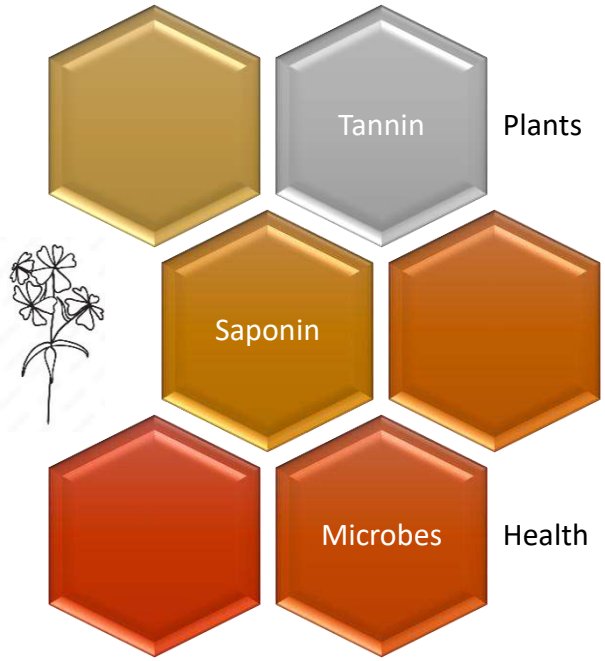
PLANTS & SECONDARY METABOLITES

VOLUME IX

Dr. Rakhee Dimri • Dr. Rajeev Kumar Singh



Ambika Prasad Research Foundation, India

Plants and Secondary Metabolites

VOLUME 9



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PREFACE

The study of plants and their secondary metabolites has been a cornerstone of medicinal research for centuries. Plants have been a rich source of bioactive compounds, many of which have been developed into life-saving medicines. Despite the significant progress made in the field of medicinal chemistry, there is still much to be discovered about the complex chemistry of plants and their potential applications. The book entitled "**Plants and Secondary Metabolites, Volume 9**" aims to contribute to the growing body of knowledge on plant secondary metabolites, highlighting their importance in the development of new medicines and other valuable products. The chapters in this volume showcase the diversity of plant species that have been studied for their phytochemical properties and potential medicinal applications. From the cytotoxic potential of *Maclura spinosa* to the medicinal values of *Antidesma ghaesembilla*, each chapter provides a unique perspective on the complex relationships between plants, their chemistry, and human health. The book brings together the work of researchers from various fields, including phytochemistry, pharmacology, and botany, to provide a comprehensive overview of the current state of knowledge on plant secondary metabolites. Editors hope that this book will be a valuable resource for researchers, students, and professionals in the fields of botany, phytochemistry, pharmacology, and medicine. The chapters in this volume demonstrate the importance of continued research into the chemistry and biology of plant secondary metabolites, and we believe that this book will inspire further studies and discoveries in this fascinating field. By exploring the potential of plants to provide new medicines and other valuable products, we can work towards a future where human health and well-being are improved through the power of nature.

(Editors)

INTRODUCTION

The world is facing an unprecedented crisis in healthcare, with rising rates of chronic diseases, antimicrobial resistance, and emerging pandemics. In this context, the importance of traditional medicine and medicinal plants cannot be overstated. For centuries, plants have been a rich source of bioactive compounds, many of which have been developed into life-saving medicines. However, the knowledge and use of medicinal plants are often passed down through generations, and much of this valuable information remains undocumented. The loss of traditional knowledge and the decline of biodiversity pose a significant threat to the discovery of new medicines and the development of sustainable healthcare solutions. Medicinal plants have been an integral part of traditional medicine in many cultures, providing a natural and often effective way to prevent and treat various ailments. The documentation of medicinal plants is crucial for several reasons. Firstly, it helps to preserve traditional knowledge and cultural heritage. Secondly, it provides a valuable resource for researchers and scientists to discover new medicines and develop evidence-based treatments. Finally, it promotes the conservation and sustainable use of plant resources, which is essential for maintaining biodiversity and ecosystem health. Despite the importance of medicinal plants, many species are facing threats such as over-harvesting, habitat loss, and climate change. The loss of medicinal plant species not only erodes traditional knowledge but also limits our access to potential new medicines. Therefore, it is essential to document and conserve medicinal plant species, as well as to promote sustainable practices and community-based conservation initiatives. By doing so, we can ensure the long-term availability of these valuable resources and support the development of new medicines and treatments. This book aims to contribute to the documentation and conservation of medicinal plants by providing a comprehensive overview of the phytochemical properties and medicinal uses of various plant species. The chapters in this volume highlight the importance of medicinal plants in traditional medicine and their potential applications in modern healthcare. By documenting the medicinal properties of these plants, we hope to promote further research and development of new medicines, as well as to support conservation efforts and sustainable practices that protect these valuable resources for future generations.

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Chapter 1

Phytochemical, cytotoxicity and chromatographic analysis of leaves of *Maclura spinosa* (Willd.) C.C. Berg (Moraceae): a less known medicinal plant of Odisha, India

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Abstract: Screening of medicinal plants is crucial in contemporary times to discover novel therapeutic agents, validate traditional claims, and develop evidence-based phytomedicines to address emerging diseases and growing antibiotic resistance. Therefore, leaves of *Maclura spinosa* was selected for qualitative phytochemical analysis, cytotoxicity, and chromatographic analysis. It is a less known medicinal plant of Odisha state, India. Results revealed that leaves showed the presence of tannins, saponins, flavonoids, phenolic compounds, and alkaloids. Cytotoxicity evaluation using brine shrimp nauplii also showed significant mortality rates for n-hexane and ethanol extracts, while the aqueous extract exhibited varying degrees of mortality. TLC (Thin Layer Chromatography) analysis identified distinct compound profiles in hydroethanolic extract. Present study provides a base line data on its medicinal and pharmacological potential.

Keywords: Bioactive compounds, Moraceae, pharmacological properties

Introduction

The resurgence of interest in traditional medicine has led to a renewed focus on screening medicinal plants for their potential therapeutic benefits (Ekor, 2014; Jena et al., 2025). With the growing threat of antibiotic resistance and the need for novel treatments for emerging diseases, exploring the bioactive compounds present in plants has become increasingly important (Kumar et al., 2013; Kumar and Jena, 2014; Kumar et al., 2017; Chinemerem-Nwobodo et al., 2022). Medicinal plants have been a rich source of leads for drug discovery, and continued research in this area is essential for identifying new therapeutic agents (Sahoo et al., 2021; Devi et al., 2025). The Moraceae family, which includes plants such as mulberries, figs, and jackfruits, has been recognized as a valuable source of bioactive compounds (Satapathy and Kumar, 2017; Kumar et al., 2022). Members of this family have been used in traditional medicine for various purposes, including treating fever, inflammation, and infections (Dangarembizi et al., 2012; Madrigal-Santillán et al., 2024). Given the potential of the Moraceae family as a source of bioactive compounds, it is essential to investigate the medicinal properties of its members, like *Maclura spinosa* (Figure 1). It is an unexplored medicinal plant and used to treat teeth problems in Odisha. By screening *M. spinosa* for its phytochemical composition and evaluating its cytotoxicity, present study aims to contribute to the understanding of its medicinal properties and potential therapeutic applications.

Methodology

The leaves of *M. spinosa* were collected from nearby Mahanadi areas of CDA-13, Cuttack, Odisha, India. The plant species was identified by the authors. The Maceration extraction method was adopted using three solvents (n-hexane, ethanol and aqueous) for phytochemical analysis (Figure 2). Detection of nine

secondary metabolites was conducted using standard methods (Devi et al., 2023; Jena et al., 2024; Marndi et al., 2024; Das et al., 2025). A herbarium specimen is deposited to the Herbarium unit, Ambika Prasad Research Foundation, Odisha (Figure 3). Details of phytochemical analysis is given below.



Figure 1: Leaves and flowers of *M. spinosa*



Figure 2: Filtrate extracts of *M. spinosa* leaves for experimental analysis



Figure 3: Herbarium specimen of *M. spinosa*

Test for tannin: 1 ml of leaves extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 leaves extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

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

Test for terpenoids: To 1 ml of the leaves 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 leaves 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. 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 leaves extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaves extract was taken, and 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 leaves 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62mg ml^{-1}, 31.25 mg ml^{-1}, 62.5mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *M. spinosa* leaves extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ºC for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *M. spinosa* leaves revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, phenolic compounds, and alkaloids (Table 1; Figures 4-6). These compounds were found to be present in selected three different extracts. The presence of these bioactive compounds suggests that *M. spinosa* leaves may possess medicinal properties.

Table 1: Qualitative phytochemical analysis of *M. spinosa* leaves using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Positive
Terpenoids	Absent	Present	Present
Phenolic compounds	Absent	Present	Present
Reducing sugars	Absent	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Present	Present
Carbonyl compounds	Absent	Absent	Absent

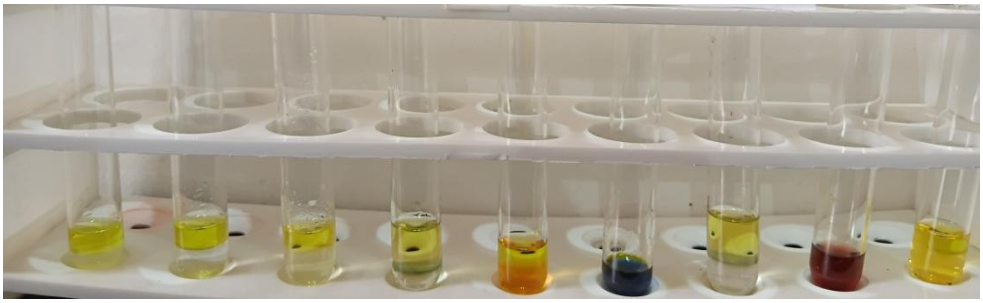


Figure 4: Detection of secondary metabolites of *M. spinosa* leaves using n-hexane extract

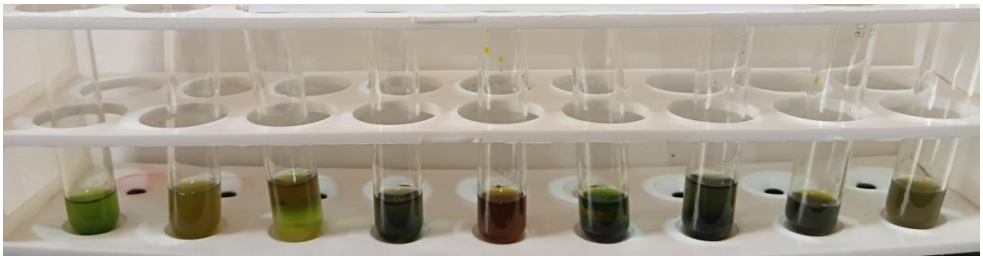


Figure 5: Detection of secondary metabolites of *M. spinosa* leaves using ethanolic extract



Figure 6**:** Detection of secondary metabolites of *M. spinosa* leaves using aqueous extract

The cytotoxicity evaluation of *M. spinosa* leaf extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 7-9). In contrast, the aqueous extract exhibited varying degrees of mortality, suggesting

a possible dose-dependent effect. These findings suggest that *M. spinosa* leaves may contain certain bioactive compounds which are responsible for toxicity.

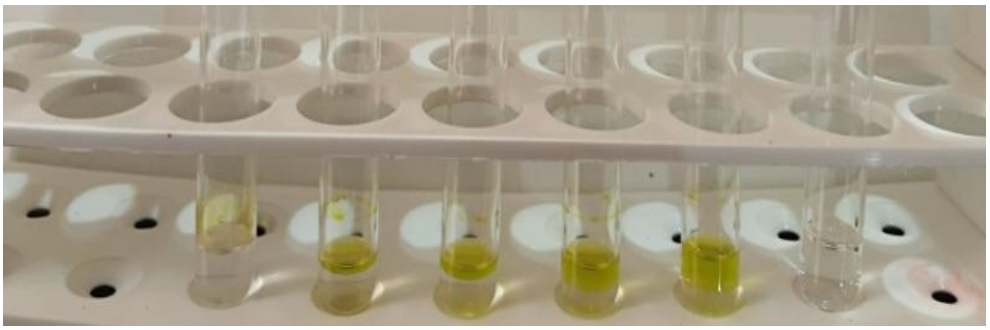


Figure 7: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *M. spinosa* leaves

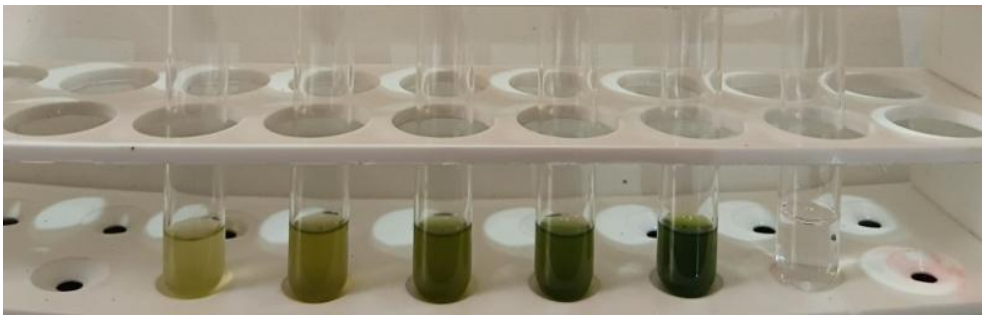


Figure 8: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *M. spinosa* leaves

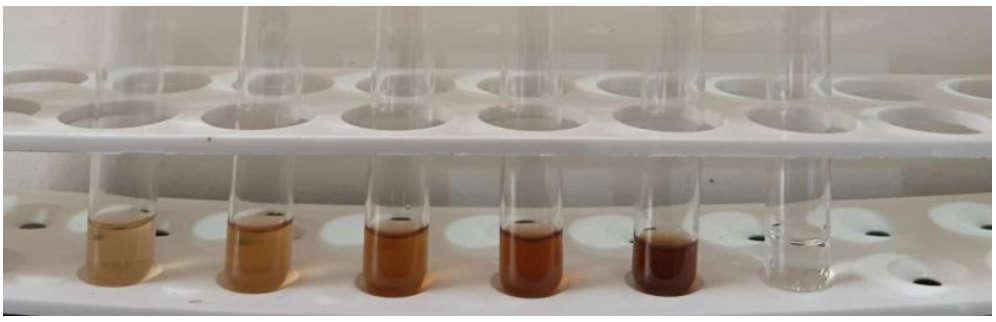


Figure 9: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *M. spinosa* leaves

Table 2: Cytotoxicity analysis of *M. spinosa* leaves using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	10	1	1	1	10
	31.25	10	10	10	10	10	0
	62.5	10	10	1	1	1	10
	125	10	10	1	1	1	10
	250	10	9	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

The TLC analysis of ethanolic and aqueous extracts of *M. spinosa* leaves revealed distinct compound profiles, with different mobile phases separating various phytochemicals based on their Rf values. The presence of distinct spots in the TLC plates confirms the presence of bioactive compounds in *M. spinosa* leaves, which may be responsible for its medicinal properties (Table 3; Figures 10-11).

Table 3: TLC analysis of *M. spinosa* leaves using different mobile phases

	Mobile Phases	Rf value of Spots					
Ethanolic aqueous	Methanol: Chloroform (1:1)	0.71	0.66	0.63	0.61	0.57	0.48
	Ethyl acetate: Chloroform (1:9)	0.7	0.67	Nil	Nil	Nil	Nil
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.72	0.52	0.41	Nil	Nil	Nil

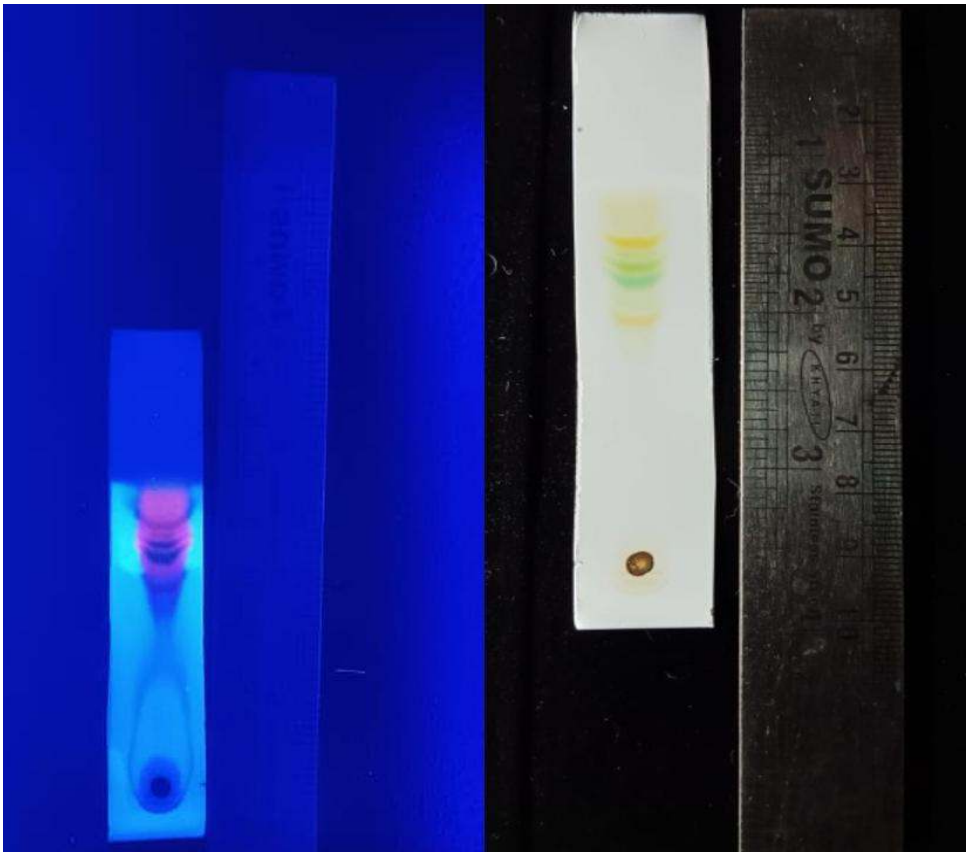


Figure 10: TLC analysis of hydroethanolic extract of *M. spinosa* leaves (Mobile phase: Chloroform: Methanol)

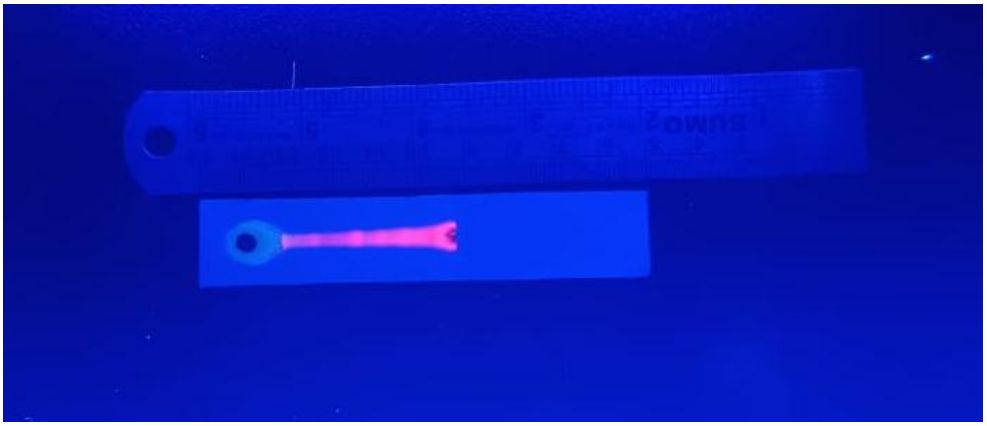


Figure 11: TLC analysis of hydroethanolic extract of *M. spinosa* leaves (Mobile phase: Ethyl acetate: Water: Acetic acid)

Literature survey revealed that very less reports on medicinal uses of *M. spinosa* is available. Therefore, present study is an important communication. Venkatesh et al., (2015) reported synergistic caseinolytic activity and differential fibrinogenolytic action of multiple proteases of *M. spinosa*. Kulkarni et al., (2020) documented that protease of *M. spinosa* accelerates healing of subcutaneous dermal wounds. Recently, Younes et al., (2025) documented antioxidant, anti-inflammatory and antidiabetic activities of *M. spinosa* from Egypt.

Conclusion

Present study confirms that *Maclura spinosa* leaves contain diverse bioactive compounds with potential therapeutic benefits, highlighting their promise as a medicinal resource. The study's findings on phytochemistry and cytotoxicity indicate that these leaves could yield valuable leads for isolation of new therapeutic agents. To fully exploit their potential, further research is needed to isolate and identify the active compounds, elucidate their modes of action, and assess their safety and effectiveness through rigorous preclinical and clinical testing.

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Chapter 2

Phytochemical analysis, toxicity to *Artemia salina* and Thin Layer Chromatography of leaves of *Chrozophora rottleri* (Geiseler) Spreng. (Euphorbiaceae)

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Abstract: The increasing prevalence of cancer and the limitations of current treatments highlight the need to explore alternative therapeutic strategies, including the screening of medicinal plants for anticancer properties. *Chrozophora rottleri*, a plant species of the Euphorbiaceae family, has been traditionally used to treat various ailments and exhibits a rich diversity of bioactive compounds. This study focuses on the phytochemical analysis, cytotoxicity evaluation using Artemia salina, and Thin Layer Chromatography (TLC) of *C. rottleri* leaves, aiming to identify potential anticancer leads and contribute to the development of novel cancer therapies.

Keywords: Bioactive compounds, cancer, chromatography, herbs

Introduction

Cancer is a leading cause of death worldwide, and the development of effective treatments remains a significant challenge (Baskar et al., 2012; Nagai and Kim, 2017). Despite advances in modern medicine, many cancer therapies are

associated with severe side effects, and the search for safer, more targeted treatments continues (Schilsky et al., 2010; Kroschinsky et al., 2017; Anand et al., 2024). Plants have been a rich source of bioactive compounds with potential therapeutic benefits (Pradhan et al., 2025), and screening plant extracts for anticancer properties has become an important area of research (Khan et al., 2019; Devi et al., 2024). Plants have been used for centuries in traditional medicine (Chandrashekar et al., 2025), and many modern cancer treatments are derived from plant-based compounds (Shrihastini et al., 2021). The diversity of plant species and the complexity of their phytochemical profiles (Murmu et al., 2016) offer a vast array of potential leads for cancer therapy.



Figure 1: Habitat of *Chrozophora rottlerin* near Mahanadi River areas

Chrozophora rottleri (Figure 1), a less known plant species of the Euphorbiaceae family (Prema et al., 2023). It has ethnomedicinal uses (Prema et al., 2023) and

in Odisha state, India, leaves paste is used to cure skin infections. As per its ethnomedicinal uses and less unexplored, an attempt has been made to carry out qualitative phytochemical analysis, cytotoxicity, and TLC studies of the leaf of *C. rottlerin*.



Figure 2: Leaves of *C. rottlerin* & collection for experimental works

Methodology

The leaves of *C. rottleri* were collected from nearby Mahanadi areas of Cuttack district, Odisha, India (Figure 2-3). The plant species was identified by the authors. The Maceration extraction method was adopted using three solvents (n-hexane, ethanol and aqueous) for phytochemical analysis (Figure 3-4). Detection of nine secondary metabolites was conducted using standard methods (Devi et al., 2023; Jena et al., 2024; Marndi et al., 2024). A herbarium

specimen is deposited to the Herbarium unit, Ambika Prasad Research Foundation, Odisha (Figure 5). Details of phytochemical analysis is given below.



Figure 3: Dried leaves powder of *C. rottleri*



Figure 4: Filtrate extracts of *C. rottleri* leaves for experimental analysis

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

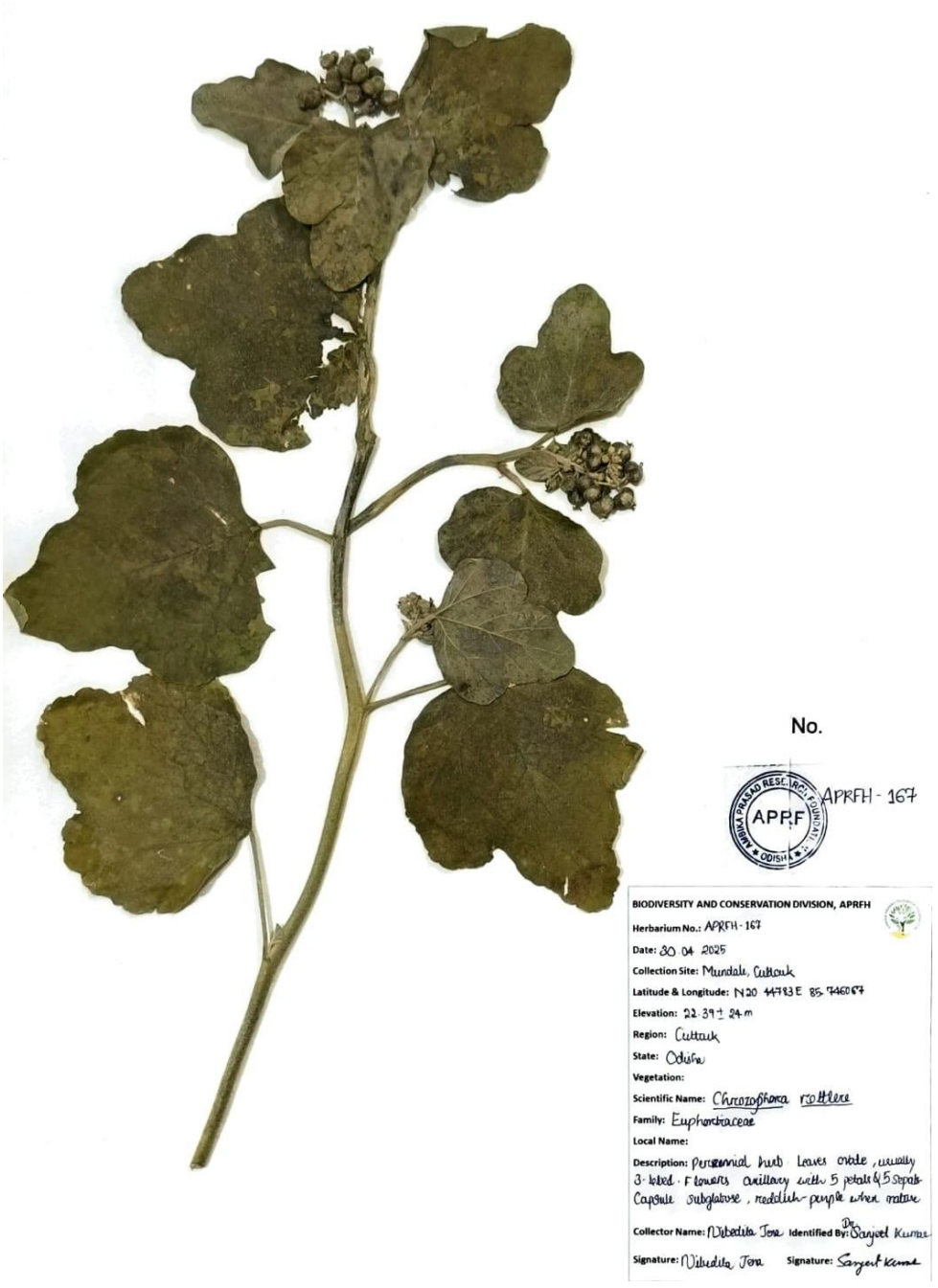


Figure 5: Herbarium specimen of *C. rottleri*

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

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

Test for terpenoids: To 1 ml of the leaves 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 leaves 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. 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 leaves extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaves extract was taken, and 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 leaves 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35ºC) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62mg ml^{-1}, 31.25 mg ml^{-1}, 62.5mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *C. rottleri* leaves extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ºC for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024).

Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *C. rottleri* leaves revealed the presence of various bioactive compounds, including tannins, saponins, reducing sugars, and alkaloids in different extracts (Table 1; Figures 6-8). The aqueous extract showed the presence of phenolic compounds and terpenoids, which are known for their medicinal properties. The presence of these compounds suggests that *C. rottleri* leaves may possess potential therapeutic benefits.

Table 1. Qualitative phytochemical analysis of *Chrozophora rottleri* leaves using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Present
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Present	Present	Absent
Carbonyl compounds	Absent	Absent	Absent

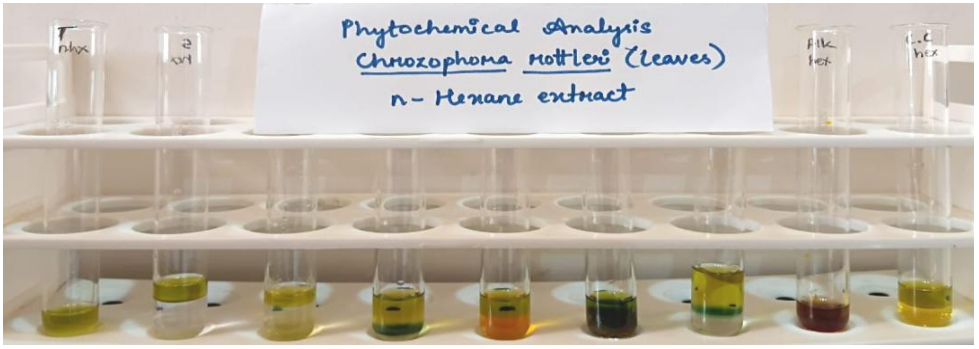


Figure 6: Detection of secondary metabolites of *C. rottleri* leaves using n-hexane extract

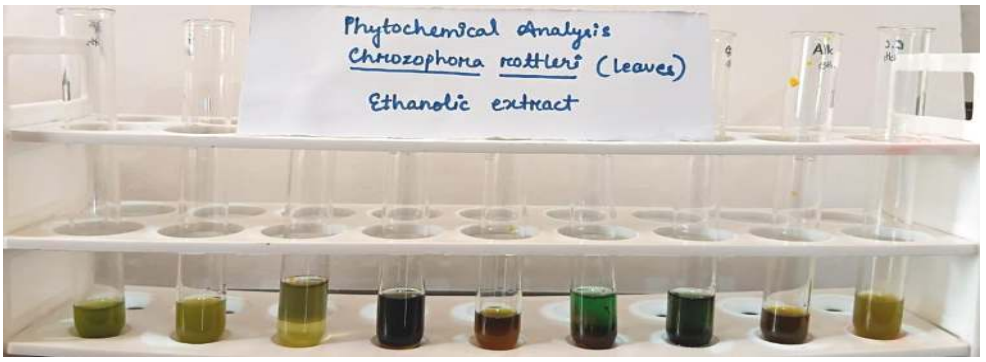


Figure 7: Detection of secondary metabolites of *C. rottleri* leaves using ethanolic extract

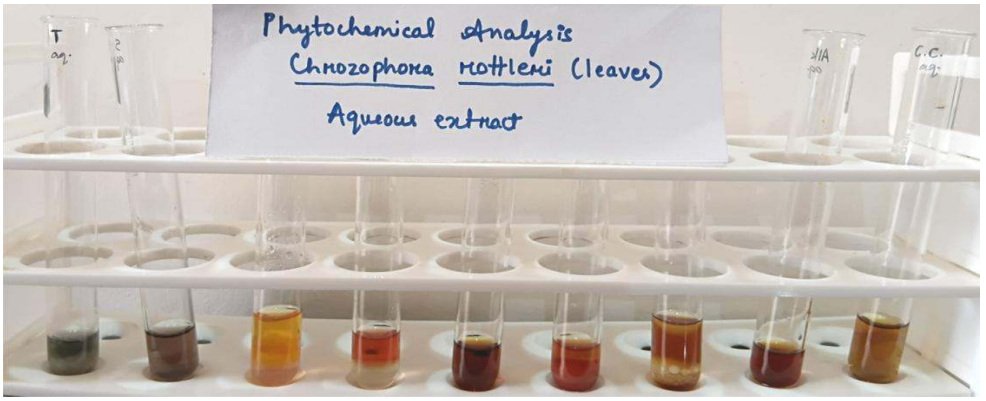


Figure 8: Detection of secondary metabolites of *C.rottleri* leaves using aqueous extract

The cytotoxicity evaluation using *Artemia salina* showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer properties (Table 2; Figures 9-11). In contrast, the aqueous extract exhibited minimal cytotoxicity, suggesting that the bioactive compounds present in this extract may have a more targeted or gentle effect (Figure 12). These findings highlight the importance of further investigating the potential therapeutic applications of *C. rottleri*.

Table 2: Cytotoxicity analysis of *Chrozophora rottleri* leaves using Brine shrimp nauplii

Extracts	**Concentration (in mg/ml)**	**Initial number of Nauplii**	**Number of Deaths of Nauplii (after hours)**			**Death rate (%)**
			1	**2**	**3**	
n-hexane	15.62	10	10	10	10	100
	31.25	10	10	10	10	100
	62.5	10	10	10	10	100
	125	10	10	10	10	100
	250	10	10	10	10	100
	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100
Ethanol	15.62	10	10	10	10	100
	31.25	10	10	10	10	100
	62.5	10	10	10	10	100
	125	10	10	10	10	100
	250	10	10	10	10	100
	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100
Aqueous	15.62	10	0	0	0	0
	31.25	10	0	0	0	0
	62.5	10	0	0	0	0
	125	10	0	0	1	10
	250	10	0	2	2	20

	3.5% Saline	10	0	0	0	0
	Vincristine sulphate	10	10	10	10	100

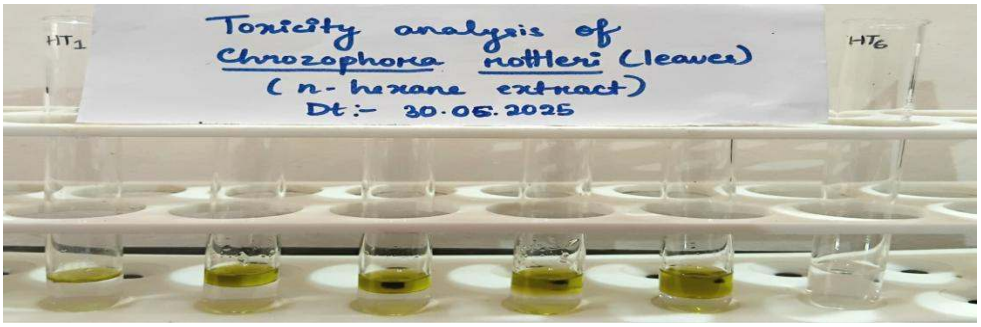


Figure 9: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *C. rottleri* leaves

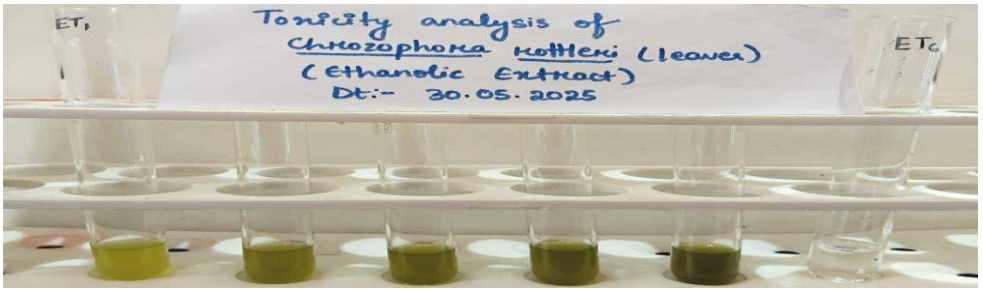


Figure 10: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *C.rottleri* leaves

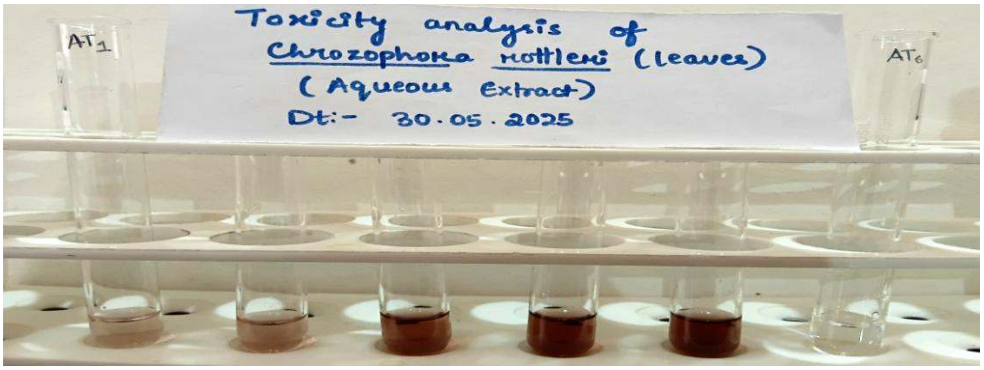


Figure 11: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *C. rottleri* leaves

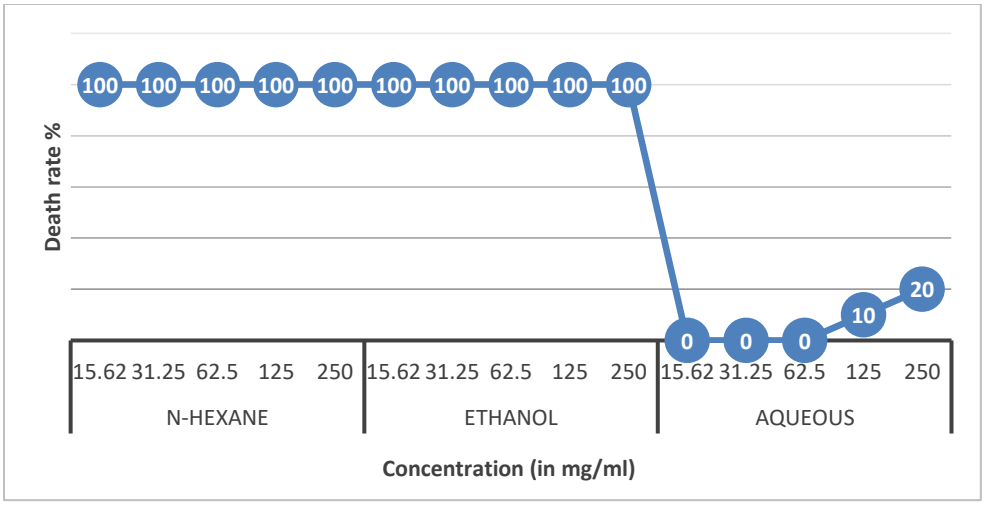


Figure 12: Death rate % of selected extracts against *Artemia salina*

The TLC analysis of the hydroethanolic extract using methanol: chloroform (1:1) as the mobile phase revealed distinct spots with different Rf values, indicating the presence of specific bioactive compounds (Table 3). The absence of spots with other mobile phases suggests that the compounds present in the extract may have specific polarities or properties.

Table 3: TLC analysis of *Chrozophora rottleri* leaves using different mobile phases

	Mobile Phases	Rf value of Spots				
		S1	S2	S3	S4	S5
Hydroethanolic	Methanol: Chloroform (1:1)	0.5	0.52	0.57	0.61	0.68
	Ethyl acetate: Chloroform (1:9)	Nil	Nil	Nil	Nil	Nil
	Ethyl acetate: Water: Acetic acid (8:1:1)	Nil	Nil	Nil	Nil	Nil

Conclusion

The phytochemical analysis and cytotoxicity evaluation of *Chrozophora rottleri* leaves suggest that this plant species possesses potential therapeutic benefits, particularly in the context of cancer treatment. The presence of bioactive compounds and significant cytotoxicity exhibited by certain extracts highlight the importance of further research to isolate and characterize the specific compounds responsible for these effects. These findings provide a foundation for future studies to explore the potential applications of *C. rottleri* in the development of novel cancer therapies.

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Chapter 3

Food and medicinal values of *Antidesma ghaesembilla* Gaertn. (Phyllanthaceae)

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Abstract: Wild edible fruits are valuable sources of nutraceuticals, contributing significantly to human health and nutrition while addressing food security challenges. In this context, the present study investigates the food and medicinal potential of *Antidesma ghaesembilla* Gaertn. through an integrative approach involving field surveys and laboratory analyses. Ethnobotanical fieldwork confirmed the edibility of the fruits and their traditional use in local communities. Phytochemical screening revealed a rich composition of primary and secondary metabolites, suggesting notable nutritional and therapeutic potential. Cytotoxicity assays demonstrated promising anticancer activity, and thin-layer chromatography (TLC) profiling indicated a diverse spectrum of bioactive constituents. These findings underscore the importance of *A. ghaesembilla* as a wild edible resource with significant health benefits, emphasizing the need for its sustainable utilization and conservation.

Keywords: Anti-cancer agents, Food problems, Global health issues, Phytochemicals

Introduction

Across the world, food insecurity, limited access to effective medicines, and climate change are among the most pressing challenges of our time (Rossati, 2017). Addressing these interconnected issues may require a shift in focus toward forests and indigenous or tribal communities, where nature still offers a wealth of underutilized resources (Addas, 2023). Forest ecosystems and their dwellers possess invaluable traditional knowledge and biodiversity that, if harnessed sustainably, could provide solutions to global warming, nutritional deficiencies, and healthcare needs. Despite the growing crisis of food scarcity worldwide (Guine, 2024), modern food systems remain heavily dependent on a narrow range of crops, primarily about twelve staple food plants, along with a limited variety of vegetables and fruits (Colgrave et al., 2021). In contrast, forests harbor numerous plant species with significant food, medicinal, and nutraceutical potential that remain largely untapped or poorly understood.



Figure 1: Fruits and leaves of *Antidesma ghaesembilla*

Recognizing and integrating these wild resources into mainstream systems could play a vital role in promoting food diversity, improving public health, and mitigating climate impacts. In this context, documenting wild edible fruits with

both nutritional and medicinal value is of critical importance, not only for human health but also for forest conservation and the well-being of the wildlife that depend on these ecosystems. Many wild fruits that are edible for humans also serve as vital food sources for birds and animals, reinforcing the ecological interconnectedness of forest systems. With this perspective, the authors conducted ethnobotanical surveys in two Indian states (Odisha and Jharkhand) and identified a commonly occurring wild fruit-bearing plant, *Antidesma ghaesembilla* (Figure 1). This species is known locally for its edibility and traditional uses. Therefore, the present study aimed to evaluate its food and medicinal potential through qualitative phytochemical analysis for primary and secondary metabolites, cytotoxicity assessment using *Artemia salina*, and thin-layer chromatography (TLC) profiling. The findings underscore the relevance of wild edible fruits in addressing contemporary global challenges related to food security, public health, and environmental sustainability.



Figure 2: Collection of fruits for experimental works

Methodology

During various floristic explorations in the Indian states of Odisha and Jharkhand, information on the food value of wild plants was collected from local tribal communities using standard ethnobotanical methods (Jain, 1987; Jain and Mudgal, 1999; Jena et al., 2025a). Fruits of *Antidesma ghaesembilla* were specifically collected from the Cuttack region of Odisha (Figure 2). The detection of primary metabolites was carried out using aqueous extracts, following the procedures outlined by Jena *et al.* (2025b). Secondary metabolite profiling was conducted using three solvent extracts (n-hexane, ethanol and aqueous; Figure 3) based on their polarity index (Chandrashekar et al., 2025). For toxicity and TLC, maceration method of extraction was adopted to get desired extracts (Chandrashekar et al., 2025; Figure 3). *Artemia salina* was used to test the cytotoxicity and 3 mobile phases were used to carry out the TLC (Chandrashekar et al., 2025).



Figure 3: Filtrate extracts of *A. ghaesembilla* fruits for experimental analysis

Results and discussion

The survey conducted in selected regions of Odisha and Jharkhand revealed that the ripe fruits of *Antidesma ghaesembilla* are commonly consumed by tribal and rural populations as a part of their traditional diet (Table 1; Figure 4). Based on their local food usage, further analysis was performed to assess the plant's nutritional and medicinal potential. Phytochemical screening of the fruit extracts confirmed the presence of primary metabolites, including carbohydrates and amino acids.



Figure 4: Authors collected fruits with tribal children for consumption

Additionally, secondary metabolites such as tannins, saponins, phenolic compounds, and reducing sugars were also detected (Table 2; Figure 5), with a notably high concentration of phenolic compounds observed. Given the abundance of phenolic compounds, often associated with antioxidant and anticancer properties, a cytotoxicity assay using *Artemia salina* was conducted.

Table 1: Food values of *Antidesma ghaesembilla*

State	Location	Food values
Odisha	Mayurbhanj	Ripen fruits are edible.
	Cuttack	Ripen fruits are edible.
	Khordha	Ripen fruits are edible.
	Keonjhar	Ripen fruits are edible.
	Sundargarh	Ripen fruits are edible.
Jharkhand	Latehar	Ripen fruits are edible.
	Jamtara	Ripen fruits are edible.

Table 2: Qualitative phytochemical analysis of *Antidesma ghaesembilla* fruits using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Present	Present	Present
Reducing sugars	Absent	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Absent
Carbonyl compounds	Absent	Absent	Absent

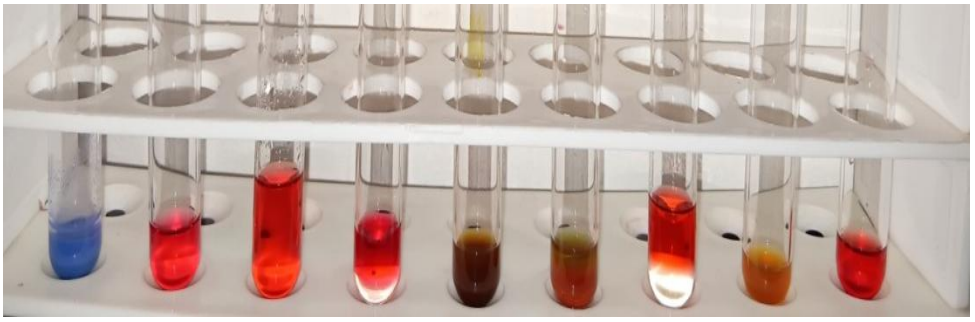


Figure 5: Detection of secondary metabolites of *A. ghaesembilla* fruits using aqueous extract

Remarkably, all three selected extracts (aqueous, ethanol, and n-hexane) exhibited 100% mortality at the tested concentrations (Table 3; Figure 6).

Table 3: Cytotoxicity analysis of *Antidesma ghaesembilla* fruits using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)		Death rates (%)
			1	2	
n-hexane	15.62	10	5	10	100
	31.25	10	1	10	100
	62.5	10	3	10	100
	125	10	10	10	100
	250	10	10	10	100
	3.5% Saline	10	0	0	0
	Vincristine sulphate	10	10	10	100
Ethanol	15.62	10	10	10	100
	31.25	10	10	10	100
	62.5	10	10	10	100
	125	10	10	10	100
	250	10	10	10	100
	3.5% Saline	10	0	0	0
	Vincristine sulphate	10	10	10	100
Aqueous	15.62	10	10	10	100
	31.25	10	10	10	100
	62.5	10	10	10	100
	125	10	10	10	100
	250	10	10	10	100
	3.5% Saline	10	10	10	100
	Vincristine sulphate	10	10	10	100

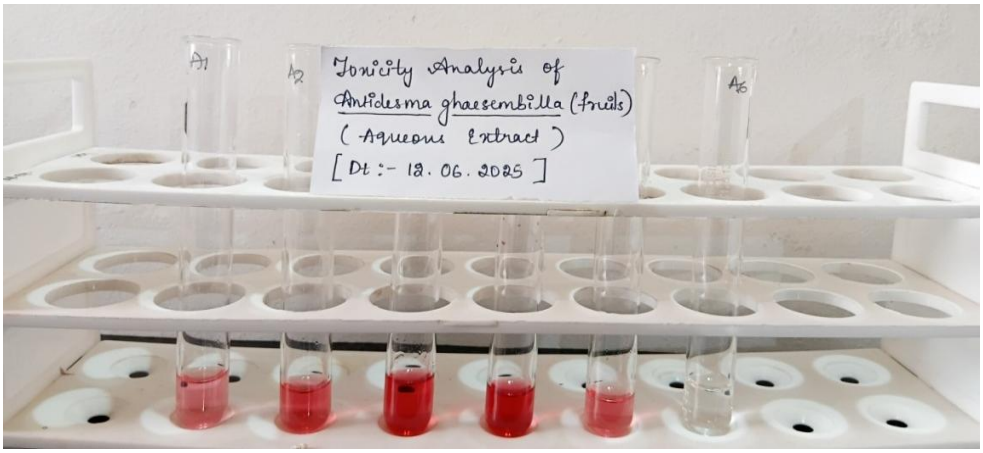


Figure 6: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *A. ghaesembilla* fruits

The TLC analysis showed that 8 spots were detected in three selected mobile phases of hydroethanolic extracts of fruits (Table 4). It showed fruits have different active constituents which maybe be act as anticancer agent.

Table 4: TLC analysis of *Antidesma ghaesembilla* fruits using different mobile phases

	Mobile Phases	Rf value of Spots		
		S1	S2	S3
Hydroethanolic	Methanol: Chloroform (1:1)	0.67	0.71	0.81
	Ethyl acetate: Chloroform (1:9)	0.8	0.97	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.95	0.97	0.98

Previous studies have highlighted the antioxidant potential of *Antidesma ghaesembilla*. Anantachoke et al. (2016) documented the presence of antioxidant

enzymes in the plant, indicating its role in combating oxidative stress. Similarly, Tiranakwit et al. (2023) reported significant antioxidant activity of *A. ghaesembilla* within the framework of traditional Thai medicine. Despite these findings, a comprehensive literature survey indicates that ethnopharmacological research on this species remains limited. There is a noticeable gap in detailed studies on its phytochemical diversity, therapeutic mechanisms, and broader nutraceutical applications. This underlines the need for further scientific investigation into the food and medicinal values of *A. ghaesembilla*, especially within the context of traditional knowledge and modern health challenges.

Conclusion

The present study underscores the significance of Antidesma ghaesembilla as a wild edible fruit plant with notable food and medicinal potential. Phytochemical analysis confirmed the presence of various bioactive compounds, including primary and secondary metabolites. The cytotoxicity assay demonstrated strong biological activity, suggesting the fruit's potential as a preventive or therapeutic anticancer agent. Furthermore, TLC profiling revealed a diverse range of phytoconstituents, reinforcing its value as a nutraceutical resource. These findings highlight the critical importance of documenting, conserving, and sustainably utilizing wild edible plants. Such underutilized species not only support local food and healthcare systems but also offer promising solutions to broader global health and environmental challenges.

Acknowledgements: The authors express their sincere gratitude to the local communities of the study areas for generously sharing their traditional knowledge regarding the food values of *Antidesma ghaesembilla*. The authors also extend their thanks to the forest officials of Odisha and Jharkhand for their support and assistance during fieldwork activities.

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Chapter 4

Evaluation of Food and medicinal values of fruits of *Ziziphus rugosa* Lam. (Rhamnaceae)

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Abstract: Present study investigated the phytochemical analysis (primary & secondary) of *Ziziphus rugosa* fruits using different extracts. Qualitative analysis revealed the presence of various metabolites, including tannins, saponins, flavonoids, and carbohydrates. The ethanol extract showed a rich presence of primary metabolites, including proteins, starch, and amino acids. The findings suggested that *Z. rugosa* fruits has potential medicinal and nutritional value which make it a good nutraceutical food.

Keywords: Phytochemical composition, Bioactive compounds, Pharmacological applications

Introduction

Plants have been a vital source of medicine and nutrition for centuries, with many species still being used today in traditional practices (Kumar, 2015). The use of plants for food and medicinal purposes is based on the presence of

bioactive compounds, which can have therapeutic effects on the human body (Kumar et al., 2013). With the increasing interest in natural products and nutraceuticals, there is a growing need to explore and understand the phytochemical composition of wild edible plants (Alemu et al., 2024). *Ziziphus rugosa* (Figure 1-2) is a wild edible fruiting plant species that has been traditionally used for its medicinal and nutritional properties (Manjunatha et al., 2020). The fruit of the plant is consumed by tribal communities, and is believed to have several health benefits.



Figure 1: Fruits of *Z. rugosa*

Despite its potential benefits, the phytochemical composition of *Z. rugosa* fruits remains understudied. Further research is needed to identify and characterize the metabolites present in the fruits.



Figure 2: Collection of fruits by authors

Therefore, present study aims to investigate the metabolites of *Z. rugosa* fruits, with a focus on identifying and characterizing primary and secondary metabolites. By exploring the medicinal and nutritional properties of the fruit, present study hopes to contribute to the understanding of the potential health benefits of *Z. rugosa*. The findings of present study may also have implications for the development of new natural products and nutraceuticals.

Methodology

Fruits of *Z. rugosa* were specifically collected from the Latehar Forest Division, Jharkhand (Figure 2). The detection of primary metabolites was carried out using selected extracts (Figure 3), following the procedures outlined by Jena *et al.* (2025).



Figure 3: Filtrate extracts of *Z. rugosa* fruits for experimental analysis

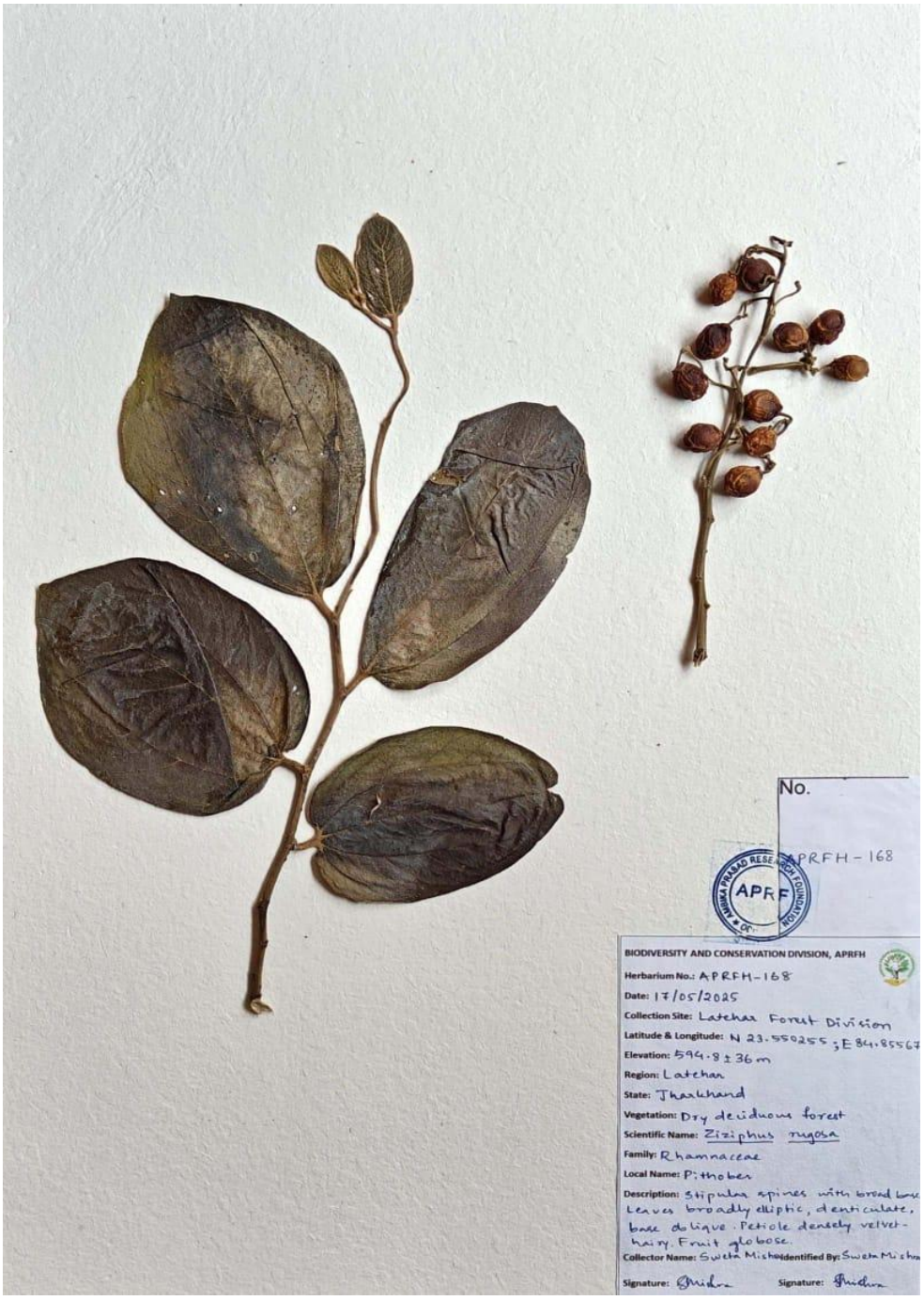


Figure 4: Herbarium specimen of *Z. rugosa*

Secondary metabolite profiling was conducted using three solvent extracts (n-hexane, ethanol and aqueous) based on their polarity index (Chandrashekar et al., 2025). A herbarium specimen (Figure 4) is submitted to the Herbarium unit, Ambika Prasad Research Foundation, Odisha, India.

Results and discussion

The phytochemical analysis of *Z. rugosa* fruits revealed the presence of various bioactive compounds, including tannins, saponins and flavonoids (Table 1; Figure 5). These compounds were detected in different extracts, with the ethanol extract showing a rich presence of primary metabolites such as proteins, starch, and amino acids (Table 2; Figure 6). The presence of these metabolites suggested that *Z. rugosa* fruits may have potential nutraceutical value.

Table 1: Qualitative phytochemical analysis of *Z. rugosa* fruits for detection of secondary metabolites using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Present
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Absent
Reducing sugars	Present	Absent	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Absent
Carbonyl compounds	Absent	Absent	Absent

Table 2: Qualitative phytochemical analysis of *Z. rugosa* fruits for detection of primary metabolites using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Protein	Absent	Present	Present
Starch	Absent	Present	Absent

Carbohydrate	Present	Present	Present
Sucrose	Absent	Absent	Absent
Lipid	Absent	Absent	Absent
Amino acid	Absent	Present	Absent

The presence of tannins and saponins in the fruit extracts is particularly noteworthy, as these compounds have been shown to have antioxidant and anti-inflammatory properties (Fraga-Corral et al., 2021).

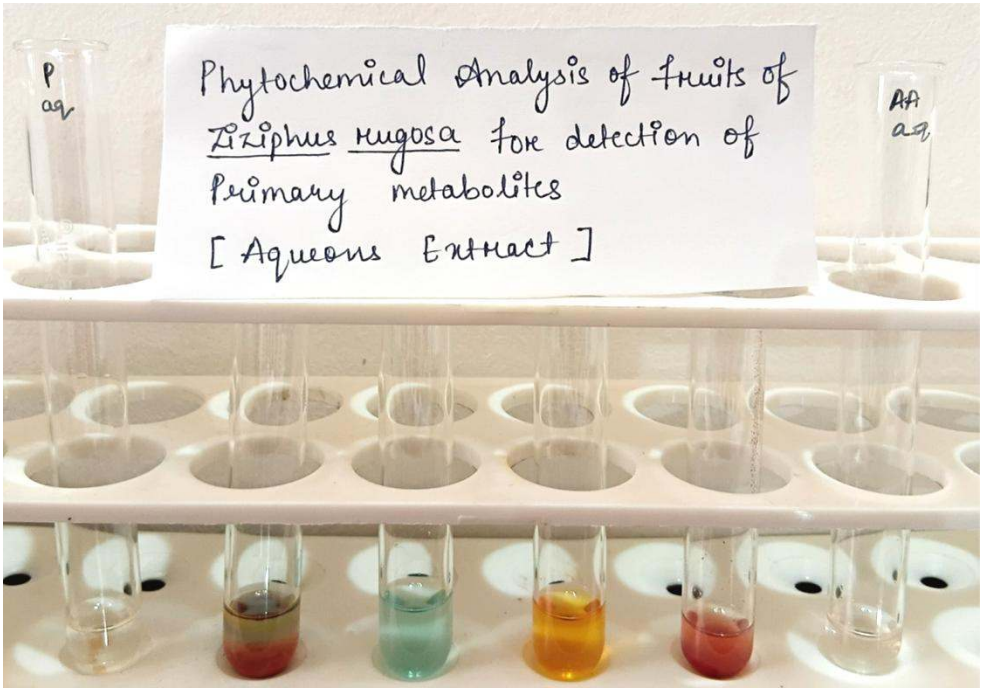


Figure 5: Detection of primary metabolites of *Z. rugosa* fruits (aqueous extract)

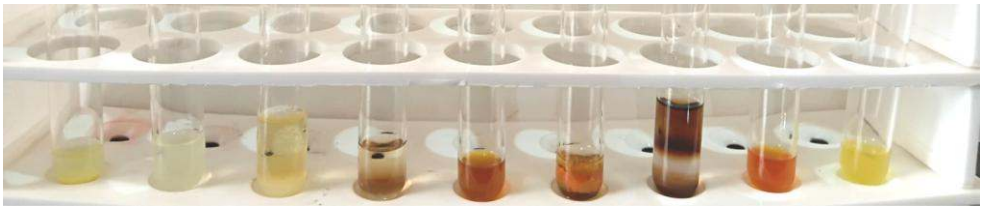


Figure 6: Detection of secondary metabolites of *Z. rugosa* fruits (aqueous extract)

Flavonoids, which were also detected in the fruit extracts, have been reported to have antimicrobial and anticancer activities (Kopustinskiene et al., 2020). The presence of these bioactive compounds supports the traditional use of *Z. rugosa* fruits for medicinal purposes and highlights their potential as a source of natural products. The findings of this study have implications for the development of new natural products and therapies. The bioactive compounds present in *Z. rugosa* fruits may be useful in the prevention and treatment of various diseases, including those related to oxidative stress and inflammation. Further research is needed to fully explore the potential health benefits of *Z. rugosa* fruits and to identify the specific bioactive compounds responsible for their medicinal properties.

Conclusion

Present study demonstrated the presence of various bioactive compounds in *Ziziphus rugosa* fruits. The findings suggest that *Z. rugosa* fruits have potential medicinal and nutritional value, supporting their traditional use and highlighting their potential as a source of nutraceutical. Further research is needed to fully explore the health benefits and therapeutic applications of *Z. rugosa* fruits.

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Chapter 5

Phytochemical, cytotoxicity and TLC evaluation of leaves of *Hibiscus vitifolius* L. (Malvaceae)

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Abstract: Present study investigated the phytochemical composition, cytotoxic potential, and thin-layer chromatography (TLC) profile of the leaves of *Hibiscus vitifolius* L. of Malvaceae family. Preliminary phytochemical screening revealed the presence of bioactive constituents such as tannins, saponins, flavonoids and reducing sugars using different extracts (n-hexane, ethanol and aqueous). The cytotoxic activity was assessed using the brine shrimp lethality assay, indicating significant mortality rates for n-hexane and ethanol extracts, while aqueous extract exhibited moderate cytotoxicity. TLC analysis was performed using hydroethanolic extract to establish a phytochemical fingerprint, highlighted distinct spots corresponding to various secondary metabolites. The findings suggest that *H. vitifolius* leaves possess significant phytochemical and cytotoxic properties, warranting further investigation for potential therapeutic applications.

Keywords: Bioactive constituents, cytotoxic activity, phytochemical fingerprint, therapeutic applications

Introduction

In the past few decades, the world has witnessed a significant rise in chronic and lifestyle-related diseases such as cancer, diabetes, antibiotic-resistant infections, cardiovascular and neurodegenerative disorders (Senapati et al., 2015; Balwan and Kour 2021; Jain et al., 2024). Additionally, emerging infectious diseases, environmental pollution, and drug resistance have added complexity to global health challenges (Coque et al., 2025). Many synthetic drugs used to treat these conditions come with adverse side effects, high costs, and limited accessibility, especially in developing regions (Gunjan et al., 2015). This growing burden on modern healthcare systems highlighted the urgent need for alternative approaches to disease prevention and treatment. Sustainable utilization of medicinal plants offered a promising solution to these challenges (Kumar et al., 2018). Medicinal plants are reservoirs of diverse bioactive compounds such as tannins, saponins, flavonoids, reducing sugars and alkaloids that exhibited a wide range of pharmacological activities including antimicrobial, anti-inflammatory, antioxidant, anticancer, and antidiabetic properties (Ramawat et al., 2009). By promoting ethnobotanical research, phytochemical screening, and pharmacological validation, it is possible to discover new therapeutic agents that are both effective and safer than many synthetic drugs. The Malvaceae family, comprising over 200 genera and around 2,300 species, includes several plants known for their medicinal value (Raj et al., 2022). Members of this family are widely distributed in tropical and subtropical regions (Das and Islam, 2019) and have been used traditionally to treat a variety of ailments such as inflammation, wounds, infections, and gastrointestinal disorders (Abat et al., 2017).



Figure 1: Leaves and flower of *H. vitifolius*

Among them, *H. vitifolius* L., commonly found in parts of Asia and Africa, has drawn attention for its potential pharmacological properties (Jejurkar and Chavan, 2023). Traditionally, different parts of this plant have been used in folk medicine for treating fever, ulcers, and skin diseases (Kapoor et al., 2021). However, scientific validation of these uses through phytochemical and cytotoxic evaluations remained limited. By studying the leaf extracts of *H.*

vitifolius, present study aims to identified the key bioactive compounds and assessed their cytotoxicity, providing a foundation for further pharmacological investigations.

Methodology

The leaves of *H. vitifolius* were collected from nearby Mahanadi areas of CDA-13, Cuttack, Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 2). Detection of nine secondary metabolites was conducted using standard methods (Hossain et al., 2025; Rani et al., 2025). Details of phytochemical analysis is given below.

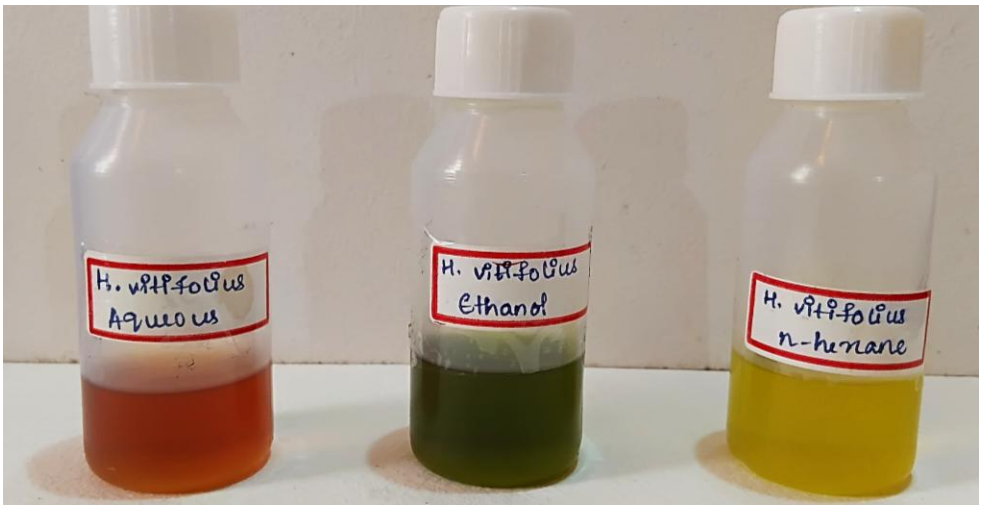


Figure 2: Filtrate extracts of *H. vitifolius* leaves for experimental analysis

Test for tannin: 1 ml of leaves extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 leaves extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

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

Test for terpenoids: To 1 ml of the leaves 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 leaves 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. 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 leaves extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaves extract was taken, and 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 leaves 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35ºC) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *H. vitifolius* leaves extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ºC for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024).

Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *H. vitifolius* leaves revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids and reducing sugars (Table 1: Figures 3-5). These compounds were found to be present in different extracts having various pharmacological potential. The presence of wide range of phytoconstituents suggests that *H. vitifolius* leaves may possess medicinal properties.

Table 1. Qualitative phytochemical analysis of *H. vitifolius* leaves using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Absent	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Present
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Absent
Carbonyl compounds	Absent	Absent	Absent

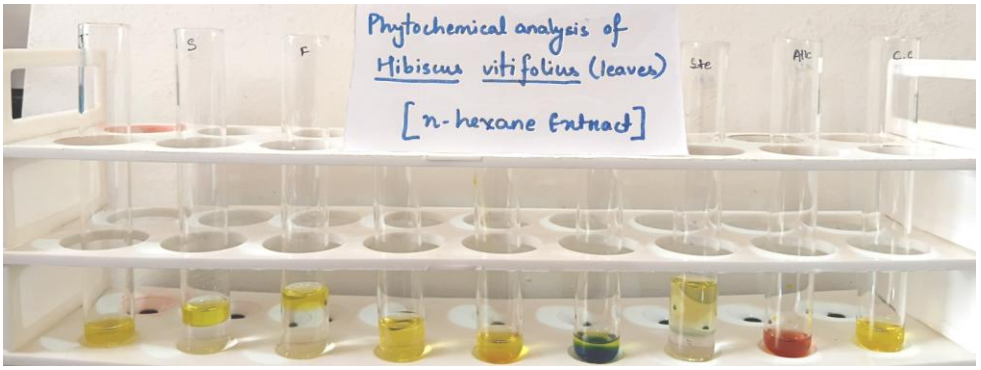


Figure 3: Detection of secondary metabolites of *H. vitifolius* leaves using n-hexane extract

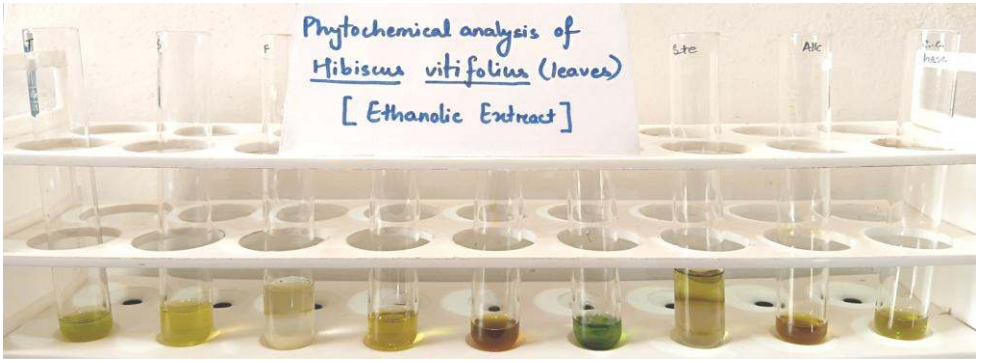


Figure 4: Detection of secondary metabolites of *H. vitifolius* leaves using ethanolic extract

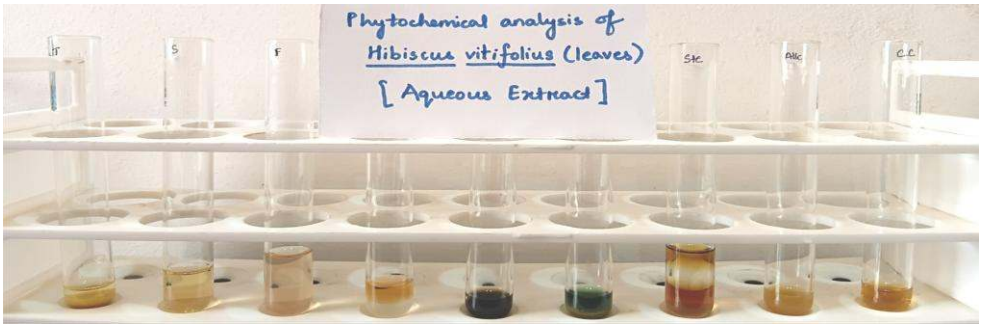


Figure 5: Detection of secondary metabolites of *H. vitifolius* leaves using aqueous extract

The cytotoxicity analysis of *H. vitifolius* leaf extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 6-8). In contrast, the aqueous extract exhibited moderate to less lethality response. Present findings suggest that *H. vitifolius* leaves may possess specific bioactive compounds that contribute to toxicity.

Table 2: Cytotoxicity analysis of *H. vitifolius* leaves using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	2	2	2	2	20
	31.25	10	0	2	2	2	20
	62.5	10	0	0	0	0	0
	125	10	0	0	0	0	0
	250	10	0	0	0	0	0
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

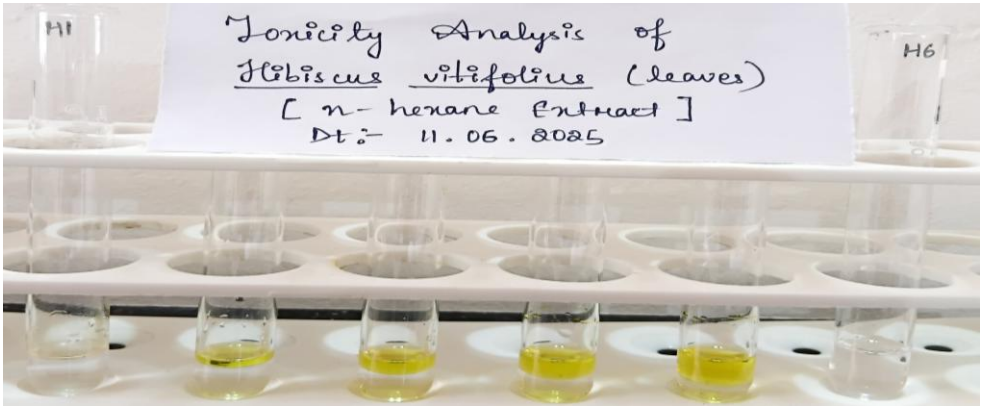


Figure 6: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *H. vitifolius* leaves



Figure 7: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *H. vitifolius* leaves



Figure 8: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *H. vitifolius* leaves

Table 3: TLC analysis of *Hibiscus vitifolius* leaves using different mobile phases

	Mobile Phases	Rf value of Spots					
		S1	S2	S3	S4	S5	S6
Ethanolic aqueous	Methanol: Chloroform (1:1)	0.64	0.68	0.72	0.75	0.82	0.91
	Ethyl acetate: Chloroform (1:9)	0.81	0.82	0.84	-	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.57	0.71	0.82	0.91	-	-

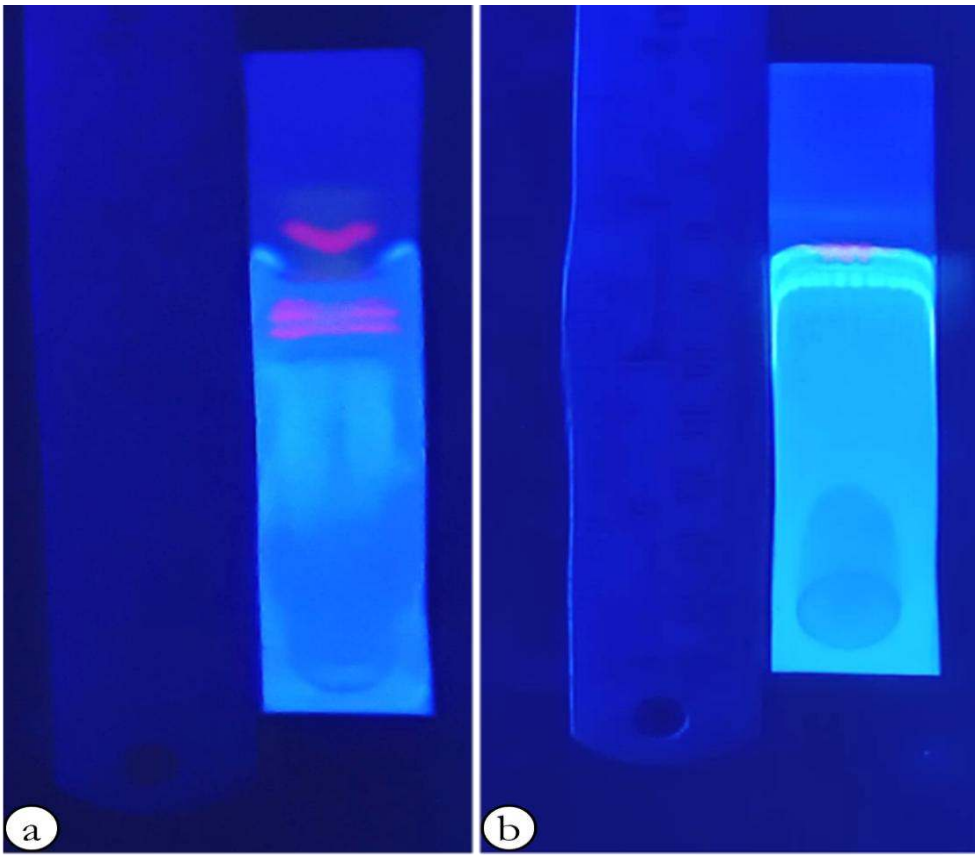


Figure 9: TLC analysis of hydroethanolic extract of *H. vitifolius* leaves (mobile phase: a) Ethyl acetate: Chloroform and b) Chloroform: Methanol

The chromatographic analysis of hydroethanolic extract of *H. vitifolius* leaves revealed distinct bioactive components, with different mobile phases separating various phytochemicals based on their Rf values. The presence of different spots in the TLC plates confirmed the presence of bioactive compounds in *H. vitifolius* leaves, which may be responsible for its medicinal properties (Table 3; Figure 9).

Conclusion

Present study on the leaves of *H. vitifolius* highlighted its potential as a valuable source of bioactive compounds with medicinal significance. Phytochemical analysis confirmed the presence of key secondary metabolites, while the cytotoxicity assay indicated significant biological activity, supporting its traditional uses in herbal medicine. Additionally, the TLC profiling provided a preliminary chemical fingerprint that can aid in further isolation and characterization of active constituents. These findings bring out the importance of *H. vitifolius* as a candidate for future pharmacological research and support the sustainable exploration of medicinal plants for drug discovery and therapeutic development.

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Chapter 6

Phytochemical, cytotoxicity and TLC analysis of *Heliotropium ovalifolium* Forssk. (Boraginaceae)

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Abstract: Herbs are most preferred sources for the formulation of drugs to treat different health problems. *Heliotropium ovalifolium*, a herb, usually grow near river areas. Pharmacologically, it is not more explored. Therefore, it is selected for evaluation of phytochemical analysis, cytotoxicity and TLC (Thin Layer chromatography). The results revealed that it contents secondary metabolites like tannin, saponin, phenolic compounds, reducing sugar and alkaloids. Cytotoxicity analysis revealed that n-hexane and ethanol showed 100 % death rate. Hydroethanolic extract showed 6 spots on TLC plate in three selected mobile phases. Present study highlights the medicinal and pharmacological potential of *H. ovalifolium*.

Keywords: Bioactive compounds, herbs, pharmacology

Introduction

Herbs are abundantly available in and around human habitats throughout the world (Srivastava, 2018). They are most preferred plant species are used as food, medicines, spices, ornamental and cultural having ecological & economic potentials (Giannenas et al., 2020). The early human has learned to use them in day-to-day life and during course of time, they learned them to use as a source of therapeutic agents (Scherrer et al., 2023). Globally, humans are facing lot of health problems and continuous need to screen the plants for development of drugs (Thomford et al., 2018). In this aspect, *Heliotropium ovalifolium* is an erect herb belonging to Boraginaceae family grows near moist areas.



Figure 1: Stem and leaves of *H. ovalifolium*

The leaves are densely softly white-silky. The flowers are white, funnel-shaped (Brahmam and Saxena, 1995; Figure 1). It has ethnomedicinal uses but less

explored (Kulkarni-Almeida et al., 2008). Therefore, stem and leaves are used together to evaluate its pharmacological values. The chapter highlights the importance of ethnopharmacological potentials of *H. ovalifolium*.

Methodology

The stem and leaves of *H. ovalifolium* were collected from the bank of Mahanadi River, Cuttack, Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 2). Detection of nine secondary metabolites was conducted using standard methods (Chauhan et al., 2025; Kapil et al., 2025; Sharma et al, 2025; Vinayak et al., 2025). Details of phytochemical analysis is given below.

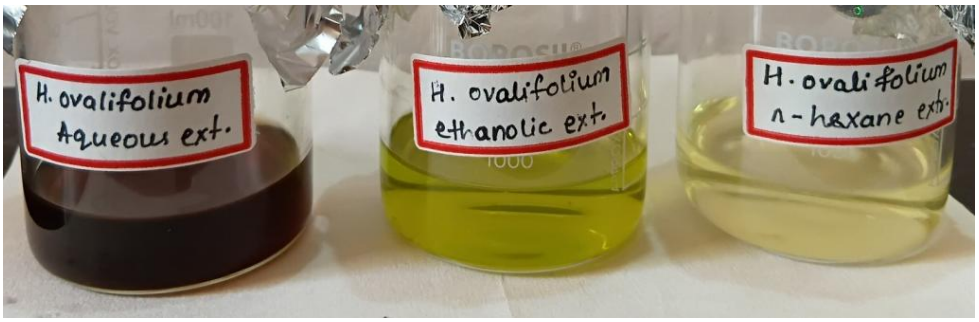


Figure 2: Filtrate extracts of *H. ovalifolium* stems & leaves for experimental analysis

Test for tannin: 1 ml of leaves extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 stem and leaves extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

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

Test for terpenoids: To 1 ml of the stem and leaves 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 stem and leaves 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 stem and leaves 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 stem and leaves extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the stem and leaves extract was taken, and 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 stem and leaves 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *H. ovalifolium* extracts were taken and 3.5% saline water were used to make the volume 2 ml in each test tube. 1 % DMSO (Dimethyl sulfoxide) 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ^{o}C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *H. ovalifolium* stem and leaves revealed the presence of various bioactive compounds, including tannins, saponins, alkaloids and reducing sugars (Table 1: Figures 3-5). These compounds were found to be present in different extracts having various pharmacological potential. The presence of wide range of phytoconstituents suggests that *H. ovalifolium* stem and leaves may possess significant medicinal properties.

Table 1: Qualitative phytochemical analysis of *H. ovalifolium* stems & leaves using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Present
Carbonyl compounds	Absent	Absent	Absent

The cytotoxicity analysis of *H. ovalifolium* stems and leaves extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 6-8).

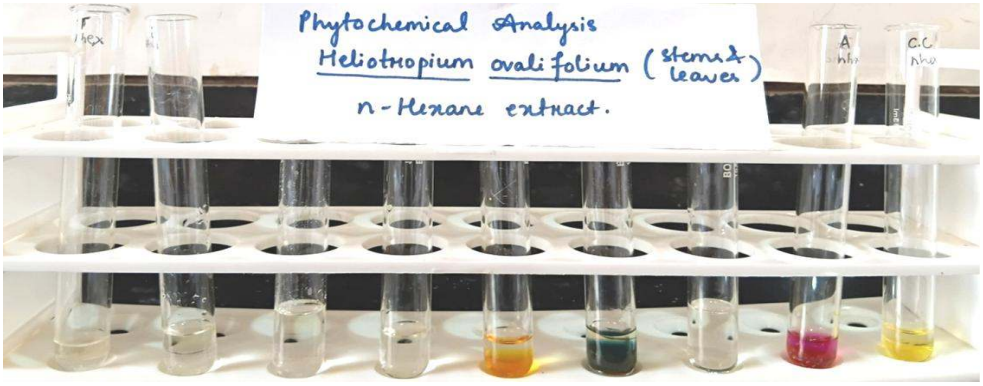


Figure 3: Detection of secondary metabolites of *H. ovalifolium* stems & leaves using n-hexane extract



Figure 4: Detection of secondary metabolites of *H. ovalifolium* stems & leaves using ethanolic extract



Figure 5: Detection of secondary metabolites of *H. ovalifolium* stems & leaves using aqueous extract

In contrast, the aqueous extract exhibited moderate lethality response for cytotoxicity analysis. Present findings suggested that *H. ovalifolium* stem and leaves may possess specific bioactive compounds that contribute to toxicity.

Table 2: Cytotoxicity analysis of *H. ovalifolium* stems & leaves using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	3	10	10	10	100
	31.25	10	2	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	2	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	1	2	3	3	30
	31.25	10	6	5	7	7	70
	62.5	10	5	5	5	5	50
	125	10	4	9	9	9	90
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

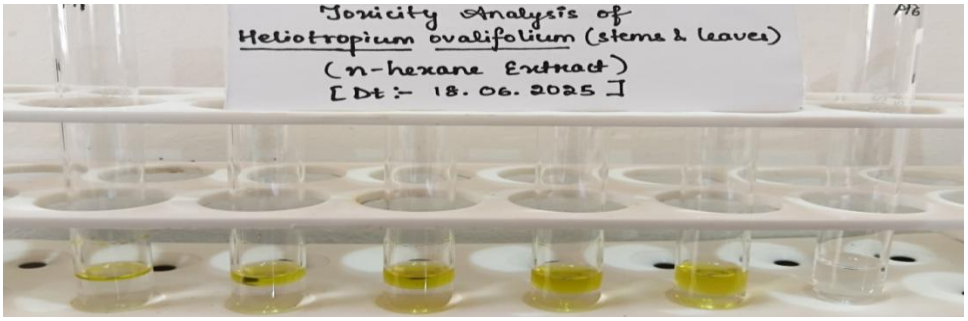


Figure 6: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *H. ovalifolium* stems & leaves

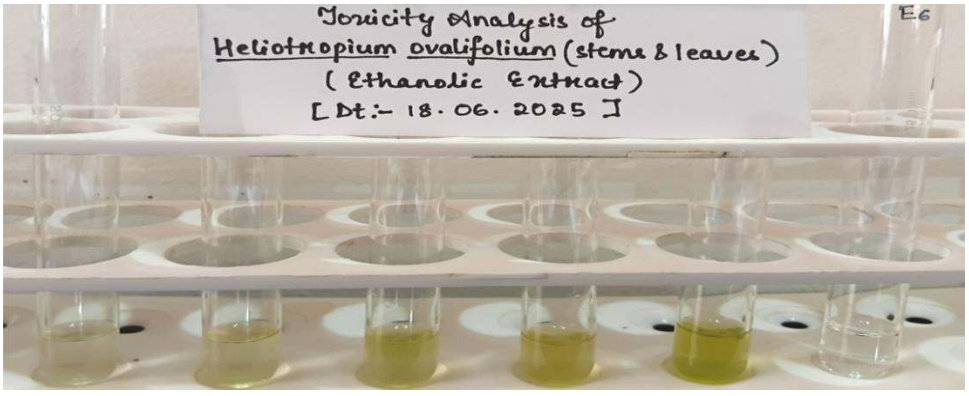


Figure 7: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *H. ovalifolium* stems & leaves

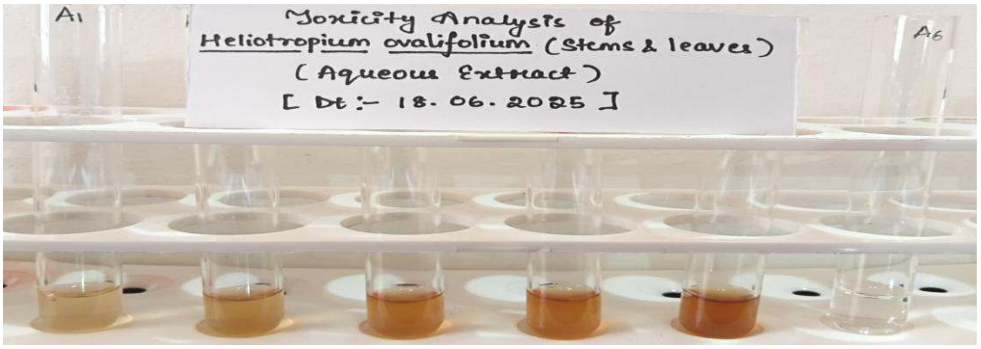


Figure 8: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *H. ovalifolium* stems & leaves

The chromatographic analysis of hydroethanolic extract of *H. ovalifolium* stem and leaves revealed distinct bioactive components, with different mobile phases separating various phytochemicals based on their Rf values. The presence of different spots in the TLC plates confirmed the presence of bioactive compounds in *H. ovalifolium* stem and leaves, which may be responsible for its medicinal properties (Table 3).

Table 3: TLC analysis of *H. ovalifolium* stems & leaves using different mobile phases

	Mobile Phases	Rf value of Spots		
Hydroethanolic	Methanol: Chloroform (1:1)	0.67	0.71	-
	Ethyl acetate: Chloroform (1:9)	0.75	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.32	0.44	0.52

Conclusion

In global health scenario, human need lot of medicines due to frequent changes in biotic and abiotic component of the earth. In this aspect, ethnopharmacology researchers are engaged to screen different types of natural products. The present study of *H. ovalifolium* drawn attention towards its ethnopharmacological potential to develop different types of therapeutic drugs. The reported pharmacological potentials are very basic and furthr advanced research works are needed using different parts of *H. ovalifolium*.

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Chapter 7

Evaluation of phytochemical constituents, cytotoxic potential, and chromatographic profiling of fruits of *Alphonsea lutea* (Roxb.) Hook.f. & Thomson

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Abstract: Present study investigated the phytochemical composition, cytotoxic potential, and thin-layer chromatography (TLC) profile of the fruits of *Alphonsea lutea* (Roxb.) Hook.f. & Thomson, a lesser-known member of the Annonaceae family. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, reducing sugar, phenolics, and saponins. TLC analysis was employed to develop a chemical fingerprint of the fruit extracts, highlighting key phytoconstituents. The cytotoxic activity was assessed using the brine shrimp lethality assay, demonstrating moderate to significant toxicity, suggesting the presence of bioactive compounds with potential pharmacological applications. These findings provide a foundation for further investigation into the therapeutic potential of *A. lutea* fruits.

Keywords: Chemical fingerprint, phytoconstituents, therapeutic potential

Introduction

Alphonsea lutea (Roxb.) Hook.f. & Thomson, belonging to the family Annonaceae, is a lesser known yet potentially valuable medicinal plant native to parts of South and Southeast Asia, including India (Srivastava and Mehrotra, 2025). It is a deciduous or semi-evergreen tree, typically found in tropical and subtropical forest regions (Katole et al., 2018). Though not extensively studied, *A. lutea* is recognized in some traditional systems of medicine for its therapeutic properties (Bakri et al., 2017). Local communities have used different parts of the plant including fruits, bark, and leaves for treating ailments such as fever, inflammation, digestive disorders, and microbial infections (Kumar and Dash, 2012). Despite its traditional relevance, scientific validation of its phytochemical profile and biological activities remains limited, creating an opportunity for further Pharmacognostic exploration.



Figure 1: Fruits of *A. lutea*

Recent global health challenges, particularly those related to antimicrobial resistance, chronic diseases, and the rising burden of cancer, call for the discovery of novel, plant-derived bioactive compounds (Chaachouay and Zidane, 2024). *A. lutea*, with its reservoir of secondary metabolites such as alkaloids, flavonoids, tannin, saponins and reducing sugar, holds promise as a source of natural therapeutic agents (Sahu et al., 2021). This study aims to bridge the gap between ethnomedicinal knowledge and scientific validation, supporting the global pursuit of sustainable and accessible healthcare alternatives.

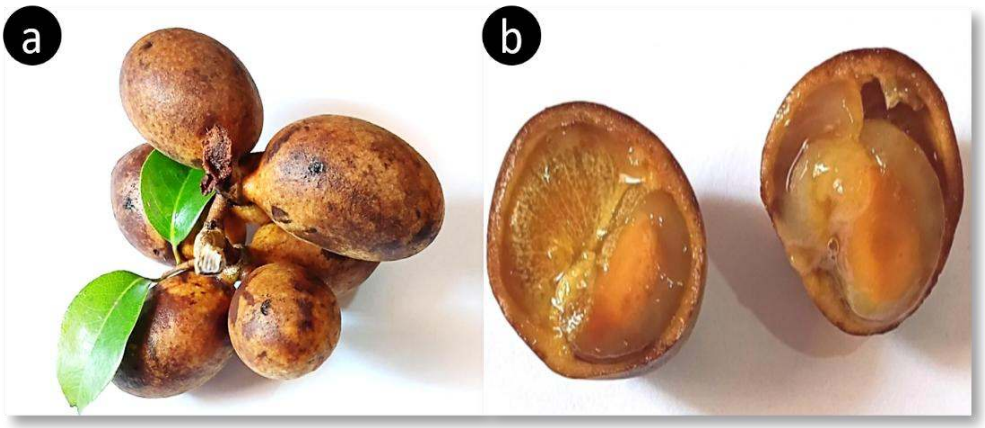


Figure 2: Fruits of *A. lutea* and their parts; a) Fruits and b) Fruit pulp and seeds

Methodology

The fruits of *A. lutea* were collected from Khordha district of Odisha, India (Figure 2). The plant species was identified by the authors by the help of relevant taxonomic literature (Saxena and Brahmam, 1994; Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 3). Detection of nine secondary metabolites was conducted using standard methods (Jena et al., 2025; Majhi et al., 2025; Nithish et al., 2025). Details of phytochemical analysis is given below.



Figure 3: Filtrate extracts of *A. lutea* fruits for experimental analysis

Test for tannin: 1 ml of fruit extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 was observed, confirming the presence of saponin.

Test for flavonoids: About 1 ml of the fruit extract was taken, and 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The color initially turned to an intense yellow color with NaOH solution and later became colorless, 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 to it. 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, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the fruit extract was taken, and 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *A. lutea* fruit extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ^{o}C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The phytochemical analysis of *A. lutea* fruits revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, alkaloids and reducing sugars (Table 1: Figures 4-6). These compounds were found to be present in different extracts having various ethnomedicinal uses and pharmacological potential. Traditionally, *Alphonsea* species are medicinally used to treat rheumatism and edema (Bakri et al., 2017). The presence of wide range of phytoconstituents suggests that *A. lutea* fruits may possess medicinal properties.

Table 1: Qualitative phytochemical analysis of *Alphonsea lutea* fruits using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Present	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Absent
Reducing sugars	Absent	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Present
Carbonyl compounds	Absent	Absent	Absent



Figure 4: Detection of secondary metabolites of *A. lutea* fruits using n-hexane extract

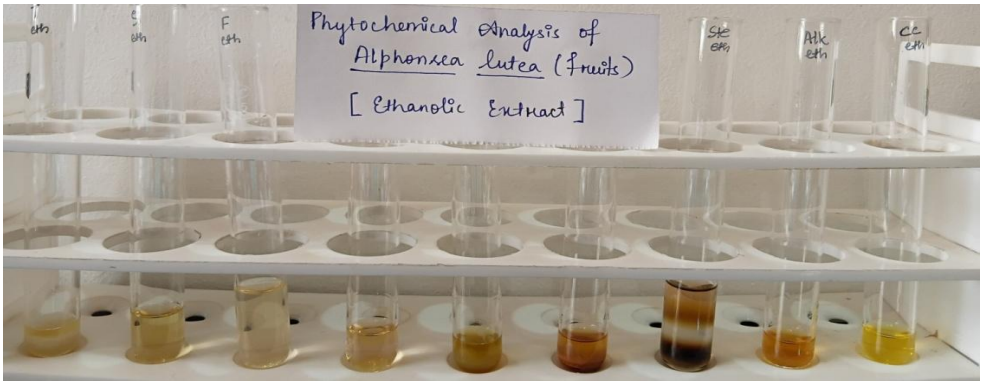


Figure 5: Detection of secondary metabolites of *A. lutea* fruits using ethanolic extract

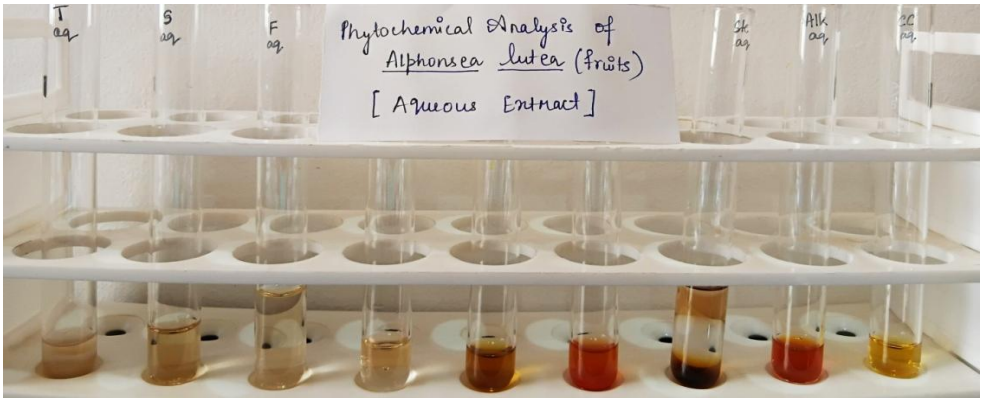


Figure 6: Detection of secondary metabolites of *A. lutea* fruits using aqueous extract

The cytotoxicity analysis of *A. lutea* fruit extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 7-9). In contrast, the aqueous extract exhibited moderate lethality response. Present findings suggest that *A. lutea* fruit may possess specific bioactive compounds that contribute to toxicity.

Table 2: Cytotoxicity analysis of *Alphonsea lutea* fruits using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	2	8	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	1	10	10	10	100
	125	10	10	10	10	10	100
	250	10	1	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100

	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	0	2	2	2	20
	31.25	10	4	10	10	10	100
	62.5	10	5	10	10	10	100
	125	10	5	10	10	10	100
	250	10	4	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

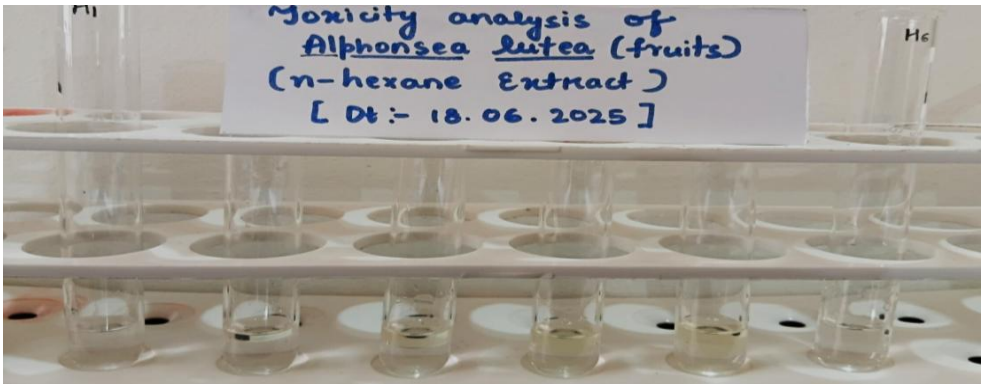


Figure7: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *A. lutea* fruits

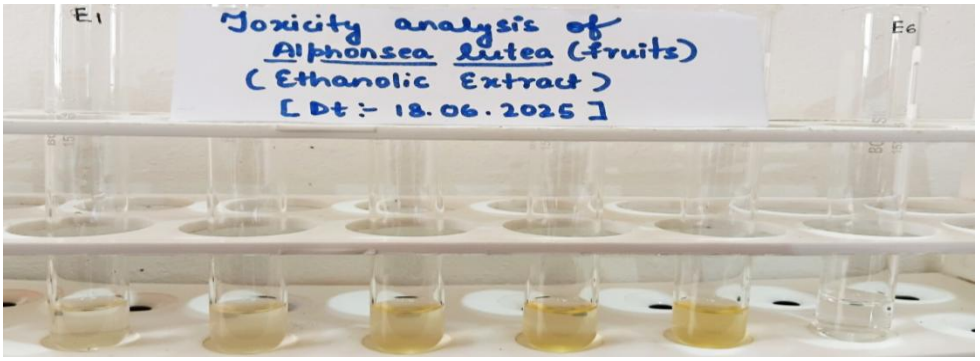


Figure 8: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *A. lutea* fruits



Figure 9: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *A. lutea* fruits

The chromatographic analysis of hydroethanolic extract of *A. lutea* fruit revealed distinct bioactive components, with different mobile phases separating various phytochemicals based on their Rf values. The presence of different spots in the TLC plates confirmed the presence of bioactive compounds in *A. lutea* fruits, which may be responsible for its medicinal properties (Table 3).

Table 3: TLC analysis of *Alphonsea lutea* fruits using different mobile phases

	Mobile Phases	Rf value of Spots	
		S1	S2
Hydroethanolic	Methanol: Chloroform (1:1)	-	-
	Ethyl acetate: Chloroform (1:9)	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.6	0.67

Conclusion

Present study highlighted the phytochemical richness of *A. lutea* fruits, which supports its traditional medicinal uses and suggests potential applications in pharmaceutical and therapeutic research. The observed cytotoxic activity further indicated the plant's potential role in anticancer or antimicrobial drug development. Biological potential of *A. lutea* stands as a strong candidate for

further pharmacological investigations that can contribute to the discovery of novel natural products against the global health challenges.

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Chapter 8

Phytochemical screening, cytotoxic potential and chemical fingerprinting of leaves of *Viscum monoicum* Roxb. ex DC. (Santalaceae)

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Abstract: Present study explored the medicinal potential of *Viscum monoicum* leaves Roxb. ex DC. by evaluating its bioactive constituents. Preliminary phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, steroids, tannins, saponins, phenols and reducing sugars, which are known for their therapeutic effects. The cytotoxic potential of leaf extracts was assessed, demonstrating significant activity which suggests possible anticancer properties. Furthermore, chemical fingerprinting using chromatographic techniques provided a detailed profile of the extract's phytochemical composition, laying the groundwork for further isolation and identification of active compounds. This integrated analysis highlighted the promise of *V. monoicum* as a source of bioactive molecules for pharmaceutical development.

Keywords: Anticancer, bioactive constituents, explore, pharmaceutical development

Introduction

Viscum monoicum Roxb. ex DC., a hemiparasitic plant belonging to the family Santalaceae, is widely distributed in tropical and subtropical regions of Asia, including India (Saxena and Brahmam, 1995). Traditionally recognized for its therapeutic significance, this species has been used in various indigenous systems of medicine (Khatun et al., 2016; Ahmed et al., 2023). Ethnomedicinally, different parts of the plant, particularly the leaves, have been employed to treat ailments such as ulcers, inflammation, respiratory issues, and skin disorders. The presence of a wide range of secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds contributes to its medicinal efficacy. Local communities have long relied on this plant, underlining its cultural and pharmacological importance (Melo et al., 2023). Pharmacological studies have indicated that *Viscum monoicum* possesses a broad spectrum of bioactivities, including antioxidant, antimicrobial, anti-inflammatory, and cytotoxic effects (Ahmmed et al., 2013; Olas, 2024). These properties positioned the plant as a promising candidate for developing novel therapeutic agents, particularly in the context of rising antibiotic resistance and the global burden of chronic diseases such as cancer. Furthermore, the exploration of its phytochemical diversity through chemical fingerprinting can lead to the identification of novel compounds with significant biomedical potential. The increasing demand for plant-based therapeutics and functional foods, *V. monoicum* offers an opportunity for sustainable utilization through integrated approaches. Its incorporation into nutraceutical and herbal formulations can not

only provide natural health solutions but also support biodiversity and traditional knowledge systems, contributing to global health resilience and sustainable development.



Figure 1: Leaves and flowers of *V. monoicum*

Methodology

The leaves of *V. monoicum* were collected from Khordha district of Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using different solvents (acetone, ethanol and aqueous) for experimental analysis (Figure 2). Detection of nine secondary metabolites was conducted using standard methods (Kshirsagar et al., 2025; Jena et al., 2025). Details of phytochemical analysis is given below.



Figure 2: Filtrate extracts of *V. monoicum* leaves for experimental analysis

Test for tannin: 1 ml of leaf extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 leaf extract 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, and 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The color initially turned to an intense yellow color with NaOH solution and later became colorless, which confirmed the presence of flavonoids.

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, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the leaf extract was taken, and 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.

Cytotoxicity analysis: For cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *V. monoicum* leaf extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) 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 analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ^{o}C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

The preliminary phytochemical screening of *V. monoicum* leaf extracts revealed the presence of various bioactive secondary metabolites, including alkaloids, steroids, tannins, saponins, phenols, and reducing sugars (Table 1; Figures 3-5). These compounds were found to be present in different extracts having various ethnomedicinal uses and pharmacological potential. The presence of wide range of phytoconstituents suggested that *V. monoicum* leaf may possess medicinal properties. These compounds are well-documented for their pharmacological activities, which correlate with the plant's traditional medicinal uses (Melo et al, 2023). The qualitative tests indicated a strong presence of tannins, suggesting significant antioxidant and anti-inflammatory potential (Khatun et al., 2016).

Alkaloids and saponins, which were also detected in moderate to high amounts, are known to contribute to cytotoxic and antimicrobial effects, supporting the plant's therapeutic relevance.

Table 1: Qualitative phytochemical analysis of *V. monoicum* leaves using different extracts

Bioactive compounds	Acetone	Ethanol	Aqueous
Tannin	Present	Present	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Present	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Present	Present
Alkaloids	Present	Present	Present
Carbonyl compounds	Absent	Absent	Absent



Figure 3: Detection of secondary metabolites of *V. monoicum* leaves using acetone extract

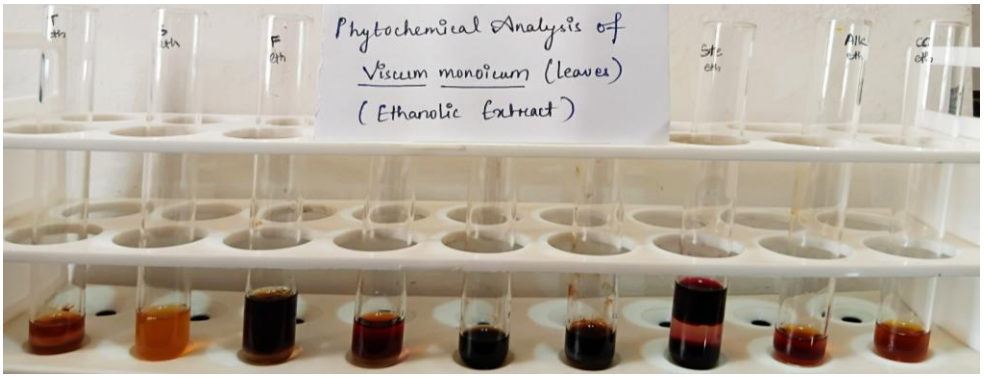


Figure 4: Detection of secondary metabolites of *V. monoicum* leaves using ethanolic extract



Figure 5: Detection of secondary metabolites of *V. monoicum* leaves using aqueous extract

The cytotoxicity analysis of *V. monoicum* leaf extracts using brine shrimp nauplii showed significant mortality rates for acetone and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 6-8). The observed cytotoxicity may be attributed to the presence of bioactive phytochemicals, which are known to interfere with cancer cell proliferation and induce apoptosis. Further studies involving mechanism-based assays and in vivo evaluations are needed to the pathways involved in its anticancer activity.

Table 2: Cytotoxicity analysis of *V. monoicum* leaves using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
Acetone	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	2	5	5	5	50
	31.25	10	0	0	5	5	50
	62.5	10	0	5	8	10	100
	125	10	2	3	7	7	70
	250	10	2	5	7	7	70
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

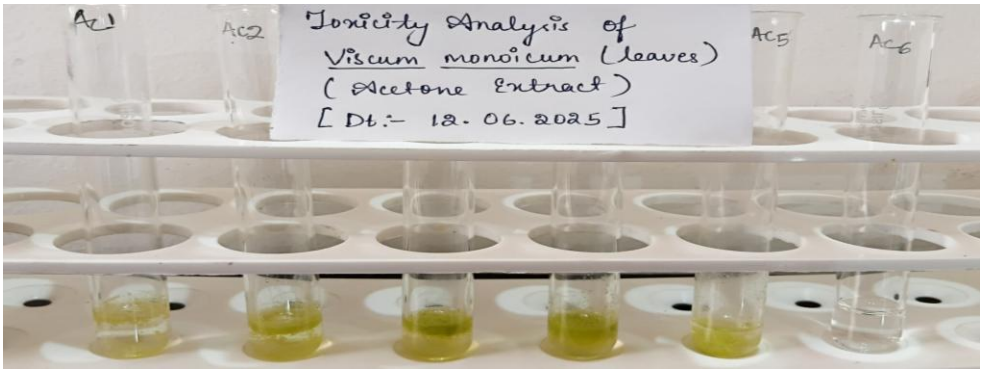


Figure 6: Toxicity analysis using Brine shrimp nauplii of acetone extract of *V. monoicum* leaves



Figure 7: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *V. monoicum* leaves

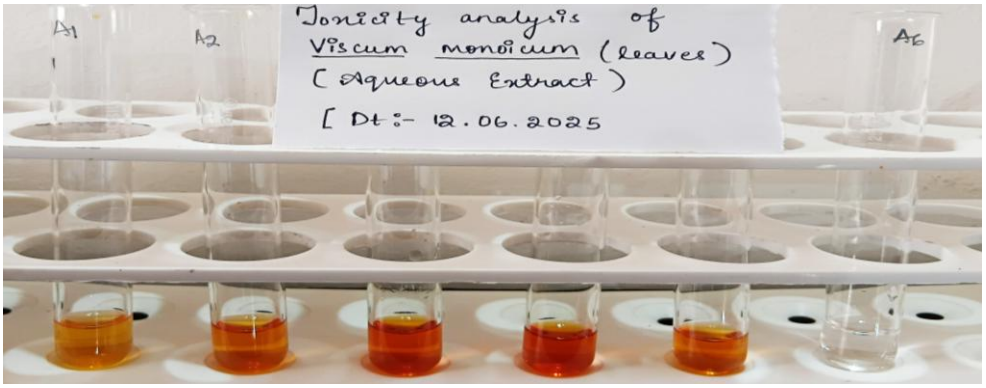


Figure 8: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *V. monoicum* leaves

Thin Layer Chromatography (TLC) profiling of the *V. monoicum* leaf extracts provided a preliminary chemical fingerprint, revealed the presence of multiple phytoconstituents. Using different mobile phases of varying polarity, distinct bands were observed in UV chamber (Table 3; Figure 9).

Table 3: TLC analysis of *V. monoicum* leaves using different mobile phases

	Mobile Phases	Rf value of Spots		
		S1	S2	S3
Hydroethanolic	Methanol: Chloroform (1:1)	0.72	0.77	0.82
	Ethyl acetate: Chloroform (1:9)	0.95	0.96	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.56	0.71	0.98

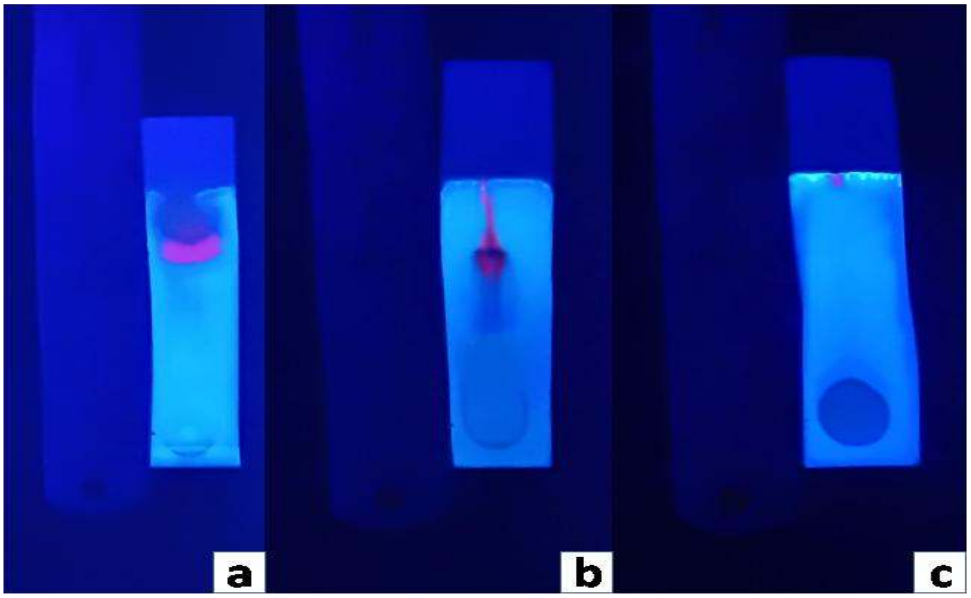


Figure 9: TLC analysis of hydroethanolic extract of *V. monoicum* leaves a) Chloroform: Methanol b) Ethyl acetate: Water: Acetic acid and c) Ethyl acetate: Chloroform

Conclusion

V. monoicum Roxb. ex DC. emerged as a valuable medicinal plant with significant ethnomedicinal, pharmacological, and phytochemical potential. Future research should focus on isolating and characterizing its active constituents, exploring their mechanisms of action, and developing standardized healthcare formulations.

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Chapter 9

Investigation of bioactive compounds in *Sphaeranthus indicus* L. whole plant through phytochemical screening, cytotoxicity testing and chromatographic analysis

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Abstract: *Sphaeranthus indicus* L., a well-known medicinal plant in Ayurvedic and traditional medicine, is recognized for its diverse therapeutic properties including antimicrobial, anti-inflammatory, and anticancer activities. Present study aimed to investigate the bioactive constituents of the whole plant through comprehensive phytochemical screening, cytotoxicity testing, and chromatographic analysis. The results revealed significant phytoconstituents including flavonoids, alkaloids, terpenoids, and phenolic compounds, with notable cytotoxic activity, supporting the plant's potential in drug discovery and therapeutic applications.

Keywords: Antimicrobial, drug discovery, traditional medicine

Introduction

Sphaeranthus indicus L., commonly known as East Indian globe thistle, is an aromatic, erect, and branched herb belonging to the family Asteraceae (Galani et al., 2010). This is widely distributed throughout the Indian subcontinent and parts of Southeast Asia, this plant thrives in moist and marshy environments, often found near water bodies and paddy fields (Arya et al., 2022). Morphologically, it is characterized by its purple-pink flower heads and deeply lobed leaves (Figure 1). Traditionally, the whole plant comprising roots, leaves, flowers, and stem has been utilized in Ayurvedic, Unani, and Siddha systems of medicine (Makhija et al., 2011). Ethnomedicinally, it is employed for the treatment of a broad range of ailments including skin disorders, respiratory infections, epilepsy, gastrointestinal issues, and even psychological conditions such as anxiety and depression (Mahajan et al., 2015). Pharmacological studies have confirmed a wide spectrum of biological activities associated with *S. indicus*, including antimicrobial, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective, and anticancer effects (Ramachandran, 2013; Dintu et al., 2024; Selvamoorthy et al., 2024). These therapeutic properties are attributed to the plant's rich reservoir of secondary metabolites, such as flavonoids, alkaloids, sesquiterpene lactones, phenolics, and essential oils. The increasing burden of chronic diseases and the growing resistance to synthetic drugs, there is an urgent need to explore effective plant-based alternatives (Muteeb et al., 2023). *S. indicus*, with its multi-targeted bioactivity, represents a promising candidate for developing novel phytopharmaceuticals to combat pressing global health challenges like cancer, antibiotic resistance, and metabolic syndromes. Looking forward, the integration of advanced analytical techniques such as high-throughput screening, metabolomics, and molecular docking could pave the way for isolating and characterizing the most potent bioactive constituents of *S.*

indicus. Combined with in vivo validation and clinical trials, this approach could transform traditional knowledge into evidence-based therapeutic applications. The current investigation, therefore, aims to bridge the gap between ethnomedicine and modern pharmacology through systematic phytochemical screening, cytotoxicity testing, and chromatographic analysis of the whole plant of *S. indicus*.



Figure 1: Leaves and flower of *S. indicus*

Methodology

The whole plant of *S. indicus* was collected from the bank of Mahanadi River, Cuttack district of Odisha, India. The plant species was identified by the authors (Figure 1). The maceration extraction method was adopted using different solvents (n-hexane, ethanol and aqueous) for experimental analysis (Figure 2). Detection of nine secondary metabolites was conducted using standard

methods (Jena et al., 2025; Jena and Kumar, 2025; Kshirsagar et al., 2025; Verma et al., 2025). Details of phytochemical analysis is given below.



Figure 2: Filtrate extracts of *S. indicus* (whole plant) for experimental analysis

Test for tannin: 1 ml of whole plant extract was taken in a test tube, and 3-5 drops of 10% lead acetate solution was 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 whole plant extract and shaken well. The formation of persistent froth was observed, confirming the presence of saponin.

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

Test for terpenoids: To 1 ml of the whole plant 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 whole plant 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 whole plant 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 whole plant extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added. The appearance of upper red and lower yellow with green fluorescence indicated the presence of steroids.

Test for alkaloids: About 1 ml of the whole plant extract was taken, and 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 whole plant 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 cytotoxicity test, hatching of Brine shrimp cysts was the initial process. Standardization of the optimum salinity was done at 2.0, 3.5 and 5.0 % saline concentration to check the optimum salinity for proper hatching of Brine shrimp (*A. salina*) cysts (Kumar et al., 2012). The brine shrimp cysts are then incubated in 3.5 % saline water with proper aeration, room temperature or between (28-35^{o}C) and light for 72 hours. Proper hatching could be observed within 24-72 hours depending upon the quality of cysts, proper aeration and light provided. Different concentrations (15.62 mg ml^{-1}, 31.25 mg

ml^{-1}, 62.5 mg ml^{-1}, 125 mg ml^{-1} and 250 mg ml^{-1}) of the *S. indicus* whole plant extracts were taken and 3.5% saline water were 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 3.5% saline water and vincristine sulphate (5 mg ml^{-1}) respectively with a volume of 2 ml. The live larvae are highly mobile and thus differentiates from the unhatched cysts. The survivor nauplii were counted and analyzed the death % (Nayak et al., 2021).

Chromatographic analysis: TLC plates were prepared on 9 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 poured over the slides. The slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50 ^{o}C for 20 minutes before running the TLC (Mishra and Bhatnagar, 2024). Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: water: acetic acid (8:1:1) were used as mobile phases. Then spots were visualized under UV chamber and recorded the RF (Retention Factor) values (Kumar and Jena, 2014).

Results and discussion

Preliminary phytochemical screening of *S. indicus* whole plant extracts revealed the presence of various bioactive components, including tannin, saponin, phenolic compounds, reducing sugars and alkaloids (Table 1; Figures 3-5).

The phytochemical analysis of *S. indicus* whole plant revealed the presence of various bioactive compounds, including tannins, saponins, flavonoids, alkaloids

and reducing sugars (Table 1: Figures 4-6). These compounds were found to be present in different extracts having various ethnomedicinal uses and pharmacological potential. The presence of wide range of phytoconstituents suggests that *S. indicus* s may possess medicinal properties.

Table 1: Qualitative phytochemical analysis of *S. indicus* (whole plant) using different extracts

Bioactive compounds	n-hexane	Ethanol	Aqueous
Tannin	Absent	Absent	Present
Saponin	Present	Present	Present
Flavonoids	Absent	Absent	Absent
Terpenoids	Absent	Absent	Absent
Phenolic compounds	Absent	Absent	Present
Reducing sugars	Present	Present	Present
Steroids	Absent	Absent	Absent
Alkaloids	Absent	Absent	Present
Carbonyl compounds	Absent	Absent	Absent

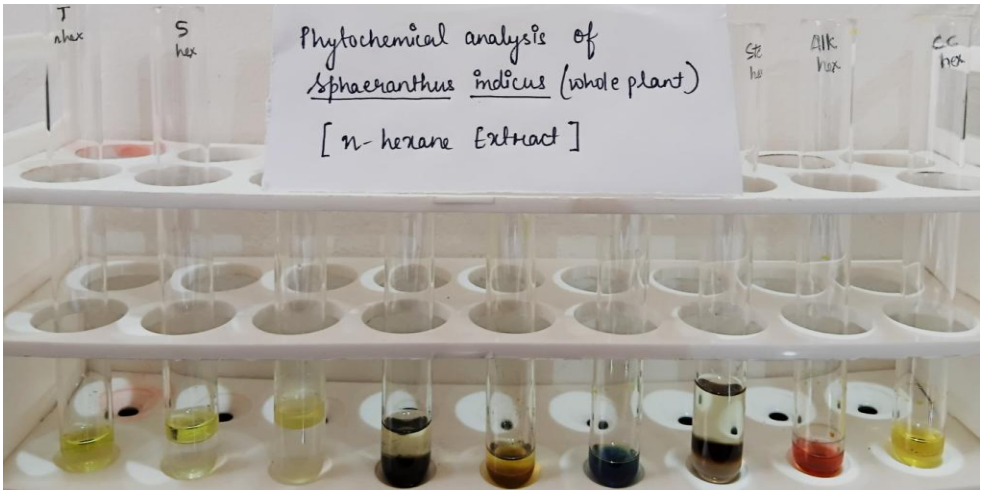


Figure 3: Detection of secondary metabolites of *S. indicus* (whole plant) using n-hexane extract

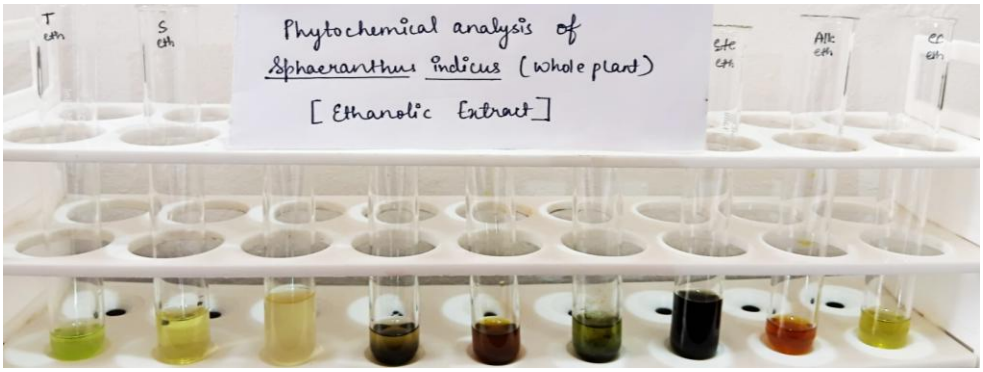


Figure 4: Detection of secondary metabolites of *S. indicus* (whole plant) using ethanolic extract

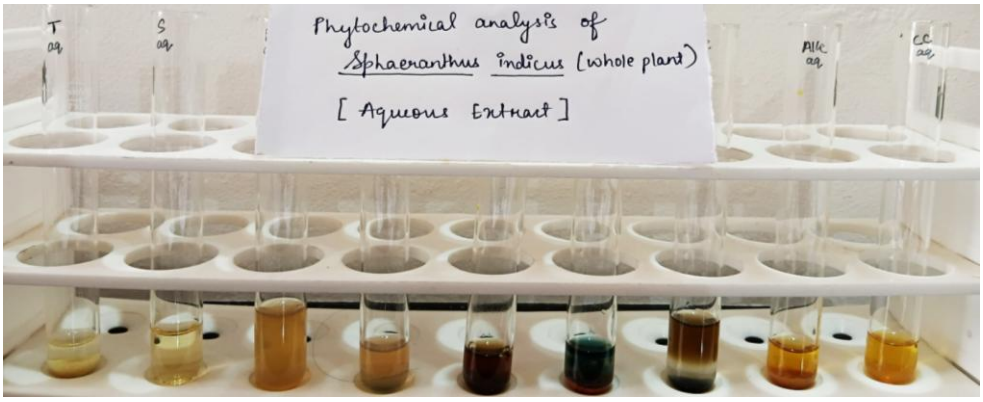


Figure 5: Detection of secondary metabolites of *S. indicus* (whole plant) using aqueous extract

The cytotoxicity analysis of *S. indicus* whole plant extracts using brine shrimp nauplii showed significant mortality rates for n-hexane and ethanol extracts, indicating potential anticancer or antimicrobial properties (Table 2; Figures 6-8; Krishnaraju et al., 2005). The observed cytotoxicity may be attributed to the presence of bioactive phytochemicals, which are known to interfere with cancer cell proliferation and induce apoptosis. Further studies involving mechanism-based assays and in vivo evaluations are needed to the pathways involved in its anticancer activity.

Table 2: Cytotoxicity analysis of *S. indicus* (whole plant) using Brine shrimp nauplii

Extracts	Concentration (in mg/ml)	Initial number of Nauplii	Number of Deaths of Nauplii (after hours)				Death rates (%)
			1	2	3	4	
n-hexane	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Ethanol	15.62	10	10	10	10	10	100
	31.25	10	10	10	10	10	100
	62.5	10	10	10	10	10	100
	125	10	10	10	10	10	100
	250	10	10	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100
Aqueous	15.62	10	0	0	0	0	0
	31.25	10	0	3	3	3	30
	62.5	10	7	10	10	10	100
	125	10	10	10	10	10	100
	250	10	7	10	10	10	100
	3.5% Saline	10	0	0	0	0	0
	Vincristine sulphate	10	10	10	10	10	100

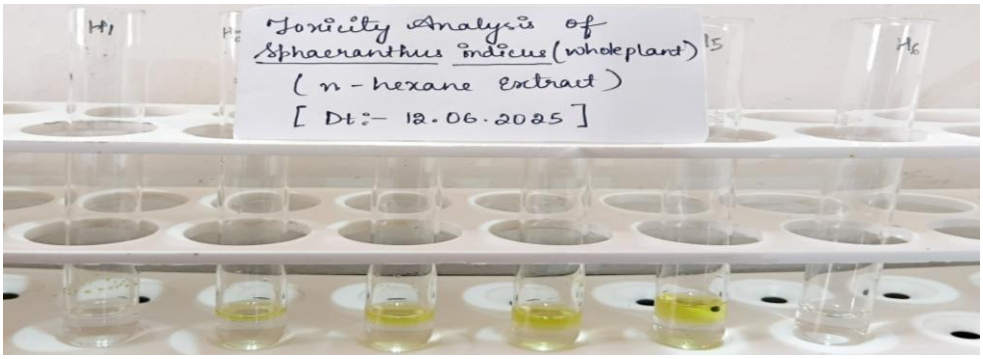


Figure 6: Toxicity analysis using Brine shrimp nauplii of n-hexane extract of *S. indicus* (whole plant)



Figure 7: Toxicity analysis using Brine shrimp nauplii of ethanolic extract of *S. indicus* (whole plant)

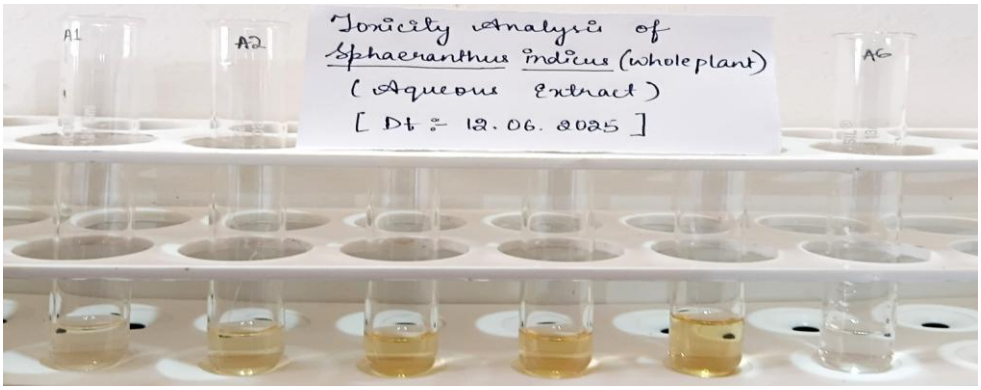


Figure 8: Toxicity analysis using Brine shrimp nauplii of aqueous extract of *S. indicus* (whole plant)

Thin Layer Chromatography (TLC) profiling of the *S. indicus* whole plant extracts provided a preliminary chemical fingerprint, revealed the presence of multiple phytoconstituents. Using different mobile phases of varying polarity, distinct bands were observed in UV chamber. In methanol: chloroform mobile phase, maximum bands are shown (Table 3; Figure 9).

Table 3: TLC analysis of *Sphaeranthus indicus* (whole plant) using different mobile phases

	Mobile Phases	Rf value of Spots				
		S1	S2	S3	S4	S5
Ethanolic aqueous	Methanol: Chloroform (1:1)	0.61	0.65	0.7	0.77	0.88
	Ethyl acetate: Chloroform (1:9)	0.68	0.88	-	-	-
	Ethyl acetate: Water: Acetic acid (8:1:1)	0.8	0.82	-	-	-

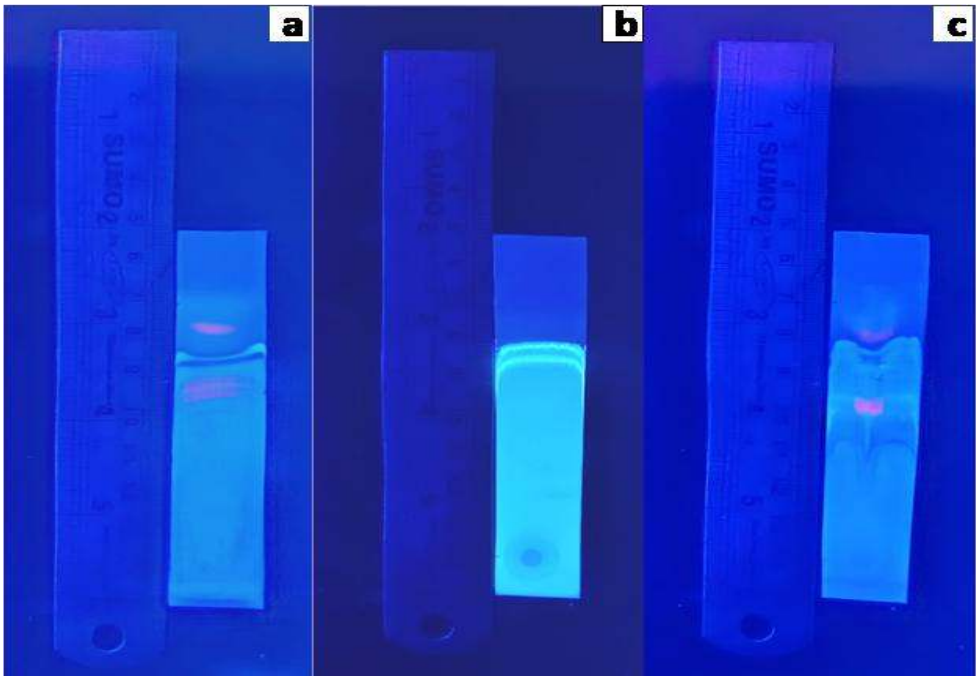


Figure 9: TLC analysis of hydroethanolic extract of *S. indicus* (whole plant) using a) Chloroform: Methanol b) Ethyl acetate: Water: Acetic acid and c) Ethyl acetate: Chloroform

Conclusion

Current research has effectively identified a range of bioactive compounds present in the whole plant of *S. indicus* L. reinforced the traditional medicinal applications of the plant. The cytotoxicity findings suggested encouraging anti-cancer properties, necessitating further exploration. In vivo pharmacological investigations and molecular docking techniques may assist in confirming the mechanisms of action of these compounds which could facilitate the advancement of innovative plant-derived therapeutics.

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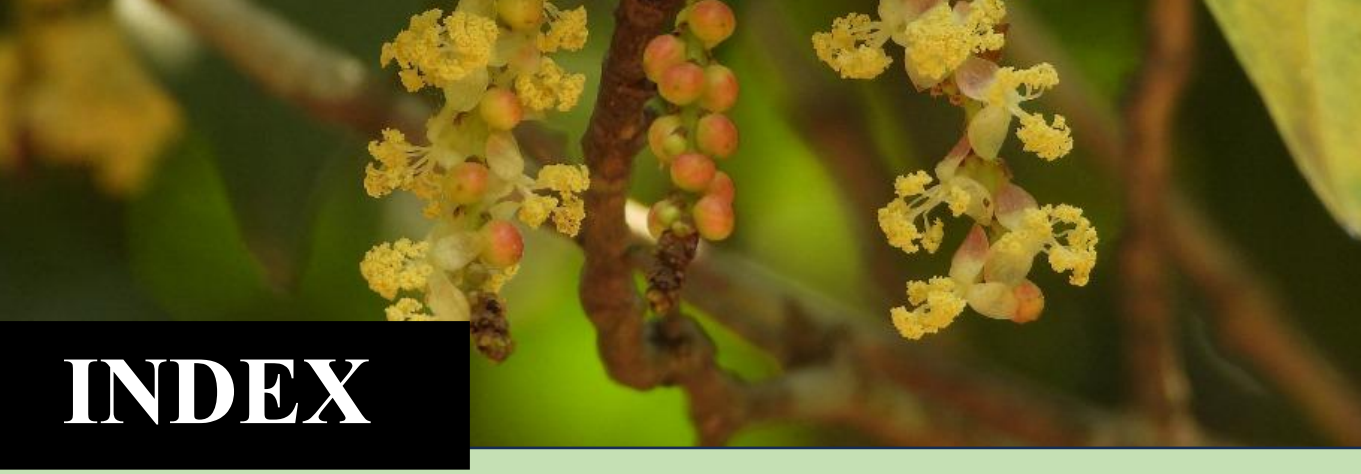
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Dr. Rajeev Kumar Singh, He is a botanist at Botanical Survey of India. He has made immense contributions in plant systematics, ethnobotany and plant nomenclature. He has revised the genus *Ischaemum* L., *Lobelia* L. and *Oropetium* Trin. for Indian flora and completed floristic studies of Mookambika Wildlife Sanctuary, Karnataka, Netravali Wildlife Sanctuary, Goa, Valmiki Tiger Reserve and National Park, Bihar. He also contributed many angiosperm families for the Indian state flora. He has explored and collected plant specimens from Himalayan regions of India, Central India and Western Ghats, India and described 18 taxa of angiosperm and rediscovered 8 species of angiosperm. He has also discovered the largest flattened plateau of Karnataka in Mookambika Wildlife Sanctuary, Udupi district. He has published 5 books, 7 book chapters and 215 research papers in peer reviewed national and international journals.

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'Plants & Secondary Metabolites, Volume 9' offers a comprehensive exploration of ten lesser-known plants, unveiling their botany, phytochemistry, traditional medicinal uses, and potential pharmacological applications, making it an invaluable resource for researchers and botanists.



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