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CHEMICAL COMPOSITION and ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Anthemis wiedemanniana* FROM TURKEY

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The chemical composition and antimicrobial activity of the essential oil from aerial parts of Anthemis wiedemanniana, *an endemic taxon of Turkey, were investigated. Linalool (12.75%), 1,8-cineole (8.49%), hexadecanoic acid (6.09%), and chrysanthenone (5.67%) were found to be the main components among the 122 compounds characterized in the essential oil of* Anthemis wiedemanniana. *Antimicrobial activities were reported against 12 microorganisms and five yeast-like fungi by the disc diffusion method.*

Key words: *Anthemis wiedemanniana,* Asteraceae, essential oil, antimicrobial activity.

The genus *Anthemis* L. of *Asteraceae* (Compositae), most of them being endemic, is represented by 50 species in Turkey. One of the endemic species, *Anthemis wiedemanniana* Fisch. et Mey., is distributed in western and southern regions of Turkey. *A. wiedemanniana* is about 10–45 cm tall and grows especially on calcareous soil [1].

This plant is called "Papatya" in the western part of Turkey and infusions of *Anthemis wiedemanniana* are used in Turkish Traditional medicine especially for abdominal pain [2]. Papatya is a common name given to plants whose flowers resemble those of Roman and German chamomile. Many *Anthemis* spp. are used as herbal tea and for food flavoring, as well as cosmetics and in the pharmaceutical industry [3–5]. Extracts, tinctures, salves, and tisanes are widely used as antispasmodic, anti-inflammatory, and antibacterial in Europe. The occurrence of sesquiterpene lactones, flavonoids, and essential oils in various *Anthemis* species has been reported in previous works [6–12].

To the best of our knowledge, there is no published reports on the phytochemical composition and antimicrobial activity of *A. wiedemanniana* essential oil. Therefore, we focused our study on the composition of the oil by GC-MS analysis, and the antimicrobial activity was determined using the agar disc diffusion method.

The results of analysis of *A. wiedemanniana* oil obtained by hydrodistillation are shown in Table 1; GC/MS analysis of the oil was done. 122 components representing 88.1% of *A. wiedemanniana* oil were characterized.

According to our results, the common main constituents of the essential oil from aerial parts of *A. wiedemanniana* are linalool (12.8%), 1,8 cineole (8.5%), hexadecanoic acid (6.1%), and chrysanthenone (5.7%).

Oil compositions of different *Anthemis* species have been reported. *A. carpatica* was reported to contain α-thujone (40.2%), β-thujone (13.3%), yomogi alcohol (18.5%), and terpinen-4-ol (9.7%) as the main components [13]. The occurrence of α-thujone (46.9%), β-thujone (16.0%), and *trans*-chrysanthemyl acetate (11.3%) was reported in the oils of *A. montana* [6]. The studies carried out on *A. nobilis* showed that the two main components of the flower oil were isobutyl angelate (33%) and isoamyl angelate (20%) [14]. In the oil of *A. tinctoria* 1,8-cineole (7.9%), β-pinene (7.3%), decanoic acid (5.4%), and α-pinene (4.4%) were found as the main constituents [15]. Grace et al. found the main components of the essential oil of *A. melampodina* to be santolinatriene (27.3%), β-pinene (76.4%), and sabinene (6.1%) [16]. In the essential oil obtained from the flowerheads of *A. xylopoda*, borneol (31.8%), carvacrol (12.7%), 1,8-cineole (5.5%), and 2,5,5-trimethyl-3,6-heptadien-2-ol (5.1%) were characterized while its leaf oil contained borneol (30.2%), 1,8-cineole (16.7%), α- and β-thujone (12.1%), 2,5,5-trimethyl-3,6-heptadien-2-ol (8.5%), and carvacrol (5.2%) [17].

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TABLE 1. Essential Oil Composition of *Anthemis wiedemanniana*

Compound	RRI	%	Compound	RRI	%
α-Pinene	1032	1.47	Benzaldehyde	1541	Tr.
α-Thujene	1035	0.13	(*E*)-2-nonenal	1548	0.10
Santholina triene	1043	2.09	Linalool	1553	12.75
Camphene	1076	0.46	*trans*-*p*-Menth-2-en-1-ol	1571	0.53
2-Methylpropyl propionate	1090	0.06	Pinocarvone	1586	0.59
Hexenal	1093	0.12	Bornyl acetate	1590	0.36
Isobutyl isobutyrate	1100	Tr.	Hexadecane	1600	0.06
β-Pinene	1118	1.39	β-Elemene	1601	0.21
Sabinene	1132	0.53	6-Methyl-3,5-heptadien-2-one	1610	Tr.
2-Methylbutyl acetate	1134	Tr.	β-Caryophyllene	1612	0.53
Thuja-2,4(10)-diene	1136	Tr.	Terpinen-4-ol	1618	1.32
Myrcene	1174	0.06	Hotrienol	1623	0.15
Isobutyl 2-methyl butyrate	1185	Tr.	4-Terpinenyl acetate	1630	0.07
α-Terpinene	1188	0.17	*cis*-*p*-Menth-2-en-1-ol	1638	0.49
3-Methylbutyl propionate	1196	0.91	Thuj-3-en-10-al	1642	0.30
3-Methyl-2-methylpropyl butyrate (isobutyl isovalerate)	1198	0.28	Myrtenal	1648	0.30
			Isobornyl propionate	1662	0.16
2-Methylbutyl isobutyrate	1200	0.08	*cis*-Verbenol	1663	0.09
Limonene	1203	0.28	*trans*-Pinocarveol	1670	0.18
1,8-Cineole	1213	8.49	Methylchavicol	1677	1.52
cis-Anhydrolinalool oxide	1220	Tr.	*trans*-Verbenol	1683	0.67
(*E*)-2-Hexenal	1232	Tr.	(*E*)-β-Farnasene	1695	0.22
iso-Chrysantenone	1234	0.54	Salicylaldehyde	1703	0.23
2-Pentylfuran	1244	0.13	α-Terpineol	1706	1.63
γ-Terpinene	1255	0.34	Borneol	1719	2.97
Butyl isovalerate	1259	Tr.	Germacrene D	1726	1.20
(*E*)-β-Ocimene	1266	Tr.	β-Bisabolene	1741	Tr.
2-Methylbutyl butyrate	1275	Tr.	β-Selinene	1742	0.38
p-Cymene	1280	1.21	Sesquicineole	1757	0.05
Isoamyl isovalerate	1285	Tr.	*cis*-Piperitol	1758	0.52
2-Methylbutyl 2-methyl butyrate	1286	0.17	*cis*-Chryzanthenol	1764	2.59
1,2,4-Trimethylbenzene	1294	1.02	Cuminaldehyde	1802	0.41
Octanal	1296	0.05	Myrtenol	1804	0.26
2-Methylbutyl isovalerate	1299	0.79	Isogeraniol	1820	0.33
1,2,3-Trimethylbenzene	1355	0.84	(*E*,*E*)-2,4-Decadienal	1827	0.26
(*Z*)-3-Hexenyl propionate	1393	0.06	*trans*-Carveol	1845	0.23
Nonanal	1400	0.39	Geraniol	1857	0.24
(*E*)-2-Octenal	1441	0.08	Isopiperitenone	1865	0.75
Filifolone	1451	2.25	Isobutylphenyl acetate	1909	0.06
1-Octen-3-ol	1452	0.27	2-Methylbutyl benzoate	1911	0.09
1-Heptanol	1463	0.08	Phenylethyl propionate	1912	0.13
Eucarvone	1465	0.19	(*E*)-beta-Ionone	1958	0.24
trans-Sabinene hydrate	1474	0.22	2-Phenylethylisovalerate	1992	Tr.
cis-Linalool oxide	1478	Tr.	Caryophyllene oxide	2008	2.56
Isonerol oxide-I	1487	0.32	Humulene epoxide II	2071	0.17
2-Ethylhexanol	1496	0.06	Octanoic acid	2084	0.52
α-Campholene aldehyde	1499	0.22	Elemol	2096	0.14
Decanal	1506	0.17	Hexahydrofarnesylacetone	2131	0.51
Chrysanthenone	1522	5.67	Spathulenol	2144	0.52
Camphor	1532	1.72	3,4-Dimethyl-5-pentylidene-2 (5H)furanone	2179	0.32

TABLE 1. (Continued)

Compound	RRI	%	Compound	RRI	%
Nonanoic acid	2185	0.31	Dodecanoic acid	2503	0.75
T-Cadinol	2187	0.24	1-Octadecanol	2596	Tr.
Eremoligenol	2211	0.77	Hexacosane	2600	0.10
β-Eudesmol	2257	0.64	Tridecanoic acid	2617	0.15
Decanoic acid	2299	3.63	Phytol	2622	0.15
Tricosane	2300	0.05	(*Z*)-Octadec-9-en-18-olide	2688	0.04
Caryophylladienol-I	2316	0.22	Heptacosane	2700	0.39
Caryophylladienol-II	2324	0.68	Tetradecanoic acid	2710	1.77
Caryophyllenol-I	2389	0.15	Pentadecanoic acid	2822	0.24
Eudesmo-4(15)-7-dien-1-E-ol	2391	0.11	Nonacosane	2900	Tr.
Caryophyllenol-II	2392	0.37	Hexadecanoic acid	2931	6.09
Pentacosane	2500	Tr.	Total		88.07

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RRI: relative retention indices calculated against *n*-alkanes. Percentage calculated from TIC data; trace (<0.1%).

TABLE 2. The Antimicrobial Activity of Essential Oil of *Anthemis wiedemanniana*

Microorganisms	Source No.	Inhibition Zone (mm)*			
		Essential oil	Standard antibiotics		
			CF20	SAM20	NS20
		Gram-positive			
Bacillus cereus	ATCC 7064	8	11	8	Nt.
Bacillus subtilis	ATCC 6633	7	24	13	Nt.
Enterococcus faecalis	ATCC 29212	10	17	19	Nt.
Staphylococcus aureus	ATCC 6538/P	10	24	23	Nt.
Stapylococcus epidermidis	ATCC 12228	8	12	19	Nt.
		Gram-negative			
Enterococcus cloae	ATCC 13047	-	10	17	Nt.
Escherichia coli	ATCC 11230	11	21	13	Nt.
Escherichia coli	ATCC 29998	9	22	12	Nt.
Klebsiella pneumoniae	CCM 2318	-	25	11	Nt.
Proteus vulgaris	ATCC 6897	-	30	26	Nt.
Pseudomonas aeroginosa	ATCC 27853	8	30	-	Nt.
Salmonella tyhimurium	CCM 5445	9	20	15	Nt.
		Fungi			
Candida albicans	ATCC 60193	-	Nt.	Nt.	17
Candida albicans	CDC 311	-	Nt.	Nt.	17
Candida albicans	ATCC 10239	-	Nt.	Nt.	19
Candida krusei	ATCC 6258	-	Nt.	Nt.	14
Candida tropicalis	RSSK 665	-	Nt.	Nt.	15

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The essential oil of A. wiedemanniana (20 mL); CF20: Ceftazidime (20 mg); SAM20: Sulbactam (10 mg)/Ampicillin (10 mg); NS 20: Nystatin (20 mg); Nt.: not tested; -: not active.

*Includes diameter of disc (6 mm).

Results from the antimicrobial screening tests are shown in Table 2. As clearly seen in Table 2, the essential oil of *A. wiedemanniana* inhibited the growth of nine out of twelve microorganisms but had no effect on the growth of *Enterobacter cloacae* ATCC 13047, *Klebsiella pneumoniae* CCM 2318, and *Proteus vulgaris* ATCC 6897. In this study, the antimicrobial activities of essential oil of 20 μL/discs are compared with standard antibiotics such as ceftazidime (CF20), sulbactam/ampicilin (SAM20), and nystatin (NS20) used as positive controls. Previous studies showed that linalool and 1,8-cineole are well-known antimicrobial compounds isolated from different plant species [18–22]. The antimicrobial activity of the oil could, in part, be associated with linalool and 1,8-cineole.

EXPERIMENTAL

Aerial parts were collected from Izmir-Bozdag, in May 2003 and identified by E. Cetin of Harran University. The voucher specimens (No. 1325) are deposited in the Herbarium of the Faculty of Pharmacy, Ege University, in Izmir. The essential oil of *A. wiedemanniana* was obtained by hydrodistillation of the comminuted above-ground parts of the plant for 4 h.

GC/MS Analysis of the Essential Oil. The oil was analyzed by GC/MS using a Hewlett-Packard MSD system. An HP-Innowax FSC column (60 m × 0.25 mm; 0.25 μm film thickness) was used with helium as carrier gas (1 mL/min). The injector temperature was at 250°C. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C temperature at a rate of 4°C/min, and then kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Split ratio was adjusted at 50:1. MS was performed at 70 eV. Mass range was from *m/z* 35–425. Library search was carried out using Wiley GC/MS Library and Baser Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by the computer.

Microorganisms. Gram-positive and gram-negative bacteria and yeastlike fungi were used for antimicrobial activity studies. Gram-negative bacteria used were *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998, *Escherichia coli* ATCC11230, *Klebsiella pneumoniae* CCM 2318, *Proteus vulgaris* ATCC 6897, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella thyphimurium* CCM 5445. Gram-positive bacteria used were *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538/P, and *Staphylococcus epidermidis* ATCC 12228. Yeastlike fungi used were *Candida albicans* ATCC 10239, *Candida albicans* ATCC 60193, *Candida albicans* CDC 311, *Candida krusei* ATCC 6258, and *Candida tropicalis* RSSK 665. Lyophilised bacteria and yeasts were obtained from the Standard ATCC bacteria strain and Standard ATCC fungi strain collection of the Science Faculty of Ege University, Department of Basic and Industrial Microbiology, Faculty of Science, Ege University.

Antimicrobial Studies. The essential oil was tested for antimicrobial activity by the disc diffusion method according to the National Committee for Clinical Laboratory Standards [23, 24] using 100 μL of a suspension of the tested microorganisms, containing 2.0×10^6 CFU/ml for bacteria and 2.0×10^5 CFU/mL spore for fungal strains. Mueller-Hinton agar (MHA) (Oxoid) and Sabouraud Dextrose Agar (SDA) (Difco) sterilized in a flask and cooled to 45–50°C were distributed on sterlized Petri dishes with a diameter of 9 cm (15 mL). The filter paper discs (6 mm in diameter) were individually impregnated with 20 μL of the oil and then placed onto the agar plates which had previously been inoculated with the tested microorganisms. The petri dishes were kept at 4°C for 2 h. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°C for 48 h for fungal strain. The diameters of the inhibition zones were measured in millimetres. The standard antibacterial agents Ceftazidime (20 μg/disc) and Sulbactam (10 μg)/Ampicillin (10 μg) were used as a positive control for bacteria, and the standard antifungal agent nystatin (20 μg/disc) was used as the positive control for yeast. All experiments were done in triplicate.

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