Research article

The Effect of Seasonal Variation on *Rosmarinus officinalis* (L.) Essential Oil Composition

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Abstract. Rosemary,(*Rosmarinus officinalis* L.) belongs to Lamiaceae family, is an aromatic plant, widely distributed in Mediterranean region. Rosemary has been used in folk medicine, cosmetics, and in food endustry. In this study the herbs of rosemary were collected from Campus of Kahramanmaraş Sütçü İmam University. Plant materials were obtained in summer, autumn, winter and spring. The essential oil of the dried plant material was obtained by distillation method. Essential oil of the samples were analysed by GC-MS. As the results of the analyses twenty seven component were determined. The major components were 1.8 cineole (41.25%-45.96%), isoborneol (11.96%-14.89%), α-pinene (9.28%-11.22%) and α-terpineol (4.65%-8.41%). Camphene (3.29%-3.51%), limonene (2.89%-3.61%), p-cymene (3.64%-4.26%) and bornyl acetate (2.02%-3.13%) found as moderately high compounds. As the results, amount of the main components showed significant diversity by seasonal variation and chemotype of the *R. officinalis* determined as 1.8 cineole.

Mevsimsel Varyasyonun *Rosmarinus officinalis* (L.) Uçucu Yağ Bileşenlerine Etkisi

Özet. Biberiye (*Rosmarinus officinalis* L.) Lamiaceae familyasına ait olup, Akdeniz Bölgesi'nde yaygın olarak bulunan aromatik bir bitkidir. Biberiye, halk tıbbında, kozmetikte ve gıda endüstrisinde kullanılmaktadır. Bu çalışmada biberiyenin topraküstü kısımları Kahramanmaraş Sütçü İmam Üniversitesi Kampüsü'nden yaz, sonbahar, kış ve ilkbahar mevsimlerinde toplanmıştır. Kurutulmuş bitki materyallerinin uçucu yağları distilasyon metodu ile elde edilmiştir. Uçucu yağlar GC-MS cihazı ile analiz edilmiştir. Analiz sonuçlarına göre yirmi yedi bileşen tespit edilmiştir. Ana bileşenler 1.8 sineol (%41.25-%45.96), izoborneol (%11.96-%14.89), α-pinen (%9.28-%11.22), α-terpineol (%4.65-%8.41) olarak tespit edilmiştir. Kampen (%3.29-%3.51), limonen (%2.89-%3.61), p-simen (%3.64-%4.26) ve bornil asetat (%2.02-%3.13) yüksek oranlı bileşenler olarak bulunmuştur. Çalışmanın sonucunda, biberiyenin uçucu yağ bileşenleri miktarının mevsimlere göre önemli değişiklik gösterdiği ve kemotipinin 1.8 sineol olarak belirlendiği tespit edilmiştir.

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INTRODUCTION

The genus *Rosmarinus* belonging to the Lamiaceae family, includes two species*, R. officinalis* L. and *R. eriocalyx* Jordan & Fourr. *R. officinalis* known as rosemary. *R. officinalis* grows naturally in the Mediterranean regions such as Spain, Morocco, Tunisia, France, Italy and Turkey. Plants, including herbs and spices with an intense pleasent smell reminiscent of pine wood. The herb is economically important that cultivated or imported and sold in many markets all over the world. Rosemary is used fresh or dried. Its essential oil and herbs were widely used in folk medicine, cosmetics, phytocosmetics, flavouring and conservation of food products (Arnold *et al.*, 1997; Pintone *et al.*, 2002; Del Pilar Sánchez-Camargo and Herrero, 2017). The plant has antiinflammatory, chemopreventive, anti-cancer, anti-proliferative, antimicrobial activities, protective towards neurological disorders. The rosemary tea used for decreasing the risks related to obesity, diabetes and metabolic syndrome (Del Pilar Sánchez-Camargo and Herrero, 2017). The oil of rosemary used in traditional medicine as a pulmonary antiseptic, a choleretic, a colagoguic and in cosmetic sector as soaps, perfumes, room sprays and deodorants. It has also stomachic, antidiarrhoic and antirheumatic properties (Arnold *et al.*, 1997; Pintone *et al.*, 2002; Özcan and Chalchat, 2008; Tawfeeq *et al.*, 2016). Rosemary oil main components especially 1.8 cineole showed insecticidal effect on third instar larvae of the cabbage looper (Tak *et al.*, 2016) and including other components as, camphor, α-pinene or borneol have also been related to generally antioxidant and antimicrobial activities (Del Pilar Sánchez-Camargo and Herrero, 2017).

Rosemary oil can be distinguished with respect to two major types of main constituents: oils with over 40% of 1,8-cineole (Morocco, Tunisia, Turkey, Yugoslavia, Greece, Italy, France) and oils with approximately equal ratios (20–30%) of 1.8-cineole, α-pinene and camphor (Spain, France, Italy, Bulgaria, Greece). Generally, α-pinene chemotypes are offen from Spain, bornyl acetate type from France, myrcene chemotype from Porteque and verbenone chemotype known from Eygpt (Tisserand and Young, 2012; Satyal *et al.*, 2017). The external uses of rosemary are similarly numerous among the local people. In this study, the effect of seasonal variation on essential oil components of *R. officinalis* was detected by GC-MS.

MATERIALS AND METHODS

Plant Material

The herbs of rosemary were collected from Kahramanmaraş Sütçü İmam University Campus (altitude 530 m from sea level) between January and December, 2016. The average climatic values of the research year for Kahramanmaraş are given in Table 1 (Anonymous, 2016). The growing area of the plant is irrigated regularly in the summer. The soil structure of the campus area consists of clay, silt and sand (loamy soil) and average ph is 7.28 (Karagöktaş and Yakupoğlu, 2014). Plant materials were obtained in summer, autumn, winter and spring at different eleven months except for Agust. The flowering period was started early spring (March) and had flowers until autumn (end of October). Dried herba (leaves, flowers) of the plant (30 g) hydrodistilled for 3h using a Clevenger type apparatus. Essential oil of the samples was analysed by GC-MS.

GC-MS Analyses

Analysis were conducted in the Plant Physiology Laboratory in Biology Dept. of KSU. Qualification of the oil was analyzed on an Agilent 5975C Mass Spectrometer coupled with Agilent GC-6890II series. The GC was equipped with HP-88 capillary column (100 m x 250 μm x 0.20 μm film thickness) and flow rate of carrier gas was 1.0 mL/min. Oven temperature of GC was programmed as follows: 70 °C (1 min), 230 °C at of 10 °C/min and then kept at 230 °C at 20 min. The injection part temperature was 250 °C. The mass spectrometer was operating in EI mode at 70 eV. Split ratio was 20:1. Mass range 35-400 m/z; scan speed (amu/s): 1000. 10 μL of the oil was shaked in 0.5 ml diethyl ether and 1 μL of the mixture injected into the column. The components were identified by mass spectra with those of pure authentic samples and Flavor2, Willey7n.1 and NIST98 libraries reference compounds. Retention indices were computed from gas chromatograms by logarithmic interpolation between n–alkanes. The homologous series of n–alkanes C7–C40, Supelco, USA were used as standard. Retention indices calculated as HP–88 capillary column. All samples were repeated three times for GC/MS analysis.

Statistical Analysis

The main components statistical analyses were performed using SPSS 15.0 programme with Waller-Duncen Test.

Table 1. Mean temperature and total precipitation values of Kahramanmaraş 2016.
Çizelge 1. Kahramanmaraş 2016 yılına ait ortalama sıcaklık ve toplam yağış değerleri

	Summer		Autumn			Winter			Spring		
	J	J	S	O	N	D	J	F	M	A	M
Temp. Cº	26.7	30.0	24.8	20.5	11.2	4.5	4.00	11	12.8	19.3	20.6
Precip. mm	17.8	0.00	29.3	13.6	38.3	146.6	140.5	30.1	61.3	17.6	18.1

RESULTS AND DISCUSSION

The essential oil composition of rosemary herba according to seasons and GC-MS chromatogram of main components were given in Table 2 and Figure 1, respectively. The major compounds were found as α-pinene, 1.8-cineole, α-terpineol, isoborneol and shown on the chromatogram as 1,2,3,4, respectively (Figure 1). As the results of GC-MS, 97.68 %, 98.60 %, 96.98 %, 96.48 % of the volatile components identified in rosemary essential oil from summer to spring. Rosemary oil was also dominated by monoterpenoids, especially oxygenated monoterpenes such as 1.8 cineole, α-terpineol, isoborneol. Only, β-caryophyllene and caryophyllene oxide found as sesquiterpenes.

Major compounds statistical analysis according to seasons were given in Figure 2. Seasonal variations showed significant differences as statistically (P<0.01). The main components 1.8 cineole and α-pinene were increased with starting of hot temperature at spring and autumn while α-terpineole and isoborneol were decreased.

Many studies about *R. officinalis* growing in Turkey have been conducted so firstly we discussed similar climatic locations with Kahramanmaraş province. Çeliktaş *et al.* (2007) analysed the essential oil composition from Mersin location at four different seasons and main components were found as 1.8 cineole with highest percentage (61.4%, 60.9%, 50.7%, 58.1%) and α-pinene contents were moderately high (10.2%, 7.8%, 9.4% and 8.8%) at December, March, June and September, respectively. Gülbaba *et al.* (2002) determined the rosmary chemotypes as 1.8 cineole (41.7%-56.4%, 54.3%-44.4%, 48.2%-55.6% at April, July, October, respectively) distributed between Adana-Mersin locations. Our findings shown similarity to Çeliktaş *et al.* (2007) and Gülbaba ve ark. (2002) reports. Özcan and Chalchat (2008), determined the main components of rosemary as p-cymene (44.02%), linalool (20.5%), γ-terpinene (16.62%), in May from Mersin. Başkaya *et al.* (2016) determined the essential oil composition of *R. officinalis* at three vegetative period and found the main components as borneol (12.69%-20.45%), 1.8 cineole (15.82%-17.93%) and

phor (11.50%-14.36%) at Hatay province. Kırpık (2005), determined the volatile compounds of 15 genotype *R. officinalis* growing in Çukurova dryland and highland conditions and main components (α-pinen, camphen, myrcene, cymol, limonene, 1.8 cineole, campher, borneol) were shown differences according to genotype. As the studies according to different geographical region from Turkey, Topal *et al.* (2008) determined the main components as b- camphor (12.94%) and borneol (14.21%), Orhan *et al.* (2008) found as 1,8-cineole (44.42%) chemotype from Balıkesir province and Gürbüz *et al.* (2016) determined the chemotype of rosmary collected from İzmir, Aydın, Antalya, Mersin, Adana and Hatay but growing in Ankara ecologic conditions. Mersin and İzmir genotype determined as 1.8 cineole but the others camphor. Çeliktaş *et al.* (2007) studied differences of chemical compounds of rosemary according to seasons from Çanakkale and İzmir beside Mersin location, reffered above. The main component 1.8 cineole content was found higher in İzmir than Çanakkale and Çanakkale oils were richer about camphor and verbonene.

The studies about *R. officinalis* essential oil from other countries; 1.8-cineole chemotype detected from Grecee, Morocco, UK (Papageorgiou *et al.*, 2008; Ait-Ouazzou *et al.*, 2011; Tawfeeq *et al.*, 2016), camphor chemotype reported from İtaly, Spain and Brazil (Arnold *et al.,* 1999; Porte *et al.,* 2000) and α-pinene chemotype determined from Lebanon, Italy, Iran (Diab *et al.*, 2002; Pintore *et al.,* 2002; Jamshidi *et al.*, 2009). Jordan *et al.* (2013), collected 150 rosmary plant from different bioclimatic zones at Spain and determined the chemotypes as 1.8-cineole, camphor and α-pinene type and were not shown statistically significant differences between the zones. Diab *et al.* (2002) detected that main component α-pinene was reached maximum level at flowering period. Papageorgiou *et al.* (2008) investigated the differences of chemical composition and observed that the highest component 1.8-cineole (50.6%-58.7%) had been in higher amount at May and Agust than February. These findings were supported our results.

Table 2. Chemical composition of *R. officinalis* herba according to seasons.
Çizelge 2. R. officinalis toprak üstü kısımlarının mevsimlere göre kimyasal kompozisyonu.

Components	RT	RI	Summer	Autumn	Winter	Spring
α-pinene	10.81	1012	11.22	9.55	9.28	9.87
camphene	11.26	1085	3.39	3.33	3.29	3.51
β-pinene	11.57	1126	1.72	2.05	2.78	2.82
myrcene	11.69	1141	1.21	1.18	1.18	1.18
α-phellandrene	11.90	1166	0.18	0.13	0.13	0.15
limonene	12.09	1188	3.61	3.17	2.89	3.09
ocimene	12.29	1210	0.38	0.24	0.29	0.31
sabinene	12.50	1231	0.92	0.72	0.93	1.02
1.8 cineole	12.98	1278	43.61	44.54	41.25	45.96
p-cymene	13.27	1305	4.19	4.26	3.64	3.67
1-octen-3-ol	14.71	1427	1.60	0.68	0.64	0.65
linalol	15.79	1430	1.14	1.3	1.01	1.35
β-caryophyllene	16.53	1582	0.75	0.50	1.14	0.87
fenchyl alcohol	16.82	1606	0.27	0.28	0.35	0.27
bornyl acetate	17.05	1626	3.13	2.02	2.95	3.13
exo-methyl-camphenilol	17.40	1655	0.10	0.24	0.23	0.36
α-terpineol	17.90	1696	6.05	8.41	7.11	4.65
ısoborneol	18.27	1730	13.00	14.52	14.89	11.96
myrtenal	18.86	1781	0.16	0.17	0.10	0.10
cis-verbenol	19.11	1803	not found	0.11	0.28	0.03
geranyl acetone	19.66	1855	0.12	0.27	0.63	0.24
methyl eugenol	21.47	1974	not found	0.19	0.20	0.13
benzothiazole	21.82	1993	0.33	0.06	0.47	0.12
thymol	21.94	1999	0.13	0.09	0.26	0.15
caryophyllene oxide	22.20	2031	0.40	0.45	0.88	0.80
carvacrol	22.64	2083	0.07	0.11	0.10	0.06
eugenol	23.15	2147	not found	0.03	0.08	0.03
Total			97.68	98.60	96.98	96.48
Monoterpene hydrocarbons			26.82	24.63	24.41	25.62
Oxygenated monoterpenes			67.41	71.54	68.58	67.63
Sesquiterpene hydrocarbons			0.75	0.50	1.14	0.87
Oxygenated sesquiterpenes			0.40	0.45	0.88	0.80
Phenylpropanoids			not found	0.22	0.28	0.16
Others			2.30	1.26	1.69	1.40

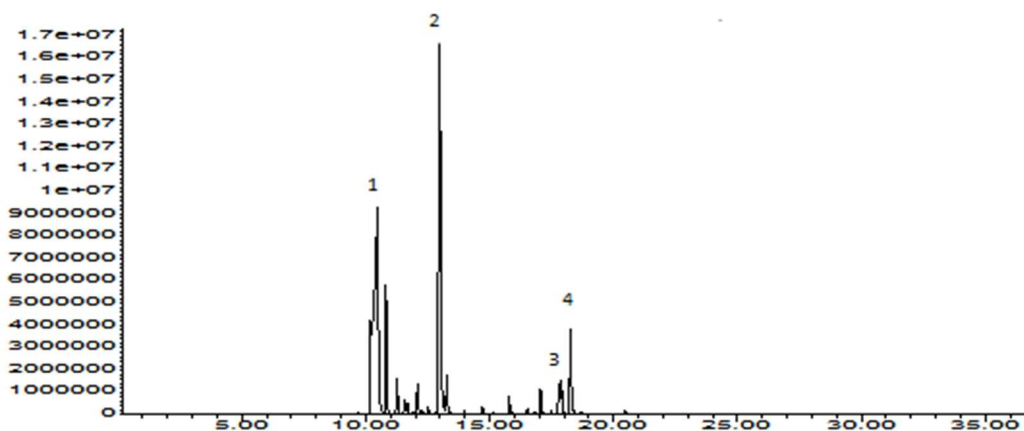RT: Retention Time.   RI: Retention Index.

Figure 1. GC-MS chromatogram of *R. officinalis* essential oil.
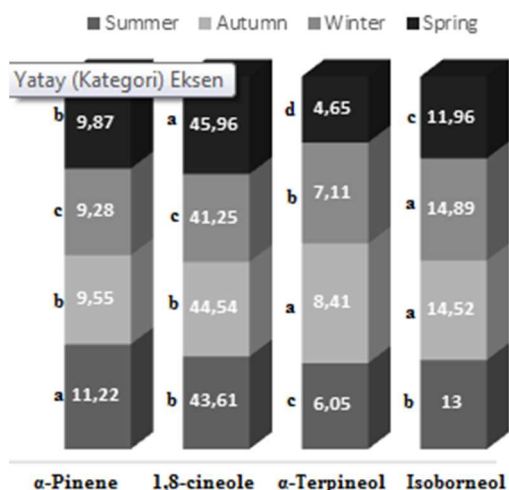*Şekil 1. R. officinalis uçucu yağının GC-MS kromatogramı.*

Figure 2. The percentage of the *R. officinalis* main components according to seasons.
Şekil 2. R. officinalis ana bileşenlerinin mevsimlere göre oranı.

CONCLUSION

To the best of our knowledge, this is first report about chemical composition of rosmary, growing in Kahramanmaraş province. As the result of this study, *R. officinalis* essential oil shown 1.8 cineole chemotype and the seasons affected the main components amount. This result confirmed the Tisserand and Young (2012) chemotype classification according to geographical region. As conclusion, we discussed the reports on essential oil composition of *R. officinalis* from different regions of the world and Turkey and the stuides were shown that 1.8-cineole, camphor, α-pinene, camphene, bornyl acetate, α-terpineol and verbenone were found as main components and these components amount was shown differences between the locations and different chemotypes could be seen in the same region.

Differences on the components and percent of the components may be affected from soil, climate, altitude and vegative period of the plant. In Turkey, rosemary growing at Adana-Mersin province preffered by customers. *R. officinalis* from Kahramanmaraş shown similar chemotype with Adana-Mersin samples. As a result, *R. officinalis* can be grown in these area and preffered to harvest at spring for high 1.8 cineole content.

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