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HPTLC fingerprint analysis of *Pedilanthus tithymaloides* or *Euphorbia tithymaloides*

Neetu Soni and Rajani Srivastava

Abstract
Background: The plant *Euphorbia tithymaloides* L. *or Pedilanthus tithymaloides* L. is well distributed plant globally and known in India with various common names as Vilaayati-sher in Maharashtra, Naagaphani in Uttar Pradesh, Naagadaman and Naagadone in Madhya Pradesh.
Objectives: To establish HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole shrub of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L.
Materials and Methods: In the present study petroleum ether (ETP1, ETP2) and chloroform extracts (ETC1, ETC2) of *Pedilanthus tithymaloides* were selected for HPTLC fingerprinting. The best resolutions for both plant extracts were obtained with solvent system of toluene: ethyl acetate: formic acid in ratio of 10:3:1.WIN CATS planer chromatography manager software utilised for HPTLC fingerprinting. CAMAG Linomat 5 applicator used for sample application, CAMAG glass Twin Trough Chamber 20x10cm used for plate development and CAMAG TLC Scanner-3 with mercury remission florescence lamps used for detection of separated compounds at wavelength 254 nm and 366 nm.
Result: The result revealed presence of three (03) components in chloroform extracts (ETC1) and four (04) components in chloroform extracts (ETC2) of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* while petroleum ether extract (ETP1, ETP2) revealed nine (09) components.
Conclusion: HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole plant of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L. was indicator of presence of phyto constituents and expression of qualitative and quantitative measures in terms of Rf values with corresponding percentage area.

Keywords: HPTLC Fingerprint, *Pedilanthus tithymaloides* L., *Euphorbia tithymaloides* L

1. Introduction
Pedilanthus tithymaloides L. is a synonym of *Euphorbia tithymaloides* L. (Euphorbiaceae), a native plant of tropical America and has wide distribution in tropical and subtropical parts of world. This plant is a succulent erect shrub, well distributed in various parts of India like Uttar Pradesh, Madhya Pradesh, Bihar, Jharkhand, and Maharashtra etc. The plant *Euphorbia tithymaloides* L. or *Pedilanthus tithymaloides* (L.) known in India with various common names as Vilaayati-sher in Maharashtra, Naagaphani in Uttar Pradesh, Naagadaman and Naagadone in Madhya Pradesh. This shrub is cultivated as an ornamental plant and used in gardening to make fences. Common names at International level are Japanese poinsettia, red bird flower, bird-Cactus, slipper Plant, slipper flower, devil's backbone, etc.
Indian traditional healers used *Pedilanthus tithymaloides* as traditional remedy for various types of diseases. This plant also reported in various traditional system of medicines like ayurveda, siddha and unani system of medicine. *Pedilanthus tithymaloides* is latex producing plant and its latex used in warts, leucoderma, venereal diseases, its Roots have emetic effect and used as substitute of Ipecacuanha (ipecac root) in West Indies.
The plant *Pedilanthus tithymaloides* reported to have antiviral [1], antibacterial [2], anti-inflammatory [3], antioxidant [3], haemostatic activity [4], larvicidal [5], wound healing [6], Anthelmintic Activity [7], anti-diabetic [8, 9], anticancer [10], anti-filarial [11], analgesic [12], antipyretic [12], anti-inflammatory [12], antimalarial [13] and antituberculosis [13] activities, antihemorrhagic, abortive, antihypertensive, antifungal effects etc.
Phytoconstituents reported in plant were flavonoids, phenols, steroids, glycosides, resins, saponins, and tannins; other significant constituents reported were: proteolytic enzyme, pedilanthain, triterpenoids, poly-o-acylated jatrophane diterpenoids and long-chain alcohols, 5V-S-methylthioadenosine, pyrogallol, 1,4-dihydroquinone, nicotinamide, proline, Butyric, 2-hexenoic, caproic, cinnamic, dihydroxycinnamic, p-hydroxycinnamic, gluconic, palmitic, 3,4-dimethoxycinnamic, m-methoxy-p-hydroxycinnamic, docosenoic, hexaeicosanoic and

octaeicosanoic acids, gallic acid and rutin.
Kaempferol, quercitrin, isoquercitrin and scopoletin [14] Pedilstatin [10]. The milky juice of the roots, stems and leaves contains euphorbol and diterpene esters. The leaves extracts reported to have natural compounds: epifriedelanyl acetate, friedelanol, β-sitosterol, ursolic acid, luteolin and a new compound 1, 2-tetradecanediol 1-(hydrogen sulfate) sodium salt.
Euphorbia tithymaloides L. *or Pedilanthus tithymaloides* L. is plant with wide pharmacological activities, attributed due to presence of significant and novel phytoconstituents. The literature survey was performed by utilising digital e-library services of Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS). On the basis of literature survey, the scientific study of this plant in relevance with herbal drug standardisation found to be lacking. The focus of present study targeted towards phytochemical standardisation of *Euphorbia tithymaloides* L. *or Pedilanthus tithymaloides* L.
The main aim of this work was to establish HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole plant of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L.
The phytochemical standardization of present study achieved by HPTLC fingerprinting analysis. The biochemical characterization via HPTLC could be helpful in qualitative and quantitative determination of phytochemicals. These studies could be helpful in identification and authentication of the plant source as well as maintenance of quality of herbal drugs as starting drug substances, for development of quality herbal drug formulations. The correct identification of the herbal materials is essential for quality of herbal medicine.

2. Materials and Methods

2.1 Plant material collection, identification and extraction

Pedilanthus tithymaloides L. or *Euphorbia tithymaloides* whole plant was collected in month of March from natural habitat of company garden Allahabad. The plants were further identified by pharmacognosy department and plant herbarium was submitted for further reference of plant. The stems & leaves were cut down into small pieces and left for shade drying at room temperature, for 3 days. The dried plant materials were made to coarse power and packed in thimble flask, subjected to hot continuous extraction by using Soxhlet apparatus. The petroleum ether, chloroform used for continuous extraction, after completion of extraction solvents evaporated, dried up to constant weight. The extractive values for petroleum ether, chloroform were calculated and subjected to preliminary phytochemical screening as per WHO guidelines. Solvents and all chemicals used for study were analytical grade.

2.2 HPTLC fingerprinting

2.3 Selection of solvent systems

A number of combinations of solvents in different proportions were tried through TLC, in attempt to develop standard solvent system which could be capable of separating the components best in perfect resolution and to find out suitable solvent system for selection and separation of phytochemicals by using HPTLC. Toluene: ethyl acetate: formic acid in different ratios (7:3:1, 7:2:1, 7:1:1, 10:3:1) were tried through Thin Layer Chromatography (TLC) for separation of phyto-constituents.
In the present study petroleum ether (ETP1, ETP2) and chloroform extracts (ETC1, ETC2) of *Pedilanthus tithymaloides* were selected for HPTLC fingerprinting. The selection of suitable solvent system for HPTLC fingerprinting based on TLC fingerprinting analysis. The best resolutions for both plant extracts were obtained with solvent system of toluene: ethyl acetate: formic acid in ratio of 10:3:1.

2.4 Sample application and development

The sample applications of plant extracts were performed with CAMAG Linomat 5 applicator. The total numbers of tracks used were four (ETP1, ETP2, ETC1, ETC2), and samples were applied with application volume of 5 and 10 micro litres for both plant extracts. Application of samples summarize in given table number-1.

Table 1: Application of samples

S. No	Application position	Application volume	Sample ID (Plant extract)	Activity
1	15.0 mm	5.0 µl	(ETC1)	Yes
2	38.3 mm	10.0 µl	(ETC2)	Yes
3	61.6 mm	5.0 µl	(ETP1)	Yes
4	84.9 mm	10.0 µl	(ETP2)	Yes

The samples were spotted with 100 µl syringe in the form of band on pre-coated plates. The plates were developed in CAMAG glass Twin Trough Chamber 20x10cm in linear ascending direction with solvents system of toluene: ethyl acetate: formic acid in ratio of 10:3:1 for both plant extracts.

2.5 Detection of separated phyto-components

Developed plates were dried in hot air oven at 60°C for 5 minutes for evaporation of solvents. The detection of spots was carried out by using CAMAG TLC Scanner-3 with mercury remission florescence lamps at wavelength of 254 nm and 366 nm. The plates were placed inside illumination instrument or photo-documentation chamber of CAMAG Reprostar 3 and captured the images with digital camera under ultra violet light at 254 nm and 366 nm scanning wavelength. The retention factor (Rf) values and finger print data were recorded by WIN CATS planer chromatography manager software.

3. Result

The HPTLC fingerprinting of petroleum ether (ETP1, ETP2) and chloroform extracts (ETC1, ETC2) of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* by using solvent system of toluene: ethyl acetate: formic acid in ratio of 10:3:1 revealed successful separation of number of phyto-constituents corresponding to retention factors (Rf) and chromatographic peaks.
The result revealed presence of three (03) components in chloroform extracts (ETC1) and four (04) components in chloroform extracts (ETC2) of *Pedilanthus tithymaloides* (L.) or *Euphorbia tithymaloides* while petroleum ether extract (ETP1, ETP2) revealed nine (09) components. The number of constituent peaks and Rf values, max %, area % for petroleum ether and chloroform extracts were summarised in table 2, 3, 4, 5.

Table 2: ETC1 numbers of constituents (Peaks) and their Rf values

Peak	Start position Rf	Start position Height	Max Rf	Max Height	Max %	End position Rf	End position Height	Area	Area %	Assigned substance
1	-0.04	1.8	-0.03	21.0	32.06	-0.01	0.1	170.0	36.37	unknown
2	0.51	0.7	0.52	19.5	29.71	0.54	0.7	138.7	29.68	unknown
3	0.87	0.8	0.90	25.0	38.23	0.91	3.0	158.7	33.95	unknown

Table 3: ETC2 numbers of constituents (Peaks) and their Rf values

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	0.0	-0.03	36.9	48.15	-0.01	0.2	303.8	29.00	unknown *
2	0.40	2.3	0.43	13.2	17.20	0.47	1.8	287.4	27.43	unknown *
3	0.59	2.1	0.62	14.0	18.29	0.64	3.4	229.9	21.94	unknown *
	0.66	2.5	0.69	12.5	16.37	0.72	0.4	226.6	21.63	unknown *

Table 4: ETP1 numbers of constituents (Peaks) and their Rf values

Peak	Start position Rf	Start position Height	Max Rf	Max Height	Max %	End position Rf	End position Height	Area	Area %	Assigned substance
1	-0.04	4.4	0.02	114.1	11.80	0.03	12.3	2042.1	10.3	*
2	0.23	5.8	0.31	18.0	1.86	0.32	16.6	682.6	3.44	*
3	0.32	16.9	0.35	150.9	15.62	0.38	11.2	3077.5	15.53	*
4	0.38	11.4	0.43	508.4	52.61	0.47	9.7	9734.2	49.11	*
5	0.47	9.7	0.49	17.1	1.77	0.52	5.1	409.5	2.07	*
6	0.52	5.1	0.55	40.3	4.17	0.59	10.2	1059.6	5.35	*
7	0.59	10.2	0.61	30.8	3.18	0.65	6.7	792.9	4.00	*
8	0.65	6.8	0.68	64.5	6.67	0.74	1.0	1885.1	9.51	*
9	0.80	0.1	0.82	22.4	2.31	0.85	0.1	138.5	0.70	*

Unknown *

Table 5: ETP2 numbers of constituents (peaks) and their Rf values

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	0.0	-0.02	149.0	9.76	0.03	24.0	3137.5	9.51	unknown *
2	0.03	24.1	0.04	27.0	1.77	0.10	10.2	822.5	2.49	unknown *
3	0.23	10.3	0.35	252.4	16.54	0.38	22.8	6487.7	19.66	unknown *
4	0.39	24.3	0.43	755.8	49.51	0.46	23.4	14849.0	45.01	unknown *
5	0.46	23.4	0.48	55.4	3.63	0.52	16.1	1511.1	4.58	unknown *
6	0.52	16.1	0.55	78.2	5.13	0.59	22.8	2156.5	6.54	unknown *
7	0.59	22.9	0.61	53.5	3.51	0.65	16.7	1332.8	4.04	unknown *
8	0.65	16.7	0.67	76.5	5.01	0.69	64.3	1195.9	3.62	unknown *
9	0.69	65.1	0.70	78.7	5.16	0.75	0.2	1499.5	4.54	unknown *

The HPTLC chromatogram of ETC1and ETC2 represented in figure 1 and 2 respectively. Similarly HPTLC chromatogram of ETP1and ETP2 represented in figure 3 and 4 respectively.

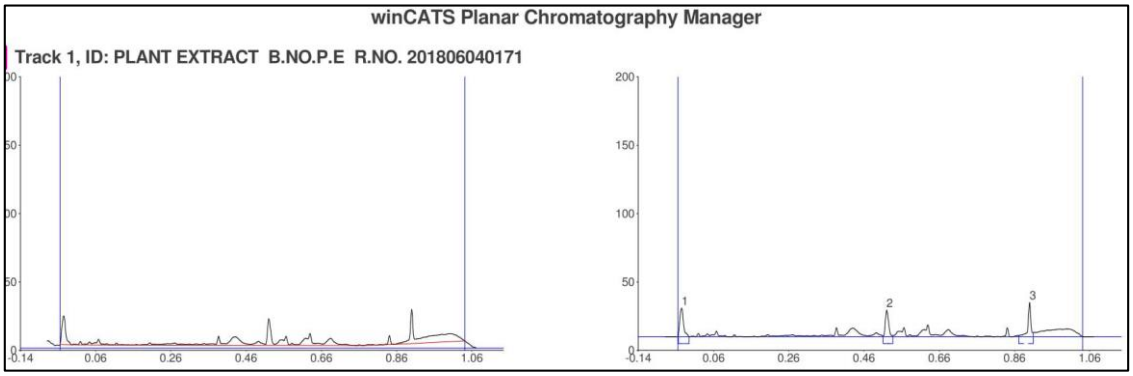

Fig 1: The HPTLC chromatogram of ETC1

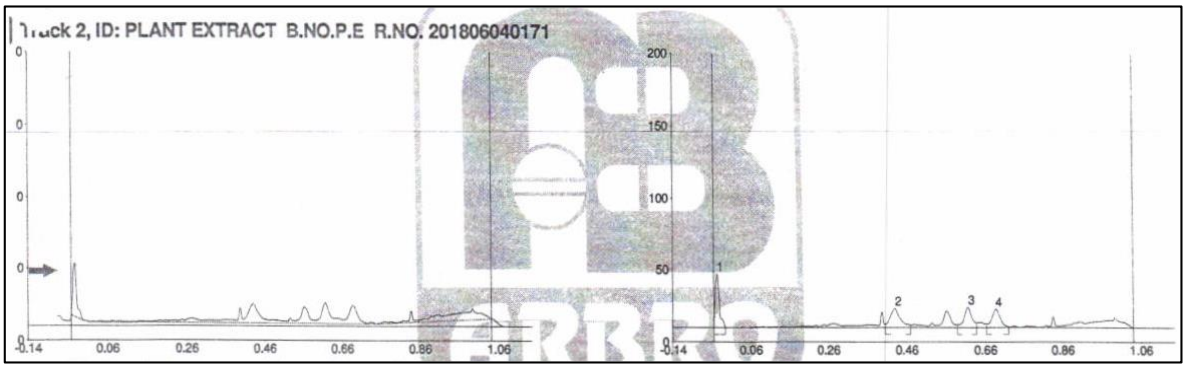

Fig 2: The HPTLC chromatogram of ETC2

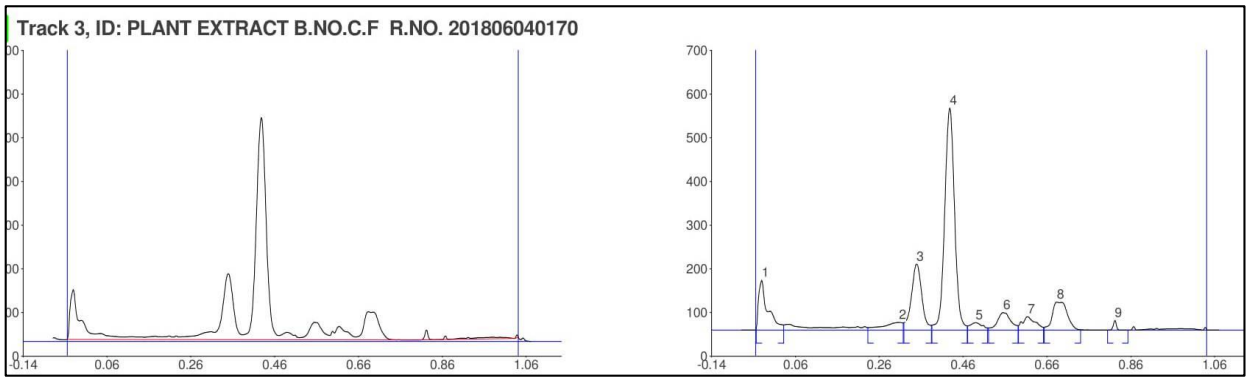

Fig 3: The HPTLC chromatogram of ETP1

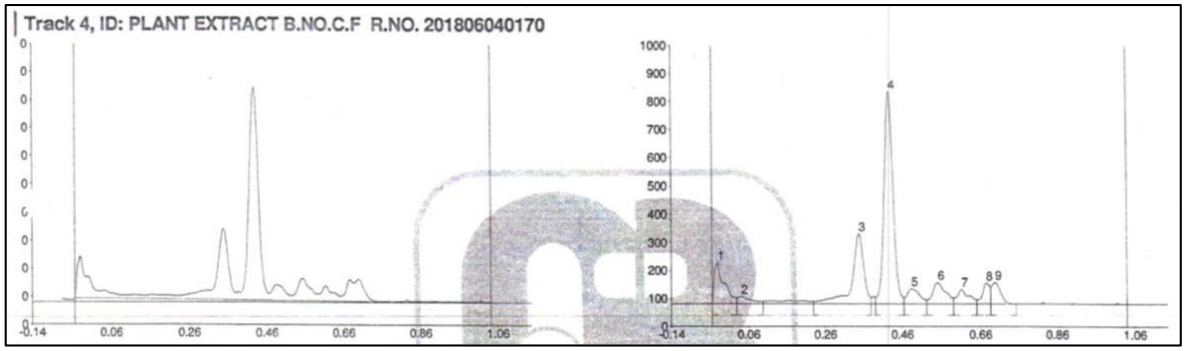

Fig 4: The HPTLC chromatogram of ETP2

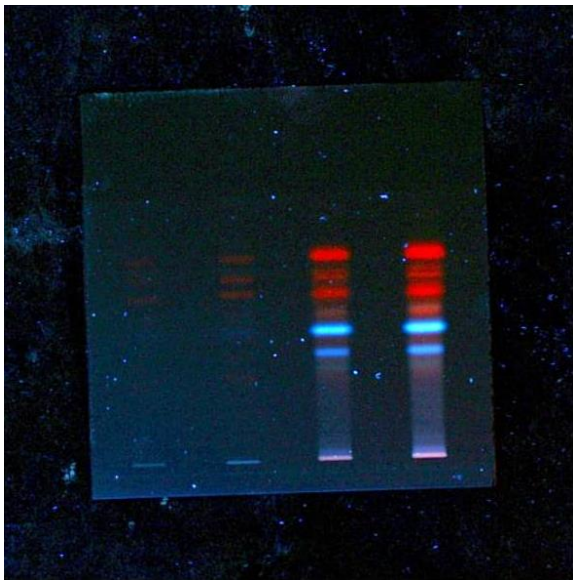

Fig 5: Chromatographic profiles at 366 nm

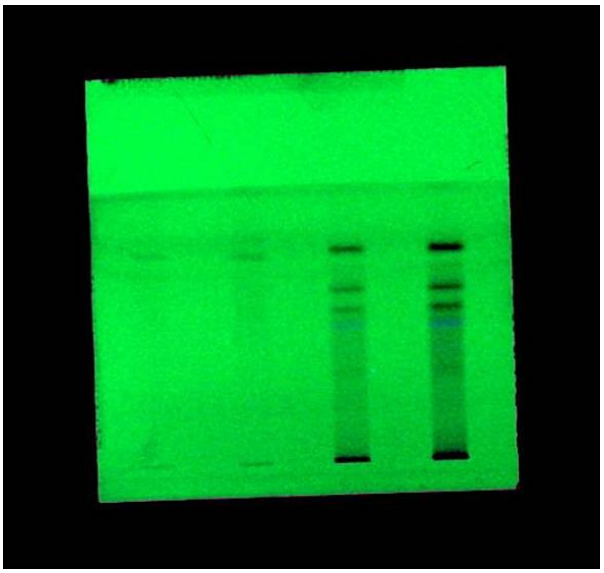

Fig 6: Chromatographic profiles at 254 nm

Chromatographic profile for ETC1, ETC2, ETP1 and ETP2 extract samples, under 366 nm and 254 nm shown by figure 5 and 6.

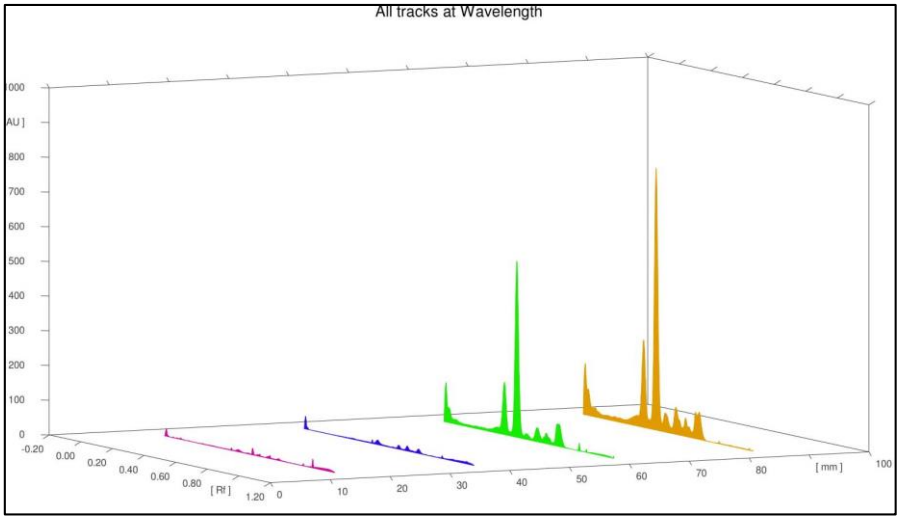

Fig 7: Three dimensional representation of all tracks at different wavelength.

4. Discussion

The main aim of this work was to establish HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole plant of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L. Fingerprinting by HPTLC (High performance thin layer chromatography) commonly perform to get preliminary information regarding phytochemicals present in plant extract as well as sophisticated advanced tool for establishing identity of selected plant species. HPTLC (High performance thin layer chromatography) is very much selective and reproducible technique for separation of complicated mixtures of plant extracts.

5. Conclusion

HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole plant of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L. was performed and presence of three component at low concentration (ETC1) and four components at high concentration (ETC2) of plant chloroform extracts were confirmed for selected plant species. Similarly presence of nine components was confirmed for petroleum ether extract of selected plant species. HPTLC Fingerprint analysis of chloroform and petroleum ether extracts of whole plant of *Pedilanthus tithymaloides* L. or *Euphorbia tithymaloides* L. was indicator of presence of phyto constituents and best expression of qualitative and quantitative measure in terms of Rf values with corresponding percentage area.

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