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Comparison of essential oils and antimicrobial activities of *Ferulago mughlae* Peşmen (Apiaceae) growing in Turkey

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ABSTRACT: *Ferulago* species have been utilized dated from ancient times as antihelmentic, carminative, digestive, sedative, aphrodisiac, along with as salads and spice in view of their exclusive odors. *F. mughlae* Peşmen was investigated for its chemical compositions of essential oils and antimicrobial activity. Antimicrobial activities of essential oils were performed via TLC bioautography methods and essential oils were analysed via GC and GC/MS. α-pinene (53.0%), myrcene (3.9%), limonene (6.0%), β-phellandrene (11.0%) were shown to be as primary components of fruit. Primary components of aerial part were found as α-pinene (48.5%), camphene (10.6%), β-pinene (4.8%) and limonene (3.0%). α-pinene (37.3%), camphene (9.1%), limonene (5.3%), terpinolene (3.4%), β-caryophyllene (3.6%), borneol (9.5%), kessan (8.0%), germacrene B (4.0%), caryophyllene oxide (3.7%) and 2,3,6-trimethylbenzaldehyde (3.7%) were shown to be the primary components of root. Aerial part and fruit essential oils of *F. mughlea* contain active compounds against *Staphylococcus aureus* ATCC 6558 while these essential oils did not show any activites against *Candida albicans* ATCC 90028 and *Escherichia coli* NRRL B-3008 strains. Root essential oil of *F. mughlea* did not show any antimicrobial activities against tested all microorganisms. The antimicrobial activities against these microorganisms from this species may be based upon the existence of the primary compounds in the essential oils.

KEYWORDS: Antimicrobial; Apiaceae; bioautography; essential oil; *Ferulago mughlae*.

1. INTRODUCTION

Members of *Ferulago* W. Koch. genus belong to Apiaceae family and they are perennial species. It is typified 34 taxa (19 of them are endemics) and they are known as "Çakşır, cağşır, günlükotu, kılkuyruk, kuzukemirdi asaotu" in Turkey. Consequently Anatolia is figured as the gene central of *Ferulago* [1,2]. *F. mughlae* Peşmen (Apiaceae) is a glaucous, perennial, glabrous, endemic species and it grows in Marmaris, Mugla, West Anatolia, Turkey [3]. *Ferulago* species have been utilized since primeval days for the curation of haemorrhoids, as antihelmentic, tonic, sedative and digestive. Besides, they are utilized against headache, ulcers, splenic diseases and snake bites [4] and gums gained from the incision of the roots from several species are utilized as spice and degasifier [5]. Nevertheless, in Turkey *Ferulago* species are in particular well recognised for the aphrodisiac properties [6]. It is reported that the plants include quinones, coumarins, flavonoids, coumarin esters and sesquiterpenes [7, 8].

Antimicrobial resistance has being increased rapidly against current drugs during the last decades, however new antimicrobial drug development has slow down. This situation leads health authorities to search for natural antimicrobial active substances and/or to combine them with existing approved drugs. Treatment with plants is actually a traditional method known from antic ages long before the development of modern medicine [9,10]. Antimicrobial activity of the plants comes from mostly by aromatic or phenolic substances [11].

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2. RESULTS

Percent yields of essential oil from the fruit, aerial part and roots of *Ferulago mughlea* were 2.564, 0.17 and 0.09, respectively. Identified constituents and results of antimicrobial activities were presented in Table 2 and Figure 1. In general, yield of the roots were low compared to the fruits and aerial parts. For best results were obtained in fruit. A total of twenty eight compounds finding 99.9% from the oil were defined in the essential oil from fruit. α-pinene, myrcene, limonene and β-phellandrene were the primary components, amounting to 53.0%, 3.9%, 6.0% and 11.0%, in order of. The analysis of the aerial part from *F. mughlae* showed in the identification of thirty one essential compounds finding 99.9% of the oil. α-pinene at 48.5% was the most abounding compound in the essential oil, followed by camphene (10.6%), β-pinene (4.8%) and limonene (3.0%).

Eighteen compounds were found at the oil from the roots of *F. mughlae* demonstrating 99.9% from the oil. The primary constituents were established to be α-pinene (37.3%), camphene (9.1%), limonene (5.3%), terpinolene (3.4%), β-caryophyllene (3.6%), borneol (9.5%), kessan (8.0%), germacrene B (4.0%), caryophyllene oxide (3.7%) and 2,3,6-trimethylbenzaldehyde (3.7%).

Aerial part and fruit essential oils of *F. mughlae* contained active compounds against *S. aureus* ATCC 6558. But this essential oils did not showed any activity against *C. albicans* ATCC 90028 and *E. coli* NRRL B-3008 strains. Essential oil of root showed no antimicrobial activity against *S. aureus* ATCC 6558, *C. albicans* ATCC 90028 and *E. coli* NRRL B-3008 strains. TLC separation of the essential oils were shown by applied of Vanillin/H_2SO_4 reagent in Figure 1.

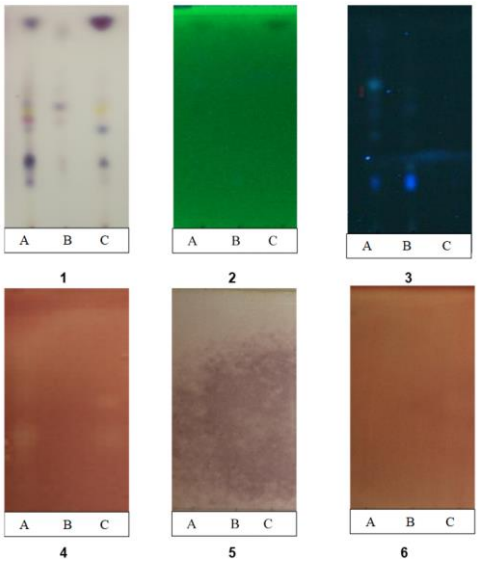

Figure 1. TLC separation of essential oils from the of; A: Aerial parts of *F. mughlae*, B: Root of *F. mughlae*, C: Fruit of *F. mughlae*. 1: TLC plate with applied vanilin/H_2SO_4 reagent. 2: TLC plate chromatogram at 254 nm. 3: TLC plate chromatogram at 364 nm. 4: TLC plate chromatogram inoculated with *Staphylococcus aureus* ATCC 6538 (pale yellow). 5: TLC plate chromatogram inoculated with *Candida albicans* ATCC 90028 (not detected activity). 6: TLC plate chromatogram inoculated with *Esherichia coli* NRRLB-3008 (not detected activity).

3. DISCUSSION

Compared content of essential oils from *Ferulago mughlae,* some compounds such as sabinene, α-phellandrene, α-terpinene, δ-3-carene, (*Z*)-β-ocimene, β–cubebene, δ-elemene, trans-p-menth-2-en-1-ol, cis-p-menth-2-en-1-ol, β-elemene and goa-6,10(14)-dien-4β-ol were only found in the fruit; some components such as pinocarvone, trans-pinocarvone, trans-verbenol, β-selinene, α-calocorene, hexahydro farnesyl acetone, spathulenol, murola-4,10(14)-dien-4β-ol and cadalane, alismol were only found in the aerial part; some components such as 1,2,4-trimethyl benzene, camphora, bornyl acetate, kessan, germacrene B and 2,5-dimethoxy-p-cymene were only found in the root. 86.2, 71.8 and 57.5% essential oils from fruit, aerial part and root, respectively were found as monoterpene hydrocarbons.

Sesquiterpene hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes were mostly found in root part (17.1, 13.6 and 37%, respectively). Fatty acid (hexadecanoic acid) was found only at aerial part (3.1%).

Comparison of the primary constituents of *F. mughlae* showed that every part had a diversified set of predominant constituents in Table 2. In addition to this, recent investigations on the essential oils from varied *Ferulago* species demonstrated that eight compounds have been indicated in high proportions, namely 2,3,6-trimethyl benzaldehyde, cis-chrysanthenyl acetate, (Z)-β-ocimene, nonacosane, sabinene, α-pinene, δ-cadinene and p-cymene have also been defined as primary components in many other species, also we found that bornyl acetate and spathulenol are primary constituents of this genus [12].

Previous studies demonstrated the major components of essential oils *F. platycarpa*, *F. pachyloba* and *F. longistylis* were nonacosane (7.7%), cis-chrysanthenyl acetate (24.2%) and α-pinene (4.2%); sabinene (6.3%), (Z)-β-ocimene (25.7%), cis-chrysanthenyl acetate (24.2%), α-pinene (9.8%), and δ-cadinene (5.6%); α-pinene (4.2%), 2,3,6-trimethylbenzaldehyde (29.8%), nonacosane (7.7%) in order of. Another investigations about essential oils from *Ferulago* species are given at Table 1.

α-pinene was found as the major compound of all parts from *F. mughlea*. Another studies indicated that α-pinene were found in *F. platycarpa*, *F. pachyloba*, *F. longistylis, F. campestris*, *F. asparagifolia, F. aucheri, F. confusa, F. galbanifera, F. humilis, F. idaea, F. macrosciadia, F. mughlae, F. sandrasica, F. silaifolia, F. sylvatica and F. trachycarpa* species [13-16]. The amount of α-pinene in *F. mughlea* was found to be higher than these species. Also limonene was found as the another major compound of all parts from *F. mughlea*. However limonene was not found in *F. platycarpa*, *F. pachyloba*, *F. longistylis, F. campestris, F. asparagifolia, F. aucheri, F. confusa, F. galbanifera, F. humilis, F. idaea, F. macrosciadia, F. mughlae, F. sandrasica, F. silaifolia, F. sylvatica and F. trachycarpa* species [13-16].

Differences come from their contents and quantities among species. It is important to find a species that has a prevalent range of antimicrobial activity on the genus. Nowadays, due to the rapid increases in resistance to antibiotics, researches are shifting to create new combinations of active compounds derived from natural products. Besides, consumers prefer foods with natural preservatives.

In this research, we found that chemical composition of essential oils composition were different. Recent studies demonstrated that chemical constituents from the essential oils of different *Ferulago* species indicate no much qualitative and quantitative conformability. The findings gained in this study recommend that this chemical diversification could be helpful in taxonomical classification.

Moreover, bioautography method was utilized to assess the accurate tested microorganism(s) in antimicrobial screening. Within our knowledge, this is the initial report on antimicrobial activity of essential oils from *F. mughlae*.

Table 1. Previous studies about essentail oils from *Ferulago* species

Plants	Compounds	Used part	Ref
F. platycarpa, *F. pachyloba* and *F. longistylis*	cis-chrysanthenyl acetate (24.2%), nonacosane (7.7%) and α-pinene (4.2%); (Z)-β-ocimene (25.7%), α-pinene (9.8%), sabinene (6.3%), and δ-cadinene (5.6%); 2,3,6-trimethylbenzaldehyde (29.8%), cis-chrysanthenyl acetate (24.2%), nonacosane (7.7%) and α-pinene (4.2%)	Aerial part	13
F. campestris	α-pinene, myrcene and γ –terpinene	Flower	13
F. campestris	myrcene (33.4–39.7%), α -pinene (22.7–23.0%) and γ -terpinene (8.1–10.9%)	Fruit	14
F. campestris	α -pinene (58.3–75.0%)	Root	14
F. campestris	myrcene (33.4–39.7%), α-pinene (22.7–23.0%), and γ-terpinene (8.1–10.9%)	Fruit	15
F. asparagifolia, *F. aucheri*, *F. confusa*, *F. galbanifera*, *F. humilis*, *F. idaea*, *F. macrosciadia*, *F. mughlae*, *F. sandrasica*, *F. silaifolia*, *F. sylvatica* and *F. trachycarpa*	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	Fruit	16

Table 2. The composition of the essential oils of *Ferulago mughlae*.

RRI	Compound	Fruit %	Aerial part %	Root %
1032	α-pinene	53.0	48.5	37.3
1076	camphene	2.4	10.6	9.1
1118	β-pinene	1.8	4.8	-
1132	sabinene	1.4	-	-
1151	δ-4-carene	0.8	-	-
1174	myrcene	3.9	1.3	1.6
1176	α-phellandrene	3.4	-	-
1188	α-terpinene	0.3	-	-
1203	limonene	6.0	3.0	5.3
1218	β-phellandrene	11.0	2.4	-
1246	(*Z*)-β-ocimene	0.2	-	-
1255	γ-terpinene	0.2	-	tr
1280	*p*-cymene	1.5	1.2	0.8
1290	terpinolene	0.3	-	3.4
1294	1,2,4-trimethyl benzene	-	-	2.8
1479	δ-elemene	0.9	-	-
1497	α-copaen	0.9	1.2	-
1532	camphora	-	-	2.9
1549	β–cubebene	0.3	-	-
1571	*trans*-p-menth-2-en-1-ol	0.2	-	-
1586	pinocarvone	-	0.8	-
1591	bornyl acetate	-	-	1.2
1600	β -elemene	0.2	-	-
1612	β-karyofilen	1.8	2.4	3.6
1638	*cis*-p-menth-2-en-1-ol	0.1	-	-
1670	*trans*- pinocarvol	-	0.6	-
1683	*trans*-verbenol	-	0.7	-
1687	α-humulene	0.7	0.6	-
1704	γ-murolene	-	tr	-
1707	δ-selinen	1.2	0.6	-
1719	borneol	-	0.8	9.5
1726	germakren D	1.8	1.1	-
1742	β-selinene	-	0.4	-
1773	δ- cadinene	3.0	2.1	1.5
1776	γ- cadinene	0.6	0.1	-
1786	kessan	-	-	8.0
1854	germacrene-B	-	-	4.0
1878	2,5-dimethoxy-p-cymene	-	-	1.5
1941	α-calacorene	-	1.0	-
2008	caryophyllene oxide	-	2.7	3.7
2019	2,3,6-trimethylbenzaldehyde	-	-	3.7
2071	humulene epoxide-II	-	1.0	-
2080	1,10-diepi-cubenol	1.6	1.6	-
2131	hexahydro farnesyl acetone	-	0.7	-
2144	spathulenol	-	1.0	-
2161	murola-4,10(14)-dien-4β -ol	-	1.2	-
2256	cadalene	-	0.8	-
2264	alismol	-	2.6	-
2269	goa-6,10(14)-dien-4β–ol	0.4	-	-
2900	nonacosane	-	1.0	-
2931	hexadecanoic acid	-	3.1	-
	Total	99.9	99.9	99.9
	Monoterpene hydrocarbons	86.2	71.8	57.5
	Oxygenated monoterpenes	0.3	2.9	13.6
	Sesquiterpene hydrocarbons	11.4	10.3	17.1
	Oxygenated sesquiterpenes	2.0	10.1	37
	Fatty acid	-	3.1	-
	Others	-	1.7	8.0

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

4. CONCLUSION

We think that results of our study will contribute to the investigations in new antibiotic combinations or food preservatives. It is hoped that the research and development studies on the antimicrobial effects of plant-derived compounds in relation to the use of current technological conditions, will broaden the scope of the solution field.

5. MATERIALS AND METHODS

5.1. Plant material

The author Fatmagül Delimustafaoğlu Bostanlık collected the plant in 2013 from Marmaris and the plant was identified by a plant taxonomist (Prof. Dr. Hayri Duman, in the Department of Biology, Faculty of Science, Gazi University). The voucher specimens are conserved at the Herbarium of Ankara University, Faculty of Pharmacy (AEF 26356).

5.2. Isolation of the essential oil

Powdered fruit, aerial part and root of the species were put to hydrodistillation for 3 h by use of a Clevenger-type equipment in conformity with the method advised at the European Pharmacopoeia. Gained oil was dried over anhydrous sodium sulfate and conserved in sealed vials at +4°C temperature in the dark till analyzed and tested and transparent oil had faint yellow color.

5.3. GC–MS analysis

GC-MS was assessed with an Agilent 5975 GC-MSD system and 60 m x 0.25 mm, 0.25 µm film thicknessed Innowax FSC column was utilized. Helium was also utilized as carrier gas at 0.8 ml/min. The temperature of GC oven was kept at 60°C temperature for 10 minute, then it was arranged to 220°C temperature at a rate of 4°C/min, and changeless at 220°C for 10 min. Then it was programmed to 240°C temperature at a rate of 1°C/min and split ratio was configured at 40:1. Injector temperature was kept at 250°C temperature and mass spectra were entered at 70 eV. Mass interspace was from m/z 35 to 450.

5.4. GC analysis

GC was assessed by use of an Agilent 6890N GC system and temperature of FID detector was 300°C temperature. To gain the similar elution with GC-MS, concurrently auto-injection was made on a two times of the similar column implementing the same operational circumstances. Relative proportion amounts of the separated constituents were calculated from FID chromatograms and seen in Table 2. Determining of the essential oil ingredient was assessed via way of classing with relative retention times of compounds with those of authentic specimens or via classing with relative retention index (RRI) of compounds to series of n-alkanes. Computer matchup against in-house "Başer Library of Essential Oil Constituents" constituted via genuine ingredient of recognized oils, besides MS literature data [17, 18] and trading [19, 20] were utilized for the definition.

5.5. Antimicrobial activity assays

5.5.1. Preparation of microorganisms and TLC bioautography method

The microorganisms used in this study were obtained from the culture collection of the Anadolu University, Faculty of Pharmacy, Pharmacognosy Department. *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC BAA 1026 and *Candida albicans* ATCC 24433 were utilized for bioautography.

Chromatography was carried out 0.2 mm silica gel 60 F_{254} aluminium sheet TLC plates and essential oils were administered to the TLC plate with minicaps capillary pipettes. TLC plates were developed with mobile phase as toluene: ethyl acetate, 93:7 and TLC plate for bioautography was prepared in parallel . After the development, TLC plates were assessed at UV 254 nm and 366 nm for determination of fluorescent components and alcoholic vanillin–sulphuric acid reagent was used to visualize the separated compounds and warmed up at 110°C for 3 minute. After TLC separation, antimicrobial activity of the essential oils were demonstrated with direct bioautography [21, 22]. Microbial suspensions were grown overnight in double strength and Mueller hinton broth (MHB) were standardized to 10^8 CFU/ml (equaled to McFarland no:0.5). TLC plates were put on Nutrient Agar plates and Molten Agar culture medium consisting of inocula was spread on TLC plates and incubated for 24 hours at 37°C. Later the incubation, 2,3,5-triphenyl-2H-tetrazolium

chloride (TTC) solution was sprayed on TLC plates and the treated plates were incubated for 2 hours at 37°C and later incubation, the inhibition zones were visionary as pale spots against a red background.

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