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Original Article

Compositions of the essential oils and antimicrobial activities of the rediscovered Turkish endemic *Salvia freyniana* and *Salvia quezelii* (Lamiaceae)

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ABSTRACT

Background and Aims: This study aims to determine the chemical compositions and antibacterial activity of the rediscovered Turkish endemic *Salvia freyniana* Bornm. and *Salvia quezelii* Hedge & Afzal-Rafii.

Methods: The study simultaneously uses gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) systems, to analyze the hydrodistilled essential oils of *S. freyniana* and *S. quezelii*. The study examines the antibacterial activity of *Salvia* essential oils against the human pathogens *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC B888, and *Salmonella typhimurium* ATCC 13311 and evaluates the oils' minimum inhibitory concentrations (MIC).

Results: The major components were found as 1,8-cineole (21.9%), β-pinene (14.2%), camphor (8.7%), α-pinene (8.0%), and valeranone (7.0%) for *S. freyniana* and limonene (20.8%), pinocamphone (11.4%), α-pinene (10.6%), camphor (9.1%), β-pinene (7.5%), camphene (7.1%) and isopinocamphone (6.7%) for *S. quezelii*. *S. typhimurium* was inhibited at a concentration of 5 mg/mL by both essential oils, with a MIC value of 5 mg/mL being found against *S. aureus* by *S. freyniana*. The other tested samples show rather moderate inhibitory effects (20 mg/mL).

Conclusion: To the best of this study's knowledge, this is the first report to show the antimicrobial activity of the essential oils of *S. freyniana* and *S. quezelii*.

Keywords: antibacterial activity, GC-FID, GC-MS, *Salvia freyniana, Salvia quezelii*, rediscovery

INTRODUCTION

The genus *Salvia* L. is one of the largest members of the Lamiaceae family (tribe: Mentheae) and contains approximately 1000 species spread all over the world (Harley et al., 2004). Türkiye is a main center of diversity for the genus in Asia, which is represented by 99 species, 52 of which are endemic to Türkiye (Hedge, 1982; Celep, Kahraman, Atalay & Dogan, 2014).

Most of the species from the genus *Salvia* have medicinal significance, as they produce many useful natural constituents including terpenes and flavonoids. Some of the essential oils and phenolic compounds of plants belonging to this genus possess a long list of medicinal uses for being spasmolytic, antiseptic, and/or astringent, and they have also shown excellent antimicrobial activity and antioxidant capacity, with some used as anticancer agents or having a hypoglycemic effect (Kelen & Tepe, 2008; Kintzios, 2000; Tepe, Daferara, Sökmen, Sökmen & Polissiou, 2005; Khalil & Zheng-Guo, 2011). Some *Salvia* species are also used as flavoring agents in perfumery and cosmetics. Many wild-growing *Salvia* species are also used in the traditional medicine of different nations in place of sage or as an adulteration (Bisset & Wichtl, 2001). Chemical compounds and biological activities in the essential oils of some *Salvia* species have been reported in Türkiye (Demirci, Başer & Tümen, 2002; Başer, 2002; Demirci, Başer, Yıldız & Bahçecioglu, 2003; Kaya, Demirci & Başer, 2003; Kaya, Demirci & Başer 2009; Karik, Sağlam, Kürkçüoğlu & Başer, 2011; Kaya, Dinç, Doğu & Demirci, 2017).

S. freyniana Bornm. (Figure 1) is, an endemic, perennial suffruticose herb with a woody rootstock whose stems ascend to erect at 15-35 cm. Its leaves are pinnatisect. Inflorescence is

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racemose. The calyx is campanulate at 7-11 mm, and its corolla is lilac-blue with a white center (Bagherpour, Celep, Doğan & Kahraman, 2009).

Figure 1. *Salvia freyniana* Bornm

S. quezelii Hedge & Afzal-Rafii (Figure 2) is an endemic perennial herb with a woody rootstock. Stems are procumbent-ascending at 6-45 cm, and its leaves are pinnate. The inflorescence is few-flowered at 5–35 cm. The calyx is green, and the corolla is white cream-colored (Celep et al., 2014).

Figure 2. *Salvia quezelii Hedge and Afzal-Rafii*

The specimens of *S. freyniana* were first collected and described from Yenipazar in Yozgat Province by Bornmueller in 1892. Later, it was rediscovered by Bagherpour, Celep, Doğan & Kahraman (2006), who gave a detailed account of the morphological and pollen-mericarp micromorphological characteristics of S. freyniana (Bagherpour, Celep, Doğan & Kahraman, 2009).

The specimens of *S. quezelii* were first collected from Mersin in 1931 by Eig and Zohary and then collected again from Namrun in Mersin Province (formerly Içel) as a type specimen by Quezel in 1970. Later, a population of *S. quezelii* was rediscovered between Anamur and Ermenek by Celep et al. in 2009, who gave a detailed account of the morphological, anatomical, pollen, mericarp, myxocarpy, and trichome micromorphological characteristics of the little-known *S. quezelii* (Celep et al., 2014). *S. freyniana* is known as *göl şalba* in Türkiye, while *S. quezelii* is known as *limon adaçayı* (Güner, Aslan, Ekim, Vural, & Babaç, 2012).

A literature search revealed no reference to any previous work on the essential oils of *S. freyniana* and *S. quezelii*. This study reports for the first time on the chemical constituents of the essential oils and the antimicrobial activity of the rediscovered *S. freyniana* and *S. quezelii*.

MATERIALS AND METHODS

Materials

Salvia freyniana and *Salvia quezelii* samples were collected during their flowering period (June, 2018) from Türkiye's Yozgat Province (Büyükören village of Yenipazarı municipality, 1000-1250 m) and Mersin Province (Km 40 on Ermenek road in Anamur municipality, 1050 m,), respectively. Voucher specimens were deposited in the herbarium of the Faculty of Education of Necmettin Erbakan University in Konya, Türkiye (NEÜ Herb.). The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulfate and stored at +4°C in the dark until analyzed and tested.

Analysis of volatile components

The processes for the gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analyses were performed with reference to Demirci, Yusufoglu, Tabanca, Temel, Bernier, Agramonte, Alqasoumi, Al-Rehaily, Başer, & Demirci, (2017).

Antibacterial activity assay

The microorganisms' strains used for the evaluation of antibacterial activity were obtained from the American Type Culture Collection (ATCC) in lyophilized form. Mueller-Hilton Agar

(MHA) was used as a medium for growing the bacterial strains. The prepared media were stored at +4°C for a maximum of 2 weeks. Their purity was controlled, and the microorganisms stored in 15% glycerol solution at -85°C were inoculated into the prepared media and allowed to multiply by incubating in a bacteriological incubator at 37°C for 24 h.

The antibacterial activity of both essential oils was evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute (2006) method, as previously described. Gram-negative strains (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC B888, and *Salmonella typhimurium* ATCC 13311) and a Gram-positive strain (*Staphylococcus aureus* ATCC 6538) were used as the standard test microorganisms. The essential oils (20-0.01 mg/mL) were dissolved in sterile dimethyl sulfoxide (DMSO) for the initial stock solution. 100 µL of essential oil was applied to 96-well microplates, and 2-fold serial dilutions were then performed. Strains were incubated in Mueller-Hinton Broth (MHB) overnight at 37°C for 24h. After the dilutions, 50 µL aliquots of turbidometrically adjusted microorganisms were inoculated (10^5-10^6 CFU/mL) onto the plates. After incubation at 37°C for 24 h, the first well was treated with 20 µL of resazurin, which ensured the minimum inhibitory concentrations (MIC) where the lowest concentration of the samples prevented visible growth was present on all microplate wells. The standard antibiotic ciprofloxacin (2.5-0.1 µg/ml) was used as the standard control. Solvent and microbial controls were also added to the assay plate. Antibacterial assays were repeated at least three times for all test samples (CLSI, 2006).

RESULTS AND DISCUSSION

The water-distilled essential oils from the aerial parts of *S. freyniana* and *S. quezelii* were characterized using GC-FID and GC-MS. The compounds identified from the essential oils along with their relative percentages are listed in Table 1. Totals of 64 and 55 compounds were identified from the respective essential oils of *S. freyniana* and *S. quezelii*, representing 99.1% and 98.9% of their respective oils. Oil components can be grouped under six main chemical classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, fatty acids, and others. The oils of the *S. freyniana* and *S. quezelii* are characterized by a high content of monoterpene hydrocarbons (35.1% and 51.0%, respectively) and oxygenated monoterpenes (40.1 % and 44.3% respectively). The major components were found as 1,8-cineole (21.9%), β-pinene (14.2%), camphor (8.7%), α-pinene (8.0%), and valeranone (7.0%) for *S. freyniana* and limonene (20.8%), pinocamphone (11.4%), α-pinene (10.6%), camphor (9.1%), β-pinene (7.5%), camphene (7.1%), and isopinocamphone (6.7%) for *S. quezelii*.

According to the results in Table 1, oxygenated monoterpenes are present in almost equal amounts in the oils of *S. freyniana* and *S. quezelii*, while *S. quezelii* is characterized by a high content of monoterpene hydrocarbons in its oil. However, some important differences were found regarding the constituents of the oils. As can be seen in Table 1, *S. freyniana* is characterized by high oxygenated sesquiterpenes content in its oil.

The essential oil composition of the 64 *Salvia* taxa from Türkiye has already been studied, with monoterpene hydrocarbons (Group 1), oxygenated monoterpenes (Group 2), sesquiterpene hydrocarbons (Group 3), oxygenated sesquiterpenes (Group 4), and phenylpropanoid (Group 5) being reported as the main groups of the constituents in the *Salvia* taxa's essential oils (Başer, 2002). According to the current study's results, *S. freyniana* and *S. quezelii* have many monoterpene hydrocarbons and oxygenated monoterpenes. As such, they can be categorized into the first and second groups, respectively.

The study has examined the antibacterial activity of *Salvia* essential oils against *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC B888, and *S. typhimurium* ATCC 13311. S. aureus and *S. typhimurium* were inhibited at a concentration of 5 µg/ml by *S. freyniana* as the best performance among the tested samples. In addition, *S. typhimurium* was inhibited at a concentration of 5 µg/ml by *S. quezelii* essential oil. The other tested samples showed rather moderate inhibitory effects. The comparative results are given in Table 2.

Table 1. The composition of the essential oils of *Salvia freyniana* and *Salvia quezelii*

RRI[a]	Compound	*S. frey.* %[b]	*S. quez.* %	IM[d]
1014	Tricyclene	0.2	0.3	MS
1032	*a*-Pinene	**8.0**	**10.6**	RRI, MS[e]
1035	*a*-Thujene	1.0	-	MS
1072	*a*-Fenchene	-	0.2	RRI, MS
1076	Camphene	**3.8**	**7.1**	RRI, MS
1118	*b*-Pinene	**14.2**	**7.5**	RRI, MS
1132	Sabinene	1.9	-	RRI, MS
1159	*d-3*-Carene	-	0.1	MS
1174	Myrcene	0.7	0.8	RRI, MS
1188	*a*-Terpinene	0.2	0.1	RRI, MS
1203	Limonene	2.3	**20.8**	RRI, MS
1213	1,8-Cineole	**21.9**	-	RRI, MS
1218	*b*-Phellandrene	-	0.6	RRI, MS
1246	(*Z*)-*b*-Ocimene	tr[c]	-	MS
1255	*g*-Terpinene	0.6	-	RRI, MS
1266	(*E*)-*b*-Ocimene	0.1	-	MS
1280	*p*-Cymene	2.0	2.9	RRI, MS
1290	Terpinolene	0.1	-	RRI, MS
1400	Nonanal	tr	-	MS
1406	*a*-Fenchone	-	tr	RRI, MS
1450	*trans*-Linalool oxide (*Furanoid*)	-	tr	MS
1452	1-Octen-3-ol	0.2	0.2	MS
1466	*a*-Cubebene	tr	-	RRI, MS
1474	*trans*-Sabinene hydrate	0.4	-	MS
1478	*cis*-Linalool oxide (*Furanoid*)	-	tr	MS
1493	*a*-Ylangene	-	0.3	MS
1497	*a*-Copaene	0.2	0.3	RRI, MS
1499	*a*-Campholene aldehyde	0.1	0.1	MS
1532	Camphor	**8.7**	**9.1**	RRI, MS
1535	*b*-Bourbonene	0.6	-	MS
1536	Pinocamphone	-	**11.4**	RRI, MS
1553	Linalool	0.2	0.6	RRI, MS
1556	*cis*-Sabinene hydrate	0.4	-	MS
1562	Isopinocamphone	-	**6.7**	MS
1571	*trans-p*-Menth-2-en-1-ol	0.1	-	MS
1586	Pinocarvone	0.3	0.4	RRI, MS

Table 1. Continued

1591	Bornyl acetate	4.2	1.1	RRI, MS
1597	*b*-Copaene	0.1	-	MS
1598	Camphene hydrate	-	0.3	MS
1601	Nopinone	-	0.1	MS
1611	Terpinen-4-ol	0.6	1.9	RRI, MS
1612	*b*-Caryophyllene	1.2	0.2	RRI, MS
1617	6,9-Guaiadiene	0.7	-	MS
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	-	0.2	MS
1648	Myrtenal	0.4	1.5	MS
1661	*trans*-Pinocarvyl acetate	-	0.2	RRI, MS
1668	(*Z*)-*b*-Farnesene	2.2	-	MS
1670	*trans*-Pinocarveol	0.4	3.5	RRI, MS
1683	*trans*-Verbenol	0.4	-	RRI, MS
1704	Myrtenyl acetate	-	1.5	MS
1704	*g*-Muurolene	0.2	tr	MS
1706	*a*-Terpineol	0.3	1.9	RRI, MS
1719	Borneol	0.9	2.0	RRI, MS
1726	Germacrene D	0.3	-	MS
1740	*a*-Muurolene	-	0.2	MS
1741	*b*-Bisabolene	0.2	-	RRI, MS
1751	Carvone	-	0.1	RRI, MS
1755	Bicyclogermacrene	0.3	-	MS
1773	*d*-Cadinene	0.2	0.2	MS
1776	*g*-Cadinene	0.2	0.1	MS
1804	Myrtenol	0.5	1.3	MS
1845	*trans*-Carveol	0.3	0.1	RRI, MS
1849	Calamenene	tr	0.5	MS
1882	*cis*-Carveol	-	0.2	RRI, MS
1896	*cis*-*p*-Mentha-1(7),8-diene-2-ol	-	0.2	MS
1900	*epi*-Cubebol	0.1	-	MS
1941	*a*-Calacorene	-	0.1	MS
1957	Cubebol	0.1	-	MS
2001	Isocaryophyllene oxide	0.3	-	MS
2008	Caryophyllene oxide	6.1	0.1	RRI, MS
2029	Perilla alcohol	-	0.1	MS
2071	Humulene epoxide-II	0.4	-	MS
2104	Viridiflorol	0.7	-	MS
2131	Hexahydrofarnesyl acetone	0.6	-	MS

Table 1. Continued

2145	Valeranone	**7.0**	-	MS
2173	6-*epi*-Cubenol	-	0.5	MS
2187	T-Cadinol	0.3	0.1	MS
2192	Nonanoic acid	0.1	-	RRI, MS
2250	*a*-Eudesmol	-	tr	MS
2255	*a*-Cadinol	0.1	0.2	MS
2256	Cadalene	-	0.4	MS
2278	Torilenol	0.1	-	MS
2324	Caryophylla-2(12),6(13)-dien-5*a*-ol (=*Caryophylladienol II*)	0.4	-	MS
2392	Caryophylla-2(12),6-dien-5*b*-ol (=*Caryophyllenol II*)	0.6	-	MS
2503	Dodecanoic acid	0.1	-	RRI, MS
2670	Tetradecanoic acid	0.1	-	RRI, MS
2931	Hexadecanoic acid	0.2	-	RRI, MS
	Monoterpene Hydrocarbons	35.1	51.0	
	Oxygenated Monoterpenes	40.1	44.3	
	Sesquiterpene Hydrocarbons	6.4	2.3	
	Oxygenated Sesquiterpenes	16.2	1.0	
	Fatty acids	0.5	-	
	Others	0.8	0.3	
	Oil Yield (%)	0.5	0.98	
	Total	**99.1**	**98.9**	

[a] Relative retention indices calculated against *n*-alkanes; [b] Percentages calculated from FID data; [c] Trace (< 0.1 %); [d] Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column and/or mass spectra (MS) identified based on computer matching of MS[e] with those of the Wiley and MassFinder libraries and comparison with literature data.

Table 2. MIC values (μg/ml)

	***E. coli* ATCC 8739**	***S. aureus* ATCC 6538**	***P. aeruginosa* ATCC B888**	***S. typhimurium* ATCC 13311**
***S. freyniana* essential oil**	20	5	20	5
***S. quezelii* essential oil**	20	10	20	5
Ciprofloxacin	0.1	0.1	0.1	0.1

CONCLUSION

When comparing the literature, no study is found regarding the antimicrobial activities of the essential oils of *S. freyniana* and *S. quezelii*. To the best of this study's knowledge, this is the first report to show the antimicrobial activity of the essential oils of *S. freyniana* and *S. quezelii*. More in detail evaluations on biological activity both on in vitro and in vivo levels are needed to exhaust the potential of essential oils from *S. freyniana* and *S. quezelii*. Further work is ongoing.

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