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Original Article

PHARMACOGNOSTIC AND HPTLC BASED COMPARATIVE STUDY ON LEAVES OF MERREMIA EMARGINATA BURM. F. AND CENTELLA ASIATICA (L.) URBAN

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ABSTRACT

Objective: In this study, an attempt was made to generate information based on botanical, physicochemical and HPTLC data needed for proper identification and authentication of *M. emarginata* and *C. asiatica* belonging to two different families.

Methods: Botanical study comprises of macroscopy, microscopy and powder microscopy of leaves of both crude drugs. The physicochemical parameters such as water-soluble extractive, alcohol soluble extractive and loss on drying at 105□, total ash, acid insoluble ash, and volatile oil were determined according to standard methods. HPTLC studies of chloroform extracts of leaves of both drugs were conducted at 254 nm, 366 nm and 575 nm after derivatisation using vanillin-sulphuric acid and the results were documented.

Results: The present study reveals that microscopy and most of the physicochemical parameters of both the plant materials are different. Anatomy of the leaves showed two main characteristic differences. First plenty of trichome with trichome base and calcium oxalate crystal is common in *M. emarginata,* which is not observed in *C. asiatica*. Both plants have different venation patterns and leaf constants. The total ash content and the solubility in alcohol and water for leaves of *C. asiatica* are higher than that of *M. emarginata*. The HPTLC fingerprinting pattern obtained for both drugs are different.

Conclusion: All the results obtained from this study help in determining differences and similarities of leaves of *M. emarginata and C. asiatica* and thereby preventing adulteration and substitution and emphasizing the importance of standardization.

Keywords: *Merremia emarginata*, *Centella asiatica*, Morphological characters, Microscopy, Physico-chemical analysis and HPTLC fingerprinting

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INTRODUCTION

Merremia emarginata Burm. F (Convolvulaceae) and *Centella asiatica* (L.) (Apiaceae) are two important drugs mentioned in Siddha system of medicine. *M. emarginata* and *C. asiatica* are perennial, much-branched creepers. *M. emarginata* is also known as *Ipomoea renifomis* and it is widely distributed all over India, especially in damp places and railway tracks. *C. asiatica* is locally known as Vallarai. It is found throughout India growing in moist places up to an altitude of 1800 m. About 20 species grow in most parts of the tropic or wet pantropical areas such as rice paddies, and also in rocky, higher elevations [1]. These plants have different vernacular names [2-3] (table 1).

Both plants have been used as a medicine in India since ancient time. *M. emarginata* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of the nose, and fever due to enlargement of the liver and also for treating cancer. *C. asiatica* widely known as Brahmi has been used as a rasayana (adaptogenic) drug since ancient time for enhancing cognitive function by revitalizing the nerve and brain cells. It has been used for revitalizing the nerves and brain cells, hence primarily known as a "Brain food" or "Memory enhancer"in India [4]. It is commonly known as mandukparni or Indian pennywort or jalbrahmi [5]. In China, known as gotukola, it is one of the reported "miracle elixirs of life" known over 2000 y ago [6].

Both plants resemble externally. The similar morphological characteristics of the leaves of both herbs with subtle differences lead to substitution. These are also frequently adulterated or substituted with other species. In this study an attempt was made to generate information based on botanical, physicochemical and HPTLC data needed for proper identification and authentication of *M. emarginata* and *C. asiatica* belonging to two different families.

Table 1: Vernacular name of *M. emarginata and C. asiatica*

S. No.	Regional language	*M. emarginata*	*C. asiatica*
1	Tamil	Elikkathilai	Vallarai
2	Malayalam	Elichevi	Kudangal
3	Sanscrit	Ākhuparni	Mandukaparni
4	Kannada	Althigida	Brahmisoppu
5	Hindi	Muskani	Ballari

MATERIALS AND METHODS

(A). Plant material

The fresh leaves of *M. emarginata* and *C. asiatica* were collected and authenticated. The plant materials were cut, crushed dried, and kept in airtight containers and used for all other experimental purposes. The fresh samples were used for anatomical studies.

(B). Macroscopy

Macroscopic characters of leaves of *M. emarginata* and *C. asiatica* were recorded systematically. The arrangement, size, shape, base,

texture, margin, apex, venation, colour, odour, taste of leaves were observed. Macroscopic characters were studied [7].

(C). Microscopy

Microscopic studies of both plant materials were carried out by preparing thin sections of leaves. The thin sections were further washed with water, stained with safranin and mounted in glycerine for observation [8].

(D). Powder microscopy

The powder microscopy of the powered leaves of both drugs was studied using standard procedure [9] by capturing the images of different fragments of tissues and diagnostic characteristic features were recorded.

(E). Determination of leaf constants

A number of leaf measurements are used to distinguish some closely related species not easily characterized by general microscopy. Stomatal number, stomatal index, palisade ratio, trichome number, vein islet and veinlet termination of leaves of both plants were observed under 4X, 20X objective of the microscope as per standard protocol.

(F). Physico-chemical study

The physicochemical parameters such as ash content, acid insoluble ash, volatile oil, solubility in water and alcohol, loss on drying at 105˚C and foreign matter were determined as per WHO guidelines [10].

(G). High-performance thin layer chromatographic analysis (HPTLC)

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials and is the simplest separation technique today available to the analyst [11]

a. Preparation of extract of the drug materials for HPTLC analysis

4 gm each of the dried and powdered leaves of *M. emarginata and C. asiatica* was soaked in 40 ml chloroform at room temperature for overnight. The contents were filtered through separate filter papers and each filtrate was concentrated on a water bath to 4 ml. These extracts were used for chromatographic studies [12].

b. Development of HPTLC

4 µl and 8 µl of chloroform extract of each plant material were spotted in the form of bands with Camag microliter syringe on a pre-coated silica gel 60 F_{254} (Merck) plates with Automatic TLC Sampler 4 (ATS4). Mobile phase selected for the study was Toluene: Ethyl acetate: Formic acid (6: 2: 0.1). Linear ascending development was done in twin trough glass chamber saturated with the specified mobile phase. The plate was air dried and kept under UV 254 nm and 366 nm and white light after derivatization using vanillin-sulphuric acid reagent and photo documentation were done. The plates were scanned at UV 254 nm, 366 nm and 575 nm after derivatization using TLC Scanner 4 with win CATS software for interpretation of data.

Fig. 1: Habit (a*) M. emarginata,* (b) *C. Asiatica*

RESULTS AND DISCUSSION

(A). Macroscopic characters

Morphology of leaves of *M. emarginata* and *C. asiatica* are discussed below *M. emarginata* is sometimes confused with *C. asiatica* which has the same habit. (fig. 1.) These are clonal perennial and prostrate. *M. emarginata* has slender stems 30-75 cm long that usually form roots at the nodes. Leaves alternate, reniform to broadly ovate, 0.3-3 cm long. Often slightly wider, cordate with broadly rounded sinus and a basal lobes entire, glabrous. Yellow flowers with very short peduncle, capsular, fruits with black seeds. Habitat is in open grasslands and fields, along railroads and waste places.

C. asiatica is faintly aromatic, stoloniferous, creeper herb, flourishes extensively in shady, marshy, damp and wet places such as paddy fields, river banks forming a dense green carpet**.** Leaves of *C. asiatica* are cordate or hastate, 1-3 from each node of stems, long petioled, 2-6 cm long and 1.5-5 cm wide, orbicular-reniform, sheathing leaf base, crenate margins, glabrous on both sides, leaf blades are, crenate with thick radiate veins, pink flowers with umbel inflorescence. Fruits of *C. asiatica* are compressed mericarps. Using this distinction, the plant material with small globose fruit capsules was identified as *M. emarginata* [13]. The leaves of *M. emarginata* are consumable as pot-herb [14]. In Salem district it is consumed in the name of *Vallarai* with the consumers expecting the benefits of *C. asiatica*.

(B). Microscopy

The T. S of *M. emarginata* and *C. asiatica* leaves were differentiated in to epidermis, mesophyll and vascular tissues. In *M. emarginata* the epidermal layer consists of semi-circular, thin walled cells with thick cuticle on the flat outer tangential wall. But in *C. asiatica* both epidermises were uniseriate, composed of compactly arranged rectangular cells with moderately striated outer walls. Cuticle appeared either completely absent or poorly developed in *C. asiatica.* Non-cuticular striated epidermis which may facilitate the steady absorption of water from the surrounding [15]. Beneath the epidermis of the *M. emarginata* small cluster of collenchymatous cells is located in the adaxial conical part. In *C. asiatica* abaxial epidermis contained a patch or band of sclerenchymatous tissues. Anomocytic stomata were embedded throughout the upper and lower epidermis of *M. emarginata* but paracytic in *C. asiatica.* Many of which contain crystals of calcium oxalate. In *C. asiatica* mesophyll having a compact palisade parenchyma with one layer of elongated, and barrel shaped cells and

oval to rectangular spongy parenchyma cells with wide intercellular spaces. A parenchymatous bundle sheath was encircled the vascular strand. In *M. emarginata* 2 to 3 layers of elongated palisade cells and spongy mesophyll with loosely arranged cells. Bicollateral bowl shaped vascular bundle. In both cells of the mesophyll were found filled with plenty of chloroplasts (fig. 3).

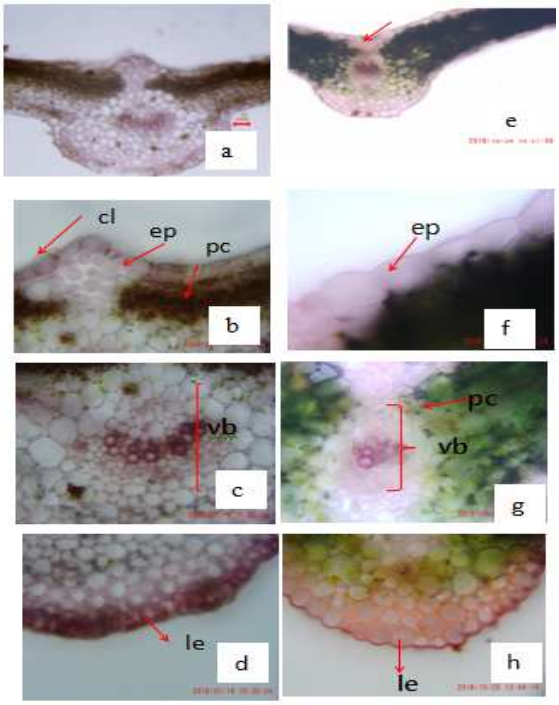


Fig. 2:T. S of Leaf (1) *M. emarginata* (a–d) (2*) C. asiatica* (e-h), aande: portion enlarged, b: Cuticle (cl), epidermis (ep), palisade cell (pc), c: vascular bundle (vb), dandh: lower epidermis (le), f: epidermis (ep), g: vascular bundle (vb), palisade cell (pc)

T. S of *M. emarginata* petiole was semi-circular on the abaxial side and the adaxial side has wide shallow concavity. Petiole of *C. asiatica* had a pentagonal shape and dorsiventeral differentiation. Trichome with thin epidermal layer was followed by collenchyma and parenchymatous ground tissue in M. *emarginata.* In *C. asiatica* trichomes are absent and a prominent enteral hollow core of air canal present. Bicollateral bundles are centrally located in *M. emarginata.* But in *C. asiatica*five vascular bundles were arranged in the corner. Vascular tissues form a continuous cylinder with a periphloematicsclereids arch over each vascular strand. Crystal idioblasts were embedded in the cortical parenchyma of the *C. asiatica* petiole (fig. 4).

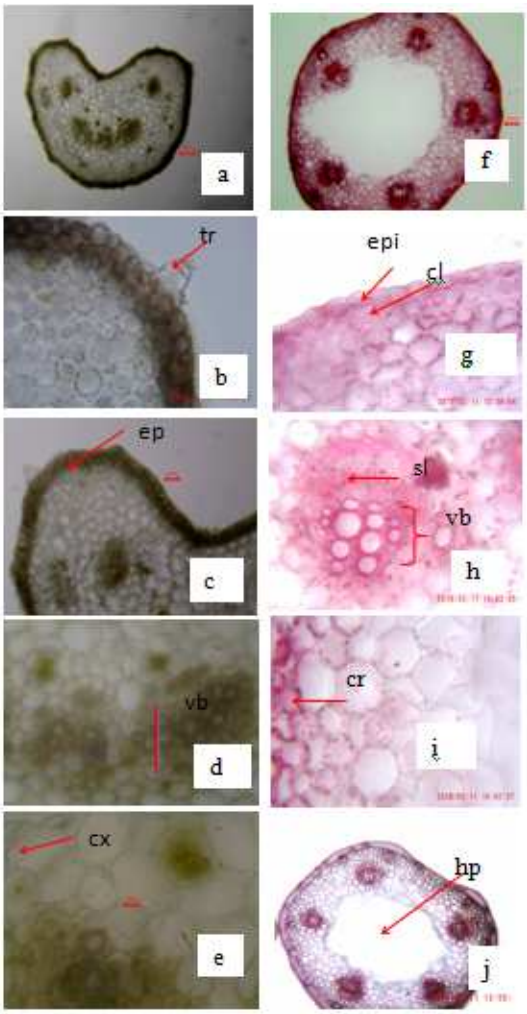
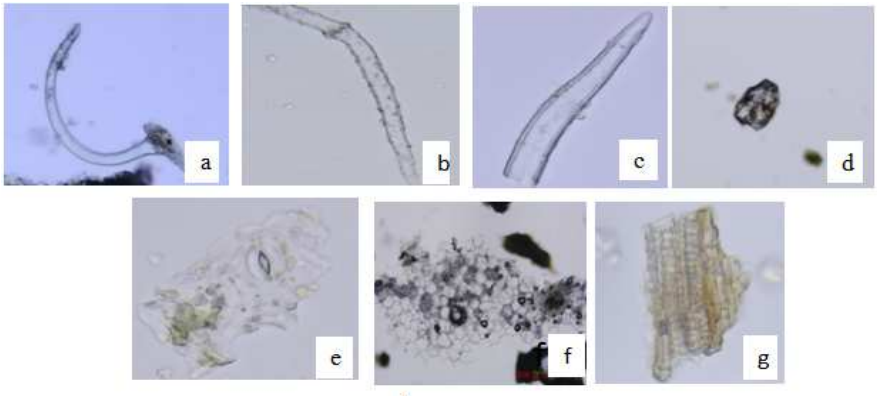


Fig. 3: T. S of Petiole (1) *M. emarginata* (a–e) (2*) C. asiatica* (f-j), aandf: enlarged portion, b: trichome (tr), c: epidermis (ep), d: vascular bundle, e: cortex (cx), g: epidermis (ep), collenchyma (cl), h: vascular bundle (vb), sclereids (sl), i: crystal (cr), j: hollow pith

(C). Powder microscopy

Powder microscopy of leaves of *M. emarginata* and *C. asiatica* showed in fig. 5**.** *M. emarginata* and *C. asiatica* were differentiated into various characters. Different types of trichomes, such as glandular and warty trichomes and anomocytic stomata were noted in *M. emarginata*. Whereas in *C. asiatica,* trichomes were absent and paracytic stomata present. Calcium oxalate crystals present only in *M. emarginata*.



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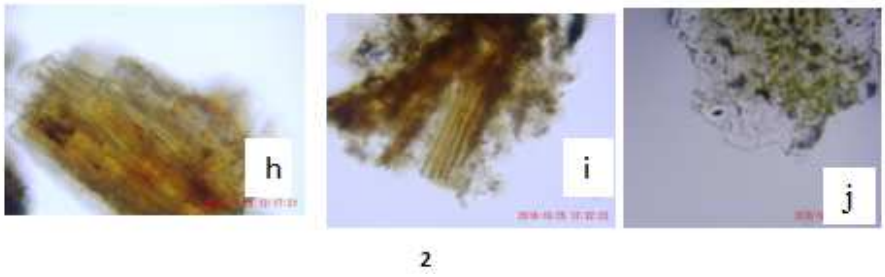


Fig. 4: Powder microscopy (1) *M. emarginata* (a-g) (2) *C. asiatica* (a-c), a-c: trichomes, d: calcium oxalate crystal, e: anomocytic stomata, f: spongy parenchyma with chloroplast, gandi: tracheid with spiral thickening, h: parenchyma cell, j: paracytic stomata

(D). Determination of leaf constants

Leaf constants of *M. emarginata* and *C. asiatica are* showed in fig. 6 and table 2

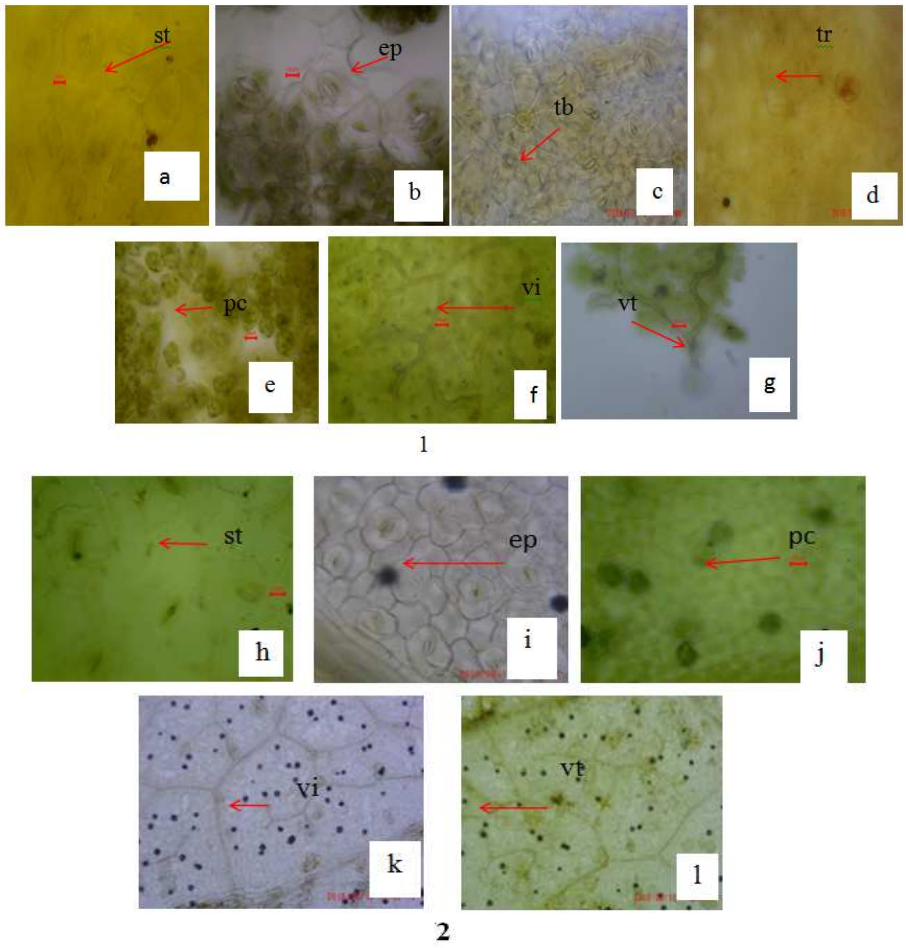


Fig. 5: Leaf constants (1) *M. emarginata* (a–g) (2) *C. asiatica* (h–l), a: anomocytic stomata (st), bandi: epidermis (ep), c: trichome with base (tb), d: trichome, eandj: palisade cell (pc), fandk: vein islet (vi), gandl: veinlet termination (vt), h: paracytic stomata

Table 2: Leaf constants of *M. emarginata* and *C. asiatica*

S. No.	Parameters	*M. emarginata*	*C. asiatica*
1	Stomatal no.	10	10.6
2	Stomatal index	21.4	22.3
3	Palisade ratio	4.1	2
4	Epidermal no.	146.6	69
5	Vein-islet no.	52	20
6	Veinlet termination	42.6	40
7	Trichome no.	14.6	Nil

M. emarginata and *C. asiatica* have some morphological similar characters and different anatomical characters. Comparison of morpho-anatomical characters is showed in the table 3.

Table 3: Comparison of morpho-anatomical characters

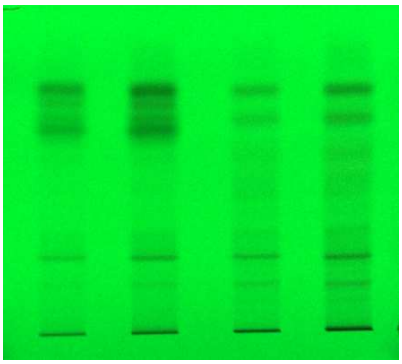
S. No.	Characters	*M. emarginata*	*C. asiatica*
1	Habit	Branched herb (creeper)	Branched herb (creeper)
2	Macroscopy		
	Root	Taproot	Tap root
	Stem	Pubescent, glabrous	Reddish, glabrous
	Leaf	Simple alternate, reniform to broadly ovate	Simple alternate, reniform elongated petiole.
	Flower	Yellow colour	Pink colour
	Inflorescences	Axillary	Umbel
	Fruit	Fruit globes capsule	Cremocarp
3	Microscopy		
	Leaf	Thin-walled cells with thick cuticle. Beneath	Uniseriate, Cuticle absent or poorly developed.
	1. Cuticle	the epidermis	
	2. Epidermis	Compactly arranged rectangular cells.	Compactly arranged rectangular cells.
	3. Mesophyll tissue	2 to 3 layers of elongated palisade cell.	2 layers of barrel-shaped palisade cell.
	3. Vascular bundle	Bicollateral, bowl-shaped vascular bundle.	Bicollateral, parenchymatous bundle sheath was encircled the vascular strand.
	Petiole		
	1. shape	Semi-circular	pentagonal
	2. trichomes	present	absent
	3. pith	parenchymatous	hollow pith
	4. Vascular bundle	5 or 6 in number	5 in number
	Sclereids	Sclereids absent	Periphloematic Sclereids
4	Powder microscopy		
	1. Stomata	Anomocytic	paracytic
	2. Trichomes	Present	absent
	3. Crystal	Calcium oxalate	no

E*).* Physico-chemical analysis

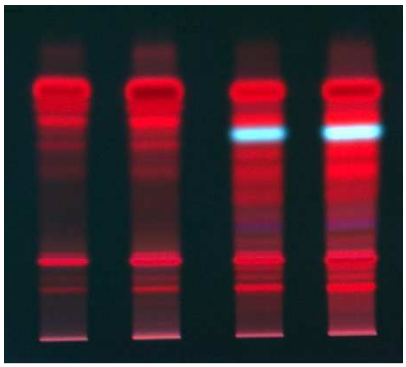
The results of the physicochemical analysis of leaves of *M. emarginata* and *C. asiatica* leaves are pictorially represented in table 4. Total cash value of the plant material indicates the amount of minerals and earthy materials attached to it. Acid-insoluble ash usually represents the amount of silica present as sand and dust and indicates contamination [16]. Loss on drying at 105 °C shows the presence of moisture content and volatile oil in the drug. The water-soluble extractive value indicates the presence of more polar constituents such as tannin, sugar, plant acid, mucilage and glycosides. The alcohol-soluble extractive values indicated the presence of phenols, alkaloids, steroids, glycosides, flavonoids etc. The water-soluble extractive value of leaf *M. emarginata* was 9.36% and that of the *C. asiatica* was 26.30%. The respective alcohol-soluble extractive values (2.26 % and 18.86%) are less when compared to water-soluble extractive values of the plant materials. Presence of volatile oil was not detected in both the plant materials. The study reveals that most of the physicochemical parameters of both plant materials are different. The extractive values in alcohol and water for *C. asiatica* was higher than that of *M. emarginata*. These values are a measure of the quantity of the chemical constituents soluble in the solvents. Ash values are also higher for *C. asiatica* indicating the presence of more inorganics in the leaf than in the leaf of *M. Emarginata.*

Table 4: The physicochemical parameters of *M. emarginata* and *C. asiatica*

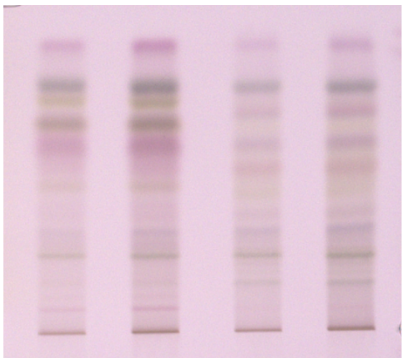
S. No.	Parameters	M. emarginata (leaf)	C. asiatica (leaf)
1	Total ash (%)	9.46	10.38
2	Acid insoluble ash (%)	1.6	2.1
3	Loss on drying (%)	14.82	8.89
4	Water soluble extractive (%)	9.36	26.30
5	Alcohol soluble extractive (%)	2.26	18.86
7	Volatile oil	nil	nil
8	Foreign Matter	<2	<2



Viewed under UV 254



Viewed under UV 366



Viewed under white light after derivatisation using vanillin-sulphuric acid

Fig. 6: HPTLC photo documentation profile of the chloroform extract of leaves of *M*. emarginata and *C. asiatica*. Solvent system: Toluene: Ethyl acetate: Formic acid (6: 2: 0.1)

(F). High-Performance thin layer chromatographic analysis (HPTLC)

HPTLC photo documentation

HPTLC photo documentation profile of the chloroform extracts of both plant materials are given in fig. 6

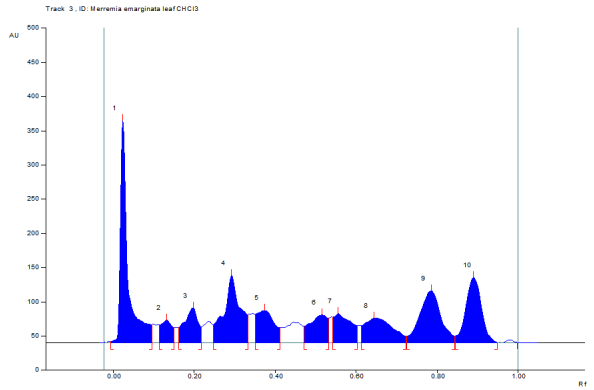
Track 1 and 2: *C. asiatica*; track 3 and 4: *M.* emarginata *(*Track 1 and 3-4 µl each: Track 2 and 4–8 µl each)

R_f values and colour of bands of chloroform extracts of leaves of *M. emarginata* and *C. asiatica* under UV 254, UV 366 and UV 575 nm are represented in the table 5.

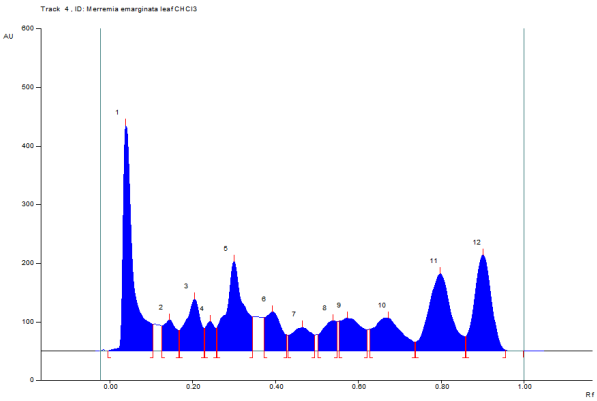
Table 5: R_f values and colour of bands

Name of plant	Wave length					
	254 nm		366 nm		575 nm (after derivatization)	
	R_f values	Colour	R_f values	Colour	R_f values	Colour
C. asiatica (Leaf)	0.18	Light green	0.18	Red	0.09	Light purple
	0.28	Light green	0.22	Brown	0.12	Light purple
	0.74	Dark green	0.29	Brown red	0.26	Light purple
	0.83	Dark green	0.56	Brown	0.35	Light purple
	0.89	Dark green	0.65	Bright red	0.51	Light yellow
	-	-	0.70	Bright red	0.66	Purple
	-	-	0.78	Bright red	0.73	Yellowish green
	-	-	0.85	Bright red	0.82	Dark green
	-	-	0.92	Brown	0.96	purple
M. emarginata (Leaf)	0.13	Light green	0.20	Bright red	0.17	Light purple
	0.20	Light green	0.24	Bright red	0.26	Light green
	0.30	Dark green	0.32	Brown	0.35	Light purple
	0.66	Light green	0.37	Violet	0.49	Light yellow
	0.80	Dark green	0.43	Brown	0.58	Light purple
	0.90	Dark green	0.56	Bright red	0.68	Light yellow
	-	-	0.62	Fluorescent blue	0.79	Dark greenish
	-	-	0.69	Bright red	0.96	Purple
	-	-	0.76	Bright red	-	-
	-	-	0.87	Brown	-	-

The HPTLC fingerprint profile and R_f table of chloroform extract of leaves of *M. emarginata* and *C. asiatica* at UV 254 nm are shown in fig. 7(1) and fig. 7(2) respectively.

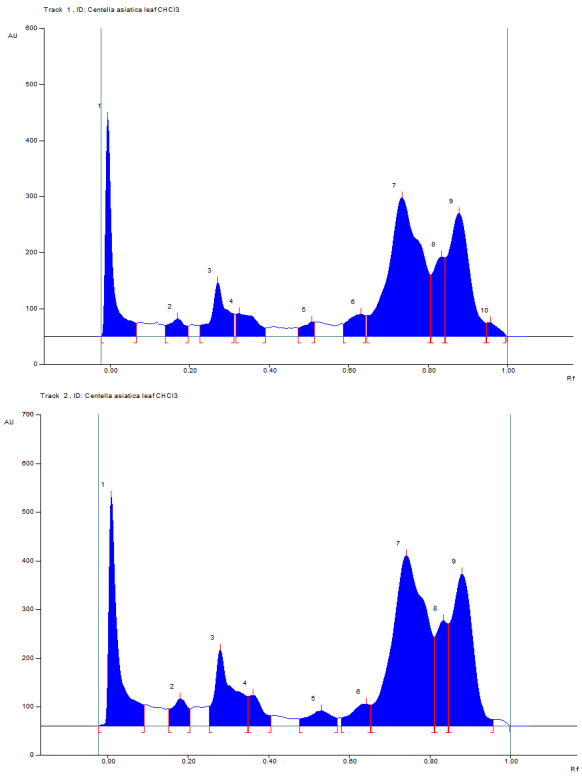


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	1.7 AU	0.03 Rf	324.7 AU	38.46 %	0.10 Rf	26.6 AU	4242.4 AU	21.28 %
2	0.12 Rf	26.3 AU	0.13 Rf	33.0 AU	3.91 %	0.15 Rf	22.5 AU	650.8 AU	3.26 %
3	0.16 Rf	22.2 AU	0.20 Rf	51.0 AU	6.04 %	0.22 Rf	23.3 AU	1181.5 AU	5.93 %
4	0.25 Rf	26.4 AU	0.29 Rf	98.0 AU	11.61 %	0.34 Rf	41.2 AU	2806.9 AU	14.08 %
5	0.35 Rf	41.9 AU	0.38 Rf	47.2 AU	5.59 %	0.41 Rf	22.1 AU	1453.0 AU	7.29 %
6	0.47 Rf	24.0 AU	0.52 Rf	40.7 AU	4.82 %	0.53 Rf	36.0 AU	1254.0 AU	6.29 %
7	0.54 Rf	37.4 AU	0.56 Rf	42.5 AU	5.04 %	0.61 Rf	25.3 AU	1310.1 AU	6.57 %
8	0.62 Rf	25.2 AU	0.64 Rf	36.3 AU	4.29 %	0.73 Rf	9.4 AU	1748.5 AU	8.77 %
9	0.73 Rf	9.4 AU	0.79 Rf	75.9 AU	8.99 %	0.85 Rf	9.7 AU	2706.1 AU	13.57 %
10	0.85 Rf	9.9 AU	0.89 Rf	95.1 AU	11.26 %	0.95 Rf	0.2 AU	2582.2 AU	12.95 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	0.5 AU	0.04 Rf	385.6 AU	29.76 %	0.10 Rf	45.4 AU	6187.8 AU	18.76 %
2	0.13 Rf	42.5 AU	0.14 Rf	52.6 AU	4.06 %	0.17 Rf	35.1 AU	1164.7 AU	3.53 %
3	0.17 Rf	35.3 AU	0.20 Rf	88.4 AU	6.82 %	0.23 Rf	38.8 AU	2117.6 AU	6.42 %
4	0.23 Rf	38.9 AU	0.24 Rf	50.5 AU	3.90 %	0.26 Rf	38.9 AU	799.6 AU	2.42 %
5	0.26 Rf	39.8 AU	0.30 Rf	153.4 AU	11.83 %	0.34 Rf	58.2 AU	4389.7 AU	13.31 %
6	0.37 Rf	56.7 AU	0.39 Rf	66.6 AU	5.14 %	0.43 Rf	27.5 AU	1779.3 AU	5.39 %
7	0.43 Rf	26.8 AU	0.47 Rf	40.3 AU	3.11 %	0.50 Rf	27.3 AU	1319.7 AU	4.00 %
8	0.50 Rf	27.7 AU	0.54 Rf	51.5 AU	3.97 %	0.55 Rf	50.1 AU	1249.3 AU	3.79 %
9	0.56 Rf	50.8 AU	0.57 Rf	55.6 AU	4.29 %	0.62 Rf	36.0 AU	2037.5 AU	6.18 %
10	0.63 Rf	37.0 AU	0.67 Rf	56.1 AU	4.33 %	0.74 Rf	14.8 AU	2584.6 AU	7.84 %
11	0.74 Rf	15.0 AU	0.80 Rf	131.6 AU	10.15 %	0.86 Rf	24.3 AU	4840.5 AU	14.67 %
12	0.86 Rf	24.7 AU	0.90 Rf	163.8 AU	12.64 %	0.96 Rf	1.6 AU	4514.4 AU	13.69 %

Fig. 7 (1): Fingerprint profile and R_f table of chloroform extract of *M. emarginata* (leaf) at 254 nm

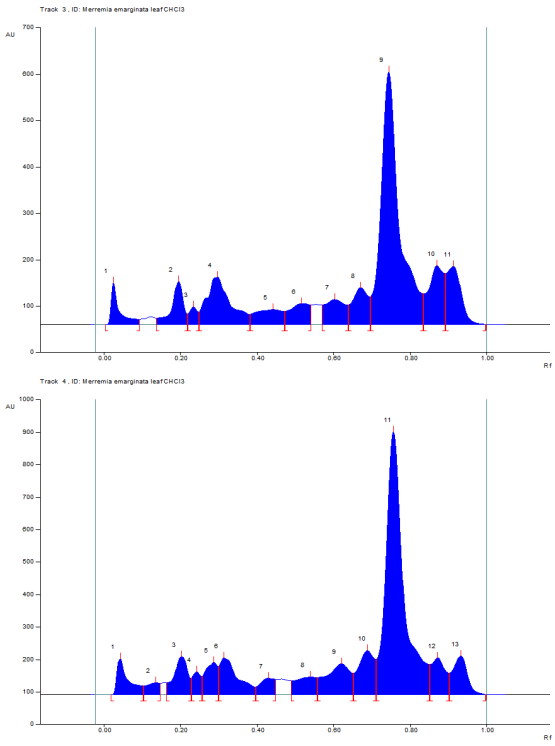


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.8 AU	-0.00 Rf	389.4 AU	30.97 %	0.07 Rf	23.6 AU	4804.0 AU	13.30 %
2	0.14 Rf	19.5 AU	0.17 Rf	31.1 AU	2.47 %	0.20 Rf	18.0 AU	847.5 AU	2.35 %
3	0.23 Rf	20.2 AU	0.27 Rf	95.7 AU	7.61 %	0.31 Rf	40.2 AU	2456.9 AU	6.80 %
4	0.32 Rf	40.2 AU	0.33 Rf	41.0 AU	3.26 %	0.39 Rf	15.4 AU	1422.2 AU	3.94 %
5	0.47 Rf	14.9 AU	0.51 Rf	26.1 AU	2.07 %	0.51 Rf	25.2 AU	519.8 AU	1.44 %
6	0.59 Rf	21.9 AU	0.63 Rf	39.6 AU	3.15 %	0.64 Rf	37.3 AU	1138.8 AU	3.15 %
7	0.65 Rf	36.8 AU	0.74 Rf	248.0 AU	19.73 %	0.81 Rf	09.3 AU	13597.0 AU	37.65 %
8	0.81 Rf	109.3 AU	0.83 Rf	142.0 AU	11.29 %	0.84 Rf	40.6 AU	2871.1 AU	7.95 %
9	0.85 Rf	140.7 AU	0.88 Rf	219.7 AU	17.47 %	0.95 Rf	23.8 AU	7958.6 AU	22.04 %
10	0.95 Rf	23.9 AU	0.96 Rf	24.6 AU	1.96 %	1.00 Rf	2.9 AU	495.2 AU	1.37 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.1 AU	0.01 Rf	470.6 AU	27.62 %	0.09 Rf	43.3 AU	7592.5 AU	14.17 %
2	0.15 Rf	34.9 AU	0.18 Rf	56.1 AU	3.30 %	0.20 Rf	35.6 AU	1418.6 AU	2.65 %
3	0.25 Rf	40.4 AU	0.28 Rf	157.3 AU	9.23 %	0.35 Rf	61.0 AU	4896.1 AU	9.14 %
4	0.35 Rf	61.3 AU	0.36 Rf	63.1 AU	3.70 %	0.41 Rf	21.0 AU	1531.5 AU	2.86 %
5	0.48 Rf	15.0 AU	0.53 Rf	31.7 AU	1.86 %	0.57 Rf	16.3 AU	1307.4 AU	2.44 %
6	0.58 Rf	17.0 AU	0.64 Rf	45.4 AU	2.67 %	0.65 Rf	43.1 AU	1438.8 AU	2.69 %
7	0.65 Rf	43.2 AU	0.74 Rf	350.3 AU	20.56 %	0.81 Rf	82.9 AU	19839.0 AU	37.03 %
8	0.81 Rf	183.2 AU	0.83 Rf	216.4 AU	12.70 %	0.85 Rf	10.1 AU	4312.3 AU	8.05 %
9	0.85 Rf	210.5 AU	0.88 Rf	312.7 AU	18.35 %	0.96 Rf	13.3 AU	11245.3 AU	20.99 %

Fig. 7(2): Fingerprint profile and R_f table of chloroform extract of *C. asiatica* (leaf) at 254 nm

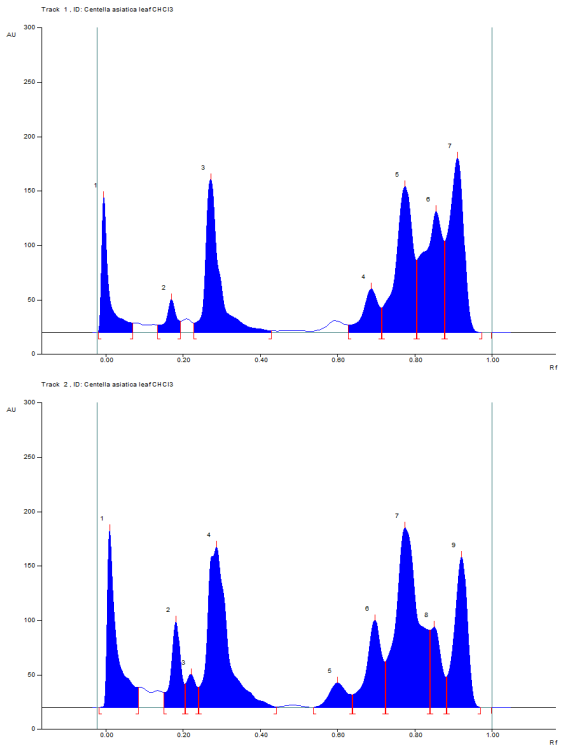
The HPTLC fingerprint profile and R_f table of chloroform extract of leaves of *M. emarginata* and *C. asiatica* at UV 366 nm are represented in fig. 8(1) and fig. 8(2) respectively.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	90.4 AU	6.79 %	0.09 Rf	11.3 AU	1344.8 AU	3.32 %
2	0.14 Rf	13.8 AU	0.19 Rf	92.4 AU	6.95 %	0.22 Rf	22.6 AU	1895.2 AU	4.67 %
3	0.22 Rf	22.8 AU	0.23 Rf	37.9 AU	2.85 %	0.25 Rf	26.9 AU	560.4 AU	1.38 %
4	0.25 Rf	27.4 AU	0.30 Rf	102.0 AU	7.67 %	0.38 Rf	22.1 AU	4378.7 AU	10.80 %
5	0.38 Rf	22.3 AU	0.44 Rf	32.3 AU	2.43 %	0.47 Rf	28.4 AU	1602.1 AU	3.95 %
6	0.47 Rf	28.5 AU	0.52 Rf	45.4 AU	3.41 %	0.54 Rf	42.5 AU	1621.9 AU	4.00 %
7	0.57 Rf	42.6 AU	0.61 Rf	53.9 AU	4.05 %	0.64 Rf	42.1 AU	1958.4 AU	4.83 %
8	0.64 Rf	42.3 AU	0.67 Rf	79.6 AU	5.98 %	0.70 Rf	59.7 AU	2240.2 AU	5.53 %
9	0.70 Rf	60.3 AU	0.75 Rf	544.3 AU	40.91 %	0.83 Rf	66.6 AU	18166.8 AU	44.80 %
10	0.84 Rf	66.6 AU	0.87 Rf	126.6 AU	9.52 %	0.89 Rf	09.9 AU	3520.0 AU	8.68 %
11	0.89 Rf	110.1 AU	0.92 Rf	125.7 AU	9.44 %	1.00 Rf	0.1 AU	3258.1 AU	8.04 %

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	162.6 AU	18.08 %	0.09 Rf	18.5 AU	2800.1 AU	11.59 %
2	0.15 Rf	13.8 AU	0.18 Rf	78.7 AU	8.74 %	0.20 Rf	21.6 AU	1285.8 AU	5.32 %
3	0.21 Rf	21.8 AU	0.22 Rf	30.6 AU	3.40 %	0.24 Rf	18.6 AU	525.9 AU	2.18 %
4	0.24 Rf	18.7 AU	0.29 Rf	147.8 AU	16.43 %	0.44 Rf	0.6 AU	5333.5 AU	22.07 %
5	0.54 Rf	0.2 AU	0.60 Rf	22.7 AU	2.53 %	0.64 Rf	11.9 AU	720.8 AU	2.98 %
6	0.64 Rf	12.0 AU	0.70 Rf	80.0 AU	8.90 %	0.73 Rf	42.0 AU	2205.4 AU	9.13 %
7	0.73 Rf	42.4 AU	0.78 Rf	165.2 AU	18.37 %	0.84 Rf	71.2 AU	6700.1 AU	27.73 %
8	0.84 Rf	71.2 AU	0.85 Rf	73.9 AU	8.21 %	0.88 Rf	27.8 AU	1440.3 AU	5.96 %
9	0.88 Rf	28.2 AU	0.92 Rf	138.1 AU	15.36 %	0.97 Rf	0.1 AU	3149.5 AU	13.04 %

Fig. 8(1): Fingerprint profile and R_f table of chloroform extract of *M. emarginata* (leaf) at 366 nm

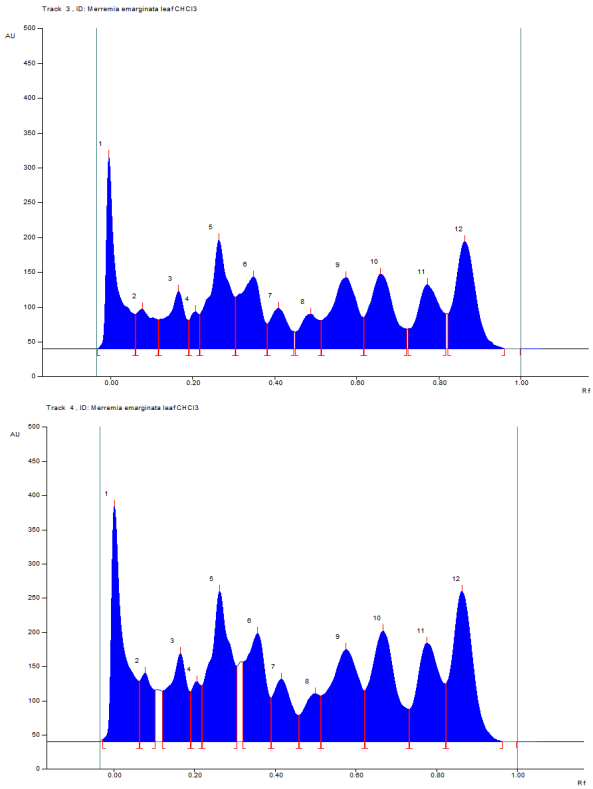


Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.02 Rf	0.4 AU	-0.00 Rf	124.1 AU	16.74 %	0.07 Rf	8.9 AU	1663.1 AU	8.86 %
2	0.14 Rf	6.9 AU	0.17 Rf	30.6 AU	4.13 %	0.19 Rf	10.6 AU	538.4 AU	2.87 %
3	0.23 Rf	8.8 AU	0.27 Rf	140.7 AU	18.98 %	0.43 Rf	1.5 AU	3501.8 AU	18.65 %
4	0.63 Rf	6.8 AU	0.69 Rf	40.1 AU	5.41 %	0.71 Rf	22.5 AU	1126.5 AU	6.00 %
5	0.72 Rf	22.7 AU	0.78 Rf	134.2 AU	18.10 %	0.81 Rf	66.4 AU	4009.1 AU	21.35 %
6	0.81 Rf	66.5 AU	0.86 Rf	111.2 AU	15.00 %	0.88 Rf	84.0 AU	3777.2 AU	20.12 %
7	0.88 Rf	84.1 AU	0.91 Rf	160.5 AU	21.65 %	0.98 Rf	0.1 AU	4157.9 AU	22.15 %

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	162.6 AU	18.08 %	0.09 Rf	18.5 AU	2800.1 AU	11.59 %
2	0.15 Rf	13.8 AU	0.18 Rf	78.7 AU	8.74 %	0.20 Rf	21.6 AU	1285.8 AU	5.32 %
3	0.21 Rf	21.8 AU	0.22 Rf	30.6 AU	3.40 %	0.24 Rf	18.6 AU	525.9 AU	2.18 %
4	0.24 Rf	18.7 AU	0.29 Rf	147.8 AU	16.43 %	0.44 Rf	0.6 AU	5333.5 AU	22.07 %
5	0.54 Rf	0.2 AU	0.60 Rf	22.7 AU	2.53 %	0.64 Rf	11.9 AU	720.8 AU	2.98 %
6	0.64 Rf	12.0 AU	0.70 Rf	80.0 AU	8.90 %	0.73 Rf	42.0 AU	2205.4 AU	9.13 %
7	0.73 Rf	42.4 AU	0.78 Rf	165.2 AU	18.37 %	0.84 Rf	71.2 AU	6700.1 AU	27.73 %
8	0.84 Rf	71.2 AU	0.85 Rf	73.9 AU	8.21 %	0.88 Rf	27.8 AU	1440.3 AU	5.96 %
9	0.88 Rf	28.2 AU	0.92 Rf	138.1 AU	15.36 %	0.97 Rf	0.1 AU	3149.5 AU	13.04 %

Fig. 8(2): Fingerprint profile and R_f table of chloroform extract of *C. asiatica* (leaf) at 366 nm

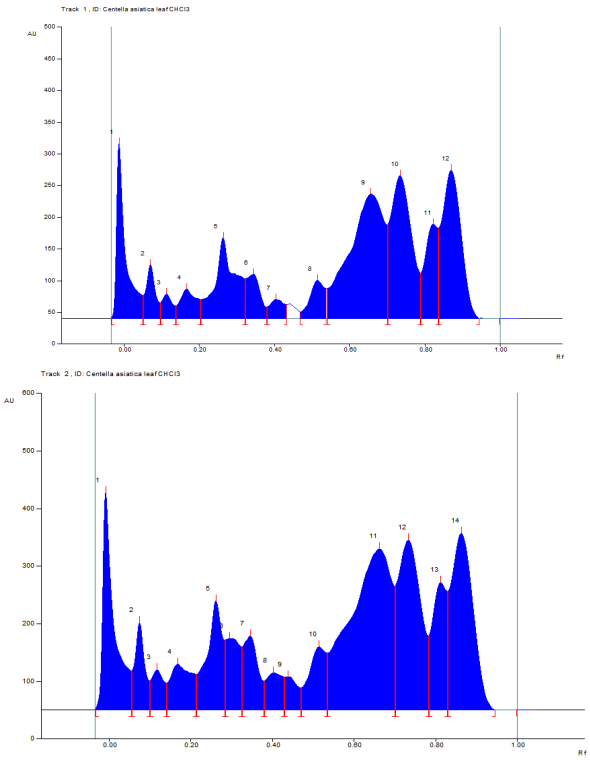
The HPTLC fingerprint profile and R_f table of chloroform extract of leaves of *M. emarginata* and *C. asiatica* at UV 575 nm after derivatization with vanillin-sulphuric acid are represented in fig. 9(1) and fig. 9(2) respectively.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	0.2 AU	-0.00 Rf	276.0 AU	21.36 %	0.06 Rf	49.5 AU	4881.2 AU	12.44 %
2	0.06 Rf	49.7 AU	0.08 Rf	57.1 AU	4.42 %	0.12 Rf	41.8 AU	1664.6 AU	4.24 %
3	0.12 Rf	41.9 AU	0.17 Rf	82.6 AU	6.39 %	0.19 Rf	40.8 AU	2443.7 AU	6.23 %
4	0.19 Rf	41.1 AU	0.21 Rf	53.1 AU	4.11 %	0.22 Rf	49.6 AU	789.2 AU	2.01 %
5	0.22 Rf	50.0 AU	0.26 Rf	156.2 AU	12.09 %	0.31 Rf	74.4 AU	5044.6 AU	12.85 %
6	0.31 Rf	74.7 AU	0.35 Rf	103.4 AU	8.01 %	0.38 Rf	36.0 AU	3624.7 AU	9.23 %
7	0.38 Rf	36.2 AU	0.41 Rf	58.4 AU	4.52 %	0.45 Rf	24.9 AU	1752.2 AU	4.46 %
8	0.45 Rf	24.5 AU	0.49 Rf	49.9 AU	3.86 %	0.51 Rf	40.8 AU	1526.8 AU	3.89 %
9	0.51 Rf	40.9 AU	0.57 Rf	102.2 AU	7.91 %	0.62 Rf	45.2 AU	4428.6 AU	11.28 %
10	0.62 Rf	45.6 AU	0.66 Rf	107.0 AU	8.28 %	0.72 Rf	28.6 AU	4236.8 AU	10.79 %
11	0.73 Rf	28.8 AU	0.77 Rf	92.2 AU	7.14 %	0.82 Rf	50.6 AU	3498.1 AU	8.91 %
12	0.82 Rf	50.4 AU	0.87 Rf	153.8 AU	11.91 %	0.96 Rf	0.2 AU	5361.7 AU	13.66 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	3.7 AU	0.00 Rf	344.7 AU	18.50 %	0.06 Rf	88.7 AU	7367.0 AU	12.43 %
2	0.06 Rf	89.1 AU	0.08 Rf	100.3 AU	5.38 %	0.10 Rf	76.0 AU	2123.6 AU	3.58 %
3	0.12 Rf	74.3 AU	0.17 Rf	129.1 AU	6.93 %	0.19 Rf	73.3 AU	3964.5 AU	6.69 %
4	0.19 Rf	73.7 AU	0.21 Rf	87.8 AU	4.71 %	0.22 Rf	82.1 AU	1409.4 AU	2.38 %
5	0.22 Rf	82.6 AU	0.26 Rf	220.0 AU	11.81 %	0.31 Rf	10.8 AU	7630.5 AU	12.88 %
6	0.32 Rf	116.5 AU	0.36 Rf	158.4 AU	8.50 %	0.39 Rf	63.9 AU	5138.2 AU	8.67 %
7	0.39 Rf	64.1 AU	0.42 Rf	91.6 AU	4.92 %	0.46 Rf	38.7 AU	2888.2 AU	4.87 %
8	0.46 Rf	39.0 AU	0.50 Rf	70.0 AU	3.76 %	0.51 Rf	67.3 AU	1895.4 AU	3.20 %
9	0.51 Rf	67.4 AU	0.58 Rf	135.3 AU	7.26 %	0.62 Rf	74.8 AU	6634.2 AU	11.19 %
10	0.63 Rf	75.3 AU	0.67 Rf	162.1 AU	8.70 %	0.73 Rf	47.5 AU	6805.9 AU	11.48 %
11	0.74 Rf	47.8 AU	0.78 Rf	144.4 AU	7.75 %	0.82 Rf	84.9 AU	5584.1 AU	9.42 %
12	0.82 Rf	85.3 AU	0.87 Rf	219.7 AU	11.79 %	0.96 Rf	0.3 AU	7820.5 AU	13.20 %

Fig. 9(1): Finger print profile and R_f table of chloroform extract of *M. emarginata* (leaf) at 575 nm after derivatization



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	1.8 AU	-0.01 Rf	276.1 AU	17.92 %	0.05 Rf	37.1 AU	4628.8 AU	9.24 %
2	0.05 Rf	37.1 AU	0.07 Rf	84.8 AU	5.50 %	0.10 Rf	25.6 AU	1455.0 AU	2.91 %
3	0.10 Rf	25.8 AU	0.11 Rf	38.6 AU	2.50 %	0.14 Rf	20.5 AU	745.2 AU	1.49 %
4	0.14 Rf	20.6 AU	0.17 Rf	46.7 AU	3.03 %	0.20 Rf	29.9 AU	1357.0 AU	2.71 %
5	0.20 Rf	30.0 AU	0.26 Rf	127.5 AU	8.28 %	0.32 Rf	63.0 AU	4785.7 AU	9.56 %
6	0.32 Rf	63.2 AU	0.34 Rf	70.3 AU	4.56 %	0.38 Rf	18.1 AU	1736.1 AU	3.47 %
7	0.38 Rf	18.4 AU	0.41 Rf	30.5 AU	1.98 %	0.43 Rf	22.5 AU	819.5 AU	1.64 %
8	0.47 Rf	10.8 AU	0.51 Rf	60.8 AU	3.94 %	0.54 Rf	47.9 AU	1658.1 AU	3.31 %
9	0.54 Rf	47.7 AU	0.66 Rf	196.5 AU	12.75 %	0.70 Rf	48.1 AU	12535.0 AU	25.03 %
10	0.70 Rf	148.8 AU	0.74 Rf	225.5 AU	14.64 %	0.79 Rf	72.2 AU	8556.7 AU	17.09 %
11	0.79 Rf	72.3 AU	0.82 Rf	149.2 AU	9.69 %	0.84 Rf	42.9 AU	3547.7 AU	7.09 %
12	0.84 Rf	143.3 AU	0.87 Rf	234.2 AU	15.20 %	0.94 Rf	0.3 AU	8246.2 AU	16.47 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.03 Rf	2.3 AU	-0.01 Rf	376.6 AU	15.36 %	0.05 Rf	67.9 AU	7261.8 AU	9.14 %
2	0.06 Rf	68.5 AU	0.08 Rf	151.6 AU	6.18 %	0.10 Rf	50.7 AU	2567.8 AU	3.23 %
3	0.10 Rf	51.3 AU	0.12 Rf	69.8 AU	2.85 %	0.14 Rf	46.8 AU	1462.7 AU	1.84 %
4	0.14 Rf	47.0 AU	0.17 Rf	79.6 AU	3.24 %	0.21 Rf	61.9 AU	2857.8 AU	3.60 %
5	0.22 Rf	61.9 AU	0.26 Rf	189.2 AU	7.72 %	0.28 Rf	21.5 AU	5083.2 AU	6.40 %
6	0.29 Rf	121.9 AU	0.30 Rf	123.8 AU	5.05 %	0.33 Rf	09.5 AU	2993.3 AU	3.77 %
7	0.33 Rf	109.8 AU	0.35 Rf	128.4 AU	5.23 %	0.38 Rf	49.9 AU	3113.4 AU	3.92 %
8	0.38 Rf	50.2 AU	0.40 Rf	64.2 AU	2.62 %	0.43 Rf	57.0 AU	1783.7 AU	2.25 %
9	0.43 Rf	57.0 AU	0.44 Rf	57.6 AU	2.35 %	0.47 Rf	38.6 AU	1256.3 AU	1.58 %
10	0.47 Rf	38.9 AU	0.51 Rf	109.3 AU	4.46 %	0.54 Rf	98.6 AU	3136.1 AU	3.95 %
11	0.54 Rf	98.7 AU	0.66 Rf	279.6 AU	11.40 %	0.70 Rf	15.0 AU	19662.5 AU	24.76 %
12	0.70 Rf	216.0 AU	0.74 Rf	295.1 AU	12.03 %	0.78 Rf	28.6 AU	11348.4 AU	14.29 %
13	0.79 Rf	129.7 AU	0.81 Rf	221.3 AU	9.02 %	0.83 Rf	05.8 AU	5372.6 AU	6.77 %
14	0.83 Rf	206.2 AU	0.87 Rf	306.5 AU	12.50 %	0.95 Rf	0.1 AU	11515.8 AU	14.50 %

Fig. 9(2): Fingerprint profile and R_f table of chloroform extract of *C. asiatica* (leaf) at 575 nm after derivatization

HPTLC for identification of chemical constituents in the plant extracts typically produces fingerprints, ie. sequence of zones that have specific positions, colours and intensity. The fingerprints obtained for the chloroform extracts of the leaves of *M. emarginata and C. asiatica* were compared and found that they are chemically different. These results are used to differentiate the two plant materials even though they are having similar morphological characteristics.

CONCLUSION

The macro and microscopical characters and HPTLC fingerprinting profile developed along with the physicochemical parameters can be used as a diagnostic tool to identify and to determine the quality and purity of the leaves of *Merremia emarginata* and *Centella asiatica.* HPTLC fingerprinting profile is a very important parameter of standardization for the proper identification of medicinal plants. The given results showed significant differences between the leaves of *Merremia emarginata* and *Centella asiatica* which help in accurate identification and thereby avoiding adulteration or substitution of these medicinally important plants.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declare none

REFERENCES

1. Bown D. Encyclopaedia of herbs and their uses. London: Dorling Kindersley; 1995. p. 361-5.
2. Nadkarni AK. Dr. K. M. Nadkarni's Indian Material Medica. Bombay popular prakashan; 1982. p. 1229.
3. http://envis.frlht.org/plantdetails/048580ccb4ee657eddf1228b869a024f/c30fc5062cf03808c4fc3b1245f3dc9b [Last accessed on 10 Jan 2019]
4. Nalini K, Aroor AR, Karanth KS, Rao A. Effect of *Centella asiatica* fresh leaf aqueous extract on learning memory and biogenic amine turnover in albino rats. Fitoterapia 1992;63:232-7.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement) New Delhi: Council of Scientific and Industrial Research; 1986. p. 51–83.
6. Diwan PC, Karwande I, Singh AK. Anti-anxiety profile of mandukparni *Centella asiatica* Linn in animals. Fitoterapia 1991;62:255–7.
7. Khandelwal KR, Nirali Prakashan. Practical pharmacognosy. 19th edn. Pune, India. Prakashan; 2008. p. 49-70.
8. Evans WC. Trease and evans pharmacognosy. 15th ed. London, United Kingdom: Saunders; 2002. p. 245-7.
9. Tyler V, Brady L, Robber J. Pharmacognosy, Varghese Company, India; 1977. p. 103-14.
10. WHO Quality control methods for medicinal plants materials. Geneva; 1998.
11. Camag. Application notes on instrumental thin layer chromatography; 2015. p. 1996.
12. Wagner H, Bladt S. Plant drug analysis-a thin layer chromatography atlas. Springer Verlage Berlin 1996;364:3-4.
13. Padmasornasubramanian M, Appranthan T, Chelladurai V. Proceedings of the workshop on standardization of siddha drugs. Chennai: Central Research Institute Siddha; 1996.
14. Anonymous The Wealth of India. VI. New Delhi CSIR; 1962. p. 347.
15. Sudhakaran MV. Botanical pharmacognosy of holostemmaada-kodien. Schult Pharmacogn J 2017;9:163-70.
16. Rizvi A, Mishra A, Mahdi AA, Wahab S, Kaleem S. Pharmacognostic evaluation and establishment of quality parameters of seeds of *Cuminum cyminum* L. Indian J Nat Prod Res 2015;6:138-42.