

Chapter 7

Qualitative phytochemical analysis and TLC profiling of flowers of *Chromolaena odorata*

Emdad Hossain^{1*}, J Jeline Rani², Brajesh Kumar Sahu³, Shikha Thakur⁴, Nibedita Jena⁵ and Sanjeet Kumar⁵

¹Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

²Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, India

³Department of Botany, P.M. College of Excellence, Government College Vidisha, Madhya Pradesh, India

⁴Department of Biotechnology, School of Applied and Life Sciences, Uttaranchal University, Dehradun, Uttarakhand, India

⁵Ambika Prasad Research Foundation, Odisha, India

*Email-Id: emdad@rediffmail.com

ORCID: <https://orcid.org/0000-0001-9110-6449>



Licensed under a Creative Commons Attribution 4.0 International License

ABSTRACT

Invasive species, despite posing a threat to the environment, can serve as a valuable source of therapeutic agents. By utilizing them as raw materials, their population can be controlled in a profitable manner, offering a potential solution to mitigate their negative impact. Therefore, present research investigates the bioactive compounds of *Chromolaena odorata* flowers through qualitative phytochemical analysis and thin layer chromatography (TLC). The qualitative phytochemical analysis revealed the presence of tannins, saponins, phenols, and reducing sugars in the hydroethanolic and acetone extracts. TLC using different mobile phases further confirmed the presence of various bioactive compounds, with distinct R_f values. These findings suggest that *C. odorata* flowers possess potential medicinal properties and further advance research and exploration of their therapeutic applications are needed.

Keywords: Bioactive compounds, flowers, invasive species, traditional uses

INTRODUCTION

Invasive species have become a significant concern globally, causing ecological, economic, and environmental problems (Kumar and Singh, 2020). These non-native species can outcompete native species for resources, alter ecosystems, and disrupt

biodiversity (Leger and Espeland, 2010). However, while their impact is often viewed as solely negative, invasive species can also serve as a valuable resource (Peter *et al.*, 2021). One potential use of invasive species is as a source of raw materials for the isolation of bioactive compounds, which can be used in the development of herbal drug formulations (Pereira *et al.*, 2021). Many invasive species contain unique compounds that have medicinal properties, making them a potential source of new drugs (Uzelac *et al.*, 2023). By utilizing invasive species as a resource, we can reduce their populations and mitigate their negative impacts. The plant *Chromolaena odorata* (L.) R.M. King & H. Rob. is referred to as Jack in the Bush. This blooming shrub belongs to the Asteraceae family and grows in tropical and subtropical regions. It is a perennial shrub that scrambles, with shallow, fibrous roots, and straight, pithy, brittle stems that branch easily. Its ovate-triangular, three-veined leaves are arranged opposite one other. At the tips of branches, panicles of 10 to 35 white to pale pinkish tubular flowers bloom (Zachariades *et al.*, 2009). West African and Asian countries use *C. odorata* as a source of medicine in their traditional medical treatments. The plant is renowned for its therapeutic qualities, particularly in wound care. In various sub-Saharan nations, *C. odorata* has long been used as a folk remedy for a variety of ailments, including colds and coughs, skin infections, wounds, dysentery, headaches, toothaches, malaria fever, stomach issues, fungal infections, stomach ulcers and as an antiseptic. Additionally, people in numerous West African nations, including Cameroon, have discovered a variety of traditional uses for this plant (Omokhua *et al.*, 2016). Traditional Indian medicine uses *C. odorata* to treat eye issues and diabetes (Onkaramurthy *et al.*, 2013). Therefore, present study explores the potential of using *Chromolaena odorata* (Figure 1), an invasive species. There is limited research on the bioactive compounds of *C. odorata* flowers. Hence, flowers are selected for phytochemical & TLC analysis. Therefore, the current findings indicate that flowers of *C. odorata* could be a significant source of bioactive compounds for application in herbal drug formulations.



Figure 1: Flowers of *C. odorata*

METHODOLOGY

The flowers of *C. odorata* were collected from nearby Mahanadi areas of CDA 13 of Cuttack district, Odisha, India (Figure 2). The plant species was identified by the authors (Figure 1; Nayak and Kumar, 2023). The Soxhlet extraction method was adopted using three extracts for phytochemical analysis (Devi *et al.*, 2023; Marndi *et al.*, 2024; Jaiswal *et al.*, 2024; Hossain *et al.*, 2025).

Test for tannin: 1 ml of flower extract was taken in a test tube and added 3-5 drops of 10% lead acetate solution into it. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of the flowers extract was added to 1 ml of distilled water and shaken well. The formation of persistent froth was observed confirming the presence of saponin.

Test for flavonoids: About 1 ml of the flowers extract was taken. Added 2 ml of NaOH solution and 3-5 drops of dilute HCl to it. The colour initially turned to an

intense yellow colour with NaOH solution and later became colourless which confirmed the presence of flavonoids.



Figure 2: Collected flowers of *C. ododrata* for experimental work

Test for terpenoids: To 1 ml of the flower extract, 5-6 drops of chloroform were added. Then 4-6 drops of concentrated sulphuric acid were added into it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the flowers extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the flowers extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the

water bath for few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroid: To 1 ml of the flowers extract, added 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

Test for alkaloid: About 1 ml of the flowers extract was taken. Added 3-4 drops of Dragendroff's reagent to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of flowers extract was taken and added 3-4 drops of 2,4- Dinitrophenylhydrazine (DNPH) reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds.

TLC (Thin layer chromatography) analysis

TLC plates were prepared on 6 cm glass slides using silica gel powder. Slides were washed with clinical laboratory detergent and distilled water, followed by drying. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constantly stirring, and finally was poured over the slides and slides were left undisturbed till the drying of silica layer. Then the slides were activated at 105° C for 15-20 minutes before running the TLC. Methanol: chloroform (50%), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were marked, recorded the R_f (Retention Factor) values and visualized under UV chamber (Kumar and Jena 2014; Mishra and Bhatnagar 2024).

RESULTS AND DISCUSSION

The present study identified various bioactive compounds in *C. odorata* flowers (Table 1). The qualitative analysis revealed the presence of tannins, saponins, phenols, and reducing sugars in the hydroethanolic and acetone extracts. These

compounds are known for their potential medicinal properties (Sorrenti *et al.*, 2023; Mehra *et al.*, 2025).

Table 1: Qualitative analysis of secondary metabolites of *Chromolaena odorata* using hydroethanolic extract

Bioactive compounds	n-hexane	Acetone	Hydroethanolic
Tannin	Not detected	Detected	Detected
Saponin	Not detected	Detected	Detected
Flavonoids	Not detected	Not detected	Not detected
Terpenoids	Not detected	Not detected	Not detected
Phenols	Not detected	Detected	Detected
Reducing sugars	Not detected	Detected	Detected
Steroids	Not detected	Not detected	Not detected
Alkaloids	Not detected	Not detected	Not detected
Carbonyl compounds	Not detected	Not detected	Not detected

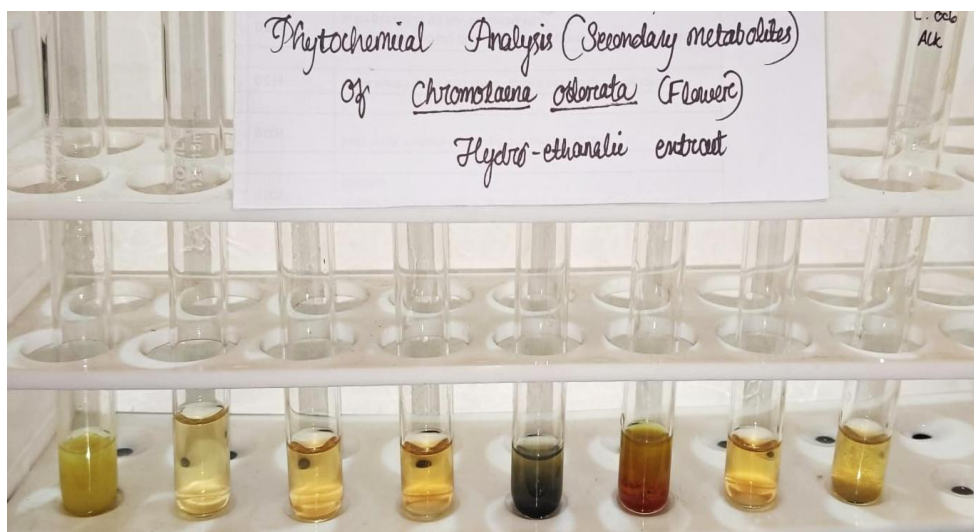


Figure 3: Detection of secondary metabolites of *C. odorata* flowers using hydroethanolic extract

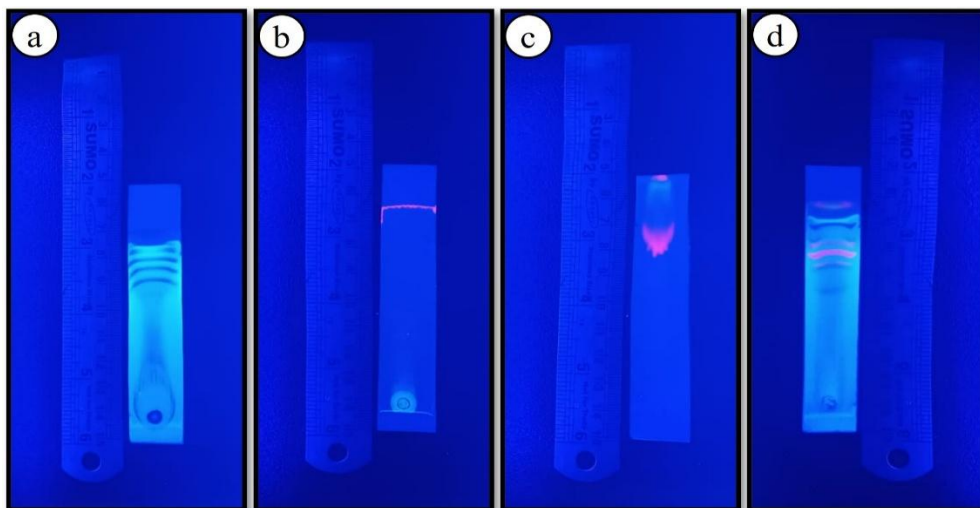


Figure 4: Spots on TLC plates under UV light; a) (Chloroform: methanol) acetone extract; b) (Chromophyll: methanol) hydroethanolic extract; c) (Ethyl acetate: water: acetic acid) hydroethanolic extract & d) acetone extract

Table 2: TLC analysis of *Chromolaena odorata* flowers using different mobile phases

Mobile phases	Extracts	R _f values			
		S ₁	S ₂	S ₃	S ₄
Chloroform: methanol (1:1)	Acetone	0.67	0.73	0.78	0.87
	Hydroethanolic	0.76	Nil	Nil	Nil
Ethyl acetate: chloroform (1:9)	Acetone	0.94	Nil	Nil	Nil
	Hydroethanolic	0.88	Nil	Nil	Nil
Ethyl acetate: water: acetic acid (8:1:1)	Acetone	0.78	0.83	0.96	0.98
	Hydroethanolic	0.81	0.96	Nil	Nil

The TLC analysis using different mobile phases further confirmed the presence of bioactive compounds in the flowers (Table 2). Hydroethanolic and acetone extracts showed distinct R_f values, indicating the presence of different compounds. The results suggest that *C. odorata* flowers may have potential therapeutic applications. The phytochemical analysis of *C. odorata* leaves showed that it contains alkaloids, flavonoids, saponins, tannins and steroids which will contribute to its medicinal values (Usunomena and Efosa, 2016). Ugwoke and his colleagues reported that the leaf, stem and root extracts of *C. odorata* have significant antimicrobial effect against *A. niger* and *C. albicans* (Ugwoke *et al.*, 2017). Further research is needed to isolate and characterize the bioactive compounds present in *C. odorata* flowers. This could lead to the development of new herbal medicines or other products with potential health benefits. The study highlights the importance of exploring invasive species as a source of raw materials for the isolation of bioactive compounds.

CONCLUSION

The phytochemical analysis of *Chromolaena odorata* flowers has revealed the presence of various bioactive compounds, including tannins, saponins, phenols, and reducing sugars. These findings suggest that *C. odorata* may be a valuable source of bioactive compounds for use in herbal drug formulations. Further research is needed to isolate and characterize the bioactive compounds present in *C. odorata* flowers, and to explore their potential therapeutic applications.

REFERENCES

- Devi RS, Satapathy KB and Kumar S. (2023). Validation of tribal claims for formulation of future drugs through evaluation of ethnopharmacological values of *Ludwigia adscendens*. Medicinal Plants. 15(4): 691-697.
- Hossain E, Roy BC, Jena N and Kumar S. (2025). *Plants and Secondary Metabolites, Volume IV*. Ambika Prasad Research Foundation, India. DOI: 10.5281/zenodo.14845411

- Jaiswal A, Hossain E, Kumar N and Kumar S. (2024). *Plants & Secondary Metabolites, Volume II*. Ambika Prasad Research Foundation, Odisha, India. DOI: 10.5281/zenodo.13995335
- Kumar RP and Singh JS. (2020). Invasive alien plant species: their impact on environment, ecosystem services and human health. *Ecological Indicator*. 111:106020. DOI: 10.1016/j.ecolind.2019.
- Kumar S and Jena PK. (2014). Chromatography, antibacterial and FT-IR analysis of *Dioscorea pentaphylla* L. tuber extracts. *Plant Science Research*. 36(1 & 2): 83-90.
- Leger EA and Espeland EK. (2010). Coevolution between native and invasive plant competitors: implications for invasive species management. *Evolutionary Applications*. 3(2):169-178.
- Marndi S, Barik M and Kumar S. (2024). Phytochemical and cytotoxicity analysis of *Eulophia explanata* Lindl. (Tuber extracts). *African Journal of Biological Sciences*. 6(4): 2751-2756.
- Mehra S, Hossain E, Nayak NR, Sahu BK, Jena N and Kumar S. (2025). Phytochemical and cytotoxicity analysis of resin of *Gardenia gummifera* L.f. (Rubiaceae). In: *Medico-Biowealth of India, Volume 19*. Ambika Prasad Research Foundation, India.
- Mishra S and Bhatnagar S. (2024). Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. *World Journal of Pharmaceutical Life Science*. 10(2): 116-118.
- Nayak S and Kumar S. (2023). Medicinal plants used by tribals of Odisha. State Medicinal Plants Board, Bhubaneswar, Odisha & Ambika Prasad Research Foundation, Odisha, India.
- Omokhua AG, McGaw LJ, Finnie JF, Staden JV. (2016). *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. *Journal of Ethnopharmacology* 183: 112-122. DOI: 10.1016/j.jep.2015.04.057
- Onkaramurthy M, Veerapur VP, Thippeswamy BS, Reddy TNM, Rayappa H, Badami S. (2013). Anti-diabetic and anti-cataract effects of *Chromolaena odorata* Linn., in

streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology* 145: 363-372.
DOI: 10.1016/j.jep.2012.11.023

Pereira AG, Fraga-Corral M, Garcia-Oliveira P, Lourenço-Lopes C, Carpena M, Prieto MA and Simal-Gandara J. (2021). The use of invasive algae species as a source of secondary metabolites and biological activities: Spain as case-study. *Marine Drugs*. 19(4):178. DOI: 10.3390/md19040178

Peter A, Zlabur JS, Surie J, Voca S, Purgar DD, Pezo L and Voca N. (2021). Invasive plant species biomass-evaluation of functional value. *Molecules*. 26(13):3814. DOI: 10.3390/molecules26133814

Sorrenti V, Buro I, Consoli V and Vanella L. (2023). Recent advances in health benefits of bioactive compounds from food wastes and by-products: biochemical aspects. *International Journal of Molecular Sciences*. 24(3):2019. DOI: 10.3390/ijms24032019

Ugwoke CEC, Orji J, Anze SPG and Ilodibia CV. (2017). Quantitative phytochemical analysis and antimicrobial potential of the ethanol and aqueous extracts of the leaf, stem and root of *Chromolaena odorata* (Asteraceae). *International Journal of Pharmacognosy and Phytochemical Research*. 9(2): 207-214.

Usunomena U and Efosa EG. (2016). Phytochemical analysis, mineral composition and in vitro antioxidant activities of *Chromolaena odorata* leaves. *ARC Journal of Pharmaceutical Sciences*. 2(2): 16-20.

Uzelac M, Sladonja B, Sola I, Dudas S, Bilie J, Famuyide IM, McGaw LJ, Eloff JN, Mikulic-Petkovsek M and Poljuha D. (2023). Invasive alien species as a potential source of phytopharmaceuticals: phenolic composition and antimicrobial and cytotoxic activity of *Robinia pseudoacacia* L. leaf and flower extracts. *Plants*. 12(14):2715. DOI: 10.3390/plants12142715

Zachariades C, Day M, Muniappan R, and Reddy GVP. (2009). *Chromolaena odorata* (L.) King and Robinson (Asteraceae) In: Muniappan R, Reddy GVP, Raman A (Eds.). *Biological Control of Tropical Weeds Using Arthropods*. Cambridge University Press. Cambridge, UK.