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Status and Conservation Threats of *Picrorhiza scrophulariiflora* Pennell. (Scrophulariaceae): An Endangered High Valued Medicinal Plant of the Indo-China Himalayan Region

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ABSTRACT

Picrorhiza scrophulariiflora Pennell. is an endangered medicinal herb of the Indo-China Himalayan region which urgently needs to be conserved *in situ* by preventing animal grazing as well as unsystematic harvesting of its rhizomes. Habitat preferences, population structures, existing status, conservation threats, correlation of soil characters with aerial and underground biomass as well as ethno-medicinal values of Sikkim Himalayas were studied. The present study revealed that analytical features such as density, frequency, abundance, relative density, relative frequency, abundance-frequency ratio, etc., were low in all populations, which indicates the requirement of immediate conservation planning. Open pastures, rocks, crevices, rocky terrain and moist areas were found to be the preferred habitat of this species. However, animal grazing, random collections of its rhizome as well as other associated rare medicinal species such as *Bergenia ciliata*, *Rhododendron anthopogan*, *Rheum australe* and *Saussurea nepalensis* by locals were the major threats to existing populations which has led to a drastic change in the original phytosociological structure of *P. scrophulariiflora*, resulting in rapid habitat loss. However, in order for conservation and sustainable utilization through cultivation, an attempt has been made to identify elite germplasm with higher underground biomass. Additionally a correlation between aerial and underground biomass growth and important soil characteristic such as available nitrogen, organic carbon, moisture content, soil pH and altitude gradients was also established.

Keywords: ethnobotany, morphological parameters, population studies, phytosociological study

INTRODUCTION

Picrorhiza scrophulariiflora Pennell. (Scrophulariaceae), commonly known as Nepalease Kutki in Hindi, is a threatened medicinal plant of the Eastern Himalayas (Olsen 1998). It had been included in the CITES Appendix II during the 10th meeting of the plant committee, Shephardstown, USA (11th-15th Dec., 2000). Due to overexploitation for medicinal purposes along with unorganized as well as unscientific collection practices (Manandhar 1999), the population of this taxon has been dwindling in the wild to such a critical level that a ban on its collection from the wild habitat has been recommended in India (Nayer and Shastry 1990) and is one of the 388 species included in the China Plant Red Data List (Anon. 2000a).

The rhizome of this plant has immense implementation in pharmaceuticals due to presence of several active constituents, which are summarized in **Table 1**. The dried rhizome is used for curing fever, malnutrition due to digestive disturbance, jaundice, diarrhoea, and dysentery (Zhang *et al.* 1994). The rhizome of this species is also used as an adulterant of, or as substitute for *Gentiana kurrooa*, whose common properties include the ability to stimulate the appetite and gastric secretions (Jain 1968). It is used as a cathartic and in the case of dyspepsia, as a purgative as well as in the treatment of scorpion bites (Anon. 2000b). Besides, the rhizome of this species is widely used as a traditional medicine in several countries such as India, Nepal, China and Bhutan (Olsen 1998; Chhetri *et al.* 2005).

Hara *et al.* (1982) claimed that Sikkim, Nepal and China are the only places where *P. scrophulariiflora* occurs naturally. However, distribution and taxonomy of *P. kurrooa*

Table 1 Some of the uses of active principles of *P. scrophulariiflora* in pharmacy.

Componnd	Used as/or against	References
Cucurbitacins I	Cytotoxic, carcinoma	Miro 1995
Cucurbitacin D, I and E	Anti-tumor	Gitter *et al.* 1961; Fuller *et al.* 1994
Cucurbitacin B	Anti-inflammatory	Yesilada *et al.* 1988, 1989
Iridoids	Cardiovascular, antihepatotoxic, choleretic, hypoglycemic, hypolepidemic, anti-inflammatory, antispasmodic, antitumor, antiviral, purgative, immunomodulatory, antioxidant, anti-phosphodiesterase, neuritogenic, molluscicidal, leishmanicidal activities	Ghisalberti 1998
Picroliv	Antihepatotoxic, hepatoregenerative, choleretic	Dhawan 1995
Kutkin	Anti-inflammatory	Singh *et al.* 1993
Picroside II and pikuroside	Anti-inflammatory	Jia *et al.* 1999
Picroliv	Hypoxia	Gaddipati *et al.* 1999
Plantamajoside	Inhibitor of phosphodiesterase and 5-lipoxygenase	Zhou *et al.* 1998
phenylethanoids	Antibacterial, reactive oxygen species scavengers	Calis *et al.* 1999

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and *P. scrophulariiflora* is a matter of controversy. According to Olsen (1998) and Smit (2000), the distribution of *P. kurrooa* is restricted to the Western Himalayas, from Kashmir to Kumaon, whereas *P. scrophulariiflora* is found in the Eastern Himalayas, from Garwal to Sikkim or even up to Bhutan. Taxonomically the genus *Picrorhiza* was considered monotypic with only species *kurrooa* until Pennell (1943) distinguished another species, based on information in the "Flora of British India" (Hooker 1885). Hooker described the flower of *P. kurrooa* as dimorphic, which was so far not known in the family Scrophulariaceae. He further described a form with long stamens and short corolla with five sub-equal lobes, and a form with short stamens and a bilabiate corolla, of which the upper lip is longer and lower lip consists of three shorter lobes. However, based on the collections made by Smith and Cave (1911) from the Zemu glacier in Sikkim, Pennell (1943) noted the presence of shorter stamens than those described by Hooker. Furthermore, he noted that all collections seen from the Western Himalayas are in agreement with the form having long stamens, while those from the Eastern Himalayas and Yunnan provinces of China correspond to the form with shorter stamens. Therefore, Pennell (1943) characterized two species, one from the dry Western Himalayas as *P. kurrooa* and the other one from the moist Eastern Himalayas as *P. scrophulariiflora*.

A thorough literature survey indicated that except for a pharmaceutical study (Olsen 1998), no systematic effort to reveal population status, conservation threats and plant-animal interactions of *P. scrophulariiflora* has been made anywhere in the world, including the Sikkim Himalayas, until now, although several reports have indicated the need of conservation, sustainable utilization as well as cultivation of this species (Olsen 1998; Subedi 2000). Thus, in this study an attempt has been made to find out (1) the population status, (2) the threat category, (3) phytosociological associations, (4) probable conservation threats, (5) ethno-botany, (6) the correlation between different parameters of soil and underground as well as aerial biomass and (7) selection of superior germplasm of *P. scrophulariiflora* in Sikkim Himalayas.

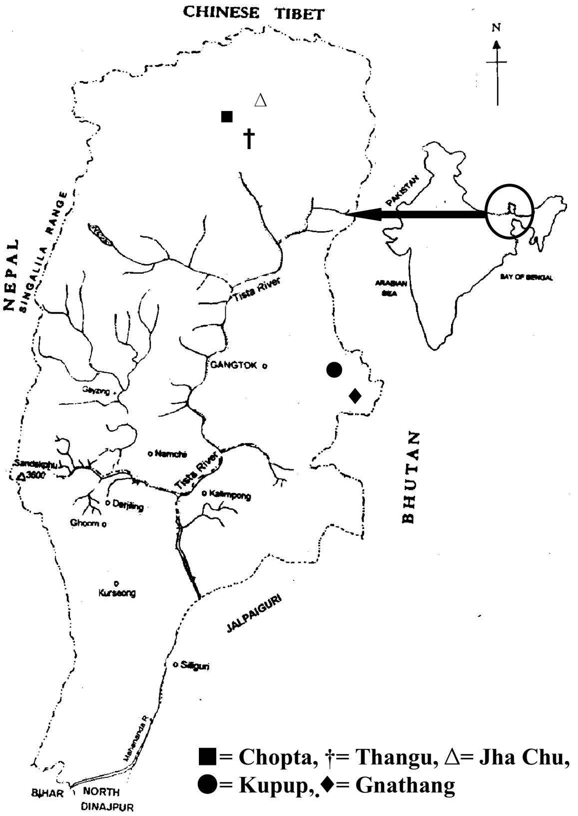

Fig. 1 Map of eastern Indian Himalayan region showing the different sites of experiments.

Table 2 Site characteristics of the selected populations of *P. scrophulariiflora* in Sikkim Himalayas.

Code	Location	Altitude (masl)	Aspect	Habitat
N1	Thangu I	4180 m	South facing	Habitat dominated by *Salix calyculata* and *Rhododendron anthopogan* in sandy and loam soil in moist condition, population good in sandy soil. Rocky terrain and boulders were the permanent habitat in steep slopes.
N2	Thangu II	4180 m	South facing	Rocky terrain and boulders were the permanent habitat in steep slopes. *Bergenia ciliata* and *Rhododendron setosum* predominated in open and moist pastures.
N3	Thangu III	4180 m	South facing	*Cassiope fastigiata* and *Rhododendron anthopogan* predominant and they are found to grow well on rock crevices.
N4	Chopta I	4250 m	South-West facing	*Cassiope fastigiata*, *Bistorta vaccinifolia* were most predominant associates and they grow well in sandy moist soil. However, population density was better in gentle slopes with moist boulders and rocky terrain.
N5	Chopta II	4250 m	South-West facing	*Bergenia ciliata*, *Rhododendron anthopoga* and *R. setosum* were predominant associates and they are found to be grown well in steep slopes, rock crevices as well as in moss laden moist rocks.
N6	Chopta III	4250 m	South-West facing	*Bistorta vaccinifolia, Persicaria polystachys* and *Salix calyculata* dominated moist slopes with wet sandy soil.
N7	Ja Chu I	4315 m	South-West facing	*Bistorta vaccinifolia, R. setosum*and and *Persicaria polystachys* were the most dominant associates. Open pasture having moist sandy loam soil and boulders were the preferred habitat.
N8	Ja Chu II	4315 m	South-West facing	*Bistorta* spp, *R. anthopogan* and *Salix calyculata* were the dominant associates and population was good in open pasture with moist and sandy soil.
N9	Ja Chu III	4315 m	South-West facing	*Bistorta* spp, *Rumex nepalensis* and *Rhadodendron* spp. were the dominant species and fairly good population was found in moist and sandy soil in open pasture.
E1	Kupup I	4200 m	North-West facing	Nearby road sides open pasture and steep slopes were the preferred habitat with *Bistorta* spp., *Rhododendron* spp. and *Saussurea nepalensis* dominated places.
E2	Kupup II	4200 m	North-West facing	As above.
E3	Kupup III	4200 m	North-West facing	Rocky crevices, boulders and moss laden moist rocks where *Bistorta* spp., *Cassiope fastigiata* and *Rhodendron setosum* showed dominant associates.
E4	Gnathang I	4100 m	North-West facing	*Bistorta* spp., *Cassiope fastigiata* and *Rhodendron setosum* as dominant associates. Open pasture with gentle slopes, moss laden moist rocks and rocky crevices with sandy loam soil were the preferred habitat and population was good at underneath of *R. anthopogan.*
E5	Gnathang II	4200 m	North-West facing	Rocky terrain, moist boulders and open pasture *Bistorta* spp., *Cassiope fastigiata* and *Rhodendron setosum* as dominant associates.
E6	Gnathang III	4200 m	North-West facing	Moist rocky terrain, moss laden rocks and gentle slopes well as open pasture with *Bistorta* spp., *Salix caliculata* and *Rhododendron anthopogan*.

MATERIALS AND METHODS

The area under the present study (**Table 2; Fig. 1**) falls between 27° 54.072′ N latitude and 88° 29.719′ E longitude, 4100 to 4315 msl, which covers three catchments, Chopta, Jha chu and Thangu in the north and Kupup as well as Gnathang in the east. Relevant specimens of *P. scrophulariiflora* Pennell. (Scrophulariaceae) were collected and authenticated at the Botanical Survey of India, Sikkim Himalayan Circle. The areas were surveyed extensively and finally nine sites (population) of North Sikkim and six sites of East Sikkim (**Table 2**) were identified on the basis of habitat, population size and accessibility for data collection. Indigenous, especially elderly people, of those areas were interviewed by one of the authors regarding the medicinal usage of the species. Intensive investigations of selected populations were carried out throughout the year and phytosociological studies were carried out during the rainy season i.e., July to August, 2006, the peak period for the growth of sub-alpine and alpine plants in these regions. For determining the threat category, pockets of 100 m^2 of *P. scrophulariiflora* were identified and marked on each site. On the basis of plant diversity (population estimation) and extent of occurrence (number of pockets in which *P. scrophulariiflora* was present), the status of the species was determined for all alpine regions of Sikkim Himalayas on the basis of (i) the extent of occurrence and (ii) population estimation according to IUCN Red List Categories (2004). Vegetation sampling was conducted through vertical belt transects (Michael 1990). One transect (20 m wide and 100 m long) was laid across each population and each population constitutes one locally demarcated geographical area, where studied species were present. Three stands (20 m^2) were marked at different positions (i.e., top, middle and base) of each transect. Fifteen quadrates (each 1 m^2) were laid at random within each stand. Individuals of all the species were counted in each quadrate. Analytical features such as relative frequency (RF) and relative density (RD) were calculated following the protocol of Curtis and McIntosh (1950) as well as Muller-Dombois and Ellenberg (1974). Other analytical features such as density, abundance, frequency and important value index (IVI) were calculated following the protocol of Misra (1968) and the Abundance Frequency ratio (A/F) was calculated following the method of Whitford (1949). In each stand, 25 individuals per transect were sampled randomly for morphological traits (rhizome diameter and rhizome length) and biomass study. These 25 selected plants per transect were brought to the laboratory; subsequently the aerial as well as underground parts were separated by cutting with a sharp knife washed carefully with water and then oven-dried at 80°C until constant weight was reached. Net dry weight was used as biomass and total biomass was calculated as per the protocol described by Airi *et al.* (1997). The remaining aerial parts were utilized to record leaf length, leaf breadth, numbers of fruit and/or flowers per inflorescence. Numbers of sheep and shepherds as well as the numbers of yaks and yak-sheds, which were present within each population, were recorded. Their movements and grazing behaviors in each population were observed carefully to understand the plant-animal interactions. This will enable us to analyze the impact of animal interactions in the depletion of this plant in the natural habitat. To correlate soil characteristics and biomass production, soil samples were collected (Jackson 1967) and analyzed for available nitrogen (Subbiah and Asija 1956), soil pH, organic carbon and moisture content (Allen 1989). Data from all 15 sites (population) on aerial as well as below ground biomass were pooled and statistically analyzed for correlating them with respect to soil characteristic and altitude gradients (Das 2000).

RESULTS AND DISCUSSION

Repeated interactions with local people of North as well as East Sikkim ultimately resulted in a warm relationship with them and they felt free to divulge the local traditional usage of the species. Sikkimese Bhutia and Sherpas are the two dominant inhabitants of alpine zones of both North and East Sikkim. Both these ethnic groups use this species to treat common diseases and disorders. Most of the uses are recorded for the first time unless otherwise mentioned. The dried rhizome is administered orally with water to treat chronic cold, fever and body ache. Small pieces of dried rhizome are taken to cure head-aches, jaundice and related stomach disorders in a minute amount (various from locality to locality). Sometimes it is also used as an antidote, especially during food poisoning. Rarely the entire plant, either green or in dry condition, is used against scorpion sting. About half a tea spoonful of rhizome powder is given orally in the case of high palpitation and other such heart-related problems. Rai *et al.* (2000) also recorded that the dried rhizome is boiled and that the decoction is given as a tonic. It is also effectively administered in the case of dyspepsia.

On the basis of extensive field visits, several pockets of *P. scrophulariiflora* were identified (**Fig. 2A-D**) and population density of the species was measured to access the mature individuals in each stand and site to determine the threat categories of the taxon. A maximum of 8 pockets at N1, N3, N5, E1, E2, E3 and E4 followed by 6 pockets at N4, 5 pockets at N6, N8, N9, E5 and E6, and finally a minimum of 4 pockets each at N2 and N7 were recorded (**Table 3**). Based upon the population density, a maximum of 5221 and minimum of 890 mature individuals of *P. scrophulariiflora* were recorded at E4 and N2 sites, respectively (**Table 3**). The extent of occurrence and population estimation is the major criteria used to assign threat categories besides population reduction and probability of extinction (IUCN 2004). On the basis of extent of occurrence, the status of *P. scrophulariiflora* is endangered at N2, N4, N6, N7, N8, N9, E5 as well as E6 and vulnerable at N1, N3, N4, N5, E1, E2, E3 and E4. However, on the basis of estimated population (**Table 3**), N1, N3, N5, E1, E2, E3, E4 and E5 are vulnerable, the rest are endangered. Therefore, for Sikkim Himalayas, the species is vulnerable, which is in agreement with Nayar and Sastry (1990) who also reported *P. scrophulariiflora* of Sikkim Himalayas as a vulnerable species while preparing the Red list of Indian plants.

Rock crevices, rocky terrain (**Fig. 2A**) with moist soil were the preferred habitat within the sub-alpine and alpine zones of Sikkim Himalayas (**Table 2**). The species was found to be habitat-specific with low ecological amplitude and sparsely distributed. Its population was noted exclusively on west and south-west facing slopes due to presence of moist sandy loam soil with enough leaf mould. In almost all cases, species presence was noted exclusively under the canopy of *Rhododendron anthopogan* and *R. setosum* (**Table 2**). Its growth was relatively better (**Table 7**) in the association with *Rhodendron anthopogan*, *R. setosum*, *Cassiope frastigiata* and *Prinsepia utilis* in the N3 population (**Table 4**). The diversity of the associated species was found to be a maximum of 25 species at N5 to a minimum of 14 species at N8 (**Table 3**). One of the reasons for this low variation of species diversity may be due to the steepness or rocky ter-

Table 3 Determination of threat status of *P. scrophulariiflora* in Sikkim Himalayas.

Code*	№ of species/m^2	Mature population estimated	EOO	Status IUCN (2004)
N1	24	2480	8	Vu^{1-2}
N2	17	890	4	En^{1-2}
N3	22	2291	8	Vu^{1-2}
N4	20	2065	6	Vu^{1} En^{2}
N5	25	2100	8	Vu^{1-2}
N6	16	2354	5	En^{1-2}
N7	17	1975	4	En^{1-2}
N8	14	2662	5	En^{1} Vu^{2}
N9	15	1725	5	En^{1-2}
E1	18	1812	8	Vu^{1-2}
E2	16	1870	8	Vu^{1-2}
E3	19	2437	8	Vu^{1-2}
E4	23	5221	8	Vu^{1-2}
E5	20	2711	5	En^{1} Vu^{2}
E6	15	1630	5	En^{1-2}

* as described in **Table 2**
En = endangered, EOO = extent of occurrence, Vu = vulnerable
^{1} = on the basis of EOO, ^{2} = on the basis of population estimation

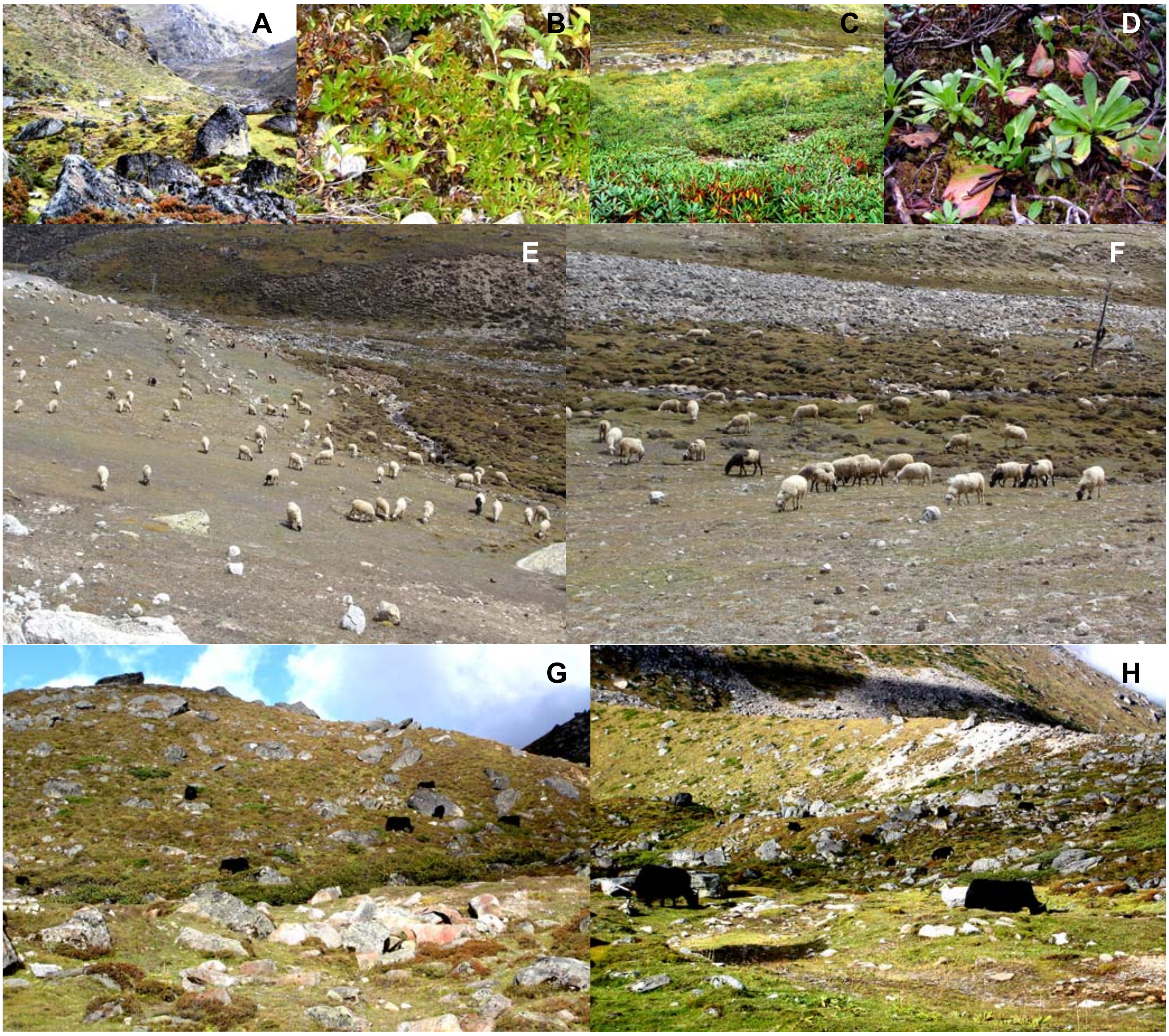

Fig. 2 Showing different locations and habitat of *P. scrophulariiflora*. (**A-C**) Natural habitat of *P. scrophulariiflora* at Jah Chu, Thangu and Chopta, respectively. (**D**) Close up view of *P. scrophulariiflora* at Chopta. (**E-F**) Sheep grazing at the alpine regions of North Sikkim and (**G-H**) Yak grazing at different places of North Sikkim.

rain, which is common in the alpine region of Sikkim Himalayas. The presence of boulders on gentle slopes was very common on almost all the sites, which may be one of the other potent factors of low species diversity. Animal grazing, another important parameter, were recorded to be maximum at N2 (437 yaks and 160 sheeps) (**Table 5**), where the recorded associated species was merely 17 in number (**Table 3**). This indicates that species richness mainly depends on two factors in these regions, which may be (i) surface terrain of the habitat and (ii) the extent of animal grazing.

Based on the values of IVI, it was found that while the population structure was quite better at N4, it was comparatively low at N5. However, relative density of the taxon was overall low in all the populations varying from a lowest of 7.93 at N9 to a maximum of 17.22 at N1 of North Sikkim and a minimum of 8.20 at E1 to a maximum of 14.86 at E6 of East Sikkim (**Table 4**). This indicates low availability of this species in these regions. Distribution pattern of species is determined by the abundance frequency ratio which varies from a minimum of 0.55 at N2 to maximum of 10.33 at N4, suggesting that the distribution of the species is very random and severely fragmented. At the same time, the frequency of *P. scrophulariiflora* varies from a minimum of 13.33% at N4 to a maximum of 53.33% at N6, which also suggests an uneven distribution. On the other hand, relative abundance also showed considerable fluctuations among the populations varying from 7.78 at N2 to 29.26 at N4. However, relative frequency is considerably low with a minimum of 1.67 at N4 to a maximum of 10.06 at N2 indicating the presence of rareness and fragmented populations of a taxon in Sikkim Himalayas. Thus the low value of all the analytical features such as frequency, abundance, etc., in the present study (**Table 4**) indicates the status of vulnerability of this species, in agreement with earlier reports of CITES appendix II (Olsen 1998) and China Red Data List (Anon. 2000a) where it is described as vulnerable. In some similar studies, to identify the conservation threats of *Nardostachys jatamansi* (Airi *et al.* 1997) and *Podophyllum hexandrum* (Nautiyal *et al.* 2003), two endangered Indian medicinal plants, it was found that their populations are also dwindling in the natural habitat due to habited destruction, over exploitation and some other human activities which further support our findings in the present study.

The phytosociological study revealed that highest relative density of *P. scrophulariiflora* was recorded as 23.26 at E4, where the dominant associates were *Bistorta affinis*, *B. macrophylla* and *Bergenia ciliata* as indicated by IVI data (**Table 4**). However, on the basis of frequency percentage, two major dominant associates were *Rhododendron anthopogan* and *R. setosum*. Importantly, among the associated

Table 4 Phytosociological attributes of *P. scrophulariiflora* at different selected populations in Sikkim Himalayas.

Code*	F%	RF	RD	RA	IVI	A/F ratio	Dominant associates: Based on F%	Dominant associates: Based on IVI
N1	20.00	5.76	17.22	15.05	38.03	2.29	*Rhododendron anthopogan* (80.00), *Salix caliculata* (60.00) and *Cassiope fastigiata* (53.33)	*Pedicularis oederi* (44.33), *Cassiope fastigiata* (42.18) and *Salix caliculata* (27.78)
N2	37.78	10.06	14.75	7.78	32.60	0.55	*Rhododendron setosum* (97.78), *Bergenia ciliata* (75.56) and *Rhodendron antopogan* (62.22)	*Bergenia ciliata* (73.48), *Stellaria decumbens* (47.90) and *Rhodedendron setosum* (37.74)
N3	22.22	3.22	10.67	16.18	30.07	4.17	*Rhododendron anthopogan* (80.00), *Prinsepia utilis* (68.89), *Cassiope fastigiata* (62.22) and *Pedicularis oederi* (62.22)	*Cassiope fastigiata* (42.65), *Prinsepia utilis* (30.11) and *Bistorta affinis* (29.28)
N4	13.33	1.67	8.33	29.26	39.22	10.33	*Bistorta vaccinifolia* (82.22), *Saussurea nepalensis* (80.00), *Cassiope fastigiata* (73.33) and *Prinsepia utilis* (73.33)	*Cassiope fastigiata* (47.62), *Anaphalis contorta* (37.63) and *Bistorta vaccinifolia* (33.78)
N5	37.78	4.43	8.63	11.48	24.54	1.32	*Rhododendron setosum* (84.44), *R. anthopogan* (82.22) and *Saussurea nepalensis* (80.00)	*Bistorta affinis* (53.74), *B. vaccinifolia* (43.40) and *Cassiope fastigiata* (29.80)
N6	53.33	7.14	11.08	12.20	30.43	0.77	*Rhododendron anthopogan* (91.11), *Bistorta vaccinifolia Rheum nobie* (68.89) and *Salix calyculata* (68.89)	*Bistorta vaccinifolia* (36.45), *Persicaria polystachys* (35.71) and *B. affinis* (33.02)
N7	42.22	6.13	13.99	16.52	36.64	0.99	*Rhododendron anthopogan* (91.11), *R. setosum* (86.67) and *Bistorta vaccinifolia* (75.56)	*Bistorta vaccinifolia* (53.36), *Persicaria polystachys* (50.70) and *Bergenia ciliata* (36.37)
N8	40.00	4.90	11.27	16.82	33.00	1.51	*Rhododendron anthoogan* (93.33), *R. setosum* (86.67), *Bistorta affinis* (73.33) and *B. vaccinifolia* (73.33)	*Bistorta vaccinifolia* (36.45), *Persicaria polystachys* (35.71) and *B. affinis* (33.02)
N9	22.22	4.05	7.93	17.02	28.99	3.11	*Rhododendron setosum* (84.44), *R. anthopogan* (82.22) and *Bistorta vaccinifolia* (73.33)	*Bistorta affinis* (66.90), *B. vaccinifolia* (47.91) and *Cassiope fastigiata* (33.95)
E1	35.56	4.89	8.20	11.85	24.95	1.30	*Rhododendron anthopogan* (88.89), *R. setosum* (80.00) and *Saussurea nepalensis* (68.89)	*Bistorta affinis* (57.83), *B. vaccinifolis* (53.36) and *B. macrophylla* (46.13)
E2	33.33	5.12	8.36	12.96	26.43	1.50	*Rhodendron setosum* (84.44), *Saussurea nepalensis* (80.00) and *Bistorta affinis* (71.11)	*Bistorta affinis* (54.98), *B. macrophylla* (49.69) and *B. vaccinifolia* (48.85)
E3	33.33	4.24	10.66	15.73	30.62	1.95	*Rhododendron anthopogan* (80.00), *Saussurea nepalensis* (77.78) and *Bistorta affinis* (73.33), *B. vaccinifolia* (73.33)	*Bistorta affinis* (53.26), *B. macrophylla* (47.99) and *B. vaccinifolia* (41.56)
E4	44.44	4.76	23.26	25.52	53.54	2.80	*Rhododendron anthopogan* (86.67), *R. setosum* (75.56), *Bergenia ciliata* (68.89) and *Bistorta affinis* (68.89)	*Bistorta affinis* (47.96), *B. macrophylla* (34.83) and *Bergenia ciliata* (29.05)
E5	42.22	4.87	11.47	14.05	30.39	1.40	*Rhododendron anthopogan* (82.22), *R. setosum* (82.22), *Bistorta affinis* (73.33) and *B. vaccinifolia* (68.89)	*Bistorta affinis* (50.63), *B. macrophylla* (44.03) and *B. vaccinifolia* (37.48)
E6	26.67	4.53	14.86	23.63	43.01	2.08	*Rhododendron anthopogan* (68.89), *Bistorta vaccinifolia* (64.44), *B. affinis* (64.44) and *R. setosum* (64.44)	*Bistorta affinis* (56.73), *B. vaccinifolia* (47.56) and *B. macrophylla* (43.47)

*Code as described in **Table 2**; F%= frequency percent, RF= relative frequency, RD= relative density, IVI= importance value index, A/F ratio= abundance/frequency percent ratio.

species found in this study, *R. anthopogan*, *R. setosum*, *B. affinis* and *Cassiope fastigiata* (**Table 4**) showed greater ecological amplitude i.e. most abundant in experimental study sites. Further, visual observation revealed that *P. scrophulariiflora* was found to grow exclusively under the canopy of either *R. setosum* or *R. anthopogan.* On the other hand the relative density was lowest at N9 with 7.93, where the dominant associates, on the basis of IVI were *Bistorta affinis*, *B vaccinifolia* as well as *Cassiope fastigiata* and *Rhododendron setosum*, *R anthopogan* and *Bistorta vaccinifolia* on the basis of frequency percentage (**Table 4**).

Our observation revealed that there is extremely high animal grazing in open pastures and hill slopes, which were a permanent habitat of *P. scrophulariiflora* (**Fig. 2E-H**). Grazing by 400 or more yaks at a time (**Fig. 2G-H**) in a common location definitely has ill effects on populations and also to its natural habitat. There were as many as 4 yak-sheds at N4 raring 509 yaks (**Table 5**). Local people are engaged in taking these animals to distant locations for grazing, which not only destroys the natural populations of the species but the populations of other associated species is also hampered due to destruction of habitat quality. Additionally, sheep rearing at higher altitudes is also a very common phenomena, especially in North Sikkim (**Fig. 1E-F**). On average, a shepherd takes over 300 sheep in a common place for grazing and it was noted that such practice is very common in North Sikkim. Due to such overgrazing, the habitat of N2 was damaged in such a critical manner that as few as 890 mature individuals were recorded (**Table 3**). Though restrictions in the collection of rare and endangered medicinal plants from wild habitats are imposed by Department of Wild life and Forest, Govt. of Sikkim, in Sikkim Himalayas, a great deal of illegal collection of such species still exists there (Rai *et al.* 2000) by the sheep- and yak-rearing people because the exact locations of occurrence of these economically important medicinal plants are known to them. Such people also collect several others rare medicinally important species such as *Rhododendron anthopogan*, *Bergenia ciliata*, *Nardostachys jatamansi*, *Saussurea nepalensis*, *Dactylorhiza hatagirea*, *Podophyllum hexandrum*, etc., along with *P. scrophulariiflora* (Nayar and Sastry 1990; no newer statistics available) which fetch a good amount of revenue (approx. 1-1.5 $US/kg dried rhizome) for their sustenance. Therefore gradual depletion of these associated species of *P. scrophulariiflora* contributed to alter the phytosociological association as most of them are the dominant associates which also contribute to the dwindling population of this taxon. Even though the correct period for collection is July to October, we observed that people have been actively engaged in harvesting rhizomes as early as the third week of April, which not only prevents seed set but also hinders sucker growth. Such activity was also common for *P. scrophulariiflora* in Nepal (Subedi 2000).

Table 5 Determination of animals (yak, yak shed, shepherd and sheep) threats in the habitat of *P. scrophulariifora* in Sikkim Himalayas.

Code*	Yaks		Sheep	
	№ of sheds	**№ of yaks**	**№ of shepherds**	**№ of sheep**
N1	-	-	2	320
N2	2	437	1	160
N3	-	-	2	150
N4	4	509	-	-
N5	1	132	3	267
N6	-	-	3	350
N7	3	362	2	170
N8	2	196	-	-
N9	2	187	-	-
E1	-	42	-	-
E2	-	36	-	-
E3	-	-	-	-
E4	-	82	-	-
E5	-	79	-	-
E6	-	88	-	-

* as described in **Table 1**

Table 6 Soil characteristics of studied sites.

Site	pH	C%	N_2 kg/ha	Moisture %
N1	4.56	7.72	166.27	37.28
N2	4.15	8	173.56	42.37
N3	4.14	8.21	175.29	47.21
N4	5.77	5.21	142.22	42.39
N5	4.48	7.76	166.89	38.28
N6	4.91	7.2	162.32	46.28
N7	4.45	7.92	170.31	36.28
N8	5.72	5.82	147.52	39.29
N9	5.82	5.1	140.76	37.76
E1	5.62	6.27	154.78	30.76
E2	5.76	6.57	161.33	34.29
E3	5.32	6.27	152.83	30.34
E4	5.22	5.8	150.66	31.68
E5	4.50	7.6	165.82	32.02
E6	4.73	7.22	160.22	30.72

* code as described in **Table 1**; C% = organic carbon percent, N_2 kg/ha = available nitrogen (kg/ha)

The pH of the soil among the surveyed area was acidic, with values varying from 4.14 to 5.77 (**Table 6**). Organic carbon was found to fall within the range of 5.1% at N9 to 8.21% at N3. However, available soil nitrogen was in the range of 140.76 kg/ha at N9 to 175.29 kg/ha at N3 and soil moisture were also recorded a minimum of 30.34% at E3 to a maximum of 47.21% at N3. Therefore we concluded that the taxon prefers to grow in acidic, moist soil rich in carbon and nitrogen. Similarly, aerial as well as underground biomass was analyzed separately (**Table 7**). A morphological study revealed that the N3 population was best as it had the highest values for most of the aerial parameters such as plant height (18.2 cm), number of leaves (15.6), leaf width (4.2 cm), leaf length (15.3 cm), number of flowers per inflorescence (19.7), although maximum fruit set (18.8) was recorded equally at N2 and N3. On the other hand, the collective value for morphological parameters such as plant height (10.3 cm), number of leaves (9.7), leaf length (9.2 cm), leaf width (2.4 cm), number of flowers per inflorescence (12.2) and number of fruits (11.4) were lowest at N9 (**Table 7**). Maximum belowground biomass as expressed by rhizome diameter (0.96 cm) and dry weight of rhizome (2.2 g) was recorded at N3, though maximum rhizome length (11.33 cm) was equal in the N2 and N3 populations (**Table 7**). On the other hand, while the minimum rhizome diameter (0.46 cm) was recorded at N9 and E6, the minimum rhi-

Table 7 Morphological parameters of different population of *P. scrophulariiflora.*

Code*	Biomass									
	Aerial						Below ground			
	Plant height ** (cm)	**№ of leaves**	**Leaf length (cm)**	**Leaf width (cm)**	**№ of flowers/ inflorescence**	**№ of fruit**	**Diameter (cm)**	**Length (cm)**	**Dry weight (gm)**	**Total biomass # (kg/ha)**
N1	17.1 (±1.92)	14.3 (±0.74)	14.1 (±0.82)	3.7 (±0.14)	18.2 (±1.56)	16.2 (±1.64)	0.76 (±0.12)	8.66 (±0.88)	1.58 (±0.1)	349.97
N2	18.0 (±2.31)	15.2 (±0.89)	15.0 (±1.22)	4.0 (±0.15)	19.5 (±1.3)	18.8 (±1.24)	0.80 (±0.06)	11.33 (±2.03)	1.89 (±0.2)	148.78
N3	18.2 (±1.64)	15.6 (±0.89)	15.3 (±0.67)	4.2 (±0.17)	19.7 (±1.52)	18.8 (±0.84)	0.96 (± 0.12)	11.33 (±1.20)	2.12 (±0.3)	436.93
N4	11.1 (±3.07)	9.8 (±1.35)	10.4 (±0.89)	2.9 (±0.12)	14.2 (±1.56)	12.3 (±0.82)	0.53 (±0.15)	10.00 (±1.53)	1.11 (±0.1)	203.20
N5	17.6 (±1.68)	14.8 (±0.89)	14.4 (±0.77)	3.9 (±0.13)	18.6 (±1.75)	16.8 (±1.08)	0.66 (±0.12)	9.00 (±1.53)	1.68 (±0.2)	315.84
N6	13.1 (±2.3)	13.1 (±0.79)	12.7 (±1.26)	3.5 (±0.25)	16.7 (±2.25)	14.6 (±0.57)	0.56 (±0.09)	10.33 (±1.20)	1.45 (±0.1)	315.88
N7	17.6 (±1.99)	14.8 (±0.42)	14.8 (±0.69)	4.1 (±0.15)	19.0 (±0.82)	17.5 (±0.96)	0.66 (±0.15)	11.33 (±0.67)	1.74 (±0.1)	305.99
N8	12.6 (±0.84)	10.3 (±0.76)	10.7 (±0.82)	3.2 (±0.15)	14.6 (±1.3)	13.0 (±1.17)	0.60 (±0.12)	8.33 (±0.33)	1.20 (±0.2)	289.07
N9	10.3 (±1.9)	9.7 (±0.65)	9.2 (±0.67)	2.4 (±0.19)	12.2 (±1.34)	11.4 (±1.15)	0.46 (±0.09)	7.33 (±0.88)	0.91 (±0.1)	139.69
E1	15.7 (±2.79)	13.3 (±1.2)	11.9 (±0.96)	3.1 (±0.34)	15.7 (±1.56)	14.1 (±0.82)	0.83 (±0.03)	8.66 (±0.90)	1.32 (±0.1)	217.65
E2	16.3 (±1.41)	12.8 (±1.08)	12.7 (±0.74)	3.5 (±0.32)	16.4 (±1.02)	14.7 (±1.10)	0.67 (±1.5)	5.50 (±0.76)	1.43 (±0.1)	238.65
E3	16.1 (±2.46)	12.3 (±1.25)	12.1 (±0.86)	3.4 (±0.22)	15.7 (±1.52)	13.4 (±1.60)	0.70 (±0.06)	6.27 (±0.656)	1.35 (±0.0)	292.5
E4	16.2 (±2.51)	12.5 (±1.1)	11.6 (±0.38)	3.2 (±0.18)	15.2 (±0.94)	12.0 (±1.37)	0.50 (±0.06)	5.57 (±0.23)	1.24 (±0.1)	684.76
E5	16.9 (±1.97)	13.2 (±0.89)	13.7 (±0.66)	3.7 (±0.07)	17.8 (±1.24)	15.7 (±1.44)	0.67 (±0.07)	6.4 (±0.95)	1.58 (±0.2)	395
E6	17.2 (±2.15)	13.7 (±1.52)	13.2 (±0.34)	3.6 (±0.23)	17.6 (±1.2)	15.7 (±1.29)	0.46 (±0.07)	6.13 (±1.46)	1.52 (±0.1)	224.96

X data pooled from separate experiments ± = Standard Error; kg/ha= Kilogram per hectare *=code as described in table 1
**= only aerial portion i.e., without rhizome
#= below ground biomass only

Table 8 Correlation between soil characters and aerial as well as belowground biomass.

Plant parameters / Soil characters	Plant height (cm)	№ of leaves	Leaf length (cm)	Leaf breadth (cm)	№ of flowers/ inflorescence	№ of fruits/ inflorescence (cm)	Rhizome diameter (cm)	Rhizome length (cm)	Rhizome dry weight (g)	Total belowground biomass (kg/ha)
Organic carbon %	0.82	0.65	0.70	0.75	0.59	0.65	0.58	0.44	0.94	0.12
Available nitrogen	0.84	0.71	0.68	0.72	0.51	0.54	0.65	0.44	0.96	0.14
Moisture	-0.21	-0.12	0.11	0.22	0.17	0.19	0.24	0.77	0.33	-0.12
Altitude	-0.55	-0.35	-0.05	-0.13	-0.35	-0.42	-0.11	0.45	-0.27	-0.46
pH	-0.76	-0.62	-0.56	-0.64	-0.63	-0.68	-0.46	-0.46	-0.90	-0.24
Leaf area	--	--	--	--	--	--	--	--	--	0.36

zome length (5.50 cm) was recorded at E2 and minimum rhizome dry weight (0.91 g) at N9 (**Table 7**). In terms of both belowground as well as aerial biomass, the N3 population was better for biomass production and may be considered as a superior line as the price per rhizome (approx. 1-1.5 $US/kg clean, dried rhizome) is related to the weight of biomass.

Thus the present study indicates that the species prefers to grow in acidic, nutrient-rich and moderately moist soil. Both available nitrogen and organic carbon values of the plant population approach high values for alpine soil (Ram and Singh 1994). Available nitrogen in the soil is an essential nutrient and has a significant effect on both underground as well on aerial biomass of the species. In order to find out the correlation between soil characters and biomass production of the species, soil pH, available nitrogen, organic carbon percent, and soil moisture was analyzed (**Table 8**). The plant height (r = 0.84), number of leaves (r = 0.71), leaf length (r = 0.68), leaf width (r = 0.72), number of flowers per inflorescence (r = 0.51), number of fruits per inflorescence (r = 0.54), rhizome diameter (r = 0.65), length of rhizome (r = 0.44) and finally rhizome biomass (r = 0.12) were positively correlated with available nitrogen in the soil (**Table 8**). Similarly the plant height (r = 0.82), number of leaves (r = 0.65), leaf length (r = 0.70), leaf width (r = 0.75), number of flower per inflorescence (r = 0.59), number of fruits per inflorescence (r = 0.64), rhizome diameter (r = 0.58), rhizome length (r = 0.44) and rhizome dry weight (r = 0.94) were also positively correlated with organic carbon of the soil (**Table 8**). However, while the leaf length (r = 0.11), leaf width (r = 0.21), number of flowers per inflorescence (r = 0.17), number of fruits per inflorescence (r = 0.19), rhizome diameter (r = 0.24) and rhizome length (r = 0.77) is positively correlated with soil moister content, it is also has negatively correlated with plant height (r = -0.21) and number of leaves (r =-0.12) (**Table 8**). Among the soil characters, soil pH greatly influences the growth of plant species (Airi *et al.* 1997). Aerial biomass such as plant height (r = -0.76), number of leaves (r = -0.62), leaf length (r = -0.56), leaf width (r = -0.64), number of flowers per inflorescence (r = -0.63) and the number of fruit (r = -0.68) as well as under ground biomass such as rhizome diameter (r = -0.0.46), rhizome length (r = -0.46), rhizome dry weight (r = -0.90) and total underground biomass (r = -0.24) were negatively correlated with soil pH. However, leaf area of the species was positively correlated with underground biomass (r = 0.36) (**Table 8**). These findings agree with the fact that biomass production is better correlated with the absolute amount of resource absorbing surface (total leaf area, total rhizome length, total rhizome mass, etc.) and biomass allocation patterns have been refined not only by studying the allocation biomass to roots and shoots but also the morphological characters of leaves and roots (Aerts *et al.* 1991).

It is known that altitude influences the vegetation characters of a plant population (Druitt *et al.* 1990). A similar trend was registered in morphological characters in the present study (**Table 8**). Among different morphological characters such as plant height (r = -0.55), number of leaves (r = -0.35), leaf width (r = -0.13), number of flowers per inflorescence (r = -0.35), number of fruits per inflorescence (r = -0.42), rhizome diameter (r = -0.11) and rhizome length (r = -0.45) exhibited an absolutely negative correlation with altitude (**Table 8**). This suggests that the species performs relatively better at lower elevation limits. Airi *et al.* (1997) also observed similar trends in *Podophyllum hexandrum* in Kumuon Himalayas. Additionally, a decrease in plant height and leaf size with an increase in altitude observed here concurred with the findings of other groups reported in other species such as *Rumex nepalensis* and *Podophyllum hexandrum* (Clausen *et al.* 1940; Bhadula and Purohit 1994).

CONCLUSION

Picrorhiza scrophulariiflora is an endangered species with a restricted habitat of sub-alpine and alpine vegetations of eastern Himalayas belonging to the Indo-Burma biodiversity hotspot which prefers to grow in acidic, moist soil of south-west aspect rich in nitrogen and carbon. It needs to be conserved at the earliest *in situ* rather than in *ex situ* conservation. In this regard, this present finding may be well utilized to optimize the performance of the propagules *in situ* of the N3 population, as the diversity of this species is being maintained by governmental agency such as State Medicinal Plant Board, Government of Sikkim, India. Thus this identification of preferred habitats, altitudinal range and the elite line with respect to belowground biomass may give new way for sustainable conservation of the species. In order to protect the natural habitat of the entire alpine zone of Sikkim and to conserve this threatened taxon along with other endangered species, animal grazing and random harvesting of rhizomes has to be minimized immediately. Furthermore, local communities should be educated about the real need of conservation of this species through community involvement and organizing action-oriented workshops as well as personal interactions through the nodal agencies such as the State Medicinal Plant Board, Government of Sikkim, India. Presently we are working to develop a composite cultivation package including micropropagation for the production of a large number of this highly valued endangered medicinal plant which may be used, as said earlier, to reintroduce in its natural habitat in order to restore its decreasing population.

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