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

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

**Amaç:** *Ferula orientalis* L.’nin toprak üstü kısımlarından, *Ferulago sandrasica* Peşmen ve Quézel’in köklerinden ve *Hippomarathrum microcarpum* Petrov’un uçucu yağlarının içeriğini ve antimikrobiyal aktivitelerini belirlemektir.

**Gereç ve Yöntemler:** Bu çalışmada türlerden elde edilen uçucu yağların içerikleri gaz kromatografisi ve gaz kromatografisi/kütle spektrometresi ile analiz edilmiştir. Antimikrobiyal aktivite biyootografi yöntemiyle incelenmiştir.

**Sonuç:** *Ferula orientalis* L.’nin toprak üstü kısımlarının uçucu yağında %75.9 ve %3.4’lü *α*-pinen ve *β*-pinen, *Ferulago sandrasica* Peşmen ve Quézel köklerinin uçucu yağında ise %28.9, %15.6 ve %13.9’lu limonene, *F. orientalis* ve *F. sandrasica* köklerinin uçucu yağında ise %31.4 ve %23.1’lü *β*-karyofillen ve karyofillen oksit bulunmuştur. *F. orientalis* ve *F. sandrasica* uçucu yağlarının *Staphylococcus aureus* ve *Candida albicans* türlerine karşı etkisi, *H. microcarpum* uçucu yağının ise *Pseudomonas aeruginosa*, *S. aureus*, *C. albicans* ve *E. coli*’ye karşı etkisi hemen hemen ilk öne çıkmıştır.

**Anahtar kelimeler:** Antimikrobiyel, biyootografi, *Ferula*, *Ferulago*, *Hippomarathrum*
INTRODUCTION

The genus Ferula L. is a member of the family Apiaceae and has been found to be a rich source of gum resin.¹ Ferula species are known in Turkey as “çakşır”, “asaotu”, “kıngor”, “heliz” etc.,² and Ferula orientalis is known as “heliz”,³ and they have been used as a carminative, sedative, laxative, antispasmodic, digestive, expectorant, diuretic, aphrodisiac, anti-septic, antihelmintic, analgesic,⁴ and stimulant.⁵ Ferula species have been found to contain sesquiterpenes and sesquiterpene coumarins.⁶ Fresh peeling stems of F. orientalis L., known as “at kasnisi” are used by local people to give flavor to pickles.⁷ 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.⁸ Ferulago W. Koch. is represented by approximately 83 taxa throughout the world and is a perennial genus of Apiaceae.⁹ Ferulago species are known as “çakşır”, “şeytanteresi”, and “kişniş” in Turkey and Ferulago sandrasica is known as “kuzu kınişi”.⁸ Since ancient times Ferulago species have been used for the treatment of intestinal worms and hemorrhoids; as a tonic, aphrodisiac, digestive, and sedative; and against ulcers, snake bites, spleen diseases, and headache. These species have been found to contain coumarins, quinones, flavonoids, and sesquiterpenes.⁹ F. sandrasica Peşmen and Quézel is an endemic glabrous species, 30-35 cm high; it grows on rocky serpentine slopes at 2000 m and its flowering time is in June and July.⁹

The genus Hippomarathrum link is a member of the family Apiaceae and it has five species. Hippomarathrum is an erect, much-branched perennial genus, 50-100 cm high, and distributed on rocky slopes and in fields. Hippomarathrum sandrasica is also used as food and is known as “çakşır” or “çarş” by local people in Eastern Anatolia in Turkey.¹⁰ The species of this genus have long been used as spices in ethnobotany.¹¹ H. microcarpum Petrov is a gray shrub with yellowish flowers and it is reported that coumarins and furanocoumarins are found in the roots and fruits of the genus Hippomarathrum.¹² Essential oils or their components have been shown to exhibit antimicrobial, antiviral, antymycotic, antitoxicogenic, anti-parasitic, and insecticidal properties. It is considered that these characteristics are related to the function of these compounds in plants.¹³

The aim of the present study was to present and compare the chemical compositions of the essential oils of the aerial parts of F. orientalis, roots of F. sandrasica, and aerial parts of H. microcarpum growing wild in Turkey. We determined the chemical composition of the essential oils by gas chromatography (GC) and GC/mass spectrometry (MS) analysis and examined the antimicrobial activities of the 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 the essential oils in 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). The localities where these species were found are given in Table 1.

Table 1. Localities of the species

| Species               | Locality                                      | Herbarium number |
|-----------------------|-----------------------------------------------|------------------|
| Ferula orientalis     | B9: Between Ağrı and Erzurum, Mount Tahir, 2475 m, 13.07.2014 | AEF 10966        |
| Ferulago sandrasica   | C2: Mount Sandras 3 km to Lake Kartal, 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        |

Isolation of the essential oil

The 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. The oils obtained were dried in anhydrous sodium sulfate and stored in sealed vials at +4°C in the dark until analyzed and tested. All oils were pleasant smelling and transparent with a faint yellow and greenish color. The essential oil % yields of the aerial parts of F. orientalis, roots of F. sandrasica, and aerial parts of H. microcarpum were 0.022%, 0.019%, and 0.048%, respectively.

GC/MS analysis

GC/MS analysis was performed with an Agilent 5975 GC-MSD system. An Innowax FSC column (60 m×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 to 40:1 and injector temperature was set to 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. The temperature of the flame ionization detector (FID) detector was 300°C. In order to obtain the same elution order as GC/MS, simultaneous auto-injection was done on a duplicate of the same column conformed with the same operational conditions. Relative percentage quantities of the separated compounds were calculated from FID chromatograms. The results of the analysis are given in Table 2. Identification of the essential oil components was performed by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index to series of n-alkanes. Computer matching against commercial sources¹⁴ and the in-house “Başer Library of Essential Oil Constituents” established with genuine compounds and components of
known oils, alongside MS literature data, was used for the identification.

**Determination of antimicrobial compounds of the essential oils by TLC-bioautography assay**

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

**Preparation of microorganisms and the TLC-bioautography assay**

After TLC separation, the antimicrobial activity of the essential oils was determined by direct bioautography. 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 Mueller-Hinton broth standardized to 10⁸ 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 overlaid on the TLC plates and they were incubated at 37°C for 24 h. Then, by incubation, 2,3,5-triphenyl-2H-tetrazolium chloride solution was sprayed on the TLC plates. The treated plates were incubated at 37°C for 2 h and after incubation the inhibition zones were visible as pale spots against a red background.

**RESULTS**

Thirteen compounds were identified in the essential oil of the aerial parts of *F. 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 the major components. The analysis on the roots of *F. sandrasica* resulted in the identification of 69 essential compounds representing 96.0% of the oil. Limonene (28.9%) was the most abundant compound in the essential oils, 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 *H. microcarpum*, representing 98.7% of the oil. The major constituents were 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 the main compounds in the three species. Trans-verbenol was the main compound in the essential oils of *F. orientalis* and *F. sandrasica*. Thuja-2,4(10)-diene, pinocarvone, trans-pinocarveol, myrtenol, and cuparene were only found in the essential oils of the aerial parts of *F. orientalis*.

Sabinene, α-phellandrene, (Z)-β-octimene, γ-terpinene, (E)-β-octimene, terpinolene, α-olefine, bornyl acetate, α-humulene, germacrene D, δ-cadinene, caryophyllene oxide, and humulene epoxide-II were the main compounds in the essential oils of *F. sandrasica* and *H. microcarpum*. Caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II) was only found in the 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.

The results for antimicrobial activity by bioautography showed that essential oils from the aerial parts of *F. orientalis* and roots of *F. sandrasica* were active against *S. aureus* and *C. albicans* strains. However, they were not active against the *E. coli* strain. Similarly, essential oil from the aerial parts of *H. microcarpum* was found to contain compounds active against *S. aureus* and *C. albicans*. The essential oil was more effective against *C. albicans* than against *S. aureus*. However, it did not have good activity against *E. coli*. The essential oils did not give any inhibition zone against *P. aeruginosa*. The TLC evaluation of the essential oils is shown in Figure 1.

**DISCUSSION**

Monoterpene hydrocarbons (P-cymene, myrcene, γ-terpinene, limonene, terpinolene, and (Z)-β-octimene), oxygenated monoterpenes (carvacrol methyl ether, 2,5-dimethoxy-p-cymene, trans-chrysanthenyl acetate, cis-chrysanthenyl acetate, and ferulagone), aldehydes (like 2,3,6-trimethylbenzaldehyde, (E)-2-decenal, and octanal), alkane derivatives (hexadecanoic acid), sesquiterpene hydrocarbons (α-humulene, 4,6-guaiadene, and 7-epi-1,2-dehydrosesquicinole), and oxygenated sesquiterpenes (like cubenol, humuleneepoxide II, and spathulenol) were the major components of some *Ferula* species.

Some *Ferula* species contain monoterpene hydrocarbons (β-pinene, sabine, camphene, β-phellandrene, and (E)-β-octimene), alkane derivatives (nonane), sesquiterpene hydrocarbons (germacrene D, germacrene B, δ-cadinene, (Z)-
Table 2. Chemical composition of the essential oil of *Ferula orientalis*, *Ferulago sandrasica*, and *Hippomarathrum microcarpum*

| RRI  | Compound                | Ferula orientalis % | Ferulago sandrasica % | Hippomarathrum 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-Dimethylstyrine    | -                   | 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 | Fenchyl 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                           |
|   |   |   |   |
|---|---|---|---|
| RRI: Relative retention indices, calculated against n-alkanes, % calculated from flame ionization detector data, tr: trace (<0.1%) |   |   |   |
| Total | 96.6 | 96.0 | 98.7 |
Δ-3-carene (6.7%).

Comparing these results with previous studies of β-pinene (75.9%) was a major component in our study. In addition, esters like bornyl acetate were major components of some Ferula and Ferulago species.

Previous studies demonstrated that the major components of the essential oil of leaves from F. sandrasica were ocimene (30.5%), carene-β-3 (27.4%), and α-pinene (17.8%).

The major components of essential oils of some Ferula species were reported as phenol, 2-methyl-5-(1-methylthyl) (18.2%), cyclopropa[α] naphthalene-octahydro-tetramethyl (6.6%), and α-bisabolol (10.4%) (3), α-pinene (18.3%), β-pinene (50.1%), and Δ-3-carene (6.7%).

The major components of essential oils of some Ferula species 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 sabine (15.8%), (2)-β-ocimene (32.4%), p-cymene (18.4%), carvacrol methyl ether (78.1%), α-pinene (25.4%), α-pinene (40.8%), trans-chrysanthenyl acetate (83.5%), p-cymene (45.8%), and (2)- β-ocimene (30.7%).

The results showed that the essential oil was active against all tested microorganisms. It was previously reported that the essential oil of H. microcarpum was studied for antimicrobial activity. The results showed that the essential oil of H. microcarpum had antimicrobial activity against C. albicans A117 and S. aureus ATCC-29213 but had no activity against E. coli A1 or Pseudomonas sp. Our finding concur with this study. Bioautography is a suitable method for evaluating essential oils because they contain mixtures of compounds. Therefore, there is a need for the detection of common antimicrobial compounds in essential oils. Additionally, this method is rapid, easy, economical, and inexpensive. In the present study, our aim was the 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. The antimicrobial activity test 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 or E. coli strains.

CONCLUSIONS

These data provide an abundance of information on the essential oil compositions of F. orientalis, F. sandrasica, and H. microcarpum and their antimicrobial activities against some pathogenic microorganisms. As far as we know, this is the first report on the antimicrobial activity of essential oils by TLC-bioautography. The antimicrobial activities against S. aureus and C. albicans of these species may be attributed to the presence of the main components in the essential oils. A comprehensive study should be conducted including the main compounds isolated from the essential oils or their combinations against different pathogenic microorganisms.

Conflict of Interest: No conflict of interest was declared by the authors.

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