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Composition and Antimicrobial Activity of the Essential Oils of Some *Achillea* L. Species in Turkey

Türkiye'deki bazı *Achillea* L. Taksonlarının Uçucu Yağlarının Bileşimi ve Antimikrobiyal Aktivitesi

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Abstract

The composition and antimicrobial activity of essential oils obtained from *Achillea millefolium* L. subsp. *pannonica* (Scheele) Hayek, *A .millefolium* L. subsp. *millefolium*, *A. crithmifolia* Waldst&Kitt. and *A. kotschyi* Boiss. subsp. *kotschyi* of Turkish flora were investigated. 1,8-cineole (43.3%) in *A. millefolium* L. subsp. pannonica, artemisia alcohol (37.2%) in *A. millefolium* subsp. *millefolium*, ascaridole (27.2%) in *A. crithmifolia* and 1,8-cineole (20.8%) in *A. kotschyi* essential oil were identified as major components by GC and GC/MS analysis. The essential oils showed antibacterial and antifungal effect even with low concentrations.

Keywords: Achillea; Asteraceae; essential oil; antimicrobial activity.

Introduction

Achillea L. (Asteraceae) genus is represented by 42 species in the flora of Turkey and the rate of endemism is 50% (Huber-Morath, 1975; Duman, 2000). This genus is a group that comprises hardly distunguishable species and subspecies and studies on the composition of their essential oils have been tentatively used as an additional characteristic of differentiation (Figueiredo *et al.*, 1992). As known from folk medicine various species of *A. millefolium* group are used as herbal remedies for antiinflammatory, spasmolytic, hemostatic, digestive, cholagogue and antimicrobial effects (Kastner *et al.*, 1995; Chandler *et al.*, 1982) *A. millefolium* and related species are also frequently used against diarrhoea, abdominal pain and stomachache in Turkish traditional medicine (Yeşilada *et al.*, 1993; Fujita *et al.*, 1995; Honda *et al.*, 1996). Although the essential oils of *A.millefolium* L. and closely related species have already been a subject of extensive studies mainly due to its medicinal properties (Kastner U. *et al.*, 1992; Figueiredo *et al.*, 1992; Afsharypuor S. *et al.*, 1996;

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Pino J.A. *et al.*, 1998; Chalchat J.C. *et al.*, 1999; Skagen *et al.*, 2000; Nemeth E. *et al.*, 2000), no previous investigation on the composition of the essential oils of *A. millefolium*, *A. crithmifolia* and *A. kotschyi* growing in Turkey have been accomplished. The present study was carried out to determine the composition and antimicrobial activity of essential oils of these plants growing in the flora of Turkey.

Material and Method

The plants were collected at flowering time from the following places: *A. millefolium* subsp. *pannonica* from Yozgat, Akdağmağdeni, 2000m., *A. millefolium* subsp. *millefolium* from Edirne, Pazarkule, 100m, *A.crithmifolia* from Kırklareli, Demirköy, 280m, and *A. kotschyi* subsp. *kotschyi* from Erzurum, Oltu, 1250m in 2001. Voucher specimens were deposited in the Herbarium of E.U., Faculty of Pharmacy, Izmir (IZEF) with numbers 5515, 5489, 5483 and 5505, resp. The dried flower heads of plants were subjected to hydrodistillation for 3h using a Clevenger-type apparatus. The essential oils were analysed by GC and GC/MS [HP 6890 GC, HP 5973 MS, 5m x 0.2mm HP-1 capillary column (0.33μm coating), oven temperature: 50°C to 300°C (10°C/min), injector temperature:150°C, dedector temperature:250°C, carrier gas: helium (20mL/min)]. Identification of the compounds of the essential oils was assigned by comparison of their Retention Indices (RI) relative to C_8-C_{32} *n*-alkanes mixture (Adams, 1995). Computerized search was carried out using Wiley 275 L. GC/MS library and ARGEFAR GC/MS library created with authentic samples. All of the essential oils were investigated for in vitro antibacterial activity for 3 gram-positive, 5 gram-negative bacteria and *Candida albicans* ATTC 10239 with microdilution method among the Broth dilution methods (Table 2) (NCCLS,1990).

Results and Discussion

The yields of the essential oils of *A. millefolium* subsp. *pannonica*, *A. millefolium* subsp. *millefolium*, *A. crithmifolia* and *A. kotschyi* subsp. *kotschyi* were 0.72%, 0.25%, 0.45% and 0.15% (v/w), resp. 1,8-cineole (43.3%) in *A. millefolium* subsp. *pannonica*, artemisia alcohol (37.2%) in *A. millefolium* subsp. *millefolium*, ascaridole (27.2%) in *A. crithmifolia* and 1,8-cineole (20.8%) in *A. kotschyi* subsp. *kotschyi* essential oil were identified as major components by GC and GC/MS analysis (Table 1). Although a great variation of the presence of proazulene content is known in the members of the Millefolium group (Haggag *et al.*, 1975), no azulenes were detected in the analysed essential oils. The occurrence of relatively large amounts of artemisia alcohol in *A. millefolium* subsp. *millefolium* is remarkable. Results identify a new chemotype of *A. crithmifolia* rich in ascaridole.

All of the tested essential oil dilutions (5%v/v) exhibited antifungal activity against *C. albicans* (Table 2). Only the essential oils of *A. millefolium* subsp. *millefolium* and *A.millefolium* subsp. *pannonica* showed activitiy against the Gram-negative bacterias *P. vulgaris* and *S. thypimurium*.

Antimicrobial activities of the essential oils may be related to the high amounts of 1,8-cineole, artemisia alcohol and camphor and also these results support the use of these medicinal plants in traditional remedies.

Table 1. Constituents of the essential oil of *Achillea* L. species.

Compound	RI	1	2	3	4
α-Thujene	927	t	t	t	t
α-Pinene	936	0.8	t	1.2	2.3
Camphene	950	1.9	t	1.1	t
β-Pinene	977	0.6	0.1	t	2.7
Yomogi alcohol*	994	0.1	17.7	-	-
α-Terpinene	1016	0.4	-	12.0	1.8
1,8-Cineole	1032	43.3	4.6	5.7	20.8
Artemisia keton	1053	-	-	3.5	-
(E)-β-Ocimene*	1054	11.9	-	-	-
γ-Terpinene	1057	-	-	-	1.0
Artemisia alcohol	1082	0.7	37.2	-	-
Terpinolene	1093	0.4	-	-	-
Linalool	1096	-	-	2.7	-
α-Thujone*	1114	-	-	-	1.4
Chrysanthenone	1118	-	-	13.5	-
Camphor	1141	10.4	-	18.8	4.9
Pinocarvone*	1145	-	3.8	-	4.1
Chrysanthenol	1160	-	-	-	3.9
Borneol	1169	16.9	2.5	1.7	2.0
Terpinene-4-ol	1178	3.1	-	2.3	4.5
α-Terpineol	1189	2.3	-	-	2.6
Myrtenol	1196	-	-	-	1.4
Ascaridole	1238	-	-	27.2	-
Bornyl acetate	1284	0.7	8.8	-	-
cis-Jasmone	1390	-	-	0.5	-
β-Caryophyllene	1441	-	-	0.6	1.0
Elemol	1559	-	2.9	-	-
(*E*)-Nerolidol	1567	-	1.7	-	5.2
Caryophyllene oxide[1]	1608	1.9	2.5	1.8	7.4
β-Eudesmol	1650	-	2.3	-	-
α-Bisabolol oxide B*	1654	-	-	-	3.9
dihidro-Eudesmol*	1656	0.7	-	-	3.2
β-Bisabolol*	1669	1.4	1.5	-	4.1
α-Bisabolol oxide A*	1674	-	3.6	-	4.7
Total		97.5	89.2	92.6	82.9

1 *A.millefolium* subsp. *pannonica*, 2. *A.millefolium* subsp. *millefolium*, 3. *A.critmifolia*, 4. *A.kotschyi* subsp. *kotschyi*. RI: Retention Indice on a HP-1 capillary column, t: trace (≤0.1%), [1]Identification based on mass spectra only, *Identification based on RI value only.

Table 2. Antimicrobial activity of the essential oil of *Achillea* L. species.

Microorganisms	1	2	3	4
Staphylococcus aureus 6538/P	-	-	-	-
Staphylococcus epidermidis ATCC 12228	-	-	-	-
Escherichia coli ATCC 29998	-	-	-	-
Pseudomonas aeroginosa ATCC 27853	-	-	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-
Enterobacter cloacae ATCC 13047	-	-	-	-
Proteus vulgaris ATCC6897	+	+	-	-
Salmonella thypimurium CMM 5445	+	+	-	-
Candida albicans ATCC 10239	+	+	+	+

1 *A.millefolium* subsp. *pannonica*, 2. *A.millefolium* subsp. *millefolium*, 3. *A.crithmifolia*, 4. *A.kotschyi* subsp. *kotschyi* essential oil (5% v/v). - : no inhibition, + : inhibition

Özet

Achillea millefolium L. subsp. *pannonica* (Scheele) Hayek, *A .millefolium* L. subsp. *millefolium*, *A. crithmifolia* Waldst&Kitt. ve *A. kotschyi* Boiss. subsp. *kotschyi*'den elde edilen uçucu yağların bileşimi ve antimikrobiyal aktivitesi incelenmiştir. GC ve GC/MS analizi ile *A. millefolium*. subsp. pannonica uçucu yağında 1,8-cineol (%43,3), *A. millefolium* subsp. *millefolium*'da artemisia alcohol (37.2%), *A. crithmifolia*'da ascaridole (27.2%) ve *A. kotschyi* subsp. *kotschyi*'de ise 1,8-cineole (20.8), ana bileşenler olarak tanımlanmıştır. Uçucu yağlar düşük konsantrasyonlarda bile antifungal ve antibakteriyal etki göstermiştir.

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