ejpmr, 2021,8(4), 394-403

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

PHYSICO-CHEMICAL, CHROMATOGRAPHIC AND SPECTROSCOPIC EVALUATION OF ERUKKU (LEAVES, FLOWER AND LATEX)

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Article Received on 23/01/2021 **Article Revised on 13/02/2021** **Article Accepted on 03/03/2021**

ABSTRACT

Calotropis gigantea (L.) R. Br. Ex Ait. (Erukku). (Fam: Asclepiadaceae) is an important medicinal plant used in the Siddha System of Medicine. The leaf of the plant is used to treat skin ulcers, leprosy, intermittent fevers and eye problems. The medicinal use of the flower is reported as bile suppression, elimination of intestinal worms, coughs, colds and asthma and latex of the plant is used to treat dysentery, leprosy, elephantiasis, epilepsy, asthma. The latex of the plant is used as a violent purgative, gastrointestinal irritant and abortion inducer. It has also been used in the treatment of toothache, earache, headache, sprain and stiff joints. This study is aimed to standardize leaves, flowers and latex of *C. gigantea*. Different methods were used to authenticate these plant materials based on physicochemical, chromatographic and spectroscopic characteristics. The physico-chemical parameters like determination of loss on drying at 105°C, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives and volatile oil were carried out by standard methods. The chloroform extracts of leaf, flower and latex of *C. gigantea* were subjected to HPTLC and Ultra Violet-Visible spectroscopic analysis. The physico-chemical parameters, HPTLC finger prints and the spectra obtained can be considered as unique for the chloroform extract of leaf, flower and latex of *C. gigantea.*

KEY WORDS: Erukku, *Calotropis gigantea*, physico-chemical parameters, HPTLC profile, UV-Vis spectra.

INTRODUCTION

Siddha system of medicine is one of the oldest traditional systems of medicine, which has been originated from India and is practiced mostly in the southern part of this country for treating various diseases including chronic conditions. Siddha has safe herbal and herbo mineral drugs for the treatment of very common and rare diseases. Herbal medicine has been commonly used over the years for treatment and prevention of diseases, health promotion and enhancement of the span and quality of life. The most important things for consumers about medications are purity, safety, potency, and efficacy. Standardisation helps to avoid adulteration and improper substitution of medicinal plants. This study is aimed to standardize an important medicinal plant, *Calotropis gigantea* (L.) R. Br. Ex Ait. (Erukku). Leaves, flowers and latex of this plant were taken for the study and different methods were used to authenticate these plant materials based on physicochemical, chromatographic and spectroscopic characteristics.

C. gigantea (Fam: Asclepiadaceae) is native to Asia and South-East Asia and has been introduced in the Pacific Islands, Australia, Central and northern South America and Africa as an ornamental herb.[1] It is a 3–4 m tall shrub with milky latex. Bark: ash coloured, leaves: opposite, decussate, sessile or subsessile; flower: 2–4 cm across, purplish white, complete, fruit: follicles, seed numerous, broadly ovate, plano-convex. *C. gigantea* has been used as a folk medicine in India for many years, and has been reported to have a variety of uses. Traditionally the milky juice of *C. gigantea* has been used as a violent purgative, gastrointestinal irritant and abortion inducer.[2,3,4] It has also been used in the treatment of toothache, earache, headache, sprain and stiff joints.[5] Various parts of the plant contain immense medicinal property. The leaf of the plant is used to treat skin ulcers, leprosy, intermittent fevers and eye problems. The medicinal use of the flower is reported as bile suppression, elimination of intestinal worms, coughs, colds and asthma and latex of the plant is used to treat dysentery, leprosy, elephantiasis, epilepsy, asthma.[6,7,8] The powdered root bark is soaked in its own milky juice from which bougies are prepared and their fumes are inhaled to treat cough in Siddha system of medicine. Its leaf juice is used in external swellings.[9]

The aqueous stem bark extract had been found to be effective on bronchial irritation by ammoniac in guinea pig.[10] Exposure of the latex of this plant may cause some toxic effect such as corneal endothelial cytotoxicity[11] and local inflammation.[12] The vernacular names of the plant is English - Madar, Giant Milk-weed; Sanskrit - Arka, Mandara; Gujrati - Akado; Hindi - Safed aak**;** Kannada – Arkagida; Malayalam – Erukku; Marathi – Rui; Tamil – Erukku; Telugu – Jilledu; Bengali – Akanda; Punjabi – Ak; Arab – Ushar; Persian – Kharak.[13,14,15]

Fig. 1: *Calotropis igantean* (L.) R. Br. Ex Ait.

MATERIALS AND METHODS

The leaves, flower and latex of *C. gigantea* were collected from the premises of Siddha Regional Research Institute, Thiruvananthapuram and identified by Botany expert. The plant materials –leaves, flower and latex were shade dried, powdered and stored in air tight containers. These dried plant materials were used for all experimental purposes.

Physico-chemical analysis

The physico-chemical parameters like determination of loss on drying at 105°C, total ash, acid insoluble ash, water soluble extractives, alcohol soluble extractives and volatile oil were carried out by standard methods[16].

High performance thin layer chromatography (HPTLC) studies

Preparation of Plant extract

Chloroform extracts of the plant materials were prepared for HPTLC analysis. 1 g of each powdered sample was soaked in separate conical flask containing 10 ml of chloroform, kept the solutions overnight and refluxed for 10 minutes. The extracts were filtered and concentrated to 1 ml.[17]

Instrumentation and chromatographic conditions

Each sample was applied as bands on the aluminium plates coated with silica gel 60 F254 (Merck, Germany). The spotting of the extracts was performed by using Camag ATS4 instrument. Ascending development to a distance of 80 mm was performed in a Camag glass twin-trough chamber previously saturated with mobile phase vapour at room temperature. A number of solvent systems were tried for each extract. The solvent system which gave better resolution and maximum number of spots were selected for each extract. After development, the plates were air dried, visualized and scanned at UV short and long wavelength (254 nm and 366 nm) with a Camag TLC scanner with WinCAT software. Then the plates were derivatized using Vanillin -sulphuric acid reagent and the plates were dried by placing on a hot plate. The dried plates were visualised under white light and scanned at 575 nm. All the results obtained were documented.

Ultra Violet-Visible (UV-Vis) spectroscopy

The chloroform extracts of leaf, flower and latex of *C. gigantea* were subjected to Ultra Violet-Visible spectroscopic analysis. The extracts were scanned at wave length ranging from 200 to 900 nm using UV-VIS spectrophotometer (Model: UV3120) and the characteristic peaks were detected and recorded.

RESULTS AND DISCUSSION

The physico-chemical data obtained for the leaves, flower and latex of *C. gigantea* are given in Table 1.

Table 1: Physico-chemical parameters of leaves, flower and latex of *C. gigantean.*

Sl. No.	Parameters	Leaves	Flower	Latex
1	Foreign Matter	Nil	Nil	Nil
2	Loss on Drying at 105^0C (%)	13.37	13.78	18.95
3	Total Ash Content (%)	10.64	9.91	8.09
4	Acid Insoluble Ash (%)	0.84	0.78	0.74
5	Water Soluble Extractive (%)	23.56	23.00	20.90
6	Alcohol Soluble Extractive (%)	13.07	12.29	9.88
7	Volatile oil (%)	Nil	Nil	Nil

These parameters are useful in establishing the profile quality of the plant materials and are important for its evaluation. The loss on drying was found maximum in the latex of the plant (18.95 %) which may be due to the presence of high moisture content in the material. It was observed that the ash content is high for leaves (10.64%). Total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash-which is derived from the plant tissue itself and non physiological ash –which is the residue of sand and soil adhering to the plant surface. Acid insoluble ash particularly indicates the contamination with siliceous material. Acid insoluble ash values were found to be low for all the plant materials, which might be an indicative of purity and absence of silicaceous materials in the selected plant parts. Most of the high polar secondary

metabolites could be extracted with water and alcohol. The water soluble extractive values were found to be within a short range for leaves, flower and latex of the plant. The alcohol soluble extractives were observed to be lesser in latex than the other parts of the plant.

HPTLC study can be considered as an important tool in routine drug analysis. In the present study HPTLC fingerprinting was employed as a parameter for standardisation of the samples.

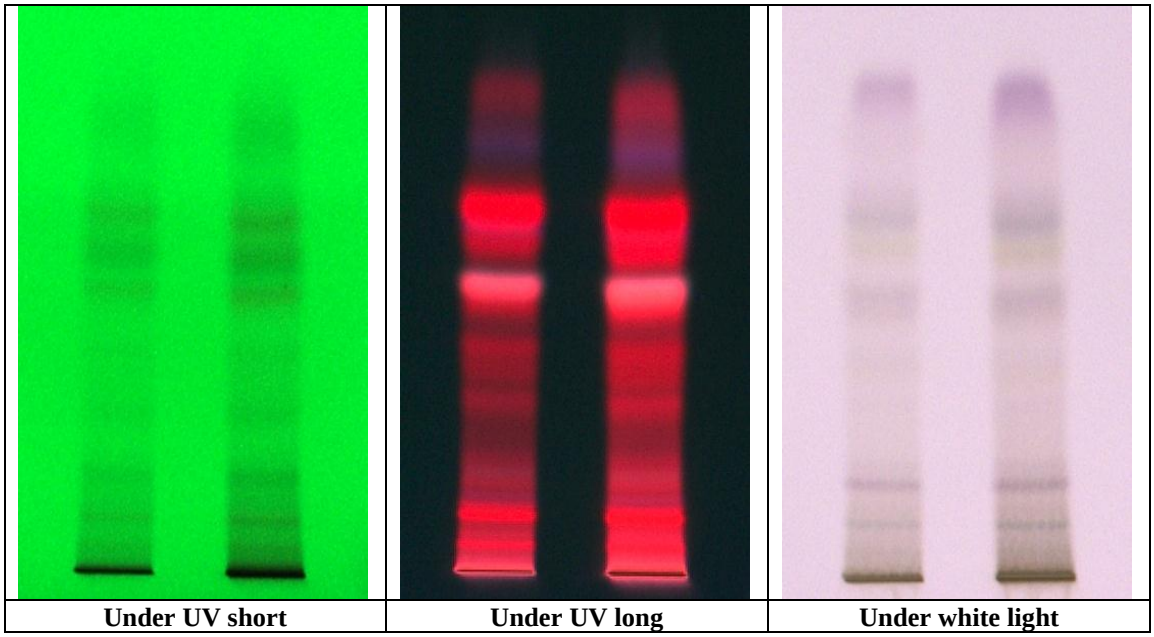
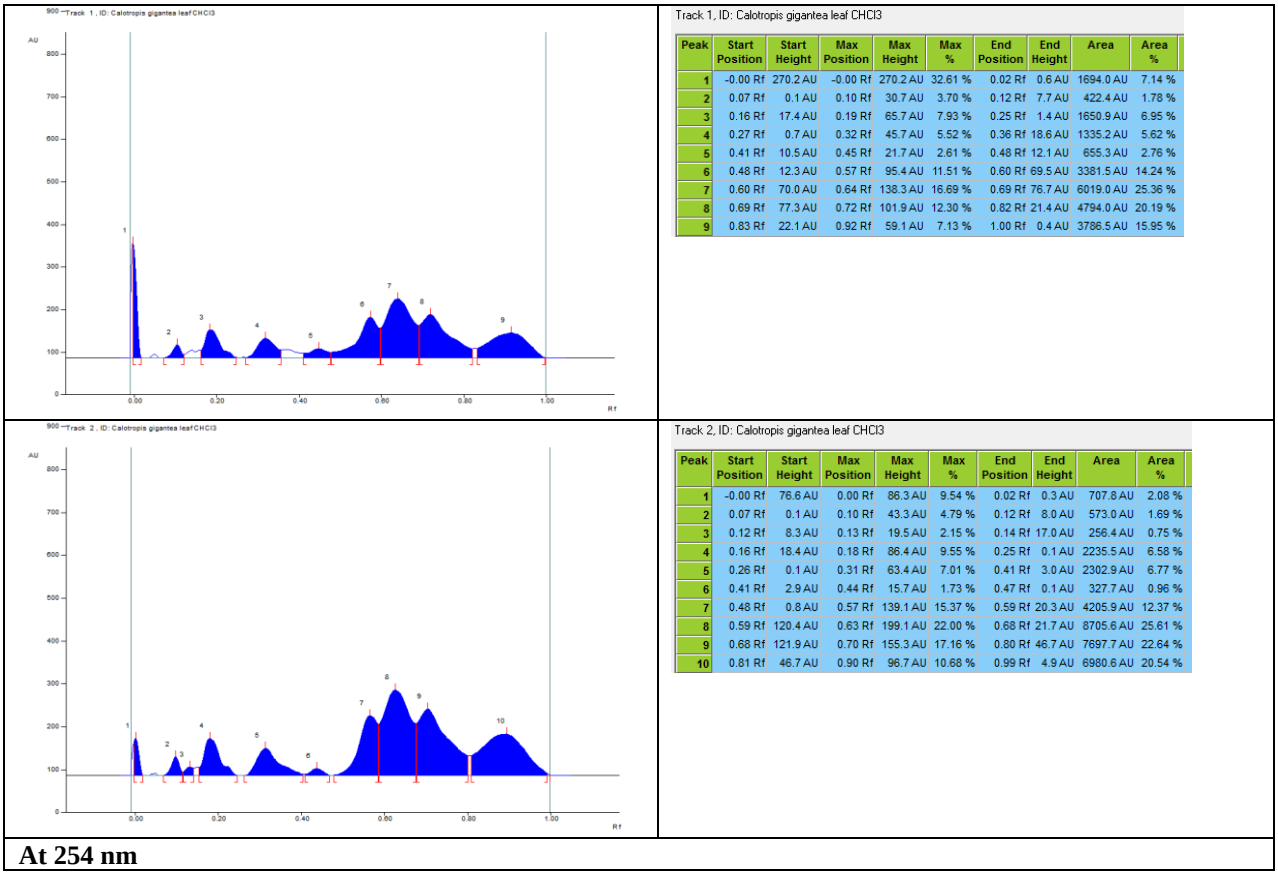


Fig. 2: HPTLC profile of chloroform extract of *Calotropis gigantea* (leaf) viewed in UV short; viewed in UV long; viewed in visible light after derivatisation using vanillin-sulphuric acid; Solvent system – Toluene: Ethyl acetate: Formic acid (5: 1: 0.1); Volume applied; Track 1- 5 µl: Track 2 – 10 µl.



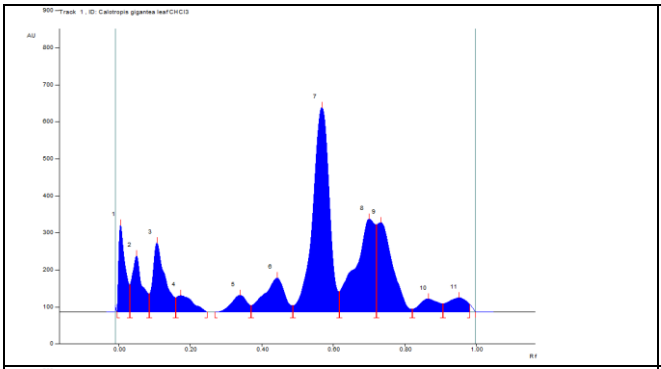
Track 1, ID: Calotropis gigantea leaf CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	270.2 AU	-0.00 Rf	270.2 AU	32.61 %	0.02 Rf	0.6 AU	1694.0 AU	7.14 %
2	0.07 Rf	0.1 AU	0.10 Rf	30.7 AU	3.70 %	0.12 Rf	7.7 AU	422.4 AU	1.78 %
3	0.16 Rf	17.4 AU	0.19 Rf	65.7 AU	7.93 %	0.25 Rf	1.4 AU	1650.9 AU	6.95 %
4	0.27 Rf	0.7 AU	0.32 Rf	45.7 AU	5.52 %	0.36 Rf	18.6 AU	1335.2 AU	5.62 %
5	0.41 Rf	10.5 AU	0.45 Rf	21.7 AU	2.61 %	0.48 Rf	12.1 AU	655.3 AU	2.76 %
6	0.48 Rf	12.3 AU	0.57 Rf	95.4 AU	11.51 %	0.60 Rf	69.5 AU	3381.5 AU	14.24 %
7	0.60 Rf	70.0 AU	0.64 Rf	138.3 AU	16.69 %	0.69 Rf	76.7 AU	6019.0 AU	25.36 %
8	0.69 Rf	77.3 AU	0.72 Rf	101.9 AU	12.30 %	0.82 Rf	21.4 AU	4794.0 AU	20.19 %
9	0.83 Rf	22.1 AU	0.92 Rf	59.1 AU	7.13 %	1.00 Rf	0.4 AU	3786.5 AU	15.95 %

Track 2, ID: Calotropis gigantea leaf CHCl3

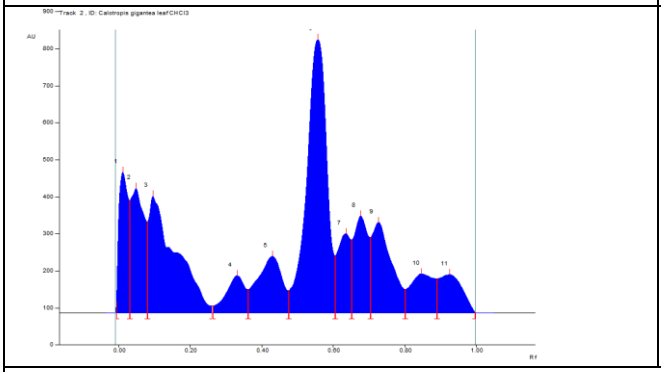
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	76.6 AU	0.00 Rf	86.3 AU	9.54 %	0.02 Rf	0.3 AU	707.8 AU	2.08 %
2	0.07 Rf	0.1 AU	0.10 Rf	43.3 AU	4.79 %	0.12 Rf	8.0 AU	573.0 AU	1.69 %
3	0.12 Rf	8.3 AU	0.13 Rf	19.5 AU	2.15 %	0.14 Rf	17.0 AU	256.4 AU	0.75 %
4	0.16 Rf	18.4 AU	0.18 Rf	86.4 AU	9.55 %	0.25 Rf	0.1 AU	2235.5 AU	6.58 %
5	0.26 Rf	0.1 AU	0.31 Rf	63.4 AU	7.01 %	0.41 Rf	3.0 AU	2302.9 AU	6.77 %
6	0.41 Rf	2.9 AU	0.44 Rf	15.7 AU	1.73 %	0.47 Rf	0.1 AU	327.7 AU	0.96 %
7	0.48 Rf	0.8 AU	0.57 Rf	139.1 AU	15.37 %	0.59 Rf	20.3 AU	4205.9 AU	12.37 %
8	0.59 Rf	120.4 AU	0.63 Rf	199.1 AU	22.00 %	0.68 Rf	21.7 AU	8705.6 AU	25.61 %
9	0.68 Rf	121.9 AU	0.70 Rf	155.3 AU	17.16 %	0.80 Rf	46.7 AU	7697.7 AU	22.64 %
10	0.81 Rf	46.7 AU	0.90 Rf	96.7 AU	10.68 %	0.99 Rf	4.9 AU	6980.6 AU	20.54 %

At 254 nm



Track 1, ID: Calotropis gigantea leaf CHCl3

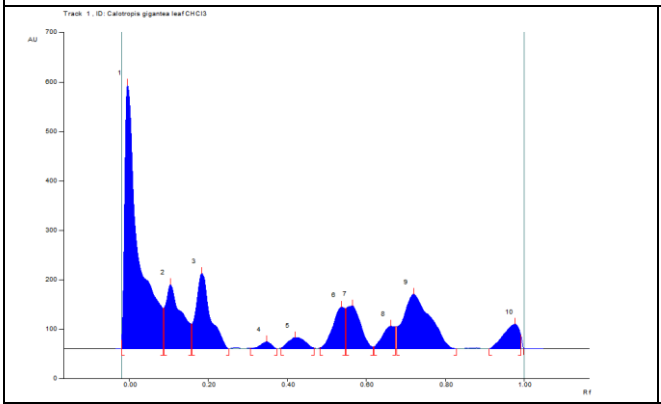
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	1.1 AU	0.01 Rf	234.1 AU	12.51 %	0.03 Rf	72.9 AU	2962.6 AU	5.47 %
2	0.03 Rf	73.8 AU	0.05 Rf	152.1 AU	8.13 %	0.09 Rf	49.5 AU	2916.3 AU	5.38 %
3	0.09 Rf	49.7 AU	0.11 Rf	186.2 AU	9.95 %	0.16 Rf	38.8 AU	4420.6 AU	8.16 %
4	0.16 Rf	39.1 AU	0.17 Rf	44.0 AU	2.35 %	0.25 Rf	0.1 AU	1394.2 AU	2.57 %
5	0.27 Rf	0.0 AU	0.34 Rf	45.2 AU	2.41 %	0.37 Rf	17.4 AU	1306.9 AU	2.41 %
6	0.37 Rf	17.5 AU	0.44 Rf	91.7 AU	4.90 %	0.49 Rf	16.8 AU	3567.5 AU	6.58 %
7	0.49 Rf	16.9 AU	0.57 Rf	552.3 AU	29.51 %	0.62 Rf	55.0 AU	17941.8 AU	33.11 %
8	0.62 Rf	55.6 AU	0.70 Rf	250.9 AU	13.41 %	0.72 Rf	35.9 AU	9654.3 AU	17.82 %
9	0.72 Rf	236.2 AU	0.74 Rf	241.3 AU	12.89 %	0.82 Rf	7.4 AU	7322.5 AU	13.51 %
10	0.82 Rf	7.5 AU	0.87 Rf	35.2 AU	1.88 %	0.91 Rf	22.5 AU	1301.9 AU	2.40 %
11	0.91 Rf	22.5 AU	0.95 Rf	38.5 AU	2.06 %	0.98 Rf	20.5 AU	1397.3 AU	2.58 %



Track 2, ID: Calotropis gigantea leaf CHCl3

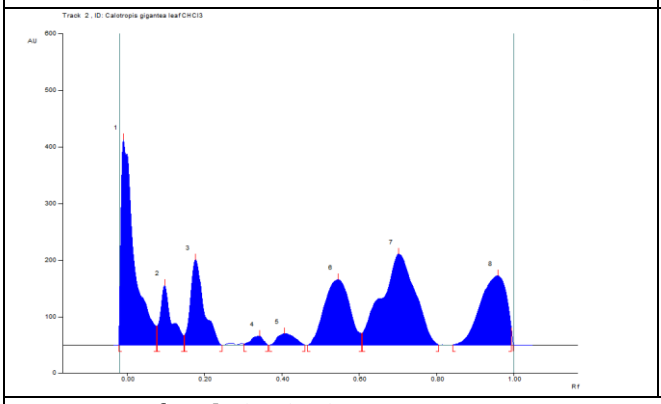
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	30.7 AU	0.01 Rf	379.9 AU	12.86 %	0.03 Rf	03.7 AU	6961.7 AU	6.70 %
2	0.03 Rf	304.3 AU	0.05 Rf	335.9 AU	11.38 %	0.08 Rf	45.8 AU	8579.8 AU	8.26 %
3	0.08 Rf	246.2 AU	0.10 Rf	315.6 AU	10.69 %	0.26 Rf	19.2 AU	16774.0 AU	16.15 %
4	0.26 Rf	19.1 AU	0.33 Rf	101.2 AU	3.43 %	0.36 Rf	63.5 AU	3636.7 AU	3.50 %
5	0.36 Rf	63.7 AU	0.43 Rf	153.0 AU	5.18 %	0.48 Rf	60.6 AU	7333.9 AU	7.06 %
6	0.48 Rf	61.2 AU	0.56 Rf	737.9 AU	24.99 %	0.61 Rf	54.1 AU	28984.1 AU	27.90 %
7	0.61 Rf	154.5 AU	0.64 Rf	213.9 AU	7.24 %	0.65 Rf	97.1 AU	5213.1 AU	5.02 %
8	0.65 Rf	197.4 AU	0.68 Rf	261.9 AU	8.87 %	0.70 Rf	03.7 AU	7344.2 AU	7.07 %
9	0.71 Rf	204.6 AU	0.73 Rf	244.7 AU	8.28 %	0.80 Rf	63.7 AU	9437.1 AU	9.08 %
10	0.80 Rf	63.8 AU	0.85 Rf	106.0 AU	3.59 %	0.89 Rf	92.1 AU	4950.4 AU	4.77 %
11	0.89 Rf	92.2 AU	0.93 Rf	103.3 AU	3.50 %	1.00 Rf	2.1 AU	4663.2 AU	4.49 %

At 366 nm



Track 1, ID: Calotropis gigantea leaf CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	17.0 AU	-0.00 Rf	533.3 AU	43.36 %	0.09 Rf	82.0 AU	13042.9 AU	39.63 %
2	0.09 Rf	82.4 AU	0.10 Rf	129.9 AU	10.56 %	0.16 Rf	50.4 AU	3653.2 AU	11.10 %
3	0.16 Rf	50.5 AU	0.19 Rf	153.0 AU	12.44 %	0.25 Rf	0.1 AU	3713.6 AU	11.28 %
4	0.31 Rf	0.8 AU	0.35 Rf	14.0 AU	1.14 %	0.38 Rf	0.1 AU	265.6 AU	0.81 %
5	0.38 Rf	1.6 AU	0.42 Rf	22.9 AU	1.87 %	0.47 Rf	1.8 AU	706.2 AU	2.15 %
6	0.49 Rf	2.4 AU	0.54 Rf	84.6 AU	6.88 %	0.55 Rf	81.8 AU	1802.3 AU	5.48 %
7	0.55 Rf	82.0 AU	0.57 Rf	86.8 AU	7.05 %	0.62 Rf	3.6 AU	2180.4 AU	6.63 %
8	0.62 Rf	3.9 AU	0.66 Rf	45.7 AU	3.72 %	0.68 Rf	44.9 AU	991.5 AU	3.01 %
9	0.68 Rf	44.8 AU	0.72 Rf	110.2 AU	8.96 %	0.83 Rf	0.2 AU	5171.4 AU	15.71 %
10	0.91 Rf	0.0 AU	0.98 Rf	49.5 AU	4.03 %	0.99 Rf	26.9 AU	1384.2 AU	4.21 %

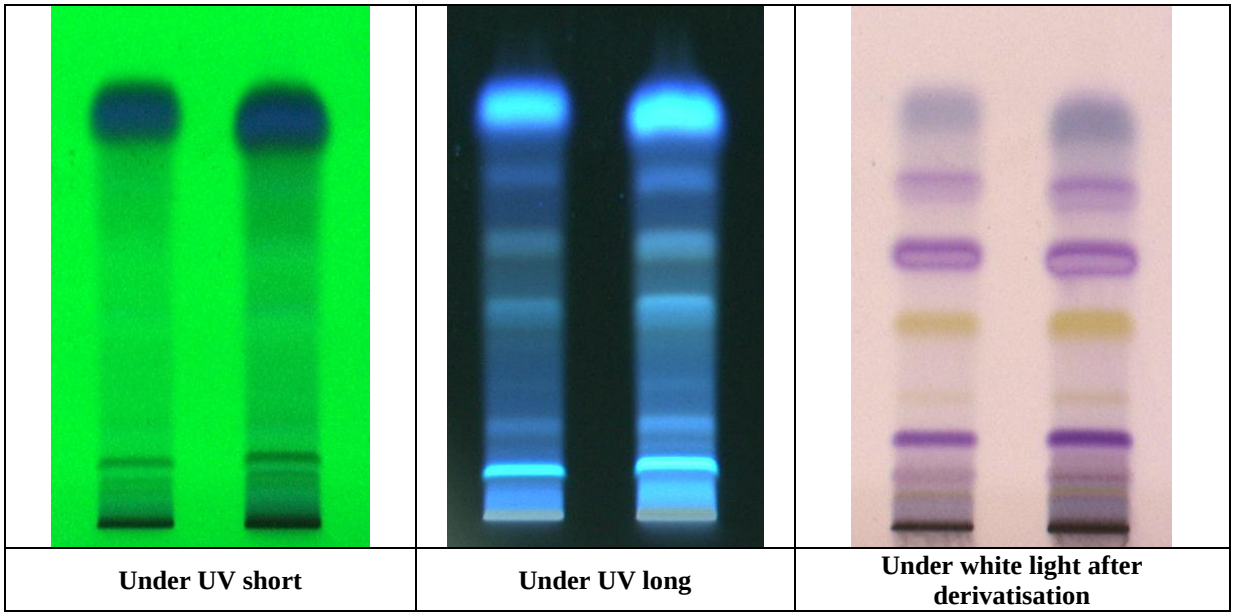


Track 2, ID: Calotropis gigantea leaf CHCl3

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.0 AU	-0.01 Rf	364.1 AU	34.46 %	0.08 Rf	33.5 AU	8733.2 AU	24.11 %
2	0.08 Rf	33.5 AU	0.10 Rf	105.3 AU	9.96 %	0.15 Rf	16.8 AU	2177.4 AU	6.01 %
3	0.15 Rf	17.5 AU	0.18 Rf	151.4 AU	14.33 %	0.25 Rf	0.0 AU	3546.8 AU	9.79 %
4	0.30 Rf	2.0 AU	0.34 Rf	15.9 AU	1.51 %	0.37 Rf	0.1 AU	331.2 AU	0.91 %
5	0.37 Rf	0.1 AU	0.41 Rf	20.6 AU	1.95 %	0.46 Rf	0.2 AU	644.9 AU	1.78 %
6	0.47 Rf	0.1 AU	0.55 Rf	115.8 AU	10.96 %	0.61 Rf	20.8 AU	5338.2 AU	14.74 %
7	0.61 Rf	21.3 AU	0.70 Rf	161.0 AU	15.24 %	0.81 Rf	0.3 AU	9676.5 AU	26.72 %
8	0.84 Rf	0.5 AU	0.96 Rf	122.5 AU	11.59 %	1.00 Rf	29.9 AU	5770.1 AU	15.93 %

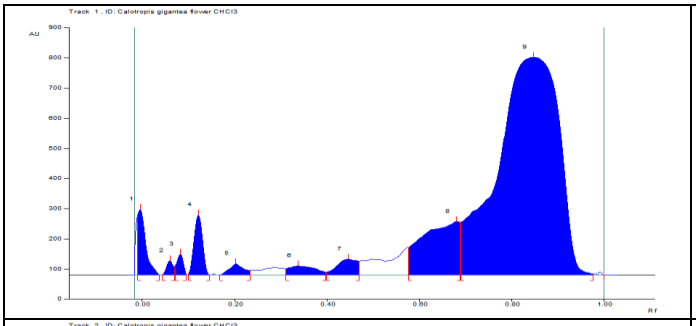
At 575 nm after derivatisation

Fig. 3: HPTLC finger print profile of 5 µl and 10 µl of chloroform extract of *Calotropis gigantea* (leaf) at 254 nm, 366 nm and at 575 nm after derivatisation.

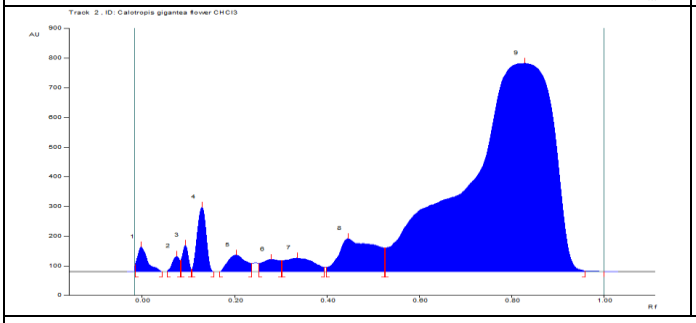


Under UV short	Under UV long	Under white light after derivatisation

Fig. 4: HPTLC profile of chloroform extract of *Calotropis gigantea* (flower) viewed in UV short; viewed in UV long; viewed in visible light after derivatisation using vanillin-sulphuric acid; Solvent system – Toluene: Chloroform (5: 2); Volume applied: Track 1- 5 µl: Track 2 – 10 µl.

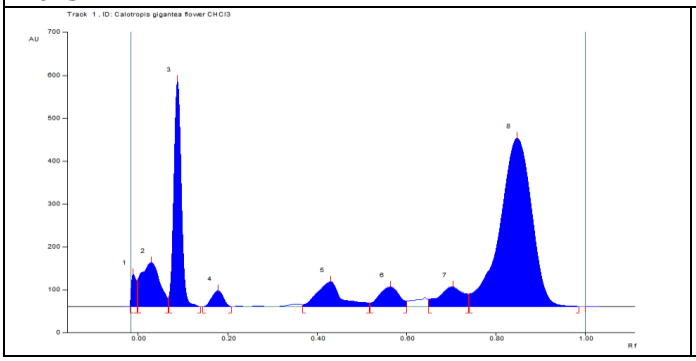


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	194.5 AU	-0.00 Rf	217.9 AU	14.07 %	0.04 Rf	0.7 AU	2797.1 AU	3.06 %
2	0.05 Rf	0.4 AU	0.06 Rf	46.4 AU	2.99 %	0.07 Rf	26.8 AU	436.8 AU	0.48 %
3	0.08 Rf	29.0 AU	0.09 Rf	67.9 AU	4.38 %	0.10 Rf	2.5 AU	676.8 AU	0.74 %
4	0.10 Rf	1.2 AU	0.13 Rf	199.0 AU	12.85 %	0.15 Rf	0.5 AU	2658.2 AU	2.91 %
5	0.18 Rf	0.2 AU	0.21 Rf	36.6 AU	2.36 %	0.25 Rf	14.8 AU	830.6 AU	0.91 %
6	0.33 Rf	20.7 AU	0.35 Rf	30.2 AU	1.95 %	0.42 Rf	9.6 AU	1280.2 AU	1.40 %
7	0.42 Rf	9.6 AU	0.47 Rf	51.8 AU	3.35 %	0.49 Rf	45.2 AU	1662.3 AU	1.82 %
8	0.61 Rf	90.7 AU	0.71 Rf	177.2 AU	11.44 %	0.72 Rf	75.1 AU	10227.4 AU	11.20 %
9	0.73 Rf	175.4 AU	0.89 Rf	721.9 AU	46.61 %	1.02 Rf	3.5 AU	70775.9 AU	77.48 %

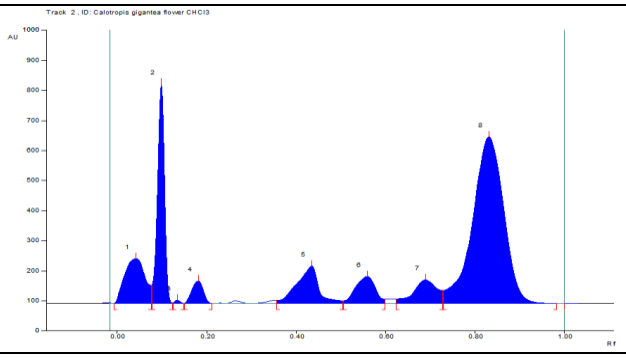


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	24.0 AU	-0.00 Rf	83.0 AU	5.94 %	0.05 Rf	0.0 AU	1316.6 AU	1.18 %
2	0.06 Rf	0.8 AU	0.08 Rf	51.6 AU	3.69 %	0.09 Rf	37.3 AU	578.2 AU	0.52 %
3	0.09 Rf	39.7 AU	0.10 Rf	88.7 AU	6.35 %	0.11 Rf	6.7 AU	810.4 AU	0.73 %
4	0.11 Rf	7.0 AU	0.14 Rf	217.5 AU	15.57 %	0.16 Rf	0.1 AU	3020.0 AU	2.70 %
5	0.18 Rf	0.1 AU	0.22 Rf	56.2 AU	4.02 %	0.25 Rf	28.0 AU	1588.1 AU	1.42 %
6	0.26 Rf	28.0 AU	0.29 Rf	41.1 AU	2.94 %	0.32 Rf	36.7 AU	1168.6 AU	1.05 %
7	0.32 Rf	36.7 AU	0.35 Rf	45.7 AU	3.27 %	0.41 Rf	15.0 AU	2118.0 AU	1.90 %
8	0.42 Rf	15.1 AU	0.47 Rf	111.0 AU	7.95 %	0.55 Rf	79.7 AU	6316.7 AU	5.66 %
9	0.55 Rf	79.9 AU	0.87 Rf	702.2 AU	50.26 %	1.01 Rf	2.0 AU	94737.4 AU	84.85 %

At 254 nm

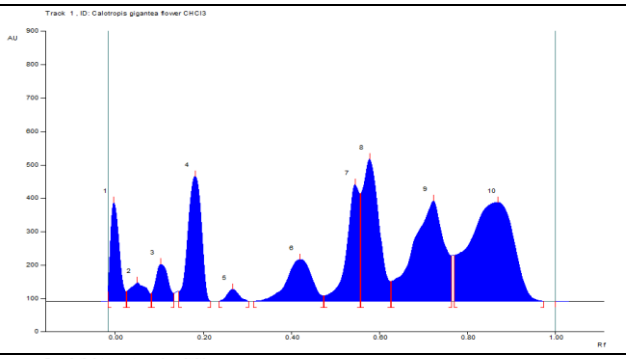


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.0 AU	-0.01 Rf	75.9 AU	5.91 %	-0.00 Rf	61.1 AU	631.0 AU	1.72 %
2	0.00 Rf	63.3 AU	0.03 Rf	102.7 AU	7.99 %	0.07 Rf	19.5 AU	3095.4 AU	8.43 %
3	0.07 Rf	22.2 AU	0.09 Rf	525.6 AU	40.91 %	0.15 Rf	0.0 AU	6332.3 AU	17.25 %
4	0.15 Rf	0.0 AU	0.19 Rf	37.1 AU	2.89 %	0.22 Rf	0.2 AU	691.3 AU	1.88 %
5	0.39 Rf	5.0 AU	0.45 Rf	58.5 AU	4.55 %	0.54 Rf	8.7 AU	2271.4 AU	6.19 %
6	0.55 Rf	8.7 AU	0.59 Rf	45.7 AU	3.56 %	0.63 Rf	13.3 AU	1515.8 AU	4.13 %
7	0.68 Rf	17.3 AU	0.74 Rf	46.6 AU	3.62 %	0.78 Rf	29.6 AU	1855.5 AU	5.05 %
8	0.78 Rf	29.7 AU	0.89 Rf	392.8 AU	30.57 %	1.03 Rf	0.6 AU	20322.5 AU	55.35 %

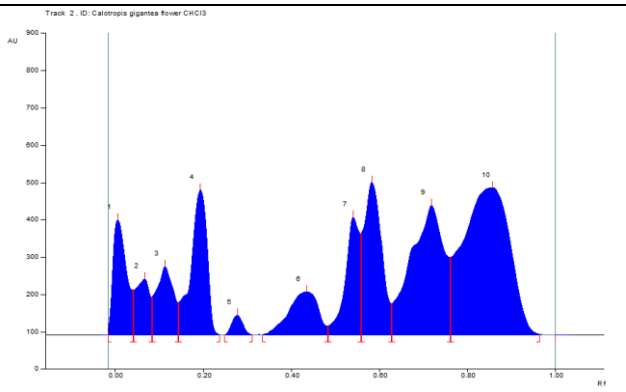


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.01 Rf	0.3 AU	0.05 Rf	149.0 AU	8.23 %	0.08 Rf	60.5 AU	4933.6 AU	9.25 %
2	0.08 Rf	62.1 AU	0.10 Rf	729.2 AU	40.27 %	0.13 Rf	1.8 AU	8317.7 AU	15.60 %
3	0.13 Rf	2.7 AU	0.14 Rf	10.5 AU	0.58 %	0.16 Rf	0.3 AU	93.1 AU	0.17 %
4	0.16 Rf	0.7 AU	0.19 Rf	74.8 AU	4.13 %	0.22 Rf	0.1 AU	1396.1 AU	2.62 %
5	0.38 Rf	9.6 AU	0.46 Rf	124.7 AU	6.89 %	0.53 Rf	8.9 AU	4357.5 AU	8.17 %
6	0.53 Rf	9.0 AU	0.59 Rf	89.4 AU	4.94 %	0.63 Rf	13.9 AU	2944.6 AU	5.52 %
7	0.65 Rf	14.9 AU	0.73 Rf	78.5 AU	4.33 %	0.76 Rf	42.7 AU	3040.3 AU	5.70 %
8	0.77 Rf	42.9 AU	0.87 Rf	554.7 AU	30.63 %	1.03 Rf	0.0 AU	28227.3 AU	52.95 %

At 366 nm



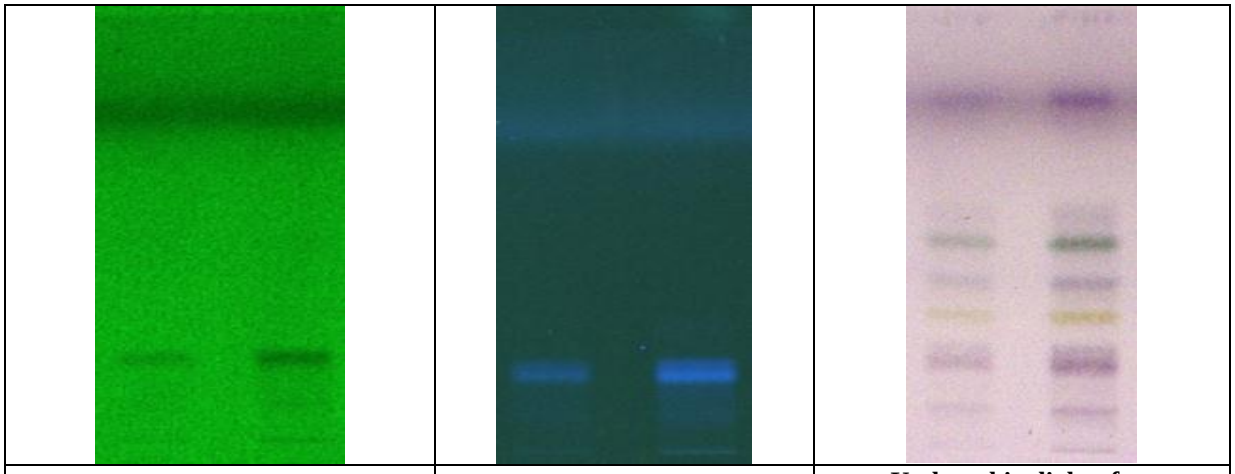
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	28.3 AU	-0.00 Rf	295.2 AU	12.46 %	0.03 Rf	29.0 AU	4219.5 AU	5.41 %
2	0.03 Rf	29.6 AU	0.05 Rf	55.6 AU	2.35 %	0.09 Rf	21.8 AU	1450.7 AU	1.86 %
3	0.09 Rf	23.4 AU	0.11 Rf	110.7 AU	4.67 %	0.14 Rf	24.7 AU	2274.5 AU	2.92 %
4	0.15 Rf	30.0 AU	0.19 Rf	374.7 AU	15.81 %	0.23 Rf	0.4 AU	8245.3 AU	10.58 %
5	0.25 Rf	0.2 AU	0.28 Rf	35.9 AU	1.52 %	0.32 Rf	0.3 AU	690.8 AU	0.89 %
6	0.33 Rf	0.1 AU	0.44 Rf	125.1 AU	5.28 %	0.50 Rf	16.1 AU	5096.1 AU	6.54 %
7	0.50 Rf	16.1 AU	0.57 Rf	349.5 AU	14.75 %	0.58 Rf	22.6 AU	7463.1 AU	9.57 %
8	0.59 Rf	323.0 AU	0.61 Rf	425.1 AU	17.94 %	0.66 Rf	60.0 AU	11461.4 AU	14.70 %
9	0.66 Rf	60.2 AU	0.76 Rf	301.3 AU	12.72 %	0.80 Rf	36.5 AU	15261.1 AU	19.58 %
10	0.81 Rf	138.0 AU	0.91 Rf	296.6 AU	12.52 %	1.02 Rf	0.2 AU	21788.0 AU	27.95 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	11.7 AU	0.01 Rf	308.9 AU	11.57 %	0.04 Rf	20.0 AU	7031.9 AU	6.89 %
2	0.05 Rf	120.3 AU	0.07 Rf	150.4 AU	5.63 %	0.09 Rf	01.3 AU	3386.4 AU	3.32 %
3	0.09 Rf	102.8 AU	0.12 Rf	183.6 AU	6.88 %	0.15 Rf	86.8 AU	5157.4 AU	5.05 %
4	0.15 Rf	87.3 AU	0.20 Rf	388.2 AU	14.55 %	0.25 Rf	0.5 AU	10572.6 AU	10.35 %
5	0.26 Rf	0.1 AU	0.29 Rf	53.0 AU	1.99 %	0.33 Rf	0.3 AU	978.2 AU	0.96 %
6	0.35 Rf	0.4 AU	0.46 Rf	115.7 AU	4.34 %	0.51 Rf	24.2 AU	5630.0 AU	5.51 %
7	0.51 Rf	24.5 AU	0.57 Rf	316.9 AU	11.87 %	0.59 Rf	72.1 AU	7624.5 AU	7.47 %
8	0.59 Rf	273.4 AU	0.61 Rf	409.8 AU	15.36 %	0.66 Rf	84.2 AU	11761.1 AU	11.52 %
9	0.66 Rf	84.7 AU	0.75 Rf	347.3 AU	13.01 %	0.80 Rf	08.4 AU	19207.3 AU	18.81 %
10	0.80 Rf	208.7 AU	0.90 Rf	395.0 AU	14.80 %	1.01 Rf	2.0 AU	30768.3 AU	30.13 %

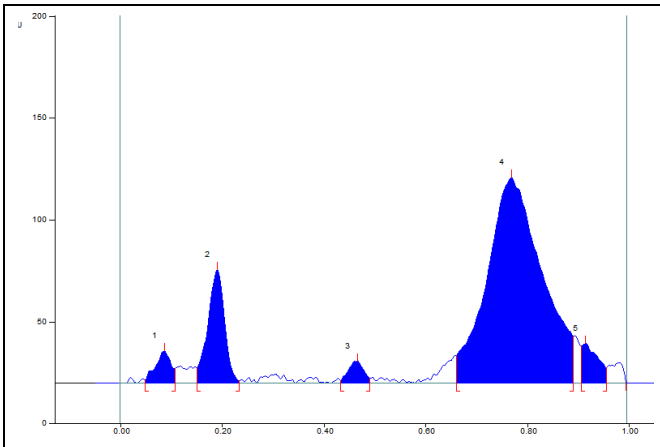
At 575 nm after derivatisation

Fig. 5: HPTLC finger print profile of 5 µl and 10 µl of chloroform extract of *Calotropis gigantea* (flower) at 254 nm, 366 nm and at 575 nm after derivatisation.

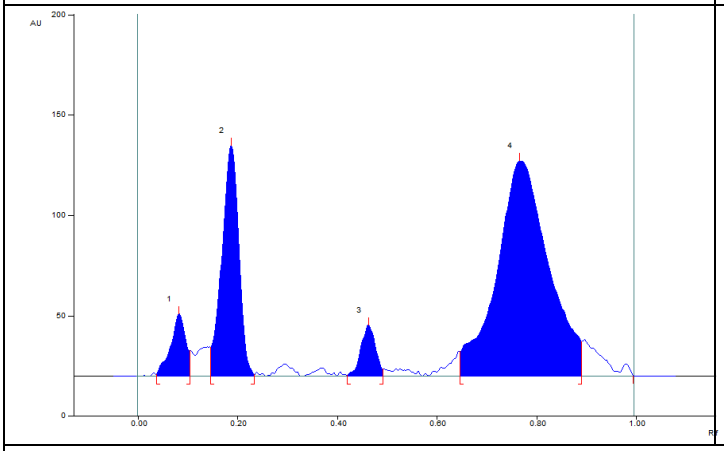


Under UV short	Under UV long	Under white light after derivatisation

Fig. 6: HPTLC profile of chloroform extract of *Calotropis gigantea* (latex defatted) viewed in UV short; viewed in UV long; viewed in visible light after derivatisation using vanillin-sulphuric acid; Solvent system – Toluene: Ethyl acetate: Methanol (4: 8: 1); Volume applied: Track 1- 5 µl: Track 2 – 10 µl.

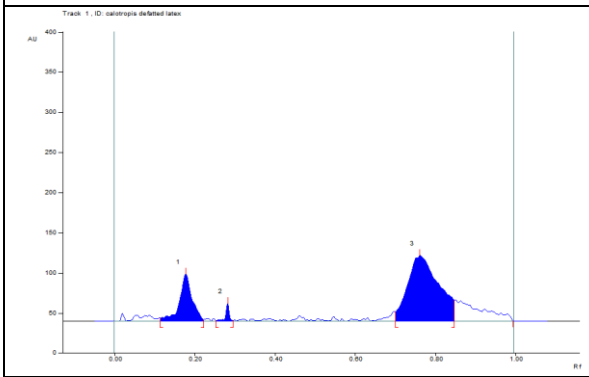


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.4 AU	0.09 Rf	15.7 AU	7.77 %	0.11 Rf	7.2 AU	350.4 AU	3.45 %
2	0.15 Rf	7.5 AU	0.19 Rf	55.8 AU	27.57 %	0.23 Rf	1.2 AU	1308.4 AU	12.88 %
3	0.43 Rf	1.2 AU	0.47 Rf	10.7 AU	5.27 %	0.49 Rf	2.4 AU	231.8 AU	2.28 %
4	0.66 Rf	13.6 AU	0.77 Rf	101.0 AU	49.85 %	0.89 Rf	23.0 AU	7805.7 AU	76.87 %
5	0.91 Rf	18.3 AU	0.91 Rf	19.3 AU	9.54 %	0.96 Rf	7.5 AU	458.2 AU	4.51 %

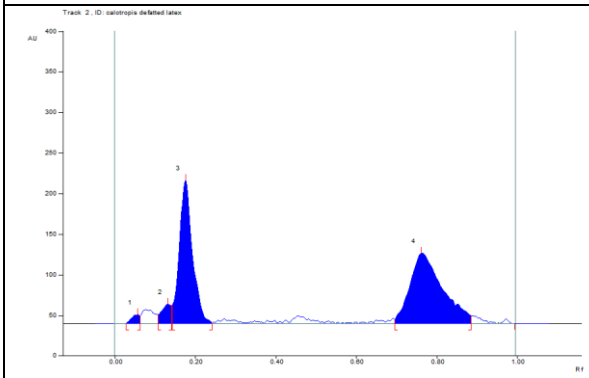


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.7 AU	0.08 Rf	31.1 AU	11.15 %	0.10 Rf	12.6 AU	677.4 AU	5.68 %
2	0.15 Rf	14.5 AU	0.19 Rf	114.9 AU	41.21 %	0.23 Rf	0.7 AU	2775.8 AU	23.27 %
3	0.42 Rf	0.4 AU	0.46 Rf	25.6 AU	9.17 %	0.49 Rf	3.4 AU	518.7 AU	4.35 %
4	0.65 Rf	12.4 AU	0.77 Rf	107.2 AU	38.47 %	0.89 Rf	17.2 AU	7956.5 AU	66.70 %

At 254 nm

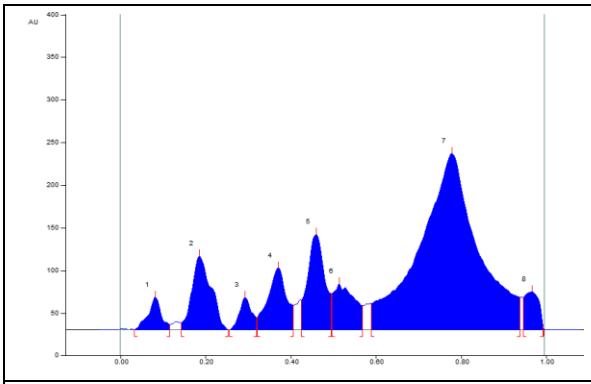


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	4.0 AU	0.18 Rf	58.7 AU	36.17 %	0.22 Rf	1.9 AU	1308.3 AU	21.92 %
2	0.25 Rf	0.6 AU	0.28 Rf	21.9 AU	13.49 %	0.30 Rf	0.8 AU	137.8 AU	2.31 %
3	0.70 Rf	11.6 AU	0.76 Rf	81.6 AU	50.33 %	0.85 Rf	26.2 AU	4521.6 AU	75.77 %

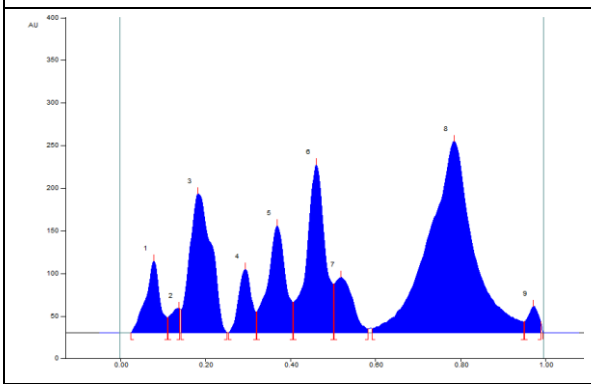


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.2 AU	0.06 Rf	11.0 AU	3.69 %	0.06 Rf	9.5 AU	160.7 AU	1.67 %
2	0.11 Rf	11.4 AU	0.13 Rf	23.6 AU	7.93 %	0.14 Rf	22.3 AU	415.9 AU	4.31 %
3	0.14 Rf	22.8 AU	0.18 Rf	176.2 AU	59.24 %	0.24 Rf	1.0 AU	4042.4 AU	41.90 %
4	0.70 Rf	5.1 AU	0.76 Rf	86.6 AU	29.13 %	0.89 Rf	9.8 AU	5027.8 AU	52.12 %

At 366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.2 AU	0.08 Rf	38.3 AU	5.88 %	0.12 Rf	6.2 AU	847.2 AU	2.57 %
2	0.14 Rf	8.3 AU	0.19 Rf	86.3 AU	13.23 %	0.25 Rf	0.1 AU	2766.2 AU	8.40 %
3	0.26 Rf	0.1 AU	0.29 Rf	37.9 AU	5.80 %	0.32 Rf	14.4 AU	765.8 AU	2.33 %
4	0.32 Rf	14.4 AU	0.37 Rf	72.3 AU	11.09 %	0.41 Rf	28.9 AU	2273.4 AU	6.90 %
5	0.43 Rf	35.2 AU	0.46 Rf	111.9 AU	17.15 %	0.50 Rf	42.5 AU	3131.6 AU	9.51 %
6	0.50 Rf	42.6 AU	0.51 Rf	54.2 AU	8.30 %	0.57 Rf	28.7 AU	1941.2 AU	5.90 %
7	0.59 Rf	30.6 AU	0.78 Rf	206.8 AU	31.71 %	0.94 Rf	38.4 AU	20099.5 AU	61.04 %
8	0.95 Rf	37.8 AU	0.97 Rf	44.6 AU	6.83 %	0.99 Rf	3.9 AU	1101.8 AU	3.35 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.1 AU	0.08 Rf	84.4 AU	8.48 %	0.11 Rf	17.8 AU	2008.3 AU	5.16 %
2	0.11 Rf	18.1 AU	0.14 Rf	28.8 AU	2.89 %	0.14 Rf	28.4 AU	442.0 AU	1.14 %
3	0.14 Rf	27.4 AU	0.18 Rf	163.2 AU	16.40 %	0.25 Rf	0.4 AU	5896.6 AU	15.16 %
4	0.25 Rf	0.1 AU	0.30 Rf	74.4 AU	7.48 %	0.32 Rf	24.3 AU	1571.0 AU	4.04 %
5	0.32 Rf	24.6 AU	0.37 Rf	125.6 AU	12.63 %	0.41 Rf	36.1 AU	3706.9 AU	9.53 %
6	0.41 Rf	36.2 AU	0.46 Rf	197.5 AU	19.85 %	0.50 Rf	57.3 AU	5913.3 AU	15.21 %
7	0.50 Rf	57.4 AU	0.52 Rf	65.1 AU	6.54 %	0.58 Rf	4.4 AU	1967.0 AU	5.06 %
8	0.59 Rf	5.1 AU	0.79 Rf	224.5 AU	22.57 %	0.95 Rf	12.9 AU	16820.7 AU	43.26 %
9	0.95 Rf	13.2 AU	0.97 Rf	31.3 AU	3.15 %	0.99 Rf	12.5 AU	557.2 AU	1.43 %

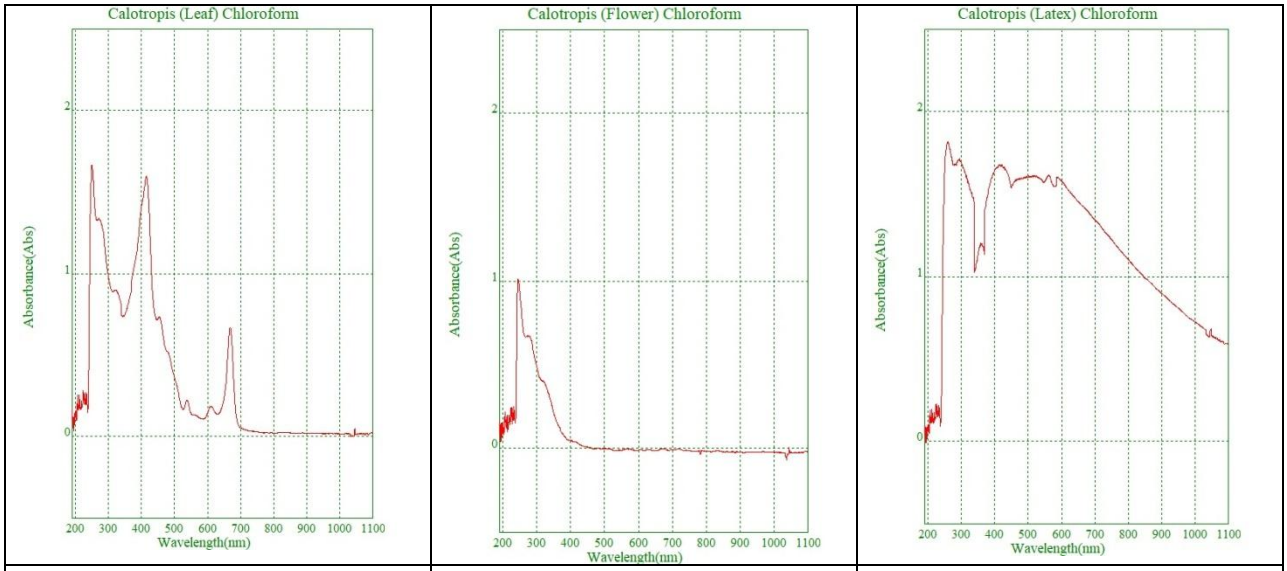
At 575 nm after derivatisation

Fig. 7: HPTLC finger print profile of 5 µl and 10 µl of chloroform extract of *Calotropis gigantea* (latex defatted) at 254 nm, 366 nm and at 575 nm after derivatisation.

The HPTLC fingerprinting patterns of chloroform extracts of the leaf, flower and latex of *C. gigantea* was developed at 254nm, 366nm and at 575nm after derivatisation with vanillin – sulphuric acid. HPTLC finger print showed prominent peaks in all the three plant parts ie., leaf, flower and latex. It was found from the HPTLC photo documentation images that all sample constituents were clearly separated without any tailing and diffuseness. The solvent system- Toluene: Ethyl acetate: Formic acid (5: 1: 0.1) effectively resolved the chemical constituents in the chloroform extract of leaf of *C. gigantea*. HPTLC photo documentation profile of the chloroform extract of leaf of the plant at 254nm, 366nm and after derivatisation is given in Fig. 2 and the finger printing profile and the R_f value and percentage area of the peaks are shown in Fig. 3. On observation 9 bands were appeared under short UV with R_f 0.10, 0.13, 0.18, 0.31, 0.44, 0.57, 0.63, 0.70 and 0.90. Out of which R_f value at 0.63 has the maximum area 25.61% indicating the presence of highest concentration of the phytoconstituents. TLC pattern at 366nm showed 10 bands at R_f value 0.05, 0.10, 0.33, 0.43, 0.56, 0.64, 0.68, 0.73, 0.85 and 0.93. Out of these, peak at R_f 0.56 was found to be more prominent. Similarly the peak at R_f 0.70 was appeared more prominent after derivatisation. This implies that these chemical constituents were present in significant quantity in the crude extract. The study of chloroform extract of the flower of *C. gigantea* showed best result in the solvent system- Toluene: Chloroform (5: 2). The HPTLC results shown in the Figure 4 & 5 revealed the presence of 8 bands at 254 nm, 7 bands at 366 nm and 9 bands at 575 nm after derivatisation. Each band indicates the presence of phytoconstituent present in the extract. The peak at R_f 0.87 was appeared more prominent in 254 nm and 366 nm with the percentage area 84.85 % and 52.95 % respectively indicating the presence of highest concentration of the phytoconstituents. In the case of latex of *C. gigantea*, the solvent system- Toluene: Ethyl acetate: Methanol (4: 8: 1) gave more clear separation for the chloroform extract even though so many systems were tried. After scanning and visualizing the plates in absorbance mode at 254nm, 366 nm and 575 nm after spraying with vanillin- sulphuric acid reagent, more phytoconstituents were shown at 575 nm. The results obtained from HPTLC finger print scanned at wavelength 575 nm revealed the presence of nine diffrent phytoconstituents with the R_f values ranged from 0.06 to 0.97 with different colours. It is evident from the figures (Figures 6 & 7) that out of nine components, the components with R_f values 0.18, 0.46 and 0.79 were found to be more predominant as the percentage area is more with 15.16%, 15.21% and 43.26 % respectively[18]. The developed chromatogram and R_f values of the extracts are specific with the selected solvent systems.

Ultra Violet-Visible (UV-Vis) Spectroscopy

The UV-VIS spectrum of chloroform extract of the leaf, flower and latex of *C. gigantea* are shown in Fig. 8. The qualitative UV-VIS spectrum of the extract was recorded from wavelength 200 to 1100 nm. These typical spectra can be considered as unique for the chloroform extract of leaf, flower and latex of *C. gigantea*.



Leaf	Flower	Latex

Fig. 8: Ultra Violet-Visible Spectrum of chloroform extract of leaf, flower and latex of *C. gigantean.*

CONCLUSION

The plant *Calotropis gigantea* is having important role in the Siddha system of medicine. In the present study, leaf, flower and latex of *C. gigantea* were thoroughly investigated to analyze their quality, safety and standardization for their use. The different physico-chemical parameters, the developed HPTLC chromatogram and UV-Vis spectrum obtained from this study help in the correct identification and authentication of these medicinal plant materials and may help in preventing its adulteration. HPTLC fingerprint helps in the proper identification and quality control of the parts of the plant and also provides semi quantitative information about the major active phytoconstituents present in the plant extracts. Thus, the present study provides sufficient information about phytoconstituents present in the chloroform extracts of leaf, flower and latex of *C. gigantea* and also in the identification, standardization and quality control of these medicinal plant materials. These data can also be considered along with the other parameters for fixing standards to leaf, flower and latex of *C. gigantea*.

ACKNOWLEDGEMENT

Authors are grateful to Prof. (Dr.) K. Kanakavalli, Director General, Central Council for Research in Siddha for providing necessary facilities to carry out the work.

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