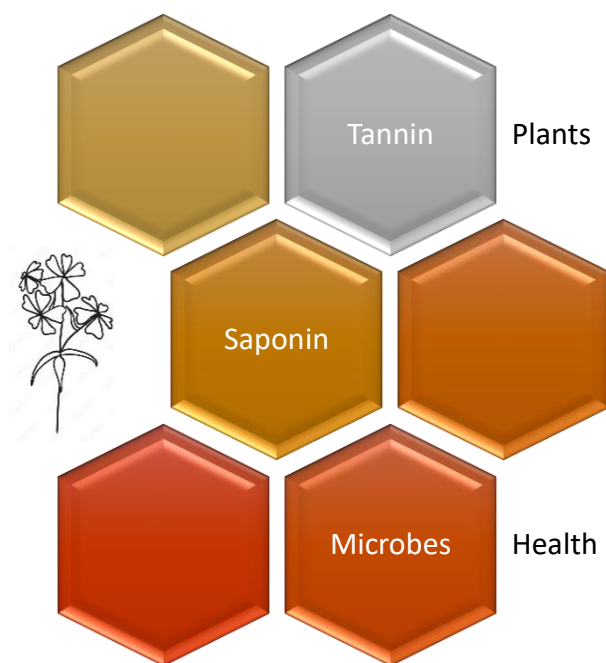


# PLANTS & SECONDARY METABOLITES

## VOLUME VII

Nibedita Jena • Gajender Singh • Sanjeet Kumar



Ambika Prasad Research Foundation, India

# Plants & Secondary Metabolites

Volume 7



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# PREFACE

Plants have been a cornerstone of traditional medicine for centuries, providing a rich source of bioactive compounds with immense therapeutic potential. The study of plants secondary metabolites has gained significant attention in recent years, driven by the need for novel and sustainable solutions to address pressing global health challenges. This book, “Plants and Secondary Metabolites, Volume 7” is a compilation of research chapters that delve into the phytochemical analysis and toxicity evaluation of various plant species, shedding light on their medicinal properties and potential applications.

The chapters in this volume cover a diverse range of plant species, including unexplored and understudied plants, highlighting the vast untapped potential of plant-based medicine. Through rigorous scientific investigation, the contributors to this volume have uncovered the phytochemical profiles of these plants, revealing a treasure trove of bioactive compounds with potential therapeutic applications. From the mangrove-associated plant *Acanthus ilicifolius* to the wild fern *Hemionitis arifolia*, each chapter provides a unique insight into the phytochemical composition and potential medicinal properties of these plant species.

This book is a valuable resource for researchers, scientists, and students in the fields of phytochemistry, pharmacology, and botany. The research presented in this volume contributes to the growing body of evidence on the importance of plant secondary metabolites in medicine and highlights the need for further exploration and conservation of plant biodiversity. We hope that this book will inspire further research and innovation in the field of plant-based medicine, ultimately leading to the development of novel therapeutic solutions that benefit humanity.

(Editors)

# BACKGROUND

Plants have been a vital source of medicine for centuries, with many traditional remedies derived from plant extracts. The study of plant secondary metabolites has become increasingly important in modern medicine, as these compounds have been found to possess a wide range of bioactive properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. The diversity of plant species, coupled with advances in analytical techniques, has enabled researchers to explore the phytochemical composition of various plants, leading to the discovery of novel compounds with potential therapeutic application.

The exploration of plant secondary metabolites has significant implications for human health, agriculture, and conservation. As the world grapples with emerging diseases, climate change, and environmental degradation, the importance of plant-based medicine and sustainable natural resource management cannot be overstated. This book contributes to the growing body of research on plant secondary metabolites, highlighting the phytochemical diversity of various plant species and their potential applications in medicine, agriculture, and beyond. By advancing our understanding of plant chemistry and its applications, this research has the potential to inform new approaches to disease prevention, treatment and conservation.



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# Chapter 1

## Phytochemical and toxicity evaluation of *Vincetoxicum fasciculatum* fruits: an unexplored plant of Apocynaceae

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**Abstract:** *Vincetoxicum fasciculatum*, a plant species of Apocynaceae family with potential medicinal properties, was subjected to phytochemical analysis and cytotoxicity evaluation. Phytochemical screening of the hydroethanolic extract of the fruits revealed the presence of tannins, saponins, flavonoids, phenolic compounds, and reducing sugars. Cytotoxicity analysis using brine shrimp nauplii showed that the extract exhibited significant cytotoxic activity, with a death rate of 100 % at concentrations of 50-200 mg/mL after 3 hours of exposure. These findings suggest that *V. fasciculatum* possesses bioactive compounds with potential therapeutic applications and draw attention towards further investigation in pharmacological aspects.

**Keywords:** Bioactive compounds; phytochemicals; wild fruits

## INTRODUCTION

The Apocynaceae family, comprising about 5300 species (Straub et al., 2020) and a rich source of medicinal plants with a long history of traditional use (Lv et al., 2020). Many members of this family have been employed in various folk medicine

practices (Bhadane et al., 2018) to treat a range of ailments, including cancer (Wong et al., 2011), diabetes (Bindu and Narendhirakannan, 2019) and cardiovascular diseases (Bachheti et al., 2022). The Apocynaceae family is particularly notable for its high diversity of bioactive compounds, including alkaloids, glycosides, and terpenoids, which have been shown to possess potent medicinal properties (Almagro et al., 2015; Fanta-Yadang et al., 2019; Kanwal et al., 2020; Naidoo et al., 2021; Mollica et al., 2022). Genus *Vincetoxicum* Wolf, an unexplored member of the Apocynaceae family, with potential medicinal applications (Prameela et al., 2024). Therefore, *V. fasciculatum* (Buch. -Ham. ex Wight) Kuntze is selected for present study. It is an unexplored plant and the secondary metabolites and biological activities remain undiscovered.



Figure 1: Fruit of *Vincetoxicum fasciculatum*

Present study aimed to investigate the phytochemical profile and cytotoxicity of the hydroethanolic extract of *V. fasciculatum* fruits, with a view to identifying potential therapeutic applications and contributing to the discovery of new medicinal agents from the Apocynaceae family.

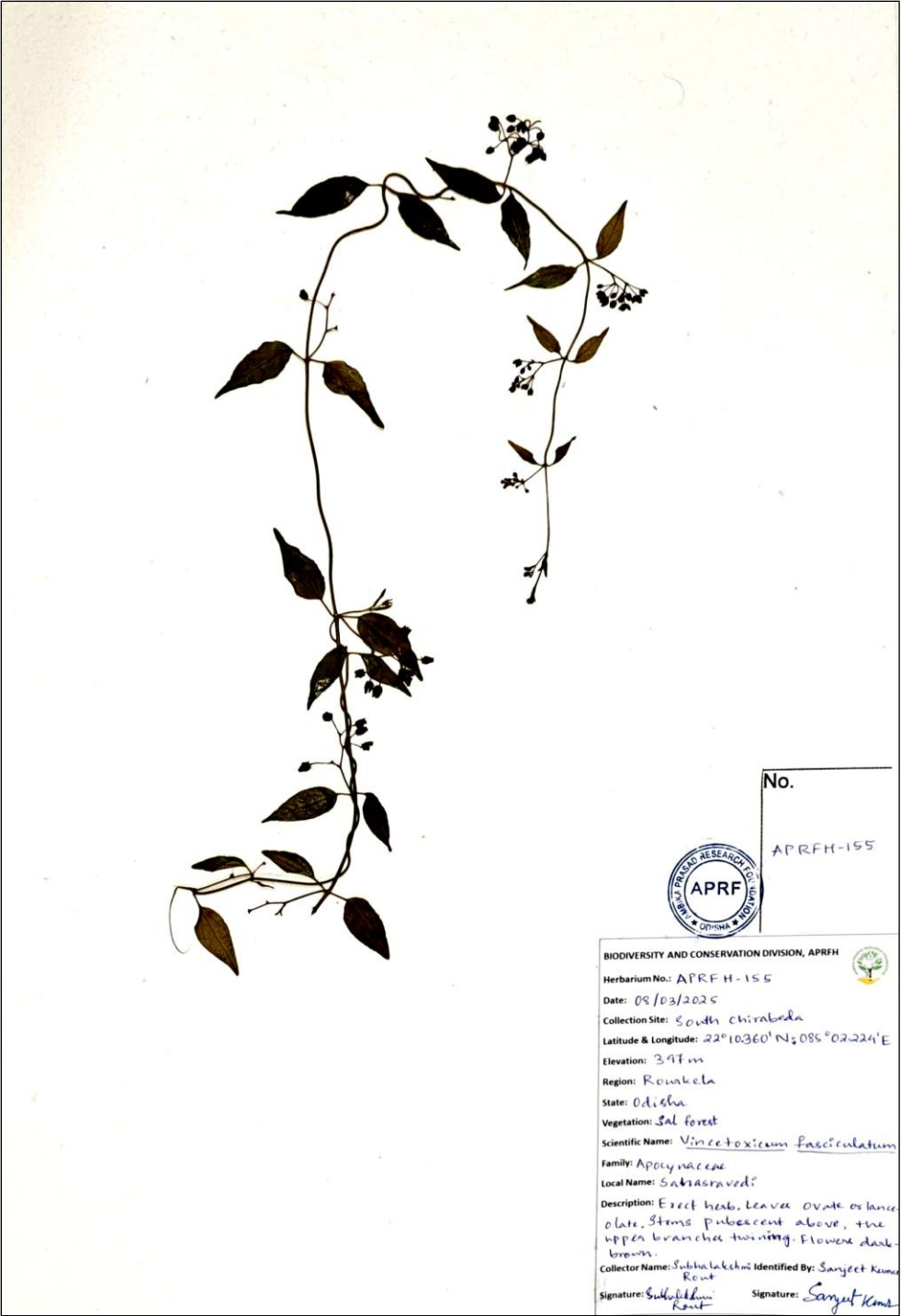


Figure 2: Herbarium specimen of *Vincetoxicum fasciculatum*

## MATERIALS AND METHODS

The fruits of *V. fasciculatum* were collected from the Rourkela Forest Division of Sundargarh district of Odisha, India (Figure 1). The plant species was identified by the authors and a specimen was submitted at APRFH (Figure 2). The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024). For toxicity analysis, hatching of Brine cysts was the initial process. Standardization of the optimum was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration, room temperature between 28-35°C and light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the fruit extracts were taken and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/ml) or potassium dichromate and 3.5% saline water respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiate from the unhatched cysts. The survivor nauplii were counted and analysed the death percentage (Kumar et al., 2012).

## RESULTS & DISCUSSION

The phytochemical analysis of the hydroethanolic extract of *V. fasciculatum* fruits revealed the presence of several bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, and reducing sugars (Table 1).

Table 1: Phytochemical analysis of fruits of *Vincetoxicum fasciculatum* using hydroethanolic extract

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | +ve                    |
| Flavonoids          | +ve                    |
| Terpenoids          | -ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |
| Steroids            | -ve                    |
| Alkaloids           | -ve                    |
| Carbonyl compounds  | -ve                    |

(+ve: Present; -ve: Absent)

Table 2: Cytotoxicity analysis of fruits of *Vincetoxicum fasciculatum* using hydroethanolic extract

| Extract                        | Concentration<br>(in mg/ml) | Initial<br>number<br>of Nauplii | Number of deaths of<br>Nauplii |                  |                  | Death<br>rates<br>(%) |
|--------------------------------|-----------------------------|---------------------------------|--------------------------------|------------------|------------------|-----------------------|
|                                |                             |                                 | After 1<br>hour                | After 2<br>hours | After 3<br>hours |                       |
| Hydro-<br>ethanolic<br>extract | 25                          | 10                              | 4                              | 7                | 10               | 100                   |
|                                | 50                          | 10                              | 7                              | 10               | 10               | 100                   |
|                                | 100                         | 10                              | 8                              | 10               | 10               | 100                   |
|                                | 200                         | 10                              | 10                             | 10               | 10               | 100                   |
|                                | 3.5% Saline                 | 10                              | 0                              | 0                | 0                | 0                     |
|                                | Vincristine<br>sulphate     | 10                              | 10                             | 10               | 10               | 100                   |

The presence of these compounds is consistent with previous reports on the phytochemical composition of other Apocynaceae species. The cytotoxicity analysis of the hydroethanolic extract against brine shrimp nauplii showed significant cytotoxic activity, with a death rate of 100 % at concentrations of 50-200 mg/ml after 3 hours of exposure (Table 2). The LC<sub>50</sub> value of the extract was found to be 25 mg/ml, indicating potent cytotoxic activity. The cytotoxic activity

of the extract may be attributed to the presence of bioactive compounds (Kumar et al., 2013; Hossain et al., 2025). The results of present study suggest that *V. fasciculatum* may be a potential source of anticancer agents (Kumar et al., 2012). The results are also consistent with previous reports on the medicinal properties of other Apocynaceae species. The presence of bioactive compounds such as tannins, saponins, and flavonoids in the extract may contribute to its medicinal properties (Aradhana et al., 2022). Further studies are needed to isolate and characterize the bioactive compounds responsible for the cytotoxic activity and to evaluate the extract's anticancer potential in vitro and in vivo. Other researchers have also reported the medicinal values.

Saheb (2014) reported that leaves of *Tylophora fasciculata* are used to treat wounds and ulcers during the survey of medicinal climbers from Nallamalais, Andhra Pradesh, India. Bhakshu et al., (2016) observed the anticandidal activity in *T. fasciculata*, and it showed inhibitory activity against *Candida albicans*. He also reported that the leaf pastes of *T. fasciculata* were applied externally to the wounds for treatment. Lakshmi et al., (2023) reported the presence of alkaloids, flavonoids, glycosides, saponins, and volatile oils in *T. fasciculata*. Mostafa et al., (2023) documented the ethno-medicinal uses of *T. fasciculata*. The above reports and present study indicate that there is a need for more explorer and bioactivity analysis works.

## CONCLUSION

The present study demonstrates the phytochemical and cytotoxic properties of *Vincetoxicum fasciculatum* and highlights its potential as a source of medicinal agents. Further research is needed to fully explore the medicinal properties of this plant species.

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## Chapter 2

### Phytochemical analysis of *Limnophila repens* (Benth.) Benth. (Plantaginaceae): a wetland plant

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**Abstract:** *Limnophila repens*, a traditionally used medicinal wetland plant, was subjected to qualitative phytochemical analysis using n-hexane, ethanol, and distilled water. The results revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, and alkaloids. The ethanol and aqueous extracts showed a broader range of bioactive compounds compared to the n-hexane extract. This study provides valuable insights into the secondary metabolites of *L. repens*, which may contribute to formulate new therapeutic agents.

**Keywords:** Bioactive compounds; medicinal plants; wetlands

## INTRODUCTION

Wetlands are vital ecosystems that provide numerous ecological, economic, and social benefits (Mazzotta et al., 2019). They support a wide range of plant and animal species, maintain water quality, and regulate water cycles (Devi et al., 2024a). Wetlands also play a crucial role in human well-being, providing food, fiber, and medicines, as well as recreational and tourism opportunities (Devi et al.,

2024b). Among the wetland plants, *Limnophila repens* (Benth.) Benth., an aquatic plant species has medicinal uses (Gunji and Seru, 2020). Despite its importance, *L. repens* remains understudied, and its secondary metabolites are not well documented. This study aims to investigate the phytochemical constituents of *L. repens* and provide a scientific basis for its traditional therapeutic uses. The findings of this study will contribute to the conservation and sustainable use of *L. repens* and highlight the importance of wetland ecosystems in supporting human well-being.



Figure 1: Whole plant of *Limnophila repens*

## MATERIALS AND METHODS

Whole plants of *L. repens* were collected from Cuttack, Odisha. The plant species was identified by the authors using published literature (Kumar *et al.*, 2022; Figure

1). The Soxhlet extraction method was adopted using three solvents for phytochemical analysis (Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024).

## RESULTS AND DISCUSSION

The phytochemical analysis of the whole plant of *L. repens* revealed the presence of various bioactive compounds in the n-hexane, ethanol, and aqueous extracts (Table 1). The n-hexane extract showed the presence of tannins, terpenoids, reducing sugars, and alkaloids. The ethanol extract contained tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The aqueous extract revealed the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids (Table 1).

Table 1: Phytochemical analysis of the whole plant of *Limnophila repens* using different extracts

| Bioactive compounds | n-Hexane | Ethanol | Aqueous |
|---------------------|----------|---------|---------|
| Tannin              | +ve      | +ve     | +ve     |
| Saponin             | -ve      | -ve     | +ve     |
| Flavonoids          | -ve      | +ve     | +ve     |
| Terpenoids          | +ve      | -ve     | -ve     |
| Phenolic compounds  | -ve      | +ve     | +ve     |
| Reducing sugar      | +ve      | +ve     | +ve     |
| Steroids            | -ve      | -ve     | -ve     |
| Alkaloids           | +ve      | +ve     | +ve     |
| Carbonyl compounds  | -ve      | -ve     | -ve     |

(+ve: Present; -ve: Absent)

The results indicate that the ethanol and aqueous extracts have a broader range of bioactive compounds compared to the n-hexane extract. The phytochemical analysis of *L. repens* revealed a diverse range of bioactive compounds, including tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, and alkaloids.

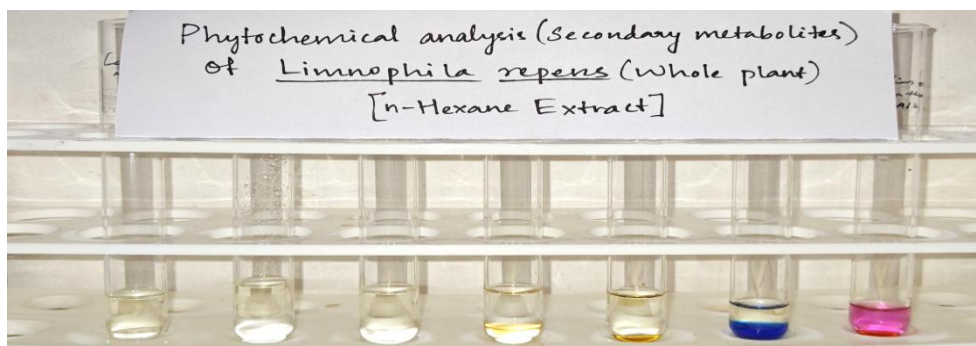


Figure 2: Phytochemical analysis of the whole plant of *L. repens* using n-hexane extract

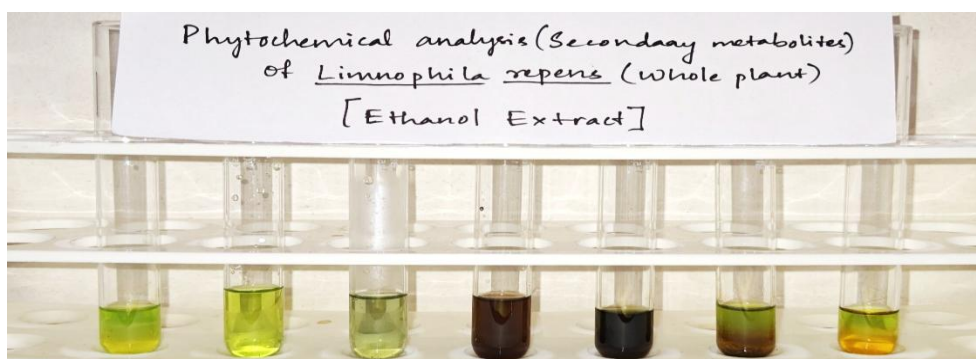


Figure 3: Phytochemical analysis of the whole plant of *L. repens* using ethanol extract

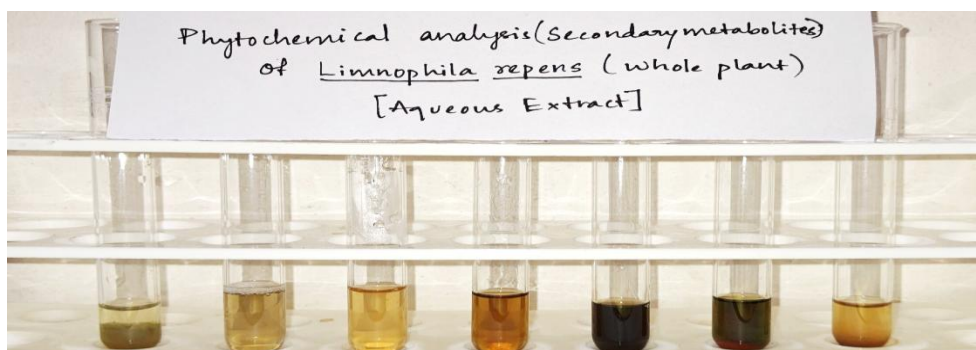


Figure 4: Phytochemical analysis of the whole plant of *L. repens* using aqueous extract

The presence of these compounds may contribute to the medicinal uses of *L. repens* (Kumar et al., 2013; Aradhana et al., 2022). The ethanol and aqueous extracts showed a broader range of bioactive compounds compared to the n-hexane extract. This suggests that polar solvents like ethanol and water may be more effective in extracting bioactive compounds from *L. repens* (Figures 2-4).

## CONCLUSION

The phytochemical analysis of *L. repens* revealed a diverse range of bioactive compounds, including tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, and alkaloids. The presence of these compounds may contribute to the traditional medicinal uses of *L. repens*. This study provides a scientific basis for the traditional medicinal uses of *L. repens* and highlights its potential as a source of natural products with pharmacological activities. Further studies are needed to fully explore the medicinal properties of *L. repens*.

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## Chapter 3

### Phytochemical analysis of *Hemionitis arifolia* (Burm.f.) T.Moore (Pteridaceae): a wild fern

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**Abstract:** Phytochemical analysis of *Hemionitis arifolia* was performed using n-hexane, ethanol, and distilled water. The results revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The aqueous extract showed a unique profile with the presence of saponins and flavonoids. This study provides valuable insights into the phytochemical composition of *H. arifolia*, which may contribute to its medicinal properties.

**Keywords:** Bioactive compounds; medicinal plants; wetlands

## INTRODUCTION

Ferns are one of the oldest and most diverse groups of plants on Earth, with a rich history dating back to the Devonian period (Vasco et al., 2013; Nitta et al., 2022). These non-flowering, vascular plants have been a vital component of terrestrial ecosystems, playing a crucial role in maintaining ecological balance and supporting biodiversity (Aros-Mualin et al., 2021). Beyond their ecological

significance, ferns have also been a valuable source of medicinal agents, with many species being used in traditional medicine (Cao et al., 2017). Most of the ferns are underexplored group of plants in terms of their secondary metabolites. *Hemionitis arifolia* (Burm.f.) T.Moore, (Figure 1) a species of fern commonly found in wet areas (Kumar et al., 2022). However, its bioactive compounds and medicinal properties are not much explored. Therefore, present study aimed to investigate the secondary metabolites of *H. arifolia* and evaluate its potential as a source of bioactive compounds with therapeutic applications.

## MATERIALS AND METHODS

Whole plants of *H. arifolia* was collected from Sundargarh, Odisha (Figure 2). The plant species was identified by the authors using published literature (Kumar *et al.*, 2022). The Soxhlet extraction method was adopted using three solvents for phytochemical analysis (Kumar et al., 2013; Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024). An herbarium specimen was submitted to APRFH (Figure 3).

## RESULTS AND DISCUSSION

The phytochemical analysis of the whole plant of *H. arifolia* revealed the presence of various bioactive compounds in the n-hexane, ethanol, and aqueous extracts (Table 1). Tannins were found to be present in all three extracts (Figures 4-6). Saponins and flavonoids were detected only in the aqueous extract. Phenolic compounds were present in the ethanol extract, while reducing sugars were found in all three extracts.

Table 1: Phytochemical analysis of the whole plant of *Hemionitis arifolia* using different extracts

| Bioactive compounds | n-Hexane | Ethanol | Aqueous |
|---------------------|----------|---------|---------|
| Tannin              | +ve      | +ve     | +ve     |
| Saponin             | -ve      | -ve     | +ve     |
| Flavonoids          | -ve      | -ve     | +ve     |

|                    |     |     |     |
|--------------------|-----|-----|-----|
| Terpenoids         | -ve | -ve | -ve |
| Phenolic compounds | -ve | +ve | -ve |
| Reducing sugar     | +ve | +ve | +ve |
| Steroids           | -ve | -ve | -ve |
| Alkaloids          | +ve | +ve | -ve |
| Carbonyl compounds | -ve | -ve | -ve |

(+ve: Present; -ve: Absent)



Figure 1: Whole plant of *H. arifolia*



Figure 2: Dried sample of *H. arifolia*

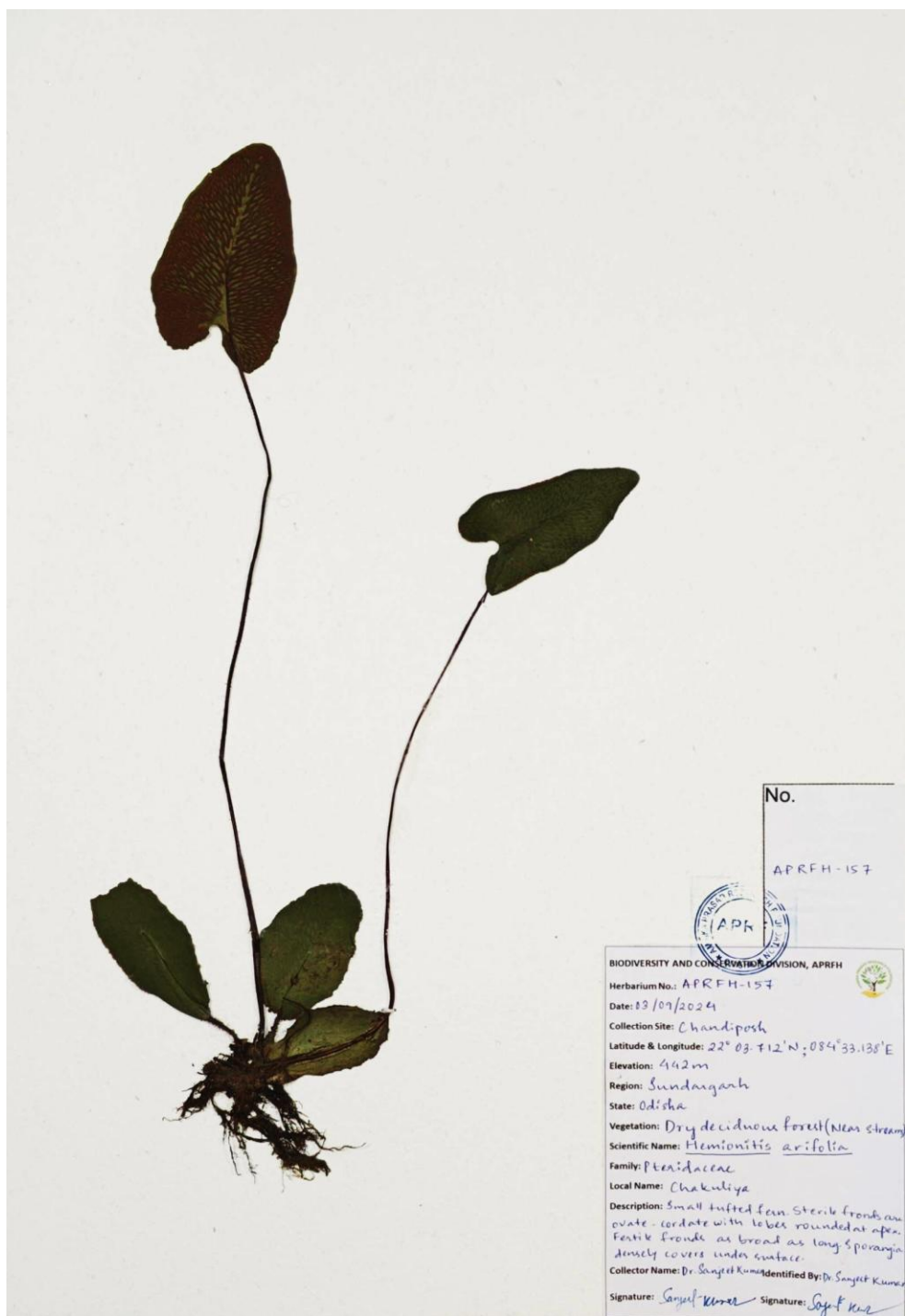


Figure 3: Herbarium specimen of *H. arifolia*

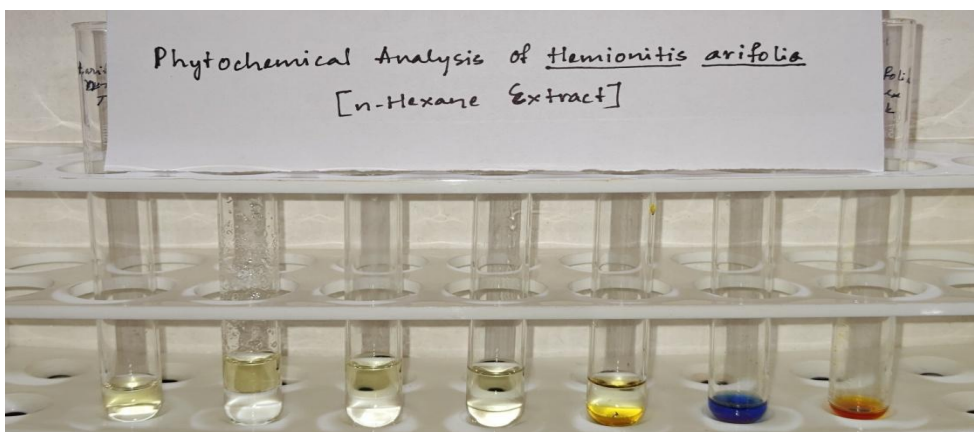


Figure 4: Phytochemical analysis of the whole plant of *H. arifolia* using n-hexane extract

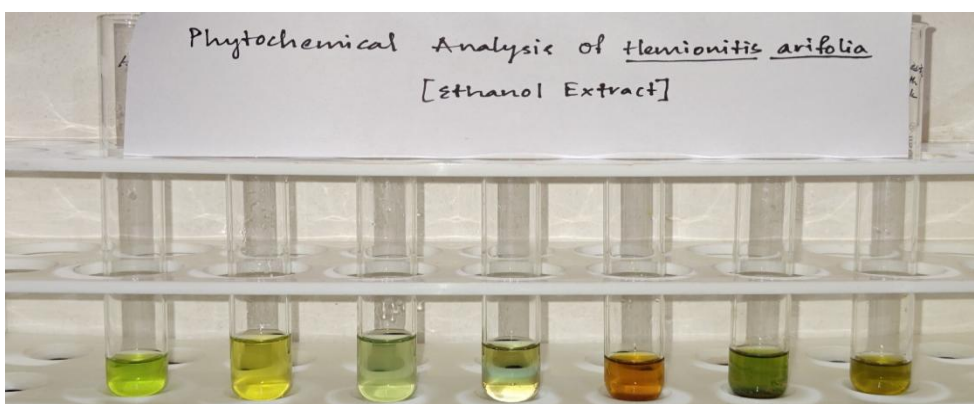


Figure 5: Phytochemical analysis of the whole plant of *H. arifolia* using ethanol extract



Figure 6: Phytochemical analysis of the whole plant of *H. arifolia* using aqueous extract

Alkaloids were detected in the n-hexane and ethanol extracts, but were absent in the aqueous extract. Steroids, terpenoids, and carbonyl compounds were not detected in any of the extracts (Table 1). The phytochemical analysis of *H. arifolia* revealed a diverse range of bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The presence of these compounds may contribute to the medicinal properties of *H. arifolia*. The absence of steroids, terpenoids, and carbonyl compounds in all extracts may be due to the specific solvent systems used or the plant's phytochemical composition. Further studies are needed to fully characterize the phytochemical profile of *H. arifolia*. The results of this study provide a scientific basis for the traditional medicinal uses of *H. arifolia*. The bioactive compounds present in the plant may have potential applications in the development of new drugs or herbal remedies.

## CONCLUSION

Phytochemical analysis of *Hemionitis arifolia* revealed a diverse range of bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The presence of these compounds may contribute to the medicinal properties of *H. arifolia*. This study provides a scientific basis for the traditional medicinal uses of *H. arifolia* and highlights its potential as a source of natural products with pharmacological activities. Further studies are needed to fully characterize the phytochemical profile of *H. arifolia* and to explore its potential applications in medicine.

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## Chapter 4

### Phytochemical analysis of leaves of *Eranthemum nervosum* (Vahl) R.Br. ex Roem. & Schult. (Acanthaceae)

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**Abstract:** *Eranthemum nervosum* leaves were subjected to qualitative phytochemical screening using n-hexane, ethanol, and aqueous extracts. The results revealed the presence of saponins, tannins, phenols, and alkaloids. Saponins were detected in all three extracts, while tannins and phenols were present in the ethanol and aqueous extracts. Alkaloids were found only in the n-hexane extract. The study provides a scientific basis for the therapeutic uses of *E. nervosum* leaves and highlights its potential as a source of future medicinal agent with therapeutic applications.

**Keywords:** Acanthaceae; bioactive compounds; local medicinal plants

## INTRODUCTION

The plant species of The Acanthaceae family are rich source of secondary metabolites with diverse medicinal properties (Akbar et al., 2021). Plants belonging to this family have been used in traditional medicines by different tribal communities (Shinwari et al., 2017). Among the species of this family, *Eranthemum nervosum*, is a medicinal plant used in traditional medicine to treat various

ailments (Nayak and Kumar, 2023). Although, *E. nervosum* is a medicinally significant plant, its secondary metabolites have not been extensively investigated. Therefore, present study aimed to investigate the bioactive compounds of *E. nervosum* leaves and evaluate their potential therapeutic applications. Present findings will enhance our understanding of the Acanthaceae family as a rich source of secondary metabolites, emphasizing the need for conservation and sustainable utilization of these valuable plant species.



Figure 1: Habitat, leaves and flowers of *E. nervosum*

## MATERIALS AND METHODS

The leaves of *E. nervosum* were collected from the nearby area of Mahanadi River, Cuttack district of Odisha, India. The plant species was identified by the authors using published literature (Figure 1; Nayak and Kumar, 2023; Jena et al., 2025). The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Devi et al., 2024; Jena et al., 2024). The identification of secondary metabolites was performed using established protocols (Marndi et al., 2024; Rani et al., 2024; Kshirsarg et al., 2025) through a series of standardized tests.

**Test for tannin:** About 1 ml of the filtrate extract was taken. 3-5 drops of 10% lead acetate solution were added to it. The gelatinous precipitate formation confirmed the presence of tannin.

**Test for saponin:** About 1 ml of the filtrate extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth was observed confirming the presence of saponin (Jayalakshmi *et al.*, 2025).

**Test for flavonoids:** About 1 ml of the leaves extract was taken. 2 ml of 2% NaOH solution and 3 to 4 drops of dilute HCl were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This colour change appearance confirmed the presence of flavonoids (Thapa *et al.*, 2025).

**Test for terpenoids:** About 1 ml of the filtrate was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of concentrated sulphuric acid were added. The reddish- brown interface confirmed the presence of terpenoids (Das *et al.*, 2025).

**Test for phenolic groups:** About 1 ml of the filtrate 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 leaves extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar (Roy *et al.*, 2025).

**Test for steroids:** About 1 ml of the leaves extract was taken. 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid was added into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

**Test for alkaloids:** About 1 ml of the leaves extract was taken and added 3 to 4 drops of Dragendroff's reagent. The formation of a reddish-brown precipitate confirmed the presence of alkaloids (Kumar *et al.*, 2025).

**Test for carbonyl compounds:** About 1 ml of the filtrate leaves extract was taken and added 3 to 4 drops of 2,4- Dinitrophenylhydrazine (DNPH) reagent. The formation of yellow crystal confirmed the presence of carbonyl compounds (Darshani et al., 2025).

## RESULTS AND DISCUSSION

The qualitative phytochemical screening of *E. nervosum* leaves was performed using n-hexane, ethanol, and aqueous extracts (Table 1). The phytochemical analysis revealed the presence of saponins in all three extracts (Figures 2-4). Tannins and phenols were detected in the ethanol and aqueous extracts, but were absent in the n-hexane extract. Alkaloids were present only in the n-hexane extract. Reducing sugars were detected only in the ethanol extract. Flavonoids, terpenoids, steroids, and carbonyl compounds were not detected in any of the extracts (Table 1). These findings suggest that *E. nervosum* leaves contain a range of bioactive compounds with potential medicinal properties. The detection of these compounds in different extracts suggests that *E. nervosum* leaves may possess diverse medicinal properties (Kumar et al., 2013).

Table 1: Qualitative phytochemical screening of *E. nervosum* leaves

| Bioactive components | Extracts |         |         |
|----------------------|----------|---------|---------|
|                      | n-hexane | Ethanol | Aqueous |
| Tannin               | Absent   | Present | Present |
| Saponin              | Present  | Present | Present |
| Flavonoids           | Absent   | Absent  | Absent  |
| Terpenoids           | Absent   | Absent  | Absent  |
| Phenols              | Absent   | Present | Present |
| Reducing sugars      | Absent   | Present | Absent  |
| Steroid              | Absent   | Absent  | Absent  |
| Alkaloid             | Present  | Absent  | Absent  |
| Carbonyl compounds   | Absent   | Absent  | Absent  |



Figure 2: Detection of secondary metabolites using n-hexane extract



Figure 3: Detection of secondary metabolites using ethanolic extract



Figure 4: Detection of secondary metabolites using aqueous extract

### RECOMMENDATIONS ON FUTURE WORKS

Authors have recommended following works:

1. **Isolation and characterization of bioactive compounds:** Future studies should focus on isolating and characterizing the bioactive compounds present in *E. nervosa* leaves to determine their structural elucidation and biological activities.
2. **In-vivo and in-vitro studies:** Further studies are needed to evaluate the pharmacological activities of *E. nervosa* extracts and isolated compounds using *in-vivo* and *in-vitro* models.
3. **Toxicity and safety evaluations:** Toxicity and safety evaluations of *E. nervosa* extracts and isolated compounds should be conducted to determine their potential risks and benefits.
4. **Pharmacokinetic and pharmacodynamic studies:** Pharmacokinetic and pharmacodynamic studies are necessary to understand the absorption, distribution, metabolism, and excretion of *E. nervosa* extracts and isolated compounds.
5. **Clinical trials:** Clinical trials should be conducted to evaluate the efficacy and safety of *E. nervosa* extracts and isolated compounds in humans.

6. **Conservation and sustainable use:** Efforts should be made to conserve and sustainably use *E. nervosum* populations to ensure their continued availability for medicinal and other purposes.

## CONCLUSION

Phytochemical analysis of *Eranthemum nervosum* leaves revealed the presence of various bioactive compounds, including saponins, tannins, phenols, and alkaloids. These findings provide a scientific basis for the traditional medicinal uses of *E. nervosum* leaves and highlight its potential as a source of natural products with therapeutic applications. Further studies are needed to isolate and characterize the bioactive compounds present in *E. nervosum* leaves and to evaluate their potential medicinal applications. The results of this study contribute to the growing body of knowledge on the phytochemical composition and medicinal properties of *E. nervosum*, and underscore the importance of conserving and sustainably using this valuable plant resource.

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## Chapter 5

### Phytochemical and toxicity evaluation of flowers of *Trichosanthes costata* Blume (Cucurbitaceae)

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**Abstract:** *Trichosanthes costata* flowers were analysed for their secondary metabolites and cytotoxic activity. Phytochemical screening revealed the presence of tannins, saponins, flavonoids, terpenoids, phenolic compounds, and reducing sugars. Cytotoxicity analysis using brine shrimp lethality assay showed that the hydroethanolic extract of the flowers exhibited significant cytotoxic activity, with a 100% death rate at concentrations ranging from 12.5 to 200 mg/mL after 24 hrs. The findings of the study suggest that *T. costata* flowers may possess potential anticancer properties and further there is a need of more advance works in this aspect. Study also highlights the importance of less known medicinal plants in the development of future drug formulations & to minimize the impacts of antimicrobial resistance.

**Keywords:** Bioactive compounds; climbers; Cucurbitaceae; secondary metabolites

## INTRODUCTION

*Trichosanthes costata* Blume (Figure 1) is a wild climber belonging to the Cucurbitaceae family, commonly found near forest fringes and tribal villages. It comes under less known medicinal plants. Its plant parts have pharmacological values (Singh and Kumar, 2024). Kumar et al., (2017) reported the antibacterial activity of leaf extracts of this plant against *Streptococcus mutans*, *S. pyogenes*, *Vibrio cholerae*, *Shigella flexneri* and *Salmonella typhi*. Recently, Saradar et al., (2024) documented the bioactive compounds of fruits of *T. costata*. Previous studies have overlooked the flowers of *T. costata* for phytochemical and cytotoxicity analysis. Present study seeks to fill this knowledge gap by examining the bioactive compounds and cytotoxic properties of *T. costata* flowers, (Figure 1) potentially unlocking their value as a source of novel therapeutic agents. The findings of present research will provide foundational data for developing future drugs against deadly diseases and antimicrobial resistance.



Figure 1: Flowers of *T. costata*

## MATERIALS AND METHODS

The flowers of *T. costata* were collected from the Khordha district of Odisha, India. The plant species was identified by the authors and a specimen was submitted at APRFH (Figure 2). The Soxhlet extraction method was adopted using different solvents for phytochemical analysis (Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024). For toxicity analysis, hatching of brine cysts was the initial process. Standardization of the optimum was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature between 28-35°C and light for 48 hours. Proper hatching could be observed within 24-48 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of the flower extracts were taken and 3.5 % saline water was used to make the volume 2 ml in each test tube. 1 % DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/ml) and 3.5 % saline water respectively with a volume of 2 ml. The survivor nauplii were counted and analysed the death percentage (Kumar et al., 2012).

## RESULTS AND DISCUSSION

The hydroethanolic extract of *T. costata* flowers was found to contain various secondary metabolites (Table 1). The cytotoxic activity of the hydroethanolic extract of *T. costata* flowers was evaluated using the brine shrimp lethality assay (Table 2). The hydroethanolic extract exhibited significant cytotoxic activity, with a 100% death rate at concentrations ranging from 12.5 to 200 mg/mL (Figure 3). The positive control, vincristine sulphate, also showed 100% cytotoxicity, while the negative control, 3.5 % saline, showed no cytotoxicity.

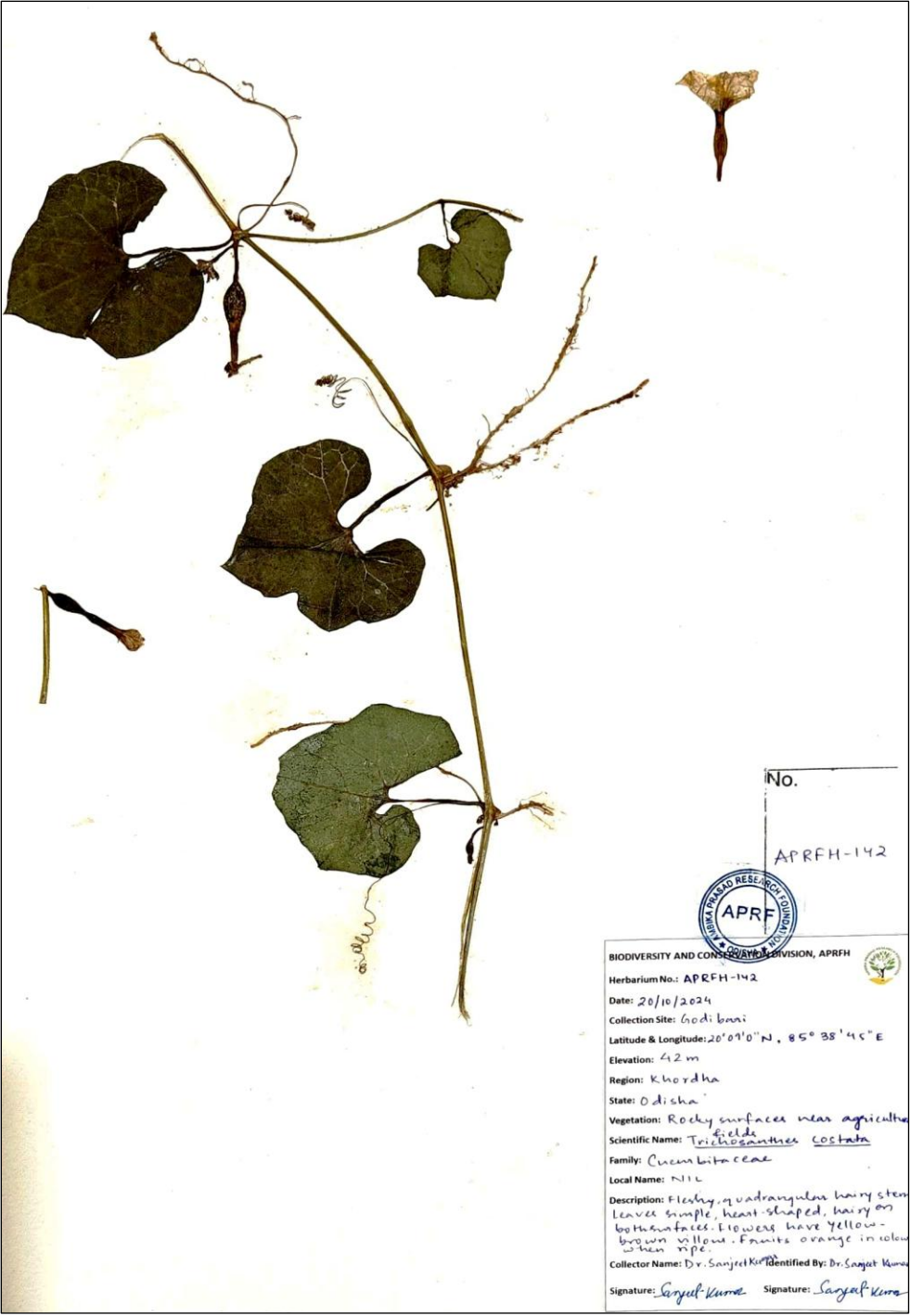


Figure 2: Herbarium specimen of *T. costata*

The present study investigated the phytochemical constituents and cytotoxic activity of *T. costata* flowers. The phytochemical screening revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, terpenoids, phenolic compounds, and reducing sugars. These compounds have been reported to possess various biological activities (Kumar et al., 2013; Sahoo et al., 2021; Devi et al., 2024).

It is suggested that the extract possesses potential anticancer properties, which may be attributed to the presence of detected bioactive compounds. Further studies are needed to isolate and characterize the bioactive compounds present in *T. costata* flowers and to evaluate their anticancer activity *in-vitro* and *in-vivo*.

Table 1: Phytochemical analysis of flowers of *Trichosanthes costata* using hydroethanolic extract

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | +ve                    |
| Flavonoids          | +ve                    |
| Terpenoids          | +ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |
| Steroids            | -ve                    |
| Alkaloids           | -ve                    |
| Carbonyl compounds  | -ve                    |

(+ve: Present; -ve: Absent)

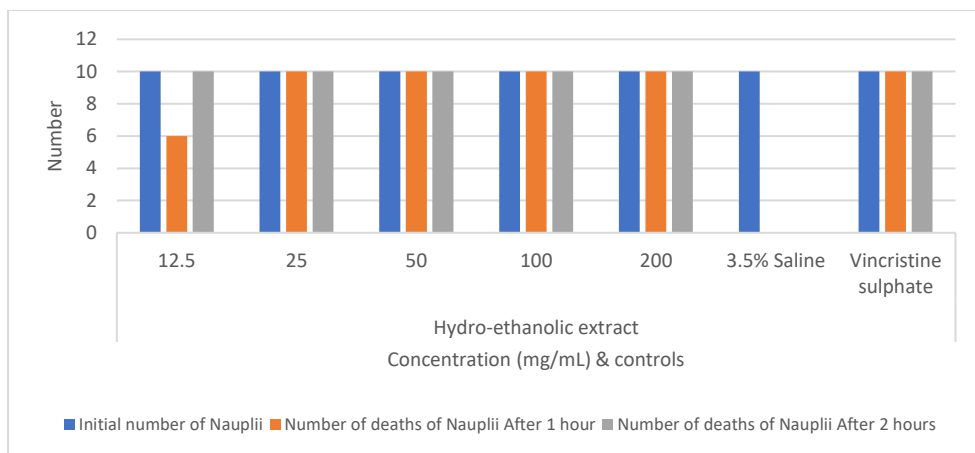


Figure 3: Cytotoxicity analysis of flowers of *T. costata*

Table 2: Cytotoxicity analysis of flowers of *T. costata* using hydroethanolic extract

| Extract                 | Concentration (in mg/ml) | Initial number of Nauplii | Number of deaths of Nauplii |               | Death rates (%) |
|-------------------------|--------------------------|---------------------------|-----------------------------|---------------|-----------------|
|                         |                          |                           | After 1 hour                | After 2 hours |                 |
| Hydro-ethanolic extract | 12.5                     | 10                        | 6                           | 10            | 100             |
|                         | 25                       | 10                        | 10                          | 10            | 100             |
|                         | 50                       | 10                        | 10                          | 10            | 100             |
|                         | 100                      | 10                        | 10                          | 10            | 100             |
|                         | 200                      | 10                        | 10                          | 10            | 100             |
|                         | 3.5% Saline              | 10                        | 0                           | 0             | 0               |
|                         | Vincristine sulphate     | 10                        | 10                          | 10            | 100             |

## CONCLUSION

Present study reveals that *Trichosanthes costata* flowers are rich in bioactive compounds and exhibit substantial cytotoxic activity. The observed cytotoxic effects may be attributed to the presence of these bioactive compounds. Our findings suggest that *T. costata* flowers hold promise as a potential source of anticancer agents, warranting further in-depth investigation to fully explore their therapeutic potential.

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## Chapter 6

### Phytochemical analysis of *Guazuma ulmifolia* Lam., leaves & fruits (Malvaceae)

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**Abstract:** *Guazuma ulmifolia*, a plant species with traditional medicinal uses, was subjected to phytochemical screening to identify its bioactive compounds. Leaves and fruits of the plant were extracted using n-hexane, ethanol, and aqueous solvents, and the extracts were analysed for the presence of various bioactive compounds. The results showed that the leaves and fruits of *G. ulmifolia* contain a range of bioactive compounds, including tannins, saponins, phenols, reducing sugars, and alkaloids. The study provides valuable information on the phytochemical composition of *G. ulmifolia*, which could be useful for the development of new medicinal products and pharmacological applications.

**Keywords:** Bioactive compounds, phytochemical analysis, pharmacological potentials

#### INTRODUCTION

As the world grapples with the challenges of drug resistance and antimicrobial failure (Kumar and Jena, 2014), scientists are turning to unconventional sources for new therapeutic agents (Sahoo et al., 2021). Ornamental trees, long overlooked for their medicinal potential, are emerging as a promising avenue for discovery (Das et al., 2025a). These trees boast a rich diversity of secondary metabolites, which can be leveraged to develop innovative drugs (Roy et al., 2025a). Despite their potential, ornamental trees have been largely ignored due to

misconceptions about their medicinal value. This study seeks to rectify this oversight by shedding light on the bioactive compounds present in *Guazuma ulmifolia*, a widely cultivated ornamental tree species. By exploring the leaves and fruits of *G. ulmifolia* using three solvent extracts, this research aims to highlight the untapped potential of ornamental trees as a source of novel therapeutics.

## MATERIALS AND METHODOLOGY

The leaves and fruits of *G. ulmifolia* were collected from the nearby Mahanadi River area from Athagarh sub-division of Cuttack district in Odisha, India. The plant species was identified by the authors (Figures 1 & 2; Rani et al., 2024; Jena et al., 2025). The Soxhlet extraction method was adopted using different solvent for phytochemical analysis (Devi et al., 2024; Jena et al., 2024; Marndi et al., 2024; Kumar et al., 2025). Detection of nine secondary metabolites were conducted using standard methods (Darshani et al., 2025).

**Test for tannin:** About 1 ml of the filtrate extract was taken. About 3-5 drops of 10% lead acetate solution were added to it. The gelatinous precipitate formation confirmed the presence of tannin.

**Test for saponin:** About 1 ml of the filtrate extract was taken and 1 ml of distilled water was added and shaken well. The formation of persistent froth was observed confirming the presence of saponin (Table 1; Thapa et al., 2025).

**Test for flavonoids:** About 1 ml of the sample extract was taken. About 2 ml of 2% NaOH solution and 3 to 4 drops of dilute HCl were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This colour change appearance confirmed the presence of flavonoids (Jayalakshmi et al., 2025).

**Test for terpenoids:** About 1 ml of the filtrate was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of

concentrated sulphuric acid were added. The reddish- brown interface confirmed the presence of terpenoids.

**Test for phenolic groups:** About 1 ml of the sample 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 filtrate extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar (Roy et al., 2025b).

**Test for steroids:** About 1 ml of the sample extract was taken. 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added into it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

**Test for alkaloids:** About 1 ml of the filtrate extract was taken and 3 to 4 drops of Dragendroff's reagent were added. The formation of a reddish-brown precipitate confirmed the presence of alkaloids (Das et al., 2025b; Kumar et al., 2025).

**Test for carbonyl compounds:** About 1 ml of the filtrate extract was taken and 3 to 4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent were added. The formation of yellow crystals confirmed the presence of carbonyl compounds (Kshirsagar et al., 2025).

## RESULTS AND DISCUSSION

The phytochemical screening of *G. ulmifolia* leaves revealed the presence of various bioactive compounds (Table 1). The n-hexane extract of the leaves showed the presence of saponins and alkaloids, while the ethanol extract revealed the presence of tannins, phenols, and reducing sugars. The aqueous extract showed

the presence of tannins, saponins, phenols, and reducing sugars (Figures 3-5). The phytochemical screening of *G. ulmifolia* fruits also revealed the presence of various bioactive compounds (Table 2). The n-hexane extract of the fruits showed the presence of saponins and reducing sugars, while the ethanol extract revealed the presence of tannins, phenolic groups, and alkaloids. The aqueous extract showed the presence of tannins, saponins, phenolic groups, and reducing sugars (Figures 6-8).

Table 1: Qualitative phytochemical screening of *Guazuma ulmifolia* leaves using different extracts

| Bioactive compounds | Extracts     |              |              |
|---------------------|--------------|--------------|--------------|
|                     | N-Hexane     | Ethanol      | Aqueous      |
| Tannin              | Not detected | Detected     | Detected     |
| Saponin             | Detected     | Detected     | Detected     |
| Flavonoids          | Not detected | Not detected | Not detected |
| Terpenoids          | Not detected | Not detected | Not detected |
| Phenols             | Not detected | Detected     | detected     |
| Reducing sugar      | Not detected | Detected     | detected     |
| Steroid             | Not detected | Not detected | Not detected |
| Alkaloid            | Detected     | Not detected | Detected     |
| Carbonyl compounds  | Not detected | Not detected | Not detected |

Table 2: Qualitative phytochemical screening of *Guazuma ulmifolia* fruits using different extracts

| Bioactive compound | Extracts |         |         |
|--------------------|----------|---------|---------|
|                    | N-Hexane | Ethanol | Aqueous |
| Tannin             | Absent   | Present | Present |
| Saponin            | Present  | Present | Present |
| Flavonoids         | Absent   | Absent  | Absent  |
| Terpenoid          | Absent   | Absent  | Absent  |
| Phenolic groups    | Absent   | Present | Present |
| Reducing sugars    | Present  | Present | Present |
| Steroids           | Absent   | Absent  | Absent  |

|                    |        |         |         |
|--------------------|--------|---------|---------|
| Alkaloids          | Absent | Present | Present |
| Carbonyl compounds | Absent | Absent  | Absent  |



Figure 1: Leaves of *G. ulmifolia*



Figure 2: Fruits of *G. ulmifolia*



Figure 3: Detection of secondary metabolites of leaves using n-hexane extract



Figure 4: Detection of secondary metabolites of leaves using ethanolic extract



Figure 5: Detection of secondary metabolites of leaves using aqueous extract

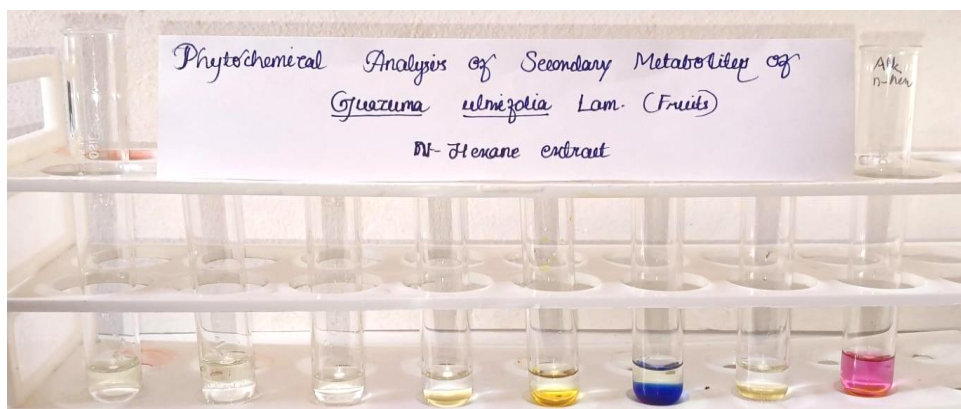


Figure 6: Detection of secondary metabolites of fruits using n-hexane extract



Figure 7: Detection of secondary metabolites of fruits using ethanol extract



Figure 8: Detection of secondary metabolites of fruits using aqueous extract

## CONCLUSION

The present study investigated the phytochemical composition of *Guazuma ulmifolia* leaves and fruits using different solvent extracts. The results showed that both the leaves and fruits of *G. ulmifolia* contain a range of bioactive compounds, including tannins, saponins, phenols, reducing sugars, and alkaloids. The presence of these bioactive compounds in *G. ulmifolia* supports its traditional medicinal uses and suggests its potential as a source of new medicinal agents. Further studies are needed to isolate and characterize the specific bioactive compounds present in *G. ulmifolia* and to evaluate their pharmacological activities.

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## Chapter 7

### Phytochemical and toxicity evaluation of seeds of *Trichosanthes costata*

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**Abstract:** Present study investigated the phytochemical composition and cytotoxic activity of *Trichosanthes costata* seeds. Phytochemical analysis revealed the presence of tannins, saponins, phenolic compounds, and reducing sugars in the hydroethanolic extract. The extract exhibited potent cytotoxic activity, with 100% mortality of nauplii at all concentrations tested. These findings suggest that *T. costata* seeds may be a valuable source of natural compounds with potential therapeutic applications.

**Keywords:** Cucurbits, secondary metabolites, unexplored plants

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## INTRODUCTION

The search for new and effective therapeutic agents from natural sources has been an ongoing endeavour, driven by the need to address various health challenges

(Dzobo, 2022; Hossain et al., 2025). Plants have been a rich source of bioactive compounds, with many modern medicines derived from plant-based sources (Kumar and Satapathy, 2011; Kumar et al., 2017; Sahoo et al., 2021; Jena et al., 2024; Devi et al., 2024; Jena et al., 2025). However, despite the vast array of plants available, many remain unexplored for their medicinal potential (Kumar and Dash, 2012; Das et al., 2020; Das et al., 2022; Devi et al., 2022; Mishra et al., 2022; Saha et al., 2022; Agarwal et al., 2023; Nayak and Kumar, 2023). Screening new and unexplored plants can lead to the discovery of novel compounds with therapeutic properties, providing new avenues for the treatment and management of diseases (Kumar et al., 2013; Nayak et al., 2021; Sahu et al., 2024). In this context, wild cucurbits can be a good selection. Among the wild cucurbits, *Trichosanthes costata*, is an unexplored plant (Figure 1). Less work is carried out using different parts of *T. costata*. Therefore, seeds of this plant are selected for the present work. The present study aims to contribute to the understanding of the medicinal potential of *T. costata* by examining its phytochemical profile and cytotoxic activity, to identify potential therapeutic applications.

## MATERIALS AND METHODS

Seeds of *T. costata* were collected from Cuttack district, Odisha (Figure 2). The plant species was identified by the authors using published literature (Kumar et al., 2022). The Soxhlet extraction method was adopted using hydroethanolic extract for phytochemical analysis (Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024). For toxicity analysis, hatching of Brine cysts was the initial process. Standardization of the optimum was done at 2.0, 3.5, and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration, room temperature between 28-35°C and light for 48 hours. Proper hatching could be observed within 24-48

hours, depending upon the quality of cysts, proper aeration, and light provided. Different concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the seed extracts were taken, and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/ml) and 3.5% saline water, respectively, with a volume of 2 ml. The live larvae are highly mobile and thus differentiate from the unhatched cysts. The survivor nauplii were counted and analysed for the death percentage (Kumar et al., 2012).

## RESULTS AND DISCUSSION

The phytochemical analysis of *T. costata* seeds revealed the presence of tannins, saponins, phenolic compounds, and reducing sugars in the hydroethanolic extract. Conversely, flavonoids, terpenoids, steroids, alkaloids, and carbonyl compounds were not detected (Table 1). The cytotoxicity analysis demonstrated that the hydroethanolic extract exhibited potent cytotoxic activity, with 100% mortality of nauplii at all concentrations tested (12.5-200 mg/ml). This cytotoxic effect was comparable to the positive control, vincristine sulphate, indicating the extract's potential as a source of bioactive compounds with therapeutic applications (Table 2).

Table 1: Phytochemical analysis of seeds of *Trichosanthes costata* using hydroethanolic extract

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | +ve                    |
| Flavonoids          | -ve                    |
| Terpenoids          | -ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |

|                    |     |
|--------------------|-----|
| Steroids           | -ve |
| Alkaloids          | -ve |
| Carbonyl compounds | -ve |

(+ve: Presence; -ve: Absence)

Table 2: Cytotoxicity analysis of seeds of *Trichosanthes costata* using hydroethanolic extract

| Extract                 | Concentration (in mg/ml) | Initial number of Nauplii | Number of deaths of Nauplii | Death rates (%) |
|-------------------------|--------------------------|---------------------------|-----------------------------|-----------------|
|                         |                          |                           | After 1 hour                |                 |
| Hydro-ethanolic extract | 12.5                     | 10                        | 10                          | 100             |
|                         | 25                       | 10                        | 10                          | 100             |
|                         | 50                       | 10                        | 10                          | 100             |
|                         | 100                      | 10                        | 10                          | 100             |
|                         | 200                      | 10                        | 10                          | 100             |
|                         | 3.5% Saline              | 10                        | 0                           | 0               |
|                         | Vincristine sulphate     | 10                        | 10                          | 100             |

Kumar et al., 2017 detected saponins, phenolic compounds, flavonoids, tannins, glycosides, etc., and evaluated anti-bacterial activity against human pathogenic bacteria (*Streptococcus mutans*, *S. pyogenes*, *Vibrio cholerae*, *Shigella flexneri*, and *Salmonella typhi*) by agar well diffusion (AWD), disc diffusion (DD), and broth dilution methods. Saradar et al., 2024, worked on the phytochemical analysis of *T. costata* fruits and reported the presence of bioactive compounds like tannins, saponins, flavonoids, phenols, reducing sugar, etc., using three different extracts. Tambe et al., 2025 have documented a range of secondary metabolites found in *T.*

*costata* flowers, along with toxicity analysis of the plant using the brine shrimp lethality assay.



Figure 1: Habitat of *T. costata*



Figure 2: Collection of seeds of *T. costata* for experimental work

## CONCLUSION

Hydroethanolic extract of *Trichosanthes costata* seeds exhibits potent cytotoxic activity, likely due to the presence of bioactive compounds such as tannins, saponins, and phenolic compounds. These findings suggest that *T. costata* seeds may be a valuable source of natural compounds with potential therapeutic applications, warranting further investigation and characterization.

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## Chapter 8

### Phytochemical analysis of leaves of *Artemisia nilagirica* (C.B. Clarke) Pamp.

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**Abstract:** The phytochemical composition of *Artemisia nilagirica* leaves was examined using n-hexane, ethanol, and aqueous extracts. The analysis detected several bioactive compounds, such as tannins, saponins, terpenoids, phenols, reducing sugars, and alkaloids, among the different extracts. These findings indicate the potential medicinal value of *A. nilagirica* leaves, highlighting the need for additional research to explore their therapeutic potential.

**Keywords:** *Artemisia*, bioactive compounds, leaf

## INTRODUCTION

Plants have been a cornerstone of traditional medicine for centuries, with many species exhibiting potent therapeutic properties (Jaiswal *et al.*, 2024; Hossain *et al.*, 2025). Among them, *Artemisia nilagirica*, a member of the Asteraceae family, is one such plant that has been used in traditional medicine to treat various ailments (Bisht *et al.*, 2021). Given the growing health concerns, including microbial resistance, globally, researchers are increasingly looking to plants as a

potential source of bioactive compounds that could address these issues (Kumar et al., 2013; Kumar et al., 2021; Jena et al., 2024a; Jena et al., 2025). Therefore, leaves of *A. nilagirica* (Figure 1) is selected for present study to explore the availability of bioactive compounds and medicinal potential of plant. Present study aims to investigate the secondary metabolites of *A. nilagirica* leaves using different extracts, providing a foundation for further research into its medicinal properties.



Figure 1: Leaves of *A. nilagirica*

## **MATERIALS AND METHODS**

The leaves of *A. nilagirica* were collected from Khordha district of Odisha (Figures 2-3). The plant species was identified by the authors. The Soxhlet extraction method was adopted using n-hexane, ethanol, and distilled water for phytochemical screening. Detection of nine secondary metabolites was

conducted using standard methods (Devi *et al.*, 2023; Devi *et al.*, 2024; Jena *et al.*, 2024b; Marndi *et al.*, 2024; Rani *et al.*, 2024; Roy *et al.*, 2025).



Figure 2: Collected leaves of *A. nilagirica* for experimental work

#### **Test for tannin**

About 1 ml of the leaf extract was taken. 3-5 drops of 10% lead acetate solution were added to it. The gelatinous precipitate formation confirmed the presence of tannin.

**Test for saponin:** About 1 ml of the leaf 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 leaf extract was taken. About 2 ml of NaOH solution and 3-5 drops of dilute HCl were added 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 3: Dried leaves powder of *A. nilagirica*

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

**Test for phenols:** About 1 ml of the leaf 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 leaf 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 a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

**Test for steroids:** To 1 ml of the leaf extract, about 1 ml of chloroform, followed by 1 ml of concentrated sulphuric acid, was added. The appearance of upper red and lower yellow with green fluorescence indicates the presence of steroids.

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

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

## RESULTS AND DISCUSSION

The phytochemical analysis of *A. nilagirica* leaves revealed the presence of various bioactive compounds in different extracts. Tannins, saponins, phenols, and reducing sugars were detected in ethanol and aqueous extracts, while terpenoids were found only in the n-hexane extract. Alkaloids were present only in the aqueous extract. Conversely, flavonoids, steroids, and carbonyl compounds were absent in all extracts (Table 1; Figures 4-6). These findings provide insights into the plant's potential medicinal properties and highlight the importance of solvent selection in phytochemical analysis.

Table 1: Qualitative phytochemical screening of *Artemisia nilagirica* leaves using different extracts

| Bioactive compounds | Extracts |         |         |
|---------------------|----------|---------|---------|
|                     | n-Hexane | Ethanol | Aqueous |
| Tannin              | Absent   | Present | Present |
| Saponin             | Absent   | Present | Present |
| Flavonoids          | Absent   | Absent  | Absent  |
| Terpenoids          | Present  | Absent  | Absent  |
| Phenols             | Absent   | Present | Present |
| Reducing sugars     | Absent   | Present | Present |
| Steroid             | Absent   | Absent  | Absent  |
| Alkaloid            | Absent   | Absent  | Present |
| Carbonyl compounds  | Absent   | Absent  | Absent  |

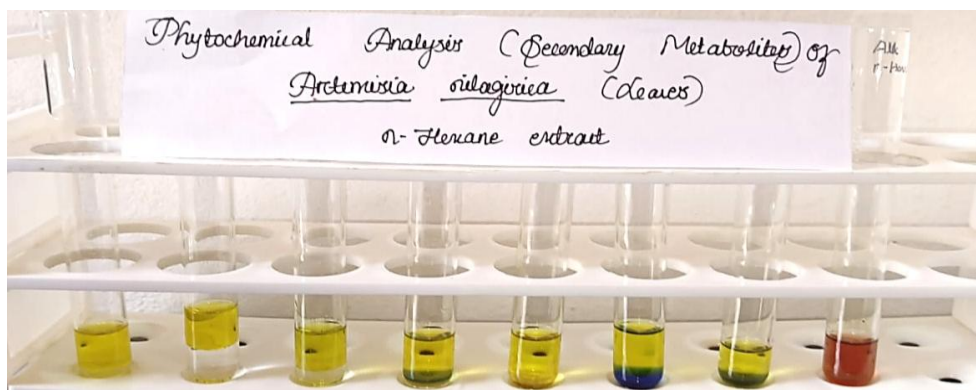


Figure 4: Detection of secondary metabolites of *A. nilagirica* leaves using n-hexane extract

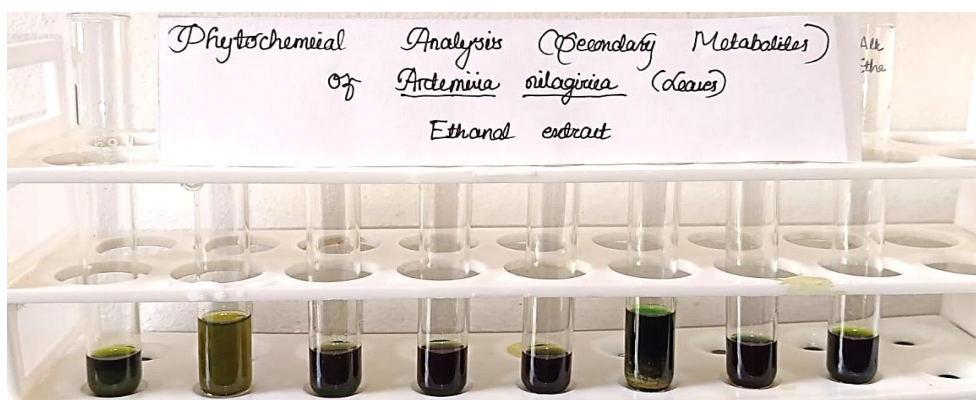


Figure 5: Detection of secondary metabolites of *A. nilagirica* leaves using ethanol extract



Figure 6: Detection of secondary metabolites of *A. nilagirica* leaves using aqueous extract

Other researchers have also reported the phytochemistry and pharmacological potentials of *A. nilagirica*. Ahameethunisa and Hopper, (2010) documented the antibacterial activity of *A. nilagirica* leaf. Bisht et al., (2021) reported phytochemistry and pharmacological activity of the genus *Artemisia*. Albaqami et al., (2022) studied phytochemical composition and in vitro antioxidant, anti-inflammatory, anticancer, and enzyme-inhibitory activities of *A. nilagirica*. Tripathi et al., (2025) documented phytochemical profiling, antioxidant, and antimicrobial properties of Kumaun Himalayan *A. nilagirica*.

## CONCLUSION

The present study demonstrated the presence of various bioactive compounds in *Artemisia nilagirica* leaves, including tannins, saponins, terpenoids, phenols, reducing sugars, and alkaloids. The findings support the traditional use of this plant in medicine and highlight its potential therapeutic applications. Further research is warranted to isolate and characterize the bioactive compounds responsible for the observed medicinal properties, paving the way for the development of new treatments and therapies.

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## Chapter 9

### Phytochemical screening and TLC analysis of flowers of *Acanthus ilicifolius* L. (Acanthaceae)

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**Abstract:** Mangrove plants are mostly unexplored in the aspects of secondary metabolites screening. Flowers of *Acanthus ilicifolius* was selected for phytochemical screening and Thin-layer chromatography (TLC) profiling using hydroethanolic extract. Phytochemical analysis revealed the presence of tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. TLC analysis showed different spots in different mobile phases. The findings suggest that *A. ilicifolius* flowers possess diverse phytochemicals with potential therapeutic applications.

**Keywords:** Bioactive compounds, coastal, mangroves

## INTRODUCTION

Evaluation of medicinal uses of a plant species depends on their available bioactive compounds (Dincheva et al., 2023). In current scenario, where whole world facing different types of health problems and drug failures (Sun et al., 2022). In this aspect, there is a need to screen unexplored and special group of plants and mangroves are quite suitable (Ghizelini et al., 2012). Therefore, flowers of *Acanthus ilicifolius*, a mangrove associated shrub was selected for present study (Hossain et al., 2025;

Figure 1). The plant parts of *A. ilicifolius* is used to treat many health problems (Singh and Aeri, 2013; Hossain et al., 2025).



Figure 1: Flower of *A. ilicifolius*

Despite its traditional use, there is limited scientific evidence on the bioactive compounds and potential therapeutic applications of flowers of *A. ilicifolius*. Hence, present study aims to investigate the phytochemical profile and chromatographic characteristics of *A. ilicifolius* flowers using hydroethanolic extract, providing insights into its potential medicinal properties and applications.

### METHODOLOGY

The flowers of *A. ilicifolius* were collected from the nearby areas of Rajnagar, Kendrapara district of Odisha, India (Figure 2). The plant species was identified by the authors. The Soxhlet extraction method was adopted using hydroethanolic solvent for phytochemical analysis. Detection of nine secondary metabolites were conducted using standard methods (Kumar et al., 2013a; Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024).



Figure 2: Collection of flowers of *A. ilicifolius* for experimental work

TLC plates were prepared on 6 cm glass slides using silica gel powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). Slides were washed with clinical laboratory detergent and dried. 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 (Kumar et al., 2013b). Then the slides were activated at 50°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 RF (Retention Factor) values and visualized under UV chamber (Kumar and Jena, 2014).

## RESULTS AND DISCUSSION

The phytochemical analysis of *A. ilicifolius* flowers using hydroethanolic extract revealed the presence of various bioactive compounds (Table 1; Figure 3). The results showed positive tests for tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. These compounds are known for their diverse biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties.

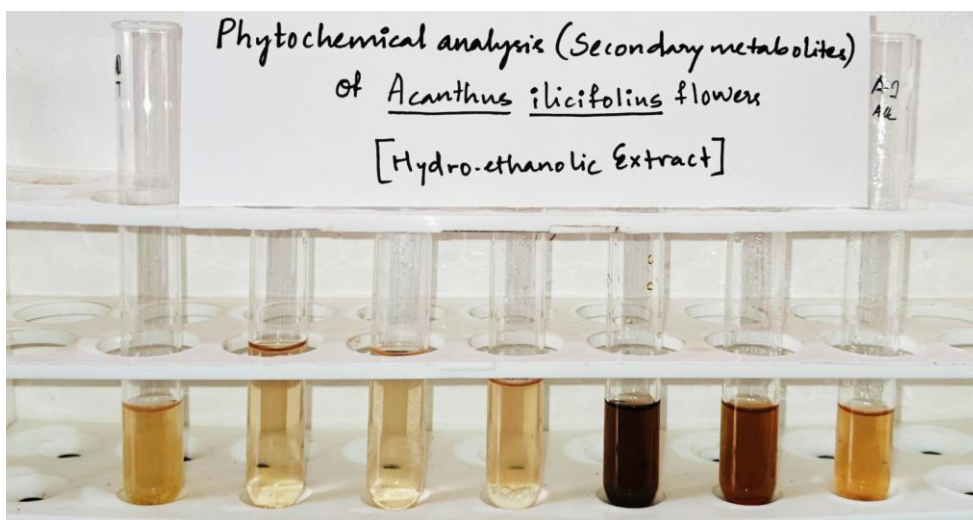


Figure 3: Phytochemical analysis of the flowers of *A. ilicifolius* using hydroethanolic extract  
The TLC profiling of the hydroethanolic extract using different mobile phases showed distinct Rf values, indicating the presence of various phytochemicals (Table

2). The results suggest that the plant contains a complex mixture of compounds, which may contribute to its medicinal properties. The presence of tannins, flavonoids, and phenolic compounds in *A. ilicifolius* flowers is consistent with previous studies on mangrove plants, which have reported similar phytochemical profiles. These compounds are known for their antioxidant and anti-inflammatory activities, which may contribute to the plant's traditional use in treating various ailments. The TLC profiling results indicate that the hydroethanolic extract contains multiple compounds with different polarities, which may be responsible for the plant's medicinal properties.

Table 1: Phytochemical analysis of flowers of *Acanthus ilicifolius* using hydroethanolic extract

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | +ve                    |
| Flavonoids          | +ve                    |
| Terpenoids          | -ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |
| Steroids            | -ve                    |
| Alkaloids           | +ve                    |
| Carbonyl compounds  | -ve                    |

(+ve: Present; -ve: Absent)

Table 2: TLC analysis of flowers of *Acanthus ilicifolius* using hydroethanolic extract

| Mobile phase                       | Extracts       | Rf value of spots |                |                |
|------------------------------------|----------------|-------------------|----------------|----------------|
|                                    |                | S <sub>1</sub>    | S <sub>2</sub> | S <sub>3</sub> |
| Chloroform: methanol<br>(1:1)      | Hydroethanolic | 0.7               | -              | -              |
| Ethyl acetate:<br>chloroform (1:9) | Hydroethanolic | 0.95              | -              | -              |

|                                                        |                |      |      |      |
|--------------------------------------------------------|----------------|------|------|------|
| Ethyl acetate: distilled<br>water: acetic acid (8:1:1) | Hydroethanolic | 0.68 | 0.88 | 0.95 |
|--------------------------------------------------------|----------------|------|------|------|

## CONCLUSION

This study demonstrates the presence of diverse phytochemicals in *A. ilicifolius* flowers, including tannins, saponins, flavonoids, phenolic compounds, reducing sugars, and alkaloids. The TLC profiling reveals a complex mixture of compounds with different polarities. These findings provide a foundation for further research on the medicinal properties and potential applications of *A. ilicifolius*. Future studies can focus on isolating and characterizing individual compounds, evaluating their biological activities, and exploring their therapeutic potential.

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## Chapter 10

### Phytochemical screening and TLC analysis of whole plant of *Malachra capitata* (L.) L. (Malvaceae)

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**Abstract:** Whole plant of *Malachra capitata* was selected for qualitative phytochemical analysis using hydroethanolic extract. Present analysis revealed the presence of tannins, flavonoids, phenolic compounds, reducing sugars, and alkaloids, while saponins, terpenoids, steroids, and carbonyl compounds were absent. Thin-layer chromatography (TLC) analysis using different mobile phases showed distinct R<sub>f</sub> values, indicating the presence of various bioactive compounds. These findings provide valuable insights into the phytochemical profile of *M. capitata*, which may have implications for their potential medicinal properties and applications.

**Keywords:** Malvaceae, secondary metabolites, TLC

## INTRODUCTION

*Malachra capitata*, a perennial under shrub of the Malvaceae family, native to Tropical and Subtropical America, thrives in seasonally dry tropical biomes (Gopi et al., 2012; Figure 1). In traditional Indian medicine, its roots are utilized to treat epilepsy (Deodhar, 2016). Despite its medicinal use, there is a scarcity of reports on its secondary metabolites. To address this gap, we conducted a phytochemical analysis of the whole plant using hydroethanolic extract and TLC profiling. Present

study underscores the potential of *M. capitata* as a source of diverse therapeutic agents for drug development, paving the way for further research and exploration.

## METHODOLOGY

The whole plant of *M. capitata* was collected from Rajnagar, Kendrapara district of Odisha, India. Hydroethanolic solvent was used for phytochemical analysis (Figure 2). Detection of secondary metabolites were conducted using standard methods (Kumar et al., 2013a; Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024). TLC plates were prepared on 6 cm glass slides using silica gel powder (SILICA GEL G, CAS 112926-00-8, Spectrochem Pvt. Ltd.). Slides were washed with clinical laboratory detergent and dried. 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 (Kumar et al., 2013b). Then the slides were activated at 50° 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 RF (Retention Factor) values and visualized under UV chamber (Kumar and Jena, 2014).

## RESULTS AND DISCUSSION

The phytochemical analysis of *M. capitata* whole plant revealed the presence of tannins, flavonoids, phenolic compounds, reducing sugars, and alkaloids in the hydroethanolic extract (Figure 3). Conversely, saponins, terpenoids, steroids, and carbonyl compounds were found to be absent. Thin-layer chromatography (TLC) analysis showed distinct Rf values, with two spots observed in the chloroform: methanol (1:1) mobile phase and single spots in the ethyl acetate: chloroform (1:9)

and ethyl acetate: distilled water: acetic acid (8:1:1) mobile phases, indicating the presence of various bioactive compounds.

Table 1: Phytochemical analysis of whole plant of *Malachra capitata*

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | -ve                    |
| Flavonoids          | +ve                    |
| Terpenoids          | -ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |
| Steroids            | -ve                    |
| Alkaloids           | +ve                    |
| Carbonyl compounds  | -ve                    |

(+ve: Present; -ve: Absent)

Table 2: TLC analysis of whole plant of *Malachra capitata*

| Mobile phase                                           | Extracts       | Rf value of spots |                |                |
|--------------------------------------------------------|----------------|-------------------|----------------|----------------|
|                                                        |                | S <sub>1</sub>    | S <sub>2</sub> | S <sub>3</sub> |
| Chloroform: methanol<br>(1:1)                          | Hydroethanolic | 0.06              | 0.52           | -              |
| Ethyl acetate:<br>chloroform (1:9)                     | Hydroethanolic | 0.95              | -              | -              |
| Ethyl acetate: distilled<br>water: acetic acid (8:1:1) | Hydroethanolic | 0.94              | -              | -              |



Figure 1: Whole plant of *M. capitata*



Figure 2: Hydro-ethanolic extract of *M. capitata* (whole plant) for experimental works

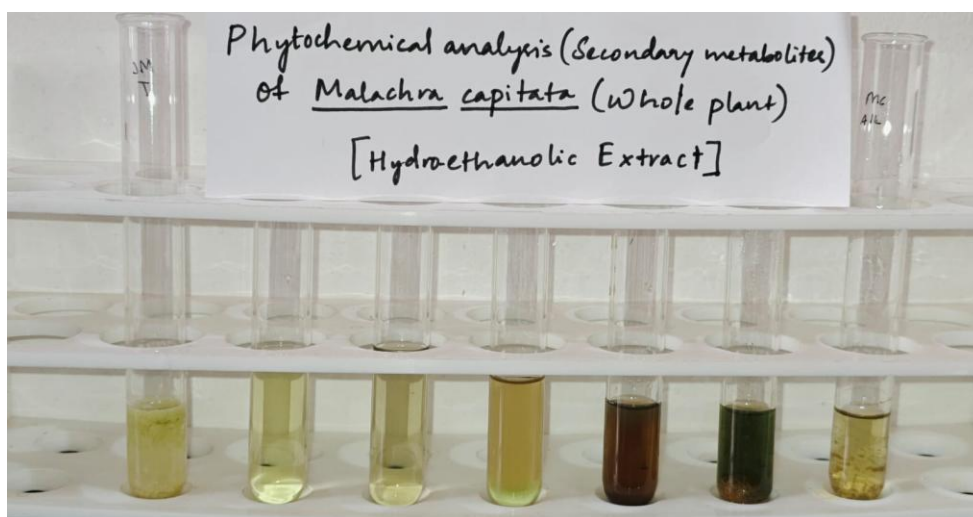


Figure 3: Phytochemical analysis of the whole plant of *M. capitata*

## CONCLUSION

Present study provides valuable insights into the phytochemical composition of *Malachra capitata* whole plant, highlighting the presence of bioactive compounds with potential medicinal properties. The findings of this study can serve as a

foundation for further research on the therapeutic applications and pharmacological effects of this herb

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## Chapter 11

### Phytochemical screening of fruits of *Casearia graveolens*

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**Abstract:** Qualitative phytochemical analysis of *Casearia graveolens* fruit extracts was carried out using acetone, ethanol, and distilled water solvents. Our results show the presence of tannins, phenolic compounds, reducing sugars, and steroids in various extracts, while saponins, flavonoids, terpenoids, alkaloids, and carbonyl compounds were absent. These findings provide valuable insights into the bioactive compounds present in *C. graveolens* fruits, which may have implications for their potential medicinal properties and applications

**Keywords:** Bioactive compounds, trees, riparian

## INTRODUCTION

For centuries, plants have been fundamental to traditional medicine, and trees are especially valued for their healing properties (Sahu et al., 2024). They are a treasure trove of bioactive compounds, showcasing antioxidant, anti-inflammatory, and antimicrobial properties (Roy et al., 2025). The varied phytochemicals in trees have paved the way for developing many pharmaceuticals and nutraceuticals, demonstrating their crucial role in

uncovering new therapeutic leads (Bolling et al., 2011). The screening of bioactive compounds from trees has gained significant attention in recent years, driven by the need for novel and sustainable sources of medicines (Batiha et al., 2022). Trees have evolved complex defence mechanisms to protect themselves against pathogens and environmental stressors, resulting in the production of a wide range of phytochemicals with potential medicinal applications (War et al., 2012). By exploring the secondary metabolites of trees, researchers can identify novel compounds with therapeutic potential, which can be developed into new treatments for various diseases. *Casearia graveolens* Dalzell, (Figure 1) a tree species belonging to Salicaceae family and known for its medicinal properties, has been studied for its bioactive compounds (Shin et al., 2018). The fruit of this tree has been reported to exhibit various biological activities (Panda et al., 2017). Present study aims to investigate the phytochemical composition of *C. graveolens* fruit extracts, with a focus on identifying bioactive compounds that may have potential medicinal applications. By exploring the phytochemical profile of this tree species, we hope to contribute to the discovery of novel therapeutic agents and further highlight the importance of trees in the screening of bioactive compounds.

## METHODOLOGY

The fruits of *C. graveolens* were collected from the Mahanadi River areas, Cuttack district, Odisha, India (Figure 1-2). The Soxhlet extraction method was adopted using acetone, ethanol and distilled water. Detection of selected secondary metabolites were conducted using standard methods (Kumar et al., 2013a; Devi et al., 2023; Marndi et al., 2024; Jena et al., 2024).

Table 1: Phytochemical analysis of fruits of *Casearia graveolens* using different extracts

| Bioactive compounds | Acetone extract | Ethanol extract | Aqueous extract |
|---------------------|-----------------|-----------------|-----------------|
| Tannin              | +ve             | +ve             | +ve             |
| Saponin             | -ve             | -ve             | -ve             |
| Flavonoids          | -ve             | -ve             | -ve             |
| Terpenoids          | -ve             | -ve             | -ve             |
| Phenolic compounds  | +ve             | +ve             | +ve             |
| Reducing sugar      | +ve             | +ve             | +ve             |
| Steroids            | +ve             | +ve             | -ve             |
| Alkaloids           | -ve             | -ve             | -ve             |
| Carbonyl compounds  | -ve             | -ve             | -ve             |

(+ve: Present; -ve: Absent)



Figure 1: Fruits of *C. graveolens*



Figure 2: Powder of fruits of *C. graveolens*

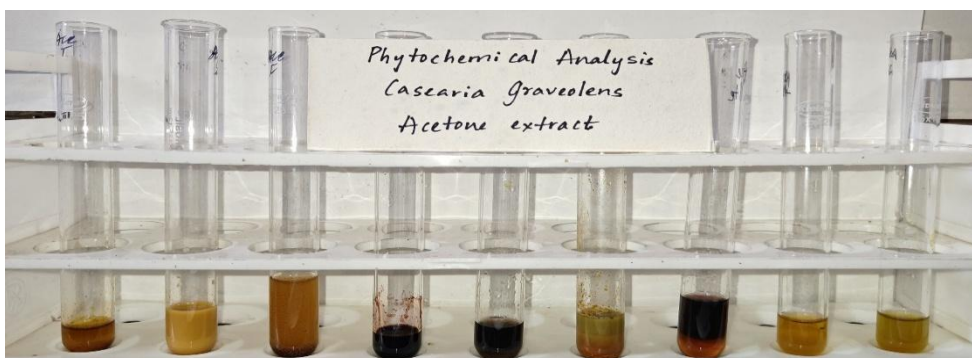


Figure 3: Phytochemical analysis of the fruits of *C. graveolens* using acetone extract

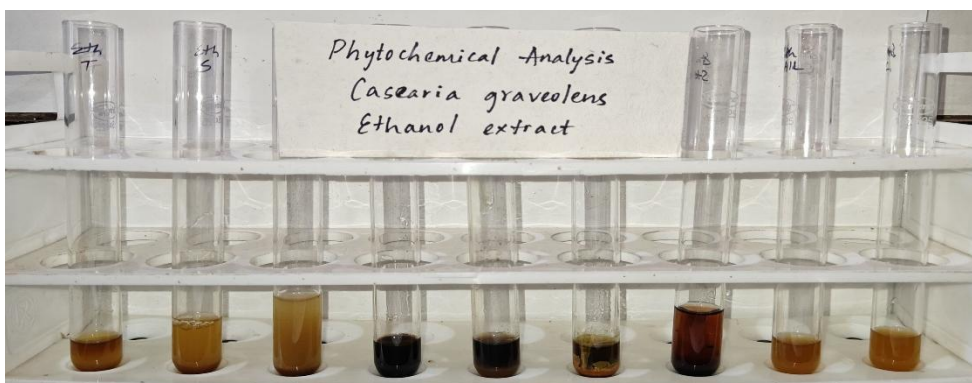


Figure 4: Phytochemical analysis of the fruits of *C. graveolens* using ethanol extract

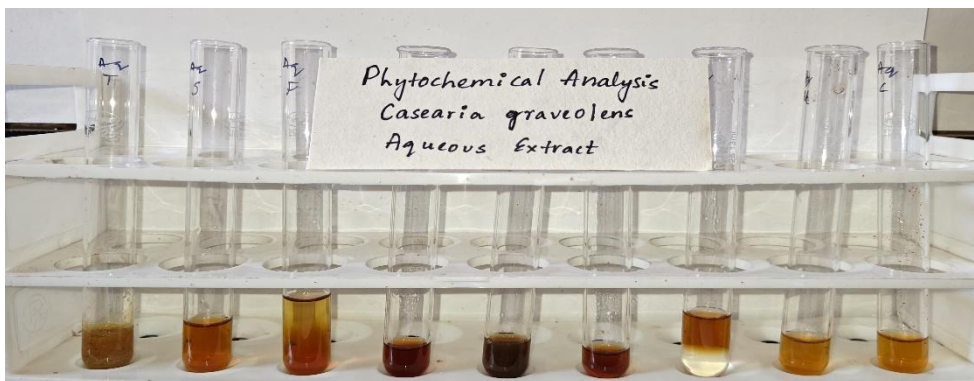


Figure 5: Phytochemical analysis of the fruits of *C. graveolens* using aqueous extract

## RESULTS AND DISCUSSION

The phytochemical analysis of *Casearia graveolens* fruit extracts showed that tannins, phenolic compounds, and reducing sugars were consistently present across acetone, ethanol, and aqueous extracts (Figures 3-5). Meanwhile, steroids were found only in acetone and ethanol extracts, and several compounds, including saponins, flavonoids, terpenoids, alkaloids, and carbonyl compounds, were not detected. The presence of these bioactive compounds suggests that *C. graveolens* fruits may possess medicinal properties, including antioxidant, anti-inflammatory, and antimicrobial effects.

## CONCLUSION

Present study confirms the presence of bioactive compounds in *Casearia graveolens* fruit extracts, underscoring their potential health benefits and validating traditional usage. Additional research is needed to investigate therapeutic uses and clarify the pharmacological impacts of these compounds.

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## Chapter 12

### Phytochemical and toxicity evaluation of hydroethanolic extract of *Trichosanthes costata* fruits

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**Abstract:** Current research explores the phytochemical profile and cytotoxic effects of *Trichosanthes costata* fruit extract, identifying key compounds like tannins, saponins, flavonoids, phenolic compounds, and reducing sugars, and demonstrating strong cytotoxic activity against brine shrimp nauplii, indicating possible uses in therapy.

**Keywords:** Bioactive compounds, brine shrimp, cytotoxic effect

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#### INTRODUCTION

Wild cucurbits, belonging to the Cucurbitaceae family, have been a valuable resource for traditional medicines and foods (Tripathy et al., 2015; Kumar et al., 2017). These plants are rich in bioactive compounds, including flavonoids, phenolic acids, and terpenoids, which contribute to their medicinal properties. Wild cucurbits have been used to treat various ailments, highlighting their potential as a source of therapeutic agents (Chakroborthy et al., 2017). *Trichosanthes costata*, a wild cucurbit species, has been used in traditional medicine for its reported health benefits (Kumar et al., 2017). Despite its potential, there is limited scientific

research on the bioactive compounds and biological activities of this plant (Kumar and Tripathy, 2017).



Figure 1: Fruit of *T. costata*

Exploring the phytochemical profile and bioactivity of *T. costata* (Figure 1) can provide valuable insights into its medicinal properties and potential applications. Present study of wild cucurbits like *T. costata* can lead to the discovery of new bioactive compounds with pharmaceutical and medicinal applications. With the

growing interest in natural products and traditional medicine, research on wild cucurbits can contribute to the development of novel therapeutic agents and promote the conservation and sustainable use of these valuable plant resources.

## METHODOLOGY

The fruits of *T. costata* were collected from the Khordha district of Odisha (Figure 2). The Soxhlet extraction method was adopted for phytochemical screening to get a hydroethanolic extract. Nine secondary metabolites were detected using standard methods (Devi et al., 2023; Devi et al., 2024; Jena et al., 2024; Marndi et al., 2024).

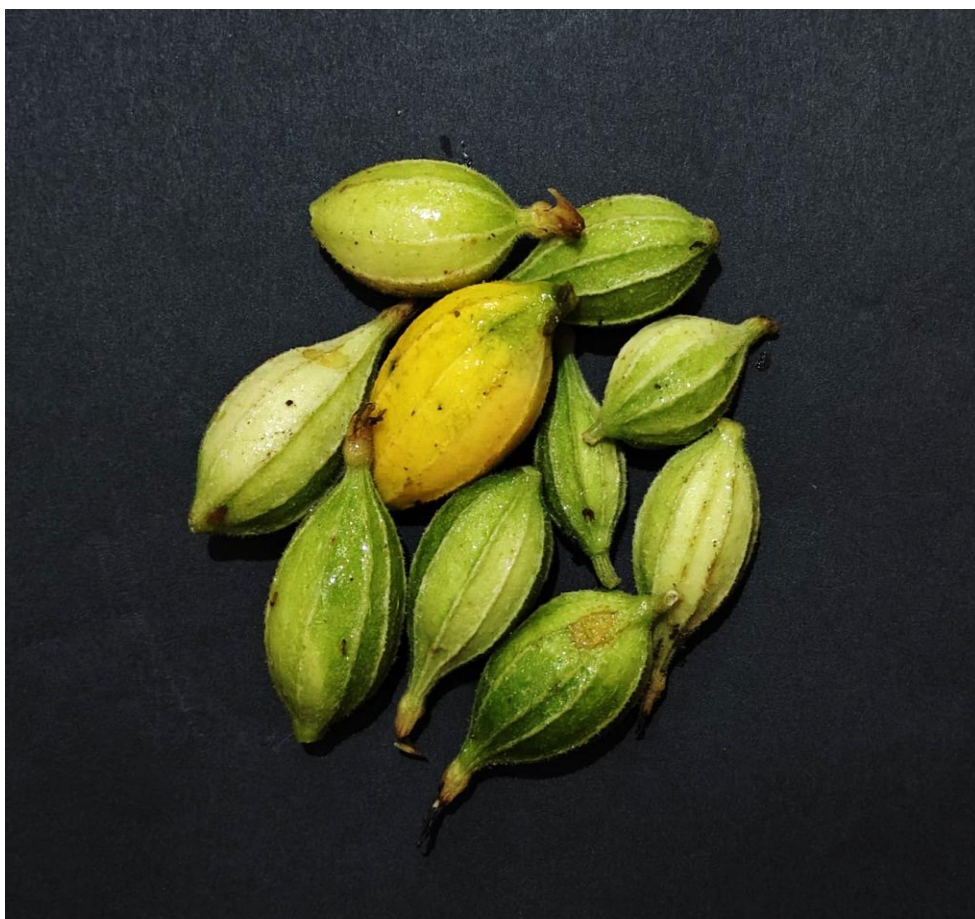


Figure 2: Collection of *T. costata* fruits for experimental works

**Test for tannin:** About 1 ml of the fruit extract was taken. 3-5 drops of 10% lead acetate solution were added to it. The gelatinous precipitate formation confirmed the presence of tannin.

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

**Test for flavonoids:** About 1 ml of the fruit extract was taken. About 2 ml of NaOH solution and 3-5 drops of dilute HCl were added. The colour initially turned to an intense yellow colour with NaOH solution, and later became colourless, which confirmed the presence of flavonoids.

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

**Test for phenols:** About 1 ml of the fruit 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 fruit 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 a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

**Test for steroids:** To 1 ml of the fruit extract, about 1 ml of chloroform, followed by 1 ml of concentrated sulphuric acid, was added. The appearance of upper red and lower yellow with green fluorescence indicates the presence of steroids.

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

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

**Cytotoxicity analysis:** For toxicity analysis, hatching of brine cysts was the initial process. Standardisation of the optimum was done at 2.0, 3.5, and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*Artemia salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5% saline water with proper aeration, room temperature between 28-35°C and light for 48 hours. Proper hatching could be observed within 24-48 hours, depending upon the quality of cysts, proper aeration, and light provided. Different concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml) of the fruit extract were taken, and 3.5% saline water was used to make the volume 2 ml in each test tube. 1% DMSO was used to dissolve the crude extracts. Ten nauplii were selected and introduced to five test tubes of the extracts. Positive and negative controls were prepared using vincristine sulphate (5 mg/ml) and 3.5% saline water, respectively, with a volume of 2 ml. The live larvae are highly mobile and thus differentiate from the unhatched cysts. The survivor nauplii were counted and analysed for the death percentage (Kumar et al., 2012).

## RESULTS AND DISCUSSION

The phytochemical analysis of *T. costata* fruit extract revealed the presence of tannins, saponins, flavonoids, phenolic compounds, and reducing sugars, while terpenoids, steroids, alkaloids, and carbonyl compounds were absent (Table 1). The cytotoxicity analysis showed 100% mortality of brine shrimp nauplii at all

tested concentrations (12.5-200 mg/ml), indicating potent cytotoxic activity comparable to the positive control vincristine sulphate (Table 2).

Table 1: Phytochemical analysis of fruits of *T. costata* using hydroethanolic extract

| Bioactive compounds | Hydroethanolic extract |
|---------------------|------------------------|
| Tannin              | +ve                    |
| Saponin             | +ve                    |
| Flavonoids          | +ve                    |
| Terpenoids          | -ve                    |
| Phenolic compounds  | +ve                    |
| Reducing sugar      | +ve                    |
| Steroids            | -ve                    |
| Alkaloids           | -ve                    |
| Carbonyl compounds  | -ve                    |

(+ve: Presence; -ve: Absence)

Table 2: Cytotoxicity analysis of fruits of *T. costata* using hydroethanolic extract

| Extract                 | Concentration (in mg/ml) | Initial number of Nauplii | Number of deaths of Nauplii (After 1 hour) | Death rates (%) |
|-------------------------|--------------------------|---------------------------|--------------------------------------------|-----------------|
| Hydro-ethanolic extract | 12.5                     | 10                        | 10                                         | 100             |
|                         | 25                       | 10                        | 10                                         | 100             |
|                         | 50                       | 10                        | 10                                         | 100             |
|                         | 100                      | 10                        | 10                                         | 100             |
|                         | 200                      | 10                        | 10                                         | 100             |
|                         | 3.5% Saline              | 10                        | 0                                          | 0               |
|                         | Vincristine sulphate     | 10                        | 10                                         | 100             |

Kumar et al., (2017) studied about a lesser-known climber of Eastern Ghats i.e., *Gymnopetulum chinense*, which leaf extracts have significant antimicrobial activity against selected human pathogenic bacteria. Saradar et al., (2024) have reported in their study that *T. costata* fruits have been consumed as a vegetable and as

condiment. The plant has potential medicinal values for the treatment of ophthalmia, rheumatism, and tetanus.

## CONCLUSION

In conclusion, present study demonstrates the presence of bioactive compounds in *T. costata* fruit extract and its potent cytotoxic activity, suggesting its potential as a source of therapeutic agents. Further research is warranted to isolate and characterize the bioactive compounds and explore their applications in pharmaceutical and medicinal fields.

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## Chapter 13

### Evaluation of medicinal values through TLC & phytochemical analysis of *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch., flowers

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**Abstract:** Present study investigates the phytochemicals of *Dendrobium aphyllum* flowers using different extracts and TLC analysis, revealing the presence of diverse bioactive compounds, providing insights into the secondary metabolites of this orchid species.

**Keywords:** Bioactive compounds, orchids, therapeutics

## INTRODUCTION

For decades, global researchers have been searching for new therapeutic agents to develop effective formulations due to growing microbial resistance and drug failure (Kumar et al., 2013; Kumar and Jena, 2014; Jena et al., 2024). In this context, exploring traditional practices and plant uses is crucial. Orchids, featured in mythology and folk medicine, have been recognized for their potential in indigenous therapeutic formulations (Chakraborty et al., 2022) and unique eco-medico food values (Kumar and Thorat, 2018; Kumar et al., 2022; Sharma et al., 2024). Despite their unique potential, orchids remain largely unexplored (Kumar and Kumar, 2020; Kumar et al., 2021). Present study aims to document the

secondary metabolites of *Dendrobium aphyllum* flowers, an epiphytic orchid (Kumar et al., 2022; Figure 1). Phytochemical screening of n-hexane, ethanol, and aqueous extracts was conducted, along with thin-layer chromatography, providing baseline data for future bioactivity research.

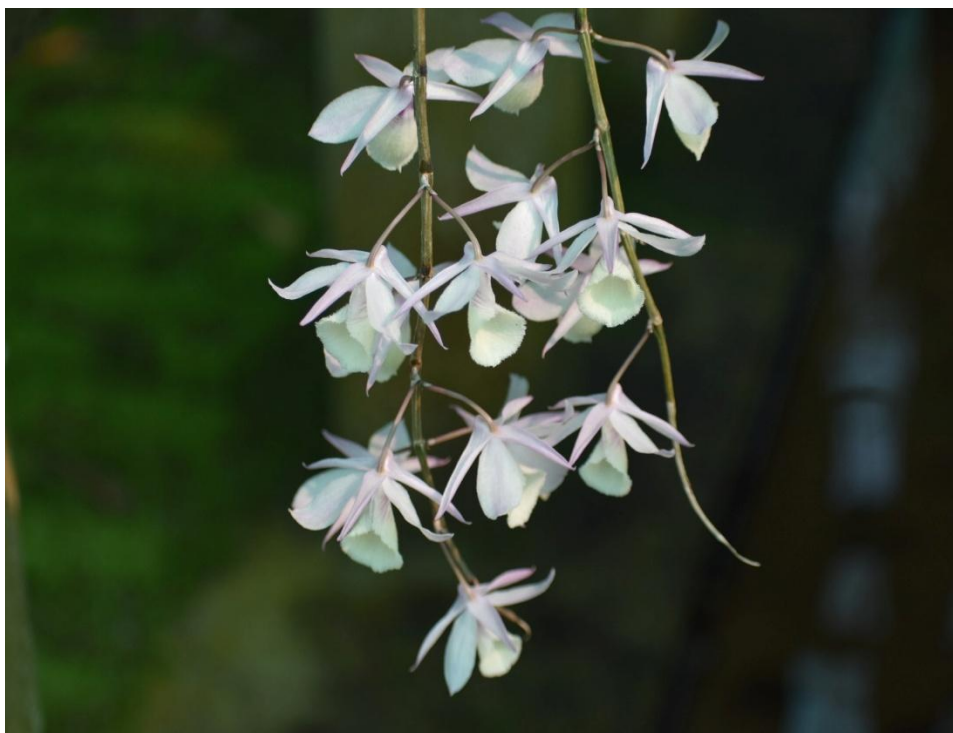


Figure 1: Flowers of *D. aphyllum*

## METHODOLOGY

The flowers of *D. aphyllum* were collected from Khordha district of Odisha, India (Figure 2). The Plant species was identified by Dr. Sanjeet Kumar (Figure 1). The Soxhlet extraction method was adopted using n-hexane, ethanol, and distilled water for phytochemical analysis. Detection of selected secondary metabolites were conducted using standard methods (Devi et al., 2023; Ankari et al., 2024; Marndi et al., 2024; Darshani et al., 2025; Das et al., 2025; Jayalakshmi et al., 2025;

Kshirsagar *et al.*, 2025; Kumar *et al.*, 2025; Malwal *et al.*, 2025; Nayak *et al.*, 2025; Patra *et al.*, 2025; Sharma *et al.*, 2025; Yadav *et al.*, 2025).



Figure 2: Collected flowers of *D. aphyllum* for experimental work

**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.

**Test for terpenoids:** To 1 ml of the flowers 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 steroids:** 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 dried. 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 50° 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 RF (Retention Factor) values, and visualized under UV chamber (Mishra and Bhatnagar, 2024).

## RESULTS AND DISCUSSION

The phytochemical screening of *Dendrobium aphyllum* flowers revealed the presence of tannins, saponins, phenols, reducing sugars, and alkaloids in various extracts. Saponins and reducing sugars were detected in all extracts (n-hexane, ethanol, and aqueous), while tannins, phenols, and alkaloids were found in ethanol and aqueous extracts (Table 1; Figures 4-6). The TLC analysis of the hydroethanolic extract showed distinct Rf values for different mobile phases, indicating the separation of bioactive compounds. The Rf values ranged from 0.76 to 0.91, with multiple spots observed in some mobile phases, suggesting the presence of diverse phytochemicals in *D. aphyllum* flowers (Table 2).

Table 1: Qualitative phytochemical screening of *Dendrobium aphyllum* flowers using different extracts

| Bioactive compounds | Extracts     |              |              |
|---------------------|--------------|--------------|--------------|
|                     | n-Hexane     | Ethanol      | Aqueous      |
| Tannin              | Not detected | Detected     | Detected     |
| Saponin             | Detected     | Detected     | Detected     |
| Flavonoids          | Not detected | Not detected | Not detected |
| Terpenoids          | Not detected | Not detected | Not detected |
| Phenols             | Not detected | Detected     | Detected     |
| Reducing sugar      | Detected     | Detected     | Detected     |
| Steroid             | Not detected | Not detected | Not detected |
| Alkaloid            | Not detected | Detected     | Detected     |

|                   |              |              |              |
|-------------------|--------------|--------------|--------------|
| Carbonyl compound | Not detected | Not detected | Not detected |
|-------------------|--------------|--------------|--------------|

Table 2: TLC analysis of *D. aphyllum* flowers using different mobile phases

| Mobile phases                             | Extracts       | Rf value of spots |                |
|-------------------------------------------|----------------|-------------------|----------------|
|                                           |                | S <sub>1</sub>    | S <sub>2</sub> |
| Chloroform: methanol (1:1)                | Hydroethanolic | 0.76              | 0.79           |
| Ethyl acetate: chloroform (1:9)           | Hydroethanolic | 0.81              | Nil            |
| Ethyl acetate: water: acetic acid (8:1:1) | Hydroethanolic | 0.87              | 0.91           |



Figure 4: Detection of secondary metabolites of *D. aphyllum* flowers using n-hexane extract



Figure 5: Detection of secondary metabolites of *D. aphyllum* flowers using ethanolic extract



Figure 6: Detection of secondary metabolites of *D. aphyllum* flowers using aqueous extract

## CONCLUSION

The study provides valuable insights into the phytochemical composition of *Dendrobium aphyllum* flowers, highlighting the presence of bioactive compounds such as tannins, saponins, phenols, reducing sugars, and alkaloids. These findings suggest the potential medicinal properties of this orchid species, warranting further research to explore its therapeutic applications and pharmacological effects.

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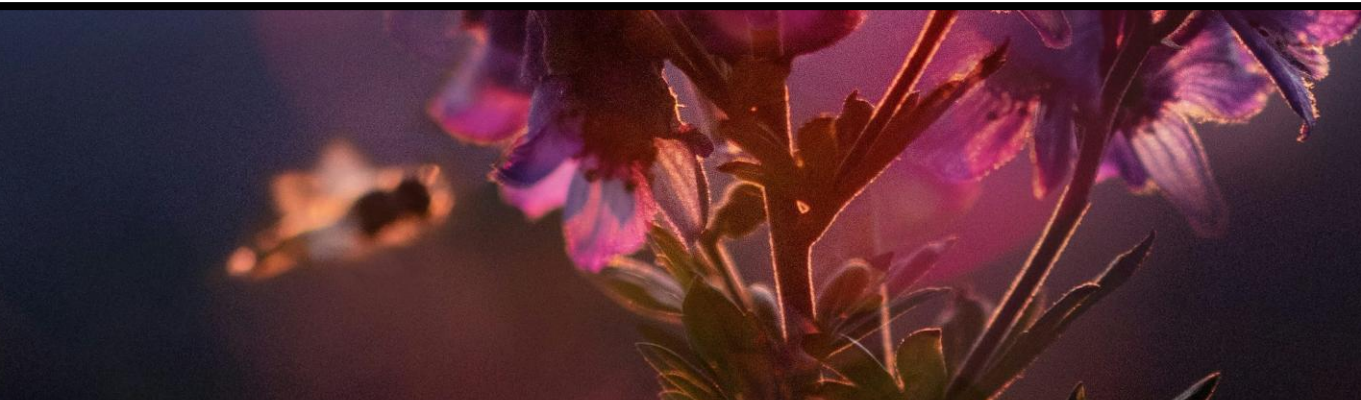
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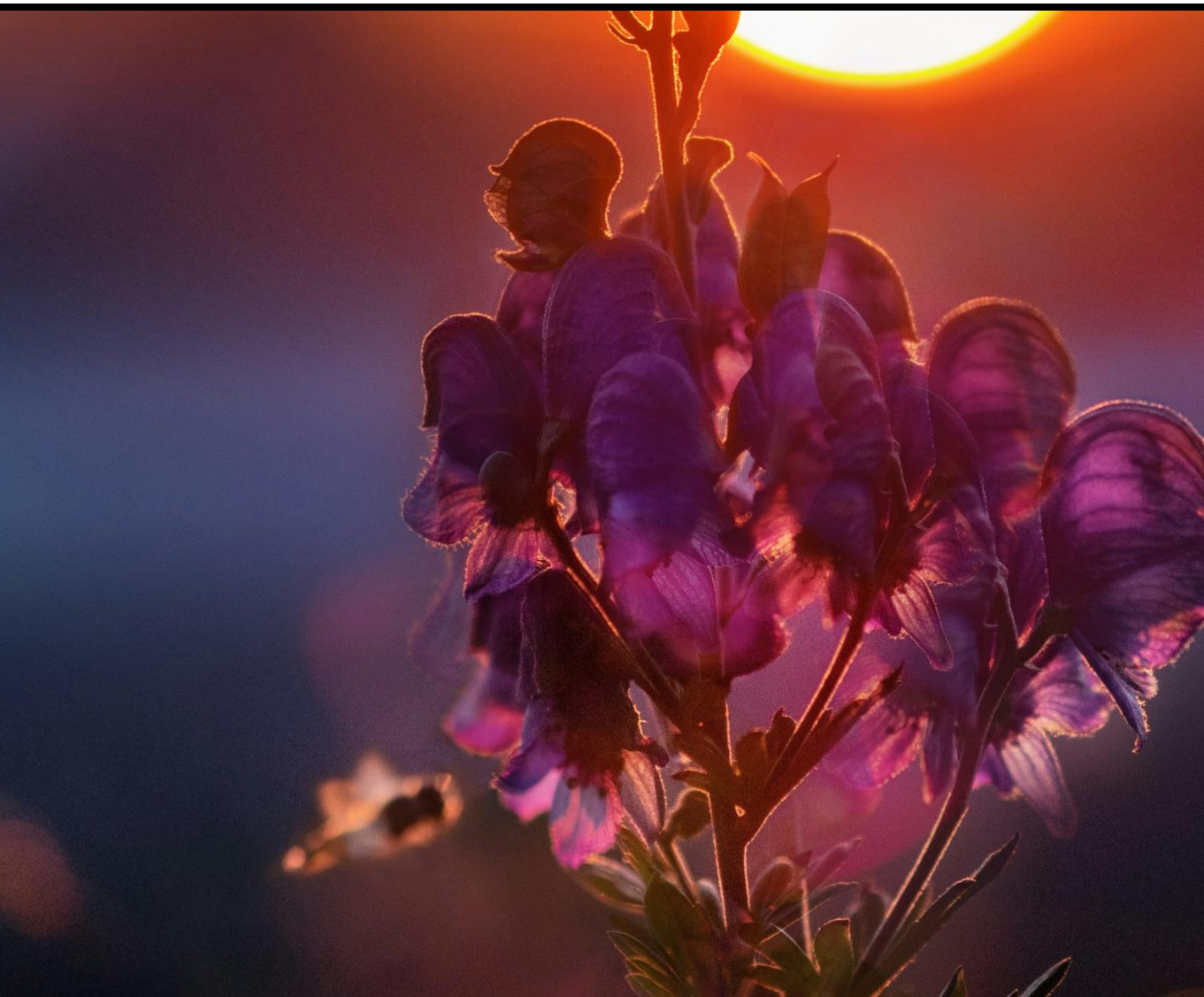


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