https://doi.org/10.48047/AFJBS.6.15.2024.1166-1175

African Journal of Biological Sciences

Research Paper Open Access

Studies on Bioregulatory Effect, Chemo-Microscopic Screening, and Qualitative Phytochemical Analysis of The Leaves of *Mikania micrantha*

Surajeet Konwar[1], Darshita Sinha[1*], Ankur Jyoti Dutta[1], James Sanong[1], Tridisha Borgohain[2], Smita Doley[2], Rituporna Doimari[3]

[1]Department of Life Sciences, Dibrugarh University, Dibrugarh, India.

[2]Department of Botany, Dhakuakhana College, Lakhimpur, Assam, India.

[3]Department of Life Sciences, D.R. College, Golaghat, Assam, India.

[*]Corresponding author email: rs_darshitasinha@dibru.ac.in

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.1166-1175

Abstract

Mikania micrantha, commonly known as Japani Lota in Assam, is an invasive weed native to South and Central America. Despite its reputation as a harmful plant due to its rapid growth and detrimental effects on local ecosystems, it possesses numerous phytochemicals with potential medicinal properties. This study investigates the phytochemical composition of *M. micrantha* leaves, focusing on their antioxidant, bioregulatory, and chemomicroscopic properties. The leaves were collected from the Golaghat district in Assam, and extracts were prepared using various solvents, including petroleum ether, chloroform, methanol, and water. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, terpenoids, and steroids, with methanolic extracts showing the highest diversity of compounds. Additionally, the bioregulatory effect of aqueous leaf extracts was tested on the germination of *Cicer arietinum* (chickpea) seeds, revealing significant inhibitory effects at higher concentrations. Chemomicroscopic analysis of the stem further identified carboxylated polysaccharides, tannins, and lipids. These findings suggest that *M. micrantha* possesses bioactive compounds that could be harnessed for therapeutic applications, despite its invasive nature. The study highlights the need for further research into the plant's potential health benefits and its allelopathic effects on other species, which could contribute to developing novel treatments for various ailments, including diabetes, cancer, and cardiovascular diseases.

Keywords: *M. micrantha*, Chemoscreening, Bioregulatory effect, germination.

1. Introduction

Mikania micrantha Kunth(Asteraceae), a perennial vine native to the subtropical regions of South and Central America, has spread widely across tropical areas of Asia and America (De Godoy et al., 2019). Often referred to as the bitter vine, creeping hemp vine, or "Japani Lota" in Assam, India, this species is recognized for its aggressive growth and invasive potential. It thrives in high-humidity environments and can easily colonize less fertile soils, contributing to its widespread distribution (Khan et al., 2023). The plant's seeds are light and feathery, enabling them to be dispersed over large areas by wind, with a single stalk capable of producing between 20,000 and 40,000 seeds in a single season. The invasive nature of *M. micrantha* poses significant threats to agricultural and forest ecosystems, where it blankets tree canopies, obstructs sunlight, reduces biodiversity, and disrupts nutrient cycling, ultimately leading to substantial economic losses (Bora et al., 2023). Despite its status as an invasive species, *M. micrantha* has been utilized in traditional medicine for centuries. It is commonly used to treat a variety of ailments, including fever, rheumatism, influenza, and respiratory conditions (Muceneeki et al., 2009; Cabral et al., 2001). The medicinal properties of *M. micrantha* are largely attributed to its rich phytochemical profile, which includes alkaloids, flavonoids, phenolics, tannins, terpenoids, and steroids (Dev et al., 2015; Dong et al., 2017). These compounds have been linked to a range of pharmacological activities, such as antimicrobial, anti-diabetic, anti-inflammatory, and anti-cancer effects (Facey et al., 2010, 2012; Wan Nurhayati et al., 2013; Dou et al., 2014; Matawali et al., 2016).However, despite the documented therapeutic benefits of *M. micrantha*, there remains a significant gap in the understanding of its antioxidant potential. Antioxidants are crucial in mitigating oxidative stress, which is implicated in the development of various chronic diseases, including diabetes, cancer, cardiovascular disorders, and neurodegenerative conditions (Sharma et al., 2024). The antioxidant capacity of a plant is often influenced by its phytochemical composition, which can vary depending on the specific part of the plant and the solvents used for extraction. Given the increasing interest in plant-based antioxidants as safer alternatives to synthetic compounds, which can have harmful or mutagenic effects, it is important to explore the antioxidant properties of *M. micrantha*. This study aims to bridge the existing knowledge gap by investigating the phytochemical content of *M. micrantha* leaves extracted using petroleum ether, chloroform, methanol, and aqueous solvents. Additionally, the study will assess the bioregulatory effects of these extracts on the germination of *Cicer arietinum* (chickpea) seeds, providing insights into the potential applications of *M. micrantha* in both traditional medicine and agricultural practices. This research not only contributes to the growing body of knowledge on the medicinal uses of *M. micrantha* but also highlights the importance of exploring its bioactive compounds for potential therapeutic applications. Understanding the antioxidant properties of this plant could lead to the development of novel treatments for oxidative stress-related diseases, offering a natural alternative to synthetic antioxidants with fewer side effects.

2. Materials & Methods

2.1.Study area: The Bioregulatory effect, Chemomicroscopic screening and qualitative phytochemical analysis of the leaves of *M. micrantha* as the basis for the current investigation. The fresh leaves were collected for the study from Golaghat district in Assam. Golaghat, the study area, stretches from 26°4584° N to 93°9878° E.

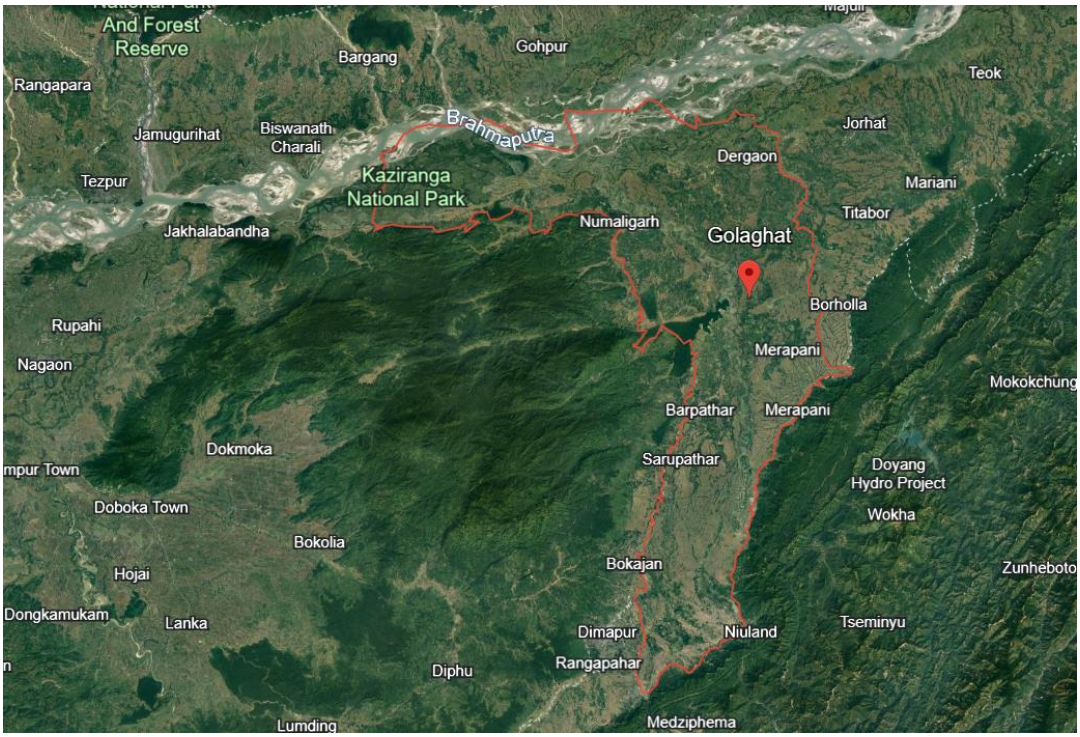

Fig 1. Map of Assam showing Golaghat district from where the samples are collected (Google Earth).

2.2.Collection of sample and Preparation of the leaf extract: Fresh leaves of *M. micrantha* were collected from different local areas of Golaghat in the month of June-August, 2024. Fresh *M. micrantha* leaves were properly cleaned under running tap water, then sterile distilled water, and then let dry in the shade for 10-15 days. We pulverized the dried plant material into a powder and stored it in airtight vessels. The extracts were made with methanol and chloroform, two distinct organic solvents, and sterile distilled water. A 16 gm sample of the plant was obtained, dissolved in 10 ml of methanol and chloroform, and left to absorb for 1-2 days. After that, the extract is filtered through filter paper, let air dry, and then concentrated until it's completely dry in a petri dish at room temperature.

2.3.Chemo-microscopic screening of the stem of *M. micrantha:* The stem of the plant species is taken and cut into thin transverse sections with the help of a sharp blade. Later the stem sections are treated with different types of reagents to perform different tests that are mentioned below (Evans, 2009)

2.3.1. Test for carboxylated polysaccharides: This test is performed by using the reagent Toluidine Blue. This reagent is prepared by dissolving 0.1 gm toluidine blue in 100 ml of Distilled water. The transverse plant section is taken in a glass slide and 2-3 drops of toluidine blue are applied on the section and waited for about 1 minute and observed it under the compound microscope. Blue coloration is observed which detects the presence of carboxylated polysaccharide

2.3.2. Test for Tannins: This test is performed by using FeCl3, as the reagent which is prepared by using 10% of FeCl3 in 10ml of alcohol. The plant section is taken in a glass slide and 1-2 drops of the reagent is poured on the section in drop wise manner and waited for 1-2 minutes. The slide is then observed under a microscope and blackish-green colour is observed. This results in the presence of tannins

2.3.3. Test for Lipid/fats: Test for lipids is done with the help of Sudan III reagent which is prepared by dissolving of 10mg of Sudan III in 10ml of alcohol. The plant section is taken and treated with this reagent and allowed to wait for 1-2 minutes. Presence of lipids is detected by the observation of red colour coloration of the plant section which confirms the presence of lipids.

2.4.Qualitative Phytochemical Screening of Extract: For the purpose of conducting a preliminary phytochemical analysis, the aqueous and methanolic extracts of *Mikania micrantha Kunth*leaves were subjected to a qualitative analysis for phytochemicals such as alkaloids, flavonoids, saponins, terpenoids, carbohydrates, and tannins following the methods of Dev *et al.* 2015.

2.5.Seed germination: Seed germination percentage of *C. arietinum*, where the effect of aqueous leaf extract of *M. micrantha* against the seed of chick pea (*C. arietinum)* was seen following the methods used by Sahu and Devkota, 2013.

3. Results

Table 1. Results of chemo-microscopic screening of the stem of *M. micrantha*

Serial No	**Name of the test**	**Observations**	**Result**
1	Toluidine Blue test (for carboxylated polysaccharide)	Blue coloration is observed.	+
2	FeCl3 test (test for Tannins)	Blackish green color is observed.	+

3	Sudan III test (test for lipid/fats)	Red color is observed	+
4	ZnCl2 test (test for cellulosic cell wall)	Blue violet color is observed	+

Table 2. Results of the Phytochemical Screening of *M. micrantha* in three different plant extracts (+ve indicates present; -ve indicates absent)**.**

Serial no	**Name of the Test**	**Observation**			
		Petroleum ether extract	**Chloroform extract**	**Aqueous extract**	**Methanolic extract**
1	**Test for carbohydrates** A. Molisch's test	-	-	+	+
2	**Test for reducing sugar** Fehling's test	-	-	-	+
	Benedict test	-	-	-	+
3	**Test for steroids** A. Lieberman test	-	-	-	+
	B. Salkowski test	-	-	-	+
4	**Test for flavanoids** A. Alkaline reagent test	-	-	-	+
	B. FeCl3 test	-	-	-	+
5	**Test for Alkaloids** A. Mayer's test	-	+	-	+
	B. Wagner's test	-	-	-	+

6	**Test for Saponins** A. Foam test	+	+	+	+
7	**Test for glycosides** A. Liebermann's test	-	-	+	+
8	**Test for terpenoids** A. Salkowski test	-	-	-	-
9	**Test for Anthraquinone**	-	-	-	-

Table 3. Effect of aqueous leaf extract of *M. micrantha* against the seed of chick pea (*C. arietinum*)

Treatments	Germination after 10 days	Radicle length (cm)	Hypocotyl length (cm)
Control	78%	1.23	1.87
1%	56%	0.76	1.12
5%	29%	0.39	0.54
10%	8%	0.22	0.1

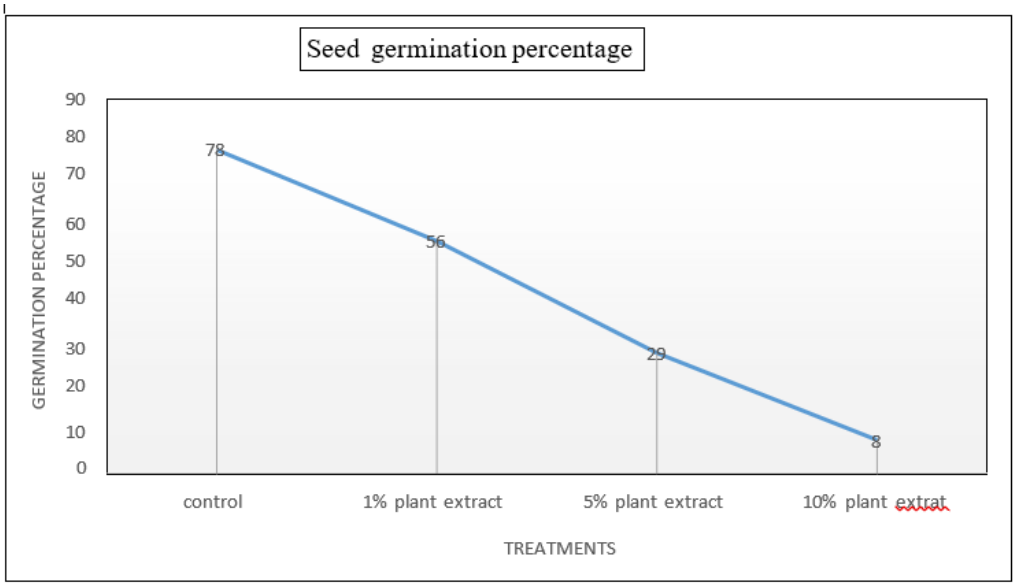

Fig 2. Seed germination percentage of *C. arietinum*

The chemo-microscopic screening of *Mikania micrantha Kunth*was conducted using various chemical tests as described by Evans (2009). The results, presented in Table 1, confirm the presence of carboxylated polysaccharides, tannins, lipids, and cellulosic cell walls in the stem cross-sections of *M. micrantha*. These findings emphasize the importance of secondary metabolites in plants, which play crucial roles in their

biological functions and interactions with the environment. In this study, the phytochemical screening of *M. micrantha* was performed using different solvents, revealing a diverse array of bioactive compounds. The methanolic extract, in particular, was found to contain steroids, alkaloids, flavonoids, glycosides, terpenoids, reducing sugars, and carbohydrates (Table 2). This contrasts with the aqueous extract, which exhibited only carbohydrates, saponins, and glycosides. Similarly, the chloroform extract revealed the presence of alkaloids and saponins, while the petroleum ether extract was limited to saponins. These results align with previous studies, such as those by Dev et al. (2009) and Ikbal et al. (2020), who also identified similar phytochemicals in *Mikania* leaf extracts, including alkaloids, glycosides, phenols, tannins, and flavonoids.The study also examined the effect of *M. micrantha* leaf extract on the germination and growth of Cicer arietinum seeds. Table 2 shows that the control group had the highest germination rate, while the group treated with a 10% concentration of the extract had the lowest germination rate (8%). Furthermore, Table 3 displays the radicle and hypocotyl lengths of the seedlings, with the control group exhibiting the longest lengths and the 10% treatment group showing the shortest. Notably, seedlings treated with the highest concentration (10%) sprouted after ten days, but no plumule development was observed, in contrast to the control, 1%, and 5% treated seeds, which germinated within 1-2 days. These findings suggest that *M. micrantha* leaf extract has a significant inhibitory effect on the germination and growth of *C. arietinum* seedlings, particularly at higher concentrations, as depicted in Figure 2. The inhibitory effects of *M. micrantha* are consistent with previous research that has documented the growth-suppressing effects of invasive plants on native species, including reductions in seed germination and seedling growth (Sánchez-Moreiras et al., 2006). The observed concentration-dependent inhibition implies that the bioactive compounds in the extract, likely allelochemicals, are responsible for this effect. Supporting this hypothesis, studies by Hoque et al. (2003). The allelopathic or bioregulatory properties of *M. micrantha* may be attributed to the presence of phenolic compounds, which have been shown to inhibit the growth of nitrogen-fixing bacteria, as reported by Pei et al. (2023). These allelochemicals can be found in various parts of the plant and may be released into the soil through root exudates, leaf litter, or other plant debris, where they break down and are absorbed by the soil (John and Sarada, 2012).This study provides evidence of the strong inhibitory effects of M. micrantha on the germination and growth of *C. arietinum* seeds, likely due to the presence of allelochemicals in the plant. These findings highlight the importance of considering the allelopathic potential of invasive species like M. micrantha, which can significantly impact the growth and development of other plants in their vicinity. Further research is needed to isolate and characterize the specific allelochemicals responsible for these effects and to explore their potential applications in agriculture and weed management.

4. Conclusion

Numerous investigations ought to be conducted on the phytochemical screening of *M. micrantha* by many investigators already. *M. micrantha* has shown possible therapeutic benefits for a number of ailments including diabetes, infections, and malignancies, based on earlier research. Terpenoids, the main components extracted from *M. micrantha*, are among the several phytochemicals that have been shown to contribute to the plant's medicinal qualities. Nevertheless, additional studies are required to bolster the conventional wisdom and scant scientific data regarding *M. micrantha*'s potential health benefits. In actuality, research on the toxicological impacts of different *M. micrantha* sections ought to be done because of concerns about quality and safety. To gain more insight into the potential therapeutic and preventative benefits of *M. micrantha*, studies including both human and animal participants ought to be conducted.

References:

1. Bora, A. R., Babu, D. S., Chetry, S., & Kalita, S. (2023). Management options of Mikania micrantha: A review. *Agricultural Reviews*, *44*(3), 393-396.
2. Pei, L., Gao, Y., Feng, L., Zhang, Z., Liu, N., Yang, B., & Zhao, N. (2023). Phenolic acids and flavonoids play important roles in flower bud differentiation in Mikania micrantha: transcriptomics and metabolomics. *International Journal of Molecular Sciences*, *24*(23), 16550.
3. De Godoy, S. M., Da Silva, J. F. M., Ruas, P. M., Ritter, M. R., De Paula, G. B. N., Maffei, E. M. D., Góes, B. D., & De Fátima Ruas, C. (2019). Multilocus approach reveals a complex evolutionary history of the invasive mile-a-minute plant, *Mikania micrantha Kunth*(Asteraceae), in its natural habitat. *Botanical Journal of the Linnean Society*, *191*(2), 188–215. https://doi.org/10.1093/botlinnean/boz045
4. Khan, M. A., El-Kersh, D. M., Islam, M. S., Khan, S. A., Kamli, H., Sarkar, C., Bhuia, M. S., Islam, T., Shill, M. C., Gobe, G. C., Gürer, E. S., Setzer, W. N., Sharifi-Rad, J., & Islam, M. T. (2023). *Mikania micrantha Kunth*Kunth: An ethnopharmacological treasure trove of therapeutic potential. *Chemistry & Biodiversity*, *20*(11). https://doi.org/10.1002/cbdv.202300392
5. Cabral, L. M., dos Santos, T. C., & Alhaique, F. (2001). Development of a profitable procedure for the extraction of 2-H-1-benzopyran-2-one (coumarin) from *Mikania glomerata*. *Drug Development and Industrial Pharmacy*, *27*(1), 103-106.
6. Ikbal, A. M. A., Rajkhowa, A., Singh, P. C., Choudhury, P. D., & Sahu, R. K. (2020). Assessment of phytochemical and anthelmintic activity of some selected ethnomedicinal plants from Barak Valley region of Assam. *Biomedical and Pharmacology Journal*, *13*(4), 1825-1831.
7. Sánchez-Moreiras, A. M., de la Peña, T. C., & Roger, M. J. R. (2006). Cell cycle analyses for understanding growth inhibition. *Allelopathy, a physiological process with ecological implications. Springer, Dordrecht*, 141-156.
8. Dev, U. K., Hossain, M. T., & Islam, M. Z. (2015). Phytochemical investigation, antioxidant activity

and anthelmintic activity of *Mikania micrantha Kunth*leaves. *World Journal of Pharmaceutical Research, 4*(5), 121-133.

9. Dong, L., Jia, X., Luo, Q., Zhang, Q., Luo, B., Liu, W., Zhang, X., Xu, Q., & Tan, J. (2017). Phenolics from Mikania micrantha Kunthand Their Antioxidant Activity. Molecules, 22(7), 1140. https://doi.org/10.3390/molecules22071140
10. Dou, X., Zhang, Y., Sun, N., Wu, Y., & Li, L. (2014). The anti-tumor activity of Mikania micrantha Kunthaqueous extract in vitro and in vivo. *Cytotechnology*, *66*, 107-117.
11. Facey, P. C., Peart, P. C., & Porter, R. B. R. (2010). The antibacterial activities of mikanolide and its derivatives. *West Indian Medical Journal, 59*(3), 249.
12. Sahu, A., & Devkota, A. (2013). Allelopathic effects of aqueous extract of leaves of Mikania micrantha KunthHBK on seed germination and seedling growth of Oryza sativa L. and Raphanus sativus L. *Scientific World*, *11*(11), 90-93.
13. Li, J., & Jin, Z. (2020). Potential allelopathic effects of *Mikania micrantha Kunth*on the seed germination and seedling growth of other plant species. *Plant Growth Regulation, 91*(3), 341-351.
14. Matawali, A., Chin, L. P., Eng, H. S., & Gansau, J. A. (2016). Antibacterial and phytochemical investigations of Mikania micrantha KunthHBK (Asteraceae) from Sabah, Malaysia. *Transactions on Science and Technology*, *3*(1-2), 244-250.
15. Muceneeki, R. S., Amorim, C. M., Cesca, T. G., Biavatti, M. W., & Bresolin, T. M. (2009). A simple and validated LC method for the simultaneous determination of three compounds in Mikania laevigata extracts. *Chromatographia, 69*, 219-223.eki, R. S., et al. (2009). A simple and validated LC method for the simultaneous determination of three compounds in *Mikania laevigata* extracts. *Chromatographia, 69*, 219-223.
16. Ríos, E., et al. (2014). Sesquiterpene lactones from *Mikania micrantha Kunth*and *Mikania cordifolia* and their cytotoxic and anti-inflammatory evaluation. *Fitoterapia, 94*, 155-163.
17. Sharma, S., Semwal, B. C., & Mazumder, A. (2024). Microscopic, pharmacognostic and phytochemical evaluation of Sesbania grandiflora leaves. *Journal of Applied Pharmaceutical Research*, *12*(3), 99-106.
18. Wan Nurhayati, W. H., Arlizan, T. N., & Nurdiana, S. (2013). Effect of *Mikania micrantha Kunth*leaf extract on the level of blood glucose and hepatic glycogen in normal and alloxan-induced diabetic rats. *Natural Products an Indian Journal, 9*(10), 398-402.
19. Evans, W. C. (2009). *Trease and Evans pharmacognosy* (16th ed., pp. 553–557). Saunders Elsevier Publishers, University of Nottingham.
20. Hoque, A. T. M. R., Ahmed, R., Uddin, M. B., & Hossain, M. (2003). Allelopathic effect of different concentration of water extracts of Acacia auriculiformis leaf on some initial growth parameters of five common agricultural crops. *Pakistan journal of agronomy*, *2*(2), 92-100.

21. John, J., & Sarada, S. (2012). Role of phenolics in allelopathic interactions. *Allelopathy Journal*, *29*(2), 215-230.