Research Article

Ethnobotany and Ethnomedicinal Uses, Chromosomal Status and Natural Propagation of Some Plants of Lahaul-Spiti and Adjoining Hills

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The present study documented the ethnobotanical and medicinal uses of plants from an ecologically fragile cold desert area of Lahaul-Spiti (Himachal Pradesh, India). Local people use plants for curing the stomach troubles, pain reliever, cough, gastric disorders, and aphrodisiac and other household purposes. In addition, chromosome numbers, male meiosis, and natural propagation were also investigated in these ethnobotanically used plants. Present investigations also form the basis for exploitation of intraspecific chromosomal variation/new cytotypes recorded in some of the presently studied species to detect biochemical diversity in the medicinally important plants. For documentation of ethnobotanical information, personal observations and interviews were conducted with medicine men, hakims, farmers, shepherds, local healers, and old aged people. This study identified 40 plant species under 33 genera belonging to 17 families which have been used locally for curing various diseases and other purposes. All the chromosome counts are new to the study area. On worldwide basis, meiotic chromosome counts of $n = 14$ and $n = 8$ in *Rosularia alpestris* and *Corydalis govaniana*, respectively, are the first ever reports. The present study indicates that the people of the area possess good knowledge about the different uses of plants in the area. It has been noticed that due to the lack of interest among younger generations in the preservation of invaluable ethnic knowledge, there is every possible chance of losing such a rich heritage of knowledge. It is very urgent to conserve such invaluable ethnic knowledge before it gets lost.

1. Introduction

Present study was conducted in Lahaul-Spiti, a cold and desolate alpine region in northwest Himalayas which is known for its seclusion, Buddhist culture, hostile climate, and unexplored, formidable, and breathtaking scenic beauty of the high snow clad mountains. It is a part of the Indian cold deserts which is situated between $31^{\circ}44'57''$ and $32^{\circ}59'57''$ N latitudes and between $76^{\circ}29'46''$ and $78^{\circ}41'34''$ E longitudes. Climatic conditions are typical of dry temperate and alpine zones. The vegetation which is mainly composed of perennial herbs and prostrate shrubs survives extreme variation in temperature including months buried under several feet of snow. Using plants for different purposes and in curing and healing is as old as man himself [1]. It was very well known to primitive people that plants contain beneficial and medicinal properties which they used in some form or the other. Even in

the ancient time, people classified plants on the basis of their medicinal uses. No comprehensive attempt has been made so far to gather ethnobotanical information and to assess the cytological diversity and natural propagation of the plants growing in the higher altitude and cold desert regions of Lahaul-Spiti. Presently an attempt has been made to collect information on the ethnobotanical uses and to examine the cytomorphological diversity and natural propagation in the species of Lahaul-Spiti and adjoining high hills.

1.1. Topography and Vegetation. The topography of the area is entirely different from the adjoining regions of northwest Indian Himalayas. The cold desert plants of Lahaul-Spiti show a wide range of habitat diversity which includes alpine pastures and slopes, moist shady and exposed places, forests and forest undergrowth, water courses, scree slopes, rock crevices, rocks/boulders, roadsides, riverbeds, and sandy

FIGURE 1: (a) Alpine pasture. (b) A part of Lahaul Valley near Baralacha Pass with scanty vegetation. (c) Dry temperate forests near Keylong. (d) Sheep grazing on alpine moist slopes.

areas (Figures 1(a)–1(d)). The Lahaul-Spiti which is the largest district of Himachal Pradesh with an area of ca.13835 km² consists of two regions (Lahaul Valley and Spiti Valley) which are different in many aspects. One such aspect is the vegetation which is quite different. The Lahaul Valley is on the greener side compared to the barren Spiti region where climate is much drier. Since the district possesses a great range of elevation, ranging from 2400 to 6500 m, the vegetation is diverse, unique, and plentiful. It comprises dry temperate to high alpine type except for some parts of Pattan Valley (Lahaul) adjoining the Pangi area of Chamba district, where it is of dry temperate to moist temperate type (Figure 1(c)).

2. Materials and Methods

2.1. Information Collection, Fixation of Material for Cytological Studies, Identification of Plants, and Male Meiotic Chromosome Counts. As the valley remains cut off from

other parts of country for more than six months due to heavy snow fall, observations on ethnobotanical information and natural propagation and collection of material for cytological studies were made between June and October in the years 2006–2009. During the intensive cytomorphological and ethnobotanical surveys, 40 plant species used by the local people of Lahaul-Spiti had been collected from different localities (Figure 2). For documentation of ethnobotanical information, personal observations and interviews were conducted with medicine men, hakims, farmers, shepherds, local healers, and old aged people. Plant identification was done by consulting the Flora of Lahaul-Spiti [2], the Flora of Kullu district [3], and Flora of Chamba district [4]. Besides, the plant accessions were also compared to the samples lying in the Herbaria of the Department of Botany, Punjabi University, Patiala (PUN), Botanical Survey of India (BSI), and Forest Research Institute (FRI) at Dehra Dun. Voucher

FIGURE 2: Study area: Lahaul Spiti, Chamba, and Kullu districts of Himachal Pradesh (India).

specimens of the cytologically worked-out specimens were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN). For male meiotic studies, floral buds of suitable sizes from the wild plants were fixed in Carnoy's fixative (mixture of ethanol, chloroform, and glacial acetic acid in volume ratio of 6:3:1) for 24 h and preserved in 70% ethanol in a refrigerator until analysed. For chromosome counts and detailed meiotic behavior in pollen mother cells (PMCs) at early prophase-I, metaphase-I (MI), anaphase-I/II (AI/II), telophases-I/II (TI/II), and sporad stage developing anthers were squashed in 1% acetocarmine. Pollen fertility was estimated through stainability tests by smearing mature anthers from different flowers in glycerol-acetocarmine mixture (1:1) and aniline blue dye (1%). Best plates of chromosome counts, meiotic abnormalities, sporads, and pollen grains were photographed from the temporary mounts with digital imaging system of Nikon Eclipse 80i microscope and Leica QWin. Field photographs of plants and landscape were taken with Nikon Coolpix L3 camera.

3. Results

3.1. Ethnobotany. In the present investigation, 40 plant species under 33 genera belonging to 17 families have been reported to be used for ethnobotanical and medicinal purposes. Data given in Table 1 includes the name of the family to which a particular species belongs, their meiotic chromosome number, ploidy level, vernacular name, locality and its altitude, voucher specimen number, habit and habitat, natural regeneration, flowering and fruiting period, plant part used, and ethnobotanical uses (Figures 3(a)–3(g)). Out of the 40 species, the maximum number of species belongs to Ranunculaceae and Leguminosae with 7 species each and 4 species to Apiaceae and Rosaceae each. The most common plant parts used in different preparations are the roots and leaves. Other plant parts used as herbal medicine are whole plant, seeds, fruits, flowers, and rhizomes. Based on the information

gathered from different sources, the local people used a good number of plant species for curing the stomach troubles, pain reliever, cough, gastric disorders, and aphrodisiac; as fodder and fuel; to obtain different dyes; and in religious ceremonies and as offerings to deities (Figures 4(a)–4(d)).

3.2. Chromosomal Status. In addition to the gathering of ethnobotanical information, chromosome number and details of male meiosis have also been investigated in all the species. Of the 40 cytologically studied species, 30 (75%) exist at diploid level with basic chromosome number ranging between $x = 5$ (*Biebersteinia odora* Steph. ex Fish., $2n = 10$) and 18 (*Cerastium cerastoides* (L.) Britt., $2n = 36$), while nine (22.5%) of them were found to be at polyploid level ($4x, 6x, 12x$). One species, *Ranunculus hirtellus* Royle ($2n = 16, 32$), existed at both diploid and tetraploid levels based on $x = 8$. All the chromosome counts are new to the study area. On worldwide basis, meiotic chromosome counts of $n = 14$ (Figure 5(a)) and $n = 8$ (Figure 5(b)) in *Rosularia alpestris* (Kar and Kir) Boriss. and *Corydalis govaniana* Wall., respectively, are the first ever chromosomes counts. While in some other species new cytotypes have been added to the already existing diploid (*Thalictrum foetidum* L., new, $2n = 6x = 42$ (Figure 5(c)), previous, $2n = 2x = 14$; *Geranium pratense* L., new $2n = 4x = 56$ (Figure 5(d)), previous $2n = 2x = 28$) or polyploid cytotypes (*Malva verticillata* L., new, $2n = 104$, previous, $2n = 76, 84, 126$). New/additional intraspecific cytotypes for India have also been established in *Indigofera gerardiana* Wall. ex Baker ($n = 24, 6x$), *Epilobium angustifolium* L. ($n = 18, 4x$), *Cerastium cerastoides* (L.) Britt. ($n = 18, 2x$), *Geranium wallichianum* D. Don ex Sweet ($n = 14, 2x$, Figure 5(e)), and *Impatiens sulcata* Wall. (Don) ($n = 9, 2x$, Figure 5(f)). Present study also recorded additional intraspecific diploid/polyploid cytotypes from India in *Ranunculus hirtellus* Royle ($n = 8, 2x; n = 16, 4x$) and *Potentilla atosanguinea* Lodd.var. *argyrophylla* (Wall. ex Lehm.) Griens. and Long ($n = 21, 6x; n = 42, 12x$). As many as eight species, namely, *Angelica glauca* Edgew. ($n = 11$), *Astragalus bicuspidis* Fischer ($n = 8$), *Biebersteinia odora* Steph. ex Fish. ($n = 5$, Figure 5(g)), *Bergenia stracheyi* Engl. ($n = 17$), *Chaerophyllum aromaticum* L. ($n = 11$), *Datisca cannabina* L. ($n = 11$), *Rosa webbiana* Wall. ex Royle ($n = 7$), and *Trigonella emodi* Benth. ($n = 8$) have been counted chromosomally for the first time from India.

3.3. Abnormal Cytological Events during Male Meiosis and Pollen Fertility. Besides new chromosome reports and additional cytotypes, in some of the species meiosis was abnormal and was characterized by the presence of multivalents and/or univalents, impaired synapsis, cytomixis, and its associated meiotic aberrations such as laggards and bridges, chromatin stickiness, pycnotic chromatin, irregular distribution of chromosomes, and micronuclei at telophases/sporads consequently leading to abnormal microsporogenesis and variable amount of pollen sterility (Figures 5(h)–5(p)).

Among the abnormal taxa, $12x$ cytotype of *P. atosanguinea* Lodd.var. *argyrophylla* (Wall. ex Lehm.) Griens. and Long is an imbalanced polyploid. Owing to the presence of high chromosome number ($2n = 84$), it depicted a

TABLE 1: Family, species and its vernacular name, meiotic chromosome number, ploidy level, locality and altitude, habit and habitat, natural regeneration, flowering and fruiting period, plant part used and ethnobotanical uses in the wild plants of Lahaul-Spiti, and adjoining high altitude hills.

Family/species (vernacular name)/meiotic chromosome number “n”/ploidy level	Locality and altitude in meters (m)	Voucher specimen number (PUN)	Habit and habitat	Natural regeneration	Flowering and fruiting period	Plant part used	Ethnobotanical and ethnomedicinal uses
Apiaceae							
(1) <i>Angelica glauca</i> Edgew. (<i>Choru, Chora</i>) n = 11 (2x)	Dhancho, 3,030 m	51529 PUN	Tall perennial herb; shady moist places	Seeds, rootstock	July–September	Whole plant, roots	Stomach troubles and stimulant Spices and condiments Bronchitis
(2) <i>Bupleurum falcatum</i> L. n = 8 (2x)	Bharmour, 2,130 m	51524 PUN	Perennial herb; dry slopes	Seeds, rootstock	June–September	Roots	Liver troubles
(3) <i>Chaerophyllum aromaticum</i> L. n = 11 (2x)	Koksar, 3,140 m	51546 PUN	Herb; moist slopes	Seeds, rootstock	June–September	Roots	Plant eaten raw or used as pot herb Along with mint is used as flavouring agent
(4) <i>Heracleum lanatum</i> Michx. (<i>Raswal</i>) n = 11 (2x)	Trilokinath, 3,020 m	51542 PUN	Perennial herb; along water courses and amongst stones and open slopes	Seeds, rootstock	June–September	Leaves, tender shoots, and fruits	Check the excessive menses and bleeding, abdominal pain and gastric disorders Fodder Aphrodisiac
Balsaminaceae							
(5) <i>Impatiens sulcata</i> Wall. (Don) n = 9 (2x)	Sissu, 3,170 m	51627 PUN	Annual herb; along streams	Seeds	June–August	Whole plant	Paste made by mixing it with lichens and <i>Rumex</i> sp. is used as nail polish
(6) <i>I. brachycentra</i> Royle n = 7 (2x)	Jhalama, 3,070 m	51632 PUN	Annual herb; glacial streams and moist shady places	Seeds	July–August	Leaves	Red dye obtained is used as nail paint
Biebersteiniaceae							
(7) <i>Biebersteinia odora</i> Steph. ex Fish. (Figure 3(a)) n = 5 (2x)	Baralacha Pass, 5,030 m	51587 PUN	Perennial herb; alpine meadows and dry rocky and scree slopes	Seeds, rootstocks	July–October	Rootstocks	Antiseptic and blood purifier
Brassicaceae							
(8) <i>Lepidium latifolium</i> L. (<i>Tharag-Thokpa</i>) n = 12 (2x)	Bahu, 2,830 m	51618 PUN	Perennial herb; open slopes and around cultivated fields	Seeds, rootstocks	July–September	Aerial parts	Rheumatic pain

TABLE 1: Continued.

Family/species (vernacular name)/meiotic chromosome number “n”/ploidy level	Locality and altitude in meters (m)	Voucher specimen number (PUN)	Habit and habitat	Natural regeneration	Flowering and fruiting period	Plant part used	Ethnobotanical and ethnomedicinal uses
Caryophyllaceae							
(9) <i>Cerastium cerastoides</i> (L.) Britt. n = 18 (2x)	Batal, 3,960 m	51479 PUN	Perennial herb; moist slopes and meadows	Seeds, stolon	June–September	Whole plant	Decoction for cough
(10) <i>Silene vulgaris</i> (Moench) Garcke (<i>Ghandoli</i>) n = 12 (2x)	Udaipur, 2,740 m	51713 PUN	Perennial herb; grassy slopes and meadows	Seeds, rootstocks	June–September	Fresh young leaves and twigs	Vegetable
Crassulaceae							
(11) <i>Rosularia alpestris</i> (Kar & Kir) Boriss. (<i>Pyau Chakti</i>) n = 14 (2x)	Kukumsheri, 2800 m	51163 PUN	Perennial herb; rock crevices and dry sandy slopes	Seed, offsets	June–September	Whole plant	Juice—considered good for health
Datiaceae							
(12) <i>Datisca cannabina</i> L. n = 11 (2x)	Jahlama, 3,070 m	49418 PUN	Perennial robust herb; dry sandy slopes	Seeds, rootstocks	July–September	Roots	Yellow dye
Fumariaceae							
(13) <i>Corydalis govianiana</i> Wall. n = 8 (2x)	Gauri Kund, 3,930 m	51673 PUN	Perennial herb; rock crevices and glacial moraines	Seeds, rootstocks	July–September	Whole plant	Leprosy and rheumatism
Geraniaceae							
(14) <i>Geranium pratense</i> L. (<i>Porlo, Likatur</i>) n = 28 (4x)	Lossar, 3,900 m	51573 PUN	Perennial herb; moist places	Seeds, rootstocks	July–September	Whole plant, roots flowers	Cough, jaundice, and gastric disorders Poultice to bruises. Black dye obtained by boiling roots along with <i>Naktsur</i> (sulfurous raw mineral) Offered to deities
(15) <i>G. wallichianum</i> D. Don ex Sweet n = 14 (2x)	Rohtang Pass, 3,980 m	51190 PUN	Perennial herb; moist slopes	Seeds, rootstocks	July–September	Whole plant	Stomachaches, cough, cold, and throat infections
Leguminosae							
(16) <i>Astragalus bicuspidis</i> Fischer n = 8 (2x)	Gondhla, 3,160 m	51179 PUN	Under shrub: open, dry, and sandy slopes	Seeds, rootstocks	July–August	Whole plant	Soil binder

TABLE 1: Continued.

Family/species (vernacular name)/meiotic chromosome number “n”/ploidy level	Locality and altitude in meters (m)	Voucher specimen number (PUN)	Habit and habitat	Natural regeneration	Flowering and fruiting period	Plant part used	Ethnobotanical and ethnomedicinal uses
(17) <i>A. himalayanus</i> Klotzsch (<i>Kayabachhutup</i>) n = 8 (2x)	Zingzingbar, 4,350 m	51288 PUN	Herb; meadows, dry slopes, and around cultivated areas	Seeds	July–September	Seeds, flowers	Strangury
(18) <i>A. rhizanthus</i> Royle ex Benth. (Figure 6(a)) (<i>Zomoshing</i>) n = 8 (2x)	Chandartal Lake, 4,300 m	51276 PUN	Perennial herb; dry slopes, riverbeds, and among stones	Seeds, rootstock	June–September	Roots	Fodder
(19) <i>Cicer microphyllum</i> Benth. (Figure 3(b)) (<i>Nayarcha, Chiri</i>) n = 8 (2x)	Chandartal Lake, 4,300 m	51219 PUN	Perennial herb; sandy and dry slopes	Seeds, rootstocks	June–September	Aerial parts, seeds	Treatment of sore mouth in cattle “Khur” disease of sheep and goats Dry as well as fresh leaves are used as vegetable or eaten raw
(20) <i>Indigofera gerardiana</i> Wall. ex Baker n = 24 (6x)	Tindi, 2,600 m	51312 PUN	Shrub; shady and sometimes also on open moist slopes	Seeds, new sprouts, and suckers begin from dormant buds on stems, rootstocks	May–August	Leaves and young shoots	Fodder and fuel
(21) <i>Medicago falcata</i> L. (<i>Kathoama</i>) n = 8 (2x)	Keylong, 3,340 m	51328 PUN	Perennial herb; moist shady places	Seeds	July–September	Aerial parts	Used as a good fodder Cuts and wounds Vegetable Plant considered being poisonous if fed before flowering Insect repellent Dried plants as perfume and fresh flowers worn as garland
(22) <i>Trigonella emodi</i> Benth. (<i>Kuchona, Buksup</i>) n = 8 (2x)	Sundrasi, 3,370 m	51160 PUN	Herb; moist places, riverbeds, and around cultivated fields	Seeds	June–August	Young leaves, shoots, and flowers	
Malvaceae							
(23) <i>Malva neglecta</i> Wall. n = 21 (2x)	Keylong, 3,340 m	51696 PUN	Perennial herb; moist places and roadsides	Seeds, rootstocks	June–September	Whole plant	Decoction for malaria Dried plants for kidney and bladder disorders
(24) <i>M. verticillata</i> L. (<i>Mikanchi</i>) n = 52 (polyploid)	Kaza, 3,800 m	51492 PUN	Perennial herb; moist places	Seeds, rootstocks	June–September	Seeds	Kidney and bladder disorders

TABLE 1: Continued.

Family/species (vernacular name)/meiotic chromosome number “n”/ploidy level	Locality and altitude in meters (m)	Voucher specimen number (PUN)	Habit and habitat	Natural regeneration	Flowering and fruiting period	Plant part used	Ethnobotanical and ethnomedicinal uses
Onagraceae							
(25) <i>Epilobium angustifolium</i> L. (Dharshak) n = 18 (4x)	Miyar, 3,000 m	51665 PUN	Perennial herb; moist places, amidst stones, and boulders	Seeds, rootstocks	July–September	Roots	Crushed roots used as a detergent
(26) <i>E. latifolium</i> ssp. <i>Speciosum</i> (Decne.) Raven n = 18 (4x)	Batal, 3,960 m	51654 PUN	Perennial herb; dry slopes and on rocks	Seeds, rootstocks	July–September	Flowers	Ornamental
Papaveraceae							
(27) <i>Meconopsis aculeata</i> Royle (Figure 3(c)) (Chharbongcha Chharmen) n = 28 (4x)	Gauri Kund, 3,930 m	49361 PUN	Herb; among boulders in alpine rocky areas	Seeds	June–September	Aerial parts roots	Swelling of legs after long distance walking Narcotic and poisonous
Podophyllaceae							
(28) <i>Podophyllum hexandrum</i> Royle (Figure 3(d)) (Omo-Shey) n = 6 (2x)	Gulaba 3,400 m	51708 PUN	Perennial herb; moist slopes	Seeds, rootstocks	July–September	Roots, fruits	Purgative Tuberculosis and cough
Ranunculaceae							
(29) <i>Aconitum heterophyllum</i> Wall. ex Royle (Figure 3(e)) (Boa) n = 8 (2x)	Manimahesh Lake, 4,300 m	51398 PUN	Herb; subalpine and alpine grassy meadows and moist slopes	Seeds	June–September	Roots	Diarrhoea, coughs, astringent, and aphrodisiac Cough, high fever stomach problems, snake and scorpion bites, contagious infections, and intestine inflammation
(30) <i>A. violaceum</i> Jacq. Ex Stapf. (Yangtso) n = 8 (2x)	Manimahesh Lake, 4,300 m	51402 PUN	Small herb; alpine Himalayan regions	Seeds, rootstocks	June–September	Roots	
(31) <i>Aquilegia fragrans</i> Benth. (Lande, Kumuk) n = 7	Chhatru, 3,360 m	51377 PUN	Perennial herb; moist slopes	Seeds, rootstocks	July–September	Flowers	Hair follicle infection
(32) <i>Caltha palustris</i> L. n = 16 (4x) (Figure 3(f))	Trilokinath, 3,020 m	51333 PUN	Perennial herb; marshy areas along water streams and glacial moraines	Seeds, rootstocks	June–September	Leaves Roots	To keep maggots out of cattle wounds. To clean the hands Gonorrhoea

TABLE 1: Continued.

Family/species (vernacular name)/meiotic chromosome number “n”/ploidy level	Locality and altitude in meters (m)	Voucher specimen number (PUN)	Habit and habitat	Natural regeneration	Flowering and fruiting period	Plant part used	Ethnobotanical and ethnomedicinal uses
(33) <i>Delphinium denudatum</i> Wall. ex Hook. f. & Thomson <i>n</i> = 8 (2x)	Dibri, 2,300 m	51384 PUN	Perennial herb; margins of Cedrus and Pine forest, dry scrubby slopes	Seeds, rootstocks	July–September	Roots	Toothache
(34) <i>Ranunculus hirtellus</i> Royle <i>n</i> = 8 (2x), 16 (4x)	Keylong, 3,340 m	51374 PUN	Perennial herb; moist places and alpine meadows	Seeds, rootstocks	June–September	Whole plant	Counter irritant Swelling in testes
(35) <i>Thalictrum foetidum</i> L. <i>n</i> = 21 (6x)	Trilokinath, 3,020 m	51335 PUN	Perennial herb; dry slopes, among stones and boulders	Seeds, rootstocks	June–September	Flowers	Religious ceremonies
Rosaceae							
(36) <i>Cotoneaster microphyllus</i> Wall. ex Lindl. (<i>Roghtali</i>) <i>n</i> = 17 (2x)	Kothi, 2,400 m	49218 PUN	Trailing evergreen shrub on rocky slopes, rocky mountain areas, thickets, and river valleys	Seeds, new shoots from dormant bud on old stem	June–September	Whole plant, fruits	Eaten raw Walking sticks and baskets Used for fuel
(37) <i>Potentilla atrosanguinea</i> Lodd. var. <i>argyrophylla</i> (Wall. ex Lehm.) Griens. & Long <i>n</i> = 21 (6x), 42 (12x)	Kilingsarai, 4,500 m	51101 PUN	Perennial herb; alpine meadows and glacial moraines	Seeds, rootstock	June–October	Roots	Toothache and gingivitis
(38) <i>Rosa webbiana</i> Wall. ex Royle (Figures 3(g), 4(a), and 4(b)) (<i>Shaybala</i>) <i>n</i> = 7 (2x)	Kaza, 3,800 m	51450 PUN	Deciduous prickly shrub, open sandy and rocky slopes	Seeds, rootstocks	June–September	Fruits, stems, and flowers	Eaten Used as biofence Brooms made—used to remove stones and debris from roads Used as fuel Offered in gompas, stomach ache
(39) <i>Spiraea canescens</i> D. Don <i>n</i> = 9 (2x) Saxifragaceae	Kothi, 2,400 m	51421 PUN	Shrub: dry slopes	Seeds, rootstocks	June–September	Slender branches	Rim of baskets
(40) <i>Bergenia stracheyi</i> Engl. (Figures 6(b), 6(c), and 6(d)) (<i>Shilpada</i>) <i>n</i> = 17 (2x)	Gramphoo, 3,160 m	51682 PUN	Perennial herb; moist rocky slopes, among boulders	Seeds, rootstocks	May–September	Leaves, rootstocks, and roots	Used as fuel wood Diuretic

FIGURE 3: (a) *Biebersteinia odora* plants in a group, coming out of withered remains of stems. (b) *Cicer microphyllum* growing on rock. (c) An individual of *Meconopsis aculeata* growing among the boulders. (d) *Podophyllum hexandrum*. (e) *Aconitum heterophyllum* with greenish blue flowers having purple veins growing on moist slopes. (f) *Caltha palustris* with bright yellow flowers and large reniform leaves growing near water. (g) A shrub of *Rosa webbiana* with pink flowers.

typically irregular meiotic behaviour (presence of multivalents and/or univalents, laggards, and irregular distribution of chromosomes), abnormal microsporogenesis, and pollen sterility (14–46%) which is the characteristic feature in such kind of imbalanced polyploids. Cytomixis which involves

the interpollen mother cell transfer of chromatin material through narrow and broad cytomictic channels is existent in *Astragalus bicuspidatus* Fischer ($n = 8$), *A. himalayanus* Klotzsch ($n = 8$), *A. rhizanthus* Royle ex Benth. ($n = 8$), *Caltha palustris* L. ($n = 16$), *Geranium pratense* L. ($n = 28$),

FIGURE 4: (a) Spiny branches of *Rosa webbiana* wrapped around the stem of *Salix* tree as a biofence. (b) A broom made of twigs of *Rosa webbiana* to remove stones and debris from road. (c) An old person carrying a bundle of young twigs of *Indigofera gerardiana* on his back to be used as a fodder and fuel. (d) Timber of some species used in house construction (arrowed).

Meconopsis aculeata Royle ($n = 28$), *P. atrisanguinea* Lodd. var. *argyrophylla* (Wall. ex Lehm.) Griens. and Long ($n = 21$, 42), *Trigonella emodi* Benth. ($n = 8$), *Rosularia alpestris* (Kar and Kir) Boriss. ($n = 14$), *Silene vulgaris* (Moench) Garcke ($n = 12$), *Thalictrum foetidum* L. ($n = 21$), and *Ranunculus hirtellus* Royle ($n = 8$, 16). Impaired synapsis owing to the lack of pairing of homologous chromosomes in *Rosularia alpestris* (Kar and Kir) Boriss. resulted in the presence of all chromosomes as univalents during early prophase stages of meiosis-I and consequently abnormal microsporogenesis and pollen sterility (28%). Besides, in *Bergenia stracheyi* Engl. high pollen sterility (22%) was recorded without the occurrence of any meiotic irregularity which might be attributed to irregularities in postmeiotic events during pollen development.

3.4. Natural Propagation. Natural propagation observations made on all the 40 species revealed that majority of the

species are perennial and propagate through asexual (vegetative propagation) means such as rootstocks, offsets, root suckers, and stolons (Figures 6(a)–6(d)).

4. Discussion

A steady stream of papers over the years have recognized the fact that the reproductive stage in the life cycle of plants is highly sensitive to different abiotic stresses [5–8] especially the low temperature conditions [9–16]. It has been observed that when plants of this area are about to enter the flowering stage, the temperature is very low (sub-zero to 15°C) which seems to disrupt the meiotic process by causing various meiotic abnormalities and consequently reduced viability of gametes. Ultimately these events hinder natural propagation of plants through sexual means.

Natural propagation observations revealed that majority of the species are perennial and propagate through asexual

FIGURE 5: Meiotic chromosome number. (a) *Rosularia alpestris*, $n = 14$ at metaphase-I. (b) *Corydalis govaniana*, $n = 8$ at metaphase-I. (c) *Thalictrum foetidum*, $n = 21$ at metaphase-I. (d) *Geranium pratense*, 28:28 chromosomes distributions at metaphase-II. (e) *Geranium wallichianum*, $n = 14$ at diakinesis. (f) *Impatiens sulcata*, $n = 9$ at metaphase-I. (g) *Biebersteinia odora*, $n = 5$ at metaphase-I. Meiotic abnormalities in pollen mother cells (PMCs) and sterile pollen grains. (h) A PMC showing univalents (arrowed) and multivalents (arrowhead). (i) PMCs in a group showing inter-PMC chromatin transfer (arrowed) and chromatin stickiness (arrowhead). (j) A PMC showing laggards at anaphase-II. (k) A PMC showing a chromatin bridge at anaphase-II. (l) Pycnotic chromatin material scattered in the cytoplasm of PMCs. (m) A PMC showing unequal distribution of chromatin material at poles. (n) Micronuclei at telophase-I. (o) Micronuclei at sporad stage. (p) Apparently sterile (arrowed) and fertile pollen grains (arrowhead).

FIGURE 6: Natural propagation. (a) A plant grown from old rootstock (arrowed) in *Astragalus rhizanthus*. (b) New plants of *Bergenia stracheyi* coming out of the rootstocks (encircled) underlying withered leaves. (c) *B. stracheyi* plants in full bloom with white pinkish flowers. (d) A new sprout can be seen growing on the old rootstock (arrowed) of *B. stracheyi*.

means such as rootstocks, offsets, root suckers, and stolons. Thus, it is assumed that in order to survive the harsh environmental conditions and gametic sterility caused by irregular meiotic events, the plants of the area have adopted alternate mechanism of reproduction through vegetative means. Vegetative means of propagation are important for species like *Meconopsis aculeata* Royle, *Potentilla atosanguinea* Lodd. var. *argyrophylla* (Wall. ex Lehm.) Griens. and Long, *Bergenia stracheyi* Engl., *Rosularia alpestris* (Kar and Kir) Boriss., and so forth, where high pollen grain sterility have been observed. Occurrence of sterility on female side in the species may pose serious problem for species existence, especially, which are rare and engendered and are overexploited for medicinal purposes. Problem becomes more serious in species like *Bergenia stracheyi*, Engl. and *Rosularia alpestris* (Kar and Kir) Boriss. which propagate through vegetative means, and perennating parts are directly collected from the wild sources. Till now these species have not been included in any of the local cultivation programme.

To date numerous valuable herbal drugs have been discovered by following the knowledge of ancient folk healers [17]. The medicinal plants used to cure various diseases by the traditional healers are gradually becoming extinct. The two factors which have been identified as major threats to the floristic diversity are the overexploitation and habitat [18–21]. Majority of the plants (*Aconitum heterophyllum* Wall. ex Royle, *Angelica glauca* Edgew., *Biebersteinia odora* Steph. ex Fish., *Bergenia stracheyi* Engl., *Delphinium denudatum* Wall. ex Hook. f. and Thomson, *Indigofera gerardiana* Wall. ex Baker, *Meconopsis aculeata* Royle, *Podophyllum hexandrum* Royle, and *Spiraea canescens* D. Don) which have been studied presently are restricted to specific pockets with very low population density and belong to rare, critically endangered, endangered, vulnerable, threatened, and newly threatened category because of the fragile ecological conditions of this Himalayan region [20–22]. Some of the other factors which can also lead to the extinction of the local valuable herbs include agricultural expansion, heavy grazing, road

building, increasing entry of tourists, and transport vehicles. So to study and examine different aspects of survival of the plants of the area in their natural habitats will be of colossal significance. Thus it becomes very urgent to collect information about the plants growing in such ecologically fragile region before it gets too late to respond. Immediate steps must be taken up on priority basis to preserve the ethnic knowledge and the plants of the region. The gradual exposure of younger generations to western civilization and limited employment opportunities are two important threats that may lead to serious losses of traditional knowledge, and it will not be surprising that after a decade or so nobody will be able to tell about the plants that were once used traditionally for ethnobotanical and medicinal purposes. In view of the above points it is suggested that an institute dealing with the conservation of plants and their traditional uses must be set up in the region. The benefits arising from ethnic knowledge must be shared among the local people and employment must be provided to local youth. Present investigations also form the basis for exploitation of intraspecific chromosomal variation/new cytotypes recorded in some of the presently studied species to detect biochemical diversity in these medicinally important plants.

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References

- [1] I. Hedberg, "Research on medicinal and poisonous plants of tropics," in *Past, Present and Future in Medicinal and Poisonous Plants of the Tropics*, A. J. M. Leewenerg, Ed., pp. 9–15, International Book Distributors, Dehra Dun, India, 1987.
- [2] B. S. Aswal and B. N. Mehrotra, *Flora of Lahaul-Spiti (A Cold Desert in North West Himalaya)*, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1994.
- [3] D. S. Dhaliwal and M. Sharma, *Flora of Kullu District (Himachal Pradesh)*, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1999.
- [4] H. Singh and M. Sharma, *Flora of Chamba District (Himachal Pradesh)*, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 2006.
- [5] J. T. Baker, L. H. Allen, and K. J. Boote, "Temperature effects on rice at elevated CO₂ concentration," *Journal of Experimental Botany*, vol. 43, no. 7, pp. 959–964, 1992.
- [6] V. A. Fuzinatto, M. S. Pagliarini, and C. B. Valle, "Evaluation of microsporogenesis in an interspecific *Brachiaria* hybrid (Poaceae) collected in distinct years," *Genetics and Molecular Research*, vol. 7, no. 2, pp. 424–432, 2008.
- [7] M. Rezaei, A. Arzani, and B. E. Sayed-Tabatabaei, "Meiotic behaviour of tetraploid wheats (*Triticum turgidum* L.) and their synthetic hexaploid wheat derivatives influenced by meiotic restitution and heat stress," *Journal of Genetics*, vol. 89, no. 4, pp. 401–407, 2010.
- [8] S. Sato, M. Kamiyama, T. Iwata, N. Makita, H. Furukawa, and H. Ikeda, "Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological processes in male reproductive development," *Annals of Botany*, vol. 97, no. 5, pp. 731–738, 2006.
- [9] F. Bretagnolle and J. D. Thompson, "Tansley Review No. 78. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants," *New Phytologist*, vol. 129, no. 1, pp. 1–22, 1995.
- [10] A. Hedhly, "Sensitivity of flowering plant gametophytes to temperature fluctuations," *Environmental and Experimental Botany*, vol. 74, no. 1, pp. 9–16, 2011.
- [11] P. Kumar and V. K. Singhal, "Chromosome number, male meiosis and pollen fertility in selected angiosperms of the cold deserts of Lahaul-Spiti and adjoining areas (Himachal Pradesh, India)," *Plant Systematics and Evolution*, vol. 297, no. 3–4, pp. 271–297, 2011.
- [12] A. S. Mason, M. N. Nelson, G. Yan, and W. A. Cowling, "Production of viable male unreduced gametes in *Brassica* interspecific hybrids is genotype specific and stimulated by cold temperatures," *BMC Plant Biology*, vol. 11, article 103, 2011.
- [13] J. Ramsey and D. W. Schemske, "Pathways, mechanisms, and rates of polyploid formation in flowering plants," *Annual Review of Ecology and Systematics*, vol. 29, pp. 467–501, 1998.
- [14] V. K. Singhal and P. Kumar, "Variable sized pollen grains due to impaired male meiosis in the cold desert plants of Northwest Himalayas (India)," in *Pollen: Structure, Types and Effects*, B. J. Kaiser, Ed., pp. 101–126, Nova Science, New York, NY, USA, 2010.
- [15] V. K. Singhal, P. K. Rana, and P. Kumar, "Syncytes during male meiosis resulting in 2n pollen grain formation in *Lindelfia longiflora* var. *falconeri*," *Journal of Systematics and Evolution*, vol. 49, no. 5, pp. 406–410, 2011.
- [16] M. te Beest, J. J. Le Roux, D. M. Richardson et al., "The more the better? The role of polyploidy in facilitating plant invasions," *Annals of Botany*, vol. 109, pp. 19–45, 2012.
- [17] N. Ekka and V. K. Dixit, "Ethno-pharmacognostical studies of medicinal plants of Jashpur district, Chattisgarh," *International Journal of Green Pharmacy*, vol. 1, pp. 2–4, 2007.
- [18] C. P. Kala, "Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya," *Biological Conservation*, vol. 93, pp. 371–379, 1999.
- [19] C. P. Kala and N. Manjrekar, "Ethno-medicobotany of Indian trans-Himalaya: a case study from Spiti," *Journal Economic and Taxonomic Botany*, vol. 23, pp. 177–183, 1999.
- [20] M. S. Rana and S. S. Samant, "Threat categorisation and conservation prioritisation of floristic diversity in the Indian Himalayan region: a state of art approach from Manali Wildlife Sanctuary," *Journal for Nature Conservation*, vol. 18, no. 3, pp. 159–168, 2010.
- [21] S. S. Samant, U. Dhar, and R. S. Rawal, "Biodiversity status of a protected area in West Himalaya: askot wildlife sanctuary," *International Journal of Sustainable Development and World Ecology*, vol. 5, no. 3, pp. 194–203, 1998.

- [22] T. K. Ved and V. Tandon, *CAMP Report: Conservation Assessment and Management Plan*, Workshop held at Kullu district in Himachal Pradesh, FRLHT, Bangalore, India, 1998.