

Investigation of pharmacognostical characteristics and antimicrobial potential of *Mikania micrantha* leaves using ethanolic extract

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ABSTRACT

Background: The *Mikania Micrantha* (a highly competitive invasive climber) is considered a major ecological threat by many; however, it also contains various bioactive secondary metabolites that have medicinal potential. This plant has been used in ethnomedicine in South America and Southeast Asia for its use in the treatment of wounds, fever, inflammation, and skin problems.

Methods: Powdered fresh leaves were prepared and studied for their morphological characteristics through the use of microscopic and cross-sectional examination. A powdered example of the sample that had been extracted by Soxhlet extraction with 95% ethanol was then used for qualitative phytochemical studies as well as thin-layer chromatography in order to determine secondary metabolites present in the sample and assess the effectiveness of the secondary metabolites identified and the efficacy against Gram-negative and Gram-positive bacteria

Results: The microscopy and cross-sections we were able to find some very important anatomical features. An extractive value of 19.85% was achieved through Soxhlet extraction. A qualitative screening for compounds and thin-layer chromatography (TLC) demonstrated that the plant contains carbohydrates, glycosides, flavonoids, terpenoids, tannins, saponins, and alkaloids. Additionally, TLC identified one compound as having a significant $R_f = 0.976$. In vitro antibacterial study describes stronger effectiveness against the Gram-negative bacterium *Escherichia coli* (20 mm zone) in comparison to the Gram-positive bacterium *Bacillus subtilis* (6 mm), while gentamycin exhibited inhibition zones ranging from 34 to 40 mm. The antibacterial properties were linked to the presence of sesquiterpene lactones, phenolics, and flavonoids, highlighting the antimicrobial potential of *M. micrantha*.

Conclusion: Ethanol-soluble leaf extract of *Mikania micrantha* contains Gram-positive and Gram-negative antibacterial phytochemical compounds that are effective for antibacterial properties. These may be used as a synthetic drug. The detailed information from this study would be helpful for the authentication of this medicinal plant. These pharmacognostic and preliminary phytochemical observations can be considered for further studies.

Keywords: *Mikania micrantha*, extraction, phytochemical screening, TLC, antimicrobial activity

1. INTRODUCTION

Mikania micrantha is recognized globally as one of the 100 most problematic alien species and ranks among the 32 most aggressive invasive plants worldwide, highlighting its significant ecological and environmental threat, commonly referred to as mile-a-minute (1). It is a multi-branched, climbing herb with a nearby rounded, hairy stem with 5 ribbed leaves that are simple, opposite (2), acuminate, triangular in shape, measuring between 11.5 and 6.6 cm by 1.8 and 0.7 cm (3). It produces flowers from October to late November in north-east India and dies during December to January (4), seed dispersal via wind, water, or animal during October to April (5). In China, flowering takes place from November to December (6). It is a highly invasive and noxious weed that can have serious effects on crops, particularly those of tea and rubber (7). The species of the genus *Mikania micrantha* was reported in the year 1985 by Robinson and King in (8). *Mikania micrantha* has its origin in central and South America. Then aggressively distributed to tropical moist areas by seed dispersal via wind, water, or animals (5). Commonly found at roadsides, forests, wet areas, bushy places, and on hedges and roadside electric poles (9). Via sea route, it first invaded Hong Kong and then Guangdong (10). To protect soil erosion, it was introduced in Taiwan in the 1970s, and the plant's distribution extends across regions located between 22–24° N latitude and 111–117° W longitude, with a height ranging from 50 to 200 meters (11). It was introduced in South and Southeast Asia during World War II as ground cover. It is distributed to some moist tropical places of north-east India (12). *Mikania micrantha* has been found to have an abundant and varied amount of phytochemicals found throughout its various parts of the plant; this diverse array of bioactive molecules are described by numerous researchers as including; sesquiterpene lactones and dilactones (for example, mikanolides and previously unreported germacranolide derivatives), flavonoids (i.e., mikanin, luteolin, eupafolin, etc., and their respective glycosides); terpenes (terpenoids) in the form of essential oils (i.e., α - and β -pinene, camphene, linalool, geraniol, terpineol, and thymol); and a large variety of phenolic compounds, as well as thymol and caffeic acid derivatives (13, 14). Traditionally, it is used to treat insect bites or scorpion stings. In Malaysia extraction of *Mikania micrantha* is used to treat diabetes, stroke, hypertension, stomach diseases, and also as a water bath during a woman's confinement (4, 15). Apart from that, the extract is also used in traditional medicine to alleviate respiratory disorders, reduce fever, and relieve symptoms of the common cold (16). In Bangladesh, it is used as an antiseptic and widely recognized in Fijian ethnomedicine for its application in treating superficial wounds and arresting bleeding. Due to the presence of a higher number of Terpenoids, it is used to treat ulcers (4, 17).

Therefore, the purpose of this study was to investigate pharmacognostical and ethnopharmacological studies of the leaves of *Mikania micrantha*, which mainly focused on microscopic, physiochemical, and phytochemical evaluation, providing proper identification and quality assurances. Also, the study includes antibacterial properties of the plant, which may have significant potential as a natural source of antibacterial agents.

2. METHODS

2.1. Collection & Authentication

The plant *Mikania micrantha* leaves were collected from Uluberia, Howrah locality, in the middle of December 2023, and left them in the shade to dry for 12 days. The coarse powder of the dried leaves was made by passing them through a high-capacity grinding machine and used in several investigations. Authentication is carried out by the Central National Herbarium of the Botanical Survey of India. Specimen No. CIPT/2023/SN-01.

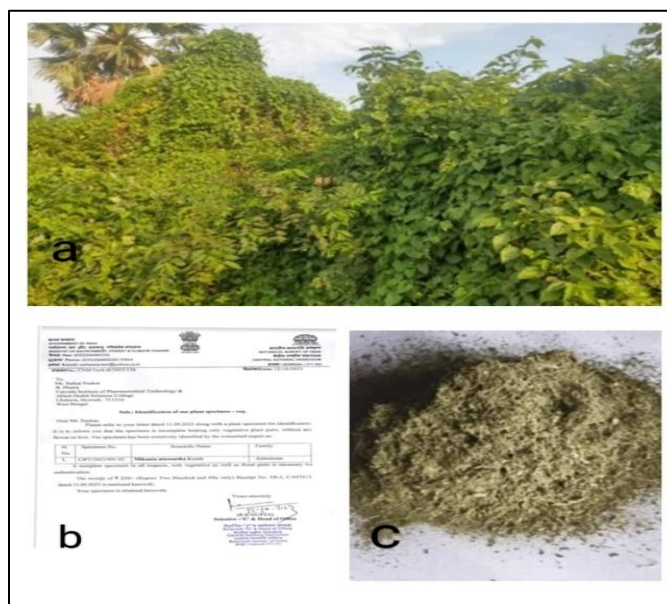


Fig. 1: (a) Plant in natural habitat at Uluberia, Howrah, (b) Authentication report, (c) Powdered drug sample

2.2. Pharmacognostical studies

Organoleptic properties (colour, odor, taste) of the leaf are investigated to obtain information about their characteristics, and organoleptic properties are also determined. Plant samples were immersed overnight in water to soften them. Sections cut across the plant sample, as well as powder microscopy of the dried plant, were performed (18).

2.3 Physiochemical studies

For regulated heating, the cylindrical flask with a round bottom was placed on top of a heating mantle and filled with 300 mL of ethanol. Then set the Soxhlet extractor on the RBF. A thimble was made up of filter paper and filled with 20 g of *Mikania micrantha* leaf powder. Place the thimble into the Soxhlet extractor. A condenser was placed on the extractor, and the inlet and outlet were set. The solvent was vaporized, travelled up the Soxhlet arm, and refluxed into the thimble containing the plant material. This cycle was continued, allowing the solvent to repeatedly extract the compounds from the plant material until it became colourless. The ethanol containing the extracted compounds was accumulated in the round-bottom flask. Then the extractive value was calculated (19).

2.3. Phytochemical studies

Phytochemical analysis was performed on the leaf part of the plant, which is a very useful part of the plant with several medicinal properties. Loss on drying, alcohol-soluble was determined. The extract was tested for the presence of various constituents such as alkaloids, tannins, flavonoids, glycosides, carbohydrates, steroids, and saponins.

2.4. Thin layer chromatography

Thin-layer chromatography made it possible to separate and profile the components of the sample. A developing chamber was saturated with a mobile phase of n-hexane and ethyl acetate in a ratio of 7:5 such that the level of the solvent was below baseline to prevent contact between the sample spots and the solvent directly. The relative

retention factor (Rf) of every component was calculated as the ratio of the migration distance of the compound to the migration distance of the solvent front. The current TLC technique is one of the fast, reproducible, and efficient approaches to preliminary analysis of the qualitative character of the sample, including determining its composition, purity, and content of bioactive substances. It is a stable and easy-to-use reagent that makes it a fundamental requirement of phytochemical research and a valuable preliminary procedure prior to the use of a more complex chromatographic or spectroscopic means of identifying natural products (20).

2.5. Antibacterial activity study

10 mg of the plant extract was dissolved in 10 milliliters of acetone to create a 1% (w/v) solution, which was used for the antibacterial assay. The microorganisms used for the studies were *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative). Gentamycin was taken as the standard drug. Mueller–Hinton agar in a quantity of 5.85 g was precisely dissolved in 150 mL of distilled water in a sterile conical flask to form the culture medium, and then the pH was adjusted to 7.2–7.5. A cotton stopper and some aluminum foil wrapped the flask, and it had been sterilized in an autoclave at 121 °C and 15 psi for 15 minutes. Bacterial cultures were incubated on the plates by placing sterile loops on them and ensuring even distribution of sterile spreaders so that growth is even. The plates were incubated at 37 °C with 24 hours to allow the bacteria to grow and identify the inhibitory effects. The estimation of antibacterial activity was done through measuring the zones of inhibition, in millimetres (21).

3. RESULTS

3.1. Organoleptic properties

Texture – Fine

Colour – Dark green

Taste – Bitter

Odor – Aromatic to slightly pungent

3.2. Macroscopic study

It is a multi-branched, climbing herb with a nearby, rounded, hairy stem with 5 ribbed leaves that are simple, opposite, acuminate, triangular in shape, measuring between 11.5 and 6.6 cm by 1.8 and 0.7 cm. The plant exhibits axillary inflorescences arranged in paniced corymbs, each comprising cylindrical capitula measuring approximately 1.5 mm in diameter.

3.3. Microscopic study

Mikania micrantha leaf transverse section (T.S.) microscopy was performed to describe the structural features of the leaf as part of a pharmacognostic analysis. Microscopic observation revealed that the outer protective layer was well-differentiated epidermis, and this was supported by collenchyma tissue that provides its mechanical strength and flexibility. The mesophyll consisted of tightly packed palisade and loosely packed parenchyma that allowed the mesophyll to be as photosynthetically active and gaseous as possible. The mesophyll had large vascular bundles (xylem and phloem) into which water, nutrients, and metabolites could be transported effectively. It was considered to be the central pith, which was parenchymatous tissue that helped in the storage of the plant and made it strong. The structure of these tissues, epidermis, collenchyma, vascular bundles, and pith, is a taxonomic and pharmacognostic determinant in the identification of the species. Such a transverse anatomy description not only aids in the verification of *M. micrantha* leaves, in addition has a controlling mechanism of the quality and detects adulteration levels in herbal products. Overall, T.S. analysis gives basic anatomical background information to support further phytochemical and pharmacological studies.

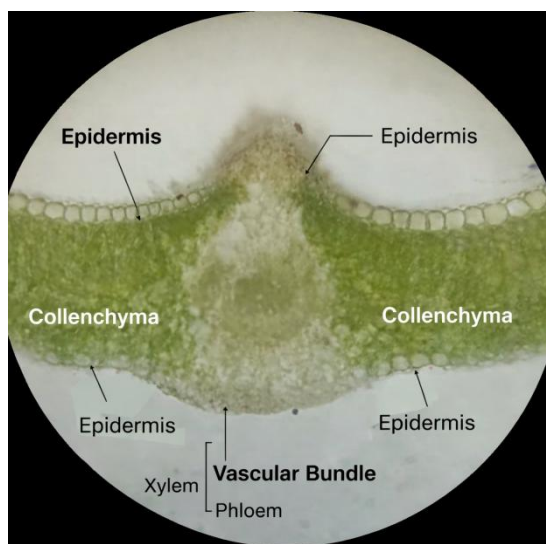


Fig. 2: Transverse section of *Mikania micrantha* leaf

Powder microscopyMajor diagnostic features of pharmacognostic and quality control were analyzed by conducting powder microscopy of leaf samples of *Mikania micrantha*. Examination by light microscope revealed the presence of single-cell trichomes, distended extensions of the epidermal, and species recognition hair-like extensions called trichomes. It was also observed to have lignified cork cells, which contributed to the structural strength and protective nature of the leaf. In addition, such evidence as residual epidermal cells, thickened cell walls, and occasional fragments of xylem vessels were also observed, which provided more taxonomic and microscopic evidence. The combined use of those characters not only helps in the morphological authentication of the plant but also gives an effective reference that helps in identifying adulteration in powdered herbal formulation. Such minute microscopic analyses are required to be standardized and ensure uniformity in the ensuing phytochemical and pharmacological follow-up analysis.

3.4. Physiochemical and phytochemical studies

The qualitative analysis of the plant powder was done, the total ash value of *Mikania micrantha* is 10.04% w/w, representing the plant residue and external material after ignition. The loss on drying determines the moisture content, and any other matter of a sample was 5.02% w/w.

Table 1: Physicochemical analysis of *Mikania micrantha*

Sl No	Test Parameters	Results
1	Loss on drying	5.02% w/w
2	Total ash	10.04% w/w
3	Alcohol soluble extractive value	19.85% w/w

The extractive potential of the leaves of *Mikania micrantha* was evaluated to determine the quantity of solubilized phytoconstituents in order to evaluate the quality as well as the amount of phytoconstituents obtained in the chosen solvent. A total of twenty (20) grams of powdered leaves were accurately measured and then macerated with appropriate solvents for a specific time duration. After filtration and evaporation, the concentrated extract was obtained. The extractive value of 19.85% w/w depicted the presence of high levels of medicinally active compounds in the leaf matrix. In further phytochemical and pharmacological studies, ensure the reproducibility and standardization of the extraction process, which determines valuable information.

The phytochemical screening shows the presence of flavonoids, carbohydrates, glycosides, saponins, and alkaloids. Whereas it shows negative results for steroids and terpenoids.

Table 2: Phytochemical analysis of *Mikania micrantha*

<i>Sl No</i>	<i>Biochemical Test</i>	<i>Qualitative Phytochemical Test</i>	<i>Alcoholic Extract</i>
1	Carbohydrates	Molisch test	+
		Benedict's test	+
		Fehling's test	+
2	Alkaloids	Mayer's test	-
		Dragendorff's test	+
		Wagner's test	-
		Hager's test	+
3	Glycosides	Keller-Kiliani test	+
		Lieberman test	+
4	Terpenoids	Salkowski test	-
5	Flavonoids	Shinoda test	+
6	Tannins	Ferric chloride test	+
7	Saponins	Foam test	+
8	Steroids	Lieberman-Burchard test	-

3.5. Thin layer Chromatography

A TLC plate was spotted by drawing a baseline close to its lower margin, onto which small concentrated drops of the sample solution were spotted with a capillary tube. The TLC plate was developed in a tank that contained a 7:5 mixture of n-hexane and ethyl acetate, keeping the level of the solvent below the baseline to avoid direct contact with the spots. Capillary action allowed the migration of the solvent in the upward direction, causing separation on the basis of the differential affinities of the components. After the solvent front reached near the top, the plate was dried and viewed under a UV chamber at 254 nm. The length between the baseline and the center of the spot was 4.1 cm. Distance from the baseline to the solvent front was 4.2 cm. And the R_f value of the separated component was found to be 0.976.

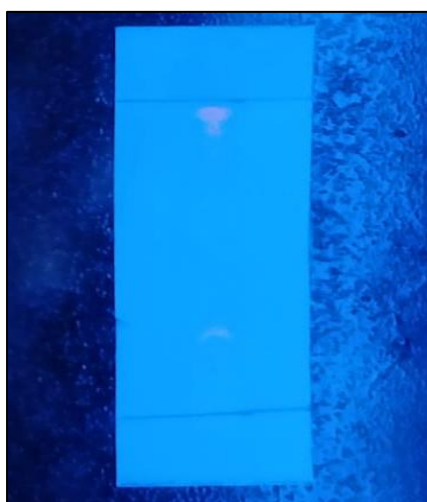


Fig. 3: Thin Layer Chromatography for sample solution

3.6. Anti-Bacterial Study

The antibacterial activity of *Mikania micrantha* leaf extract was evaluated against Gram-positive and Gram-negative bacteria through the standardised disc diffusion assay under reference control with gentamycin. Against

Gram-positive *Bacillus subtilis*, the control antibiotic showed an apparent zone of inhibition of 40 mm, while that of the leaf extract was relatively smaller at 6 mm. In contrast, for Gram-negative *Escherichia coli*, gentamycin had a 34 mm zone of inhibition, whereas the leaf extract showed a more pronounced effect with a 20 mm inhibitory zone. These results indicate that the leaf extract has selective antibacterial activity, displaying much higher effectiveness against Gram-negative bacteria than against Gram-positive strains. The moderate level of inhibition indicates the occurrence of bioactive phytoconstituents that can interfere with bacterial growth and cell functions, though their effectiveness is relatively low compared to the standard antibiotic sample. The differential sensitivity between Gram-positive and Gram-negative bacteria could be due to differences in cell wall composition and permeability, affecting the access of the extract to intracellular targets. The preliminary study sheds light on the potential of the *M. micrantha* leaf extract as a natural source of antibacterial substances. Even though there is moderate inhibitory activity, the results provide a foundation on which future research involving isolation, identification, and mechanism research on active principles can be done. This type of research can potentially lead to the development of novel plant-based antimicrobials, which will create the possibility of alternative treatment against bacterial infection. Based on the results of the anti-bacterial experiment, we can conclude that *Mikania micrantha* leaf extract has a higher antimicrobial activity against Gram-negative bacteria than Gram-positive bacteria.

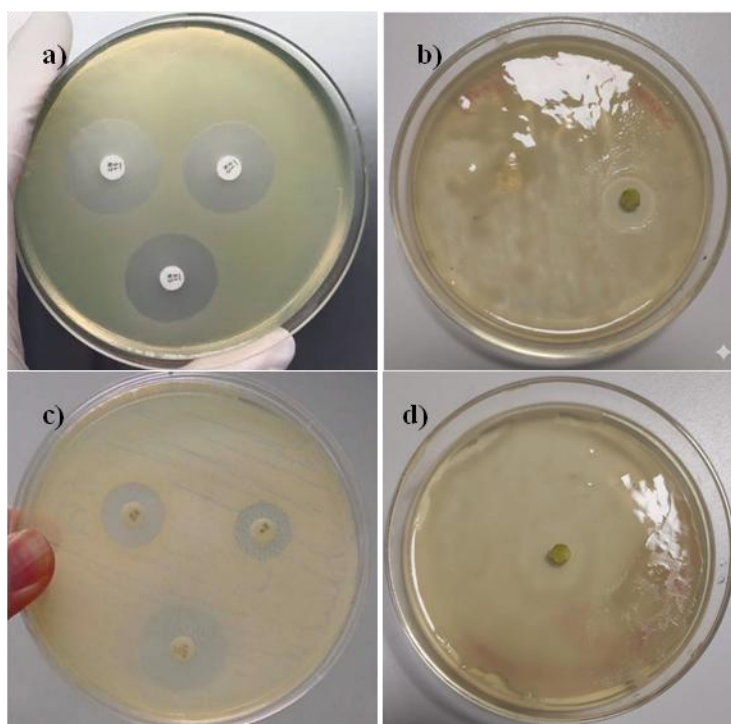


Fig. 4: a) Anti-bacterial activity of Gentamycin Gram-positive bacteria b) Anti-bacterial activity of *Mikania micrantha* leaf extract Gram-positive bacteria C) Anti-bacterial activity of Gentamycin Gram-negative bacteria d) Anti-bacterial activity of *Mikania micrantha* leaf extract Gram-positive bacteria.

Table 3: Anti-bacterial studies of *Mikania micrantha*

Sl. No.	Drug Sample	Diameter of Zone of Inhibition (mm)	
		For Gram-Positive Bacteria	For Gram-Negative Bacteria
1	Standard drug	40	34
2	Test solution	6	20

4. DISCUSSION

This study establishes the characteristics of *Mikania micrantha*. These outcomes can be used as an appropriate analysis and quality assurance of the herb. The Pharmacognostical investigation such as histological, morphological, physiochemical, and phytochemical screening, helps in the identification of different types of plant secondary metabolites, which are essential for pharmacological activities. The detailed evaluation of *Mikania micrantha* and its antibacterial properties holds significant potential as a natural source of antibacterial agents. The fact that it can be obtained naturally is also beneficial, as it can be considered sustainable and may possess fewer harmful side effects than synthetic compounds. It determines the precise bioactive compounds that contribute to its antibacterial properties, as well as to understand how it acts. The study will also be part of the body of research that has led to the emergence of a number of new perspectives for developing antibacterial treatments; this could include *Mikania micrantha* as a potential candidate for such development. Moreover, continuing to investigate the field holds potential to fully exploit the therapeutic benefits of the plant and to address the critical global issue of antibiotic resistance.

5. FUTURE PROSPECT

The study involved a qualitative assessment of the phyto-constituents that are actively present in the leaves of *Mikania micrantha*, and it has anti-bacterial properties. Future studies should focus on quantitative analysis of bioactive compounds and other pharmacological evaluations. As it shows potential anti-bacterial properties, it can be further used for commercial purposes.

6. CONCLUSION

Comprehensive analysis of antibacterial properties of *M. micrantha* through extraction and assessment has shown that *M. micrantha* is a good natural source for antibacterial agents. Additionally, since *M. micrantha* grows naturally in nature, it could potentially be a sustainable option with fewer negative side effects than those found in synthetic products. Further studies are required for the determination of bioactive compounds that ensure the antibacterial properties and can be useful in the new antibacterial treatment development perspectives. Furthermore, continual investigation in this field has the potential to fully maximize the therapeutic benefits of this plant and address the critical global challenge of antibiotic resistance.

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