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Ethnobotanical, Phytochemical, and Cytomorphological Evaluation of some *Viola* Species from Riparian Vegetation along the Beas River of Himachal Pradesh

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KEYWORDS

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ABSTRACT:

Introduction: Viola, commonly known as violets, is a genus of flowering plants belonging to the family Violaceae. It is the largest genus of the family, encompassing more than 695 recognized species.

Objectives: The present study evaluated the ethnobotanical, cytogenetic, and phytochemical characteristics of Viola biflora, Viola canescens, and Viola serpens from riparian habitats along the Beas River, Himachal Pradesh.

Methods: Information was collected through simple interviews, open-ended questionnaires, friendly discussions, and field walks where informants showed the plants directly in their natural habitats.

Results: Ethnobotanical surveys revealed that these species are traditionally used by local communities to manage respiratory ailments, inflammation, fever, cough, and cold. Cytogenetic analysis demonstrated stable diploid chromosome numbers in V. biflora and V. canescens (2n = 12), whereas V. serpens exhibited two cytotypes (2n = 12 and 2n = 16), reflecting intraspecific chromosomal variability. Meiotic behavior was largely regular, resulting in high pollen fertility across species. Phytochemical profiling using GC–MS of methanolic extracts of V. serpens from two populations revealed significant inter-population variation. The Mandi population showed fifteen metabolites dominated by n-hexadecanoic acid (28.75%) and 10-undecyn-1-ol (26.97%), whereas the Kullu population exhibited thirty-two compounds, with 9,12,15-octadecatrienoic acid, methyl ester (19.57%), 9,12,15-octadecatrienoic acid (11.44%), and n-hexadecanoic acid (10.63%) as major constituents, indicating greater chemical diversity.

Conclusions: The findings highlight the ethnobotanical importance, cytogenetic stability, and phytochemical richness of these species, providing a basis for conservation, pharmacological research, and the sustainable utilization of Himalayan Viola species

1. Introduction

Viola, widely referred to as violets, represents a genus of flowering plants that belongs to the family Violaceae. It represents the largest genus within the family, comprising more than 695 recognized species [1]. Most species occur primarily in the temperate regions of the Northern Hemisphere, but some also extend into geographically distant areas, including Hawaii, Australasia, and the Andes [2]. *Viola* species may be annual or perennial in nature and are predominantly herbaceous, although a few species can grow as shrubs and, in very rare cases, as small tree-like forms [3]. The family is largely composed of perennial herbs and shrubs, and its members typically have simple leaves that may be arranged alternately or oppositely, often showing palmate or deeply lobed patterns. The flowers are bisexual and may be zygomorphic or actinomorphic, with five sepals and five petals, the front petal being larger and spurred. The androecium has five stamens, and the gynoecium has three fused carpels with a superior ovary. The fruit is a capsule, and the family has limited economic importance [4].

Viola species represent an important group of medicinal plants widely distributed across temperate and sub-temperate regions of the world. In the riparian region of Himachal Pradesh, species such as *Viola biflora*, *Viola canescens*, and *Viola serpens* are traditionally used for treating respiratory ailments, skin infections, cold and cough, astringent, demulcent, febrifuge, purgative, diaphoretic, and inflammatory conditions, and various

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other disorders [5]. Their rich ethnobotanical value has been documented across different communities, which utilize aerial parts, roots, and floral components for preparing herbal remedies. Despite their long-standing traditional use, scientific investigations on their phytochemical constitution and cytomorphological characteristics remain limited, particularly in the context of Himalayan riparian ecosystems [6].

Riparian vegetation, forming a transitional zone between terrestrial and aquatic environments, supports unique ecological conditions that influence plant diversity, morphology, and chemical composition. Himachal Pradesh supports a diverse assemblage of medicinal flora, including several *Viola* species that thrive in the cool, moist, and shaded microhabitats typical of riparian zones [7]. These environmental gradients may induce variations in morphological traits, cytological behavior, and secondary metabolite accumulation in plant species, making riparian populations important for comprehensive biological investigations [8].

Viola species are recognized for containing a diverse range of bioactive phytochemicals, including rutin, coumarins, saponins, and diverse phenolic antioxidants [9]. However, comparative phytochemical evaluations of different *Viola* species growing within the same ecological habitat are still limited. Cytomorphological studies—including chromosome counts, meiotic behavior, pollen fertility, and morphological variation—also play an important role in understanding species differentiation and genetic diversity [10]. In the present investigation, *Viola biflora* and *Viola canescens* exhibited a chromosome number of 2n = 12, while *Viola serpens* displayed two cytotypes with 2n = 12 and 2n = 16.

Given the ecological significance and medicinal relevance of *Viola* species, there is a need for integrated assessments that combine ethnobotanical documentation with phytochemical and cytomorphological analyses. The present study evaluates *Viola biflora*, *Viola canescens*, and *Viola serpens* collected from various regions of Himachal Pradesh. By documenting traditional uses, analyzing phytochemical constituents, and examining cytomorphological features, this work provides a comprehensive understanding of the biological potential and genetic diversity of these species. Such information is valuable for conservation, sustainable utilization, and future pharmacological research on Himalayan *Viola* species.

2. Materials and Methods

2.1 Study area

The present research was carried out in the riparian zone of the Beas River in Himachal Pradesh, India. Extending for approximately 460 km, it originates from the Beas Kund near Rohtang Pass at an elevation of about 3,700 meters in the Pir Panjal range. In Himachal Pradesh, the river extends for about 256 km, whereas in Punjab it flows for nearly 214 km. Flowing southward through the districts of Kullu, Mandi, Hamirpur, and Kangra, the river traverses diverse terrains, including alpine zones, dense forests, agricultural valleys, and wide floodplains. Its course forms a dynamic riparian ecosystem that supports rich plant and animal diversity.

2.2 Ethnobotanical studies

The ethnobotanical study was carried out to record how local people used *Viola* species for traditional healing. Informants such as elderly villagers, traditional healers, women, and shepherds were selected through snowball sampling because they had good knowledge of local plants. Information was collected through simple interviews, open-ended questionnaires, friendly discussions, and field walks where informants showed the plants directly in their natural habitats. Details on local names, useful plant parts, methods of preparation, ways of administration, and diseases treated were noted carefully. Plant specimens were collected, pressed, dried, and identified with the help of floras and experts, and voucher samples were kept in the herbarium for future reference. Throughout the study, prior informed consent was taken, and ethical guidelines were followed to respect the knowledge and privacy of local communities.

2.3 Cytological studies

Flower buds at suitable meiotic stages were collected early in the morning and fixed in Carnoy's solution (ethanol: chloroform: acetic acid, 6:3:1) for 24 hours. The fixed buds were stored in 70% ethanol until use. Anthers were squashed in 1% acetocarmine to prepare temporary slides for observing meiotic stages. Chromosome counts, meiotic configurations, and abnormalities were examined under a light microscope, and photomicrographs were captured for documentation.

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Pollen fertility was assessed using glycerol–acetocarmine staining, scoring well-stained and uniformly shaped pollen as fertile.

2.4 Phytochemical studies

2.4.1 GC-MS analysis

The extracts were analyzed using a Shimadzu QP-2010 Ultra GC–MS system operated in splitless mode. The instrument ran at 61.3 kPa with a linear velocity of 36.7 cm/sec, maintaining a purge flow of 3.0 mL/min and a split ratio of 10:0, resulting in a total flow of 14.0 mL/min and a column flow of 1.0 mL/min. The interface and ion source temperatures were set to 260 °C and 200 °C. The oven program began at 70 °C, with the injector temperature at 250 °C, and a solvent cut-off of 4.50 minutes. Compound concentrations were calculated from individual peak areas relative to the total chromatogram, and identification was performed by matching mass spectra to the NIST library.

3. Results

3.1 Ethnobotanical studies

In the Himalayan region, certain native herbs have long been valued for their medicinal and therapeutic properties. Among these, *Viola biflora*, *Viola canescens*, and *Viola serpens* are traditionally used by local communities to treat respiratory ailments such as cough, cold, bronchitis, and asthma, owing to their expectorant and soothing effects. Decoction, paste, and extract prepared from their leaves, flowers, and roots are also applied for skin infections, wounds, and inflammatory conditions. Additionally, these species are used as febrifuges, diuretics, and analgesics, and are incorporated into herbal remedies for digestive and urinary disorders. Their widespread traditional use highlights their ethnobotanical importance and potential for pharmacological investigation (Table 1).

Table 1: Medicinal uses, chromosome number, and pollen fertility of some *Viola* species

S.No.	Name	Habit	Parts used	Medicinal uses	Chromosome number	Pollen fertility (%)
1.	*Viola biflora* L.			The whole plant is traditionally used in local healthcare systems for its antiseptic and antispasmodic properties and is administered to manage colds, coughs, fever, and several skin disorders such as psoriasis and leucoderma. In folk medicine, the plant is also used as a laxative and emetic. Various preparations are made depending on the ailment: whole-plant decoctions or infusions are taken for respiratory complaints and fever, while pastes of the fruits mixed with water are consumed to alleviate excessive sweating and intestinal pain.	12	96.45
2.	Viola canescens Wall.			The whole plant of canescens is widely valued in traditional medicine. Local communities commonly use it for treating cough, malaria, cold, fever, and flu, and it is also administered for its reputed anticancer properties. The plant is generally prepared as a decoction or herbal infusion, where the dried or fresh plant material is boiled in	12	97.29

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				water and consumed orally. In some regions, the powdered form of the entire plant is mixed with warm water or honey and taken to relieve respiratory ailments.		
3.	*Viola serpens* Wall	Herb	Whole plant	The whole plant is widely used in traditional medicine as an antipyretic, demulcent, diaphoretic, and mild diuretic. It is taken for asthma, cough, fever, headaches, constipation, bleeding piles, throat ailments, and skin diseases. In local practice, the dried plant is powdered and consumed with warm water or honey, or prepared as a decoction for respiratory and febrile conditions. Plant paste or extract is also applied externally for skin disorders, reflecting its broad therapeutic use in folk healthcare.	12, 16	97.17

3.2 Cytomorphological analysis

3.2.1 *Viola biflora* L.

It is a perennial, rhizome-based herb with round to kidney-shaped leaves and one or two yellow flowers, sometimes marked with purple-brown streaks. It grows in shaded rocky or grassy sites and produces a capsule fruit that splits to release seeds. Flowering and fruiting occurred in June–July.

Meiotic observations showed that the species possessed a chromosome number of 2n = 12 (Fig. 1/1b) in its pollen mother cells at different meiotic stages, aligning with earlier findings from India as well as other regions. However, chromosome numbers such as 2n = 18, 20, and 35 have also been documented for this species in various parts of the world. The meiotic progression appeared regular, and the species exhibited a high pollen fertility rate of 96.45%.

3.2.2 *Viola canescens* Wall.

It is a perennial herb with a very short or absent stem and a branched, cylindrical rootstock. Its leaves are ovate-cordate to nearly reniform, serrated, and covered with grey hairs. The plant bears hermaphroditic, zygomorphic flowers that are pale violet or white with dark streaks on the lower petal; the lower petal also forms a short, compressed spur. It is a medicinally important species growing in moist, shaded Himalayan habitats. Flowering and fruiting occurred from May to July.

Both accessions showed a stable chromosome count of 2n = 12 during metaphase I (Fig. 1/2b), aligning with earlier findings from India as well as other regions. The meiotic divisions progressed normally, and the accessions exhibited a high level of pollen fertility (97.29%).

3.2.3 *Viola serpens* Wall

It was a small perennial herb with a short, partly underground rootstock and fibrous roots. Basal leaves formed a rosette and were cordate to ovate with finely serrated margins. The plant bore solitary white to pale lilac flowers on slender pedicels. Its globose to ellipsoid capsule contained several seeds that were released on dehiscence. Flowering and fruiting occurred from April to September.

Both accessions examined in the present study showed chromosome numbers, 2n = 12 (Fig. 1/3b) and 2n = 16 (Fig. 1/3c), which were consistent with the reports of Kishore (1951) from India. Earlier studies from the country also documented this species with chromosome numbers of 2n = 18 and 2n = 48. Meiosis proceeded normally, resulting in a high pollen fertility of 97.17%.

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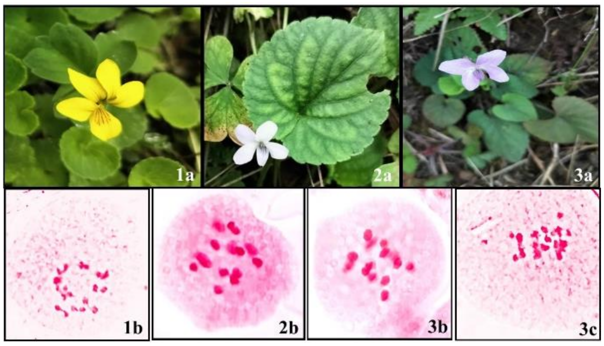

Fig 1: Showing Chromosome number. **(1a)** ***Viola biflora***, **(1b)** Metaphase-I showing 6_{II}, **(2a)** ***Viola canescens***, **(2b)** Metaphase-I showing 6_{II}, **(3a)** ***Viola serpens,*** **(3b)** Metaphase-I showing 6_{II}, **(3c)** Metaphase-I showing 8_{II}.

3.3 Phytochemical analysis of *Viola serpens* Wall

GC–MS analysis of methanolic extracts of the whole plant of two *Viola serpens* populations showed clear phytochemical variation. The TICs of both groups contained multiple peaks, with the Mandi plants showing fifteen peaks and the Kullu plants showing thirty-two, indicating higher chemical diversity in the Kullu population. In Mandi samples, major metabolites were n-Hexadecanoic acid (28.75%), 10-Undecyn-1-ol (26.97%), Neophytadiene (7.07%), Hexasiloxane (5.97%), and Heptasiloxane (4.99%) (Table 2, Fig. 2). The Kullu population contained a broader range of compounds, dominated by 9,12,15-Octadecatrienoic acid, methyl ester (19.57%), 9,12,15-Octadecatrienoic acid (Z, Z, Z) (11.44%), n-Hexadecanoic acid (10.63%), Linoelaidic acid (6.83%), and Hexadecanoic acid, methyl ester (2.27%) (Table 3, Fig. 2). Minor constituents included Neophytadiene (1.45%), Hexasiloxane (2.93%), Silicic acid (0.54%), and Ethoxy(phenyl)silanediol, 2TMS (0.44%). Overall, both populations shared some metabolites, but the Kullu plants displayed greater chemical diversity, likely influenced by ecological or geographical factors.

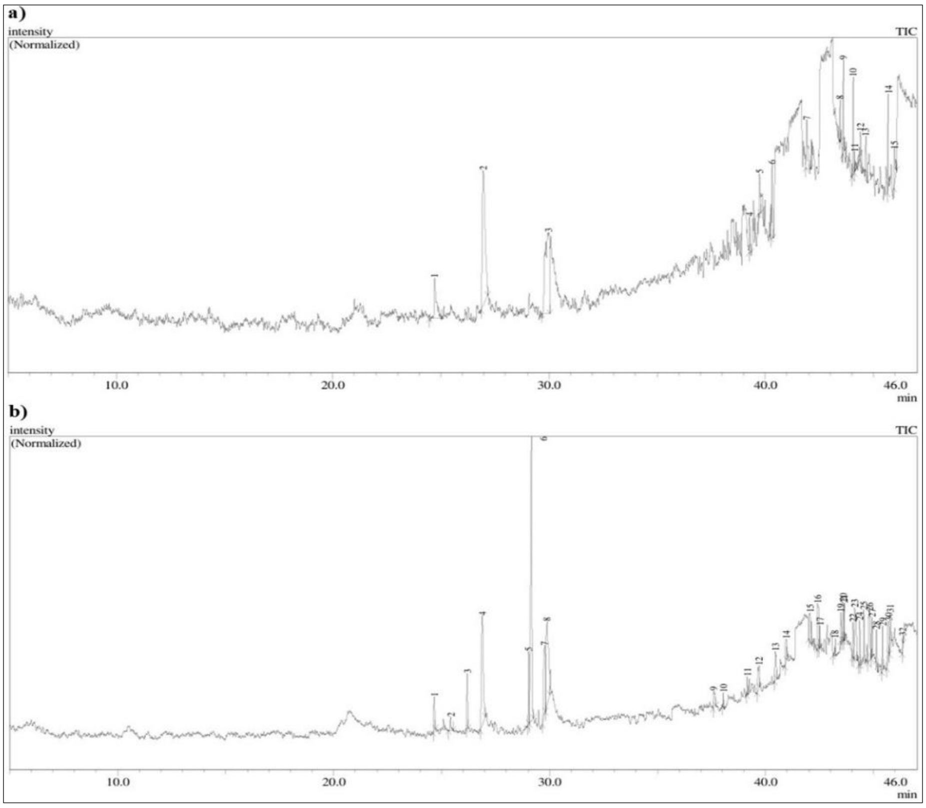

Fig 2: GC–MS profiles of whole-plant methanolic extracts of two populations of *Viola serpens*, showing differences in peak number and phytochemical diversity.

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Table 2: GC–MS analysis of methanolic extracts of *Viola serpens* populations from Mandi and Kullu showing identified phytochemical compounds and their relative percentages

Peak	Retention time	Height %	Area %	Name
1	24.721	4.48	7.07	Neophytadiene
2	26.957	14.93	28.75	n-hexadecanoic acid
3	29.997	9.01	26.97	10-Undecyn-1-ol
4	39.268	3.98	1.83	5,9,13,17-Tetramethyl 4,8,12,16-octad
5	39.727	4.38	2.62	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
6	40.310	8.14	5.97	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
7	41.900	5.28	4.99	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
8	43.444	4.56	1.12	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
9	43.601	10.33	3.72	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
10	44.052	10.89	5.12	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
11	44.125	2.14	0.63	Ethylhomovanillate, TMS derivative
12	44.392	3.45	2.20	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-deca
13	44.633	3.97	2.44	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
14	45.668	10.61	3.83	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
15	45.958	3.87	2.74	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-styrylhexadecahydrocyclopenta

Table 3: GC–MS analysis of methanolic extracts of *Viola serpens* populations from Kullu showing identified phytochemical compounds and their relative percentages.

Peak	Retention time	Height %	Area %	Name
1	24.656	2.25	1.45	Neophytadiene
2	25.416	0.89	0.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
3	26.184	3.64	2.27	Hexadecanoic acid, methyl ester
4	26.874	7.26	10.63	n-Hexadecanoic acid
5	29.028	4.84	3.12	9,11-Octadecadienoic acid, methyl ester
6	29.153	18.88	19.57	9,12,15-Octadecatrienoic acid, methyl ester
7	29.767	4.61	6.83	Linoelaidic acid
8	29.876	5.60	11.44	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
9	37.601	1.11	1.33	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
10	38.033	0.95	0.36	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11

11	39.151	1.20	0.41	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
12	39.696	1.52	2.02	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
13	40.449	1.92	1.92	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
14	40.939	1.58	1.55	Silicic acid, diethyl bis(trimethylsilyl) ester
15	42.051	1.92	2.58	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11
17	42.503	1.47	0.49	Silicic acid, diethyl bis(trimethylsilyl)
19	43.479	1.91	1.48	Heptasiloxane, hexadecamethyl
32	46.350	0.77	0.44	Ethoxy(phenyl)silanediol, 2TMS

4. Discussion

The ethnobotanical findings of the present study show a strong agreement with earlier research conducted across the Western and Central Himalaya. Previous studies reported that *Viola canescens*, *V. biflora*, and *V. serpens* were widely used by Himalayan communities for managing respiratory ailments such as cough, cold, bronchitis, and asthma, which supports the observations recorded in this study [5,11,12]. The use of leaf and flower pastes of *V. canescens* and *V. biflora* for skin infections, wounds, and inflammatory disorders has also been documented in earlier ethnobotanical surveys from Himachal Pradesh and Uttarakhand, indicating similar therapeutic patterns across regions [13,14]. The convergence of the present results with previous ethnobotanical studies underscores the cultural and medicinal importance of these species across Himalayan communities. Moreover, the established antioxidant and antimicrobial activities reported for *Viola* extracts strengthen the argument for further pharmacological validation and bioactive-compound isolation. Considering increasing demand and habitat pressures, conservation measures, including tissue-culture propagation techniques recently developed for *V. canescens*, are crucial for ensuring sustainable availability of these medicinally significant species. Overall, the agreement between current observations and earlier studies highlights the therapeutic potential of *Viola* species and reinforces their relevance in traditional healthcare systems of the Himalaya.

The cytogenetic findings of the present study closely correspond with earlier reports on the genus *Viola*. The diploid chromosome number observed in *V. biflora* ($2n = 12$) agrees with the consistent diploid cytotype documented across Europe and Asia, indicating that this species maintains remarkable chromosomal stability throughout its distribution range [15]. Similarly, the stable diploid count of $2n = 12$ recorded for *V. canescens* matches previous cytological studies from India and adjoining Himalayan regions, which also reported regular meiotic behaviour and a uniform diploid configuration [16]. In contrast, *V. serpens* exhibited two cytotypes ($2n = 12$ and $2n = 16$) in the present investigation, a pattern consistent with earlier Indian studies that documented a wider cytological spectrum, including $2n = 18$ and $2n = 48$ [17]. Such numerical variation within *V. serpens* and other Himalayan *Viola* species has been attributed to polyploidy, aneuploidy, or localized chromosomal differentiation, reflecting the cytogenetically dynamic nature of the genus [18]. Overall, the present cytological data support the existence of a stable diploid condition in *V. biflora* and *V. canescens*, while reaffirming the cytotype variability characteristic of *V. serpens* across its Himalayan range.

The phytochemical variation observed between the two *Viola serpens* populations in the present study is consistent with earlier reports showing that Viola species often exhibit significant chemical diversity across different ecological zones. Previous GC-MS-based investigations on Himalayan *Viola* species also documented variability in fatty acids, terpenoids, and phenolic derivatives, with compounds such as n-hexadecanoic acid, methyl linolenate, and neophytadiene commonly reported but occurring in different concentrations depending on locality and environmental conditions [19]. Similar population-level differences were noted in GC–FID and essential-oil analyses of *Viola*

species, where altitude, soil chemistry, and microclimatic factors were identified as major drivers of metabolite fluctuations [14]. These findings parallel the current results, where the Kullu population exhibited higher chemical richness than the Mandi population, suggesting that geographical or ecological factors may contribute to the observed metabolite diversity in *V. serpens*.

Conclusion

The present investigation provides an integrated assessment of the ethnobotanical relevance, cytogenetic characteristics, and phytochemical diversity of *Viola biflora*, *Viola canescens*, and *Viola serpens* from riparian habitats of Himachal Pradesh. Ethnobotanical records highlighted that local communities have relied on these species for many years to manage respiratory ailments, dermatological, and inflammatory disorders, underscoring their cultural and medicinal significance in Himalayan communities. Cytological analyses revealed predominantly stable diploid chromosome numbers in *V. biflora* and *V. canescens*, while *V. serpens* exhibited multiple cytotypes, reflecting its broader cytogenetic variability within the region. Normal meiotic behaviour and high pollen fertility across species further indicated cytological stability in the examined populations. Phytochemical profiling through GC–MS demonstrated notable inter-population variation, particularly in *V. serpens*, where the Kullu population exhibited greater chemical richness than the Mandi population, suggesting potential environmental or genetic influences on metabolite expression. Overall, the study highlights the biological, ecological, and pharmacological potential of these *Viola* species and provides a foundational framework for future research aimed at conservation, biochemical characterization, and the development of plant-based therapeutics.

Conflict of interest: The authors declare that there is no conflict of interest associated with this research work.

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