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Comparison of Volatiles of *Sideritis caesarea* Specimens Collected from Different Localities in Turkey

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Sideritis caesarea H. Duman, Aytaç & Başer of the Lamiaceae is an Anatolian endemic species, and is mainly used as herbal tea and folk medicine. This present study aimed to determine and compare the chemical compositions of the volatile components of *S. caesarea* specimens collected from different localities in Kayseri province, Turkey: Sarız town (samples A and B), Pınarbaşı-Kaynar (sample C) and Pınarbaşı-Şirvan Mountain (sample D), respectively. Initially, the essential oils of the aerial parts were obtained by hydrodistillation and analysed both by gas chromatography-flame ionization detector (GC-FID), gas chromatography/mass spectrometer (GC/MS), simultaneously. Major volatile components of samples A and D were characterized as hexadecanoic acid (19.7 and 20.5%), caryophyllene oxide (6.7 and 20.2%), *β*-caryophyllene (6.5 and 12.6%), respectively. In sample B, hexadecanoic acid (14.6%), *β*-caryophyllene (11.5%) and caryophyllene oxide (8.3%) were detected as the major components. Whereas caryophyllene oxide (13.7%), hexadecanoic acid (8.5%) and spathulenol (6.1%) were the main components of the essential oil of sample C. Besides the chemical profiling, *in vitro* antimicrobial effects of samples were evaluated against a panel of six pathogenic microorganisms. Inhibition zones of the tested samples varied against bacteria with low to moderate activity, where no susceptibility against *Candida albicans* was observed.

Keywords: *Sideritis caesarea*, Volatiles, GC-FID and GC/MS profiling, *in vitro* Antimicrobial evaluation.

The genus *Sideritis* L., which includes nearly 150 species, is one of the largest of the Lamiaceae family. The members of this genus are distributed worldwide, mostly in the Mediterranean basin [1-3]. *Sideritis* sp. have long been used traditionally against bronchitis, pulmonary emphysema, cough, hypertension, 'worm in the eyes' syndrome, urinary tract infections, intestinal diseases, gastric ulcer, wounds, low back pain, eye infection or as appetite stimulant, antiseptic, expectorant, digestive, anti-inflammatory and as anti-rheumatic *etc*. [2, 4]. Turkey is one of the richest countries with 45 *Sideritis* species, which are commonly named as 'dağ çayı, yayla çayı, adaçayı, Alanya adaçayı, balbaşı, çay çalbası, sivri çayı' in different regions. In Turkish folk medicine they are mainly used against abdominal pain, cough, common cold and as carminative, stimulant, expectorant and as diuretic [1, 4-6].

Phytochemical studies revealed that *Sideritis* species contain terpenes, flavonoids, iridoids, coumarins, lignans, flavonoids, sterols and volatile components among others. These compounds were associated with anti-inflammatory, antioxidant, anti-ulcerogenic, analgesic, antimicrobial, antiproliferative, anti-HIV, antifeedant, antinociceptive, selective estrogen receptor modulatory and anti-herpes activity according to biological and pharmacological studies [2, 7].

The study material *S. caesarea* is an Irano-Turanian element and endemic species growing in Kayseri, Kahramanmaraş, Malatya, Adana and Yozgat. A literature survey resulted in relatively limited reports on this species [7-15].

In the frame of a folk medicinal survey, data on *S. caesarea* was reported for the first time by Özkan and Koyuncu (2005) [16]. According to this work, decoction of the aerial parts of this endemic plant was used as a remedy against stomach ache and intestinal spasms in Pınarbaşı, district of Kayseri province. Based upon this folk use claim, decoction of the aerial parts of this plant was investigated against ethanol induced ulcerogenesis, and 95.8% gastroprotective activity was shown *in vivo* [7, 16]. Furthermore, the *in vitro* antimicrobial activity of *S. caesarea* methanol extract against 19 microorganisms was previously reported showing varying inhibitory ranges [12, 13]. The antioxidant activity of the infusion of *S. caesarea's* aerial parts against trichloroacetic acid toxicity in rat was reported [11]. It was found that the extract has high level of antioxidant activity in case of chemically-induced oxidative injury. Sağdıç *et al*. (2008) also demonstrated by DPPH assay that methanol extract of aerial parts of *S. caesarea* has 72% antioxidant activity at 100 ppm concentration [13, 17-19].

Additionally, four diterpenoids (eubol, eubotriol, *ent*-7*α*,18-dihydroxy-15-oxokaur-16-ene, epoxysiderol), thirteen flavonoids [include (-)-epicatechin, (+)-catechin, penduletin, apigenin, naringin, luteolin, rutin, eriodictiol, isoscutellarein and hypolaetin derivatives] and other phenolic compounds [gallic acid, caffeic acid, and rosmarinic acid] were identified in *S. caesarea* [8-10]. Fatty acid composition of the seeds were also investigated by GC-MS. Oil yield was determined as 35.4% and linoleic acid (55%), oleic acid (18.5%) and 6-octadecynoic acid (12.9%) were reported as the main components [14]. In another study from one location conducted in 1999, the volatile components were investigated by GC-MS, and it was found rich in sesquiterpenes; mainly in *β*-caryophyllene and caryophyllene oxide [15].

In this present study, volatile components and antimicrobial activity of four specimens of *S. caesarea* collected from different locations in different times of the year were investigated in order to determine the potential chemical variations. The volatile chemical composition of four *S. caesarea* specimens collected from different sites in Turkey were analysed and compared by GC-FID and GC-MS analyses, which were listed in Table 1.

Table 1: The composition of the volatile components in essential oils obtained from different *S. caesarea* samples.

RRI	Compound	A	B	C	D
1118	*β*-Pinene	1.0	-	-	-
1400	Nonanal	0.6	-	-	-
1452	1-Octen-3-ol	2.1	1.7	2.1	-
1495	Bicycloelemene	0.8	0.9	0.4	-
1497	*α*-Copaene	0.7	0.4	-	-
1535	*β*-Bourbonene	0.4	tr	0.4	-
1548	(*E*)-2-Nonenal	tr	0.4	-	-
1553	Linalool	0.7	0.6	0.9	1.7
1562	Octanol	0.4	0.5	0.5	-
1586	Pinocarvone	0.5	0.3	0.6	-
1610	Calarene (=*β-gurjunene*)	-	0.1		-
1612	***β*-Caryophyllene**	**6.5**	**11.5**	**5.5**	**12.6**
1638	*β*-Cyclocitral	-	0.4	-	-
1648	Myrtenal	0.5	0.4	0.6	-
1655	(*E*)-2-Decenal	0.3	-	-	-
1664	Nonanol	-	-	0.5	-
1668	(*Z*)-*β*-Farnesene	1.5	2.3	2.1	-
1670	*trans*-Pinocarveol	0.6	0.5	1.0	-
1687	*α*-Humulene	0.4	0.6	-	-
1726	Germacrene D	1.0	-	-	-
1741	*β*-Bisabolene	-	0.4	-	-
1755	Bicyclogermacrene	1.7	1.8	-	-
1764	*(E)*-2-undecenal	0.4	0.6	-	-
1766	1-Decanol	-	-	0.9	-
1773	*δ*-Cadinene	0.7	0.7	-	-
1804	Myrtenol	0.5	0.5	0.7	-
1857	Geraniol	-	0.1	0.5	-
1868	(*E*)-Geranyl acetone	0.8	0.5	1.0	-
1882	Aplotaxene	-	0.1	-	-
1941	*α*-Calacorene	0.2	-	-	-
1958	(*E*)-*β*-Ionone	0.7	0.7	0.4	0.5
1965	2-Ethyl hexanoic acid	-	-	-	1.1
1973	1-Dodecanol	0.2	-	0.3	-
1981	Heptanoic acid	-	-	-	0.9
2008	**Caryophyllene oxide**	**6.7**	**8.3**	**13.7**	**20.2**
2037	Salvial-4(14)-en-1-one	-	0.1	-	-
2041	Pentadecanal	-	-	0.5	-
2084	Octanoic acid	0.2	-	-	0.3
2100	Heneicosane	0.4	0.7	0.5	-
2131	Hexahydrofarnesyl acetone	2.1	1.2	2.4	-
2144	**Spathulenol**	3.4	4.2	**6.1**	3.9
2148	(*Z*)-3-Hexen-1-yl benzoate	0.3	0.4	0.5	-
2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone	0.5	-	0.6	-
2186	Eugenol	0.2	0.5	-	-
2192	Nonanoic acid	1.2	1.0	0.6	0.8
2200	Docosane	-	0.4	-	-
2209	T-Muurolol	0.2	-	-	-
2210	Copaborneol	-	0.1	-	-
2247	*trans-α*-Bergamotol	**-**	**0.2**		**-**
2298	Decanoic acid	0.8	0.3	1.3	0.3
2300	Tricosane	1.0	1.0	1.0	-
2312	9-Geranyl-*p*-cymene	1.6	2.2	0.9	-
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol I*)	0.4	-	1.4	-
2337	Kaur-15-ene	-	0.3	0.2	-
2384	Farnesyl acetone	0.7	0.7	0.9	-
2389	Caryophylla-2(12),6-dien-5*α*-ol (=*Caryophyllenol I*)	0.6	0.2	1.2	1.1
2392	Caryophylla-2(12),6-dien-5*β*-ol (=*Caryophyllenol II*)	1.3	1.4	3.1	-
2500	Pentacosane	0.9	1.2	0.9	-
2503	Dodecanoic acid	1.0	0.5	0.9	-
2600	Hexacosane	-	0.7	-	-
2607	1-Octadecanol	-	0.2	-	-
2617	Tridecanoic acid	0.4	-	-	-
2622	Phytol	2.2	1.6	3.7	3.5
2655	Benzyl benzoate	0.5	0.5	1.8	2.3
2670	Tetradecanoic acid	2.9	1.7	1.9	2.6
2679	Manool	-	0.2	-	-
2700	Heptacosane	2.9	4.0	3.4	2.6
2722	Geranylgeraniol	5.3	3.1	1.0	-
2822	Pentadecanoic acid	1.1	0.7	0.6	-
2857	Palmito-*γ*-lactone	0.4	-	-	-
2900	Nonacosane	-	2.0	2.6	-
2931	**Hexadecanoic acid**	**19.7**	**14.6**	**8.5**	**20.5**
	Total	82.1	80.2	78.6	74.9

RRI: Relative retention indices calculated against *n*-alkanes. %: calculated from FID data. tr: Trace (< 0.1 %).

The overall number of the detected volatile compounds of samples A, B and C were similar (53, 55, 43, respectively) and accounted for 82.1%, 80.2%, 78.6% of the total compositions. Approximately 74.9% of the volatile composition of sample D was characterized, and only 16 compounds were identified.

As seen in the Table 1, major volatile components obtained from four samples were the same with minute differences. The major volatile components of samples A, B and D were characterized as hexadecanoic acid (19.7%, 14.6% and 20.5%), caryophyllene oxide (6.7%, 8.3% and 20.2%) and *β*-caryophyllene (6.5%, 11.5% and 12.6%). On the other hand, spathulenol (6.1%) was among the major constituents of sample C besides caryophyllene oxide (13.7%) and hexadecanoic acid (8.5%). Although the samples A and B were collected from the same location with a one-year interval, *β*-caryophyllene ratio of sample B was found nearly two fold more than that of the sample A. In addition, there were obvious differences and variations in volatile contents of samples that collected from different locations in the same year (Sample A, C and D). In sample C, hexadecanoic acid ratio was quite lower than those of sample A and D. Spathulenol was one of the major compounds only detected in sample C. Relatively high proportion of caryophyllene oxide in sample D was another significant difference. Major common compounds of each sample analysed were namely hexadecanoic acid, caryophyllene oxide and *β*-caryophyllene, however, having different ratios.

In addition, antimicrobial activities of volatiles obtained from four samples were studied against a panel of five human pathogenic bacteria and one microfungus (Table 2). As seen in Table 2, the test samples displayed varying degrees of growth inhibition (inhibition zones between 0.7-1.3) against the bacteria tested. Highest inhibition zones (1.3 mm) were observed with sample B on *Staphylococcus aureus* strain. Nevertheless, sample B was inactive against *Escherichia coli.* Samples showed no inhibition against *Candida albicans* at the tested concentrations.

Table 2: Inhibition zones (mm) of volatile samples (A-D)

Test Sample	*Escherichia coli* NRRL B-3008	*Staphylococcus aureus* ATCC 6538	*Pseudomonas aeruginosa* ATCC 27853	*Bacillus subtilis* NRRL B-4378	*Staphylococcus epidermidis* ATCC 14990	*Candida albicans* ATCC 90028
A	0.8	1	0.9	0.8	0.8	0
B	0	1.3	1.2	0.9	1	0
C	1	1.1	0.9	0.9	0.9	0
D	0.9	0.9	0.7	0.7	0.8	0
Chloramp.	0.9	1.8	0	NT	NT	NT
Amph B.	NT	NT	NT	NT	NT	2.0

Chloramp: Chloramphenicol, Amph. B: Amphotericin B, NT: Not tested

Kırımer *et al*. (1999) reported *S. caesarea* volatiles only from one locality, where *β*-caryophyllene (8.3%), caryophyllene oxide (7.4%), spathulenol (4.3%) and hexadecanoic acid (4.1%) were determined as predominant volatile components [15]. Supporting this, hexadecanoic acid was the major component of three samples in our results. In addition, bicycloelemene, *α*-copaene, calarene, (*Z*)-*β*-farnesene, 1-decanol, *δ*-cadinene, aplotaxene, *α*-calacorene, 2-ethyl hexanoic acid, 1-dodecanol, salvial-4(14)-en-1-one, eugenol, nonanoic acid, T-muurolol, copaborneol, *trans-α*-bergamotol, 9-geranyl-*p*-cymene, caryophylla-2(12),6(13)-dien-5*β*-ol, kaur-15-ene, caryophylla-2(12),6-dien-5*α*-ol, hexacosane, 1-octadecanol, tridecanoic acid were identified for the first time in *S. caesarea* volatiles in this present study. However, 48 compounds previously reported [15] were not identified in this present study.

Sideritis species were classified into six groups according to the major volatile components, namely; monoterpene hydrocarbon-rich, oxygenated monoterpene-rich, sesquiterpene hydrocarbon-rich, oxygenated sesquiterpene-rich, diterpene-rich and others [20, 21]. According to Kırımer *et al*. (1999), *S. caesarea* may be placed in the sesquiterpene-rich group, which is also consistent with our findings [15].

In a previous work reported by Cha *et al*., *β*-caryophyllene showed low antimicrobial activity (MIC 12.8 mg/mL) against various microorganisms [22]. Due to the panel containing *Escherichia coli, Staphylococcus aureus* and *Staphylococcus epidermidis* among the microorganisms tested, the results of this study can be considered as partly comparable with our work. On the other hand, Donati *et al*. also investigated antimicrobial activity of the same compound against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *and Candida parapsilosis* by disc diffusion method and reported no activity against the tested microorganisms [23]. According to these findings, antibacterial activity of *S. caesarea*'s could be associated with other volatile compounds.

Antimicrobial activity of spathulenol, the other major constituent of *S. caesarea* was previously studied against 9 microorganism including *E. coli*, *S.aureus* and *Staphylococcus epidermidis*. The results revealed that spathulenol excerted antibacterial activity against the panel of three bacteria [24]. Therefore, inhibitory activity against *E. coli*, *S. aureus* and *S. epidermidis* observed in our study can be associated with spathulenol. Previous reports on ineffective spathulenol against *Candida albicans* supports our results [24].

Costa *et al*., suggested that high caryophyllene oxide proportion in the essential oil, may result in stronger antifungal activity [25]. In our study, volatile content of *S. caesarea* did not exhibit any activity against *Candida albicans.* [25]. In a different research, at a dose of 15 mg/disc, caryophyllene oxide showed 11.5, 14.6 and 14.3 mm inhibition zone diameters against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*, respectively. No inhibitory effects on *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Escherichia coli.* 6 mg/mL MIC values were obtained against *Aspergillus fumigatus* and *Candida albicans* and 0.1 and 0.625 mg/mL MLC values were obtained against *Trypanosoma brucei brucei* and *Trichomonas vaginalis,* respectively [26]. Rasoarivelo *et al*. evaluated the caryophyllene oxide's antibacterial activity against 31 reference and 22 clinical strains. MIC values were found between 0.75 to >200 mg/L [27]. In another work, caryophyllene oxide showed weak activity on *Mycobacterium tuberculosis* [28] and antifungal activity against *Paracoccidioides brasiliensis* and *P. lutzii* isolates, respectively [29].

With the contribution of above mentioned literature results, we can conclude that occurrence of *β*-caryophyllene, caryophyllene oxide and spathulenol as the major volatile components of *S. caesarea* highlights the importance of chemical composition of the essential oil in view of antimicrobial effect. There are previous reports that focused on antibacterial and antifungal activity of *S. caesarea* methanol extracts [12, 13]; but to the best of our knowledge, antimicrobial activity of *S. caesarea'*s volatile content was evaluated first time in our research.

Over all as a conclusion, the volatile composition of four *S. caesarea* samples collected from different periods and localities were determined comparatively. Major volatile components of the evaluated samples were hexadecanoic acid, caryophyllene oxide, *β*-caryophyllene, and spathulenol in varying ratios. Differences in the volatile components according to their locality and periods were reported in this present work.

Experimental

Plant material: Aerial parts of *S. caesarea* were collected in different locations in different times (Table 3). Plants were dried in the shade at room temperature and kept under appropriate conditions until experiment were carried out. Herbarium specimens were stored in Gazi University Faculty of Pharmacy Herbarium (GUE).

Table 3: Herbarium information of *S. caesarea* samples.

Samples	Collected locality	Date	GUE no
A	Kayseri, Sarız District, Dayıoluk village	04.07.2013	3229
B	Kayseri, Sarız District, Dayıoluk village	07.07.2014	3232
C	Kayseri, Pınarbaşı District, Kaynar burg	16.07.2013	3230
D	Kayseri, Pınarbaşı District, Şirvan Mountain	16.07.2013	3228

Isolation and analysis of volatile components: The volatile components of dried plant materials were obtained by hydrodistillation for 3 hours, using a Clevenger-type apparatus to produce a small amount of volatile components which was trapped in *n*-hexane. The obtained oils were dried over anhydrous Na_2SO_4 and stored at 4°C in the dark until analysed.

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

GC-FID analysis: The GC analysis was carried out using an Agilent® 6890N GC system. The flame ionisation detector (FID) temperature was set to 300°C. To obtain the same elution order as GC-MS, simultaneous auto-injection was performed on a duplicate of the same column where the same operational conditions were applied. Relative percentages (%) of the separated compounds were calculated from FID chromatograms.

Identification and characterization of components: Identification of the volatile components was carried out by comparison of their relative retention times with those of authentic samples or by comparing their relative retention index (RRI) to series of *n*-alkanes. Computer matching against a commercial (Wiley GC/MS Library, MassFinder 3 Library) and in-house Baser Library of Essential Oil Constituents built genuine compounds and components of known oils, as well as MS literature data was used for identification [30-33].

Antimicrobial activity

Preparation: Bacterial cultures were kept at -20°C or -85°C, prior incubation on Mueller Hilton Agar (MHA) or Mueller Hinton Broth (MHB) at 37°C for 24 hours, while *Candida albicans* strain was incubated on Potatoes Dextrose Agar (PDA) and RPMI at 37°C for 24 hours. After incubation, microorganism culture densities were adjusted turbidometrically to McFarland No: 0.5 tube [34, 35]. Test samples were dissolved initially in dimethyl sulfoxide and diluted with distilled water.

Agar disc diffusion: 100 µL bacterial strains were inoculated to Petri dishes contain 20 mL MHA or PDA. 20 µL of the sample was added to discs with 6 mm diameter placed onto agar surface. After incubation at 37°C for 24 hours, inhibition zones were measured in triplicates and reported as mean mm [36].

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