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Effect of Harvesting Time on Chemical Components of Sage (*Salvia officinalis* L.) From Iran

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ABSTRACT

Sage (*Salvia officinalis* L.) is perennial shrub and is one of the most important medicinal plants of the Lamiaceae family. The objective of this study was to evaluate the effect of different harvesting stages on contents, and essential oil components of sage. The essential oil from the aerial parts of the plant that were collected at different times was extracted by hydro-distillation in 2021 in the province of Isfahan in center Iran. Plants were harvested in four stages, i.e. the before blooming, beginning of blooming, full blooming and fruit stage. Results of mean comparisons revealed that the highest oil percentage (1.7%) was obtained at the stage of before blooming and the lowest oil percentage (0.36%) was obtained at the stage of fruit set. Based on results obtained from GC/MS analysis, in total, 39, 36, 32 and 37 constituents were identified in the essential oils of sage in the before blooming, beginning of blooming, full blooming and fruit stages, respectively. The major identified essential oil compounds were α-thujone (26.18–39.53%), camphor (10.39–19.78%), β-thujone (4.65–14.12%) and 1,8-cineol (7.75–13.98%). α-thujone as one of the major constituents of all samples was lower in the stage of before blooming (26.18%) and gradually increased in subsequent harvesting times to reach a maximum in the fruiting set (39.53%). Camphor was another compound where the highest (19.78%) was observed in beginning of blooming stage. The results showed that the harvesting time may have a significant effect on the essential oil yield and composition of sage. Our findings in sage, may pave the way to optimize the quality and quantity of sage essential oil to identify the best harvest time for pharmaceutical industries.

Keywords: *Salvia officinalis* L., Chemical constitutes, Essential oil, Harvest time.

INTRODUCTION

Salvia spp., with more than 1000 species, is categorized as the largest and important genus belongs to the Mentheae tribe within the Nepetoideae subfamily of the Lamiaceae family. The genus salvia in three regions of the world: Central and South America (500 spp.), Western Asia (200 spp.) and Eastern Asia (100 spp.). The genus Salvia in Iran includes 58 species, 17 of which are endemic (Walker *et al.*, 2004; Kharazian, 2014). Sage (*S. officinalis* L.) is an herbaceous, perennial plant, evergreen with woody stems and gray-green leaves. Sage is native to the Middle East and the Mediterranean region and cultivated throughout the world (Golparvar and Hadipanah, 2013).

The leaves of sage, contain essential oil widely used as a raw material in pharmaceutical, perfumery, and food industries (Martins *et al.,* 2015). According to the reviewed literature, the sage plant has shown various biological activities including antibacterial, antiviral, antifungal, antioxidant, anti-diabetes, anticholinesterase, antispasmodic, anti-inflammatory properties, and also sage essential oil is traditionally used to treat diseases including bronchitis, menstrual disorders, colds, bleeding and tuberculosis (Eidi and Eidi, 2009; Abu-Darwish *et al.,* 2013; Kontogianni *et al.,* 2013; Dammak *et al.,* 2019; Golparvar *et al.,* 2017; Ilić *et al.,* 2023). According to the results of phytochemical analysis of sage plant, the main constitutes of the oils are monoterpenes (α–and β-thujone, 1,8–cineole, camphor, borneol and etc.), sesquiterpenes (caryophyllene oxide and viridiflorol), and other compounds include alkaloids and phenolics (coumarins and flavonoids) (Lu and Foo, 2000; Vosoughi *et al.,* 2018; Ilić *et al.,* 2023).

Harvesting time can be suggested according to area and its environmental conditions. So that, the type and amount of essential oil compounds are functions of environmental factors (Hadipanah *et al.,* 2011). For example, Mirza *et al.* (2011) reported the major constituents of essential oils in (*S. officinalis* L.) at early, full and after flowering stages were α-thujene (20.8%, 27.1%, 35/9%) camphor (29.2%, 14.6%, 17.2%) and β-thujene (15.1%, 14.6%, 4.1%), respectively. In addition, results a study by Baranauskiene *et al.* (2011) indicated that α-thujone as one of the major constituents of sage were from May 23 to June 20 steadily increased from 29.4 to 39.7% and also viridiflorol was another compound where from May 23 to June 7 increased at the same period. In a study by Mohammadi-Cheraghabadi *et al.* (2023) the highest essential oil yield and concentrations of borneol, camphor and α-thujone of *S. officinalis* were higher in summer than spring. These quantitative characters may depend on the cultivation the climatic conditions (the soil water amount, the temperature, the light intensity) (Letchamo *et al.,* 1994) or may be genetically determined (Ložien and Venskutonis, 2003). Controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest (Canter *et al.,* 2005). Thus, the main objective of this study was to determine the optimal harvest time for sage essential oil production.

MATERIALS AND METHODS

Plant material

Sage (*S. officinalis*) seeds were obtained from Pakan Bazr Company, Isfahan, Iran. In the spring 2021, the seedlings of *S. officinalis* were transplanted to research farm at Islamic Azad University, Khorasgan (Isfahan) (Isfahan, Iran) with the geographical coordinates of 32° 38' N, 51° 47' E (1550 m above sea level). The climate of the area of study is classified as arid and warm region (according to the Koppen climate classification). According to the soil analysis, the soil at the experimental site was categorized as a clay loam (based on the soil texture triangle). The results of soil chemical analysis were; pH 7.25, E.C. = 2.5 dS m^{-1}, and contains total N (0.43 %), total P_2O_5 (26 ppm) and total K_2O (287 ppm).

Experimental design and treatments

The experiment was done in a split plot arrangement based on the randomized complete block design (RCBD) with three replications. Each experimental plot was 2.5×5 m, and plants were grown in 5 rows, with a spacing of 30 cm in rows 50 cm apart per replication. During the growth time, irrigation was performed according to the crop's needs in relation to the soil properties. Weeding was performed by hand. There were no pests or diseases observed during the plant growth. Finally, the aerial parts of sage harvested in four stages, i.e. the before blooming, beginning of blooming, full blooming and fruit set in September 2022.

Essential Oil Extraction

In order to measure the essential oil content of the harvested material, the aerial parts of the plants were cut at a height of 10 cm above soil levels and dried under shade at room temperature (25 ± 5 °C) for 10 days. For about 100 g of dried aerial part of sage for three hours in Clevenger-type apparatus were subjected with 500 mL distilled water to hydro-distillation, then for drying obtained oils, anhydrous sodium sulphate applied and before analysis stored in sealed vials at 4 °C. The obtained oils essential oils were clear and yellow liquid.

GC/MS analysis

Compositions of the essential oils were determined by GC–MS. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used with helium as carrier gas with flow rate of 1.0 mL/min. The oven temperature was kept 20°C at 50°C for 4 min and programmed to 280°C at a rate of 5°C /min, and kept 20°C constant at 280 °C for 5 min, at split mode. The injector temperature was at 20°C at 280°C. Transfer 20 line temperatures 280°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450. Identification of the essential oil components was accomplished

based on comparison of retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system) (Adams, 2017).

Statistical analysis

The data was statistically analyzed by $SPSS_{16}$ software, the experiment was done in a split plot arrangement based on the randomized complete block design (RCBD) with three replications. For means comparison analysis was used Duncan's multiple range test method (DMRT) at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Results of this study indicated that harvesting time affected ($P < 0.01$) on plant height, fresh weight and dry weight of sage (Fig. 1A & B). According to Figure 1A and B, results of mean comparisons plant height, fresh weight and dry weight revealed that the highest plant height (32.29 cm), fresh weight (3467.7 kg/ha) and dry weight (1236.65 kg/ha) were observed at the stage of fruit set and the lowest plant height (19.57 cm), fresh weight (1451.5 kg/ha) and dry weight (391.6 kg/ha) were obtained at the stage of before blooming setting.

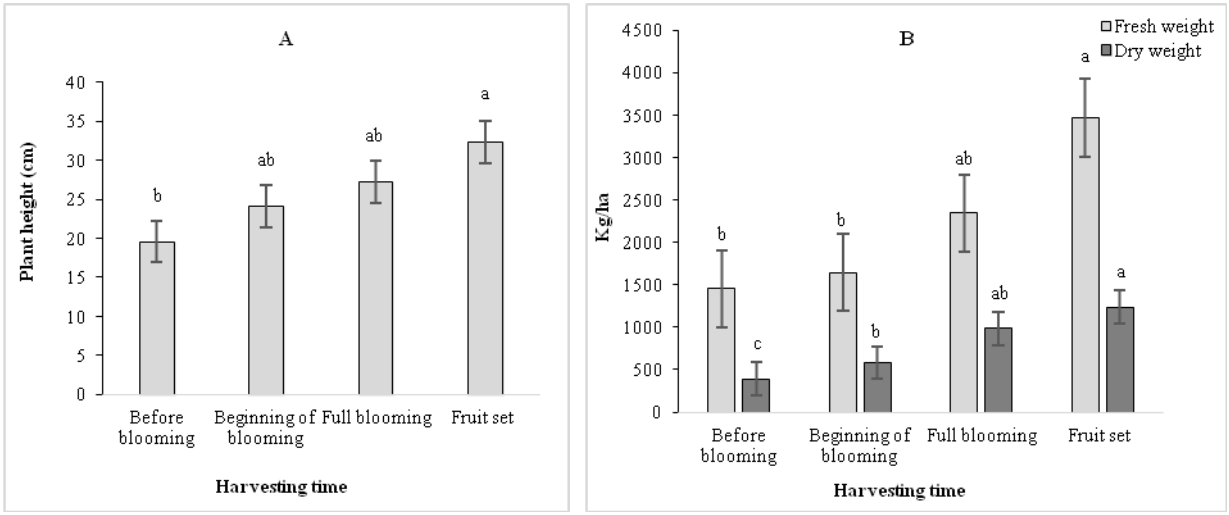

Figure 1. Effect of harvesting time on plant height (A), fresh weight and dry weight (B) in sage (*Salvia officinalis* L.). Different letters above the bars indicate statistically significant differences ($P < 0.01$).

The essential oil extracted from the sage aerial parts was colorless or pale yellow. Therefore, based on the extraction of essential oils by the hydro-distillation method with a Clevenger-type apparatus, a significant difference was observed in the amount of essential oil yielded in different harvesting time. The higher yield of essential oil was observed from before blooming (1.7 %) and the lowest amount of essential oil was observed from fruit stage (0.36 %). The results of the analysis of the constituents of the essential oil are reported in Table 1. Based on results obtained from GC/MS analysis, a total of 39, 36, 32 and 37 compounds were identified in the essential oils of sage in the before blooming, beginning of blooming, full blooming and fruit stages, respectively. The results of the experiment showed

that the quality of the essential oil was affected by the harvest time at different stages. Results of this study demonstrated that main volatile oil components were monoterpenes. The major identified essential oil compounds were α-thujone (26.18–39.53%), camphor (10.39–19.78%), β-thujone (4.65–14.12%) and 1,8-cineol (7.75–13.98%).

Table 1. The effect of harvesting time on chemical compositions of the essential oil from *S. officinalis*

No	Compound	RI	Before blooming	Beginning of blooming	Full blooming	Fruit set	ANOVA
	Monoterpene hydrocarbons						
1	Cis-Salvene	852	0.33 ± 0.03	1.21 ± 0.05	0.00 ± 0.00	0.17 ± 0.07	
2	Trans-Salvene	859	0.08 ± 0.02	0.94 ± 0.05	0.24 ± 0.04	0.02 ± 0.01	
3	Tricyclene	915	0.00 ± 0.00	0.28 ± 0.03	0.03 ± 0.01	0.21 ± 0.07	
4	Thujene	928	0.21 ± 0.04	0.06 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	
5	α-Pinene	937	3.89 ± 0.21^{c}	7.63 ± 0.64^{a}	5.43 ± 0.54^{b}	6.91 ± 0.84^{ab}	$p \leq 0.01$
6	Camphene	955	4.58 ± 0.12^{a}	3.86 ± 0.11^{ab}	2.98 ± 0.09^{b}	3.76 ± 0.06^{ab}	$p \leq 0.01$
7	Sabinene	974	0.15 ± 0.02	0.12 ± 0.03	0.25 ± 0.05	0.49 ± 0.04	
8	β-Pinene	978	1.03 ± 0.04	2.47 ± 0.03	0.95 ± 0.06	0.36 ± 0.05	
9	1-Octan-3-ol	985	0.65 ± 0.02	0.00 ± 0.00	0.21 ± 0.01	0.04 ± 0.02	
10	Myrcene	992	0.44 ± 0.11	0.67 ± 0.02	0.47 ± 0.03	0.52 ± 0.01	
11	3-Octanol	995	0.07 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	
12	α -Phellandrene	999	0.28± 0.04	0.05 ± 0.01	0.23 ± 0.02	0.41 ± 0.04	
13	α-Terpinene	1008	1.19 ± 0.00	0.37 ± 0.01	0.05 ± 0.02	0.01 ± 0.00	
14	p-Cymene	1021	1.57± 0.23	1.21 ± 0.12	0.01 ± 0.01	0.52 ± 0.02	
15	Limonene	1025	1.95± 0.26	1.17 ± 0.15	0.97 ± 0.05	0.65 ± 0.07	
16	1,8-Cineole	1027	7.75 ± 0.36^{b}	8.65 ± 0.47^{b}	11.73 ± 0.74^{ab}	13.98 ± 0.98^{a}	$p \leq 0.01$
17	Cis -Ocimene	1030	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
18	γ-Terpinene	1044	0.61 ± 0.02	0.67 ± 0.03	0.85 ± 0.05	0.35 ± 0.01	
19	Cis- Sabinene hydrate	1050	0.05 ± 0.01	0.18 ± 0.02	0.00 ± 0.00	0.21 ± 0.01	
20	Linalool oxide	1076	0.12 ± 0.02	0.42 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	
21	α-Terpinolene	1083	0.23 ± 0.02	0.61 ± 0.06	1.24 ± 0.21	0.68 ± 0.11	
22	Trans-Sabinene hydrate	1093	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.24 ± 0.02	
23	Linalool	1099	0.82 ± 0.02	0.01 ± 0.01	1.26 ± 0.35	0.95 ± 0.22	
24	α-Thujone	1107	26.18 ± 1.05^{b}	27.67 ± 0.87^{b}	34.47 ± 1.35^{ab}	39.53 ± 1.15^{a}	$p \leq 0.01$
25	β-Thujone	1115	14.12 ± 0.97^{a}	10.89 ± 1.24^{ab}	9.36 ± 0.64^{ab}	4.65 ± 0.09^{b}	$p \leq 0.01$
26	α-Campholene aldehyde	1119	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	
27	Camphore	1126	18.41 ± 0.68^{a}	19.78 ± 1.23^{a}	15.45 ± 0.98^{ab}	10.39 ± 0.87^{b}	$p \leq 0.01$
28	Borneol	1163	5.47 ± 0.41^{ab}	2.69 ± 0.11^{b}	6.36 ± 0.25^{a}	6.85 ± 0.33^{a}	$p \leq 0.01$
29	Terpinene-4-ol	1177	0.62 ± 0.03	0.17 ± 0.01	0.68 ± 0.02	0.41 ± 0.01	
30	α-Terpineol	1188	0.34 ± 0.04	0.46 ± 0.05	0.04 ± 0.01	0.32 ± 0.02	
31	Trans-Sabinyl acetate	1289	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.06 ± 0.02	
32	Carvacrol	1300	0.41 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
33	Carvacryl acetate	1352	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	
	Sesquiterpenes hydrocarbons						
34	*β*-Caryophyllene	1418	1.99 ± 0.25	0.32 ± 0.08	0.72 ± 0.04	0.28 ± 0.02	
35	α-Humulene	1452	0.67 ± 0.05	0.38 ± 0.03	0.02 ± 0.01	0.01 ± 0.01	
36	Naphthalene	1480	0.25 ± 0.02	0.01 ± 0.01	0.41 ± 0.03	0.05 ± 0.01	
37	Ledene	1499	0.01 ± 0.00	0.08 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	
	Oxygenated Sesquiterpenes						
38	Caryophyllene oxide	1582	0.31 ± 0.09	1.57 ± 0.21	0.01 ± 0.01	0.35 ± 0.04	
39	Viridiflorol	1591	3.82 ± 0.45^{ab}	2.39 ± 0.21^{b}	4.02 ± 0.84^{a}	$4.32 \pm 0.45_{a}$	$p \leq 0.01$
40	β-Selinene	1604	0.08 ± 0.01	0.82 ± 0.07	0.06 ± 0.02	0.01 ± 0.01	
41	Humullene epoxide Π	1606	0.01 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	
42	Manool	2056	0.75 ± 0.02	0.41 ± 0.03	0.01 ± 0.01	0.01 ± 0.00	
	Total		99.47	98.25	98.52	97.78	
	Oil yield (%)	-	1.7^{a}	0.98^{ab}	0.57^{b}	0.36^{c}	

Means ± SD with different letter in a row are statistically significant at 5% level probability by DMRT (Duncan's multiple range test) method; RI = Retention indices in elution order from DB-5 column.

Many environmental factors, such as plant genetic, geographical origin, weather conditions, geobotanical conditions, cultivation method, stress type, time of plant collection, storage method, plant age, extraction method, herbivore or microbial attack, and genetic changes, can play an important role in changing the composition of the extracted essential oils from medicinal plants (Letchamo *et al.*, 1994; Ložien and Venskutonis, 2003; Thompson *et al.*, 2003; Golparvar and Hadipanah, 2024). While the percentage of each major constituent in

the oil was found to vary during all the harvesting times. Among the terpenoids, α-thujone had the highest value among the identified compounds. Among the harvest time, the highest (39.53%) and lowest (26.18%) values of α-thujone were related to fruit stage and before blooming stage, respectively. In addition, camphore was another dominant compound in all stages of the harvesting times. The highest (19.78%) and lowest (10.39%) values of this compound were obtained from beginning of blooming stage and fruit stage, respectively. Another dominant compound was β-thujone, according to results showing the highest (14.12 %) and lowest (4.65 %) amounts were related to before blooming stage and fruit stage, respectively. 1,8-cineole and borneol were other compounds whose highest values were obtained in fruit stage with 13.98% and 6.85% values, respectively. Other identified aroma compounds included α-pinene, camphene and viridiflorol, but all in much smaller quantities. To the best of our knowledge, two harvest times in sage, including fruit stage and before blooming stage are suggested, considering the yield, quantity and quality of the essential oil. Moreover, it is known that terpenoid synthesis proceeds through a variety of intermediates and that thujones (is a ketone and a monoterpene that occurs in two diastereomeric forms: α-thujone and β-thujone) and camphor originate from different reactive carbocations. Dominance of one or the other compound in the essential oil of sage should be reasonably correlated with the activation of the specific metabolic pathway (Croteau and Karp, 1976).

For example, Hazrati *et al.* (2022) reported that the effect of harvesting times (day/night) have a significant effect on essential oil yield and composition of (*Salvia officinalis*). They stated the quantitative and qualitative properties of essential oil in sage are subjected to significant hourly changes (different hours of the day and night) during the day/night time. In addition, results a study by Arraiza *et al.* (2012) indicated that the highest oil yield of (*Salvia officinalis* L.) was obtained in the stage of full flowering and the highest concentration of α-thujone (40.1 - 46.5%) in the period of initial flowering. In another study the yield of essential oil of sage in different stages was floral budding (0.9%), vegetative (0.7%), flowering (0.5%), immature fruit (0.4%) and ripen fruit (0.2%) (Hossein Mirjalili *et al.,* 2006). Results of a study by Golparvar *et al.* (2012) indicated that the highest thymol content of *Thymus daenensis* obtained at the stage of before blooming, also Salehi *et al.* (2014) reported the optimum of harvest time of *Thymus vulgaris* was extracted at the beginning of flowering stage. A comparison of our results with different reports, differences in the volatile composition of the plants could be attributed to genetic (genus, species, and ecotype), chemotype, distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.

CONCLUSION

The results obtained in our study indicated that the highest plant height, fresh weight and dry weight obtained at fruit set stage. Also, the results showed that the harvesting time have a significant effect on the essential oil yield and composition of sage. To gain the highest essential oil yield, the best harvesting time was found at before blooming stage. α-thujone was

detected at the highest amount when the plants were collected at fruit stage and lowest value was obtained at before blooming stage. In addition, camphor was another dominant compound, and the highest amount of this compound was obtained in beginning of blooming stage. Our findings clearly demonstrate that there are notable changes to the essential oil's quantitative and qualitative characteristics during harvesting time.

REFERENCES

Abu-Darwish M, Cabral C, Ferreira I, Gonçalves M, Cavaleiro C, Cruz M. Al-bdour T. Salgueiro L. 2013. Essential oil of common sage (*Salvia officinalis* L.) from Jordan: Assessment of safety in mammalian cells and its antifungal and anti-inflammatory potential. BioMed research international. https://doi.org/10.1155/2013/538940.

Adams RP. 2017. Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing.

Arraiza MP, Arrabal C, López JV. 2012. Seasonal variation of essential oil yield and composition of sage (*Salvia officinalis* L.) grown in Castilla-La Mancha (Central Spain). Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 40(2): 106-8.

Baranauskiene R, Dambrauskiene E, Venskutonis PR, Viskelis P. 2011. Influence of harvesting time on the yield and chemical composition of sage (*Salvia officinalis* L.). Foodbalt. 104-109.

Canter PH, Thomas H, Ernst E. 2005. Bringing medicinal plants into cultivation: opportunities and challenges for biotechnology. TRENDS in Biotechnology. 23(4): 180-185.

Croteau R, Karp F. 1976. Biosynthesis of monoterpenes: Enzymatic conversion of neryl pyrophosphate to 1, 8-cineole, α-terpineol, and cyclic monoterpene hydrocarbons by a cell-free preparation from sage (*Salvia officinalis*). Archives of biochemistry and biophysics. 176(2): 734-746.

Dammak I, Hamdi Z, El Euch SK, Zemni H, Mliki A, Hassouna M, Lasram S. 2019. Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis, Lavandula dentata* and *Laurus nobilis* essential oils and a major monoterpene constituent 1, 8-cineole against Aspergillus carbonarius. Industrial Crops and Products. 128: 85-93.

Eidi A, Eidi M. 2009. Antidiabetic effects of sage (*Salvia officinalis* L.) leaves in normal and streptozotocin-induced diabetic rats. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 3(1): 40-44.

Golparvar AR, Ghasemi Pirbalouti A, Zinaly H, Hadipanah A. 2012. Effect of harvest times on quantity (morphological) and quality characteristics of *Thymus daenensis* Celak. in Isfahan. Journal of Medicinal Herbs. 2(4): 245-254.

Golparvar AR, Hadipanah A, Gheisari MM, Naderi D, Rahmaniyan S, Khorrami M. 2017. Chemical composition and antimicrobial activity of essential oil of *Salvia officinalis* L. and *Salvia virgata* Jacq. Journal of Medicinal Herbs. 8(2): 71-78.

Golparvar AR, Hadipanah A. 2024. Effect of ecological factors on plant properties of *Thymus daenensis* Celak in different regions in Southwest and Central Iran. Agriculturae Conspectus Scientificus. 89(2): 105-111.

Golparvar AR, Hadipanah A. 2013. Identification of the components of sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) cultivated in Isfahan climatic conditions. Electronic Journal of Biology. 9(2): 42-45.

Hadipanah A, Golparvar AR, Pirbalouti A, Zaynali H. 2011. Determine optimum of harvest time on the quantity/quality of essential oil and thymol of thyme (*Thymus vulgaris* L.) in Isfahan. Journal of Herbal Drugs. 2(1): 23-32.

Hazrati S, Beidaghi P, Beyraghdar Kashkooli A, Hosseini SJ, Nicola S. 2022. Effect of harvesting time variations on essential oil yield and composition of Sage (*Salvia officinalis*). Horticulturae. 8(2): 149.

Hossein Mirjalili M, Salehi P, Sonboli A, Mohammadi Vala M. 2006. Essential oil variation of *Salvia officinalis* aerial parts during its phenological cycle. Chemistry of natural compounds. 42: 19-23.

Ilić ZS, Kevrešan Ž, Šunić L, Stanojević L, Milenković L, Stanojević Milenković A, Cvetkoviić D. 2023. Chemical profiling and antioxidant activity of wild and cultivated sage (*Salvia officinalis* L.) essential oil. Horticulturae. 9(6): 624.

Kharazian N. 2014. Chemotaxonomy and flavonoid diversity of Salvia L.(Lamiaceae) in Iran. Acta Botanica Brasilica. 28: 281-92.

Kontogianni VG, Tomic G, Nikolic I, Nerantzaki AA, Sayyad N, Stosic-Grujicic S. 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. Food chemistry. 136(1): 120-9.

Letchamo W, Marquard R, Hölzl J, Gosselin A. 1994. Effects of water supply and light intensity on growth and essential oil of two *Thymus vulgaris* selections. Angew. Bot. 68: 83–88.

Ložien K, Venskutonis PR. 2003. Chemical composition of the essential oil of different varieties of thyme (*Thymus pulegioides*) growing wild in Lithuania. Biochemical Systematics and Ecology. 31(3): 249-59.

Lu Y, Foo LY. 2000. Flavonoid and phenolic glycosides from *Salvia officinalis*. Phytochemistry. 55(3):263-7.

Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira IC. 2015. Evaluation of bioactive properties and phenolic compounds in different extracts prepared from *Salvia officinalis* L. Food chemistry. 170: 378-85.

Mirza M, Ghoraishi F, Bahadori A. 2011. Effect of harvesting time on essential oils content and composition of *Salvia officinalis* L. and *Mentha piperita* L. in Khuzestan province. Iranian Journal of Medicinal and Aromatic Plants. 26(4): 531-543.

Mohammadi-Cheraghabadi M, Modarres-Sanavy SAM, Sefidkon F, Mokhtassi-Bidgoli A, Hazrati S. 2023. Harvest time explains substantially more variance in yield, essential oil and quality performances of *Salvia officinalis* than irrigation and putrescine application. Physiology and Molecular Biology of Plants. 29(1): 109-20.

Salehi S, Golparvar AR, Hadipanah A. 2014. Effect of harvest time on yield and quality of *Thymus vulgaris* L. essential oil in Isfahan province, Iran. Agriculturae Conspectus Scientificus. 79(2): 115-8.

Thompson JD, Chalchat J-C, Michet A, Linhart YB, Ehlers B. 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. Journal of chemical ecology. 29: 859-80.

Vosoughi N, Gomarian M, Pirbalouti AG, Khaghani S, Malekpoor F. 2018. Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. Industrial crops and products. 117:366-74.

Walker JB, Sytsma KJ, Treutlein J, Wink M. 2004. Salvia (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of Salvia and tribe Mentheae. American Journal of Botany. 91(7): 1115-25.