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Research Article

GC-MS Based Phytochemical Screening and Characterisation of Invasive weed *Mikania micrantha* Kunth

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ABSTRACT

This study investigated the phytochemical composition and bioactive potential of the invasive weed *Mikania micrantha* Kunth through qualitative screening and GC-MS analysis of leaf extracts. Fresh leaves were extracted with methanol, ethyl acetate, and hexane, with methanol yielding the highest extract yield and the most diverse phytochemical profile, including alkaloids, phenolics, flavonoids, terpenoids, tannins, glycosides, and saponins. GC-MS analysis of the methanolic extract identified twenty-nine compounds, including major constituents such as isoxazolidine, 4-ethyl-2,5-dimethyl-, cis-, d-Glycero-d-ido-heptose, and N-Acetylenehydrazine, many of which exhibit documented antimicrobial, antioxidant, anti-inflammatory, anticancer, and herbicidal activities. These results underscore the extensive phytochemical resources of *M. micrantha*, which contribute to its invasive capabilities and indicate its potential as a source of new therapeutic compounds and environmentally friendly biopesticides. The study provides a foundation for further isolation and pharmacological evaluation of individual compounds from this plant.

Keywords: Invasive, Phytochemical, Bioactivity, Leaf, Extraction, GC-MS, Methanol.

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INTRODUCTION

Mikania micrantha Kunth is a highly destructive invasive species belonging to the family Asteraceae. This perennial weed is characterised by its rapid growth rate and creeping, vine-like nature, enabling it to spread quickly over large areas. Native to Central and South America, *M. micrantha* thrives in fertile, organic-rich soils with abundant moisture and high humidity. This plant significantly impedes the growth of native vegetation by overshadowing them, effectively reducing light availability, and outcompeting other species for essential resources. Due to its extensive ecological impact, the IUCN has classified *M. micrantha* as one of the top 100 invasive alien species globally (Deori et al., 2017). It was first recorded in India in the early 20th century when it was introduced as an ornamental plant. Since its introduction, this species has spread extensively, particularly in northeastern states such as Arunachal Pradesh, Assam, and Meghalaya. *M. micrantha* is known by several common names across regions, reflecting its widespread distribution and cultural significance. These include American rope, Chinese creeper, and mile-a-minute. In West Bengal, it is locally called 'Chhagalbati' or 'Japanilata' (Saha et al., 2015). *M. micrantha* often possesses phytochemically active traits that provide it with superior defense mechanisms and competitive advantages over native species. Numerous higher plant extracts have been found to exhibit antibacterial, antifungal, and insecticidal properties in laboratory settings, underscoring their potential utility in biocontrol and pharmaceutical applications (Lockwood et al., 2001). Plant metabolites and plant-derived pesticides are increasingly favoured as environmentally sustainable alternatives to synthetic chemicals due to their lower toxicity to non-target organisms and reduced environmental persistence (Varma et al., 1999). *M. micrantha* has been reported to offer a wide range of health benefits attributed to its rich phytochemical composition. Studies have demonstrated its antimicrobial activity against various pathogens (Facey et al., 2010; Chetan et al., 2012; Chetia et al., 2014), as well as anti-diabetic effects (Wan Nurhayati et al., 2013). Additionally, it exhibits anti-dermatophytic properties useful for treating fungal skin infections (Jyothilakshmi et al., 2015), anti-stress effects (Ittiyavirah & Sajid, 2014), and anti-inflammatory actions (Pérez Amador et al., 2010). Research has also indicated potential anti-proliferative and anti-cancer activities (Ríos et al., 2014; Dou et al., 2014; Matawali et al., 2016), alongside antiallergic and antioxidant properties (Rufatto et al., 2012). The phytochemicals present in this plant are abundant and include terpenoids, alkaloids, flavonoids, steroids, reducing sugars, saponins, phenolics, and tannins, all of which contribute to its diverse bioactivity. Therefore, further phytochemical investigations are essential to isolate and characterise these bioactive compounds, which could serve as leads for pharmaceutical development. This study focuses on the qualitative analysis of secondary metabolites extracted from *M. micrantha* leaves to highlight its medicinal potential. In addition, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to identify the specific phytochemicals present in the plant. These findings contribute to the search for novel natural products with significant therapeutic applications,

supporting the development of plant-based drugs and environmentally friendly pesticides.

Materials and methods

Collection of plant material

Fresh, healthy leaves of *M. micrantha* were collected from the college campus for in-depth phytochemical analysis. The leaves were collected on a clear day to ensure optimal conditions for analysis. The precise GPS coordinates of the collection site are 22.71° latitude and 88.52° longitude.

Extraction of plant sample

The leaves were rinsed with running distilled water, air-dried at room temperature for 15 days, and subsequently ground into a fine powder using a blender. The powdered sample (100 g) was extracted separately with 500 ml each of methanol, ethyl acetate, and hexane in a rotary shaker for 72 hours, maintaining a constant speed of 180 rpm at fixed intervals. After completing the extraction, the mixture was filtered using Whatman No. 1 filter paper. All extracted fractions were then evaporated to dryness at room temperature to produce solvent-free residues. The dry extracts were scraped off from the petri dish. The extracts were named methanolic leaf extract, ethyl acetate leaf extract, and hexane leaf extract. The solvent-free extracts were collected and stored in vials at 4°C for future analysis. The yield of the extract from the plant was calculated using the following formula:

$$\text{leaf extract yield (\%)} = \frac{\text{Dry weight of leaf extract}}{\text{Dry weight of powdered leaf taken before extraction}} \times 100$$

Preliminary phytochemical screening

To evaluate the various chemical groups in the extracts, qualitative phytochemical analyses were performed following the established protocols outlined by Raaman (2006).

Detection of alkaloids

Mayer's Test: To a small amount of the extract, a few drops of Mayer's reagent were added along the side of the test tube. The appearance of a white, creamy precipitate indicated a positive result. To prepare Mayer's reagent, 1.358 g of mercuric chloride was dissolved in 60 ml of water, and 5.0 g of KI was dissolved in 10 ml of water. The two solutions were then combined and diluted with water to a total volume of 100 ml.

Detection of Glycosides

Keller-Killiani test: A 50 mg extract was dissolved in distilled water and filtered using a filter paper. To 2 ml of the resulting filtrate, 1 ml of glacial acetic acid, a drop of FeCl₃, and a drop of concentrated H₂SO₄ were added. The development of a green-blue hue in the upper layer and a reddish-brown hue at the boundary between the two layers indicates the presence of cardiac glycosides.

Detection of Flavonoids

Alkaline reagent test: A few drops of NaOH solution were added to the test solution. The formation of an intense yellow colour, which turned colourless after adding a few

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drops of dilute acid, indicated the presence of flavonoids.

Detection of Triterpenoids and Steroids

Salkowski test: The extract was mixed with a few drops of concentrated H₂SO₄, thoroughly shaken, and left to settle for a while. A red colour in the lower layer indicated the presence of steroids, whereas a yellow colour in the lower layer indicated the presence of triterpenoids.

Detection of Tannins

10% NaOH test: A mixture was prepared by combining 4 ml of 10% NaOH solution with 0.4 ml of leaf extract. The mixture was then shaken vigorously. The formation of an emulsion indicates the presence of tannins in the extract.

Detection of Phenolic Compounds

Ferric chloride Test: A few drops of a neutral 5% ferric chloride solution were added to the extract. The appearance of a dark green colour indicated the presence of phenolic compounds.

Detection of Saponins

Foam test: An extract weighing approximately 50 mg was mixed with 20 ml of distilled water. The mixture was agitated in a graduated cylinder for 15 min. The formation of a foam layer indicates the presence of saponin.

Detection of oils and fats

Spot test: A small amount of extract was squeezed between two filter papers. The appearance of an oil stain on the paper suggests the presence of fixed oils.

GC-MS analysis

The bioactive compounds in the extract were analysed using the GC-MS method developed by Agilent Technologies. An electron ionisation device, calibrated to an ionisation energy of 70 eV, facilitated the GC-MS detection. Helium was employed as the carrier gas, and its flow rate was precisely maintained at 1 ml/min. The injector's temperature was set at 250°C. Initially, the oven temperature was held at 70°C for 3 minutes, then gradually increased at a rate of 5°C per minute until it reached 300°C, where it was held for 8 minutes. The samples were introduced in split mode at a 10:1 ratio. The relative quantities of the compounds in the methanolic extracts of *M. micrantha* were expressed as percentages, calculated from the peak areas in the chromatogram. Each peak represents a specific phytochemical constituent, and its area is directly proportional to the concentration of that compound in the extract.

Identification of component

The bioactive compounds were identified by aligning their retention indices and mass spectra with those in the NIST database. The percentage of each component was calculated by comparing the average peak area to the total area. A comprehensive library search report was generated, detailing the names, molecular weights, and molecular formulas of the test materials.

Results

Phytochemical analysis

Table 1. Phytochemicals present in the methanol, ethyl acetate, and hexane leaf extracts from *M. micrantha*.

Phytochemical	Methanolic extract	Ethyl acetate extract	Hexane extract
Alkaloids	Present	Present	Present
Phenolics	Present	Present	Present
Terpenoids	Present	Absent	Absent
Flavonoids	Present	Present	Present
Tannins	Present	Present	Absent
Glycosides	Present	Absent	Absent
Saponins	Present	Present	Absent
Steroids	Absent	Absent	Absent
Oils and fats	Present	Present	Present

Leaf extracts of *M. micrantha*, obtained using methanol, ethyl acetate, and hexane, were examined for various phytochemical compounds (Table 1). All three extracts contained alkaloids, phenolics, flavonoids, and oils and fats. However, the presence of terpenoids, tannins, glycosides, and saponins varied among the samples. Terpenoids were found solely in the methanolic extract. Tannins appeared in both the methanolic and ethyl acetate extracts but were absent from the hexane extract. Glycosides and saponins were present in the methanolic and ethyl acetate extracts, while saponins were absent from the hexane extract, and glycosides were absent from both the ethyl acetate and hexane extracts. Steroids were not detected in any of the extracts. Overall, the methanolic extract of *M. micrantha* contained the highest number of phytochemicals compared to the other extracts, followed by the ethyl acetate and hexane extracts.

Table 2. Yield (%) from methanol, ethyl acetate and hexane leaf extract of *M.micrantha*.

Extract of <i>M.micrantha</i>	Initial dry weight (gm) before extraction	Final dry weight (gm) after extraction	Yield (%)	Colour
Methanolic	100	7.83±0.90	7.83	Black
Ethyl acetate	100	3.46±0.23	3.46	Deep green
Hexane	100	1.86±0.30	1.86	Lemon yellow

Table 2 outlines the yield percentages and appearances of each extract. In the extraction process of *M. micrantha* leaves, methanol emerged as the most effective solvent, yielding the highest percentage, with ethyl acetate and hexane following in order. The methanolic extract achieved the highest yield of 7.83%.

GC MS analysis

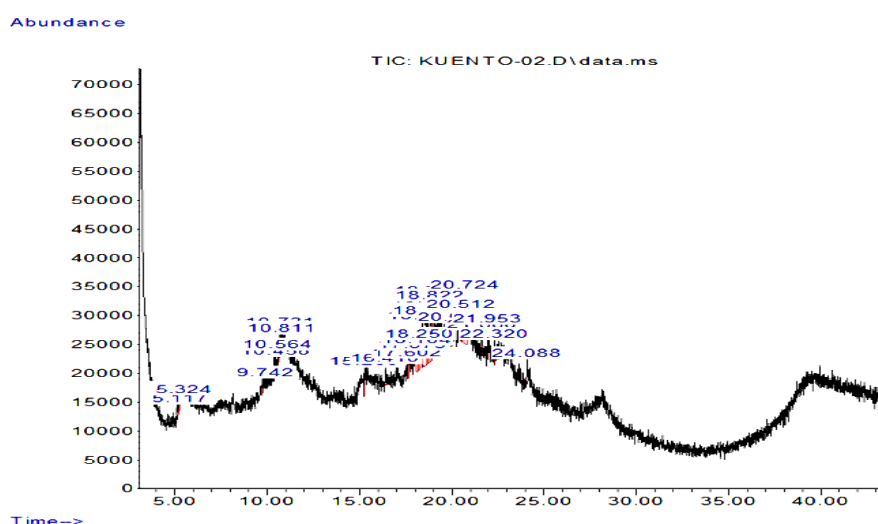


Fig 1. GC MS chromatogram of methanolic leaf extract of *M.micrantha*.

Table 3. Biologically active phytochemicals of methanol extract from *M. micrantha* leaves.

Peak no	Compound name	Mol wt.	Rt (Min)	Formula	Area (%)	Biological activity
1	Pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo-	182.05	5.12	C ₈ H ₁₂ N ₂ OS	1.51	Antimicrobial, anti-inflammatory, antioxidant, and anticancer ((Leoni et al., 2014))
2	Ethylamine	45.058	5.25	C ₂ H ₇ N	1.61	Antimicrobial (Aswar et al., 2015).
3	alpha. -D- Mannopyranoside, methyl 3,6-anhydro-	176.06	5.32	C ₇ H ₁₄ O ₆	0.09	Inhibition of bacterial adherence (Aronson et al.,1979)
4	4-Oxopentyl formate	130.06	9.74	C ₆ H ₁₀ O ₃	1.30	anti-inflammatory, antioxidant, antibacterial, and anticancer (Armoncu et

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						al., 2015; Choubey et al., 2018; Arokiyaraj et al., 2018).
5	2-Thiouracil	128.0	10.38	C ₄ H ₄ N ₂ OS	2.56	Antithyroid (Shiroozu et al., 1983; Nakashima et al., 1978; Raby et al., 1990).
6	3-Pentanone, dimethylhydrazone	128.13	10.45	C ₇ H ₁₆ N ₂	0.79	Not reported
7	N,N-Dimethylformamide ethylene acetal	117.07	10.56	C ₅ H ₁₁ NO ₂	0.74	Hepatotoxic (Scailteur and Lauwerys, 1984; Mraz et al., 1993).
8	1-Hexanamine	101.12	10.72	C ₆ H ₁₅ N	3.87	Cytotoxic and antimicrobial ((Rasoul et al., 2019)
9	Carbamic acid, ethylnitroso-, ethyl ester	146.06	10.78	C ₅ H ₁₀ N ₂ O ₃	0.67	Antimicrobial (Owen et al., 1996).
10	Arginine	174.11	15.26	C ₆ H ₁₄ N ₄ O ₂	3.17	Wound healing and tissue repair (Galeotalanza et al., 2011).
11	Norpseudoephedrine	151.1	16.41	C ₉ H ₁₃ NO	1.70	Sympathomimetic agents (Lam and Shaffer, 2020))
12	N-Isopentyl-N-nitroso-pentylamine	186.17	17.60	C ₁₀ H ₂₂ N ₂ O	1.57	Alkylating agent (Rao et al., 1984)
13	N-Methoxy-aziridine-2-carboxamide, N-(2-aminoethyl)	159.10	17.87	C ₆ H ₁₃ N ₃ O ₂	1.83	inhibitor of angiotensin-converting enzyme 2 (ACE2) (Huentelman et al., 2004)
14	1,1-Dodecanediol, diacetate	286.21	18.10	C ₁₆ H ₃₀ O ₄	2.80	Not reported
15	Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl-	211.05 9	18.25	C ₈ H ₉ N ₃ O ₄	4.43	Tumor imaging agent (Motaleb et al., 2018).
16	Paromomycin	615.29	18.40	C ₂₃ H ₄₅ N ₅ O ₁₄	4.34	Antibacterial (Ibrahim et al., 2019)
17	Isoxazolidine, 4-ethyl-2,5-dimethyl-, cis-	129.11	18.50	C ₇ H ₁₅ NO	13.36	Not reported

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18	Guanidineacetic acid	117.05	18.63	C ₃ H ₇ N ₃ O ₂	8.42	Antioxidant (Nasiroleslami et al., 2018)
19	Acetamide, N-(3-oxo-4-isoxazolidinyl)-, (R)-	144.05	18.69	C ₅ H ₈ N ₂ O ₃	2.75	Not reported
20	d-Glycero-d-ido-heptose	210.07	18.78	C ₇ H ₁₄ O ₇	9.07	Antibacterial (Durka et al., 2011) and immunostimulatory (Inuki et al., 2017).
21	N-Acetythylenediamine	102.07	18.82	C ₄ H ₁₀ ON ₂	8.83	Antimicrobial (Dang et al., 2018)
22	N-Acetylcycloserine	144.05	20.03	C ₆₄ H ₁₁₃ N ₁₁ O ₁₃	1.28	Antioxidant, Antimicrobial (Pei et al., 2018), Anti-inflammatory (Tenório et al., 2021)
23	Undecanoic acid, 11-amino-	201.17	20.45	C ₁₁ H ₂₃ NO ₂	3.0	Antimicrobial (Muthamil et al., 2018)
24	Pentanol, 5-amino-	103.1	20.48	C ₅ H ₁₃ NO	1.22	Not reported
25	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	144.07	20.50	C ₇ H ₁₂ O ₃	2.04	antitumor, antibacterial, antifungal, antioxidant, anticancer, and anti-inflammatory properties (Javaid et al., 2021).
26	Ethanamine, 2-phenoxy-	137.08	20.72	C ₈ H ₁₁ NO	8.80	Larvicidal (Kozyra and Pitucha, 2022)
27	2-Propenoic acid, 2-(acetylamino)-	129.04	21.67	C ₅ H ₇ NO ₃	1.22	Not reported
28	3-Piperidinol, 1,4-dimethyl-, trans-	129.11	21.95	C ₁₇ H ₁₅ NO	3.21	Antimicrobial (Gupta et al., 2019)

29	Diacetyl(methoxyimino)methane	143.05	24.09	C ₆ H ₉ NO ₃	1.84	Not reported

The GC-MS chromatogram of the methanolic leaf extract of *M. micrantha* revealed 29 peaks, indicating the presence of twenty-nine distinct compounds (Fig.1). Table 3 lists the name of each compound, along with its molecular weight, retention time (RT), molecular formula, and peak area percentage. The peak heights corresponded to the relative quantities of chemicals in the extract. The analysis identified Isoxazolidine, 4-ethyl-2,5-dimethyl-, cis- (13.36%), d-Glycero-d-ido-heptose (9.07%), N-acetylenediamine (8.83%), Ethanamine, 2-phenoxy- (8.8%), and Guanidineacetic acid (8.4%) as the five primary constituents, with retention times of 18.5, 18.78, 18.82, 20.72, and 18.63 min, respectively, occupying a larger concentration area (>5%) in the extract. The ten compounds, including Butanenitrile, 2,3-dioxo-, dioxime, O,O'-diacetyl- (4.43%), Paromomycin (4.34%), 1-Hexanamine (3.87%), 3-Piperidinol, 1,4-dimethyl-, trans- (3.21%), arginine (3.17%), undecanoic acid, 11-amino- (3%), 1,1-Dodecanediol, diacetate (2.8%), Acetamide, N-(3-oxo-4-isoxazolidinyl)-, (R)- (2.75%), 2-Thiouracil (2.56%), and 2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester (2.04%), exhibited retention times of 18.25, 18.4, 10.72, 21.95, 15.26, 20.45, 18.1, 18.69, 10.38, and 20.5 min, respectively, and accounted for a moderate concentration range of area coverage, between 2% and 5%. The remaining fourteen compounds, including Diacetyl(methoxyimino)methane (1.84%), N-Methoxy-aziridine-2-carboxamide, N-(2-aminoethyl) (1.83%), Norpseudoephedrine (1.7%), Ethylamine (1.61%), N-Isopentyl-N-nitroso-pentylamine (1.57%), Pyridine-3-carboxamide, 1,2-dihydro-4,6-dimethyl-2-thioxo- (1.51%), 4-Oxopentyl formate (1.3%), N-Acetylcycloserine (1.28%), Pentanol, 5-amino- (1.22%), 2-Propenoic acid, 2-(acetylamino)- (1.22%), 3-Pentanone, dimethylhydrazone (0.79%), N,N-Dimethylformamide ethylene acetal (0.74%), Carbamic acid, ethylnitroso-, ethyl ester (0.67%), and alpha.-D-Mannopyranoside, methyl 3,6-anhydro- (0.09%), were identified as minor constituents, as they were present in relatively minor quantities (< 2%) in the plant extract. In addition, the methanolic leaf extract of *M. micrantha* exhibits a range of biological properties, including antimicrobial effects that prevent bacterial adherence, as well as antioxidant, herbicidal, anti-inflammatory, anticancer, antithyroid,

hepatotoxic, wound healing, cytotoxic, sympathomimetic, alkylating agent, ACE2 inhibition, immunostimulatory, antitumor, tumor imaging, antifungal, and larvicidal activities. (Table 3).

Discussion

The comprehensive analysis of methanolic, ethyl acetate, and hexane leaf extracts underscores the richness of secondary metabolites in this species, particularly in the methanolic extract, which exhibited the highest yield and broadest range of compounds. Preliminary phytochemical screening confirmed the presence of alkaloids, phenolics, flavonoids, and oils and fats in all three extracts. However, methanol was the most effective solvent, extracting a greater diversity of phytochemicals, including terpenoids, tannins, glycosides, and saponins, compared to ethyl acetate and hexane. These observations are consistent with previous reports of Zohmachhuana et al. (2022), Devkota and Sahu (2018) and Raka et al. (2019). The exclusive detection of terpenoids and glycosides in the methanolic extract suggests that methanol is a more effective solvent for extracting polar and semi-polar compounds, consistent with previous findings on plant metabolite solubility. The yield data further supports methanol's superior extraction efficiency, yielding 7.83% of extractable compounds, which correlates with the greater phytochemical diversity observed. The variation in phytochemical presence across solvents highlights the importance of solvent polarity in targeting specific classes of secondary metabolites for medicinal and biocontrol applications. GC-MS analysis of the methanolic extract identified twenty-nine distinct compounds, with Isoxazolidine, 4-ethyl-2,5-dimethyl-, cis-, d-Glycero-d-ido-heptose, and N-Acetylenediamine as the most abundant constituents. Many of these compounds possess documented biological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, and herbicidal properties, which align with the traditional uses and pharmacological effects of *M. micrantha*. The presence of compounds such as paromomycin and guanidineacetic acid highlights the potential of this plant as a source of antimicrobial and wound-healing agents. The detection of bioactive molecules with diverse therapeutic potentials supports the invasive success of *M. micrantha*, which may be partly due to its phytochemical arsenal, which confers competitive advantages through allelopathic effects

and resistance to pathogens and herbivores. Moreover, the identification of compounds with larvicidal and immunostimulatory activities suggests potential applications beyond traditional medicine, including environmentally friendly pest management. Overall, this study confirms the rich phytochemical profile of *M. micrantha* and validates the use of methanol as a suitable solvent for the extraction of bioactive constituents. The qualitative and GC-MS analyses collectively provide a foundation for further isolation, characterisation, and pharmacological evaluation of individual compounds. These findings contribute to the growing interest in exploiting invasive species as renewable sources of natural products for pharmaceutical and agricultural applications.

Conclusion

In conclusion, the findings of this study reinforce the value of invasive plants like *Mikania micrantha* as promising sources of pharmacologically active compounds. The broad array of phytochemicals identified provides a scientific basis for further exploration and utilisation of this species in the search for novel therapeutic agents and environmentally friendly biopesticides. Continued research will contribute to the management of invasive species and the advancement of natural product-based drug discovery.

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