

Phytochemical analysis of *Vitex negundo* L. (Lamiaceae): A biopesticide of India

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Abstract

This research examines the phytochemical profile of *Vitex negundo* (Lamiaceae) leaves and stem through various extraction methods. The qualitative analysis identified a range of bioactive compounds, including tannins, saponins, flavonoids, phenols, reducing sugars, and alkaloids. The study sheds light on the phytochemical composition of *V. negundo*, suggesting its potential as a natural source of biopesticides. The results contribute to the development of environmentally friendly pest management strategies and underscore the significance of phytochemical screening in identifying plant-based biopesticides.

Key words: Biopesticides, eco-friendly pest control, phytochemical analysis

Introduction

The increasing use of synthetic pesticides has led to environmental pollution, human health hazards, and the development of pesticide-resistant pest populations (Pathak *et al.*, 2022; Ahmad *et al.*, 2024). As a result, there is a growing need to identify alternative, eco-friendly pest control methods. Biopesticides, derived from natural sources such as plants, offer a promising solution (Bhat *et al.*, 2021; Mishra *et al.*, 2023; Tadesse *et al.*, 2024). Plants have evolved complex defence mechanisms against pests and pathogens, and

many of these defence compounds have potential as biopesticides (Sengul and Canpolat, 2022). Phytochemical screening is a crucial step in the search for biopesticides from plants.



Figure 1: Different plant parts of *V. negundo*

Phytochemical screening can help identify plants with potential pest control properties, and provide valuable insights into the structure and function of bioactive compounds (Khameneh *et al.*, 2021). By screening plants for phytochemicals, researchers can discover new leads for the development of biopesticides (Divekar *et al.*, 2022). *Vitex negundo* L. (Lamiaceae; Figure 1) is a plant species that has been traditionally used in Indian medicine for its various therapeutic properties (Gill *et al.*, 2018; Nayak and Kumar, 2023). Previous studies have reported the insecticidal and repellent properties of *V. negundo*, making it a potential source of biopesticides (Kumar *et al.*, 2022). However, a comprehensive phytochemical analysis of this plant is lacking. The present study aims to investigate the phytochemical composition of *V. negundo* leaves and stem using different extracts. The study will provide valuable insights into the bioactive compounds present in this plant, and their potential as biopesticides. The findings of this study will contribute to the search for eco-friendly pest control methods and highlight the importance of phytochemical screening in the discovery of biopesticides from plants.

Materials and methods

The leaves and stems of *V. negundo* were collected from the village of Badampahar of Mayurbhanj district, Odisha in June 2024 (Figure 2-3; Kumar *et al.*, 2021). Authors have identified the plant specimen (Jena *et al.*, 2025). Leaves and stem extracts of aqueous, ethanol and methanol were prepared followed by nine different bioactive compounds were detected using standard methods (Devi *et al.*, 2023; Marndi *et al.*, 2024; Devi *et al.*, 2024). An herbarium specimen (APRFH 123; Figure 4) is deposited to the Herbarium unit of Biodiversity and Conservation Lab of Ambika Prasad research Foundation, Cuttack, Odisha (Basole *et al.*, 2024).



Figure 2: Dried leaves powder of *V. negundo* for experimental analysis



Figure 3: Dried stem powder of *V. negundo* for experimental analysis

Detection of nine secondary metabolites were conducted using standard methods (Tripathi *et al.*, 2024; Jena *et al.*, 2024; Tallur *et al.*, 2024).

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

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

Test for flavonoids: About 1 ml of the filtrate extract was taken. 2 ml of 2% NaOH solution and 3 to 4 drops of dilute hydrochloric acid were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless. This colour change appearance confirmed the presence of flavonoids.

Test for terpenoids: About 1 ml of the filtrate was added with 6 drops of chloroform and placed in the water bath for a few minutes. Then 6 drops of concentrated H₂SO₄ were added. The reddish- brown interface confirmed the presence of terpenoids.

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

Test for reducing sugars: About 1 ml of the filtrate extract was taken and 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for some time. The presence of red-orange precipitate confirmed the presence of reducing sugar.

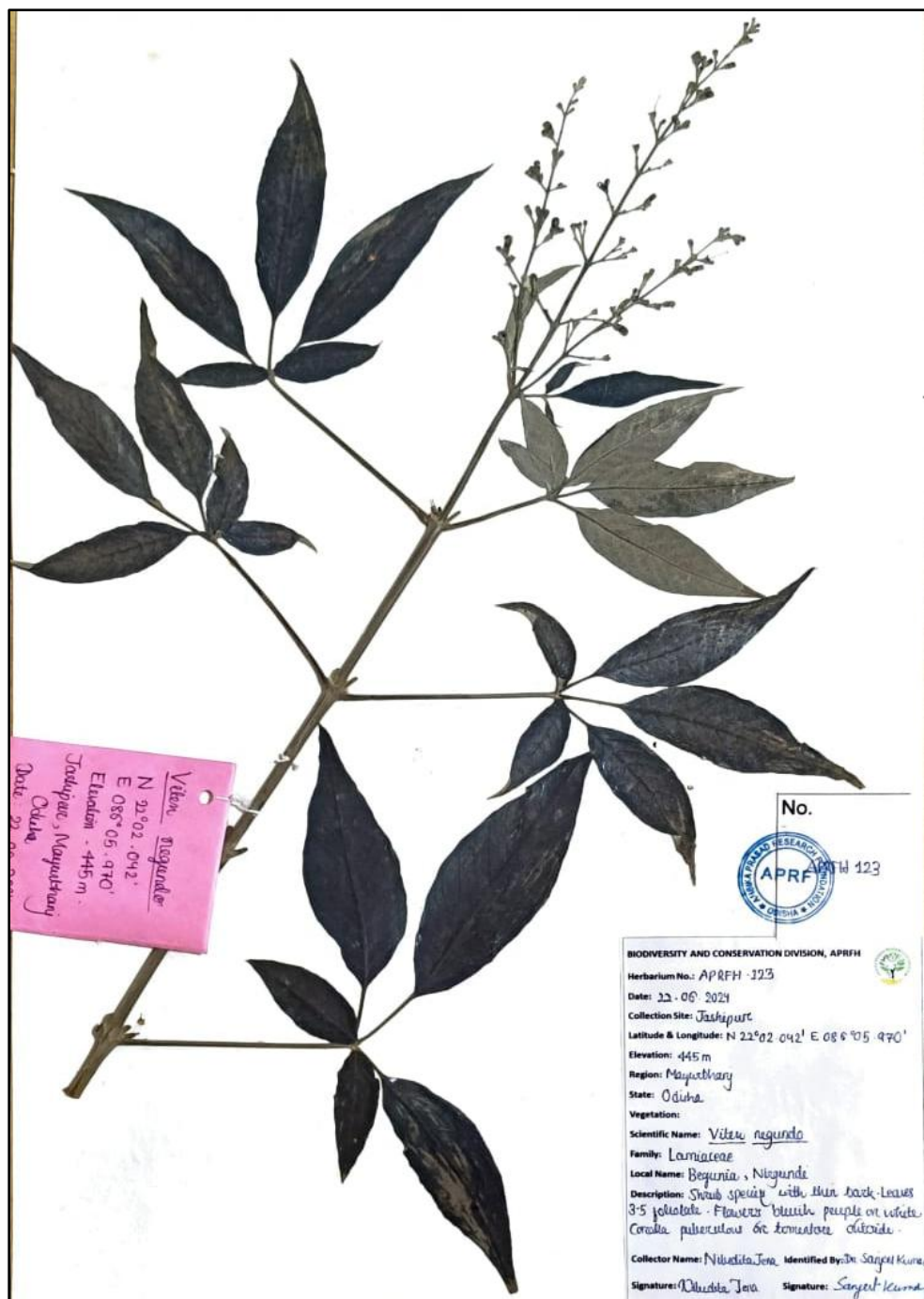


Figure 4: Herbarium specimen of *V. negundo*

Test for steroids: About 1 ml of the filtrate extract was taken. 1 ml of chloroform and 1 ml of concentrated sulphuric acid was added gently into it touching test tube mouth. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids (Hebbar *et al.*, 2024).

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

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

Results and discussion

The qualitative phytochemical analysis of *V. negundo* leaves and stem revealed the presence of various bioactive compounds (Table 1-2). The results showed that tannin, saponin, flavonoids, phenols, reducing sugar, and alkaloids were present in different extracts of the plant.

Table 1: Qualitative phytochemical analysis of *Vitex negundo* leaves using different extracts

Bioactive compounds	Leaves		
	Aqueous	Methanol	Ethanol
Tannin	+	+	-
Saponin	+	+	+
Flavonoids	-	-	+
Terpenoids	-	-	-
Phenols	+	+	+
Reducing sugar	+	-	-

Steroid	-	-	-
Alkaloid	+	-	-
Carbonyl compounds	-	-	-

(+: Positive; -: negative)

Table 2: Qualitative phytochemical analysis of *Vitex negundo* stem using different extracts

Bioactive compounds	Stem		
	Aqueous	Methanol	Ethanol
Tannin	+	+	-
Saponin	+	+	+
Flavonoids	-	-	-
Terpenoids	-	-	-
Phenols	-	-	-
Reducing sugar	+	-	-
Steroid	-	-	-
Alkaloid	+	-	-
Carbonyl compounds	-	-	-

(+: Positive; -: negative)

The aqueous and methanol extracts of leaves and stem showed the presence of tannin, saponin, and phenols, while the ethanol extract of leaves showed the presence of flavonoids. The phytochemical analysis also revealed that the bioactive compounds were present in varying concentrations in different parts of the plant. The leaves were found to contain a higher concentration of bioactive compounds compared to the stem. The results suggest that *V. negundo* has the potential to be used as a source of biopesticides. The presence of bioactive compounds such as tannin, saponin, flavonoids, phenols, reducing sugar, and alkaloids in *V. negundo* indicates its potential as a biopesticide.

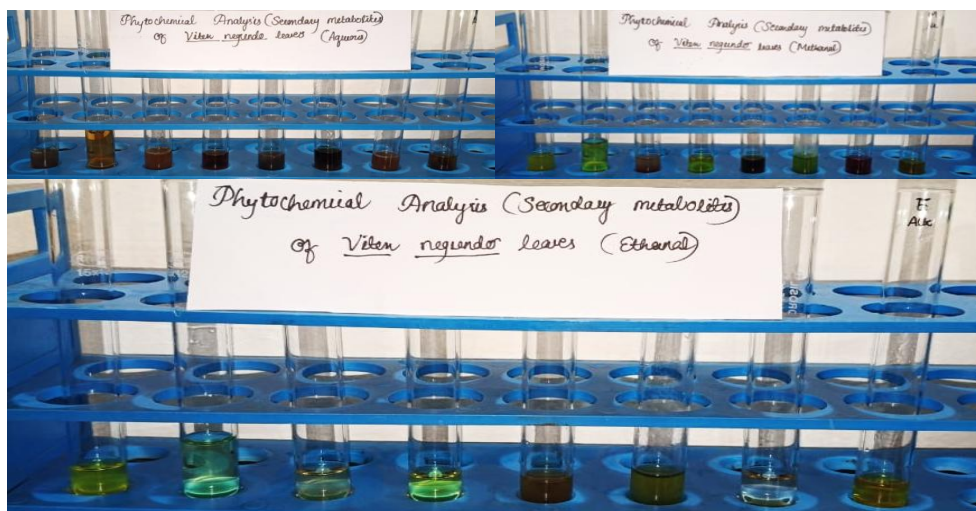


Plate 5: Detection of secondary metabolites of *V. negundo* leaves using different extracts

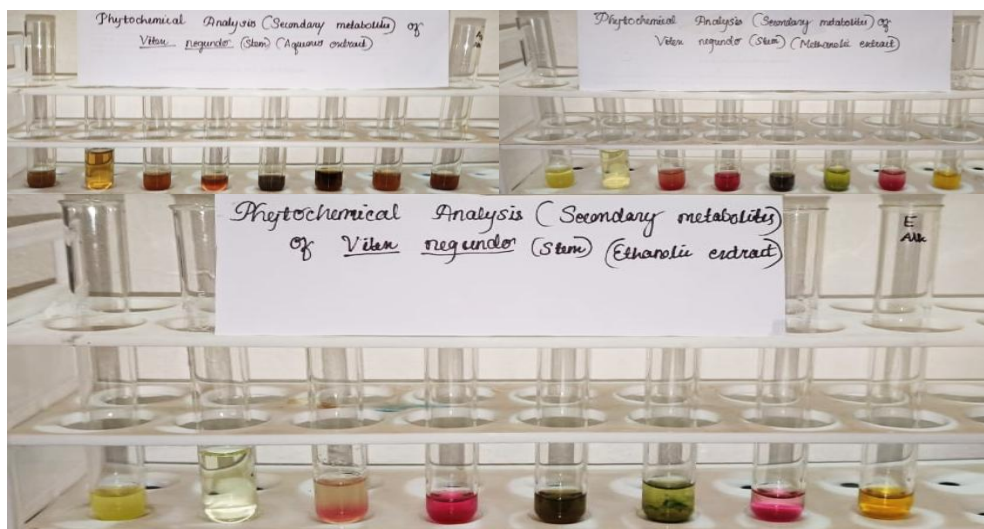


Plate 6: Detection of secondary metabolites of *V. negundo* stem using different extracts

These compounds have been reported to possess insecticidal, repellent, and antimicrobial properties, making them useful for pest control. The presence of these compounds in varying concentrations in different parts of the plant suggests that *V. negundo* could be a valuable source of bioactive compounds

for the development of eco-friendly pest control methods. The phytochemical analysis of *V. negundo* also highlights the importance of secondary metabolites in plant defense. The presence of tannin, saponin, and phenols in the plant suggests that these compounds play a crucial role in protecting the plant against pests and pathogens. The results of this study provide valuable insights into the phytochemical composition of *V. negundo* and its potential as a source of biopesticides, and emphasize the need for further research into the development of eco-friendly pest control methods using plant-based bioactive compounds. The authors have also noted that neem leaves and cow dung cake ashes are traditionally used to preserve seeds. Additionally, the first author has personal experience with using biopesticides on her farm. Specifically, she prepares a biopesticide solution by finely chopping leaves of Sitahal, Aam, and Neem, and soaking them in cow urine for a week. The mixture is then filtered and diluted with water in a 1:10 ratio before being sprayed as a biopesticide (Present study).

Conclusion

The present study demonstrates the presence of various bioactive compounds in *V. negundo* leaves and stem, highlighting its potential as a source of biopesticides. The phytochemical analysis reveals that the plant contains a range of secondary metabolites, including tannin, saponin, flavonoids, phenols, reducing sugar, and alkaloids. These compounds have been reported to possess insecticidal, repellent, and antimicrobial properties, making *V. negundo* a valuable resource for the development of eco-friendly pest control methods. Further research is needed to isolate and characterize the bioactive compounds present in *V. negundo* and to evaluate their potential as biopesticides.

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