

Seasonal and Spatial Variability Among Secondary Metabolites of *Senna italica* Across Ecological Zones in Saudi Arabia

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Abstract

This investigation analyzes the influence of seasonal and spatial changes on the secondary metabolite contents in *Senna italica* growing in nine ecologically distinct regions in Saudi Arabia. The findings indicate a strong relationship between environmental factors and the levels of sennosides A and B, total flavonoid content (TFC), total phenolic content (TPC), and antioxidant activity. Temperature, humidity, and soil composition—particularly the concentrations of sodium, magnesium, and phosphorus—had an immense impact on metabolite production. Results suggest that spring and winter are the optimal periods for post-emergent harvesting and highlight the roles of elevation and climate in phytochemical production.

Keywords: *Senna italica*; sennosides; flavonoids; phenolics; antioxidants

Saudi Arabia covers approximately 2,500,000 km², with the Arabian Peninsula constituting the majority of its landmass (Khalik et al., 2013). The country's location in terms of latitude significantly affects its climate, which is predominantly characterized by aridity or dryness in most regions (Azaizeh et al., 2003). This climate is marked by hot daytime temperatures and rapid cooling at night (Al-Yemeni, 2000). One of the important plant families with many pharmacologically active species is the Leguminosae (Fabaceae) family. This plant family is widely utilized in traditional medicine for both economic and therapeutic benefits, with its medicinal properties recognized in the medical practices of India and the UK, as well as within the pharmaceutical industries of Germany and the US (Al-Dakan et al., 1995; Bown, 1995; Willuhn, 1997). The *Senna* species in Saudi Arabia is well known for its ability to grow under tough environmental conditions and its remedial attributes against various ailments (Yusuf et al., 2014). Notably, *Senna italica* is recognized for its tolerance to environmental stress, believed to be due to the elicitation of secondary metabolites (SMPs) that enable its survival in extreme conditions. The secondary metabolites of *S. italica*, including anthraquinones, glycosylated anthraquinones, and flavones, are known to play significant biological roles as laxatives and antidiarrheal agents (Adjou et al., 2021). SMPs are essential for the plant's adaptation to biotic and abiotic stress and confers it with resistance to pathogens and herbivores (Akula & Ravishankar, 2011).

Plant secondary metabolites are structured compounds, such as flavonoids, phenolic acids, alkaloids, and several other bioactive compounds, that enhance the defense mechanisms of plants (Yang et al., 2018). The synthesis of SMPs is influenced by environmental conditions, including geographical location, seasonal changes, and climatic fluctuations (Alqahtani et al., 2015; Demasi et al., 2018). *S. italica* is a small herbaceous plant with glabrous, green stems, decumbent branches, and a mild odor (Dabai et al., 2012). It typically grows as a deciduous shrub, measuring about 50-75 cm tall (Olorukooba et al., 2022). The generic name, *Senna*, is derived from an Arabic word meaning brightness, sparkle, or glow (Soladoye, 2010).

In Saudi Arabian traditional medicine, *S. italica* is used to treat various diseases, including gastrointestinal disorders, constipation, diabetes, rheumatism, and urinary system diseases (Sulieman et al., 2017). It is also employed in managing skin infections and edema (Abdoulahi et al., 2022). In traditional medicine, the whole plant or leaves are boiled to produce a pale-yellow extract, which is then used to prepare purgatives or laxatives (Singh et al., 2013). Compounds from some *Senna* species have been reported to possess anti-inflammatory (Susunaga-Notario et al., 2014), antibacterial (Bukar et al., 2009), antioxidant, and antifertility properties (Mokgotho et al., 2013). Additionally, *S. italica* leaves are used in managing sexually transmitted infections (Sermakkani & Thangapandian, 2012). Every part of *S. italica* has medicinal value, with the leaves used for skin disorders such as ulcers and burns and roots used for conditions such as cholecystitis, hepatic disorders, dysmenorrhea, and urinary tract infections (Masoko et al., 2010). The leaves and pods are also considered laxatives (Rajesham et al., 2013). Studies have shown that the leaves of *S. italica* exhibit antioxidant and antibacterial activities (Lekganyane et al., 2012), and its SMPs exert antimicrobial action, which may support efforts to combat antibiotic resistance and reduce antibiotic misuse (Dabai et al., 2012). This study aims to investigate the effects of seasonal variation and soil types on the natural habitat of *S. italica* across different geographic locations in Saudi Arabia, with the goal of determining the best time and site for harvesting *S. italica* to maximize the yield of beneficial SMPs.

Materials and Methods

Study Areas

Aerial parts of *S. italica* were collected from nine localities throughout the Kingdom of Saudi Arabia: Thadiq, Hotat Bani Tamim, Taif, Arafat, Baish, Khaybar, Al Quwarah, Al-Baha, and Wadi Al-Disah.

Sample Collection

S. italica was chosen for this study due to its prevalence in diverse topographic regions that constitute the majority of the Saudi Arabia's climatic zones, including arid and semi-arid regions. Aerial parts of *S. italica* were collected across the four seasons.

Physical Analysis of Soil

Physical Components of Soil. The textural classes of the soils were determined using the hydrometer method as described by Bouyoucos (1962).

Soil Texture. Soil textural analysis was also conducted using the hydrometer method (Bouyoucos, 1962).

Chemical Analysis of Soil

pH. The pH of the saturated soil paste extract was determined for each sample using a pH meter, according to McLean (1983) with slight modifications.

Electrical Conductivity (EC). The treated saturated soil pastes (extracts) were analyzed for their electrical conductivity using an electrical conductivity meter. Readings were expressed as mhos/cm (Rhoades, 1983).

Chloride. The soluble chloride in the saturated soil paste extract was determined through titration, following the method outlined by Richards (1954).

Organic Matter. Organic matter was estimated using the loss-on-ignition method, as described by Dean (1974).

Potassium and Sodium. Potassium and sodium contents were determined using flame photometry, following the method described by Bisergaeva and Sirieva (2020).

Calcium and Magnesium. Calcium and magnesium concentrations were determined using the atomic absorption flame procedure as described by Richards (1954).

Phosphorus. Phosphorus levels were measured using an inductively coupled plasma atomic emission spectrometer (APHA, 1998).

Nitrogen. Nitrogen levels were calculated using the Kjeldahl method (Bremner & Mulvaney, 1982).

Estimation of Pharmaceutical Compounds

Sennosides A and B. Sennosides A and B were analyzed using HPLC with an Alliance chromatograph system (Waters Associates, Millipore, Milford, MA, USA). Stock solutions of sennosides A and B in methanol (500 µg/mL) were prepared. Two standard solutions were prepared to contain three gradient levels through serial dilution with 90% methanol and 10% distilled water. Standard solutions of sennosides A and B were prepared in serial dilutions of 2-100 µg/ml. Calibration curves were constructed by plotting the peak area (y) ratio of sennosides A and B versus standard concentration (x).

Total Flavonoid Contents. Total flavonoid contents were determined using spectrophotometry in accordance with the aluminum chloride method (El Far & Taie, 2009).

Total Phenolic Contents. Total phenolic contents in each extract were determined using the Folin-Ciocalteu reaction with slight modifications (Slinkard & Singleton, 1977).

Total Antioxidant Activity. Total antioxidant activity was determined using the DPPH method. The total antioxidant content was evaluated in a WELL 96-PLATE according to Zahratunnisa (2002), with minor modifications.

Statistical Analysis. The study was conducted based on a two-factorial design. One factor was the location of the nine *S. italica* sites, and the other was the seasons, including winter, spring, summer, and autumn. Each site and season was replicated three times.

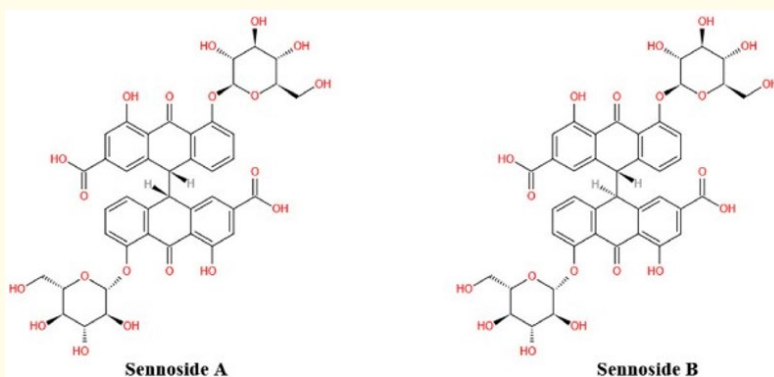


Figure 1: Shows the chemical structure of sennosides A and B (Le et al., 2021).

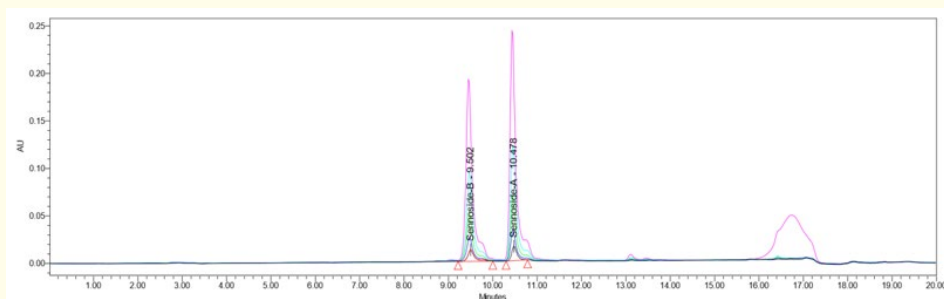


Figure 2: Chromatogram of the standard compound's sennoside A and B using HPLC by serial dilution of 90% methanol/water. Acidified water (1% acetic acid, v/v) and acetonitrile were used as mobile phases at an absorbance of 280 nm.

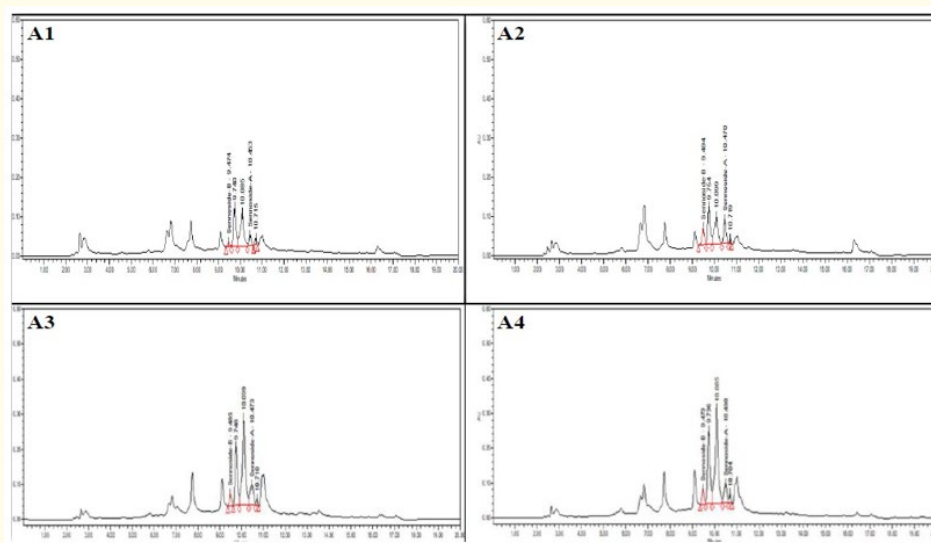


Figure 3: Chromatogram of the standard compound's sennoside A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as mobile phases at an absorbance of 280 nm. A1 represents the Al-Baha region for summer, A2 for autumn, A3 for winter, and A4 for spring.

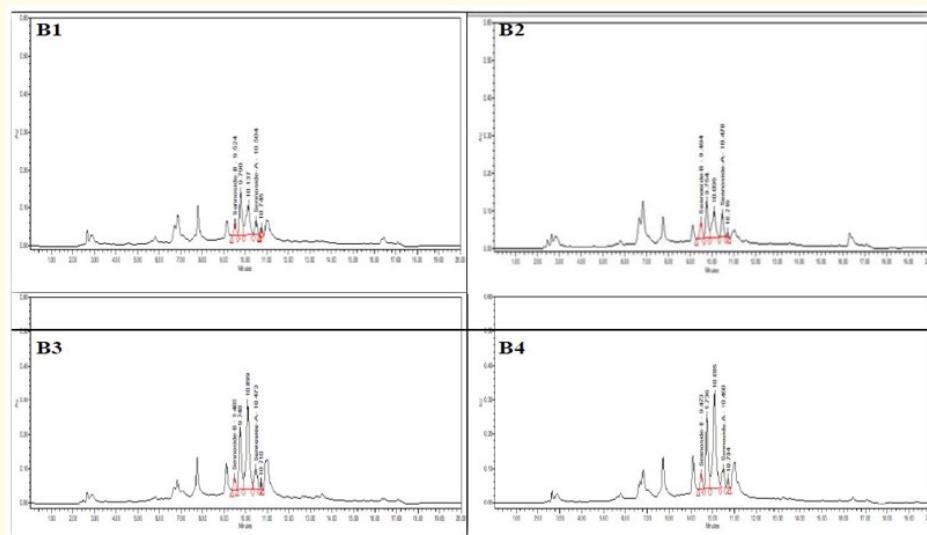


Figure 4: Chromatogram of the standard compound's sennoside A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as the mobile phases at an absorbance of 280 nm. B1 represents the Bish region for summer, B2 for autumn, B3 for winter, and B4 for spring.

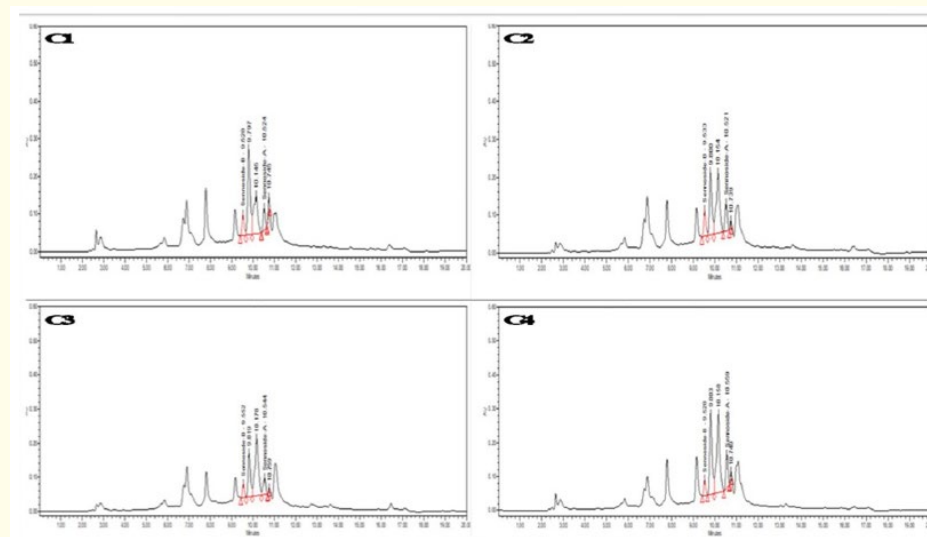


Figure 5: Chromatogram of the standard compound's sennoside A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as the mobile phases at an absorbance of 280 nm. C1 represents the Thadiq region for summer, C2 for autumn, C3 for winter, and C4 for spring.

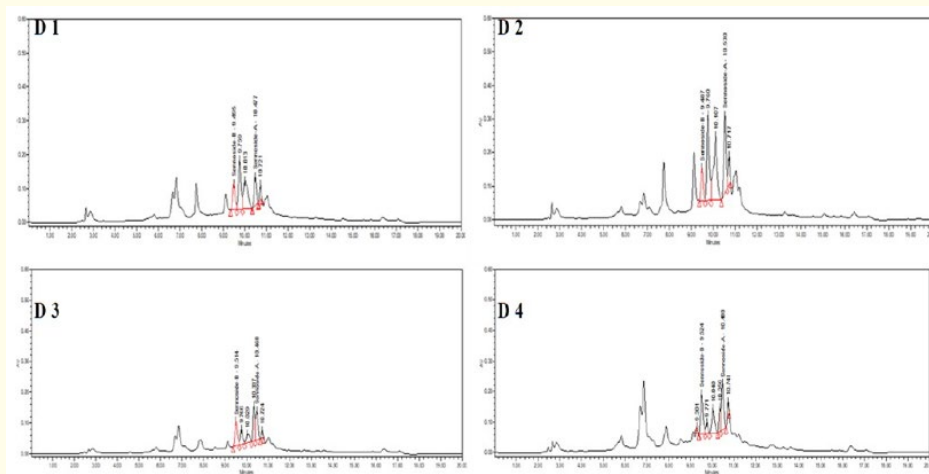


Figure 6: Chromatogram of the standard compound's sennosides A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as the mobile phases at an absorbance of 280 nm. D1 represents the Hawtah B.Tamim region for the summer, D2 for the autumn, D3 for the winter, and D4 for the spring.

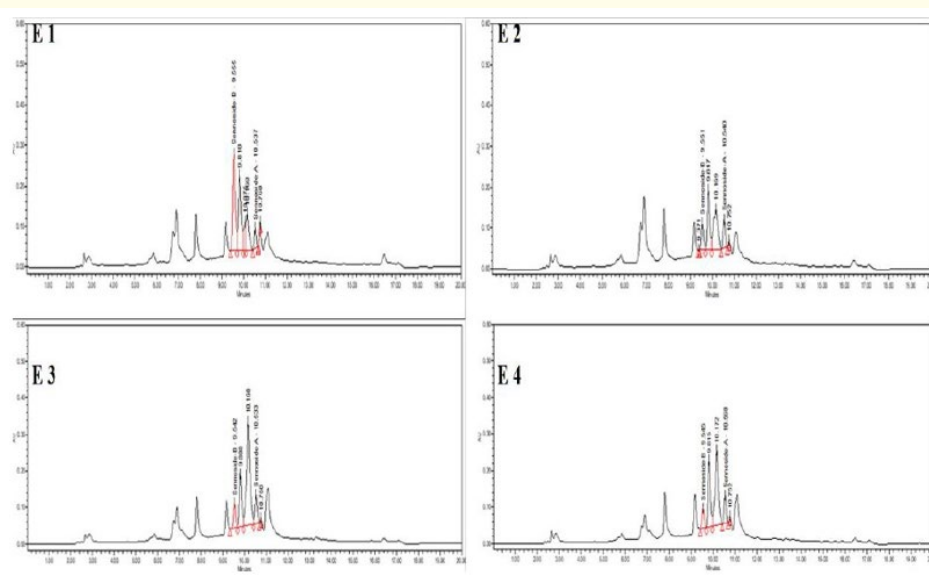


Figure 7: Chromatogram of the standard compound's sennoside A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as the mobile phases at an absorbance of 280 nm. E1 represents the Khaybar region in the summer, E2 in the autumn, E3 in the winter, and E4 in the spring.

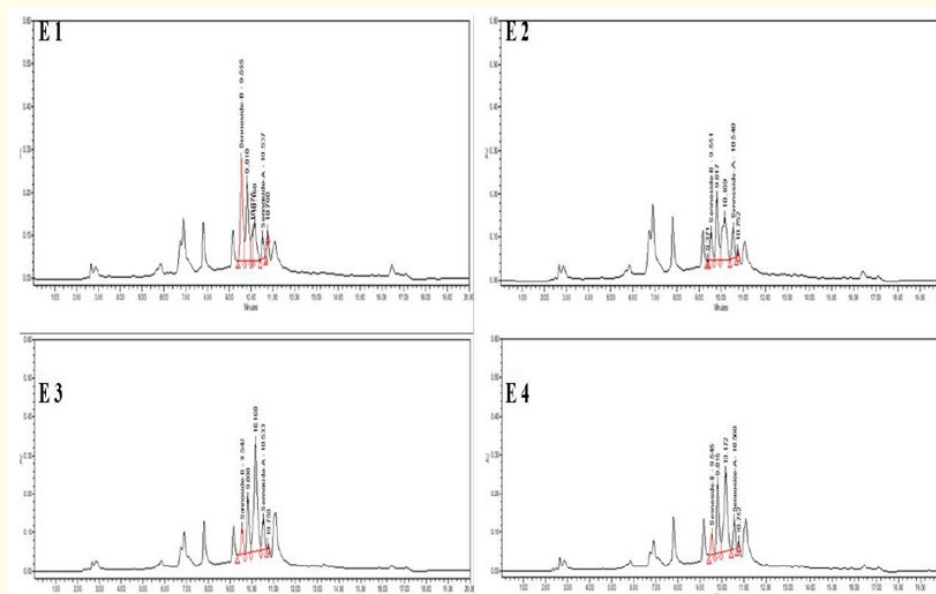


Figure 8: Chromatogram of the standard compound's succinate and succinate A and B using HPLC. 1% acetic acid, v/v, and succinate and acetonitrile were used as mobile phases at an absorbance of 280 nm. F1 represents Wadi Al-Disah in the summer region, F2 represents the autumn, F3 represents the winter, and F4 represents the spring.

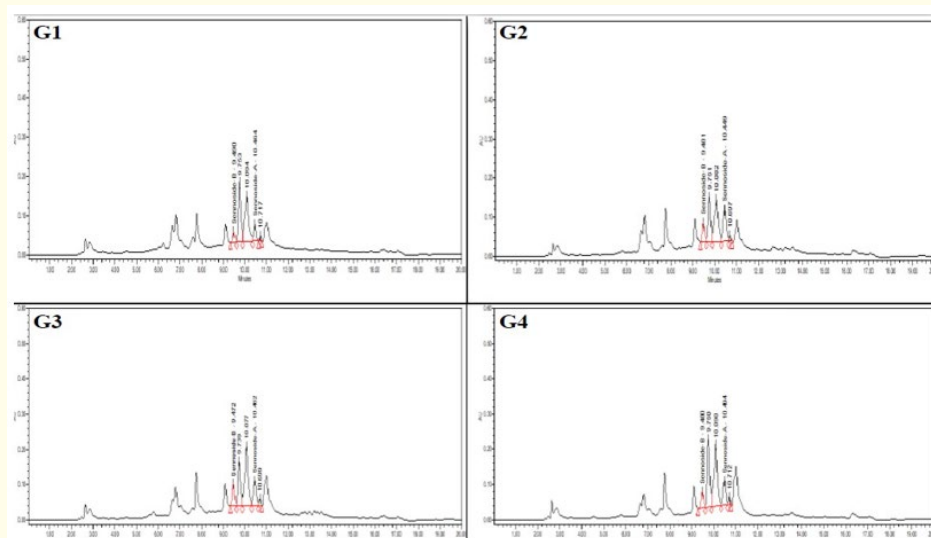


Figure 9: Chromatogram of the standard compound's succinate and succinate A and B using HPLC. 1% acetic acid, v/v, and acetonitrile were used as mobile phases at an absorbance of 280 nm. G1 represents the Taif region for the summer season, G2 for the autumn season, G3 for the winter season, and G4 for the spring season.

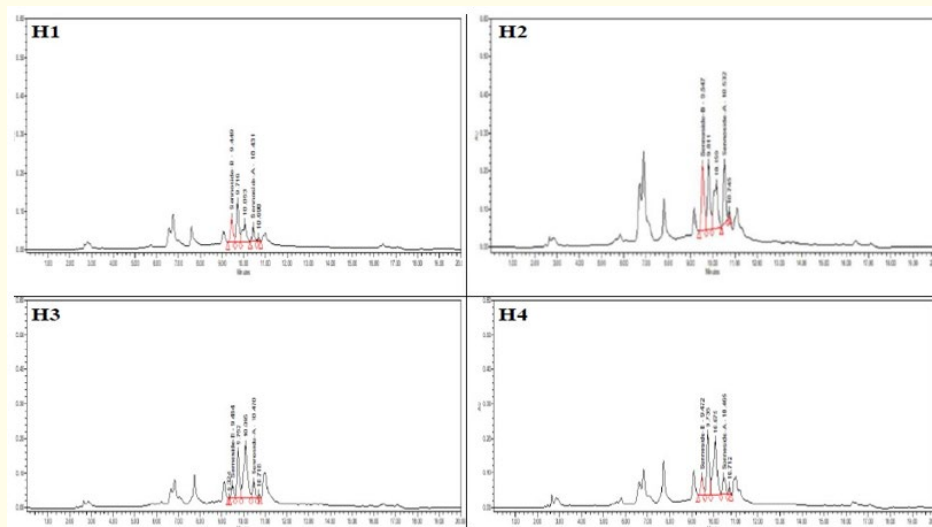


Figure 10: Chromatogram of the standard compounds succinate and succinate using HPLC. 1% acetic acid, v/v, and acetonitrile were used as mobile phases at an absorbance of 280 nm. H1 represents the Arafat region for the summer season, H2 for the autumn season, H3 for the winter season, and H4 for the spring season.

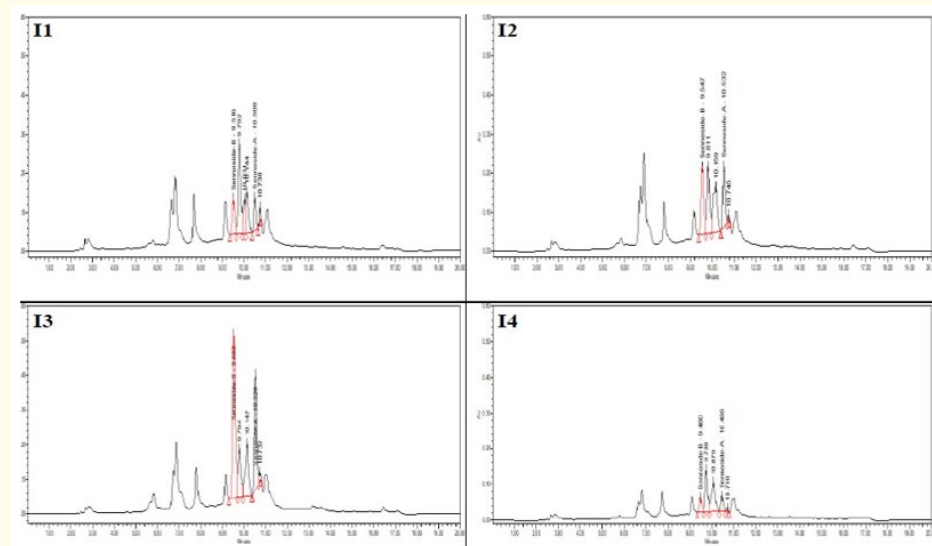


Figure 11: Chromatogram of the standard compounds succinate and succinate using HPLC. 1% acetic acid, v/v, and acetonitrile were used as mobile phases at an absorbance of 280 nm. I1 represents the Al-Qawarah region for the summer, I2 for the fall, I3 for the winter, and I4 for the spring.

Results

Physical Analysis of Soil

Soil Texture. Soil texture was determined based on the soil texture triangle (Table 1). In Al-Baha, the soil types were sandy loam in summer, fall, and spring and loamy sand in winter. The soil textures in the Khaybar area were loamy sand during summer and fall, sandy in winter, and sandy loam during spring. The textures of the soil at the Thadiq site was loamy sand in summer, autumn, and winter and sandy loam in spring. In Arafat, soil textures were loamy sand in summer and winter and sandy loam in autumn and spring. The soil texture in Al Quwarah, Taif, and Baish was sandy loam across all seasons. The soils of Hotat Bani Tamim and Wadi Al-Disah also exhibited sandy loam texture across all seasons. Soil texture changed during some seasons, as flooding in the study areas is common in the valleys.

<i>Region</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>
Khaybar	Loamy sand	Loamy sand	Sandy	Sandy loam
Thadiq	Loamy sand	Loamy sand	Loamy sand	Sandy loam
Al Quwarah	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Taif	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Hotat B.Tamim	Loamy sand	Loamy sand	Loamy sand	Loamy sand
Al-Baha	Sandy loam	Sandy loam	Loamy sand	Sandy loam
Arafat	Loamy sand	Sandy loam	Loamy sand	Sandy loam
Baish	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Wadi Al-Disah	Loamy sand	Loamy sand	Loamy sand	Loamy sand

Table 1: Soil texture variations across climatic seasons at the nine study locations.

Soil Moisture. The mean value was determined from measurements performed at three sampling sites in each season, totaling 108 samples across the four seasons. Soil moisture content according to site and season is presented in Table 2. The maximum soil moisture content was recorded in Al Quwarah (15.08%) during summer, while the minimum was observed in Thadiq (0.69%). The soil moisture content in Hotat Bani Tamim reached a peak of 31.39% during autumn, whereas the lowest amount was recorded in Thadiq (1.90%).

<i>Location</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>	<i>Mean</i>
Khaybar	4.09	13.32	0.95	2.16	5.13
Thadiq	0.69	1.90	2.77	2.97	2.8
Al-Qawara	15.08	15.68	8.02	7.50	11.57
Al-Taif	9.72	27.46	0.95	1.64	9.94
Hotat B.Tamim	2.53	31.39	0.77	0.56	8.81
Al-Baha	13.82	10.87	3.45	0.80	7.24
Arafat	9.74	4.15	3.45	1.85	4.80
Baish	11.67	29.19	4.15	3.96	12.24
Wadi Al-Disah	2.21	28.97	0.98	3.28	8.86
LSD Location			0.044		
Overall Mean	7.73	18.1	2.83	2.75	
LSD Seasons			0.0296		

Table 2: Soil moisture content (%) across nine locations during different climatic seasons ($\pm$ Standard error).

Chemical Analysis

pH. pH was determined in saturation using a pH meter, and the results are presented in Table 3. During summer, pH in Hotat Bani Tamim was 8.91, while the lowest pH was recorded in Al-Baha at 8.40. In autumn, the highest pH was observed in Hotat Bani Tamim (8.77), whereas the lowest was in Al Quwarah (8.38). During winter, the maximum pH was recorded in Wadi Al-Disah (8.87), while the minimum was in Al Quwarah (8.09).

Location	Summer	Autumn	Winter	Spring	Mean
Khaybar	8.86	8.74	8.74	8.63	8.74
Thadiq	8.76	8.54	8.24	8.58	8.53
Al-Qawara	8.69	8.38	8.35	8.09	8.38
Al-Taif	8.67	8.41	8.25	8.28	8.40
Hotat B.Tamim	8.91	8.77	8.64	8.58	8.72
Al-Baha	8.40	8.46	8.46	8.27	8.40
Arafat	8.81	8.69	8.55	8.72	8.69
Baish	8.62	8.46	8.33	8.41	9.46
Wadi Al-Disah	8.74	8.68	8.87	8.51	9.70
LSD Location			0.028		
Overall Mean	8.72	8.57	8.49	8.45	
LSD Seasons			0.0188		

Table 3: Soil pH across nine locations during different climatic seasons.

Soil Organic Matter. Organic matter percentages were recorded at nine specific points, averaging three samples from each area in one season (Table 4). The highest percentage of organic matter in summer was recorded in Taif (3.38%), while the lowest was in Wadi Al-Disah (0.77%). In autumn, the maximum organic matter percentage was recorded in Taif (2.74%) while the minimum was in Wadi Al-Disah (0.67%). The trend of the highest organic matter content (3.38%) in Taif in winter was consistent with the lowest (0.77%) in Wadi Al-Disah. The highest organic matter content (2.91%) in spring was also found in Taif, while the lowest was in Wadi Al-Disah (0.62%).

Location	Summer	Autumn	Winter	Spring	Mean
Khaybar	1.63	1.37	1.63	1.44	1.52
Thadiq	1.58	1.09	1.58	0.83	1.27
Al-Qawara	1.79	1.40	1.79	1.12	1.53
Al-Taif	3.38	2.74	3.38	2.91	3.10
Hotat B.Tamim	1.64	1.03	1.64	0.94	1.31
Al-Baha	0.99	1.38	0.99	1.32	1.17
Arafat	1.41	1.35	1.41	1.58	1.44
Baish	2.47	2.09	2.47	1.98	2.25
Wadi Al-Disah	0.77	0.67	0.77	0.62	0.71
LSD Location			0.0525		
Overall Mean	1.74	1.46	1.74	1.42	
LSD Seasons			0.035		

Table 4: Percentage of organic matter (OM) across nine locations during different climatic seasons.

Soil Electrical Conductivity. Soil electrical conductivity at the nine sites was recorded for each season, represented with the average of three samples per site, denoted in micromhos/cm ($\mu\text{mhos/cm}$) (Table 5). The highest electrical conductivity (EC) level was observed during summer in Al Quwarah (589.33 $\mu\text{mhos/cm}$), while the lowest EC was recorded in Khaybar (142.67 $\mu\text{mhos/cm}$). The highest electrical conductivity in autumn was also obtained in Al Quwarah (773.33 $\mu\text{mhos/cm}$), while the lowest value was observed in Baish (135.33 $\mu\text{mhos/cm}$). During winter, the highest EC reading was once again recorded in Al Quwarah (778.33 $\mu\text{mhos/cm}$), whereas the lowest was in Al-Baha (94.67 $\mu\text{mhos/cm}$). The highest EC value recorded in spring was also in Al Quwarah (952.00 $\mu\text{mhos/cm}$), with the lowest in Baish (93.00 $\mu\text{mhos/cm}$).

Location	Summer	Autumn	Winter	Spring	Mean
Khaybar	142.67	169.33	195.33	170.33	169.42
Thadiq	166.33	213.00	370.33	102.00	121.92
Al-Qawara	589.33	773.33	778.33	952.00	773.25
Al-Taif	248.00	236.00	258.33	203.00	236.33
Hotat B.Tamim	198.00	149.00	122.67	127.67	149.33
Al-Baha	281.33	192.00	94.67	203.00	192.75
Arafat	190.67	143.00	112.33	129.00	134.75
Baish	183.00	135.33	128.67	93.00	135.00
Wadi Al-Disah	168.67	142.33	134.00	125.33	142.58
LSD Locations			2.373		
Overall Mean	240.89	239.26	243.85	233.93	
LSD Seasons			1.583		

Table 5: Electrical conductivity (E.C) in $\mu\text{mhos/cm}$ for nine locations across the four climatic seasons.

Chloride. Chloride (Cl^-) concentrations were measured in milliequivalents per liter (mEq/L), as shown in Table 6. In summer, the highest chloride concentration was recorded in Al Quwarah (4.40 mEq/L), while the lowest was observed in Hotat Bani Tamim (2.17 mEq/L). Al Quwarah recorded 3.95 mEq/L in autumn, with the lowest reading in Wadi Al-Disah (1.08 mEq/L). In winter, the highest concentration of 3.52 mEq/L was observed in Al Quwarah, while the lowest was in Al-Baha (0.45 mEq/L). In spring, Al Quwarah recorded a chloride concentration of 4.10 mEq/L, with the lowest found in Taif (0.33 mEq/L).

Location	Summer	Autumn	Winter	Spring	Mean
Khaybar	2.20	1.75	0.85	0.202	1.74
Thadiq	2.65	1.43	1.32	0.45	1.46
Al-Qawara	4.40	3.95	3.52	4.10	3.99
Al-Taif	2.20	1.32	1.32	0.33	1.29
Hotat B. Tamim	2.17	1.20	0.87	0.43	1.17
Al-Baha	3.08	1.32	0.45	0.42	1.32
Arafat	3.52	1.73	0.73	0.85	1.71
Baish	2.65	1.32	0.80	0.43	1.30
Wadi Al-Disah	2.30	1.08	0.80	0.85	1.26
LSD Location			0.148		
Overall Mean	2.80	1.68	1.18	1.13	
LSD Seasons			0.987		

Table 6: Chloride salts (Cl^-) concentration in mEq/L for nine locations across the four climatic seasons.

Sodium. Sodium (Na) concentrations were measured in mg/L at the nine studied locations, with results shown in Table 7. In summer, the highest concentration was recorded in Al Quwarah (55.95 mg/L), while the lowest was in Khaybar (3.83 mg/L). In autumn, the highest level was again observed in Al Quwarah (62.03 mg/L), while the lowest was recorded in Thadiq (6.45 mg/L). In winter, Al Quwarah once again recorded the highest concentration (61.40 mg/L), while the lowest was found in Hotat Bani Tamim (2.89 mg/L). In spring, Al Quwarah showed the highest concentration (68.24 mg/L), while the lowest was in Wadi Al-Disah (2.90 mg/L).

<i>Location</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>	<i>Mean</i>
Khaybar	3.83	15.55	20.73	22.01	15.53
Thadiq	5.15	6.45	9.71	4.55	6.47
Al-Qawara	55.95	62.03	61.40	68.24	61.91
Taif	9.83	12.22	5.81	20.90	12.19
Hotat B.Tamim	9.19	6.79	2.89	8.30	6.79
Al-Baha	7.74	7.16	5.13	9.14	7.29
Arafat	17.15	11.03	8.44	8.31	11.23
Bish	10.25	7.60	8.31	4.47	7.66
Wadi Al-Disah	8.60	8.21	13.42	2.90	8.28
LSD Location			0.147		
Overall Mean	14.19	15.23	15.09	16.54	
LSD Seasons			0.098		

Table 7: Sodium (Na) concentration (mg/L) across nine locations during the climatic seasons.

Potassium. Potassium (K) concentrations were measured in milligrams per liter (mg/L) at the nine studied locations, with results shown in Table 8. In summer, Taif recorded the highest concentration (6.16 mg/L), while the lowest was found in Khaybar (2.42 mg/L). In autumn, Al Quwarah recorded the highest level (5.60 mg/L), while the lowest was in Baish (2.41 mg/L). In winter, Taif once again recorded the highest concentration (8.38 mg/L), while the lowest was in Wadi Al-Disah (1.82 mg/L). In spring, Khaybar recorded the highest level (6.02 mg/L), while the lowest was in Arafat (1.91 mg/L).

<i>Location</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>	<i>Mean</i>
Khyber	2.42	4.55	4.95	6.02	3.97
Thadiq	5.72	5.13	7.81	1.92	6.22
Al-Qawarah	5.78	5.60	5.15	5.94	5.51
Taif	6.16	4.62	8.38	4.94	6.39
Hotat B.Tamim	5.46	3.66	5.75	2.93	4.96
Al-Baha	3.64	3.30	1.92	5.44	2.95
Arafat	4.72	3.33	2.44	2.91	3.50
Bish	2.62	2.41	1.89	1.92	2.31
Wadi Al-Disah	3.13	3.54	1.82	5.70	2.83
LSD Location		0.053			
Overall Mean	4.41	4.02	4.46	4.19	
LSD Seasons			0.0352		

Table 8: Potassium (K) concentration (mg/L) in the nine study locations during the climatic seasons.

Calcium. Calcium (Ca) concentrations were measured in milligrams per liter (mg/L) at the nine studied locations, with results shown in Table 9. In summer, the highest concentration was recorded in Al Quwarah (15.35 mg/L), while the lowest was found in Khaybar (5.15 mg/L). Al Quwarah again recorded the highest level in autumn (16.30 mg/L), with the lowest in Khaybar (3.78 mg/L). In winter, the highest concentration was observed in Thadiq (20.81 mg/L), while the lowest was in Wadi Al-Disah (2.60 mg/L). In spring, the highest level was recorded in Al Quwarah (17.37 mg/L), and the lowest was in Taif (2.63 mg/L).

Location	Summer	Autumn	Winter	Spring	Mean
Khyber	5.15	3.78	2.61	3.71	3.81
Thadiq	8.66	11.27	20.81	4.42	11.29
Al-Qawarah	15.	16.30	16.52	17.37	16.38
Taif	13.16	10.62	16.25	2.63	10.67
Hotat B.Tamim	11.14	7.69	6.60	5.53	7.74
Al-Baha	15.34	10.41	4.42	11.50	10.42
Arafat	6.92	5.63	4.44	5.51	5.62
Bish	8.42	6.11	5.53	4.42	6.12
Wadi Al-Disah	6.30	5.12 ±	2.60	6.60	5.16
LSD Location		0.0436			
Overall Mean	10.05	8.55	8.86	6.85	
LSD Seasons		0.0291			

Table 9: Calcium (Ca) concentration (mg/L) in the nine study locations during the climatic seasons.

Magnesium. Magnesium (Mg) concentrations were measured in milligrams per liter (mg/L) at the nine studied locations, with results shown in Table 10. In summer, the highest concentration was recorded in Al Quwarah (14.35 mg/L), while the lowest was found in Khaybar (1.41 mg/L). In autumn, Al Quwarah again recorded the highest level (15.00 mg/L), with the lowest in Wadi Al-Disah (1.70 mg/L). The highest concentration in winter was also observed in Al Quwarah (14.52 mg/L), while the lowest was in Wadi Al-Disah (1.10 mg/L). In spring, the highest level was again recorded in Al Quwarah (16.10 mg/L), while the lowest was in Baish (1.21 mg/L).

Location	Summer	Autumn	Winter	Spring	Mean
Khyber	1.41	5.22	2.61	3.71	2.56
Thadiq	3.13	2.02	1.81	1.22	2.04
Al-Qawarah	14.35	15.00	14.52	16.10	14.99
Taif	3.33	2.71	2.25	2.63	2.73
Hotat B.Tamim	3.14	2.69	2.50	2.53	2.71
Al-Baha	2.27	2.21	1.21	3.10	2.20
Arafat	6.60	3.63	2.14	2.51	3.72
Bish	2.82	2.17	2.53	1.21	2.18
Wadi Al-Disah	1.41	1.70	1.10	2.52	1.68
LSD Location			0.0835		
Overall Mean	4.27	3.85	3.41	3.95	
LSD Seasons			0.0557		

Table 10: Magnesium (Mg) concentration (mg/L) in the nine study locations during the climatic seasons.

Nitrogen. Nitrogen (N) levels were measured in milligrams per liter (mg/L) at the nine studied locations, with results shown in Table 11. In summer, the highest concentration was recorded in Arafat (15.20 mg/L), while the lowest was found in Wadi Al-Disah (6.82 mg/L). In autumn, the highest level was recorded in Al-Baha (12.27 mg/L), with the lowest in Khaybar (8.62 mg/L). In winter, the highest concentration was observed in Taif (14.25 mg/L), while the lowest was in Arafat (7.62 mg/L). In spring, the highest level was recorded in Wadi Al-Disah (13.42 mg/L), whereas the lowest was in Al-Baha (8.70 mg/L).

Location	Summer	Autumn	Winter	Spring	Mean
Khyber	7.22	8.62	8.82	9.91	8.64
Thadiq	9.53	10.52	10.23	11.82	10.53
Al-Qawarah	11.72	10.63	9.82	10.27	10.61
Taif	7.53	10.22	14.25	8.81	10.20
Hotat B.Tamim	8.70	11.07	13.40	10.80	10.99
Al-Baha	12.33	12.27	11.81	8.70	11.28
Arafat	15.20	11.22	7.62	10.82	11.12
Bish	12.22	11.68	10.90	11.78	11.65
Wadi Al-Disah	6.82	9.67	8.60	13.42	9.63
LSD Location			0.0378		
Overall Mean	10.14	10.65	10.60	10.70	
LSD Seasons			0.0252		

Table 11: Total Nitrogen (Total N) concentration (mg/L) in the nine study locations during the climatic seasons ($\pm$ standard error).

Climate Data

Climate data were collected from the National Center of Meteorology, with monthly records for the year of sampling.

Temperatures. The mean temperatures for the seasons are presented in Table 14. During summer, both Hotat Bani Tamim and Thadiq registered the highest average temperature (44.23°C), while the lowest average temperature was recorded in Al-Baha (35.33°C). In autumn, Al-Baha recorded the lowest average temperature (29.87°C), while Arafat registered the highest average temperature (39.90°C). During winter, Arafat experienced the highest average temperature (32.50°C), and Wadi Al-Disah recorded the lowest average temperature (20.67°C). In spring, average temperatures varied from 39.73 °C in Arafat to 31.13°C as the lowest in Al-Baha.

Location	Summer	Autumn	Winter	Spring
Khyber	42.13	35.73	24.43	35.63
Thadiq	44.23	34.57	22.40	35.70
Al-Qawarah	42.57	33.97	21.53	34.47
Taif	35.73	31.43	24.33	31.73
Hotat B.Tamim	44.23	34.57	22.40	35.70
Al-Baha	35.33	29.87	24.47	31.13
Arafat	43.27	39.90	32.50	39.73
Bish	37.60	36.30	31.07	35.23
Wadi Al-Disah	39.20	33.10	20.67	31.87
Overall Mean	40.48	34.38	24.87	34.85

Table 12: Average Maximum Temperatures in Celsius degree (°C) During the Seasons at the Nine Study Locations.

<i>Location</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>
Khyber	14.67	22.33	37.33	14.33
Thadiq	16.33	28.33	48.00	21.00
Al-Qawarah	22.33	36.33	68.67	30.00
Taif	23.00	32.00	49.33	32.00
Hotat B.Tamim	16.33	28.33	48.00	21.00
Al-Baha	32.33	37.33	61.33	44.00
Arafat	31.33	47.00	54.67	40.00
Bish	66.33	69.00	73.67	66.00
Wadi Al-Disah	18.67	25.67	43.00	19.33
Overall Mean	26.81	36.26	83.78	31.96

Table 13: Average Relative Humidity (%) During the Seasons at the Nine Study Locations.

<i>Location</i>	<i>Summer</i>	<i>Autumn</i>	<i>Winter</i>	<i>Spring</i>
Khyber	0.00	8.77	12.10	3.30
Thadiq	0.00	1.90	4.17	1.10
Al-Qawarah	0.00	4.03	23.50	0.00
Taif	9.43	0.00	19.47	38.53
Hotat Bani Tamim	0.00	1.90	4.17	1.10
Al-Baha	34.70	0.73	0.67	13.93
Arafat	4.17	26.60	14.53	15.10
Bish	90.37	6.97	27.40	2.00
Wadi Al-Disah	0.00	0.00	11.00	0.33
Overall Mean	15.41	5.66	13	8.38

Table 14: Average Rainfall in Millimeters (mm) During the Seasons at the Nine Study Locations.

Relative Humidity. Table 15 presents the humidity levels for the seasons. In summer, the mean relative humidity for the nine studied sites varied from 66.33% in Baish to 14.67% in Khaybar. The highest relative humidity in autumn was recorded in Baish (69.00%), while the lowest was recorded in Khaybar (22.33%). Average relative humidity in winter ranged from 73.67% in Baish to 37.33% in Khaybar. During spring, relative humidity ranged between 14.33% in Khaybar and 66.00% in Baish.

<i>Location</i>	<i>Summer ($\mu\text{g}/\text{mg}$)</i>	<i>Autumn ($\mu\text{g}/\text{mg}$)</i>	<i>Winter ($\mu\text{g}/\text{mg}$)</i>	<i>Spring ($\mu\text{g}/\text{mg}$)</i>	<i>Mean</i>
Khyber	21.93 $\pm$ 0.68	14.15 $\pm$ 0.32	25.79 $\pm$ 0.42	21.43 $\pm$ 0.47	20.82
Thadiq	22.62 $\pm$ 0.37	18.42 $\pm$ 0.56	18.66 $\pm$ 0.42	26.96 $\pm$ 0.86	21.67
Al-Qawarah	47.72 $\pm$ 0.74	24.75 $\pm$ 0.65	67.31 $\pm$ 0.32	52.22 $\pm$ 0.57	48.00
Taif	34.21 $\pm$ 0.31	12.64 $\pm$ 0.46	28.44 $\pm$ 0.59	24.48 $\pm$ 0.57	24.94
Hotat B.Tamim	46.43 $\pm$ 0.84	25.91 $\pm$ 0.84	30.15 $\pm$ 0.67	34.21 $\pm$ 0.59	34.18
Al-Baha	18.48 $\pm$ 0.55	8.65 $\pm$ 0.42	25.98 $\pm$ 0.45	22.11 $\pm$ 0.89	18.81
Arafat	15.64 $\pm$ 0.75	12.80 $\pm$ 0.78	17.27 $\pm$ 0.82	19.07 $\pm$ 0.91	16.20
Bish	8.80 $\pm$ 0.31	11.45 $\pm$ 0.41	14.36 $\pm$ 0.41	37.84 $\pm$ 0.90	18.11
Wadi Al-Disah	17.53 $\pm$ 0.46	20.67 $\pm$ 0.62	27.91 $\pm$ 0.76	21.26 $\pm$ 0.55	21.85
LSD Location			0.872		

Overall Mean	25.93	16.61	28.43	28.84	
LSD Seasons			0.5816		

Table 15: Average Sennoside A (SA) Content ($\mu\text{g}/\text{mg}$) During the Seasons at the Nine Study Locations ($\pm$ standard error).

Precipitation. Seasonal rainfall in millimeters (mm) is presented in Table 16. The average precipitation during summer for the nine investigated areas varied between 90.37 mm in Baish and 4.17 mm in Arafat; other locations recorded no precipitation. The highest and lowest rainfall recorded was in Arafat (26.60 mm) and Al-Baha (0.73 mm), respectively, with no precipitation recorded in Taif and Wadi Al-Disah. Mean precipitation varied from 27.40 mm in Baish to 0.67 mm in Al-Baha during winter, while in spring, it ranged between 0.33 mm in Al Quwarah and 38.53 mm in Taif.

Location	Summer ($\mu\text{g}/\text{mg}$)	Autumn ($\mu\text{g}/\text{mg}$)	Winter ($\mu\text{g}/\text{mg}$)	Spring ($\mu\text{g}/\text{mg}$)	Mean
Khyber	21.41 $\pm$ 0.61	50.90 $\pm$ 0.37	22.48 $\pm$ 0.59	18.71 $\pm$ 0.59	28.37
Thadiq	22.85 $\pm$ 0.42	21.71 $\pm$ 0.78	12.25 $\pm$ 0.23	13.90 $\pm$ 0.42	17.68
Al-Qawarah	54.30 $\pm$ 0.42	33.28 $\pm$ 0.80	82.54 $\pm$ 0.37	42.16 $\pm$ 0.69	53.07
Taif	16.94 $\pm$ 0.34	8.03 $\pm$ 0.42	21.78 $\pm$ 0.57	15.94 $\pm$ 0.56	15.67
Hotat B.Tamim	31.44 $\pm$ 0.70	30.69 $\pm$ 0.78	29.04 $\pm$ 0.88	40.39 $\pm$ 0.62	32.89
Al-Baha	14.70 $\pm$ 0.54	3.20 $\pm$ 0.40	10.34 $\pm$ 0.29	15.47 $\pm$ 0.38	10.93
Arafat	14.14 $\pm$ 0.37	26.24 $\pm$ 0.57	12.19 $\pm$ 0.49	18.90 $\pm$ 0.76	17.87
Bish	7.84 $\pm$ 0.48	9.61 $\pm$ 0.62	9.92 $\pm$ 0.38	53.53 $\pm$ 0.32	20.22
Wadi Al-Disah	20.60 $\pm$ 0.38	28.68 $\pm$ 0.66	18.67 $\pm$ 0.50	20.86 $\pm$ 0.96	22.20
LSD Location			0.7932		
Overall Mean	22.69	23.59	24.36	26.65	
LSD Seasons			0.5288		

Table 16: Mean Sennoside B (SB) Content ($\mu\text{g}/\text{mg}$) During the Seasons at the Nine Study Locations ($\pm$ standard error).

Location	Summer (mg/g)	Autumn (mg/g)	Winter (mg/g)	Spring (mg/g)	Mean
Khaybar	30.82 $\pm$ 0.46	39.46 $\pm$ 0.15	41.07 $\pm$ 1.47	33.95 $\pm$ 0.19	36.33
Thadiq	36.07 $\pm$ 0.99	26.17 $\pm$ 2.10	32.06 $\pm$ 1.05	38.99 $\pm$ 0.52	33.32
Al-Qawara	61.44 $\pm$ 0.71	35.07 $\pm$ 1.79	54.62 $\pm$ 1.90	53.91 $\pm$ 0.61	51.26
Al-Taif	24.98 $\pm$ 0.01	25.09 $\pm$ 0.17	32.40 $\pm$ 0.01	37.01 $\pm$ 0.07	29.87
Hotat B.Tamim	31.71 $\pm$ 0.41	34.18 $\pm$ 0.34	29.34 $\pm$ 2.42	45.14 $\pm$ 0.94	35.09
Al-Bahah	40.69 $\pm$ 2.61	24.20 $\pm$ 0.46	34.32 $\pm$ 2.73	31.41 $\pm$ 2.32	32.66
Arafat	28.88 $\pm$ 3.26	20.82 $\pm$ 0.13	27.37 $\pm$ 1.19	33.33 $\pm$ 0.69	27.60
Baysh	31.03 $\pm$ 1.65	24.70 $\pm$ 1.41	56.02 $\pm$ 1.48	32.46 $\pm$ 0.21	36.05
Wadi Al-Disah	30.96 $\pm$ 1.20	28.70 $\pm$ 0.42	36.96 $\pm$ 0.40	38.66 $\pm$ 0.44	33.82
LSD Location			1.899		
Overall Mean	35.18	28.71	38.24	38.32	
LSD Seasons			1.2658		

Table 17: Mean Total Flavonoids (TF) content (mg/g) across the four seasons at nine locations ($\pm$ standard error).

Secondary Metabolic Products

Sennoside A. Sennoside A was analyzed using HPLC, and the results in $\mu\text{g}/\text{mg}$ are shown in Table 14. In summer, the maximum sennoside A content was recorded in Al Quwarah ($47.72 \mu\text{g}/\text{mg}$), while the minimum was observed in Baish ($8.80 \mu\text{g}/\text{mg}$). In autumn, the maximum sennoside A content ($25.91 \mu\text{g}/\text{mg}$) was recorded in Hotat Bani Tamim, while the minimum ($8.65 \mu\text{g}/\text{mg}$) was recorded in Al-Baha. During winter, the highest sennoside A content ($67.31 \mu\text{g}/\text{mg}$) was reported in Al Quwarah, with the lowest ($14.36 \mu\text{g}/\text{mg}$) in Baish (Table 3). In spring, the highest content was again observed in Al Quwarah ($52.22 \mu\text{g}/\text{mg}$), while the lowest was in Arafat ($19.07 \mu\text{g}/\text{mg}$). A greater accumulation of sennoside A was found in spring ($28.84 \mu\text{g}/\text{mg}$), followed by winter ($28.43 \mu\text{g}/\text{mg}$), summer ($25.93 \mu\text{g}/\text{mg}$), and autumn ($16.61 \mu\text{g}/\text{mg}$). Sennoside A concentration in the highest alkaloid content was observed in Al Quwarah ($48 \mu\text{g}/\text{mg}$), while the lowest concentration was in Arafat ($16.20 \mu\text{g}/\text{mg}$).

Sennoside B. Sennoside B levels (micrograms per mg: $\mu\text{g}/\text{mg}$) were determined using HPLC (Table 18). In summer, Al Quwarah recorded the highest value of sennoside B ($54.30 \mu\text{g}/\text{mg}$), while the lowest was in Baish ($7.84 \mu\text{g}/\text{mg}$). Khaybar recorded the highest-level during autumn ($50.90 \mu\text{g}/\text{mg}$), with the minimum value observed in Al-Baha ($3.20 \mu\text{g}/\text{mg}$). The highest value of sennoside B in winter was also detected in Al Quwarah ($82.54 \mu\text{g}/\text{mg}$), while the lowest was in Baish ($9.92 \mu\text{g}/\text{mg}$). During spring, the highest content of sennoside B was detected in Baish ($53.53 \mu\text{g}/\text{mg}$), while the lowest was in Thadiq ($13.90 \mu\text{g}/\text{mg}$). The most sennoside B was retained during spring, with an average of $26.65 \mu\text{g}/\text{mg}$, while winter and autumn showed averages of 24.36 and $23.59 \mu\text{g}/\text{mg}$, respectively. Least values were recorded for summer ($22.69 \mu\text{g}/\text{mg}$). The highest level of sennoside B accumulation ($53.07 \mu\text{g}/\text{mg}$) was observed in Al Quwarah, while the lowest level ($10.93 \mu\text{g}/\text{mg}$) was recorded in Al-Baha.

Location	Summer (mg/g)	Autumn (mg/g)	Winter (mg/g)	Spring (mg/g)	Mean
Khaybar	76.75 ± 2.20	86.83 ± 9.44	90.75 ± 13.73	89.25 ± 17.03	85.89
Thadiq	74.83 ± 1.53	69.91 ± 5.01	147.75 ± 3.31	96.00 ± 2.48	97.12
Al-Qawara	125.50 ± 5.36	101.41 ± 6.42	184.25 ± 3.31	77.16 ± 6.08	122.08
Al-Taif	71.91 ± 5.07	61.00 ± 2.95	75.08 ± 4.55	65.25 ± 7.47	68.31
Hotat B.Tamim	46.75 ± 5.67	74.91 ± 4.55	143.83 ± 4.55	74.75 ± 7.27	85.06
Al-Bahah	125.91 ± 4.57	116.16 ± 3.79	158.50 ± 3.79	123.91 ± 8.38	131.12
Arafat	108.08 ± 4.26	91.00 ± 1.86	159.83 ± 5.24	68.83 ± 9.88	106.93
Baysh	120.16 ± 2.33	67.75 ± 5.73	159.66 ± 5.98	125.25 ± 9.72	118.21
Wadi Al-Disah	127.25 ± 3.61	53.16 ± 13.61	147.33 ± 10.17	112.75 ± 4.43	110.12
LSD Location			11.8399		
Overall Mean	97.46	80.24	140.77	92.57	
LSD Seasons			7.8933		

Table 18: Mean Total Phenolic Content (PC) (mg/g) across the four seasons at nine locations ($\pm$ standard error).

Location	Summer	Autumn	Winter	Spring	Mean
Khaybar	58.25 ± 0.34	46.46 ± 2.24	64.20 ± 0.74	60.83 ± 2.34	57.44
Thadiq	54.66 ± 0.68	39.62 ± 0.40	56.23 ± 0.67	57.91 ± 2.86	52.10
Al-Qawara	59.26 ± 0.70	49.38 ± 0.45	59.93 ± 0.67	59.15 ± 0.40	56.93
Al-Taif	63.41 ± 0.88	62.63 ± 0.39	64.42 ± 0.68	47.70 ± 0.30	59.54
Hotat B.Tamim	63.86 ± 1.07	40.51 ± 1.51	61.28 ± 0.51	49.38 ± 0.56	53.76
Al-Bahah	43.55 ± 0.30	61.95 ± 0.39	67.11 ± 0.49	66.67 ± 2.44	59.82
Arafat	64.98 ± 2.06	45.68 ± 0.68	66.33 ± 5.39	62.85 ± 0.45	59.96
Baysh	66.89 ± 0.79	50.06 ± 0.79	66.22 ± 1.27	66.55 ± 0.22	62.43

Wadi Al-Disah	45.90 ± 0.90	43.77 ± 1.08	62.63 ± 0.19	59.71 ± 0.11	53.00
LSD Location			1.998		
Overall Mean	87.86	48.90	36.15	85.97	
LSD Seasons			1.332		

Table 19: Antioxidant activity (%) across seasons at the nine study locations ($\pm$ standard error).

Total Flavonoid Contents. Total flavonoid contents (TFC) were detected using the aluminum chloride colorimetric method with quercetin as the reference standard (mg/g). The mean of three readings was recorded and is presented in Table 16. The maximum TFC during summer was recorded at Al Quwarah (61.44 mg/g), while the minimum was observed in Taif (24.98 mg/g). In autumn, Khaybar recorded the highest TFC (39.46 mg/g), while Arafat recorded the lowest (20.82 mg/g). In winter, Baish recorded the highest TFC (56.02 mg/g), while Arafat recorded the lowest (27.37 mg/g). In spring, Al Quwarah reported a maximum TFC (34.91 mg/g), while Al-Baha had the minimum (3.91 mg/g). The highest flavonoid content was obtained in spring (38.32 mg/g), while the lowest was in autumn (28.71 mg/g). Among all areas, Al Quwarah had the maximum amount of TF (51.26 μ g/mg), while Arafat had the minimum (27.60 μ g/mg).

Phenolic Contents. Phenolic contents (PC) were recorded using a UV-visible spectrophotometer and expressed as gallic acid equivalents. The highest phenolic content during summer was found in Wadi Al-Disah (127.25 mg/g), while the lowest was in Hotat Bani Tamim (46.75 mg/g). In autumn, the highest was in Al-Baha (116.16 mg/g), while the lowest was in Wadi Al-Disah (53.16 mg/g). In winter, the highest content was recorded in Al Quwarah (184.25 mg/g), while Taif recorded the lowest (75.08 mg/g). In spring, Baish recorded the highest (125.25 mg/g), while Taif had the lowest (65.25 mg/g). The highest total phenolic content (140.77 mg/g) was observed in winter, while the lowest was recorded in autumn (80.24 mg/g). Spring saw higher phenolic content (132.92 mg/g) than that during autumn but lower than that during winter (80.24 mg/g) (Table 1). The Al-Bahah area had the highest total mean phenolic content (131.12 mg/g), while Taif had the lowest (68.31 mg/g) among the studied regions.

Total Antioxidants. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method at different concentrations (1000, 500, 250, 125, 62.5, and 31.25 mg/ml). We established a mean of 250 mg/ml from three readings per season and background, which provided the most reliable measurements. The results are expressed as inhibition percentage compared to that of Rutin, used as a reference (Table 17).

During summer, the highest antioxidant percentage was recorded in Baish (66.89%), while the lowest was observed in Al-Baha (43.55%). In autumn, Taif reported the highest percentage (62.63%), while the lowest was noted in Thadiq (39.62%). The highest antioxidant activity of the methanol extracts in winter was seen in Al-Baha (67.11%), while the lowest was observed in Thadiq (56.23%). In spring, Al-Baha again reported the highest percentage (66.67%), while the lowest was reported in Taif (47.70%).

The highest antioxidant content (63.15%) was noted in winter, while the lowest percentage was observed in autumn (48.90%). Among the studied locations, Baish showed the best antioxidant activity (62.43%), whereas the lowest antioxidant content was in Thadiq (52.10%).

Discussion

Sennoside A (SA)

The highest contents of the bioactive compound sennoside A (SA) were observed during the winter and spring seasons, particularly at the Al-Qawara site. This trend is attributed to the moderate climatic conditions during these seasons, as relatively low temperatures and high humidity facilitate the activation of enzyme pathways involved in SA biosynthesis. SA is an anthraquinone compound synthesized through the shikimate pathway, and its accumulation is influenced by various environmental factors, including temperature, humidity, and soil properties. The highest SA levels were observed at the Al-Qawara site, which has high sodium and magnesium

levels in the soil (Table 1). These ions may improve the plant's resistance against oxidative burst, as hypothesized by Zandalinas et al. (2017). There were also seasonal patterns. The total SA content varied based on the following seasonal changes: the highest amounts in winter and spring, a decline in summer, and the lowest levels in autumn. The reduced sunlight in fall was a contributing factor; a 25% reduction in solar radiation was observed at Taif, which experienced increased cloud cover. This decrease in solar irradiation exposure resulted in lesser sennoside A formation, consistent with findings from another study on *Cassia angustifolia* (Raju et al., 2013). SA accumulation was also affected by soil texture. Sandy loam soils like those in Khaybar, Thadiq, and Al-Qawara increased SA content compared with loamy sand soils. For example, SA increased by 54.84% following a transition from loamy sand to sandy in Khaybar from autumn to winter. There was a strong negative association between temperature and SA accumulation in Wadi Al-Disah, as the highest levels of SA were accumulated in winter and the levels decreased in summer. This finding aligns with Ratnayaka et al. (2002), who indicated that lower temperatures favor SA production. High soil sodium concentration was also associated with high SA accumulation, observed in both Wadi Al-Disah and Hotat Bani Tamim. This may reflect an adaptive mechanism of the plants in response to soil moisture deficit, as plants may increase secondary metabolite contents under stress conditions (Arshi et al., 2006). Variations in SA accumulation may represent the growth stage of the plant, mediated by changes in soil water content due to seasonality. This finding is consistent with the findings of Singh et al. (2018) in *Cassia angustifolia*, where differences in SA accumulation between plantations based on environmental conditions during planting and harvesting were compared. Water stress may lead to decreased protein synthesis in melon leaves than in roots when compared with the control. The authors also observed that during the vegetative stage, water shortages resulted in shorter melon plants, reduced photosynthesis rates, fewer leaves, less fruit development, and decreased branching due to low soil moisture. Therefore, water stress-induced sodium accumulation may be a plant-controlled response to soil water deficit, consequently influencing the allocation of secondary metabolites, such as increased SA content. Sennoside A (SA) levels were highest in winter and spring due to moderate temperatures, high humidity, and soils rich in sodium and magnesium. Furthermore, sandy loam soil and low temperatures favor SA production.

Sennoside B (SB)

In contrast to SA, no evident relationships were observed between environmental factors, seasonal changes, and SB accumulation. However, a strong association was found between the botanical season in distinct locations and SB content. SB accumulation in autumn was higher in Khaybar and Wadi Al-Disah, likely due to the spatial effect on the maturity period of leaves and fruits during that season. This aligns with findings by Singh et al. (2018), who observed higher accumulation of sennosides after leaf and fruit ripening in *Cassia angustifolia*. Soil water content is a crucial factor in SB accumulation. A significant positive relationship was found between soil moisture and SB contents, where SB inducement was 59.90% and 28.17% in Khaybar and Wadi Al-Disah, respectively, when comparing the autumn and summer seasons. However, in most studied sites, negatively serrated SB was more pronounced with reduced soil water content than under potential conditions, except in Khaybar and Wadi Al-Disah. This result is comparable to studies on *Cassia acutifolia* and *Cassia angustifolia*, where sennoside accumulation was enhanced with irrigation water deficit (El Mula Ahmed et al., 2014; Lohar et al., 1979). Under cool temperatures, SB accumulation was generally reduced, whereas SA accumulation increased. SB accumulation was also influenced by spatial relationships. The accumulation in winter was lesser in Thadiq, Hotat Bani Tamim, Arafat, and Wadi Al-Disah, much like in autumn in Al Quwarah, Taif, and Al-Baha. SB levels were lowest during spring in Khaybar. The effects of accessions and location on SB accumulation behavior also contributed, as per the observation of Morris et al. (2019), who noted similar effects of genetics and environment on sennoside production. Overall, SB accumulation appears to be more flexible and context-dependent than SA accumulation, with soil moisture, species phenology, and plant growth stage playing significant roles. There was no clear seasonal trend; the highest SB levels were recorded in autumn at some stations (Khaybar and Wadi Al-Disah). Soil moisture and crop age are key factors, with lower water content correlating with higher SB levels. SB accumulation is however suppressed by low temperatures.

Flavonoid Content (FC)

The analysis demonstrated unique seasonal variation in flavonoid accumulation, influenced by temperature and light conditions. At 15° C and/or high temperatures, TFC increased at most sites, except for Al Quwarah. The highest TF levels were detected during winter

at Khaybar, followed by Baish, with mean maximum temperatures ranging from 24.43 to 31.07° C. This is consistent with conclusions by Shui-Yuan et al. (2010), who found that temperature promotes the accumulation of flavonoids in *Ginkgo biloba* leaves. Conversely, high temperatures resulted in reduced flavonoid content, as indicated by Virjamo et al. (2014) in *Picea abies* bark, even at a mean maximum summer temperature of 42.57 °C at the Al-Qawara site.

Spring was identified as the most suitable season for flavonoid production, with Thadiq, Taif, Hotat Bani Tamim, Arafat, and Wadi Al-Disah exhibiting the highest flavonoid activity. The mean maximum temperatures in these sites varied between 31.73° C and 39.73° C during this season. Maximum flavonoid accumulation occurred on days with high light intensity and photosynthesis, coinciding with days of maximum daylight hours, which corresponds with Akula and Ravishankar (2011), who noted that light induces flavonoid production as a protective mechanism against UV radiation. Diurnal temperature fluctuations were observed, with the least flavonoid accumulation recorded during autumn and summer in most regions, while Hotat Bani Tamim maintained constant flavonoid levels. The content of all five flavonoids was significantly related to various environmental factors, as shown by statistical analyses. Although correlations were low for individual location analyses, both Khaybar and Taif indicated high precipitation and FC when high precipitation rates were recorded ($r = 0.99327$ and $r = 0.95459$, respectively, both $p < 0.05$). When assessing the overall impact of environmental factors, strong correlations were also found for total dissolved solids ($r = 0.53315$), electrical conductivity ($r = 0.52844$), magnesium ($r = 0.57131$), and sennoside B (SB) ($r = 0.54526$) at $p > 0.005$. Highly significant relationships were obtained for sodium ($r = 0.59347$) and SA ($r = 0.59978$) at $p < 0.0001$. These results agree with Taibi et al. (2016), who reported enhanced FC in *Phaseolus vulgaris* L. due to NaCl soil application, and with Hussain et al. (2019), who observed increased FC accumulation in *Raphanus sativus* L. after MgO application. Flavonoid concentrations from different geographical locations were remarkable, with the Al-Qawarah area accumulating higher content throughout the study. This phenomenon may be associated with the soil mineral composition, particularly a high content of magnesium and potassium—components that activate flavonoid-synthesizing enzymes (Gharibi et al., 2019). Further, evidence of soil composition's effect on flavonoid composition could be drawn from the correlation between organic matter content and concentrations of flavonoids and minerals in Taif and Al-Baha. These spatial patterns confirm the interactions between environmental and soil factors that shape flavonoid production. The study also revealed several strong relationships between flavonoid content and other metabolites. FC was positively and significantly correlated with SA ($r = 0.59978$, $p > 0.0001$) and SB ($r = 0.54526$, $p > 0.005$), indicating positive synergisms in the secondary metabolism pathways of the plant. These correlations illustrate the integrated nature of defensive compound formation in response to allelopathic signals.

Total Phenolic Content (TPC)

The TPC of all investigated populations was highest in winter, suggesting that low temperatures played an advantageous role. This finding aligns with previous research on *Juglans regia* L., where phenolic contents were found to be higher during colder seasons and decreased in warmer seasons (Sin et al., 2011). The mean winter temperatures ranged from 21 to 33° C, with average relative humidity between 74% and 34%, which may account for the observed increase in total phenolic content. The results support previous research indicating that low temperatures increase phenolic content in plants, as demonstrated in studies on *Camellia sinensis* grown in Australia (Yao et al., 2005) and in Turkey (Erturk et al., 2010). Conducting this study during the monsoon season may have induced elevated phenolic contents besides decreasing temperatures. Phenolic contents decreased with low relative humidity and high temperatures, with the lowest values found in summer and spring, except in Al Quwarah during spring. This indicates that higher temperatures and lower humidity correlate with decreased phenolic content. The increased levels of phenolics found during cooler months may be due to enhanced secondary metabolism in the leaves, characterized by high carbohydrate levels at low temperatures (Sampaio et al., 2011). For instance, in *Glycine max*, an increase in phenolic compounds was observed after 24 hours of exposure to low temperatures (da Costa Zonetti et al., 2013). Janská et al. (2010) also noted that phenolics accumulate due to cold stress, as these compounds are integrated into the cell wall as lignin and suberin. The significant differences are not uniform across locations in statistical terms. The Hotat Bani Tamim site exhibited the highest correlation with other studied components, including sodium ($r = -0.97491$), nitrogen ($r = 0.96614$) (Table 1), average upper temperature ($r = -0.98397$), average relative humidity ($r = 0.97654$), and average precipitation ($r = 0.97872$) at $p < 0.05$. In Khaybar, phenolic content was significantly related to sodium and phosphorus levels at $p < 0.05$, with $r =$

0.98239 and 0.97779, respectively. The significant positive correlation between sodium and PC concentration in Khaybar is expected, as plants from this location are exposed to high water salinity due to the geological matrix in the area. Salts in the soil increase as water evaporates (Sonbul, 2016). A study by Parida et al. (2004) on *Aegiceras corniculatum* also confirmed this result, showing a more than twofold increase in total phenol content in plants receiving 250 millimolar sodium chloride compared with controls. Furthermore, increased soil phosphorus at the Khaybar site resulted in elevated phenolic content, consistent with findings from a study on *Iris* species conducted in Ukraine, Lithuania, and Latvia, which observed that phenolic content increased with elevated phosphorus soil levels (Mykhailenko et al., 2020). In Arafat and Wadi Al-Disah, silt showed negative significant relationships with TPC at $p < 0.05$, with $r = -0.95728$ and $r = -0.96579$, respectively. Additionally, clay showed a negative correlation with phenolic content at $p < 0.0005$ in Al Quwarah and Arafat ($r = -0.99533$ and -0.99856 respectively). The only negatively significant correlation between TPC and soil water content was observed in Wadi Al-Disah ($r = -0.95896$, $p < 0.05$), which was also in parallel with SB ($r = -0.98686$). The Al-Baha site reported the highest phenolic content, associated with high organic matter and phosphorus levels in the soil. This agrees with the report by Elsafy et al. (2014), which indicated that phosphorus-rich soils promote phenolic biosynthesis via the enzyme PAL (phenylalanine ammonia lyase) in this pathway. The highest levels of phenolic compounds were found during winter, a season characterized by cold temperatures and environmental stresses (e.g., drought or cold at night). These findings are consistent with those of Lattanzio et al. (2006) who reported an increase in phenolic production due to environmental stressors as a part of the plant's resistance phenomenon. This suggests that the senescence stage of *S. italica* could influence phenolic contents, as the plant matures during winter. This observation aligns with findings on pomegranate species, where differences in total phenolic content were explained by the plant's growth stage (Di Stefano et al., 2020). Additionally, Ahmad et al. (2011) indicated lower concentrations of phenolic compounds in winter as a consequence of both low temperatures and plant maturity.

Antioxidants

The study revealed clear patterns in antioxidant accumulation across different locations and seasons. Altitude above sea level significantly affected antioxidant levels in plant species (Chrysargyris et al., 2021), with mountainous areas (Khaybar, Taif, Al-Bahah, Arafat, and Wadi Al-Disah) exhibiting the highest antioxidant content in winter, while the content in the plains (Thadiq, Hotat Bani Tamim, and Baish) peaked in summer and spring. These results align with findings on *Salvia officinalis* L., where mountain locations demonstrated the highest antioxidant content in winter, while plains had peak levels during summer (Chrysargyris et al., 2021). Interestingly, although Al Quwarah is classified as a plain area, it recorded the highest antioxidant content in winter, suggesting unique local factors at play.

Seasonal analysis indicated that antioxidant content was generally highest in winter and lowest in autumn, with Taif being an exception, showing negligible accumulation in spring. The altitude of this area likely contributed to this anomaly. Supporting evidence comes from Mudau et al. (2008), who found that *Athrixia phylicoides* had the highest total antioxidant content during winter, followed by summer, with spring exhibiting the lowest levels. Similarly, *Baccharus dentata* recorded the highest antioxidant activity in summer and winter (Sartor et al., 2013).

Statistical correlations ($p < 0.05$) revealed important relationships:

1. In Taif, positive correlations with calcium ($r = 0.95131$) and electrical conductivity ($r = 0.95596$) were observed.
2. In Khaybar, a strong negative correlation between soil moisture and antioxidants ($r = -0.99448$) was noted.
3. In Al-Bahah, a significant negative correlation with chloride ($r = -0.99127$) was observed.

The positive correlation with calcium aligns with studies on sage, which show significant positive values related to altitude and season (Chrysargyris et al., 2021). The correlation with electrical conductivity in Taif indicates salt-tolerance mechanisms, consistent with findings on *Mesembryanthemum edule* (Falleh et al., 2012) and *Cassia angustifolia* (Agarwal & Pandey, 2004). The negative correlation with chloride suggests that increased chloride levels may reduce photosynthesis.

DPPH measurements indicated the highest antioxidant capacity in winter, followed by spring, mirroring the patterns of phenolic and flavonoid accumulation. This supports Mokgotho et al.'s (2013) finding that phenolics are primarily responsible for antioxidant activity in medicinal plants. The Baish site exhibited the strongest antioxidant activity, likely due to an optimal balance of soil moisture, organic matter, and salt content.

Geographical comparisons revealed the following:

1. Mountainous regions generally outperformed plains in antioxidant production during winter.
2. Al Quwarah was an exception among the plains, with a peak observed in winter.
3. Baish's balanced soil created ideal conditions for antioxidant accumulation.

These findings demonstrate that antioxidant production in *S. italica* involves complex interactions among altitude, seasonality and soil chemistry, with winter conditions and specific soil balances being particularly favorable for antioxidant accumulation. The study provides comprehensive evidence that environmental factors significantly influence plant antioxidant profiles, with implications for the cultivation and medicinal use of *S. italica*.

Conclusion

This study investigated five secondary metabolic products of *S. italica* that grows naturally in Saudi Arabia across nine locations and four seasons, comparing the results with various factors that may impact secondary metabolite production. The best season for sennoside A accumulation was summer, while it was least in autumn. Al Quwarah recorded the highest sennoside A accumulation among the studied locations, whereas Arafat reported the lowest. For sennoside B, the highest accumulation was observed in spring, while the least was in winter. Again, the highest sennoside B accumulation occurred in Al Quwarah, while Al-Bahah reported the least.

Spring also accounted for the highest total flavonoid content, with autumn showing the least accumulation among the seasons. By location, Al Quwarah exhibited the highest total flavonoid contents, while Arafat had the lowest. Total phenol content peaked in winter, with a significant difference from the other seasons, and recorded the lowest content in autumn. Al-Bahah reported the highest total phenolic content, while Hotat Bani Tamim had the lowest. Lastly, antioxidant content was highest in spring and lowest in autumn, with Baish recording the highest percentage of antioxidants and Thadiq the lowest.

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Conflict of Interest

I declare that I have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Abdoulahi MII., et al. "Ethno Botanical, Pharmacology and Phytochemistry of widely used medicinal plants in Niger: A Review". *Journal of Medicinal Plants* 10.4 (2022): 46-60.
2. Adjou ES., et al. "Phytochemical profile and potential pharmacological properties of leaves extract of *Senna italica* Mill". *American Journal of Pharmacological Sciences* 9.1 (2021): 36-39.
3. Agarwal S and Pandey V. "Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*". *Biologia Plantarum* 48.4 (2004): 555-560.
4. Ahmad I., et al. "Seasonal variation in some medicinal and biochemical ingredients in *Mentha longifolia* (L.) Huds". *Pak J Bot* 43.4 (2011): 69-77.

5. Akula R and Ravishankar GA. "Influence of abiotic stress signals on secondary metabolites in plants". *Plant signaling & behavior* 6.11 (2011): 1720-1731.
6. Al-Dakan AA, al-Tuffail M and Hannan MA. "Cassia senna inhibits mutagenic activities of benzo[a]-pyrene, aflatoxin B1, shamma and methyl methanesulfonate". *Pharmacology & toxicology* 77.4 (1995): 288-292.
7. Alqahtani A., et al. "Seasonal Variation of Triterpenes and Phenolic Compounds in Australian *Centella asiatica* (L.) Urb". *Phytochemical analysis: PCA* 26.6 (2015): 436-443.
8. Al-Yemeni MN. "Ecological studies on Sand dunes vegetation in Al-Kharj region". *Saudi Arabia Saudi Journal of Biological Sciences* 7.1 (2000): 64-87.
9. APHA. "Standard methods for the examination of water and wastewater". 20th edition (1998).
10. Arshi A, Abdin MZ and Iqbal M. "Sennoside content and yield attributes of *Cassia angustifolia* Vahl. as affected by NaCl and CaCl₂". *Scientia horticulturae* 111.1 (2006): 84-90.
11. Azaizeh H., et al. "Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region". *Fitoterapia* 74.1-2 (2003): 98-108.
12. Bisergaeva RA and Sirieva YN. "Determination of calcium and magnesium by atomic absorption spectroscopy and flame photometry". In *Journal of Physics: Conference Series* 1691.1 (2020): 012055.
13. Bouyoucos GJ. "Hydrometer method improved for making particle size analyses of soils 1". *Agronomy journal* 54.5 (1962): 464-465.
14. Bown D. "Encyclopedia of herbs and their uses". London: Dorling Kindersley (1995): 240-241.
15. Bremner JM and Mulvaney CS. Total nitrogen In: Page, AL, RH Miller, and DR Keeney (Eds). *Methods of Soil Analysis. Part 2.* Amer. Soc. Agron. Madison, WI USA (1982): 595-624.
16. Bukar A, Mukhtar M and Hassan A. "Phytochemical screening and antibacterial activity of leaf extracts of *Senna siamea* (Lam) on *Pseudomonas aeruginosa*". *Bayero Journal of Pure and Applied Sciences* 2.1 (2009): 139-142.
17. Chrysargyris A, Evangelides E and Tzortzakakis N. "Seasonal variation of antioxidant capacity, phenols, minerals and essential oil components of sage, spearmint and sideritis plants grown at different altitudes". *Agronomy* 11.9 (2021): 1766.
18. da Costa Zonetti P, et al. "Root growth and lignification of glyphosate susceptible and resistant soybean at low temperatures". *Seminars: Agricultural Sciences* 34.2 (2013): 509-515.
19. Dabai YU, Kawo AH and Aliyu RM. "Phytochemical screening and antibacterial activity of the leaf and root extracts of *Senna italica*". *African Journal of Pharmacy and Pharmacology* 6.12 (2012): 914-918.
20. Dean WE. "Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition; comparison with other methods". *Journal of Sedimentary Research* 44.1 (1974): 242-248.
21. Demasi S., et al. "Latitude and Altitude Influence Secondary Metabolite Production in Peripheral Alpine Populations of the Mediterranean Species *Lavandula angustifolia* Mill". *Frontiers in plant* (2018).
22. Di Stefano V, et al. "Effect of sunlight exposure on anthocyanin and non-anthocyanin phenolic levels in pomegranate juices by high resolution mass spectrometry approach". *Foods* 9.9 (2020): 1161.
23. El Far MM and Taie HA. "Antioxidant activities, total anthocyanins, phenolics and flavonoids contents of some sweetpotato genotypes under stress of different concentrations of sucrose and sorbitol". *Australian Journal of Basic and Applied Sciences* 3.4 (2009): 3609-3616.
24. El Mula Ahmed MF, et al. "Effect of Soil Moisture Content and Irrigation Intervals on Sennoside Content of Alexandrian Senna (*Cassia acutifolia* L.)". In *The 5 th Annual Conference Agricultural and Veterinary Research* 105 (2014).
25. Elsafy M., et al. "Unveiling the influences of P fertilization on bioactive compounds and antioxidant activity in grains of four sorghum cultivars". *Plos one* 19.10 (2024): e0311756.
26. Erturk Y, et al. "Seasonal variation of total phenolic, antioxidant activity and minerals in fresh tea shoots (*Camellia sinensis* var. *sinensis*)". *Pakistan journal of pharmaceutical sciences* 23.1 (2010): 69-74.
27. Falleh H., et al. "Effect of salt treatment on phenolic compounds and antioxidant activity of two *Mesembryanthemum edule* provenances". *Plant physiology and biochemistry : PPB* 52 (2012): 1-8.

28. Gharibi S., et al. "The effect of drought stress on polyphenolic compounds and expression of flavonoid biosynthesis related genes in *Achillea pachycephala* Rech". f. *Phytochemistry* 162 (2019): 90-98.
29. Hussain F, Hadi F and Akbar F. "Magnesium oxide nanoparticles and thidiazuron enhance lead phytoaccumulation and antioxidative response in *Raphanus sativus* L". *Environmental science and pollution research international* 26.29 (2019): 30333-30347.
30. Janská A., et al. "Cold stress and acclimation-what is important for metabolic adjustment?". *Plant Biology* 12.3 (2010): 395-405.
31. Khalik KA, El-Sheikh M and El-Aidarous A. "Floristic diversity and vegetation analysis of wadi Al-Noman, Mecca, Saudi Arabia". *Turkish Journal of Botany* 37.5 (2013): 894-907.
32. Lattanzio V, Lattanzio VM and Cardinali A. "Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects". *Phytochemistry: Advances in research* 661.2 (2006): 23-67.
33. Le J., et al. "Pharmacology, toxicology, and metabolism of sennoside A, A medicinal plant-derived natural compound". *Frontiers in pharmacology* 12 (2021): 714586.
34. Legkanyane MA., et al. "The phytochemical, antibacterial and antioxidant activity of five medicinal plants against the wound infecting bacteria". *African Journal of Biotechnology* 11.68 (2012): 13210.
35. Lohar DR., et al. "Seasonal variation in the content of sennoside in *Senna* leaves". *Pharmaceutical weekly* 1.1 (1979): 206-208.
36. Mahmood A., et al. "Ethno medicinal survey of plants from district Bhimber Azad Jammu and Kashmir, Pakistan". *Journal of Medicinal Plants Research* 5.11 (2011): 2348-2360.
37. Masoko P., et al. "Evaluation of the antioxidant, antibacterial, and antiproliferative activities of the acetone extract of the roots of *Senna italica* (Fabaceae)". *African Journal of Traditional, Complementary and Alternative Medicines* 7.2 (2010).
38. McLean EO. "Soil pH and lime requirement". *Methods of soil analysis: Part 2 Chemical and microbiological properties* 9 (1983): 199-224.
39. Mokgotho MP., et al. "Isolation and Chemical Structural Characterisation of a Compound with Antioxidant Activity from the Roots of *Senna italica*". *Evidence-based complementary and alternative medicine: eCAM* (2013): 519174.
40. Morris JB, Tonnis BD and Wang ML. "Variability for Sennoside A and B concentrations in eight *Senna* species". *Industrial Crops and Products* 139 (2019): 111489.
41. Mudau FN., et al. "Seasonal variation of total antioxidant contents of wild bush tea". In *IV International Symposium on Applications of Modelling as an Innovative Technology in the Agri-Food-Chain: Model-IT 802* (2008): 273-276.
42. Mykhailenko O., et al. "Effect of ecological factors on the accumulation of phenolic compounds in *Iris* species from Latvia, Lithuania and Ukraine". *Phytochemical Analysis* 31.5 (2020): 545-563.
43. Olorukooba AB., et al. "Antimalarial activities of the methanol leaf extract of *Senna italica* mill. in *Plasmodium berghei* infected mice". *Bayero Journal of Pure and Applied Sciences* 13.1 (2022): 142-148.
44. Parida AK., et al. "Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*". *Aquatic botany* 80.2 (2004): 77-87.
45. Rajesham C., et al. "Studies on the medicinal plant biodiversity in forest ecosystem of Mahadevpur forest of Karimnagar (A.P) India". *Bioscience Discovery* 4.1 (2013): 81-88.
46. Raju S, Shah S and Gajbhiye N. "Effect of light intensity on photosynthesis and accumulation of sennosides in plant parts of *senna* (*Cassia angustifolia* Vahl.)". *Indian Journal of Plant Physiology* 18.3 (2013): 285-289.
47. Ratnayaka HH, Meurer-Grimes B and Kincaid D. "Sennoside yields in Tinnevely *senna* affected by deflowering and leaf maturity". *HortScience* 37.5 (2002): 768-772.
48. Rhoades JD. "Cation exchange capacity". *Methods of soil analysis: Part 2 chemical and microbiological properties* 9 (1983): 149-157.
49. Richards LA. (Ed.). "Diagnosis and improvement of saline and alkali soils (No. 60)". US Government Printing Office (1954).
50. Sampaio BL., et al. "Effect of the environment on the secondary metabolic profile of *Tithonia diversifolia*: a model for environmental metabolomics of plants". *Scientific reports* 6.1 (2016): 29265.
51. Sartor T., et al. "Seasonal changes in phenolic compounds and in the biological activities of *Baccharis dentata* (Vell.) GM Barroso". *Industrial crops and products* 51 (2013): 355-359.

52. Sermakkani M and Thangapandian V. "GC-MS analysis of *Cassia italica* leaf methanol extract". *Asian J Pharm Clin Res* 5.2 (2012): 90-94.
53. Shui-Yuan C, Feng X and Yan W. "Advances in the study of flavonoids in *Ginkgo biloba* leaves". *Journal of Medicinal Plants Research* 3.13 (2010): 1248-1252.
54. Sina COSMULESCU and Ion TR. "Seasonal variation of total phenols in leaves of walnut (*Juglans regia* L.)". *Journal of Medicinal Plants Research* 5.19 (2011): 4938-4942.
55. Singh AK, Singh A and Singh S. "Impact of sowing and harvest times and irrigation regimes on the sennoside content of *Cassia angustifolia* Vahl". *Industrial Crops and Products* 125 (2018): 482-490.
56. Singh S, Singh SK and Yadav A. "A review on *Cassia* species: Pharmacological, traditional and medicinal aspects in various countries". *American Journal of Phytomedicine and Clinical Therapeutics* 1.3 (2013): 291-312.
57. Slinkard K and Singleton VL. "Total phenol analysis: automation and comparison with manual methods". *American journal of enology and viticulture* 28.1 (1977): 49-55.
58. Soladoye MO., et al. "Morphometric study of the genus *Senna* Mill. in South-western Nigeria". *African Journal of Plant Science* 4.3 (2010): 44-52.
59. Sonbul AR. "Hydrological and hydrochemical studies of the groundwater aquifer of Harrat Khaybar area, North of Saudi Arabia". *Int. J. Sci. Eng. Res* 7.7 (2016): 492-502.
60. Sulieman AME., et al. "Evaluation of antimicrobial activity of *Senna* (*Senna italica* Mill) plant and its synergistic effect with anti-biotic drugs". *Evaluation* 5.1 (2017).
61. Susunaga-Notario A.delC., et al. "Bioassay-guided chemical study of the anti-inflammatory effect of *Senna villosa* (Miller) H.S. Irwin & Barneby (Leguminosae) in TPA-induced ear edema". *Molecules (Basel, Switzerland)* 19.7 (2014): 10261-10278.
62. Taibi K., et al. "Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L". *South African Journal of Botany* 105 (2016): 306-312.
63. Viegas Junior C., et al. "Chemical, biological, and ethnopharmacological aspects of the genus *Cassia*". *New Chemistry* 29 (2006): 1279-1286.
64. Virjamo V, Sutinen S and Julkunen-Tiitto R. "Combined effect of elevated UVB, elevated temperature and fertilization on growth, needle structure and phytochemistry of young Norway spruce (*Picea abies*) seedlings". *Global change biology* 20.7 (2014): 2252-2260.
65. Willuhn G. "*Petasitidis folium*". *Tea Herbs and Phytopharmaceuticals*, 3rd Edition. Scientific Publishing Company, Stuttgart (1997).
66. Yang L., et al. "Response of plant secondary metabolites to environmental factors". *Molecules* 23.4 (2018): 762.
67. Yao L., et al. "Seasonal variations of phenolic compounds in Australia-grown tea (*Camellia sinensis*)". *Journal of agricultural and food chemistry* 53.16 (2005): 6477-6483.
68. Yusuf M., et al. "Diversity of Medicinal Plants in the Flora of Saudi Arabia 3: An inventory of 15 Plant Families and their Conservation Management". *International Journal of Environment* 3.3 (2014): 12-320.
69. Zahratunnisa N, Elya B and Noviani A. "Inhibition of Alpha-Glucosidase and antioxidant test of stem bark extracts of *garcinia fruticosa* lauterb". *Pharmacognosy Journal* 9.2 (2017).
70. Zandalinas SI., et al. "Plant adaptations to the combination of drought and high temperatures". *Physiologia plantarum* 162.1 (2018): 2-12.