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Original article

Composition of Volatile Oil of the Aerial Parts, Flowers and Roots of *Ferulago blancheana* Post. (Apiaceae) Growing in Turkey and Determination of Their Antimicrobial Activities by Bioautography Method

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Volatile oils from the aerial parts, flowers and roots of an endemic species *Ferulago blancheana* Post, were obtained by hydrodistillation and analyzed by GC and GC/MS. Main components were found to be bornyl acetate (11.7%) and β-caryopyllene (10.2%); sabinene (23.2%), myrcene (17.5%); (E)-2-decenal (20.3%), caryopyllene oxide (17.8%) for the aerial parts, flowers and roots, respectively. Antimicrobial activity study with bioautography method was performed against three different microorganisms; *Pseudomonas aeruginosa* ATCC 13388, *Escherichia coli* NRRL, *Staphylococcus aureus* ATCC BAA 1026, and *Candida albicans* ATCC 24433. The results showed that essential oils obtained from the aerial parts, flowers and roots were active against *S. aureus* and *C. albicans*, however essential oils obtained from the aerial parts and roots were found to be less effective against *S.aureus*. In addition, no antibacterial effects were observed against *P. aeruginosa*.

Key words: *Ferulago blancheana*, Apiaceae, Volatile oil, Bioautography method, GC/MS

Türkiye'de Yetişen Ferulago blancheana Post. (Apiaceae) Türünün Toprak Üstü, Çiçek ve Köklerinden Elde Edilen Uçucu Yağların İçeriklerinin ve Antimikrobiyal Aktivitesinin Biyootografi Yöntemiyle Tanımlanması

Endemik bir tür olan *Ferulago blancheana* Post. türünün hidrodistilasyon yöntemiyle toprak üstü, çiçek ve köklerinden uçucu yağları elde edilmiştir. GC ve GC/MS analizleriyle ana bileşenleri sırasıyla toprak üstü kısmında bornil asetat (%11.7) ve β-karyofillen (%10.2); çiçekte sabinen (%23.2), mirsen (%17.5) ve kökte (E)-2-desenal (%20.3), karyofillen oksit (%17.8) olarak saptanmıştır. Elde edilen uçucu yağların biyootografi yöntemi kullanılarak *Pseudomonas aeruginosa* ATCC 13388, *Escherichia coli* NRRL, *Staphylococcus aureus* ATCC BAA 1026 ve *Candida albicans* ATCC 24433 suşlarına karşı antimikrobiyal etkisi araştırılmıştır. Toprak üstü, çiçek ve köklerden elde edilen uçucu yağların *S. aureus* ve *C. albicans*'a karşı etkili olduğu tespit edilmiş, buna karşın toprak üstü ve köklerden elde edilen uçucu yağların *S. aureus*'a karşı nispeten az etkili olduğu görülmüştür. *P. aeruginosa*'ya karşı antimikrobiyal etki saptanmamıştır.

Anahtar kelimeler: *Ferulago blancheana*, Apiaceae, Uçucu yağ, Biyootografi yöntemi, GC/MS

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INTRODUCTION

Ferulago W. Koch. is a perennial genus of Apiaceae and is represented by approximately 50 taxa throughout the world (1, 2). *Ferulago* species are known as "Çakşır" or "Çağşır" in Turkey and according to recent records the genus is represented by 35 taxa in Turkey, 18 of which are endemics. This suggests that Anatolia is the gene centre of this genus (3). *Ferulago* species have been used since ancient times for the treatment of intestinal worms, hemorrhoids and as tonic, digestive and sedative. In addition, they are used against snake bites, ulcers, spleen diseases and headache (4). Gums obtained from the incision of the roots of some species are used as seasoning and as a carminative (5). However, the plants are mostly well known for their aphrodisiac activities in Turkey (6). *Ferulago* species have been found to contain coumarins, flavonoids, quinones, sesquiterpenes, coumarin esters and furanocoumarins (7, 8). Essential oil is one of the major components of genus and recently these components in different studies from several species have been showed (4, 5, 8-18). The aim of this study is to present and compare the chemical composition of the essential oils of aerial parts, flowers, and roots of *F. blancheana* Post. growing wild in Turkey. We performed GC and GC/MS analysis to determine the constituents of the essential oil and also determined the antimicrobial activities of these oils by TLC-bioautography thin-layer, a simple and inexpensive tool for simultaneous chemico-biological screening of natural sources. To the best of our knowledge, this is the first report on the chemical analysis and antibacterial activity of *F. blancheana.* Identified constituents are presented in Table 1.

MATERIALS AND METHODS

Plant material

The plant materials were collected from the below mentioned locality and 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).

Collection locality: B6: Kayseri: Between Pınarbası-Sarız, 4 km left to Sarız, rocky slopes, 1696 m, 13.07.2014 (AEF 26672).

Isolation of the essential oil

Aerial parts (61.79 g), flowers (33.88 g) and roots (31.38 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia. Obtained oils were dried over anhydrous sodium sulfate and stored in sealed vials at +4°C in the dark until analyzed and tested. All oils were pleasant smelling, transparent with a faint yellow color. Essential oil yields of the aerial parts, flowers and roots of the studied plant are 0.032%, 0.15% and 0.032%, respectively.

GC/MS analysis

GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 □m 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. 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

GC analysis was carried out using an Agilent 6890N 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. The analysis results are given in Table 1.

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

Table 1. Composition of essential oils of aerial parts, flowers and roots of *F. blancheana*

RRI	**Compound**	A%	F%	R%
1032	α-Pinene	5.4	14.0	-
1035	α-Thujene	tr	0.2	-
1076	Camphene	1.4	-	-
1118	β-Pinene	0.5	1.3	-
1132	Sabinene	7.2	23.2	-
1159	γ-3-Carene	2.8	3.4	-
1174	Myrcene	3.5	17.5	-
1188	α-Terpinene	-	0.4	-
1203	Limonene	3.4	5.4	-
1218	β-Phellandrene	tr	0.8	-
1246	(*Z*)-β-Ocimene	6.1	4.5	-
1255	γ-Terpinene	tr	1.0	-
1266	(E)-β-Ocimene	1.6	5.9	-
1280	*p*-Cymene	1.4	0.9	-
1286	Isoterpinolene	-	0.2	-
1290	Terpinolene	0.2	1.7	-
1296	Octanal	-	-	3.2
1548	(E)-2-Nonenal	-	-	tr
1553	Linalool	0.4	-	-
1591	Bornyl acetate	11.7	5.4	11.5
1600	β-Elemene	0.4	tr	-
1594	trans-β-Bergamotene	0.7	-	-
1602	6-Methyl-3,5-heptadien-2-one	0.1	-	-
1612	β-Caryophyllene	10.2	3.0	10.5
1655	(E)-2-Decenal	-	-	20.3
1658	Sabinyl acetate	0.4	-	-
1683	*trans*-Verbenol	0.5	-	-
1687	α-Humulene	0.7	0.3	-
1704	γ-Curcumene	-	0.3	-
1719	Borneol	0.4	-	-
1726	Germacrene D	2.3	2.2	-
1733	Neryl acetate	0.3	-	-
1740	α-Muurolene	0.3	-	-
1755	Bicyclogermacrene	1.8	0.6	-
1765	Geranyl acetate	1.0	-	-
1773	δ-Cadinene	1.2	0.4	-
1776	γ-Cadinene	tr	-	-
1786	*ar*-Curcumene	0.6	0.3	-
1857	Geraniol	0.3	-	-
1864	*p*-Cymen-8-ol	0.3	-	-
2001	Isocaryophyllene oxide	0.5	-	-
2008	Caryophyllene oxide	3.2	0.5	17.8
2096	Elemol	6.2	0.9	-
2084	Octanoic acid	-	-	6.8
2103	Guaiol	2.6	tr	-

2103	Guaiol	2.6	tr	-
2144	Spathulenol	6.4	1.0	11.2
2145	Valeranone	-	0.3	-
2185	γ-Eudesmol	1.1	0.1	-
2187	T-Cadinol	0.8	tr	-
2202	Germacrene D-4-ol	1.9	0.6	-
2209	T-Muurolol	1.3	0.1	-
2250	α-Eudesmol	1.6	0.1	-
2255	α-Cadinol	4.8	0.8	12.0
2298	Decanoic acid	-	-	0.5
	Total	97.5	97.3	93.8

RRI: Relative retention indices calculated against *n*-alkanes; % calculated from FID data

tr: Trace (< 0.1 %); A: Aerial parts, F: Flowers, R: Roots

Library) (19, 20) and in - house "Başer Library of Essential Oil Constituents" built up by genius Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (21, 22), was used for the identification.

Determination of antimicrobial compounds from essential oils by TLC-Bioautography thin-layer chromatography (TLC)

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

Preparation of microorganisms and TLC bioautography method

After TLC separation, the antimicrobial activity of the essential oil was detected with direct bioautography (23, 24). *Pseudomonas aeruginosa* ATCC 13388, *Escherichia coli* NRRL, *Staphylococcus aureus* ATCC BAA 1026 and *Candida albicans* ATCC 24433 were used for bioautography. Microbial suspensions were grown overnight in double strength Mueller-Hinton broth (MHB) were standardized to 10^8 CFU/mL (corresponding to McFarland no: 0.5). TLC plates were put on nutrient agar plates. And molten agar culture medium containing inocula was spread on TLC plates and incubated at 37°C for 24h. After 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. After incubation, the inhibition zones were visible as pale spots against a red background.

RESULTS

A total of fortyfive compounds representing 97.5% of the oil were identified in the essential oil of aerial parts of *F. blancheana*. Bornyl acetate, β-caryopyllene, sabinene, spathulenol, elemol, (Z)-β-ocimene were the major components, amounting to 11.7%, 10.2%, 7.2%, 6.4%, 6.2% and 6.1%, respectively. The analysis on the flowers of *F. blancheana* resulted in the identification of thirty-five volatile compounds representing 97.3% of the oil. Sabinene at 23.2% was the most abundant compound in the volatile oil, followed by myrcene (17.5%), α-pinene (14%), (E)-β-ocimene (5.9%) and bornyl acetate (5.4%). Ten compounds were characterized in the oil of the roots of *F. blancheana* representing 93.8% of the oil. The major constituents were found to be (E)-2-decenal (20.3%), caryopyllene oxide (17.8%), bornyl acetate (11.5%), spathulenol (11.2%). The oils obtained from different parts of this species did not show much qualitative and quantitative similarity. Some components such as camphene, linalool, trans-verbenol,

sabinyl acetate, borneol, isocaryophyllene oxide, α-muurolene, geranyl acetate, α-cadinene, geraniol, *p*-cymen-8-ol and neryl acetate were only found in the essential oil of the aerial parts and some components such as α-terpinene, γ-curcumene, valeranone were only found in the essential oils of the flowers and some components such as octanal, (E)-2-decenal, octanoic acid and decanoic acid were only found in the essential oil of the roots. The composition of the essential oils obtained from different parts of this species and their relative percentages are given in Table 1. Antimicrobial effect was investigated using TLC-bioautographic method.

Bioautography is a laboratory technique to detect substances affecting the growth rates of test organisms in complex mixtures and matrices and the method belongs to a large group of screening methods for the detection of antimicrobial activity. It is based on the biological activity of the analyte, which can be antibacterial, antifungal, antitumour, antiprotozoae etc. Diffusion and dilution methods are also used. The main benefit of bioautography is its providing information about antimicrobial activities of substances separated from a mixture. Bioautography is proving its worth as a simple and inexpensive tool for simultaneous chemico-biological screening of natural sources. In other word, it offers the simplest mean of bioassay guided lead discovery from natural products.

TLC bioautography assay was performed against three different microorganisms, *P. aeruginosa* ATCC 13388, *S. aureus* ATCC BAA 1026 and *C. albicans* ATCC 24433. Essential oils have very active components againts *S. aureus* ATCC BAA 1026 and showed good inhibiton. Essential oils of the aerial part and roots were found active against *C. albicans* ATCC 24433, however inhibition zones were less visible than *S. aureus*. Essential oils did not give any inhibiton zone against *P. aeruginosa* ATCC 13388. Compared with another study carried out on a different *Ferulago* species (*F. sandrasica* Pesmen & Quezel) performed by bioautography method showed that the essential oil of *F. sandrasica* of the roots was active against *S. aureus* ATCC 6558 and *C. albicans* ATCC 90028 strains; however it was not active against *Escherichia coli* NRRL B-3008 strain. The essential oil of the aerial part and flowers showed higher antibacterial activity against *S. aureus* than the roots. TLC

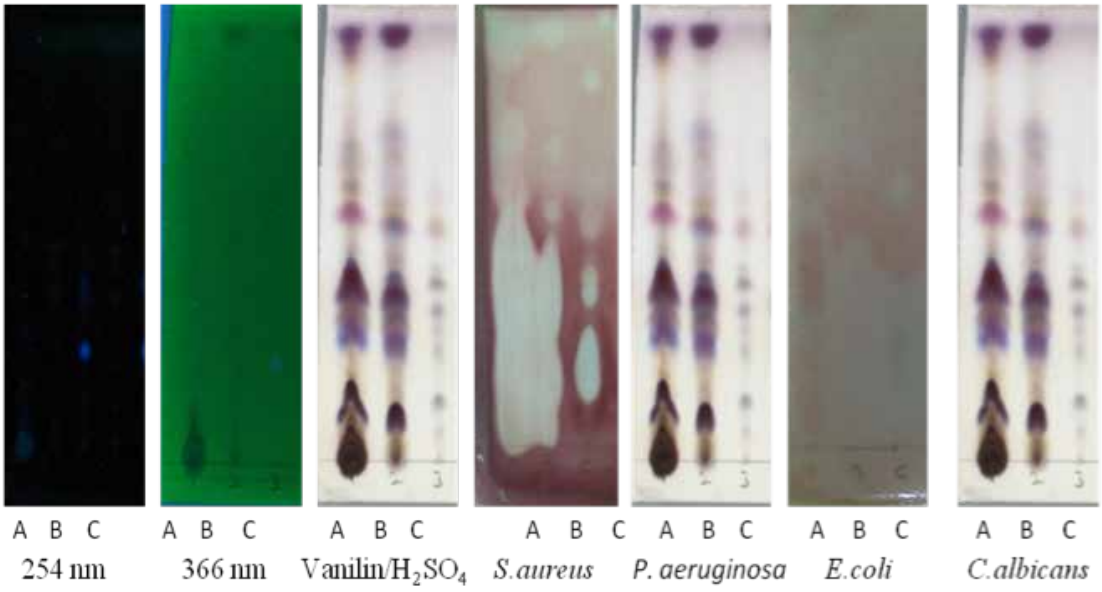

Figure 1. TLC separation of the essential oil from the different parts of *F. blancheana* on silica gel 60 F_{254}.: lane A: Aerial parts, lane B: Flowers, C: Roots

evaluation of the essential oils is reported in Figure 1.

DISCUSSION

Comparison of the main constituents of different parts of this species shows that each part has a different set of dominant compounds in Table 1. However, previous studies of the oils of different *Ferulago* species (5, 25) revealed that eight compounds have been detected in high percentages, namely (Z)-β-ocimene, 2,3,6-trimethylbenzaldehy, cis-chrysanthenyl acetate, nonacosane, sabinene, δ-cadinene, α-pinene and p-cymene have also been defined as major components in many other species. Erdurak et al. reported main components of essential oil of the fruit and root of *F. isaurica* and the root oil of *F. syriaca* (8) in 2006. They were characterized as terpinolene (42.1%) and myrcene (27%) for the root oil of *F. isaurica* and bornyl acetate (69.4%) and terpinolene (12.5%) for the root oil of *F. syriaca*. Another study showed that the major components of essential oils *F. pachyloba*, *F. platycarpa* and *F. longistylis* were (Z)-β-ocimene (25.7%), α-pinene (9.8%), sabinene (6.3%), and δ-cadinene (5.6%); cis-chrysanthenyl acetate (24.2%), nonacosane (7.7%) and α-pinene (4.2%); 2,3,6-trimethylbenzaldehyde (29.8%), cis-chrysanthenyl acetate (24.2%), nonacosane (7.7%) and α-pinene (4.2%) respectively.

Previous studies demonstrated monoterpene hydrocarbons constituted the main fraction of the essential oils of *F. campestris*, with α-pinene, myrcene and *γ* -terpinene as the major components of the flowers (11); myrcene (33.4–39.7%), α-pinene (22.7–23.0%) and γ -terpinene (8.1–10.9%) as the major components of the fruits, and α -pinene (58.3–75.0%) as the predominant compound in the oil from the roots (26); monoterpene hydrocarbons (78.8–80.3%), with myrcene (33.4–39.7%), α-pinene (22.7–23.0%), and γ-terpinene (8.1–10.9%) as the major components of the fruits essential oils (27). Baser et al. (2002), 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 studies indicated that the main essential oil components are 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%); 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%); (Z)- β-ocimene (30.7%) respectively (28). Previous studies indicated that chemical composition of the essential oils of several *Ferulago* species did not show much qualitative and quantitative similarity, but it can be said that major constituents of the oils of *Ferulago* species are β-ocimene, α-pinene, α- and β-phellandrene, limonene, myrcene and p-cymene.

The results obtained in this investigation suggest that this chemical diversity may be useful in taxonomical classification. In addition, bioautography method was used to evaluate the correct test organism(s) in antimicrobial screening. Agar disc diffusion technique and broth dilution methods are also used to screen plant extracts for antimicrobial activity. However we choose this method since it is efficient, simple and inexpensive. Antimicrobial activity study performed against three different microorganisms, *P.aeruginosa* ATCC 13388, *S. aureus* ATCC BAA 1026 and *C. albicans* ATCC 24433 showed that the essential oils ontained from the aerial parts, roots and flowers were active against *S. aureus* ATCC 6558 and *C. albicans* ATCC 24433, however inhibition zones obtained with the essential oils obtained from the aerial parts and roots were found to be less visible than the ones obtained against *S. aureus*. In addition, no volatile oil yielded an inhibition zone against *P. aeruginosa* ATCC 13388.

CONCLUSION

In summary, the present study, reported the composition of the essential oils of *F.*

blancheana aerial parts, flowers and roots for the first time and showed that they are mainly composed of terpenoids and benzenoid derivatives.

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