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Geographical and seasonal variations in the chemical composition of essential oils of wild Libyan *Salvia fruticosa* Mill. from Al-Jabal Al-Akhdar area

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Abstract
Salvia fruitcosa Mill. (Syn. *S. triloba* L.) is a native species of the eastern Mediterranean basin, that is one of the most used medicinal plant in Al- Jabal Al-Akhdar, Libya. Plant materials were collected seasonally (the four seasons) from three different sites (Alwasita, Sidi Khaled and Wadi Al-kuf) of Al- Jabal Al-Akhdar area. Essential oils (EOs) were isolated from fresh aerial parts of the plant species growing wild by hydroidstillation. The chemical composition of EOs has been carried out by Gas Chromatography and GC Mass spectrometry (GC/MS). One-way ANOVA method was conducted to analyze the obtained data using with Least Significant Difference (LSD) test. The results indicated that oil percentages obtained from the altitudes of Alwasita (360 m), Sidi Khaled (385 m) and Wadi Al-kuf (600 m) were (2.24%, 2.00%, and 1.55%, respectively), so that Alwasita area revealed the greatest quantity, and Wadi Al-kuf area represented the smallest quantity. EOs percentage and the main components of EOs from different regions were compared. As the results of the analyses 13 major components were determined:1,8-cineole (57.53-3.27%), camphor (4.61-13-32%), α-pinene (1.70-7.21%), caryophyllene (1.35-2.08%), β-pinene (1.24-3.06%), camphene (1.03-3.55%), α-thujene (1.17-4.11%), myrcene (1.04-2.41%), α-terpinene (1.02-6.52%), limonene (0.85-1.75%), linalool (0.78-1.70%), bornyl acetate (0.80-2.29%), borneol (1.02-5.87%) based on different areas and seasons. A significant seasonal variation in EO percentage and the composition of the EO and their concentrations was observed. This findings noted on seasonal variation may be useful in selecting the best season for optimal yield of this species for the production of the plant active components with high medicinal significance. Moreover, the percentages of the main components of volatile oil were differed as results of changing the geographical regions**.** Statistical analysis showed that the geographical origin of plants did have significant influence on the variation in chemical composition of the EO. The study highlighted that both seasonal and geographical variation can influence the chemical composition of essential oil from *S fruitcosa.*

Keywords: *Salvia fruticosa*, essential oil, season, region, chemical composition

Introduction

Libya has immense prosperity of medicinal aromatic plants spread all over a huge area particularly in Al- Jabal Al-Akhdar district. The region of Al-Jabal Al-Akhdar, lies on the Mediterranean coast in north eastern Libya (Cyrenaica) which is known as (Green Mountain), three terraces hill which rises up gradually, the highest terrace reaches about 880 m above sea level (El-Barasi and Saaed, 2013) [16].

Medicinal plants investigated in the region was 179 species belonging to 166 genera and 72 families. Some important plant families are Apiaceae, Asteraceae, Lamiaceae, Poaceae, Fabaceae, Brassicaceae and Abiaceae (El-Mokasabi *et al*., 2018) [18]. At present, there is an imminent threat of genetic damage of the medicinal species in Al-Jabal Al-Akhdar due to over grazing, over-exploitation for traditional medicine, over cultivation, recurrent drought conditions and dangers (El-Darier and El-Mogaspi, 2009 [17]; Almajdoub *et al*., 2019) [4].

The genus *Salvia* L. is the largest genus in the Lamiaceae, symbolizes a vast and broad-based collection of nearly 1000 species worldwide of which 36 were found in Europe (as cited in Alimpić *et al*., 2015 [2]; Shehu and Zekaj, 2018) [42] and 10 in Libya (Jafri and El-Gadi, 1985) [22]. These 10 species are: *Salvia fruticosa, S. aegyptiaca, S. lanigera, S. verbenaca, S. chudaei, S. spinosa, S. viridis, S. officinals, S. coccinea, S.splendens,* (Jafri and El-Gadi, 1985) [22]. High consideration has been given to *Salvia* species because of the broad range of its biological activities (Askun *et al*., 2010). Many researchers have concentrated on the biological features of the EOs acquired from *Salvia* species and their main compounds such as antibacterial, cytostatic (Janssen *et al*., 1987 [23]; Stankovic, 2020), antiviral (Alim *et al*., 2009 [3]; Martins *et al*., 2016) [31] and antioxidant activities (Sarrou *et al*., 2016) [41]. Furthermore, they are commonly utilized in traditional medicine to cure diarrhea, eye diseases, gonorrhea; they possess antiseptic and antispasmodic activities. An extraordinarily large number of valuable secondary metabolites belonging to diverse chemical groups, such as EOs, terpenoid compounds, and phenolic derivatives, have been segregated from the genus *Salvia*, which included markedly in the pharmacopoeias of many countries all the world (Lu and Foo, 2002; Ipek *et al*., 2012).

Salvia fruitcosa Mill. (Syn. *S. triloba* L.) is a native species of the eastern Mediterranean basin (Louhaichi *et al*., 2011 [29]; Cvetkovikj *et al*., 2015 [13]; Bahadirli, 2020 [7]). This herb (especially the leaves) was used in folk medicine in the eastern Mediterranean region for the cure of different skin, blood, and infectious diseases in addition to illnesses of the digestive, circulatory, respiratory, and osteomuscular

systems (Ali-Shtayeh *et al*., 2000 [5]; Carmona *et al*., 2005 [11]; Süzgeç-Selçuk *et al*., 2021) [47]. The essential oils of *S.fruticosa* exhibited excellent antimicrobial activity against food borne bacteria (Delamare *et al*., 2007 [15]; Sarrou *et al*., 2016) and has antifungal activity (Pitarokili *et al*., 2003 [38]; Sarrou *et al*., 2016 [41]; Boukharay *et al*., 2018 [10]).

The current study was undertaken because *S. fruticosa* is one of wild aromatic plants widely spread in AL-Jabal Al-Akhdar area (Libya). Furthermore, to the best of our knowledge, no study has been conducted on the seasonal and regional variations of the chemical composition of the EOs extracted from wild Libyan sage and finally because there is little or no data among local harvesters for choosing an appropriate time for harvesting to maximize the medicinal potency of this plant species. Therefore, the objectives of this work are: (i) to determine and compare the EOs of wild *S. fruticosa* Mill. collected from three regions (Alwasita, Sidi Khaled and Wadi Al-kuf) from Al-Jabal Al-Akhdar area along the four seasons at three months intervals of the years (2021-2022). Using Gas Chromatography/Mass spectrometry (GC-MS) technique and (ii) to determine the best time for harvesting *S. fruticosa* to gain highest amounts of EOs.

Materials and methods

Study areas

Figure (1) and table (1) show compilation of *S. fruticosa* from three different sites (Alwasita, Sidi Khaled and Wadi Al-kuf) of Al- Jabal Al-Akhdar area collected along the four seasons at three months intervals of the years (2021-2022). The sites divided according to different elevation using (GPS reading).

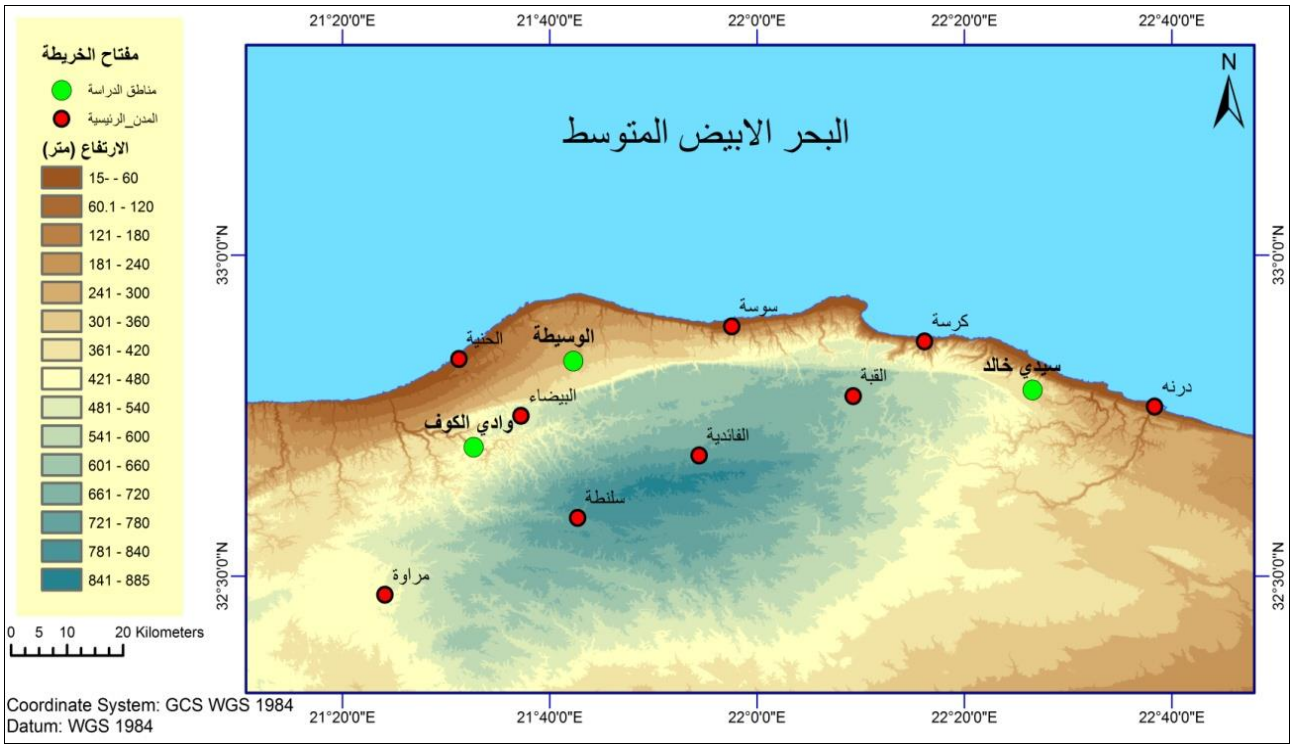

Fig 1: A map showing the geographical distribution of three study areas.

Table 1: The GPS data for the three different locations.

Sites	Elevation (m/amsl)	Latitude	Longitude
Alwasita	360	32 47'360"N	21 39' 479"E
Sidi Khaled	385	32 44' 815"N	22 28' 072"E
Wadi Al-kuf	600	32 41' 435"N	21 38' 764"E

Table 2: Means of the meteorological data of Alwasita location during the four seasons of 2021-2022.

Month	Air temperature (c^0)	Precipitation (mm)	Relative Humidity (%)	Specific Humidity (g/kg)
Spring	13.28	0	62.38%	*g/kg*5.86
Summer	26.9	0	69.88%	*g/kg*15.14
Autumn	22.88	0.06	66.94%	*g/kg*11.66
Winter	13.71	3.74	77.12%	*g/kg*7.57

Table 3: Means of the meteorological data of Sidi Khaled location during the four seasons of 2021-2022.

Month	Air temperature (c^0)	Precipitation (mm)	Relative Humidity (%)	Specific Humidity (g/kg)
Spring	16.11	2.16	73.5%	*g/kg*8.3
Summer	26.83	0	71.56%	*g/kg*15.75
Autumn	22.55	0	64.44%	*g/kg*10.93
Winter	15.55	0.01	74.56%	*g/kg*8.18

Table 4: Means of the meteorological data of Wadi Al-kuf location during the four seasons of 2021-2022.

Month	Air temperature (c^0)	Precipitation (mm)	Relative Humidity (%)	Specific Humidity (g/kg)
Spring	15.87	0	40.75 %	*g/kg*4.7
Summer	25.69	0	60.38 %	*g/kg*12.08
Autumn	19.73	0	60.94%	*g/kg*8.24
Winter	8.19	33.23	91.81 %	*g/kg*6.59

Plant material

Plant samples collected at various growing seasons of the years (2021-2022) from the studied sties. New aerial parts from one plant collected from three regions. The identification of specimens was carried out by *Sylphum Herbarium* of the Botany Department, Sciences Faculty, Omar AL-Mukhtar University, El-Bayda, Libya.

Essential oils extraction of *S. fruticosa*

The essential oil was obtained by hydrodistillation of 100 g of air-dried aerial parts of *S. fruticosa* using a Clevenger-type apparatus for 2 h according to the British Pharmacopoeia specifications (Pharmacopoeia, B 1980) [37]. The oil was stored in dark glass vials at 4˚C of *S. fruticosa*. The production of oils (v/w) will be calculated on a fresh weight basis.

Gas Chromatography/Mass Spectrometry (GC\MS)

At the National Research Center (NRC), Cairo – Egypt, quantitative analyses of the oils were performed using GC-MS. The GC-MS analysis was carried out by a HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in an m/z range of 40-700. Identification of the individual oil components was accomplished by comparison of the retention times with standard substances and by matching mass spectral data with those held in the Wiley 275 library of mass spectra. Confirmation was performed using AMDIS software and literature (Adams, 2007) [1]. For the purpose of quantitative analysis, area percents obtained by FID were used as a base. Quantities of the essential oils were determined according to the European Pharmacopoeia 4th Ed (Council of Europe, 2002) [12].

Statistical analysis

Data obtained for three replicates from plant samples from each region during four seasons, was statistically analyzed by one – way analysis of variance (ANOVA) using Minitab Statistical Package (Minitab Inc., State College, PA, USA. 2010) [33]. Mean comparisons was done by Least Significant Difference (LSD) test at 5% level of significance ($p \leq 0.05$).

Results

Essential oil amount and essential oil major components

One-way ANOVA test proved that the region of study largely affected ($p \leq 0.05$) the studied components. EO percentages varied significantly among regions and seasons. They were highest in the Alwasita area in summer season and lowest in Wadi AL-Kuf area in winter. Our findings are in consistent with previous studies, which showed that with increasing altitude, a decrease was determined in the amounts of EO in the plants (Delazar *et al*., 2011 [14]; Mohamadi and Rajaei, 2016) [34], this decrease was found to be statistically significant.

In the current research, between 43 and 50 different EO components were identified in *S. fruticosa* plants. Thirteen 13 major chemical constituents 1,8-cineole, camphor, α-pinene, caryophyllene, β-pinene, camphene, α-thujene, myrcene, α-terpinene, limonene, linalool, bornyl acetate, borneol were detected. 1,8-cineole was the highest component (table 6, figure 2) and linalool was the lowest as clarified by (table7, figure 3).

Table 5: Effect of different locations on essential oil percentage of *Salvia fruticosa* during the four seasons.

Area	Essential Oil %
Alwasita	2.24^a
Sidi Khaled	2.00^b
Wadi AL-Kuf	1.55^c
p -Value = 0.000	
Seasons	Essential Oil %
Spring	1.82^b
Summer	2.78^a
Autumn	^{c}*1.94*
Winter	1.19^d
p -Value = 0.000	

Different letters in the same column indicate statistical difference ($p \leq 0.05$).

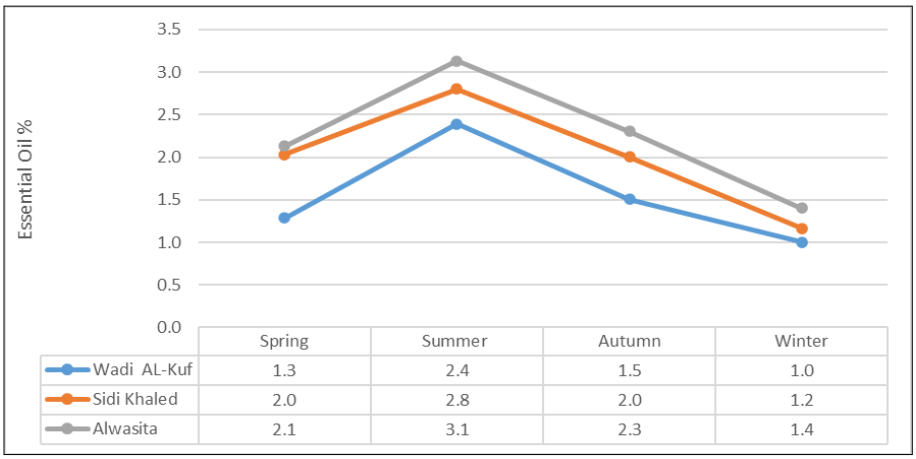

Fig 1: Effect of different locations on essential oil percentage of *Salvia fruticosa* during the four seasons.

Table 6: 1, 8-Cineole percentage of *S. fruticosa* at the three different locations according to seasons.

Area	**1,8 Cineole**
Alwasita	27.18[a]
Sidi Khaled	24.38[b]
Wadi AL-Kuf	26.67[a]
p -Value = 0.027	
Seasons	1,8 - Cineole
Spring	23.65[b]
Summer	57.53[a]
Autumn	19.86[c]
Winter	3.27[d]
p -Value = 0.000	

Different letters in the same column indicate statistical difference (p≤0.05).

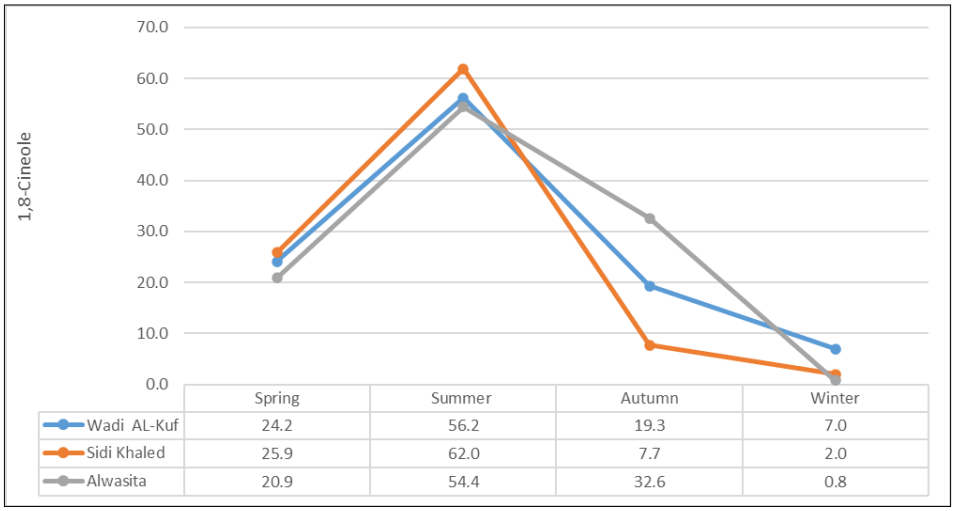

Fig 2: 1, 8-Cineole percentage of *S. fruticosa* at the three different locations according to seasons.

Table 7: Linalool percentage of *S. fruticosa* at the three different locations according to seasons.

Area	**Linalool**
Alwasita	1.34[a]
Sidi Khaled	1.35[a]
Wadi AL-Kuf	1.07[a]
p -Value = 0.244	
Seasons	Linalool
Spring	1.70[a]
Summer	0.84[b]
Autumn	1.69[a]
Winter	0.78[b]
p -Value = 0.000	

Different letters in the same column indicate statistical difference (p≤0.05).

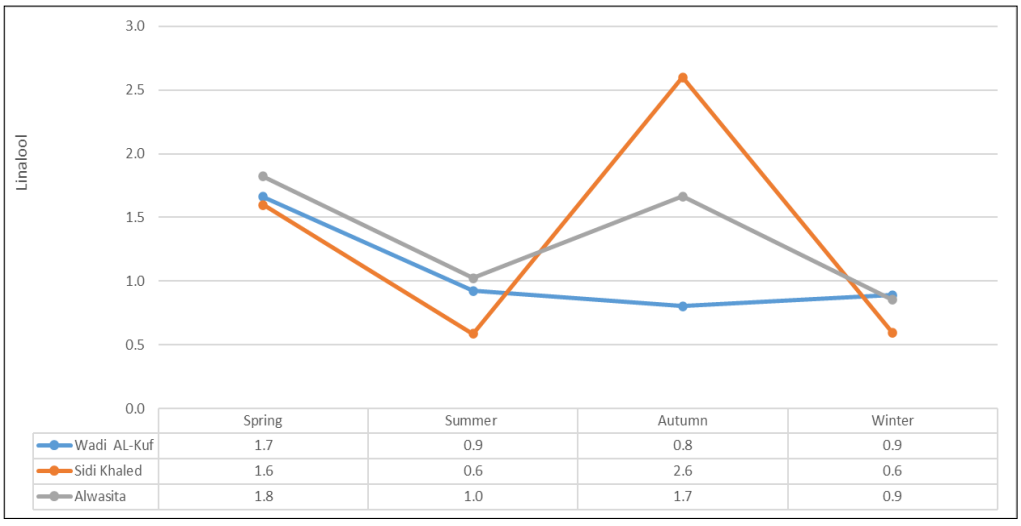

Fig 3: Linalool percentage of *S. fruticosa* at the three different locations according to seasons.

The highest amounts in Alwasita area (i.e. the lowest altitude) were two components only (1, 8-cineole, caryophyllene). Similarly, the highest amounts in Sidi Khaled area were two components (camphor, α-pinene). In comparison, the highest amounts in Wadi Al-kuf area (i.e. the highest altitude) were six components (β-pinene, camphene, α-thujene, myrcene, α-terpinene, borneol). There were no significant differences among regions in the remaining three components (bornyl acetate, linalool, limonene), whereas, there were significant differences among seasons in their amounts.

In previous studies, 1, 8-cineole was found to be the most common component (Bayrak and Akgul, 1987 [8]; Leontaritou *et al*., 2020) [28]. Our findings are in agreements to these results. 1, 8-cineole was found to be in the highest proportions at all three altitudes in summer season. The 1, 8 cineol content of EOs was found to vary from country to country (Skoula *et al.,* 2000 [43]; Delamare *et al.,* 20007 [15]; Giweli *et al.,* 2013 [19]; Karık *et al.,* 2018 [24]; Kocabaş and Kaplan, 2023) [26]. These changes in EOs compositions may vary under the influence of the environment (climatic, seasonal, geographical) (Bellomaria *et al.,* 1992 [9]), genetic factors (Perry *et al.,* 1999) [36] and agricultural practices (Sorrou *et al.,* 2016) and growth stages of the plant (Zigheb *et al*., 2019).

Influence of geographical locations on the major components of *Salvia fruticosa* essential oil

One-way ANOVA test demonstrated that the region of harvest largely affected (p≤0.05) the studied components and permitted to conclude that 10 components (1, 8-cineole (p≤0.027), caryophyllene (p≤0.001), camphor (p≤0.000), α-pinene (p≤0.000), β-pinene (p≤0.000), camphene (p≤0.000), α-thujene (p≤0.000), myrcene (p≤0.014), α-terpinene (p≤0.007) and borneol (p≤0.000) varied significantly among the regions. Three components (bornyl acetate (p≤0.590), linalool (p≤0.244), and limonene (p≤0.339) did not vary among regions. This conclusion verifies the result of (Giweli, *et al*., 2013) [19] ratifying that *S. fruticosa* EO chemical composition differs in line with different geographical locations. Chemical polymorphism produced by the region is connected to geographical and environmental factors, such as altitude, climate, humidity, photoperiod, temperature, soil composition or genetic factors and botanical diversity (Topçu *et al*., 2013). In comparison, Cvetkovikj *et al*. (2015) [13] concluded that EO composition of Greek sage was not influenced by the geographical locations, which contradict with our findings.

Influence of harvesting time on the major components of *Salvia fruticosa* essential oil

To investigate and underline the relationship and differences between samples collected at different seasons, the application of One-way ANOVA test permitted to emphasize the effect of the collection month on the major 13 chemical constituents of *S. fruticosa*. 1, 8-cineole was significantly higher in summer, and lower in winter (α= p≤0.05, p≤0.000) and caryophyllene was also higher in summer, and lower in winter, but there is no significant differences among season (p≤0.524). Camphor was significantly higher in autumn, and lower in winter (p≤0.000). α-pinene was significantly higher in autumn, and lower in spring (p≤0.00). α-thujene (p≤0.000), myrcene, (p≤0.000), β-pinene, (p≤0.181), α-terpinene (p≤0.000), and borneol (p≤0.000) were significantly higher in autumn, and lower in summer. Camphene was significantly higher in winter, and lower in summer (p≤0.000). Limonene and bornyl acetate, were significantly higher in spring, and lower in summer (p≤0.000). Linalool was significantly higher in spring and lower in winter (p≤0.000).

Discussion

To the best of our knowledge, there is no study on the chemical composition of wild *S. fruticosa* from Libya with regard to both their location and harvesting time. With regard to EO percentages, our results are in complete agreements with previous work, which found that EO component displays a rhythmic rise in oil production during the growing season and then a stable decrease towards the winter therefore, late summer was suggested as the best harvesting time for plants (Verma *et al*., 2011) [48]. In comparison, another research determined that winter as a best season for harvesting plants (Kritika *et al*., 2013) [27]. Similar to our results, previous researches (Putievsky *et al*., 1986 [40]; Hussain, 2009 [20]; Sarrou *et al*., 2016 [41]; Zgheib *et al*., 2019) [49] have established that the harvesting season can alter the chemical composition of the EOs of *S. fruticosa*. The EOs showed a seasonal and altitudinal variations in their chemical components contents. Therefore, the best season and altitude is crucial for collecting plants. These data may be useful in choosing the optimal collecting time for the production of the plant active components with high medicinal significance. (Majdoub *et al*., 2022) [32]. Different major chemical components showed significant variations among regions and seasons. Soni *et al*. (2015) mentioned that it is not only EO that affected by the season but other main components like polyphenol, flavonoids, glycosides, alkaloids etc. are similarly influenced significantly by seasons. There is no general rule for the collection time for enhanced production of particular secondary metabolites. Though, numerous researches have been issued demonstrating the influence of seasons on secondary metabolites but there is no assembled data denoting the optimized season for the collection of essential constituents from diverse plant sources (Soni *et al*, 2015) [44].

1, 8-cineole was higher in Alwasita area and lower in Wadi Al-Kuf area, whereas it was higher in summer and lower in winter season. Our results are in full agreement with most of the researches into the EO composition of *S. fruticosa*, which stated that 1, 8-cineole was the main compound of this medicinal plant (Skoula *et al*., 2000 [43]; Pitarokili *et al*., 2003 [38]; Kosar *et al*., 2005 [25]; Papageoriou *et al*., 2008) [35]. In contrast, Delamare *et al*. (2007) [15] and Pierozan *et al*. (2009) [39] found α-thujone as a major compound in their investigated populations, which is not in agreement with our results and other available data. According to our study, it is recommended to investigate the environmental conditions of the location which aromatic plants will be cultivated.

This is likely that the detected chemical variations are due to the various climatological and geographical circumstances especially altitude related (slope side, slope percent, altitude itself, soil characteristics) factors. Also, the forces of different plant growing stages on these alterations are unavoidable. In addition to above-mentioned causes, effects of different plant genetic, biochemical and physiological capacity at different growing stages and wild growing habitats on the compartmentalization of diverse biochemical

ways is commendable of great attention (Kocabaş and Kaplan, 2023) [26].

Similar to our findings, preceding studies (Putievsky *et al*., 1986 [40]; Hussain, 2009 [20]; Sarrou *et al*., 2016 [41]; Zgheib *et al.*, 2019) [49] have confirmed that the harvesting season can change the chemical composition of the EOs of *S. fruticosa*. The EOs showed a seasonal and altitudinal variations in the concentration of their chemical components. Hence, an optimum season and altitude is essential for harvesting plants.

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