Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Medicinal Plants

ISSN 1819-3455
DOI: 10.3923/rjmp.2020.43.52

Research Article
Influence of Altitude on Secondary Metabolites and Antioxidant Activity of *Coleus forskohlii* Root Extracts

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Abstract

Background and Objectives: Secondary metabolites like phenols, flavonoids and terpenoids plays an important role in plants defense system against free radicals. Increasing altitude magnifies the intensity of UV radiation and the decreasing temperature results in stressful conditions for the plants. *Coleus forskohlii* is a well-known medicinal plant that grows along a wide altitudinal range of 500-2300 m. Considering the globally accepted medicinal value of *Coleus forskohlii*, the aim of the study was to evaluate the effect of altitudes on the production of secondary metabolites and antioxidant activity in the wild populations. **Materials and Methods:** The plant samples were collected from 5 different locations of varying altitudes viz., Srinagar (606 m), Rudraprayag (727 m), Gopeshwar (1488 m), Pipalkoti (1339 m) and Joshimath (1986 m) in the Garhwal region of Uttarakhand, India. Standard spectrophotometric methods were employed to determine the total phenolic, flavonoid, terpenoid content and antioxidant activity of the plant roots. **Results:** A significant increase ($p<0.05$) was observed in the phenol, flavonoid and terpenoid content with the increasing altitude. The highest value for phenols was observed in the sample collected from Joshimath (1986 m) and similar trends were reported for flavonoids, terpenoids and antioxidant activity as well. The neighbor joining cluster and principal component analysis also confirms the high altitude population as better cultivar. **Conclusion:** The study confirms that change in growing altitudes affects the quantity of secondary metabolites in *Coleus forskohlii* as well as its antioxidant potential. Thus, the wild populations of *Coleus forskohlii* from a higher altitude may serve better in order to grow it and to exploit its therapeutic potential.

Key words: *Coleus forskohlii*, secondary metabolites, altitude, antioxidants, phenols, flavonoids, terpenoids, elite population

Citation: Pawan Singh Rana, Pooja Saklani and Chandresh Chandel, 2020. Influence of altitude on secondary metabolites and antioxidant activity of *Coleus forskohlii* root extracts. Res. J. Med. Plants, 14: 43-52.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

All the living organisms get affected by their surroundings in one way or another. Plants being sessile in nature have no escaping strategy so they have to face harsh environmental conditions. The chemical interaction between plants and their environment is mediated by the secondary metabolites[1]. They are produced as by-products of the primary metabolic pathways. Usually they have no role in the primary metabolic activities like photosynthesis, respiration and excretion but are important for adaptation to the stress conditions and defense mechanism[2]. Secondary metabolites are not only important for plant as defense system but also possesses high medicinal value for human beings against various ailments[3]. The environment plays a key role in determining the amount of secondary metabolites produced by different plant species. Difference in the level of secondary metabolites (phenolics, alkaloids, saponins and tannins) have been reported in *Primula denticulate* with changing altitude[4]. The phenolic content was found increasing with the increasing altitude in *Hedychium spicatum*[5]. Similarly, habitat and altitude were found to have an effect on the antioxidant activity and secondary metabolite content in *Valeriana jatamansi*[6].

Coleus forskohlii (family Lamiaceae, commonly known as patharchur) is highly rated for its medicinal properties in Ayurveda. The plant is native to the Indian sub-continent and grows under sub-tropical conditions all along the South East Asia, Brazil and East Africa. In India it is distributed throughout the Himalayan range extending from Shimla to all along Garhwal and Kumaon hills at an altitude range[7] of 500-2300 m. It is cultivated in some parts of Gujarat, Maharashtra, Karnataka and Tamil Nadu for its tuberous roots. In Gujarat the roots are also used to as condiments and for making pickle[8]. In the hilly areas, it grows wild on the dry exposed steep slopes in crevices of rocky outcrop, dry barren hills, margins of agricultural fields and wastelands. Traditionally it has been used by local people in Garhwal and Kumaon regions for the treatment of various ailments such as to kill intestinal worms, roots are ground in mustard oil and the paste is applied on eczema and skin infections, as a remedy for heart, abdominal and respiratory disorders[9,10]. Coleus is currently being used clinically in the treatment of hypertension, congestive heart failure, cerebrovascular disease, thyroid under activity, chronic obstructive airways disease, poor digestion and psoriasis[11]. *Coleus forskohlii* is the only source of an important diterpenoid, forskolin[12]. Forskolin is Cyclic-Adenosine Mono Phosphate (cAMP) genic as it activates the adenylate cyclase. This compound has a very high medicinal value as it is known to be hypotensive, regulates the body metabolism and also assists fat mobilization leading to weight loss[13]. All these properties make this plant highly precious in terms of medicinal utilities. Although a lot of information about the medicinal properties of this plant are available but the information on the commercial potential of its wild genotypes are very meager. Therefore, considering the medicinal importance of *C. forskohlii*, the present study was designed to select promising source of secondary metabolites and antioxidants among different altitudes of Uttarakhand, India.

MATERIALS AND METHODS

Study area: The plant samples were collected in the duration of August, 2017-January, 2018. All the experiments were carried out in the Plant Biochemistry and Biotechnology Unit of the Department of Zoology and Biotechnology of HNB Garhwal University, Uttarakhand, India, from February-July, 2018. The data analysis was done in the same lab from July-August, 2018.

Sample collection: The plant samples for the present study were collected from 5 different locations ranging from 600-2000 m (a.m.s.l.) altitude (Table 1). The roots were detached, washed thoroughly under running tap water and shade dried. Further, these roots were completely dried in hot air oven at 40°C. The dried roots were crushed to fine powder and tightly packed in sterile containers at 4°C. Botanical identity of the sample was authenticated by Botanical Survey of India (Dehradun), Accession No. 118603.

Extract preparation: The root powder (100 g) was extracted with 300 mL of solvent using Soxhlet extraction at 40-50°C and continued till the liquid entering back in the round bottom flask get colorless. Finally, around 200-250 mL of colored solvent (containing phytochemicals) was obtained. The extracts were prepared in 5 different solvents in an order of increasing polarity (petroleum ether, chloroform, acetone, methanol and water). The extracts so obtained were further concentrated by evaporation on a water bath at a temperature 25-30°C resulted in sticky viscous substance left in the flask. This viscous substance was further dried using lyophilizer. Same process was repeated with the samples from each of the 5 locations, for five solvents resulting in a total of 25 extracts. All the extracts were kept at -20°C until required for further use[14].

Table 1: Site characteristics of selected sampling locations

Sites	Locations	Altitudes (m)	Latitudes	Longitudes	Habitats
S1	Srinagar	606	30°15'	79°18'	Dry slopes
S2	Rudraprayag	727	30°17'	79°00'	Chir pine forest
S3	Gopeshwar	1488	30°25'	79°18'	Mixed forest
S4	Pipalkoti	1339	30°26'	79°25'	Rocky crevices
S5	Joshimath	1986	30°33'	78°53'	Pine forest dry slope

Quantitative estimation of 3 important metabolite group: Total phenol, flavonoid and terpenoid content were estimated by using the standard spectrophotometric methods as:

Quantification of Total Phenolic Content (TPC): Total Phenolic Content (TPC) was estimated by using the method described by Singleton *et al.*[15]. The extracts were dissolved in methanol at a concentration of 1 mg mL^{-1} and 0.5 mL of it was taken into a test tube. To it 2.5 mL of 10% Folin-ciocalteu reagent was added and it was mixed by shaking with 2.5 mL of Na_2CO_3 (7.5%). All the test tubes were kept for incubation at 45°C. After 45 min absorbance was recorded at 765 nm on the UV spectrophotometer (Lab India UV-2000$^+$). The Total Phenolic Content (TPC) in each extract was calculated by using the equation of line from standard gallic acid calibration curve and was expressed as mg GAE g^{-1} DW.

Quantification of Total Flavonoid Content (TFC): Total flavonoids content were estimated by the protocol described by Zhishen *et al.*[16]. The extract (500 µL) was mixed to 150 µL of 5% Sodium nitrite and left at room temperature for 5 min. Further, 150 µL of 10% $AlCl_3$ was added to the reaction mixture and left for 6 min at room temperature and 2 mL of 4% NaOH was added to it. The final volume was made to 5 mL by adding distilled water and left for 15 min at room temperature. The absorbance was measured at 510 nm using a UV-VIS spectrophotometer. The Total Flavonoid Content (TFC) in each extract was calculated by using the equation of line from standard quercetin calibration curve and was expressed as mg QE g^{-1} DW.

Quantification of Total Terpenoid Content (TTC): Total terpenoids content were estimated following Ghorai *et al.*[17]. The crude extracts were centrifuged (4000 g for 15 min at room temperature) and 1.5 mL of chloroform was added to the supernatant. All the test tubes were vortexed and left for 3 min and 100 µL H_2SO_4 was added to each of the test tube. The final reaction mixture was left at room temperature for 1.5-2 h. A reddish brown precipitate so obtained in each test tube was dissolved in methanol (95%) and vortexed to dissolve it completely. Ultimately, all the standards and test samples were measured by spectrophotometer at 538 nm using methanol as blank. Total Terpenoid Content (TTC) was calculated by using the line equation from the calibration curve of Linalool standard and the values were expressed as mg LE g^{-1} DW.

Antioxidant activity assays: The antioxidant potential of the root powder extracts of *C. forskohlii* was estimated by 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Superoxide anion scavenging, Ferric Reducing Antioxidant Power (FRAP) and 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assays. The IC_{50} values were calculated for DPPH and Superoxide Anion Scavenging (SAS) assay while the FRAP value Ascorbic Acid Equivalent (AAE) and percentage radical inhibition was calculated for FRAP and ABTS, respectively.

DPPH Inhibition assay: Free radical scavenging activity is determined according to the method of Khamsah *et al.*[18]. In the presence of antioxidants, the purple colour of DPPH decays and the change of absorbance were monitored at 517 nm against reagent and sample blank.

The antioxidant activity of the samples is defined as:

$$\text{DPPH scavenging activity (\%)} = 1 - \left(\frac{\text{Atest}}{\text{Acontrol}}\right) \times 100$$

Where:

$A_{control}$ = Absorbance of the control (2 mL DPPH solution +1 mL of 70% ethanol solution)

A_{test} = Absorbance of the DPPH solution in the presence of extract (2 mL DPPH+1 mL of extract)

The 50% radical scavenging or IC_{50} was calculated from the dose-response plot of DPPH absorbance versus concentration of the extracts.

Superoxide Anion Scavenging (SAS) activity: Superoxide Anion Scavenging (SAS) activity was measured by the method described by Liu *et al.*[19], with slight modifications. The reaction mixture was prepared by adding 2 mL of nitroblue tetrazolium (50 mM) to the 3 mL Tris-HCl buffer (16 mM, pH 8.0) with 1 mL of NADH (60 mM) and methanol solution of the extracts (1 mg mL^{-1}) were mixed. To the reaction mixture 1 mL of phenazine methosulfate (PMS) solution (10 mM) was mixed to start the reaction and it was incubated for 5 min at 25°C. The oxidation of Nicotinamide Adenine Dinucleotide (NADH) leads to the generation of superoxide free radicals in the PMS-NADH system and the inhibition of these free radicals is assayed by the reduction of nitroblue tetrazolium. The absorbance was recorded at 560 nm. A decrease in

absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following equation:

$$\text{Inhibiton (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

Where:
A_o = Absorbance of the control
A_1 = Absorbance of plant extracts

The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture.

FRAP assay: The FRAP assay was performed to measure the ferric reducing ability of plasma[20]. For this, 3 mL of freshly prepared FRAP solution (0.3 M acetate buffer containing 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) and 40 mM $FeCl_3.6H_2O$) and 100 µL of extract (prepared as for phenol determination) was incubated at 37°C for 4 min and the absorbance was measured at 593 nm and compared with the change in absorbance of 1 mM solution of L-ascorbic acid standard at different concentrations and the results were expressed as µmol AAE g^{-1} DW.

ABTS assay: Free radical scavenging activity of plants extracts were determined by ABTS radical cat-ion decolourization assay[21]. The radicals were generated by making a reaction mixture of 7 mM ABTS in 2.45 mM phosphate buffer (1:1) and was stored in dark for 12-16 h at room temperature before final use. This solution as diluted with methanol to get an absorption of 0.706±0.07. After adding 5 µL extract to 3.995 mL of diluted ABTS solution the absorbance was measured after 30 min of initial mixing. The inhibition of ABTS (%) by extracts was calculated using the following equation:

$$\text{ABTS scavenging effect} = \frac{AB - AA}{AB} \times 100$$

Where:
AB = OD of ABTS reagent in methanol
AA = OD of ABTS reagent in methanol+extract

Selection of most promising location: Neighbor-joining cluster analysis and Principal Component Analysis (PCA) was performed for selection of the most promising location and elite cultivars for higher phytochemical content and antioxidant activity[22].

Statistical analysis: All the data presented is the mean of triplicates (n = 3), was subjected to analysis of variance (ANOVA) and the correlation (r) were performed using Microsoft excel, 2016. All the graphs were made using GraphPad Prism 8.1.1. The Principal Component Analysis (PCA) and Neighbour-Joining (NJ) cluster analysis were done with the help of the PAleontological Statistics (PAST, version 3.22).

RESULTS

Quantitative estimation of 3 important metabolite group: The amount of 3 important phytoconstituent groups (phenols, flavonoids and terpenoids) present in different extracts of all the 5 populations are shown in Table 2-4, respectively. Changing altitude has significant effect on the quantity of studied phytochemicals because significant variation ($p<0.05$) was observed in the amount of all the 3 metabolites (phenols, flavonoids and terpenoids) with changing altitudes. Total phenolic content (59.29 mg GAE g^{-1} DW) and total flavonoid content (95.67mg QE g^{-1} DW) were found highest in the methanol extract of Joshimath population (Table 2, 3), while the highest value for total terpenoid content (29.03 mg LE g^{-1} DW) was observed in the chloroform extract of the Joshimath population. The lowest value was observed in the petroleum ether extract of Srinagar population for phenols (10.31 mg GAE g^{-1} DW) and flavonoids (18.29 mg QE g^{-1} DW) while for terpenoids, it was lowest (5.66 mg LE g^{-1} DW) in the acetone extract of Srinagar population. Across all the populations methanol extracts were

Table 2: Total phenolic content in all the extracts of each population

		Total phenolic content (mg GAE g^{-1} DW)				
Locations	Altitude (m)	ME	CE	AcE	PeE	AE
Srinagar	606	21.63±0.66	14.69±0.93	18.08±1.55	10.31±0.29	11.03±0.77
Rudraprayag	727	37.37±1.61	27.48±0.41	23.22±0.90	12.67±0.83	15.57±1.15
Pipalkoti	1339	55.19±1.42	31.69±1.71	23.82±0.24	14.02±2.05	17.37±0.77
Gopeshwar	1488	56.50±0.98	33.30±0.50	27.97±0.34	14.59±0.22	18.77±0.59
Joshimath	1986	59.29±0.90	36.67±0.52	27.87±0.87	16.05±0.74	19.56±1.00

All the values are mean of n = 3 and represented as Mean±SD, PeE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 3: Total flavonoid content all the extracts of each population

		Total flavonoid content (mg QE g^{-1} DW)				
Location	Altitude (m)	ME	CE	AcE	PeE	AE
Srinagar	606	72.33±0.73	41.92±0.97	22.00±0.45	18.29±0.36	29.63±0.22
Rudraprayag	727	73.00±0.50	43.46±0.14	26.58±0.69	20.46±0.58	35.08±1.00
Pipalkoti	1339	94.71±0.48	48.08±0.31	28.46±0.26	22.21±0.52	37.63±0.33
Gopeshwar	1488	95.08±0.26	48.07±1.96	28.38±0.43	22.21±0.56	37.33±0.26
Joshimath	1986	95.67±0.19	49.83±0.19	29.83±0.26	23.46±0.38	39.04±0.31

All the values are mean of n = 3 and represented as Mean±SD, PeE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 4: Total terpenoid content in all the extracts of each population

		Total terpenoid content (mg LE g^{-1} DW)				
Locations	Altitudes (m)	ME	CE	AcE	PeE	AE
Srinagar	606	20.00±0.23	26.30±0.23	11.18±0.18	5.66±0.52	7.30±0.27
Rudraprayag	727	22.51±0.27	26.63±0.18	11.97±0.14	7.03±0.16	7.72±0.32
Pipalkoti	1339	23.03±0.23	27.33±0.19	11.57±0.19	6.87±0.23	5.93±0.23
Gopeshwar	1488	23.75±0.30	28.48±0.05	11.97±0.16	7.57±0.10	7.72±0.14
Joshimath	1986	24.21±0.23	29.03±0.19	12.87±0.19	7.81±0.37	8.63±0.42

All the values are mean of n = 3 and represented as Mean±SD, PeE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 5: IC_{50} for DPPH inhibition

		IC_{50} (μg mL^{-1})				
Locations	Altitudes (m)	PeE	CE	AcE	ME	AE
Srinagar	606	398.75	314.20	306.94	211.67	261.96
Rudraprayag	727	340.88	307.14	302.79	199.21	244.34
Gopeshwar	1488	296.08	276.86	292.14	176.03	222.90
Pipalkoti	1339	284.50	267.64	253.45	173.05	193.96
Joshimath	1986	279.64	283.90	278.46	164.06	249.04

PE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 6: IC_{50} values of extracts against superoxide scavenging

		IC_{50} (μg mL^{-1})				
Location	Altitude (m)	PeE	CE	AcE	ME	AE
Srinagar	606	341.85	294.75	255.23	192.24	215.40
Rudraprayag	727	331.47	270.31	241.10	172.12	201.19
Gopeshwar	1488	307.63	244.80	217.59	154.35	187.15
Pipalkoti	1339	314.49	249.06	213.79	163.37	192.00
Joshimath	1986	304.00	238.54	194.86	135.69	185.29

PeE: Petroleum ether extract, CE: Chloroform extract, Ac: Acetone extract, Me: Methanol extract, Aq: Aqueous extract

reported with highest total phenolics and flavonoids content while the petroleum ether extract showed lowest values for both of them (Table 2, 3). Highest quantity of terpenoids was reported in the chloroform extract across all the populations while lowest were registered in the acetone extracts (Table 4).

Antioxidant activity

DPPH and SAS activity analysis: A higher antioxidant potential corresponds to low IC_{50} value. In the present study IC_{50} values were calculated for DPPH and superoxide anion scavenging (SAS) assay in both the cases methanol and aqueous extracts were found far more potent against the free radicals as compared to others (Table 5). The lowest IC_{50} value was recorded for the methanol extract of Joshimath population (1986 m) indicating towards the high potential of it to encounter the free radicals produced in the plant cells. Superoxide anion scavenging also provides information about the antioxidant potential of the plant extracts. The lowest IC_{50} values for superoxide scavenging were observed in the methanol and aqueous extracts for all the populations. It can be observed that with the increase in altitude the IC_{50} values tends to decrease (Table 6) similar to DPPH inhibition activity assay.

Table 7: FRAP values of all the extracts of *C. forskohlii*

		Extracts				
Location	Altitudes (m)	ME	AcE	CE	PeE	AE
Srinagar	606	190.63	172.22	166.09	108.36	177.22
Rudraprayag	727	194.27	172.00	165.18	109.50	177.68
Gopeshwar	1488	267.00	197.77	184.95	130.18	192.22
Pipalkoti	1339	237.65	194.04	183.81	127.45	189.72
Joshimath	1986	322.68	253.36	188.36	143.81	199.95

All the values are mean of n = 3, PeE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 8: Inhibition (%) of ABTS free radical by *C. forskohlii* extracts

		Extracts				
Location	Altitudes (m)	ME	CE	AcE	PeE	AE
Srinagar	606	65.72	57.68	61.77	51.40	62.48
Rudraprayag	727	67.13	58.95	62.05	52.32	63.04
Gopeshwar	1488	74.47	63.75	69.11	58.81	70.38
Pipalkoti	1339	75.31	64.31	70.09	60.08	72.21
Joshimath	1986	81.80	68.82	72.35	64.88	74.47

All the values are mean of n = 3, PeE: Petroleum ether extract, CE: Chloroform extract, AcE: Acetone extract, ME: Methanol extract, AE: Aqueous extract

Table 9: Correlation between altitude, phytochemicals and antioxidant activity for methanol extracts

Parameters	Altitude	TPC	TFC	TTC	DPPH IC_{50}	SAS IC_{50}	FRAPA	ABTSA
Altitude	1							
TPC	0.908598	1						
TFC	0.91617	0.942681	1					
TTC	0.863542	0.957681	0.818907	1				
DPPH IC_{50}	-0.95844	-0.98071	-0.95811	-0.91703	1			
SAS IC_{50}	-0.95827	-0.91856	-0.83045	-0.94922	0.934734	1		
FRAPA	0.985136	0.835193	0.846279	0.814791	-0.89677	-0.94666	1	
ABTSA	0.990302	0.901724	0.910091	0.842252	-0.96508	-0.94177	0.96458	1

TPC: Total phenolic content, TFC: Total flavonoid content, TTC: Total terpenoid content, DPPH IC_{50}: DPPH inhibitory concentration 50, SAS IC_{50}: Superoxide anion scavenging inhibitory concentration 50, FRAPA: Ferric reducing ability of plasma activity, ABTSA: ABTS scavenging activity

FRAP activity: Table 7 shows the FRAP values for different extracts to the corresponding altitudes. The Joshimath population showed highest FRAP value (322.68 AAE) in the methanol extract.

ABTS scavenging activity: Table 8 shows the percentage of ABTS free radical scavenged by the plants extracts. The values suggest a trend of increasing antioxidant potential with the increasing altitude. The plant extracts of a higher altitude plant sample show enhanced ability to scavenge the free radicals.

Correlation among altitude, phytochemical contents and antioxidant activity: The total phenolic (r = 0.908), flavonoids (r =0.942) and terpenoid (r = 0.863) contents were found positively correlated to the increasing altitude (Table 9). The FRAP values also found positively correlated to the altitude (r = 0.985), phenolic content (r = 0.835), flavonoids (r = 0.846) and terpenoids (r = 0.814). The ABTS activity was also found positively correlated to altitude (r = 0.990), TPC (r = 0.901), TFC (r = 0.910) and TTC (r = 0.842). All the three metabolites were found negatively correlated with IC_{50} values of DPPH and SAS activity. The r-value for DPPH IC_{50} showed high negative correlation with terpenoids (r = -0.917), flavonoids (r = -0.958) and phenolics (r = -0.980), IC_{50} value of superoxide scavenging activity (SAS) also showed high negative correlation with phenolics (r = -0.958), flavonoids (r = -0.980) and terpenoids (r = -0.830).

Selection of most promising location: Neighbor-Joining cluster analysis was performed for selection of the most promising location and elite cultivars for higher phytochemical content and antioxidant activity. Two clusters were observed in the plot cluster 1 showing the locations with lowest values of the secondary metabolite contents and antioxidant potential while cluster 2 with the locations having higher value for secondary metabolites and antioxidant potential. Joshimath population stands out the best among all the sampled populations (Fig. 1). In PCA plot *C. froskohlii* populations from different altitude are placed distantly in different co-ordinates shows that there is high variance among the populations. Thus, it supports the fact that the changes in altitude have a significant impact on phytochemical content and antioxidant activity in *C. forskohlii* (Fig. 2).

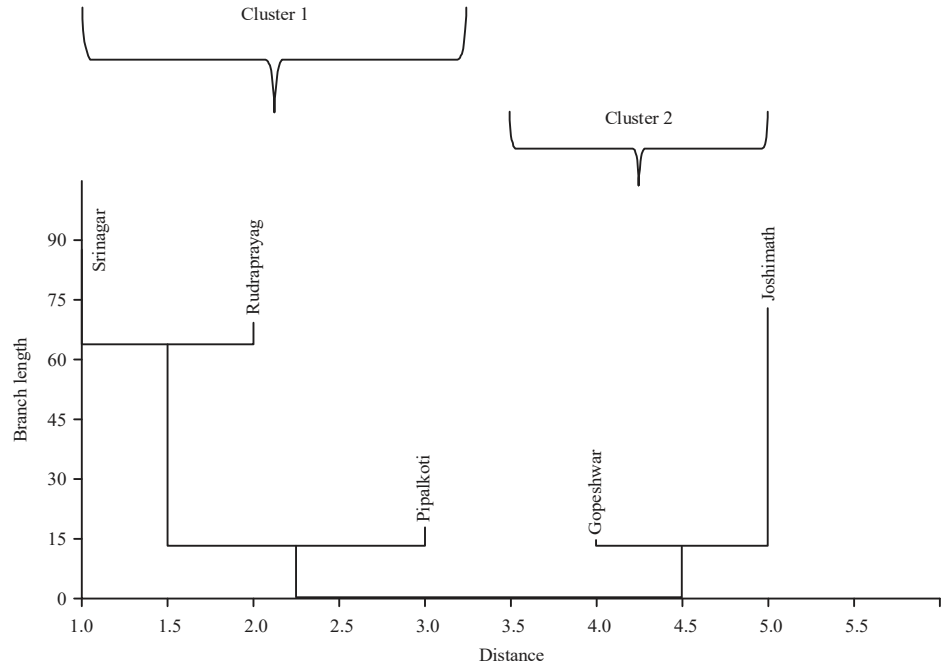

Fig. 1: Selection of Elite location for *C. forskohlii* cultivars by Neighbor-joining (NJ) cluster analysis based on the phytochemical content and antioxidant activity

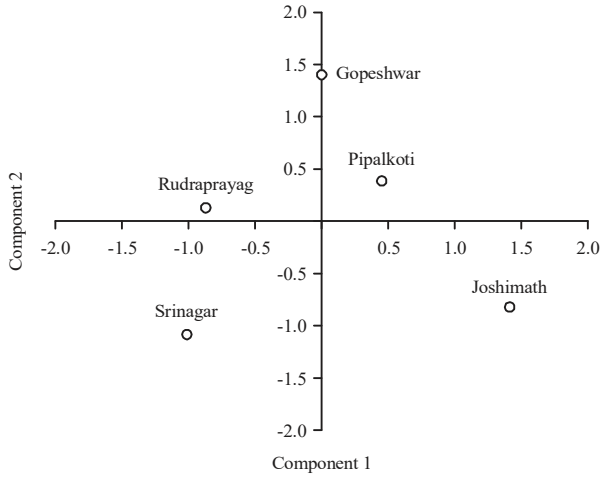

Fig. 2: Identification of best locations of *C. forskohlii* cultivars by Principal Component Analysis (PCA) of the based on the phytochemical content and antioxidant activity

DISCUSSION

Among all organisms living on the earth, plants are much more diverse as they adapt to their environment much more readily. Plants can't move so they have to develop strategies for adaptation to their surrounding environment for survival. The plants are well known for their secondary metabolites, which are produced as by-products in almost all important primary biochemical pathways. Of these secondary metabolites some groups (viz., phenolics, flavonoids, terpenoids) plays an important role in scavenging the free radicals generated from irradiation or during the metabolic processes. With an increase in altitude the intensity of radiations (especially UV-B) increases[23] which tend to generate more free radicals in the cellular system and hence an organism living in such conditions must have well adapted defense mechanism. At higher altitudes plants usually adapt by avoiding or overcoming the stress conditions by means of various physiological and biochemical mechanisms. Different plants can show different responses to the particular stress condition which depends mainly on their morphology, anatomy and life cycle[24]. Increasing altitude also results in decreasing atmospheric temperature which tends to trigger the production of phenolics even in the absence of UV radiations[25]. The present study was planned keeping in mind that the changing altitude may have effect on the quantity of different phytochemicals and antioxidant activity of *C. forskohlii.* Hence, in the present study the plant samples of *C. forskohlii* were collected from different altitudes and analyzed for the quantity of phenolics, flavonoids and terpenoids and their antioxidant potential.

It was observed that *C. forskohlii* samples collected from a higher altitude have high phenolic, flavonoid and terpenoid content. Concomitant increase in the content of these metabolites was observed from 606-1986 m in the present study. Altitude is known to play an important role in the production of secondary metabolites, that ultimately affect the free radical scavenging ability, altitudinal differences in the flavonoid and phenolic acids content were reported in the flowering heads of three plant species *Hypochaeris radicata*, *Hieracium pilosella* and *Crepis capillaries*[26]. The production of saponins was found increasing with the increasing altitude in *Quillaja saponica* was attributed to the stressful environmental conditions[27]. The increasing elevation leads to a higher exposure of plants to the UV radiation which accelerate the production of harmful free radicals. The plants at high mountains are equipped with the altered synthesis of secondary metabolites such as phenols, flavonoids and essential oils. Higher amount of phenolic antioxidants in *Arnica montana* was attributed to altitude while increased level of rosmarinic and carnosic acids had been reported in the rosemary plants treated with enhanced level of UV-B radiations[28,29]. The higher production of secondary metabolites (phenolics and flavonoids) that can scavenge the free radicals may be a part of the defence mechanism of the plant[30]. High alkaloid and flavonoid content in the *A. fragratssima* and *T. polium*, respectively at higher altitudes[31] also supports the results of the current study.

In the present study the antioxidant potential of the wild cultivars of *Coleus forskohlii* collected from varying altitudes of Uttarakhand, was also explored. The sample from a higher elevation showed higher antioxidant potential than those from a lower elevation for all the four assays. It is a well-known fact that, Reactive Oxygen Species (ROS) are generated in the cells as by-products of many metabolic reactions and are potential threat for the health of individual in they are produced. These ROS are free radicals which can initiate many undesired reactions in the cells that may lead to cellular damage in a certain way or another (may lead to the most threatening cancer like diseases in humans). In the process to combat the reactive oxygen species, plants produce antioxidants, in the form of secondary metabolites. The hydroxyl groups and decrease in glycosylation in these metabolites equip them with higher antioxidant potential and hence the increasing amount of secondary metabolites leads to higher antioxidant potential[32].

A positive correlation of phenolics, flavonoids and terpenoids content with the increasing altitude supported by the findings that plant produce more essential oils as a defence strategy against low temperature and high UV radiation exposure at higher altitudes[33,34]. While, negative correlation between the increasing altitude and DPPH IC_{50} values indicated towards a greater antioxidant potential of high altitude growing *C. forskohlii* population. Similar results were reported in *Calluna vulgaris* as well[23]. The positive correlation of total terpenoid content to the antioxidant activities indicate towards their antioxidant potential[35].

The effect of changing altitudes on the phenolics, flavonoids, terpenoids and thereby the antioxidant activity was further supported by the PCA and NJ cluster analysis. The secondary metabolite content and antioxidant activity differed from site to site, indicating towards the effect of the changing altitude, this was confirmed by PCA. In the PCA plot none of the sites were lying close to each other in the ordination space, confirms that at a higher altitude plants produce higher amount of secondary metabolites and have a greater antioxidant potential[22].

The positive correlation of radical scavenging to the TPC, TFC and TTC with the increasing altitude is suggestive of the thought at higher altitudes the plants produce high amount of secondary metabolites that shield them from the reactive oxygen species.

CONCLUSION

A concomitant rise in the level of secondary metabolites with the increasing altitude was observed in the study. Among the five solvents used for extraction of secondary metabolites methanol was found better for the extraction of phenolics and flavonoids while chloroform was found most suitable for terpenoid extraction from *C. forskohlii* roots. Results of the antioxidant assays also indicated an increasing trend in the antioxidant potential of the plant with the increasing altitude, this was further confirmed by the PCA and Neighbor-Joining Cluster.

SIGNIFICANCE STATEMENT

This study discovers the effect of changing altitude on the secondary metabolite content and antioxidant potential of *C. forskohlii* populations where the population of a higher altitude stands out the best (Joshimath, 1986 m). It could be beneficial to grow it as an elite germplasm for the commercial cultivation of *Coleus forskohlii* from Uttarakhand, India. Further, this study will help the researchers to identify newer cultivar of the *Coleus forskohlii* and to apply similar analytical approaches to other plants also so that novel and better cultivar of important medicinal plants can be developed.

ACKNOWLEDGMENT

We gratefully acknowledge the financial assistance provided by the Uttarakhand Council for Biotechnology (UCB), Govt. of Uttarakhand in the form of project Ref. No. UCB/R&D Project/2017/47.

Authors also thankful to the Research Journal of Medicinal Plants for publishing this article FREE of cost and to Karim Foundation for bearing the cost of article production, hosting as well as liaison with abstracting and indexing services and customer services.

REFERENCES

1. Sampaio, B.L., R. Edrada-Ebel and F.B. Da Costa 2016. Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: A model for environmental metabolomics of plants. Sci. Rep., Vol. 6. 10.1038/srep29265.
2. Heldt, H.W. and F. Heldt, 2005. Plant Biochemistry. 3rd Edn., Elsevier Academic Press, Burlington, USA., ISBN-13: 9780120883912, Pages: 630.
3. Wink, M., 2015. Modes of action of herbal medicines and plant secondary metabolites. Medicines, 2: 251-286.
4. Aslam, K., I.A. Nawchoo and B.A. Ganai, 2015. Altitudinal variation in some phytochemical constituents and stomatal traits of *Primula denticulata*. Int. J. Adv. Sci. Res., 1: 93-101.
5. Rawat, S., I.D. Bhatt and R.S. Rawal, 2011. Total phenolic compounds and antioxidant potential of Hedychium spicatum Buch. Ham. ex D. Don in west Himalaya, India. J. Food Compos. Anal., 24: 574-579.
6. Jugran, A.K., A. Bahukhandi, P. Dhyani, I.D. Bhatt, R.S. Rawal and S.K. Nandi, 2016. Impact of altitudes and habitats on valerenic acid, total phenolics, flavonoids, tannins and antioxidant activity of *Valeriana jatamansi*. Applied Biochem. Biotechnol., 179: 911-926.
7. Sharma, N., K.P.S. Chandel and V.K. Srivastava, 1991. *In vitro* propagation of *Coleus forskohlii* Briq., a threatened medicinal plant. Plant Cell Rep., 10: 67-70.
8. Jagtap, M., H.M. Chandola and B. Ravishankar, 2011. Clinical efficacy of Coleus forskohlii (Willd.) Briq. (Makandi) in hypertension of geriatric population. AYU., 32: 59-65.
9. Shah, V. and B.S. Kalakoti 1996. Development of *Coleus forskohlii* as a medicinal crop. Proceedings of the International Conference on Domestication and Commercialization of Non-Timber Forest Products in Agroforestry Systems, February 19-23, 1996, Nairobi, Kenya, pp: 212-216.
10. Srivastava, S.K., M. Chaubey, S. Khatoon, A.K.S. Rawat and S. M ehrotra, 2002. Pharmacognostic evaluation of *Coleus forskohlii*. Pharm. Biol., 40: 129-134.
11. Dubey, M.P., R.C. Srimal, S. Nityanand and B.N. Dhawan, 1981. Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*. J. Ethnopharmacol., 3: 1-13.
12. Valdes, L.J., S.G. Mislankars, A.G. Paul, 1987. *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (Coleonol). Econ. Bot., Vol. 41. 10.1007/BF02908139.
13. Anonymous, 2006. *Coleus forskohlii*. monograph. Altern. Med. Rev., 11: 47-51.
14. Azwanida, N.N., 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants, Vol. 4. 10.4172/2167-0412.1000196.
15. Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299: 152-178.
16. Zhishen, J., T. Mengcheng and W. Jianming, 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 64: 555-559.
17. Ghorai, N., S. Chakraborty, S. Gucchait, S.K. Saha and S. Biswas, 2012. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Protoc. Exch. 10.1038/protex.2012.055
18. Khamsiah, S.M., G. Akowah and I. Zhari, 2006. Antioxidant activity and phenolic content of *Orthosiphon stamineus* Benth from different geographical origin. J. Sustain. Sci. Manage., 1: 14-20.
19. Liu, F., V.E.C. Ooi and S.T. Chang, 1997. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sci., 60: 763-771.
20. Benzie, I.F.F. and J.J. Strain, 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Method Enzymol., 299: 15-27.
21. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26: 1231-1237.
22. Bahukhandi, A., P. Dhyani, I.D. Bhatt and R.S. Rawal, 2018. Variation in polyphenolics and antioxidant activity of traditional apple cultivars from West Himalaya, Uttarakhand. Hortic. Plant J., 4: 151-157.
23. Rieger, G., M. Müller, H. Guttenberger and F. Bucar, 2008. Influence of altitudinal variation on the content of phenolic compounds in wild populations of *Calluna vulgaris*, *Sambucus nigra* and *Vaccinium myrtillus*. J. Agric. Food Chem., 56: 9080-9086.
24. Kuss, F.R., 1986. A review of major factors influencing plant responses to recreation impacts. Enviorn. Manage., 10: 637-650.

25. Bilger, W., M. Rolland and L. Nybakken, 2007. UV screening in higher plants induced by low temperature in the absence of UV-B radiation. Photochem. Photobiol. Sci., 6: 190-195.
26. Zidorn, C., B. Schubert and H. Stuppner, 2005. Altitudinal differences in the contents of phenolics in flowering heads of three members of the tribe Lactuceae (Asteraceae) occurring as introduced species in New Zealand. Biochem. Syst. Ecol., 33: 855-872.
27. Copaja, S.V., C. Blackburn and R. Carmona, 2003. Variation of saponin contents in *Quillaja saponica* Molina. Wood Sci. Technol., 37: 103-108.
28. Spitaler, R., A. Winkler, I. Lins, S. Yanar, H. Stuppner and C. Zidorn, 2008. Altitudinal variation of phenolic contents in flowering heads of Arnica montana cv. ARBO: A 3-year comparison. J. Chem. Ecol., 34: 369-375.
29. Luis, J.C., R.M. Pérez and F.V. González, 2007. UV-B radiation effects on foliar concentrations of rosmarinic and carnosic acids in rosemary plants. Food Chem., 101: 1211-1215.
30. Kolb, C.A., M.A. Käser, J. Kopecký, G. Zotz, M. Riederer and E.E. Pfündel, 2001. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol., 127: 863-875.
31. Khan, M.N., M. Mobin, K.A. Zahid and K.A. Al Mutairi, 2016. Impact of varying elevations on growth and activities of antioxidant enzymes of some medicinal plants of Saudi Arabia. Acta Ecol. Sin., 36: 141-148.
32. Kishore, G., S. Ranjan, A. Pandey and S. Gupta, 2010. Influence of altitudinal variation on the antioxidant potential of tartar buckwheat of Western Himalaya. Food Sci. Biotechnol., 19: 1355-1363.
33. Ioannidis, D., L. Bonner and C.B. Johnson, 2002. UV B is required for normal development of oil glands in *Ocimum basilicum* L. (Sweet Basil). Ann. Bot., 90: 453-460.
34. Lianopoulou, V., A.M. Bosabalidis, A. Patakas, D. Lazari and E. Panteris, 2014. Effects of chilling stress on leaf morphology, anatomy, ultrastructure, gas exchange and essential oils in the seasonally dimorphic plant *Teucrium polium* (Lamiaceae). Acta Physiol. Planta., 36: 2271-2281.
35. Grassmann, J., 2005. Terpenoids as plant antioxidants. Vitam. Horm., 72: 505-535.