, precise and accurate method for compound identification and authentication. Thus, retention factor (Rf value) obtained from ethanolic extracts at different concentration of each samples can be used to identify each component due to their specificity and uniqueness.

In the present study we have used ethanolic extracts at different concentration starting from 3µl,5µl and 10µl of given samples. We have found combine extracts of root part of *Hemidesmus indicus*(L.)R.Br and stem part of *Tinospora cordifolia* (Wild.)Miers detected 7 peaks at 254nm in chromatograms with major number of spots were visualized. Therefore, combine mixture has higher no. of secondary metabolites at higher concentration of 5µl and 10µl than other samples.

PHC>EESTC>EERHI

Due to higher number of secondary metabolites one additional information we achieved from HPTLC, is its biological potential is more than other two samples. Therefore, it can also provide better synergistic effect.

The developed HPTLC method and its outcome can be used as a quality control tool for rapid authentication of wide variety of herbal samples

The HPTLC fingerprinting is helpful in differentiating the species from the adulterant. Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality

control of a particular species but also provide basic information for the isolation, purification, characterization and identification of chemical marker compounds of the species.[27]

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