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Research Article

Screening and Evaluation of Superior Chemotypes of *Podophyllum hexandrum* Royle from Different Geographical Locations of North-west Himalayas

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Abstract

Podophyllum hexandrum (Bankakdi) is a very important high valued temperate medicinal plant which is known for its medicinal property in treatment of specific types of cancers. The species is in great demand and is being harvested from the wild indiscriminately. The species needs immediate attention for its protection and conservation. Keeping in view of the above facts, after extensive survey, 30 sites were identified for collection of *Podophyllum hexandrum* species from different geographical locations of Himachal Pradesh and Jammu & Kashmir states in India. Each site was geo- referenced along with characterization of micro-habitat. On the basis of high podophyllotoxin content, the superior chemotypes of *P. hexandrum* were identified.

Keywords: *Podophyllum* hexandrum; Superior chemotypes; Characterization; Active ingredients; Geographical locations; North-west Himalayas

Introduction

Podophyllum hexandrum Royle belongs to family 'Berberidaceae' and it is a perennial herb bearing the common names 'Himalayan May apple'. The genus '*Podophyllum*' is represented by three species viz., *P. hexandrum*, *P. peltatum*, and *P. sikkimensis* [1]. *P. hexandrum* is found in the Himalayan regions of Asia and *P. peltatum* is found in North America [2]. The whole plant has got great importance in traditional systems of medicines including Ayurveda, Unani and Tibetian systems for curing several diseases. The rhizomes and roots of *Podophyllum* species have gained much importance throughout the world as being the main source or the starting material for an aryltetralin lignan 'podophyllotoxin' and its semi synthetic compounds viz., etoposide, teniposide, and etoposide phosphate since these are used in treatment of specific types of cancers. It is categorized as globally rare plant in IUCN red list and has endangered status in India [3]. It is distributed in very restricted pockets in the Himalayan zone at altitudes ranging from 2000 to 4000 m a.s.l [4], mostly found in Alpine region. It has been reported to be used through the ages and in modern times as a cure for allergic and inflammatory conditions of the skin; biliary fever; burning sensation; cold; constipation; cancer of the brain, bladder and lung; erysipelas; Hodgkin's disease; insect bites; mental disorders; monocytoid leukemia non-Hodgkin's lymphoma; rheumatism; septic wounds; plague; and venereal warts [5,6,7,8,9].

Podophyllum hexandrum needs study of its variability and population under different locations with scientific basis and its *ex-situ* and *in-situ* conservation. Medicinal plants are valued for their various active ingredients contents which are used by the pharmaceutical companies for various formulations. Therefore, it is of paramount importance to identify the superior stock (high active ingredient contents) from the natural condition and then multiply the same for commercial cultivation. In this context, it is necessary to clearly establish the natural sources of superior genetic material of important medicinal plants species and to conserve the same by establishing Field Gene Bank for future use and to initiate appropriate conservation and resource augmentation measures for long-term availability of these selected medicinal plants.

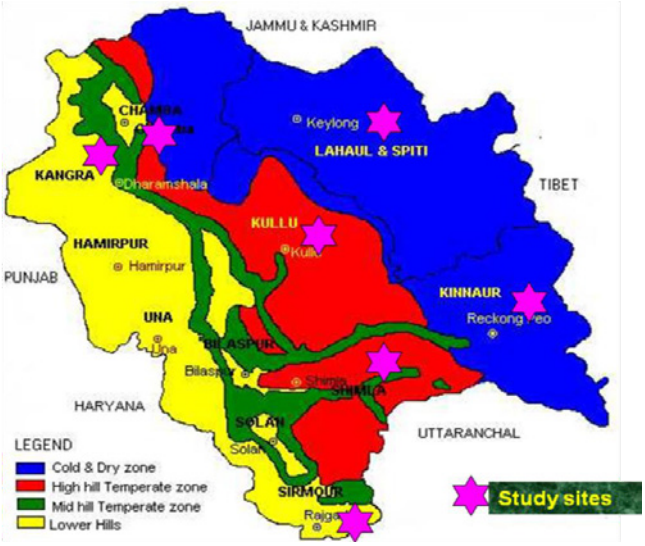

Figure 1: Map showing identified sites for *P. hexandrum* in different districts of H.P.

Materials and Methods

After extensive surveys, 30 sites were identified for collection of *Podophyllum hexandrum* species from most probable different geographical locations of H.P. state and Ladakh Valley of J&K state (Table 1, Figure 1 & 2). The population of *P. hexandrum* was found

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Table 1: Geo-referencing of Identified sites for *Podophyllum hexandrum* in different geographical locations of H.P. and J&K.

S. No.	State	District	Division	Source/Place	Latitude	Longitude	Altitude (m)
1	J&K	Kargil	Shankoo	Satkpa	N 34°18.'094"	E 75°55'.645"	3146
2	J&K	Kargil	Shankoo	Beemaputri	N 34°12'353"	E 75°55'699"	3231
3	H.P.	Chamba	Killar	Suralbhaturi	N 33°08'594"	E 76°27'015"	3320
4	H.P.	Chamba	Killar	Hudanbhaturi	N 33°05'980"	E 76°29'223"	3580
5	H.P.	Chamba	Khajjiar	Kalatop	N 32°33'052"	E 76°01'168"	2356
6	H.P.	Chamba	Bhandal	Thathidhar	N 32°49'905"	E 75°58'600"	2899
7	H.P.	L&S	Keylong	Kangsar	N 32°32'721"	E 76°58'463"	3240
8	H.P.	Kangra	Bir	Joardu Forest	N 32°08'715"	E 77°45'526"	2675
9	J&K	Kargil	Shankoo	Shangra	N 34°12'984"	E 75°058'219"	3148
10	J&K	Kargil	Shankoo	Satkpa Dras Road	N 34°18'.042"	E 75°55'.610"	3188
11	H.P.	L&S	Lahaul	Kardang	N 32°33'918"	E 77°01'088"	3148
12	H.P.	Kullu	Naggar	Parot	N 32°11'654"	E 77°17'012"	3032
13	H.P.	Kullu	Patlikul	Shangchar	N 32°10'502"	E 77°08'576"	3056
14	J&K	Kargil	Shankoo	Panikher	N 34°07'119"	E 75°58'002"	3306
15	H.P.	Kullu	Naggar	Kulri (Bansheru)	N 32°10'64"	E 77°13'415"	3079
16	H.P.	Kullu	Manali	Gulaba	N 32°19'114"	E 77°12'310"	3664
17	H.P.	Kullu	Kullu	Majhoni (Tirthan)	N 31°45'640"	E 77°27'870"	3460
18	H.P.	Kullu	Sainj	Khoruthach	N 31°45'976"	E 77°26'732"	2580
19	H.P.	Kinnaur	Sarahan	Katgaon	N 31°33'061"	E 77°58'489"	2750
20	H.P.	Kinnaur	Nichar	Chotkanda	N 31°308'77"	E 77°58'704"	3400
21	J&K	Kargil	Sankoo	UMBA 2	N 34°18'096"	E 75°55'677"	3142
22	H.P.	Shimla	Rohru	Maila (Diudi)	N 31°22'014"	E 78°01'129"	2990
23	J&K	Leh	Sankoo	Thangbu-1	N 34°12'337"	E 75°55'743"	3228
24	H.P.	L&S	Keylong	Chimrit	N 32°43'249"	E 76°40'870"	2718
25	H.P.	Chamba	Killar	Karyuni	N 33°03'271"	E 76°26'300"	3064
26	H.P.	Kullu	Naggar	Bansherudhar	N 32°10'795"	E 77°12'994"	2866
27	H.P.	L&S	Udaipur	Trilokinath	N 32°06'741"	E 76°41'471"	2951
28	H.P.	Kullu	Anni	Jalorijot	N 31°32'236"	E 77°22'391"	3140
29	H.P.	Kangra	Bir	Palachak	N 32°08'076"	E 76°45'416"	2760
30	J&K	Kargil	Shankoo	Prachik-2	N 34°04'129"	E 75°58'667"	3497

occurring at an altitude ranging from 2356 meter (masl) to 3664 meter in Himachal Pradesh (H.P). And from 3142 to 3497 meter in Jammu & Kashmir (J.K) (Laddakh Valley). The samples (roots and rhizomes) were collected in the month of October-November 2012 in H.P. while keeping in view of feasibility of accessibility in Laddakh valley (J&K), samples were collected in the month of July-August. Samples of *P. hexandrum* were brought to laboratory for processing and coding. The samples were washed and dried at room temperature. The dried material was packed in brown paper bags and sent to IHBT, Palampur for carrying out a.i. analysis for screening and identification of superior chemotypes. For each site, 3 replications (Sample Packets with coding) were sent for analysis of active ingredients to draw valid statistical conclusion.

Characterization of micro-habitat of selected sites

The observations were recorded for characterization of micro-habitat of the each selected sites of *P. hexandrum* for important parameters viz., Habitat (Moist habitat/Dry Habitat/ Refractory Habitat/ Degraded Area/ Open Area/ Closed Canopy/ Along Road Side/ Along River Side/ Nallas/ Rocky Areas/ Under Thick Bush etc.), Aspect, Slope, Associated species, Main Tree Species, Soil Color, Forest Type and Physical Boundaries etc. to have a good idea of the micro-habitat conditions of the selected sites (Table 2).

Analysis of active ingredients of *P. hexandrum*

The standard methodology was adopted for active ingredient analysis of *Podophyllum hexandrum* [10]. The plant material ranging from 1 g to 2 mg was weighed and extracted thrice with methanol. The extracts were combined, filtered and dried under vacuum. The concentrate was re-dissolved in HPLC-grade methanol for quantitative analysis. Methanol extract of the plant material ranging from 1 g to 10 mg was resinified with acidulated water. The precipitated resin was redissolved in HPLC grade methanol for podophyllotoxin analysis. HPLC analysis was carried on Waters HPLC system (600

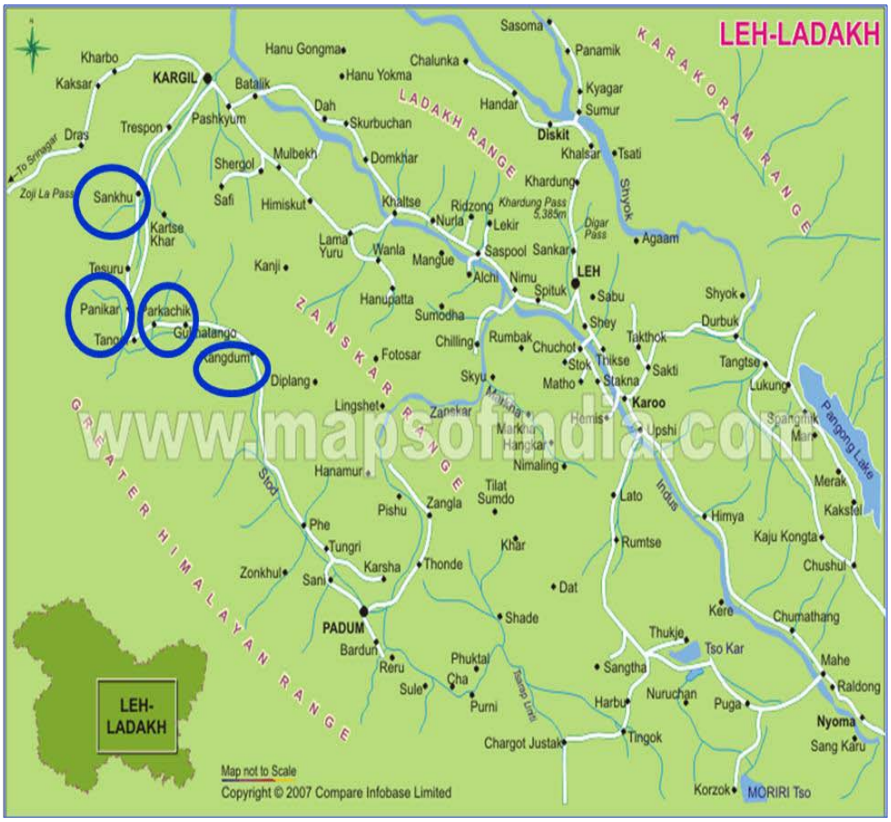

Figure 2: Map showing identified sites for *P. hexandrum* in different districts of J&K (Ladakh).

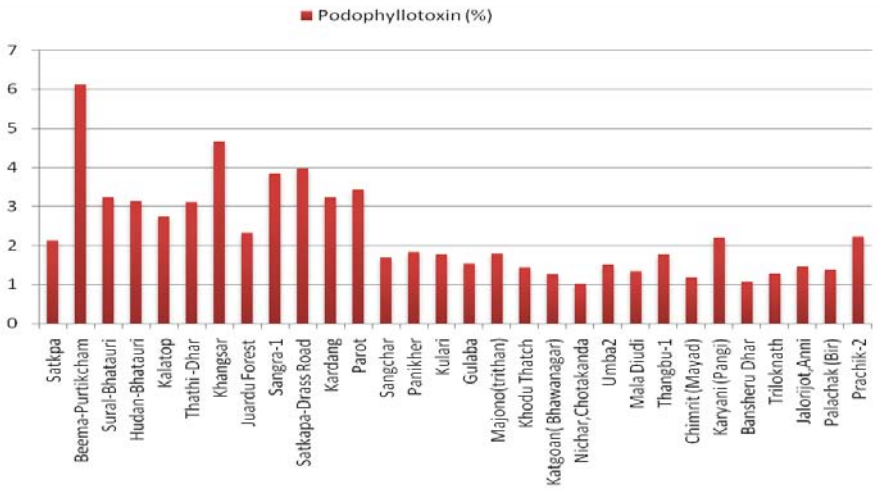

Figure 3: Podophyllotoxin content variation in *P. hexandrum* samples collected from different sources of H.P. and Ladakh Valley (J&K).

Gradient pump; 7725i Rheodyne Injector; 996 PDA Detector; Millennium32 version 3.05.1). All samples were filtered through 0.2 mm (Millipore) membranes. Column used was LichrospherR 100 5-RP-18e (250 mm´ 4 mm´ 5 mm), flow rate: 1 ml/min, run time: 20 min, detector wavelength 230 nm. HPLC-grade solvents were filtered through a 0.45 mm Millipore filter. The solvent system used was as solvent A: acetonitrile, solvent B: water in ratio of 4:6. The analysis of podophyllotoxin content in extracts and resins was performed by the external standard method, using podophyllotoxin (Sigma-Aldrich) as the standard compound. Stock solutions of 1 mg/ml were further diluted to 0.03, 0.06, 0.125, 0.25, 0.50 and 1 mg/ml for the formation of calibration curves. Each determination was carried out in triplicate. The regression equation for methanol extract was y = 0.979×-0.0175, coefficient of determination 0.9941, RT 7.20 ± 0.9, Limit of Detection (LOD) 30 pg, Limit of Quantitation (LOQ) 110 pg while for resin these were y = 0.0154×-0.0058, 0.9687, 7.21 ± 0.5, 100 pg and 615 pg respectively.

Table 2: Characterization of micro-habitat of *P. hexandrum* sites in H.P. and J&K.

S. No.	Name of District	Area / (Site)	Aspect	Slope	Major associated Spp.	Tree spp.	Habitat	Soil colour	Forest type
1	Kargil	Satkpa	S	*Steep*	*Achillea millifolium, Berginia ciliata*	*Salix alba*	Moist	Black	Alpine
2	Kargil	Beemaputri	W	-	*Salix alba, Aconitum heterophyllum*	*Salix alba*	Moist	-	-
3	Chamba	Suralbhaturi	NE	Gentle	*Heracleum candicans*	*Aesculs indica*	Moist	Dark brown	Open forest
4	Chamba	Hudanbhaturi	NE	Moderate	*Prunus cornuta, Rhododendron campanulatum, Juniperus spp.*	*Betula utilis*	Rocky moist	Dark brown	Betula forest
5	Chamba	Kalatop	NE	Moderate	*Viola serpens, Valeraina jatamansi, Trifolium repens*	*Cedrus deodara*	Moist	Blackish	Moist deodar forest
6	Chamba	Thathidhar	NW	Gentle	*Berginia ciliata , Achiellia spp.*	*Picea smithiana*	Moist	Black	
7	L&S	Kangsar	-	-	*Sausauria costus,Heracleum candicans*	*Rhododendron spp., Salix spp.*	Moist	Dark Brown	Alpine
8	Kangra	Joardu Forest	N	Steep	-	*Abies pindrow*	Open	Brown	Conifer
9	Kargil	Shangra	N	Steep	*Hippophae salicifolia, Aconitum heterophyllum*	-	-	Dark Brown	-
10	Kargil	Satkpa Drass Road	W	Steep	*Heracleum candicans*	*Betula utilis*	Moist	Dark brown	Open Betula forest area
11	L&S	Kardang	NW	Steep	*Dactylorhiza hategirea, Heracleum candicans, Hippophae salicifolia*	*Salix alba,*	Moist	Black	Broad leaved Alpine forest
12	Kullu	Parot	-	Steep	*Trillium govanianum, Valeriana jatamansi*	*Acer spp., Prunus cornuta, Cedrus deodara, Quercus spp.*	Moist rocky	Black	Mixed
13	Kullu	Shangchar	N/W	Gentle	*Berginia ciliata, Heracleum candicans,Berberis spp.*	*Cotoneaster spp., Picea smithiana*	Moist rocky	Black	Mixed
14	Kargil	Panikher	N	Steep	*Geranium nepalense, Aconitum heterophyllum*	*Rhododendron campanulatum, Betula utilis*	Dry rocky	Brown	Sub alpine fores
15	Kullu	Kulri (Bansheru)	NE	Steep	*Juniperus spp., Betula utilis, Prunus spp.*	*Betula utilis*	Moist	Golden Brown	Open Beula forest
16	Kullu	Gulaba	N	Gentle	*Viola spp., Berginia spp., Heracleum candicans*	*Abies pindrow, Cedrus deodara*	Moist	Brown	Scanty
17	Kullu	Majhoni (Tirthan)	N	Gentle	*Rhododendron spp., Fragaria spp., Viola spp., Berginia spp., Heracleum candicans*	*Betula utilis, Pinus wallichiana, Abies pindrow, Cedrus deodara*	Moist	Blackish	Mixed coniferous forest
18	Kullu	Khoruthach	NW	Gentle	*Berginia ciliata, Angelica glauca*	*Pinus wallichiana, Sterculia foetida, Juglans regia, Acer oblongum, Asculus indica*	Rocky open	Red Black	Mixed temperate forest
19	Kinnaur	Katgaon	S	Steep	*Viola odorata, Betula utilis, Brginia ciliata, Berberis spp.*	*Abies pindrow, Picea smithiana, Betula utilis, Taxus wallichiana*	Moist	Temperate dark brown	Alpine
20	Kinnaur	Chotkanda	-	Gentle	*Polygonatum spp, Trillium govanianum*	*Cedrus deodara, Prunus spp., Taxus baccata*	Temperate	Blackish forest soil	Mixed coniferous forest
21	Kargil	UMBA 2	NE	Moderte	*Rosa webbiana,Hippophae spp.*	*Salix spp.*	Moist	Gray	Alpine
22	Shimla	Maila (Diudi)	N	Steep	*Angelica glauca, Berginia ciliata, Polygonatum verticillatum*	*Juglans regia, Cedrus deodara, Abies pindrow, Picea smithiana*	Moist temperate	Black	Mixed conferous forest
23	Leh	Thangbu-1			*Rosa webbiana*	*Salix spp.*	Moist	Brown	Alpine
24	L&S	Chimrit	NE	Gentle	*Heracleum candicans*	*Salix spp., Pinus wallichiana*	Rocky	Grayish	Mixed

25	Chamba	Karyuni	NE	Steep	*Pteridophytes, Impatiens,Rosa microphylla*	--	Rocky	Blackish	Alpine
26	Kullu	Bansherudhar	S	Steep	*Heracleum candicans, Berginia ciliata*	*Pinus wallichiana, Cedrus deodara, Abies pindrow*	Moist Rocky	Black	Mixed
27	L&S	Trilokinath	NW	Gentle	*Dactylorhiza hategirea, Heracleum candicans, Rosa webbiana*	*Juglans regia, Pinus wallichiana,Cedrus deodara*	Road side Rocky area	Black	Grassland
28	Kullu	Jalorijot	N	Moderate	*Viola serpens, Trifolium repens*	*Cedrus deodara, Abies pindrow, Picea smithiana , Pinus wallichiana*	Moist	Temperate forest soil	Mixed coniferous forest
29	Kangra	Palachak	NE	Gentle	*Primula spp., Valeriana jatamansi*	*Pinus wallichiana* and *Abies pindrow*	Rocky moist	Dark brown	Open area
30	Kargil	Prachik-2	N	Gentle	*Rosa webbiana, Aconitum heterophyllum, Primula spp.*	*Salix spp.*	Moist	Grey	Alpine

Table 3: Podophyllotoxin content variations in samples of *P. hexandrum*, collected from different sources of H.P. and Ladakh valley (J&K).

S. No	Accession No.	Collection No.	District	State	Altitude (m)	Source Name	Podophyllotoxin content (% dry wt.) (mean±SE)
1	*	HFRI/PH/09/STP	Leh	J&K	3146	Satkpa	2.12±0.11
2	IC-0597505R	HFRI/PH/09/BP	Leh	J&K	3231	Beema-Purtikcham	6.13±0.42
3	IC-0597474	HFRI/PH/01/SB	Chamba	H.P.	3320	Sural-Bhatauri	3.23±0.24
4	IC-0597473	HFRI/PH/01/HB	Chamba	H.P.	3580	Hudan-Bhatauri	3.13±0.68
5	IC-0597497	HFRI/PH/01/KT	Chamba	H.P.	2356	Kalatop	2.73±0.49
6	IC-0597488	HFRI/PH/01/THD	Chamba	H.P.	2899	Thathi -Dhar	3.10±0.11
7	IC-0597501	HFRI/PH/05/KGR	Lahul	H.P.	3240	Khangsar	4.67±0.99
8	IC-0597510	HFRI/PH/02/JDF/201	Kangra	H.P.	2675	Juardu Forest	2.31±0.27
9	IC-0597507	HFRI/PH/09/SG-1	Leh	J&K	3148	Sangra-1	3.83±0.15
10	*	HFRI/PH/09/SDR	Leh	J&K	3188	Satkapa-Drass Road	3.97±0.56
11	IC-0597503	HFRI/PH/05/KDG	Lahul	H.P.	3148	Kardang	3.23±0.14
12	IC-0597492	HFRI/PH/04/PRT	Kullu	H.P.	3032	Parot	3.42±0.03
13	IC-0597491	HFRI/PH/03/SGR	Kullu	H.P.	3056	Sangchar	1.70±0.10
14	IC-0597506	HFRI/PH/09/PKR	Leh	J&K	3306	Panikher	1.83±0.25
15	IC-0597493	HFRI/PH/03/KLRI	Kullu	H.P.	3079	Kulari	1.76±0.15
16	IC-0597494	HFRI/PH/03/GLB	Kullu	H.P.	3664	Gulaba	1.53±0.15
17	IC-0597495	HFRI/PH/03/MJ(NP)	Kullu	H.P.	3460	Majono (trithan)	1.80±0.17
18	IC-0597487	HFRI/PH/03/KT(NP)	Kullu	H.P.	2580	Khodu Thatch	1.43±0.15
19	IC-0597496	HFRI/PH/02/BHW-Y	Kinnour	H.P.	2750	Katgoan (Bhawanagar)	1.26±0.15
20	IC-0597477	HFRI/PH/02/CKN	Kinnour	H.P.	3400	Nichar,Chotakanda	1.03±0.21
21	IC-0597483	HFRI/PH/09/L-2(2)	Leh	J&K	3142	Umba2	1.52±0.49
22	IC-0597499	HFRI/PH/07/MLA	Shimla		2990	Mala Diudi	1.33±0.057
23	IC-0597480	HFRI/PH/09/L-1(S)	Leh	J&K	3228	Thangbu-1	1.76±0.43
24	IC-0597471	HFRI/PH/05/MDV/	L&S	H.P.	2718	Chimrit (Mayad)	1.17±0.83
25	IC-0597472	HFRI/PH/01/PNG (2)	Chamba	H.P.	3064	Karyani (Pangi)	2.19±0.18
26	IC-0597470	HFRI/PH/04/BDR	Kullu	H.P.	2866	Bansheru Dhar	1.06±0.36
27	IC-0597476	HFRI/PH/05/TLN	L&S	H.P.	2951	Triloknath	1.29±0.85
28	IC-0597489	HFRI/PH/04/JL(A)	Kullu	H.P.	3140	Jalorijot,Anni	1.45±0.75
29	IC-0597485	HFRI/PH/01/PCK	Kangra	H.P.	2760	Palachak (Bir)	1.38±0.92
30	IC-0597504	HFRI/PH/09/PRK-2	Leh	J&K	3497	Prachik-2	2.23±0.32
						SEM± CD at 0.05%	0.26 0.53

*Accession no. for this collection could not be obtained from NBPGR, New Delhi as the same collections were made later.

Results

Most of the identified habitat for *P. hexandrum* in H.P. and Ladakh (J&K) were moist and rocky moist having gentle to steep slope which shows habitat specificity of species. *Achillea millefolium*, *Berginia ciliata*, *Dactylorhiza hatagirea*, *Heracleum candicans*, *Salix spp*., *Rosa webbiana*, *Primula spp*., *Aconitum heterophyllum* and *Angelica glauca* etc. were commonly species associated with *P. hexandrum* at most of the sites.

Among the 30 sources identified in H.P and Ladakh valley (J&K), podophyllotoxin content was in the range of 1.03% to 6.13%. On dry wt. basis highest podophyllotoxin content was found in Beema-Purtikcham (HFRI/PH/09/BP (6.13%) while minimum (1.03%) in Nichar, Chotakanda (HFRI/PH/09/CKN) (Table 3). It was observed that, out of 30 sources, there were 12 groups in which there were significant variation in podophyllotoxin contents from different sources of H.P. and Ladakh Valley (J&K) (Table 4). Higher podophyllotoxin content (>3.10%) was found in case of 09 sources viz. HFRI/PH/01/THD (Thathidar), HFRI/PH/01/HB (Hudan Bhatauri), HFRI/PH/05/KDG (Kardang), HFRI/PH/01/SB (Sural Bhatauri), HFRI/PH/04/PRT (Parot), HFRI/PH/09/SG-1(Sangra-1), HFRI/PH/09/SDR (Satkapa Drass Road), HFRI/PH/05/KGR (Khangsar), HFRI/PH/09/BP (Beema Purtikcham) while in other 21 sources total Podophyllotoxin content varied from 1.03% to 2.73% (Figure3, Table 3).

Discussion

It is reported that *Podophyllum hexandrum* the highest podophyllotoxin content (8.86 to 9.93% on dry wt. basis) is in the roots obtained from Lahoul Forest division and lowest values (3.02 to 4.75%) from Parvati forest division [11]. However in the present study the highest podophyllotoxin content was determined for the

Table 4: Means for different sources in homogeneous subsets.

Source	N	Subset											
		1	2	3	4	5	6	7	8	9	10	11	12
20	3	1.03											
26	3	1.06											
24	3	1.17	1.17										
19	3	1.26	1.26										
27	3	1.29	1.29										
22	3	1.33	1.33										
29	3	1.38	1.38										
18	3	1.43	1.43										
28	3	1.45	1.45	1.45									
21	3	1.52	1.52	1.52	1.52								
16	3	1.53	1.53	1.53	1.53								
13	3	1.70	1.70	1.70	1.70	1.70							
23	3		1.76	1.76	1.76	1.76							
15	3		1.76	1.76	1.76	1.76							
17	3		1.80	1.80	1.80	1.80							
14	3		1.83	1.83	1.83	1.83							
1	3			2.12	2.12	2.12	2.12						
25	3				2.19	2.19	2.19						
30	3					2.23	2.23						
8	3					2.31	2.31						
5	3						2.73	2.73					
6	3							3.10	3.10				
4	3							3.13	3.13				
11	3							3.23	3.23	3.23			
3	3							3.23	3.23	3.23			
12	3								3.42	3.42	3.42		
9	3									3.83	3.83		
10	3										3.97		
7	3											4.67	
2	3												6.13

samples from Beema-Purtikcham (Ladakh Valley, J&K) and the lowest value for the samples from Nichar, Himachal Pradesh.

The Indian *Podophyllum hexandrum* contains three times more podophyllotoxin than the American species [12]. The rhizome of the plant yields the highest amount of the compound (4.3%) of total dry weight [13], so the plant is uprooted to harvest the bioactive compound by solvent method. Considerable variation in morphological characters such as plant height, leaf characteristics, fruit weight, seed weight and color etc., and in biochemical characters such as podophylloresin and podophyllotoxin content in rhizomes also been reported in *P. hexandrum* plants from the Garhwal Himalayas [10,14,15]. The RAPD study of *P. hexandrum* has indicated the existence of high inter- and intra-population variations [16]. It is reported that for populations in the same forest division as well as between the forest divisions, the podophyllotoxin content increased with increase in altitude [17]. However, in our study such trend was not observed.

Podophyllotoxin rich accessions are of particular interest because biomass with high purity represents a significant economy in the process of purification and these accessions were proven to be stable podophyllotoxin chemotypes and confirmed as chemotypes by cultivating them in different growing conditions for three consecutive years and harvesting their blades for lignan extraction [16]. The superior chemotypes needs to be further tested for stability and yield under varied conditions through multi-location trials.

Conclusion

The rhizomes and roots of *Podophyllum* species have gained much importance being the main source or the starting material for the alkaloid podophyllotoxin, which is used in the treatment of specific types of cancers. In recent years, the frequency of *Podophyllum hexandrum* in nature has declined because the plant species are collected in large quantities by uprooting from wild to meet the ever-increasing demand of phamaceutical industry. Therefore, immediate thrust has to be given for generating the reliable conventional protocols of mass cultivation of *P. hexandrum*. However, before taking up commercial cultivation of the species, it is of paramount importance that superior genetic stock of the same should be identified and multiplied the same stock for realization of maximum profit to the farming communities. The present study has identified the superior chemotypes of this species by screening different geographical locations of Himachal Pradesh and Ladakh valley (J&K).

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