WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 8, 545-556 Research Article ISSN 2278 – 4357

A COMPARISON OF PHYSICO-CHEMICAL AND HPTLC STUDIES ON LEAVES AND FRUITS OF *CADABA FRUTICOSA* (L.) DRUCE

Sakkeena A.[1], Anitha John*[2], Gayathri Devi V.[2], Manju K.C.[3], Neethu Kannan B.[4], Kanagarajan A.[5]

[1]Senior Research Fellow (Chemistry), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

[2]Research Officer (Chemistry), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

[3]Senior Research Fellow (Botany), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

[4]Research Assistant (Botany), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

[5]Assistant Director (Siddha), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala.

Article Received on
25 May 2018,

Revised on 15 June 2018,
Accepted on 05 July 2018

DOI: 10.20959/wjpps20188-12045

***Corresponding Author**
Anitha John
Research Officer (Chemistry), Siddha Regional Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

ABSTRACT

Cadaba fruticosa (L.) Druce (Family- Capparaceae**)** is a commonly used plant in Siddha, Ayurveda and other Indian traditional systems of medicine and is considered as an important remedy for the treatment of several diseases. *Cadaba* is a shrub that grows up to 5m in height and widely distributed in the Indian subcontinent especially in scrub jungles and rocky areas. The aim of the present work is the comparitive study of the physico-chemical parameters and HPTLC fingerprinting of leaf and fruit of *C. fruticosa*. The physico–chemical parameters such as water soluble extractive, alcohol soluble extractive, moisture content, total ash, acid insoluble ash and volatile oil were carried out according to standard methods. HPTLC studies of leaf and fruit of *C. fruticosa* were conducted at 254 nm, 366 nm and 575 nm after derivatisation using vanillin- sulphuric acid. The physic-chemical parameters indicated that the fruit is having more solubility and moisture content than the leaf of *Cadaba fruticosa*. HPTLC finger print of the leaf and fruit of *Cadaba fruticosa* were documented in visible, UV

short and UV long light and they were observed in different pattern. The physico-chemical parameters and the HPTLC fingerprinting profile helped to determine the quality and purity of the leaf and fruit of *Cadaba fruticosa*. These findings may provide useful information with regard to its physico- chemical parameters and HPTLC finger printing studies in future.

KEYWORDS: *Cadaba fruticosa*, Capparaceae, physico-chemical, HPTLC finger printing.

INTRODUCTION

Cadaba fruticosa (L.) Druce or the Indian Cadaba is a climbing shrub belonging to the Capparaceae family. The shrub is widely distributed in the Indian subcontinent, commonly seen in scrub Jungles and Rocky areas. *Cadaba fruticosa* has been used to treat various diseases since time immemorial. It is commonly used in Siddha medicine in the northern districts of Tamilnadu. Leaf juice is used as a remedy for dysentery, stimulant, purgative, fever, and cough and lungs problem (Watt et al., 1962). It was reported to possess stachydrine, 3-hydroxystachydrine from the stem, roots and cadabine from leaves (Uddin, 1975; Yousif, 1984) Cadabalone, cadabaicine were isolated from the leaf (Chattopadhyay, 2002). Leaves are reported to possess antimicrobial activity (Arokiyaraj, 2008) anti pyretic activity (Mythreyi, 2008), anti-diabetic activity (Arokiyaraj, 2008). Leaf aqueous extract study revealed the presence of terpenoids, flavones, proteins, furans; anthraquinones and sugars. Alcoholic extract possesses steroids, alkaloids, gums and saponins (Arokiyaraj, 2008). Therefore, the presence of these biologically active components in *C. fruticosa* may be a reason for their bioactivities. The leaves and fruits are used to treat worm infestation, swellings, eczema and constipation (Arokiyaraj, 2008; Sankaranarayanan *et al*. 2010). Ganesan et al. (2005) reported that leaf paste mixed with castor oil is applied for bone fracture in human being and livestock.

C. fruticosa is a shrub with a height up to 5 m. Flower solitary or raceme. Sepals 4, unequal in two whorls, outer 2 valvate. Petals 4 or 2, clawed, hypogynous. Disk large, coloured, encircling the gynophore with its tubular stalk and expanded trumpet wise at the top or spathulate. Stamens 4-6 inserted unilaterally on the slender gynophore. Ovary1-celled; stigma sessile; ovules many on 2-4 parietal placentas. Fruit a fleshy slender cylindrical berry or sometimes dehiscing ultimately by two valves which fall away from placentas. Seeds globose; testa horny; cotyledons convolute.

Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agents as well as new sources of economic materials like oil and gums. Chromatographic and spectral fingerprint analysis plays an important role in the quality control of complex herbal medicines. Thin layer chromatography (TLC) is the preliminary step to identify the phytochemical constituents in a sample. High performance thin layer chromatography (HPTLC) can provide an electronic image of the chromatographic fingerprint and a densitogram to detect the presence of various compounds in a plant sample. Both the techniques are efficient, faster, reliable and reproducible (Gong et al., 2005). The present study aims at the comparative study of physicochemical and HPTLC fingerprinting of *C. fruticosa* leaf and fruit.

MATERIALS AND METHODS

Plant Material

The fresh leaves and fruits of *C. fruticosa* were collected, dried and supplied by Siddha Medicinal Plants Garden, Mettur Dam. The plant materials were chopped, crushed and kept in airtight containers and used for all experimental purposes.

Physico-chemical parameters

The physico-chemical parameters like determination of ash content, acid insoluble ash, volatile oil, solubility in water and alcohol and loss on drying, were carried out by standard methods (SPI, 2011; WHO guidelines, 1998).

Total ash

A silica crucible is ignited, cooled and weighed. Take about 2 g of powdered drug in the crucible and accurately weighed. Incinerate the drug until free from carbon, cooled and weighed. The percentage of total ash has been calculated.

Acid insoluble ash

Total ash was taken in the crucible and 25 ml 6N Hydrochloric acid was added and boiled for five minutes. Filtered through an ash less filter paper. Further washed with hot water until the filtrate is free from acid. Transferred the filter paper containing the insoluble matter into the same crucible and ignited to constant weight.

Loss on drying

A 100 ml beaker is accurately weighed. About 4g of powdered drug was taken in the beaker. The sample was placed in Hot air oven at 105˚C for 5hrs and weighed. The process was continued until constant weight is obtained.

Water soluble and Alcohol soluble extractive

For both water and alcohol soluble extractive 4 g of powdered drug in each was taken and accurately weighed. Transferred it in a glass stoppered conical flask and 100 ml distilled water and alcohol was added. After 18 hours with occasional shaking filtered the sample and 25 ml was pippeted out from the filtrate. Kept it in air oven at 105˚C for 6 hours and further cooled and calculated the percentage of water and alcohol soluble extractive (Uma et al., 2010).

Volatile oil

20 g powdered drug was taken in a 1 liter round bottom flask. The flask is connected to a Clevenger apparatus. The contents of the flask are heated and boiled for 2 hrs or until distillation is completed. The percentage of volatile oil is calculated from the volume of oil collected.

Preparation of extract of the drug material for HPTLC analysis

4 g each of the powder of leaf and fruit of *C. fruticosa* were soaked in separate conical flasks containing 40 ml chloroform at room temperature for overnight. The contents were filtered through filter paper and the filtrates were then concentrated on a water bath to 1 ml. These extracts were used for chromatographic studies (Wagner & Bladt, 1996).

High performance thin layer chromatography (HPTLC)

HPTLC is an analytical separation and determination method which has a wide application in herbal drug analysis. Chloroform extracts of the plant materials were spotted in the form of bands using Camag microliter syringe on precoated silica gel 60 F_{254} (Merck) plates with Automatic TLC Sampler 4 (ATS4) separately. Mobile phase used was Toluene: Ethyl acetate (5: 0.4) for leaf and Toluene: Ethyl acetate: Formic acid (5:0.4: 0.1) for fruit. Linear ascending developments were done in twin trough glass chambers saturated with each mobile phase. The plates were air dried and kept under UV 254 nm and 366 nm, and derivatised using vanillin-sulphuric acid reagent and photo documentations were done. The plates were scanned in UV 254 nm, 366nm and in white light (575nm) after derivatisation using TLC

Scanner 4 with winCATS software for interpretation of data (Wagner et al., 1996; Harborne, 1998).

RESULTS AND DISCUSSION

Physico- chemical analysis

The results of the physico- chemical analysis are represented in Table 1.Total ash value of the material indicated the amount of minerals and earthy material attached to the plant material. Acid insoluble ash usually represents the amount of silica present as sand and dust. Loss on drying at 105°C shows the presence of moisture content and volatile oil (if any) present in the drug. The water soluble extractive value indicates the presence of polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids etc.

The loss on drying of leaf and fruit of *C. fruticosa* are 11.25% and 17.1% respectively. The moisture retaining capacity of fruit is more when compared to the leaf. The total ash value of leaf and fruit are 12.92 % and 5.43 % respectively. The acid insoluble ash of leaf and fruit are 1.61% and 0.86% respectively. The water soluble extractive values of leaf (31.86%) and fruit (36.98%) indicate the possible presence of high polar compounds in the materials. The ethanol soluble extractive values are 10.54 % and 19.61 % respectively. The *C. fruticosa* leaf and fruit do not contain volatile oil. The physico-chemical parameters of leaf and fruit of *C. fruticosa* indicate that there are differences in ash content, moisture content and the solubility in water and alcohol.

Table I: Comparison of the physico-chemical parameters of leaf and fruit of *C. fruticosa.*

Sl no.	Physico-chemical parameters	Leaf (%w/w)	Fruit (%w/w)
1	Total ash	12.92	5.43
2	Acid insoluble ash	1.61	0.86
3	Loss on drying	11.25	17.1
4	Water soluble extractive	31.86	36.98
5	Alcohol soluble extractive	10.54	19.61
6	Volatile oil	Nil	Nil

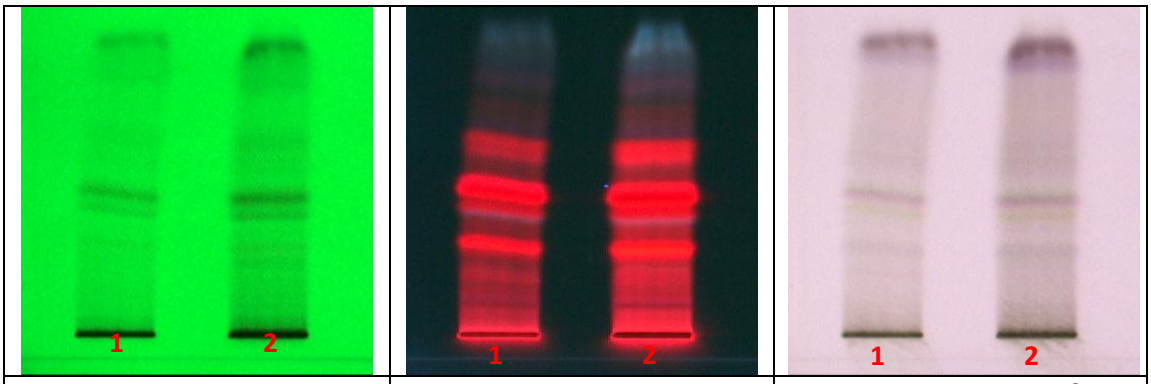
High performance thin layer chromatographic analysis (HPTLC)

Most of the available monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of

active constituents in the plant material may be useful for proper standardization of herbals and its formulations.

High-performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. HPTLC also facilitates repeated detection of chromatogram with same or different parameters.

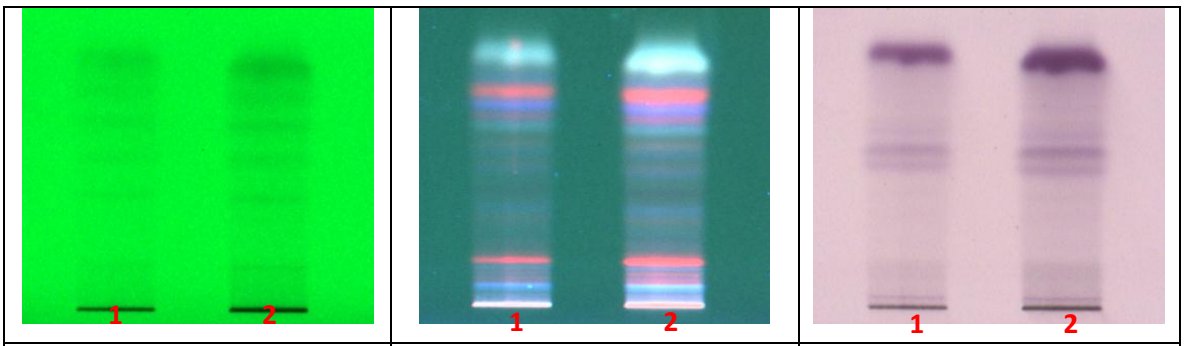
The HPTLC fingerprinting patterns of chloroform extracts of the leaf and fruit of *C. fruticosa* were developed at 254nm, 366nm and after derivatisation with vanillin – sulphuric acid at 575nm (Fig. 1; Fig. 2).



Under UV 254	Under UV 366	Under white light after derivatisation

Track 1: 2µl; Track 2: 4µl
Solvent system - Toluene: Ethyl acetate (5: 0.4)

Fig. 1: HPTLC photo documentation profile of the chloroform extract of leaf of *C. fruticosa*.



Under UV 254	Under UV 366	Under white light after derivatisation

Track 1: 2µl; Track 2 : 4µl
Solvent system - Toluene: Ethyl acetate: Formic acid (5:0.4: 0.1)

Fig. 2: HPTLC photo documentation profile of the chloroform extract of fruit of *C. fruticosa*.

R_f values and colour of spots of chloroform extract under UV 254, UV 366 and 575 nm were represented in the table 2 and 3 denote the total number of bands obtained with their characteristics colours. The present study showed the development of dark green, light purple, red and light yellow bands for the chloroform extract of the *C. fruiticosa* leaves suggesting the presence of a pool of secondary metabolites. Similarly, the *C. fruiticosa* fruits also showed prominent bands of green, purple and blue colour.

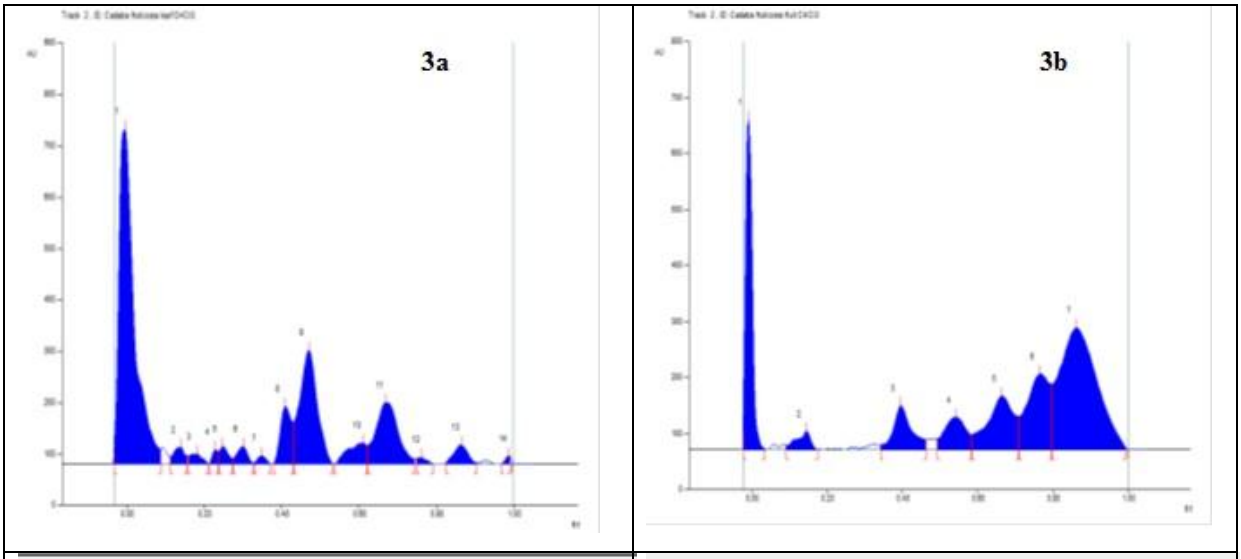
Table II: R_f values and colour of spots of chloroform extract of *C. fruticosa* leaf.

Sl No.	*C. fruticosa* leaf					
	UV 254nm		UV 366 nm		575 nm	
	Rf	colour	Rf	colour	Rf	colour
1	0.39	Dark green	0.13	Red	0.29	Light purple
2	0.48	Dark green	0.22	Red	0.45	Light purple
3	0.67	Light green	0.31	Red	0.62	Light yellow
4	0.74	Light green	0.52	Red	0.65	Light purple
5	0.86	Light green	0.63	Red	0.74	Light purple
6	0.97	Dark green	0.87	Red	0.95	Light purple

Table III: R_f and colour of spots of chloroform extract of *C. fruticosa* fruit.

S NO	*C. fruticosa* fruit					
	UV 254nm		UV 366 nm		UV 366 nm	
	R_f	Colour	R_f	Colour	R_f	Colour
1	0.16	Light green	0.07	Light blue	0.35	Light purple
2	0.41	Light green	0.17	Pink	0.51	Light purple
3	0.56	Dark green	0.64	Light green	0.57	Light purple
4	0.67	Dark green	0.72	Purple	0.63	Light purple
5	0.79	Dark green	0.79	Purple	0.75	Light purple
6	0.92	Dark green	0.93	Light blue	0.92	Light purple

The HPTLC finger print profile of leaf of *C. fruticosa* at UV 254 (Fig. 3(a)) showed fourteen peaks among which the peak at R_f 0.47 is the major peak with an area of 18.40% followed by the peak at R_f 0.41, 0.99 with areas of 5.97% and 0.35% respectively. Other peaks appeared at R_f 0.14,0.18,0.23,0.25,0.30,0.35,0.61,0.67,0.76,0.87 with area of 1.75%, 1.17%, 0.73%, 1.32%, 1.74% 0.64%,3.75%,12.71%,0.55% and 2.52%. The HPTLC finger print profile of fruit *of C. fruticosa* at UV 254 (Fig.3(b)) showed seven peaks among which the peak at R_f 0.77 is the major peak with an area of 14.75 % followed by the peak at R_f 0.55, 0.67with areas of 6.08 % and 11.97 % respectively. Other peaks appeared at R_f 0.01, 0.15 and 0.40 with areas of 19.22 %, 2.26 % and 6.83 %. The R_f value with major peak area percentage may corresponds to the predominant compound present in the plant materials.



Track 2, ID: Cadaba fruticosa leaf CHCl3

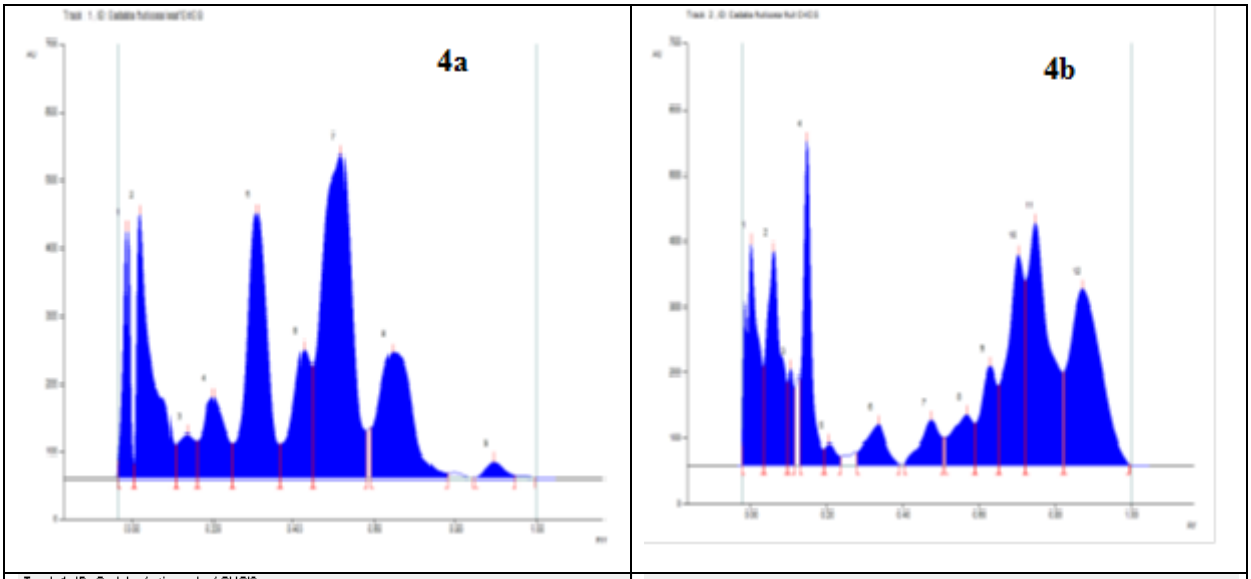
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	19.9 AU	-0.00 Rf	652.4 AU	47.50 %	0.09 Rf	29.2 AU	18160.0 AU	48.42 %
2	0.11 Rf	14.0 AU	0.14 Rf	32.4 AU	2.36 %	0.16 Rf	15.9 AU	655.5 AU	1.75 %
3	0.16 Rf	15.5 AU	0.18 Rf	18.9 AU	1.37 %	0.21 Rf	0.5 AU	437.8 AU	1.17 %
4	0.22 Rf	0.6 AU	0.23 Rf	27.5 AU	2.00 %	0.24 Rf	24.3 AU	274.6 AU	0.73 %
5	0.24 Rf	24.9 AU	0.25 Rf	33.4 AU	2.43 %	0.27 Rf	10.4 AU	493.7 AU	1.32 %
6	0.28 Rf	10.6 AU	0.30 Rf	33.9 AU	2.47 %	0.33 Rf	2.2 AU	651.0 AU	1.74 %
7	0.33 Rf	2.6 AU	0.35 Rf	15.4 AU	1.12 %	0.37 Rf	0.1 AU	238.9 AU	0.64 %
8	0.38 Rf	0.5 AU	0.41 Rf	112.1 AU	8.16 %	0.43 Rf	82.2 AU	2237.2 AU	5.97 %
9	0.43 Rf	82.4 AU	0.47 Rf	222.2 AU	16.17 %	0.53 Rf	0.4 AU	6899.1 AU	18.40 %
10	0.54 Rf	0.3 AU	0.61 Rf	41.0 AU	2.99 %	0.62 Rf	35.8 AU	1406.9 AU	3.75 %
11	0.63 Rf	36.3 AU	0.67 Rf	119.6 AU	8.71 %	0.75 Rf	10.1 AU	4765.5 AU	12.71 %
12	0.75 Rf	10.9 AU	0.76 Rf	12.6 AU	0.92 %	0.79 Rf	0.3 AU	206.5 AU	0.55 %
13	0.82 Rf	2.6 AU	0.87 Rf	36.8 AU	2.68 %	0.90 Rf	2.3 AU	944.5 AU	2.52 %
14	0.97 Rf	0.3 AU	0.99 Rf	15.5 AU	1.13 %	0.99 Rf	14.4 AU	130.8 AU	0.35 %

Track 2, ID: Cadaba fruticosa fruit CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	34.0 AU	-0.01 Rf	590.0 AU	48.76 %	0.04 Rf	1.5 AU	7404.7 AU	19.22 %
2	0.09 Rf	7.7 AU	0.15 Rf	33.3 AU	2.75 %	0.18 Rf	0.2 AU	872.1 AU	2.26 %
3	0.34 Rf	8.8 AU	0.40 Rf	78.7 AU	6.50 %	0.46 Rf	18.0 AU	2632.3 AU	6.83 %
4	0.49 Rf	19.4 AU	0.55 Rf	58.5 AU	4.83 %	0.58 Rf	26.6 AU	2343.6 AU	6.08 %
5	0.59 Rf	26.8 AU	0.67 Rf	96.7 AU	7.99 %	0.71 Rf	59.6 AU	4612.2 AU	11.97 %
6	0.71 Rf	59.7 AU	0.77 Rf	135.1 AU	11.17 %	0.80 Rf	17.6 AU	5684.0 AU	14.75 %
7	0.80 Rf	117.7 AU	0.86 Rf	217.8 AU	18.00 %	1.00 Rf	4.9 AU	14974.5 AU	38.87 %

Fig. 3: Finger print profile and R_f table of Chloroform extract of Cadaba fruticosa leaf Under 254 nm.

The HPTLC finger print profile of leaf of *Cadaba fruticosa* at UV 366 (Fig. 4(a)) showed nine peaks among which the peak at R_f 0.52 is the major peak with an area of 32.32 % followed by the peaks at R_f 0.31 and 0.64 with areas of 17.52 % and 0.87 % respectively. Other peaks appeared at R_f 0.01, 0.02, 0.14, 0.20 and 0.43 with areas of 4.7 %, 13.85 %, 2.61 %, 5.90 % and 7.99 % respectively. The peak at R_f 0.89 has not been taken into account since it is near the solvent front. The HPTLC finger print profile of fruit *of Cadaba fruticosa* at UV 366 (Fig. 4(b)) showed twelve peaks among which the peak at R_f 0.06 (10.31%), 0.15 (9.41 %), 0.63 (5.51 %), 0.70 (12.99 %) and 0.75 (19.14 %) are the major peaks. Other peaks appeared at R_f 0.11 (2.09 %), 0.21 (0.87 %), 0.34 (3.00 %), 0.48 (3.12 %) and 0.57 (3.75 %) respectively.



Track 1, ID: Cadaba fruticosa leaf CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	26.8 AU	-0.01 Rf	364.9 AU	16.57 %	0.00 Rf	19.6 AU	3510.8 AU	4.70 %
2	0.01 Rf	21.0 AU	0.02 Rf	388.7 AU	17.65 %	0.11 Rf	49.6 AU	10346.8 AU	13.85 %
3	0.11 Rf	49.7 AU	0.14 Rf	64.8 AU	2.94 %	0.16 Rf	55.5 AU	1950.5 AU	2.61 %
4	0.17 Rf	55.6 AU	0.20 Rf	119.4 AU	5.42 %	0.25 Rf	51.3 AU	4404.5 AU	5.90 %
5	0.25 Rf	51.4 AU	0.31 Rf	389.9 AU	17.70 %	0.37 Rf	50.6 AU	13092.4 AU	17.52 %
6	0.37 Rf	50.6 AU	0.43 Rf	188.8 AU	8.57 %	0.45 Rf	65.8 AU	5972.3 AU	7.99 %
7	0.45 Rf	166.1 AU	0.52 Rf	476.4 AU	21.63 %	0.58 Rf	68.6 AU	24149.6 AU	32.32 %
8	0.59 Rf	73.2 AU	0.64 Rf	185.3 AU	8.41 %	0.78 Rf	6.9 AU	10490.6 AU	14.04 %
9	0.85 Rf	0.3 AU	0.89 Rf	24.3 AU	1.10 %	0.95 Rf	3.3 AU	797.4 AU	1.07 %

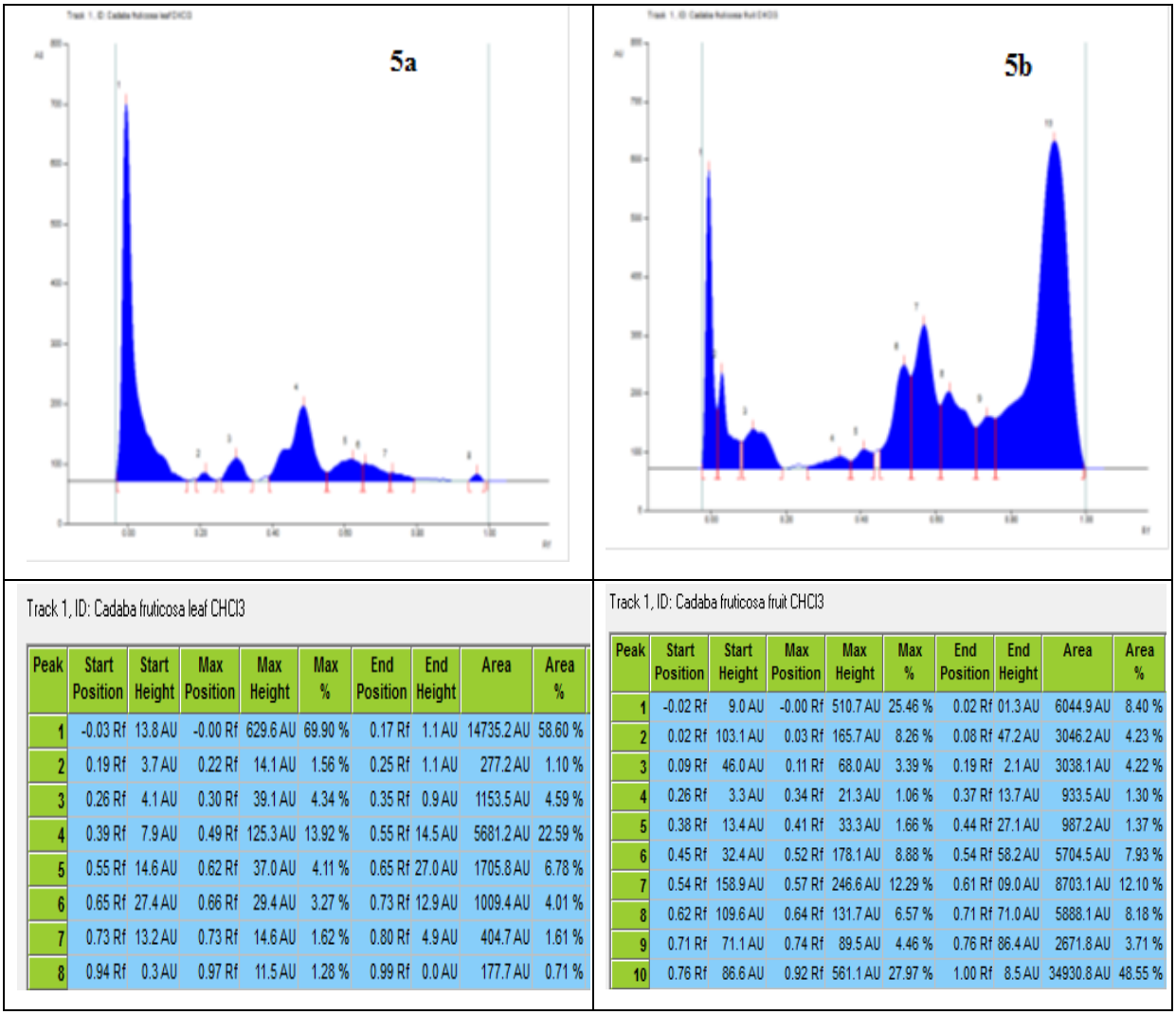
Track 2, ID: Cadaba fruticosa fruit CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	30.1 AU	0.00 Rf	335.5 AU	12.76 %	0.04 Rf	46.0 AU	7431.9 AU	9.82 %
2	0.04 Rf	148.8 AU	0.06 Rf	324.5 AU	12.34 %	0.10 Rf	25.1 AU	7799.0 AU	10.31 %
3	0.10 Rf	126.1 AU	0.11 Rf	144.2 AU	5.48 %	0.12 Rf	17.5 AU	1579.9 AU	2.09 %
4	0.13 Rf	129.8 AU	0.15 Rf	490.2 AU	18.64 %	0.19 Rf	24.7 AU	7117.7 AU	9.41 %
5	0.19 Rf	25.0 AU	0.21 Rf	32.2 AU	1.23 %	0.24 Rf	13.5 AU	660.0 AU	0.87 %
6	0.28 Rf	19.5 AU	0.34 Rf	60.5 AU	2.30 %	0.39 Rf	0.5 AU	2267.3 AU	3.00 %
7	0.41 Rf	0.9 AU	0.48 Rf	67.4 AU	2.56 %	0.51 Rf	40.4 AU	2364.4 AU	3.12 %
8	0.51 Rf	39.9 AU	0.57 Rf	76.0 AU	2.89 %	0.59 Rf	61.6 AU	2839.4 AU	3.75 %
9	0.59 Rf	61.8 AU	0.63 Rf	148.3 AU	5.64 %	0.65 Rf	20.9 AU	4167.7 AU	5.51 %
10	0.65 Rf	121.2 AU	0.70 Rf	319.2 AU	12.14 %	0.72 Rf	77.9 AU	9757.6 AU	12.89 %
11	0.73 Rf	278.9 AU	0.75 Rf	365.7 AU	13.90 %	0.82 Rf	38.5 AU	14482.7 AU	19.14 %
12	0.82 Rf	139.0 AU	0.87 Rf	266.2 AU	10.12 %	1.00 Rf	1.7 AU	15202.7 AU	20.09 %

Fig. 4: Finger print profile and R_f table of Chloroform extract of Cadaba fruticosa leaf Under 366 nm.

The HPTLC finger print profile of leaf of *Cadaba fruticosa* after derivatization with vanillin-sulphuric acid (Fig. 5(a)) showed eight peaks among which the peak at R_f 0.49 (22.59 %) is the major peak. Other peaks appeared at R_f are 0.22 (1.10 %), 0.30 (4.59 %), 0.62 (6.75 %), 0.66 (4.01 %) and 0.73 (1.61 %) respectively. 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 fruit *of Cadaba fruticosa* after derivatization with vanillin-sulphuric acid (Fig.5 (b)) showed ten peaks among which the peak at Rf 0.57(12.10 %) is the major peak. Other peaks appeared at R_f 0.03(4.23 %), 0.11(4.22 %), 0.34 (1.30 %), 0.41 (1.37 %), 0.52 (7.93 %), 0.64 (6.16 %) and 0.74 (3.71 %) respectively. The peaks at R_f 0.00 and 0.92 have not been taken into account since first one is at loading position and latter one is near the solvent front**.**



Track 1, ID: Cadaba fruticosa leaf CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	13.8 AU	-0.00 Rf	629.6 AU	69.90 %	0.17 Rf	1.1 AU	14735.2 AU	58.60 %
2	0.19 Rf	3.7 AU	0.22 Rf	14.1 AU	1.56 %	0.25 Rf	1.1 AU	277.2 AU	1.10 %
3	0.26 Rf	4.1 AU	0.30 Rf	39.1 AU	4.34 %	0.35 Rf	0.9 AU	1153.5 AU	4.59 %
4	0.39 Rf	7.9 AU	0.49 Rf	125.3 AU	13.92 %	0.55 Rf	14.5 AU	5681.2 AU	22.59 %
5	0.55 Rf	14.6 AU	0.62 Rf	37.0 AU	4.11 %	0.65 Rf	27.0 AU	1705.8 AU	6.78 %
6	0.65 Rf	27.4 AU	0.66 Rf	29.4 AU	3.27 %	0.73 Rf	12.9 AU	1009.4 AU	4.01 %
7	0.73 Rf	13.2 AU	0.73 Rf	14.6 AU	1.62 %	0.80 Rf	4.9 AU	404.7 AU	1.61 %
8	0.94 Rf	0.3 AU	0.97 Rf	11.5 AU	1.28 %	0.99 Rf	0.0 AU	177.7 AU	0.71 %

Track 1, ID: Cadaba fruticosa fruit CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	9.0 AU	-0.00 Rf	510.7 AU	25.46 %	0.02 Rf	01.3 AU	6044.9 AU	8.40 %
2	0.02 Rf	103.1 AU	0.03 Rf	165.7 AU	8.26 %	0.08 Rf	47.2 AU	3046.2 AU	4.23 %
3	0.09 Rf	46.0 AU	0.11 Rf	68.0 AU	3.39 %	0.19 Rf	2.1 AU	3038.1 AU	4.22 %
4	0.26 Rf	3.3 AU	0.34 Rf	21.3 AU	1.06 %	0.37 Rf	13.7 AU	933.5 AU	1.30 %
5	0.38 Rf	13.4 AU	0.41 Rf	33.3 AU	1.66 %	0.44 Rf	27.1 AU	987.2 AU	1.37 %
6	0.45 Rf	32.4 AU	0.52 Rf	178.1 AU	8.88 %	0.54 Rf	58.2 AU	5704.5 AU	7.93 %
7	0.54 Rf	158.9 AU	0.57 Rf	246.6 AU	12.29 %	0.61 Rf	09.0 AU	8703.1 AU	12.10 %
8	0.62 Rf	109.6 AU	0.64 Rf	131.7 AU	6.57 %	0.71 Rf	71.0 AU	5888.1 AU	8.18 %
9	0.71 Rf	71.1 AU	0.74 Rf	89.5 AU	4.46 %	0.76 Rf	86.4 AU	2671.8 AU	3.71 %
10	0.76 Rf	86.6 AU	0.92 Rf	561.1 AU	27.97 %	1.00 Rf	8.5 AU	34930.8 AU	48.55 %

Fig. 5: Finger print profile and R_f table of Chloroform extract of *Cadaba fruticosa* leaf under 575 nm.

The observations in the study showed that HPTLC fingerprints patterns of *C. fruticosa* leaf and fruit were different. The R_f value with major peak area percentage may corresponds to the predominant compound present in the plant materials. Based on the results obtained from this study, it can be inferred that the chloroform extract of *Cadaba fruticosa* leaf and fruit have considerable amount of secondary metabolites, some of which could be developed as pharmaco therapeutic agent in future. The fingerprints developed in this study are likely to aid in the quality control and standardization of these plant materials.

CONCLUSION

The different physico-chemical parameters and the developed HPTLC chromatogram obtained from the study help to evaluate the differences and similarities of leaf and fruit of *C. fruticosa*. Standardization is essential measure for quality, purity and sample identification.

HPTLC fingerprinting profile is a very important parameter of standardization for the proper identification of medicinal plants. The fruit of *C. fruticosa* possesses more solubility in water and alcohol than the leaf of *C. fruticosa* since it contains more number/concentration of chemical constituents. The HPTLC fingerprinting profile developed along with the physico-chemical parameters can be used as a diagnostic tool to identity and to determine the quality and purity of the leaf and fruit of *C. fruticosa*.

ACKNOWLEDGEMENT

The authors are highly thankful to the Director General and Central Council for Research in Siddha, Chennai for providing necessary facilities to carry out this work.

REFERENCES

1. The Siddha Pharmacopoeia of India (SPI). CCRS. Ministry of Health & Family Welfare. Dept. of Ayush. Govt. of India, 2011; 1,2: 181-184.
2. Arokiyaraj S, Radha R, Martin S, Perinbam K. Phytochemical analysis and anti-diabetic activity of *Cadaba fruticosa* R.Br. Indian J. Sci. Technol, 2008; 1(6): 1-4.
3. Chatterjee TK. Handbook of Laboratory Mice and Rats, 1st ed, Chatterjee Publications, Calcutta, 1993; 151.
4. Chattopadhyay D, Arunachalam G, Mandal A.B, Mandal S.C, Evaluation of antipyretic activity leaf extracts of *Mallotu speltatus* (Geist) Muell. Arg.Var. *acuminatus*, folk medicine, Phytomed, 2002; 9: 727-730.
5. Ganesan S, Venkateshan G, Banumathy N, Medicinal plants used by ethnic group Thottianaickans of Semmalai hills (reserved forest), Tiruchirappalli district Tamil Nadu, Indian J. Trad. Know, 2006; 5(2): 245-252.
6. Gong F, Wang BT, Chau FT, Liang YZ, HPLC data preprocessing for chromatographic fingerprint of herbal medicine with chemometric approaches. Anal Lett., 2005; 38: 2475–92.
7. Harborne J.B, Phytochemical methods. 3rd Ed, Chapman and Hall, London, 1998.
8. Mythreyi R, Sasikala E, Geeta A, Madhavan V, Antipyretic activity of leaves of *Cadaba fruticosa*. Pharmacol. Online, 2008; 3: 136-142.
9. Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, Dhamotharan R, Dananjeyan Sathya Bama S, Ethno botanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J. Med. Plants Res., 2010; 4(12): 1089-1101.
10. Uddin VA, Anwar B, Rahman A, Identification and C-13-NMR spectrum of stachydreine from *Cadaba frutiicosa*. Phytochem, 1975; s1 4: 292-293.

11. Uma KS, Parthiban P, Kalpana S, Pharmacognostical and preliminary phytochemical screening of Aavaarai Vidhai Chooranam, Asian J. Pharm. Clin. Res., 2017; 10(10): 111-116.
12. Wagner H, Bladt S, Plant drug analysis - A Thin Layer Chromatography Atlas, Springer - Verlage Berlin, 1996; 364: 3-4.
13. Watt JM, Breyer-Brandwijk MG, Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. E&S Livingston Ltd London, 1962; 160.
14. World Health Organization (WHO), Quality control Methods of Medicinal Plant Materials Geneva, 1998; 8: 28-34, 45-46.
15. Yousif G, Iskander GM, Eisa EB, Alkaloids of *Cadaba farinosa* and *Cadaba rotundifolia*. Fitoterapia, 1984; 55: 117-118.