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Altitudinal variation in phytochemical, physicochemical, and morphological aspects of *Justicia adhatoda* L. plant growing wildly in Western Himalayas

Mehak Jamwal[1]*, Sunil Puri[1], Radha[1], Niharika Sharma[1], Suraj Prakash[1], Ashok Pundir[2]

[1]School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India.
[2]School of Mechanical and Civil Engineering, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India.

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ABSTRACT

Fluctuations in environmental circumstances with varying altitudinal gradients account for variations in plants. This study was performed to scrutinize the impact of varying altitude on medicinal plant, namely, *Justicia adhatoda* L. for 2 consecutive years. The phytochemical, physicochemical constituents, and morphological aspects of *Justicia adhatoda* L. were studied and statistically compared. Significant variations were observed among plant height, crown spread, branches, leaves, inflorescence, carbohydrates, phenols, flavonoids, total sugar, and reducing sugar across different elevational sites where the observed variations showed that *Justicia adhatoda* L. plant chemically adapted itself to sustain at high altitudinal sites by exhibiting change in its phytochemical and morphological aspects. The quality and therapeutic efficacy of medicinal plants are influenced by their biochemical composition, which, in turn, depends on the environment in which they grow. The various biotic and abiotic stresses operating in the environment across altitudinal gradient undeniably perform as a driving force behind these variations and ultimately the survival of medicinal plants. The assessment of biochemical composition of medicinal plants growing at different elevational sites can contribute to selecting the best genotype and better altitude for commercial cultivation of medicinal plants.

1. INTRODUCTION

Geographical regions positioned far from one another possess diverse climatic conditions which thereby impact the production of plant's physiology. An extensive series of environmental aspects change with the varying elevation, for example, plants in high-altitude surroundings are exposed to numerous environmental strains, mainly in temperature and radiation extremes [1]. Elevation could be the main predictor of changes in phytochemical defense strategy of plants [2]. Altitude, in relation to several climatic trends, is accountable for adjustment in the biochemical and biological properties of the plant and also in determination of the phytochemical composition of the plant [3]. The temperature, pressure, and light regimes under which the plants grow affect various the plant attributes [4-6]. For instance, plants at higher altitudes face higher amount of light intensities as well as lower mean temperatures, suggesting that they might have established capability to prevent themselves from the damage created by factors such as chilling and photo destruction [7-10]. Provenance variations are significant in a wide range of traits in plants [11,12]. Variations could be beneficial or unprofitable to the plant [13]. Morphological and physiological variations can be associated with change in habitat conditions at higher elevation sites [14-17]. Along the challenging environmental gradients, plants modify their biological performances to indulge into a certain environmental situation [18]. For instance, in a study carried on different medicinal herbs, growing along an elevational gradient (3,000−4,600 m) in China, it was observed that the adaptive strategy of plants to adjust in environment was reflected by variation in their leaf traits, namely, leaf thickness, stomatal density, and mesophyll tissue thickness, at different altitudes thereby suggesting morphological and anatomical plasticity [19]. In a study carried in Italy, significant differences were observed in morphological and physiological traits of *Crepis pygmaea* and *Isatis apennina* as a result of differences occurred in their elevations [20]. A study of *M. polymorpha* revealed that several characteristics such as ecophysiological behavior and anatomical features were chiefly prompted by the environment and the other characteristics such as leaf size and internode length were seen decreasing with rise in elevation and were retained even when grown in similar environmental conditions suggesting alteration in their genetic background [21]. Plant develops well in its favorable environmental conditions. In a study, *O. vulgare* population exhibited larger and thicker leaves with vastly developed internal tissues, namely, palisade and spongy parenchymas and also the photochemical efficacy of PS II appeared to be highest in the mid altitude where environmental

*Corresponding Author:
Mehak Jamwal, School of Biological and Environmental Sciences, Shoolini University of Biotechnology and Management Sciences, Solan 173229, India.
E-mail: mehak29jamwal @ gmail.com

conditions were more favorable [22]. In a study carried on roots of *Desmodium gangeticum* L., the quantity of lupeol was estimated by HPTLC densitometric quantification and observed to be present in high amounts in plants that belonged to high altitude [23]. Significant variations in phenols, valerenic acid, and antioxidant activity in aerial and root portions were observed in inhabitants of Valeriana *jatamansi* sampled from Uttarakhand, West Himalaya [24]. These phytochemicals serve as a major source for pharmaceutical products [25]. In a study done on *Adhatoda zeylanica* in different phytogeographical regions of South India, four distinctive morphotypes were identified based and they exhibited significant variation in quantitative their vasicine content [26]. Plants may counter multiple stress conditions in various ways [27]. Explicitly, the plants are challenged to fight surplus abiotic and biotic stresses operating at different elevations and the worthwhile adaptation of plants to their sessile lifestyle can be ascribed to their capability to adapt and respond to various types of stresses. In this perspective, it is of utmost importance to highlight physiological mechanisms adapted by plants as a response to changing altitudes and coping with environmental stress.

2. MATERIALS AND METHODS

2.1. Study Site

The present study was performed on *Justicia adhatoda* L. which is an evergreen sub herbaceous bush mostly distributed up to altitude of 1300 m above sea level. The present study was done for two consecutive years in nine different provenances selected on the basis of different altitudes of Jammu, namely, 330 m, 336 m, 358 m, 661 m, 688 m, 696 m, 1302 m, 1311 m, and 1330 m (masl). The sampling was done from three different sites of each provenance. In case of morphological analysis, measurements of five plants from each sampling site were taken. The collected plant specimens were verified from Botanical Survey of India, Dehradun and submitted in the herbarium of Shoolini University under the voucher numbers, namely, SUBMS/BOT-4827 (Akhnoor, 330 m), SUBMS/BOT-4800 (Vijaypur, 336 m), SUBMS/BOT-4828 (Samba, 358 m) SUBMS/BOT-4801 (Harotkot, 688 m), SUBMS/BOT-4830 (Sarangdhar, 661 m), SUBMS/BOT-4829 (Salal, 696 m), SUBMS/BOT-4831 (Bashat, 1302 m), SUBMS/BOT-4832 (Mantalai, 1311 m), and SUBMS/BOT-4802 (Khetriar, 1330 m).

2.2. Morphological Analysis

To study the morphological variability among plants, following growth characteristics, namely, plant height, crown spread, inflorescence size, and leaf length as well as leaf width were measured and expressed in centimeters whereas number of leaves, number of branches, and number of inflorescences were counted.

2.2.1. Leaf area

To measure the leaf area, protocol described by Green-armytage [28] was followed, the collected leaves were placed on a graph paper having 1 cm^2 square grid boxes and then boundary was outlined. After that following formula was applied:

Leaf area = No. of grids covered + No. of grids incompletely covered/2

2.2.2. Crown spread

The average crown spread was measured by the method described by Blozan [29]. Measurements of crown from both the directions of plant, that is, longest spread (A1) and longest cross spread (A2) were taken and average was determined.

Average crown spread = (A1 + A2)/2

2.3. Biochemical Analysis

For biochemical analysis, the leaves were hand harvested from each plant. Fifth and sixth mature leaves from the top of the plant were taken. Leaves were collected from all the four directions of each plant. The collected leaves were cleaned and shade dried and after that, the composite leaf samples were stored and analyzed in the laboratory for different parameters using standard techniques given below:

2.3.1. Carbohydrate content

Carbohydrates were estimated by following protocol of Thangaraj [30]. 100 mg of the leaf sample was placed in a boiling water bath to heat it along with 2.5 N hydrochloric acid for 3 h. The amount was kept 5 ml. After that, sodium carbonate was added to it to make its total volume up to 100 ml. The contents were further centrifuged at 10,000 rpm for 5 min. 4 ml of anthrone reagent was poured to the collected supernatant after centrifugation. On the other hand, different concentrations of standards were prepared with the help of glucose solution and it was treated with the same set of chemicals expect the leaf sample. All the contents were heated at least for 8 min inside the water bath and the absorbance was recorded at 630 nm in a spectrophotometer.

2.3.2. Protein content

The protein content was estimated by following Lowry's method [31]. Leaf sample (100 mg) was ground well in 1−5 ml of the phosphate buffer. After that the centrifugation of the sample was performed for 5 min and at 7000 rpm. To the 1 ml of collected supernatant, 2 ml of alkaline copper solution was added and mixed. After that, 0.2 ml of Folin-Ciocalteu reagent was poured to it and the solutions were positioned at room temperature for incubation in the dark and the process took 30 min. OD was taken in spectrophotometer at 660 nm. A standard graph was drawn using BSA.

2.3.3. Amino acids

Amino acids were estimated by following protocol of Moore and Stein [32]. Leaf sample (100 mg) was taken and ground fine with acid washed sand. 2 ml of 80% ethanol was added to it. Centrifugation was performed for 5 min and at 7000 rpm. To 100 µl of extract, 900 µl of ninhydrin solution, and 1 ml distilled water was added and the contents were mixed. The solution was left for 15 min until the bluish-purple color appeared. OD was recorded in spectrophotometer taking lecine as standard at 570 nm.

2.3.4. Reducing sugar

The reducing sugars were estimated by the method described by Usunobun and Okolie [33]. 100 mg of powdered leaf sample was dissolved in 5 ml distilled water and thereafter filtered. The reducing sugar content was assayed by adding 2 ml of the 3,5-dinitrosalicyclic acid reagent in 1 ml of the sample. The dinitrosalicyclic reagent was made by adding 1.8 g of 3,5-dinitrosalicyclic acid in 20 ml of 1.0 M NaOH followed by further addition of 60 ml distilled water. After heating in water bath for 5 min and the solution was cooled swiftly. The absorbance was taken at 540 nm in a spectrophotometer. Glucose served as standard.

2.3.5. Total sugar

The total sugar content was estimated by protocol given by Jain [34]. To 1 ml of the leaf extract, 1 ml of 5% phenol solution was added. The contents were further mixed with 5 ml of 96% sulfuric acid and left for 10 min. The solution was heated at 35°C in a water bath for 20 min. Its absorbance was taken in a colorimeter at 490 nm. Glucose served as standard solution.

2.3.6. Non-reducing sugar

Non reducing sugars were estimated by following the method of Otache [35]. It was estimated by subtracting the reducing sugars from that of the total sugars.

2.3.7. Crude fiber

Crude fiber was estimated by following the protocol of Bosha [36]. 2 g of leaf sample was taken and 1.25% sulfuric acid solution (100 ml) was added to it and further heated for 30 min. The contents were then filtered and 1.25% NaOH (100 ml), 1 % HNO_3 solution was added. The residue obtained was heated to 105°C and weighed (M1). It was then treated at 55°C in a muffle furnace for 5 h. The ash was obtained and it was weighed (M2). The % crude fiber was calculated as:

Crude fiber (%) = 100 × (M2-M1)/weight of original sample

2.3.8. Crude fat

Crude fat was estimated by following the method of A.O.A.C. [37]. 5 g of dry leaf sample was extracted with petroleum ether in soxhlet apparatus. The extract obtained was further cooled and weighed. Washings with petroleum ether were also given 4−5 times. The contents were repeatedly dried and weight until the difference between first and last readings was <1 mg. Crude fat was estimated as:

Crude fat (%) = 100 × weight of flask containing dried extract − weight of flask/weight of obtained dried sample

2.3.9. Ash content

Ash content was measured by the protocol given by Kumar [38]. The leaf sample was weighed and heated at 600°C in muffle furnace for 5 h. The ash obtained was cooled swiftly and weighed. This procedure was subsequently repeated unless the weight of obtained ash became constant.

Ash (%) = 100 × weight of ash/weight of sample

2.3.10. Moisture content

The moisture content was estimated by following standard protocol of Kumar [38]. The fresh leaves were weighed after collection (B1) and then leaves were kept overnight at 150°C in an oven for drying. After that, the samples were cooled and weighed (B2).

Moisture (%) = 100 × (B1−B2)/B1

2.3.11. Potassium and sodium

Potassium and sodium were determined by following the protocol of Singh [39]. To 500 mg leaf sample, 10 ml of conc. HNO_3 was added and left overnight. After that, 2−3 ml of $HClO_4$ were also added after pre-digestion and the solution was kept on hot plate at 200°C. The solution was filtered using Whatman no. 42 filter paper and washings with distilled water were also given. NaCl and KCl were taken as standards and the concentrations were determined using flame photometer.

2.3.12. Calcium

Calcium was determined by following protocol of Oser [40]. 25 ml of ash solution was prepared by dry ashing of the leaf samples and 150 ml of water was added to it. Some amount of methyl red indicator was poured to it and the contents were neutralized awaiting the pale pink color turning to yellow. The contents were heated and 10 ml of ammonium oxalate was added which was left for boiling again for 5 min. Further, glacial acetic acid was added and the solution was filtered. Washings with warm water were given. 5−10 ml of dilute H_2SO_4 was also added and heated at 70°C. Finally, the obtained solution was titrated against 0.01 N $KMnO_4$ to a permanent pale pink color.

Calcium mg/100 mg = Titre value × Normality of $KMnO_4$ × 0.2004 × total volume of ash solution × 100/ml of ash solution taken for estimation× weight of sample taken for ashing.

2.3.13. Alkaloid content

Alkaloids were determined by following method of Harborne [41]. 0.5 g leaf sample was taken (D1) and was extracted with 50 ml of 10% acetic acid. The extract was filtered and transferred to a China dish. It was further boiled on a water bath for 2 h to reduce its volume. To the extract, concentrated ammonium hydroxide was added until the precipitates were formed. Further, washings with 1% ammonium hydroxide were given and the extract was dried at 80°C in oven. The content left at last was alkaloids (D2).

Alkaloid (%) = D2/D1 × 100

2.3.14. Flavonoid content

Total flavonoid content was determined using the protocol of Zao [42]. 1 ml leaf extract prepared in methanol and distilled water (4 ml) was added which was followed by further addition of 5% sodium nitrite (1 ml). After 5 min, 1 ml of 10 % aluminum chloride and 8 ml of sodium hydroxide were also added to the mixture. In this method, the aluminum chloride formed a complex with hydroxyl groups of flavonoids present in the sample and orange yellowish color was developed. Absorbance of that mixture was taken using spectrophotometer at 510 nm against blank containing all reagents except plant extract. The calibration curve of rutin was prepared to find out the flavonoid content.

2.3.15. Phenolic content

Phenolic content was determined by following method of Sethi and Sharma [43]. 1 ml leaf extract prepared in ethanol was taken and 1 ml of Folin-Ciocalteu reagent was added in it. 20% sodium carbonate solution (2 ml) was also added to it. After shaking the contents of the solution, it was boiled for 2 min and cooled swiftly. 25 ml of distilled water was added and O.D was taken in spectrophotometer at 750 nm. In the same way, gallic acid which was taken as a standard phenol was processed and O.D was taken. The blank solution was prepared by all reagents except the plant extract. Finally, total amount of phenol was calculated referring the O.D of standard phenol with standard curve.

2.3.16. Tannin content

Tannin content was estimated by following the protocol of Saxena [44]. 100 mg of the powdered leaf material was weighed and 1 ml of distilled water was added to it. The material was heated and centrifuged for a period of 20 min and at 2000 rpm and the supernatant was taken. Folin-Denis reagent (60 µl) and sodium carbonate solution (100 µl) were added and again diluted. For the preparation of standard curve, standard tannic acid solution was made. The absorbance was taken after 30 min and at 700 nm.

2.3.17. Saponin content

Saponins were determined by following the method described by Mir [45]. 5 g of leaf sample was taken (E1) and 20% of 50 ml ethanol was added to it. It was then heated for about 4 h at a temperature of 55°C and constant stirring was done. The volume of filtered extract was reduced up to 40 ml by keeping it at a temperature of 90°C. After that, 5 ml diethyl ether was added to it in a separating funnel and shaken well. The aqueous layer was recovered and ether layer was removed. Added to it n-butanol (15 ml). The contents were washed by 2.5 ml of 5% aqueous NaCl. After that, the contents of the solution were evaporated and the residue was obtained and weighed (E2).

Total saponin (%) = E2/E1 × 100

2.4. Statistical Analysis

All the data were subjected to two-way ANOVA analysis and Bonferroni multiple comparison test.

3. RESULTS AND DISCUSSION

In morphological analysis, there was a significant change in plant height, the plants from high altitudes were comparatively short in size [Figure 1]. The maximum plant height was recorded in mid altitude (661 masl), namely, 169.6 cm and minimum at 1311 masl, that is, 13.1 cm. Accordingly, crown spread also decreased with increasing altitude with maximum value, namely, 205.2 cm at 661 masl and minimum value at 1311 masl (139.4 cm) [Figure 1]. Plant's capability to adjust to the troubling environmental circumstances can be related to the morphological or physiological properties displayed by its organs [46-48]. The results reveal that altitudinal gradients and concomitant variables hold a significant impact on plant's height. The height is a crucial component of a plant, various traits such as ability to compete for light, metabolic rate, and carbon gain strategy are dependent on the height of a plant [49]. Low altitude plants have to tolerate the high temperature and dry climatic conditions, harsh radiations, and limited precipitation whereas high altitude plants deal with low temperatures, high irradiance, etc., [50]. This significant reduction is in agreement with several other studies that have found decreasing plant height along environmental gradients [51] as higher elevations are often considered more stressful for plant growth and survival. Nutritional requirements and competition pose a remark on plants of all the sizes for instance the competition for light possess a noteworthy consequence on the growth of plants. In a study, growth of mountain beech trees was affected because of the competition for light and nutrients, tree growth dropped with increasing elevation [52]. This can bargain with the fact that trees become undersized at high elevations [53,54]. However, short stature of plants is also known to possess some benefits for instance, provides protection against stresses like winds [55-57]. Reduction in size is also considered as an important policy of plants at high altitude to endure stress, namely, reduced nutrient availability and low temperature. Reduction in cell size and intercellular spaces that ultimately led to reduction in whole plant size has been observed at high altitudes [58-61]. The decrease in temperature with elevation adds to less availability of prerequisite heat that is to be utilized by plants during the period of short summer and as a consequence of which the rates of many temperature dependent reactions get hindered [62]. The small stature could also be a genetic adaptation of the plant [63] and it could be possible that when a heat tolerant plant like *Justicia adhatoda* L. grows under low temperature at high altitudinal regions, it retains the changes in its morphological and physiological features to thrive in unsuitable conditions. Thus, the small height could be a strategy to cope up with the prevailing environmental conditions for instance staying near the warmer microclimatic conditions close to the ground and safeguard from the wind [64]. The crown spread also varied and showed a significant reduction at the higher elevation in comparison to the lower ones. Maximum value was observed in 661 masl, that is, 205.2 cm and minimum value was observed in 1311 masl, that is, 139.4 cm. Similarly, in a study, noticeable variations in the morphological traits of *Boswellia* trees in Eritrea were observed. Tree height, crown diameter, and depth all decreased with increasing altitude [65,66]. The number of primary branches was found to be significantly low at 1330 masl, that is, 94.4 and high at 336 masl, that is, 139.8 and maximum number of secondary branches were found at 1330 masl, namely, 122.6. No significant change was observed in number of tertiary branches [Table 1]. With decreasing height, mechanical damage in branches and trunks of the trees was also observed in some studies suggesting that regardless of the thermal conditions, mechanical damage for instance, by strong winds also contributes to these variations [67]. Wind, although gentle but can obligate an important effect on plant's height, plant's growth can also be chiefly influenced by the amount of water available in the soil [68]. Number of inflorescence were observed to be highest in 688 masl, namely, 148.2 whereas 1330 masl had lowest number of inflorescence count, that is, 92.4. This significant reduction in the total number of inflorescences at high altitude is in agreement with the study done on *Polylepis indicana* where the number of inflorescences showed a marginally significant decrease at higher altitude [69]. The leaf width and inflorescence size showed statistically significant variation [Table 1] [70-79]. The amount of solar radiation captured by plants is intensely affected by the leaf morphology [73]. The previous studies have revealed decrease in leaf size with the increasing altitude [74,75]. In a study, leaf traits of *Pleioblastus amarus* were evaluated along varying altitudes where leaf length and width decreased significantly with increasing altitude and increase in environmental stresses, that is, solar radiation, nutrient loss, low temperatures, and other abiotic pressure were held responsible for those variations [70]. In a study, all the morphological characters were affected with increasing altitude but a few of them were retained when the plants were maintained in their common environment thereby indicating heritable variation for those morphological characters [76]. The number of leaves decreased as altitude increased, maximum number of leaves were found at 358 masl, namely, 2855 and minimum number of leaves were present at 661 masl, namely, 1566.2 [Table 1]. Several other studies have found decrease in number of leaves [19,74]. The environment in which the leaves are produced has a significant influence on leaf morphology [77,78].

Total carbohydrates were seen decreasing significantly with increasing altitude for both the years with maximum value at 330 masl (31.465 mg/g) during 1st year and minimum value at 1311 masl (19.264 mg/g) during 2nd year, that is, the carbohydrate content was observed to be highest in low and mid elevations and lowest in high elevation [Table 2] [Figure 2]. However, the previous studies have also reported that plants have adopted to stress by altering several metabolic pathways for instance, carbohydrate synthesis and increased sugar content that act in osmoregulation, accumulation of sucrose during cold stress [60,79,80]. Hence, it is possible that the amount of carbohydrates is less which are to be invested for metabolic processes, hence, altering plant growth at high elevations.

Plants can turn an ample amount of assimilated carbon and energy into the synthesis of other organic molecules that are included in the plant defense [81]. Plants produce secondary metabolites by undergoing various metabolic pathways as a response to stress conditions and these pathways are in turn prompted from the primary metabolite pathways which create the crucial precursors of secondary metabolites [82]. Total sugar, reducing sugar, non-reducing sugar, and ash also significantly varied with elevation [Table 2]. In some studies, significant variation in total amino acids and total proteins among different provenances has been observed [83,84] but in our study, variations were not statistically significant [11,85,86]. Insignificant changes in calcium content, alkaloid content, saponin content, phosphorus, potassium, and sodium were observed in the study [Table 2] [Figure 2] [87,88]. Crude fiber and crude fat in our study were observed in higher amounts at middle altitude in comparison to the higher and low elevation which was in agreement with the study done on red clover cultivars, where crude fat content was higher in mountain region in comparison to the low land region [1,89] but the outcomes in our study were not statistically significant [Table 1].

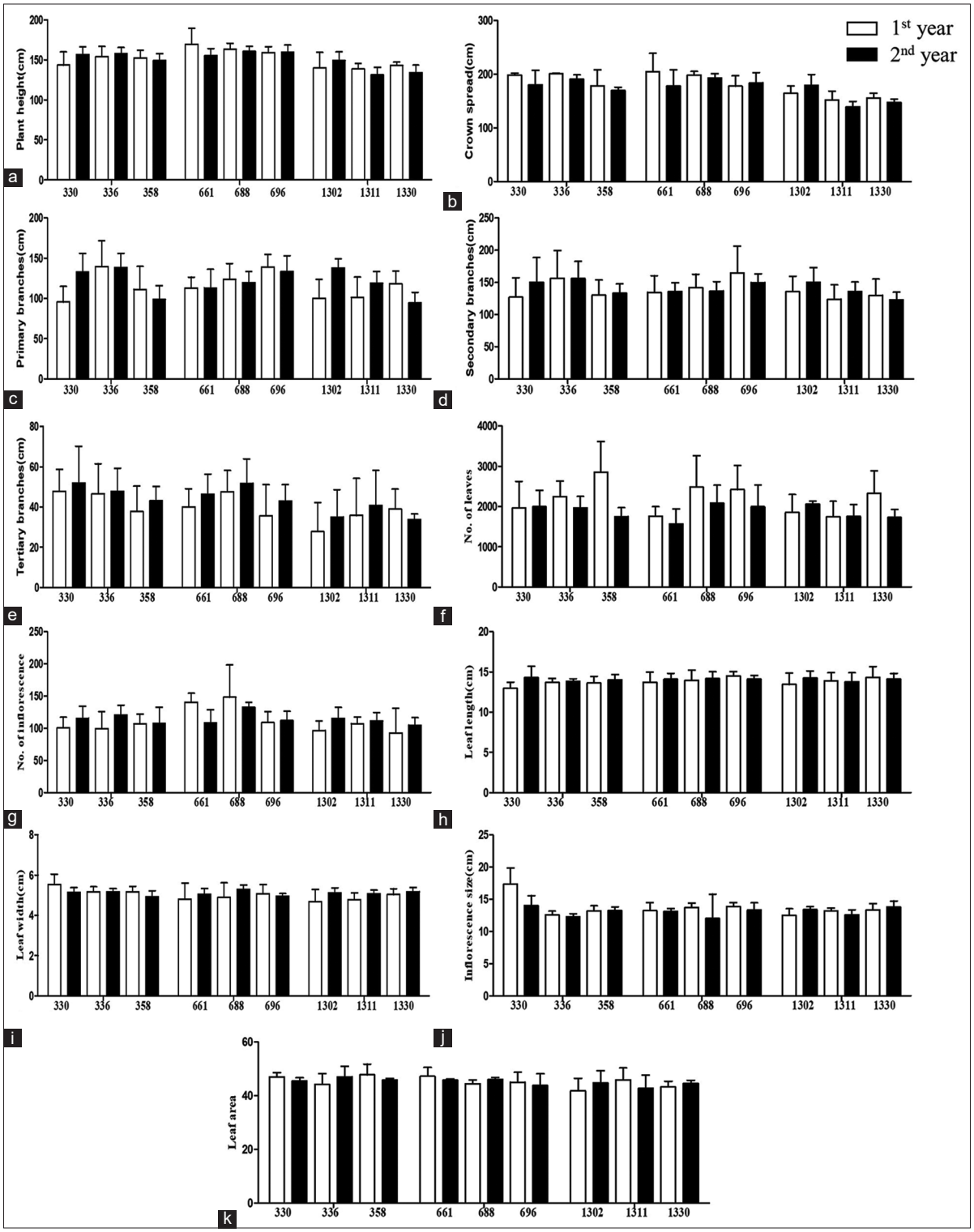

Figure 1: Plant height (a), crown spread (b), primary branches (c), secondary branches (d), tertiary branches (e), number of leaves (f), number of inflorescence (g), leaf length (h), leaf width (i), inflorescence size (j), and leaf area (k) of *Justicia adhatoda* L. growing in different altitudes of Jammu.

In comparison to low altitude, concentration of phenols increased at high altitudes in our study. The highest amount of phenols was observed to be 3.006 mg/g present in 1330 masl, the phenol content of mid and high altitude area was comparatively high as compared to the low altitude area and minimum phenols were found to be present at 688 masl, that is, 1.186 mg/g [Table 2]. This is in agreement with the fact that phenols were found in high levels in the plants collected from high altitudes in a study and that increase in phenol concentration with altitude was ascribed as a response to increasing intensity of UV radiations [90]. The previous studies also suggest that decreased temperatures and increase in intensity of UV radiation activate the amplified production of UV-absorbing

Table 1: Variation in morphological attributes of *Justicia adhatoda* L. growing in different altitudes.

Morphological analysis	Year	Study Site 1 (330 masl)	Study Site 2 (336 masl)	Study Site 3 (358 masl)	Study Site 4 (661 masl)	Study Site 5 (688 masl)	Study Site 6 (696 masl)	Study Site 7 (1302 masl)	Study Site 8 (1311 masl)	Study Site 9 (1330 masl)
Plant height (cm)	1st Year	143.6±16.5	154±12.94	152.4±9.96	169.6±19.93	163.2±7.66	159.2±7.46	140±19.65	138.8±7.04	143.2±4.14
	2nd Year	156.8±9.88	157.8±7.85	149.4±8.44	155.2±9.038	160.8±6.22	159.6±9.07	149.4±10.784	131.4±9.343	134.2±9.36
Crown spread (cm)	1st Year	198.6±3.2	200.8±1.64	178.2±30.17	205.2±33.83	198.6±6.76	177.8±19.4	164.6±13.84	152.2±16.39	155.8±9.06
	2nd Year	180.2±27.26	190.6±8.2	169.8±6.09	178.4±30.07	193±8.18	184±19.22	179.4±20.18	139.4±10.06	147.6±5.94
Primary branches	1st Year	95.6±19.26	139.8±32.08	111.2±28.78	112.8±13.62	123.6±19.88	139±15.5	100.2±23.56	101.4±25.10	118±15.92
	2nd Year	133±23.01	138.6±17.41	98.8±17.07	113±23.28	119.8±13.73	133.4±19.69	137.6±11.63	118.8±14.61	94.4±13.22
Secondary branches	1st Year	127±30.14	156.4±43.04	130.2±23.38	134.4±25.85	141.8±20.71	164.6±41.48	135.6±23.89	123.8±22.39	129.8±25.38
	2nd Year	150.2±38.44	156±26.58	133.2±14.30	135.8±13.49	136.8±14.41	149.6±13.55	150±22.80	135.6±14.87	122.6±12.17
Tertiary branches	1st Year	47.8±10.96	46.6±14.92	37.8±12.55	40±9.08	47.6±10.59	35.6±15.46	27.8±14.34	35.8±18.45	39±9.92
	2nd Year	52±18.22	47.8±11.47	43.2±7.04	46.4±9.93	51.8±12.09	43±8.15	35±13.61	40.8±17.49	33.8±2.77
Total number of leaves	1st Year	1962.4±661.79	2247±384.72	2855±760.73	1762.8±233.21	2486.6±776.08	2420.8±598.16	1852.8±444.53	1749.2±379.99	2328±558.87
	2nd Year	1993.8±406.55	1963.8±286.28	1750±222.50	1566.2±374.42	2087.8±444.21	1994.2±537.94	2064.8±67.18	1754.6±293.30	1728.4±198.58
Leaf length (cm)	1st Year	12.96±0.76	13.695±0.49	13.63±0.82	13.705±1.29	13.975±1.25	14.525±0.52	13.435±1.41	13.87±1.03	14.3±1.38
	2nd Year	14.301±1.4	13.84±0.32	14±0.67	14.1±0.71	14.175±0.85	14.105±0.47	14.24±0.88	13.785±1.09	14.11±0.71
Leaf width (cm)	1st Year	5.54±0.50	5.17±0.25	5.18±0.26	4.805±0.79	4.89±0.72	5.08±0.45	4.69±0.59	4.78±0.33	5.035±0.29
	2nd Year	5.143±0.23	5.18±0.15	4.931±0.29	5.045±0.29	5.29±0.21	4.945±0.13	5.1248±0.24	5.079±0.17	5.175±0.20
Leaf area	1st Year	47±1.58	44.2±4.08	47.8±3.96	47.2±3.34	44.4±1.51	45±3.7	41.8±4.65	45.9±4.44	43.2±2.16
	2nd Year	45.6±1.14	47±3.93	45.8±0.49	45.8±0.44	46±0.70	43.8±4.38	44.8±4.54	42.7±5.02	44.6±1.14
Inflorescence size (cm)	1st Year	17.365±2.49	12.6±0.55	13.19±0.80	13.235±1.24	13.71±0.69	13.885±0.61	12.5±1.03	13.215±0.41	13.355±0.98
	2nd Year	14.035±1.53	12.274±0.48	13.24±0.57	13.11±0.45	12.04±3.75	13.32±1.123	13.385±0.46	12.605±0.75	13.81±0.93
Total number of Inflorescences	1st Year	100.8±16.48	99.4±26.08	106.8±15.44	140.6±13.68	148.2±50.34	108.8±16.63	96.6±14.74	107.2±10.42	92.4±38.82
	2nd Year	115.6±18.60	120.4±15.46	107.4±25.16	108.6±20.41	132.4±8.20	111.8±14.54	115.2±17.09	111.6±12.97	104.8±11.81

Data is represented as mean±standard deviation.

Table 2: Variation in physico and phytochemical elements of *Justicia adhatoda* L. growing in different altitudes.

Biochemical Analysis	Year	Study Site 1 (330 masl)	Study Site 2 (336 masl)	Study Site 3 (358 masl)	Study Site 4 (661 masl)	Study Site 5 (688 masl)	Study Site 6 (696 masl)	Study Site 7 (1302 masl)	Study Site 8 (1311 masl)	Study Site 9 (1330 masl)
Carbohydrate (mg/g)	1st Year	31.4±4.5	27.1±3.13	30±4.88	29.6±5.85	20.8±3.96	21±1.33	20.3±2.94	21.3±2.29	19.4±0.99
	2nd Year	30.5±1.57	28±2.34	25.8±2.9	28.6±1.32	24.7±4.08	27.18±3.08	23±1.32	19.2±1.81	21±5.28
Protein (mg/g)	1st Year	19.4±0.96	17.6±2.37	15.9±1.87	18±1.94	16.7±3.64	18.6±0.68	16.5±2.28	15.9±1.37	17.1±2.83
	2nd Year	17.8±0.67	15.7±1.14	19.1±2.07	16.9±1.96	17.3±1.33	17.8±1.75	16.8±0.78	15.1±5.23	17.2±1.41
Crude Fiber (%)	1st Year	5.3±0.61	5.5±0.55	5.6±0.75	6.1±0.79	5.4±1.15	6.2±0.91	5.4±0.7	5.7±0.9	5.5±0.77
	2nd Year	5.6±0.8	5.9±1.02	4.9±0.58	5.03±0.66	5.1±1.34	5.9±0.62	5.5±0.77	4.7±0.9	5.6±0.57
Ash Content (%)	1st Year	3.7±0.45	3.6±1.03	3.4±1.1	4.1±0.75	4.3±1.25	3.7±0.11	5.4±0.3	4.3±1.17	4.9±0.23
	2nd Year	4.6±0.76	5.4±0.8	4.6±0.65	5.2±0.55	4.7±0.45	4.9±0.55	5±0.72	4.3±0.2	4.93±0.55
Moisture content (%)	1st Year	4.7±0.45	5.3±0.5	4.9±0.2	5.8±0.83	5.7±0.9	6.1±0.6	5.6±0.45	4.4±0.69	5±0.23
	2nd Year	5±1.41	5.3±1.24	5.8±0.83	4.4±1.85	5±0.66	3.7±0.9	3.8±1.15	5.3±2.57	5.7±0.72
Crude fat (%)	1st Year	5.3±0.61	5.5±0.55	5.6±0.75	6.1±0.79	5.4±1.15	6.2±0.91	5.4±0.7	5.7±0.9	5.5±0.75
	2nd Year	5.6±0.8	5.9±1.02	4.9±0.5	5±0.66	5.1±1.34	5.9±0.62	5.5±0.77	4.7±0.9	5.6±0.57
Reducing sugar (mg/g)	1st Year	32±3.33	38.4±0.96	39.2±4	30±2.61	33.1±2.35	35.2±6.88	26.1±2.07	33.2±6.12	35.1±9.94
	2nd Year	40.3±5.34	38.6±2.17	43.9±1.85	34.2±6.14	33.9±2.5	36.3±10.2	27±9.8	26.6±2.12	24.6±2.88
Non-reducing sugar (mg/g)	1st Year	20.8±1.52	20.5±2.4	21.3±2.67	19.1±6.49	19.7±8.09	15±4.15	11.2±3.18	12.7±12.66	10.6±11.79
	2nd Year	20.7±6.98	13.2±2.46	18.8±5.6	9.3±19.37	23±7.59	19±13.27	16±15.23	19.1±9.21	14.1±11.7
Amino acid (mg/g)	1st Year	13.1±1.98	13.4±0.7	14.3±0.69	12.7±0.48	13.9±0.94	14.7±0.54	12±1.53	14.7±0.2	13.7±1.1
	2nd Year	13±1.39	13.9±0.79	13.8±0.7	13.8±1.07	13.7±1.28	13.9±0.94	13.9±0.44	14.4±0.3	14±0.44
Alkaloid (%)	1st Year	46.9±0.8	44.5±0.87	43.8±2.9	47.2±1.27	47±3.95	45.5±4.28	45±3	46.4±1.35	45.9±3
	2nd Year	41.4±5.06	45.2±2.35	45.5±0.6	49.4±8.65	46.2±2.68	45.9±3.35	49±9.81	50.6±2.06	46.4±6.46
Flavonoid (mg/g)	1st Year	3.1±0.49	2.9±1.69	1.7±1.88	4.2±1.57	3.3±1.44	4.5±0.36	2.8±1.34	4.4±0.51	4.5±0.16
	2nd Year	3.2±0.73	3.2±1	2.7±0.67	4.4±0.32	4.1±0.19	4.2±0.47	2.7±1.2	4.4±1	4.4±0.85
Tannin (mg/g)	1st Year	3.7±0.07	3.9±0.255	3.68±0.05	3.7±0.04	3.7±0.06	3.7±0.03	3.8±0.06	3.8±0.06	3.9±0.22
	2nd Year	4.1±0.38	3.8±0.14	3.9±0.19	3.8±0.09	3.8±0.07	3.7±0.05	3.6±0.46	3.8±0.14	3.9±0.11
Phenol (mg/g)	1st Year	1.5±0.18	1.8±0.17	1.2±0.18	1.8±0.51	1.1±0.06	2.1±0.27	2.5±0.07	2.1±0.47	2.3±0.13
	2nd Year	1.3±0.45	1.5±0.15	1.2±0.03	2.5±0.45	1.7±0.29	1.4±0.11	2.7±0.33	2.4±0.14	3±0.08
Saponin (%)	1st Year	22.9±2.79	23.1±6.1	31.9±5.1	33.1±1.91	26.6±4.96	31.1±6.74	30.3±8.95	31.5±2.36	29.2±2.13
	2nd Year	28.4±5.71	31.1±7.49	29.1±7.57	28.7±5.31	32.4±3.13	29.2±7.03	29.2±6.27	26.4±5.67	29.1±5.49
Calcium (mg/100 g)	1st Year	13.6±0.3	13.5±1.15	14±2.49	13.6±2.59	14.2±2.7	13.5±2.15	13.2±1.41	14.3±2.02	15.8±1.45
	2nd Year	14.5±3.38	14.5±1.4	14.8±0.8	14.7±1.3	13.9±2.68	15.6±2.3	13.4±2.53	13.6±1.98	14.5±3.33
Potassium (ppm)	1st Year	2.1±0.51	2.9±0.61	2.3±0.42	2.2±0.46	2.6±0.35	1.3±0.37	2±0.16	1.9±0.03	2.3±0.4
	2nd Year	2.3±0.71	2±0.4	2.7±0.1	2.5±0.58	2.2±0.4	2.6±0.4	2.4±0.3	3±0.32	2.5±0.69
Sodium (ppm)	1st Year	0.14±0	0.14±0	0.26±0.2	0.26±0.2	0.26±0.2	0.14±0	0.14±0	0.14±0	0.26±0.2
	2nd Year	0.14±0	0.26±0.2	0.14±0	0.26±0.2	0.14±0	0.26±0.2	0.14±0	0.26±0.2	0.26±0.2
Phosphorus (mg/g)	1st Year	1.9±0.45	1.9±0.04	1.7±0.47	1.6±0.26	1.6±0.3	1.4±0.14	1.7±0.53	1.6±0.04	1.5±0.2
	2nd Year	1.8±0.22	1.8±0.17	1.3±0.26	1.5±0.39	1.4±0.2	1.4±0.12	1.5±0.12	1.5±0.2	1.7±0.28
Total sugar (mg/g)	1st Year	52.9±4.63	59±1.71	60.5±3.66	49.2±6.23	52.9±8.9	50.3±7.4	37.4±1.22	45.9±7.77	45.8±12.3
	2nd Year	61.1±5.94	51.8±1.03	62.8±3.95	43.6±15.2	57±5.1	55.4±3.05	43.1±9.17	45.8±11.2	38.8±8.8

Data are represented as mean±standard deviation.

and various antioxidant compounds, namely, phenols in plants, this could be probably the phenomenon happening to the phenols there [91-93]. Plants at high altitudes possess UV protective systems to cope up with the increasing harmful UV radiations with altitude. Phenols play a vital part in defense and other mechanisms of the plant for instance, hydrogen scavenging system [94] which could function less effectively when the temperature remains less and therefore, more phenolic compounds are required to avoid the loss happening to plants at low temperatures [95]. Total flavonoid content increased in high altitude areas. Total flavonoid content of the plant also significantly increased with increasing altitude, maximum amount of flavonoids was observed at 696 masl, that is, 4.543 mg/g and least present at 358 masl, namely, 1.71 mg/g [Table 2]. Some studies have revealed that due to the various environmental factors happening at high altitudes, flavonoid accumulation was induced as a consequence of UV radiations. Flavonoid is considered as a foremost

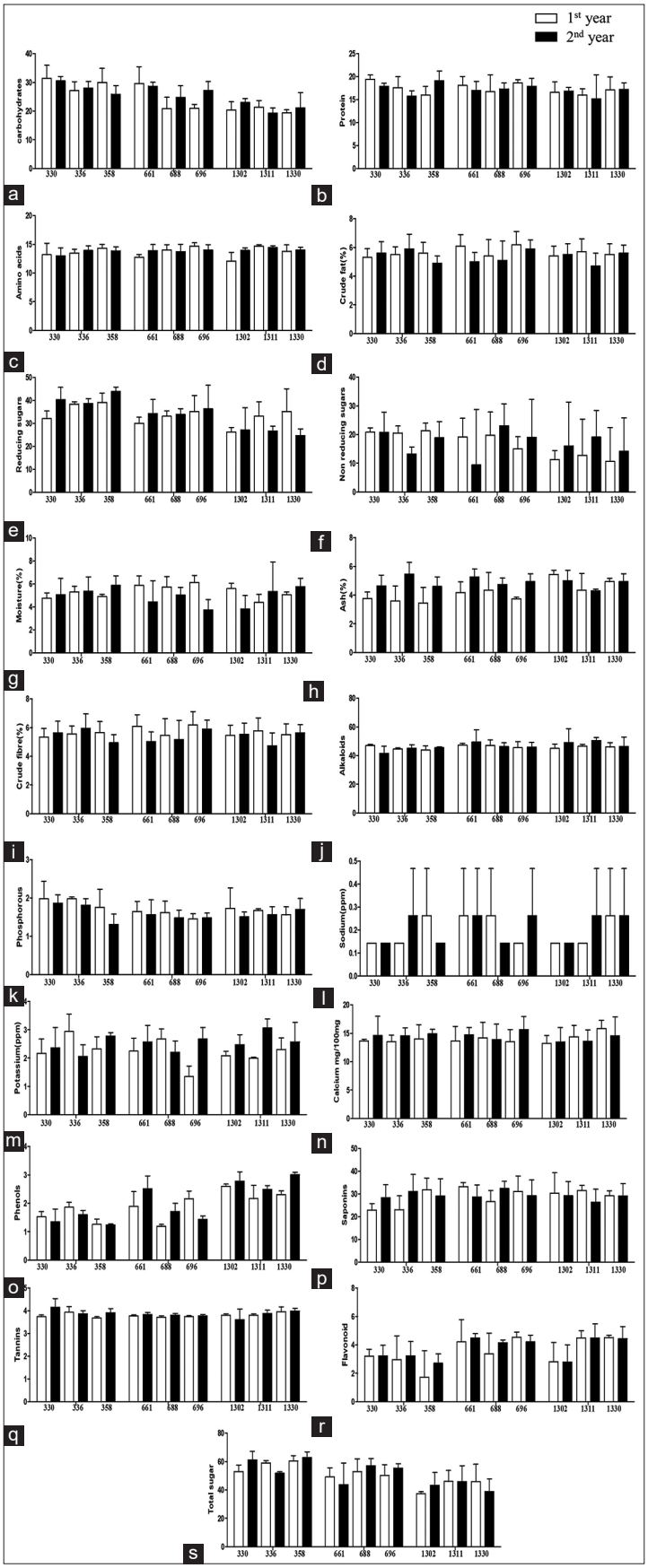

Figure 2: Carbohydrate (a), protein (b), amino acid (c), crude fat (d), reducing sugar (e), non-reducing sugar (f), moisture (g), ash (h), crude fiber (i), alkaloid (j), phosphorus (k), sodium (l), potassium (m), calcium (n), phenol (o), saponin (p), tannin (q), flavonoid (r), and total sugar (s) of *Justicia adhatoda* L. growing in different altitudes of Jammu.

UV protectant and free radical scavenging compound [96]. Phenolic acids and flavonoids are considerably produced in plants at high elevation sites [97]. It is possible that other abiotic and biotic factors could also be contributing to the observed altitudinal variations in phenols. There could be various other factors like exposure to strong wind regimes at high altitudes [98,99], air temperature [100,101], soil mineralization and nitrification with increasing altitude [102-104], transpiration rates [105,106], rate of photosynthesis [107,108], and disturbances in light intensity as the photoperiod holds an impact on the biosynthesis of several secondary metabolites in a number of plant species [109] which can contribute to these variations for instance, in a study, soil and leaves of herbs, shrubs, and trees of Ecuadorian tropical montane forest were accessed at various altitudes where foliar nutrient concentrations were observed to be highest in low altitude and also there was slow mineralization of plant litter and less availability of nutrients at high altitudes [110]. Plant trait variations are mostly environmentally based but sometimes, these can have a genetic background. In a study, variations were found in the morphological attributes and still retained when the plants were grown under similar environmental conditions which suggested a possible genetic background for those traits [111]. Hence, the findings of the present study illustrated the general phenomenon that the plants possess the ability to deal with the environmental stress by alternate mechanisms be it morphological, physicochemical, or phytochemical.

4. CONCLUSION

Plants are not randomly distributed in a particular elevational site, their distribution depends on the environment in which they grow and are able to functionally adapt themselves. Plant's ability to adapt to various ecological conditions relies on the morphological and physiological properties exhibited by its organs. Phytochemical variability helps plants to cope up with unsuitable environment for instance, in this study, phenols and flavonoids which are also powerful antioxidants increase their levels in *Justicia adhatoda* L. plants growing at higher elevations to combat stress. *Justicia adhatoda* L. chemically adapted itself along the altitudinal range by regulation of its phytochemicals. Plants at high altitudes are equipped with defense system which activates biosynthesis of secondary metabolites, and the results demonstrate that plants can adapt to varying environmental circumstances happening along altitudinal gradient.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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