PERIÓDICO TCHÊ QUÍMICA
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ESTUDO SOBRE COMPOSIÇÕES DE ÓLEO ESSENCIAL DE SALVIA (*SALVIA NEMOROSA L.*) COLHIDA DO NOROESTE DO IRÃ EM DIFERENTES ESTÁGIOS DE CRESCIMENTO

STUDY ON ESSENTIAL OIL COMPOSITIONS OF SAGE (*SALVIA NEMOROSA L.*) COLLECTED FROM THE NORTH WEST OF IRAN AT DIFFERENT GROWTH STAGES

مطالعه ترکیبات روغن‌های فرار مریمگلی (*SALVIA NEMOROSA* L.) جمع‌آوری شده از شمال غرب ایران در مراحل مختلف رشد

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RESUMO

Salvia nemorosa L. ou sálvia é uma fonte rica de metabólitos antimicrobianos e antioxidantes. Considerando a importância dessa planta medicinal e da diversidade fitoquímica entre suas populações para o consumo local e os adubos de reprodução, este estudo foi realizado para determinar e comparar a composição de óleo essencial (OE) das plantas sálvia coletadas em quatro regiões do noroeste do Irã, incluindo Ahar, Zonouz, Urmia e Ardabil em dois estágios de crescimento (vegetativo e florido) para finalmente demonstrar os efeitos do crescimento e localização nas características da OE. Os resultados mostraram que o teor de OE das flores nas regiões estudadas foi o mais alto em comparação com as fases vegetativa e florística. A porcentagem e o número de composições voláteis no OE de flores foram as que apresentaram a maior quantidade. Em Zonouz, 87,13% e 12 composições enquanto em Aahar, 80,20% e 19 composições. A menor porcentagem e o número de composições voláteis no OE da flor foi visto em Urmia, 78,56% e 13 composições enquanto em Ardabil, esses números foram 68,61% e 10 composições, respectivamente. O óxido de cariofileno teve o maior teor em todos os óleos essenciais extraídos, sendo o mais alto no estágio de floração das regiões de Zonouz, Ardabil e Urmia, respectivamente. Em Ahar, diferentemente de outras regiões, a maior porcentagem desse composto estava nas folhas da fase vegetativa. Os sesquiterpenos oxigenados aumentaram o teor de OE nas flores das regiões Urmia (46,31%), Zonouz 42,59% e Ardabil (45,60%). Em contraste com outras regiões, para a região de Ahar, a maior quantidade (36,18%) de sesquiterpenos oxigenados foi observada no OE das folhas do estágio de floração. Pode-se concluir que diferentes estágios de crescimento das plantas, época de colheita das plantas, condições ambientais, habitat principal e diferenças nas condições climáticas podem contribuir na concentração, tipo e porcentagem de compostos voláteis no OE da sálvia.

Palavras-chave: *Salvia nemorosa, Metabólitos secundários, Óleos essenciais, Óxido de cariofileno, Sesquiterpenos*

ABSTRACT

Salvia nemorosa L. or wood sage is a rich source of antimicrobial and antioxidant metabolites. Considering the importance of this medicinal plant and phytochemical diversity among its populations for local consumption and breeding porpuses this study was performed to determine and compare essential oil (EO) compositions of sage plants collected from four regions in the northwest of Iran including Ahar, Zonouz, Urmia, and Ardabil at two growth stages (vegetative and flowering) to finally demonstrate the effects of growth and location on EO features. The results showed the EO content of flowers in the studied regions were the highest in comparison with vegetative and flowering stages leaves. The percentage and the number of volatile compositions in the OE of flowers were those that presented the highest quantity. In Zonouz, 87.13% and 12 compositions while in Aahar,

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80.20%, and 19 compositions. The lowest percentage and the number of volatile compositions in the OE of the flowers were seen in Urmia, 78.56%, and 13 compositions, while in Ardabil, these numbers were 68.61% and 10 compositions, respectively. Caryophyllene oxide had the highest content in all essential oils extracted, being the highest in the flowering stage leaves of the regions of Zonouz, Ardabil, and Urmia, respectively. In Ahar, unlike other areas, the most significant percentage of this compound was in the leaves of the vegetative stage. The oxygenated sesquiterpenes increased in the EO content of the flowers of the Urmia (46.31%), Ardabil (45.60%) and Zonouz (42.59%) regions. In contrast to other areas, for the Ahar region, the highest amount (36.18%) of oxygenated sesquiterpenes was observed in the EO of the leaves of the flowering stage. It can be concluded that different plant growth stages, plant harvest time, environmental conditions, primary habitat, and differences in climatic conditions can contribute to the concentration, type, and percentage of volatile compounds in the *salvia* EO.

Keywords: *Salvia nemorosa, Secondary metabolites, Essential oils, Caryophyllene oxide, Sesquiterpenes*

چکیده

Salvia nemorosa L. یا مریم‌گلی چوبی دارای فعالیت‌های ضدمیکروبی، آنتی‌اکسیدانی و منبعی غنی از متابولیت‌های فعال می‌باشند. با در نظر داشتن اهمیت این گیاه دارویی برای مصرف کننده‌گان محلی و اهداف اصلاحی این بررسی در راستای تعیین و مقایسه ترکیبات اسانس در گیاهان مریم گلی جمع‌آوری شده در چهار منطقه مختلف شامل زنوز، ارومیه، اردبیل و اهر و در دو مرحله رشد، رویشی و گل‌دهی صورت گرفت تا نهایتا اثر مرحله رشد و مناطق رشد روی اجزای روغن‌های فرار تعیین گردد. نتایج نشان داد که درصد اسانس سرشاخه‌های گلدار در هر چهار منطقه مورد مطالعه در مقایسه با برگ‌های مراحل رویشی و گل‌دهی از بالاترین میزان برخوردار بودند. بیشترین درصد و تعداد ترکیبات فرار روغن‌های فرار در اندام زایشی مشاهده گردید. با 87.13% و 12 ترکیب در منطقه زنوز، 80/20% و 19 ترکیب در منطقه اهر بالاترین میزان ترکیباب برخوردار بودند. کم‌ترین میزان درصد اسانس با 78.56% و 13 ترکیب در منطقه ارومیه و با 68.61% و 10 ترکیب در منطقه اردبیل مشاهده شد. کاریوفیلن اکساید بالاترین میزان ترکیبات فرار شناسایی شده به ترتیب در اسانس گل‌ها، برگ‌های مراحل رویشی و گل‌دهی در هر چهار منطقه بود. بالاترین درصد کریوفیلن اکساید در برگ مرحله گل‌دهی هر سه منطقه زنوز، اردبیل و ارومیه دیده شد. از سوی دیگر بالاترین درصد این ترکیب در منطقه اهر برخلاف سایر مناطق در برگ مرحله رویشی بود. سسکوئی‌ترپن‌های اکسیژن‌دار در اسانس گل های مناطق ارومیه (46.31%) ، اردبیل (45.60%) و زنوز (42.59%) افزایش داشتند. در منطقه اهر بر خلاف سایر مناطق، بالاترین میزان سسکوئی‌ترپن‌های اکسیژن‌دار در اسانس برگ مرحله گل‌دهی (36.18%) مشاهده شد. می‌توان این‌گونه نتیجه گرفته که مراحل مختلف رشد گیاه، زمان برداشت گیاه، تاثیر شرایط‌های محیطی، زیستگاه اصلی و اختلاف در شرایط اقلیمی می‌توانند بر نوع و درصد ترکیبات فرار اسانس گیاهان مریم گلی تاثیر داشته باشند.

کلمات کلیدی: *Salvia nemorosa*، متابولیت‌های ثانویه، روغن‌های فرار، کاریوفیلن اکسید، سسکویی ترپن‌ها

1. INTRODUCTION:

Salvia L. is the largest genus of Lamiaceae (tribe Mentheae) with nearly 1000 species (Walker and Sytsma, 2007, Sarac and Ugur, 2007). The genus Salvia is derived from the Latin word “Salvare” meaning “to heal” or “to be safe and unharmed,” referring to the medicinal properties of the genus (Walker *et al*., 2004, Giuliani and Maleci Bini, 2008). The members of the genus display a remarkable diversity in growth form, floral morphology, pollination biology, and secondary compounds; they are distributed all around the world (Walker *et al*., 2004,). The genus Salvia is represented in Iran by 58 species, 17 of which are endemic (Mozaffarian, 1996, Ipek, 2012).

Many species of Salvia are used in traditional medicine throughout the world. In Iranian folk medicine, several Salvia species have been used as antiseptic for wounds, as a diuretic, stomach tonic, antiflatulent, and constituent, and for the treatment of eye disorders, diarrhea, dyspepsia, fever, rheumatism, excessive menstruation, common cold, coughing, pertussis, and sinusitis (Naghibi, 2005, Ozkan, 2008, Raal *et al*., 2007). *Salvia nemorosa* L., commonly known as wood sage, is growing in central Europe and Western Asia (Skala and Wysokinska, 2004).

Leaves of *S. nemorosa* are traditionally used in Turkish folkloric medicine for stop bleeding when applied externally (Takeda *et al*., 1997; Topcu, 2006). Also, in the Bulgarian traditional medicine, *S. nemorosa* is used mainly for the treatment of stomach ache, diarrhea, hemorrhages, and furuncles (Daskalova, 2004, Farimani *et al*., 2013). In Russia, *S. nemorosa* is utilized for much the same ailments as *S. officinalis.* Studies have shown that *S. nemorosa* is a rich source of bioactive metabolites (flavonoids and oxygenated sesquiterpenes). Also, *S. nemorosa* exhibited considerable antibacterial, antioxidant, and enzyme inhibitory activities. In conclusion, *S. nemorosa* is a valuable source of natural products and could be used for preparing novel functional foods, cosmetics, and pharmaceutical ingredients (Bahadori, 2017, Bahadori and Mirzaei, 2015, Wu *et al*., 2012). In studies conducted by Coisin *et al* (2010) in the volatile oil extracted from *Salvia nemorosa* L. in plant development stage, 22 compounds have been identified. The main chemical compounds belong to monoterpenes group with having antiseptic (bactericide), carminative, antispastic and diuretic properties such as: sabinene (33.93%) ß-cariophyllene (19.86%), ß-cubebene (11.87%), a-cariophyllene (6.37%) and other compounds included cariophyllene oxid, γ-terpinene, Limonene, a-tujone, ß-mircene, ß-pinene, γ-terpinene, cimol, elixene, terpinolene, ß-burbonene, alloaromadendrene oxid (1) γ-cadinene , alloaromadendrene oxid (2) a-pinene r-cadinen , pulegone, a-terpinil-acetate (Coisin *et al*, 2010).

Also, a study on *S. nemorosa* showed that the chemical compositions in leaves and flowers essential oils were sesquiterpenes hydrocarbons, oxygenated sesquiterpenes, and monoterpene hydrocarbons in the highest amount and oxygenated monoterpenes, aliphatic compounds, monoterpene hydrocarbons, sabinene, and limonene were in the lowest amount. In addition, phenolic compounds of salvia nemorosa are gallic acid, protocatechuic acid catechin, p-hydroxybenzoic acid, caffeic acid, epicatechin, vanillin, p-coumaric acid, ferulic acid, sinapic acid, benzoic acid, O-coumaric acid, rutin, naringin, hesperidin, rosmarinic acid, trans-cinnamic acid, quercetin, luteolin, kaempferol, apigenin (Bahadori *et al*, 2017).

The aim of this study was to evaluate the composition and comparison of *Salvia nemorosa* essential oils of plants collected plants in four different regions (northwest of Iran) and at two growth stages. This study will allow the realization of two key scientific points in which one is determining the best time of harvest with aromatic properties (quality) or optimal therapeutic properties and the most useful plant origin on its phytochemical compositions. This may help increase the economic efficiency of the production of essential oils and the acquisition of new scientific data on the change in secondary volatile metabolites of sage plants during its phenological cycle that can be utilized by local consumers as well as medicinal plant breeders.

2. MATERIALS AND METHODS:

Sage plants (*salvia nemorosa* L.) were collected at two vegetative and flowering stages from four regions in the northwest of Iran including Ahar (75 km from Tabriz to Ahar) (longitude: 38.26 ° E' , latitude: 47.04° N and altitude:1391m) and Zonouz gardens (longitude: 38.26° E' , latitude: 45.46° N and altitude:1550 m) in East Azarbaijan, Urmia, Qasemlu village (longitude: 37.65 ° E', latitude: 47.05° N and altitude:1328m) in western Azarbaijan and Ardabil, Abbasabad village (85 km from khalkhal to Ardabil) (longitude: 38.13° E' , latitude: 48.19° N and altitude:1335 m) in Ardabil province (Figures 1, 2 and Table 3). Plant collection times at the vegetative stage were May 12th to May 26th, 2017, and collection times at the flowering stage were May 19th to June 5th, 2017. After collecting the plants, they were dried at room temperate.

2.1. Extraction and analysis of essential oils by GC-MS

After collecting plants at two growth stages (vegetative and flowering) from different regions and after drying them in the shade, essential oils extracted by water distillation using the Clevenger apparatus and then analysis by GC-MS. Therefore, flowers and leaves of plants (vegetative and flowering stages) underwent distillation using Clevenger apparatus for 4 hours. Then, essential oils were kept in the refrigerator at the 4°C and when analyzing they were separated from water and dried over anhydrous sodium sulfate and for identification of essential oils composition was injected to GC-MS (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 3°.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 were obtained and the mass spectra of various compounds were examined. Then, the spectra were identified and studied. The individual compounds were identified by MS and their identity was confirmed by comparing their retention indices relatives to C8 –C32 n-alkanes and by comparing their mass spectra and retention times with those of authentic samples or with data already available in the NIST library and literature Adams (Adams, 2017).

Identification of GC/MS chromatogram spectra was performed using mass database (Adams, 2017), inhibition index, study of mass spectra of each essential oil component and comparison with standard spectra and the Quatts formula was used to calculate the inhibition index.

3. RESULTS AND DISCUSSION:

The results of the composition of sage (*Salvia nemorosa* L.) essential oils in two phenological stages of vegetative and flowering stages showed that in each regions (Ahar, Zonouz, Ardabil and Urmia) separately, essential oils of sage flowers had the highest percentage of components in comprising with essential oils of sage vegetative stage leaves and flowering stage leaves, in which according to Figure 3, the essential oil of *S. nemorosa* flowers had the highest percentage in comparison with the essential oils of vegetative stage leaves and flowering stage leaves in Zonouz, Ahar, Urmia and Ardabil regions so that, the essential oil of sage flowers in Zonouz region had the highest percentage and in Ardabil region showed the lowest percentage. In addition, by comparing the number, type and percentage of identified volatile compound in the essential oil of *S. nemorosa* flowers in every four regions was seen that, the essential oils of flowers of Zounoz region with 12 compounds had the highest of the percentage of essential oils (87.13%) comprising of the essential oils of flowers in other regions while, in the essential oils of flowers of Ardabil region, there were 10 compounds with 68.61% of total oils. In the essential oils of flowers of Ahar region 20 compounds with 80.20% of total oils and in the essential oils of flowers of Urmia region 14 compounds were identified with 78.56% of total oils (Tables 1 and 2; Figure 5 a, b, c, d).

The essential oil of vegetative stage leaves in Urmia and Ahar regions had the lowest percentage and the essential oil of vegetative stage leaves in Ardabil and Zonouz regions increased in comparison with flowering stage leaves (Tables 1 and 2; Figures 3 and 7 a, b, c, d).

Among identified volatile compounds in the essential oils of flowers in Ahar, Zouoz, Urmia and Ardabil regions, respectively, caryophyllene oxide, spathulenol and trans caryophyllene were the main compounds of essential oils. In addition to, compounds such as para cymene, sabinene, terpinen-4-ol, were another main compounds in the essential oils of flowers in Ahar region, para cymene, 1- octen-3-ol were another main compounds in the essential oils of flowers in Urmia region, terpinen-4-ol, camphor and para cymene were another main compounds in the essential oils of flowers in Zounoz region and 1-octen-3-ol was another main compound in the essential oils of flowers in Ardabil region (Table 1 and 2).

In the study of Kashef *et al* (2019) on growth compatibility and medicinal potential of four *Salvia* species in Semnan climatic conditions, was observed that, in *S. nemorosa* species in seedling stage, caryophyllen (43.91%), caryophyllen oxide (19.65%) and farnesene (12.13%), in meddle of growth seasen, trans-caryophyllen (11.93%), caryophyllen oxide (37.89%) and trans- β farnesene (11.49%) and in the end of growth seasen, trans- caryophyllen (28.94%), caryophyllen (30.13%) and α- cadinol (5.69%) were the main of composition. In *S. sclarea* and *S. officinalis* plants in seedling stage caryophyllen, caryophyllen oxide, germacrene D, in middle of growth seasen caryophyllen, caryophyllen oxide, trans-β farnesene and β- bourdonen, in the end of growth seasen caryophyllen oxide, β farnesene and germacrene D with different percentage were the main of composition (Kashef *et al*, 2019).

In the essential oils of flowering stage leaves in Ahar region 14 volatile compounds with 69.18% of total essential oils, and in Zounoz region only one volatile compound was identified with 8.48% of total essential oils. Also, In the essential oils of flowering stage leaves in the two regions of Urmia and Ardabil, 7 and 4 of volatile compounds, respectively, were identified with almost 58.89% of total oils (Tables 1 and 2; Figure 6 a, b, c, d).

Among the identified volatile compounds in the essential oils of flowering stage leaves in each four studied regions, caryophyllene oxide then Beta- ionone had the highest percentage so that, the highest amount of caryophyllene oxide was seen with 39.40% in the essential oils of flowering stage leaves in Ardabil region and the lowest amount of caryophyllene oxide was seen with 8.48% in the essential oils of flowering stage leaves in Zounoz region. In addition to, 1-octen-3-ol and camphor in the essential oils of *S. nemorosa* L. in Ardabil and Urmia regions and camphor, trans caryophyllene ,1-Octen-3-ol in the essential oil of sages plants in Ahar region were main compound (Table 1 and 2).

Extraction and identification of essential oils volatile compounds showed existence of 10 volatile compounds with 55.54% and 63.89% of total essential oils in vegetative stage leaves of sage collected from Ahar and Ardabil regions. While, essential oil of vegetative stage leaves in Urmia region showed only two volatile compounds with 24.62% of total essential oils. In addition to, essential oils of vegetative stage leaves in Zonouz region showed three volatile compounds with 41.29% of total essential oils. Among identified volatile compounds caryophyllen oxide showed the highest percentage in essential oils of vegetative stage leaves each four regions (Ahar, Zonouz, Urmia and Ardabil). So that, the highest percentage of caryophyllen oxide was in essential oil of vegetative stage leaves in Ardabil region (31.35%) and the lowest percentage caryophyllen oxide was in essential oils of vegetative stage leaves in Urmia (18.19%). In addition, trans-beta caryophyllen was part of main compound in essential oils of vegetative stage leaves in Ahar, Ardabil and Zonouz regions (Table 1 and 2).

It was reported that existence of compounds such as caryophyllen oxide, beta-caryophyllen, germacrene D and germacrene B were the main compounds of essential oils in *S. nemorosa* and *S. virgata* harvested from Iran (Sefidkon and Mirza,1999).

By comparing of chemical groups in essential oils of flowers, vegetative stage leaves and flowering stage leaves in all regions (Ahar, Zonouz, Urmia and Ardabil) was observed that oxigenated sesquiterpenes in essential oils were the highest percentage of chemical groups (Figure 4). So that, the highest percentage of oxigeneted sesquiterpenes were with 46.31% in essential oils of flowers of sage plants in Urmia region and the lowest percentage of oxigeneted sesquiterpens were seen with 25.60% in essential oils of sage plants in Ahar region (Table 1 and 2, Figure 4).

In essential oils of flowering stage leaves, the highest amount oxygenated sesquiterpenes were with 44.05% in essential oils of sage plants in Ardabil region and the lowest amount this chemical groups were observed with 8.48% in Zonouz region. Also, oxygenated sesquiterpenes showed the highest amount with 37.21% and 34.3%, respectively, in essential oils of vegetative stage leaves in Ardabil and Ahar regions and showed lowest amount with 18.19% in essential oils of vegetative stage leaves in sage plants in Urmia region (Table 1 and 2, Figure 4).

In a study on the composition of essential oils of some wild salvia species growing in Serbia was observed that in the essential oil of *S. nemorosa* flowers, caryophyllen oxide, beta-caryophyllen, sabinene and germacrene D and in the essential oil of *S. glutinosa* flowers, caryophyllen oxide, spathulenol, humulene epoxide were major compositions. In addition, in the essential oils of *S. nemorosa* and *S. glutinosa* plants, sesquiterpenes were the main chemical groups (Chalchat, 2004).

In Yayli *et al* (2010) study on constituents of the essential oil from the flower, leaf and stem of *Salvia viridis* L. grown in Turkey, sesquiterpenes hydrocarbons were the main constituents in organs and the major components in the essential oils of leaf were ß-pinene, ß-copaene , trans-muurola-4(14),5-dien which, showed significant difference in the type and percentage of identified compound of *S. nemorosa* essential oil in this study (Yayli *et al*., 2010).

Comparing the identified compounds in this study with other reports shows the similarities and differences in terms of the type and amount of identified composition. These differences may be due to a variety of reasons, including the time of harvested and the ecological conditions of the sage plants, which can affect the amount and type of compound and it could be due to the collection of *S. nemorosa* plants from different natural environments, which can be said that the results obtained are due to the interaction of genetic factors and the environment (Masksimovic *et al*., 2007; Figueiredo *et al*., 2008).

The quantity of active ingredients in medicinal plants is mainly affected by natural variables in the environment. Although the amount 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 (Ebrahimpour *et al*., 2009, Figueriredo *et al*., 2008, Russo *et al*., 2013).

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 (Bakhshi *et al*., 2010, Cheynier *et al*., 2013, Maksimovic *et al*., 2007). 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 characteristics between them (Omidbeigi, 2009, Li *et al*., 2015, Ben farhad, 2009). Furthermore, edaphic and climatic variations and genetic characteristics may have a strong influence on the morphological, agronomic and essential oil chemical characteristics (Mossi *et al*., 2011, Papageorgiou *et al*., 2008).

As a result, in this study, different stage of growth of *S. nemorosa* plants, harvest times, different in climatic condition, major habitat and direct relation between ecological and genetic factors have led to differences in the type and percentage of identified composition in the essential oils of sage plants in Ahar, Ardabil, Zonouz and Urmia regions.

4. CONCLUSIONS:

On the basis of all the analyses, it could be concluded that oxygenated sesquiterpenes were the highest percentage of compounds identified in the essential oils of flowers, vegetative stage leaves, flowering stage leaves in four regions. Also, Sesquiterpene hydrocarbons, oxygenated monoterpenes, and monoterpene hydrocarbons there were in a lower percentage. The main oxygenated sesquiterpene was caryophyllene oxide in essential oils. The most number and the highest rate of the volatile compound identified was seen in essential oils of *S. nemorosa* flowers collected from Zonouz region. It confirms the influence of environmental conditions (harvested time, original habitat and differences in climatic conditions) on the nature of plant chemical composition that has the vital role in plant adaptation and speciation.

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***Table 1**. Comparision of flowers, vegetative stage leaves, flowering stage leaves essential oils of S.memorosa collected from Ahar and Ardabil regions*

Components	Ahar						Ardabil					
	Flowers		Flowering stage leaf		Vegetative stage leaf		Flowers		Flowering stage leaf		Vegetative stage leaf	
	KI	%	KI	%	KI	%	KI	%	KI	%	KI	%
Heptane	-	-	-	-	-	-	701	1.57	700	2.58	-	-
α-Thujene	930.1	5.02	-	-	-	-	929.3	1.30	-	-	-	-
α -Pinene	939.9	0.52	-	-	-	-	-	-	-	-	-	-
1-Octen-3-ol	967.6	4.43	968.4	5.94	965.6	1.13	965.2	8.57	964.3	9.10	967.2	11.89
Sabinene	975.3	5.66	-	-	-	-	973.7	1.34	-	-	-	-
β-pinene	982.7	1.04	-	-	-	-	-	-	-	-	-	-
Benzene 1-metri-2	-	- -	1020.1	1.40	-	-	1019.2	2.44	-	-	-	-
1-methylethyl) Para-cymene	1020.9	8.12	-	-	-	-	-	-	-	-	1019.6	0.92
γ-terpinene	1056.9	3.28	-	-	-	-	-	-	-	-	-	-
Cis-sabinene hydrate	1065.4	3.21	-	-	-	-	-	-	-	-	-	-
3,5-heptadien-2-one-6-methyl	1082.8	0.52	-	-	-	-	-	-	-	-	-	-
α-terpinolene	1087	1.27	-	-	-	-	-	-	-	-	-	-
Linalool	-	-	1090.4	1.67	-	-	-	-	-	-	-	-
Trans-sabinene hydrate	-	-	-	-	-	-	-	-	-	-	-	-
α-thujone	-	-	1098.8	1.77	1097	0.86	-	-	-	-	-	-
β-thujone	1109.6	1.14	-	-	-	-	-	-	-	-	-	-
Camphor	1136.7	2.97	1138.5	4.88	1137	3.50	-	-	1135.8	3.16	1136.2	4.09
Terpinene-4.ol	1176.8	7.31	1175.9	1.44	1174	1.14	1174.1	1.76	-	-	1174.1	2.07
β-cyclocitral	-	-	1210.6	1.04	-	-	-	-	-	-	-	-
Hexadecanoic acid	-	-	-	-	1357	4.18	-	-	-	-	-	-
Trans-β-damasenone	-	-	1371.4	2.22	-	-	-	-	-	-	1369.6	1.02
Cis-caryophyllen	-	-	-	-	1417	1.46	-	-	-	-	-	-
Geranyl-aceton	-	-	1428.8	3.73	1434	1.82	-	-	-	-	1425.5	1.34
β-caryophyllene	1435.3	5.93	1436.4	6.29	1434	7.15	1433.2	6.03	-	-	1433.2	5.35
Allo-Aromadendrene	1453.8	0.59	1453.8	2.62	-	-	-	-	-	-	-	-
β-Ionone	-	-	1472.8	7.32	1469	5.95	1468.5	1.70	1468.5	4.65	1469.5	3.28
α-Humulene	1467.4	0.52	-	-	-	-	-	-	-	-	-	-
Spathulenol	1588.4	9.34	-	-	-	-	1582.5	10.83	-	-	-	-
Caryophyllen oxide	1596.6	12.7	1597.7	25.45	1593	28.37	1591.9	33.07	1589.5	39.40	1593	31.35
Caryophylla 4 (12),8(13)-dien-5-Betaol	-	-	1631.7	3.41	-	-	-	-	-	-	1629.1	2.58
Caryophyllenol	1625.7	3.47	-	-	-	-	-	-	-	-	-	-
Oxygenated sesquiterpene	25.6		36.18		34.3		45.6		44.05		37.21	
Sesquiterpene hydrocarbons	7.04		8191		8.61		6.03		-		5.35	
Oxygenated monoterpenes	17.7		16.75		7.32		1.76		3.16		8.52	
Monoterpene hydrocarbons	24.91		1.40		-		5.08		-		0.92	
Others	4.95		5.94		5.31		10.14		11.68		11.89	
All components	80.20		69.18		55.54		68.61		58.89		63.89	

KI: kovats Index on DB-5 in reference to n-alkanes.

Table 2. *Comparision of flowers, vegetative stage leaves, flowering stage leaves essential oils of S.memorosa collected from Urmia and Zonouz regions*

Components	Urmia						Zonouz					
	Flowers		Flowering stage leaf		Vegetative stage leaf		Flowers		Flowering stage leaf		Vegetative stage leaf	
	KI	%	KI	%	KI	%	KI	%	KI	%	KI	%
Heptane	-	-	701	2.47	-	-	-	-	-	-	-	-
Thujene	929.3	1.58	-	-	-	-	-	-	-	-	-	-
1-octen-3-ol	966.8	7.30	965.2	6.78	965.2	6.43	966	4.64	-	-	-	-
Sabinene	974.1	1.63	-	-	-	-	973.3	2.18	-	-	-	-
Para cymene	1019.2	4.48	-	-	-	-	1019.2	4.74	-	-	-	-
Gamma-terpinene	1056.1	1.63	-	-	-	-	1056.1	2.13	-	-	-	-
Cis-sabinene hydrate	1063.5	1.01	-	-	-	-	1063.7	2.49	-	-	-	-
Trans sabinene hydrate	-	-	-	-	-	-	1093.8	1.29	-	-	-	-
Camphor	1136.7	3.31	1136.2	4.41	-	-	1136.7	5.54	-	-	-	-
Terpinen-4-ol	1175	4.57	-	-	-	-	1174.1	7.05	-	-	-	-
Neryl-acetone	1425.5	0.92	1425.5	2.55	-	-	-	-	-	-	-	-
Cis-caryophyllen	-	-	-	-	-	-	1417.9	1.95	-	-	-	-
Trans-β-Damascenen	-	-	-	-	-	-	-	-	-	-	1369.6	2.29
Beta-Ionone	1467.9	1.59	1468.5	4.81	-	-	-	-	-	-	1468.5	4.20
Spathulenol	1588.4	14.84	-	-	-	-	1584.9	14.46	-	-	-	-
Caryophyllen oxide	1595.4	26.41	1590.7	33.54	1590.7	18.19	1594.2	28.13	1590.1	80.48	1589.5	26.30
Caryophylla 4(12)8(15)- dien	1630.01	3.47	1629.1	2.66	-	-	-	-	-	-	-	-
Oxygenated sesquiterpenes		46.31		41.01		18.19		42.59		8.48		30.50
Sesquiterpene hydrocarbons		5.82		2.41		-		14.48		-		80.50
Oxygenated monoterpenes		9.81		6.96		-		16.34		-		2.29
Monoterpene hydrocarbons components		9.32		-		-		9.05		-		-
Others		7.30		9.25		6.43		4.64		-		-
All components		78.56		59.63		24.62		87.13		8.48		41.29

KI: kovats Index on DB-5 in reference to n-alkanes.

***Table 3**: Geographical characteristics of S. nemorosa collection regions*

Collection regions	Longitude	Latitude	Altitude
Zonouz (EastAzarbaijan)	38.26ºE'	45.46ºN	1550m
Qasemlu village urmia (westernAzarbaijan)	37.65ºE'	47.05ºN	1328m
Ahar (East Azarbaijan)	38.26ºE'	47.04ºN	1391m
Abbasabad village Ardabil (Ardabil province)	38.13ºE'	48.19ºN	1335m

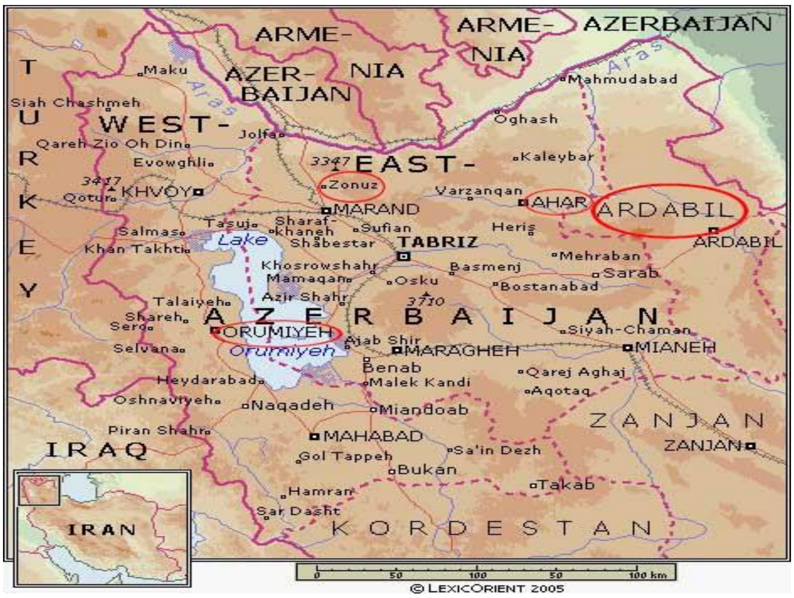


***Figure 1.** Map of the collection regions of s. nemorosa from the northwest of Iran (Zonouz, Ahar, Ardabil and Urmia)*

Figure 2. *Salvia nemorosa* L.

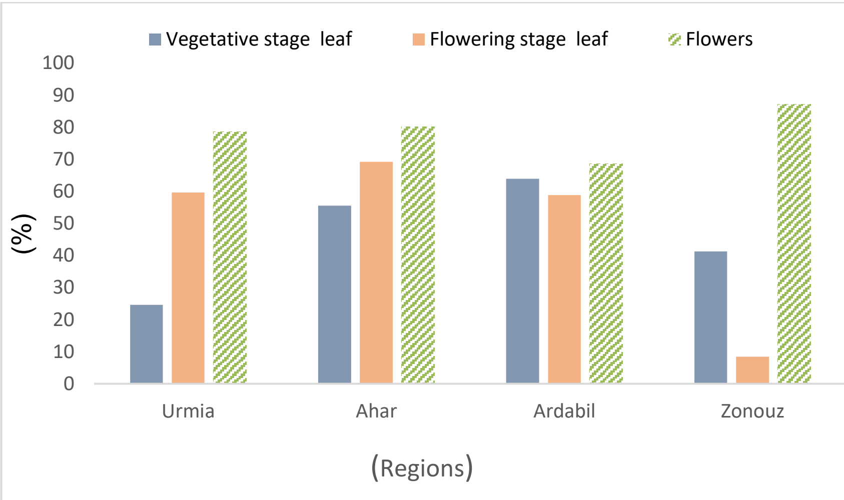


Figure 3. Comparison of the amount of essential oil compounds in *S. nemorosa* L. collected from four regions at different stages of growth.

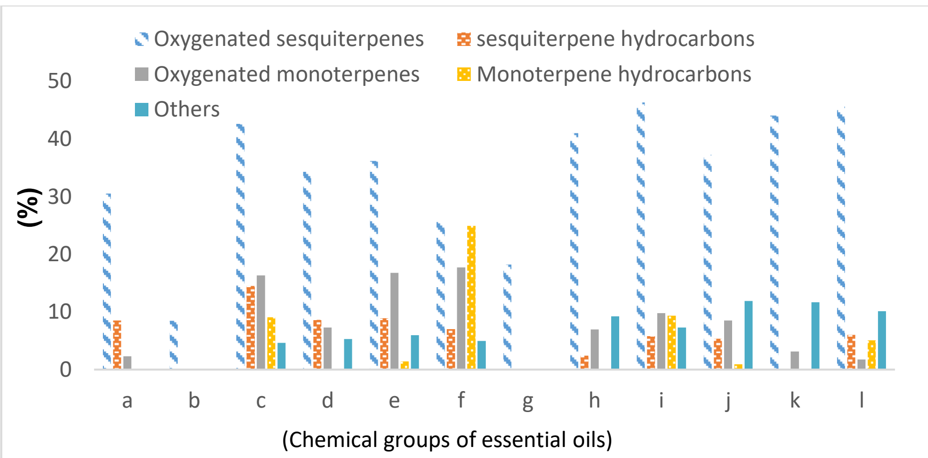


Figure 4. Comparison of chemical groups in the essential oil of *S. nemorosa* L. collected from four regions at different stages of growth

Note. a: Zonouz; vegetative stage leves, b: Zonouz; flowering stage leves, c: Zonouz; flowers,d: Ahar; vegetative stage leves, e: Ahar; flowering stage leves, f: Ahar; flowers, g: Urmia; vegetative stage leves, h: Urmia; floweing stage leves, i: Urmia; flowes, j: Ardabil; vegetative stage leves, k: Ardabil; flowering stage leves, l: Ardabil; flowers.

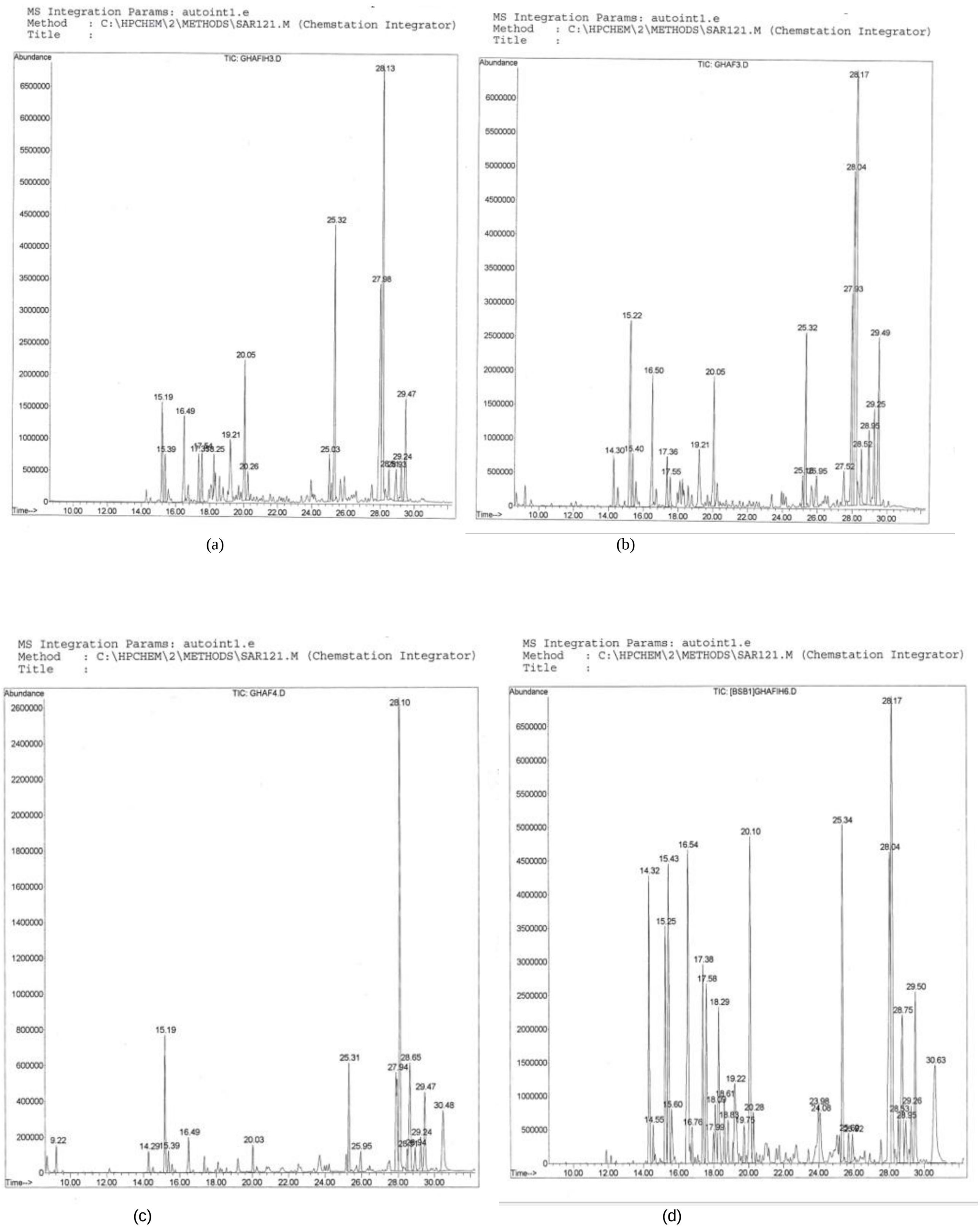
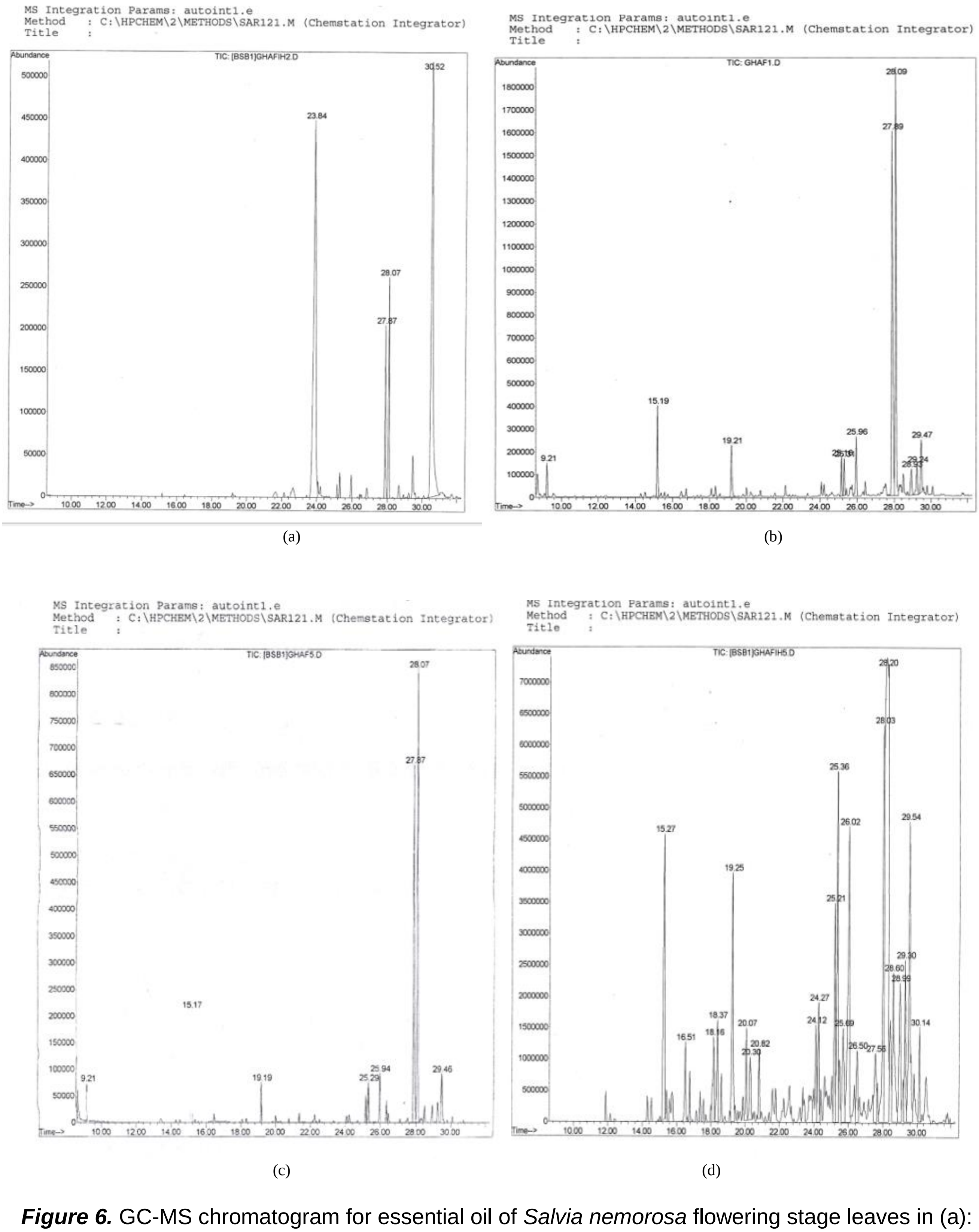


Figure 5. *GC-MS chromatogram for essential oil of Salvia nemorosa flowers in (a): Zonouz, (b): Urmia, (c): Ardabil and (d): Ahar regions.*



(a) (b) (c) (d)

Figure 6. GC-MS chromatogram for essential oil of *Salvia nemorosa* flowering stage leaves in (a): Zonouz, (b): Urmia, (c): Ardabil and (d): Ahar regions.

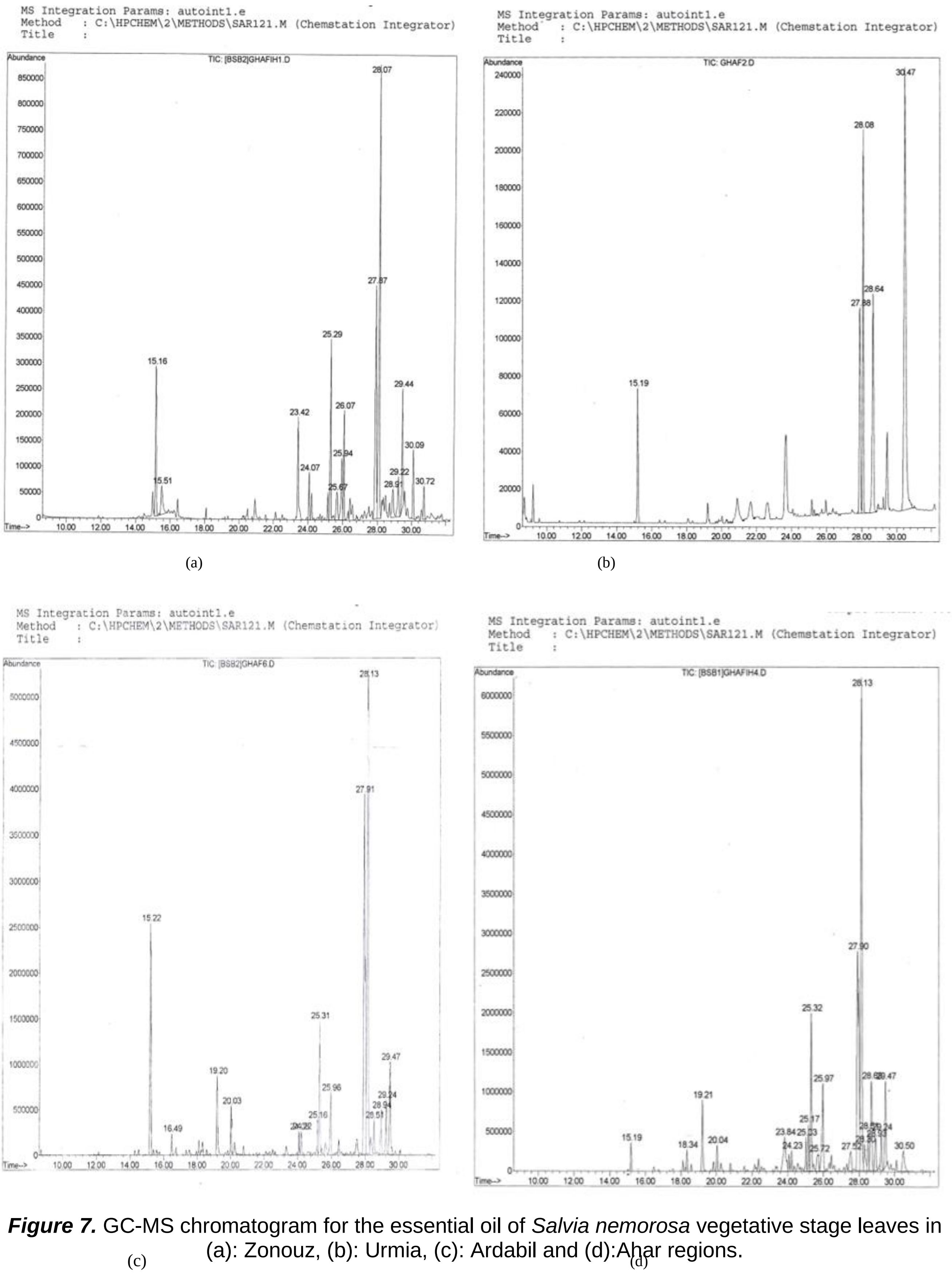


Figure 7. GC-MS chromatogram for the essential oil of *Salvia nemorosa* vegetative stage leaves in (a): Zonouz, (b): Urmia, (c): Ardabil and (d):Ahar regions.