

## First Global Report of Alkaloids Tubotaiwine and Vinervine in *Vinca herbacea*: Potential Pharmacological Applications

Mohammad Kordkatouli <sup>1,2\*</sup>, Ali Varasteh Moradi <sup>3</sup>, Mohammad Amin Javid<sup>4</sup>, Aryan Sateei <sup>2,5\*</sup>, Audrius Dulskas <sup>6,7</sup>, Daiva Luniene <sup>8</sup>

1

1. Department of Genetics, Faculty of Advanced Sciences and Technology, TeMS.C, Islamic Azad University, Tehran, Iran

2. Medicinal Plants Research Center, Go.C, Islamic Azad University, Gorgan, Iran

3. Department of Chemistry, Go.C, Islamic Azad University, Gorgan, Iran

4. Integrative Oncology Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran

5. Department of Plant Science, Go.C, Islamic Azad University, Gorgan, Iran

6. Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

7. Department of Abdominal and General Surgery and oncology, National Cancer Institute, Vilnius, Lithuania

8. SMK College of Applied Sciences, Vilnius, Lithuania

\* Corresponding authors:

Mohammad Kordkatouli  
Department of Genetics, Faculty of Advanced Sciences and Technology, TeMS.C, Islamic Azad University, Tehran, Iran  
Tel: + 989128394042  
E-mail: mohammad.kordkatouli@iau.ir

\* Corresponding authors:

Aryan Sateei  
Department of Plant Science, Go.C, Islamic Azad University, Gorgan, Iran  
Tel: + 989113754290  
E-mail: aryan.sateei@iau.ac.ir

### ABSTRACT

Dear Editor,

*Vinca herbacea* Waldst. & Kit. is a perennial herbaceous species 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 countries including Iran, Turkey, Iraq, Lebanon, Syria, Palestine, Armenia, Ukraine, Russia, Hungary, Germany, Austria, and Romania. However, data from the Global Biodiversity Information Facility (GBIF) indicate that its confirmed presence in Iran is minimal, recorded only in a few locations in the northern provinces and on the slopes of the Alborz Mountains. This restricted distribution highlights the need for further phytochemical and pharmacological investigations [1,2,3].

The Apocynaceae family includes medicinally important plants producing monoterpenoid indole alkaloids (MIAs). Among them, Tubotaiwine and Vinervine have significant clinical relevance and act as precursors of anticancer agents used in the treatment of leukemia, lymphoma, and various solid tumors. These alkaloids exhibit cytotoxic and antitumor activities, as well as antimicrobial, antioxidant, and regulatory effects on critical cellular pathways, potentially supporting conventional chemotherapy. Due to the scarcity of natural sources of these compounds, identifying new species capable of their biosynthesis is of high pharmacological and biotechnological importance [2,3].

In June 2024, aerial parts (leaves, flowers, and stems) of *Vinca herbacea* were collected from the Baleskuh protected area, Tonekabon County, Mazandaran Province, Iran. Verified plant specimens were obtained from the Iranian Biological Resource Center (IBRC) under code IBRC P1006834 (Figure 1). The samples were collected at 36°38'21.6" N latitude and 50°44'27.5" E longitude, at an elevation of 1,095 meters. This region has a mild and humid climate, with average temperatures ranging from 20 to 30°C in spring and summer, and a relative humidity of between 70–80%, conditions that are favorable for secondary metabolite production. The dried and powdered plant material was extracted using 96% ethanol by cold maceration [3,4,5].

The resulting extracts were analyzed using liquid chromatography coupled with mass spectrometry (LC-MS). The LC-MS analysis was performed using a Waters Alliance 2695 HPLC coupled with a Micromass Quattro micro-API mass spectrometer under



ESI+ mode. Separation was achieved on a Eurospher C18 column (4.6 × 120 mm, 5 μm) at 35 °C with a flow rate of 0.3 mL/min, using a gradient mobile phase of acetonitrile and water (both containing 0.1% formic acid). Extracts were dissolved in methanol, sonicated, centrifuged, filtered (0.22 μm), and 5 μL was injected for analysis

- Vinervine (m/z 339.25) was detected in leaves at a retention time of 14.20 min. In stems, two peaks at the same m/z were observed at 12.46 and 14.21 min. This compound was not detected in flowers.
- Tubotaiwine (m/z 325.32) was identified in stems at 18.38 min, in flowers at m/z 325.31 at 18.69 min, and in leaves at m/z 325.33 at 18.43 min.

Data analysis using MassLynx confirmed that these peaks have molecular ions and isotopic patterns consistent with previously reported data for Tubotaiwine and Vinervine. High similarity in molecular mass, mass-to-charge ratio, and isotopic profile strongly supports the presence of these pharmacologically relevant alkaloids in the ethanolic extracts of *Vinca herbacea*.

To our knowledge, this is the first global report of Tubotaiwine and Vinervine in *Vinca herbacea*. These findings expand the phytochemical profile of this rare species and highlight its potential as a natural source for pharmacological applications. The alkaloids identified exhibit anticancer, antimicrobial, and antioxidant properties, suggesting their potential use in the development of therapeutic agents [2,6].

While our initial LC-MS data strongly suggest the presence of these alkaloids, we acknowledge that further confirmatory studies are essential. Our immediate future work will focus on:

1. **LC-MS/MS Analysis:** To obtain fragmentation spectra and compare them with literature data or authentic standards for definitive identification.
2. **Isolation and Purification:** Using preparative HPLC to isolate these compounds for unequivocal structural elucidation by NMR spectroscopy.
3. **Bioactivity Assays:** Evaluating the purified compounds for their purported anticancer, antimicrobial, and antioxidant activities in vitro.



**Figure 1:** Voucher specimen of *Vinca 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). The specimen has been verified and deposited at the Iranian Biological Resource Center under code IBRC P1006834

4. **Population Study:** Analyzing samples from other geographical populations of *Vinca herbacea* to assess chemotypic variations and the influence of environmental factors on alkaloid production.

Considering the limited distribution of *Vinca herbacea* in Iran, conservation efforts alongside these detailed chemical and pharmacological studies are also of high importance.

In conclusion, our preliminary LC-MS results indicate that *Vinca herbacea*, a rare species in Iran, may contain Tubotaiwine and Vinervine, alkaloids with known anticancer and antimicrobial properties. This discovery, if confirmed through further rigorous analysis, suggests the biotechnological and medicinal potential of this species and underscores the importance of phytochemical research on lesser-known members of the Apocynaceae family.

#### **Acknowledgment:**

The authors sincerely express their gratitude to the Islamic Azad University, Gorgan Branch, for its valuable spiritual support and continuous encouragement, as well as for providing access to laboratory facilities and equipment. Special thanks are also extended to the Medicinal Plants Research Center, Islamic Azad University, Gorgan Branch, for generously providing research facilities and sustained support.

We gratefully acknowledge the ISLAMIC REPUBLIC OF IRAN Meteorological ORGANIZATION for kindly supplying the meteorological data of Tonekabon County. We also wish to thank the Iranian Biological Resource Center for providing the plant samples, their continuous support and assistance, and for supplying additional information on the geographical distribution and the confirmation of the herbarium specimen.

#### **References:**

1. Kordkatouli M, Sateei A, Khoshbakht T. Antitumor Potential of *Vinca herbacea* and Its Molecular Pathways in Inhibiting Cancer Cell Growth. *Immunoregulation*. 2024;7(1).
2. Vrabec, R., Drašar, P., Opletal, L. et al. Alkaloids from the genus *Vinca* L. (Apocynaceae): a comprehensive biological and structural review. *Phytochem Rev*. 2025;24(1). <https://doi.org/10.1007/s11101-025-10102-z>
3. Kordkatouli M, Sateei A. The First Global Report on the Alkaloid Aspidospermidine in *Vinca herbacea*: A Potential Source of Anticancer Alkaloid Precursors. *Future Natural Products*. 2025;10(2):109-13.
4. Mahmood Janlou MA, Kordkatouli M, Sateei A, Koohsari H. Gc-Ms-based phytochemical profiling and antibacterial efficacy of terminalia chebula extract against gastrointestinal pathogens resistant to  $\beta$ -lactam antibiotics. *Journal of Patient Safety & Quality Improvement*. 2025;13(3):169-78.
5. Koohsari H, Kordkatouli M, Sateei A, Janlou MA. GC-MS Analysis and Antibacterial Activity of Terminalia chebula Against  $\beta$ -lactam-resistant *Kelebsiella pneumoniae*. *Immunoregulation*. 2024 ;7(1).
6. Joshi S, Huo C, Budhathoki R, Gurung A, Bhattarai S, Sharma KR, Kim KH, Parajuli N. HPLC-ESI-HRMS/MS-Based Metabolite Profiling and Bioactivity Assessment of *Catharanthus roseus*. *Plants*. 2025;14(15):2395.