Research Article

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ANATO-PHARMACOGNOSTIC STUDIES OF *MIKANIA MICRANTHA* KUNTH: A PROMISING MEDICINAL CLIMBER OF THE FAMILY ASTERACEAE

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ABSTRACT

Present investigation aims to set some parameters for quality control of the crude drug obtained from *Mikania micrantha* Kunth, a traditionally used medicinal plant for several health conditions throughout the world. Different pharmacognostic parameters like foliar micromorphology, stem and petiole anatomy, xylem element characters, preliminary phytochemical screening, physical constants and UV fluorescence characters have been evaluated following the standard methods. The epidermal cells are irregular in shape and cell wall outline is wavy. The stomata are of anomocytic type. Stomatal index in lower epidermis is 14.48 which is higher than the stomatal index of upper epidermis (4.53). Palisade ratio is 2.47. Trichomes are of glandular and non-glandular types with more than one cell. Vessel elements are with oblique, simple perforation plate. Fibre is a typical libriform type. Tannins, saponins, flavonoids, reducing sugars, etc. have been detected in methanolic extract of the plant powder. Total ash content is 9.05%. In most of the cases findings have been confirmed with previous works on Asteraceae. Some diagnostic features have been identified here which can be employed for easy identification of *M. micrantha* in its fresh as well as dried form. This study will help in proper identification and in controlling the standard of the crude drug obtained from this ethnomedicinal plant.

Key words: Anato-pharmacognostic studies, crude drug, *Mikania micrantha* Kunth

INTRODUCTION

The family Asteraceae or Compositae is an exceptionally large, highly evolved, easily recognised and wide spread family of Phanerogams represented by more than 22,750 currently accepted species. Numerous species of the family Asteraceae are used in popular medicine and the genus *Mikania* of this family is known as one of the best-selling natural products in the world. There are nearly 430 species under this genus and only 12% of it has been evaluated for their chemical and pharmacological diversity[1]. *Mikania micrantha* Kunth (Figure 1), a cosmopolitan weed, has been listed as one of the world's 100 worst invasive alien species[2] which is known to have immense medicinal importance. The plant is used traditionally for several health conditions in the Northern and Central America as well as Asian continent including India and Bangladesh[1,3,4]. Due to its broad spectrum healing potential, it became a valuable research material among the scientists throughout the world. Perusal of literature has revealed that a number of scientific studies have been done on *M. micrantha* for its phytochemistry and a wide range of biological activity[1,5-11].

Use of micromorphology and anatomy is now a recognised tool in the field of plant systematics[12-15]. Importance of epidermal characters in general and those of trichomes in particular and comparative wood anatomy are widely recognised in taxonomic consideration of angiosperms including medicinal plants[16-22]. Stomatal structure, its ontogeny and other epidermal features have also been considered as diagnostic character for identification of the members of many angiospermic families[23-26]. Many members of the family Asteraceae have been studied anatomically by the earlier researchers highlighting their leaf epidermal micromorphology[27-29]. But no anatomical as well as pharmacognostic work on *M. micrantha* has been carried out earlier in detail. In this scenario, present work has been under taken to investigate the foliar micromorphology, vegetative anatomy, stem xylem elements characters, preliminary phytochemical screening and physical evaluation of this ethnomedicinally important member under the family Asteraceae.

MATERIAL AND METHODS

Investigated plant: *Mikania micrantha* Kunth
Family: Asteraceae.
Herbarium voucher number: S. Saha 12 (Visva-Bharati Herbarium, Department of Botany, Visva-Bharati, Santiniketan, India).
Common English name: Mile-a-minute weed, climbing hemp weed.
Local name (Bengali): In different parts of West Bengal, the plant is called in local dialects with various names. In Birbhum district, it is known as 'Chhagalbati' because of its morphological similarity with *Naravelia zeylanica*, a climber which is also known as 'Chhagalbati'. In East and West Midnapore districts, the plant is called in various names like 'Banchhalata', 'Tarulata', 'Taralata' or 'Germalata'[30-31]. On the other hand, in Koch Bihar district it is known as 'Japanilata'[32].

Etymology: *Mikania* is named after Joseph Gottfried Mikan (1743-1814), a Professor of Botany and Chemistry at the University of Prague. The specific epithet *micrantha* means "small flowered".

Distribution: Native to Central and South America, Mexico and West Indies. It is naturalized in India, Pakistan, Sri Lanka, Southeast Asia, Pacific Islands and United States.

Habit and habitat: Extensive climber without tendrils; frequently found in wet places, swampy woods, bushes of moist places, on hedges and roadside electric poles, sometimes at high elevations, forest borders and clearings, along streams and rivers, etc.

Botanical characters: Perennial, multi-branched, climbing herb. Stem nearly rounded, hairy, sometimes inconspicuously five ribbed. Leaves simple, opposite, petiolate, cordate or hastate, acuminate, broadly dentate-serrate; size 11.5 - 6.6 cm × 1.8 - 0.7 cm. Heads very small, white, arranged in corymbose panicles. Fruits achene, narrowly oblong, 5 angled. Pappus is capillary, uniseriate, and connate at the base.

Flowering and fruiting time: August - February.

Parts used: Aerial parts, mainly leaves.

Medicinal uses

Ethnomedicinal uses: Different parts of the plant *M. micrantha* are used to treat fever, jaundice, dysentery, rheumatism, colds, respiratory diseases and scorpion stings[1,33]. The leaves have been reported as good haemostatic agent[3,4,34-36]. In India, the plant is used by different ethnic communities for the treatment of dysentery, body sprain, diabetes, snake bites, itches, gout, flatulence, rheumatism and cancer[3,35,37-39].

As Ethnoveterinary medicine: In India, the plant is used to treat diarrhoea of veterinary animals and to repeal body lice of poultry birds[40-41].

Chemical constituents: The plant is enrich with several sesquiterpene lactones and phenolic compounds[1,9-11]. The most common phytosterols detected in aerial parts of this plant are stigmasterol, lupeol and sitosterol[42-43]. Terpenoids like amyrin and friedelin are abundant in *M. micrantha*[44]. Flavonoids identified so far in this plant are eupalitin, eupafolin, luteolin, mikanin, alpinetin, etc.[10,42-43,45]. Ten components of essential oil have been isolated from it are linalool, α-pinene, β-pinene, camphene, α–felandrene, β–ocimene, geranyl acetate, terpineol, geraniol and thymol[7]. The plant has been identified as a rich source of vitamin A, B and C.

Biological activity: A wide range of biological activity studies have been performed with different solvent extracts of *M. micrantha* which include anti-inflammatory, anti-stress, anti-spasmodic, anti-microbial, anthelmintic and antioxidant activities[5-8,46-50]. Antibacterial potential of Mikanolide and its derivatives extracted from this plant has been established[51]. In a recent study, aqueous extract of it showed anti-tumour activity against two kinds of human cancer cell lines, K562 and Hela[52].

Methods

For the study of foliar epidermis, leaf samples were cleared following Bokhari's method[53]. The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin, and observed under compound light microscope. For xylem elements study, the stem pieces were macerated following the standard method[54] and macerated stem sample washed several times with distilled water. A bit of macerated sample teased with needles, stained in safranin, mounted on the slide with 10% glycerine and observed under microscope. The drawings of the leaf epidermal cells as well as other structures and stem xylem elements were made with the help of camera lucida, and measurements were taken with standardized ocular micrometer in each case. Finally, the plant powder was extracted (Soxhlet extraction) with 90% methanol and the extracts were screened for detection of different phytochemical groups by different chemical colour reaction tests. Physical constants including UV fluorescence nature of the plant powder were studied following the standard methods[55-57].

RESULTS

Foliar micromorphology

General descriptions along with measurement of the foliar epidermal cells, stomata and trichomes have been represented in Tables I, II and III.

Epidermis: Epidermal cells are irregular in shape and cell wall outlines are strictly wavy on both the surfaces (Figure 2). Cell wall outline of lower epidermis is much wavy than the upper epidermis. Size of the upper epidermal cells is 90.16 µm × 63.08 µm and it is 87.36 µm × 61.81 µm on the lower surface. Cell frequency in both upper and lower surfaces is 738.31/mm^2, and 814.56/mm^2 respectively. Palisade ratio is 2.47 (Table 1).

Stomatal complex: Stomata are present on both surfaces of the leaf i.e. leaves are amphistomatic. Stomata are of anomocytic type (Figure 2). Size of the stomata in upper surface is 39.06 µm × 22.07 µm and for the lower surface, it is 35.24 µm × 19.9 µm. Stomatal frequencies are 89.79/mm^2 and 215.53/mm^2 on upper and lower surfaces, respectively. Stomatal index is 4.53 in upper surface and it is 14.48 in the lower surface of the leaf (Table 2).

Trichomes: Trichomes are of both nonglandular and glandular types. Nonglandular, multicellular, uniseriate trichomes are present in few numbers on both upper and lower surfaces of the leaves, whereas this kind of trichomes are present abundantly on the petiole and stem. Number of cells present in the trichome of the leaf varies from 3-4 cells and their size is 83.33 µm × 19.7 µm. Contributory cell number of such type of trichome present in the stem and petiole ranges from 6 – 17 and size of these trichomes is 338.32 µm × 21.36 µm (Figure 3).Trichome index for such type of nonglandular, uniseriate trichomes is 0.13 and 0.02 on upper and lower surfaces of the leaves, respectively. Glandular trichomes are of two types - one type is multicellular, uniseriate, elongated with a glandular cell at the tip and another type is multicellular, conical in shape with two large glandular cells at the tip (Figure 4 & 5). Elongated glandular trichomes are distributed in two ways - one group of trichomes found on the stem and petiole, but other group is distributed exclusively on both surfaces of the leaf. Number of the contributory cells in this type of leaf specific trichomes ranges from 3-4 and average size of

these trichomes is 64.04 µm × 14.97 µm. Where as, cell number in other group of such non-foliar trichomes varies from 2-3 and trichome size is of 51.1 µm × 16.43 µm. Trichome index of leaf specific type is 0.95 and 1.22 on the upper and lower surfaces, respectively. In conical glandular trichome, there is a distinct biseriate stalk where each row of stalk contains 2-3 cells. These stalk cells are bit smaller than the two swollen glandular cells present at the tip of trichome. Such type of trichomes is found abundantly on both surfaces of the leaf, but absent in the stem and petiole. Size of this kind of trichome is 41.98 µm × 37.23 µm and diameter is 128.93 µm (in top view). Trichome index of such type is 0.75 and 0.59 in the upper and lower leaf surfaces, respectively (Table 3).

Crystals: No crystals found here in this species.

Xylem elements

General descriptions along with measurements of the xylem elements of stem have been represented in Table 4. The vessel elements are with simple, slightly oblique or transversely placed perforation plate (Figure 6). Pits are simple. Tails are present in some of the vessel elements. Size is 290 µm × 215 µm and frequency is 22.22/mm^2. Tracheids are with condensed spiral sidewall thickening. Width of the tracheid is 45.5 µm and frequency is 8.12 /mm^2. Fibres are typically libriform type with pointed end (Figure 7). Septa and pits are completely absent. Size of the fibre is 428.8 µm × 8.8 µm and frequency is 69.66 /mm^2.

Stem anatomy: Transverse section of the stem is more or less circular in outline with obscurely 5 ridges. Following tissue organization from periphery towards centre was observed (Figure 8).

Epidermis: It is uniseriate with compactly arranged barrel shaped epidermal cells and cuticle is thin here. Both nonglandular and glandular trichomes are present on the epidermis.

Cortex: The cortex is massive and differentiated into three distinct zones. The first zone is collenchymatous hypodermis, of two- cell thick, lying just below the epidermal layer. Two layers of chlorenchyma cell are present beneath the hypodermal layer, which constitute the middle cortex. Characteristic continuous single layer starch sheath present next to the chlorenchyma zone.

Vascular bundles: They are collateral, conjoint and open type with phloem and xylem. Vascular bundles are arranged in a continuous ring. Each vascular bundle is with a patch of sclerenchyma above the phloem forming the bundle cap.

Pith: At the centre, massive pith is present. Cells are very large, isodiametric and parenchymatous.

Petiole anatomy

The outline of petiole, in transverse section, is horse-shoe shaped or concavo-convex (Figure 9). Epidermis is single layered and cells are compactly arranged. Cuticle is thin. Non-glandular trichomes are found in the epidermis. Three to four layers of collenchyma cells present just below the epidermal layer. A total of 7 vascular bundles are observed in the petiole. They are smaller and larger in size and are arranged alternately in 'U' shaped manner. A quite massive parenchymatous ground tissue is there at the centre.

Organoleptic features of the powdered drug

Colour: Light olive green, Odour: Faintly aromatic
Taste: Pungent, Texture: Fibrous

Microchemical screening of the powdered drug

Through phytochemical tests of methanolic extract of the aerial part (leaf and stem), the important phytochemical groups like tannins, alkaloids, flavonoids, reducing sugars, saponins, glycoside, anthraquinones, etc. have been detected which basically indicate the medicinal properties of this plant. Among these detected phytochemical groups, flavonoids and tannins are present in high concentration (Table 5).

Histochemical study

Histochemical study of the stem part highlighted presence of various phytochemical groups localized in different tissue zones of it. Phytochemical groups like flavonoids, proteins, alkaloids, etc. have been detected in various tissue zones of the stem (Table 6). It has been observed that vascular bundles and cortical zone are the main active sites for synthesis or storage of different phytochemical groups detected.

Physical constants

Ash Value (% w/w):
Total ash: 10.55, Acid insoluble ash: 0.59
Water soluble ash: 3.13, Moisture Content: 87.63% (in fresh form).

Fluorescence analysis

The drug powder treated with different chemical reagents gives characteristic colour when seen under UV light (365 nm) and it is compared with the colour observed under visible light (Table 7). In presence of acetone, methanol and ethanol, the powdered drug in visible light showed olive green, faint green and straw colour, respectively (Figure 10). But under UV light the same drug samples gave prominent bright reddish pink, lemon yellow and bluish yellow fluorescence, colours respectively (Figure 11) which are quite distinct from the colour observed in visible light.

Table 1: Foliar epidermal cell characters of *M. micrantha

Leaf surface	Cell shape	Cell length (µm)	Cell width (µm)	Cell wall outline	Cell frequency (No./mm^2)	Palisade ratio
Upper	Irregular	90.16	63.08	Wavy	738.31	2.47
Lower	Irregular	87.36	61.81	Sinuous	814.56	

*Data presented in the table are averages of 20 observations.

Table 2: Stomatal features of *M. micrantha

Leaf surface	Stomatal type	Stomatal length (µm)	Stomatal width (µm)	Stomatal frequency (No./mm^2)	Stomatal Index (%)
Upper	Anomocytic	39.06	22.07	89.79	4.53
Lower	Anomocytic	35.24	19.9	215.53	14.48

*Data presented in the table are averages of 20 observations.

Table 3: Trichome features of *M. micrantha

Trichome types	Occurrence	Length (µm)	Width (µm)	Diameter (µm)	No. of cells / trichome	Trichome Index (%)
Non-glandular	Petiole & Stem	338.32	21.36	-	6 - 17	-
	Leaf	83.33	19.7	-	3 - 4	0.13 (upper) 0.02 (lower)
Multicellular, uniseriate, glandular	Petiole & Stem	51.1	16.43	-	2 - 3	-
	Leaf	64.04	14.97	-	3 - 4	0.95 (upper) 1.22 (lower)
Conical, stalked glandular	Leaf	41.98	37.23	128.93	-	0.75 (upper) 0.59 (lower)

*Data presented in the table are averages of 20 observations.

Table 4: Xylem elements characters of *M. micrantha

Structures	Parameters	Types & Measurements
Vessel elements	Types of perforation plate	Simple
	Arrangement of perforation plate	Transverse or slightly oblique
	Pit	Simple
	Tail	Sometime present
	Length (µm)	290
	Breadth (µm)	215
	Frequency (No. /mm^2)	22.22
Tracheids	Wall thickening	Condensed spiral
	Width (µm)	45.5
	Frequency (No. /mm^2)	8.12
Fibres	Ends	Tapering & Pointed
	Pits	Absent
	Septa	Absent
	Length (µm)	428.8
	Diameter (µm)	8.8
	Frequency (No. /mm^2)	69.66

*Data presented in the table are averages of 20 observations.

Table 5: Microchemical tests of the methanolic powder extract of *M. micrantha*

Tests/ Reagents	Tests for	Changes of colour	Degree of changes
Dragendroff's reagent	Alkaloids	Yellow ppt.	++
Wagner's reagent	Alkaloids	Reddish brown ppt.	++
Hager's solution	Alkaloids	Yellow ppt.	+
Shinoda's test	Flavonoids	Prominent Magenta colour	+++
10% NaOH	Flavonoids	Prominent Magenta colour	+++
Benedict's reagent	Reducing sugars	Brick red ppt.	+
Fehling's reagent	Reducing sugars	Red ppt.	+++
Molish test	Carbohydrate	Violet ring	-
Ninhydrin	Amino acids	Purple colour	+
Lugol's solution	Amino acids	Faint pink	++
Millon's reagent	Protein	White ppt.	++
10% $K_2Cr_2O_7$ solution	Tannins	Yellowish-brown ppt.	++
10% $Pb(C_2H_3O_2)_2$ solution	Tannins	White ppt.	+++
5% aqueous $FeCl_3$ solution	Tannins	Greenish-black ppt.	+++
Kedde reagent	Glycoside	Blue colour	++
Phloroglucinol	Lignin	Reddish brown	++
1% lead acetate	Saponins	White ppt.	+
Borntrager's test	Anthraquinones	Pink colour	+
Salkowski test	Steroids	Reddish blue colour in chloroform layer and green fluorescence in acid layer	++

+ = Present; - = Absent

Table 6: Histochemical localization tests of the stem T.S. of *M. micrantha*

Tests / Reagents	Tests for	Nature of colour change	Tissue zones
Wagner's reagent	Alkaloids	Light yellow Reddish yellow	Bundle cap Xylem
Lugol's solution	Proteins	Orange yellow Reddish yellow	Bundle cap Xylem
Phloroglucinol	Lignin	Rose red Lemon Yellow Red	Xylem Bundle cap Few cells of upper hypodermal layer
1% aqueous KI	Starch	Blue	Starch sheath
10 % NaOH	Flavonoids	Grayish black	Inter-cellular zones and xylem vessels

Table 7: UV fluorescence nature of the plant powder

Materials and treatment	In fluorescence light (365 nm)	In visible light
Plant powder as such	Fluorescent green	Green
Powder scratched on the filter paper	Lemon yellow	Light green
Treated with 50% HNO_3	Reddish orange	Orange
Treated with 5% KOH	Chocolate colour	Lemon yellow
Treated with 1N HCl	Sky blue	Olive green
Treated with 80% H_2SO_4	Maroon	Blackish green
Treated with $SbCl_3$	Lemon yellow	Straw
Treated with acetone	Reddish pink	Olive green
Treated with methanol	Lemon yellow	Faint green
Treated with ethanol	Bluish yellow	Straw


Figure 1

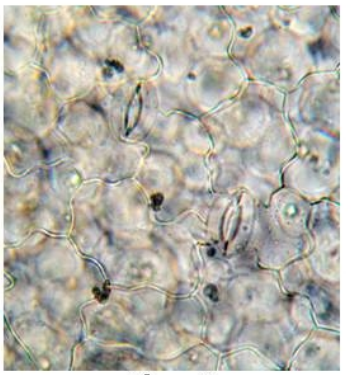
Figure 2


Figure 3


Figure 4

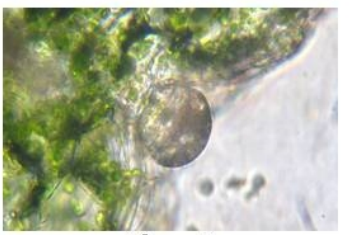
Figure 5


Figure 6

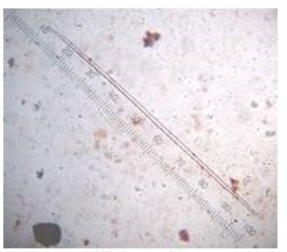
Figure 7

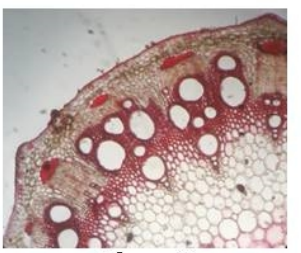
Figure 8


Figure 9


Figure 10


Figure 11

Figure 1: The plant *Mikania micrantha* Kunth; Figure 2: Epidermis with anomocytic stomata; Figure 3: Nonglandular uniseriate trichome on petiole; Figure 4: Stalked, uniseriate glandular trichome; Figure 5: Conical glandular trichome; Figure 6: Vessel element; Figure 7: A fibre; Figure 8: Stem T.S.; Figure 9: Petiole T.S.; Figure 10: Crude drug under visible light; Figure 11: Crude drug under UV light.

DISCUSSION

From this study it has been found that some of the characters of vegetative anatomy, foliar micromorphology, xylem elements and physical evaluation of *M. micrantha* are of great importance in identification of this medicinal plant in its fresh as well as dried form.

The foliar epidermal cells of this plant are with sinuous anticlinal walls which kind of cell wall was also found in several species of *Mikania*[58]. Studies in stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants[22-24]. Anisocytic and anomocytic stomata are predominant in Asteraceae[27] but in *M. micrantha*, exclusively anomocytic stomata are observed which was also found in *M. lanuginosa*[58]. There is a marked difference between stomatal indices of upper (4.53%) and lower (14.48%) surfaces of the leaf which may consider as distinct feature of this plant. Foliar trichome features have been found very reliable for proper identification of plant taxa and their respective leaf drugs[14,16]. Here in *M. micrantha*, multicellular uniseriate glandular trichomes have been encountered which is considered as a typical character of the genus *Mikania*[58]. A very typical type of Conical glandular trichome has been observed in both surfaces of the leaf of *M. micrantha* which was not found in other species of the genus *Mikania* so far studied[58,59].

Vessel elements of stem show a characteristic feature in respect of their size. Presence of very wide and short type of vessel elements confirms taxonomic inclusion of *M. micrantha* in a highly evolved family like Asteraceae.

Anatomical feature of petiole has been recognised as a marker character for identification of plant species. Transverse section of petiole of this plant shows a characteristic 'U' shaped or 'semi-circular' arrangement of 7 unequal vascular bundles.

Chemical analyses and biological assays are very important aspects in pharmacognostic evaluation of medicinal plants[60-62]. Preliminary phytochemical screening of methanolic extract of this plant highlighted that it is rich in phytochemical groups like flavonoids and tannins. Presence of such two important classes of polyphenolic compounds in *M. micrantha* indicates its wide range of medicinal properties including anti-tumour, anti-inflammatory and anti-bacterial activity[49,63-64].

In Pharmacognosy, physical constant like ash value is considered as one of the diagnostic features for crude drug evaluation[56]. Ash value of *M. micrantha* is 9.05% which is very distinct to this species of *Mikania* and may be used in proper identification of crude drug obtained from this medicinal plant.

UV fluorescence character of drug powder is also used as effective parameter for proper identification of it[56]. Under UV light, drug powder of *M. Micrantha* treated with different solvents gives characteristic pattern of colours which will ultimately help in identification of its genuine drug. Acetone treated drug powder of this plant appears olive green colour in visible light, but under UV light it fluoresces reddish pink colouration.

Finally, some diagnostic features have been provided here which can be employed for easy identification of *Mikania micrantha* Kunth in its fresh as well as dried form.

Diagnostic features

- Palisade ratio- 2.47.
- Stomatal index- 4.53% in upper and 14.48% in lower leaf surface.
- Trichome index of multicellular, uniseriate glandular trichome- 0.95 in upper and 1.22 in lower leaf surface.
- In petiole, number of vascular bundles is 7 which are arranged in a semicircular manner.
- Ash value is 9.05%.
- UV fluorescence character: powdered drug in acetone gives olive green in visible light and bright reddish pink under UV light.

CONCLUSION

This study will be very helpful in controlling the standard of crude drug obtained from *Mikania micrantha*. Some diagnostic features have been established here that can be used as marker for easy identification of this ethnomedicinal plant in its fresh as well as dried powder form. Further phytochemical analysis has to be carried out to understand the chemical profile of crude drug of this plant. The results of this study can be taken as a standard reference for further research work.

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