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Research article

Analytical and HPTLC studies on *Coldenia procumbens* Linn. whole plant

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Key words: Boraginaceae, Ceruppatai, Tripunkhi, Tripakṣī, rheumatism, abscess.

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Abstract

Coldenia procumbens Linn. (Boraginaceae): is procumbent herb found wild in fields, dried lakes and roadsides in warmer parts of India. The whole plant of this herb is used in Indian systems of medicine. In this paper, authors intend to investigate the dried whole plant of the herb collected from three different places of Tamil Nadu, a Southern state of India. The investigations include physico-chemical parameters, viz., total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive, alkalinity and pH. Also developed the thin layer chromatographic profile and high performance thin layer chromatographic finger print profiles.

Introduction

Coldenia procumbens Linn. belongs to the family Boraginaceae. The herb is used to cure rheumatism and for abscess in Ayurvedic system and the dose is 3-6 g powder [1]. The ground paste of fresh leaves of the herb is applied on rheumatic swellings as the folklore medicine[2]. The dried plant powdered with equal quantity of fenugreek seeds are applied to mature abscesses[3]. In the recent past decades, there is a universal demand for herbal drugs due to their safety and efficacy. Hence identifying a herbal drug with efficacy will be helpful for the herbal drug industry. *C. procumbens* is reported for antioxidant [4,5], anti-inflammatory [5,6], antibacterial [7-9], antidiabetic [10], hepatoprotective [11-13], CNS depression [14] and anthelmintic [15] activities which are evident for the efficacy of the plant. The chemical constituents present in the herbs are responsible for their efficacies. In this plant, wedelolactone [16], ehretioside A1 and lithospermoside [17] are the phytochemicals previously reported by other researchers. Pharmacognostic studies on the leaves of the plant were also reported [18]. The aerial parts of the plant were studied for physico-chemical, fluorescence analysis, and preliminary phytochemical analysis by other authors [19]. However thin layer chromatographic photo documentation and high performance thin layer chromatographic finger print profiles are not reported for this plant. Hence authors aim to analyze three samples collected from different places, document their TLC profiles and HPTLC finger print profiles of hexane extract of whole plant. The results would be useful for developing the pharmacopoeial standards for this plant which is mandatory for quality control of the any drug.

Experimental

Plant Material

The whole plant in flowering season was collected in the month of March from Mettur, Pudukottai and Srivilliputhur, Tamil Nadu, India. They were authenticated by Dr. Sasikala Ethirajulu, Department of Pharmacognosy, Siddha Central Research Institute, Chennai. The plant materials were cut into small pieces and dried in shade. 100 g of each of the plant were powdered coarsely and stored in an airtight container till the completion of the study.

Reagents and chemicals

The chemicals and reagents were purchased from Merck and analytical grade.

Physico-chemical parameters

Loss on drying at 105°C, total ash, water soluble ash, alkalinity of water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive, pH values and successive extraction with hexane, chloroform and ethanol were carried out as per the standard procedures [20].

Heavy metal analysis

The lead, cadmium, arsenic and mercury are considered as heavy metals and their concentration in the plant samples were estimated using atomic absorption spectrophotometer by following the methods of Ansari et al, 1999 [21] and Sahito et al., 2001 [22] which is rapid and the percentage recovery of elements is better than other methods.

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Microbial load and Pathogens

Enterobacteriacea, E. coli, Salmonella spp. Pseudomonas aeruginosa, Staphylococcus aureus, total bacterial count and total fungal count were determined as per the WHO methods.

Preparation of extract

4 g of the plant powder was soaked in hexane (100ml) at room temperature for overnight. The content was filtered through filtered paper and filtrate was concentrated by vacuum rotary evaporator at 40°C. Finally crude metabolites were obtained and stored at 4°C until further use.

TLC Solvent system

For a better resolution many solvent systems were attempted. The solvent system Toluene: Ethyl acetate: Formic acid (5:1.5:0.1, v/v/v) showed a better separation than the other solvent systems tried. This solvent system was used for developing the extracts in the TLC plate.

Visualizing reagent

The most commonly used visualizing reagent namely Vanillin-sulphuric acid reagent (one gram vanillin dissolved in the mixture of ethanol: sulphuric acid in the ratio 95:5) was used.

Instrument

The CAMAG's twin chamber was used for developing the TLC plate. Linomat IV (CAMAG, Muttenz, Switzerland) applicator was used for the application of the extract. Aluminium plate precoated with silica gel $60F_{254}$ of 0.2 mm thickness (Merck) was used TLC plate. Bands with a width of 8 mm and 6 mm distance between tracks were applied on 6 x10 cm plate. CAMAG TLC scanner 030618 attached with WINCATS software were used for finger print analysis under UV 254 nm. CAMAG visualizer was used for photo documentation at UV 254 nm, 366 nm; and invisible lights after dipping in vanillin-sulphuric acid reagent followed by heating in an air circulated oven till the development of coloured spots.

Procedure for TLC& HPTLC

The extract was applied on the TLC plate as 7 µl, 10 µl and 12 µl bands of 8 mm width and 6 mm distance in between tracks using Linomat IV applicator and developed in the above mentioned solvent system. The developed TLC plate was air dried and photographs were taken under UV 254 and 366 nm. The plate was scanned under UV 254 nm, UV 366 nm using the scanner. The finger print was recorded. Then the plate was dipped in vanillin-sulphuric acid reagent, heated in an oven at 105°C till the development of coloured spots and photograph taken and again scanner for finger printing.

Results and Discussion

The results of the physico-chemical analysis are pictorially represented in Fig. 1. The loss on drying of *C. procumbens* collected from Mettur, Pudukottai and Srivilliputhur were calculated as 10.33 %, 13.00 % and 14.77 % respectively. The moisture retaining capacity of the plant collected from Srivilliputhur is more when compared to Mettur.

The total ash value of 12.25 %, 12.46 % and 12.26 % respectively of *C. procumbens* says that the plant has higher amount of inorganic matter while their respective water soluble ash value of 6.63 %, 5.15 % and 5.03 % represents that the nearly half of the total inorganic compound is soluble in water. Their alkalinity values of 4.10, 3.73 and 3.93 ml of 0.1N HCl/g of the sample show that the basicity of water soluble inorganic content is considerably high when compared to other plants. pH values of 7.80, 7.84 and 7.90 indicate that the plants are slightly basic in nature which may be due to the presence of water soluble salts. The water soluble extractive values of 13.25 %, 12.65 % and 9.25 % indicate the possible presence of high polar compounds.

The ethanol soluble extractive values of 6.60 %, 7.00 % and 5.80 % show that the values are less when compared to water soluble extractive and the higher loss on drying value may be the reason for lesser ethanol soluble extractive value. Similarly the higher total ash content is also the hidden reason for lesser ethanol soluble extractive value. However the total hot extraction values with hexane and chloroform are lesser than 3 % which is an indication that the plant is rich in high polar compounds like glycosides, tannins, etc.

The physico-chemical parameters of *C. procumbens* indicate that there are some differences in the solubilities and ash contents. There may be different phytochemicals derived from same or different biochemical pathways. The heavy metals (Table 1), viz., lead, cadmium, mercury and arsenic as well as microbial load and other pathogens (Table 2) are within the WHO permissible limits. Hence this plant is safe as an internal medicine.

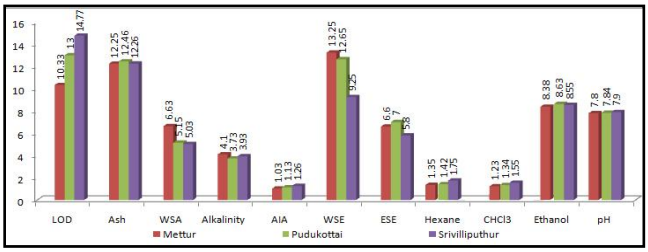


Figure 1. Graphical representation of physicochemical values of *C. procumbens.*

Table 1. Heavy metal content of *C. procumbens* whole plant

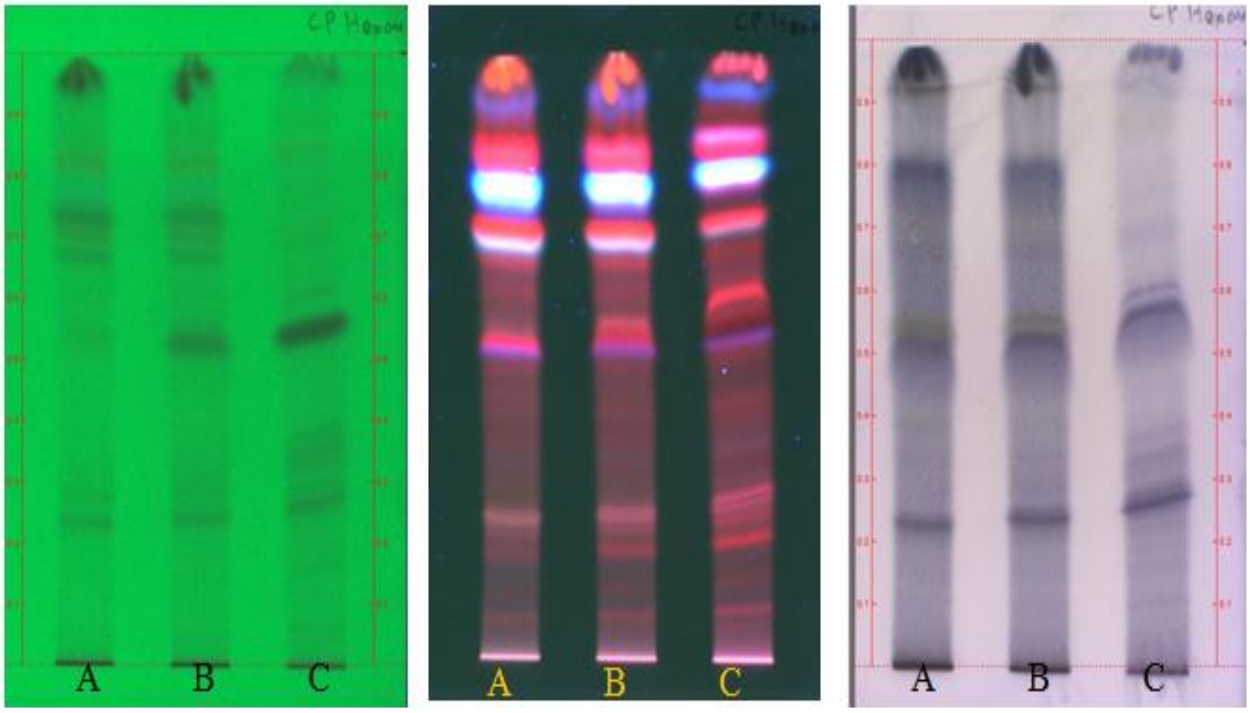
S. No.	Parameter	Value	WHO Limit (in ppm)
1.	Lead	Not detected	10
2.	Cadmium	Not detected	0.3
3.	Mercury	Not detected	1
4.	Arsenic	Not detected	3

Table 2. Microbial load in *C. procumbens* whole plant

S. No.	Parameter	Value	WHO Limit (CFU/g)
1.	Total Bacterial count	$<10^4$	10^5
2.	Total Fungal count	$<10^2$	10^3
3.	Enterobacteriacea	$<10^2$	10^3
4.	*E. coli*	Absent	10
5.	*Salmonella* spp.	Absent	None
6.	*Pseudomonoas aeruginosa*	Absent	Absent
7.	*Staphylococcus aureus*	Absent	Absent

The TLC pattern of hexane extract at UV 254 nm of these three plants are almost similar in all R_f except that at 0.06, 0.15, 0.38, 0.61 and 0.90 which are exclusively present in Srivilliputhur sample. Similarly, the TLC pattern at UV 366 nm are also similar. The spot at R_f 0.56/0.57 of Pudukottai/ Srivilliputhur is not present in Mettur sample.

The TLC pattern of Srivilliputhur sample after derivatization with vanillin-sulphuric acid is differing from other two plants in spots at R_f 0.11, 0.33, 0.58, 0.61, 0.70 and 0.87. At the same time, the spot at R_f 0.56 is present in Mettur and Pudukottai and not present in Srivilliputhur sample. TLC pattern of hexane extract at UV 254 nm, 366 nm and after derivatization are shown in Fig. 2 and the R_f value with colour in Table 3-5.



UV 254 nm **UV 366 nm** **White light after derivatization with Vanillin-Sulphuric acid**

Figure 2. HPTLC photo documentation of 10 µl of hexane extract of *C. procumbens* whole plant A. Mettur; B. Pudukottai; C. Srivilliputhur

Table 3. R_f and colour of spots of hexane extract under UV 254 nm

Sl. No.	Mettur		Pudukottai		Srivilliputhur	
	R_f	Colour	R_f	Colour	R_f	Colour
1.	-	-	-	-	0.06	Green
2.	-	-	-	-	0.15	Green
3.	0.26	Green	0.26	Green	0.26	Green
4.	0.30	Green	0.30	Green	0.30	Green
5.	0.34	Green	0.34	Green	0.34	Green
6.	-	-	-	-	0.38	Green
7.	0.55	Green	0.55	Green	0.55	Green
8.	-	-	-	-	0.61	Green
9.	0.66	Green	0.66	Green	0.66	Green
10.	0.71	Green	0.71	Green	0.71	Green
11.	0.73	Green	0.73	Green	0.73	Green
12.	0.82	Green	0.82	Green	0.85	Green
13.	-	-	-	-	0.90	Green

Table 4. R_f and colour of spots of hexane extract under UV 366 nm

Sl. No.	Mettur		Pudukottai		Srivilliputhur	
	R_f	Colour	R_f	Colour	R_f	Colour
1.	0.08	Pink	0.08	Pink	0.08	Pink
2.	0.13	Pink	0.13	Pink	0.13	Pink
3.	0.20	Pink	0.20	Pink	0.20	Pink
4.	0.23	Pink	0.23	Pink	0.23	Pink
5.	0.26	Pink	0.26	Pink	0.26	Pink
6.	0.28	Pink	0.28	Pink	0.28	Pink
7.	0.35	Pink	0.35	Pink	0.35	Pink
8.	0.42	Pink	0.42	Pink	0.42	Pink
9.	0.50	Pale Blue	0.50	Pale Blue	0.50	Pale Blue
10.	0.53	Pink	0.53	Pink	0.53	Brown
11.	-	-	0.56	Pink	0.57	Pink
12.	0.60	Pink	0.60	Pink	0.60	Pink
13.	0.68	Pink	0.68	Pink	0.68	Pink
14.	0.78	Bluish white	0.78	Bluish white	0.78	Bluish white
15.	0.84	Pink	0.84	Pink	0.84	Pink
16.	0.90	Pale blue	0.90	Pale blue	0.90	Pale blue

Table 5. R_f and colour of spots of hexane extract at 575 nm

Sl.No	Mettur		Pudukottai		Srivilliputhur	
	R_f	Colour	R_f	Colour	R_f	Colour
1.	-	-	-	-	0.11	Purple
2.	0.25	Purple	0.25	Purple	0.25	-
3.	-	-	-	-	0.33	-
4.	0.39	Purple	0.39	Purple	0.37	Purple
5.	0.52	Purple	0.52	Purple	0.53	Purple
6.	0.56	Green	0.56	Green	-	-
7.	-	-	-	-	0.58	Purple
8.	-	-	-	-	0.61	Purple
9.	0.66	Green	0.66	Green	-	-
10.	-	-	-	-	0.70	Purple
11.	0.79	Purple	0.79	Purple	0.76	Purple
12.	-	-	-	-	0.87	Purple

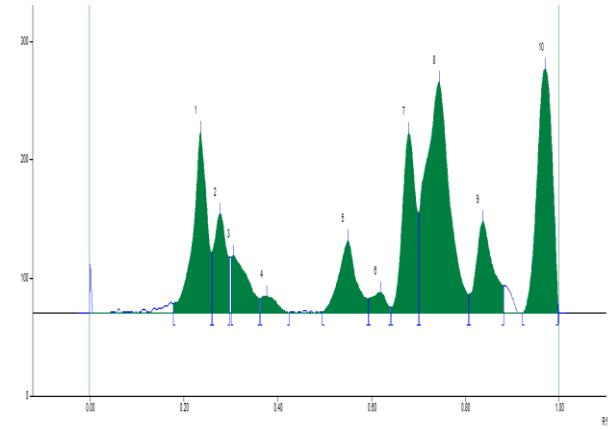
The HPTLC finger print profile of Mettur sample at UV 254 (Fig. 3) showed ten peaks among which the peak at R_f 0.75 is the major peak with an area of 27.88 % followed by the peak at R_f 0.24, 0.68 with areas of 12.78% and 12.14 % respectively. Other peaks appeared at R_f 0.84, 0.55, 0.28, 0.31, 0.62 and 0.38 with area of 7.89 %, 6.46 %, 6.16 %, 4.83 %, 1.50 % and 1.32 %. The peak at R_f 0.97 has not been taken into account since it is near the solvent front.

The HPTLC finger print profile of Pudukottai sample at UV 254 (Fig. 4) showed twelve peaks among which the peak at R_f 0.53 is the major peak with an area of 25.51 % followed by the peak at R_f 0.75, 0.24 with areas of 19.01% and 13.98 % respectively. Other peaks appeared at R_f 0.68, 0.31, 0.29, 0.84, 0.63, 0.88 and 0.38 with areas of 8.32 %, 8.17 %, 5.34 %, 4.20 %, 1.55 %, 0.99 % and 0.91 %. The peak at R_f 0.98 has not been taken into account since it is also near the solvent front.

The HPTLC finger print profile of Srivilliputhur sample at UV 254 (Fig. 5) showed thirteen peaks among which the peak at R_f 0.56, 0.35 and 0.27 are the major peaks with area of 41.08 % 21.17% and 20.32 % respectively. Other peaks appeared at R_f 0.62, 0.71, 0.16, 0.86, 0.91, 0.66, 0.81 and 0.06 with areas of 4.30 %, 2.97 %, 2.22 %, 1.30 %, 1.10 %, 0.99 %, 0.91 and 0.81 % respectively. The peak at R_f 0.98 has not been taken into account since it is also near the solvent front. The 3D chromatogram of all three samples under 254 nm is shown in Fig.6.

The HPTLC finger print profile of Mettur sample at UV 366 (Fig. 7) showed eight peaks among which the peak at R_f 0.71 is the major peak with an area of 25.96 % followed by the peaks at R_f 0.84, 0.24 and 0.53 with areas of 24.12 %, 12.56 % and 10.17 % respectively. Other peaks appeared at R_f 0.77, 0.28 and 0.39 with areas of 7.80 %, 6.67 % and 2.12 %. The peak at R_f 0.98 has not been taken into account since it is also near the solvent front.

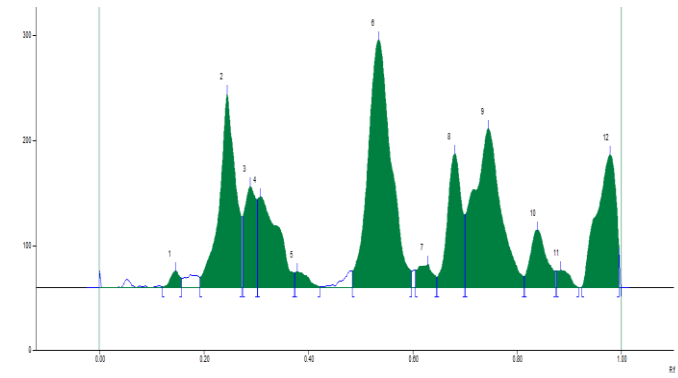
The HPTLC finger print profile of Pudukottai sample at UV 366 (Fig. 8) showed twelve peaks among which the peak at R_f 0.84 (22.43%), 0.71 (20.97 %), 0.54 (18.52 %) and 0.24 (11.71 %) are the major peaks. Other peaks appeared at R_f 0.78 (7.55 %), 0.95 (3.32 %), 0.39 (1.70 %), 0.67 (1.40 %), 0.32 (1.19 %) and 0.18 (1.03 %). The peak at R_f 0.98(5.38 %) has not been taken into account since it is also near the solvent front.



Track 1, ID: C procumbens Hexane extract Mettur

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.18 Rf	8.3 AU	0.24 Rf	152.3 AU	15.12 %	0.26 Rf	51.1 AU	4243.4 AU	12.78 %
2	0.26 Rf	51.6 AU	0.28 Rf	83.7 AU	8.30 %	0.30 Rf	47.4 AU	2046.0 AU	6.16 %
3	0.30 Rf	47.4 AU	0.31 Rf	48.4 AU	4.81 %	0.36 Rf	11.8 AU	1602.8 AU	4.83 %
4	0.36 Rf	11.9 AU	0.38 Rf	14.5 AU	1.44 %	0.42 Rf	0.3 AU	439.5 AU	1.32 %
5	0.50 Rf	0.8 AU	0.55 Rf	60.9 AU	6.04 %	0.59 Rf	11.7 AU	2145.6 AU	6.46 %
6	0.59 Rf	11.9 AU	0.62 Rf	17.4 AU	1.72 %	0.64 Rf	4.5 AU	499.8 AU	1.50 %
7	0.64 Rf	4.6 AU	0.68 Rf	151.7 AU	15.06 %	0.70 Rf	83.7 AU	4032.4 AU	12.14 %
8	0.70 Rf	85.0 AU	0.75 Rf	195.0 AU	19.35 %	0.81 Rf	15.4 AU	9259.8 AU	27.88 %
9	0.81 Rf	15.6 AU	0.84 Rf	77.3 AU	7.68 %	0.88 Rf	23.0 AU	2618.9 AU	7.89 %
10	0.92 Rf	0.0 AU	0.97 Rf	206.2 AU	20.47 %	1.00 Rf	10.5 AU	6320.6 AU	19.03 %

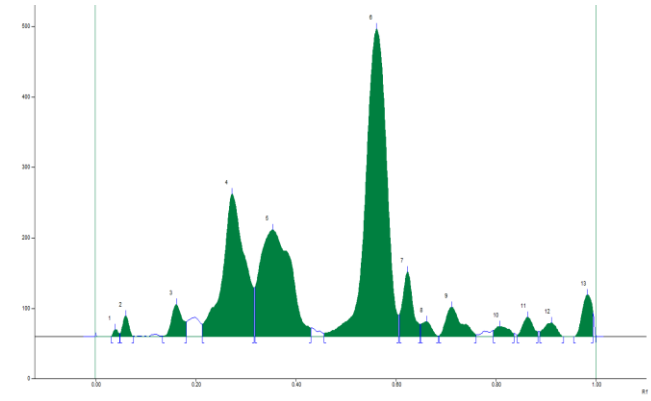
Figure 3. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Mettur) under UV 254 nm.



Track 2, ID: C procumbens Hexane extract Pudukottai

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.12 Rf	0.7 AU	0.15 Rf	15.7 AU	1.38 %	0.16 Rf	9.1 AU	262.3 AU	0.68 %
2	0.19 Rf	9.7 AU	0.24 Rf	184.3 AU	16.28 %	0.27 Rf	67.1 AU	5390.5 AU	13.98 %
3	0.27 Rf	67.5 AU	0.29 Rf	96.3 AU	8.51 %	0.30 Rf	83.7 AU	2057.1 AU	5.34 %
4	0.30 Rf	84.4 AU	0.31 Rf	86.3 AU	7.63 %	0.37 Rf	13.7 AU	3150.1 AU	8.17 %
5	0.37 Rf	14.0 AU	0.38 Rf	15.2 AU	1.34 %	0.42 Rf	1.1 AU	350.1 AU	0.91 %
6	0.48 Rf	15.5 AU	0.53 Rf	236.0 AU	20.85 %	0.60 Rf	15.4 AU	9833.8 AU	25.51 %
7	0.61 Rf	16.5 AU	0.63 Rf	22.1 AU	1.95 %	0.65 Rf	9.9 AU	598.7 AU	1.55 %
8	0.65 Rf	9.9 AU	0.68 Rf	127.2 AU	11.23 %	0.70 Rf	69.1 AU	3209.7 AU	8.32 %
9	0.70 Rf	69.1 AU	0.75 Rf	151.3 AU	13.37 %	0.81 Rf	10.2 AU	7328.2 AU	19.01 %
10	0.82 Rf	10.4 AU	0.84 Rf	54.8 AU	4.84 %	0.88 Rf	15.5 AU	1618.4 AU	4.20 %
11	0.88 Rf	15.6 AU	0.88 Rf	16.3 AU	1.44 %	0.92 Rf	0.0 AU	383.0 AU	0.99 %
12	0.93 Rf	0.5 AU	0.98 Rf	126.4 AU	11.17 %	1.00 Rf	37.9 AU	4374.3 AU	11.35 %

Figure 4. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Pudukottai) under UV254 nm.



Track 3, ID: C procumbens Hexane extract Sri Villiputthur

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.3 AU	0.04 Rf	10.1 AU	0.88 %	0.05 Rf	4.0 AU	92.3 AU	0.22 %
2	0.05 Rf	4.8 AU	0.06 Rf	29.3 AU	2.55 %	0.08 Rf	0.8 AU	336.8 AU	0.81 %
3	0.13 Rf	0.9 AU	0.16 Rf	45.6 AU	3.97 %	0.18 Rf	20.9 AU	920.5 AU	2.22 %
4	0.21 Rf	17.4 AU	0.27 Rf	202.0 AU	17.58 %	0.32 Rf	68.9 AU	8418.9 AU	20.32 %
5	0.32 Rf	69.9 AU	0.35 Rf	151.3 AU	13.17 %	0.43 Rf	12.1 AU	8771.1 AU	21.17 %
6	0.46 Rf	4.4 AU	0.56 Rf	436.1 AU	37.95 %	0.61 Rf	30.2 AU	17023.1 AU	41.08 %
7	0.61 Rf	30.8 AU	0.62 Rf	91.8 AU	7.99 %	0.65 Rf	17.5 AU	1781.9 AU	4.30 %
8	0.65 Rf	17.7 AU	0.66 Rf	21.3 AU	1.86 %	0.69 Rf	0.8 AU	406.3 AU	0.98 %
9	0.69 Rf	1.3 AU	0.71 Rf	41.8 AU	3.63 %	0.76 Rf	3.0 AU	1230.0 AU	2.97 %
10	0.80 Rf	8.5 AU	0.81 Rf	14.0 AU	1.22 %	0.84 Rf	4.3 AU	379.0 AU	0.91 %
11	0.84 Rf	2.1 AU	0.86 Rf	27.5 AU	2.39 %	0.88 Rf	7.3 AU	539.0 AU	1.30 %
12	0.89 Rf	6.8 AU	0.91 Rf	19.3 AU	1.68 %	0.94 Rf	0.3 AU	456.5 AU	1.10 %
13	0.96 Rf	0.0 AU	0.98 Rf	59.0 AU	5.14 %	1.00 Rf	33.9 AU	1078.6 AU	2.60 %

Figure 5. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Srivilliputhur) under UV254 nm

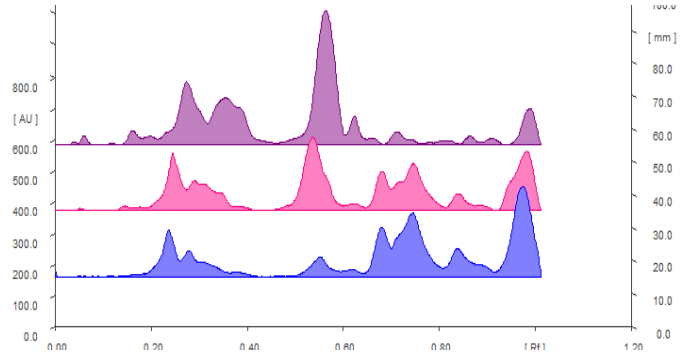
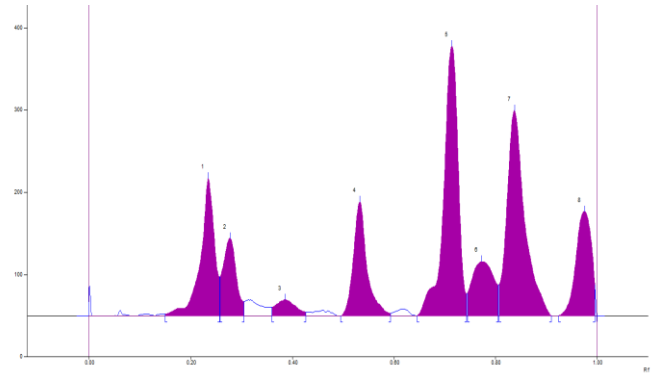


Figure 6. 3D Chromatogram of 10 µl of hexane extracts of *C. procumbens* whole plant under 254 nm. A. Mettur; B.Pudukottai; C. Srivilliputhur

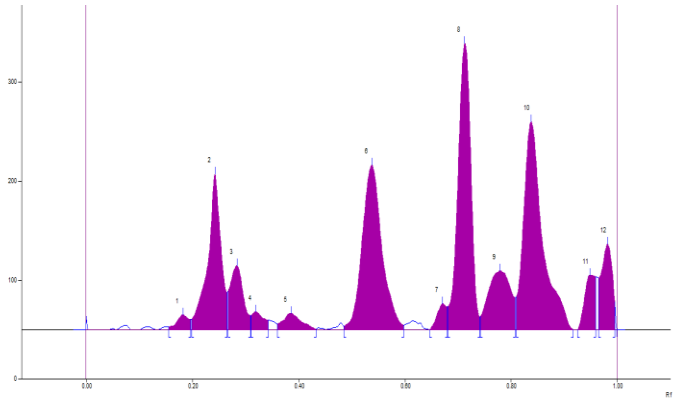
The HPTLC finger print profile of Srivilliputhur sample at UV 366 (Fig. 9) showed twelve peaks among which the peak at R_f 0.84 (22.43%), 0.71 (20.97 %), 0.54 (18.52 %) and 0.24 (11.71 %) are the major peaks. Other peaks appeared at R_f 0.78 (7.55 %), 0.95 (3.32 %) and 0.39 (1.70 %), 0.67 (1.40 %), 0.32 (1.19 %) and 0.18 (1.03 %). The peak at R_f 0.98 (5.38 %) has not been taken into account since it is also near the solvent front. The 3D Chromatogram of all three samples under UV 366 nm is shown in Fig.10.



Track 1, ID: C procumbens Hexane extract Mettur

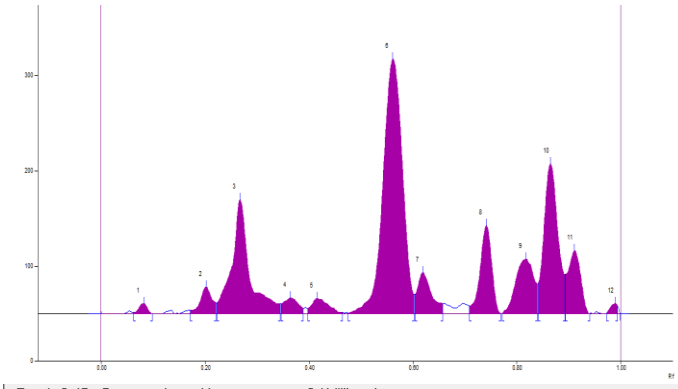
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.15 Rf	2.2 AU	0.24 Rf	167.3 AU	14.05 %	0.26 Rf	47.2 AU	4329.7 AU	12.56 %
2	0.26 Rf	47.8 AU	0.28 Rf	94.4 AU	7.93 %	0.30 Rf	17.1 AU	2297.9 AU	6.67 %
3	0.36 Rf	10.6 AU	0.39 Rf	19.7 AU	1.66 %	0.43 Rf	4.4 AU	730.5 AU	2.12 %
4	0.50 Rf	0.1 AU	0.53 Rf	138.8 AU	11.65 %	0.59 Rf	3.0 AU	3505.1 AU	10.17 %
5	0.65 Rf	0.2 AU	0.71 Rf	328.2 AU	27.56 %	0.74 Rf	26.7 AU	8947.4 AU	25.96 %
6	0.75 Rf	27.5 AU	0.77 Rf	66.0 AU	5.54 %	0.81 Rf	37.2 AU	2687.9 AU	7.80 %
7	0.81 Rf	37.8 AU	0.84 Rf	249.5 AU	20.95 %	0.91 Rf	0.3 AU	8312.1 AU	24.12 %
8	0.93 Rf	0.1 AU	0.98 Rf	127.0 AU	10.66 %	1.00 Rf	38.0 AU	3651.6 AU	10.60 %

Figure 7. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Mettur) under 366 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.16 Rf	3.1 AU	0.18 Rf	15.4 AU	1.32 %	0.20 Rf	10.7 AU	329.5 AU	1.03 %
2	0.20 Rf	10.8 AU	0.24 Rf	157.7 AU	13.51 %	0.27 Rf	37.6 AU	3732.4 AU	11.71 %
3	0.27 Rf	38.3 AU	0.28 Rf	64.7 AU	5.54 %	0.31 Rf	14.5 AU	1527.2 AU	4.79 %
4	0.31 Rf	14.7 AU	0.32 Rf	18.1 AU	1.55 %	0.34 Rf	9.5 AU	379.8 AU	1.19 %
5	0.36 Rf	6.2 AU	0.39 Rf	16.9 AU	1.45 %	0.43 Rf	0.5 AU	540.7 AU	1.70 %
6	0.49 Rf	3.7 AU	0.54 Rf	166.4 AU	14.25 %	0.60 Rf	4.5 AU	5901.6 AU	18.52 %
7	0.65 Rf	0.1 AU	0.67 Rf	26.6 AU	2.28 %	0.68 Rf	23.1 AU	447.3 AU	1.40 %
8	0.68 Rf	23.4 AU	0.71 Rf	289.6 AU	24.81 %	0.74 Rf	13.1 AU	6682.1 AU	20.97 %
9	0.74 Rf	13.3 AU	0.78 Rf	59.7 AU	5.12 %	0.81 Rf	32.6 AU	2405.0 AU	7.55 %
10	0.81 Rf	32.9 AU	0.84 Rf	210.2 AU	18.01 %	0.92 Rf	0.2 AU	7146.6 AU	22.43 %
11	0.93 Rf	0.3 AU	0.95 Rf	55.4 AU	4.74 %	0.96 Rf	53.7 AU	1058.7 AU	3.32 %
12	0.97 Rf	52.2 AU	0.98 Rf	86.6 AU	7.42 %	1.00 Rf	27.8 AU	1714.7 AU	5.38 %

Figure 8. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Pudukottai) under 366 nm



Track 3, ID: C procumbens Hexane extract Sri Villiputthur

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.06 Rf	1.5 AU	0.08 Rf	10.9 AU	1.23 %	0.10 Rf	0.1 AU	163.0 AU	0.63 %
2	0.17 Rf	3.5 AU	0.20 Rf	28.1 AU	3.17 %	0.22 Rf	11.5 AU	606.0 AU	2.35 %
3	0.22 Rf	11.6 AU	0.27 Rf	120.0 AU	13.52 %	0.35 Rf	10.2 AU	3907.0 AU	15.14 %
4	0.35 Rf	10.2 AU	0.36 Rf	16.8 AU	1.89 %	0.39 Rf	5.4 AU	445.4 AU	1.73 %
5	0.40 Rf	5.5 AU	0.42 Rf	16.0 AU	1.80 %	0.47 Rf	1.2 AU	494.9 AU	1.92 %
6	0.48 Rf	0.7 AU	0.56 Rf	267.8 AU	30.17 %	0.60 Rf	20.0 AU	9523.9 AU	36.92 %
7	0.60 Rf	20.1 AU	0.62 Rf	43.3 AU	4.87 %	0.66 Rf	10.8 AU	1116.4 AU	4.33 %
8	0.71 Rf	8.3 AU	0.74 Rf	92.8 AU	10.46 %	0.77 Rf	0.2 AU	1959.2 AU	7.59 %
9	0.77 Rf	0.0 AU	0.82 Rf	57.2 AU	6.44 %	0.84 Rf	31.2 AU	1906.2 AU	7.39 %
10	0.84 Rf	31.7 AU	0.87 Rf	157.9 AU	17.79 %	0.89 Rf	41.1 AU	4023.0 AU	15.59 %
11	0.89 Rf	41.1 AU	0.91 Rf	66.5 AU	7.49 %	0.94 Rf	0.1 AU	1520.3 AU	5.89 %
12	0.97 Rf	0.5 AU	0.99 Rf	10.4 AU	1.17 %	1.00 Rf	8.4 AU	132.2 AU	0.51 %

Figure 9. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Srivilliputhur) under 366 nm.

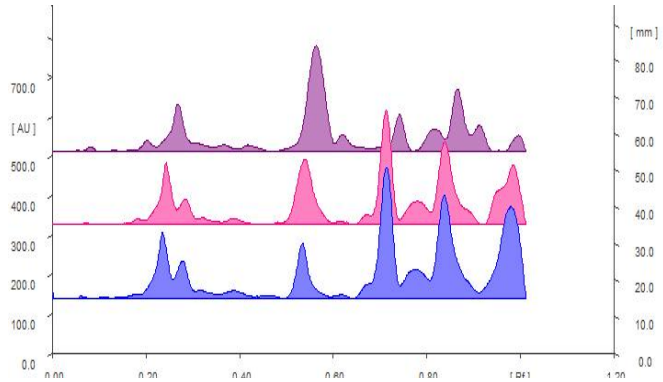
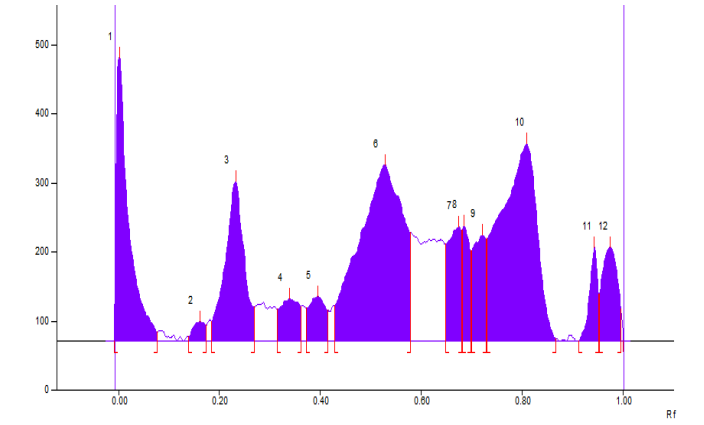


Figure 10. 3D Chromatogram of 10 µl of hexane extract of *C. procumbens* whole plant under 366 nm. A. Mettur; B.Pudukottai; C. Srivilliputhur.

The HPTLC finger print profile of Mettur sample after derivatization with vanillin-sulphuric acid (Fig. 11) showed twelve peaks among which the peak at R_f 0.53 (26.56 %), 0.81 (24.71 %) and 0.23 (10.31 %) are the major peaks. Other peaks appeared at R_f 0.67 (5.3 %), 0.72 (4.60 %), 0.69 (2.91 %), 0.34 (2.73 %), 0.94 (2.53 %), 0.40 (2.49 %) and 0.16 (0.81 %). The peaks at R_f 0.00 and 0.97 have not been taken into account since first one is at loading position and latter one is near the solvent front. The HPTLC finger print profile of Pudukottai sample after derivatization with vanillin-sulphuric acid (Fig. 12) showed thirteen peaks among which the peak at R_f 0.54 (24.80 %), 0.81 (18.34 %) and 0.25 (12.91 %) are the major peaks. Other peaks appeared at R_f 0.69 (8.95 %), 0.57 (8.72 %), 0.30 (4.28 %),

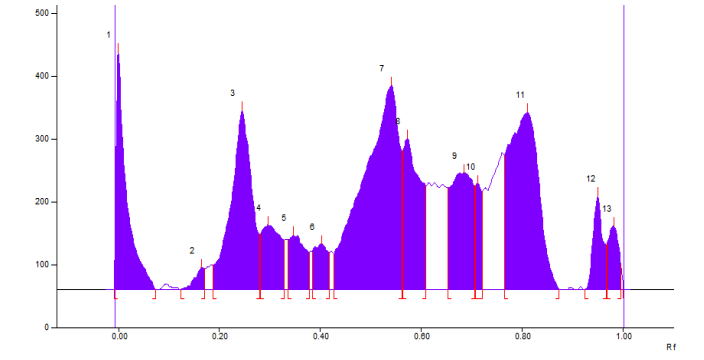
0.95 (3.04 %), 0.71 (2.27 %), 0.40 (2.23 %) and 0.17 (0.73 %). The peak at R_f 0.98 (2.28 %) has not been taken into account since it is also near the solvent front and the peak at loadking position has also not been considered.



Track 1, ID: C procumbens Hexane extract Mettur

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	0.00 Rf	410.5 AU	19.62 %	0.08 Rf	13.7 AU	9578.3 AU	12.18 %
2	0.14 Rf	6.3 AU	0.16 Rf	27.7 AU	1.33 %	0.17 Rf	23.1 AU	633.6 AU	0.81 %
3	0.18 Rf	28.8 AU	0.23 Rf	230.9 AU	11.04 %	0.27 Rf	49.0 AU	8109.4 AU	10.31 %
4	0.32 Rf	46.4 AU	0.34 Rf	61.9 AU	2.96 %	0.36 Rf	50.4 AU	2145.9 AU	2.73 %
5	0.37 Rf	47.6 AU	0.40 Rf	64.4 AU	3.08 %	0.41 Rf	43.9 AU	1961.8 AU	2.49 %
6	0.43 Rf	50.7 AU	0.53 Rf	255.3 AU	12.20 %	0.58 Rf	56.4 AU	20884.9 AU	26.56 %
7	0.65 Rf	140.5 AU	0.67 Rf	164.9 AU	7.88 %	0.68 Rf	59.9 AU	4169.0 AU	5.30 %
8	0.68 Rf	161.1 AU	0.69 Rf	166.7 AU	7.97 %	0.70 Rf	30.4 AU	2285.6 AU	2.91 %
9	0.70 Rf	130.9 AU	0.72 Rf	152.9 AU	7.31 %	0.73 Rf	47.7 AU	3616.0 AU	4.60 %
10	0.73 Rf	148.1 AU	0.81 Rf	285.1 AU	13.63 %	0.87 Rf	3.8 AU	19435.8 AU	24.71 %
11	0.91 Rf	0.7 AU	0.94 Rf	136.1 AU	6.50 %	0.95 Rf	67.6 AU	1986.4 AU	2.53 %
12	0.95 Rf	68.3 AU	0.97 Rf	135.7 AU	6.49 %	1.00 Rf	46.3 AU	3836.0 AU	4.88 %

Figure 11. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Mettur) at 575 nm.

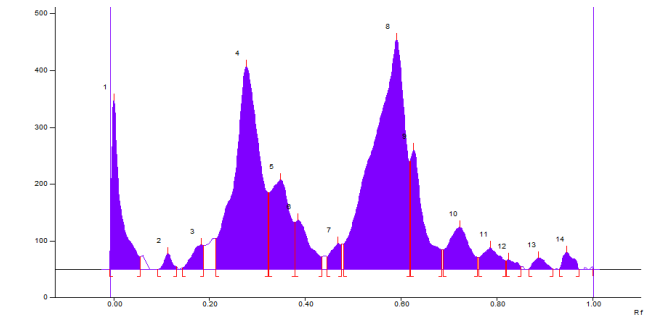


Track 2, ID: C procumbens Hexane extract Pudukottai

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	0.00 Rf	377.6 AU	15.68 %	0.07 Rf	0.4 AU	7253.5 AU	8.38 %
2	0.12 Rf	0.0 AU	0.17 Rf	35.1 AU	1.46 %	0.17 Rf	32.7 AU	632.7 AU	0.73 %
3	0.19 Rf	38.6 AU	0.25 Rf	285.3 AU	11.85 %	0.28 Rf	88.4 AU	11173.3 AU	12.91 %
4	0.28 Rf	88.5 AU	0.30 Rf	102.2 AU	4.24 %	0.33 Rf	78.6 AU	3705.6 AU	4.28 %
5	0.34 Rf	79.2 AU	0.35 Rf	86.5 AU	3.59 %	0.38 Rf	59.2 AU	2650.3 AU	3.06 %
6	0.38 Rf	59.9 AU	0.40 Rf	72.0 AU	2.99 %	0.42 Rf	59.4 AU	1927.3 AU	2.23 %
7	0.43 Rf	57.1 AU	0.54 Rf	323.7 AU	13.44 %	0.56 Rf	19.3 AU	21463.3 AU	24.80 %
8	0.56 Rf	220.0 AU	0.57 Rf	239.5 AU	9.95 %	0.61 Rf	65.1 AU	7543.6 AU	8.72 %
9	0.65 Rf	161.4 AU	0.69 Rf	185.8 AU	7.72 %	0.71 Rf	64.2 AU	7745.0 AU	8.95 %
10	0.71 Rf	164.8 AU	0.71 Rf	168.2 AU	6.99 %	0.72 Rf	56.0 AU	1965.1 AU	2.27 %
11	0.76 Rf	212.8 AU	0.81 Rf	281.5 AU	11.69 %	0.87 Rf	0.3 AU	15875.6 AU	18.34 %
12	0.93 Rf	0.6 AU	0.95 Rf	148.8 AU	6.18 %	0.97 Rf	71.2 AU	2630.4 AU	3.04 %
13	0.97 Rf	71.7 AU	0.98 Rf	101.6 AU	4.22 %	1.00 Rf	38.9 AU	1973.8 AU	2.28 %

Figure 12. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Pudukottai) at 575 nm.

The HPTLC finger print profile of Srivilliputhur sample after derivatization with vanillin-sulphuric acid (Fig. 13) showed fourten peaks among which the peak at R_f 0.59 (35.97 %) and 0.28 (25.22 %) are the major peaks. Other peaks appeared at R_f 0.35 (8.47 %), 0.63 (7.69 %), 0.72 (4.30 %), 0.39 (3.53 %), 0.79 (1.84 %), 0.13 (1.43 %), 0.47 (1.37 %),0.95 (0.89 %), 0.89 (0.69 %), 0.11 (0.57 %) and 0.82 (0.47 %). The peak at loading point has not been considered. The 3D chromatogram of all three samples at 575 nm is shown in Fig. 14.



Track 3, ID: C procumbens Hexane extract Sri Villiputhur

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.0 AU	0.00 Rf	297.8 AU	16.45 %	0.06 Rf	21.8 AU	5239.8 AU	7.58 %
2	0.09 Rf	0.2 AU	0.11 Rf	27.5 AU	1.52 %	0.13 Rf	4.9 AU	397.2 AU	0.57 %
3	0.14 Rf	2.5 AU	0.18 Rf	43.3 AU	2.39 %	0.19 Rf	41.8 AU	986.2 AU	1.43 %
4	0.21 Rf	54.1 AU	0.28 Rf	357.3 AU	19.74 %	0.32 Rf	35.6 AU	17446.4 AU	25.22 %
5	0.32 Rf	135.6 AU	0.35 Rf	157.5 AU	8.70 %	0.38 Rf	82.4 AU	5861.0 AU	8.47 %
6	0.38 Rf	82.5 AU	0.39 Rf	86.9 AU	4.80 %	0.43 Rf	22.3 AU	2434.7 AU	3.52 %
7	0.45 Rf	22.7 AU	0.47 Rf	45.6 AU	2.52 %	0.48 Rf	44.3 AU	944.2 AU	1.37 %
8	0.48 Rf	44.5 AU	0.59 Rf	404.4 AU	22.34 %	0.62 Rf	89.5 AU	24882.3 AU	35.97 %
9	0.62 Rf	190.7 AU	0.63 Rf	210.6 AU	11.63 %	0.69 Rf	34.7 AU	5322.3 AU	7.69 %
10	0.69 Rf	34.7 AU	0.72 Rf	74.1 AU	4.09 %	0.76 Rf	21.5 AU	2973.3 AU	4.30 %
11	0.76 Rf	21.1 AU	0.79 Rf	38.1 AU	2.10 %	0.82 Rf	15.3 AU	1270.5 AU	1.84 %
12	0.82 Rf	15.3 AU	0.82 Rf	17.2 AU	0.95 %	0.85 Rf	5.5 AU	323.3 AU	0.47 %
13	0.87 Rf	0.4 AU	0.89 Rf	20.1 AU	1.11 %	0.92 Rf	1.0 AU	475.2 AU	0.69 %
14	0.93 Rf	0.5 AU	0.95 Rf	30.1 AU	1.66 %	0.97 Rf	0.1 AU	612.2 AU	0.89 %

Figure 13. Finger print profile and R_f table of 10 µl of hexane extract of *C. procumbens* whole plant (Srivilliputhur) at 575 nm.

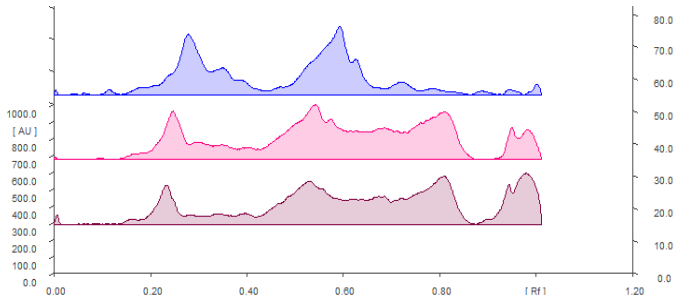


Figure 14. 3D Chromatogram of 10 µl of hexane extract of *C. procumbens* whole plant at 575 nm. A. Mettur; B.Pudukottai; C. Srivilliputhur

Conclusion

From the physico-chemical analysis it is evident that the three samples of *C. procumbens* whole plant have similarities in all parameters except in ash content and water soluble extractives. However, pharmacopoeial standards can be derived from the higher value of ash and lower value of water soluble extractive. From the TLC and HPTLC studies

it is understood that the sample collected from Srivilliputhur is differing from other two samples. Since hexane extract was investigated for chromatographic studies, probably the low polar compounds present in Srivilliputhur sample may not be same like Mettur and Pudukottai samples. Further isolation and characterization study may be conducted for exploring the phytochemicals.

Acknowledgement

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