Comparison of Essential Oils *of Ferula orientalis* L., *Ferulago sandrasica* Peşmen & Quézel and *Hippomarathrum microcarpum* Petrov and Their Antimicrobial Activity

Ferula orientalis L., *Ferulago sandrasica* Peşmen & Quézel ve *Hippomarathrum microcarpum* Petrov'un Uçucu Yağ ve Antimikrobiyal Etkilerinin Karşılaştırılması

ABSTRACT

Objectives: To determine the chemical composition and antimicrobial activity essential oils of aerial parts of *Ferula orientalis L.,* roots of *Ferulago sandrasica* Peşmen & Quézel and aerial parts of *Hippomarathrum microcarpum* Petrov.

Materials and Methods: Essential oils were analysed by GC and GC/MS. The antimicrobial activity of the essential oils were determined with bioautography assay.

Results: α-pinene (75.9%) and β-pinene (3.4%) were found as major components for aerial parts of *F. orientalis;* limonene (28.9%), α-pinene (15.6%) and terpinolene (13.9%) for *F. sandrasica;* β-caryophyllene (31.4%) and caryophyllene oxide (23.1%) for aerial parts of *H. microcarpum*, respectively. Essential oils from aerial parts of *F. orientalis* and roots of *F. sandrasica* and from aerial parts of *H. microcarpum* were active against *Staphylococcus aureus* and *Candida albicans* strains. However, essential oils were not active against *Pseudomonas aeruginosa* and *Escherichia coli*.

Conclusion: The antimicrobial activities against *Staphylococcus aureus* and *Candida albicans* of these species may be attributed to the presence of the main components in the essential oils.

Key words: Antimicrobial, Bioutography, *Ferula, Ferulago*, *Hippomarathrum*

ÖZET

Amaç: *Ferula orientalis* L.'nin toprak üstü kısımlarından, *Ferulago sandrasica* Peşmen & Quézel'in köklerinden ve *Hippomarathrum microcarpum* Petrov'un toprak üstü kısımlarından elde edilen uçucu yağların içeriğini ve antimikrobiyal aktivitelerini belirlemek.

Gereç ve Yöntemler: Bu çalışmada türlerden elde edilen uçucu yağların içerikleri GC ve GC/MS ile analiz edilmiştir. Antimikrobiyal aktivite biyootografi yöntemiyle incelenmiştir.

Bulgular: Sırasıyla; α-pinen (%75.9) ve β-pinen (%3.4) *F. orientalis'*in toprak üstü kısımlarının; limonen (%28.9), α-pinen (%15.6) ve terpinolen (%13.9) *F.* sandrasica'nın köklerinin; β-karyofillen (%31.4) ve karyofillen oksit (%23.1) *H. microcarpum'un* toprak üstü kısımlarının ana bileşenleri olarak bulunmuştur. *F. orientalis*'in toprak üstü kısımlarından ve *F. sandrasica*'nın köklerinden elde edilen uçucu yağlar *Staphylococcus aureus* ve *Candida albicans* türlerine karşı etkili olduğu görülürken, *Pseudomonas aeruginosa* ve *Escherichia coli*'ye karşı etkisiz olduğu görülmüştür. *H. microcarpum*'un toprak üstü kısımlarının *P. aeruginosa*, *S. aureus, C. albicans* ve *E. coli*'ye karşı etkisiz olduğu tespit edilmiştir.

Sonuç: Bu türlerin *Staphylococcus aureus* ve *Candida albicans*'a karşı antimikrobiyal aktiviteleri uçucu yağlarında bulunan ana bileşenlerin varlığından kaynaklanabilir.

Anahtar kelimeler: Antimikrobiyal, Biyootografi, *Ferula, Ferulago*, *Hippomarathrum*

INTRODUCTION

Ferula L. genus is the member of Apiaceae family and has been found to be a rich source of gum-resin.[1] *Ferula* species are known as "Çakşır, asaotu, kıngor, heliz etc." [2], *F. orientalis* is known as "heliz" in Turkey[3] and have been used as carminative, sedative, laxative, antispasmodic, digestive, expectorant, diuretic, aphrodisiac, antiseptic, anthelminitic, analgesic[4] and stimulants.[5] *Ferula* species have been found to contain sesquiterpenes and sesquiterpene coumarins.[6] Fresh peeling stems of *Ferula orientalis* L., known as ''at kasnisi'' is used by local people to give flavor to the pickles.[5] It is 100–150 cm high, grows on rocky slopes at 1600–2900 m and has distinguished yellow flowers, with a flowering time in late May and June.[7] *Ferulago* W. Koch. is represented by approximately 83 taxa throughout the world and is a perennial genus of Apiaceae.[8] *Ferulago* species are known as "Çakşır, şeytanteresi, kişniş" and *F. sandrasica* is known as "kuzu kişnişi" in Turkey.[2] Since ancient times

Ferulago species have been used for the treatment of intestinal worms, hemorrhoids, as tonic, aphrodisiac, digestive, sedative, ulcers, against snake bites, spleen diseases and headache. These species have been found to involve coumarins, quinones, flavonoids and sesquiterpenes.[9] *F. sandrasica* Peşmen & Quézel is an endemic glabrous species, 30-35 cm high, grows on rocky serpentine slopes, at 2000 m and it is flowering time in June and July.[7]

The genus of *Hippomarathrum* Link is a member of Apiaceae family and it has five species. *Hippomarathrum* is an erect, much-branched perennial genus, 50-100 cm high and distributed in rocky slopes and fields. *H. microcarpum* is also used as food and is known "çakşır" or "çaşır" by local people in Eastern Anatolia of Turkey.[10] The species of this genus have long been used as spice in ethnobotany.[11] *H. microcarpum* Petrov is a gray shrub filled with yellowish flowers[7] and it is reported that the coumarins and furanocoumarins found in the roots and fruits of the genus of *Hippomarathrum*.[12] Essential oils or their components have been shown to exhibit antimicrobial, antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties. It is considered that these characteristics are related to the function of these compounds in plants.[13]

The aim of this study was to presented and compared the chemical composition of the essential oils of aerial parts of *F. orientalis*, roots of *F. sandrasica* and aerial parts of *H. microcarpum* growing wild in Turkey. We determined chemical composition of the essential oils by GC and GC/MS analysis and examined the antimicrobial activities of essential oils by thin-layer chromatography (TLC)-bioautography assay. To the best of our knowledge, this is the first report on the chemical composition and antimicrobial activity of essential oils for *F. orientalis, F. sandrasica* and *H. microcarpum*.

MATERIALS AND METHODS

Plant Material

The plant materials were collected from different parts of Turkey and were identified by Prof. Dr. Hayri Duman (Gazi University, Faculty of Science, Department of Biology) and the voucher specimens are kept in AEF (Herbarium of Ankara University Faculty of Pharmacy). Locality of these species are given in Table 1.

Isolation of the essential oil

Roots and aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus in accordance with the method recommended in the European Pharmacopoeia. Obtained oils were dried in anhydrous sodium sulphate and stored in sealed vials at +4°C in the dark till analysed and tested. All oils were pleasant smelling, transparent with a faint yellow and greeny colour. Essential oil % yields of the aerial parts of *Ferula orientalis, roots of Ferulago sandrasica* and aerial parts of *Hippomarathrum microcarpum* are 0.022%, 0.019% and 0.048% respectively.

GC/MS analysis

GC/MS analysis was performed with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 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 and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1 and injector temperature was set at 250°C. Mass spectra were recorded at 70 eV and mass range was from m/z 35 to 450.

GC analysis

GC analysis was performed with an Agilent 6890N GC system. Temperature of FID detector was 300°C. In order to obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column conforming the same operational conditions. Relative percentage quantities of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 2. Identification of the essential oil components were performed 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 [14,15] and in-house “Başer Library of Essential Oil Constituents” established by genuine compounds and components of known oils, alongside MS literature data[16,17] was used for the identification.

Determination of Antimicrobial Compounds from Essential oils by TLC-Bioautography Assay

Chromatography was carried out on 0.2 mm silica gel 60 F_{254} aluminum sheet TLC plates. 10 µl essential oils were applied with minicaps capillary pipette to the TLC plates. TLC plates were developed with toluene:ethyl acetate, 93:7, as a mobile phase and TLC plate for bioautography was prepared in parallel. After the development, TLC plates were valued at UV 254 nm and 366 nm for determination of fluorescent compounds. To visualize alcoholic vanillin–sulphuric acid reagent was used the separated compounds and heated for 3 min at 110°C.

Preparation of Microorganisms and TLC Bioautography Assay

After TLC separation, the antimicrobial activity of the essential oils were determined with direct bioautography.[18,19] *Pseudomonas aeruginosa* ATCC 13388, *Staphylococcus aureus* ATCC BAA 1026, *Candida albicans* ATCC 24433 and *Escherichia coli* NRRL B-3008 strains were used for bioautography. Microbial suspensions were grown overnight in double strength and Mueller-Hinton broth (MHB) were standardized 10^8 CFU.mL^{-1} (corresponding to McFarland no: 0.5). TLC plates were placed on Nutrient Agar plates and Molten Agar culture medium containing inocula was overlayed TLC plates and incubated at 37°C for 24h. Then by incubation, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) solution was sprayed on TLC plates. The treated plates were incubated at 37°C for 2 h and afterwards incubation, the inhibition zones were visible as pale spots against a red background.

RESULTS

A total of thirteen compounds were identified for essential oil of aerial parts of *Ferula orientalis*, representing 96.6% of the oil. α-pinene (75.9%), β-pinene (3.4%), trans-verbenol (3.0%) and β-caryophyllene (2.5%), were found as major components, respectively. The analysis on the roots of *Ferulago sandrasica* resulted in the identification of sixty-nine essential compounds representing 96.0% of the oil. Limonene (28.9%) was found the most abundant compound in the essential oil, followed by α-pinene (15.6%), terpinolene (13.9%), camphene (2.6%), myrcene (2.8%), p-cymene (2.8%) and 2,3,6-trimethylbenzaldehyde (3.2%).

Twenty-one compounds were characterized in the oil of the roots of *Hippomarathrum microcarpum* representing 98.7% of the oil. The major constituents were found as, β-caryophyllene (31.4%), caryophyllene oxide (23.1%), bornyl acetate (9.1%), α-humulene (4.9%), germacrene D (4.2%), β-phellandrene (4.6%), α-pinene (3.0%) and caryophylla-2(12),6-dien-5β-ol (=caryophyllenol II) (3.0%). The essential

oils obtained from these species did not show much qualitative and quantitative similarity. α-pinene, camphene, β-pinene, limonene, β-phellandrene, p-cymene and β-caryophyllene were determined main compounds for three species. Trans-verbenol was observed main compound for essential oils of *F. orientalis* and *F. sandrasica.* Thuja-2,4(10)-diene, pinocarvone, trans-pinocarveol, myrtenol and cuparene were only found for essential oils of the aerial parts of *F. orientalis*.

Sabinene, α-phellandrene, (Z)-β-ocimene, γ-terpinene, (E)-β-ocimene, terpinolene, α-copaene, bornyl acetate, α-humulene, germacrene D, δ-cadinene, caryophyllene oxide and humulene epoxide-II were determined main compounds for essential oils of *F. sandrasica and H. microcarpum.* Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*) was only found for essential oils of the aerial parts of *H. microcarpum.* The composition of the essential oils obtained from these species and their relative percentages are given in Table 2.

Results of antimicrobial activity by bioautography method showed that essential oil of from aerial parts of *F. orientalis* and root of *F. sandrasica* were active against *S. aureus* and *C. albicans* strains. However, they were not active against *E. coli* strain. Similarly, essential oil of from aerial parts of *H. microcarpum* was found that contain active compounds against *S. aureus* and *C. albicans*. Essential oil was determined more effective againts *C. albicans* than *S. aureus.* But it had not good activity againts *E. coli*. Essential oils did not give any inhibiton zone against *P. aeruginosa*. TLC evaluation of the essential oils was shown in Figure 1.

DISCUSSION

Monoterpene hydrocarbons; P-cymene, myrcene, γ-terpinene, limonene, terpinolene and (Z)-β-ocimene, oxygenated monoterpenes; carvacrol methyl ether, 2,5-Dimethoxy-pcymene, trans-Chrysanthenyl acetate, cis-Chrysanthenyl acetate and ferulagone, aldehydes like; 2,3,6-Trimethylbenzaldehyde, (E)-2-Decenal and octanal and alkane derivatives; hexadecanoic acid, sesquiterpene hydrocarbons; α-humulene, 4,6-guaiadiene and 7-Epi-1,2-dehydrosesquicineole and oxygenated sesquiterpenes like cubenol, humuleneepoxide II and spathulenol were found as major components of some *Ferulago* species.

Some *Ferula* species contain monoterpene hydrocarbons; β-Pinene, sabinene, camphene, β-phellandrene and (E)-β-ocimene, alkane derivatives;

nonane,–sesquiterpene hydrocarbons; germacrene D, germacrene B, δ-Cadinene, (Z)-β-Farnesene, dehydrosesquicineole and eremophilene; and oxygenated sesquiterpenes; germacrene D-4-ol, α-Cadinol, shyobunone, epi-shyobunone, 6-Epi-shyobunone, β-Eudesmol and α-Eudesmol were found as major components of some *Ferula* species.

In addition; esters like bornyl acetate was found to be major component of some *Ferula* and *Ferulago* species.[20]

Previous studies demonstrated that the major components of essential oil of leaves from *Ferulago sandrasica* were ocimene (30.5%), carene-δ-3 (27.4%) and α-pinene (17.8%).[19] Baser et al. studied twelve *Ferulago* (*F. asparagifolia* Boiss., *F. aucheri* Boiss. , *F. confusa* Velen, *F. galbanifera* (Mill.) W.D. J. Koch, *F. humilis* Boiss., *F. idaea* Özhatay & Akalın, *F. macrosciadia* Boiss. & Balansa, *F. mughlae* Peşmen, *F. sandrasica* Peşmen & Quézel, *F. silaifolia* (Boiss.) Boiss., *F. sylvatica* (Besser) Rchb. and *F. trachycarpa* Boiss.) species growing in Turkey and this study showed that the major components of essential oils were 2, 3, 6-trimethylbenzaldehyde (38.9%) and myrcene (18.2%); α-pinene (35.9%); 2,5-dimethoxy-p-cymene (63.4%); α-pinene (31.8%) and sabinene (15.8%); (Z)-β-ocimene (32.4%); pcymene (18.4%); carvacrol methyl ether (78.1%); α-pinene (25.4%); α-pinene (40.8%); trans-chrysanthenyl acetate (83.5%); pcymene (45.8%); (Z)- β-ocimene (30.7%) respectively.[21]

Major components of essential oils some *Ferula* species were demonstrated as; phenol, 2-methyl-5-(1-methylethyl) (18.2%), cyclopropa [a] naphthalene-octahydro-tetramethyl (6.6%), α–bisabolol (10.4%) (3); α-pinene (18.3%), β-pinene (50.1%), δ-3-carene (6.7%).[22] Comparing these results with previous studies that of *F. orientalis* showed that the major components were; nonane (45.6%) and 2-methyloctane (19.4%).[23] Furthermore; essential oils from aerial parts of *F. orientalis* were obtained; β-phellandrene (24%), (E)-β-ocimene (14%), α-pinene (13%), α-phellandrene (12%), and dehydrosesquicineole (10%)[24] but α-pinene (75.9%) was showed as a major component in our study.

Comparing these results with previous studies that of *H. boissieri* from Turkey[18] showed that the major components of essential oils from both species were β-caryophyllene (31.4% for aerial parts oil of *H. microcarpum*, 25.6% for aerial parts oil of *H. boissieri*). Another study showed that the major components of essential oils

leaf and flower of *H. microcarpum* were α-caryophyllene(26.4%), γ-muurolene (19.0%), and linalool (6.1%); (18.5%), γ-muurolene (19.2%), thymol (6.9%), and linalool (5.9%), respectively.[10] The results gained in this investigation suggest that this chemical diversity may be useful in taxonomic classification.

There is not enough data on antimicrobial activity for these species. In a previous survey, *F. sandrasica* essential oil was tested againts *E. coli* MC 400, *E. coli* ATCC 25922, *E. coli* 0157 H7, *E. colaecea* ATCC 23355, *E. feacalis* ATCC 19433, *P. aeruginosa* NRRL B-2679, *S. aureus* ATCC 25923, *S. aureus* ATCC 33862, *B. cereus* NRRL B-3711, *B. subtilis* ATCC 6633, *B. subtilis* NRRL B-209, *B. nischenoformis* NRRL B-1001, *Micrococcus luteus* NRRL B-1013, and *Listeria monocytogenes* ATCC 7644 by disc diffusion method. The results showed that the essential oil was active against all tested microorganisms.[18] It was previously reported that the essential oil of *H. microcarpum* was studied for antimicrobial and antifungal activity. The results showed that the essential oil of *H. microcarpum* had antimicrobial activity against *Candida albicans* A117 and *Staphylococcus aureus* ATCC-29213 but had no activity againts *Escherichia coli* A1 and *Pseudomonas* sp.[10] Our finding concur with this study.

Bioautography is suitable method for evaluating essential oils because of they contain mixture of compounds. Therefore, there is a need for the detection of common antimicrobial compounds in essential oils. Additionally, this method is rapid, easy economic and inexpensive.[25] The present study, we aimed to chemical characterization of the essential oils of *F. orientalis, F. sandrasica* and *H. microcarpum* and the detection of antimicrobial activity of essential oils and their main components against some pathogenic bacteria and yeast by TLC bioautography. For the purpose of antimicrobial activity was performed against four different microorganisms showed that the essential oils were active against *S.aureus* and *C. albicans* strains; however they were not active against *P. aeruginosa* and *E. coli* strains.

CONCLUSION

In conclusion, these data have provided a abundance of information on the essential oils composition of *F. orientalis, F. sandrasica* and *H. microcarpum* and their antimicrobial activities againts some pathogenic microorganisms. As far as we know,

this is the first report on antimicrobial activity of essential oils by TLC bioautography method. The antimicrobial activities againts *Staphylococcus aureus* and *Candida albicans* of these species may be attributed to the presence of the main components in the essential oils. A comprehensive study should be studied, including main compounds isolated from the essential oils or their combinations againts different pathogenic microorganisms.

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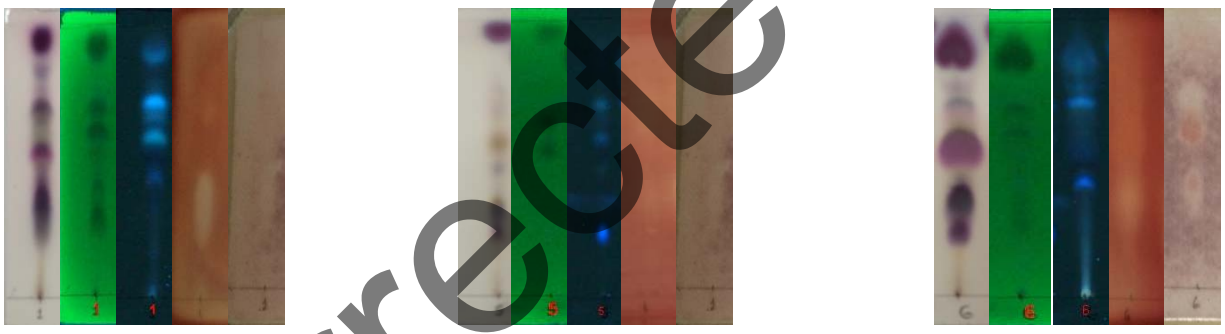

V/H_2S0_4 366nm 254nm SA CA V/H_2S0_4 366nm 254nm SA V/H_2S0_4 366nm 254nm SA CA

V/H_2S0_4: Vanillin/H_2S0_4 reagent, SA: *Staphylococcus aureus* ATCC 6538, CA: *Candida albicans* ATTC 90028

Figure 1. TLC separation of the essential oil from the *Ferula orientalis, Ferulago sandrasica and Hippomarathrum microcarpum* on silica gel 60 F254.

Table 1. Locality of the species

Species	Locality	Herbarium number
Ferula orientalis	B9: Between Ağrı-Erzurum, Mountain of Tahir, 2475 m, 13.07.2014.	AEF 10966
Ferulago sandrasica	C2: Mountain of Sandras 3 km to Kartal lake, Under *Pinus nigra* trees, in Muğla, 1675 m, 10.6.2013	AEF 26274
Hippomarathrum microcarpum	C5: Adana, south of Tufanbeyli, 13.07.2014	AEF 26699

Table 2. Chemical composition of the essential oil of *F. orientalis, F. sandrasica and H. microcarpum.*

RRI	Compound	*F. orientalis* %	*F. sandrasica* %	*H. microcarpum* %
1032	α-Pinene	75.9	15.6	3.0
1072	α-Fenchene	0.6	0.3	-
1076	Camphene	3.4	2.6	1.5
1093	Hexanal	-	0.1	-
1118	*β*-Pinene	-	0.3	tr
1132	Sabinene	-	0.1	1.1
1135	Thuja-2,4(10)-diene	2.0	-	-
1151	δ-4-Carene	-	tr	-
1159	*δ*-3-Carene	-	0,1	-
1174	Myrcene	-	2.8	-
1176	α -Phellandrene	-	-	2.0
1188	α-Terpinene	-	0.2	-
1203	Limonene	1.4	28.9	1.9
1218	β-Phellandrene	1.3	0.6	4.6
1244	2-Pentyl furan	-	0.1	-
1246	(Z)- β-Ocimene	-	0.8	tr
1255	γ-Terpinene	-	1.9	tr
1266	(*E*)-β-Ocimene	-	tr	2.0
1280	*p*-Cymene	2.2	2.8	1.9
1290	Terpinolene	-	13.9	1.4
1294	1,2,4-Trimethyl benzene	-	0.1	-
1452	α,*p*-Dimethylstyrene	-	0.8	-

1468	*trans*-1,2-Limonene epoxide	-	0.3	-
1479	δ-Elemene	-	0.3	-
1497	α-Copaene	-	0.2	0.6
1532	Camphor	-	0.3	-
1538	*trans*-Chrysanthenyl acetate	-	0.2	-
1586	Pinocarvone	tr	-	
1591	Fencyl alcohol	-	1.3	9.1
1598	Camphene hydrate	-	0.1	-
1600	β-Elemene	-	0.6	-
1612	*β*-Caryophyllene	2.5	0.8	31.4
1614	Carvacrol methyl ether	-	1.3	-
1670	*trans*-Pinocarveol	2.0	-	-
1683	*trans-Verbenol*	3.0	-	-
1684	Isoborneol	-	1.3	-
1683	*trans*-Verbenol	-	0.2	-
1687	α-Humulene	-	0.3	4.9
1704	γ-Muurolene	-	0.2	-
1706	α-Terpineol	-	1.8	-
1707	δ-Selinene	-	0.3	-
1719	Borneol	-	0.8	-
1726	Germacrene D	-	1.4	4.2
1742	β-Selinene	-	0.3	-
1744	α-Selinene	-	0.3	-
1751	Carvone	-	0.1	-
1773	δ-Cadinene	-	1.6	0.6
1776	γ-Cadinene	-	0.9	-
1779	(*E,Z*)-2,4-Decadienal	-	0.1	-

1786	*ar*-Curcumene	-	0.3	-
1796	Selina-3,7(11)-diene	-	0.4	-
1804	Myrtenol	1.6	-	-
1807	α-Cadinene	-	0.4	-
1827	(*E,E*)-2,4-Decadienal	-	0.4	-
1849	Cuparene	0.7	-	-
1864	*p*-Cymen-8-ol	-	0.2	-
1878	2,5-Dimethoxy-*p*-cymene	-	0.3	-
1918	β-Calacorene	-	tr	-
1941	α-Calacorene	-	0.1	-
1945	1,5-Epoxy-salvial(4)14-ene	-	0.2	-
2008	Caryophyllene oxide	-	0.3	23.1
2019	2,3,6-Trimethylbenzaldehyde	-	3.2	-
2037	Salvial-4(14)-en-1-one	-	0.1	-
2071	Humulene epoxide-II	-	0.1	2.4
2073	β-Caryophyllene alcohol	-	0.1	-
2080	Cubenol	-	0.6	-
2080	Junenol (=*Eudesm-4(15)-en-6-ol*)	-	0.2	-
2096	Elemol	-	0.1	-
2130	Salviadienol	-	0.2	-
2144	Spathulenol	-	0.1	-
2209	T-Muurolol	-	0.1	-

2255	α-Cadinol	-	0.5	-
2256	Cadalene	-	0.2	-
2269	Guaia-6,10(14)-dien-4β-ol	-	0.2	-
2369	Eudesma-4(15),7-dien-4β-ol	-	0.2	-
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	-	-	3.0
	Total	96.6	96.0	98.7

Notes: [a] RRI: Relative retention indices calculated against *n*-alkanes.[b] % calculated from FID data. [c] tr: trace (< 0.1%)