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A probe into the medicinal potential of *Viola canescens* – A threatened medicinal plant from Himalaya

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ABSTRACT

Viola canescens Wall. ex Roxb. is a perennial herb belonging to family Violaceae, and it is almost cosmopolitan in distribution. This plant is widely used in Ayurveda and Unani medicinal systems for curing various ailments, most commonly for cough and cold. Phytochemical studies releaved that this plant is rich in secondary metabolites. This plant revealed significant antimicrobial, anti-inflamatory, antioxidant, hepatoprotective, laxative, analgesic as well as antitumor activities. Due to all these important pharmacological activities, market demand of *Viola canescens* is increasing day by day and this plant is facing tremendous over exploitation and becomes a threatened plant according to International Union for Conservation of Nature and Natural Resources. The present review compiles that the ethnobotanical, phytochemical and pharmacological aspects of the plant need to be conserved.

1. Introduction

1.1. Habitat and distribution

Viola canescens Wall. Ex Roxb. (*V. canescens*) is native to India, China and Bhutan. It is distributed throughout Himalayas from Kashmir to Northeast India at altitude of about 1 500–2 400 m. In Pakistan, *V. canescens* is present in Razmak, Dara Adam Khel, Fizagut, Bajour (Khyber Agency), Teera (Orakzai Agency), Kaalam (Malakand Agency), Parachinar (Kurrum Agency) and Northern and Southern Waziristan (Shawal and Miran Shah, respectively)[1].

1.2. Common names of the plant

V. canescens belongs to family Violaceae. The synonym of *V. canescens* is *Viola serpens* Wall. Ex Ging. var. *canescens* (Wall.) Hook F. and Thomson. It is commonly known as banafsha in Urdu and Himalayan white violet in English as it is mostly found in Himalayan region. It is also commonly called as Savar phal in Khyber Pakhtunkhwa, vanafsha and banfasa in India and ghatteghaans in Nepal is. Family Violaceae is also known as other names such as Alsodeiace, Leoniaceae and Retrosepalaceae[2-8].

1.3. Viola species in Pakistan

Overall the Violaceae family consists of about 20 genera and around 800 species distributed worldwide. In Pakistan, the family is represented with its one of the most important genus "*Viola*" with its 17 species including *Viola odorata, Viola biflora, Viola cinerea, Viola betoicifolia, V. canescens, Viola turkestanica, Viola pilosa, Viola kunawurensis, Viola falconeri, Viola stocksii, Viola reichenbachiana, Viola tricolor, Viola macroceras, Viola kashmiriana, Viola makranica and Viola rupestris*[2,9].

1.4. Taxonomic and morphological characteristics

V. canescens is subglabrous or hairy almost perennial prostrate herb. Its roots are cylindrical, profusely branched as well as long. Leaves are broad, ovate and reniform and may be with cordate to acute tip. Leaf margins are serrate to crenate. The length of the leaf petiole is almost twice the lamina. Leaves are pubescent and stipules are freely present. The approximate width and length of

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lanceolate leaves is 5.0–20.0 and 1.0–3.0 mm. At base, they are reddish. Stems are absent. Flowers are deliberate with the size of 1.0–1.8 cm approximately. The colors of its flowers range from pale violet to violet and often almost white. Lateral spur is almost 10 mm in length. Sepals are 5 in number and are almost 2 mm wide, dentate near its base. Petals are up to 15 mm long and its width is 4.0 mm. Other characteristics of petals include obtuse and obovate tip, and the two upper petals are wedge-shaped and the two lateral petals are narrow and hairy at their base and dark clear streaks are found on them. Himalayan white violet's style is club-shaped and the ovary shape is ovate with hairs. Capsule is globose, has many seeds and may be glabrous or hairy. Its flowering period is from March to June. *V. canescens* produces beautiful flowers with pale violet to violet or white in color and flowers are small sized mostly (Figure 1)[10].

Figure 1. Floral representation of *V.* canscens.

1.5. Active constituents of the plant

A qualitative analysis of the ethanolic plus methanolic extract of *V. canescene* revealed the presence of many compounds such as flavonoids, anthocyanins, phenols, alkaloid tannins, phytosterols and triterpenoids and saponins. The photochemicals presenting in *V. canescens* are methyl salicylate, glycoside *Viola* quercitrin, alkaloid, glucosides and saponins. An alkaloid known as violin found in the roots of *V. canescens* which is similar to emetine but having change properties from emetine. Violin is present in a combined form with malic acid in *V. canescens*. Some scientists believe that violin is poisonous and remarkably active. Violaquercitrin, a glucoside, is comparable to another compound quercitrin. The compound violin is an alkaloid and an emetocathartic and its chemical composition is yet unknown. Violin is a cream colour powder having bitter-taste; on heating it melts and burns like resin. Since the chemical structure of violin is yet unknown, it is in doubt that if it resembles to emetine or not. Violin is less soluble in organic solvents suah as alcohol and ether, while it is more soluble in polar solvent such as water. Violin is annoying and irritant substance for the alimentary canal causing diarrhoea and vomiting. *Viola* species possess compounds violin, *viola* quercitrin a natural glycoside and is similar to saponin, methyl salicylate and rutin[5,6,11].

2. Biological activities of the plant

2.1. Laxative activity

Recently, it has been reported that the ethanolic crude extract of the leaves of *V. canescens* possesses significant activities. A dose-dependent purgative effect has been observed in BALB/c mice using charcoal meal paradigm. The crude methanolic extract of *Viola serpense* found to be a good laxative in a dose-dependant manner. The plant extract possesses diuretic property and explores that potassium ion and sodium ion levels and urine production were additional in aqueous extract form in comparison of control specimen at a prescription level of dose 400 mg/kg. Aqueous and alcoholic extracts at prescription of 400 mg/kg and 200 mg/kg respectively have significantly laxative effect[12],

2.2. Antioxidant activity

Antioxidants are usually plant-originated substances that neutralize and lessen the effect of free radicals and have a significant function for the prevention of cardiovascular diseases, cancer and neurodegenerative disorders including Parkinson's diseases and Alzheimer's disease. The sweet violet antioxidant property is attributed to anthocyanins, a groups of flavonoids. Anthocyanins are found in all tissues including roots, stems, leaves and flowers. Antioxidants such as polyphenols, phenolic acids and flavonoids and so on show effects by scavenging free radicals, preventing the production of reactive oxygen species or by activating detoxifying proteins. Banafsha possesses tremendous antioxidant properties. The *in vitro* data clearly depicted the antioxidant effectiveness of all extracts. The flower extracts exposed antixidant potential by means of scavenging of 2,2-diphenyl-1-picrylhydrazyl radical[13].

2.3. Sedative and pre-anesthetic

The leaves extract showed pre-anesthetic and sedation effects at prescription of about 100–400 mg/kg and increasing extract dose elevated the sedative effect. The leaves extract showed better pre-anesthetic and sedative effects as compared to diazepam, a well-known classical benzodiazepine[14].

2.4. Antipyretic activity

The chloroform and hexane extracts of the plant produced a noteworthy verbal antipyretic property in rabbits. Antipyretic

property was more significant in those portions of plants which were hexane-soluble[15].

2.5. Hepatoprotective activity

High-pressure liquid chromatography showed the presence of two flavonoids isorhamnetin and luteolin in the plant. Both these flavonoids are responsible for the hepatoprotective activity of this plant. Banafsha revealed a strong hepatoprotective activity in rats in regards to paracetamol-induced hepatic injury[16].

2.6. Anticancer activity

The entire aerial portion of the plant including flowers, leaves and stems possess anticancer properties. The plant was reported as a pharmacological tool and perhaps prime to antitumor tool cycloviolacin, a cyclotide from the plant having antitumor properties and causing cell death by membrane permeabilization. Numerous cyclotides with significant cytotoxicity are reported to be capable of chemosensitizing activity in contrast to drug-resistant breast cancer[17,18].

2.7. Antimicrobial activity

Cyclotides in the plant showed an effective antimicrobial activity in contrast to Gram-negative plant pathogenic bacteria. Cycloviolacin is a cyclotide with powerful functions against Gram-negative bacteria. Ethanoic and methanolic extracts obtained from the banafsha leaves were proved to be effective against the whole experimental bcterial strains while fungi are resistant towards all extracts. Ethanol extract exhibited a greater inhibition to *Bacillus subtilis* (20 mg/mL), *Escherichia coli* (10 mg/mL), *Staphylococcus aureus* (20 mg/mL) and *Pseudomonas aeruginosa* (40 mg/mL). So this plant is used to treat cystitis, bronchitis and tonsillitis[19,20].

2.8. Antidyslipidemic and antihypertensive activities

It was foound that the plant extract had potential in lowering blood pressure. Weight loss and antidyslipidemic effects were also shown by the plant. These activities are due to the reduction in synthesis and absorption of lipids as well as antioxidant activities. So this is the pharmacologic reason of the medicinal use of this plant in hypertension and dyslipidemia cycloviolacin, a plant peptide of the cyclotide family obtained from the plant is found to have potent effects against fouling barnacles[21,22].

2.9. Cytotoxic, mosquito repellent and molluscicidal activities

Cycloviolacin in plant shows strong cytotoxic properties which varies according to a dose-dependent sequence[18]. The essential oil of the plant induces a protection interval of 8 h maximumly and 100% repellent against *Anopheles*, *Aedes* and *Culex* mosquitoes[23]. Raw cyclotide extracts of the plant revealed a significant molluscicidal activity as compared to the synthetic molluscicide compound metaldehyde[24].

2.10. Anti-inflammatory activity

An aqueous extract of banafsha strongly showed an anti-inflammatory activity which is comparable with hydrocortisone. Plant extract prophylactically given is partially effective in preventing lung damage, equal to the effect of hydrocortisone in aiding the resolution of formalin-induced lung damage[25].

2.11. Larvicidal and antiprotozoal activities

Plant essential oil in 50 mg/L oil solution has 86.7% larvicidal activity against *Aedes aegypti* larvae with time period of 24 h[26]. The antiprotozoal activity was initially recorded from India (Garhwal region). Petroleum ether extract of Himalayan white violet showed a good function against *Leishmania donovani*. This extract also possesses a well-defined function against *Trypanosoma cruzi*[6].

2.12. Cytotoxic activity

The cytotoxic activity was also initially recorded from Garhwal, India. This property was tested on the infected rat skeletal myoblasts (L-6 cells) to obtain information about the selectivity of the extracts. All the extracts were classified as noncytotoxic[6].

2.13. Antimalarial activity

The antiplasmodial activity of *V. canescens* petroleum ether extract explores to be 2.76 g/mL[6]. All types of extracts of *V. canescens* are noncytotoxic but petroleum ether extract of Himalayan white violet shows cytotoxic activity against L-6 cells of mice skeletal myoblasts. It inhibits the function of pathogen (*Plasmodium falciparum*) in the blood in comparison to the control organism[11].

3. Medicinal uses of plant

The whole plant extract is used to treat insomnia, exhaustion, headaches and dizziness. The leaf paste of *V. canescens* with brown sugar is traditionally used to cure cough as well as several respiratory problems. Flowers of *V. canescens* are mostly used for medicinal purposes. Decoction of flowers along with cinnamon, clove and fennel is used to cure respiratory tract infections. The whole plant of *V. canescens* is used against malaria. The entire aerial parts of the plant including flowers, leaves and stem are used for cough, cold, sneezing, asthma and bronchitis[27].

4. Viola essential oil composition and uses

Essential oil of *Viola* species is extracted by using hydrodistillation-solvent extraction method and then analyzed using gas chromatography-mass spectrometer technique. The chemical analysis showed that generally *Viola* oil is composed of 25 compounds. The chief components includes butyl-2-ethylhexylphthalate and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone. The *Viola* essential oil is also commonly used in perfume industry. This essential also possesses tremendous antioxidant and antibacterial activities as well as antiseptic properties [28].

5. Status of *V. canescens* in Pakistan

V. canescens is susceptible to extinction. In Pakistan, *V. canescens* is harvested from Margalla Hills National Park and Ayyubia National Park illegally. The presence of *V. canescens* is highly under threat in future because of its excessive utilization for medicinal purposes. In Swat, Himalayan white violet is under great pressure because of it's over collection[29-31].

6. Identification issue and trade of the plant

Viola species are difficult to distinguish. Market demand of *V. canescens* is increasing and due to it's over exploitation it is getting endangered. Due to it's over harvesting for drug industry, other species of Violaceae such as *Viola* biflora are supplied in market with the name of *V. canescens*. It is quite necessary for medicinal industries to correctly identify the species of *Viola* before making drugs. The primitive methods of plant identification are not much accurate for the identification of different species. DNA barcoding is a novel method which can be utilized for *Viola* species identification. In this technique, the conserved DNA sequences are used for identification. Interspecific as well as intraspecific sequences can be used for identification. Molecular markers and polymerase chain reaction technology are used for correct identification of *Viola* species in drug industry[32].

7. Threats to the plant and conservation measures

V. canescens is a plant of high altitudes facing great risks of extinction due to its over exploitation for medicinal purposes. In Pakistan, it is illegitimately harvested in Ayyubia National Park as well as Margalla Hills National Park. However, it is reported as secured in Neelum Valley. In many areas of Khyber Pakhtunkhwa like swat its over collection is frequent so this plant is becoming rare in those areas. Natural population of this plant is declining day by day and numerous factors for this reduction are soil erosion, unchecked grazing, biopollution as well as over harvesting. Habitat loss due to deforestation for industrial purposes is also responsible for the extinction of various medicinally important plant species. For conservation of this precious plant both *in situ* and *ex situ* techniques should be adopted. *In situ* technique is more favorable because it deals with the conservation of habitat as well. Public awareness must be initiated to harvest plant carefully for medicinal purposes. Uprooting of the entire plant must be banned. Human intervention should be checked, introduction of exotic species must be checked and proper monitoring programs should be developed for plant protection[33,34].

8. Conclusion

V. canescens belonging to family Violaceae is an important medicinal plant in traditional medicinal system such as Ayurvedic and Unani medicinal systems. Members of family Violaceae are commonly known as violets. This plant is commonly known as banafsha. This plant revealed tremendous medicinal potentials and it is used to cure cough, cold, flu, fever, juandice also acts as laxative, purgative, febrifuge, diuretic, antioxidant agent, antimicrobial and larvicidal drugs as well. Flowers of this plant are sweet scented and found used in perfume industry. This plant is not commercially cultivated at large scale. So now this plant is under threat due to over exploitation for medicinal purposes. Modern biotechnological techniques should be adopted to conserve the germplasm of this precious plant otherwise this plant will soon face extinction. It is also necessary to develop commercial market for this plant and the cultivation of this plant must be promoted to conserve the plant in wild state.

Conflict of interest statement

We declare that we have no conflict of interest.

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