Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(2): 37-42
Received: 19-01-2020
Accepted: 23-02-2020

Manosi Das
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Sundar Sahu
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Avijit Banerji
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Ch. V. Narasimhaji
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Jayram Hazra
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Corresponding Author
Manosi Das
Central Ayurveda Research Institute for Drug Development, CN Block, Sector V, Kolkata, West Bengal, India

Pharmacognostical evaluation and HPTLC finger-printing profile of fresh and dried leaves of rabonlata (*Mikania micrantha* Kunth.)

Manosi Das, Dip Sundar Sahu, Avijit Banerji, Ch. V. Narasimhaji and Jayram Hazra

Abstract
Recently, there has been a global surge of interest in herbal drugs, which have been in use for centuries in traditional Asian schools of medicine. Current research on plant-based drugs continue to provide new remedies to mankind. The weed Rabonlata (*Mikania micrantha* Kunth.) has medicinal properties and is widely used in ethnomedical practice in India and other south-east Asian countries. The present study reports comparative HPTLC finger-printing studies of the fresh and dried leaves of Rabonlata (*Mikania micrantha* Kunth.) and pharmacognostical studies.

Keywords: Fresh and dried leaves of rabonlata, powder microscopy, *Mikania micrantha*, HPTLC

1. Introduction
In the last few decades, the popularity of plant-based drugs, used in traditional Asian systems of medicine, has seen phenomenal growth; globally these now take a significant share of health care. As herbal medicinal products are complex mixtures that originate from biological sources, great efforts are necessary to guarantee constant and adequate quality. Quality control and standardisation of botanical products for ensuring global acceptability of these drugs is therefore of vital importance. The authentication of the drugs by phyto-pharmacognostical studies and HPTLC fingerprinting proceed simultaneously with the search for chemical components, which contribute to the biological properties of drugs.

Mikania micrantha Kunth. (Division: Magnoliophyta; Class: Magnoliopsida; Order: Asterales; Family: Asteraceae; Tribe: Eupatorieae) [1, 2, 3, 4, 5, 6] is a perennial climber originating from tropical Central and South America. The genus *Mikania* originates from the tropical areas of Central and South America, it is widely known as guaco. It is reported to comprise about 300 identified species, but only about 20 of them have been reportedly studied [4]. It is synonymous with *viz. Eupatorium denticulatum* Vahl, *Kleinia alata* G. Meyer, *Mikania alata* (G. Mey.) DC., *Mikania cissampelina* DC, *Mikania cordata* var. *indica*, *Mikania denticulata* (Vahl.) Willd., *Mikania sinuata* Rusby, *Willoughbya cissampelina* (DC.) Kuntze, *Willoughbya micrantha* (Kunth.) Rusby.

Mikania micrantha is also known as bitter vine, climbing hemp vine, Chinese creeper, climbing Hempweed. It is a common vine in the district of Hooghly in West Bengal, termed *Rabonlata* (*Ravanalata*) or *Tarulata* in Bengali. It is also fairly common in outlying areas of Kolkata city. In Malayalam it is known as *Vayara*, in Manipuri as *Oori Hingchabi*, in Mizo as *Japan Hlo*.

It is a major invasive species [1, 2, 3, 4, 5, 6] in India, Sri Lanka, Bangladesh, Malaysia and other countries of South east Asia as well as the Pacific islands. It was introduced in India as ground cover in tea plantations [5] in the 1940s; it has spread to plantations of crops and to some forest areas of our country. It is used as a fodder in many countries: in Malaysia sheep preferentially grazed on Mikania, cattle also graze on it. In Kerala the weed is utilised as fodder in some parts of the state.

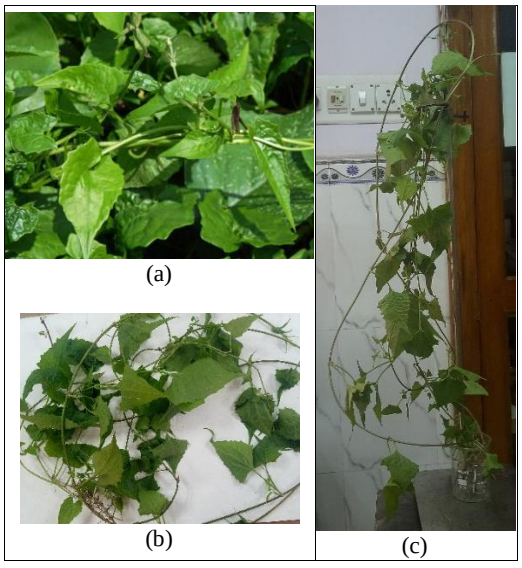
Mikania micrantha is a perennial herbaceous vine, climbing, variable development, with ribbed stems that grow up to 6 metres (20 ft) in length with leaves that have a heart-shaped base and a pointed apex. The stems are slender often highly branched and intertwined. The leaves are simple, opposite, petiole long. The inflorescence[4] is axillary panicled corymbs; cylindrical capitula, 1.5 mm across; 4 flowers per capitula; 4 involucral bracts, oblong to obovate, acute, green in colour, 1 - 3 mm long with a fifth smaller one that is 1 - 2 mm long; 5 - lobed corolla, white, often with purple tinge, 4 - 5 mm long. Botanical characteristics of the plant, its uses and control are detailed extensively in Invasive Pest Fact Sheet: *Mikania micrantha*

of the Asia - pacific forest invasive species network [4].

Allelopathy **-** Extracts from *M. micrantha* slow the germination and growth of a variety of plant species [7]. At least three sesquiterpenoids have been identified which produce this effect [8]. This causes it to suppress local plants and to spread instead as an invasive species. Another report [4] mentions the adverse effect of Mikania on crops and soil properties is through the production of phenolic and flavanoid compounds.

Medicinal uses **-** It is extensively used in ethnomedicine [1, 2, 3, 4, 5, 6]. It is used to treat wide range of ailments *viz*. respiratory diseases, fever, influenza, rheumatism. The antibacterial and antifungal properties effect of *Mikania* and its efficacy in wound healing has been reported [4]. In Fiji, it is used to heal cuts and stop minor external bleeding. Use of juice of Mikania as a curative agent for itches is reported from Malaysia. It is an extensively used ethnomedicine in the North-Eastern states of India. In Mizoram it is a popular local antiseptic drug. In Arunachal Pradesh fresh leaves are pounded and then applied over lacerations to stop bleeding and subsequent healing. Kabi tribes in Assam use the leaf juice of Mikania as an antidote for insect bite and scorpion sting. The sap of this plant is often used by rural children in minor cuts and wounds. The leaves are also used for treating stomachache in Assam. In Bangladesh it is used to treat gastric ulcer and as a local antiseptic.

At present, a thorough re-investigation of this plant is being carried out at our Institute in order to gather further information regarding various aspects to supplement the existing literature on this plant; particularly as meagre information was available on the pharmacognostical standardisation and HPTLC profiling. In this connection aspects of phytochemical screening and fluorescence studies with shade dried powdered sample was reported [9]. Leaf constants (*viz*. stomatal length - width frequency-type, stomatal index, vein-islet number, cell length width-frequency-shape, palisade ratio) were also evaluated [9]. In a second publication the results of acute dermal toxicity and wound-healing activity of *Mikania micrantha* ointment in rats were reported [10]. Other research groups have reported on antidermatophytic activity of the plant [11] and have carried out anato-pharmacognostic studies [12].



(a) (b) (c)

Fig 1a: Habit of Plant *Mikania micrantha* Kunth. showing characteristic shaped leaves.
Fig 1b, c: Whole plant uprooted and displayed in laboratory.

Fresh as well as dried leaves of *Mikania micrantha* are used as drugs. Most reports in ethnomedicine are on the use of fresh leaves to treat wounds. Hence, our interest was piqued to make a comparative analysis of the components of extracts of fresh and dried leaves. For this purpose, preliminary HPTLC analysis was undertaken with methanolic extracts of both fresh and dried leaves. To have a meaningful comparison it was necessary to make an estimate of the moisture content in the fresh compared to the shade-dried leaves, and to take fresh leaves in an appropriately larger amount to compensate for the loss in weight.

2. Materials and Methods

2.1 Plant Material collection and authentication

The matured leaves of *Mikania micrantha* Kunth. were collected from the gardens of CARIDD, Kolkata West Bengal, India in February 2019, and identified in Department of Pharmacognosy, CARIDD, Kolkata.

Plant sample processing

A total of about 300g. of the fresh leaves were collected. The plant samples were washed under running tap water, followed thrice by sterile distilled water. About half of the collected samples was used fresh for morphological study and HPTLC analysis. The rest were air-dried in the shade at ambient temperature for 7 days. Small portions of the air-dried plant samples were used for macroscopic and organoleptic studies, while the rest of the plant materials were pulverised with a grinder to obtain 60 mesh size leaf powder and stored at room temperature in air-tight light-resistant containers to avoid any contamination due to moisture as per standard guidelines [13, 14].

Solvents and Chemicals

All solvents and chemicals were of GR grade sourced from E. Merck Ltd., Mumbai, India.

Macroscopy of plant material

The organoleptic parameters *viz*. texture, shape, size, colour and odour of the plant material were noted by naked eye observation [13, 14] with a simple microscope Olympus OIC DM.

Cytomorphology of plant material

For powder microscopy the powdered samples (~2g. each) were treated with appropriate reagents, stained and mounted following standard protocol and observed under the binocular compound microscope (Olympus OIC- 07964) at projection 10× and 40× magnifications. The Camera Lucida drawing of cellular details was done.

3. Results and Discussions

Table 1: Physicochemical Evaluation of *Mikania micrantha*

S. No.	Parameters	Results
1.	Loss on drying of fresh leaves in the shade at ambient temperature for 7 days	90.1% w/w
2.	Ash value with respect to dried sample	15.8% w/w
3.	Ash value with respect to fresh leaves	1.58% w/w
4.	pH of 10% crushed fresh leaves suspension in distilled water	5.2

Pharmacognostical Study

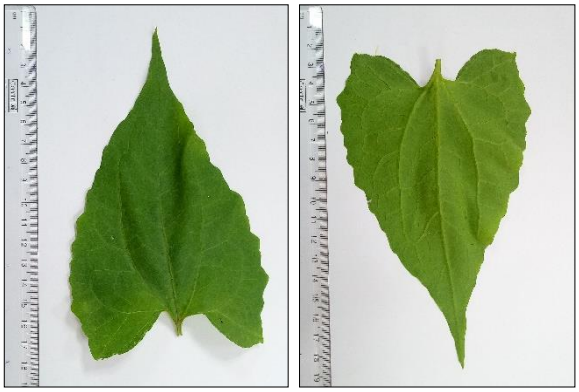
(a) Macroscopic

Fig. 2: Leaves simple, oppositely arranged, dorsiventral, thin,

heart shaped or cordate in shape with acuminate pointed apex and broad base, glabrous, petiolate, margin broadly dentate, leaf blade 12-19 cm. long, 7-8 cm. wide, 3-7 nerved. Veins reticulate, prominent at dorsal side. Small petiole about two-thirds long as the blade. Ventral surface olive green, dorsal surface slightly paler in colour compared to ventral surface, faintly aromatic odour, slightly pungent taste.

(a)

Fig 2: (**a**) Morphology of leaves (Ventral side) with petiole of *Mikania micrantha* Kunth



(b) (c)

Fig. 2: (**b, c**) Ventral and Dorsal views of both sides of the same fresh leaf.

4. Powder analysis of leaf

(a) Macroscopic

Organoleptically, fine powder of dried leaves light olive green in colour, texture fibrous, slightly pungent in taste with faint aromatic odour. The crushed fresh leaves are light green in colour with a faint aromatic odour.

(b) Powder Microscopy

Fig. 3 shows the characteristic features – patches of epidermis made up of compact epidermal cells with undulated cell walls and numerous anomocytic stomata, along with glandular trichomes (with short stalk and oval head containing volatile substances) and nonglandular trichomes (slender, tapering, tri to multicellular); simple starch grains; prismatic crystals of calcium oxalate of different shapes; groups of spiral xylem vessels; thick walled long aseptate fibres with tapering ends; irregularly shaped parenchymatous cells in group with cell contents; xylem parenchyma cells with cell content present.

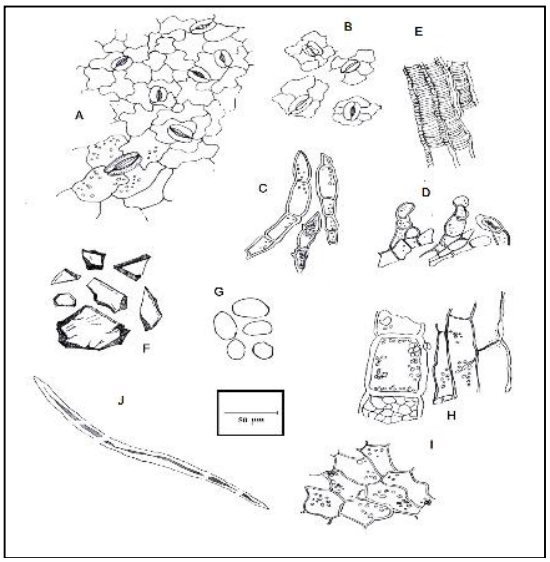


Fig 3: Camera Lucida drawing of powder microscopy of *Mikania micrantha* Kunth. dried leaves

A: epidermal cells with undulated cell wall and numerous anomocytic stomata; **B**: anomocytic stomata; **C**: glandular trichomes - with short stalk and oval head containing volatile substances; **D**: nonglandular trichomes - slender, tapering, tri to multicellular; **E**: spiral xylem vessels; **F**: differently shaped prismatic crystals of calcium oxalate; **G**: simple starch grains; **H**: xylem parenchyma; **I**: irregularly shaped parenchymatous cells with cell content; **J**: long aseptate fibre with thick lignified wall.

HPTLC Finger-printing profile of *M. micrantha* – fresh leaves and dried leaves

Chromatography experiment

Macerated fresh leaves and dried and powdered leaves were separately extracted with refluxing methanol; the extracts were subjected to HPTLC analysis.

Sample preparation

1 g of the dried and powdered leaves of *M. micrantha* were taken in a 50 ml round bottom flask and refluxed with methanol (GR grade, Merck, India, 20 ml) for 1 hr, kept overnight (18 hr) and then filtered through fluted filter paper (Whatman No. 40). Methanolic extract RLD.

6 g of the fresh leaves were crushed with 60 ml of methanol, then taken in a 100 ml round bottom flask, refluxed for 1hr, kept overnight (18 hr) and then filtered through fluted filter paper (Whatman No. 40). 50 ml of the filtrate concentrated under reduced pressure, transferred to a 10 ml volumetric flask and made up to the mark with methanol – extract RLF.

Stationary Phase: Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel $60F_{254}$, Merck.

Mobile Phase: Hexane: Chloroform: Methanol (5:3:2) v/v.

Sample application: CAMAG-HPTLC system Applied volume – 5 µL as 8 mm band and applied at 12 mm from the base of the plate, with a CAMAG ATS4. Plate size was 10 x10 cm.

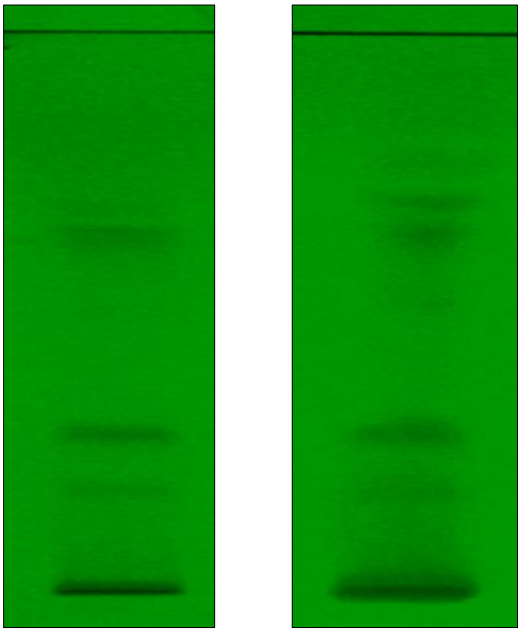
Development: Developed up to 80mm in CAMAG Twin trough chamber (20x10 cm).

Plate preconditioning – temperature 27 ℃; relative average humidity was 48%.

Observation: The chromatograms were visualised at 254 nm and 366 nm in CAMAG TLC visualiser, and scanned using a CAMAG TLC Scanner 4. The HPTLC chromatograms are given in Fig. 3 (visualisation at 254 nm) and Fig. 5 (visualisation at 366 nm).

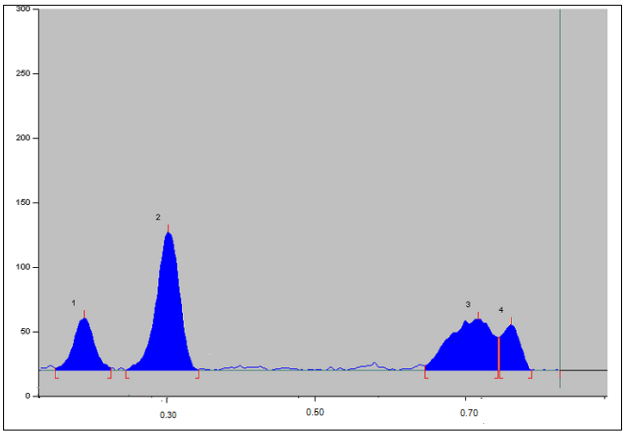
General Comments

In each case, a number of bands were obtained, few of which were overlapped. Figs. (4 a, b) and Figs. (6 a, b) depict the densitometric HPTLC finger print profiles observed at 254 nm and 366 nm respectively. The more intense and sharper bands have been listed, along with their R_f values, absolute intensities and relative areas – Tables (2a, b) for visualisation at 254 nm; Tables (3 a, b) for visualisation at 366 nm. The chromatograms showed that the components present in the methanolic extracts of fresh and dry leaves - *viz*. RLF and RLD respectively - differed in some respects; it was further noted that bands corresponding to similar components also differed in relative intensities.

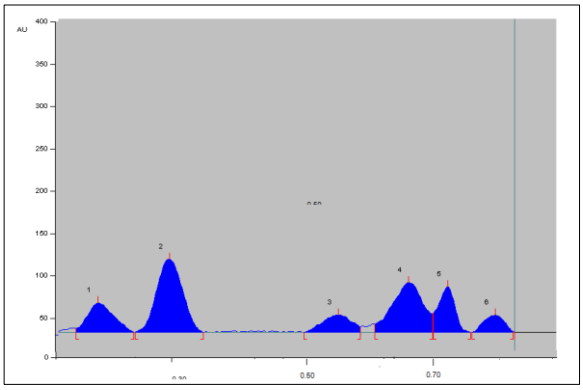


(a) RLF extract (b) RLD extract

Fig. 3: Photography of HPTLC Plate: Visualisation at 254 nm - Plate 1.
Fig. 3 (a): RLF extract (fresh leaves). **(b)** RLD extract (dried leaves).



(a) RLF 254nm



(b) RLD 254 nm

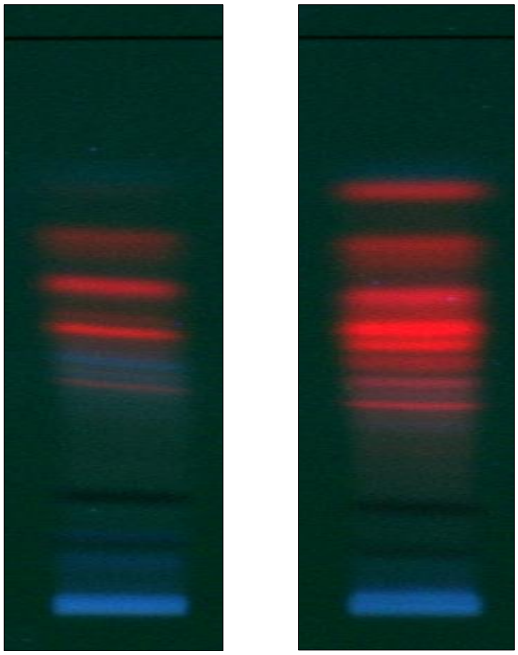
Fig. 4: Densitometric finger print profiles of Rabonlata leaves at 254 nm.
Fig. (4a): extract RLF (fresh leaves). **(4b):** extract RLD (dried leaves).

Table 2a: R_f values and relative areas of the HPTLC peaks of Rabonlata (fresh leaves) methanolic extract RLF visualised at 254 nm

S. No	R_f	Relative Area (%)	Color
1	0.18	14.86	Light black
2	0.30	43.45	Black
3	0.71	30.37	Black
4	0.76	11.32	Light black

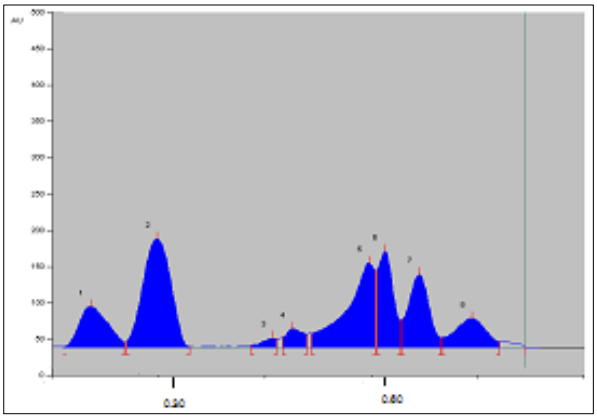
Table 2b: R_f values and relative areas of the HPTLC peaks of Rabonlata (dry leaves) methanolic extract RLD visualised at 254 nm

S. No	R_f	Relative Area (%)	Color
1	0.18	13.23	Light black
2	0.30	32.74	Black
3	0.56	8.68	Light black
4	0.66	26.35	Black
5	0.72	12.89	Light black
6	0.79	6.10	Light black

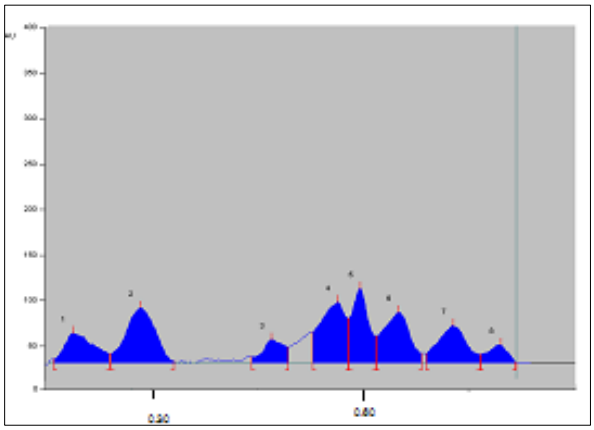


(a) RLF extract (b) RLD extract

Fig 5: Photography of HPTLC plate: visualization at 366 nm - plate 2.
Fig (5a): RLF extract (fresh leaves). **(5b):** RLD extract (dried leaves).



(a) RLF 366 nm



(b) RLD 366 nm

Fig. 6: Densitometric finger print profiles at 366 nm of Rabonlata leaves.
Fig. (6a): extract RLF (fresh leaves). **(6b):** extract RLD (dried leaves).

Table 3a: R_f values and relative areas of the HPTLC peaks of Rabonlata (fresh leaves) methanolic extract RLF visualised at 366 nm

Sl. No	R_f	Relative Area (%)	Color
1	0.18	10.74	Sky blue
2	0.27	27.69	Black
3	0.44	1.24	Red
4	0.47	3.01	Red
5	0.58	21.62	Deep red
6	0.60	13.48	Deep red
7	0.65	13.85	Deep red
8	0.72	8.38	Deep red

Table 3b: R_f values and relative areas of the HPTLC peaks of Rabonlata (dry leaves) methanolic extract RLD visualised at 366 nm

S. No	R_f	Relative Area (%)	Color
1	0.17	10.40	Sky blue
2	0.26	18.95	Black
3	0.45	5.93	Red
4	0.55	18.10	Deep red
5	0.60	14.43	Deep red
6	0.63	15.56	Deep red
7	0.72	12.41	Deep red
8	0.78	4.21	Deep red

4. Discussions

The present work on pharmacognostical study and HPTLC finger-printing profile of fresh and dried leaves of *Mikania micrantha* Kunth. will serve as ready reference for identification, and authentication of these drug materials. HPTLC studies indicated the presence of many secondary metabolites in the fresh and dried leaves of *Mikania micrantha*; these make the plant materials effective against different ailments. Of particular interest is the characteristic differences in HPTLC profiles of methanolic extracts of the fresh leaves and dried leaves – this shows shift in the components present and their concentrations, during the drying process. This is a significant finding for the development of drugs from plant species. The results indicate that further phytochemical work is warranted both on the fresh and dried leaves of this plant, and approximate estimation of the relative amounts of the major components in each case.

5. Conclusions

The present report on pharmacognostical profiling and HPTLC analysis of *Mikania micrantha* leaves provide vital diagnostic tools for identification, authentication and development of quality parameters of this botanical species. The difference in HPTLC profiles of methanolic extracts of the fresh leaves and dried leaves, shows a degree of shift in the components present and their concentrations during the drying process. We consider this to be significant finding in the development of drugs from plant species. Further, we consider that similar studies should be undertaken with other plant species used as drugs in traditional medicine in both fresh and dried forms.

6. Conflict of interest

We declare that we have no conflict of interest.

7. Acknowledgements

The authors thank the Director-General, CCRAS for providing the necessary facilities. Thanks are due to the Pharmacognosy Department, CARIDD, particularly Sreya Dutta for their support. Thanks are due to Sitaram Mandi, laboratory technician of chemistry Department for help in the Experimental work. AB thanks ISCA-DST for the award of the Sir Asutosh Mookerjee Fellowship for senior scientists for working at CARIDD.

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