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RESEARCH ARTICLE

Evaluation of the chemical composition and biological activities of *Salvia officinalis* subsp. *lavandulifolia* (Vahl) Gams essential oil

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Abstract

The main aim of the present study was to investigate the antibacterial effect of the commercial Pharma Grade *S. officinalis* subsp. *lavandulifolia* (Lamiaceae) essential oil against the skin pathogen *Staphylococcus aureus*. The chemical composition of the essential oil was confirmed by GC and GC/MS, simultaneously. Camphor (30.5%), 1,8-cineole (24.8%), α-pinene (6.5%), linalool (4.0%) and linalyl acetate (3.5%) were found as major components. The bioactivity of the essential oil and its main compounds were tested using the *in vitro* microdilution technique. Minimum inhibitory concentration (MIC) values of α-pinene, essential oil, camphor, linalool and linalyl acetate were in the range of 2.5-10 mg/mL, respectively. The results showed that the tested pathogen was only moderately susceptible against the essential oil and its main compounds when compared with standard antibiotics. In addition, the *in vitro* antioxidant activity was evaluated using the radical scavenging activity mediated by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The activity range was found more than 30 mg/mL for all tested oil samples, compared with the standard. The results suggested that the oil and its major constituents represent antimicrobial activity supporting its antiseptic use in folk medicinal use.

Keywords: *Salvia lavandulifolia* Vahl., essential oil, antibacterial activity, antioxidant activity

Introduction

The *Salvia* genus, with more than 900 species throughout the world, is one of the most widespread members of the Lamiaceae family, with many species classified as culinary or medicinal. *S. officinalis* subsp. *lavandulifolia* (Lamiaceae) commonly known as "Spanish sage" is an aromatic plant. *S. officinalis* subsp. *lavandulifolia* has commonly been used in folk medicine as spasmolytic, antiseptic, analgesic, sedative and antioxidant, among others. Furthermore, it is used in dementia therapy attributed to its sedative, antioxidant, anti-inflammatory, estrogenic and anticholinesterase activities (Perry et al., 2003). It is a small woody herbaceous perennial shrub up to 17–100 cm with mauve-blue flowers that grow preferably on the sandy–calcareous soils of mountainous areas (at 350–2000 m) of Spain, southeast of France and northwest of Africa (Kintzios, 2000; Porres-Martinez et al., 2013).

Staphylococcus aureus is a Gram-positive, ubiquitous organism that is found in both hospital and common areas. *S. aureus* is found on the skin, mucous membranes, and humans are the major reservoir for this organism. It is estimated that up to half of all adults are colonized (Fanelli et al., 2011). While *S. aureus* colonizes the skin, it can also be responsible for localized cutaneous infection such as acne (Deleo et al., 2010; Chambers & Deleo, 2009). Antibiotics are commonly used in treatment of acnes caused by *S. aureus* (Deleo et al., 2010).

In this present study, it was aimed to investigate the antimicrobial and antioxidant activity of the pharma grade *S. officinalis* subsp. *lavandulifolia* essential oil and its major components against the skin pathogenic bacterium *S. aureus.*

Materials and Methods

Materials

Pharma grade *S. lavandulifolia* essential oil was obtained from Aromapharm Company, Germany, the standard antibiotic ciprofloxacin, Gallic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH)• and major components were also acquired from commercial sources like Sigma-Aldrich (St. Louis, USA) if not otherwise stated.

Methods

Gas chromatography and gas chromatography-mass spectrometry (GC and GC-MS) analysis

The essential oils were analysed by GC using a Hewlett Packard 6890 system and an HP Innowax fused silica capillary column (FSC) (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used with nitrogen as carrier gas at 1 mL/min. Initial oven temperature was 60 °C for 10 min, and increased at 4 °C/min to 220°C, then kept constant at 220 °C for 10 min and increased at 1 °C/min to 240 °C. Injector temperature was set at 250 °C. Percentage compositions of the individual components were obtained from electronic integration using flame ionization detection (FID, 250 °C) (Demirci et al., 2008). Relative percentages of the separated compounds were calculated from FID chromatograms as cited in Table 1. GC-MS analysis was performed with a Hewlett-Packard GCD, system (SEM Ltd, Istanbul, Turkey) and Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with Helium. GC oven temperature conditions were as described above, split flow was adjusted at 50 mL/min, the injector temperature was at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 425 as previously reported (Demirci et al., 2008). Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC-MS Library, MassFinder Software 4.0) (McLafferty & Stauffer, 1989, Hochmuth, 2008), and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998) was used for the identification as also previously reported in detail (Demirci et al., 2008).

Antibacterial activity

The microbial strain used was obtained from the American Type Culture Collection (ATCC) in lyophilized form. The microorganism was stored at -85 °C in glycerol until inoculation and purity testing.

The potential antibacterial activity of both the essential oil and the main compounds, α-pinene, camphor, linalool, linalyl acetate and 1,8-cineole were evaluated by broth microdilution assay according to a modified Clinical and Laboratory Standards Institute (CLSI) method (Anonymous, 2006; Demirci et al., 2015). *Staphylococcus aureus* ATCC 11632 was used as test microorganism. The samples (20-0.019 mg/mL) were dissolved in sterile dimethyl sulfoxide (DMSO) for the initial stock solution. 100 µL of samples were applied to 96-well microplates and 2 fold serial dilutions were performed. After the dilutions, 50 µL aliquots of tubidometrically adjusted microorganism was inoculated (10^5-10^6 CFU /mL) on to the plates. After incubation at 37 °C for 24 h the first well was treated with 20 µL of resazurin, which insured on all microplates the Minimum Inhibitory Concentrations (MIC), where the lowest concentration of the samples prevented visible growth. The standard antibiotic ciprofloxacin (128-0.25 µg/mL) were used as standard control. Solvent and microbial controls were also added to the assay plate. Antibacterial assays were repeated three times for all the test samples.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Serial dilutions were carried out with stock solutions (10 mg/mL) of the essential oils to obtain the concentrations of 10-3125×10^{-4} mg/mL. Diluted solutions were mixed with DPPH and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm at room temperature using a microplate spectrophotometer. The experiment was performed three times and average absorption was noted for each concentration. Gallic acid was used as a positive control. The percentage of inhibition was calculated using equation. The EC_{50} value, which is the concentration of the test materials that inhibits 50% of the free radical concentration, was calculated as mg/mL (Kumarasamy et al., 2007).

$$\text{Percentage Inhibition} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] x100$$

Results and Discussion

Gas chromatography and gas chromatography-mass spectrometry (GC and GC-MS) analysis

The chemical composition of the essential oil *S. officinalis* subsp. *lavandulifolia* was characterized by GC and GC-MS analysis. The results are shown in Table 1. The major components were camphor (30.5%), 1,8-cineole (24.8%), *α*-pinene (6.5%), linalool (4.0%) and linalyl acetate (3.5%). Thirty-two components were identified representing 99.1% of the sample, confirming the Pharmacopoeia (PHEUR 9.0) standard (Anonymous, 2016).

Table 1. Volatile components of *S. officinalis* subsp. *lavandulifolia* essential oil

RRI[a]	Component	%[b]
1014	Tricyclene	0.3
1032	*α*-Pinene	6.5
1035	*α*-Thujone	0.1
1076	Camphene	5.2
1118	*β*-Pinene	4.6
1132	Sabinene	1.0
1174	Myrcene	2.0
1203	Limonene	4.1
1213	1,8-Cineole	24.8
1246	(*Z*)-*β*-Ocimene	0.1
1255	*γ*-Terpinene	0.2
1266	(*E*)-*β*-Ocimene	tr
1280	*p*-Cymene	0.6
1290	Terpinolene	0.2
1474	*trans*-Sabinene hydrate	0.1
1532	Camphor	30.5
1553	Linalool	4.0
1565	Linalyl acetate	3.5
1590	Bornyl acetate	0.9
1611	Terpinen-4-ol	0.4
1612	*β*-Caryophyllene	1.5
1658	Sabinyl acetate	1.9

Table 1. cont.

RRI[a]	Component	%[b]
1687	α-Humulene	0.3
1706	α -Terpineol	1.0
1709	α-Terpinyl acetate	0.9
1719	Borneol	3.4
1755	Bicyclogermacrene	0.1
1765	Geranyl acetate	0.1
1819	Geranyl isobutyrate	0.3
1857	Geraniol	0.2
1864	*p*-Cymene-8-ol	0.1
	Total	**99.1**

[a]RRI: Relative retention indices calculated against n-alkanes; [b]%: calculated from FID data; tr, identification based on the retention times (tR) of genuine standard compounds on the HP Innowax column

In a previous study, the main constituent of *S. officinalis* subsp. *lavandulifolia* essential oil was reported to contain 1,8-cineole (21.4-33.8 %), followed by α-pinene (10.5-17.5 %), β-pinene (6.0- 17.3 %), limonene (5.6-10.4 %) camphor (6.1- 9.4 %) and β-caryophyllene (4.0-8.5 %), respectively (Usano-Alemany et al., 2012). According to Pierozan (2009), the main components of *S. officinalis* subsp. *lavandulifolia* essential oil were β-thujone (19.9%), camphor (18.9%), α-thujone (18.9%), 1,8-cineole (8.1%), and β-pinene (3.9%) (Pierozan et al., 2009). In other work, among 27 identified constituents, camphor was the most abundant one (29.1%), along with 1,8-cineole (20.3%) and α-pinene (8.2%) (Nikolic et al., 2014).

Antibacterial activity

The antibacterial activity of *S. officinalis* subsp. *lavandulifolia* essential oil and major components were examined against human pathogen *Staphylococcus aureus* ATCC 11632. *S. aureus* was inhibited at a concentration of 2.5 mg/mL by α-pinene with the best performance among the tested samples. The other samples tested, except for 1,8-cineole showed rather moderate inhibitory effects. 1,8-Cineole showed the lowest inhibition against *S. aureus*. The comparative results are given in Table 2.

Table 2. Antibacterial evaluation of the oil and its major components (MIC, mg/mL)

Test samples	*Staphylococcus aureus* ATCC 11632
S. officinalis subsp. *lavandulifolia* essential oil	5
Camphor	5
α-Pinene	2.5
Linalool	10
Linalyl acetate	10
1,8-Cineole	>20
Ciprofloxacin	0.03

As a result of this present work, the *S. officinalis* subsp*. lavandulifolia* essential oil was evaluated for its *in vitro* biological activities such as antibacterial and antioxidant activities. The chemical analysis, confirmed with camphor (30.5%), 1,8-cineole (24.8%), *α*-pinene (6.5%), linalool (4.0%), linalyl acetate (3.5%), respectively, the quality. One of the major skin pathogens *S. aureus* presented higher sensitivity for the essential oil rather its major components, except for *α*-pinene. The lowest MIC was observed when *S. aureus* was exposed to 2.5 mg/mL *α*-pinene, while the lowest sensitivity with MIC of >20 mg/mL value was obtained when *S. aureus* was exposed to 1,8-cineole. It is also difficult to say that the main components act synergistically.

According to the literature, MIC= 2.3 mg/mL was observed for the pathogenic *S. aureus* strain against when interacted with *S. officinalis* subsp. *lavandulifolia* essential oil (Pierozan et al., 2009). Nikolic et al. (2014) reported that, MIC was 6**.**2 mg/mL against *S. aureus* (Nikolic et al., 2014). In addition, Tzakou et al. (2001) were determined MIC of main components, *α*-pinene and 1,8-cineole, 7.5 and 9.5 mg/mL, respectively (Tzakou et al., 2001).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was used to determine the antioxidant capacity of *S. officinalis* subsp. *lavandulifolia* essential oil. However, EC_{50} values of essential oils were found more than 30 mg/mL. EC_{50} value of gallic acid which used as a positive control, was found 0.004 mg/mL. When the compared with gallic acid, the samples were showed weak DPPH radical scavenging activities.

Recently, Cutillas et al. (2017) studied the antioxidant activity of *S. officinalis* subsp*. lavandulifolia* essential oil and its main components by DPPH method and found activity lower than 0.05 units at a maximum assay concentration of 100 mM.

As an overall result, our antibacterial and antioxidant results are comparable with literature findings. The results obtained show correlation with previous published data.

Conclusion

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 *Salvia* sp. Further work is ongoing.

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RESEARCH ARTICLE

Chemical characterization of *Schinus molle* L. essential oils from North Cyprus

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Abstract

Analysis of hydrodistilled essential oils from dried leaves and fruits of *Schinus molle* L. collected from two localities in Lefkoşa (Nicosia), Northern Cyprus were analyzed by GC-FID and GC/MS. α-Phellandrene (31.5 -31.6%), (26.7-36.3%), limonene (10.1-11.4%), (12.5-13.5%), and β-phellandrene (9.9-10.9%), (10.3-12.2%) were identified as the major constituents, respectively. Moreover, myrcene was a main constituent identified only in fruits (18.5-19.9%), while bicyclogermacrene was the main component characterized in the leaf oils (12-11.1%).

Keywords: Essential oil, *Schinus*, Anacardiaceae

Introduction

Schinus molle L. (Anacardiaceae) (Peruvian pepper tree) is an evergreen tree native to South America but used in landscape architecture in many countries in the tropical and temperate regions of the World. It is planted on road sides and gardens as a shade tree (Belhamel et al., 2008). It is native in South America, grows in America, Asia, Australia some parts of Europe and exotic in Cyprus as well as in Turkey (Orwa et al., 2009).

Leaves are imparipinnate, leaflets are linear-lanceolate. It is easily distinguished from the related *S. terebinthifolius* Raddi (Brazilian pepper tree) by having compound leaves. *S. molle* fruits are small, round berries, 5-9 mm in diameter, the colour of berries is bright red when mature, later turning to black (Orwa et al., 2009).

This plant plays an important role in Chilean folk medicine. The whole fruits of *S. molle* are soaked or fermented in hot water to make a drink which is used for many health purposes, such as diuretic, digestive, antiseptic, for pain relief and haemorrhoids. The plant has also been used in the treatment of menstrual disorders, respiratory infections, and urinary tract infections (Perez-Lopez et al., 2011). In some traditional cuisines, *S. molle* fruits have been used as a replacement for black pepper and to prepare alcoholic beverages (Maringiu et al., 2004).

There are several studies from different places around the world revealing the variation in the chemistry of *S. molle* essential oil. Water distilled oils from dried fruits and leaves of *S. molle* from Turkey revealed 80 different components representing 98.2% to 99.8% of the oil, respectively. Fruit oil contained α-phellandrene (22.1% and 38.1%), β-phellandrene (10.4% and 11.8%) and limonene (9.6%). Whereas, the main components in the leaf oil were α-phellandrene (45.7%), β-phellandrene (13.6%) and limonene (13.4%) (Baser et al., 1997). The main components of the leaf extracts from Izmir, Turkey were germacene D (20.7%) and β-caryophyllene (13.4%) (Onder et al., 2010). In Algeria, the main components were reported as α-

phellandrene (26.5%), limonene (8.6%), β-phellandrene (12.4%), elemol (8.6%) and α-eudesmol (6.1%) (Belhamel et al., 2008). From the Costa Rican material, the main components of the essential oil of the leaves were β-pinene and α-pinene (Diaz et al., 2008). The main components of essential oil extracted from *S. molle* fruit collected from Syria (Damascus) were α-phellandrene (24.8%), β-pinene (14.7%), β-phellandrene (11%) and limonene (10.5%) (Ibrahim and Al-naser, 2014). The main components of essential oils obtained from leaves, stems and fruits of the Tunisian *plant* were β-eudesmol (14.8%), elemol (13.7%), α-eudesmol (12.7%), limonene (9.2%) and spathulenol (7.2%). Whereas, the main components in stem were elemol (20.7%), 6-epi-shyobunol (20.3%) and α-eudesmol (7.0%). Fruit essential oils were characterized by 6-epi-shyobunol (16.2%), limonene (15.3%), spathulenol (8.1%) and 4-epi-cubebol (7.8%) (Abir et al., 2016).

The essential oil of *S. molle* showed the maximum fungitoxic activity as compared with some other essential oils during the screening against common storage and animal pathogenic fungi (Dikshit et al., 1986). Some oils of *S. molle* showed potential anti-tumoral effects, either alone or in combination (Diaz et al., 2008). *S. molle* essential oils showed insecticidal activity against *Trogoderma granarium* and *T. castaneum* (Abdel-sattar et al., 2008). The essential oil and hexane extract of the plant showed potential result in term of antimicrobial and insect repellent activity (Onder et al., 2010). The essential oils extracted from *S. molle* leaves showed inhibitory effects, reduced growth of *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Salmonella setubals*, and *Candida albicans* (Simionatto et al., 2011). The essential oil isolated from the fruits showed higher antioxidant activity than the oil isolated from leaves (Abir et al., 2016). Five terpenes isolated from the bark resin of *S. molle* provided significant growth inhibitory effect against human colon carcinoma (Gonzalo et al., 2017).

The main aim of this study was to determine the chemical composition of *S. molle* essential oils from different locations in North Cyprus.

Materials and Methods

Plant material

Leaves and fruits of *Schinus molle* L. were collected on 6/10/2017 from Near East University Campus, Nicosia, North Cyprus. While, the other *S. molle* leaves and fruits were collected on 10/11/2017 from Kucukkaymakli area (KK), Şehit Mehmet street. Air dried leaves and fruits were used for distillation. The plant material was identified by Prof. Baser. Herbarium vouchers are deposited at the Herbarium of the Near East University (NEUN 6896 for the Near East University sample, and NEUN 6897 for the Küçükkaymaklı sample).

Leaves and fruits of plants from two locations were separately hydrodistilled using a Clevenger type apparatus for 3 h. Yields of essential oil for NEU sample were 1.88% (leaf), 0.48% (fruit); and for Küçükkaymaklı (KK) sample 1.62% (leaf), 0.62% (fruit) on moisture-free basis.

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MS system. Innowax FSC column (60 m, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 C˚/min, and kept constant at 220 °C for 10 min. Then, programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent GC system. FID detector temperature was 300 °C to obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components

Characterization of the essential oil components was carried out by comparison of their retention times with those of authentic samples or by comparison of their Linear Retention Indices (LRI) to a series of n-alkanes. Computer matching against commercial Wiley GC/MS library (MacLafferty and Stauffer, 1989), MassFinder 3 Library (Koenig et al., 2004) and in house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain and Koenig, 1998: ESO, 2000) was used for the identification.

Results and Discussion

As shown in Table 1, in total 67 constituents were identified. The main component was α-phellandrene in leaves and fruits of both NEU and K.K locations as 31.6% and 36.3%; 31.5% and 26.7%, respectively. Limonene was the second major component in leaves and fruits of NEU (11.4% and 13.3%) and KK localities (10.1% and 12.5%), resp. The third major component was β-phellandrene in NEU (10.9% and 12.2%) and KK (10.3% and 9.9%) in leaf and fruit samples, resp. Leaf oils contained bicyclogermacrene as main constituent in NEU (11.1%) and KK (12.0) samples. Elemol content in leaf oils varied between 9.3% (KK) and 6.3% (NEU). Fruit oils from both localities contained myrcene (19.9% for NEU; 18.5% for KK) as a major constituent. Its content was lesser in leaf oils. Moreover, fruits of KK contained the highest percentage of p-cymene (8.1%). In the other samples, the percentages were not more than 4.2%.

The results of this study have been compared with the previous results reported from Turkey and elsewhere. α- Phellandrene was a main component in leaf and fruit oils from Turkey (45.7 % and 38 %), respectively (Baser et al., 1997). However, no component in the leaf oils of the other Turkey sample was more than 7 % (Onder et al., 2010). The leaf oils of Algeria contained α-phellandrene (26.5 %), β-phellandrene (12.4%), β-eudesmol (14.8 %) and elemol (13.7 %) as main components (Belhamel et al., 2008). While, the Egyptian fruit oil contained α-phellandrene (25.1 %), limonene (20.9%) and myrcene (16.4%) as main components. In contrast, the Egyptian leaf oils contained p-cymene (9.4 %) as a main component, limonene, α-pinene and myrcene contents were less than 5 % (Dalia et al., 2016). The Tunisian leaf and fruit oils contained different main components. 6-epi-shyobunol (16.2 %), limonene (15.3 %) and spathulenol (8.1%) were the main components in the Tunisian fruit oils. Whereas, the leaf oils contained β-eudesmol (14.8 %), elemol (13.7 %) and α-eudesmol as the major components (Abir et al., 2016). The leaf oil of Costa Rican origin contained α-pinene (22.7%) and β-pinene (31.1%) as main components (Diaz et al., 2008). The Syrian fruit oils showed α-phellandrene (24.8 %), β-pinene (14.7 %) and β-phellandrene (11%) as main components (Ibrahim and Al-naser, 2014). The Uruguaian leaf oil contained bicyclogermacrene (29.2 %) as main component (Carmen et al., 2011).

The result of this study showed similarity with fruit and leaf oils from Turkey, Algerian leaf oils and Syrian fruit oils by having α-phellandrene and β-phellandrene as main components. Whereas, the differences in main components were observed Uruguaian oil, which contained bicyclogermacrene; and Costa Rican oils, which contained α-pinene and β-pinene as major components.

Table 1. Chemical components of *Schinus molle* essential oil.

LRI	Compound Name	KK% Fruit	NEU% Fruit	KK% Leaf	NEU% Leaf
1020	α-pinene	3.7	4.0	3.9	5.2
1119	β-pinene	0.2	0.2	0.1	0.1
1131	sabinene	0.1	0.1	0.3	0.2
1173	myrcene	18.5	19.9	1.9	1.4
1178	α-phellandrene	26.7	36.3	31.5	31.6
1181	pseudolimonene	0.1	0.1	0.1	0.1
1212	limonene	12.5	13.3	10.1	11.4
1221	1,8-cineole	0.4	-	-	-
1223	β-phellandrene	10.3	12.2	9.9	10.9
1263	(*E*)-β-ocimene	-	-	0.1	-
1288	*p*-cymene	8.1	4.2	2.1	4.2
1299	terpinolene	0.1	0.2	0.2	0.2
1400	methyl octenoate	0.9	0.5	-	-
1499	bicycloelemene	-	0.1	1.0	0.5
1555	α-gurjunene	-	-	0.2	-
1556	linalool	0.1	-	-	0.1
1580	*cis*-sabinene hydrate	-	0.1	-	-
1581	*trans-p*-menth-2-en-1-ol	0.4	-	-	-
1605	bornyl acetate	0.1	0.1	-	-
1613	β-elemene	0.1	-	0.7	0.4
1625	terpinen-4-ol	0.2	-	-	-
1627	β-caryophyllene	-	-	0.2	0.2
1638	aromadendrene	0.1	0.2	0.3	0.4
1661	γ-elemene	-	-	0.3	0.2
1685	allo aromadendrene	-	-	0.1	0.1
1688	epi-zonarene	-	-	tr	-
1701	*trans*-piperitol	0.2	-	-	-
1702	α-humulene	-	-	0.2	0.1
1710	cryptone	0.3	0.1	-	tr
1715	carvotanacetone	0.1	-	-	-
1717	-humulene	-	-	0.1	-
1726	ledene	-	-	0.2	0.2
1742	valencene	-	-	-	0.3
1743	germacrene D	-	-	0.3	-
1752	α-muurolene	0.1	-	0.5	0.3
1756	β-selinene	-	-	-	0.2
1759	selina-4,11 diene	-	-	-	0.2
1762	phellandral	0.1	-	-	-
1769	bicyclogermacrene	1.1	2.0	12.0	11.1

1786	δ-cadinene	0.3	0.1	1.4	0.6
1692	γ-cadinene	-	-	0.2	0.1
1802	cadina-1,4-diene (=cubenene)	-	-	0.1	-
1822	cuminaldehyde	0.1	-	-	-
1836	*p*-mentha-1(7),5-dien-2-ol	2.1	0.8	0.2	-
1859	carveol	0.1	-	-	-
1871	germacrene B	-	-	0.1	-
1873	*p*-cymen-8-ol	0.1	-	-	-
1916	epicubebol	-	-	0.1	-
1931	α-phellandrene epoxide	0.5	0.1	-	-
2072	ledol	-	-	0.1	-
2085	germacrene D-4-ol	-	-	-	0.3
2092	cubenan-11-ol	-	-	0.1	-
2098	cubenol	-	-	0.1	-
2108	elemol	4.0	1.4	9.3	6.3
2124	viridiflorol	-	-	0.6	-
2148	10-epi-γ-eudesmol	-	-	0.2	-
2160	spathulenol	2.0	2.0	2.0	5.3
2205	γ-eudesmol	0.6	0.2	1.7	1.1
2217	eremoligenol	-	-	-	0.2
2222	T-muurolol	0.1	-	0.4	0.2
2226	α-guaiol	0.1	-	0.1	0.2
2231	δ-cadinol	-	-	0.1	-
2244	thymol	0.7	0.2	0.2	0.2
2261	α-eudesmol	1.2	0.7	1.8	2.2
2268	α-cadinol	0.2	-	0.8	0.3
2273	β-eudesmol	1.4	0.6	1.9	2.4
	Total %	**98.4**	**99.7**	**97.8**	**99.0**

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RESEARCH ARTICLE

Comparison of *Juniperus oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarpa* berries essential oils from Turkey

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Abstract

Juniperus oxycedrus L. and related taxa are within the natural habitat in Turkey. In this present study, essential oils from *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarpa* berries, collected from different provinces of Turkey were obtained by hydrodistillation and analyzed for their chemical compositions. GC/MS analyses revealed α-pinene (19.9-47.8%), β-myrcene (44.6-10.3%) and germacrene D (15.5-13.5%) as main constituents of the berries, respectively. The olfactory evaluation showed characteristic woody, greenish, herbal, turpentine-like and balsamic sweety nature suggesting their uses especially in men's cosmetics.

Keywords: *Juniperus oxycedrus*, *Juniperus macrocarpa*, Berry essential oil, Olfactory

Introduction

The genus *Juniperus* (Cupressaceae), well known juniper, is a very common botanical species in northern hemisphere. The family is represented with around 70 subspecies. *Juniperus* sp. consists of a wide variety in especially Mediterranean countries and is used for its wood as construction material because of resistance to pests and other pathologies (Arapi et al., 2012). *J. oxycedrus* and *J. macrocarpa* are two of the 14 species that comprise the section Juniperus throughout the world (Medini et al., 2010). Both *J. oxycedrus* and *J. macrocarpa* are most distinctive subspecies of *Juniperus* family as their attractive volatile and non-volatile chemical composition (Adams, 1998; Barrero et al., 1993). Because of their volatile and non-volatile chemical composition diversity, they have attractive olfactive effects. *Juniperus* exists in Turkey by 7 species and 14 taxa. *Juniperus oxycedrus* has two subspecies – subsp. *oxycedrus* and subsp. *macrocarpa* – in Turkey (Alan et al., 2016).

Juniperus pseudo fruits, female cones – improperly called ''berries'' are used as a spice, mainly in European cuisine; they are used in Northern European and particularly Scandinavian cuisine to impart a sharp, clear flavour to meat dishes (Taviano et al., 2013).

Berries of *J. oxycedrus* have widely been used as medicinal herbal in folk medicine in order to threat gastrointestinal disorders, common colds, as expectorant in cough, to treat calcinosis in joints and as diuretic to pass kidney stone, against urinary inflammations, hemorrhoids, and as hypoglycemic; leaves and berries are applied externally for parasitic disease (Akkol et al., 2009; Loizzo et al., 2007; Sezik et al., 1997). In addition, *Juniperus* subspecies are used for various purposes, such as sweating antiseptic, and as a tar source. These species also contain oleoresins, a triterpene known as 'resin cadinene' and phenols (guaiacol and cresol derivatives) and they are used in the treatment of some skin ailments among many other uses (Digrak et al., 1999; Baytop, 1984).

The aim of this work was to extract the essential oils from the berries, compare their chemistry and olfactory properties for possible utilization in various cosmetics.

Materials and Methods

Plant material

Air dried *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarpa* berries were obtained from south of Turkey, Antalya and Isparta, in May, 2018.

Isolation of essential oils

The oils were obtained by hydrodistillation for 3 hours using a Clevenger apparatus. The essential oils were stored at 4 °C in the dark until analyzed. The oils were analyzed by GC/MS using Agillent GC-MS system. The essential oils were obtained from two type berries (*J. oxycedrus* and *J. macrocarpa*) in 1.6% and 1.9% yields, respectively.

Gas chromatography - mass spectroscopy (GC-MS) analysis

The GC/MS analysis was carried out with an Agilent 5975 C GC/MS system. 95% Dimethylpolysiloxane HP 5 MS column (30 m x 0.25 mm x 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 minutes and programmed to 130 °C at a rate of 4 °C/minutes, and kept constant at 220 °C for 1 min. and then programmed to 170 °C at a rate of 1.5°C/minutes, then programmed to 270 ºC at a rate of 20 ºC/minutes and kept constant 10 minutes. Split ratio was adjusted 30:1. The injector temperature was at 250 °C. Mass range was from *m/z* 35 to 400.

Characterization of components

Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention indices (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library) and *in-house* "Parkim Fragrance House GC/MS Library" built up by genuine compounds and components of known oils was used for the identification of oil components.

Olfactory evaluation panel

Olfactive parameters of the essential oils were evaluated by trained perfumers. Both essential oils, *J. oxycedrus* subsp. *oxycedrus* and *macrocarpa,* olfactive notes were determined for their characteristic odors and their usage area were classified with some features such as diffusion rate and deep of the olfactive note.

Results and Discussion

The oils were analysed by GC-MS, *J. oxycedrus* subsp*. oxycedrus* berries essential oil showed 15 compounds representing 97.3 percent of the oil. α-Pinene (19.9%), β-myrcene (44.6%) and germacrene D (15.5%) were characterized as main constituents.

J. oxycedrus subsp*. macrocarpa* berry essential oil showed overall 18 compounds representing 100 percent of the oil. α-Pinene (47.8%), sabinene (7.2%), β-myrcene (10.3%) and germacrene D (13.5%) were characterized as main constituents.

Table 1. Composition of the essential oils of *J. oxycedrus* subsp*. oxycedrus* and *macrocarpa* berries

RI	***t*R**	**Compounds**	**Relative percentage (%)**	
			J. oxycedrus* subsp*. macrocarpa	***J. oxycedrus* subsp*. oxycedrus***
1087	8.5	α-Pinene	36.5	22.7
1196	11.08	Sabinene	6.7	3.4
1222	11.15	β-Pinene	2.4	-
1275	12.2	β-Myrcene	18.3	41.8
1314	14.8	Limonene	2.2	2.9
1360	12.8	β-Phellandrene	-	0.6
1502	23.6	α-Cubenene	2.0	0.3
1617	25.82	Isobornyl acetate	-	0.2
1526	29.218	α-Copaene	0.8	-
1542	24.95	Unidentified sesquiterpene	-	0.5
1711	31.52	β-Caryophyllene	2.1	3.1
1731	32.69	β-Farnesene	-	0.7
1753	32.57	α-Humulene	1.8	2.8
1776	33.371	α-Amorphene	1.5	-
1702	31.175	Germacrene D	13.2	15.1
1865	36.548	α-Muurolene	1.3	0.7
1902	42.371	γ-Cadinene	1.6	1.8
1963	41.11	δ-Cadinene	2.4	-
2264	41.75	epi-α-Cadinol	0.9	-
2638	41.84	α-Cadinol	0.7	0.7
1849	35.46	Manoyl oxide	3.8	-
1856	36.48	Abietadiene	1.8	-

RI: Retention indices. Rt: Retention times in minutes. %; calculated from the GC-MS chromatograms; main constituent of the unidentified sesquiterpene is γ-terpinene.

In this present study, we investigated the essential oil chemical composition of berries of *J. oxycedrus* subsp*. oxycedrus* and subsp*. macrocarpa* from south of Turkey. Sezik et. al (2005) performed similar study with leaves of subsp*. oxycedrus* and subsp*. macrocarpa* was collected in Turkey. Another study was performed by Lesjak et al. (2014) as antimicrobial activity of subsp. *macrocarpa*. In this study samples were collected as leaves and berries. Berries samples had GC-MS data similar to ours.

Another parameter of our study was based on olfactive evaluation of subsp. *oxycedrus* and subsp. *macrocarpa* berries essential oils. *J. oxycedrus* berries essential oil consisted of α-pinene, β-myrcene and germacrene D, respectively. These components redounded olfactive results of *J. oxycedrus* subsp. *oxycedrus* essential oil with a turpentine-like juniper odor; fresh balsam, woody terpene. *J. macrocarpa* berries essential oil of our sample was mostly consisting of α-pinene, β-myrcene and germacrene D, however, percentages were different from that of *J. oxycedrus* subsp. *oxycedrus*, demonstrated in Table 1. The top note has a turpentine type odour with the woody dryness, and *Amyris balsamifera* and the sweetness of juniper berries combined with earthy elements of patchouli. Dry woody notes absolutely dominate the heart and as the oil dries out, one is flooded with intense balsamic sweetness.

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