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Short Communication

The First Global Report of Vindolinine in *Vinca herbacea* From the Hyrcanian Floristic Region: GC–MS Identification and Potential Anticancer Relevance

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ABSTRACT

Background: *Vinca herbacea* Waldst. & Kit. is a relatively understudied species of the Apocynaceae family, distributed across temperate regions of Asia and Europe, with verified populations in northern Iran, particularly within the Hyrcanian forests. The family is well known for producing monoterpenoid indole alkaloids (MIAs), such as vinblastine and vincristine, which possess potent anticancer activities. Vindolinine, an important indole alkaloid and a biosynthetic precursor of these chemotherapeutic agents, has not been previously reported in *V. herbacea*.

Materials and Methods: In June 2024, aerial parts of *V. herbacea* (leaves, stems, and flowers) were collected from the Baleskuh Protected Area in Mazandaran Province, Iran. Plant materials were extracted using ethanol, n-propanol, and butanol through cold maceration, and all extracts were analyzed using gas chromatography–mass spectrometry (GC–MS). Compound identification was performed using National Institute of Standards and Technology (NIST) and Wiley spectral libraries.

Results: Vindolinine was detected predominantly in the ethanolic extracts of leaves and stems, with retention times of 55.22 and 55.28 minutes, respectively, and was absent in flowers. The mass spectral data showed strong similarity to reference spectra (CAS: 5980-02-9), confirming its presence. The organ-specific pattern suggests higher biosynthetic accumulation in photosynthetically active tissues.

Conclusion: This study provides the first global report of vindolinine in *V. herbacea*, highlighting northern Iran as a natural source of this rare and pharmacologically crucial alkaloid. These findings expand the phytochemical knowledge of the species and provide a basis for future biotechnological, pharmacological, and conservation-oriented studies.

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Introduction

Vinca herbacea Waldst. & Kit. is a relatively understudied member of the Apocynaceae family, distributed across temperate regions of Asia and Europe. According to the Royal Botanic Gardens, Kew (World Flora Online), this species is native to several countries, including Iran, Turkey, Iraq, Syria, Lebanon, Armenia, Russia, Hungary, Germany, Austria, and Romania. However, data from the Global Biodiversity Information Facility indicate that its confirmed presence in Iran is restricted mainly to the northern parts of the country, particularly along the slopes of the Alborz Mountains [1-4]. In Iran, verified populations of *Vinca herbacea* are predominantly found within the ancient Hyrcanian forests, spanning the provinces of Golestan, Mazandaran, and Gilan, where the species naturally occurs in the mountainous regions of the Hyrcanian forests [3-5].

The Apocynaceae family is well known for its rich diversity of monoterpenoid indole alkaloids (MIAs), including vinblastine, vincristine, vincamine, and their derivatives, which exhibit anticancer, neuroprotective, anti-inflammatory, and antioxidant activities. Among these compounds, vindolinine is a naturally occurring indole alkaloid that serves as a biosynthetic precursor of the anticancer agents vinblastine and vincristine, which are extensively used in chemotherapy. Although vindolinine has previously been reported in other Vinca species, its presence in *V. herbacea* has not yet been documented. Pharmacologically, vindolinine plays a crucial role in the biosynthetic pathway leading to these clinically important MIAs and may also possess intrinsic bioactive properties, including potential anticancer and neuroprotective effects [3-7].

Objective

This study aimed to identify and confirm the presence of the valuable indole alkaloid vindolinine in *V. herbacea* growing in the mountainous regions of the Hyrcanian forests in northern Iran. To achieve this aim, leaf, stem, and flower samples were collected from the Baleskuh Protected Area and extracted using three different solvents. The chemical profile of each extract was subsequently analyzed using gas chromatography–mass spectrometry (GC–MS) to verify the occurrence of vindolinine—a crucial biosynthetic precursor of the anticancer drugs vinblastine and vincristine—through comparison with authenticated reference spectra.

Materials and Methods

Sample collection and environmental conditions

In June 2024, aerial parts of *V. herbacea*—including leaves, stems, and flowers—were collected from the Baleskuh Protected Area in Tonekabon County, Mazandaran Province, located in the mountainous regions of the Hyrcanian forests. The collected specimens were authenticated by the Iranian Biological Resource Center (IBRC) and deposited under the code IBRC P1006834. The geographic coordinates of the collection site were 36°38′21.6″ N and 50°44′27.5″ E, at an elevation of 1,095 m above sea level (Figure 1). This area is characterized by a mild, humid climate, with average temperatures ranging from 20 °C to 30 °C and relative humidity of 70–80% during the growing season—conditions highly favorable for secondary metabolite production [3, 5].

Soil type and pH analysis

A soil sample from the same area was collected at a depth of 20 cm, air-dried, and sieved for analysis. For pH measurement, 10 g of soil was mixed with 25 mL of distilled water (1:2.5 soil-to-water ratio) and stirred for 30 minutes before measuring the supernatant using a digital pH meter (PH827, Metrohm, Switzerland). Soil texture was determined using the hydrometer (Horn) method: 50 g of soil was mixed with 100 mL of distilled water and 2 mL of 5% sodium hexametaphosphate as a dispersing agent, stirred thoroughly, and allowed to settle according to ASTM standard procedures [8].

Extraction method

Plant material was extracted using cold maceration in 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 minutes) 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, 9-11].

Analysis by GC-MS

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

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Figure 1. A Voucher specimen of *V. herbacea* Waldst. & Kit. Collected from the Baleskuh Protected Area, Tonekabon County, Mazandaran Province, Iran (36°38′21.6″ N, 50°44′27.5″ E; elevation 1,095 m)

Note: The specimen was identified and deposited in the Iranian Biological Resource Center under the code IBRC P1006834.

was used as the carrier gas at 1.0 mL/min. The oven temperature was set from 60 °C (2 min hold) to 280 °C (5 °C/min), followed by a 20-min final hold. Electron impact ionization at 70 eV was used, scanning m/z 40–500. Compound identification was performed exclusively using the National Institute of Standards and Technology (NIST) and Wiley spectral library [9-11].

Results

Soil type and pH analysis

The results showed a slightly alkaline soil (pH 7.2) with a sandy–loamy texture, comprising 60% sand, 30% silt, and 10% clay (Si-L).

Analysis by GC-MS

Stems: In the ethanolic stem extract, vindolinine was detected at a retention time of 55.28 min (Figure 2). The match quality (Qual) in the Wiley and NIST spectral library was 92%, and its relative abundance represented 1.83% of the total ion chromatogram (TIC). These results confirm the presence of the alkaloid in stem tissue at a low but clearly detectable level (Figure 3). No corresponding peak was detected in the n-propanol and butanol stem extracts.

Leaves: In the ethanolic leaf extract, vindolinine was identified at a retention time of 55.22 minutes, with a Wiley match quality (qual) of 90%. The compound accounted for 0.99% of the total chromatogram, indicating a similarly low yet reliable presence of this alkaloid in leaf tissue (Figure 3 and Figure 4). No detectable peak for this compound was observed in n-propanol and butanol leaf extracts.

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

Mass spectral data of the detected compound perfectly matched reference spectra of vindolinine (CAS: 5980-02-9) within the Wiley and NIST database, confirming accurate and unequivocal identification.

This study represents the first global report of vindolinine in *V. herbacea*. The predominant presence of the compound in photosynthetic tissues (leaves and stems), along with solvent-dependent variability, suggests organ-specific biosynthesis and accumulation patterns.

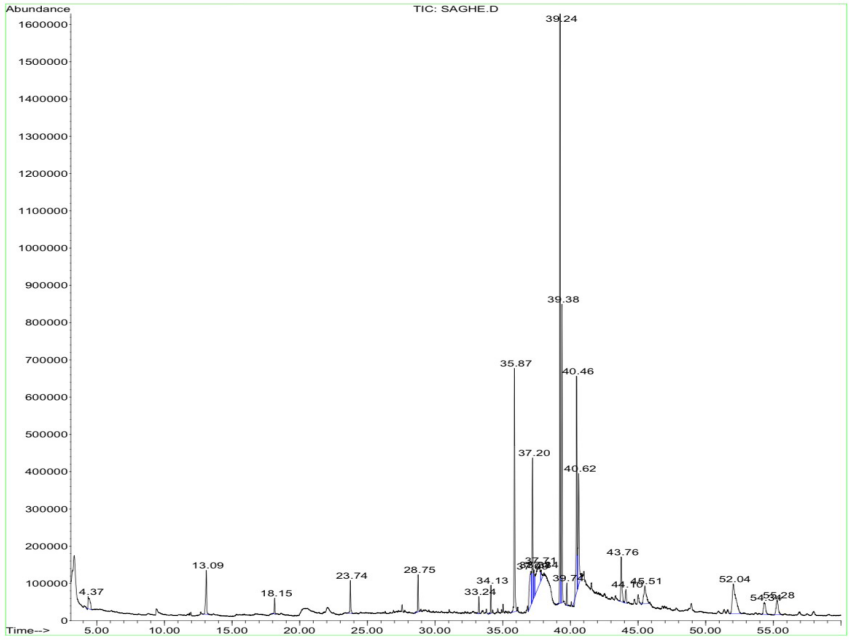

Figure 2. TIC obtained from GC–MS analysis of the ethanolic extract of *V. herbacea* stems

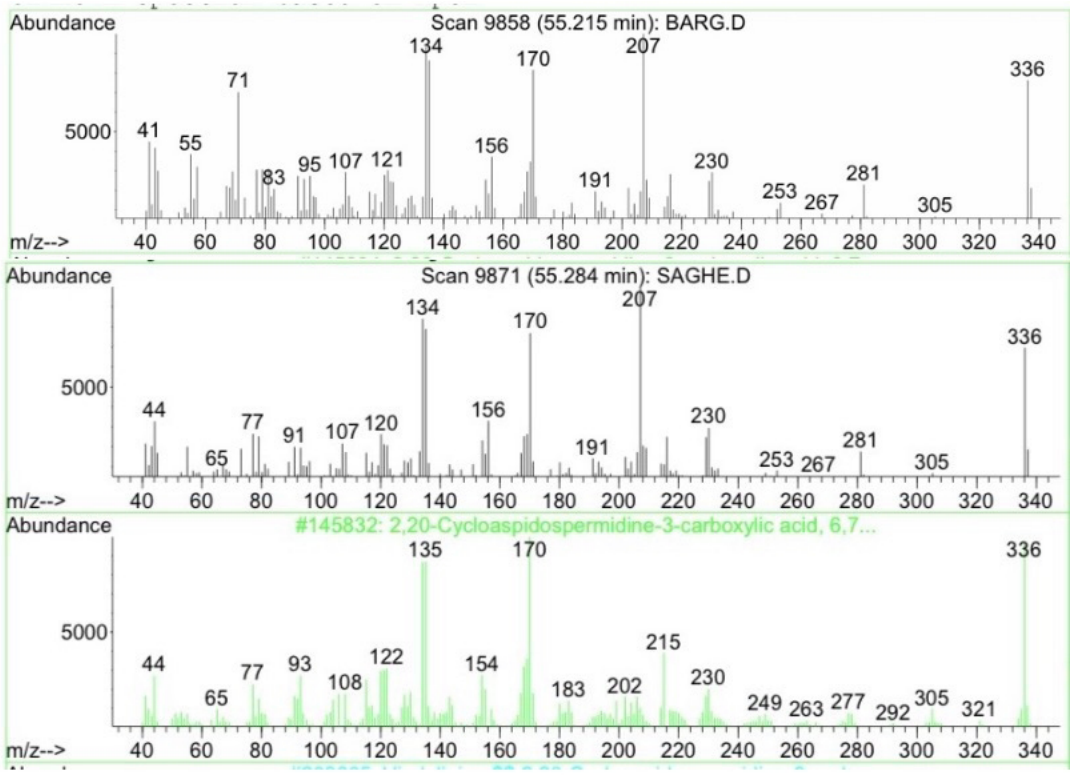

Figure 3. GC-MS analysis of the ethanolic extracts from both the stem and leaf of *V. herbacea* revealed a distinct diagnostic peak corresponding to vindolinine (CAS: 5980-02-9)

Note: The leaf extract showed a match quality of 90% at 55.22 min, while the stem extract exhibited a match quality of 92% at 55.28 min. These highly consistent chromatographic and spectral features confirm the presence of vindolinine in both tissues, suggesting that *V. herbacea* is a natural source of this indole alkaloid, with slightly higher identification confidence observed in the stem. The first image (top) relates to the leaf, the second to the stem, and the last one is for the library standard, which was the same for the NIST and Wiley libraries.

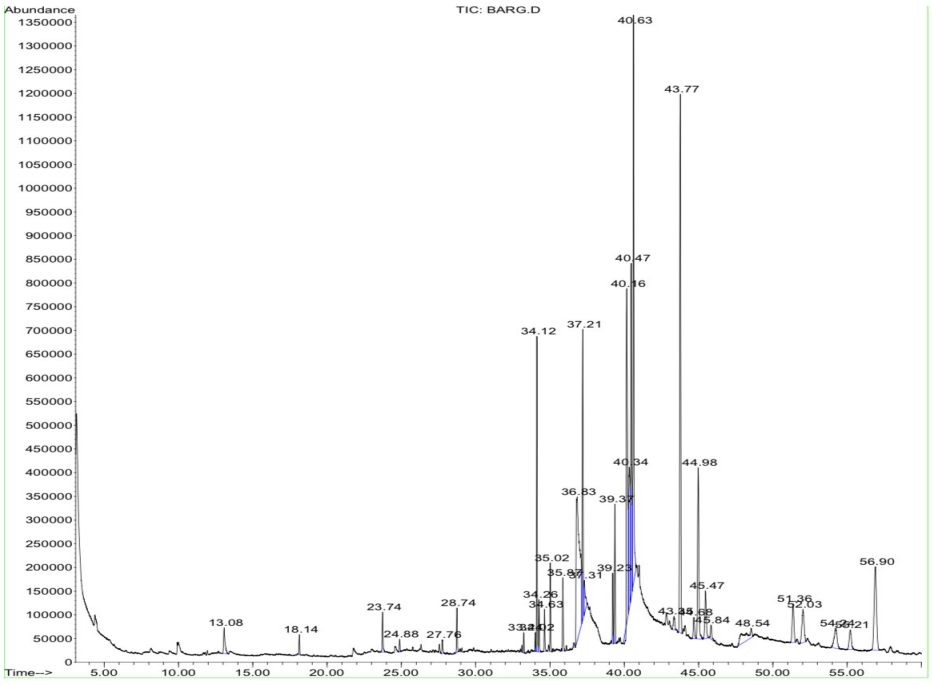

Figure 4. TIC obtained from GC–MS analysis of the ethanolic extract of V. herbacea leaves

Note: Extracts shows characteristic peaks corresponding to Vindolinine (CAS: 5980-02-9). In the leaf extract, a prominent peak was observed at 55.22 minutes, corresponding to peak 33. In the stem extract, a distinct peak appeared at a retention time of 55.28 min, corresponding to peak number 24. These results represent the overall GC–MS profile of the ethanolic extracts of the plant. b) TIC obtained from GC–MS analysis of the ethanolic extract of *V. herbacea* leaves

Final yields of plant extracts (per 1 g dry powder)

Using ethanolic extraction, the yields obtained per 1 g of dry powder were 0.046 g from leaves, 0.036 g from stems, and 0.03 g from flowers. Using n-propanolic extraction, the yields obtained per 1 g of dry powder were 0.012 g from leaves, 0.011 g from stems, and 0.013 g from flowers. Using butanolic extraction, the yields obtained per 1 g of dry powder were 0.005 g from leaves, 0.007 g from stems, and 0.004 g from flowers.

Discussion

The identification of vindolinine in the ethanolic and n-propanol extracts of *V. herbacea* represents the first global report of this indole alkaloid in this species and the first record in northern Iran, specifically within the mountainous regions of the Hyrcanian forests. Vindolinine, a MIA, is structurally characterized by a polycyclic indole–terpenoid scaffold, incorporating a highly functionalized pentacyclic framework with multiple chiral centers. Its structural complexity limits synthetic production, making natural sources essential for further study and utilization [3-7].

In the present study, vindolinine was detected in the stems and leaves of *V. herbacea*, but not in the flowers, suggesting that its biosynthesis is more active in photosynthetically active organs. This finding expands the phytochemical profile of *V. herbacea* and highlights its potential as a natural source of bioactive MIAs [3-6].

Vindolinine exhibits several biologically significant properties, including anticancer, antioxidant, anti-inflammatory, and cytoprotective activities. However, its pharmacological profile remains less explored than that of other major MIAs, such as vinblastine and vincristine. The complex, multi-ring structure and chemical sensitivity of vindolinine make laboratory synthesis impractical and economically challenging, emphasizing the importance of natural plant reservoirs. The presence of vindolinine in *V. herbacea*—a non-invasive, native species of the Hyrcanian floristic region—suggests that northern Iran may serve as a sustainable and accessible source of this rare alkaloid. Conservation of the species in its natural habitat ensures both biodiversity preservation and long-term availability of this pharmacologically valuable compound [3, 5, 7].

This discovery also opens avenues for future biotechnological and agricultural strategies, such as tissue and callus culture, cell suspension culture, and larger-scale controlled cultivation. Such approaches could allow economic utilization while maintaining ecological balance. Northern Iran, part of the UNESCO-registered Hyrcanian forests, provides a unique ecological context supporting the sustainable conservation and exploitation of *V. herbacea* [5, 12, 13].

Further studies are recommended to confirm and extend these findings, including:

Liquid chromatography–tandem mass spectrometry (LC–MS/MS) for fragmentation profiling and standard comparison, isolation and purification via preparative high-performance liquid chromatography (Prep-HPLC), structural elucidation using nuclear magnetic resonance (NMR) spectroscopy, in vitro bioassays to evaluate anticancer, neuroprotective, and antioxidant activities, and chemotype studies across different populations to assess environmental influences on vindolinine biosynthesis [3].

Conclusion

This study presents the first global report of vindolinine in *V. herbacea* and the first record of this alkaloid in populations from the Hyrcanian floristic region of northern Iran. Vindolinine was detected exclusively in the ethanolic extracts of leaves and stems, and was absent in flowers, indicating organ-specific accumulation likely associated with photosynthetically active tissues. The excellent spectral match with standard reference data confirms the reliability of this identification. Considering the structural complexity and synthetic difficulty of vindolinine, its presence in *V. herbacea* highlights this native, non-invasive species as a valuable and sustainable natural source of pharmacologically significant MIAs. These findings provide a foundation for future studies—including LC–MS/MS confirmation, compound isolation, NMR characterization, bioactivity assays, and chemotype analyses—to explore the medicinal, biotechnological, and ecological potential of this species.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and study design: Mohammad Kordkatouli, Aryan Sateei, and Ali Varasteh Moradi; Resources: Mohammad Kordkatouli, Mehr Ali Mahmood Janlou, and Mohammad Amin Javidi; Data collection: Mohammad Kordkatouli, Aryan Sateei, Muhammad Rizwan Ali Varasteh Moradi; Data analysis and interpretation: Mohammad Kordkatouli, Aryan Sateei, Muhammad Rizwan, and Ali Varasteh Moradi; Investigation and writing: All authors.

Conflicts of interest

The authors declared no conflict of interest.

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