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Comparative Physiological and Phytochemical Analysis of Two *Artemisia* Species (*A. annua* and *A. sieberi*) at Different Growth Stages in Tuskestan and Kalaleh Habitats, Golestan Province

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ABSTRACT

Artemisia spp. are widely recognized for their medicinal and aromatic properties, making the study of their phytochemical composition crucial for maximizing their potential applications. This two-year study (2021-2023) investigated the impacts of growth habitat and developmental stage on the phytochemical profiles of two *Artemisia* species (*A. annua and A. sieberi*). Plant materials were collected at three growth stages (vegetative, flowering, and seed formation stages) from two distinct habitats (Tuskestan and Kalaleh). Photosynthetic pigments, proline, total phenolics, flavonoids, anthocyanins, and essential oils were quantified using the established spectrophotometric and hydrodistillation methods. Variance analysis revealed significant effects of species, habitat, and growth stage on phytochemical content. *A. annua* generally exhibited higher photosynthetic pigment levels, reaching means of 20.67, 8.49, 29.15, and 4.73 $\mu g.g^{-1}$ FW for chlorophyll a, b, total chlorophyll, and carotenoids, respectively, during flowering in Tuskestan, while *A. sieberi* accumulated more proline, peaking at 1.75 $\mu mol.g^{-1}$ FW in Kalaleh. Anthocyanin content was also elevated in *A. sieberi*, reaching 3.3 mg $CG.g^{-1}$ FW during seed formation in Kalaleh. Total phenolic content reached 19.12 mg $GA.g^{-1}$ FW in *A. sieberi* during seed formation in Kalaleh, while flavonoid content peaked at 9.59 mg $QA.g^{-1}$ FW in *A. annua* during seed formation in Tuskestan. The essential oil content was significantly influenced by all factors, with *A. annua* demonstrating higher yields, reaching 2.34% during flowering in Kalaleh. Regression analysis identified significant relationships between the essential oil content and specific plant traits (total chlorophyll and total phenolics), soil properties (organic carbon), and climatic factors (altitude and average precipitation). GC-MS analysis of essential oil profiles revealed distinct compositional differences between the two species. The essential oils of *A. annua* and *A. sieberi* from the Tuskestan and Kalaleh habitats are characterized by Artemisia ketone as the dominant compound (5.32%–58.98%), with notable variations in other key compounds such as Borneol (16.28%-50.26%), α-fenchene (0.14%-10.75%), methyl chavicol, and linalool, showing distinct concentration patterns across growth stages. These results highlight the significant impact of ecological factors on the phytochemical composition of *Artemisia* species and suggesting potential implications for their targeted cultivation and utilization.

Keywords: *Artemisia,* Phytochemical composition, Growth habitat, Developmental stage, Essential oil

INTRODUCTION

Recently, there has been a significant increase in interest among scientists and pharmaceutical companies in the study of medicinal plants. The recent surge in the market is predominantly attributed to the increasing global demand for herbal crops. This trend has resulted in a flourishing market presence across various countries, including Germany, the United States, France, India, and China. Iran, with its unique and diverse climate and rich flora, has made substantial progress in promoting the use of medicinal plants, aligning its efforts with global initiatives aimed at harnessing the potential benefits of these natural resources [1, 2]. In this context, there is an urgent need for the global scientific community to engage in both applied and fundamental research. This research should focus on identifying native medicinal species, assessing their ecological requirements, documenting their natural habitats, and extracting active pharmaceutical and antioxidant compounds [3, 4].

Among the diverse range of medicinal plants, the genus *Artemisia* (L.) is the major in the tribe Asteraceae and is one of the most significant in the Asteraceae [5]. This perennial genus typically presents as a shrub and comprises nearly 430 identified species, of which 34 are found within the borders of Iran [6]. *Artemisia* species thrive in a variety of environments, forming dominant cover in pastures, mountains, and deserts across the country. These plants can be classified as annual or perennial, herbaceous, or semi-woody, with varying characteristics such as being hairy or hairless [7]. Notably, many *Artemisia* species are aromatic and often exhibit a strong fragrance, which enhances their appeal for both culinary and medicinal purposes. Pollination of these plants predominantly occurs through wind, a factor that contributes to their wide distribution. This genus is particularly recognized for its ability to produce terpenoids throughout all parts of the plant, making it a valuable source of essential oils that have been utilized for centuries [8, 9]. Essential oils extracted from *Artemisia* species are among the most important secondary metabolites. Historically, these oils have been valued for a wide range of properties, including antimicrobial, antioxidant, anti-inflammatory, soothing, digestive, and diuretic effects. Additionally, they play vital roles in preservation and pest control [10].

The influence of soil features and climatic factors on the spread of *Artemisia* spp. has been highlighted in various studies. Mohamadi and Rajaei [11] underscored the critical role of factors such as elevation above sea level, soil texture, and organic matter in shaping the populations of these species. Similarly, Bashari and Shahmoradi [12] emphasized the ecological significance of these parameters in their research on *A. sieberi* within the rangeland ecosystems of the Qom Province. Their findings indicate that this particular species flourishes at elevations ranging from 1,000 to 1,900 m, where annual rainfall varies between 100 and 260 mm. Furthermore, *A. sieberi* thrives in soils with varying textures and pH levels ranging from 7.2 to 8.3, commonly found in the medium to old alluvial deposits of the Qom formation. These insights underscore the fundamental role that both soil and climate conditions play in the successful growth and distribution of the *Artemisia* species.

Recent studies have explored the chemical profiles of essential oils take out from *A. sieberi* and *A. annua*, both of which are important in traditional Persian medicine. Houshmand *et al*. [13] conducted a thorough analysis using GC/MS, revealing major compounds such as camphor, sabinene, linalool, hydroxy dihydrolavandulyl acetate, and geraniol. Their research identified key chemical groups, including hydrocarbon and oxygen monoterpenes, particularly ketones, which emphasize the significance of these oils in Iran. According to findings by Zarei *et al*. [14], *A. sieberi* exhibits a broad presence across Iran's arid and semi-arid landscapes. Their study involved collecting samples, extracting essential oils via hydrodistillation, and analyzing them through GC-MS. Among the 72 identified compounds, the significant components identification of trans-thujone, cis-thujane, 1,8-cineole, camphor, santolinyl acetate, and cis-chrysanthenyl acetate occurred during the analysis. The application of chemometric methods then resulted in the formation of 6 different chemical groups.

This comprehensive examination emphasizes the substantial diversity and ecological importance of the *Artemisia* genus and highlights the necessity for further research into the impacts of climatic and geographical factors on its physiological and phytochemical traits. Such investigations are critical for elucidating the potential applications of *Artemisia* species in medicine and their contribution to sustainable resource management. The primary aim of this study was to investigate the effects of climatic and geographical factors on the physiological, biochemical, and phytochemical traits of two *Artemisia* species in Golestan Province. By analyzing essential oil composition and the variations in these traits across different growth stages, the research sought to enhance our understanding of how environmental conditions influence the growth and chemical profiles of these species. Ultimately, the study aimed to provide valuable insights into the cultivation and utilization of *Artemisia* species, particularly regarding their medicinal properties and essential oil production.

MATERIAL AND METHODS

Research Setting and Methodological Framework

This investigation occurred in two natural habitats in Golestan Province, Touskestan (H1) and Kalaleh (H2), during the years 2021-2023. Table 1 provides the climatic data for the habitats, and Table 2 outlines the soil's physicochemical properties. Throughout the two years, plants in the studied habitats were visited intermittently. Two species within the *Artemisia* genus, *A. sieberi,* and *A. annua*, were subjected to evaluation in this study. For each habitat, three plant samples per species were collected each year. After comparison with herbarium samples at the Natural Resources Research Department of Golestan Province and confirmation by a botanist, the examples were transferred to the test site for further investigation. Harvesting was conducted at three stages: peak vegetative growth (20–35 leaves), flowering, and seed formation using a completely random method from each habitat. To measure physiological and biochemical traits, including the content of photosynthetic pigments, proline, flavonoids, phenols, and anthocyanins, fresh plant samples were used, while dried samples were utilized to assess the essential oil levels.

Table 1 Climatic and geographic data of the study areas

Habitats	Longitude (°)	Latitude (°)	Average precipitation (mm)	Altitude (m)	Average annual temperature (°C)	Relative humidity (%)
H1	54.59	36.71	400	1100	13	60
H2	55.49	37.41	500	1350	15	65

H1: Tuskestan; H2: Kalaleh.

Table 2 Physicochemical properties of soil in the studied habitats

Habitats	EC (dS.m^{-1})	pH	Organic carbon (%)	P (mg.kg^{-1})	K (mg.kg^{-1})	N (%)	Zn (mg.kg^{-1})	Mn (mg.kg^{-1})	Fe (mg.kg^{-1})	Cu (mg.kg^{-1})	B (mg.kg^{-1})	Pb (mg.kg^{-1})	Cr (mg.kg^{-1})
H1	1.45	7.84	1.096	8.76	212	0.98	0.888	9.76	4.84	2.69	2.88	1.33	0.76
H2	1.95	7.6	0.96	7.56	169	0.786	0.745	8.93	3.611	2.087	2.25	1.56	0.99

H1: Tuskestan; H2: Kalaleh.

Table 3 Combined analysis of variance (mean squares) for biochemical and physiological traits of two species of *Artemisia* at different growth stages in Golestan province over two years

SOV	df	Chlorophyll a		Chlorophyll b		Total chlorophyll		Carotenoid		Proline		Anthocyanin		Phenol		Flavonoid		Essential oil	
		First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
Habitat (H)	1	84.81 ns	38.72 ns	22.01 ns	4.1 ns	193.21 ns	68 ns	0.06 ns	5.14 ns	0.21 ns	0.02 ns	0.16 ns	1.24 ns	4.29 ns	10.46 ns	19.84 ns	1.38 ns	0.21 ns	0.15 ns
Rep (H)	4	0.47	1.49	0.12	0.29	0.48	1.83	0.13	0.19	0.05	0.03	0.08	0.02	1.63	1.2	0.82	0.26	0.01	0.03
Species (S)	1	463.88 **	603.04 **	54.05 **	102.29 **	834.61 **	1202.05 **	7.64 **	28.15 **	2.2 **	1.43 *	3.17 **	8.86 **	23.22 **	340.07 **	16.96 **	98.58 **	2.18 **	4.33 **
H x S	1	18.62 *	25.35 **	2.8 ns	0.1 ns	35.84 **	28.59 *	1.4 ns	1.66 ns	0.06 ns	0.49 **	0.3 ns	0.03 ns	0.04 ns	13.8 *	5.27 *	3.28 **	0.005 ns	0.04 ns
Error S	4	1.46	0.55	1.78	1.11	1.34	2.25	0.42	0.23	0.03	0.02	0.05	0.02	3.16	1.45	0.37	0.04	0.05	0.04
Growth stages (GS)	2	2.79 ns	11.31 *	0.28 ns	0.39 ns	4.45 ns	14.67 ns	1.23 ns	1.61 ns	0.01 ns	0.1 ns	0.03 ns	0.09 ns	5.98 ns	6.78 ns	9.19 ns	2.48 ns	0.22 ns	0.11 ns
Hx GS	2	1.9 ns	0.58 ns	12.44 **	4.48 *	23.05 **	5.36 ns	3.88 **	0.1 ns	0.17 *	0.03 ns	0.24 *	0.1 ns	2.84 *	3.67 *	7.26 **	5.26 **	0.87 **	0.22 **
S x GS	2	10.15 ns	7.41 ns	0.23 ns	1.29 ns	7.71 ns	3.04 ns	0.05 ns	1.37 ns	0.04 ns	0.05 ns	0.06 ns	0.1 ns	2.4 ns	1.41 ns	10.69 ns	4.06 ns	0.03 ns	0.02 ns
H x S x GS	2	6.0 *	4.52 **	8.1 **	5.52 *	12.65 *	4.52 *	0.85 **	4.52 **	0.18 *	4.52 *	0.29 *	4.52 **	4.56*	4.82 *	10.33 **	4.52 *	0.25 **	4.52 *
Error	16	1.43	1.1	0.97	0.95	2.78	2.94	0.13	0.28	0.04	0.01	0.06	0.03	0.76	0.75	0.73	0.33	0.02	0.03
CV (%)	-	8.51	6.47	15.04	16.51	8.09	7.77	8.96	10.45	16.21	8.06	16.53	7.76	6.72	5.9	15.57	8.14	11.78	11.77

ns, *, and **: non-significant, significant at the 5% level, and significant at the 1% level, respectively.

Table 4 Comparison of mean interactive physiological and biochemical effects of two *Artemisia* species across different growth stages in various habitats of Golestan province (year 1)

Habitat	Species	Growth stages	Chlorophyll a (µg.g^{-1} FW)	Chlorophyll b (µg.g^{-1} FW)	Total chlorophyll (µg.g^{-1} FW)	Carotenoid (µg.g^{-1} FW)	Proline (µmol.g^{-1} FW)	Anthocyanin (mg CG.g^{-1} FW)	Phenol (mg GA.g^{-1} FW)	Flavonoid (mg QA.g^{-1} FW)	Essential oil (%)
H1	S1	G1	17.63 ± 1.31 b	7.63 ± 0.36 ab	25.26 ± 1.67 b	4.58 ± 0.42 ab	0.92 ± 0.09 d	1.17 ± 0.11 c	13.8 ± 1.05 a-d	8.48 ± 0.12 a	1.78 ± 0.12 ab
		G2	20.67 ± 0.53 a	8.49 ± 0.98 ab	29.15 ± 1.05 a	4.73 ± 0.22 a	0.99 ± 0.1 d	1.17 ± 0.04 c	13.6 ± 0.42 a-d	8.41 ± 0.98 a	1.33 ± 0.12 c
		G3	17.0 ± 0.63 bc	8.73 ± 0.77 a	25.76 ± 1.16 b	4.73 ± 0.22 a	0.93 ± 0.04 d	1.31 ± 0.13 bc	12.8 ± 1.16 bcd	4.97 ± 0.2 cd	1.27 ± 0.12 c
	S2	G1	13.17 ± 0.25 d	5.05 ± 0.54 d	18.21 ± 0.78 e	4.03 ± 0.28 bc	1.03 ± 0.06 d	1.24 ± 0.07 c	10.34 ± 0.11 e	4.32 ± 0.55 d	1.16 ± 0.11 cd
		G2	12.34 ± 0.23 d	8.76 ± 0.33 a	21.11 ± 0.14 d	3.03 ± 0.28 de	1.45 ± 0.07 ab	1.91 ± 0.07 a	14.2 ± 0.32 ab	4.7 ± 0.44 d	1.2 ± 0.05 c
		G3	12.58 ± 0.18 d	5.36 ± 0.69 cd	17.94 ± 0.68 e	3.03 ± 0.28 de	1.59 ± 0.06 a	1.74 ± 0.09 a	10.68 ± 0.52 e	6.42 ± 0.24 bc	0.47 ± 0.06 e
H2	S1	G1	17.97 ± 0.86 b	7.81 ± 0.39 ab	25.78 ± 0.79 b	3.56 ± 0.17cd	1.07 ± 0.06 cd	1.23 ± 0.07 c	13.7 ± 0.42 a-d	2.51 ± 0.09 e	1.14 ± 0.12 cd
		G2	17.1 ± 0.44 bc	7.11 ± 0.56 ab	24.3 ± 0.66 bc	4.76 ± 0.2 a	0.85 ± 0.12 d	1.3 ± 0.06 bc	14.53 ± 0.61 a	7.76 ± 0.75 ab	1.96 ± 0.06 a
		G3	15.27 ± 0.83 c	6.91 ± 0.57 bc	22.1 ± 0.31 cd	4.76 ± 0.2 a	1.13 ± 0.05 bcd	0.98 ± 0.14 c	13.9 ± 0.9 abc	4.84 ± 0.45 d	1.66 ± 0.05 b
	S2	G1	7.38 ± 0.57 e	5.28 ± 0.69 cd	12.6 ± 0.64f g	2.58 ± 0.28 e	1.75 ± 0.14 a	2.1 ± 0.17 a	12.42 ± 0.18 d	3.86 ± 0.22 de	0.9 ± 0.04 d
		G2	9.33 ± 0.29 e	4.96 ± 0.13 d	14.29 ± 0.25 f	4.46 ± 0.13 ab	1.63 ± 0.29 a	1.69 ± 0.09 ab	12.6 ± 0.58 cd	4.98 ± 0.48 cd	1.21 ± 0.05 c
		G3	7.86 ± 0.73 e	2.56 ± 0.14 e	10.42 ± 0.82 g	4.46 ±0.13 ab	1.41 ± 0.07 abc	2.05 ± 0.37 a	12.54 ± 0.55 cd	4.45 ± 0.31 d	1.25 ± 0.09 c
LSD = 0.05			2.06	1.7	2.88	0.62	0.34	0.42	1.5	1.47	0.26

H1: Tuskestan; H2: Kalaleh. S1: *A. annua*., S2: *A. sieberi*. G1: peak vegetative growth, G2: flowering, G3: seed formation.

The means (±SE) in each column that share common statistical letters, based on the Least Significant Difference (LSD) test, do not show significant differences at the 5% probability level.

Table 5 Comparison of mean interactive physiological and biochemical effects of two *Artemisia* species across different growth stages in various habitats of Golestan province (year 2)

Habitats	Species	Growth stages	Chlorophyll a (µg.g^{-1} FW)	Chlorophyll b (µg.g^{-1} FW)	Total chlorophyll (µg.g^{-1} FW)	Carotenoid (µg.g^{-1} FW)	Proline (µmol.g^{-1} FW)	Anthocyanin (mg CG.g^{-1} FW)	Phenol (mg GA.g^{-1} FW)	Flavonoid (mg QA.g^{-1} FW)	Essential oil (%)
H1	S1	G1	19.08 ± 0.58 cd	8.65 ± 1.06 a	27.7 ± 1.39 abc	5.33 ± 0.34 cde	1.68 ± 0.09 b	1.56 ± 0.04 e	11.02 ± 0.4 f	7.46 ± 0.19 d	2.01 ± 0.02 b
		G2	22.63 ± 0.6 a	7.66 ± 0.87 ab	30.29 ± 1.43 a	7.54 ± 0.16 a	1.95 ± 0.1 a	1.7 ± 0.04 de	13.39 ± 0.49 e	9.36 ± 0.42 ab	1.68 ± 0.04 c
		G3	19.66 ± 1 bc	7.34 ± 0.66 ab	27 ± 1.6 bc	6.75 ± 0.34 ab	1.71 ± 0.1 b	1.87 ± 0.11 de	10.66 ± 0.42 f	9.59 ± 0.31 a	1.64 ± 0.11 c
	S2	G1	15.6 ± 0.05 e	4.11 ± 0.52 c	19.71 ± 0.5 d	4.75 ± 0.39 cdef	0.99 ± 0.05 f	2.8 ± 0.16 b	14.91 ± 0.56 d	3.75 ± 0.09 h	1.17 ± 0.08 d
		G2	13.64 ± 0.62 f	6.2 ± 0.21 b	19.84 ± 0.78 d	4.04 ± 0.5 ef	1.28 ± 0.02 de	2.9 ± 0.1 b	17.6 ± 0.49 bc	6.12 ± 0.35 ef	1.25 ± 0.05 d

		G3	12.61 ± 0.57 f	3.55 ± 0.2 c	16.15 ± 0.74 e	4.24 ± 0.18 def	1.17 ± 0.05 ef	2.25 ± 0.14 c	17.28 ± 0.96 c	4.8 ± 0.12 g	1.02 ± 0.04 d
		G1	21.66 ± 0.24 a	6.85 ± 0.37 b	28.51 ± 0.59 ab	4.99 ± 0.13 cde	1.52 ± 0.12 bc	1.81 ± 0.06 de	10.96 ± 0.21 f	7.98 ± 0.46 cd	1.73 ± 0.32 bc
	S1	G2	21.11 ± 0.7 ab	7.21 ± 0.15 ab	28.32 ± 0.74 ab	5.47 ± 0.18 bcd	1.38 ± 0.08 cd	2.29 ± 0.1 c	11.75 ± 0.07 f	8.58 ± 0.34 bc	2.34 ± 0.05 a
		G3	17.41 ± 0.73 de	7.88 ± 0.57 ab	25.29 ± 0.84 c	5.6 ± 0.22 bc	1.58 ± 0.08 bc	1.98 ± 0.07 cd	11.88 ± 0.35 f	9.22 ± 0.23 ab	1.85 ± 0.03 bc
H2		G1	9.81 ± 0.44 g	3.68 ± 0.31 c	13.49 ± 0.14 e	3.51 ± 0.21 f	1.23 ± 0.04 de	2.97 ± 0.14 b	18.74 ± 0.24 abc	7.05 ± 0.05 de	1.09 ± 0.03 d
	S2	G2	10.2 ± 0.67 g	3.42 ± 0.46 c	13.62 ± 0.93 e	4.27 ± 0.11 def	1.53 ± 0.09 bc	2.95 ± 0.03 b	18.88 ± 0.82 ab	5.79 ± 0.48 fg	1.26 ± 0.06 d
		G3	10.59 ± 0.37 g	4.42 ± 0.01 c	15 ± 0.37 e	4.27 ± 0.43 def	1.22 ± 0.04 de	3.3 ± 0.02 a	19.12 ± 0.9 a	4.82 ± 0.02 g	1.28 ± 0.04 d
LSD = 0.05			1.81	1.69	2.96	1.29	0.2	0.31	1.5	0.99	0.3

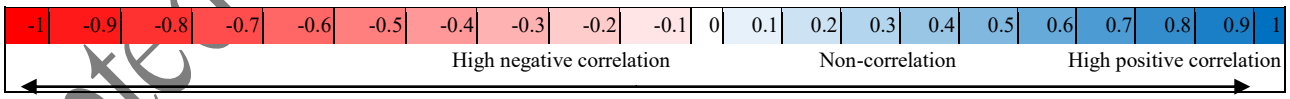
H1: Tuskestan; H2: Kalaleh. S1: *A. annua*., S2: *A. sieberi*. G1: peak vegetative growth, G2: flowering, G3: seed formation.

The means (±SE) in each column that share common statistical letters, based on the Least Significant Difference (LSD) test, do not show significant differences at the 5% probability level.

Table 6 Simple correlation between plant, soil, and climatic and geographical traits of two habitats of *Artemisia* under the influence of species type and growth stage (two-year average)

		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Proline	Anthocyanin	Phenol	Flavonoid	Essential oil
Plant attributes	Chlorophyll b	0.59								
	Total chlorophyll	0.69	0.48							
	Carotenoid	0.57	0.66	0.49						
	Proline	-0.59	-0.68	-0.38	-0.65					
	Anthocyanin	-0.68	-0.24	-0.39	-0.38	0.55				
	Phenol	-0.6	-0.47	-0.71	-0.45	0.57	0.39			
	Flavonoid	0.76	0.51	0.68	0.3	-0.62	-0.59	-0.69		
	Essential oil	-0.44	-0.49	-0.37	-0.25	0.69	0.49	0.88	-0.58	
Soil characteristics	EC	-0.65	-0.49	-0.47	-0.27	0.41	0.59	0.78	-0.48	0.57
	Acidity	-0.8	-0.86	-0.73	-0.65	0.85	0.64	0.71	-0.58	0.53
	Organic carbon	0.86	0.79	0.71	0.07	-0.8	-0.45	-0.62	0.57	0.58
	P	0.41	0.86	0.65	0.18	-0.86	-0.48	-0.86	0.69	-0.06
	K	0.71	0.63	0.78	-0.17	-0.64	-0.85	-0.8	0.89	-0.22
	N	0.55	0.45	0.63	-0.37	-0.46	-0.72	-0.66	0.77	0.41
	Zn	0.48	0.55	0.69	0.39	-0.55	-0.58	-0.57	0.57	-0.59
	Mn	0.59	0.58	0.58	0.49	-0.48	-0.59	-0.86	0.68	-0.17
	Fe	0.64	0.59	0.57	0.27	-0.69	-0.69	-0.88	0.55	-0.16
	Cu	0.76	0.42	0.58	0.31	-0.52	-0.34	-0.79	0.48	-0.27
	B	0.85	0.79	0.49	0.06	-0.48	-0.55	-0.62	0.67	0.18
	Pb	-0.79	-0.72	-0.85	0.04	0.73	0.51	0.87	-0.44	0.06
	Cr	-0.48	-0.45	-0.59	-0.4	0.66	0.48	0.57	-0.76	0.06
Climate data	Longitude	-0.58	-0.59	-0.63	-0.53	0.59	0.69	0.58	-0.67	0.85
	Latitude	-0.62	-0.39	-0.56	-0.57	0.49	0.78	0.59	-0.86	0.54
	Average precipitation	-0.71	-0.68	-0.48	-0.52	0.59	0.59	0.56	-0.57	0.46
	Altitude	-0.88	-0.75	-0.49	-0.38	0.85	0.55	0.65	-0.58	0.69
	Average temperature	-0.69	-0.81	-0.59	-0.61	0.66	0.47	0.78	-0.64	0.12
	Relative humidity	-0.59	-0.59	-0.37	-0.67	0.52	0.64	0.47	-0.51	0.18

Coefficients less than 0.25: Not significant; between 0.26 and 0.35: Significant at the 5% level; greater than 0.36: Significant at the 1% level.



Measurement of Chlorophyll and Carotenoid Content in Plant Samples

The photosynthetic pigments were measured using the Arnon method [15]. In this method, fresh plant material (0.5 g) underwent grinding in a mortar with the aid of liquid nitrogen. After centrifuged, a portion of the sample in the flask was placed in a spectrophotometer cuvette, where the absorbance was read separately. The calculations were as follows:

Chlorophyll a ($\mu g.g^{-1}$ FW) = $(19.3 \times A_{663} - 0.86 \times A_{645})$ V / 100W

Chlorophyll b ($\mu g.g^{-1}$ FW) = $(19.3 \times A_{645} - 3.6 \times A_{663})$ V / 100W

Total chlorophyll ($\mu g.g^{-1}$ FW) = Chlorophyll a + Chlorophyll b

Carotenoids ($\mu g.g^{-1}$ FW) = $[100(A_{470}) - 3.27(\text{Chl a}) - 104(\text{Chl b})]$ / 227

Measurement of Free Proline Content

Free proline content was determined using the method outlined by Bates *et al*. [16]. Leaf tissue was homogenized in sulfosalicylic acid, filtered, and reacted with ninhydrin. Following heat treatment and toluene addition, proline concentration was measured spectrophotometrically at 520 nm.

Measurement of Flavonoid and Total Phenolic Content

To extract flavonoids, 0.1 g of plant leaves were blended in 2.5 mL of 1% acidic ethanol. Once the supernatant was separated by centrifugation, it was heated for 10 minutes in a water bath set to 85 °C. Then, absorbance values were recorded at 270, 300, and 330 nm [17].

To measure total phenolic content, 0.1 g of either freeze-dried or fresh sample was mixed with 80% ethanol and centrifuged. Then, 5 mL of Folin-Ciocalteu reagent was added and varied thoroughly. After 3 min, 1 M sodium carbonate was extra and the color absorbance was determined at 765 nm. The control sample was prepared using distilled water and Folin-Ciocalteu reagent [18].

Measurement of Total Anthocyanins

Following the methodology detailed by Mazandarani *et al*. [19], total anthocyanin content was measured using the pH differential approach. For this procedure, potassium chloride (1.8 g KCl in 1 L distilled water, pH 1.0) and sodium acetate (54.3 g $CH_3COONa \cdot 3H_2O$ in 1 L distilled water, pH 4.5) buffer systems were employed, with pH adjustments using concentrated HCl. Absorbance values were obtained at 510 nm and 700 nm. The total anthocyanin content was then determined, using cyanidin-3-glucoside as a reference.

Measurement of Essential Oil Content

For the determination of essential oil content, the procedure developed by Mazandarani *et al*. [19] was utilized. To the flask holding 100 g of powdered plant material, distilled water was added, and subsequently, a Clevenger apparatus was connected. Essential oil content was designed by weighing the primary weight and the resulting essential oil. The essential oil obtained was dried using an appropriate amount of anhydrous sodium sulfate (one-tenth of the weight of the essential oil).

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Essential oil analysis was performed using a GC-MS system consisting of a Shimadzu GC-17A gas chromatograph (Kyoto, Japan) coupled with a Shimadzu QP5050 quadruple mass spectrometer. The compounds were separated on a 30 m × 0.22 mm i.d. fused-silica capillary column coated with a 0.25 µm film of BP-5 (Shimadzu). A split/splitless injector with a 1 mm internal diameter glass liner was used for the injection. Ultra-pure helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. Injector and interface temperatures were set to 280 °C and 300 °C, respectively. The mass spectrometer operated with an ionization voltage of 70 eV, and the mass range was from 35 to 450 am. The oven temperature program began at 40 °C (held for 5 min), ramped at 4°C/min to 250°C, and held for 5 min, as described above for the GC. The linear velocity of the carrier gas was approximately 37.8 cm/sec. Retention indices (RIs) were calculated using a series of n-alkanes (C8–C20) under the same chromatographic conditions. Component identification was based on the comparison of their mass spectra with those of the internal reference mass spectra library (NIST08 and Wiley 9.0) and literature data. The relative percentages of individual components were determined by peak area normalization. For compound identification, mass spectral data were matched with the NIST library and additional literature references. The chromatographic system allowed for accurate and reproducible results, providing detailed profiles of the essential oil's constituents [4,12].

Data Analysis Methodology

After data collection, a variance homogeneity test was conducted before analysis. For each experimental year, a split-plot experiment within an RCBD was employed, with species type as the main factor and different growth stages of the plant as the sub-factor. SAS software (version 9.2) was used for all statistical analyses. The LSD test was applied to compare means at a significance level of 5%. To assess the linear relationships, Pearson correlation coefficients were computed for the relationships between traits and between soil and climatic parameters and the measured traits, utilizing both SAS-9.2 and Minitab 18 software. Additionally, a stepwise regression investigation occurred with essential oil content as the dependent variable and other traits related to the plant, soil, and climate as independent variables.

RESULTS

Content of Photosynthetic Pigments

Significant variations in photosynthetic pigment content (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were observed, as indicated by the ANOVA results in Table 3, in response to the plant species. Furthermore, a notable interaction effect between growth habitat and plant species during the developmental stages was also observed, highlighting the complexity of these relationships. The comparison of means revealed that growth habitat H1 exhibited a higher content of photosynthetic pigments than H2. Additionally, among

the two species of *Artemisia*, *A. annua* exposed the maximum chlorophyll levels. In terms of the interaction effects in the first year, the peak concentrations of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were detected in samples harvested during the flowering stage of *A. annua* from the H1 habitat, with mean values of 20.67, 8.49, 29.15, and 4.73 $\mu g.g^{-1}$ FW, respectively. Conversely, the lowest mean values for chlorophyll a, chlorophyll b, and total chlorophyll were documented in samples collected during the seed formation stage of the *A. sieberi* species from the H2 habitat, with mean values of 7.86, 2.56, and 10.42 $\mu g.g^{-1}$ FW, respectively. Furthermore, the bottom mean carotenoid content was found in samples collected during the vegetative growth stage of *A. sieberi* from habitat H2, which had a mean of 2.58 $\mu g.g^{-1}$ FW (Table 4). Similar results were obtained in the second year. The species *A. annua* during the flowering stage in the H1 habitat exhibited the peak mean for chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, measuring 22.63, 7.66, 30.29, and 7.54 $\mu g.g^{-1}$ FW, respectively. Conversely, the lowest mean values for these traits were recorded in *A. sieberi* from habitat H2 across all three growth stages (Table 5).

Proline Content

Data analysis revealed that both plant species and the three-way interaction among habitat, plant species, and growth stage had significant effects on proline content in both years of the experiment (Table 3). In the first year, the peak proline content was detected in the *A. sieberi* from habitat H2 across all three growth stages, with values of 1.75, 1.63, and 1.41 $\mu mol.g^{-1}$ FW, respectively. Additionally, samples of *A. sieberi* from habitat H1 during the flowering and seed formation stages exhibited elevated proline levels. Conversely, the least proline content was achieved in samples of *A. annua* from habitat H1 across all growth stages, with values of 0.92, 0.99, and 0.93 $\mu mol.g^{-1}$ FW (Table 4). In the second year, samples of *A. annua* collected during the flowering stage from habitat H1 displayed the highest proline content at 1.95 $\mu mol.g^{-1}$ FW. In contrast, the minimum proline content was noted in samples of the *A. sieberi* at the peak vegetative growth stage from habitat H1, averaging 0.99 $\mu mol.g^{-1}$ (Table 5).

Anthocyanin Content

Anthocyanin content was significantly influenced by plant species, as well as the interaction between habitat and plant species during the growth stage in both years of the experiment (Table 3). In the first year, the *A. sieberi* exhibited the highest anthocyanin content during the flowering and seed formation stages in habitat H1, with an average of 1.91 and 1.74 mg $CG.g^{-1}$ FW, respectively. Additionally, *A. sieberi* in habitat H2 showed elevated anthocyanin levels at all three growth stages. Conversely, the lowest anthocyanin content in the first year was recorded for *A. annua* during the seed formation stage in habitat H2, with values of 0.98 mg $CG.g^{-1}$ FW (Table 4). In the second year, the highest anthocyanin content was observed in samples of *A. sieberi* during the seed formation stage in habitat H2, averaging 3.3 mg $CG.g^{-1}$ FW. In contrast, the lowest anthocyanin content for that year was found in samples of *A. annua* at the maximum vegetative growth stage in habitat H1, with values of 1.56 mg $CG.g^{-1}$ FW (Table 5).

Total Phenolic Content

Based on the results, the effects of plant species and the interaction between habitat, plant species, and growth stage on total phenolic content were significant in both years of the experiment (Table 3). In the first year, *A. annua* displayed the peak total phenolic content during the flowering stage in habitat H1 exhibited the highest total phenolic content, averaging 14.53 mg $GA.g^{-1}$ FW. In contrast, the lowest average was found in *A. sieberi* during the seed formation stage in habitat H1, with an average of 10.43 mg $GA.g^{-1}$ FW (Table 4). In the second year, the peak total phenolic content was detected in *A. sieberi* during seed formation in habitat H2, averaging 19.12 mg $GA.g^{-1}$ FW. The minimum total phenolic content for that year was recorded in *A. annua* across all three growth stages in the H2 habitat, with averages of 10.96, 11.75, and 11.88 mg $GA.g^{-1}$ FW, respectively (Table 5).

Flavonoid Content

Significant variations in flavonoid content were observed through ANOVA, revealing the influence of plant species and the three-way interaction of habitat on these species during the growth stage. Notably, the two years of the study showed marked differences (Table 3). In the first year, *A. annua* exhibited the highest flavonoid content during both the vegetative and flowering stages at habitat H1, measuring 8.48 and 8.41 mg $QA.g^{-1}$ FW, respectively. Contrariwise, the minimum flavonoid content was observed in the *A. annua* during the vegetative stage at habitat H2, at just 2.51 mg $QA.g^{-1}$ FW (Table 4). In the second year, *A. annua* in the seed formation stage in habitat H1 demonstrated the highest flavonoid content, reaching 9.59 mg $QA.g^{-1}$ FW. In contrast, the *A. sieberi* in the same developmental stage at both habitats showed the lowest flavonoid levels, averaging 4.8 and 4.82 mg $QA.g^{-1}$ FW, respectively (Table 5).

Essential Oil Content

The content of essential oils exhibited a significant change due to plant species, interaction between growth stage and habitat, and interaction of habitat with plant species and growth stage across both years of the experiment (Table 3). In the first year, *A. annua* in the flowering stage in habitat H2 exhibited the highest essential oil content, averaging 1.96%. Conversely, *A. sieberi* had the lowest essential oil content during the seed development stage at H1, with an average of 0.47% (Table 4). In the second year, *A. annua* again demonstrated the peak essential oil percentage in the flowering stage in habitat H2, averaging 2.34%. In contrast, *A. sieberi* consistently had the lowest essential oil content across all three growth stages in both habitats (Table 5).

Simple Correlation Analysis

The simple correlation analysis revealed among the various plant, soil, and climatic parameters are presented in Table 6. The analysis indicated a significant positive correlation between essential oil content and proline, anthocyanins, and total phenols. In contrast, a significant negative correlation was detected among photosynthetic pigments and flavonoids. Among the soil parameters, electrical conductivity, acidity, organic carbon, and nitrogen content demonstrated a significant direct correlation with essential oil content. Conversely, zinc and copper contents exhibited significant negative correlations with essential oil content. Climatic factors such as latitude, longitude, average precipitation, and altitude demonstrated significant positive correlations with essential oil content.

Stepwise Regression Analysis

Stepwise regression analysis, which considered essential oil content as the dependent variable and various plant (Table 7), soil (Table 8), and climatic (Table 9) parameters as independent variables, are summarized below. Among the plant-related parameters, total chlorophyll and total phenols were included in the regression model, collectively accounting for 74.17% of the variation in essential oil. Among the soil factors, only the organic carbon content was included, explaining 28.50% of the difference in essential oil. For climatic factors, altitude and average precipitation were incorporated into the regression model, accounting for 41.08% of the difference in essential oil.

Essential Oil Profiles

The essential oils of *A. annua* and *A. sieberi* from the Tuskestan and Kalaleh habitats at different growth stages reveal several key compounds, with *Artemisia ketone* standing out as the most dominant (Table 10). This compound shows an average percentage of 51.55% and 58.98% in *A. sieberi*, during the vegetative growth and flowering stages, respectively making it the primary component in both species' essential oils. The combination of Borneol, with its high concentration ranging from 16.28% to 50.26%, shows the highest average in the vegetative growth of *A. annua* in the Kalaleh region, while the lowest average of this compound was observed in the flowering of *A. sieberi* in the same region. The α-fenchene in the *A. annua* vegetative growth and *A. annua* flowering samples from the Tuskestan region had high averages of 10.75% and 10.22%, respectively. Methyl chavicol also appears prominently, especially in *A. sieberi* during the flowering and seed formation stages, with concentrations of 5.12% and 3.43% in Tuskestan. Linalool, found in *A. sieberi* during the vegetative growth (1.74%) and flowering stages (2.24%) in Tuskestan, is another important compound, although it appears in lower concentrations compared to the previous ones. Lastly, Cis-sabinene hydrate peaks at 29.66% in *A. annua* during vegetative growth in Tuskestan, making it another key component of the essential oils. The highest average concentration of essential oil compounds is observed in *A. sieberi* during the flowering stage in the Kalaleh habitat, with Artemisia ketone reaching 58.98%, while *A. annua* from the Tuskestan region during the vegetative growth stage exhibits significant concentrations of compounds like Artemisia ketone and Cis-sabinene hydrate.

Table 7 Stepwise regression for phytochemical and physiological traits affecting essential oil content in two species of *Artemisia*

Term	Coef	SE Coef	T-Value	P-Value
Constant	-1.868	0.600	-3.11	0.012
Total chlorophyll (X1)	0.2774	0.0797	3.48	0.007
Phenol content (X2)	0.1560	0.0477	3.27	0.010
Y = -1.868 + 0.2774 (X1) + 0.1560 (X2); R-sq (adj) = 74.17%				

Table 8 Stepwise regression for soil characteristics affecting essential oil content in two species of *Artemisia*

Term	Coef	SE Coef	T-Value	P-Value
Constant	11.77	5.61	2.10	0.012
Organic carbon (X3)	-1.346	0.720	-1.87	0.031
Y = 11.77 - 13.346 (X3) ; R-sq(adj) = 28.50%				

Table 9 Stepwise regression for geographical and climatic characteristics affecting essential oil content in two species of Artemisia

Term	Coef	SE Coef	T-Value	P-Value
Constant	1.959	0.939	2.09	0.047
Altitude (X4)	0.00268	0.00167	1.60	0.003
Average precipitation (X5)	-0.273	0.120	-2.28	0.049
Y = 1.959 + 0.00268 (X4) - 0.273 (X5); R-sq(adj) = 41.08%				

Table 10 Percentage composition of key compounds in the essential oils of *A. annua* and *A. sieberi* samples from Tuskestan and Kalaleh habitats at different growth stages (two-year average)

S. No.	Compounds	R.I	Tuskestan habitats					
			A. annua vegetative growth	*A. annua* flowering	*A. annua* seed formation	*A. sieberi* vegetative growth	*A. sieberi* flowering	*A. sieberi* seed formation
1	3,4-hexanedione	800					0.04	0.03
2	3-methyl-butanoic acid	833					0.13	0.27
3	Ethyl isovalerate	849	0.142				0.13	0.44
4	Santolina triene	905	0.92					
5	Butyl propanoate	907						
6	Isobutyl isobutyrate	908						
7	α- thujene	927	0.162	0.32	0.12	0.30	0.24	0.20
8	α- pinene	931		0.21		0.41	0.38	0.45
9	α- fenchene	945	10.75	10.22	0.99	1.44	1.58	2.11
10	Camphene	946	3.87				0.09	0.22
11	Benzaldehyde	951		7.52	3.55	6.79	3.68	2.73
12	Thuja-2,4 (10) -diene	953	0.03				0.03	0.06
13	β- pinene	976	1.38	0.27	0.29	0.30	0.70	0.52
14	Trans- isolimonene	980		1.83	0.36	0.96	0.69	0.15
15	Myrcene	991	4.73		0.12	0.43	0.12	0.03
16	Ethyl hexanoate	994	0.76				0.17	0.18
17	n-decane	1000				0.18	0.86	0.97
18	α-phellandrene	1004		0.22	0.63			
19	α- terpinene	1018	0.222	0.18	0.16	0.26	0.36	0.31
20	p-cymene	1020	0.121					0.61
21	Limonene	1024		0.53	0.23		0.59	1.57
22	1,8-cineole	1026				1.83	2.41	
23	(Z)-β - ocimene	1031		0.14		0.15		
24	Artemisia ketone	1056	14.74	12.94	17.08	16.08	36.26	25.75
25	n-octanol	1062				0.91	0.75	0.67
26	Cis sabiene hydrate	1065	29.66	4.97	25.17		0.17	0.04
27	Cis-linalool oxide	1068	0.222	0.25	0.33	0.38	0.03	0.18
28	Terpinolene	1083	0.111	0.47	1.04	0.13	0.14	0.10
29	Linalool	1097	0.03	0.25	1.67	1.74	2.24	2.94
30	Cis- thujone	1103	0.314			1.28		5.05
31	α-campholenal	1124	0.101					1.29
32	Chrysanthenone	1133	0.91		0.16	1.95	3.77	7.68
33	camphor	1141	0.081			0.61	2.67	3.40
34	Isoborneol	1152	0.051					
35	Cis-chrysanthenol	1160						0.76
36	δ- terpineol	1162	1.17	2.27	3.07	0.74	0.52	
37	Borneol	1170	17.29	38.19	29.47	43.88	19.83	22.02
38	p-cymen-8-ol	1181	0.131				0.13	0.68
39	3-decanone	1185	0.46	2.32	2.14	0.92	0.38	
40	α- terpineol	1189	1.02	0.12	0.26	0.16		2.09
41	Methyl chavicol	1192	0.66	1.79	0.63	3.43	5.12	
42	n- decanal	1205	0.87	1.03	1.21	2.08	1.82	1.57
43	Linalool formate	1216	1.06			0.14		
44	Cis-sabinene hydrate acetate	1220	0.061				1.04	0.54
45	Cis-carveol	1225	0.121	0.67	1.31	0.32		0.33
46	Exo- fenchyl acetate	1230					0.21	0.33
47	Isobornyl formate	1235	0.04	0.23	0.07		0.06	0.47
48	Hexyl isovalerate	1240				0.23	0.26	0.10
49	Isoamyl hexanoate	1245		0.13	0.13	0.66	0.18	0.29

50	Cis-myrtanol	1250	0.71			1.14	6.84	0.09
51	Perilla aldehyde	1269					0.08	0.03
52	α- terpinene-7-al	1280	0.04			0.23		0.07
53	Bornyl acetate	1285	0.051			0.21	0.26	0.52
54	Trans-sabinyl acetate	1289	0.04			0.20	0.20	0.09
55	n-tridecane	1300	0.101					0.06
56	α- copaene	1376	0.152				0.03	
57	Geranyl acetate	1380	0.03	0.26	0.79	2.89	0.03	0.26
58	β- elemene	1391	0.091	0.16	0.06			0.07
59	Ethyl decanoate	1399	0.04	0.10		0.48	2.66	5.19
60	n- tetradecane	1402					0.36	0.13
61	(E)- caryophyllene	1417	0.344	0.33	0.91		0.04	
62	γ- gurjunene	1475	0.172					0.14
63	Germacrene D	1484	0.324		0.16			
64	δ- selinene	1492	1.153		1.29			0.29
65	n- pentadecane	1501	0.02			0.17		0.58
66	γ- cadinene	1512	0.02			0.98	0.19	0.25
67	Spathulenol	1578	0.091		0.18	0.98	0.07	0.67
68	1-hexadecene	1589			2.91			0.32
69	Cubenol	1643	0.526					0.18

Table 10 Continued

S. No.	Compounds	R.I	Kalaleh habitats					
			A. annua vegetative growth	*A. annua* flowering	*A. annua* seed formation	*A. sieberi* vegetative growth	*A. sieberi* flowering	*A. sieberi* seed formation
1	3,4-hexanedione	800	0.03					
2	3-methyl-butanoic acid	833						
3	Ethyl isovalerate	849	0.09			1.15		1.98
4	Santolina triene	905				0.19	0.18	0.25
5	Butyl propanoate	907						0.12
6	Isobutyl isobutyrate	908				0.18		0.39
7	α- thujene	927	0.26	0.27		0.07		0.1
8	α- pinene	931	0.16	0.15	0.08	0.07		
9	α- fenchene	945	7.19	2.97	5.74	0.22		0.14
10	Camphene	946						
11	Benzaldehyde	951	6.05	6.81	0.14	1.8	1.53	2.37
12	Thuja-2,4 (10) -diene	953	0.06					
13	β- pinene	976	0.08	0.24	1.36	0.38		0.14
14	Trans- isolimonene	980	1.32	1.93	36.22	0.12	0.37	0.12
15	Myrcene	991				0.22	0.16	2.13
16	Ethyl hexanoate	994			4.39	0.14		0.17
17	n-decane	1000						
18	α-phellandrene	1004	0.17		0.16	0.30	0.77	0.76
19	α- terpinene	1018	0.17	0.14	0.22	0.49	0.14	0.42
20	p-cymene	1020						
21	Limonene	1024	0.58					
22	1,8-cineole	1026	0.05	0.63	5.23	1.63	1.08	1.59
23	(Z)-β - ocimene	1031		0.17	4.60			
24	Artemisia ketone	1056	12.17	12.96	5.32	51.55	58.98	49.19
25	n-octanol	1062	1.64	1.46	8.94	0.92	0.35	1.16
26	Cis sabiene hydrate	1065	0.15	0.19		0.39	0.16	0.32
27	Cis-linalool oxide	1068	0.18		0.17	0.62		0.83
28	Terpinolene	1083	0.07		0.10	0.17	1.53	0.17

29	Linalool	1097	0.14				0.28	
30	Cis- thujone	1103	0.04					
31	α-campholenal	1124					2.59	
32	Chrysanthenone	1133		0.19	0.18	0.52	0.18	0.56
33	camphor	1141	0.44		0.07			
34	Isoborneol	1152						0.39
35	Cis-chrysanthenol	1160		0.90	0.23	0.73		0.70
36	δ- terpineol	1162	2.67		0.49			
37	Borneol	1170	50.26	41.31		20.33	16.28	23.96
38	p-cymen-8-ol	1181		0.86	0.12	0.39	0.61	0.54
39	3-decanone	1185	2.70			0.47	0.26	
40	α- terpineol	1189		0.15	0.04		0.35	
41	Methyl chavicol	1192	1.09	0.47	0.09	2.54	1.70	1.92
42	n- decanal	1205	1.20	0.80	0.32	0.28	1.09	2.37
43	Linalool formate	1216	0.13		0.05	0.28	0.19	0.1
44	Cis-sabinene hydrate acetate	1220	1.27	0.54		1.04		0.17
45	Cis-carveol	1225						0.19
46	Exo- fenchyl acetate	1230	0.040.06			0.15		
47	Isobornyl formate	1235	0.25					
48	Hexyl isovalerate	1240	0.16	0.18				
49	Isoamyl hexanoate	1245	0.14	0.12		0.20	0.23	0.22
50	Cis-myrtanol	1250		0.08		0.10	0.11	2.46
51	Perilla aldehyde	1269				2.93	3.61	
52	α- terpinene-7-al	1280						
53	Bornyl acetate	1285	0.03			0.19	0.30	
54	Trans-sabinyl acetate	1289	0.06			0.81	0.41	0.79
55	n-tridecane	1300		0.98	6.54	0.24		
56	α- copaene	1376	0.04	1.17	3.68			
57	Geranyl acetate	1380	0.07					
58	β- elemene	1391	0.34	0.26				
59	Ethyl decanoate	1399	0.08			0.07	0.34	0.33
60	n- tetradecane	1402	0.03	2.58	0.65			
61	(E)- caryophyllene	1417	0.30	0.83	1.17	0.04		
62	γ- gurjunene	1475	0.20	0.47	0.15			
63	Germacrene D	1484		0.24	0.07	0.16	0.18	0.32
64	δ- selinene	1492	5.81	4.48		0.22		
65	n- pentadecane	1501		0.28		0.09	0.22	0.27
66	γ- cadinene	1512	0.03				0.13	
67	Spathulenol	1578				0.80	0.55	0.64
68	1-hexadecene	1589				0.16		
69	Cubenol	1643				0.14		

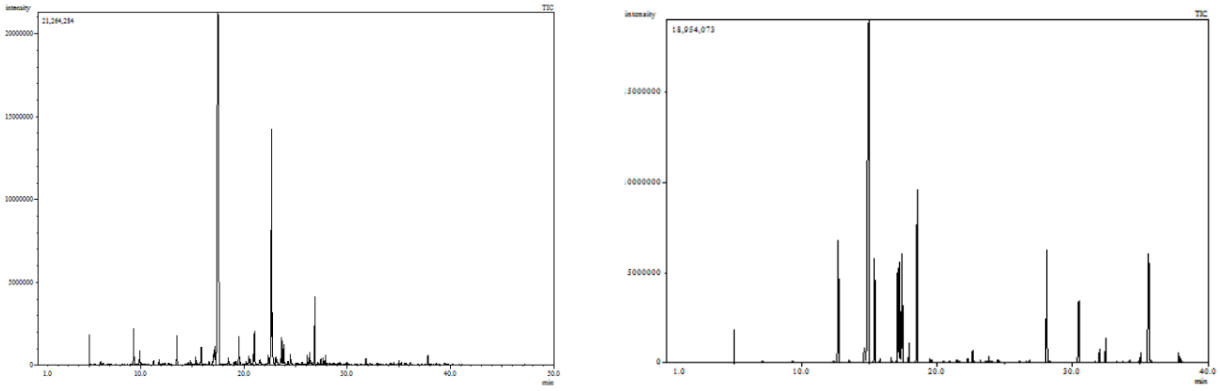


Fig. 1 GC-MS chromatogram analysis of essential oils from two species of *Artemisia* (right: *A. annua* and left: *A. sieberi*).

DISCUSSION

The results showed that the photosynthetic pigment content was significantly affected by the plant species. The higher pigment levels observed in habitat H1 compared to H2 likely reflect the more favorable environmental conditions in H1, where factors such as increased availability of light, nutrients, and water contribute to enhanced pigment production [20]. Among the two *Artemisia* species, *A. annua* exhibited higher levels of chlorophyll, which is likely related to its greater ability to absorb and utilize light. The peak concentrations of chlorophyll a and chlorophyll b, total chlorophyll, and carotenoids were observed in the flowering samples of *A. annua* from the H1 habitat. This growth stage increases the energy needs of the plant for flower and seed production, consequently elevating the production of photosynthetic pigments [21]. Conversely, the lowest levels of pigments were recorded in samples of *A. sieberi* during the seed-formation stage from the H2 habitat, possibly because of reduced photosynthetic activity in the final growth stages or environmental stresses present in H2. The results from the second year were similar to those of the first, showing that *A. annua* maintained the highest levels of photosynthetic pigments during the flowering stage in the H1 habitat.

Proline is a stress-responsive compound whose accumulation under stress conditions aids the plant in maintaining osmotic balance and protecting against cellular damage [22]. The highest proline levels were observed in *A. sieberi* from habitat H2 across all growth stages, indicating that this species experiences greater stress in this habitat. On the other hand, the lowest proline content was recorded in *A. annua* samples from habitat H1 during the growth stages, likely due to the more favorable environmental conditions in this habitat. In the second year, the highest proline levels in *A. annua* samples were observed during the flowering stage from habitat H1, which may indicate changes in environmental conditions and proline requirements of the species during this growth phase [23].

Anthocyanins are pigments that play a crucial role in protecting against environmental stresses, particularly light and temperature [24]. The highest anthocyanin levels in *A. sieberi* were observed during the flowering and seed formation stages in habitat H1, indicating the pigment's role in protecting the plant against environmental conditions during these critical growth phases. Conversely, the lowest anthocyanin levels in *A. annua* were recorded during the seed formation stage in habitat H2, which may be due to the differences in physiological needs or reduced stress exposure in this species.

Phenolic compound production plays a vital role in plant defense against environmental stresses and pests [25], and the results demonstrated that both plant species and the interaction between habitat, species, and growth stage significantly affected total phenolic content across both years of the study. The peak total phenolic content in *A. annua* was detected during the flowering stage in habitat H1, indicating the role of these compounds in providing the energy needed for flowering and reproduction. In contrast, the lowest total phenolic content in *A. sieberi* was recorded during the seed formation stage in habitat H1, which may be due to differences in the production patterns of these compounds in this species. Flavonoids such as phenolics are antioxidant compounds that play protective roles against stress [26]. The highest flavonoid levels in *A. annua* were observed during the vegetative and flowering stages in habitat H1, suggesting that this species requires more of these compounds to cope with stress during these growth phases. The lowest flavonoid levels in *A. annua* were recorded during the vegetative stage in habitat H2, potentially because of differences in environmental or genetic conditions between these two habitats. Younessi *et al*. [27] studied 50 compounds in four types of essential oils from different seasons and found camphor, thujone, and 1,8-cineole to be the key components. Monoterpenes were the most prevalent, with Shahrivar (91.87%), Azar (90.55%), Ordibehesht (96.32%), and Tir (95.4%). The highest phenolic content (49.5 mg GAE.g^{-1}) and antioxidant capacity (89.28%) were noted in Shahrivar essential oil. The phenolic compound content varies throughout the plant's life cycle in *Artemisia* plants. The highest amount of these compounds was measured during seed development, whereas the level of chlorophyll a was at its lowest during this time. A potential reason for the decrease in chlorophyll levels in plants could be changes in nitrogen metabolism. The abiotic stress led to the decreased

incorporation of glutamate, a common precursor for both chlorophyll and proline synthesis, into the chlorophyll synthesis pathway [28].

The essential oil content was significantly influenced by the interaction among habitat, plant species, and growth stage in both years. Essential oils are volatile aromatic compounds that play various roles in plants, including attracting pollinators, repelling pests, and protecting against environmental stress [29]. The maximum essential oil content in *A. annua* was observed during the flowering stage in habitat H2, likely because of the role of these compounds in attracting pollinating insects during this phase. NazarPour and Yadegari [30] identified key compounds in essential oils, including α-pinene, camphene, and caryophyllene. Their study showed significant effects of geographical region and phenological stage on oil quantity and quality. The highest oil yield (17.2%) was observed in *A. aucheri* L. in Masjed Soleyman during flowering, whereas the lowest yield (0.6%) was observed in *A. vulgaris* L. in Izeh during seed formation. Aromatic compounds peaked at the end of vegetative growth, with a shift from cooler to warmer climates, resulting in decreased monoterpenes, such as alpha-pinene and limonene, but increased sesquiterpenes, such as caryophyllene.

Essential oil content and composition in plants, particularly medicinal and aromatic species like *Artemisia*, are influenced by multiple factors. The analysis of essential oil profiles using GC-MS is crucial for identifying and assessing the quality of these plant oils. This method aids in identifying various compounds present in the oil and quantifying each of them, which is vital for different applications of essential oils, including pharmaceutical, cosmetic, and food products [13]. For example, the essential oil of *A. annua*, owing to its Artemisia ketone and other specific compounds, is utilized in the production of antimalarial drugs and other medicinal products [31]. In contrast, the essential oil of *A. sieberi*, with its 1,8-Cineole and Eucalyptol, can be used in cosmetic and health products as flavoring and preservative agents. Arvin and Firouzeh [32] explored the biochemical properties of medicinal plants, focusing on the essential oil characteristics of two sage species, *A. kopetdaghensis* and *A. sieberi*, in North Khorasan Province. Their study identified 34 compounds in the essential oils, with camphor and pinocarveol being prominent in *A. kopetdaghensis*, whereas davanone was dominant in *A. sieberi*. The essential oil yields were 0.92% for *A. kopetdaghensis* and 0.11% for *A. sieberi*. Notably, *A. kopetdaghensis* showed higher antioxidant capacity, suggesting its greater medicinal potential owing to its higher yield and antioxidant properties. In a study by Sakhaie *et al*. [33], a total of 32 components were detected in the essential oil extracted from *A. annua*. The main constituents were camphor (48%), 1,8-cineole (9.31%), camphene (6.98%) and spathulenol (4.89%). Rabiei *et al*. [34] initiate that the peak essential oil was from *A. absinthium* (92%), while the lowest was from *A. spicigera* (46%). The main components identified were Artemisia ketones (14.3%) in *A. annua*, capillene (48%) in *A. scoparia*, camphor (40%) in *A. spicigera*, and alpha-phellandrene (25.5%) in *A. absinthium*.

The essential oil content and its profiles in *Artemisia* plants are affected by genetic factors (species and genotype), environmental factors (temperature, humidity, light, and soil), and growth stages (physiological changes). *A. sieberi* produces more Artemisia ketone, while *A. annua* produces more Borneol. The growth (habitat) also affected the quantity and type of essential oil, with *A. annua* showing higher Cis sabiene hydrate levels in H1 and *A. sieberi* producing more Artemisia ketone in H2. In addition, *A. annua* produces the most essential oil in the flowering stage, and the composition of the essential oil changes at different growth stages. The investigation of essential oil profiles using GC-MS is vital for identifying compounds and determining the quality of oils and is applied across various industries. This study emphasizes that understanding the factors affecting essential oil production and studying the essential oil profiles are essential for optimizing the production and use of these valuable plants. Keivan-behjou *et al*. [35] concluded that the K parameter significantly affects atmospheric deposits, while pH, silt, and sand influence subsoil deposits of *Artemisia* plants. Their research confirms that certain soil parameters are impactful and highlight the crucial role of environmental factors in stabilizing these systems. Effective management of these factors can help mitigate damage to wetlands and vegetation. The amount and composition of active ingredients in plant essential oils are affected by several environmental factors. These include climate, soil composition, elevation, and when plants are harvested [36]. Research across seasons often yields similar results. Many studies have shown considerable variation in essential oil components within the *Artemisia* genus. The specific compounds present can differ due to factors such as soil pH, climate, and other factors. Furthermore, the volatile compounds within these plants can change depending on the plant's growth stage or the altitude at which it grows [13].

CONCLUSION

Significant variation was observed across species, habitats (Tuskestan (H1) and Kalaleh (H2)), and growth stages. A. annua generally exhibited higher photosynthetic pigment content, particularly during the flowering stage in H1, while *A. sieberi* accumulated more proline, especially in H2. Anthocyanin content was higher in *A. sieberi*, notably during the later developmental stages. Total phenolic and flavonoid contents varied significantly, with *A. annua* showing higher flavonoid levels and *A. sieberi* exhibiting greater phenolic content in some instances. The essential oil content was significantly influenced by all factors, with *A. annua* demonstrating higher yields, especially during flowering in H2. Correlation and regression analyses uncovered complex interactions between essential oil content and various plant, soil, and climatic factors, identifying total chlorophyll, total phenolics, organic carbon, altitude, and precipitation as significant predictors. GC-MS analysis of essential oil profiles revealed distinct

compositional differences between the two species, with *A. annua* rich in Artemisia ketone and Cis sabiene hydrate, and *A. sieberi* characterized by higher levels of Artemisia ketone and Borneol. These findings highlight the significant influence of environmental factors on the phytochemical composition of *Artemisia* species and suggest important implications for their targeted cultivation and utilization in various applications.

Conflict of Interest

The authors have not declared any conflict of interests.

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