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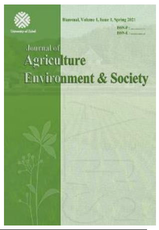

Investigating the morphological, nutritive, and secondary metabolite characteristics of *Calotropis procera* (Aiton) R. Br wild populations in certain habitats of Baluchestan, Iran

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ABSTRACT

The development and implementation of effective, beneficial programs towards restoration, conservation, and appropriate utilization of medicinal plant species are feasible only through the identification of their ecological characteristics. This research was conducted with the objective of investigating the morphological, nutritive, and secondary metabolite characteristics of calotrope wild populations in certain habitats of Baluchestan, as a completely random nested design with three repetitions throughout four cities during February/March 2014. The edaphic properties of habitats were also assessed. The obtained data were compared at a 5% significance level via DMRT. The samples were collected from various regions, including the cities of (1) Iranshahr (Mand-e Bala and Sarzeh villages), (2) Chabahar (Negour and Nalent villages), (3) Nikshahr (Bandaan and Bent villages), and Khash (Baluchkan and central Khash), were compared. Results showed that the majority of the investigated characteristics were significantly different among cities and regions. In this research, Nikshahr city had the highest number of morphological characteristics. The investigated soil in Nikshahr city was more suitable regarding P and K, as compared to other regions. The plants grown in Nikshahr had the highest level of foliar N, Ca, K, and P, the highest level of floral K, and the highest level of P and K in the roots. Forty-two compounds were identified in the aerial organs and root extracts of calotrope. Among the active ingredients, 9-Octadecenamide acid with 8.3% in the aerial organ, Benzyl alcohol with 8.7% in the root, and 2,6,10-Trimethyl,14-ethylene-14-pentadecane with 27.2% in the stem-extracted latex had the highest content in this plant.

1. Introduction

Calotrope (Calotropis procera) is a sap-producing plant of the Asclepiadaceae family that grows in the arid and semi-arid regions of Asia and Africa (Rashmi and Arya, 2011). This plant is distributed throughout the Hormozgan, Baluchestan, and Khuzestan provinces of Iran. Calotrope is considered a rubber plant that reaches a height of 3-4 m. In traditional literature, this plant is referred to as Ashar, Akras, Madaar, Ashar and is known as Karag and Kark in Baluchi language (Mirheidar, 1994). Calotrope latex is used by Baluchestan natives as an antidote for curing the effects of insect and harmful creature stings. All parts of calotrope have therapeutic properties. Calotrope leaves treat common cold and coughing, while its latex has antiseptic and laxative properties and is used for the treatment of toothache and scorpion sting, as well as fetal abortion. The plant roots are useful for treating coughs, dyspnea, and fever. Its flowers are sedative and nourishing

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(Dewan et al., 2000). In India, the different plant parts are used to treat stomach ulcers, tumors, and diseases of the spleen and liver (Kirtikar and Basu, 1935). The latex of this plant has toxic effects on insects and fungi (Cleverson et al., 2016; Ramos et al., 2014) and harmful microorganisms (Shobowale et al., 2013; Asma et al., 2019), and effectively heals colon inflammation (Vijay et al., 2019) and swelling and aching of joints (Dewan et al., 2000) as well. The aqueous extract of this plant contains an alkaloid, flavonoid, glycoside, terpenoid, tannin sterol, and saponin (Asmaa et al., 2019).

Genetic and environmental factors and their interactions are the most important factors affecting plants' secondary chemical compounds. The most important environmental factors that greatly affect the quantity and quality of plant active ingredients are light, temperature, irrigation, location height (Omidbeigi, 2005), and soil chemistry (Vaičciulyte et al., 2017). By investigating the effect of climate on certain flavonoids of the hawthorn shrub (Crataegus spp.), Hematti et al. (2007) showed that there are interaction effects between location and characteristics. They concluded that the amount of quercetin in all organs of hawthorn in Kelardasht, Mazandaran, was higher than that of Gorgan. Jelena et al. (2019) reported that according to geographical location, all samples of Thymus pannonicus collected from natural habitats contain a higher amount of essence in comparison to cultivated plants. In another investigation conducted through the extraction and measurement of flavonoid, kaempferol, and quercetin compounds in the petals of ten genotypes of Damask rose in western Iran, Jaymand et al. (2009) found that the best locations regarding the amount of kaempferol and quercetin were West Azerbaijan, Ilam, and Ardabil, respectively. Tajali and Khazaeipoor (2002) investigated the effect of altitude on the total phenol content and flavonoids of the Crataegus microphylla plant and stated that this plant contained the highest amount of the mentioned compounds at 1000 m altitude, as compared to plants that had grown at low altitudes. By investigating the ecotype diversity of Thymus daenensis in Isfahan and Chaharmahal-and-Bakhtiari provinces, Karimi et al. (2011) found that altitude increase had a positive effect on the amount of thymol and had no significant effect on carvacrol. They reported the highest amount of thymol in the Sheikh Shabãn sample at an elevation of 2747 m above sea level, and the highest amount of carvacrol was that of the Larak sample at an elevation of 2370 m above sea level. Nutrient elements significantly affect each other, which eventually impacts plant growth and productivity (Facchinelli et al., 2001). By investigating the effect of environmental factors on the level of antioxidants and phenolic and flavonoid compounds in the walnut plant, Ghasemi et al. (2011) reported that the highest level of phenolic and flavonoid compounds was achieved at the highest altitude and lowest daily average temperature.

The environment is known as the most important factor affecting the expression level of secondary metabolite biosynthesis genes in medicinal plants (Saharkhiz, 2002). Dowling et al. (1986) reported that the coverage percentage of Acacia heterophylla increases by increasing the organic matter, N, S, K, P, exchangeable Ca, and soil depth. Therefore, recognizing the factors that influence the quality and quantity of medicinal plants' active ingredients is of great importance. Considering the aforementioned facts, the present study investigates morphological, nutritive, and phytochemical characteristics, as well as the soil properties of locations where calotrope are established, in certain natural habitats of Baluchestan, Iran.

2. Materials and methods

2.1. Site description

Sistan and Baluchestan Province includes Baluchestan in the south and Sistan in the north, with a 181785 km2 area, twenty cities, and comprising over 11% of Iran. The Baluchestan region, with a total of 15 cities, comprises two districts of Sarhad (including Zahedan, Mirjaveh, and Khash cities) and Makran (including Saravan, Iranshahr, Nikshahr, Chabahar, Sarbaz, Konarak, Delgan, Mehrestan, Sib va Sooran, Qasr-e-Gand, Fanouj, and Bampour) (Figure 1).

To determine the distribution spots of Calotropis procera in Baluchestan, the habitat range of this plant was first identified using reference (Mozafarian, 2004), interviewing the Natural Resources Department experts of various cities, and field surveys. The majority of calotrope habitats are located in the Makran district in the southern region of Baluchestan.

Figure 1. Geographical situation of Sistan and Baluchistan province, Iran. Studied cities are shown on the map

This research was conducted as a completely random nested design with three repetitions and four treatments (throughout four cities) during February/March (flowering season) of 2014. The study was carried out in Mand-e Bala and Sarzeh villages of Iranshahr city; Negour and Nalent villages of Chabahar city; Bandaan and Bent villages of Nikshahr city; central region and Baluchkan Village of Khash city. To determine the soil properties of various habitats, soil samples were taken from zero to 60 cm depths and transferred to the laboratory. Leaves, flowers, roots, and calotrope latex were also collected by performing field surveys in various regions. Three plant populations were randomly sampled from each region, and their morphological characteristics were examined. Three leaves were collected from the mid-section of branches to assess leaf properties, and three inflorescences were collected from the distal part of the stem to assess flower properties. To examine root properties, three profiles were dug out in the target habitats and three samples were collected from rootlets at 30-60 cm depths. Finally, all three organs, including root, leaf, and flower, were shadow dried and prepared for the experiments to determine element percentages. To collect the latex, several leaves were chosen from the mid-sections of the branch, and after detaching them from the branch, the latex excreted from the leaf was immediately collected in dark 10-milliliter glass vials. To measure the active ingredients of the organs, the samples collected from the two regions of each city were combined and sent to the laboratory. While collecting plant samples, a GPS device was used to record the geographical location of the region, including elevation from sea level, longitude, and latitude (Table 1), and the meteorological information of the studied cities was obtained from weather stations (Table 2).

Table 1. Geographical location of the studied cities

Row	City	Area in city	Longitude	Latitude	Altitude (m)
1	Iranshahr	Sarzeh	61° 42′	28°13′	560
2		Mand-e Bala	61° 38′	28° 06′	416
3	Chabahar	Negour	60° 10′	25° 42′	11
4		Nalent	61° 24′	25° 45′	10
5	Nikshahr	Bent	61° 31′	27° 16′	340
6		Bandaan	61° 03′	27° 11′	432
7	Khash	Central Khash	61°21′	28° 21′	911
8		Baluchkan	61° 48′	28° 30′	815

Table 2. Characteristics and average climate of the studied cities

City	Climate	Av. An. Temp. (°C)	Av. An. Rain. (mm)	Av. An. Hum. (%)	Av. An. No. Frost Day	Av. An. No. Day. Above 35°	Av. Max. Da. Temp. (°C)
Khash	Semi-dry and moderately warm	20	153.0.	31	20	110	28.4
Iranshahr	Warm and dry	26.9	116.8	31	1	187	35
Nikshahr	Hot and dry desert	28	175.2	36	0	231	34
Chabahar	Hot and dry beach	26.3	114.3	76	0	13	30.2

2.2. Measurements

The soil texture was determined using the Hydrometer Method and via the Soil Texture Triangle (Klute and Dirksen, 1986). Thomas's method was implemented to measure soil pH (Thomas, 1996). To measure K and Na, saturated extracts were prepared from the soil samples, and K and Na were measured using a flame photometer device (model- Cl361) (Mostofi and Najafi, 2005). Soil P was measured using the Olsen method (Olsen and Sommers, 1982), and N was measured via a Kjeldahl apparatus (model V40) (Page et al., 1982).

Morphological characteristics, including branch length, internode distance, leaf length, leaf width, inflorescence length, inflorescence width, number of leaves, and stem diameter were measured using equipment such as a scaled ruler and a caliper device.

The phosphorus content of plant tissues was determined using the digestion method and a spectrophotometric device (Sadou et al., 2007), the potassium content was determined using the flame emission method and a flame photometer device (Sadou et al., 2007), the nitrogen content was determined using the Kjeldahl apparatus (Page et al., 1982), and the other elements were determined using an atomic absorption apparatus (Baker and Amacher, 1982).

A gas chromatography device (Agilent 6890) was used to identify the active ingredients. The device consisted of a 30-m column with an internal diameter of 0.25 mm and a layer thickness of 0.25 μm of the BPX5 kind. To identify the constituent compounds of the essence, one μl of the sample that had been diluted by n-Hexane was injected into the GC/MS device. The temperature program of the column was set as follows: Initial oven temperature of 50°C and pause at this temperature for 5 minutes, a temperature gradient of 3°C per minute, increasing the temperature up to 240°C and then with a speed of 15°C per minute, increasing the temperature up to 300°C and a 3-minute pause in this temperature and the response time was 75 minutes. The injection chamber temperature was 290°C as a split of 1 to 35, and helium gas was used as the carrier gas with a flow speed of 0.5 mm per minute. The mass spectrometer (model Agilent 5973) had an ionization voltage of 70 electronvolts, the ionization method was EI, and the ion source temperature was 220°C. The mass scanning range was set at 40 to 500. Chemstation software was used. Spectrum identification was carried out using their retention index and comparing it to the indices available in reference books and articles, using the mass spectrums of standard compounds, and using the information available at the computer library (McLafferty and Stauffer, 1989; Adams, 2004).

2.3. Statistical analysis

The data from this study were first tested for normality before being analyzed using a nested design with three repetitions. Data analysis was performed by SAS software (version 9.2), and means were compared via Duncan's multiple range test at a 5% significance level.

3. Results and Discussion

3.1. Soil edaphic properties of calotrope habitats

The edaphic properties of habitat soils investigated in this study included EC, pH, the amount of N, P, K, and Na, and the percentage of sand, silt, and clay. The level of pH, EC, Na, clay, and silt in the soil did not reveal a significant difference between cities, but the impact of city regions on all properties of the soil of the calotrope shrubs was significant at a 1% level (Table 3). The calotrope habitat soil of the Bent region in Nikshahr city had the maximum level of K and P (Table 3). The highest level of N was measured in the Negour region of Chabahar city, and the highest level of Na was measured in the Mand-e Bala region of Iranshahr city (Table 4). The level of Na was higher in Iranshahr city. A possible reason for higher soil salinity in Iranshahr habitats may be the excessive usage of water in this city which is due to the excavation of deep wells for agricultural utilization, and water salinity results in soil salinity.

Table 3. Analysis of variance of soil properties in different habitats of calotrope

S.V.	df	EC	pH	N	P	K	Na	Sand	Clay	Silt
City	3	2.81ns	0.52ns	0.01**	1.62**	156.39*	0.027ns	96.66**	10.26ns	61.95ns
Area in city	4	1.86**	0.64**	0.007**	0.7**	79.18**	0.032**	168.86**	6.42**	147.69**
Error	24	0.00001	0.00001	0.000003	0.000003	0.001	0.0001	7	1.16	0.15
CV (%)	-	0.27	0.055	1.63	0.46	0.094	3.45	7.37	8.43	3.82

*, ** and ns are significant at 5%, 1% and no difference, respectively.

Table 4. Mean Comparison of soil properties in different habitats of calotrope

City	District	EC (dS m^{-1})	pH	N (%)	P (ppm)	K (ppm)	Na (ppm)	Sand (%)	Clay (%)	Silt (%)
Iranshahr	Sarzeh	2.06 a	8.28a	0.14b	0.18 e	36.61c	155.77b	84.45a	10.815c	4.74e
	Mand-e Bala	2.38 f	7.06c	0.14b	0.21c	30.31f	156.07a	71.32d	12.18bc	16.50b
Chabahar	Negour	0.92d	8.18b	0.16a	0.11f	32.57e	108.91f	69.05d	15.11a	15.28c
	Nalent	0.93e	8.18b	0.14b	0.21d	33.17d	110.36e	78.75c	12.05b	9.04d
Nikshahr	Bent	1.62b	8.19b	0.07d	1.10b	41.12a	123.21c	83.28ab	13.14b	3.58f
	Bandaan	1.61c	8.18b	0.10c	1.05a	41.10 a	122.25d	80.10cb	12.51bc	7.39d
Khash	Central Khash	0.64g	8.18b	0.06e	0.15g	30.1g	98.11g	69.47d	12.26bc	18.27a
	Baluchkan	0.64g	8.19b	0.05f	0.09g	30.05h	98.23g	82.60abc	12.14b	5.26e

The common letters in each column indicate no significant difference.

Soil is among the most important environmental factors that play a major role in the distribution and density of plant vegetation. In fact, soil characteristics are the result of the effects of other environmental factors over a period of time. Soil types determine the plant species, and plants affect the nutrient element cycle and spatial properties of the soils (Kouchaki and Azizi, 2005). The highest amount of soil N was measured in the Negour habitat of Chabahar city, while the lowest amount of this element was measured in Khash city. One way of N removal from the soil is the leaching process (Tabatabaie, 2013). The highest amounts of K and P were measured in the Bent region of Nikshahr city. Considering the impact of soil texture on the absorption level of elements in the soil, the higher amounts of these two elements in the soil of Nikshahr city habitat can be attributed to the higher clay content in the soil of this city, such that the elements' positive charges are attracted by clay's negative charge. After evaluating the elements Zn, Cd, Na, Mg, K, Fe, and Ca in various species, Mohamed et al. (2003) concluded that the concentration level of elements differs in various species, as well as in similar species from different habitats. Ankita et al. (2016) also reported that the N, P, and K levels varied in different soil samples, and their amounts were in the ranges of 7.40–89.70 mg gm-1, 14.38–82.75 mg gm-1, and 0.76–5.46 mg gm-1, respectively.

The highest amount of Na was measured in the Mand-e Bala region of Iranshahr city. Jafari et al. (2006) found that special relationships exist between the type of plant and soil characteristics in the Howz-e Soltan region of Qom province, and the roles of salinity and texture were more influential than the other factors. Soil texture plays a role in the establishment of plant vegetation through the humidity level and the aeration of available nutrients (Jafari et al., 2002). Investigating some soil characteristics such as texture, EC, sodium adsorption ratio, organic matter, gravel percentage, and plant attributes such as density and canopy surface in 35 calotrope habitats in the southern pastures of Fars Province showed that the calotrope shrubs could grow in a wide range of soil conditions, such that up to 50% of surface gravel, up to 10 dS m-1, up to 8.56 sodium adsorption ratio, heavy, moderate to light textures, and soils with a low content of organic matter (0.1%) are not limiting factors for plant establishment (Sadeghian et al., 2010).

3.2. Morphological characteristics of calotrope in various habitats

The studied morphological characteristics of calotrope included branch length, internode distance, leaf length, leaf width, inflorescence length, inflorescence width, number of leaves, and stem diameter. A statistical difference was observed among all characteristics, except inflorescence length, in the studied cities (Table 5). Differences in all characteristics were also significant at a 1% level among the various habitats (regions of a city). According to the data presented in Table 6, Nikshahr city had the highest branch length, leaf length and width, inflorescence length, and stem diameter among all the studied cities.

Table 5. Analysis of variance of morphological traits of calotrope in different habitats of Baluchestan mean of squares

S.V.	df	Branch length	Intermediate distance	Leaf length	Leaf width	Flower length	Flower width	No. of leaves	Stem diameter
City	3	239.21**	15.5**	32.21**	13.02**	5.68ns	2.63**	672.74*	139.6**
Area in city	4	119.63**	7.35**	30.65**	9.76**	10.56**	3.35**	806.53**	65.36**
Error	24	0.23	0.01	12.55	1.07	2.95	0.55	11.05	0.07
CV (%)	-	0.24	2.24	2.09	12.43	19.96	11.04	5.63	1.82

*, ** and ns are significant at 5%, 1% and no difference, respectively.

Table 6. Mean Comparison of morphological traits of calotrope in different habitats of Baluchestan

City	District	Branch length	Intermediate distance	Leaf length	Leaf width	Flower length	Flower width	No. of leaves	Stem diameter
Iranshahr	Sarzeh	194.25e	7.0a	8.62e	5.45f	7.62c	6.89c	73.00a	16.85c
	Mand-e Bala	195.0d	7.12a	13.25b	7.51e	10.87a	8.11a	64.500b	15.75d
Chabahar	Negour	197.0c	3.97d	11.75 c	9.06c	8.0bc	6.98bc	63.0b	8.35e
	Nalant	198.25b	4.05d	8.62e	7.51e	8.75b	7.02bc	26.0d	7.80f
Nikshahr	Bent	200.25a	3.95d	15.87a	10.61a	7.37c	5.37d	63.75b	17.12a
	Bandaan	200.50a	3.92d	13.62b	8.23d	11.37a	7.10b	65.0b	16.89c
Khash	Central Khash	187.25f	5.05b	11.37dc	9.49b	7.62c	6.92bc	61.75b	17.02b
	Baluchkan	186.25g	4.6c	10.25d	8.89c	7.25c	5.40d	55.25c	16.89c

The common letters in each column indicate no significant difference

Regarding the majority of studied characteristics, Nikshahr city, with about 450 m of elevation above sea level, had the maximum measured values (Table 6). The calotrope species in Sistan and Baluchestan Province is distributed at 10-9110 m above sea level. One of the influential factors regarding the distribution or lack of distribution of plants is elevation from sea level. With the rise or decline of elevation, habitat conditions change, especially in terms of climate, and plants become established in an elevation range based on their ecological needs (Tabataba'i and Qasriani, 1992). Also, the amount of P and K nutrient elements in caloptrope decreased with elevation, and this factor can also be a reason for the reduction of morphological characteristics in high altitudes. These results are similar to those of Mellati (1995) and Salarian et al. (2009) that reported a significant relationship between elevation above sea level and canopy density and percentage. According to meteorology data (Table 2), increased altitude leads to reduced temperature, such that mean annual temperature declined from 26.3°C to 20°C in the highest altitude. Since the growth and development of plant organs decreased in low-temperature conditions, many of the morphological characteristics investigated in this study such as branch length, leaf length, and inflorescence length and width decreased. Najjar Firozjaee et al. (2014) investigated the effect of altitude on morphological and biochemical properties of nettle (Urtica dioica) leaves in Mazandaran and Gulestan Provinces and stated that high altitudes decreased the morphological characteristics of nettle, while the phytochemical properties of nettle showed better performance in high altitudes. The increase of factors such as organic matter, N, S, K, P, exchangeable Ca, and soil depth increased the canopy percentage of Acacia harpophylla (Dowling et al., 1986). The variability of soil properties in pastures may be effective on the better performance of soil in absorbing nutrient elements and promoting plant growth (Shukla et al., 2004) and these elements vary and differ depending on the type of plant and chemical properties of soil such as pH (Gilliam and Dick. 2010). Morphological characteristic identification is considered the first step of evaluating diversity and genetic structure (Zanella et al., 2011) and is also indicative of the environmental conditions and factors in each region and city. Pouyanfara et al. (2018) morphologically examined 65 samples from seven wild populations and six cultivated populations of Melissa officinalis L. from five provinces in Iran and concluded that there is great morphological diversity among the populations of different regions. The effect of habitat on morphological diversity of Melissa officinalis L. (Hadj Ali et al., 2012), Plantago psyllium (Shahriari et al., 2018), Salvia fruticosa Mill. (Peggy et al., 2020), Glycrhiza glabra (Esmaeili et al., 2020), and Chamomilla recutita L. (Ieva et al., 2020) has also been investigated. The higher number of most morphological characteristics in Nikshahr city may be due to the higher levels of K and P, as well as the percentage of sand in the soil texture of this city's habitats. The Baluchkan region of Khash city with 911 m above sea level has the least values regarding the majority of characteristics, and the necessity of tending to Nikshahr, Iranshahr, and Chabahar cities is emphasized for the preservation of these valuable genetic resources.

3.3. The percentage of elements in different organs of the calotrope in various habitats

The analysis of the variance table for element percentages showed that the Na, Ca, K, P, N content of leaf, flower, and root × city were significant at a level of 1%. Leaf P and flower K were not significant in the region × city interaction, whereas flower Na and root K were

Table 7. Analysis of variance of percentage of elements of different organs of calotrope in different habitats of Baluchestan

Elements		N			P			K		
S.V.	df	Leave	Flower	Root	Leave	Flower	Root	Leave	Flower	Root
City	3	0.651010**	3.02881146**	0.42665313**	0.00082**	0.00058983**	0.00013128**	16065.40865**	3455.12448**	982.488646**
Area in city	4	1.05398**	1.01984063**	0.06050313**	0.00004847ns	0.00007538**	0.00008434**	1220.53219**	512.36344ns	123.102812*
Error	24	0.08166458	0.03211562	0.00493646	0.00002553	0.00000212	0.00000951	69.54073	293.65885	31.837187
CV (%)	-	14.30	10.51	10.53	16.86	6.70	10.71	4.13	6.88	8.99

*, ** and ns are significant at 5%, 1% and no difference, respectively.

Table 7 (Continued)

Elements		Ca			Na		
S.V.	df	Leave	Flower	Root	Leave	Flower	Root
City	3	0.00117**	0.00018878**	0.00214275**	15362.12792**	3787.27917**	2334.546146**
Area in city	4	0.00012441**	0.00009878**	0.00016931**	667.01250**	259.02937*	303.051562**
Error	24	0.00002732	0.00000195	0.00000902	9.94562	71.77688	32.714271
CV (%)	-	6.00	7.40	10.53	3.01	11.34	9.51

*, ** and ns are significant at 5%, 1% and no difference, respectively.

Table 8. Mean comparison of percentage of elements of different organs of calotrope plant in different habitats of Baluchestan

City	Area in city	N (ppm)			P (ppm)			K (ppm)			Ca (ppm)			Na (ppm)		
		Leave	Flower	Root	Leave	Flower	Root	Leave	Flower	Root	Leave	Flower	Root	leave	flower	Root
Iranshahr	Sarzeh	1.42d	1.69dc	0.66c	0.03cb	0.01bc	0.031b	152.90e	236.30bc	52.50d	0.04a	0.026a	0.015f	159.32a	96.75a	89.72a
	Mand-e Bala	1.80cd	2.19ba	0.44e	0.03c	0.01c	0.022c	136.87f	258.77b	58.50dc	0.03b	0.022a	0.022e	132.02c	96.85a	73.90b
Chabahar	Negour	2.38ab	2.07bc	1.13a	0.02ed	0.01de	0.024c	202.65c	256.80b	68.65b	0.02c	0.021a	0.056a	143.75b	78.67ba	68.20bc
	Nalant	1.50d	2.01bc	0.88b	0.01e	0.01e	0.025c	180.77d	254.60b	63.85bc	0.02c	0.017b	0.045b	133.10c	98.90a	61.20c
Nikshahr	Bent	1.70cd	2.70a	0.52e	0.04a	0.03a	0.036a	268.27a	279.70a	70.05b	0.03b	0.012c	0.033c	48.85f	55.47c	51.45d
	Bandaan	2.71 a	1.38dc	0.50e	0.03ab	0.02ba	0.032ab	233.62b	262.52a	83.37a	0.04a	0.024a	0.028d	70.15e	45.20c	33.95e
Khash	Central Khash	2.42ab	0.70d	0.63cd	0.02ed	0.02dc	0.033ab	229.42b	228.85c	53.87d	0.02c	0.014b	0.008g	77.00d	63.80b	51.47d
	Baluchkan	2.02cb	0.86d	0.55ed	0.02cd	0.02de	0.024c	206.95c	214.02d	50.77d	0.01d	0.011c	0.019e	72.35e	61.95bc	50.82d

The common letters in each column indicate no significant difference.

significant in the region × city interaction at a level of 5%. The remaining characteristics were significant at a 1% level in the region × city interaction (Table 7). The comparison of means for the level of elements in the four studied cities indicated that the highest amount of leaf N was measured in the central region of Khash, and the lowest amount was measured in the Sarzeh region of Iranshahr. In most cases, plants dedicate more N to the leaves. One of the reasons for a higher amount of N in the leaves, compared to other organs, is increased photosynthesis under the conditions of reduced stomatal conductance and water availability (Kerkhoff et al., 2005). Nitrogen is mainly transferred via mass transfer, intermediated by transpiration. The highest amount of P and K in all three organs was measured in the

Bent region of Nikshahr city. The highest amount of leaf Ca was measured in the Bent region of Nikshahr and Sarzeh region of Iranshahr in equal amounts, whereas the highest amount of flower Ca and root Ca was measured in the Sarzeh region of Iranshahr and the Negour region of Chabahar, respectively. Also, the highest amount of leaf and root Na was measured in the Sarzeh region of Iranshahr, and the highest amount of flower Na was measured in the Nalent region of Chabahar. The lowest percentages of elements including, N, P, and K in all organs of calotrope were measured in Iranshahr city (Table 8). The observed differences of elements in various organs of calotrope, among the cities and the city regions may be due to the different ecological traits of the regions such as temperature, humidity, elevation from sea level, plant growth stage, harvesting season of plant, and other soil-related and geographical factors. Harati Rad et al. (2017) reported that different habitat conditions resulted in a significant difference of Ca, N, K, Zn percentages, and soluble carbohydrate of colocynth seeds at a level of 1%, such that the highest amount of seed ash and carbohydrate was observed in Zabol city and the highest level of elements was observed in Iranshahr city. Investigating the Dracocephalum moldavica L. plant in 5 different habitats of Iran (Salmas, Urmia, Khoy, Maragheh, and Tabriz), Yousefzadeh et al. (2018) concluded that the highest level of N (2.3%) and P (0.22%) were measured in the flowering branches of this plant in Salmas city.

Table 9. Chloroform extract of calotrope leaf leachate in different habitats of Baluchestan

Row	Substance name	Peak No.	Retention time (Min)	Iranshahr	Khash	Chabahar	Nikshahr
1	2-Tert-butyl-4-(1,1,3,3-tetramethylbutyl) phenol	1	10.72	1.1	0.9	--	1.4
2	2-Methylene-1,5-pentanediol	2	12.81	1.6	2.2	2.7	4.6
3	2,6,10-Trimethyl,14-ethylene-14-pentadecne	3	13.16	23.1	20.1	27.2	14.5
4	Bicyclo[4.1.0]heptane, 7-butyl	4	13.42	1.5	1.9	2.6	5.1
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5	13.59	5.7	7.1	3.8	10.1
6	Nonanoic acid, 7-methyl methyl ester	6	13.83	3.1	2.6	1.7	0.9
7	5-Norbornene-2-carboxylic acid	7	14.10	1.4	0.9	3.6	1.1
8	Bis-(3,5,5-trimethylhexyl) ether	8	14.39	2.5	3.7	1	0.8
9	Methyl 9-octadecenoate	9	14.55	9.3	12.6	14.3	16.7
10	6-Octen-1-ol, 3,7-dimethyl	10	14.91	3.4	5.1	2	1.1
11	(+)-(1r,2r)-2,7,7-Trimethyl-3-oxabicyclo[4.1.1.]Octan-4-one	11	15.24	--	1.7	--	1.8
12	6(e),9(z),13(e)-Pendectriene	12	15.42	1.9	1.5	1	2.3
13	Beta-L-galactopyranose, 6-deoxy-1,2,3,4-tetrakis-O-(trimethylsilyl)	13	15.60	9.2	7.3	5.7	4.4
14	D-xylopyranose, 1,2,3,4-tetrakis-o-(trimethylsilyl)	14	15.83	4.3	2.9	2.7	3.5
15	Bis-(3,5,5-trimethylhexyl) ether	15	16.41	1.5	1.8	1.4	2.5
16	Phytol	16	16.82	4.8	3.5	4.1	5.6
17	1,2-Benzenedicarboxylic acid, diisooctyl ester	17	17.69	1	1.7	1.1	2
18	1-(2-Hydroxyethoxy)-pentadecane	18	17.93	4.1	2.7	1.5	1.1
19	2,6,10-Dodecatrienoic acid, 7,11-dimethyl-3-(trifluorpmethyl)-, methyl ester	19	19.78	1.2	1.6	1.3	1.5
20	Squalene	20	26.02	1.7	1.9	1.1	1.6
21	alpha-Amyrin	21	35.73	8.3	7.1	5.5	7
22	beta-Amyrin	22	37.03	2.9	3.3	7.9	8.7
23	Oleanolic Acid	23	38.41	4.1	5.2	6.1	1.6

Referring to Table 4, it is evident that the calotrope habitat soil in Iranshahr has the highest level of EC and Na. A nutritive imbalance in saline soils is among the main reasons for the reduction of plant growth and productivity. Studying the effects of salinity on clover has shown that increased salinity results in the reduction of the total dry weight of shrub, N percentage, NUE, and K concentration, whereas Na percentage increases in the aerial organs. Furthermore, the amount of elements absorbed by plant roots from the soil varies depending on the type of plant and the chemical properties of the soil, such as pH (Gilliam and Dick, 2010). However, it should be noted that the deficiency of an element in the plant does not indicate the deficiency of that element in the soil; the same element may exist at adequate levels in the soil, but its absorption does not occur due to the lack of required ecological conditions. The soil of Nikshahr city is more alkaline, and the level of its leaf K and Ca is higher than the other cities. The different results obtained from the studied species indicate variations in the absorption level of nutrient elements among the various organs of the studied species, such that the leaf has a higher absorbing capability for the majority of the investigated elements in comparison to the flower and the root.

3.4. The impact of habitat on the active ingredients of various calotrope organs

To measure the active ingredients of various calotrope organs in each habitat, a combinational sample from the two regions of each city was assessed. Examining the ingredients with GC\MS showed that 42 compounds have been identified in the extract of aerial organs and root of calotrope. The main components of active ingredients in the ethanolic extract of aerial organs and root of calotrope

Table 10- Percentage of active ingredients of ethanolic extract of shoots and roots of calotrope in Baluchestan habitats

Row	Substance name	Peak No.	Retention time (Min.)	Aerial part				Root		
				Iranshahr	Khash	Chabahar	Nikshahr	Iranshahr	Chabahar	Nikshahr
1	Tyrantone	1	5.33	5.3	3.3	3.7	3.1	0.8	0.6	-
2	Hydroxy methyl -2,pentanone	2	5.74	-	-	2.6	1.9	4.1	2.9	5
3	2-methyl 1,3-Buten-2-ol	3	6.52	1.4	1	-	-	0.5	1.9	1.6
4	Alpha-Thujene	4	7.48	2.1	0.7	2.9	2	1.6	2.2	0.9
5	Nonanal	5	8.02	1.3	1.6	5.2	5.6	7.7	6.1	8.1
6	Benzyl alchohol	6	8.74	1.7	2.1	-	1.3	5	8.7	7.2
7	Butene-2,2,-dimethyl	7	9.55	6.5	4.7	6.8	7.7	2.1	3	1.8
8	Myrcene	8	10.61	2.2	1.3	2.5	0.9	4.7	2.9	5.5
9	Catechin	9	11.25	6.3	4.2	1.5	5.5	1	0.8	1.3
10	1,2-Benzen dicarboxilic acid	11	11.78	1.7	2.9	0.7	1.8	1.8	2.4	2
11	Alpha-Phellandren	11	12.15	2.1	3.7	2.2	1	2.2	1.7	1.3
12	Epicatechin	12	12.63	2.9	2.7	3.3	2.1	-	0.6	-
13	1-Dodecene	13	13.08	2.2	1.8	0.9	-	1.1	1.8	1.6
14	3-Hexanol	14	13.55	1.4	2.8	1.6	1.1	3.7	2.9	0.8
15	Fumaric acide	15	13.97	-	-	1.2	0.9	-	-	-
16	1,8-Cineol	16	14.30	1.1	3	2.2	1.3	2.1	2.6	3.6
17	1-Tetradecene	17	14.83	-	-	-	-	7.9	5.9	8.3
18	Hexadecanoic acide methyl ester	18	15.22	5.4	7.6	3.4	5.1	1.8	2.5	1.3
19	9-Octadecenoic acid	19	15.53	3.2	2.9	6.5	8.3	-	-	-
20	Ethyl-9,12-Octadecanoate	20	15.91	-	3.3	1.6	-	2.4	3.3	1.8
21	Alpha-D-glucopyranoside	21	16.20	2.2	1.1	0.8	1.4	2	1.5	2.7
22	1,2,3,4-Tetrakis-o-pentopyranose	22	16.63	1.8	4	6.3	2.6	1.7	2.1	1
23	1,3-Hexanediol,2-ethyl	23	17.04	-	-	1.1	0.7	1.5	-	-
24	Cyclohexanol-3-methyl	24	17.44	3.5	1.7	0.9	1.5	2	1.4	1.8
25	1-Octanol, 3-7-dimethyl	25	17.72	2.4	4.3	-	1.1	-	1.1	0.8
26	3-ethyl- 1-tetradecene	26	18.03	-	0.8	-	-	8.1	5.8	6.7
27	P-Cumaroyl hexose	27	18.80	0.9	1.7	1.1	1.6	1.7	2.2	1.4
28	Rutin	28	20.06	2.3	1.4	2.8	2.5	2.5	2.3	1.7
29	Tridecene	29	21.78	2.6	4.2	3.1	4.7	0.8	0.7	-
30	Isobutylnonane	30	22.26	1.4	2.2	1	-	-	2.1	1.7
31	3-Octadecene	31	22.31	2.1	1.8	-	1.3	2.9	1.2	1
32	Myrcetin	32	23.45	0.9	2.4	0.8	2.4	1.1	1.7	0.8
33	1-pentadecene	33	25.33	6	4.1	6.7	3.9	2.2	0.9	1.4
34	Pentadecanoic acid	34	26.24	3.8	-	-	5.8	4.3	6.7	7.1
35	Hepta decene	35	27.04	2.1	1.8	1.5	3.1	0.8	1.3	0.9
36	Luteolin	36	28.55	3	2.1	1	1.7	-	-	-
37	9-Octadecenamide	37	30.6	4.6	5.7	3.9	6.5	1.2	1.7	1.4
38	Ethyl linoleate	38	31.11	2.8	1.3	2	1.7	2	1.8	2.7
39	Apigenine	39	31.74	3.1	2.7	1.8	1.1	1.4	2.1	1.5
40	Docosane	40	31.90	-	-	0.8	0.9	6.2	7.7	4.6
41	Kampferol	41	35.21	2.4	1.3	4.7	3.6	1.9	1.5	2.6
42	β-caryophyllene	42	37.10	1.2	1.5	4.4	3.8	2.6	1.7	1.9

were nonanal (7.7% in root and 5.6% in the aerial organs, collected from the Nikshahr region), benzyl alcohol (8.7% in the root in Chabahar city), butene-2,2,-dimethyl (7.7% in the aerial organs in Nikshahr), 1-tetradecene (7.9% in the root in Iranshahr and 8.3% in the root in Nikshahr), 9-Octadecenoic acid (8.3% in the aerial organs in

Nikshahr), 3-ethyl-1-tetradecene (8.1% in root in Nikshahr), and pentadecanoic acid (7.1% in root in Nikshahr).

Also, 23 compounds were identified in the latex extracted from calootrope leaves. Among the active ingredients, the amount of 2,6,10-trimethyl,14-ethylene-14-pentadecane measured in Chabahar, Iranshahr, Khash, and Nikshahr was 27.2, 23.1, 20.1, and 14.5, respectively (Table 9). Also, the methyl 9-octadecenoate measured at 16.7%, 14.3%, 12.6%, and 9.3% in Nikshahr, Chabahar, Khash, and Iranshahr cities, respectively, was the second compound identified with the highest percentage. Overall, the maximum amount of most active ingredients identified in calotrope latex in Nikshahr and Iranshahr cities was measured at an altitude of 411–560.

Edaphic factors play a major role in the production of plants' secondary metabolites (Ankita et al., 2016). Numerous reports have revealed the positive effects of soil N, P, and K on the level of the essence and active ingredients of various medicinal plants (Omer et al., 2014 ; Sharma and Kumar, 2012; Zheljazkov et al., 2012 ;Ankita et al., 2016; Xiao-Dan et al., 2018). A reason for Nikshahr's prominence regarding the majority of active ingredients may be the higher content of P and K in the habitat soil of this city. However, investigating the effect of Salvia miltiorrhiza's secondary metabolite levels in some natural habitats of China with different topographies, Xiao-Dan et al. (2018) reported that the amount of active ingredients in this plant is more dependent on climatic factors rather than the quantity of soil micronutrients. Baghazadeh-Daryaii et al. (2017) evaluated the Morphological and genetic variations of the Iranian 101 genotypes of Christ’s-thorn (Ziziphus spina-christi (L.) Desf., Rhamnaceae from 12 habitats in the South of Iran. In total, 32 qualitative and quantitative characteristics were measured. Flavonoid quercetin and saponin content were measured for nine dominant populations. Maximum saponin content was related to the Shahdad sample (large leaves and fruits with least thorn density) with 2.6 μg ml−1. Maximum flavonoid quercetin content (3.66 mg g−1) was related to the Jahrom sample. According to Table 10, the amount of active ingredient compounds of aerial organs and roots of calotrope differs in various habitats. For instance, with the increase of elevation from sea level, the amount of benzyl alcohol in the root decreases, and the highest amount of this substance has been obtained from Chabahar city with the lowest elevation from sea level. Also, the level of butene-2,2,-dimethyl and 9-octadecenoic acid in the aerial organs of calotrope in Nikshahr city is the highest, whereas the lowest level of these active ingredients has been measured in the aerial organs of calotrope in Khash city, which has the highest elevation from sea level. The impact of altitude on the quantity of essences and type of active ingredients differs in various plants. Zargar et al. (2016) studied the Epimedium elatum for its active principle content at different habitats of Kashmir Himalayas. The results showed that the content of active principles in leaves varies significantly between plants growing at different habitats. The Icariin and Icariside-II yield (per plant) of wild populations significantly increased with a decreasein altitude of habitat. Investigating the level of active ingredients in six samples of Litsea coreana Levl. var. Lanuginose collected from various habitats, Zhao et al. (2018) concluded that altitude has the most impact on the essence level in the leaves of this plant.

The level of a plant's active ingredients is directly related to its biosynthesis, metabolism, and biological activity, which are all dependent on environmental and climate conditions (Barimani, 2008). The difference in type and percentage of essence constituent components could be the result of genetic or non-genetic variations in response to environmental differences in habitat ecosystems. In each habitat, one or more active ingredients were at higher levels compared to other regions, and, in general, the maximum amount of most identified active ingredients in calotrope latex was measured in Nikshahr and Iranshahr cities at 411–560 m above seed level.

According to the results, the best growing location for this plant for achieving optimum quantitative and qualitative efficiency of active ingredients is at an elevation of 510 m above sea level. Among the important factors influencing essence components are temperature and humidity (Salehi Surmaghi, 2008), and the higher levels of certain compounds in Chabahar city may be due to the higher levels of humidity. Plant growing location may affect the process of active ingredient formation through changes in temperature and humidity. It has also been reported that the quantitative and qualitative diversity of chemical substances in Helichrysum italicum ssp. Microphyllum is not only dependent on differences in habitat (mountainous and coastal) but also dependent on local events (Melitoa et al., 2016). Laurel et al. (1999) also reported that the highest level of hypericin accumulation in perforated St. John's-wort (Hypericum perforatum) is achieved when plant growth and production occur in regions with high relative humidity.

The higher levels of certain plant chemical compounds such as octadecanoate and alpha-phellandrene in altitudes of above 900 m indicate the plant's tolerance to cold and adverse conditions of higher altitudes and its capability of producing an optimal amount of certain medicinal active ingredients. Although in case the goal is to harvest a high percentage of active ingredients, it should be considered that selecting the plant from high altitudes and extremely cold regions is not suitable for the optimal production of active ingredients. In the present study, the difference in highest and lowest elevations from sea level in the eight studies habitats is 901 m. Hadian et al. (2011) studied on the essence obtained from the plants of 18 landraces of Zataria multiflora, collected from various regions of Iran, also showed that this plant's essence contains 56 compounds and the effect of habitat on the percentage of active ingredients was significant.

4. Conclusion

The effect of environmental factors on the absorption rate of substances by plants, morphology, and production of active ingredients in medicinal plants is very complicated and ambiguous, and the interpretation of results must be carried out with proper delicacy. All four studied cities possess the required conditions and favorable weather for the growth of calotrope plants. The maximum amount of the most active ingredients identified in the leaf latex of calotrope, which has the highest medicinal applicability in the Baluchestan region, was measured in Nikshahr and Iranshahr cities. The results of this study indicate that calotrope habitats in Nikshahr city, with an average elevation of 450 m above sea level and higher contents of P and K, are considered the primary habitats of this plant in Baluchestan.

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