Hindawi Publishing Corporation
Journal of Botany
Volume 2014, Article ID 345451, 6 pages
http://dx.doi.org/10.1155/2014/345451

Review Article

Viola canescens: Herbal Wealth to Be Conserved

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Received 7 August 2014; Revised 2 November 2014; Accepted 7 November 2014; Published 20 November 2014

Academic Editor: M. Yasin Ashraf

Viola canescens Wall. ex Roxb., commonly known as Himalayan White Violet, belongs to Violaceae family. It is found in the Himalayan regions of Pakistan, India, Bhutan, and Nepal. It is a perennial herb which mostly prefers to grow in the shady and moist places. *V. canescens* is an important medicinal plant which is mostly used in the traditional medicinal system for cough, cold, flu, fever, and malaria and is also given as anticancerous drug. So far, violin (alkaloid), viola quercitrin, methyl salicylate, and saponins are the different phytochemicals which are extracted from this plant. Molecular studies on *V. canescens* suggest that, in case of adulteration in the powdered form of *Viola* species, they can be distinguishable by the lengths of their spacer regions. Because of the overexploitation of *V. canescens* for medicinal purposes, the conservational status of *V. canescens* in different regions became endangered. It is the need of the hour to utilize different conservational strategies and save this precious medicinal wealth from extinction.

1. Introduction

1.1. Geographical Distribution. *Viola canescens* Wall. ex Roxb. is widely distributed in Pakistan, India, Nepal, and Bhutan [1]. It is present mostly in the temperate and tropical zones, restricted only to the mountainous areas [2].

In Pakistan, *Viola canescens* Wall. ex Roxb. is found in the areas of Shawal (North Waziristan), Razmak, Miran Shah (South Waziristan), Parachinar (Kurrum Agency), Teera (Orakzai Agency), Bajour (Khyber Agency), Dara Adam Khel, Fizagut, Swat, and Kaalam (Malakand Agency) [3]. The plant is found mostly at an altitude of 2000 meters all the way through temperate Himalayas [4].

Locally its presence is recorded from various regions of Pakistan. In Ayyubia National Park (ANP), five plant associations were recognized with different botanical composition. They were recognized on the basis of cluster analysis. *V. canescens* was present along with other herbs like *Podophyllum emodi*, *Polygonum*, *Euphorbia wallichii*, and *Fragaria indica* [5]. Another multivariate cluster analysis done in ANP demonstrated the presence of *V. canescens* in *Pinus wallichiana-Geranium nepalense* association. The association is distributed above 2282 meters and it extends up to 2300 meters or sometimes slightly higher at other localities. This association includes species such as *Viola canescens*, *Fragaria indica*, *Dryopteris fragilis*, *Geranium nepalense*, and *Pinus wallichiana* and all of these generally occur in the damp woods or the edges of streams on the damp to wet soils [6]. *V. canescens* is widely distributed in Swat, Buner, and Chitral valleys of Pakistan [7–9]. Its presence was also detected in Kotli Sattian, Rawalpindi District and in Neelum Valley, Azad Jammu and Kashmir, [10–14].

In India, *V. canescens* is found in Pangi Valley of Chamba District in the cold desert of Himalaya, also known as Trans Himalayan region [15]. The presence of *V. canescens* was also reported in Garhwal region of Himalaya located in Uttarakhand, India, at an altitude of 1600 m–2000 m. It is also present in Uttarakhand in the areas of Nanda Devi National Park and Nainital catchment area [16–21]. From the Dhunkharka community, Kavrepalanchowk, Nepal, *V. canescens* was also reported [22].

1.2. Taxonomy and Morphology. The complete botanical name is *Viola canescens* Wallich ex Roxburgh. Its synonym is *Viola serpens* Wall. ex Ging. var. *canescens* (Wall.) Hook F. and Thomson.

There are different common names for *V. canescens*. In English, *Viola canescens* Wall. ex Roxb. is called Himalayan White Violet because it is mostly associated with the

Table 1: Hierarchical classification of *Viola canescens* Wall. ex Roxb.

Rank/taxa	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malpighiales
Family	Violaceae
Genus	Viola
Species	*Viola canescens*

Himalayan region. In Urdu, it is called Banafsha [7, 9–13, 23–25]. In different localities of Pakistan, this plant is given different names. In NWFP province of Pakistan, it is known as Savar Phal [26]. It is called Skoramindoq by people of Baltistan [14]. It is also called Phulnaqsha, bamasha, or sweet violet in Lesser Himalayas of Pakistan. In India it is called Ratmundi or Vanaksha [15], Vanafsha [17, 21]. In Himachal Pardesh, it is called Gugluphul [27], Banaksha [28], and Banfasa [29]. In Uttarakhand, it is commonly called Vanfsa [18]. In Nepal it is locally called Ghatteghaans [22].

1.2.1. Classification. See Table 1.

1.2.2. Morphological and Taxonomic Features. *Viola canescens* Wall. ex Roxb. belongs to Violaceae family also known as Alsodeiace, Leoniaceae, or Retrosepalaceae. Family Violaceae includes 20 genera and about 800 species which are present worldwide [30]. It is represented by one genus (*Viola*) and 17 species in Pakistan [31] including *Viola betonicifolia*, *V. biflora*, *V. canescens*, *V. cinerea*, *V. falconeri*, *V. fedtschenkoana*, *V. kashmiriana*, *V. kunawurensis*, *V. macroceras*, *V. makranica*, *V. odorata*, *V. pilosa*, *V. reichenbachiana*, *V. rupestris*, *V. stocksii*, *V. tricolor*, and *V. turkestanica* [1].

Viola canescens Wall. ex Roxb is a prostrate, subglabrous, or hairy perennial herb. It has long, much branched and cylindrical *root system*. Stem is missing. *Leaves* are present towards the base. Leaves are ovate and are broad and subreniform, maybe cordate or obtuse to acute and margins are serrate-crenate. The length of the leaf petiole is approximately double the lamina. Leaves are also pubescent; stipules are present freely. Lanceolate leaves present with approximate length and width of 5.0–20 × 1.0–3.0 mm. They are reddish at base and are as long as broad. Flowers are tiny with the approximate size of 1.0–1.8 cm, and their color ranges from pale violet to violet. Flowers are present on the erect and pubescent peduncle which is 5–15 cm long; 2 bracteoles are present which may be opposite to subopposite in position and are present more or less on the middle of the peduncle. Sepals are 5 in number which may be linear or lanceolate and are acute, entire, and unequal. Lateral sepal is bigger, approximately 10 mm in length. Sepals are 2 mm wide; they are dentate near their base. The length and width of other sepals is 8.0 × 3.0 mm. Petals length is up to 15 mm and they are 4.0 mm broad. Other characters of petals include obovate and obtuse, and the two upper petals are wedge shaped while two lateral petals are narrower and much hairy at their base and clear dark streaks are present on them. The lower petals are short and they have dark stripes on them. Spur is short and blunt. Style of Himalayan white violet is club shaped, and its ovary is ovate in shape with presence of hairs. Capsule is round or globose, maybe hairy or glabrous, and has many seeds (Figure 1).

Figure 1: Flower of *V. canescens*.

1.2.3. Flowering Period and Habitat. Flowering period of *Viola canescens* Wall. ex Roxb. ranges from March to June and it produces beautiful, small pale violet to violet to white flowers during this period. It requires complete shade or semishady place for its perfect growth or it might require shady edges [26].

1.2.4. Morphology of the Pollen Grain. Based on the exine pattern, there are two discrete types of pollen recognized in family Violaceae, that is, *Viola stocksii* type and *Viola pilosa* type. In *Viola pilosa* type of the pollen, tectum is densely punctuated, while in the case of *Viola stocksii* type of the pollen, tectum is of subpsilate densely punctuating type. *Viola canescens* Wall. ex Roxb. has pollen grains of *Viola pilosa* type. Pollen grain of *V. canescens* belongs to the class of tricolporate. The ratio of the length of the polar axis (*P*) to the equatorial diameter (*E*) is 105–131. Shape of the pollen is subprolate. The apertures are having the colpus which is long and sunken with acute ends and the length of colpus is 26.25–30.10 μm. In the exine, sexine is thicker than the nexine while the ornamentation of exine is densely punctated. The thickness of exine is 1.50–2.11 μm (Figure 2) [31].

1.3. Phytochemistry of V. canescens. Many research studies have been carried out to figure out the exact number of chemicals in *V. canescens*. Qualitative testing of ethanolic and methanolic extract of this plant revealed the presence of various compounds mentioned in Table 2 [7].

The phytochemicals found in *V. canescens* include methyl salicylate (Figure 3(a)), alkaloid violin, glycoside viola quercitrin, saponins, and glucosides [18]. An alkaloid was discovered by Boullay (1828) in the roots known as violin which

Figure 2: Pollen grain of *V. canescens* [31].

Table 2: Chemical constituents of *V. canescens* [7].

Chemical constituents
Alkaloids
Phytosterols and triterpenoids
Phenol
Flavonoids
Tannins
Saponins
Anthocyanins

are similar to emetine (Figure 3(c)), but possessing different properties from emetine. It exists in the plant in combined form with malic acid. Some scientists believe that it is remarkably active and may be poisonous. A glucoside which is named as viola quercitrin was also found which was comparable to quercitrin (Figure 3(b)).

Violin is an emetocathartic alkaloid having unknown chemical composition. Violin is a cream colored, bitter-taste powder, and if it is heated it melts and then burns like resin. It is still doubtful that either the chemical structure of violin resembles or not to the emetine, so its chemical structure is still unknown. Sometime, scientists think that violin may be an impure form of emetine. Solubility tests of violin shows that it is less soluble in alcohol and ether and more soluble in water as compared to emetine. Violin is an irritant of the alimentary canal and can produce vomiting and diarrhoea. *Viola* species contain violin, viola quercitrin which is a glycoside in nature and similar to rutin, saponin, and methyl salicylate.

1.4. Pharmacological Activities. Phytopharmacological activities which were studied in *Viola canescens* Wall. ex Roxb include antimalarial activity [32] and antiprotozoal activity [17].

Antiprotozoal activity and cytotoxic activity were recorded from Garhwal region of India. Four extracts of the plant *V. canescens* were obtained. Extracts were tested at different concentrations. The petroleum ether extract of *V. canescens* showed good activity against *Leishmania donovani*. The extract also showed well-defined activity against *Trypanosoma cruzi*. The cytotoxic activity was also checked on the infected rat skeletal myoblasts (L-6 cells) to obtain information about selectivity of the extracts. All the extracts were classified as noncytotoxic. The antiplasmodial activity of the petroleum ether extract comes out to be 2.76 μg/mL [17]. The antimalarial activity of *V. canescens* was also studied in the Garhwal region of northwestern Himalaya. All extracts of *V. canescens* were noncytotoxic except petroleum ether extract of *V. canescens* which showed some cytotoxic effects against L-6 cells of rat skeletal myoblasts. Petroleum ether extract of *V. canescens* shows that it inhibits the action of parasite (*Plasmodium falciparum*) in the blood as compared to the control [32].

The *in vitro* pharmacology of *Viola canescens* Wall. ex Roxb. was also studied. The analgesic property and gastrointestinal motility were tested for this purpose. Both these effects were studied on mice. The results show that the ethanolic extract of *V. canescens* is good purgative and it is used for this purpose in traditional medicines. So the crude extract of this plant can be used as pain reliever and laxative in traditional medicinal system [7].

1.5. Ethnomedicinal Aspects of V. canescens. A misfortune of the recent times is that the valuable ethnobotanical knowledge is vanishing from our culture very fast. Modernization and breakdown of native cultures and even the destruction of whole tribal groups are responsible for fast destruction of traditional knowledge [33].

V. canescens was found to have carminative, demulcent, astringent, antipyretic, diaphoretic, and purgative properties. Plant is anticancerous in action and is used for treating different nervous disorders [12–14, 26]. In many other areas, locally, herbal practitioners utilize plant extract against eczema, epilepsy, rheumatism, and stomach acidity and as a cure for respiratory problems [8, 23]. The leaf paste of *V. canescens* is used along with brown sugar mostly to cure cough and other respiratory problems [34].

Flowers are the main parts used as medicine. Decoction of flowers along with cinnamon, clove, and fennel is used to treat respiratory tract problems [15]. The whole plant of *V. canescens* is used against malaria [20, 35]. It was reported that *V. canescens* is used in Himachal Pardesh and 8–10 gm of powdered dried flowers and leaves is used with warm water early in the morning to cure dysentery [27]. The flowers of Banaksha are boiled in water to make infusion, which is used as tea to cure for cough, cold, and fever [16, 28]. In Uttarakhand, the extract of the whole plant is given for treatment of leucorrhoea, regulating menstruation, and headache. It is also given to treat bronchial asthma and cough and is also aphrodisiac. Paste of plant is externally applied on cuts, wounds, and boils as antiseptic [18]. *V. canescens* is also used in Nepal as antipyretic, laxative for boils and leaves are emollient [22].

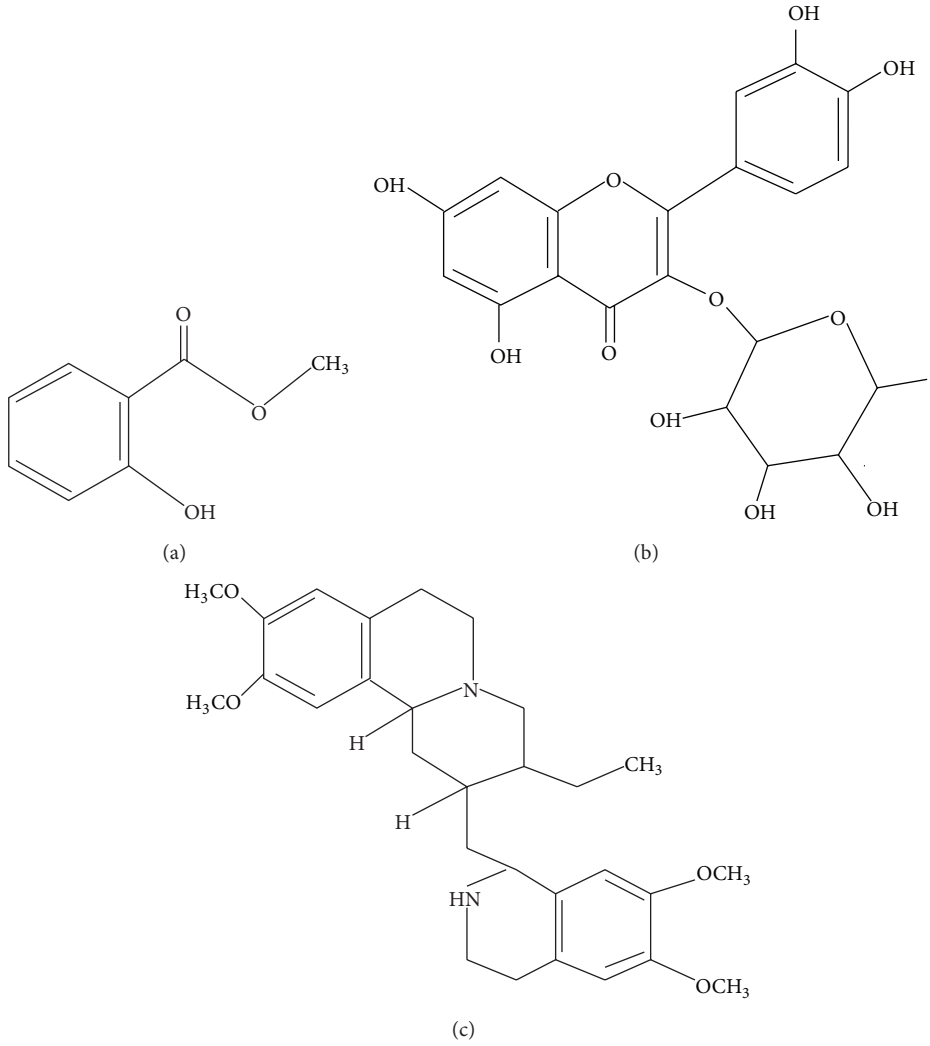

Figure 3: (a) Methyl salicylate. (b) Quercitrin. (c) Emetine.

1.6. Molecular Studies on V. canescens. It is important for industries to correctly identify the species of *Viola* before making drug but the classical methods of identification were not much accurate in identifying different species. In the case of *Viola*, the conserved sequences are used for identification. In an experiment by Singh et al. (2005), the cloning and sequencing of spacer regions between 5s rRNA genes in different species of *Viola* were determined. The differences in the sequences were determined by polymerase chain reaction, so species specific DNA sequences were given to differentiate *Viola* species (Figure 4) [2].

The chromosome count of different *Viola* species showed that the current chromosome count of *Viola canescens* Wall. ex Roxb which was $n = 6$ was the pioneer chromosome count for *V. canescens*. Its ploidy level was $2x$ and the pollen fertility was 91.69% [36].

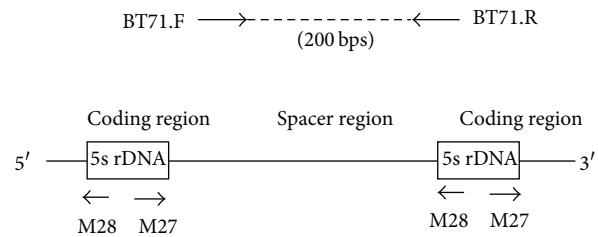

Figure 4: 5s rRNA gene and its spacer region along with the primer combinations for *V. canescens* (forward and reverse) [2].

1.7. Threats and Conservation

1.7.1. Conservational Status. Viola canescens Wall. ex Roxb. is present at an altitude of 2300 m in Battagram region which is highly exploited for its biodiversity. So due to its medicinal importance and loss of habitat, its population size is reduced to 64% and it falls under criteria A of endangered type which is based on population reduction [25].

Due to their overuse, various medicinal plants along with *V. canescens* are vulnerable to extinction [37]. In Pakistan, *V. canescens* is being illegally harvested in Ayyubia National Park [33] and in Margalla Hills National Park [9], *V. canescens*

existence is threatened in the future as there is more demand of its parts being used medicinally, so it is most vulnerable. The secured status of *V. canescens* was reported in the Neelum Valley [12]. In Swat, *V. canescens* is under immense pressure due to its over collection, so it is endangered or rare in the area [8, 23, 38].

1.7.2. Factors Responsible for Low Population. Various factors are responsible for declining population of *V. canescens* like grazing and fodder collection, soil erosion, introduction of invasive species, and overexploitation of plant species [3]. One of the main factors is deforestation as the people are dependent on the forest area for their livelihood so it is also exerting pressure on the flora of an area [33]. Excessive use of the parts of plant of *V. canescens* for medicinal purposes is causing a decline in its population. Browsing, grazing, and grass cutting are also some of the major factors responsible for lowering population of *V. canescens* [9, 37]. Some other authors have reported that loss of habitat, change in environments, overexploitation, extensive grazing, effect of invasive plants, deforestation, and attack of pathogens are liable for making *V. canescens* endangered [25].

1.7.3. Steps Needed for Conservation of Plant Species. Some measures that urgently reduce the risk of extinction include proper documentation of the native knowledge, training of the local communities, and development of monitoring programmes [12]. Other methods suggested by the authors include that local communities should be given conservation education and that medicinal plants should be used sustainably to ensure community mobilization [8, 37, 38].

To avoid the loss of various important plant species, in situ and ex situ conservation methods should be applied by protecting plants in their natural habitats and by cultivating them and again reintroducing them in the natural environment, respectively. The plants were selected from another site and brought to the other site for cultivation. Using this technique, *V. canescens* survived in the new habitat, showed dense growth, and also produced fruits and flowers. [3, 9, 23]. *V. canescens* is one of the most wanted plants in Swat and its annual sale is 1 tonne per vendor in the area. There is a dire need to conserve the plant in situ (like in nature reserves and national parks) and ex situ (like in seed banks, botanical gardens, and other germplasm collections).

Different steps which are the need of the hour include reduction of anthropogenic impacts, introduction of threatened species in the botanical gardens, and developing monitoring programmes [25].

2. Conclusion

From the above discussion, it is clear that *V. canescens* is an important plant species with respect to its ethnomedicinal importance. It is widely used in traditional health care system. So this importance builds a pressure on the plant regarding its use. So there is a need to conserve this plant species which is under threat according to the listing of IUCN (International Union for Conservation of Nature). So practical steps are needed for its conservation which include ex situ and in situ conservation.

Much more work should be done on its molecular studies and phytochemistry. The structures and composition of different chemical components present in it should be determined for recognizing its further activities. This type of information is required for drug production from this plant for treating various diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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