

# Impact of Altitudes and Habitats on Valerenic Acid, Total Phenolics, Flavonoids, Tannins, and Antioxidant Activity of *Valeriana jatamansi*

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**Abstract** The changes in total phenolics, flavonoids, tannins, valerenic acid, and antioxidant activity were assessed in 25 populations of *Valeriana jatamansi* sampled from 1200 to 2775 m asl and four habitat types of Uttarakhand, West Himalaya. Significant ( $p < 0.05$ ) variations in total phenolics, flavonoids, valerenic acid, and antioxidant activity in aerial and root portions and across the populations were observed. Antioxidant activity measured by three in vitro antioxidant assays, i.e., 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) (ABTS) radical scavenging, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, and ferric-reducing antioxidant power (FRAP) assays, showed significant ( $p < 0.05$ ) differences across the populations. However, no clear pattern was found in phytochemicals across the altitudinal range. Among habitat types, (pine, oak, mixed forest, and grassy land), variation in phytochemical content and antioxidant activity were observed. Equal class ranking, neighbor-joining cluster analysis, and principal component analysis (PCA) identified Talwari, Jaberkheth, Manjkhal, and Khirshu populations as promising sources with higher phytochemicals and antioxidant activity. The results recommended that the identified populations with higher value of phytochemicals and antioxidants can be utilized for mass multiplication and breeding program to meet the domestic as well as commercial demand.

**Keywords** Chemotypes · Elite identification · *Valeriana jatamansi* · Valerenic acid · Himalayan medicinal plant

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## Introduction

Naturally occurring biologically active compounds in plants are known for their (i) potential disease-inhibiting capabilities; (ii) ability to adapt in adverse environmental conditions; (iii) resistance to pathogens and pests; and (iv) controlling factors for taste, color, aroma, etc. [1]. Considering their effectiveness in combating several diseases, studies on plant-derived natural products are receiving greater attention in different systems of medicine [2], and Himalayan medicinal plants are being considered the best prospect/option.

*Valeriana jatamansi* Jones (family *Valerianaceae*, commonly known as “Tagar” or “Indian valerian”) is an important plant of the genus *Valeriana* that remains a subject of interest due to its extensive use in traditional and modern medicines, variability in morphology, reproductive behavior, and source of active ingredients, i.e., valerenic acid. The species is dioecious, perennial, polygamous, or occasionally polygamo monocious and grows wild in temperate Himalayan region at 1000–3000 m above sea level (asl). Traditionally, the species is used in the treatment of several diseases like sleeping disorders, obesity, epilepsy, insanity, snake poisoning, and eye and skin problems [3]. In addition, the species is source of various secondary metabolites, e.g., valepotriates [4], dihydrovaltrate [5], linarin-isovalerianate [6], sesquiterpenoids, valerenic acid derivatives (valerenic acid, acetoxy valerenic, and hydroxyvalerenic acid) [7], 6-methylpigenin and hesperidin [8], valerenic acid [9], phenolics, and flavonoids [10]. Among these, valerenic acid and valeopatriates are often used for drug preparation [9, 11]. Besides, the species is reported to be used as a substitute of *V. officinalis*, one of the widely used medicinal plants of the genus [11].

It has been reported that environmental factors are known to influence the quality and quantum of secondary metabolites [12, 13]. For example, increase in altitude showed a decreasing trend in berberine content of *Berberis asiatica* [13]. In contrast, phenolic content increased with increasing altitude in *Hedychium spicatum* [14], etc. These studies are an indicative of the fact that responses of a species for secondary metabolites do affect by environmental factors. Studies on these aspects are not systematically investigated in *V. jatamansi*. Earlier studies on the variation in morphological and genetic diversity of *V. jatamansi* suggest that environmental and genetic factors influence the diversity of the plant and its chemical constituents [15, 16]. However, a systematic investigation on the response of the species for its secondary metabolites and antioxidant activity across altitudinal range and habitat conditions is poorly known. This is important because the species grow in different habitat conditions at wide altitudinal range, where microclimatic conditions like temperature, moisture regime, and soil nutrients varied considerably [17].

All these factors are reported to have direct bearing with secondary metabolites of a plant species [18]. Thus, the present study attempts to investigate the level of chemical constituents (valerenic acid, total phenolic, flavonoid, and tannin contents) and antioxidant activity in *V. jatamansi* collected from different habitat conditions (oak, pine, oak-mixed, and grassy land) across altitudinal range. The results of this study will help in identification of promising populations, suitable habitat condition, and altitude for harnessing potential of the species.

## Materials and Methods

### Study Area and Sampling

The samples (aerial and root portions of *V. jatamansi*) were collected during February 2009 from 25 populations of *V. jatamansi* in Uttarakhand (West Himalaya) region, India, representing diverse habitat conditions and altitudinal range (Table 1). Plants (four numbers) were randomly collected and divided in two portions (aerial and root portions) were dried in hot air oven at 40 °C and grounded to fine powder using Grinder mill (Macro Scientific, India). Immediately after grounding, the samples were processed for further analysis of total phenolics, flavonoids, and tannin estimation. Botanical identity of the species authenticated from Botanical Survey of India (BSI), Dehradun, and accession number of each population was obtained. Voucher specimens were deposited at herbaria of BSI and G.B. Pant Institute of Himalayan Environment and Development (GBPIHED), Kosi Katarmal, Almora, Uttarakhand, India (Table 1). Samples were collected from different altitudes (1201–1500, 1501–1800, 1801–2100, 2101–2400, and >2401 m asl) and habitat types (pine, oak, mixed forest, and grassy land) for investigating chemical constituents and antioxidant activity.

**Table 1** Site characteristics and accession number of selected populations of *V. jatamansi*

Serial no.	Population	Altitude (m asl)	Latitude (N°)	Longitude (E°)	Habitat	Accession number
1	Daulaghat	1215	29° 46' 00"	80° 18' 00"	Grassy	113261
2	Katarmal	1250	29° 38' 25"	79° 37' 20"	Oak	BSD-112793
3	Tharali	1330	30° 04' 06"	79° 30' 08"	Oak	113206
4	Dolti	1626	30° 02' 56"	79° 29' 50"	Oak	GBPI-3201-2
5	Berinag	1672	29° 43' 17"	80° 02' 14"	Pine	113257
6	Manjkhali	1702	29° 40' 20"	79° 31' 47"	Pine	113209
7	Talwari	1785	30° 01' 46"	79° 30' 59"	Oak	113205
8	Khirsu	1810	29° 51' 17"	79° 35' 46"	Oak	113212
9	Didihat	1850	29° 46' 16"	80° 17' 59"	Oak	113253
10	Pithoragarh	1872	29° 36' 14"	80° 11' 40"	Pine	113214
11	Sitlakheth	1900	29° 35' 40"	79° 32' 42"	Pine	GBPI-3201-1
12	Gwaldam	1923	30° 00' 24"	79° 33' 30"	Oak	113213
13	Kausani	1925	29° 47' 51"	79° 26' 12"	Grassy	113210
14	Ukhimath	1985	30° 31' 00"	80° 05' 00"	Pine	113258
15	Camel back	2000	30° 27' 43"	78° 04' 26"	Grassy	113200
16	Jaberkhet	2080	30° 27' 18"	78° 06' 50"	Grassy	113260
17	Joshimath	2100	29° 47' 37"	79° 27' 38"	Mixed	113211
18	Dooagiri	2125	29° 47' 37"	79° 27' 40"	Grassy	113254
19	Buranskhand	2150	30° 27' 22"	78° 05' 59"	Oak	113207
20	Nainital	2176	29° 23' 34"	79° 27' 45"	Mixed	113208
21	Makku band	2240	30° 34' 0.0"	79° 13' 00"	Grassy	113262
22	Munsyari	2240	30° 03' 39"	80° 14' 36"	Mixed	113202
23	Malyadaur	2350	30° 08' 16"	79° 57' 56"	Mixed	113255
24	Dwali	2730	30° 10' 38"	79° 59' 46"	Mixed	113204
25	Surkunda	2775	30° 24' 21"	78° 17' 21"	Mixed	113259

## Extract Preparation for Phytochemical Analysis

Dried powder (50 mg) of each portion (aerial and root) extracted from each population was mixed separately with 25 ml 80 % (v/v) methanol, stirred gently (12 h), and sonicated (22 °C, 10 min; Model-ANIS 09001, Toshiba, New Delhi, India). The extract was centrifuged (10,000 rpm; 15 min, 22±1 °C), and supernatants were collected, filtered, and stored at 4 °C prior to use for chemical analysis within 24 h.

## Estimation of Total Phenolic, Flavonoid, and Tannin Contents

Total phenolic (TP), flavonoid (TF), and total tannin (TT) contents were determined following the methods used in earlier reports [10, 19].

## Valerenic Acid and Antioxidant Activity

Valerenic acid was estimated using the method of Upton [20] in the ethanolic extract of aerial and root portions. Powdered material (10 mg) of plant portion (aerial and root) of each population was extracted in ethanol, and valerenic acid content was determined using external standard in a HPTLC system (Camag), equipped with an automatic TLC sampler (Linomat 5), TLC scanner 3, and integrated software WinCATS version 1.3.4 [21]. The antioxidant activity was determined using different *in vitro* methods such as 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric-reducing antioxidant power (FRAP) method [10]. All the assays carried out in triplicates and average values were considered.

## Prioritization and Elite Identification

Toward prioritization of suitable population for higher chemical constituents and antioxidant activity, value of each attributes in a population was classified into equal classes. The classification was done by subtracting the minimum value from the maximum and divided by 5 (Tables A1 and A2). These data were further subjected for principal component analysis (PCA) for identification of the promising populations using SPSS software. In the PCA, data set consisted of a 25×8 matrix (for phytochemicals) and 25×6 matrix (for antioxidants), where the results obtained for each parameter were adopted as columns and the *Valeriana* populations (samples) were used as the rows. Analyses were based on correlations and variances were computed as  $SS/(n-1)$ . Eigen values higher than 1.0 were adopted to explain the projection of the assays on the factor plane. All the data were auto scaled before the analysis, which means that each column data matrix was mean-centered and scaled to unit variance and avoid the effect of different scales of the variables [22].

## Statistical Analysis

Analysis of variance and significant difference among means were tested using the Duncan's multiple-range test by one-way ANOVA with SPSS program. Correlation coefficients ( $r$ ) and coefficients of determination ( $R^2$ ) were calculated using Microsoft Excel 2007. The independent sample (unpaired)  $t$  test was used to determine the significant difference in altitudinal

range and habitat types. Toward prioritization of suitable population for phytochemical content and antioxidant activity, each population was classified into equal classes based on phytochemical content and antioxidant activity (sum of aerial and root portions). Neighbor-joining (NJ) cluster analysis was performed using PAST (Paleontological Statistics Software Package for Education and Data Analysis) packages [23] for classifying distant populations of *V. jatamansi* based on all studied secondary metabolites. PCA was performed using SPSS software version 16 to identify the major population groups with higher activity.

## Results

### Phytochemical Assessment

Significant ( $p < 0.05$ ) variation in total phenolics and flavonoids in aerial and root portions of *V. jatamansi* across all populations was observed (Table 2). Munsyari population exhibited significantly higher total phenolics (aerial –  $25.23 \pm 0.03$  mg; root portion  $27.54 \pm 0.06$  mg GAE/g dw) as compared to other populations. Flavonoid contents were significantly ( $p < 0.05$ ) higher (aerial portion  $32.67 \pm 0.34$  mg; root portion  $29.91 \pm 0.47$  mg QE/g dw) in the Jaberkheth and Talwari populations. In case of total tannins, significant ( $p < 0.05$ ) variations were recorded in aerial portion; however, no significant variations were recorded in TT content in root portion (Table 2). The tannin content was significantly ( $p < 0.05$ ) higher (aerial portion  $8.09 \pm 0.04$ ) in the Ukhimath population as compared to other populations. Significant variation ( $p < 0.05$ ) was detected in valerenic acid in aerial and root portions of *V. jatamansi* across all studied populations (Table A3). Significantly higher valerenic acid content was in the Katarmal (aerial portion  $0.57 \pm 0.04$  %) and Joshimath populations (root portion  $1.80 \pm 0.12$  %). Similarly, aerial portion of Dolti and root portion of Dwali also showed significantly higher valerenic acid content than the other populations. However, valerenic acid could not be detected in the aerial portion of some studied populations (Table A3).

### Antioxidant Activity

Antioxidant activity of *V. jatamansi* (aerial and root portions) measured by ABTS, DPPH, and FRAP assays showed significant difference ( $p < 0.05$ ) across the populations (Table A3). In case of aerial portion, maximum ( $8.63 \pm 0.06$  mM AAE/100 g) activity was recorded in the Didihat population and minimum ( $2.75 \pm 0.16$  mM AAE/100 g dw) in the Malyadaur population using ABTS assay. Root portion of the Katarmal population exhibited maximum ABTS activity ( $8.36 \pm 0.00$  mM AAE/100 g) as compared to other populations (Table A3). Talwari population exhibited the maximum ( $16.80 \pm 0.03$  mM AAE/100 g dw) antioxidant activity in aerial portion by DPPH assay. Similarly, root portion of the Didihat population exhibited the maximum antioxidant activity ( $17.53 \pm 0.04$  mM AAE/100 g dw). The population from Didihat showed significantly higher ( $15.23 \pm 0.09$  mM AAE/100 g dw) antioxidant activity in aerial portion using FRAP assay. In case of root portion, the Ukhimath population exhibited significantly higher ( $12.71 \pm 0.04$  mM AAE/100 g dw) antioxidant activity as compared to other populations of *V. jatamansi*.

**Table 2** Phytochemical contents in aerial and root portions of selected populations of *V. jatamansi*

Serial no.	Population	Plant portions					
		Aerial			Root		
		TP (mg GAE/g)	TF (mg QE/g)	TT (mg TAE/g)	TP (mg GAE/g)	TF (mg QE/g)	TT (mg TAE/g)
1	Daulaghat	8.54 ± 0.09 <sup>v</sup>	16.51 ± 0.33 <sup>f</sup>	6.42 ± 0.10 <sup>bcd</sup>	10.64 ± 0.16 <sup>f</sup>	18.14 ± 0.21 <sup>g</sup>	7.27 ± 0.04
2	Katramal	10.07 ± 0.02 <sup>s</sup>	18.79 ± 0.37 <sup>d</sup>	7.16 ± 0.61 <sup>a</sup>	11.30 ± 0.04 <sup>p</sup>	22.23 ± 0.73 <sup>bc</sup>	7.13 ± 0.30
3	Tharali	12.04 ± 0.07 <sup>n</sup>	21.12 ± 0.40 <sup>bc</sup>	6.91 ± 0.23 <sup>bcd</sup>	8.98 ± 0.05 <sup>u</sup>	19.06 ± 0.42 <sup>fg</sup>	7.33 ± 0.03
4	Dolti	14.09 ± 0.03 <sup>j</sup>	18.18 ± 0.40 <sup>de</sup>	5.00 ± 0.96 <sup>de</sup>	12.99 ± 0.05 <sup>m</sup>	15.38 ± 0.25 <sup>h</sup>	7.27 ± 0.71
5	Berinag	13.45 ± 0.04 <sup>k</sup>	17.61 ± 0.45 <sup>def</sup>	6.78 ± 0.09 <sup>bcd</sup>	14.87 ± 0.04 <sup>j</sup>	15.48 ± 0.25 <sup>h</sup>	7.50 ± 0.09
6	Manjkhal	7.54 ± 0.08 <sup>x</sup>	13.73 ± 0.49 <sup>h</sup>	3.40 ± 0.04 <sup>ef</sup>	10.39 ± 0.03 <sup>s</sup>	21.86 ± 0.37 <sup>bcd</sup>	7.44 ± 0.04
7	Talwari	19.27 ± 0.04 <sup>b</sup>	17.47 ± 0.47 <sup>ef</sup>	6.72 ± 0.98 <sup>bcd</sup>	25.05 ± 0.05 <sup>b</sup>	29.91 ± 0.47 <sup>a</sup>	7.73 ± 0.35
8	Khirshu	14.32 ± 0.05 <sup>g</sup>	11.94 ± 0.50 <sup>kl</sup>	6.99 ± 0.22 <sup>bc</sup>	15.21 ± 0.03 <sup>l</sup>	16.18 ± 0.36 <sup>h</sup>	7.27 ± 0.38
9	Didihat	17.36 ± 0.07 <sup>c</sup>	12.65 ± 0.31 <sup>kl</sup>	7.57 ± 0.73 <sup>a</sup>	18.91 ± 0.07 <sup>f</sup>	19.19 ± 0.23 <sup>fg</sup>	6.36 ± 0.79
10	Pithoragarh	15.39 ± 0.03 <sup>e</sup>	21.41 ± 0.60 <sup>bc</sup>	7.27 ± 0.08 <sup>a</sup>	23.90 ± 0.06 <sup>c</sup>	20.44 ± 0.21 <sup>e</sup>	6.50 ± 0.34
11	Sitlakheth	11.53 ± 0.08 <sup>t</sup>	6.03 ± 0.04 <sup>m</sup>	2.75 ± 0.40 <sup>f</sup>	13.83 ± 0.08 <sup>l</sup>	5.75 ± 0.03 <sup>k</sup>	3.40 ± 0.11
12	Gwaldam	12.31 ± 0.07 <sup>n</sup>	21.94 ± 0.40 <sup>b</sup>	7.61 ± 0.54 <sup>a</sup>	14.39 ± 0.03 <sup>k</sup>	25.99 ± 0.28 <sup>a</sup>	7.91 ± 0.60
13	Kausani	5.02 ± 0.02 <sup>y</sup>	18.82 ± 0.36 <sup>d</sup>	6.66 ± 0.15 <sup>bcd</sup>	6.83 ± 0.03 <sup>t</sup>	15.76 ± 0.33 <sup>h</sup>	7.79 ± 0.13
14	Ukimath	8.86 ± 0.04 <sup>t</sup>	17.67 ± 0.41 <sup>def</sup>	8.09 ± 0.04 <sup>a</sup>	11.26 ± 0.04 <sup>q</sup>	13.03 ± 0.46 <sup>i</sup>	7.35 ± 0.07
15	Camel back	8.78 ± 0.02 <sup>u</sup>	11.97 ± 0.46 <sup>kl</sup>	6.48 ± 0.69 <sup>bcd</sup>	11.93 ± 0.07 <sup>o</sup>	16.07 ± 0.31 <sup>h</sup>	7.28 ± 0.01
16	Jaberkhet	13.16 ± 0.03 <sup>l</sup>	32.67 ± 0.34 <sup>a</sup>	7.55 ± 0.47 <sup>a</sup>	23.83 ± 0.06 <sup>c</sup>	21.35 ± 0.41 <sup>cde</sup>	7.00 ± 0.28
17	Joshimath	8.29 ± 0.04 <sup>w</sup>	20.31 ± 0.22 <sup>c</sup>	6.01 ± 1.42 <sup>bcd</sup>	9.47 ± 0.04 <sup>v</sup>	20.96 ± 0.33 <sup>de</sup>	6.83 ± 1.07
18	Doonagiri	14.21 ± 0.06 <sup>hi</sup>	26.37 ± 0.27 <sup>a</sup>	6.79 ± 0.06 <sup>bcd</sup>	18.89 ± 0.05 <sup>f</sup>	18.09 ± 0.48 <sup>g</sup>	7.75 ± 0.60
19	Buranskhanda	12.29 ± 0.04 <sup>p</sup>	13.46 ± 0.31 <sup>l</sup>	7.44 ± 0.25 <sup>a</sup>	14.40 ± 0.05 <sup>k</sup>	11.78 ± 0.42 <sup>j</sup>	7.00 ± 0.65
20	Nainital	18.68 ± 0.05 <sup>d</sup>	20.74 ± 0.48 <sup>c</sup>	6.01 ± 0.51 <sup>bcd</sup>	19.57 ± 0.04 <sup>e</sup>	18.84 ± 0.33 <sup>fg</sup>	6.87 ± 0.30
21	Makku band	15.39 ± 0.05 <sup>e</sup>	18.21 ± 0.32 <sup>de</sup>	7.43 ± 0.57 <sup>a</sup>	12.70 ± 0.05 <sup>n</sup>	25.54 ± 0.27 <sup>a</sup>	6.80 ± 0.05
22	Munsiyari	25.23 ± 0.03 <sup>a</sup>	11.59 ± 0.45 <sup>l</sup>	7.38 ± 0.07 <sup>a</sup>	27.54 ± 0.06 <sup>a</sup>	12.15 ± 0.47 <sup>ij</sup>	6.52 ± 0.82
23	Malyadaur	12.30 ± 0.05 <sup>e</sup>	15.08 ± 0.25 <sup>g</sup>	6.62 ± 0.06 <sup>bcd</sup>	17.83 ± 0.06 <sup>g</sup>	19.39 ± 0.24 <sup>f</sup>	7.18 ± 0.10
24	Dwali	13.13 ± 0.02 <sup>m</sup>	17.76 ± 0.42 <sup>de</sup>	7.24 ± 0.19 <sup>a</sup>	20.06 ± 0.08 <sup>d</sup>	18.83 ± 0.29 <sup>fg</sup>	7.55 ± 0.31
25	Surkunda	14.47 ± 0.03 <sup>f</sup>	12.90 ± 0.36 <sup>jk</sup>	5.10 ± 1.15 <sup>cde</sup>	15.93 ± 0.04 <sup>h</sup>	22.54 ± 0.47 <sup>b</sup>	6.79 ± 0.32
	Mean	13.18 ± 0.93	17.40 ± 1.07	6.54 ± 0.26	15.63 ± 1.09	18.56 ± 1.01	7.03 ± 0.17

All data are reported as mean of three replicates ± standard error. No variations were recorded in case of TT in root portion. Means with different letters are significantly different ( $p < 0.05$ ) according to Duncan's multiple-range test (DMRT)

TPs total phenolics, TFs total flavonoids, TTs total tannins

## Variation in Phytochemicals and Antioxidant Activity Across Altitudinal Range and Habitat Types

Analysis using independent sample *t* test at different altitude level revealed the following (Table 3 and Table A4): (i) significant difference in total phenolics of root portion at 1201–1500 and 2101–2400 m asl ( $t = 2.618$ ,  $p < 0.05$ ) and 1201–1500 and 2101–2400 ( $t = 4.323$ ,  $p < 0.05$ ), (ii) significant difference in antioxidant activity in the aerial portion using ABTS assay at 1501–1800 and 2101–2400 m asl ( $t = 2.917$ ,  $p < 0.05$ ), DPPH assay in aerial portion at 1500–1800 and 1801–2100 ( $t = 2.497$ ,  $p < 0.05$ ) and 1501–1800 and 2101–2400 ( $t = 3.682$ ,  $p < 0.05$ ), and FRAP assay at 1201–1500 and 1501–1800 ( $t = 2.904$ ,  $p < 0.05$ ) and 1501–1800

**Table 3** Difference in phytochemicals and antioxidant activity across the altitudinal range analyzed using independent sample (unpaired) *t* test

Serial no.	Parameter	Altitudinal range (m asl)	<i>t</i> value
1	TPR	1201–1500; 2101–2400	2.618*
2	TPR	1201–1500; >2401	4.323*
3	ABTSA	1501–1800; 2101–2400	2.917**
4	DPPHA	1501–1800; 1801–2100	2.497*
5	DPPH A	1501–1800; 2101–2400	3.682**
6	FRAPA	1201–1500; 1501–1800	2.904*
7	FRAPA	1501–1800; 2101–2400	3.585**

TPR total phenolics in root portion, ABTSA ABTS activity in aerial portion, DPPHA DPPH activity in aerial portion, FRAPA FRAP activity in aerial portion

\* $p < 0.05$

\*\* $p < 0.01$

and 2101–2400 ( $t = 3.585$ ,  $p < 0.05$ ). Similarly, only samples collected from grassy and pine habitat exhibited significant difference ( $t = -2.325$ ,  $p = 0.045$ ) in FRAP activity in aerial portion. Other studied attributes among habitat type did not show any significant difference; however, level of chemical content in different habitats varied (Fig. 1a–c). Pine forest habitat was found to be the best for valerenic acid (VA) content, mixed-oak forest for TP, and grassy land for total flavonoids and total tannins. Similarly, pine forest for antioxidant activity of aerial portion and grassy land habitat for root portion using ABTS and pine forest FRAP and DPPH emerged suitable habitats (Fig. 2a–c).

### Relationship Among Phytochemicals and Antioxidant Activity

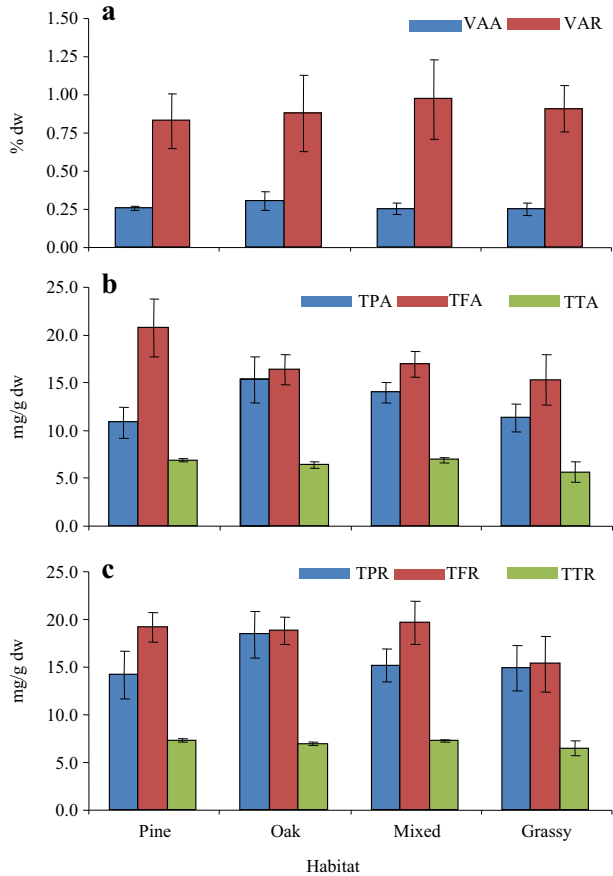
No significant ( $p < 0.05$ ) relationship was found between altitude and studied phytochemical parameters using the Pearson's correlation coefficient analysis in both plant portion (Tables 4 and 5). However, a significant ( $p < 0.05$ ) positive relationship of TP and TF contents in aerial portion ( $r = 0.429$ ,  $p < 0.05$ ) was observed. Similarly, ABTS activity exhibited significantly positive relationship with DPPH ( $r = 0.840$ ,  $p < 0.01$ ) and FRAP activity ( $r = 0.811$ ,  $p < 0.01$ ) in aerial portion, while DPPH activity showed positive relationship with FRAP activity ( $r = 0.519$ ,  $p < 0.05$ ) in the aerial portion (Table 4).

TF in root portion showed a positive correlation with TT content ( $r = 0.519$ ,  $p < 0.05$ ). ABTS activity also showed a positive relationship with DPPH ( $r = 0.482$ ,  $p < 0.05$ ) and FRAP activity ( $r = 0.637$ ,  $p < 0.01$ ). Significant positive relationship was also found in DPPH activity with FRAP activity ( $r = 0.763$ ,  $p < 0.01$ ) in the root portion of *V. jatamansi* (Table 5).

### Identification of Promising Source (Chemotypes) and Elite Identification

Neighbor-joining cluster analysis was performed to prioritize the populations and identify promising individuals/populations based on phytochemicals and antioxidant activity in aerial and root portions (Fig. 3a, b). Cluster analysis based on all studied attributes grouped the populations into two major clusters, with one cluster joining those *V. jatamansi* populations having higher value of phytochemicals [(cluster I, population no. 2–16 (78.56–107.28 total of

**Fig. 1** Variations in phytochemicals [TPs (total phenolics), TFs (total flavonoids), and TTs (total tannins)] in *V. jatamansi* across habitat types. **a** valericenic acid in aerial and root portions, **b** aerial portion, and **c** root portions

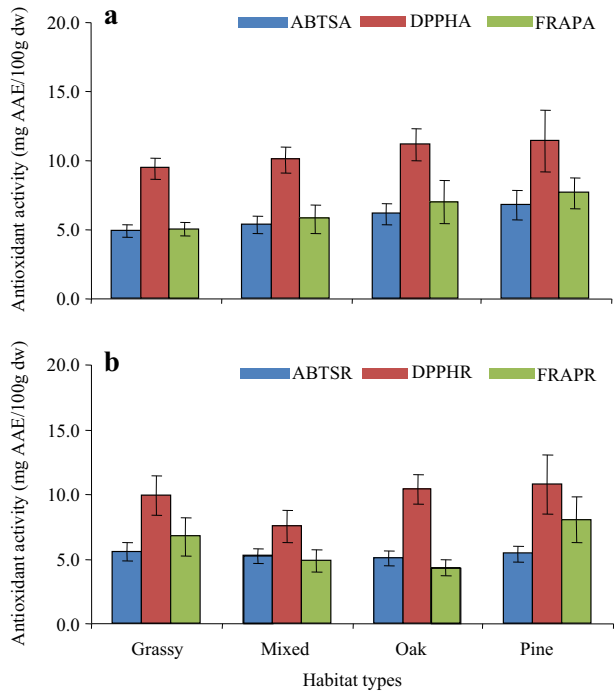


all phytochemicals)] and the second cluster joining those populations with a low phytochemical content [(cluster II, population no. 6–13 (62.16–68.45 phytochemical content; Fig. 3a)]. Similarly, neighbor-joining cluster analysis of antioxidant activity determined by all three assays grouped all populations into three major clusters, with one cluster joining the *V. jatamansi* populations which maintained higher levels of antioxidant activity (population no. 6–2; cluster no II), while remaining two cluster joining those populations with low levels of antioxidant activity (Fig. 3b). Based on neighbor-joining cluster analysis, populations 16 (Jaberkhet) and 7 (Talwari) can be prioritized for harboring maximum phytochemicals and populations 14 (Ukimath), 6 (Majkhali), and 8 (Khirshu) for maximum antioxidant activity.

Promising source(s) based on phytochemicals and antioxidant activity among different populations were identified using equal group classification method. However, shortcoming of equal group categorization (identification of various populations based on different components in higher value class) is further resolved using the PCA. For example, the Munsyari population fall in class 5 based on total phenolic content, while the Talwari, Gwaldam, and Jaberkhet populations fall in class 5 based on total flavonoids. Similarly, Joshimath population exhibited higher value of valericenic acid and fall in the class 5 category; however, 19 populations fall in class 5 based on total tannins. PCA categorized all the populations in two major chemotypes. Chemotype I includes the populations from Khirshu and Munsyari, which



**Fig. 2** Variations in antioxidant activity of *V. jatamansi* across habitat types. **a** Aerial portion and **b** root portion



showed almost similar higher level of all phytochemicals, while chemotype II includes all remaining populations of *V. jatamansi* (Fig. 4a). In addition, PCA-based analysis of phytochemicals exhibited the three first principal components, representing 98.65 % of the total variability. Figure 4a shows a classical representation of the scores on PC1 and PC2 (95.83 % of total variance). Similarly, PCA-based analysis of antioxidant activity revealed that three first principal components represented 92.56 % of the total variability. Figure 4b shows a classical

**Table 4** Relationship among phytochemicals and antioxidant activity (aerial portions) in selected populations of *V. jatamansi* (n = 25)

Serial no.	Altitude	TP	TF	TT	ABTS	DPPH	FRAP	VA
Altitude	1.000							
TP	0.275	1.000						
FLV	-0.057	-0.053	1.000					
TT	0.055	0.222	0.429*	1.000				
ABTS	-0.162	-0.003	0.149	0.051	1.000			
DPPH	-0.133	-0.008	0.327	0.160	0.840**	1.000		
FRAP	-0.176	-0.055	-0.012	-0.141	0.811**	0.655**	1.000	
VA	-0.197	-0.066	-0.101	-0.128	-0.335	-0.086	-0.293	1.000

TPs total phenolics, TFs total flavonoids, TTs total tannins, ABTS ABTS activity, DPPH DPPH activity, FRAP FRAP activity, VA valeric acid

\*Correlation is significant at the 0.05 level

\*\*Correlation is significant at the 0.01 level

**Table 5** Relationship among phytochemicals and antioxidant activity (root portions) in selected populations of *V. jatamansi* (n=25)

Serial no.	Altitude	TP	TF	TT	ABTS	DPPH	FRAP	VA
Altitude	1.000							
TP	0.385	1.000						
FLV	0.006	0.147	1.000					
TT	-0.066	-0.093	0.519**	1.000				
ABTS	-0.228	-0.126	0.132	0.001	1.000			
DPPH	-0.11	-0.051	0.199	0.272	0.482*	1.000		
FRAP	-0.005	-0.04	-0.071	-0.11	0.637**	0.763**	1.000	
VA	0.208	-0.286	-0.034	0.205	-0.113	-0.196	-0.118	1.000

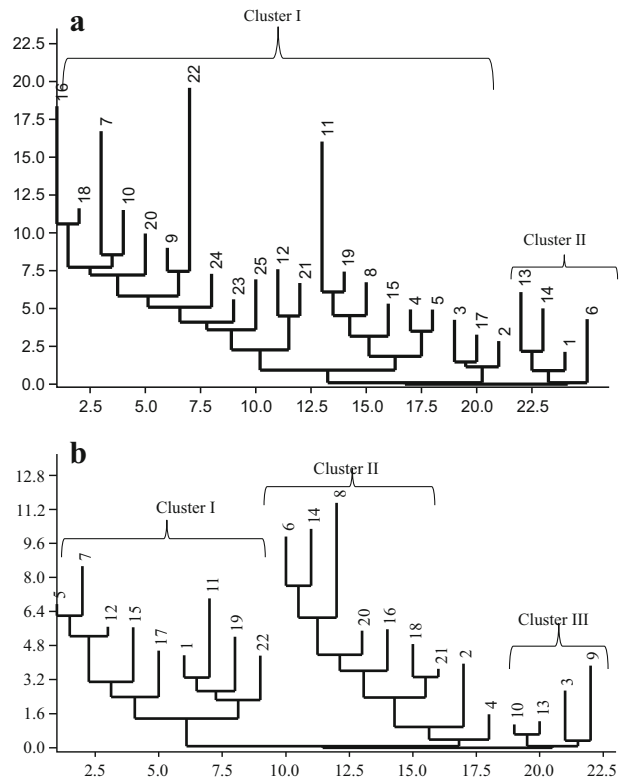
TPs total phenolics, TFs total flavonoids, TTs total tannins, ABTS ABTS activity, DPPH DPPH activity, FRAP FRAP activity, VA valeric acid

\*Correlation is significant at the 0.05 level

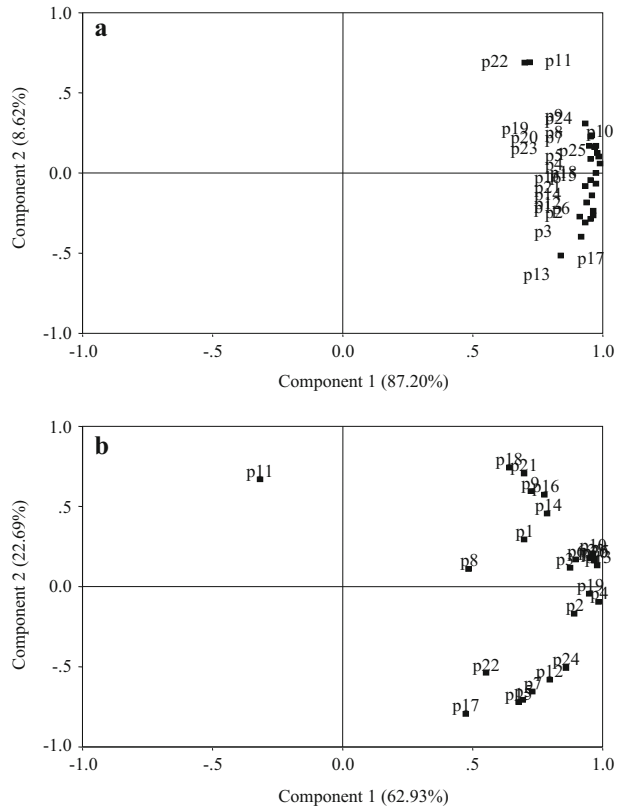
\*\*Correlation is significant at the 0.01 level

representation of the scores on PC1 and PC2 (85.63 % of total variance). PCA classified all 25 populations into two major groups; group I contains the Khirshu population while group II contains all remaining 24 populations of *V. jatamansi* (Fig. 4b).

**Fig. 3** Identification of promising source of *V. jatamansi* in aerial and root portions using NJ clustering analysis. **a** Total phytochemicals and **b** antioxidant activity



**Fig. 4** Identification of promising source of *V. jatamansi* in aerial and root portions using principal component analysis. **a** Total phytochemicals and **b** antioxidant activity



## Discussion

A marked intraspecific variability in the phytochemical content and antioxidant activity among plant samples has been reported for different species of the region including *V. jatamansi*. Causes of such variation are generally attributed either to (i) genetic character of source population/plant [19], (ii) growing season/development stages of plant [24], (iii) effect of light intensity [25], (iv) altitude of plant origin [26], and (v) microclimatic condition of the area [27]. In the present study, sample collected from different localities (25 populations) with diverse altitudinal range (1215–2775 m asl), varying habitat (oak, pine, mixed-oak, and grassy land), and environmental conditions along with different genetic background of each individual (sample) might be responsible for such variation. Such types of studies are also available elsewhere. For example, growing season found to be responsible for variations in phenolic mass fraction among different cultivars of blueberry [28] highlights that intensity in glasshouse condition is reported to increase total phenolics, flavonoids, and antioxidant activities in *Zingiber officinale* [25] latitude of plant origin that influenced the tannin and glycyrrhizin contents of the licorice (*Glycyrrhiza glabra*, Leguminosae). Anthocyanin content is influenced by soil pH and electrical conductivity [27]; seasonal changes in the shoots of *Mentha longifolia* are reported to have influence on dry matter, fiber, fat, protein, net free energy (NFE), nitrogen-free extractable substances (NFESs), macronutrients (Na, N, Ca, K, and P) and micronutrients (Zn, Mg, Fe, and Cu), and alkaloid, flavonoid, and phenolic contents [29]; and growth stages

are reported to influence phenolics and antioxidant activity in *H. spicatum* [14]. Moreover, phytochemical diversities in *Echinacea angustifolia* populations from different natural geographic areas were found to be significantly associated with latitudinal variation [30]. Similar reports are also available on other species like *Eucommia ulmodies* [31] and *Centella asiatica* [32]. Similarly, significant variations were recorded in total phenolics, flavonoids, proline, malondialdehyde,  $H_2O_2$ , and antioxidant activity of *Achillea millefolium*, *A. nobilis*, and *A. filipendulina* growing under four irrigation regimes [33]. In addition, the effect of initial temperature treatment on phytochemicals and antioxidant activity was also studied in *Azadirachta indica* [34].

The mean values for total phenolics, flavonoids, tannins, and antioxidant activity measured by all three assays in *V. jatamansi* were considerably higher as compared to earlier reports [10, 19, 35]. Similarly, the reported values in this species were higher than the value (0.34 mg/g GAE dw) in root portion of other related species of the family, e.g., *Nardostachys jatamansi* and *V. officinalis* [32]. Antioxidant activity of the essential oil and root extract derived from *V. jatamansi* using DPPH and FRAP assays suggests potential of the species as natural source of antioxidant [35, 36]. Higher amount of phytochemicals in root portion as compared to aerial portion in *V. jatamansi* corresponds with earlier reports for the same species [10]. Although studies are also available on valerenic acid quantification with respect to *V. jatamansi* [11], present study is not in agreement with the earlier study where valerenic acid was not reported in the samples collected from the western Himalaya.

Altitude plays an important role in the accumulation of secondary metabolites in plants. For example, altitudinal differences in flowering heads of three members of the species *Crepis capillaris*, *Hieracium pilosella*, and *Hypochaeris radicata* showed a positive correlation with the altitude of the growing site and the contents of flavonoid and phenolic acids [37]. In the present study, altitude did not show any significant relationship with the phytochemical content and antioxidant activity; however, higher TP, TF, and TT (aerial portion) and TP (root portion) at 2101–2400-m zone and TF (in root portion) at >2401 m were recorded. Observations from the population at higher altitude are considered obvious largely on account of higher exposure to UV radiation, lower temperature, and stressed conditions that induce production of secondary metabolites [38, 39].

Environmental factors affect the production of biologically active constituents in plants and animals at different habitat conditions. For example, the study on yeast revealed that a variety of physical and chemical stressors, including temperature, hypoxia, reactive oxygen species, and osmotic shock, triggered relatively similar changes in expression of approximately 900 genes, which represents approximately 14 % of the yeast genome [40]. Variations in phytochemical content across habitat conditions in different species of medicinal plants like *Ocimum sanctum* [41] and *Pteridium arachnoideum* [42] are also reported. In the present study, pine forest and mixed forest emerged as suitable habitat.

Strong positive relationship ( $p < 0.05$  and  $p < 0.01$ ) of antioxidant assays suggested the suitability of the assays used in this study. Relationship between tannin content and ABTS and DPPH assays in *V. jatamansi* suggests the role of tannin content in generating antioxidant activity [29, 39]. Although several reports exist on highly positive relationship between total phenols and antioxidant activity in many plant species, non-significant relationship between total phenolic content and antioxidant activity was determined in this study. It is possible that antioxidant activities in the target species may be attributed to some other compounds than the phenolics, flavonoids, and tannins. Although significant differences were recorded in total

phenolics, tannins and FRAP activity (in aerial portion), and tannin content (in root portion) among different altitudinal ranges, no correlation was found between the altitudes, phytochemicals, and antioxidant activity. These results are not in agreement with Rawat et al. [40] who showed increased phytochemical content in *H. spicatum* with the increase in altitude.

Based on NJ cluster analysis, populations Jaberkheth and Talwari were identified for harboring maximum phytochemicals and Ukimath, Majkhali, and Khirshu for maximum antioxidant activity. The NJ cluster analysis is generally used for the creation of phenograms, but it can also be used for the identification of best methods from a set of multiple procedures involving multiple variables [42]. Population with high phytochemical and antioxidant activity identified using NJ cluster analysis can be potential candidates in the breeding programs. PCA is a multivariate analysis technique that was employed to identify promising sources based on the phytochemical and antioxidant activity analysis. This is a frequently employed statistical analysis method and has been successfully applied to analytical results, both for individual compounds and component combinations [43]. All phytochemicals and antioxidant attributes were considered to identify the principal components. Generally, the two first principal components (PCs) are sufficient to explain the maximum variation in all original data. In the present study, two-dimensional score plot in the space defined by PC1 and PC2 shows that the distribution of samples does not follow a pattern that depends on PC2 (Fig. 4a, b). Earlier studies on *V. jatamansi* based on essential oil composition identified two chemotypes [44] and three chemotypes [15]. Chemotypic variability in various other species based on essential oil composition have also been reported, e.g., *Origanum vulgare* [45], *Hippophae rhamnoides* [46], *Lychnophora ericoides* [47], *Liptospermum scorparium* [48], *Anemopsis californica* [49], and *Artemisia dracunculus* [50]. However, identification of chemotypes based on the active compound, valerenic acid, and other antioxidant phytochemicals is reported for the first time. Based on the phytochemicals and antioxidant activity, Munsyari (P22) and Doongiri (P18) populations were found to be most promising sources. These sources can further be explored for focusing particular group of chemical content and obtaining the desired phytochemicals. Also, the compound specific population can be promoted for harnessing the potential of species.

## Conclusions

The present study suggests that screening of multilocal plants/populations is essential for optimizing suitable sources for higher phytochemicals and antioxidant activity. The aerial and root portions of *V. jatamansi* showed that higher phytochemicals and antioxidant activity indicate that the whole plant can be utilized to harness its potential. The results of the study support the fact that species is a promising source of various phytochemicals and antioxidant activity and found comparable with some commercially available plant species, e.g., *Origanum* spp. and tea. Also, the species can be a substitute of *V. officinalis*, one of the highly commercial species across the globe. The present study revealed that isolation of active constituents and their impact on various health improvement/control of free radical-mediated disorder through in vitro studies is needed. As the population at 1501–1800 m asl and pine forest is the best source for total phenolic content and antioxidant activity, mixed type habitat is best for other phytochemicals; thus, the cultivation on these sites is recommended. Higher amount of phytochemicals and antioxidant activity in the Munsyari and Doonagiri populations

could be used as an elite stock for mass multiplication and to meet the market demand for target species. Further, the species has potential to enhance the income of Himalayan people; therefore, breeding program on this species needs to be initiated and material for the same can be used from the identified promising sources.

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