ORIGINAL RESEARCH PAPER

Ayurveda

KEY WORDS: *Mikania micrantha*, Phamacognosy, Phytochemistry, HPTLC, Fluorescence analysis

# PHARMACOGNOSTICAL AND PHYTOCHEMICAL PROFILING OF TARULATA PATRA (*MIKANIA MICRANTHA* KUNTH LEAVES)

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**ABSTRACT**

**Introduction:** *Mikania micrantha* Kunth is commonly known as 'Tarulata' by the several ethnic groups of West Bengal. The climbing hemp weed or Mile-a-minute vine weed, *M. micrantha* is a cosmopolitan, perennial, multibranched extensive climber or weed, under family Asteraceae is known to have immense medicinal importance.
**Aims and Objectives:** To investigate the pharmacognostical, phytochemical, and HPTLC profiles of *M. micrantha* leaves.
**Materials and Methods:** The measures taken for pharmacognostical characterization were organoleptic study, macroscopy, microscopy, powder microscopy, leaf constant, fluorescence analysis, preliminary phytochemical screening and HPTLC spectra profile.
**Results:** Macroscopic and organoleptic studies found that leaves are simple, oppositely arranged, dorsiventral, thin, triangular to broadly ovate, heart shaped or cordate in shape with acuminate pointed apex and broad base, light olive green in color, with an slight pungent taste. In microscopic analysis, compact epidermal cell with undulated or wavy cell wall and numerous anomocytic stomata were found. Many angular prismatic crystals of Ca-oxalate having- different shapes (rectangular, triangular, irregular); aseptate, long, fibres with thick inner wall; glandular and non-glandular trichomes, groups of spiral xylem vessels; irregularly shaped parenchymatous cells in group with cell contents; xylem parenchyma cells with few cell content are found present. Alkoloides, flavonoids, tannins, terpenoids, steriods, reducing sugars, saponins, phenolic compounds, amino acids and proteins were found present. Analysis on the leaf constants, powder microscopy and fluorescence characteristics resulted a valuable data to establish standards for the plant. HPTLC profile provides number of constituents present in the extracts with their respective Retention Factor ($R_f$).
**Conclusions:** Present report on pharmacognostical profiling and HPTLC analysis of *M. micrantha* leaves provides a vital diagnostic tool for identification, authentication and development of quality parameters of this botanical. Data obtained by present study may be considered as standard for future studies.

## Introduction

The increasing demand for herbal medicines, both in the developing and developed countries, has inevitably led for sustaining the quality and purity of herbal raw materials and finished products. Quality of drugs has been a concern of World Health Organisation (WHO) since its inception, therefore substantiated the utilization of Pharmacognostical and photochemical standards as a protocol authentication and quality assurance of herbal drugs.[1,2] *Mikania micrantha* Kunth. (Asteraceae) is a cosmopolitan hemp weed. Eleven Pacific Ocean countries have rated this herb among their top 10 worst weeds and it is listed among 100 of the "World's Worst" invasion alien species by the Invasive Species Specialist Group (ISSG).[3] It is original from Central and South America. This genus is also reported as a weed in the tropics of Asian subcontinent including India, Bangladesh Sri Lanka, Mauritius, Thailand, Philippines, Malaysia, and Indonesia; and is widely known as guaco. It comprises about 300 identified species, but only 20 of them have been studied. *Mikania* genus is one of the best selling natural products in the world[4] and widely known for its flavonoid, phenolics and terpenes compounds.[5,6] Those classes of compound especially sesquiterpene lactones had been closely related to various biological activities including anticancer and antibacterial.[7] *M. Micrantha* is a ethnomedicinal botanical, known to have immense medicinal potential and is used to treat wide range of diseases, such as fever, rheumatism, influenza, respiratory diseases, chicken pox, skin disorders etc. Both leaves and stem bark possess antibacterial and antifungal properties.[8-10] Due to its wide range of therapeutic attributes, this botanical has become a valuable research material among the researchers throughout the world. Several reports have been available on various parts of *M. Micrantha* for its phytochemistry, pharmacology and other biological activities.[11] However meagre information is available on the pharmacognostical standardization and HPTLC profiling. Considering this, the present study was attempted to develop a quality standard for leaves of this plant.

## General description of *M. micrantha*

**Common English name:** Climbing Hempweed, American rope, Bittervine, Chinese creeper, Mikania vine, Mile-a-minute weed, climbing hemp weed.

**Bengali name**: Chhagalboti, Tarulata, Banchhalata, Germalata

Submitted : 25th April,2019 | Accepted : 12th June,2019 | Publication : 15th September, 2019

**Synonym:**
*Mikania cordata* var. *indica* Kitam, *Eupatorium denticulatum* Vahl, *Kleinia alata* G. Meyer, *Mikania denticulata* (Vahl) Willd., *Willoughbya cissampelina* (DC.) Kuntze, *Mikania alata* (G.Mey.) DC.

**Habitat and Distribution:**
Vigorous, fast growing creeping or twinning plant with numerous cordate leaves, frequently found in moist, bushy, swampy and wet places, on hedges and roadside electric poles (Figure 1). This weed grows best in soils rich in organic matter and sunny areas of high humidity. Climbing Hempweed is found at altitudes of 700-1200 m. It grows naturally in India, Sri lanka, Pakistan, South east Asia, Pacific Islands and native to south and central America, West Indies and Mexico.

**Botanical and chemical profile:**
The botanical is twinning, perennial, multi-branched, extensive climber herb. Bark grey, thick, exfoliating in irregular flakes. Stems 5 ribbed, pubescent or glabrous, internodes 7.5-21.5 cm long. Leaves are simple, opposite, 3-foliolate, petiolate, cordate or hastate, acuminate, broadly dentate-serrate, 11.5-6.66 cm × 1.8-0.7 cm sized. Strongly fragrant flowers, whitish- yellow, each 3-5 mm long, are arranged in dense corymbs in leaf axils or at the end of branches. Each flower-head is 4.5-6 mm long. Individual florets are white to greenish-white, 4 in each head (Figure 2). Fruit is obovoid drupe, achene, 5 angled. A single plant can cover over 25 square meters within a few months, reproducing rapidly vegetatively by rooting and sexually by dispersing some 40,000 seeds per year. The seed is black, linear-oblong, five-angled and about 2 mm long.[12]

*M. micrantha* is rich in several sesquiterpene lactones and phenolic compounds. The most common phytosterols detected in the aerial parts of this plant are stigmasterol, lupeol and sitosterol. Terpenoids like amyrin and friedelin are also abundant in this plant. Leaves of the plant contain compounds like peduncularaside, iridoidanguside, vitexin, triterpenoids and flavonoids. It also contains triterpenoids and flavonoids, pachypodol, ursolic acid and 2-hydroxy-ursolic acid.[11]

## MATERIALS AND METHODS

**Chemicals**
Phloroglucinol, glycerin, hydrochloric acid, chloral hydrate, potassium hydroxide and all other chemicals used in the study were of analytical grade.

**Collection and authentication of plant materials**
The matured leaves of *M. micrantha* were collected from Newtown area of Rajarhat, Kolkata, West Bengal, India, in February 2017. The plant was identified, authenticated and certified by Head, Department of Pharmacognosy, National Research Institute of Ayurvedic Drug Development, Ministry of Ayush, Kolkata.

**Preparation of samples**
These samples were washed under running tap water for 5 minutes followed by sterile distilled water for three times. Half of the drug samples used fresh for morphological study and half of them were air dried and pulverized to obtain 60 mesh size and dried in shade for 7 days and stored in airtight container to avoid any contamination due to moisture.

**Analytical study**
The macroscopy and organoleptic study of the crude drug were performed in terms of its shape, size, color, odour, taste etc. Leaf constants evaluation (viz. stomatal length-width-frequency-type, cell length-width-frequency-shape, stomatal index, vein-islet number, palisade ratio) or quantitative microscopy of leaves was done as per standard method. For powder microscopy powdered samples each was treated with different solutions, stained and mounted following standard method and observed under a compound microscope at projection 10X and 40X along with Camera Lucida Drawing. Shade dried powdered sample was also used for the fluorescence characterization and phytochemical investigations according to the standard method.[13,14]

**Development of chromatographic profile by HPTLC of *M. micrantha* (whole plant)**
The dried plant was subjected to soxhlet extraction with methanol for 6 hours and extract was filtered and concentrated and taken for the following HPTLC profile. CAMAG-HPTLC system having a sample applicator Linomat 5 was used to obtain HPTLC chromatograms using standard methods.[15] HPTLC profile of the *M. micrantha* extract was developed using Hexane:Chloroform:Methanol (5:3:2) as mobile phase [G R grade solvent used, manufactured by MERCK, India] to confirm the occurrence of different phytoconstituents. The chromatographic development processes were performed in an air-conditioned room where the temperature was maintained at 22 °C and RH was maintained at 55%. Ready to use silica gel-G precoated HPTLC aluminium plates were used for chromatographic separation. The extract (10 µL) was spotted as bands of 10 mm width with the help of the auto sampler fitted with a 100 µL Hamilton syringe. The solvent system was transferred to CAMAG Twin Trough plate development chamber lined with filter paper and pre-saturated with mobile phase (25 mL) for 30 minutes [Plate preconditioning: temperature - 25°C, relative average humidity - 54%]. The resulted plates were air dried and scanned. Developed spots on the plaes were scanned at the wavelength of 254 nm, 366 nm and white light (after derivatization) respectively. Derivatising Reagent: Dipped in 20% aqueous Sulphuric acid and charred at 105°C for 10 minutes. Retention factor (*Rf*) value for each spot found on plate were recorded.

## RESULTS AND DISCUSSION

Several sophisticated modern research tools for evaluation of the plant drugs are available now-a-days, but microscopic method is still one of the simplest and cheapest methods to start for establishing the correct identity of the source materials.[16] In the present work microscopy evaluation and phytochemical analysis of *M. micrantha* leaf were carried out. Morphological studies of the leaf will enable to identify the crude drug. The macroscopical characters of the leaf can serve as diagnostic parameters.

Macroscopic characterization revealed that *M. micrantha* leaves are simple, oppositely arranged, dorsiventral, thin, triangular to broadly ovate, heart shaped or cordate in shape with acuminate pointed apex and broad base, petiolate, petiole tendriliform, 2-3 cm long, amplustomatic, margin broadly dentate, blade 4-12 cm long, 1.8 -8 cm wide, and 3-7 nerved (Figure 3). Veins reticulate and prominent at dorsal side. At the junction of the petioles with the nodes, unusual nodal appendages, membranous, up to 5 mm long, upper surface olive green, lower surface is slightly different in colour with upper surface, odour faintly aromatic, taste slightly pungent.

Microscopic analysis of leaf powder consists of profuse patches of epidermis made up of compact epidermal cell with undulated or wavy cell wall and numerous anomocytic stomata along with (Figure 4). Two types of trichomes are found viz. glandular and non-glandular; where glandular one is with short stalk and oval head consisting volatile substances while nonglandular one is slender, tapering, tri to multicellular cellular with long pointed tips; many angular prismatic crystals of Ca-oxalate having- different shapes (rectangular, triangular, irregular); aseptate, long, fibres with thick inner wall; groups of spiral xylem vessels; irregularly shaped parenchymatous cells in group with cell contents;

xylem parenchyma cells with few cell content are found present. Mean value of various leaf constants was calculated and recorded in Table 1.

Organoleptic characters which correspond to the *panchagyanedriya pariksha* (perception by five sense organs) of *Ayurveda*, were documented for *M. Micrantha* leaf powder and stipulated in Table 2.

The fluorescence analysis of powdered drug impart a valuable role in the detection of adulterants, hence in determination of quality and purity of the drug materials. The powdered drugs when subjected to ultraviolet light and visible light in the presence of various chemical reagents, exhibit characteristic fluorescence.[17] Fluorescence report of *M. Micrantha* powdered leaf is tabulated in Table 3.

Preliminary phytochemical screening of extracts of *M. micrantha* (whole plant) were carried out to detect the bioactive compounds in the plant (Table 4). The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. It revealed the presence of alkoloides, flavonoids, tannins, terpenoids ,Steriods, reducing sugars, saponins, phenolic compounds, amino acids and proteins by using different solvent extracts with increasing polarity, such as hexane, ethylacetate, chloroform, methanol, and aqueous. Presence of wide varieties of secondary metadolites in *M. micrantha* signifies its broad spectrum of therapeutic utility.

HPTLC chromatogram of methanolic extract showed a total of 6 spots at different $R_f$ value at 254 nm, whereas 13 spots observed at 366 nm (Figure 5 and Table 5). Visualisation under white light (after derivatization revealed) 9 spots at different $R_f$ values. The HPTLC chromatogram of various extracts may be useful in the confirmation of presence of number of constituents along with the respective $R_f$ value.

The diagnostic features identified in present study can be employed for easy identification of *M. micrantha* in its fresh as well as dried form.

## CONCLUSION

Present report on pharmacognostical profiling and HPTLC analysis of *M. micrantha* leaves provides a vital diagnostic tool for identification, authentication and development of quality parameters of this botanical. Data obtained by present study may be considered as standard for future studies.

### Conflict of interest

We declare that we have no conflict of interest.

**Table 1: Leaf Constants of *M. micrantha***

| S. No. | Leaf Constants | Values |
|---|---|---|
| 1 | Stomatal type | Anomocytic |
| 2 | Stomatal length (µm) | 38.8 (Adaxial Surface)<br>36.1 (Abaxial Surface) |
| 3 | Stomatal width (µm) | 22.1 (Adaxial Surface)<br>19.8 (Abaxial Surface) |
| | Stomatal frequency (No./mm2) | 89.7 (Adaxial Surface)<br>216.1 (Abaxial Surface) |
| 4 | Stomatal Index (%) | 4.43 (Adaxial Surface)<br>14.35 (Abaxial Surface) |
| 5 | Cell shape | Irregular |
| 6 | Cell length (µm) | 90.2 (Adaxial Surface)<br>87.4 (Abaxial Surface) |
| 7 | Cell width (µm) | 63.1 (Adaxial Surface)<br>61.5 (Abaxial Surface) |
| 8 | Cell frequency (No./mm2) | 738.3 (Adaxial Surface)<br>814.6 (Abaxial Surface) |
| 9 | Vein Islet Number | 30.6 - 39.65 |
| 10 | Palisade Ratio | 2.3 – 2.45 |

**Table 2: Organoleptic characters of *M. Micrantha* leaf powder**

| Parameters | Observations |
|---|---|
| *Rupa* (Colour) | Light olive green |
| *Rasa* (Taste) | Slightly pungent |
| *Gandha* (Odour) | faint aromatic |
| *Sparsha* (Touch) | Fine, fibrous |

**Table 3: Fluorescence characteristics of *M. Micrantha* leaf powder**

| S. No. | Treatment | Visible light | In fluorescence light (366 nm) |
|---|---|---|---|
| 1 | Leaf Powder | Green | Fluorescence green |
| 2 | Leaf Powder rubbed on filter paper | Light green | Lemon yellow |
| 3 | Leaf Powder + 5% KOH | Lemon yellow | Chocolate colour |
| 4 | Leaf Powder + 1N HCl | Olive green | Sky blue |
| 5 | Leaf Powder + 50% HNO3 | Orange | Reddish orange |
| 6 | Leaf Powder + 80% H2SO4 | Blackish green | Maroon |
| 7 | Methanolic extract of leaf Powder | Faint green | Lemon yellow |
| 8 | Ethanolic extract of Leaf Powder | Straw | Bluish yellow |

**Table 4: Phytochemical screening of whole plant of *M. Micrantha***

| Sr. no. | Functional groups tested | Extracts | | | | |
|---|---|---|---|---|---|---|
| | | Hexane extract | Ethylacetate Extract | Chloroform extract | Methanol extract | Water extract |
| 1 | Alkaloids | (+)ve | (+)ve | (+)ve | (+)ve | (+)ve |
| 2 | Flavonoids | (-)ve | (-)ve | (-)ve | (+)ve | (+)ve |
| 3 | Tannin | (-)ve | (+)ve | (-)ve | (+)ve | (+)ve |
| 4 | Triterpenoids | (-)ve | (+)ve | (+)ve | (+)ve | (-)ve |
| 5 | Steriods | (-)ve | (-)ve | (+)ve | (+)ve | (-)ve |
| 6 | Reducing sugar | (-)ve | (-)ve | (-)ve | (+)ve | (-)ve |
| 7 | Amino acids and proteins | (-)ve | (-)ve | (-)ve | (+)ve | (+)ve |
| 8 | Poly phenols | (-)ve | (+)ve | (+)ve | (+)ve | (-)ve |
| 9 | Saponins | (-)ve | (+)ve | (+)ve | (+)ve | (+)ve |

*+ve: Present-ve: Absent*

**Table 5: HPTLC profile of *M. Micrantha***

| Observed at 254 nm | | Observed at 366 nm | | Observed at white light (after derivatization) | |
|---|---|---|---|---|---|
| Rf | Colour | Rf | Colour | Rf | Colour |
| 0.03 | Deep black | 0.03 | Sky Blue | 0.03 | Grey |
| 0.09 | Light black | 0.09 | Sky Blue | 0.09 | Grey |
| 0.29 | Deep black | 0.15 | Sky Blue | 0.15 | Grey |
| 0.63 | Light black | 0.20 | Black | 0.20 | Yellow |

| | | | | | |
|---|---|---|---|---|---|
| 0.70 | Deep black | 0.26 | Black | 0.32 | Grey |
| 0.77 | Deep black | 0.36 | Red | 0.36 | Grey |
| | | 0.45 | Red | 0.54 | Grey |
| | | 0.51 | Red | 0.70 | Grey |
| | | 0.54 | Deep Red | 0.77 | Grey |
| | | 0.56 | Deep Red | | |
| | | 0.63 | Deep Red | | |
| | | 0.70 | Deep Red | | |
| | | 0.77 | Deep Red | | |

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