Changes in Essential Oil Content and Composition of Salvia Limbata C.A. Mey at Different Growth Stages and Altitudes

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Abstract

Background
The present study investigates the effect of growth stage (vegetative, flowering and seed ripening) and altitude (1500, 2000 and 2500 m above sea level) on the content and chemical composition of *S. limbata* essential oil which belongs to *Lamiaceae* family.

Results
According to the oil analysis, 28 components representing 96.5% to 99.7% of the total volatile oil composition were characterized. The main compounds of *S. limbata* oils were \( \alpha \)-pinene (14.7-38.7%), \( \beta \)-pinene (12.5-26.2%), allo-aromadendrene (9.2-21.7%), germacrene D (4.2-8.3%), bicyclogermacrene (6.5-14.5 %), and spathulenol (7.5-25.4 %).

Discussion
The obtained results showed that the content and constituents of *S. limbata* essential oil strongly depend on the growth stage and altitude. Our findings revealed that the vegetative stage at 1500 m is the optimal harvest time to obtain the highest content of oil yield. Results of the current study helps to find the optimum situation to gain the highest content of *S. limbata* essential oil but more researches are needed.

1. Introduction
*Salvia* is the largest and prominent genus of the *Lamiaceae* family, which includes more than 900 medicinal and ornamental species distributed in the world [1]. This genus is found in Central and South America, Western Asia and Eastern Asia [2]. Fifty-eight species of genus *Salvia* are found in Iran which seventeen species are endemic [3,4]. *Salvia limbata* C.A. Mey, a native plant of Iran, is a perennial, herbaceous and aromatic plant (30-60 cm tall) with thick, rounded and bright green leaves. The distribution of this plant within Iran is in Azerbaijan, Lorestan, Shiraz, Kermanshah, Semnan, and Damavand [5]. The genus *Salvia* has always been noticeable in doing research around the world for diverse biological activities and compounds in its essential oil [6,7]. Since the species of genus *Salvia* contain substantial amounts of essential oils, people have been applied for thousands of years in folk medicine to improve health and treat diseases [8,9]. Modern science illustrates that *Salvia* essential oils improve memory and could be effective in treating Alzheimer’s in the future [10]. *Salvia* has also been used for treating coughs, colds and wounds and it has been considered as spasmylytic, antiseptic, astringent, and liver protective [11].

Moreover, the phenolic compounds of plants belonging to this genus have shown antiviral, antibacterial, antifungal, antioxidant, antitumor, anti diabetic, anxiolytic, sedative, and anti-inflammatory activities [12,13,14,15,16,17,18]. Active compounds such as hydrocarbon monoterpenes, hydrogenated monoterpenes, oxygenated monoterpenes, di-terpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes have been obtained from *Salvia* species [19]. The contents of these compounds are influenced by various factors such as planting conditions, harvest time, organ used and growth stage. It seems that ecological factors and genetic are the main significant factors that influence the content of the plant active compounds [20].

To the best of our knowledge, few investigations have been done in the field of autecology and phytochemistry of *S. limbata*. Since the global approach has been perusing the use of medicinal herbs and natural compounds in the pharmaceutical, cosmetic and food industries, there is a strong need to delve more into the issue and do further research to understand how to increase the yield of active ingredients in varied ecological conditions. This could be economically important for the food, cosmetic and pharmaceutical industries. Although phytochemistry of different species of this genus has already been studied in different ecological conditions (mostly in flowering stage), for the first time the effect of different phenological stages and altitudes on the essential oil content and composition of *S. limbata* was investigated in this paper.

2. Materials And Methods

2.1. Plant material
Aerial parts of *S. limbata* at several developmental stages (vegetative, flowering and seed ripening) were harvested in three replicates from its wild habitat at altitudes of 1500, 2000 and 2500 m above sea level from Taleghan rangeland (semi-humid) in Alborz province, Iran (36° 5‘ 19“ N to 36° 19‘ 19“ N and 50° 36‘ 43“ E to 50° 53‘ 20“ E) (Fig. 1). Taleghan is one of *S. limbata’s* main sites with mean relative humidity about 12%, and the average annual temperature of 11.4°C. In this area, there are 150 freezing days and annual precipitation is about 446 mm. Harvested plant materials were dried in the shade and then ground in a grinder (2mm mesh size). A voucher herbarium specimen (MP-300) was lodged at the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

2.2. Isolation of the essential oils

Samples (100 g) of the air-dried aerial parts of *S. limbata* were subjected to hydro distillation using a Clevenger-type apparatus for 4h, according to the method recommended in British Pharmacopoeia. The distilled oils were dried over anhydrous sodium sulfate and stored at 4°C in tightly closed dark vials to be analyzed.

2.3. Chemical composition of the essential oils

For characterization of the volatile oil constituents, samples were subjected to gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). GC-FID analysis was performed by a Shimadzu 15 A gas chromatograph (Dyson Instruments, Newcastle, U.K) equipped with a split/splitless injector (250°C), a DB-5 (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) capillary column and flame ionization detector (250°C). The helium gas was used as the carrier gas (1 mL/min) and the oven temperature was 60-280°C at a rate of 5°C/min. The injector temperature was 250°C and 1μL of oil sample was injected in the split mode with the split ratio of 100:1.

GC-MS analysis was carried out using a Thermo-Trace GC-MS system (Thermo Electron; San José, CA, USA). This device was equipped with a DB-5 silica column (60 m length × 0.25 mm internal diameter, 0.25 μm film thickness) containing 5%-phenyl 95%-methyl polysiloxane and a mass spectrometer detector (MS). The carrier gas was helium at a flow rate of 1 ml/ min. The column temperature was kept at 60°C for 3 min and then programmed to 250°C at a rate of 5°C/min. The injector and GC/MS interface temperatures were 290°C and 300°C, respectively. The mass spectra were taken at 70 eV in the scan range of m/z 50-550.

2.4. Identification of compounds

The constituents of the essential oils were recognized by calculation of retention indices for all the components, using retention times of C_6-C_{24} n-alkenes series as standards under the same chromatographic conditions. Identification of individual compounds were performed by comparison of their retention indices and mass spectral fragmentation patterns with those reported in the literature, Wiley library (New York, NY, USA) or published mass spectra [8,21]. The relative percentage of essential oil constituents were obtained from the GC-FID peak areas in the chromatogram without the use of correction factors.

2.5. Statistical analysis

The statistical analyses were performed using Graph Pad Prism software (San Diego, CA; version 5.0). Data are presented as mean ± standard deviation (SD) in 5 randomized replicates. One way analysis of variance (ANOVA) and Tukey post-test were used to analyze obtained results. *P*-value < 0.05 was considered as statistically significant difference.

3. Results And Discussion

As shown in Figure 2, comparison of the essential oil yield among different samples revealed that the highest content of essential oil belongs to the harvested aerial parts of *S. limbata* in the vegetative stage at an altitude of 1500 m (0.86% v/w) while no significant difference was observed in the essential oil content among other groups. In a published study authors observed the significant impact of different altitudes and phenological stages on the essential oil yield [22,23]. It was revealed that plant performance is strongly influenced by various factors such as altitude, climate, soil, developmental stages, extraction and analysis methods, genetic factors, abiotic stresses, and slope and modeling techniques can predict these factors in other areas [24,25,26,27, 28]. The results of the current study were compatible with other studies that found the highest content of essential
oil of *Origanum majorana* in the vegetative stage, so they proposed the vegetative stage as the best stage to harvest *Origanum majorana* [29,30, 31,32, 33,34,35,36]. Similar results were also found by the essential oil content of *Nepeta kotschyr* [37]. Moreover, according to a study conducted by the highest yield of essential oil in *Teucrium polium* L. was obtained in the vegetative stage [38]. These results are in agreement with our findings. The accumulation of essential oil in vegetative stage could be due to the fact that plant protection is supplied by phenolic components which are in high amount in this stage [39].

On the contrary, it was reported by that the highest essential oil in *Satureja mutica* is acquired in the flowering stage [40]. In another study [41] determined that the highest value of essential oil of *Mentha pieperata* in flowering stage, which contradicts our findings. Also, the percentage of essential oils in vegetative stage in *Thymus vulgaris* was the lowest and it rose in flowering stage [42]. One explanation for the increase in essential oil content in the flowering stage is the maintenance of the reproductive stage [43,44] and to attract insects for pollination [35]. Nevertheless, other researches have illustrated that the lowest amount of essential oils in the vegetative stage could be due to the lower activity of some enzymes in synthesizing phenolic compounds in this stage [45]. Since photosynthetic products accumulate in the endosperm during plant growth, it leads to a decrease in the amount of essential oil [46]. It is clear that phenological stages have a great impact on the essential oil metabolism, enzymatic activity and finally essential oil content [45].

As illustrated in Tables 1, 2 and 3, twenty-eight components were identified in the *S. limbata* essential oil by means of GC-FID and GC-MS analysis which represented about 96.5% to 99.7% of the total composition of the obtained essential oil. In the current study, the main identified compounds were α-pinene (14.7-38.7%), β-pinene (12.5-26.2%), allo-αromadendrene (9.2-21.7%), germacrene D (4.2-8.3%), bicyclogermacrene (6.5-14.5 %), and spathulenol (7.5-25.4 %). The molecular structures of the main identified compounds from *S. limbata* essential oil are presented in the Figure 3. Comparing the results of the current study to others, α-pinene (23.7 %), β-pinene (18.7%), sabinene (14.5%), 1, 8-cineole (9.9%) and β-caryophyllene (7.1%) as the major components of *S. limbata* essential oil in the flowering stage [47]. In another research, following GC-MS analysis of the aerial parts of *S. limbata* obtained from Turkey, 42 components were characterized representing 95.6% to 98.1% of the compounds including α-pinene (11.2-24.3%), β-pinene (10.0-20.9%) and sabinene (14.6-17.4%) as the major constituents of the essential oil [48].

Comparing of the monoterpenes and sesquiterpenes contents of the *S. limbata* essential oil at different altitudes and phenological stages in Figure 4, the amount of monoterpenes has decreased from vegetative stage to seed ripening stage; however, the obtained results for sesquiterpenes were reverse. These findings for *Artemisia herba-alba* essential oil were previously observed in another study [49]. Moreover, during the developing plants the amount of sesquiterpenes increased in *Cannabis sativa* L. which are in line with our results [50]. As we found out in our research, the highest amount of monoterpenes was related to the vegetative period at 2000 m, while the highest amount of sesquiterpenes was obtained in seed ripening stage at altitudes of 1500 and 2500 m. [51] 2002 in a research on *Thymus vulgaris* at different growth stages confirmed that the highest content of the monoterpane was related to the vegetative stage.

As shown in Figure 5, the content of α-pinene, β-pinene, alloaromadendrene, germacrene D, bicyclogermacrene, and spathulenol illustrates some changes in different altitudes and developmental stages. The highest percentage of monoterpenes including α-pinene (41.3%) and β-pinene (30.1%) was obtained in the vegetative stage at 2000 m. The contents of α-pinene and β-pinene were decreased to the lowest values in the ripening stage. Moreover, the highest content for alloaromadendrene was measured 20.6% and 20.7% in the ripening stage at 1500 m and 2500 m, respectively without any significant difference between them. However, the lowest quantity was obtained 3.5% for the vegetative stage at 2000 m. The most abundant germacrene D reached in ripening stage at 2500 m (8.3%) and the lowest amount (1.2%) achieved at 2000 m in the vegetative stage. Moreover, high value of bicyclogermacrene was attained in the ripening stage at 1000 m and 2000m (14.5% and 14.3%, respectively) while no significant difference was observed between the mentioned altitudes. On the contrary, the lowest amount was attained in the vegetative stage at 1500 m (4.2%). Furthermore, the highest and the lowest contents of spathulenol (25.4% and 7.2%) were gained in the ripening stage at 2500 m and vegetative stage at 2000 m, respectively. Variation in the percentage of compounds at different stages of phenology and altitudes could be due to the high or low synthesis of compounds by enzymes, which leads to different percentages of compounds in essential oils.

**Table 1.** The percentage of chemical compositions of *Salvia limbata* essential oil in the vegetative stage at different altitudes
| No. | Compounds               | RI  | 1500 m          | 2000 m          | 2500 m           |
|-----|------------------------|-----|-----------------|-----------------|------------------|
| 1   | α-Thujene              | 922 | Tr              | Tr              | Tr               |
| 2   | α-Pinene               | 938 | 30.4±0.1        | 41.3±0.2        | 28.5±0.8         |
| 3   | Camphene               | 952 | 1.2±0.1         | 1.4±0.2         | 0.5±0.0          |
| 4   | Sabinene               | 975 | 2.4±0.1         | 1.9±0.1         | 4.1±0.1          |
| 5   | β-Pinene               | 980 | 25.4±0.3        | 30.1±0.1        | 24.2±0.2         |
| 6   | Myrcene                | 985 | Tr              | Tr              | Tr               |
| 7   | p-Cymene               | 1025| Tr              | Tr              | Tr               |
| 8   | Limonene               | 1029| 0.9±0.1         | 0.6±0.04        | 0.4±0.0          |
| 9   | Z-β-Ocimene            | 1035| Tr              | Tr              | Tr               |
| 10  | Linalool               | 1085| Tr              | Tr              | Tr               |
| 11  | α-Campholenal          | 1103| Tr              | Tr              | Tr               |
| 12  | Trans-Pinocarveol      | 1125| Tr              | 0.2±0.0         | Tr               |
| 13  | Trans-Verbenol         | 1162| Tr              | 0.3±0.0         | 0.2±0.01         |
| 14  | Borneol                | 1186| 0.2±0.0         | Tr              | Tr               |
| 15  | Terpine-4-ol           | 1203| Tr              | 0.4±0.0         | 0.2±0.0          |
| 16  | Myrtenal               | 1216| Tr              | 0.4±0.0         | 0.2±0.0          |
| 17  | Verbenone              | 1239| 0.3±0.1         | 0.2±0.0         | 0.3±0.0          |
| 18  | Bornyl-acetate         | 1316| 1±0.1           | Tr              | Tr               |
| 19  | Eugenol                | 1340| Tr              | 0.9±0.1         | 1.4±0.2          |
| 20  | β-Caryophyllene        | 1426| Tr              | Tr              | 0.2±0.0          |
| 21  | Allo-Aromadendrene     | 1482| 9.5±0.1         | 3.5±0.2         | 12.6±0.3         |
| 22  | γ-Muurolene            | 1485| Tr              | 0.6±0.0         | Tr               |
| 23  | Germacrene D           | 1498| 3.7±0.2         | 1.2±0.1         | 4.1±0.2          |
| 24  | Bicyclogermacrene      | 1505| 4.6±0.2         | 5.5±0.3         | 8.5±0.9          |
| 25  | Eugenol-acetate        | 1521| Tr              | 0.6±0.0         | Tr               |
| 26  | Spathulenol            | 1575| 12.6±0.1        | 7.2±0.2         | 13.8±0.2         |
| 27  | Caryophyllene oxide    | 1580| 4.3±0.1         | Tr              | Tr               |
| 28  | Sclareol               | 2200| 1.9±0.1         | 0.2±0.0         | 0.5±0.0          |
|     | Monoterpene hydrocarbons |   | 60.3            | 75.3            | 57.7             |
|     | Oxygenated monoterpenes |   | 1.3             | 2.6             | 2.3              |
|     | Sesquiterpene hydrocarbons |   | 17.8            | 10.8            | 25.4             |
|     | Oxygenated sesquiterpenes |   | 18.8            | 8.0             | 14.3             |
|     | Total                  |   | 98.2            | 96.7            | 99.7             |
Data are presented as mean±SD; RI indicates retention indices relative to C$_6$-C$_{24}$ $n$-alkanes; Tr indicates trace (<0.1%)

**Table 2.** The percentage of chemical compositions of *Salvia limbata* essential oil in the flowering stage at different altitudes
| No. | Compounds          | RI  | 1500 m      | 2000 m      | 2500 m      |
|-----|--------------------|-----|-------------|-------------|-------------|
| 1   | α-Thujene          | 922 | Tr          | Tr          | Tr          |
| 2   | α-Pinene           | 938 | 21.2±0.2    | 29.5±0.4    | 21.7±0.6    |
| 3   | Camphene           | 952 | 1.3±0.1     | 1.4±0.2     | 1.8±0.2     |
| 4   | Sabinene           | 975 | 1.7±0.1     | 3.3±0.1     | 1.8±0.1     |
| 5   | β-Pinene           | 980 | 17.3±0.1    | 26.2±0.2    | 20.1±0.2    |
| 6   | Myrcene            | 985 | Tr          | Tr          | Tr          |
| 7   | p-Cymene           | 1025| Tr          | Tr          | Tr          |
| 8   | Limonene           | 1029| 1±0.1       | 1±0.1       | 0.8±0.1     |
| 9   | Z-β-Ocimene        | 1035| Tr          | 1.2±0.1     | Tr          |
| 10  | Linalool           | 1085| Tr          | Tr          | Tr          |
| 11  | α-Campholenal      | 1103| Tr          | Tr          | Tr          |
| 12  | Trans-Pinocarveol  | 1125| 0.3±0.0     | Tr          | 0.4±0.0     |
| 13  | Trans-Verbenol     | 1162| Tr          | Tr          | 0.3±0.0     |
| 14  | Borneol            | 1186| Tr          | Tr          | 0.3±0.0     |
| 15  | Terpine-4-ol       | 1203| 0.6±0.0     | 0.6±0.0     | Tr          |
| 16  | Myrtenal           | 1216| 0.6±0.0     | 0.6±0.1     | Tr          |
| 17  | Verbenone          | 1239| 0.2±0.0     | 0.3±0.0     | Tr          |
| 18  | Bornyl-acetate     | 1316| Tr          | 0.2±0.0     | Tr          |
| 19  | Eugenol            | 1340| 1.2±0.1     | 0.4±0.0     | 0.5±0.0     |
| 20  | β-Caryophyllene    | 1426| Tr          | Tr          | Tr          |
| 21  | Allo-Aromadendrene | 1482| 15.2±0.2    | 9.2±0.1     | 14.6±0.2    |
| 22  | γ-Murolene         | 1485| 0.5±0.0     | 3.2±0.1     | 0.3±0.0     |
| 23  | Germacrene D       | 1498| 5.7±0.2     | 4.4±0.1     | 4.2±0.2     |
| 24  | Bicyclogermacrene  | 1505| 10.7±0.2    | 6.5±0.1     | 13.6±0.2    |
| 25  | Eugenol-acetate    | 1521| Tr          | Tr          | Tr          |
| 26  | Spathulenol        | 1575| 18.6±0.3    | 7.5±0.1     | 15.5±0.3    |
| 27  | Caryophyllene oxide| 1580| 1±0.1       | 2.1±0.2     | Tr          |
| 28  | Sclareol           | 2200| 1.7±0.2     | Tr          | 0.4±0.0     |

Monoterpene hydrocarbons: 42.5 62.6 46.2
Oxygenated monoterpenes: 2.9 2.1 1.5
Sesquiterpene hydrocarbons: 32.1 23.3 32.7
Oxygenated sesquiterpenes: 21.3 9.6 15.9
Total: 98.8 97.6 96.3

Data are presented as mean±SD; RI indicates retention indices relative to C6-C24 n-alkanes; Tr indicates trace (<0.1%).
### Table 3: The percentage of chemical compositions of *Salvia limbata* essential oil in the ripening stage at different altitudes

| No. | Compounds                  | RI  | 1500 m     | 2000 m     | 2500 m     |
|-----|----------------------------|-----|------------|------------|------------|
| 1   | α-Thujene                  | 922 | Tr         | Tr         | Tr         |
| 2   | α-Pinene                   | 938 | 14.9±0.2   | 19.5±0.2   | 14.7±0.1   |
| 3   | Camphene                   | 952 | 0.9±0.0    | 1.6±0.2    | 0.6±0.0    |
| 4   | Sabinene                   | 975 | 1.3±0.2    | 2.2±0.2    | 1.1±0.1    |
| 5   | β-Pinene                   | 980 | 14.2±0.3   | 17.6±0.3   | 12.5±0.1   |
| 6   | Myrcene                    | 985 | Tr         | Tr         | Tr         |
| 7   | p-Cymene                   | 1025| Tr         | Tr         | Tr         |
| 8   | Limonene                   | 1029| 0.5±0.0    | 0.7±0.0    | 0.4±0.0    |
| 9   | Z-β-Ocimene                | 1035| Tr         | Tr         | Tr         |
| 10  | Linalool                   | 1085| Tr         | Tr         | Tr         |
| 11  | α-Campholenal              | 1103| Tr         | Tr         | Tr         |
| 12  | Trans-Pinocarveol          | 1125| Tr         | Tr         | Tr         |
| 13  | Trans-Verbenol             | 1162| Tr         | 0.2±0.0    | Tr         |
| 14  | Borneol                    | 1186| Tr         | Tr         | Tr         |
| 15  | Terpine-4-ol               | 1203| 0.3±0.0    | 0.5±0.0    | Tr         |
| 16  | Myrtenal                   | 1216| 0.3±0.0    | 0.5±0.0    | Tr         |
| 17  | Verbenone                  | 1239| 0.2±0.0    | 0.2±0.0    | Tr         |
| 18  | Bornyl-acetate             | 1316| Tr         | Tr         | Tr         |
| 19  | Eugenol                    | 1340| 0.6±0.0    | Tr         | 1.5±0.2    |
| 20  | β-Caryophyllene            | 1426| Tr         | 2.6±0.0    | Tr         |
| 21  | Allo-Aromadendrene         | 1482| 20.6±0.2   | 16.3±0.2   | 21.7±0.2   |
| 22  | γ-Muurolene                | 1485| 0.3±0.0    | 0.5±0.0    | Tr         |
| 23  | Germacrene D               | 1498| 7.6±0.2    | 6.3±0.2    | 8.3±0.3    |
| 24  | Bicyclogermacrene          | 1505| 14.5±0.0   | 14.3±0.2   | 12.4±0.1   |
| 25  | Eugenol-acetate            | 1521| Tr         | 0.9±0.0    | Tr         |
| 26  | Spathulenol                | 1575| 22.4±0.2   | 12.6±0.1   | 25.4±0.2   |
| 27  | Caryophyllene oxide        | 1580| Tr         | Tr         | Tr         |
| 28  | Sclareol                   | 2200| 0.7±0.0    | Tr         | 0.4±0.0    |

- Monoterpene hydrocarbons: 31.8, 41.6, 29.3
- Oxygenated monoterpenes: 1.4, 1.4, 1.5
- Sesquiterpene hydrocarbons: 43.0, 40.0, 42.4
- Oxygenated sesquiterpenes: 23.1, 13.5, 25.8
- Total: 99.3, 96.5, 99.0
Data are presented as mean±SD; RI indicates retention indices relative to C_6-C_{24} n-alkanes; Tr indicates trace

4. Conclusion

The data presented in this paper confirmed that the essential oil yield and constituents of *S. limbata* were highly influenced by phenology and altitude. A significant difference in the composition of *S. limbata* essential oil at different growing stages and altitudes was observed. It is noteworthy that the dominant compounds of *S. limbata* essential oil were α-pinene, β-pinene, allo-aromadendrene, germacrene-D, bicyclogermacrene, and spathulenol. Since higher yield of essential oil was obtained in vegetative stage at 1500 m, this stage could be considered as the best stage to harvest plant. Moreover, it was revealed that the highest content of monoterpenes and sesquiterpenes could be obtained at the vegetative stage and ripening stage, respectively. This was the first research on the yield and constituents of *S. limbata* essential oil at different developmental stages and altitudes that could be economically beneficial for the food and pharmaceutical industries as well as other researchers to examine further investigations about this plant.

Declarations

Ethic approval: Not applicable

Consent for publication: Yes

Availability of data and materials: The form of material is in Excel file. The location was in Taleghan rangeland.

Competing interests: No

Funding: No

Author's contribution: Hossein Azarnivand devised the project, the main conceptual ideas and proof outline. Dr Mohammad Ali Zare Chahouki and Dr Maryam Saffariha worked out almost all of the technical details, and performed the numerical calculations for the suggested experimented Ali Tavili and Dr Samad Nejad Ebrahimi verified the numerical results. Pr. Daniel Potter aided in interpreting the results and worked on the manuscript. Dr. Reza Jahani helped in analysing the data. All authors discussed the results and commented on the manuscript.

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References

1. Jash SK, Gorai D, Roy R (2016) Salvia genus and triterpenoids. International Journal Of Pharmaceutical Sciences And Research. 7(12):4710.

2. Jassbi AR, Asadollahi M, Masroor M, Schuman MC, Mehdizadeh Z, Soleimani M (2012) Chemical classification of the essential oils of the Iranian Salvia species in comparison with their botanical taxonomy. Chemistry & biodiversity. 9(7):1254-71.

3. Salehi P, Sonboli A, Dayeni M, Eftekhar F, Yousefzadi M (2008) Chemical composition of essential oils of *Salvia limbata* from two different regions in Iran and their biological activities. Chemistry of Natural Compounds. 44(1):102-5.

4. Raal A, Orav A, Arak E (2007) Composition of the essential oil of *Salvia officinalis* L. from various European countries. Natural product research. 21(5):406-11

5. Kaya A, Goger F, Baser KHC (2007) Morphological, anatomical and palynological characteristics of *Salvia halophila* endemic to Turkey. Nordic Journal of Botany. 25(5-6):351-8.
6. Alimpić A, Oaldje M, Matevski V, Marin P, Duletić-Laušević S (2014) Antioxidant activity and total phenolic and flavonoid contents of Salvia amplexicaulis Lam. extracts. Archives of Biological Sciences. 66(1):307-16.

7. Abu-Darwish M, Cabral C, Ferreira I, Gonçalves M, Cavaleiro C, Cruz M (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.

8. Damyanova S, Mollova S, Stoyanova A, Gubenia O (2016) Chemical composition of Salvia officinalis L. essential oil from Bulgaria. Ukrainian food journal. 5 (4):695-700.

9. Kintzios SE (2000) Sage: the genus Salvia: CRC Press.

10. Perry NS, Bollen C, Perry EK, Ballard C (2003). Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacology biochemistry and behavior. 75(3):651-9.

11. Walker JB, Sytsma KJ (2007). Staminal evolution in the genus Salvia (Lamiaceae): molecular phylogenetic evidence for multiple origins of the staminal lever. Annals of Botany. 100(2):375-91.

12. Kelen M, Tepe B.(2008). Chemical composition, antioxidant and antimicrobial properties of the essential oils of three Salvia species from Turkish flora. Bioresource technology. 99(10):4096-104.

13. Cardile V, Russo A, Formisano C, Rigano D, Senatore F, Arnold NA. (2009). Essential oils of Salvia bracteata and Salvia rubifolia from Lebanon: Chemical composition, antimicrobial activity and inhibitory effect on human melanoma cells. Journal of Ethnopharmacology. 126(2):265-72.

14. Akin M, Demirci B, Bagci Y, Baser KHC. (2010). Antibacterial activity and composition of the essential oils of two endemic Salvia sp. from Turkey. African Journal of Biotechnology. 9(15):2322-7.

15. Karatas H.(2010).Antimicrobial activities of the essential oils of four Salvia species from Turkey. Journal of Medicinal Plants Research. 4(12):1238-40.

16. Orhan IE, Senol FS, Ercetin T, Kahraman A, Celep F, Akaydin G.(2013). Assessment of anticholinesterase and antioxidant properties of selected sage (Salvia) species with their total phenol and flavonoid contents. Industrial Crops and Products. 41:21-30.

17. Esmaeili M, Kananim, Sonbolia, Sadeghi H, Karimianpour N.(2010). Evaluation of the effect of Salvia sahendica on tissue damages induced by alcohol in oxidative stress conditions in the rat: Effect on liver and kidney oxidative parameters.
26. Jahani, A, Saffariha, M. (2020). Human Activities Impact Prediction in Vegetation Diversity of Lar National Park in Iran Using Artificial Neural Network Model. Integrated Environmental Assessment and Management. https://doi.org/10.1002/ieam.4349.

27. Jahani, A., Rayegani, B. (2020). Forest Landscape Visual Quality Evaluation Using Artificial Intelligence Techniques As A Decision Support System. Stochastic Environmental Research and Risk Assessment. 34(10), 1473-1486. https://doi.org/10.1007/s00477-020-01832-x

28. Jahani, A., Goshtasb, H., Saffariha, M. (2020). Tourism impact assessment modeling in vegetation density of protected areas using data mining techniques, Land Degradation & Development 31(12): 1502-1519.

29. Zebib B, Beyrouthy ME, Sa C, Merah O.(2015). Chemical composition of the essential oil of Satureja myrtifolia (Boiss. & Hohen.) from Lebanon. Journal of Essential Oil Bearing Plants. 18(1):248-54.

30. Aldarkazali M, Rihan HZ, Came D, Fuller MP. (2019). The growth and development of sweet basil (Ocimum basilicum) and bush basil (Ocimum minimum) grown under three light regimes in a controlled environment. Agronomy. 9(11):743.

31. Roche J, Mouloungui Z, Cerny M, Merah O.(2019). Effect of Sowing Dates on Fatty Acids and Phytosterols Patterns of Carthamus tinctorius L. Applied Sciences. 9(14):2839.

32. Sarmoum R, Haid S, Biche M, Djazouli Z, Zebib B, Merah O.(2019). Effect of salinity and water stress on the essential oil components of rosemary (Rosmarinus officinalis L.). Agronomy. 9(5):214.

33. Dixon RA, Paiva NL(1995) Stress-induced phenylpropanoid metabolism. The plant cell. 7(7):1085.

34. Al-Asmari AK, Athar MT, Al-Faraidy AA, Almuhaiza MS. (2017). Chemical composition of essential oil of Thymus vulgaris collected from Saudi Arabian market. Asian Pacific Journal of Tropical Biomedicine. 7(2):147-50.

35. Fonseca LM, dos Santos Cruxen CE, Bruni GP, Fiorentini ÂM, da Rosa Zavareze E, Lim L-T.(2019). Development of antimicrobial and antioxidant electrospun soluble potato starch nanofibers loaded with carvacrol. International journal of biological macromolecules.139:1182-90.
46. Özcan MM, Chalchat JC. (2006). Effect of collection time on chemical composition of the essential oil of *Foeniculum vulgare* subsp. piperitum growing wild in Turkey. European Food Research and Technology. 224(2):279-81.

47. Rustaiyan A, Akhgar MR, Masoudi S, Nematollahi F. (2005). Chemical Composition of Essential Oils of Three *Salvia* Species Growing Wild in Iran: *Salvia rhytidea* Benth., *S. limbata* CA Mey and *S. palaestina* Benth. Journal of Essential Oil Research. 17(5):522-4.

48. Kürkçüoğlu M, Demirci B, Baser K, Dirmenci T, Tümen G, Özgen U. (2005). The essential oil of *Salvia limbata* CA Meyer growing in Turkey. Journal of Essential Oil Research. 17(2):192-3.

49. Behtari B, Gholami F, Khalid KA, Tilaki GD, Bahari R. (2012). Effect of growth stages and altitude on *Artemisia herba-alba* Asso essential oil growing in Iran. Journal of Essential Oil Bearing Plants. 15(2):307-13.

50. Abdollahi M, Sefidkon F, Calagari M, Mousavi A, Mahomoodally MF. (2020). Impact of four hemp (*Cannabis sativa* L.) varieties and stage of plant growth on yield and composition of essential oils. Industrial Crops and Products. 155:112793.

51. Hudaib M, Speroni E, Di Pietra AM, Cavrini V. (2002). GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. Journal of pharmaceutical and biomedical analysis. 29(4):691-700.