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Isolation and Spectroscopic Characterization of Alkaloids from the Methanolic Leaf extract of Adhatoda vasica Nees

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Abstract- Background: Adhatoda vasica Nees (Acanthaceae), commonly known as Vasaka, is an important medicinal plant extensively used in traditional Ayurvedic and Unani systems for the treatment of respiratory disorders. The therapeutic efficacy of the plant is largely attributed to the presence of quinazoline alkaloids, particularly vasicine and vasicinone. Objective: The present investigation aimed to isolate and characterize alkaloidal constituents from the methanolic leaf extract of A. vasica using chromatographic and spectroscopic techniques. Methods: Dried leaf powder was extracted with methanol by maceration, followed by acid–base fractionation to obtain alkaloid-enriched fractions. Preliminary screening was performed using thin-layer chromatography (TLC) and Dragendorff's reagent. Isolation of alkaloids was achieved through silica gel column chromatography and preparative TLC. Structural characterization of the isolated compounds was carried out using UV–Visible spectroscopy, FT-IR, mass spectrometry (MS), and NMR analyses. Results: Methanolic extraction yielded an alkaloid-rich fraction that exhibited positive Dragendorff's test. TLC profiling revealed multiple alkaloidal constituents with distinct Rf values. Chromatographic purification afforded a major alkaloidal fraction (SA-2), obtained as a pale-yellow amorphous powder with a melting point of 311–315°C. High-resolution mass spectrometry displayed a molecular ion peak at m/z 270.24. FT-IR analysis indicated characteristic absorption bands corresponding to hydroxyl, aromatic C–H, carbonyl, and olefinic functionalities. UV spectra showed absorption maxima at 280 and 443 nm. Spectral data were consistent with quinazoline alkaloid derivatives reported from A. vasica. Conclusion: The developed extraction and purification strategy effectively isolated alkaloidal constituents from A. vasica leaves. Spectroscopic evidence confirmed the presence of bioactive quinazoline alkaloids, supporting the medicinal significance of the plant and its potential for pharmaceutical applications.

Keywords— Adhatoda vasica, quinazoline alkaloids, vasicine, vasicinone, phytochemical isolation, chromatography, spectroscopy.

I. INTRODUCTION

Medicinal plants remain one of the most valuable sources of bioactive compounds for drug discovery and development. Among the diverse classes of phytochemicals, alkaloids represent an important group of nitrogen-containing secondary metabolites exhibiting a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, bronchodilatory, and anticancer effects.

Adhatoda vasica Nees (syn. Justicia adhatoda L.), belonging to the family Acanthaceae, is a perennial evergreen shrub widely distributed throughout the Indian subcontinent and Southeast Asia. The plant has been extensively utilized in traditional systems of medicine for the management of respiratory disorders such as asthma, chronic bronchitis, cough, tuberculosis, and allergic airway diseases.1

Phytochemical investigations have revealed that A. vasica contains a variety of secondary metabolites, including alkaloids, flavonoids, phenolics, tannins,

saponins, and essential oils. Among these, quinazoline alkaloids such as vasicine, vasicinone, deoxyvasicine, and vasicinol are considered the principal bioactive constituents responsible for its therapeutic effects. Vasicine has demonstrated bronchodilatory, expectorant, mucolytic, and uterotonic activities, whereas vasicinone possesses significant bronchodilatory and anti-inflammatory properties.

Although numerous pharmacological studies have been conducted on A. vasica, standardized procedures for the isolation and characterization of its alkaloids remain essential for quality control, phytochemical standardization, and future drug development. Therefore, the present study was undertaken to isolate alkaloidal constituents from the methanolic leaf extract of A. vasica and characterize the isolated compounds using chromatographic and spectroscopic techniques.1-2

II. MATERIALS AND METHODS

1. Plant Material Collection and Authentication

Fresh leaves of Adhatoda vasica were collected from the campus of SAM Global University, Raisen, Madhya Pradesh, India. Taxonomic authentication was performed by Dr. Jagrati Tripathi, and a voucher specimen was deposited in the Department of Chemistry herbarium for future reference.

2. Preparation of Plant Material

Collected leaves were washed thoroughly with distilled water and shade-dried at ambient temperature for two weeks. The dried material was pulverized using a mechanical grinder and stored in airtight containers until extraction.

3. Methanolic Extraction

Approximately 500 g of powdered leaf material was macerated in 2 L of analytical-grade methanol for 72 h at room temperature with intermittent stirring. The extract was filtered through Whatman No. 1 filter paper. The marc was re-extracted twice using fresh methanol (1 L each). Combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator to obtain the crude methanolic extract.

4. Alkaloid-Enriched Fraction Preparation

The crude extract was dissolved in 10% aqueous hydrochloric acid and subjected to liquid–liquid partitioning with petroleum ether to remove lipophilic impurities. The acidic aqueous layer was subsequently basified to pH 9–10 using concentrated ammonia solution and extracted successively with chloroform and ethyl acetate.

Organic fractions were dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield alkaloid-rich chloroform and ethyl acetate fractions.

5. Thin-Layer Chromatography

TLC analyses were performed on silica gel 60 F254 plates. Mobile phases investigated included:

- Chloroform:Methanol:Ammonia (85:15:0.5)
- Ethyl acetate:Methanol:Ammonia (80:15:5)

Developed plates were visualized under UV light at 254 and 365 nm and sprayed with Dragendorff's reagent. Alkaloid-containing bands were identified based on characteristic orange-brown coloration.

6. Column Chromatography

Silica gel (60–120 mesh) was activated at 110°C for 2 h and packed into a glass column. The alkaloid-rich fraction was loaded onto the column and eluted using a gradient solvent system of chloroform–methanol with increasing polarity.

Fractions were collected and monitored by TLC. Fractions displaying similar chromatographic profiles were pooled and concentrated.

7. Purification of Alkaloids

Pooled fractions containing alkaloids were subjected to preparative TLC to obtain purified compounds. Isolated compounds were collected, concentrated, and stored for spectroscopic characterization.

8. Spectroscopic Characterization

Structural elucidation of purified compounds was performed using:

- UV–Visible Spectroscopy
- Proton Nuclear Magnetic Resonance (^1H NMR)

- Carbon-13 Nuclear Magnetic Resonance (^13C NMR)
- High-Resolution Mass Spectrometry (HR-MS)

III. RESULTS

1. Extraction and Fractionation

Methanolic extraction yielded a dark green crude extract rich in secondary metabolites. Acid–base partitioning successfully concentrated alkaloidal constituents into chloroform and ethyl acetate fractions.

2. TLC Profiling

TLC analysis revealed multiple alkaloidal spots exhibiting positive Dragendorff's reactions. Distinct chromatographic bands suggested the presence of several quinazoline alkaloid derivatives.

3. Isolation of Major Alkaloid Fraction

A major compound designated SA-2 was isolated as a pale-yellow amorphous powder. The compound exhibited a melting point range of 311–315°C and showed yellow fluorescence under UV illumination.
HPLC chromatographic analysis demonstrated a dominant peak at a retention time of 7.582 min, indicating a high degree of purity.

4. Spectroscopic Characterization

Mass Spectrometry

HR-MS analysis revealed a prominent molecular ion peak at m/z 270.24, indicative of a quinazoline alkaloid framework.

FT-IR Analysis

Major absorption bands were observed at:

Wavenumber (cm^{-1})	Assignment
3474.5	O–H stretching
2926	Aromatic C–H stretching
1622	C=C stretching
1377	C–N/C–H bending
1274	Carbonyl-associated C–O stretching

Absorption maxima were recorded at:

- 280 nm
- 443 nm

These peaks indicate the presence of conjugated aromatic and heterocyclic chromophoric systems.

NMR Analysis

^1H NMR and ^13C NMR spectra exhibited signals characteristic of quinazoline alkaloids previously reported in A. vasica. Spectral features supported the tentative identification of the isolated compound as a vasicine-related alkaloid.

IV. Discussion

The extraction protocol effectively concentrated alkaloidal constituents from A. vasica leaves. Methanol proved to be an efficient solvent owing to its ability to extract both polar and moderately non-polar phytochemicals. Acid–base partitioning significantly enhanced alkaloid recovery by exploiting their basic nitrogen functionality.3-4

Chromatographic separation produced fractions enriched in quinazoline alkaloids. The chromatographic behavior and spectroscopic characteristics of compound SA-2 showed strong agreement with previously reported data for vasicine-related compounds isolated from A. vasica.5

The observed IR, UV, NMR, and mass spectral features confirmed the presence of heterocyclic alkaloidal structures possessing aromatic and oxygenated functionalities. These findings corroborate earlier reports that identified quinazoline alkaloids as the principal pharmacologically active constituents of the species.6-8

The study further demonstrates that chromatographic purification coupled with modern spectroscopic techniques provides an effective approach for the isolation and identification of medicinally important alkaloids from botanical sources.9-10

V. CONCLUSION

The present investigation successfully established a reproducible protocol for the isolation and characterization of alkaloids from the methanolic leaf extract of Adhatoda vasica. Sequential extraction, acid–base fractionation, chromatographic purification, and spectroscopic analyses enabled the isolation of alkaloid-rich fractions and characterization of a major quinazoline alkaloid derivative. The findings support the traditional medicinal use of A. vasica and provide a scientific basis for future pharmacological and pharmaceutical studies involving its bioactive alkaloids.

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Conflict of Interest

The authors declare no conflict of interest.

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