Herbal Medicines Journal
https://doi.org/10.22087/hmj.2025.563749.1030
ORIGINAL ARTICLE

The First Global Report of Isovindolinine, an Indole Alkaloid, in *Vinca herbacea* from Northern Iran: Identification by Gas Chromatography–Mass Spectrometry with Potential Pharmacological Applications

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Received: 08 May 2025 / Revised: 19 Jun 2025 / Accepted: 30 Jun 2025 / Published: 12 Jul 2025

Abstract

Vinca herbacea Waldst. & Kit., a creeping perennial herb of the Apocynaceae family, is a lesser-known species native to temperate regions of Europe and Asia, including northern Iran. The Apocynaceae family is known for producing monoterpenoid indole alkaloids (MIAs) with various pharmacological properties. Among these, Isovindolinine is a naturally occurring indole alkaloid with cytotoxic, bioactive, and antimicrobial properties and may serve as a source of novel therapeutic agents. Although it has been reported in other Vinca species, its presence in *Vinca herbacea* has not been documented. In this study, the aerial parts of *Vinca herbacea* were collected from Mazandaran Province, Iran. These parts were then extracted using ethanol, n-propanol, and butanol. GC–MS analysis of the ethanolic extracts revealed Isovindolinine in the stems (retention time 54.34 min, 93% match) and leaves (54.24 min, 91% match), accounting for 1.50% and 1.41% of the total ion chromatograms, respectively. No detectable levels were found in flowers or in n-propanol and butanol extracts. This represents the first global report of Isovindolinine in *Vinca herbacea*. The organ-specific accumulation in photosynthetic tissues suggests targeted biosynthesis, highlighting the species as a promising natural source of bioactive MIAs. Further structural and bioactivity studies are recommended to explore its medicinal potential and guide sustainable utilization.

Keywords Alkaloid, Iran, Isovindolinine, *Vinca herbacea*

Introduction

Vinca herbacea Waldst. & Kit., a creeping perennial herb from the Apocynaceae family, is considered one of the lesser-studied species within the genus Vinca. This species naturally grows in temperate regions of Europe and Asia and is characterized by slender creeping stems, evergreen ovate–lanceolate leaves, and blue–violet flowers. According to the Royal Botanic Gardens, Kew (World Flora Online), *Vinca herbacea* is native to several countries in Eurasia, including Iran, Turkey, Iraq, Syria, Lebanon, Armenia,

Russia, Hungary, Germany, Austria, and Romania. However, Global Biodiversity Information Facility (GBIF) records indicate that its confirmed presence in Iran is primarily limited to the northern regions along the Alborz mountain range. (1, 2, 3, 4, 5)

The Apocynaceae family is known for producing monoterpenoid indole alkaloids (MIAs), which are a diverse group of secondary metabolites with significant pharmacological activities. Among these compounds, Isovindolinine ($C_{21}H_{24}N_2O_2$, MW = 336.4 g/mol) is a naturally occurring monoterpenoid indole alkaloid that has demonstrated cytotoxic activity against various cancer cell lines *in vitro*. Early studies suggest its potential as an anticancer, bioactive, and antimicrobial agent, indicating that it may serve as a promising lead for the development of new therapeutic agents from natural products. However, further research is required to understand its pharmacological profile fully. (6, 7)

Although Isovindolinine has been reported in other Vinca species, its presence in *Vinca herbacea* has not yet been documented. (6, 7)

In June 2024, the aerial parts (leaves, stems, and flowers) of *Vinca herbacea* were collected from the Balesu Protected Area in Tonekabon County, Mazandaran Province, Iran (36°38′21.6″ N; 50°44′27.5″ E; elevation 1095 m). This region has a temperate, humid climate with temperatures ranging from 20–30°C and relative humidity levels of 70–80% during the growing season, creating conditions favorable for secondary metabolite biosynthesis. Collected specimens were taxonomically verified by the Iranian Biological Resource Center (IBRC) and deposited under herbarium code IBRC P1006834. (3, 4)

Plant material was extracted through cold maceration using three separate solvent systems. First, powdered plant material was extracted with 96% ethanol (1 g:10 mL) for one week at 4°C with occasional shaking. After centrifugation (4000 rpm, 20 min) and filtration, the ethanolic extract was collected. Subsequent extraction steps were performed similarly using 96% n-propanol and 96% butanol, each producing independent extracts. None of the extracts were combined. All extracts were concentrated under reduced pressure using a rotary evaporator (Heidolph Hei-VAP Expert) and stored at 4°C. (4, 8, 9)

Gas Chromatography–Mass Spectrometry (GC–MS) analyses were conducted using an Agilent 6890 GC coupled to an Agilent 5973 MSD. A 2 µL aliquot from each extract was injected (split ratio 1:5) into an HP-5MS column (30 m × 0.25 mm, 1 µm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60°C (with a 2-minute hold) up to 280°C (at 5°C/min), then held for 20 minutes. Electron-impact ionization at 70 eV was used, with scanning from m/z 40–500. Compound identification was performed exclusively using the Wiley spectral library. (8, 9).

Stems

In the ethanolic stem extract, Isovindolinine was detected at a retention time of 54.34 minutes. The match quality (Qual) in the Wiley spectral library was 93%, and its relative abundance represented 1.50% of the total ion chromatogram. These results confirm the alkaloid's presence in stem tissue at a low but clearly detectable level (Fig.2a). No corresponding peak was detected in the n-propanol and butanol stem extracts.

Leaves

In the ethanolic leaf extract, Isovindolinine was identified at a retention time of 54.24 minutes, with a Wiley match quality (Qual) of 91%. The compound accounted for 1.41% of the total chromatogram, indicating a similarly low but consistent presence of this alkaloid in leaf tissue (Fig.2b). No detectable peak for this compound was observed in n-propanol and butanol leaf extracts.

Flowers

Isovindolinine was not detected in any of the flower extracts (ethanol, n-propanol, and butanol), indicating extremely low or undetectable levels in the floral tissues.

Mass spectral data of the detected compound perfectly matched reference spectra of Isovindolinine (CAS: 6822-38-4) within the Wiley database, confirming precise and unequivocal identification.

This study provides the first comprehensive report on Isovindolinine in *Vinca herbacea*. The prominent presence of the compound in photosynthetic tissues (leaves and stems), along with solvent-dependent variability, suggests organ-specific biosynthesis and accumulation patterns.

The discovery of Isovindolinine in *Vinca herbacea* significantly expands the known phytochemical profile of this rare species and highlights its potential as a valuable natural source of bioactive indole alkaloids. Further confirmation through preparative HPLC, followed by NMR structural elucidation, is strongly recommended. Bioassays targeting antibacterial and anticancer activities, along with chemotypic comparisons across different Iranian populations, could clarify environmental factors influencing alkaloid biosynthesis. (3,4,5)

Overall, the present findings underscore the importance of *Vinca herbacea* as a promising medicinal plant and highlight the need to conserve it through tissue culture propagation and controlled cultivation to ensure sustainable utilization of this rare species.

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Fig. 1 A voucher specimen of *Vinca herbacea* collected from the Baleskuh Protected Area in Tonekabon County, Mazandaran Province, Iran (36°38′21.6″ N, 50°44′27.5″ E; elevation 1,095 m). The specimen was identified and deposited in the Iranian Biological Resource Center with the code IBRC P1006834.

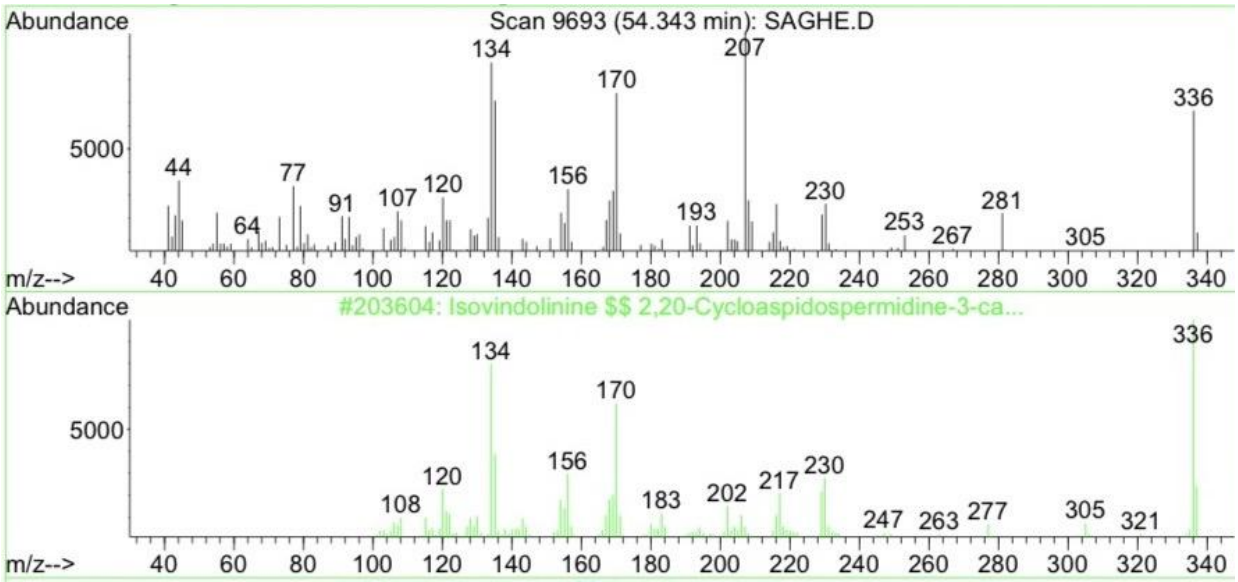

Figure.2A: Isovindolinine peak in stem extract

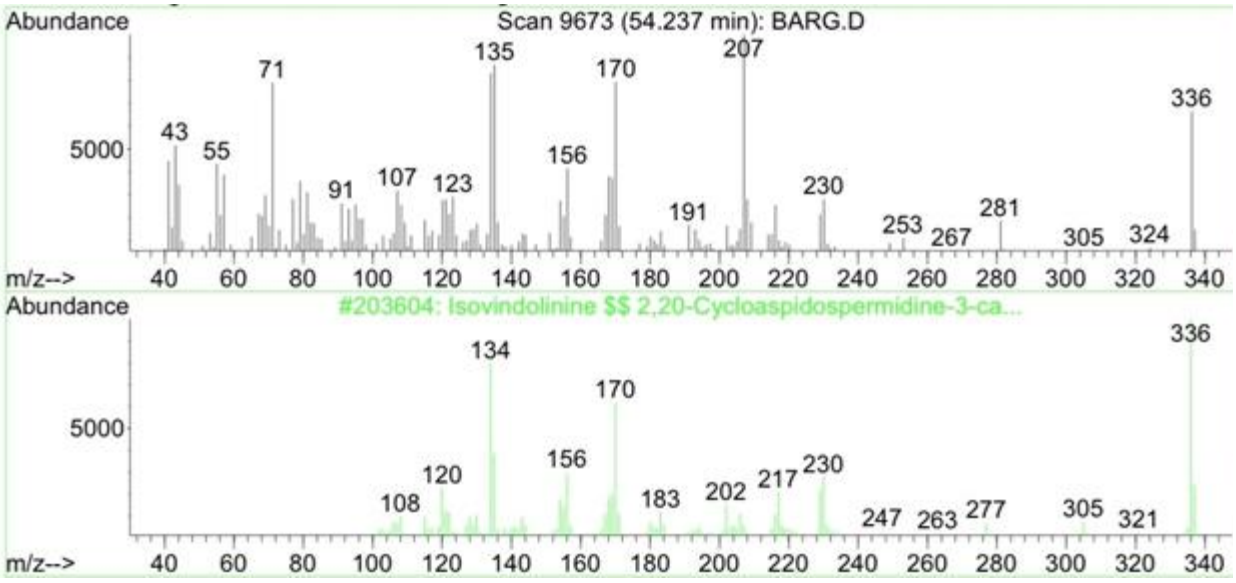

Figure 2B: Isovindolinine peak in leaf extract

Fig. 2 GC–MS analysis of ethanolic extracts from both the stem and leaf of Vinca herbacea revealed a distinct diagnostic peak corresponding to Isovindolinine (CAS: 6822-38-4). The leaf extract showed a match quality of 91% with a retention time of 54.24 minutes, while the stem extract exhibited a match quality of 93% at 54.34 minutes. These highly consistent chromatographic and spectral features confirm the presence of Isovindolinine in both tissues, suggesting that Vinca herbacea is a natural source of this indole alkaloid, with slightly higher identification confidence observed in the stem.