Short Communication

First Global Report of the Alkaloids Quebrachamine in *Vinca herbacea* From Northern Iran Using GC-MS

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ABSTRACT

Background: *Vinca herbacea* Waldst. & Kit. (Apocynaceae) is a lesser-studied perennial species native to the Hyrcanian forests of northern Iran. The genus Vinca is renowned for producing monoterpenoid indole alkaloids (MIAs) with significant pharmacological properties. Quebrachamine, a bioactive indole alkaloid with vasodilatory, neuroprotective, and antioxidant activities, has been reported in other *Vinca* species but has never been documented in *V. herbacea*.

Objective: This study aimed to identify and characterize the presence of quebrachamine in the aerial parts (leaves, stems, and flowers) of *V. herbacea* collected from northern Iran using gas chromatography–mass spectrometry (GC–MS) analysis.

Methods: Plant samples were collected from the Baleskuh Protected Area, Tonekabon, in June 2024. Cold maceration extraction was performed separately using ethanol, n-propanol, and n-butanol. GC–MS analysis was conducted using an Agilent 6890–5973 system equipped with an HP-5MS column. Compound identification was established based on the Wiley and NIST spectral libraries.

Results: Quebrachamine was detected exclusively in the ethanolic extracts of stems (6.18%, RT 52.04 min) and leaves (1.71%, RT 52.03 min), with spectral match qualities greater than 95%. No quebrachamine was detected in the n-propanol or n-butanol extracts, nor was it found in flower tissues, indicating a strict solvent- and organ-specific accumulation pattern.

Conclusion: This constitutes the first global report of quebrachamine in *V. herbacea*. Its predominant presence in vegetative and photosynthetic tissues suggests active biosynthesis in the leaves and stems. These findings highlight the species as a promising natural source of bioactive alkaloids and emphasize the need for further isolation, structural confirmation, bioactivity assays, and conservation strategies—including tissue culture and domestication—for sustainable utilization.

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Introduction

Vinca herbacea Waldst. & Kit., a creeping perennial herb belonging to the family Apocynaceae, is considered one of the less-studied species within the genus Vinca. This species naturally occurs 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 data from the royal botanic gardens, Kew (World Flora Online), *V. herbacea* is native to several Eurasian countries, including Iran, Turkey, Iraq, Syria, Lebanon, Armenia, Russia, Hungary, Germany, Austria, and Romania; however, GBIF records indicate that its confirmed presence in Iran is primarily restricted to the northern provinces along the Alborz mountain range [1-4]. Within Iran, authenticated occurrences of *V. herbacea* are largely confined to the mountainous terrains of the ancient Hyrcanian forests in Golestan, Mazandaran, and Gilan provinces [5].

Members of the family Apocynaceae are widely known for producing monoterpenoid indole alkaloids (MIAs)—a class of structurally complex secondary metabolites with potent pharmacological activities. Among these compounds, quebrachamine ($C_{19}H_{26}N_2$; MW=282.4), also known as a naturally occurring indole alkaloid, exhibits remarkable antihypertensive, vasodilatory, neuroprotective, anticancer, antibacterial, and antioxidant effects. It acts as a selective antagonist of α_1-adrenergic receptors, thereby increasing cerebral and peripheral blood flow and protecting neuronal tissues from oxidative stress and ischemic injury. Although the presence of quebrachamine has been widely reported in other Vinca species—particularly Vinca minor—its existence in *V. herbacea* had not been documented until now [4-8].

Materials and Methods

In June 2024, aerial parts (leaves, stems, and flowers) of *V. herbacea* were collected from the Baleskuh Protected Area, Tonekabon County, Mazandaran Province, Iran. This location lies within the mountainous part of the Hyrcanian forests at coordinates 36°38′21.6″ N, 50°44′27.5″ E, at an elevation of 1,095 meters above sea level. This area features a temperate humid climate with average temperatures ranging from 20 to 30 °C and relative humidity between 70–80% during the growing season—conditions favorable for the biosynthesis of secondary metabolites. The specimens were taxonomically verified by the Iranian biological resource center (IBRC) and deposited under the herbarium code IBRC P1006834 (Figure 1) [3-6].

A soil specimen was collected from the identical location at a depth of 20 cm, then allowed to air-dry and pass through a sieve before testing. For pH determination, 10 g of the prepared soil was added to 25 mL of distilled water (maintaining a 1:2.5 soil-to-water ratio) and agitated for 30 minutes. The resulting liquid's pH was measured using a digital pH meter (Model PH827, Metrohm, Switzerland). To determine soil texture, the hydrometer (Horn) technique was employed. This technique involved mixing 50 g of soil with 100 mL of distilled water and 2 mL of 5% sodium hexametaphosphate, which served as a dispersing agent. The suspension was then mixed thoroughly and allowed to separate according to ASTM standard guidelines. The soil exhibited a mildly alkaline reaction (pH 7.2) and a sandy loam texture, with a composition of 60% sand, 30% silt, and 10% clay (Si-L) [5].

The extraction of *V. herbacea* flowers was performed using the cold maceration method in 3 independent solvent phases. In the first extraction step, the powdered plant material was mixed with 96% ethanol (Merck, Germany) at a ratio of 1 g/10 mL of solvent and kept at 4 °C for 1 week with occasional shaking. After maceration, the mixture was centrifuged at 4000 rpm for 20 min, and the supernatant was filtered through Whatman Grade 1 filter paper. The ethanolic extract was collected and stored separately [9, 10].

The remaining plant residue was subjected to a second extraction step using 96% n-propanol (Merck, Germany) under the same conditions (1 g/10 mL, 4 °C, one week). After maceration, the mixture was again centrifuged and filtered, and the n-propanol extract was collected and stored independently.

In the third extraction step, the plant residue was extracted using 96% butanol (Merck, Germany) following the same procedure (1 g/10 mL, 4 °C, one week). The butanolic extract was also centrifuged, filtered, and stored separately.

Importantly, the extracts obtained from ethanol, n-propanol, and butanol were not combined. Each extract was evaporated individually under reduced pressure using a rotary evaporator (Heidolph Hei-VAP Expert, Germany), dried, and stored in tightly sealed containers at 4 °C.

The gas chromatography-mass spectrometry (GC-MS) analysis was carried out as follows. The chemical constituents of the ethanolic, propanolic, and butanolic extracts of *V. herbacea* flowers were analyzed separately using an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass selective detector. For each extract, a 2 µL aliquot of the concentrated sample was injected in split mode (1:5) into an HP-5MS capillary column (30 m×0.25 mm, 1 µm

Figure 1. A voucher specimen of *V. herbacea* waldst. & kit. collected from the Baleskuh protected area, Tonekabon County, Mazandaran Province, Iran (38°36'21.6" N, 44°50'27.5" E; elevation 1,095 m).

Note: The specimen was identified and deposited in the IBRC under the code IBRC P1006834.

film thickness). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min [3, 5, 6].

The oven temperature was programmed from an initial 60 ˚C (held for 2 min) to 280 ˚C at a rate of 5 ˚C/min, followed by a final hold of 20 min. Mass spectrometry was performed using electron impact ionization at 70 eV with a scanning range of m/z 40–500. Identification of compounds was based on comparisons of retention times and mass spectra with those in the NIST and Wiley spectral libraries [6, 9, 10].

Results

The results for each organ and solvent are as follows.

Stems

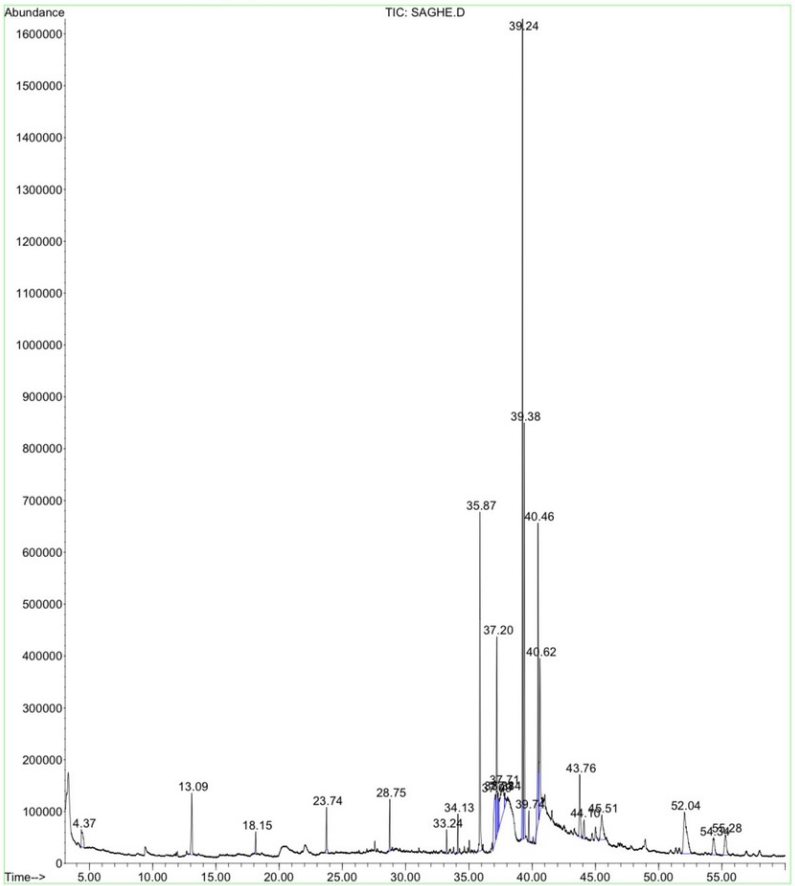
In the ethanolic stem extract, quebrachamine was identified at a retention time of 52.04 minutes. The mass spectral match quality with the Wiley library was 95% and with NIST was 96%, indicating highly accurate and reliable identification of this alkaloid in stem tissue. The relative abundance of quebrachamine in this extract was 6.18% of the total chromatogram, significantly higher than in the ethanolic leaf extract. This finding suggests that stems contain more of this alkaloid than leaves, and that ethanol is more efficient in extracting quebrachamine from stem tissue. This outcome may reflect differences in tissue structure, vascular density, or organ-specific alkaloid distribution (Figures 2A and 3A). No peak corresponding to this compound was observed in the n-propanol stem extract. Therefore, it appears that stems contain no detectable amount of quebrachamine under these analytical conditions. Similarly, no peak corresponding to this compound was detected in the n-butanol stem extract.

Leaves

In the ethanolic leaf extract, quebrachamine was identified at a retention time of 52.03 minutes with a match quality of 99% in both Wiley and NIST libraries. This high match accuracy confirms reliable identification. The relative abundance of this compound was 1.71% of the total chromatogram, indicating moderate extractability of the alkaloid from leaves by ethanol (Figures 2B and 3B).

No peak corresponding to this compound was found in either the n-propanol and butanol extracts of the leaves, suggesting that leaves contain very low or undetectable levels of Quebrachamine when extracted with these solvents.

A



B

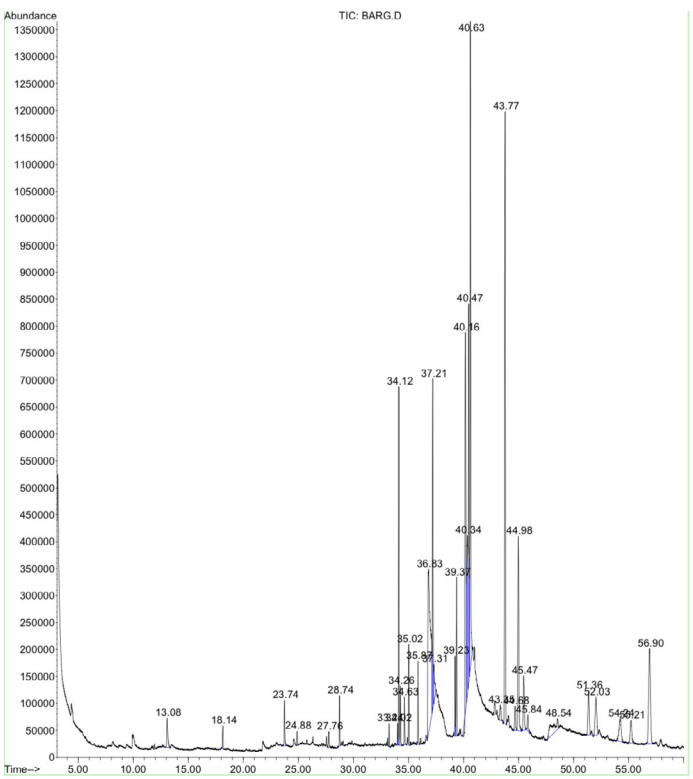


Figure 2. A) The TIC obtained from GC-MS analysis of the ethanolic extract of *V. herbacea* stems; B) TIC obtained from GC-MS analysis of the ethanolic extract of *V. herbacea* leaves

Flowers

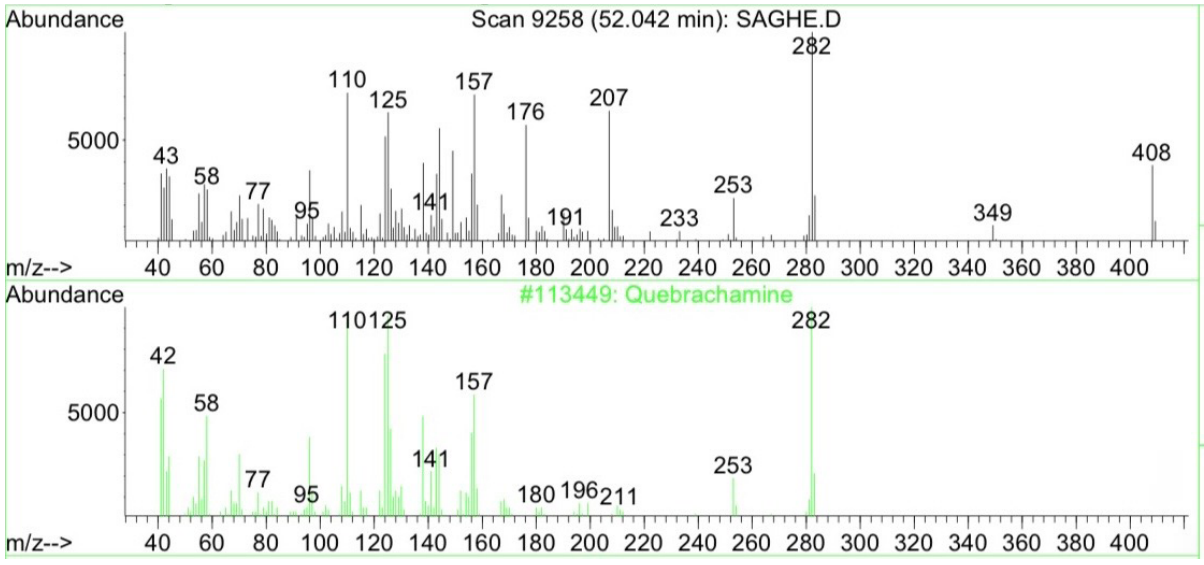
In flower tissues, quebrachamine was not detected in the ethanolic extract. This absence may indicate extremely low levels of the compound in floral tissues or low extraction efficiency of ethanol for this alkaloid in flowers.

Similarly, no peak corresponding to this compound was detected in either the n-propanol and butanol extracts of flowers, suggesting that flowers contain no detectable amount of quebrachamine under the applied GC-MS conditions.

Detailed analysis of the GC-MS results showed that the recorded mass spectrum of the detected compound perfectly matched the reference patterns of quebrachamine (CAS: 4850-21-9) in certified spectral libraries. Comparison of spectra with the Wiley and NIST libraries showed match quality (qual) values above 95% in all samples. This high spectral similarity confirms that the detected compound fully corresponds to the known structure of quebrachamine, and its presence in the plant samples is unequivocally verified.

This high-level library match not only ensures the validity of the identification but also highlights the significance of the finding, as the detection of quebrachamine in *V. herbacea* is reported for the first time and indicates the considerable phytochemical potential of this species.

A



B

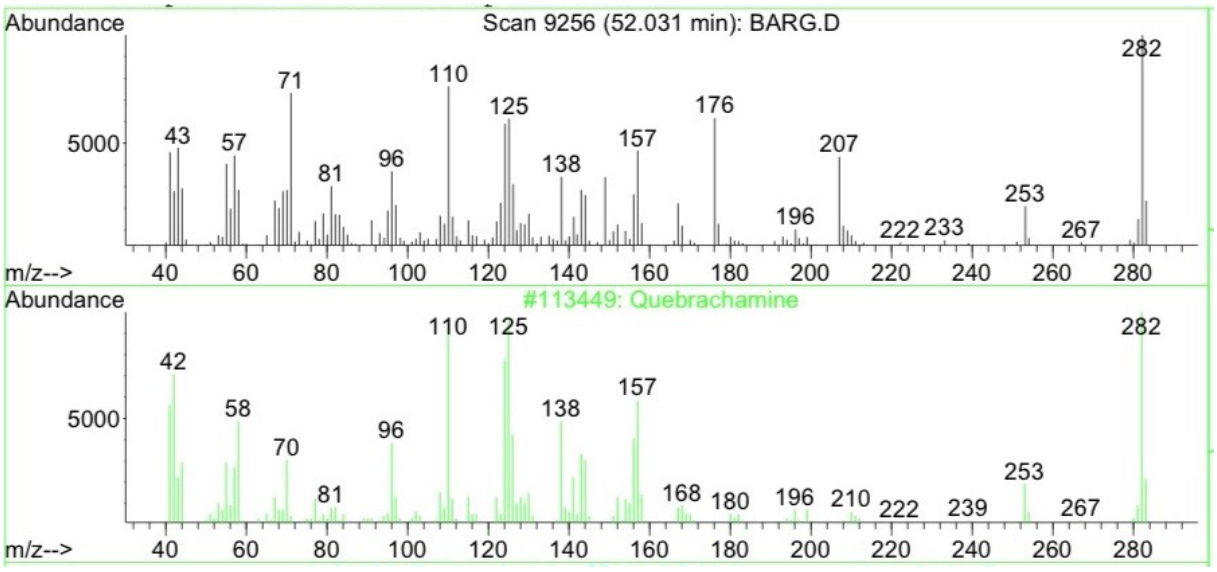


Figure 3. A) Quebachamine peak in stem extract, B) Quebachamine peak in leaf extract

Discussion

This study represents the first global report of quebrachamine in *V. herbacea* and the first record from northern Iran. Its presence predominantly in leaves and stems, and the solvent-dependent differences, suggest that biosynthesis of quebrachamine is concentrated in metabolically active, photosynthetic tissues. The minor variations observed in fragmentation patterns among different organs—especially slight differences in ion intensities—are more likely due to natural isotopic distributions and relative abundance differences, rather than enzymatic or accumulation differences [5, 6].

Overall, the available reports on the presence of quebrachamine in the genus Vinca are limited and mainly date back to older studies. These accounts include the report by Rakhimov et al. (1970) on *Vinca erecta* and the study by Mokrý et al. (1967) on *Vinca minor*. In these investigations, ethanol was generally used as the extraction solvent; however, the analytical techniques applied differ from those used in the present study. These methodological differences may to some extent limit direct comparison of the results, while at the same time highlighting the importance of employing more up-to-date approaches in the investigation of phytochemical constituents within this genus [11, 12].

The identification of quebrachamine in *V. herbacea* expands the known phytochemical profile of this understudied species and highlights its potential as a new natural source of valuable indole alkaloids. Given the strong vasodilatory, neuroprotective, and antioxidant properties of this compound, further confirmatory studies, preparative high-performance liquid chromatography (Prep-HPLC) isolation, and full structural elucidation using nuclear magnetic resonance (NMR) spectroscopy are strongly recommended. Additionally, in vitro bioassays to evaluate antihypertensive, neuroprotective, and radical-scavenging activities of purified quebrachamine, as well as chemotypic studies among different Iranian populations, could illuminate the influence of environmental factors on its biosynthesis [3-5].

Conclusion

This study provides the first global report of quebrachamine presence in *V. herbacea* and highlights the phytochemical and pharmacological potential of this lesser-known species as a promising natural source of bioactive compounds. These findings further emphasize the need for enhanced conservation measures for this rare and scattered species, including preservation through tissue culture, enabling large-scale propagation, and developing cultivation under controlled conditions such as farms, greenhouses, or as an agricultural crop to ensure sustainable and economically viable utilization. Considering that Iran represents one of the natural habitats of this species, focused efforts on its conservation, alongside comprehensive phytochemical, biological, and ecological studies, are essential to safeguard its genetic resources and to facilitate its scientific and applied exploitation.

The total ion chromatogram (TIC) obtained from GC–MS analysis of the ethanolic extracts of *V. herbacea* leaf and stem tissues revealed a distinct diagnostic peak corresponding to Quebrachamine (CAS: 4850-21-9). In the TIC profile of the leaf extract, this peak appeared at a retention time of 52.03 min with a match quality of 99%, while in the stem extract TIC, the same compound eluted at 52.04 min with a match quality of 96%. The high consistency in retention times and spectral matching across both TIC profiles confirms the presence of Quebrachamine in both tissues, demonstrating that *V. herbacea* serves as a natural source of this indole alkaloid, with a marginally higher identification confidence observed in the leaf tissue.

The GC-MS analysis of the ethanolic extracts of both the stem and leaf of *V. herbacea* revealed a clear diagnostic peak corresponding to quebrachamine (CAS: 4850-21-9). The leaf extract displayed a match quality of 99% with a retention time of 52.03 min, while the stem extract showed a match quality of 96% with a retention time of 52.04 min. These highly consistent spectral and chromatographic characteristics confirm the presence of quebrachamine in both tissues, indicating that *V. herbacea* serves as a natural source of this indole alkaloid, with slightly higher identification confidence in the leaf.

Ethical Considerations

Compliance with ethical guidelines

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

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Authors' contributions

Conceptualization: Aryan Sateei and Mohammad Kordkatouli; Data curation: Mohammad Kordkatouli and Aryan Sateei; Formal analysis and validation: Mehr

Ali Mahmood Janlou and Ali Varasteh Moradi; Funding acquisition: Mohammad Kordkatouli; Investigation and project administration Mohammad Kordkatouli, Ali Varasteh Moradi, and Aryan Sateei; Methodology: Mohammad Kordkatouli and Mehr Ali Mahmood Janlou; Resources: Mohammad Kordkatouli, Ali Varasteh Moradi; Supervision: Ali Varasteh Moradi and Aryan Sateei; Visualization and writing the original draft: Mohammad Kordkatouli and Mehr Ali Mahmood Janlou; Review and editing: Ali Varasteh Moradi and Aryan Sateei.

Conflict of interest

The authors declared no conflict of interest.

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