Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Review doi: 10.12980/jclm.4.2016J5-228

Pharmacology and biochemistry of *Polygonatum verticillatum*: A review

Saboon[1], Yamin Bibi[1], Muhammad Arshad[1], Sidra Sabir[1], Muhammad Shoaib Amjad[1,2], Ejaz Ahmed[1], Sunbal Khalil Chaudhari[1*]

[1]*Department of Botany, PMAS Arid Agriculture University, Rawalpindi, Pakistan*

[2]*Department of Botany, Women University of Azad Jammu and Kashmir, Bagh, Pakistan*

ARTICLE INFO

Article history:
Received 19 Nov 2015
Received in revised form 8 Dec, 2nd revised form 15 Feb, 3rd revised form 24 Feb 2015
Accepted 28 Dec 2015
Available online 22 May 2016

Keywords:
Polygonatum verticillatum
Solomon's seal
Threatened
Pharmacology

ABSTRACT

Polygonatum verticillatum (Linn.) All. syn. *Convallaria verticillata* Linn. is a valuable medicinal plant, distributed in the temperate Himalaya at the elevations 2 400 to 2 800 m. It is a perennial rhizomatous herb and contains various pharmacologically important secondary metabolites among which the most important are α-bulnesene, linalyl acetate, eicosadienoic, pentacosane, piperitone, docasane, diosgenin, santonin and calarene. It also possesses antimalarial, antipyretic, anti-inflammatory, anticonvulsant, lipoxygenase, urease inhibition, diuretic, tracheorelaxant, antidiarrheal, antispasmodic, antinociceptive, antifungal, antibacterial and bronchodilator activities. The plant also got importance in traditional systems of medicine due to its broad therapeutic potential especially of its rhizome. But in the past few years, over exploitation of plant parts caused the decline in the frequency of this species due to which it became threatened, endangered and vulnerable in different parts of the world. So efforts are being made in certain regions of the world for both *ex-situ* and *in-situ* conservation. This paper briefly reviewed the botanical, traditional, phytochemical, pharmacological and conservation related aspects of this plant.

1. Introduction

Polygonatum (King Solomon's-seal, Solomon's seal) is a genus of erect or decumbent perennial herbs belonging to the family Liliaceae representing about 57 species in the world. This genus is mostly distributed in the temperate regions of the northern hemisphere, most concentrated in the Himalayas. It also occurs in East Asia, where it is found mostly in China and Japan where 40 species of this genus were found[1]. In addition to this, they are also found in India, Pakistan, Korea, Nepal, Afghanistan, Bhutan, Russia and in moderate climate zones of North America and Europe. The Flora of Pakistan showed the presence of four different species of *Polygonatum*, including *Polygonatum multiflorum*, *Polygonatum geminiflorum*, *Polygonatum cirrhifolium* and *Polygonatum verticillatum* (*P. verticillatum*). These species are widely distributed in various part of the country including Hazara, Chitral, Swat and Kurram Agency[2,3]. The characteristic feature of this genus is thick, fleshy creeping sympodial rhizomes. According to Miller (1754) the generic name of *Polygonatum* is derived from its charactistic feature of rhizome which resembles to a great extent as yovi, a knee, because it has many little knees[4]. In the year 1753 Linnaeus listed three species of *Polygonatum* in his book 'Species Plantarum' under the genus *Convallaria*, namely, *Convallaria verticillata*, *Convallaria polygonatum* and *Convallaria multiflora*[5]. Later on, this was listed under the generic name *Polygonatum* by Alloni[6]. In the natural system of classification the family Liliaceae of Angiosperms was classified in the series Coronarieae by Bentham and Hooker[7,8]. The genus *Polygontum* is very impotant in term of their high medicinal value as most of their members are used in herbal medicine from thousands of years in different region of the world. All plant parts have some medicinal value but the most important of these parts is the rhizomes which contain many impotant medicinal activites like adaptogenic, antioxidant, cardiotonic, demulcent, diuretic, energizer, hypoglycemic, tonics, antibacterial and antifungal[9], and also used in the treatment of pulmonary problems for dry coughs

*Corresponding author: Sunbal Khalil Chaudhari, Department of Botany, PMAS Arid Agriculture University, Rawalpindi, Pakistan.

E-mail: khalilsunnbal@yahoo.com

The journal implements double-blind peer review practiced by specially invited international editorial board members.

and tuberculosis[10,11]. Many of their members can reduce the blood sugar level[12]. Due to high medicinal value, some of their members are overexploited and become threatened or vulnerable like *P. verticillatum* (Linn.) All. and *Polygonatum cirrhifolium* (Wall.) Royle[13].

P. verticillatum syn. *Convallaria verticillata* Linn. commonly known as whorled solomon's seal is a perennial rhizomatous herb and distinguished medicinal plant of temperate Himalaya. It is frequently distributed between the elevations 2400 to 2800 m[14]. It is a highly valuable medicinal plant specially rhizome which in the form of syrup is used for the treatment of pain, pyrexia, burning sensation and for phthisis[15] in combination with other herbs as it promotes urine discharge[16]. The plant is also used as emollient, aphrodisiac, appetizer, galactagogue and tonic for weakness[17]. Rhizome of this plant is an important ingredient of Ashtavarga, a drug used in Ayurveda as a tonic and for aphrodisiac[18,19]. The plant also exhibits antifungal activities and used in the preparation of cosmetics as a skin tonic. Its rhizome (Meda/ Mahameda) is collected from wild and traded for medicinal purposes. This is one of the reasons that *P. verticillatum* is rapidly disappearing. So there is an urgent need for conservation of this plant[20].

2. Systematic position

According to phylogenetic system of classification of Hutchinson, the systematic position is: Kingdom: Plantae, Phylum: Angiosperms, Subphylum: Monocotyledons, Divison: Corolliferae, Order: Liliales, Family: Liliaceae, Genus: *Polygonatum*, Species: *P. verticillatum*[21].

3. Distribution

P. verticillatum has worldwide distribution. Distributed from montane to alpine Himalaya, Kashmir to Northeast States; Sikkim, Southeast Tibet, West Asia, Europe (except Mediterranean region) [22] in temperate Himalayas[23] in Russia (W. Siberia, Caucasia) Afghanistan, Pakistan (Chitral, Dir, Swat, Hazara, Gilgit, Azad Jammu Kashmir), Kashmir, India, Nepal, Sikkim, Bhutan and China (Gansu, Nei Mongol, Qinghai, Shaanxi, Sichuan, Xizang). From Garhwal Himalaya the species was reported from Bhuna, Dunagiri and Niti[24], from Binsar by Gaur[22], and from Tungnath, Rudranath, Valley of Flowers and Dayara by Vashistha[25].

4. Vernacular name

The vernacular name of *P. verticillatum* is Solomon's seal in English; Nor-e-Alam in Urdu; Basuchidra, Devamani, Pandura, Shakakul, Seal, Vasuchhidra, Mahamaida in Hindi; Meda in Sanskrit; Salam mishri in Pahari; Keruwa, Khinraula in Nepali; Peramole in Pashto; Salam dana, mishri, mitha dodhu, Ra-mnye, Khol in Kashmiri; Saat Ashee in Gilgati or Balti; Lun Ye Huang Jing in Chinese.

5. Botanical description

P. verticillatum is a perennial rhizomatous herb, its rhizomes are usually tuber like, shortly branched and 0.7–1.5 cm thick. Stem usually erect, 2 to 4 feet/ 30–60 cm in height, angled and grooved, glabrous sometimes mottled. Leaves in whorls of 4 to 8, occasionally alternate near the base of stem, sometimes opposite near the apex, sessile, elliptic to narrowly lanceolate/linear, 4 to 8 × ¼ to ½ inch, or in case of lanceolate, 3½ × ¾ inch, tips usually acute but some time acuminate, margins entire, sometimes obtuse or slightly in rolled, lower surface is glaucous. Inflorescence racemes whorled, 2 to 3 flowered, peduncle 1–2 cm, bract < 1 mm or some time absent, pedicel 2.5–4.5 mm, hermaphrodite, perianth 8–9 mm or 1/3 inch, white or pale yellow, tinged with green, contracted in the middle, teeth inside, tip hairy. Stamens are epipetalous, filaments 0.5–1.0 mm, ovary 3 mm, style 2.5–3.0 mm. The flowering and fruiting takes place in the month of June to October. Fruit is in the form of berries which are red, becoming purple on maturation, 6–8 mm or ¼ inch in diameter[26].

Figure 1. Physical description of plant *P. verticillatum.*

6. Traditional uses

Ethnomedicinally, the plant is very important. Different parts of the plant in crude form or with some other ingredients are used for the

Table 1
Traditional uses of plant parts.

S. No.	Part used	Uses	Reference
1	Root	Used for urino-genital disorders, nerve tonic, general weakness, spermatorrhoea, haemorrhoid, leucorrhoea, anemia, gastric problems, wounds, rheumatism, aphrodisiac, appetiser, backache, menstrual troubles, vitaliser, rejuvenative, digestive, eaten as raw vegetable	[28,29,31-40]
2	Rhizomes	Rheumatism, general body weakness, aphrodisiac, nervine tonic, kidney trouble, wounds, emollient, vitiated condition of pitta and vata, appetizer, glactagogue, anticancer, boils, eaten mixed with dairy products and as a tonic	[41-50]
3	Tuber	Seminal weakness, strangury, anorexia, fever, general debility, tonic, promote body heat, appetizer, aphrodisiac, nerve tonic, urinary problems, edible used as vegetable	[51-55]
4	Bulb	In powdered form used for tuberculosis, general debility, as tonic, leucorrhoea, tonifying spleen, dampness, treat "xiaoke" (diabetes) and tonifying Qi	[13,56]
5	Green foliage	As nutritive item utilized as vegetable, shoots are cooked with other spring herbs	[57-60]
6	Seed	Indigestion	[61]
7	Whole herb	Cure appetite, nervine tonic, kidney trouble and also restores body strength	[30]

cure of different diseases. For example, in some parts of Gilgit the root of the plant is utilized with milk and ghee as general tonic[27], in some other regions gastric flatulence and allergies are treated by the oral administration of this plant. In India this plant is used for the cure of spermatorrhaea and piles for this the fresh roots are broken into small pieces and kept in water for overnight and then crushed in same water and taken daily in morning[28]. In another state of India the root powder of this plant is taken daily with water for leucorrhoea[29], the root paste in some area are applied on wounds. In some places the whole herb is utilized to cure appetite, as nervine tonic, for kidney trouble and to restores body strength[30]. Some other traditional uses from different areas are listed in (Table 1). In some Himalayan region this plant is consider as wild vegetable and the root of the plant is eaten raw[33,34] and in some areas the whole plant is cooked and utilized in raw form[53].

7. Phytochemistry

The plant contains many phytochemicals isolated from its different parts like the compound diosgenin isolated from rhizome of the plant[62]. The rhizome also contains glucose, galactose, sucrose and fructose[63,64]. The study showed that the aerial parts of plant contain saponins, alkaloids, phenols, flavonoids, tannins, *etc*.[65]. Chemical constituents of plant were identified in *n*-hexan fraction by using gas chromatography mass spectrometry, which showed that the aerial oily components of plant contain α-bulnesene, linalyl acetate, eicosadienoic, pentacosane, piperitone, docasane, and calarene[66]. By using mass and NMR spectra two compounds 2-hydroxybenzoic acid and β-sitosterol were isolated from the rhizome of *P. verticillatum*[67]. The rhizome of the plant also showed the presence of lysine, serine, aspartic acid, threonine, diosgenin, β-sitosterol, sucrose and glucose[68]. Two chemical compounds diosgenin and santonin were also isolated from the rhizomes of *P. verticillatum*[69]. To test the biological activities of the rhizome of this plant two different compounds, 5 hydroxymethyl-2-furaldehyde and diosgenin, were isolated from the rhizome with the help of bioactivity guided isolation[70]. The study by Khan *et al*. showed that the crude extract of *P. verticillatum* rhizome as well as its solvent extract has significant amount of alkaloid and saponin which are responsible for the bioactivity of the plant[71]. In another study the rhizome of the plant showed significant concentrations of flavonoid and phenolic compounds[72]. The aerial parts of the plant contain significant amount of alkaloid and phenolic content[70]. The study by Khan *et al*. indicated that the aerial parts of the plant contain different phytochemical including saponins, alkaloids, flavonoids, phenols, sterols, terpenoids and tannins[67]. Phytochemically, lectin has been isolated from the roots of *P. verticillatum* the lectine was obtained in purified form (120 mg/kg) that contain high percentage of asparaginic acid (28%)[73]. Both the rhizome and aerial parts of plant were analysed for their phytochemicals both the parts showed the presence of saponin, alkaloids, glycosides, phenols, flavonoids, sterols and tannins, but the rhizome also showed the presence of anthraquinones and terpenoids[74]. By the use of various modern techniques such as high resolution electron ionization-mass spectrometry, 1D and 2D NMR, two new compounds were isolated from the rhizome of plant such as propyl pentadecanoate and 2/, 3/ dihydroxypropyl pentadecanoate[75].

8. Nutritional composition

The bulb and rhizome of the plant were analyzed for their nutritional composition the mineral profile of plant extract showed that the plant contained both micro and macronutrient at concentrations of P [(100.00 ± 0.00) mg/100 g], K [(13.33 ± 0.39) mg/100 g], Na [(37.82 ± 2.19) mg/100 g], Ca [(1338.30 ± 3.83) mg/100 g], Mg [(90.10 ± 0.80) mg/100 g], Fe [(23.64 ± 0.88) mg/100 g], Cu [(0.21 ± 0.01) mg/100 g], Mn [(28.64 ± 0.37) mg/100 g] and the nutritional constituents were at concentrations of moisture [(84.53 ± 4.69) g/100 g], protein [(16.20 ± 1.62) g/100 g], fat [(0.46 ± 0.06) g/100 g], fibre [(12.33 ± 0.57) g/100 g], carbohydrates [(17.07 ± 0.00) g/100 g], ash [(7.45 ± 0.79) g/100 g], energy value [(108.23 ± 9.73) Kcal/100 g] [76]. In another study the nutrient analysis of rhizome extract showed that the plant contained significant amount of both micro (Zn, Cu, Cr, Fe, Mn, Ni) and macronutrient (Ca, Na, K) which was determined by using atomic absorption spectrometry for micronutrients and flame

photometry for macronutrients analysis. In nutritional constituents the plant contained significant amount of proteins, fats, fiber, carbohydrates, ascorbic acid along with ash and moisture content[77]. Mineral profile of the aerial parts of *P. verticillatum* was also analyzed in different plant extracts the study showed that the plants contain significant amount of both micronutrient (Zn, Cu, Cr, Fe, Pb, Mn, Ni) and macronutrient (Ca, Na, K)[78].

9. Pharmacological activities

9.1. Antioxidant activities

Free radical scavenging activities of aerial parts of the plant were analyzed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by Khan *et al*., which showed the highest activities was the crude extract (IC_{50}: 122 μg/mL) and followed by ethyl acetate extract (IC_{50}: 137 μg/mL) and *n*-butanol (IC_{50}: 167 μg/mL) fractions[66]. The rhizome extract showed significant antioxidant activity in DPPH assay with the most potent antioxidant activity observed in chloroform (IC_{50}: 90 μg/mL) followed by ethyl acetate (IC_{50}: 93 μg/mL) and then *n*-butanol (IC_{50}: 95 μg/mL)[77]. Another study was conducted on the antioxidant potential of two compound diosgenin and santonin, isolated from *P. verticillatum* rhizomes for this purpose DPPH and reducing power assays were employed the result showed that both compounds exhibit strong antioxidant activity in DPPH assay the inhibitory concentration (IC_{50}) values for diosgenin is (65.80 μg/mL) and santonin (50.03 μg/mL), respectively. Similarly in reducing power assays the IC_{50} values are diosgenin (62.10 μg/mL) and santonin (46.40 μg/mL), respectively[65].

9.2. Antimalarial activities

The antimalarial activities of different extracts of *P. verticillatum* aerial parts were checked against *Plasmodium falciparum*. The maximum potency was showed by *n*-hexane fraction (IC_{50}: 4.86 μg/mL), which was followed by chloroform fraction (IC_{50}: 5.71 μg/mL) but as compared to other the crude extract was less potent (IC_{50}: 21.67 μg/mL) against this pathogen[79]. The rhizome of *P. verticillatum* was tested for its antimalarial activity against same *Plasmodium falciparum* the result showed that the crude extract and its non polar fractions showed significant antimalarial activity[72].

9.3. Antipyretic activity

The antipyretic activity of rhizome and aerial parts of the plant were analyzed in Wistar rats and in Albino NMR imaging mice the Brewer's-yeast-induced pyrexia in test organisms the organisms were treated separately with rhizome and aerial plant extracts and the result showed that both the extract exhibited marked antipyretic activity but the rhizome extract was more effective as compared to aerial parts which is dose dependent like rhizome showed (82.20%) at 200 mg/kg and aerial parts 64% at 200 mg/kg[80].

9.4. Anticonvulsant activity

The rhizome and aerial parts of the plant were analyzed for their anticonvulsant activity the convulsion were induced by pentylenetetrazole both the extract were seprately analysed for this activity but the plant extracts didn't show any effect against this activity[74].

9.5. Anti-inflammatory activities

The anti-inflammatory activity of *P. verticillatum* rhizome was tested in rat in which the carrageenan induced rat paw edema. Result showed that the plant rhizome showed marked reduction in edema the anti-inflammatory activity were shown at the test doses of 50, 100 and 200 mg/kg. At 200 mg/kg the protection is 65.22% which is similar to aspirin[81]. For again the same activity the aerial parts of plant were analyzed in wistar rats in this study with the help of carrageenan hind paw edema is induced the methanolic extracts of plant shows that the plant exhibite significant anti-inflammatory activity and reduction in paw edema in rats were observed at the test dose of (50, 100 or 200 mg/kg) but the maximum result was observed at the concentaration of 200 mg/kg (65.22%)[67].

9.6. Tracheorelaxant activity

P. verticillatum rhizome was studied for its tracheorelaxant activity in isolated guinea-pig tracheal tissues the result shows that *P. verticillatum* rhizome caused complete inhibition of the high K^+ and carbachol-induced contractions at the dose range of 0.01–10 mg/mL which is similar to verapamil which also cause relexation of tissue[81].

9.7. Lipoxygenase activity

P. verticillatum rhizome was tested for inhibition of soybean lipoxygenase with UV absorbance based enzyme assay, the plant shows significant activity against lipoxygenase with resultant IC_{50} value of (102 ± 0.19) μg/mL which is compared with that of the standard drug, baicalaim (22.6 ± 0.09 μg/mL)[81]. Same as that of rhizome the aerial parts of the plant were also tested for the same activity in different solvent extracts the study revealed that the plant contain significant lipoxygenase activity in all extracts, but ethyl acetate extracts was the most potent inhibitor of the enzyme (IC_{50}: 97 μg/mL), followed by aqueous fraction (IC_{50}: 109 μg/mL) and crude extract showed inhibition (IC_{50}: 125 μg/mL)[66]. Again the aerial parts of plant were also tested for its lipoxygenase activities the different dilution of plant showed significant lipoxygenase with IC_{50} values of 102 mg/mL[67].

9.8. Urease inhibition activity

The urease inhibition activity of the aerial parts of the *P. verticillatum* is tested in the crude and its subsequent solvent fraction of the plant extract. The crude extract of plant exhibited significant reduction of enzyme (IC_{50}: 192.00 ± 0.09). When different fractionation were tested for the activity, *n*-butanol was the most potent fraction [IC_{50}: (166.00 ± 0.69) μg/mL] followed by the ethyl acetate [IC_{50}: (187.00 ± 0.77) μg/mL]. However, *n*-hexane and chloroform fractions were inactive in urease inhibition assay. This showed that plant exhibit significant urease inhibition activity[66].

9.9. Insecticidal activity

Aerial parts of *P. verticillatum* was tested for its *in vitro* insecticidal assay against *Tribolium castaneum*, *Sitophilus oryzea*, *Rhyzopertha dominica* and *Callosobruchus analis* result of this this activity showed that two fractions *n*-hexane (50%) and chloroform (30%) showed moderate activity against *Rhyzopertha dominica*, on the other hand neither crude extract nor its solvent fraction showed any activity against other insects[82].

9.10. Antileishmanicidal activity

The aerial parts of *P. verticillatum* was tested for its *in vitro* antileishmanicidal activity against *Leishmania major* (strain DESTO) the crude extract and different solvent extracts were tested result showed that neither crude extract nor its solvent fraction showed any significant activity against *Leishmania major*[82].

9.11. Phytotoxicity assay

To test the phytotoxicity, aerial parts of *P. verticillatum* was tested against *Lemna acquinoctialis* Welv result of this study showed that the crude as well as the different solvent fraction of this plant showed outstanding phtotoxicity against *Lemnaemna acquinoctialis* at the dose of 5, 50 and 500 μg/mL and complete growth inhibition was observed in the crude extract and aqueous fraction at the maximum dose (500 μg/mL)[82].

9.12. Antibacterial activity

The crude and different solvent extracts of plant rhizome were tested against various Gram-positive [*Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*)] and Gram-negative [*Escherichia coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi*, *Shigella flexeneri*] bacteria by using agar well diffusion method the plant extract show significant antibacterial activity against these bacteria specially against Gram-negative bacteria except *P. aeruginosa* in Gram-positive the *S. aureus* showed more sensitivity against this plant[83]. The aerial parts of plant is also analyzed for their antibacterial activities against same Gram-positive and Gram-negative bacteria in different extracts the result showed that in Gram-positive the plant extract is effective only against *B. subtilis* and in Gram-negative like rhizome the aerial plant extract are ineffective against *P. aeruginosa*[65]. The same activity was again tested by two compounds isolated from *P. verticillatum* rhizome diosgenin and santonin against various Gram-positive (*B. subtilis*, *Bacillus cereus*, *S. aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) bacteria both these compounds showed significant zone of inhibition against both the strain[69].

9.13. Antifungal activity

Different extracts of plant rhizome was tested against various fungi including (*Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microspoum canis*, *Fusarium solani*, *Candida glaberata*) by using agar tube dilution method the result of this study showed that the antifungal activity of this plant is only limited to the *M. canis* and *F. solani*[83]. The aerial parts of plant were also analyzed for their antifungal activity against the same six fungal strains by using same method the result of aerial plant extract was effective only against *Microspoum canis* and all other fungal strains were resistant to plant extract[65]. Again the antifungal activity was tested by isolated compounds diosgenin and santonin from *P. verticillatum* rhizome against different strains of fungi (*Aspergillus flavus*, *Aspergillus niger*, *Trichoderma harzianum* and *Fusarium oxysporum*) the result of this study showed that only santonin showed the marked antifungal activity against these strains of fungi[69].

9.14. Inhibition of protein denaturation

Diosgenin and santonin two isolated compounds from the rhizome of *P. verticillatum* these two compounds shows marked attenuation on heat-induced protein denaturation in a concentration dependent manner with with maximum effect of 61.55% and 67.90% at 500 mg/mL, respectively[69].

9.15. Cytotoxic activity

The cytotoxic activity of *P. verticillatum* rhizome is tested by using its crude extract and its subsequent solvent fractions by using brine shrimp cytotoxic assay the result shows that only ethyl acetate fraction showed prominent cytotoxicity (LD_{50}: 492.846 μg/mL)[70]. Different fractions of the aerial parts of plant were also analyzed for its cytotoxicity by using same brine shrimp cytotoxic assay the assay shows that except the choloform assay all other fractions are safe and no toxicity was observed[78].

Table 2
Status of plant *P. verticillatum* in different region of world.

Status	Area	References
Critically endangered	Kumaun Himalaya;	[85]
Endangered	Kumaun Himalaya; Mankial Valley Hindukush Range, Pakistan; District Swat, Pakistan; North-West Himalaya; Himachal Pradesh, India	[43,85-88]
Threatened	Changa Valley district Shangla, Pakistan; Garhwal Himalaya, India; Lohba Range of Kedarnath Forest Division, Garhwal Himalaya, India; District Kinnaur, Himachal Pradesh, India	[41,33,89,90]
Vulnerable	Kinnaur, Himachal Pradesh, India; Manali wildlife sanctuary, north western Himalaya; Trans-Himalayan, Ladakh, Jammu and Kashmir; Uttaranchal, India; Dhauli Ganga, Central Himalaya; Bhabha Valley in Western Himalaya; Uttarakhand Himalaya, India; Mornaula Reserve Forests, West Himalaya, India; Nanda Devi National Park and highland National, Indian Himalayan Region; Jammu and Kashmir; Himachal Pradesh, India; North-West Himalaya	[32,34,53-55, 87,91-99]
Rare	Kumaun state west Himalaya, India; District Shangla, Khyber Pakhtunkhwa, Pakistan	[42, 100]
Uncommon	Lohba Range of Kedarnath Forest Division, Garhwal Himalaya, India	[33]

9.16. Antinociceptive activity

Crude methanolic extract of the rhizomes of *P. verticillatum* was tested for its antinociceptive activity in various pain models in rodents at the concentrations (50, 100 and 200 mg/kg) the plant showed significant antinociceptive activity in various pain models including visceral pain model, formalin test and hot plate test[71]. The aerial parts of plant were also analyzed for the same activity the the effect of plant extract were analyzed in different pain models in all the plant showed the marked antinociceptive activity from the result the author suggested that the plant may contain some pharmacologically active substances which may interfere with the blockade of the effect or the release of endogenous substances (arachidonic acid metabolites) which are responsible for the excitation of pain nerve endings[70].

9.17. Diuretic activity

The rhizome of *P. verticillatum* was tested for its diuretic activity in male Albino rats at concentration 300 and 600 mg/kg, the result of this study showed that the plant exhibit mild diuretic activity at concentration of 300 mg/kg and no activity were showed at the high dose concentration of 600 mg/kg[71]. The aerial parts of plant were also analyzed for their diuretic activity in male Wistar rats the plant show mild diuretic activity but found insignificant in both test doses at both test doses (300 and 600 mg/kg *p.o.*) when compared with the standarerd drug hydrochlorothiazide[70].

9.18. Bronchodilator activity

The bronchodilator activity of aerial parts of *P. verticillatum* were tested in the isolated tracheal tissues of rabbits the methanolic extract of plant parts show strong bronchodilator activities when tested against carbachol and K^+ (80 mmol/L) so it induced contractions more ever it also showed Ca^{2+} channel blocker-like activity[67].

9.19. Antispasmodic activity

To test this activity the methanoilc extract of *P. verticillatum* rhizome is used in the spontaneously contracting isolated rabbit jejunum the findining of this study showed that plant extract demonstrated dose-dependent relaxation of the spontaneous contractions in rabbit jejunum and the complete relaxation is at 10 mg/mL these result are similar to that of inhibitory activity of cromakalim and verapamil. The plant extract was also tested against low K^+- and high K^+-induced contractions, the plant showed inhibition at low K^+-induced contractions, while high K^+-induced contractions were partially inhibited[80].

9.20. Antidiarrheal activity

The methanolic rhizome extract of *P. verticillatum* was tested for its antidiarrheal activity in mice diarrhea is induce in test organism with the help of castor oil the study showed that the plant exhibited marked antidiarrheal activity reduction in diarrhea was in a dose-dependent manner which is 80% at 1 000 mg/kg, *p.o.* these result are similar with that of drug loperamide[80].

9.21. Anti-insulin activity

On the bases of traditional medicine system it was reported that the plant is utilized to treat diabetes. To check this plant was analyzed for their antidiabatic activity in dexamethasone which induced insulin resistant HepG2 cells but the result showed that the plant exhibited insignificant antidiabatic activities[56].

10. Conservation and management

P. verticillatum is a perennial herb distributed worldwide especially in Europe, Turkey, Afghanistan, India, Nepal, China, in North and Central Asia and in temperate Himalayas at the altitude of 2 400 to 2 800 m. The plant is highly medicinal and used in many traditional medicines as diuretic, in pain, pyrexia, burning sensation, phthisis, weakness, Aphrodisiac, tonic and galactagogue, emollient, appetizer and for kidney trouble. The plant is also used for food in some areas of the world different parts of plant were utilized as raw vegetable. Based on their traditional uses the plant was also analyzed for their pharmacological potential and the plant showed marked potential against many pharmacological activities. The plant parts are also utilized in many traditional herbal formulations. For all this the plant was collected in large number from wild by the local community

due to which the reduction in the population will be seen another reason of the reduction of plant is the rhizome of plant which have more medicinal value as compared to other plant parts so for the collection of rhizome the whole plant was digged from the soil due to which large number of plant were destroyed. Secondly, due to lack of awareness indiscriminate cutting of grasses and bushes were done by local community moreover the cutting of plants take place along with the underground reproductive parts of the plants and the cutting also destroyed the matured seeds. This all result in the reduction in the population of these plants. Another big reason is the change in environmental conditions which is going on constantly in the different ecosystems of the world such changes also occur in Himalayans region which is the rich source of natural vegetation and home for many native plant species due to the anthropogenic destruction of natural vegetation, environmental changes and the change in the natural habitat of the plant due to the change in the geographical and climatic conditions, decreased the overall density and availability of the plants[84].

Bisht *et al*. (2012) also concluded that some plant have habitat specificity, some have narrow range of distribution, land-use disturbances by human beings, introduction of non- natives plant species or invasive species, change of habitat, climatic changes, heavy grazing pressure, explosion of human population, fragmentation and degradation of the plant density, population restriction and genetic drift are the potential causes of destruction of medicinal plant species. In some areas of the world the women carry all the activities of livestock domestication and for that they collect the food and fodder from the nearby forests and due to lack of identification they also cut the medicinal plant species along with the fodder grasses. Therefore, this is one of the reasons of threatened status of these medicinal plant species[20].

11. Status of *P. verticillatum*

In many parts of the world the natural habitat of this plant is decline due to over exploitation, harvest in an uncontrolled way, overgrazing and lack of awareness is the reason for the decline of this species (Table 2). Secondly, in some regions of the world the plant were also utilized in herbal formulations and have some market value so the local people harvest plant before maturity due to which mature seed production become very low and large number of seeds destruction also take place. Furthermore, rhizome of the plant has much medicinal value so the whole plant is uprooted from the soil which also destroys the plant. Thus, there is a need for its *in-situ* as well as, *ex-situ* conservation and propagation to conserve this important medicinal plant.

11.1. Ex-situ conservation of P. verticillatum

In recent year the ex-situ conservation of plant is done by Lohani *et al*. (2012) in that study the scientist grow the plant rhizome in Medicinal Plant Garden of Central Council for Research in Ayurveda and Siddha, Ranikhet (29°38’ 60 N, 79°25’0 E), India[101]. During the growth of the plant they supplemented it with different organic fertilizer. Three types of organic fertilizers, namely farmyard manure, forest litter and vermin compost were used to check their effects on the survival, growth and yield of the plant. Total twelve treatments were arranged. The result of this study showed that in control the yield was lower as compared to litter, farmyard manure and vermi compost which showed higher economic yield. However, yield was highest at T3 (furrow + forest litter) as compared to other beds which was seen best for the growth of that plant[101]. Again in another region the rhizome of plant was cultivated farmyard manure and vermicompost to check their yield and compared with other plants, the plant showed good yield[102].

11.2. In-vitro micropropagation of P. verticillatum

Micropropagation of *P. verticillatum* was done by using stem disc explants. The study showed the multiple shoots were initiated on Murashige and Skoog medium prepared with different concentrations and combinations of cytokinins (0.25–10.00 mg/L) along with auxins (0.5–1.0 mg/L). From the different phytohormones used, benzylaminopurine (1.0 mg/L) with 1-naphthaleneaceticacid (0.5 mg/L) was found to be the most effective in producing maximum number of shoots. When in the same medium the regular subculturing of these *in vitro* multiple shoots was done it induced profuse growth of lateral roots. Individual shoots were excised and rooted *in vitro* on half strength MS medium supplemented with auxins *viz*. indole acetic acid, indole-3-butyric acid and 1-naphthaleneacetic acid (NAA) (0.5–1.0 mg/L) result showed that only NAA and indole-3-butyric acid could induced rooting on half strength of MS basal media. Where’s 0.5 mg/L NAA in half strength MS medium reflected better and longer roots[20].

12. Conclusions

P. verticillatum is an endangered but high valuable medicinal plant from temperate Himalaya. The plant has immense importance because of its efficacy towards various serious diseases which is also an important plant species with respect to its ethnomedicinal importance, so this importance builds a pressure on the plant regarding to its use. This pressure posed the serious threat towards its extinction. So there is an urgent need to conserve this species, sustainable harvesting methods are also urgently required.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Tamura MN. Biosystematic studies on the genus *Polygonatum* (Liliaceae) morphology of staminal filaments and karyology of elevan Eusasian species. *Bot Jahrb Syst* 1993; **115**: 1-26.

[2] Flora of Pakistan. Polygonatum. 2010. [Online] Available from: http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=126394 [Accessed on 5th October, 2015]

[3] Stewart RR. [*An annotated catalogue to the vascular plants of West Pakistan and Kashmir*]. Karachi: Fakhri Printing Press; 1972, p. 56. Russian.

[4] Miller P. *The abridgement of the gardeners dictionary*. London: Botanical Society and Exchange Club of the British Isles; 1754, p. 426-36.

[5] Linnaeus C. [*Plant species presented at the proper manner to genera (1957-1959)*]. London: Ray Society; 1753. Latin.

[6] Allioni C. [*Flora Pedemontana*]. Turin: Giovanni Michele Briolo; 1785. Italian.

[7] Bentham G, Hooker JD. *Genera Plantarum*. Vol. 1-3. London: L. Reeve & Co.; 1862-1883.

[8] Ohara M, Tamura M, Hirose T, Hiei K, Kawano S. Life-history monographs of Japanese plants. 8: *Polygonatum odoratum* (Miller) Druce var. *pluriflorum* (Miq.) Ohwi (Convallariaceae). *Plant Spec Biol* 2007; **22**: 59-64.

[9] Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (Brine Shrimp Test). *Int J App Sci Eng* 2006; **4**: 115-25.

[10] Jiang S. [*Dictionary of Chinese herbal medicine*]. Shanghai: Shanghai People's Publishing Press; 1977, p. 2041-4. Chinese.

[11] Jiang S. [*Concise dictionary of traditional Chinese medicine*]. Shanghai: Shanghai Press of Science Technology; 1986, p. 551-3. Chinese.

[12] Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J. Ethnopharmacol* 2004; **92**: 1-21.

[13] Bhatt D, Kumar R, Tewari LM, Joshi GC. *Polygonatum cirrhifolium* Royle and *Polygonatum verticillatum* (L.) Allioni: Status assessment and medicinal uses in Uttarakhand, India. *J Med Plant Res* 2014; **8**: 253-9.

[14] *Healing herbs of himalaya- a pictorial & herbaria guide.* New Delhi: Department of AYUSH, Ministry of Health and Family Welfare, Government of India; 2008, p. 110058.

[15] Singh AP. Ashtavarga-rare medicinal plants. *Ethnobotanical Leaflets* 2006; **10**: 104-8.

[16] Ballabh B, Chaurasia OP, Ahmed Z, Singh SB. Traditional medicinal plants of cold desert Ladakh, used against kidney and urinary disorders. *J Ethnopharmacol* 2008; **118**: 331-9.

[17] Alam G, editor. *Database on medicinal plants.* Calcutta: CUTS Centre for International Trade, Economics and Environment; 2004, p. 33.

[18] Chaumont JP. Fungistatic activity of eight phanerogams against phyto pathogenic fungi. *Acta Bot Gallica* 1979; **126**: 537-42.

[19] Varier PS. *Indian medicinal plants: a compendium of 500 species.* New Delhi: Orient Longman; 1994, p. 336-8.

[20] Bisht S, Bisht NS, Bhandari S. *In vitro* micropropagation in *Polygonatum verticillatum* (L.) All. an important threatened medicinal herb of Northern India. *Physiol Mol Biol Plants* 2012; **18**: 89-93.

[21] Hutchinson J. *The families of flowering plants, arranged according to a new system based on their probable phylogeny.* 3rd ed. London: Oxford University Press; 1973.

[22] Gaur RD. *Flora of district Garhwal, North West Himalaya: with ethnobotanical notes.* Srinagar: TransMedia; 1999.

[23] Chauhan NS. *Medicinal and aromatic plants of Himachal Pradesh*. New Delhi: Indus Publication Company; 1999.

[24] Naithani BD. *Flora of Chamoli. Vol. 2, Botanical survey of India-flora of India series 3.* Howrah: Government of India; 1984, p. 654.

[25] Vashistha RK. Ecophysiology and agro-technology of two important Himalayan herbs: *Angelica glauca* Edgew. and *Angelica archangelica* Linn. [dissertation]. Srinagar: H.N.B. Garhwal University; 2006.

[26] Collett H. *A handbook of flowering plants of Simla and the neighbourhood.* Dehradun: Bishen Singh Mahendra Pal Singh; 1971, p. 524.

[27] Khan SW, Khatoon S. Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, Northern areas of Pakistan. *Pak J Bot* 2008; **40**: 43-58.

[28] Kaur I, Sharma S, Lal S. Ethnobotanical survey of medicinal plants used for different diseases in Mandi district of Himachal Pradesh. *Int J Res Pharm Chem* 2011; **1**: 1167-71.

[29] Phondani PC, Maikhuri RK, Rawat LS, Farooquee NA, Kala CP, Vishvakarma SCR, et al. Ethnobotanical uses of plants among the Bhotiya Tribal Communities of Niti valley in Central Himalaya, India. *Ethnobot Res Appl* 2010; **8**: 233-44.

[30] Sharma PK, Thakur SK, Manuja S, Rana RK, Kumar P, Sharma S, et al. Observations on traditional phytotherapy among the inhabitants of Lahaul valley through Amchi system of medicine—a cold desert area of Himachal Pradesh in North Western Himalayas, India. *Chin Med* 2011; **2**: 93-102.

[31] Bisht C, Badoni A. Medicinal strength of some Alpine and sub-Alpine zones of Western Himalaya, India. *New York Sci J* 2009; **2**: 41- 6.

[32] Kala CP. Medicinal and aromatic plants of Tons watershed in Uttarakhand Himalaya. *Appl Ecol Env Sci* 2015; **3**: 16-21.

[33] Radha B, Singh RD, Tiwari JK, Tiwari P, Gairola A. Wild edible plant resources of the Lohba range of Kedarnath Forest Division (KFD), Garhwal Himalaya, India. *Int Res J Biol Sci* 2013; **2**: 65-73.

[34] Pant S, Samant SS. Diversity, distribution, uses and conservation status of plant species of the Mornaula Reserve Forests, West Himalaya, India. *Int J Biodiver Sci Manag* 2006; **2**: 97-104.

[35] Phondani PC, Maikhuri RK, Bisht NS. Medicinal plants used in the health care system practiced by traditional Vaidyas in Alaknanda catchment of Uttarakhand, India. *Ethnobotanical Leaflets* 2009; **2009**: 1453-67.

[36] Bhat JA, Kumar M, Bussmann RW. Ecological status and traditional knowledge of medicinal plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya, India. *J Ethnobiol Ethnomed* 2013; **9**: 1.

[37] Gupta SK, Sharma OMP, Raina NS, Sehgal S. Ethno-botanical study of medicinal plants of Paddar valley of Jammu and Kashmir, India. *Afr J*

Tradit Complement Altern Med 2013; **10**: 59-65.

[38] Sher H, Al Yemeni M. Economically and ecologically important plant communities in high altitude coniferous forest of Malam Jabba, Swat, Pakistan. *Saudi J Biol Sci* 2011; **18**: 53-61.

[39] Kumari P, Joshi GC, Tewari LM. Indigenous uses of threatened ethno-medicinal plants used to cure different diseases by ethnic people of Almora District of Western Himalaya. *Int J Ayu Herbal Med* 2012; **2**: 661-78.

[40] Ballabh B, Chaurasia OP. Medicinal plant of cold desert Ladakh used in the treatment of stomach disorders. *Indian J Tradit Knowl* 2009; **8**: 185-90.

[41] Razaq A, Rashid A, Ali H, Ahmad H, Islam M. Ethnomedicinal potential of plants of Changa valley district Shangla, Pakistan. *Pak J Bot* 2010; **42**: 3463-75.

[42] Razzaq A, Hadi F, Rashid A, Ibrar M, Ali U. Exploration of medicinal plants and their conservation status at higher altitude of district Shangla, Khyber Pakhtunkhwa, Pakistan. *Am Eurasian J Agric Environ Sci* 2015; **15**: 328-31.

[43] Hamayun M, Khan SA, Sohn EY, Lee IJ. Folk medicinal knowledge and conservation status of some economically valued medicinal plants of District Swat, Pakistan. *Lyonia* 2006; **11**: 101-13.

[44] Akhtar N, Rashid A, Murad W, Bergmeier E. Diversity and use of ethnomedicinal plants in the region of Swat, North Pakistan. *J Ethnobiol Ethnomed* 2013; **9**: 25.

[45] Sharma P, Samant SS. Diversity, distribution and indigenous uses of medicinal plants in Parbati Valley of Kullu district in Himachal Pradesh, Northwestern Himalaya. *Asian J Adv Basic Sci* 2014; **2**: 77-98.

[46] Negi VM, Chauhan NS. Medicinal and aromatic plants wealth of a tribal district Kinnaur in Himachal Himalaya. *Indian For* 2009; **135**: 838-52.

[47] Alam G, editor. *Database on medicinal plants*. 1st ed. Culcutta: CUTS Centre for international trade, economics and environment; 2004, p. 34.

[48] Wujisguleng W, Liu YJ, Long CL. Ethnobotanical review of food uses of *Polygonatum* (Convallariaceae) in China. *Acta Soc Bot Pol* 2012; **81**: 239-44.

[49] Uniyal B. Utilization of medicinal plants by the rural women of Kullu, Himachal Pradesh. *Indian J Tradit Knowl* 2003; **2**: 366-70.

[50] Khan SM, Page S, Ahmad H, Harper D. Anthropogenic influences on the natural ecosystem of the Naran Valley in the Western Himalayas. *Pak J Bot* 2012; **44**: 231-8.

[51] Dhyani A, Nautiyal BP, Nautiyal MC. Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. *Int J Biodivers Sci Ecosyst Serv Manage* 2010; **6**: 13-9.

[52] Semwal DP, Saradhi PP, Kala CP, Sajwan BS. Medicinal plants used by local Vaidyas in Ukhimath block, Uttarakhand. *Indian J Tradit Knowl* 2010; **9**: 480-5.

[53] Pant S, Pant VS. Status and conservation management strategies for threatened plants of Jammu and Kashmir. *J Phytol* 2011; **3**: 50-6.

[54] Samant SS, Pant S, Singh M, Lal M, Singh A, Sharma A, et al. Medicinal plants in Himachal Pradesh, north western Himalaya, India. *Int J Biodivers Sci Manag* 2007; **3**: 234-51.

[55] Man SR, Samant SS. Diversity, indigenous uses and conservation status of medicinal plants in Manali wildlife sanctuary, North Western Himalaya. *Indian J Tradit Knowl* 2011; **10**: 439-59.

[56] Ma JZ, Yang LX, Shen XL, Qin JH, Deng LL, Ahmed S, et al. Effects of traditional Chinese medicinal plants on antiinsulin resistance bioactivity of DXMS-induced insulin resistant HepG2 cells. *Nat Prod Bioprospect* 2014; **4**: 197-206.

[57] Kunwar RM. Ethnobotanical notes on flora of Khaptad National Park (KNP), far-Westren Nepal. *Him J Sci* 2003; **1**: 25-30.

[58] Misra S, Maikhuri RK, Kala CP, Rao KS, Saxena KG. Wild leafy vegetables: a study of their subsistence dietetic support to the inhabitants of Nanda Devi Biosphere Reserve, India. *J Ethnobiol Ethnomed* 2008; **4**: 15.

[59] Shrestha D. Indigenous vegetables of Nepal for biodiversity and food security. *Int J Biodivers Conserv* 2013; **5**: 98-108.

[60] Dreon AL, Paoletti MG. The wild food (plants and insects) in Western Friuli local knowledge (Friuli-Venezia Giulia, North Eastern Italy). *Contrib Nat Hist* 2009; **12**: 461-88.

[61] Gaur RD, Sharma J. Indigenous knowledge on the utilization of medicinal plant diversity in the Siwalik Region of Garhwal Himalaya, Uttarakhand. *J Forest Sci* 2011; **27**: 23-31.

[62] Yesilada E. *Polygonatum verticillatum*, (L.) All. rhizomes as an additional source of diosgenin. *Gazi Univ Eczacilik Fak Derg*1987; **4**: 11-7.

[63] Shanker V, Prakash S, Uniyal MR. Ashtvarg studies. II. Chromatographic examination of sugars in the rootstock of *Polygonatum vercillatum*. *Nat Appl Sci Bull* 1970; **22**: 139.

[64] Srivastava SN, Singh A, Kapoor LD. Chemical examination of *Polygonatum verticillatum*. *Indian J Pharm* 1969; **31**: 31-2.

[65] Khan H, Saeed M, Muhammad N, Perviz S. Phytochemical analysis, antibacterial, and antifungal assessment of aerial parts of *Polygonatum verticillatum*. *Toxicol Ind Health* 2013; doi: 10.1177/0748233713512362.

[66] Khan H, Saeed M, Muhammad N, Gaffar R, Gul F, Raziq N. Lipoxygenase and urease inhibition of the aerial parts of the *Polygonatum verticillatum*. *Toxicol Ind Health* 2013; **31**: 758-63.

[67] Khan H, Saeed M, Gilani AH, Mehmood MH, Rehman N, Muhammad N, et al. Bronchodilator activity of aerial parts of *Polygonatum verticillatum* augmented by anti-inflammatory activity: attenuation of Ca^{2+} channels and lipoxygenase. *Phytother Res* 2013; **27**: 1288-92.

[68] Sagar PK. Adulteration and substitution in endangered, asu herbal medicinal plants of India, their legal status, scientific screening of active phytochemical constituents. *Int J Pharm Sci Res* 2014; **5**: 4023-39.

[69] Khan H, Saeed M, Rauf A, Khan MA, Muhammad N. Antimicrobial and inhibition on heat-induced protein denaturation of constituents isolated from *Polygonatum verticillatum* rhizomes. *Nat Prod Res* 2015; **29**: 2160-3.

[70] Khan H, Saeed M, Gilani AH, Khan MA, Khan I, Ashraf N. Antinociceptive activity of aerial parts of *Polygonatum verticillatum*: attenuation of both peripheral and central pain mediators. *Phytother Res* 2011; **25**: 1024-30.

[71] Khan H, Saeed M, Gilani AU, Khan MA, Dard A, Khan I. The

antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. *J Ethnopharmacol* 2010; **127**: 521-7.

[72] Khan H, Saeed M, Khan MA, Khan I, Ahmad M, Muhammad N, et al. Antimalarial and free radical scavenging activities of rhizomes of *Polygonatum verticillatum* supported by isolated metabolites. *Med Chem Res* 2012; **21**: 1278-82.

[73] Antoniuk VO. [Purification and properties of lectins of *Polygonatum multiflorum* (L.) All. and *Polygonatum verticillatum* (L.) All]. *Ukr Biokhim Zh (1978)* 1993; **65**: 41-8.

[74] Khan H, Saeed M, Gilani AH, Muhammad N, Haq I, Ashraf N, et al. Antipyretic and anticonvulsant activity of *Polygonatum verticillatum*: comparison of rhizomes and aerial parts. *Phytother Res* 2013; **27**: 468-71.

[75] Khan H, Saeed M, Khan MA, Haq I, Muhammad N, Ghaffar R. Isolation of long-chain esters from the rhizome of *Polygonatum verticillatum* by potent tyrosinase inhibition. *Med Chem Res* 2013; **22**: 2088-92.

[76] Sharma BD, Singh L, Kaur MJ. Nutritional composition of rare himalayan herbs constituting the world's first health food. *Int J Agric Food Sci Technol* 2014; **5**: 75-80.

[77] Khan H, Saeed M, Muhammad N, Khan F, Ibrar M, Hassan S, et al. Comprehensive nutrients analysis of rhizomes of *Polygonatum verticillatum*. *Pak J Pharm Sci* 2012; **25**: 871-5.

[78] Saeed M, Khan H, Khan MA, Khan F, Khan SA, Muhammad N. Quantification of various metals and cytotoxic profile of aerial parts of *Polygonatum verticillatum*. *Pak J Bot* 2010; **42**: 3995-4002.

[79] Khan H, Saeed M, Muhammad N, Tariq SA, Ghaffar R, Gul F. Antimalarial and free radical scavenging activities of aerial parts of *Polygonatum verticillatum* (L.) ALL. and identification of chemical constituents by GC-MS. *Pak J Bot* 2013; **45**: 497-500.

[80] Khan H, Saeed M, Gilani AH, Muhammad N, Rehman N, Mehmood MH, et al. Antispasmodic and antidiarrheal activities of rhizomes of *Polygonatum verticillatum* maneuvered predominately through activation of K^+ channels: components identification through TLC. *Toxicol Ind Health* 2013: doi: 10.1177/0748233713506956.

[81] Khan H, Saeed M, Mehmood MH, Rehman NU, Muhammad N, Haq I, et al. Studies on tracheorelaxant and anti-inflammatory activities of rhizomes of *Polygonatum verticillatum*. *BMC Complement Altern Med* 2013; doi: 10.1186/1472-6882-13-197.

[82] Saeed M, Khan H, Khan MA, Simjee SU, Muhammad N, Khan SA. Phytotoxic, insecticidal and leishmanicidal activities of aerial parts of *Polygonatum verticillatum*. *Afr J Biotechnol* 2010; **9**: 1241-4.

[83] Khan H, Saeed M, Muhammad N, Ghaffar R, Khan SA, Hassan S. Antimicrobial activities of rhizomes of *Polygonatum verticillatum*: attributed to its total flavonoidal and phenolic contents. *Pak J Pharm Sci* 2012; **25**: 463-7.

[84] Balkrishna A, Srivastava A, Mishra RK, Patel SP, Vashistha RK, Singh A, et al. Astavarga plants threatened medicinal herbs of the North-West Himalaya. *Int J Med Arom Plants* 2012; **2**: 661-76.

[85] Lohani N, Tewari LM, Kumar R, Joshi GC, Chandra J, Kshore K, et al. Population studies, habitat assessment and threat categorization of *Polygonatum verticillatum* (L.) Allioni in Kumaun Himalaya. *J Ecol Nat Environ* 2013; **5**: 74-82.

[86] Ullah A, Rashid A. Conservation status of threatened medicinal plants of Mankial Valley Hindukush Range, Pakistan. *Int J Biodivers Conserv* 2014; **6**: 59-70.

[87] Badola HK, Aitken S. Biological resources and poverty alleviation in the Indian Himalayas. *Biodiversity* 2010; **11**: 8-18.

[88] Goraya GS. Conservation concern medicinal plants for Himachal Pradesh. *ENVIS Newsl Med Plants* 2011; **3**: 15.

[89] Poonam B, Pratti P, Prasad NB. *Polygonatum verticillatum* (Linn.) All. and *Polygonatum cirrhifolium* (Wall.) Royle: two threatened vital healers from Asthaverga nurtured by Garhwal Himalaya, India. *J Plant Dev* 2011; **18**: 159-67.

[90] Verma RK, Kapoor KS. Status of plant diversity in Alpine Area of Rakchham- Chitkul Wild life sanctuary of District Kinnaur, Himachal Pradesh. *Biol Forum Int J* 2014; **6**: 5-12.

[91] Chawla A, Parkash O, Sharma V, Rajkumar S, Lal B, Gopichand, et al. Vascular plants, Kinnaur, Himachal Pradesh, India. *Check List* 2012; **8**: 321-48.

[92] Kumar GP, Kumar R, Chaurasia OP, Singh SB. Current status and potential prospects of medicinal plant sector in trans-Himalayan Ladakh. *J Med Plants Res* 2011; **5**: 2929-40.

[93] Dhar U, Manjkhola S, Joshi M, Bhatt A, Bisht AK, Joshi M. Current status and future strategy for development of medicinal plants sector in Uttaranchal, India. *Curr Sci* 2002; **83**: 956-64.

[94] Kandari LS, Phondani PC, Payal KC, Rao KS, Maikhuri RK. Ethnobotanical study towards conservation of medicinal and aromatic plants in upper catchments of Dhauli Ganga in the Central Himalaya. *J Mt Sci* 2012; **9**: 286-96.

[95] Samant SS, Joshi HC. Plant diversity and conservation status of Nanda Devi National Park and comparison with highland National Parks of the Indian Himalayan Region. *Int J Biodivers Sci Manag* 2005; **1**: 65-73.

[96] Chawla A, Rajkumar S, Singh KN, Lal B, Singh RD, Thukral AD. Plant species diversity along an altitudinal gradient of Bhabha Valley in Western Himalaya. *J Mt Sci* 2008; **5**: 157-77.

[97] Kumar A, Meenakshi, Uniyal SK, Lal B, Chawla A, Rajkumar S, et al. HimFlorIS - an information system for flora in Himachal Pradesh, India. *Curr Sci* 2010; **99**: 98-101.

[98] Butola JS, Badola HK. Threatened Himalyan medicinal plants and their conservation in Himachal Pradesh. *J Trop Med Plants* 2008; **9**: 125-42.

[99] Kala CP, Ratajc P. High altitude biodiversity of the Alps and the Himalayas: ethnobotany, plant distribution and conservation perspective. *Biodivers Conserv* 2012; **21**: 1115-26.

[100]Rawal RS, Dhar U. Sensitivioty of timberline flora in Kumaun Himalaya, India: conservation implications. *Arct Alp Res* 1997; **29**: 112-21.

[101]Lohani N, Kumar R, Tewari LM, Joshi GC. *Ex-situ* conservation of *Polygonatum verticillatum* (L.) Allioni under different types of organic treatments. *Int J Biodivers Conserv* 2012; **4**: 22-31.

[102]Butola JS. Post-cultivation evaluation of germplasm in Himalayan threatened medicinal herbs: implication for ex-cultivation and conservation. *Natl Acad Sci Lett* 2011; **34**: 49-58.