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Growth Compatibility and Medicinal Potential of Four *Salvia* Species in Semnan Climatic Conditions

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KEYWORDS

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ABSTRACT: *Salvia* is a medicinal plant native to Iran with pharmaceutical and healthcare importance. We aimed to assess the compatibility of four *Salvia* species (*officinalis*, *sclarea*, *nemorosa* and *limbata*) in Semnan, Iran climatic conditions. The experiment was conducted in a randomized complete block design with four replications. The plantlets were planted in the main field and evaluated in early, mid and late-growth seasons. The studied characteristics included plant height, leaf and flowering traits. The active ingredients of the plant were obtained in various phenological stages and measured by GC-MS. Results showed that the maximum and minimum plant height and number of leaves were observed in *officinalis* and *limbata* species, respectively. Maximum leaf length and diameter were observed in *sclarea* and *nemorosa* species, respectively. The leaf growth process was increased with the development of growth season, such that maximum leaf length and diameter were obtained in the post-flowering conditions. Depending on the species, *Salvia* flowers are formed in different growth years, such that in the four species studied in this research, *sclarea* and *officinalis* started flowering from the first and second years, respectively. According to the comparison of flowering traits measured in these two species, the number of florets, height of main flowering spikes, height of lateral flowering spikes, and height of the post-flowering plant were higher in *sclarea* than *officinalis*. However, the number of lateral flowering spikes was higher in *officinalis*. Moreover, the number of active ingredients was higher in *officinalis* than other species. Conclusively, *sclarea* was the best species in growth indices and *officinalis* species had highest active ingredients yield.

INTRODUCTION

Medicinal plants are among the natural resources of many countries, and the type, number, and variety of plant species differ based on geographic conditions and locations [1]. Iran is the origin of various many plants which have unique medicinal properties. Iran has many medicinal plant species, and about 90% of the world's medicinal plants can be produced under its five climate conditions [2]. In recent years, the possibility of increasing the active ingredients of medicinal plants through increasing the total weight of the plants (biomass) has been widely considered, because the affordability of medicinal plants in terms of the quantity and quality of primary and secondary metabolites is highly important and should reach a favorable limit. Maximum product can be attained by choosing favorable environmental factors and plant varieties [3]. Research has suggested that no obvious association can be found between the amount of product and active ingredients in medicinal plants. Although these materials are produced

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with genetic processes, their construction is significantly affected by environmental factors [4]. Thus, environmental factors cause changes in the growth of medicinal plants as well as the quantity and quality of ingredients [5].

Salvia is originated from the Latin word salvere meaning savior and healer, because of its great therapeutic properties. This plant was used in Western medicine and traditional medicines of Egypt, Greece, Rome, and India, and was utilized in ancient Egypt as a fertility medicine. In the first century AD, the Greek physician Pedanius Dioscorides used *Salvia* decoction to sterilize wounds and stop bleeding [6]. In the 9th century, *Salvia* was introduced to other parts of Europe and China [7]. In the middle ages, it was known as a panecea, and advocates of Salernitan surgery attributed wonderful therapeutic effects to it [8]. *Salvia* extraction was common in the 15th century, and people brew *Salvia* leaves for drinks in 16th century England before tea become popular. During the 17th century, it became popular in North America, and in the past 200-300 years, it has been used there as an excellent spice. Currently, its essential oil is used in pesticides, perfume making, scenting soaps, and as an antioxidant in tinned meats, sausages, and chicken meat [7]. This plant has several species [8].

Salvia is a perennial native plant to Northern Mediterranean areas. In Russia, USA, Italy, and Central European countries, a large area of arable lands is allocated to the cultivation of *Salvia*. This plant requires a warm and dry climate. It starts to grow at 12-15 ℃ with a slow growth in the first year. Flowering continues until early summer, and fruits begin to form in late August. Young plants growing from seeds require a lot of water. In the winter, this plant suffers from frostbite at temperatures lower than -15 ℃ and dries within 5-6 days. *Salvia* does not require a special soil and can properly grow in any type of soil. Warm weather and medium-textured soils with adequate levels of calcium compounds are appropriate for the cultivation of this plant and significantly increase its active ingredients. Plant propagation can be sexual and asexual [3]. *Salvia* is the most valuable medicinal species of the mint family, with important therapeutic specificities and a strong impact. Its leaf has an ergogenic effect because of the essential oil and is strong due to tannin. Moreover, it has digestion facilitative, diuretic, anticonvulsant, antipyretic, antiseptic, blood glucose level decreasing, and menstruation-inducing properties. *Salvia* has antibacterial and antimicrobial properties and is used in manufacturing perfumes, soaps, cosmetics, and air-fresheners [8]. The useful parts of *Salvia* are leaves, floral branches, and abundant oil whose extract is known as salvion and salvon. The plant contains maximum essential oil at the flowering stage [3].

Numerous studies have been conducted on this medicinal plant and its active ingredients. The essential oil of various species of the genus *Salvia* in Iran has been studied [9, 10, 11 and 12]. In the *S. atropatana* essential oil, beta-caryophyllene (16.3%), sclareol (13.3%), and hexyl octane (12.2%) compounds had maximum values [13]. Among the 29 identified compounds in *S. atropatana* essential oil, 24 compounds were recognized and the major compounds were T-cadinol and caryophyllene oxide (15.7%) [14]. *S. sclarea* had a good adaptability for cultivation and domestication in Mashhad climatic conditions [15]. In the full flowering stage, the plants contain maximum essential oil whose major components were linalool (30.03%), linalyl acetate (23.08%), and alpha-terpineol (11.13%). Moreover, it seems to be quite appropriate for cultivation in green spaces with ornamental purposes because of the beauty of leaves and flowers, abundance of perfumes at the flowering stage, lack of need for special conditions for breeding, and resistance to adverse conditions.

The biological activity and usage of essential oils in various industries depends on their chemical compounds, which are in turn affected by environmental factors, growth stage, harvest time, planting conditions, and usage organ. Studies were conducted on the variations of essential oil quantity and components in different growth stages of plants such as *Anethum graveolens* [16, 17], *Pimpinella anisum* [18], *Trachyspermum ammi* [19], *Diplotaenia cachrydifolia* and *Nepeta heliotropifolia* [20], and the results showed differences in the quantity and components of essential oils in various plants at different stages of growth. Maximum essential oil in Saliva flowers (cultivated in Karaj Botanical

Garden) can be seen 8-10 days after the start of flowering [1].

Essential oil is produced from the beginning of flower formation, increased with the evolution of flowers, and reduced at the beginning of seed formation. In *Salvia* (*S. officinalis*), it was determined that maximum essential oil can be seen at the flowering stage, in the sepals, and after the fall of petals [21]. For the first time, [22] reported major *Salvia* compounds to be linalyl acetate, linalool, and alpha-terpineol. Research reported major oil constituents of *S. mirzayanii* species to be linalool (19%), linalyl acetate (12.9%) and 1, 8-cineole (12.1%) [12]. In *S. limbata* essential oil, germacrene (25.7%), linalyl acetate (16.1%), and linalool (16.1%) were the most important components [14]. In addition, in previous reports on *S. sclarea* essential oil, 17 compounds have been identified among which linalyl acetate (77.8%) and germacrene D (9.6%) were the major components [23].

Due to the significance of *Salvia* and its important medicinal and therapeutic properties, the present research was conducted in order to investigate and compare the morphological characteristics and yields of the essential oil of four species of *Salvia* in Semnan climatic conditions.

MATERIALS AND METHODS

The present research was conducted to compare the morphological properties and quantity of essential oil in different growth stages of four *Salvia* species. The seeds of four species of *S. sclareae*, *S. limbata*, *S. officinalis*, and *S. nemorosa* were purchased from Pakan Bazr Esfahan Co. and cultivated for transplantation in terraces (with the average temperature of 25°C and average relative humidity of 70%) inside the greenhouse. The seedlings were transferred to the main field (inside 2 × 1.5cm terraces, 40 × 30 cm apart) after germination (1 month after cultivation) and full deployment. In order to strengthen the plants and improve their growth conditions, an organic fertilizer was used for nutrition in the early stages of plantlet growth. Irrigation was planned manually in primary terraces and plastic pots according to the field capacity level based on ambient temperature. It should be noted that this plant requires a lot of water in the early stages of growth. Terrace irrigation was performed in the main field twice a week during the growing stages of the plant. The irrigation water had an EC of 3.7 (µS.cm) and pH of 7.55. During these stages, water and soil samples were prepared for measurement and experimental analysis and delivered to the laboratory (Table 1).

Table 1. Soil test results.

Texture	Sand%	Clay%	Silt%	Cl^- Meq.L	HCO_3^- Meq.L	Co_3^{-2} Meq.L	C%	EC	pH	$Ca(OH)_2$%	K(ppm)	P(ppm)	N%
Sandy loam	66	6	28	15.5	3	0	0.035	3.5	7.8	25	180	13.5	0.004

During the plant growth stage, in order to evaluate specific potentials and morphological characteristics in various phenological stages (pre-flowering, flowering, and post-flowering), the following factors were examined: plant height; leaf length, diameter, and number; height of the main and lateral flower spikes (using a ruler); number of lateral flower spikes (by enumeration); and number of florets. Moreover, in order to study essential oils quantity variations at different growth stages, the flower and leaves underwent extraction separately in a number of phenological stages. The phenological sampling stages included plantlet (early growing season), mid-growth season (in case of flowering, pre-flowering and post-flowering), and the end of the growing season. Extraction was carried out by water distillation using the Clevenger apparatus. After dehydration, the essential oil were kept in the refrigerator at the temperature of 4°C until injection into the gas chromatography equipment connected to a mass spectrometer and gas chromatograph. The gas chromatography equipment connected to the mass spectrometer (GC-MS) consisted of a Varian gas chromatography equipment (Agilent, Iran), equipped with

an HP-5 column (USA) with the length of 30 m, diameter of 0.25 mm, and stationary phase thickness of 0.25 μm, and a Varian mass spectrometer detector (Varian Saturn 3). The working conditions were adjusted based on helium as the carrier gas at the rate of 2 mL.min, ionization potential of 70 eV, and mass range of 40-300 u, and column temperature programming was done with the column temperature variation of 60-280 °C at the rate of 8°.min and the injection chamber temperature of 280°C. In each case, after the injection of very small quantities of essential oil (0.1 μL), chromatograms was obtained and the mass spectra of various compounds were examined. Then, the spectra were identified and studied. The experiment was carried out in the randomized complete block design with four replications. The results were analyzed by SAS (Statistical Analysis System). In order to classify the results, means were compared using Duncan's multiple range test, and the curves were drawn using Excel.

RESULTS

The results of ANOVA showed that the rosette plant and pre-flowering plant height had a significant difference at 1% probability level among different species (Table 2, 3). Comparison of the means also revealed a different classification (Table 4 and 5).

Table 2. Variance analysis of height in rosette plant and before flowering of four *Salvia* species in Semnan climatic conditions

Sources of variation	Freedom degree	Mean square	
		Height of rosette plant	Plant Height before flowering
Species	3	464.43**	6181.4**
Block	3	12*	69.43*
Error	15	2.02	6.2
CV(%)	-	28	21.4

Table 3. Variance analysis of leave traits of four *Salvia* species in Semnan climatic conditions in three phonologic stage

Sources of variation	Freedom degree	Mean square		
		Leaf number	Leaf diameter	Leaf length
Species	3	1029**	975.33**	80608**
Sampling steps	2	1378**	254.33**	97991**
Block	3	18.15*	3.52*	47.6*
Error	22	2.5	1.05	84
CV (%)	-	18.6	21.6	22

As can be seen, the rosette plant height was higher in *officinalis* than other species (8.93 cm), and *limbata* and *sclarea* are shorter than the others (3.34 and 3.12 cm, respectively). The rosette plant height in the pre-flowering stage was higher in *officinalis* species than the other species (25.34 cm) and shorter than the rest in *limbata* (2.86 cm) (Table 4).

Table 4. Average comparison of different traits in four *Salvia* species in Semnan climatic conditions

Species	Height of rosette plant	Plant height before flowering	Leaf length	Leaf diameter	Leaf number
Officinalis	8.93 [a]	25.34 [a]	6.29 [c]	2.02 [d]	90.18 [c]
Nemarosa	4.89 [b]	11.64 [b]	10.2 [b]	7.48 [a]	49.15 [b]
Sclarea	3.34 [c]	6.65 [c]	10.95 [a]	6.58 [b]	30.59 [a]
Limbata	3.12 [c]	2.86 [d]	5.86 [d]	2.96 [c]	16.13 [d]

In examining the leaf traits, leaf length, diameter, and number were different among the species at 1% probability level. The sampling stages also showed a significant difference in each leaf trait (Table 3), and the comparison of the means revealed a different classification (Table 5).

Table 5. Average comparison of different times on leave traits in four *Salvia* species in semnan climatic conditions

Time	Leaf length	Leaf diameter	Leaf number
Before flowering	6.13^{c}	3.90^{c}	16.98^{c}
Flowering	9.79^{b}	4.99^{b}	66.69^{b}
After flowering	11.79^{a}	6.65^{a}	72.72 ^{a}

Leaf length showed a significant difference among the species; *sclarea* (10.95 cm) had highest and *limbata* (5.86 cm) had lowest leaf length (Table 4). A significant difference was shown in different sampling stages; maximum and minimum leaf lengths were observed in post-flowering (11.79 cm) and pre-flowering (6.13 cm) stages.

Leaf diameter was different among the samples; *nemorosa* (7.48 cm) had maximum and *officinalis* (2.02 cm) had minimum diameters (Table 4). There was a significant difference among samples in various sampling stages; maximum and minimum leaf diameters were seen in the post-flowering (6.65 cm) and pre-flowering (3.9 cm) stages, respectively (Table 5).

The number of leaves was different in various samples; *officinalis* (90.18) and *limbata* (16.13) showed highest and lowest numbers of leaves, respectively (Table 4). A significant difference was observed in different sampling stages; the post-flowering (72.72) and pre-flowering (16.98) stages indicated the maximum and minimum numbers of leaves, respectively (Table 5).

In investigating the flowering traits in *officinalis* and *sclarea*, it was determined that the height of main flowering spikes, height of lateral flowering spikes, height of post-flowering plant, and number of florets were higher in *sclarea* than *officinalis*, while the number of lateral spikes was lower in *sclarea* than *officinalis*.

As can be seen in Table 6, the active ingredients of plant in various phenological stages were different in various species (Figure 1). Caryophyllene and caryophyllene oxide were the main compounds in almost all samples and stages. According to the number of ingredients and samplings in the research, the most compounds found in various stages and species are: farnesene, Trans-Caryophyllene, Trans-β-Farnesene, α-Cadinol, Arisol-9-en-3, Germacrene D, and β-Bourdonene.

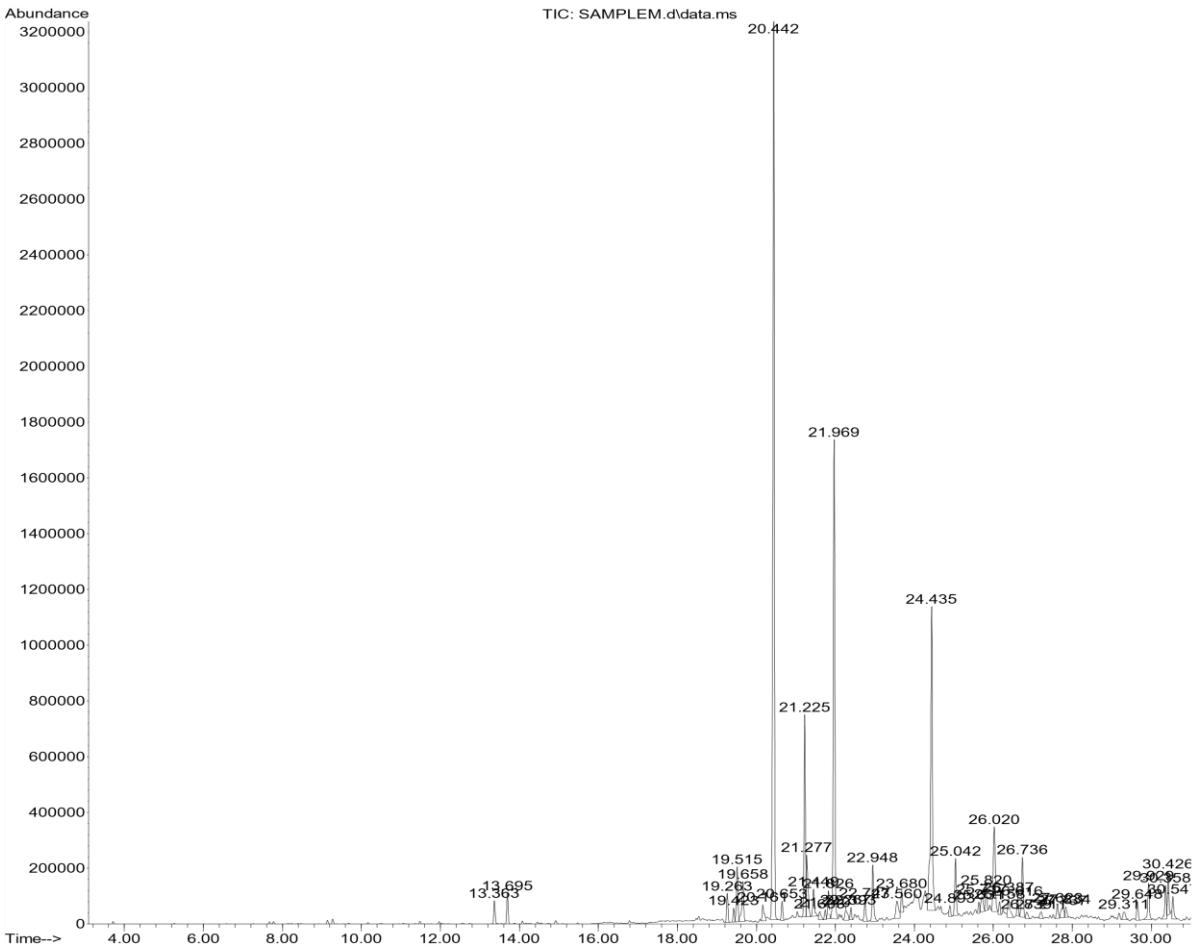

Figure 1. GC-Mas spectrum of active ingredients content for *Salvia* species in Semnan climates

Table 6. Active ingredients of four *Salvia* species in different phonologic stage in Semnan climatic conditions.

Row	Species	Sampling steps	Compounds	%
1	*nemarosa*	seedling	Caryophyllene	43.91
			Caryophyllene oxide	19.65
			Farnesene	12.13
		Middle of growth season	Trans-Caryophyllene	11.93
			Caryophyllene oxide	37.89
			Trans-β-Farnesene	11.49
			α-Cadinol	3.61
		End of growth season	Trans-Caryophyllene	28.94
			Caryophyllene	30.13
			α-Cadinol	5.64
			Arisol-9-en-3	3.58
2	*sclarea*	seedling	Caryophyllene	62.65
			Caryophyllene oxide	27.84
			Arisol-9-en-3	0.41

Table 6. Continued

		Before flowering	Caryophyllene	42.4
			Caryophyllene oxide	23.84
			α-Cadinol	16.33
		After flowering	Caryophyllene	27.56
			Caryophyllene oxide	23.62
			Trans-β-Farnesene	17.57
		End of growth season	Caryophyllene oxide	58.25
			Trans-β-Farnesene	0.5
3	*officinalis*	seedling	Caryophyllene oxide	41.02
		Before flowering	Caryophyllene oxide	27.28
			Germacrene D	17.59
			Caryophyllene oxide	9.444
		Middle of growth season	Caryophyllene	43.06
			Caryophyllene oxide	17.39
			β-Bourdonene	12.76
		End of growth season	Caryophyllene oxide	32.04
			Germacrene D	18.02
			Trans-β-Farnesene	4.3397
4	*limbata*	seedling	Caryophyllene oxide	22.20
			β-Bourdonene	15.7
			Arisol-9-en-3	7.90
			Trans-Caryophyllene	6.67
			Germacrene D	8.92
			Trans-β-Farnesene	3.84
		Middle of growth season	Caryophyllene	55.21
			Caryophyllene oxide	23.43
			Trans-β-Farnesene	1.09
		End of growth season	Caryophyllene oxide	14.94
			Caryophyllene	12.66
			β-Bourdonene	9.31
			Germacrene D	8.75

DISCUSSION

The quantity of active ingredients in medicinal plants is mainly affected by natural variables in the environment. Although the quantity of secondary metabolites is under the control of genes, their quantity and accumulation are significantly affected by environmental conditions. Climatic variations and different ecological conditions have caused diversity and richness in medicinal plants throughout Iran [24], bringing about differences in the quantity of active ingredients and production of herbal medicines [25]. The major habitat conditions in different areas include height, slope direction and percentage, latitude and longitude, temperature, humidity and annual rainfall, soil characteristics, and associated species [26].

Considering the growth location of the plant in the environment is one of the main factors of great impact on essential oil and active ingredients. Some reports have expressed associations between the habitat conditions and chemical compounds of plants, manifesting a high

correlation between the geographical origin of plants and their active ingredients [3].

Salvia has a large number of species with limited research on the comparison of agronomic and morphological characteristics between them. Furthermore, edaphic and climatic variations and genetic characteristics may have a strong influence on the morphological, agronomic and essential oil chemical characteristics. This may be especially true if we take into account that most studies regarding *Salvia* species are devoted to species cultivated in Europe, where differences in climate and soil nutritional conditions are obvious compared with other regions in the world [27]. They investigated morphological characteristics and parameters of different species of *Salvia* sp. All species were compatible with Brazilian climatic conditions and morphological differences were observed between various species. Based on the dry weight and dry weight performance, *S. faicinalis* had the highest level of essential oil and yield.

According to the results of the present study, maximum and minimum plant heights were observed in *officinalis* and *limbata*, respectively. *Salvia* species are rosette-forming, and stem extension occurs at the time of flowering. *Officinalis*, with small and dense leaves, had highest height. However, in other species, the leaves are larger with a lower density, and so the plants have lowest heights. Minimum number of leaves was seen in *limbata*. The highest leaf length and diameter were seen in *sclarea* and *nemorosa*, respectively. The growth trend of leaves was increased with the development of the growing season, such that maximum leaf length and diameter were obtained in the post-flowering conditions. Based on species, *Salvia* flowers are formed in different growing years. In the four species studied in this research, *sclarea* and *officinalis* started flowering from the first and second years, respectively. Thus, according to the time period in this research, flowering traits were examined in *sclarea* and *officinalis*. According to the comparison of flowering traits between these two species, the number of florets, height of main flowering stem, height of lateral flowering stem, and height of post-flowering plant were higher in *sclarea* than *officinalis*, while the number of lateral stems was higher in *officinalis*. Moreover, the number of active ingredients was the highest in *officinalis*.

Examining the results of the present study on four *Salvia* species in Semnan climatic conditions has determined that, according to growing traits and active ingredients, *officinalis* with a proper vegetative growth and a high number of active ingredients, and *limbata* with a poor vegetative growth and the destruction of many plants during the first year, had the highest and lowest compatibility with Semnan climatic conditions, respectively, with *sclarea* and *nemorosa* at the second and third places, respectively. In general, based on the vegetative and reproductive growth and the production of active ingredients, *officinalis* and *sclarea* can be introduced as the species compatible with the area.

Research showed the oil yield and chemical compositions of the essential oil depend on different stages of growth. The oil yield and compounds changed in various phonological stages in *Syzygium aromaticum* buds. Eugenol and Eugenyl acetate were the main compounds in all samples with lower and higher levels in the young bud stage, respectively. So, Eugenol enhanced in the further phenological stages to reach highest amount and Eugenyl acetate was decreased to attained lowest content at the full fruiting stage [28]. Methyl chavicol (81.52–91.41%), methyl eugenol (0.97–4.18%) and 1, 8-cineole (1.30–2.43%) were the most important compounds of *Ocimum ciliatum* [29]. Shadi and Saharkhiz [30] reported a total of 56, 65, 65, and 68 components were identified and quantified at the above mentioned stages of *Artemisia sieberi*, respectively. Quantity of essential oil enhancement after flowering stage, significantly. The major compounds in all phenological stages were 1,8-cineole (21.1-24%), camphor (11.8-18.3%), α-thujone (8-13%), p-cymene (3-5.4%), terpineol (3-4.9%) and camphene (2.7-4%).

In this research, the most compounds found in various stages and species were caryophyllene, caryophyllene oxide and others were consists of farnesene, Trans-Caryophyllene, Trans-β-Farnesene, α-Cadinol, Arisol-9-en-

3, Germacrene D, and β-Bourdonene in Semnan climates, generally.

CONCLUSIONS

Results showed that the maximum and minimum plant height and number of leaves were observed in *officinalis* and *limbata* species, respectively. Maximum leaf length and diameter were observed in *sclarea* and *nemorosa* species, respectively. There were higher flowering traits in *sclarea* than *officinalis*. Moreover, the number of active ingredients was higher in *officinalis* than other species. Conclusively, *sclarea* was the best species in growth indices and *officinalis* species had highest active ingredients yield.

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Conflict of interests

Non-declared.

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