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The Essential Oil of *Anthemis cretica* L. subsp. *leucanthemoides* (Boiss.) Grierson

Anthemis cretica L. subsp. *leucanthemoides* (Boiss.) Grierson' in Uçucu Yağı

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Abstract

Water distilled essential oil of *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson was analyzed by GC/MS. Ninety nine compounds were characterized representing 87.7% of the oil. Enantiomeric distribution of the main constituent camphor (19.4%) was determined by MD-GC/MS. Antimicrobial activity of the oil was also investigated.

Key words: *Anthemis cretica*, Compositae, essential oil, camphor, enantiomer, antimicrobial activity

Introduction

Anthemis (Compositae) is represented by fifty two species in Turkey. It exists in the flora in three sections; Anthemis, Maruta and Cota (Baytop, 1999; Davis, 1975; Güner *et al.*, 1975). *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson belongs to the Section *Anthemis*.

The flowering parts of *Anthemis* species are used as dying material and for medicinal purposes. Flowers of *Anthemis nobilis* L., *A. altissima* L., *A. hyalina* DC., *A. arvensis* L., *A. auriculata* Boiss. and *A. chia* L. are used as subtitutes of *Matricaria chamomilla* L. which is used as diuretic, appetizing, carminative and choleretic. Its infusion is used as a gargle against inflammation of the throat and against haemorrhoids. In addition, *A. nobilis* L. is utilized as a hair shampoo. The flowers of *A. cotula* L. are used as stimulant, carminative and to induce menses. Furthermore, *A. chia* L. and *A. tinctoria* L. var. *tinctoria* are used to dye cloth (Baytop, 1999). Decoction of *Anthemis* sp. is drunk for calculi (Tabata *et al.*, 1988).

To the best of our knowledge, this is the first report on the chemistry of *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson.

The plant material used in this work is collected in Isparta for sale and is locally known as "Papatya". Its herbal tea is reportedly used against stomachache and cough.

Material and Methods

Dried aerial parts of *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson collected from Isparta, Turkey in 1996 were distilled for 3 hours using a Clevenger apparatus. Percentage yield of the oil on moisture free basis was 0.03%. The compounds characterized in the oil are shown in Table 1.

Table 1. The results of essential oil analysis

KIP	Components	%	KIP	Components	%
1032	α-pinene	0.9	1611	terpinen-4-ol	5.7
1035	α-thujene	0.1	1638	cis-p-menth-2-en-1-ol	0.3
1076	camphene	0.1	1648	myrtenal	0.2
1118	β-pinene	1.8	1654	octyl isovalerate	0.2
1132	sabinene	0.2	1655	(E)-2-decenal	0.3
1136	isoamyl acetate	0.1	1663	cis-verbenol	0.3
1159	δ-3-carene	0.1	1664	trans-pinocarveol	0.6
1174	myrcene	0.2	1674	p-mentha-1,5-dien-8-ol	0.1
1188	α-terpinene	0.1	1682	δ-terpineol	0.2
1203	2-methyl butyl isobutyrate	0.2	1683	trans-verbenol	0.9
1203	limonene	0.9	1687	α-humulene	0.4
1213	1,8-cineole	7.2	1704	γ-muurolene	0.2
1255	γ-terpinene	0.9	1706	α-terpineol	1.3
1280	p-cymene	1.0	1719	borneol	4.5
1285	isoamyl isovalerate	0.4	1726	germacrene D	1.1
1286	2-methyl butyl 2-methyl butyrate	0.6	1737	(Z, E)- α-farnesene	0.3
1290	terpinolene	0.1	1743	α-cadinene	0.1
1299	2-methyl butyl isovalerate	2.4	1755	bicyclogermacrene	0.1
1400	nonanal	0.5	1758	cis-piperitol	0.1
1429	perillen	0.3	1766	decanol	0.3
1441	(E)-2-octenal	0.1	1773	δ-cadinene	0.5
1452	1-octen-3-ol	0.3	1776	γ-cadinene	0.1
1468	isoamyl hexanoate	0.4	1779	(E, Z)-2, 4-decadienal	0.1
1474	trans-sabinene hydrate	0.2	1802	cumin aldehyde	1.2
1499	α-campholene aldehyde	0.4	1805	α-campholene alcohol	0.2
1506	decanal	0.2	1811	p-mentha-1, 3-dien-7-al	0.2
1532	camphor	19.4	1827	(E, E)-2, 4-decadienal	0.7
1541	benzaldehyde	0.2	1845	trans-carveol	0.2
1548	(E)-2-nonenal	0.2	1853	cis-calamenene	0.2
1553	linalool	0.3	1868	(E)-geranyl acetone	0.4
1556	cis-sabinene hydrate	0.1	1893	dodecyl acetate	0.3
1562	octanol	0.1	1929	2-methyl butyl benzoate	0.4
1565	linalyl acetate	0.1	1932	isoamyl benzoate	0.2
1571	trans-p-menth-2-en-1-ol	0.2	1945	1, 5-epoxy-salvial-4(14)-ene	0.4
1586	pinocarvone	0.5	1958	(E)-β-ionone	0.5
1588	bornyl formate	0.4	1973	dodecanol	0.1
1589	isocaryophyllene	0.1	1988	2-phenyl ethyl 2-mehyl butyrate	0.3

Table 1 continued

KIP	Components	%
1992	2-phenyl ethyl isovalerate	0.3
2008	caryophyllene oxide	2.4
2016	isoamyl phenyl acetate	0.4
2030	methyl eugenol	0.3
2037	salvial-4(14)-en-1-one	0.4
2041	pentadecanal	0.4
2045	humulene epoxide-I	0.6
2050	(E)-nerolidol	0.3
2088	1-epi-cubenol	0.2
2131	hexahydrofarnesyl acetone	2.7
2144	spathulenol	4.3
2198	thymol	1.0
2239	carvacrol	3.1
2257	β-eudesmol	0.9
2298	decanoic acid	2.1
2300	tricosane	1.1
2316	caryophylladienol-I	0.3
2324	caryophylladienol-II	0.4
2500	pentacosane	0.8
2503	dodecanoic acid	1.1
2622	phytol	0.1
TOTAL		87.7

GC/ MS Analysis

Gas Chromatographic/Mass Spectrometric measurements were performed using a Hewlett Packard G1800A GCD system. HP-Innowax (60 m x 0.25 mm ∅., with 0.25 μm film thickness) column was used with Helium (0.7 mL/min) as carrier gas. GC oven temperature was kept at 60 ^{0}C for 10 min and programmed to 220 ^{0}C at a rate of 4^{0}C /min and then kept constant at 220 ^{0}C for 10 min and at a rate of 1^{0}C /min to 240 ^{0}C (Total=80 min). Split ratio was adjusted at 50:1. The injector temperature was at 250^{0}C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 425. Library search was carried out using Wiley Library and TBAM Library of Essential Oil Constituents. Relative percentage amounts of the separated compounds were calculated from Total Ion Chromatograms by the computerized integrator.

Multi Dimensional Gas Chromatography/Mass spectrometry (MD-GC/MS) Analysis

The chiral separation of camphor in the oil sample was performed on a MD-GC/MS system consisting of two Hewlett Packard GC 6890 gas chromatographs coupled through a GERSTEL Multi Column Switching (MCS) system to a Mass Selective Detector (MSD). The system was equipped with a double oven system with two independent temperature controls, one each for FID and MS detectors, Cool Injection System (CIS), Cryo-Trap System (CTS) and a Multi-Column Switching (MCS) system. The first oven was equipped with a polar FS capillary column while the second oven contained a FSC chiral column. (MD-GC/MS) analysis was performed using a HP-Innowax silica capillary column (60 m x 0.25 mm i.d., with 0.25 μm film thickness) as a precolumn. Precolumn temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C /min and then kept constant at 220°C for 10 min and at a rate of 1°C /min to 240 °C and then 240°C for 40 min (Total 120 min). Cooled Injection System (CIS) was used as an injector (40°C). FID detector was used (250°C). Lipodex E, [=Octakis (3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin], (70% in OV1701), (25 m x 0.25 mm i.d.) was used as a main column. Main column temperature was kept at 40°C for 34 min and programmed to 120 °C at a rate of 1°C /min and then 120°C for 6 min (Total 120 min). MS were taken at 70 eV. Mass range was from *m/z* 35 to 425.

Bioassay

Micro-dilution broth susceptibility assay was used for the determination of the antimicrobial activity (Koneman et al., 1997). Stock solution of the oil was prepared in DMSO. Serial dilutions were prepared in sterile distilled water in a 96-well microtiter plate (2000 – 1.94 μg/mL). Freshly grown bacterial suspensions in double strength Mueller-Hinton broth (Merck) and yeast suspension of *Candida albicans* in yeast medium were standardized to 10^8 CFU/ml. Sterile distilled water served as growth control. 100 μL of each microbial suspension was then added to each well. The last row containing only the serial dilutions of the essential oil without microorganism was used as negative control. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC). Chloramphenicol was used as antibacterial and Ketoconazole was used as an antifungal positive control in the experiment. (See Table 2.)

Table 2. MIC (μg/mL) values of the Essential oil of *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson

Microorganism	Source	E.O.	ST
Escherichia coli	ATCC 25922	125	62.5
Staphylococcus aureus	ATCC 6538	125	7.81
Pseudomonas aeruginosa	ATCC 27853	125	250
Enterobacter aerogenes	NRRL 3567	125	125
Proteus vulgaris	NRRL 123	125	31.25
Salmonella typhimurium	NRRL 4420	125	62.5
Candida albicans	O. G. Ü.	62.5	125*

E.O.: *Anthemis* ST: Chloramphenicol * : Ketoconazole

Results and Discussion

The main component of the essential oil of *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson was characterized as camphor. Ninety nine compounds were defined representing 87.7% of the oil.

Camphor which is commercially produced from Camphor tree, *Cinnamomum camphora* is distributed in the members of Lauraceae, Labiatae and Compositae. It is semi-synthetically manufactured from Pinene. Camphor is used in perfumery and in medicine for its analeptic, respiratory stimulant, topical analgesic, antipruritic and antirheumatic properties (Buckingham, 1994). It is used also in the manufacturing of plastics, especially celluloid; varnishes; moth repellents and as a preservative in pharmacy, cosmetics (Hocking, 1997). Commercial camphor has (+) enantiomer. Its (-) enantiomer occurs in *Chrysanthemum parthenium* and *Artemisia* spp. (Compositae), and in a *Lavandula* spp. (Labiatae) (Harborne and Baxter, 1993).

The existence of (1*S*)(-)-camphor in *Osmitopsis asteriscoides*, *Tanacetum parthenium*, *T. haradjani*, *Achillea grandifolia*, *A. bieberstenii*, *A. phrygia*, *A. sieheana*, *Arischrada korolkowii* and *Thymus spyleus* and the occurrence of (1*R*)(+)-camphor in *Plectranthus grandidentatus* have been reported by our group (Başer et al., 2000). Furthermore, the oil

of *Osmitopsis asterioides* rich in (-)-camphor has been shown to have antimicrobial activity while (+)-camphor was inactive (Viljoen et al., 2000).
Enantiomeric distribution of the camphor isolated from the oil was as follows:

Enantiomer	%
(1*S*)(-)-camphor	96.4
(1*R*)(+)-camphor	3.8

The essential oil was evaluated for its antimicrobial properties against various human pathogenic bacteria and fungi. The results observed in this experiment suggest that the essential oil has moderate inhibitory activity against the pathogens *Staphylococcus aureus,* and *Proteus vulgaris.* Good inhibitions were seen in the case of *Pseudomonas aeruginosa, Enterobacter aerogenes* and the fungi *Candida albicans. Escherichia coli* and *Salmonella typhimurium* were also inhibited by *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson essential oil. Observed MIC values were 62.5-125 μg/mL. The MIC values for the standard antimicrobials are also given in Table 2.

Özet

Su distilasyonu ile elde edilen *Anthemis cretica* L. var. *leucanthemoides* (Boiss.) Grierson' un uçucu yağı GC/MS ile analiz edildi. Yağın % 87.7' sini oluşturan 99 bileşik belirlendi. Ana bileşik olan kafurun enantiyomerik dağılımı MD-GC/MS yöntemi ile belirlendi. Yağın antimikrobiyal aktivitesi de incelendi.

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