Ethnomedicinal uses and phenotypic attributes of four *Viola* species from the Kashmir Region

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Research

Abstract

Background: The Violaceae family consists of the flower-bearing taxa *Viola*, which is mostly utilized in Kashmir region to treat respiratory disorders and hold substantial ecological and cultural importance. *Viola* species (*Viola odorata* L., *Viola biflora* L., *Viola canescens* Wall. ex. Roxb. and *V. indica* W. Becker) are widely distributed in the Himalayan regions of India, Pakistan, Iran, Afghanistan, Nepal, China, Bhutan, Europe and other Asian countries. Their wide geographical distribution, coupled with significant morphological diversity, has made the genus an important subject in taxonomy, phylogeny, ethnobotany and pharmacological research.

Methods: In the present study, four commonly found species of *Viola* genus which include *V. odorata*, *V. biflora*, *V. canescens* and *V. indica*, were compared based on their ethnomedicinal uses and phenotypic traits. These *Viola* species are traditionally used as herbal remedies to relieve respiratory ailments, reduce inflammation and pain and promote relaxation and sleep in local Unani and Ayurvedic practices in the Kashmir region. Further, the standardized trait descriptors (11 qualitative and 9 quantitative) were used to assess the extent of variation among the four *Viola* species.

Results: The phenotypic attributes revealed significant morphological differences among the four *Viola* species based on analysis of qualitative traits. Besides, PCoA and cluster analysis grouped four *Viola* species into two major groups. Group I comprise V. indica and group II consists of three *Viola* species (*V. canescens*, *V*. *ordorata* L. and *V. biflora*).

Conclusions: The study suggested that the Kashmir region has a rich interspecific diversity of *Viola* species, which needs to be conserved for the maintenance of ecological balance and exploring its potential therapeutic applications.

Keywords: Ethnomedicinal potential, Himalayan region, Phenotypic attributes, Trait descriptors, *Viola* species

Background

The genus *Viola* is an annual to perennial herbaceous, mostly stoloniferous, flowering plant that is cosmopolitan in distribution with over 760 species known worldwide (Kaundal & Kumar 2025). It is found in the European Union, North America, Australia and Asia, and is an important edible medicinal plant (Muhammad *et al*. 2012, Mehrvarz *et al*. 2013, Lim

2014, Marcussen *et al*. 2022). The *Viola* species are variously named in different parts of the world like "sweet violet (*Viola odorata*)", "yellow wood violet (*V. biflora*)", "Himalayan white violet (*V. canescens*) ", "Indian violet (*V. indica*) " and so on (Sher *et al*. 2021). The diversity in plants of Jammu and Kashmir shaped by the varying topography, altitude and geography underpins a rich traditional knowledge system, where distinct ethnic communities utilize different local flora in their traditional foods as productive resources and despite their relative dependence on small-scale pastoral and horticultural modes of production (Manzoor *et al*. 2023, Gillani *et al*. 2024, Mirzaman *et al*. 2023). They have been able to generate their unique cultural identities, practices, beliefs and socio-economic distinctions which throughout time and space were reinforced and reflected upon by their traditional food varieties, cooking methods and taste from the special local flora (Gillani *et al*. 2024, Kayani *et al*. 2024). The Kashmir Himalayan region of India is known for its rich biodiversity, including a diverse array of plant species with significant ethnomedicinal potential (Meena *et al*. 2019). Among these, the genus *Viola*, commonly known as violets, has been a subject of interest due to its therapeutic properties and traditional use in the region (Kaundal *et al*. 2022). Phytochemical studies have revealed that different parts of *Viola* species are rich in various active metabolites, including phenolic compounds, flavonoids, steroids, tannins, saponins, phytosterols and alkaloids (Barkatullah *et al*. 2012, Masood *et al*. 2014, Prasad 2014, Fazeenah & Quamri 2020). All plant parts of *Viola* species are used in trade and commerce in the form of dried parts of flowers, seeds, aerial parts or entire plants, mostly used for medicinal purposes in different pharmacological activities (Asheesh *et al*. 2017, Batiha *et al*. 2023, Orchard *et al*. 2023). Therefore, it is used in the traditional systems of medicine like Ayurvedic and Unani for the treatment of cough, cold, flu, fever and malaria (Gerlach *et al*. 2010, Gautam *et al*. 2012, Mahboubi & Taghizadeh 2018, Bashir *et al*. 2021). Furthermore, *Viola* species are also known to possess laxative, analgesic (Antil *et al*. 2011), antimicrobial, (Hassan & Naeem 2014, Zhang *et al*. 2023), antihypertensive (Siddiqi *et al*. 2012), anti-inflammatory (Koochek *et al*. 2003), hepato-protective and anti-cancer activities (Elhassaneen *et al*. 2013, Abdullah *et al*. 2017, Alipanah et al. 2018, Payez & Deldadeh 2022). For instance, *Viola canescens* is indigenous to the Kashmir Himalayas and shows great therapeutic potential because of its various phytochemical constituents. It contains a high concentration of alkaloids, phenols, flavonoids, saponins, tannins and tri-terpenes, all of which have therapeutic benefits (Abdullah *et al*. 2017). Moreover, the polyphenols and alkaloids content is also associated with the antioxidant activity of *Viola canescens*. For example, active principles such as emetine, quercetin and violanthin have demonstrated free radical scavenging abilities. Thus, it can be suggested that most of the hepato-protective effects of *Viola canescens* are related to its antioxidant and membrane-stabilizing activities (Ahmad *et al*. 2024). Furthermore, the antioxidant potential of *Viola canescens* is linked to its polyphenolic and alkaloid content. *In-silico* studies suggest that these compounds interact effectively with proteins involved in oxidative stress pathways, supporting their role in the plant's antioxidant activity. These findings underscore the therapeutic relevance of *Viola canescens* phytochemicals, particularly their hepato-protective and antioxidant properties, validating the traditional use of this species in treating liver disorders and related ailments (Abdullah *et al*. 2017). In the Kashmir Himalayan region, several *Viola* species exhibit significant therapeutic potential due to their rich phytochemical compositions. Beyond *Viola canescens*, species such as *Viola pilosa* and *Viola odorata* have been studied for their medicinal properties. *V. pilosa* is traditionally utilized for its antitussive, analgesic, antipyretic, antimalarial, anti-inflammatory and anti-cancer properties. Studies have shown that *V. pilosa* contains notable levels of polyphenols and flavonoids, particularly in its flowers. These compounds contribute to its antioxidant and antimicrobial activities, which vary with altitude, indicating an adaptive response to environmental conditions (Kaundal *et al*. 2022). Likewise, *V. odorata* has been traditionally used for its expectorant and anti-inflammatory effects. Phytochemical analyses reveal the presence of alkaloids, flavonoids, terpenoids, saponins and phenolics, with methanolic extracts showing significant antioxidant activity. Additionally, these extracts exhibit antimicrobial effects against pathogens such as *Bacillus subtilis*, *Escherichia coli* and *Candida albicans*, likely due to the presence of compounds like stigmasterol (Dhiman *et al*. 2024). The therapeutic relevance of these *Viola* species is closely linked to their phytochemical constituents. Polyphenols and flavonoids contribute to antioxidant properties, mitigating oxidative stress and inflammation. Alkaloids and terpenoids may enhance antimicrobial and anticancer activities. These findings support the traditional use of *Viola* species in treating various ailments and highlight their potential for developing novel therapeutic agents. The Kashmir Himalayan region is home to a variety of *Viola* species, each with unique morphological characteristics and traditional medicinal applications. Realizing the importance of these valuable medicinal plants, researchers have undertaken comprehensive studies to document the morphological features and ethnobotanical uses of the *Viola* species found in this region (Dar *et al*. 2018, Abdullah *et al*. 2023). The rationale for selecting *Viola* species from the Kashmir Himalayas lies in their rich traditional medicinal use, where local communities rely on these plants to treat respiratory, inflammatory and other ailments. In addition, the region's unique altitudinal diversity drives significant morphological and phytochemical variation that may reveal novel bioactive compounds for therapeutic applications. Therefore, the present study was undertaken to explore the ethnomedicinal significance and phenotypic diversity of four commonly occurring *Viola* species from the Kashmir region. By documenting indigenous medicinal uses alongside detailed morphological characterization, this research aims to establish species specific diagnostic markers, assess interspecific variation and understand how phenotypic attributes support ethnobotanical recognition and utilization. Such

an approach is valuable for strengthening the scientific basis of traditional medicine, promoting sustainable harvesting, conserving medicinal plant diversity and creating future opportunities for pharmacological and phylogenetic exploration of *Viola* species in the Himalayan landscape.

Materials and Methods

Study area and plant material

In this study, the plant samples of four *Viola* species (*Viola odorata , Viola biflora, Viola canescens* and *Viola indica*) in the wild habitat were collected from different sites (Doothpathri, Yousmerg, Naranag and Dachigam) of the Kashmir region for herbarium preparation and phenotypic description. For collection, drying and processing of the herbarium specimens, standardized taxonomic procedures were adopted (Bridson & Forman 1998). The herbarium specimen of *Viola* species, which include *V. odorata, V. biflora, V. canescens* and *V. indica,* plants were authenticated by the Center of Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir and deposited these Herbaria with voucher numbers as, 6012-KASH (*Viola odorata*), 6018-KASH (*Viola biflora*), 6011-KASH (*Viloa canescens*) and 6017-KASH (V. indica), of all species, were reconfirmed with KASH authentic reference numbers VO-38582, VB-38589, VC-38583 and VI-38590 Dated: 01.06.2022., which were collected from wild during February to August depending upon their phenological period. A handheld GPS MM50 and Spectra MobileMapper 50 were used to record the geo-coordinates. To prepare the distribution map, ArcGIS (version 10.8; https://www.arcgis.com/) was used. These *Viola* species were found at an altitudinal range from 1400 to 2800 meters (amsl) as depicted in Fig. 1, Table 1.

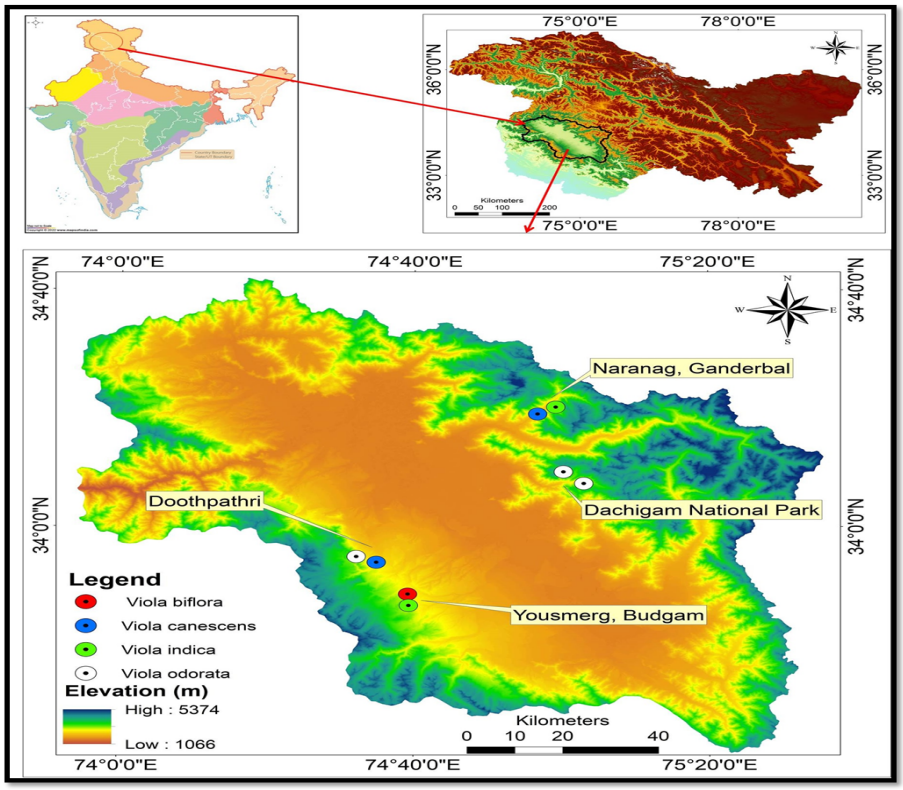

Figure 1. Survey map depicting the locations of *Viola* species (*V. odorata*, *V. biflora, V. canescens* and V. indica) from the Kashmir Himalayan region

Table 1. Collection of *Viola* species from different sites of the Kashmir region

Scientific name	Location	Latitude (N)	Longitude (E)	Altitude ranges (amsl) m
Viola odorata	Doothpathri, Budgam	33°86'046''	74°57'388''	1400-2800
Viola biflora	Yousmerg, Budgam	33°47'548''	74°67'486''	1650-2500
Viola canescens	Naranag, Ganderbal	34°31'846''	74°94'741''	1700-2300
Viola indica	Dachigam National Park, Srinagar	35°62'014''	74°97'522''	1450-2400

Specimen examined

Different herbal floras of various countries along with their herbariums were studied to act as a reference specimen to comprehend various phenotypic characteristics of different *Viola* species, domestically, nationally as well as internationally. Also, various e-resources of different countries were accessed to understand the *Viola* species/genus panorama of known pointed centers across the world. Details of these specimens along with their date of collection among authenticated institutions and persons who collected wherever present in given in Table 2. Numerous search engines, including electronic databases such as Google, Web of Sciences, Scopus, Google Scholar, SciFinder, Science Direct, PubMed, John Wiley, Springer, Taylor and Francis, etc., were used to conduct extensive literature reviews. For the intended aims, a variety of books, pertinent abstracts and full-text articles were consulted. The traditional Indian medicinal systems and Persian medicine were the sources of this plant's multi-potential therapeutic qualities. From traditional medicinal to phytochemical and pharmacological applications, a thorough scientific ethnobotanical profile of *Viola* species was examined and hypothesized accordingly in the manuscript (Table 3).

Table 2. Geographical distribution and ethnomedicinal uses of *Viola* species found in the Kashmir region

Species	Flowering time	Geographical distribution	Ethnomedicinal use
Viola odorata	March-May	**Global:** China, India, Nepal, Pakistan; North West Asia, Europe, North Africa. **Local:** Andhra Pradesh, Gujarat, Jammu & Kashmir, Karnataka, Tamil Nadu, West Bengal.	Expectorant, antimicrobial (Sher *et al*. 2021), narcotic, diaphoretic, antipyretic, antihypertensive (Siddiqi *et al*. 2012) and anti-inflammatory (Muhammad *et al*. 2012).
Viola biflora	May-August	**Global:** Afghanistan, Bhutan, China, Japan, Korea, Malaysia, Mongolia, Myanmar, Nepal, Pakistan, Russia; Europe; North America **Local:** Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Punjab, Sikkim, Uttar Pradesh, Uttarakhand, West Bengal	Diaphoretic, antipyretic, anti-cancer, epilepsy and nervous disorders (Khattak *et al*. 1985, Hamayun *et al*. 2006).
Viola canescens	March-June	**Global:** Bhutan, China, India, Myanmar, Nepal, Pakistan. **Local:** Arunachal Pradesh, Himachal Pradesh, Jammu & Kashmir, Meghalaya, Sikkim, Tamil Nadu, Uttarakhand, Uttar Pradesh, West Bengal.	Purgative, diaphoretic, antipyretic, febrifuge, and anti-cancer (Bashir *et al*. 2021, Kaundal *et al*. 2022).
Viola indica	March-May	**Global:** Afghanistan, India, Nepal, Pakistan. **Local:** Himachal Pradesh, Jammu & Kashmir, Meghalaya, Punjab, Uttar Pradesh	Demulcent, expectorant, diaphoretic, antipyretic, anticancer and antimicrobial (Muhammad *et al*. 2012).

Table 3. Vouchers of *Viola* species with details of the collection

Species	Herbarium with authentication sources	Accession/collection no.	Date of collection
Viola odorata	Naturalist data from Escuder Olivier	4608741/ 18129 4611136 / 48099	18.03.2023 09.04.2023
L.	Herbarium of Regional Research Laboratory, J & K, Indian Institute of Integrative Medicine (IIIM) by B.M. Sharma, Janaki Ammal Herbarium.	20860 and HRRL-19373	18.07.1976
	Flora of North America., Herbarium of Clemson University	HCU32723b	24.04.1988

	University of Birmingham, Dept. of Botany. Collected By H. Stuart Thompson Bequest,	BIRM015328	02.04.1920		
	University of Birmingham, collected by Dr Richard Charles L'Estrange Burges	BIRM036612	17.03.1938		
	West Kent in 1901 by William Henry Griffin Cudham, South London Botanical Institute (SLBI)	HERB3885	1901		
	Kent Ethnobotanical Herbarium, University of Kent, UK, UKC campus, Parkwood, collected by B. Arthurian	BA07	23.03.2011		
	Kent Ethnobotanical Herbarium, University of Kent, UK, Earley Wood, Nr Petham, Kent, collected by Dylen Macvicar	DM03	31.03.2008		
	Kent Ethnobotanical Herbarium, University of Kent, UK, Downs Road, Canterbury, Kent, collected By Stephanie Borios	SCB2	23.03.2002		
	Botanical Society of Britain and Ireland. Hurtmore Bottom, by Mr. William Hadden Beeby	SLBI-LC58/181C VC17 Surrey in 1885	10.01.2010		
	Central Herbarium, University of Wah, Punjab, Pakistan. Collected Mr. Jahangir Khan.	WAH000394, WAH000564	03.01.2021 (V1O/001)	(V1O/002),	17/Aug/2021
	Kashmir University Botanical Garden, Center of Taxonomy and Biodiversity, Dept. of Botany. University of Kashmir, Srinagar, J&K.	KASH-6012	02.04.2022		
	Herbarium Center of Taxonomy, Dept. of Botany. University of Kashmir, Srinagar, J&K, collected from Kashmir, J&K by, Dr. Gh. Hassan Dar.	31562/9201 (12.02.2011) 31138/524 (09.02.2011)	07.04.1987 09.06.1999		
	Herbarium Center of Taxonomy, Dept. of Botany. University of Kashmir, Srinagar, J&K, collected from Kashmir, J&K by, A. R. Naqashi	19818/6887 (13.09.94)	July.1974		
	Herbarium Center of Taxonomy, Dept. of Botany. University of Kashmir, Srinagar, J&K, collected from Kashmir, J&K by, Dr. Anzar A Khuroo	33600/326 (11.03.2011)	20.08.2002		
	Herbarium Center of Taxonomy, Dept. of Botany. University of Kashmir, Srinagar, J&K, collected from Kashmir, J&K by, Showkat Ahmad	31371 (11.02.2011)	15.09.2003		
	Herbarium Center of Taxonomy, Dept. of Botany. University of Kashmir, Srinagar, J&K, collected from Kashmir, J&K by, Akhtar Masoodi	23259/1011 (07.08.2004)	20.06.2003		
***Viola biflora* L.**	Herbarium of Regional Research Laboratory, J & K, IIIM by B.M. Sharma, Janaki Ammal Herbarium	17213	20.08.1989		
	Biodiversity India	261774	15.03.1921		
	Royal Botanical Garden Kew. Flora of Chania, Identified by Y.S. Chen (PE).	K000254205, H2003/0218066, Western China No.3217.	Jun/2006		
	Center of Taxonomy and Biodiversity, Dept. of Botany. The University of Kashmir, Srinagar, J&K. collected by Fayeem Aadil	KASH-6093	05.06.2022		
	Herbarium Institute of Plant Taxonomy. Plants of Jammu and Kashmir, Sink Valley, collected from Kashmir, J&K by, Dr. Gh. Hassan Dar.	11384/6865 (12.08.1985)	15.07.1983		
	Herbarium Institute of Plant Taxonomy. Plants of Jammu and Kashmir, Sink Valley, collected from Kashmir, J&K by, Mr. Vinod C Gupta.	9290/1631 (18.08.1981)	May.1977		

	Herbarium Institute of Plant Taxonomy. Plants of Jammu and Kashmir, Sink Valley, collected from Kashmir, J&K by, Dr. Gurcharan Singh.	6726 (24.04.1978) 1669/2238	03.08.1870
	Center of Taxonomy and Biodiversity (CTB), Dept. of Botany. University of Kashmir, Srinagar, J&K. collected by Akhtar H Malik.	30345/8315 (04.02.2011)	07.07.2008
***Viola canescens* Wall. ex Roxb.**	United States National Museums Y. S. Chen (PE)	1608081, 03019763	Oct. 2017
	Bhadwar, Kangra, Punjab. Waltr kolez.	KOLEZ4284.	25. Apr.1933
	Herbarium of Regional Research Laboratory, J & K, (IIIM) Janaki Ammal Herbarium	HRRL-16930	06.04.1990
	Herbarium of Regional Research Laboratory, J & K, IIIM by B.M. Sharma, Janaki Ammal Herbarium	20943	22.08.1977
	Herbarium JCB, Center of Taxonomic studies, St. Joseph's College, Bangalore, by Cicil. J. Saldanha, Karnataka, India.	CS1225 KFP9617	06. may.1954
	Kashmir University Botanical Garden, Center of Taxonomy and Biodiversity, Dept. of Botany. University of Kashmir, Srinagar, J&K.	KASH-6011	28.03.2022
	Herbarium Institute of Plant Taxonomy. Plants of Jammu and Kashmir, Sink Valley, collected from Kashmir, J&K by Akhtar H Malik.	40432/11964 (20.08.2013)	16.05.2010
***Viola indica* W. Becker**	Kashmir University Botanical Garden, Center of Taxonomy and Biodiversity, Dept. of Botany. University of Kashmir, Srinagar, J&K.	KASH-6017	28.05.2022
	Herbarium Institute of Plant Taxonomy. Plants of Jammu and Kashmir, Sink Valley, collected from Kashmir, J&K by, Dr. Gh. Hassan Dar.	13442/4849 (20.12.1985) 13437/325 (20.12.1985) 13347/4728 (19.12.1985) 13435/392 (20.12.1985) 15559/4113 (27.09.1988) 19856/9199 (13.09.1994) 19851/9137 (13.09.1994) 19850/9138 (13.09.94)	08.05.1983 03.03.1981 08.05.1983 18.03.1981 21.06.1985 27.03.1987 06.04.1986 06.04.1986

Phenotypic attributes

The phenotypic attributes of four *Viola* species (*V. odorata, V. biflora, V. canescens* and *V. indica*) found in the wild collected from different sites of the Kashmir region were studied by considering 20 standardized trait descriptors, 11 qualitative (leaf shape, leaf margins, leaf apex, leaf base, the position of bracteoles on the peduncle, flower color, lateral petals, petals, sepals, spur shape and seed color) and 9 quantitative (averages of fresh weight, dry weight, plant height, root length, leaf length, leaf width, petiole length, spur length and number of flowers per plant) as per the classification for *Viola* species by Marcussen *et al*. (2022). These qualitative and quantitative traits were measured by randomly selecting ten competitive plants from each *Viola* species under study.

Data analysis

The mean of quantitative traits taken was used for data analysis. Principal coordinate analysis (PCoA) and cluster analysis for hierarchical classification were performed using DARwin (Dissimilarity Analysis and Representation for Windows) version 6.0.021 software (Perrier & Jacquemoud-Collet 2006). To enhance the effectiveness of Ward's method, certain data transformations were made. For normalization, the data was adjusted to a common side without making any changes in the

range of values. Before multivariate analysis, skewed data based on quantitative traits were transformed using the logarithm function. Prior to clustering, the data was standardized, especially where the variables were on different scales. This ensures that each variable contributes equally to the distance calculations. To construct dendrograms, Ward's minimum variance clustering method was used to classify *Viola* species in different groups (Sneath & Sokal 1973) using quantitative traits. In this method, squared Euclidean distances were used to accurately measure dissimilarities among sample data.

Results

Ethnomedicinal and phenotypic attributes

The *Viola* species are stoloniferous, perennial herbaceous flower-bearing plants, commonly found beneath tall coniferous trees in forests, under the bushes, shrubs, rocks, hedgerows and mostly in the sloppy shady sides of mountains or forests. In general, the morphology of *Viola* species revealed that their leaves may be simple, ovate, ovate-triangular, cordate, serrate, crenate, stalked, obovate to heart-shaped. Additionally, they may be fimbriate, lanceolate-ovate, dentate and stipulate. Its flowers are bisexual, zygomorphic and have five sepals and petals with attached spurs, besides having long petiole length comprising almost 50 to 65% of the total plant length. Further, it has a peduncle that is bracteolate and has cone-shaped anthers that cover the ovary. The ovary is usually sessile, with a thickened top, curved bottom style and a stigma that can be lobed, straight or beaked. The fruit has three valves and is adjusted like a capsule. The seeds are spherical, ovate, smooth and shining. It has a short stem with or without rhizomes and usually grows within a year or in months. During the present study, at least ten plants of each species of *Viola* (*V. odorata*, *V. biflora*, *V. canescens* and *V. indica*) collected from different sites of the Kashmir region were taken into consideration to study comparative qualitative and quantitative morphological traits (Table 4). The morphological observations made from these *Viola* species are:

Table 4. Qualitative delimiting traits of four *Viola* species found in the Kashmir region

Species/Traits	***Viola odorata***	***Viola biflora***	***Viola canescens***	***Viola indica***
Common name	Sweet Violet	Yellow Wood Violet	Himalayan White Violet	Indian Violet
Vernacular names in J&K (UT)	Nunposh, Banafsha	Gulposh Banafsha, Dundi-Birali	Gulbanafsha, Banafsha	Banafsha, Kudkuddi
Leaf shape	Ovate-cordate, orbicular, puberulous heart-shaped, hairy	Reniform, broadly cordate ovate, sub-orbicular, puberulous	Lanceolate, ovate, cordate, apex acute, acuminate, broadly canescent, obtuse, serrate-crenate, hairy	Aristate, with a spine-like tip, mostly deltoid-cordate, simple basal, ovate-lanceolate to ovate-oblong, hairy
Leaf margins	Entire-crenate, not hairy	Repand, serrate-crenate, dentate, forwarding, pointing, hairy or puberulous	Crenate, slightly serrate, hairy	Serrate-crenate, dentate with rounded teeth
Leaf apex	Acute	Obtuse	Apiculate	Acute-apiculate, sub-acuminate
Leaf base	Cordate	Auriculate	Auriculate	Auriculate
Position of bracteoles on peduncle	Mostly in the middle lower side	Predominantly at the upper side	Predominantly at the lower part with light purplish spots.	Predominantly at the upper part
Flower colour	Deep Purple, Dark violet	Mostly yellowish	Whitish blue or light purplish slightly white, pubescent	Light blueish white, with dark black striations
Lateral petals	Entire long, pinkish-green	Glabrous or greenish not hairy	Light purplish, not hairy	Light purplish with a whitish base
Petals	Crenate-obovate long with white striations, sub-equal	Oblong-obovate entire, purplish striations, glabrous, unequal	Obovate, obtuse, sub-cordate, mostly whitish, with light	Oblong-ovate, sub-equal, lateral petal largest and spurred

			purplish striations, sub-equal	
Sepals	Acute apex, lanceolate, equal shaped, purple-green, hairy	Apex acute, linear-lanceolate, equal, greenish, hairy	Acute, acuminate, lanceolate, ovate, obovate, hairy, pinkish green base, sub-equal	Ovate, oblong-lanceolate, auriculated base, ciliate, pinkish, sub-equal
Spur shape	Straight, slightly curved, obtuse, with a dark pinkish base	Straight, cylindrical, short, obtuse, yellowish base	Straight and slightly curved with a lilac purplish base	Straight, lilac-bluish white base
Seed colour	Greenish	Yellowish	Dark brown	Brownish

***Viola odorata*:** It is popularly known as sweet violet, nunposh or banafsha. From the ethnomedicinal perspective, *V. odorata* is commonly used in the form of expectorant, narcotic, diaphoretic, choleretic, antipyretic, antihypertensive and anti-inflammatory (Table 2). The morphology of this plant species reveals that the shape of its leaves is ovate-cordate, orbicular, puberulous or heart-shaped with long petioles comprising almost 50% to 65% of the total plant length. Its petiole length ranges from 4 to 11 cm. The leaf margins are entire-crenate without hairs, the leaf apex is acute and its base is cordate. The position of bracteoles is mostly on the lower side. Further, it possesses a deep purple flower with dark violet stripes, spur is usually straight, slightly curved 5-10 mm long, obtuse, with a dark-pinkish base. Petals are crenate-obovate, long with white striations and sub-equal. The seeds are greenish and the capsule is large, globose with scattered short hairs (Fig. 2, Table 4).

Figure 2. *Viola odorata*: a— Entire plant, b—Peduncle with flower, c—Bracteoles, d—Petals shape, e—Petal with strips, f—Flower with spur (dorsal), g—Petal with spur attached, h— Spur shape, i—Petiole, j—Sepals, k—Flower with spur orientation, l—Bracteole shape, m—Carpel, n—Anthers, o—Leaf tip, p— Flower orientation, q— Hairs on petals ventral side, r—Leaf with leaf venation (dorsal side), s—Leaf with its margins (ventral side)

***Viola biflora*:** It is popularly known as yellow wood violet, gulposh banafsha, or Dundi-Birali. From the ethnomedicinal perspective, *V. biflora* is used as diaphoretic, antipyretic, febrifuge, anticancer and its flowers are used to treat eczema, epilepsy and nervous-related disorders (Table 2). The morphology of this plant species reveals that the shape of its leaves is reniform, cordate, ovate, sub-orbicular and puberulous. It has small petioles whose length ranges from 2 to 10 cm. The leaf

margins are repand, serrate-crenate, dentate, forwarding, pointing, hairy or puberulous. The leaf apex is obtuse and the base is auriculate. The position of bracteoles is predominantly on the upper side. Further, it possesses flowers that are mostly yellowish with purplish stripes, spur is usually straight, cylindrical, short and obtuse with a yellowish base. Petals are oblong-obovate entire with purplish striations, glabrous and unequal. The seeds are yellowish, and the capsule is oblong-ovoid (Fig. 3, Table 4).

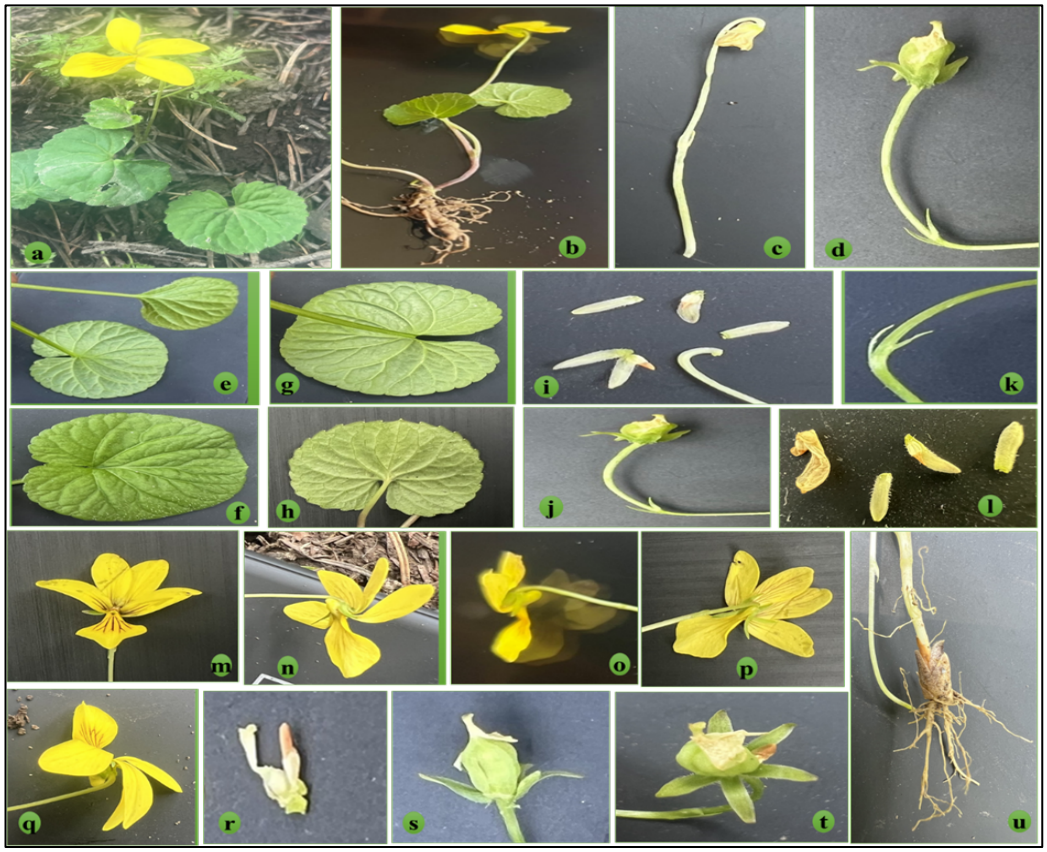

Figure 3. *Viola biflora*: a—Habit and habitat, b—Plant body with stolon, c—Peduncle, d—Capsule with sepals, e—Leaf (dorsal side), f—Leaf (ventral side), g— Leaf margins and its base, h—Venation, i—Sepals, j—Sepals with bracteoles, k—Bracteoles, l—Petals, m—Shape of petals (ventral side), n—Petals (dorsal side), o—Spur shape, p— Petals shape, q—Spur and petals, r— Carpel, s—Capsule, t—Capsule with sepals, u—Root

***Viloa canescens*:** It is popularly known as Himalayan white violet, gulbanafsha or simply banafsha. From the ethnomedicinal viewpoint, *V. canescens* is used as a purgative, astringent, demulcent, diaphoretic, antipyretic, febrifuge and anti-cancer agent (Table 2). The morphology of this plant species reveals that the shape of its leaves is lanceolate, ovate, cordate, apex acute, acuminate, broadly canescent, obtuse, serrate-crenate, hairy and has the largest leaf diameter about 5 to 10 cm. It has small petioles whose length ranges from 4 to 8 cm. The leaf margins are crenate, slightly serrated and hairy. The leaf apex is apiculate and the base is auriculate. The position of bracteoles is predominantly at the lower side with light purplish spots. Further, it possesses flowers that are mostly whitish blue, or light purplish white, pubescent and spur is straight and slightly curved with a lilac purplish base. Petals are obovate, obtuse, sub-cordate, mostly whitish, with light purplish striations and sub-equal. The seeds are dark brown and the capsule is globose, pubescent or glabrous (Fig. 4, Table 4).

Viola indica: It is popularly known as Indian violet, banafsha or kudkuddi. From the ethnomedicinal viewpoint, *V. indica* is used as a demulcent, expectorant, diaphoretic, antipyretic, anticancer and antimicrobial agent (Table 2). The morphology of this plant species reveals that the shape of its leaves is aristate, with a spine-like tip, mostly deltoid-cordate, simple basal, ovate-lanceolate to ovate-oblong, hairy and has the largest leaf diameter amongst the four *Viola* species under study. The petiole length ranges from 6 to 22 cm. Its stem is usually absent or acaulescent. The leaf margins are serrate-crenate or dentate with rounded teeth. The leaf apex is acute-apiculate or sub-acuminate and the base is auriculate. The position of the bracteoles is predominantly on the upper side. Further, it possesses flowers that are light blueish white with dark black striations at the upper side, spur is straight with a lilac bluish-white base. Petals are oblong-ovate, sub-equal, lateral petal largest and spurred. The seeds are brownish and the capsule is tri-valved, loculicidal and globose (Fig. 5, Table 4).

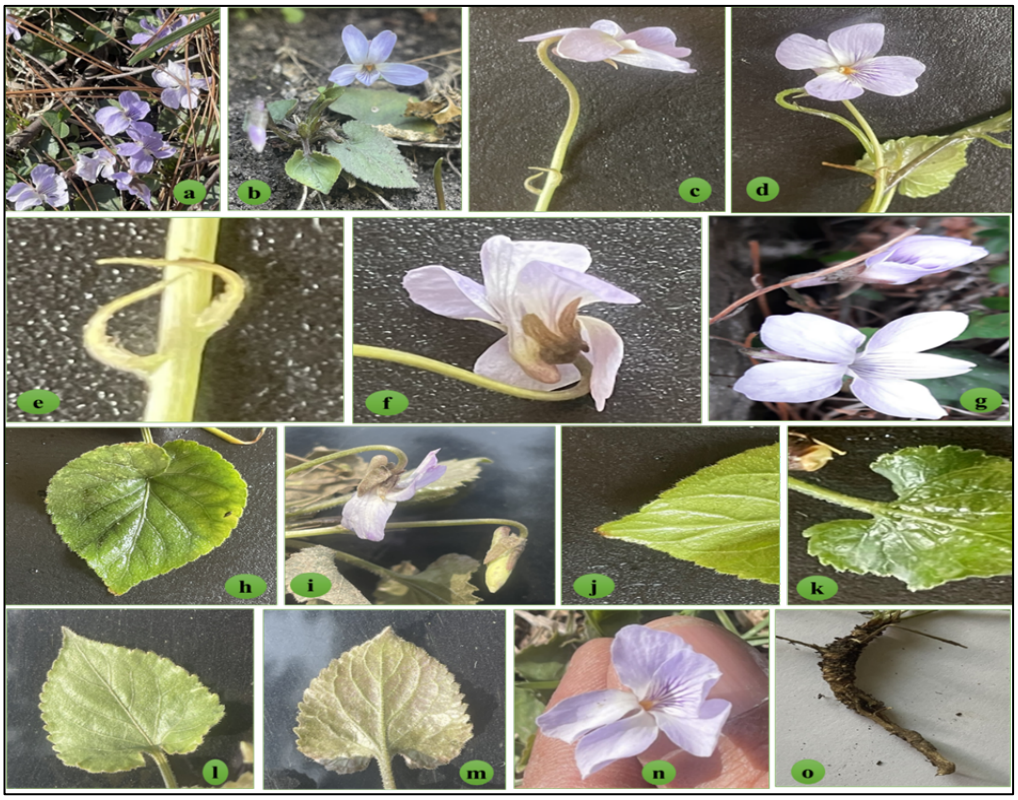

Figure 4. *Viola canescens*: a—Habit and habitat, b—Plant body with stolon, c—Peduncle with flower and bracteoles, d—Plant twig with flower, e—Bracteoles, f—Spur base, g—Flowers with petal orientation, h—Leaf venation, i—Spur shape, j—Leaf apex, k—Leaf base and margin, l—Leaf (ventral side), m—Leaf (dorsal side), n—Petals, o—Root

Figure 5. *Viola indica*: a & b— Entire plant body with stolon, c—Leaf (ventral side), d— Leaf (dorsal side) and venation, e—Petals & sepals, f—Leaf margins, g— Leaf tip, h— Sepals, i—Spur shape with petals and sepals, j—Leaf shape (ventral side), k—Petals with brownish carpels, l—Root, m—Seeds with elaiosomes

PCoA and cluster analysis of *Viola* species

Principal coordinate analysis (PCoA) is a powerful tool for exploring quantitative traits because it condenses complex, multivariate data into interpretable visual patterns, enabling clearer insights into the underlying biological structure and relationships among species. PCoA was performed on 9 important quantitative morphological traits of 4 *Viola* species (*V. odorata V. biflora*, *V. canescens* and *V. indica*) collected from different sites of the Kashmir region revealed that the most informative components accounted for 92.89% variance. It also presented the traits with greater contribution to the distribution of *Viola* species along the principal coordinate axes. Selected traits that contribute significantly to the distribution of four *Viola* species include averages of fresh weight, dry weight, plant height, root length, leaf length, petiole length, leaf width, spur length and number of flowers per plant. The principal coordinate analysis helps in the confirmation of segregation through cluster analysis as represented in Fig. 6.

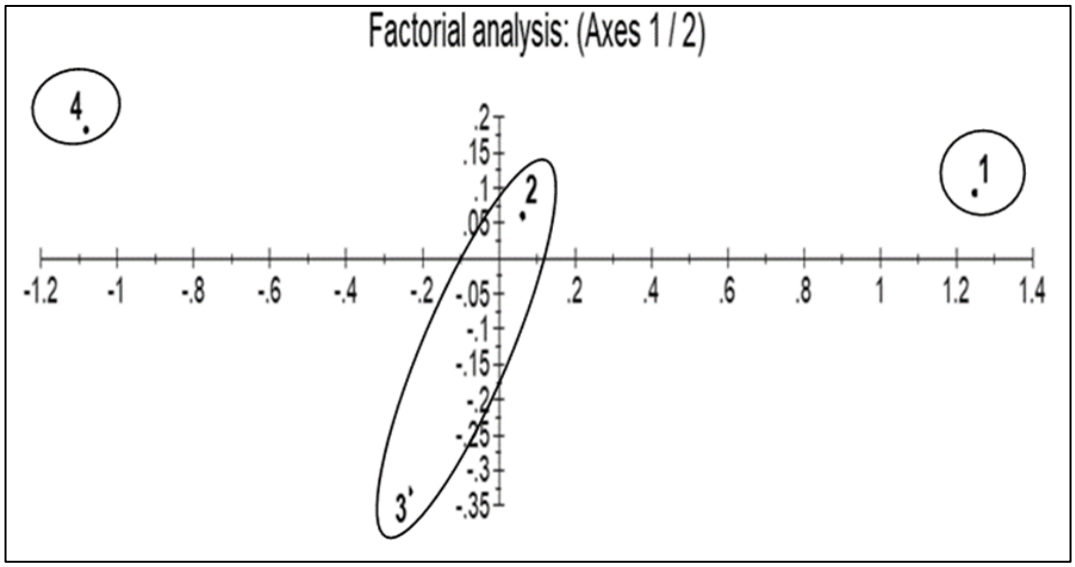

Figure 6. Principal coordinate analysis (PCoA) plot of 4 *Viola* species based on quantitative data

Further, cluster analysis using Ward's minimum variance technique classified the four *Viola* species into two major groups. Group I consist of exclusively the species of V. indica, whereas Group II comprises 3 species of *Viola* (*V. canescens*, *V. ordorata* L. and *V. biflora*). It was also observed that groups I and II were characterized based on significant differences in 9 important quantitative morphological traits analyzed among the four *Viola* species during the present investigation (Fig. 7).

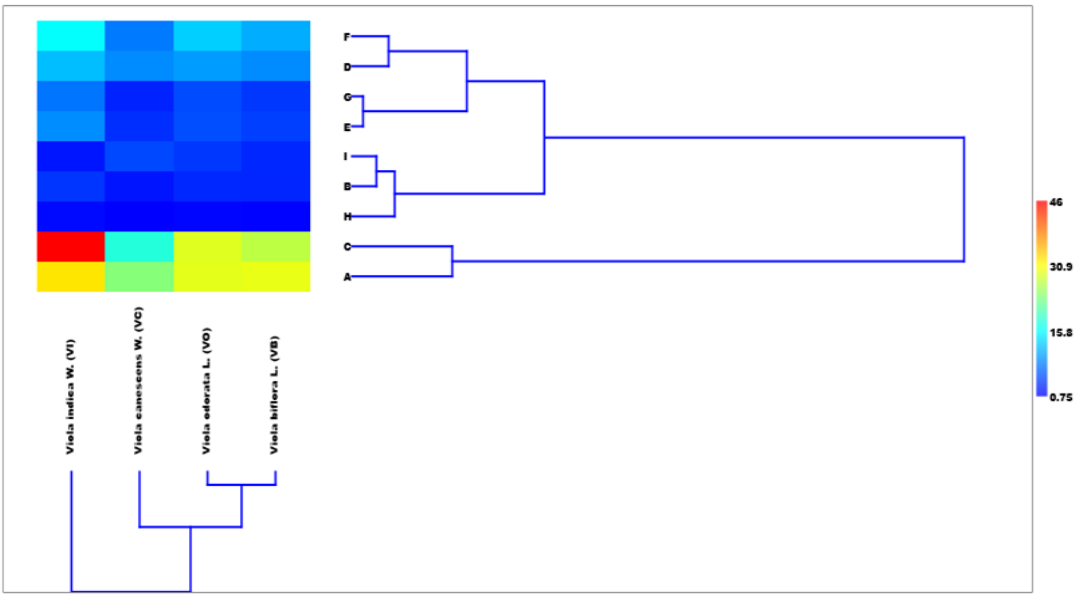

Figure 7. Ward's minimum variance dendrogram of 4 *Viola* species based on quantitative data

Discussion

Ethnomedicinal and phenotypic attributes

The present study highlights that phenotypic traits serve as a critical bridge between ethnobotanical knowledge and scientific characterization of medicinal plants in the Kashmir Himalayas. The comparative evaluation of four *Viola* species revealed that observable morphological characters, including leaf architecture, flower color, bracteole position, petal structure, spur morphology and seed color, are not merely taxonomic markers but also practical indicators used in traditional identification, selection and utilization of these species. The distribution of *Viola* species in the Kashmir Himalayan region has historical significance from the phenotypic and ethnomedicinal viewpoints as its species mostly inhabit diverse biogeographical ranges (Fig. 1). During the present study, the authors tried to assess the morphological traits and ethnomedicinal potential of the different species of *Viola* found in this region and to establish a genetic relationship amongst them. In addition, herbaria of four *Viola* species which include *V. odorata*, *V. biflora*, *V. canescens* and *V. indica* were also prepared and deposited as specimens in the Centre for Biodiversity & Taxonomy, Department of Botany, University of Kashmir, Srinagar for future studies and reference. Besides, it also helps in conserving the diversity of *Viola* species through comparative morphological, biochemical and genetic studies (Table 3). The present study revealed significant morphological differences and ethnomedicinal uses of *Viola* species found in the Kashmir region (Figs. 2-5, Table 2). In this region, *V. odorata* is commonly known as 'Nun-Posh'. Similarly, *V. canescens* is commonly known as 'banaksa', 'gulbanafsha' or 'banafsha' plant which is widely distributed. Besides, its presence has also been reported from Kashmir and Uttarakhand Himalayan regions at an altitude of 1600-2500 meters (amsl) (Rana *et al*. 2010, Fayaz *et al*. 2019). These morphological features enable them to survive under varied climatic and ecological conditions of these regions. *Viola* species have been extensively used in traditional medicine across the Kashmir region, where they are valued for their therapeutic properties (Table 2). Some of the vital ethnomedicinal applications of *Viola* species are: Infusions and decoctions made from the flowers and leaves of *V. canescens* are used to treat respiratory ailments such as colds, coughs, asthma, bronchitis and jaundice (Jan *et al*. 2009, Haq *et al*. 2011). The mucilaginous nature of the plant extracts helps soothe the respiratory tract (Gairola *et al*. 2014). Topical applications of *Viola* species such as *V. odorata* and *V. biflora* are common in treating skin disorders like eczema, acne and rashes (Batiha *et al*. 2023, Hamayun 2006). The plant's anti-inflammatory and antiseptic properties help reduce irritation and promote healing (Drozdova & Bubenchikov 2005, Rana & Samant 2011). *Viola* species like *V. odorata* are used to alleviate pain and inflammation. Similarly, decoctions made from the leaves and flowers of this plant are consumed to relieve headaches, joint pain and muscle stiffness, making them a popular remedy for arthritis and rheumatism (Sher *et al*. 2021). It has also been observed that the flowers of *V. biflora* are used to treat digestive issues such as constipation. Further, *Viola* extracts are believed to have mild laxative properties and help in maintaining digestive health (Batiha *et al*. 2023). *Viola* species such as *V. odorata* are also employed as a remedy for fevers. The plant's cooling properties are believed to help reduce body temperature and provide relief from febrile conditions (Jafari *et al*. 2018). Herbal teas made from many *Viola* species are consumed to enhance immunity and protect against various types of infections. One such study conducted in the Khumbu region of Nepal, a neighboring Himalayan area, highlighted the presence of 12 highly valuable *Viola* species with significant medicinal potential (Kaur *et al*. 2023). The bioactive compounds in the plant are thought to have antioxidant and immunomodulatory effects (Batiha *et al*. 2023, Kaur *et al*. 2023). *Viola betonicifolia* is used in traditional medicine for its diuretic properties, helping in the treatment of urinary tract infections and kidney stones by promoting urine flow and cleansing the urinary system (Husain *et al*. 2008). The whole plant of *V. canescens* is traditionally used to treat fever, cancer and pain-related issues (Masood *et al*. 2014, Batiha *et al*. 2023). *V. indica*, commonly known as the Indian violet, is a species of the genus *Viola* that is native to various parts of Asia, including India, Pakistan, China and Japan (Sher *et al*. 2021). This plant has been used in traditional medicine by various indigenous communities for centuries, and its morphological characteristics have been the subject of extensive study by botanists and phytochemists. Different plant parts of *V. indica* are used as demulcent, diaphoretic and antipyretic. It is also used as a potent remedy against various types of throat infections, cough and lung infections. Additionally, it has been widely used in ethnomedicine to treat a variety of ailments, ranging from gastrointestinal disorders to skin infections. The plant's therapeutic properties can be attributed to its diverse phytochemical composition, which includes a range of secondary metabolites such as flavonoids, terpenoids and alkaloids (Chandra *et al*. 2015). The morphological and ethnomedicinal data on *Viola* species from this study is essential for phylogenetic classification and medicinal uses in both traditional and modern systems of medicine. This study will help in the restoration and sustainable use of *Viola* species in terms of their geographical distribution and therapeutic values.

Similar kind of morphological assessments were observed in the case of *V. biflora*, *V. canescens*, *V. odorata*, *V. pilosa* Blume, *V. rupestris* F.W. Schmidt, *V. suavis* M. Bieb. collected from diverse geographical sites in Pakistan (Sher *et al*. 2021). This may be attributed to the similar type of ecological conditions inhabited by the *Viola* species as revealed during the present study. Many studies revealed that environmental factors, such as altitude, temperature and soil composition may significantly influence the phenotypic variation observed among *Viola* species in the Kashmir Himalayas, driving differences in

morphological traits and phytochemical profiles that enhance their adaptability and therapeutic potential (Kaundal *et al*. 2022). Likewise, Mittal *et al*. (2015) reported similar morphological features and pharmacological activities, while assessing the species of *V*. *odorata*. Further, morphological and taxonomical descriptions of different *Viola* species were validated using available monographs and herbarium specimens. In addition, various species of *Viola* were individually examined for trait analysis. The morphological description of *Viola* species helps in understanding group relationships, rhizome, stem, or stolon habits, stipule size and shape, leaf lamina, margins features, calycine appendage size, shape, patterns of sepals, petals, spur size and shape, style features, capsule behavior and the capacity to produce flowers seasonally (Ballard 2022). The genus *Viola* is widely distributed in temperate zones of both hemispheres and at high elevations in tropical mountain ranges (Menegoz *et al*. 2024). Besides, Masood *et al*. (2014) reported a similar kind of morphological and taxonomic assessment in the case of *V. canescens*, which is found in high altitudinal regions of Nepal, Bhutan, India and Pakistan. In these regions, *Viola* species are also utilized as herbal medicine to treat various chronic ailments (Batiha *et al*. 2023, Kaundal *et al*. 2024). In ethnobotanical systems, local communities often distinguish medicinal plants through visible phenotypic traits, which directly influence folk taxonomy, harvesting preferences and therapeutic applications. The distinct vernacular recognition of *Viola* species such as *V*. *odorata*, *V*. *canescens*, *V*. *biflora*, and *V*. *indica* in the Kashmir region reflects how morphology shapes indigenous plant knowledge and species level discrimination.

The significant qualitative and quantitative phenotypic variation observed among the studied *Viola* species suggests that these traits are valuable for authenticating medicinal taxa and preventing substitution or adulteration in traditional herbal practices. For example, variations in flower pigmentation, leaf margins, petiole dimensions and spur structure provide reliable descriptors for field identification, especially where multiple species are traded under similar local names such as 'Banafsha'. Such differentiation is essential because each species possesses distinct ethnomedicinal roles, ranging from respiratory relief and anti-inflammatory applications to febrifuge and antimicrobial uses. Therefore, phenotypic characterization strengthens the scientific basis of ethnomedicinal documentation by linking species specific morphology with therapeutic practices.

Recent phylogenetic studies have shed new light on the evolutionary relationships within the genus *Viola*, providing valuable insights into the origins and diversification of these fascinating plants. The Latin American sections of *Viola* are considered to be basal, with some researchers suggesting an Andean origin for the genus and subsequent radiations into other regions (Ballard 2022, Marcussen *et al*. 2022, Menegoz *et al*. 2024). This finding challenges the long-held belief that *Viola* originated in the Northern Hemisphere, highlighting the importance of incorporating diverse geographic regions into our understanding of plant evolution.

Within the genus, certain species have garnered particular attention due to their widespread distribution and historical significance. *V*. odorata, for instance, was shown to have some geographically structured variation in Western Eurasia, with similarities between Tenerife and continental populations (Marcussen 2006), underscoring the complex interplay between human activities and the genetic diversity of wild plant populations. Equally, intriguing are the closely related species *V*. *elatior*, *V*. *pumila* and *V*. *stagnina*, which share a continental distribution centered in the temperate zone of eastern Europe and western Siberia (Marcussen *et al*. 2022). Environmental heterogeneity across the Kashmir Himalayan landscape likely contributes to the phenotypic plasticity documented in this study. Altitudinal gradients, habitat variability and climatic factors can influence morphological adaptations, which in turn may alter phytochemical profiles and medicinal potency. This relationship between ecological adaptation and phenotypic expression is particularly relevant in ethnobotany, where local populations may preferentially select certain phenotypes perceived as more efficacious. Thus, the study supports the idea that phenotypic traits not only reflect taxonomic diversity but may also shape ethnomedicinal value through environment driven variation.

PCoA and cluster analysis of *Viola* species

The application of multivariate tools such as PCoA and cluster analysis further demonstrated that phenotypic descriptors can effectively reveal interspecific relationships and diversity patterns. Principal coordinate analysis (PCoA) performed on the four species of *Viola* (*V. odorata*, *V. biflora*, *V. canescens* and V. indica) based on the 9 quantitative traits revealed their distribution pattern in a 2D scatter plot. The distribution pattern of two *Viola* species (*V. odorata* and *V. biflora*) formed a single group that shows close affinity with the *V. canescens* along the 2D scatter plot. Contrary, V. indica forms a separate group along the 2D scatter plot confirming its significant morphological variation from the other *Viola* species during the present study (Fig. 6). Cluster analysis further confirmed the scattering and distribution patterns determined by PCoA. These morphological variations may be attributed to the diverse ecological ranges inhabited by the four *Viola* species under study. Similarly, cluster analysis based on Ward's minimum variance indicated that *V. odorata* and *V. biflora* exhibited similarity

with *V. canescens* and thus formed a single group, while V. indica shows a marked difference with the other species and therefore formed a separate group as depicted by the dendrogram (Fig. 7). The pattern obtained through cluster analysis confirmed by two-dimensional scatter plot of principal components analysis accounted for a significant portion of the cumulative variance, indicating that morphological and biochemical traits were useful in preliminary evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable *Viola* genotypes (Kumar *et al*. 2019, Marcussen *et al*. 2022).

The findings of this study are consistent with previous research on the application of multivariate analyses, including principal component analysis and cluster analysis, to assess patterns of morphological variation in plant species. For example, similar approaches have been used to characterize phenotypic diversity in buckwheat (Agbolade & Komolafe 2016), aloe (Khan *et al*. 2011) and minor legumes (Khattak *et al*. 1985). The clear segregation of *V. indica* from the remaining species indicates its distinctive morphological identity, which may correspond to unique medicinal properties or ecological adaptations. Such approaches provide a framework for integrating traditional plant knowledge with modern biodiversity assessment, offering valuable insights for conservation biology, phylogenetic studies and herbal pharmacology. Further, this research highlights the utility of these statistical techniques in understanding the genetic relationships and evolutionary trajectories of closely related plant taxa.

From an ethnobotanical perspective, documenting phenotypic diversity is essential for preserving indigenous knowledge systems, particularly in biodiversity rich yet vulnerable Himalayan ecosystems. The continued use and overharvesting of medicinal *Viola* species necessitate conservation strategies that recognize both biological diversity and cultural significance. By combining ethnomedicinal documentation with phenotypic trait analysis, the present study contributes to a more comprehensive understanding of how plant morphology underpins traditional healthcare systems and supports sustainable resource management.

Overall, the findings emphasize that phenotypic traits are fundamental tools in ethnobotany, enabling accurate species identification, supporting traditional medicinal practices, guiding pharmacological exploration and strengthening conservation priorities. Integrating phenotypic characterization with ethnobotanical research enhances the scientific credibility of indigenous medicinal knowledge while creating pathways for sustainable utilization and future therapeutic discovery.

Conclusion

Based on the present study, it can be concluded that the four *Viola* species from Kashmir Himalayan region have potent ethnomedicinal properties to treat various chronic diseases, due to the formation of diverse bioactive compounds as these plants inhabit high altitudinal ranges. Besides, there is a noticeable phenotypic variation revealed by the four *Viola* species (*V. odorata*, *V. biflora*, *V. canescens* and *Viola_indica*) observed from the different sites of the Kashmir Himalayan region. Both qualitative (leaf shape, margins, position of bracteoles, sepals, petals, spur shape, seed color, capsule etc.) and quantitative (averages of fresh weight, dry weight, plant height, root length, leaf length, petiole length, leaf width, spur length and the number of flowers/plant) trait descriptors indicated significant phenotypic differences among the four *Viola* species and with the help of principal coordinate and cluster analysis segregated them into two major groups in which V. indica formed one group and the other three species (*V. odorata*, *V. biflora*, *V. canescens*) the second group as indicated by the similarities in the qualitative and quantitative traits. The differences in their phenotype can be attributed to the type of diverse biogeographical ranges and environmental factors inhabited by these species. Therefore, it can also be concluded that effective conservation strategies for *Viola* species in the Kashmir Himalayan region should integrate *in-situ* habitat protection, *ex-situ* germplasm preservation, sustainable harvesting practices and community-led management to preserve their genetic diversity and traditional medicinal knowledge. The present study of *Viola* species helps not only in conserving their diversity across the diverse biogeographical ranges but also opens new ways in their potent application in herbal pharmaceuticals, reinforcing the importance of integrating traditional knowledge with modern science.

Declarations

Ethics approval and consent to participate: Not Applicable
Consent for publication: Not applicable
Availability of data and materials: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.
Competing interests: The authors declare no competing interests.

Funding: No funding or grant was received for any source for this entire study.

Author contributions: **F.A.** collected the species samples from different sites of the Kashmir region, prepared the map showing the species point areas and prepared the manuscript. **F.A.D.** performed the data analysis, edited and contributed to the writing and final preparation of the manuscript. **E.H.** conceived the problem and critically reviewed the manuscript and **R.U.R.** helped in formulating the experimental design, revision and approved the final version of the manuscript.

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