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Characterization and Biological Activity of *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek Essential Oils

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Abstract: The aerial parts of the endemic *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek collected from Beyşehir were investigated for their essential oil compositions and several biological activities. The essential oils were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main components were identified as 1,8-cineole (34%), camphor (11%), terpinen-4-ol (8%), and α-thujone (5%) for *A. teretifolia* essential oil, while fragranyl acetate (32%), fragranol (24%), and β-eudesmol (8%) for *A. nobilis* subsp. *neilreichii* essential oil, respectively. The essential oils were also evaluated for their in vitro antimicrobial activity and their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals using microdilution techniques as an index for the evaluation of antioxidant activity. Both essential oils were found to be effective against the tested human pathogenic microorganisms (MIC 0.5 to >2 mg/ml) and DPPH (IC_{50} > 0.5 mg/ml) assay as compared to the references.

Key Words: Asteraceae; *Achillea*; essential oil; GC and GC-MS; antimicrobial activity; antioxidant activity

Achillea teretifolia Willd. ve *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek Uçucu Yağlarının Kimyasal Bileşimi ve Biyolojik Aktivitesi

Özet: Bu çalışmada, Beyşehir'den toplanan endemik bir tür olan *Achillea teretifolia* Willd. ile *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek bitkilerinin toprak üstü kısımları uçucu yağ bileşimleri ve çeşitli biyolojik aktiviteleri yönünden incelenmiştir. Uçucu yağlar hem gaz kromatografisi (GC) ve hem de gaz kromatografisi-kütle spektrometresi (GC-MS) ile analiz edilmiştir. *A. teretifolia* uçucu yağında 1,8-sineol (% 34), kafur (% 11), terpinen-4-ol (% 8), α-tuyon (% 5), *A. nobilis* subsp. *neilreichii* uçucu yağında ise fragranil asetat (% 32), fragranol (% 24) ve β-ödesmol (% 8) başlıca bileşikler olarak tayin edilmiştir. Uçucu yağların in vitro mikrodilüsyon teknikleri kullanılarak antimikrobiyal aktivitesi ve aynı zamanda genel antioksidan aktivite değerlendirilmesinde gösterge olan 1,1-difenil-2-pikrilhidrazil (DPPH) radikallerini süpürücü kapasitesi incelenmiştir. Her iki uçucu yağın, referansla karşılaştırıldığında test edilen insan patojeni mikroorganizmalara (MIC 0,5 - > 2 mg/ml) ve DPPH (IC_{50} > 0,5 mg/ml) karşı aktiviteye sahip olduğu bulunmuştur.

Anahtar Sözcükler: Asteraceae; *Achillea*; uçucu yağ; GC ve GC-MS; antimikrobiyal aktivite; antioksidan aktivite

Introduction

The genus *Achillea* of the Asteraceae family is diversely distributed in Anatolia and represented by 42 species of which 23 are endemic for Turkey (1,2).

From a wide perspective, *Achillea* species comprise an important biological resource in Turkish folk medicine against gastro-intestinal complaints (stomachache, abdominal pain, flatulence, diarrhea, and hemorrhoids),

inflammatory disorders (rheumatic pain, for maturation on abscess, and eye inflammations), for wound healing, as emmenagogue, as diuretic, against jaundice, and for many other complaints (3-9). Particularly, yarrow (*Achillea millefolium* L.), inflorescence, or aerial part is recorded as an official plant in the European Pharmacopoeia and suggested as choleretic, antibacterial, astringent, and antispasmodic remedy in official monographs and prescribed as herbal tea, sitz bath, or pressed juice (10).

Antinociceptive and anti-inflammatory (11,12), human erythrocyte and leukocyte protective (13), antispasmodic (14), antimicrobial (15-20), and antioxidant activities (17,18,20) of different *Achillea* species growing in Turkey were previously reported. Antibacterial and antifungal activities against a wide spectrum of pathogens as well as antioxidant properties of essential oils from several *Achillea* species and their compositions were also investigated (20-23).

The essential oil of the plant is an important portion within the phytochemistry of *Achillea* species (23,24). Monoterpenes, such as pinene, 1,8-cineole, camphor, artemisia ketone and sesquiterpenes, such as caryophyllene, germacrene, azulene and derivatives as well as sesquiterpene lactones, polyenes, alkamides, flavonoids, lignans, and triterpenes are considered as important major constituents and their amounts vary within different species (20,23-27).

In the present study, the aerial parts of the endemic *Achillea teretifolia* Willd. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formanek collected from Beyşehir were investigated for their essential oil compositions and some biological activities. The essential oils were analyzed by both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The essential oils were evaluated in vitro for their antimicrobial and radical scavenging activities.

Material and Methods

General

All chemicals, solvents, culture media, and standards were purchased from Sigma-Aldrich-Fluka (Taufkirchen, Germany), Carlo Erba, Italy, or Merck (Darmstadt, Germany) and were reagent grade (purity >99%), unless indicated otherwise.

Plant material

Achillea teretifolia (endemic) and *A. nobilis* L. subsp. *neilreichii* were collected during flowering time (June 1999) from a roadside (31 km of Beyşehir-Şarkikaraağaç road and 25 km of Beyşehir-Yeşildağ road, respectively) from Konya, Turkey. Voucher specimens [*Achillea teretifolia* (99A053) *A. nobilis* L. subsp. *neilreichii* (99A045)] were stored in the Herbarium of Gazi University, Faculty of Pharmacy.

Isolation of the Essential Oils

Each plant material was air dried in shade at room temperature and the chopped aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the essential oils. The yields for *A. teretifolia* and *A. nobilis* L. subsp. *neilreichii* were found to be 0.48% and 0.27% (v/w), respectively.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm 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 increase to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to increase to 240 °C at a rate of 1 °C/min. Split ratio was adjusted at 40:1. The injector temperature was at 250 °C. Mass spectra were obtained at 70 eV. Mass range was from *m/z* 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was set to 300 °C. In order to obtain the same elution order with GC-MS, simultaneous injection was carried out using the same column and appropriate operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analyses are shown in Table 1.

Identification of the Essential Oil Components

Identification of the *Achillea* essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC-MS, MassFinder 3, Adams Library) and in-house

Table 1. The composition of the essential oils of *A. nobilis* ssp. *neilrechii* and *A. teretifolia*.

No	Compound	RRI	AN (%)	AT (%)
1	Tricyclene	1014	nd	0.1
2	α-Pinene	1032	nd	0.6
3	α-Thujene	1035	nd	1.1
4	Santolinatriene	1043	nd	2.5
5	Camphene	1076	nd	1.5
6	β-Pinene	1118	0.1	1.6
7	Sabinene	1132	nd	0.4
8	α-Phellandrene	1176	nd	0.1
9	α-Terpinene	1188	tr	1.4
10	Dehydro-1,8-cineole	1195	nd	0.1
11	Limonene	1203	nd	0.1
12	1,8-Cineole	1213	0.8	**33.7**
13	γ-Terpinene	1255	tr	2.3
14	p-Cymene	1280	0.1	2.6
15	Terpinolene	1290	nd	0.6
16	2-Methylbutyl isovalerate	1299	nd	0.2
17	α-Thujone	1437	nd	5.4
18	Filifolone	1445	tr	0.1
19	β-Thujone	1451	nd	1.0
20	Eucarvone	1465	nd	0.1
21	trans-Sabinene hydrate	1474	tr	0.4
22	α-Copaene	1497	0.1	nd
23	Chrysanthenone	1522	nd	0.1
24	Camphor	1532	nd	**11.4**
25	cis-α-Bergamotene	1545	0.2	nd
26	Linalool	1553	1.5	0.1
27	cis-Sabinene hydrate	1556	tr	0.3
28	trans-p-Menth-2-en-1-ol	1571	nd	0.9
29	cis-Chrysanthenyl acetate	1582	0.5	nd
30	Pinocarvone	1586	nd	0.3
31	Terpinen-4-ol	1611	0.1	7.9
32	β-Caryophyllene	1612	nd	0.2
33	Lavandulyl acetate	1617	0.1	nd
34	4-Terpinenyl acetate	1630	0.4	0.2
35	cis-p-Menth-2-en-1-ol	1638	nd	0.7
36	Myrtenal	1648	nd	0.1
37	Alloaromadendrene	1661	nd	0.5
38	Sesquisabinene	1669	0.1	nd
39	Fragranyl formate	1670	0.1	nd
40	δ-Terpineol	1682	nd	0.6
41	trans-Chrysanthemol	1684	nd	tr
42	trans-Piperitol (=trans-p-Menth-1-en-3-ol)	1689	nd	0.3
43	γ-Curcumene	1704	0.4	nd
44	Fragranyl acetate	1705	**31.8**	nd
45	α-Terpineol	1706	nd	1.6
46	Borneol	1719	nd	1.0
47	Germacrene D	1726	0.1	nd
48	Piperitone	1748	nd	0.2
49	Carvone	1751	nd	0.2
50	-Curcumene	1755	0.4	nd
51	cis-Piperitol	1758	nd	0.3
52	cis-Chrysanthenol	1764	nd	0.7
53	δ-Cadinene	1773	0.1	tr
54	γ-Cadinene	1776	nd	0.4
55	Fragranyl isobutyrate	1777	1.6	nd
56	ar-Curcumene	1786	0.7	0.1
57	α-campholene alcohol	1793	nd	0.1
58	Myrtenol	1804	nd	0.1
59	Fragranol	1807	**24.1**	nd
60	trans-Carveol	1845	nd	0.1
61	Fragranyl butyrate	1852	2.5	nd
62	Fragranyl 2-methylbutyrate	1863	1.5	nd
63	p-Cymen-8-ol	1864	nd	0.1
64	Fragranyl 3-methylbutyrate	1874	1.7	nd
65	cis-Carveol	1882	nd	0.1
66	Fragranyl valerate	1952	0.2	nd
67	cis-Jasmone	1969	nd	0.4
68	trans-Sesquisabinene hydrate	2000	0.4	nd
69	Caryophyllene oxide	2008	0.1	2.5
70	Methyl eugenol	2030	nd	0.1
71	Salvial-4(14)-en-1-one	2037	0.1	nd
72	Fragranyl hexanoate	2056	0.1	nd
73	13-Tetradecanolide	2056	nd	0.1
74	Humulene epoxide-II	2071	nd	0.1
75	Caryophylla-2(12), 6(13)-dien-5-one	2074	nd	0.1
76	-Oplopenone	2092	nd	0.2
77	cis-Sesquisabinene hydrate	2096	1.7	nd
78	Cumin alcohol	2113	nd	0.1
79	Spathulenol	2144	0.2	0.2
80	β-Bisabolol	2170	0.9	nd
81	γ-Eudesmol	2185	1.3	nd
82	Eugenol	2186	0.2	0.4
83	T-Cadinol	2187	nd	2.4
84	Eremoligenol	2204	0.2	nd
85	ar-Turmerol	2214	0.5	0.2
86	α-Eudesmol	2250	nd	0.6
87	β-Eudesmol	2257	7.7	nd
88	15-Hexadecanolide	2260	nd	0.2
89	Intermedeol	2264	0.2	nd
90	Torilenol	2278	0.1	nd
91	Decanoic acid	2298	nd	0.1
92	Caryophylla-2(12),6(13)-dien-5β-ol (=Caryophylladienol I)	2316	nd	0.6
93	Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol II)	2324	nd	1.9
94	Eudesma-4(15),7-dien-4β-ol	2369	0.1	nd
95	Hexadecanol	2384	0.1	nd
96	Caryophylla-2(12),6-dien-5α-ol (=Caryophyllenol I)	2389	nd	0.5
97	Caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II)	2392	nd	0.9
98	γ-Costol	2533	0.2	nd
99	1-Octadecanol	2607	0.1	tr
100	Hexadecanoic acid	2931	1.6	0.3
		Total	**85.0**	**96.4**

AN: *A. nobilis* subsp. *neilreichii* essential oil; AT: *A. teretifolia* essential oil; RRI: Relative retention indices calculated against *n*-alkanes; %: Percentages were calculated from FID data; tr: Trace (<0.1%); nd: Not detected.

"Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data, was also used for the identification (28-32).

Antimicrobial Bioassay

A micro-dilution broth susceptibility assay was used (33,34). Stock solutions of the test samples were prepared in 25% (v/v) dimethylsulfoxide (DMSO, Carlo Erba, Italy). Serial dilutions of samples were prepared up to 1.95 µg/ml by using sterile distilled water in a 96-well microtiter plate. Microbial suspensions grown as described previously (33) were standardized to 1 x 10^8 CFU ml^{-1} using McFarland No: 0.5 in double strength Mueller-Hinton broth (MHB, Merck, Germany). Then, 100 µl of each microbial suspension was added to the appropriate well. The last row, which contained only the serial dilutions of the essential oil without microorganism, was used as a negative control. To eliminate solvent effects DMSO dilutions were considered as another control. After incubation at 37 ºC for 24 h, the first well without turbidity was determined as the minimal inhibitory concentration (MIC, mg/ml). Chloramphenicol and Ketoconazole were used as standard antimicrobials. All experiments were repeated in triplicate and average MICs are given in Table 2.

Antioxidant Activity

Free Radical Scavenging Activity using DPPH: The test substances were screened for their radical scavenging activity (35,36) preliminary at 0.5 mg/ml concentration in MeOH qualitatively using a TLC assay to detect active substances. The active substances were subjected to a quantitative UV-spectroscopic microdilution assay where inhibitory concentrations were determined in comparison with standard substances.

Qualitative Assay: 1 ml of stock solution of each test sample and controls were applied onto the TLC plate - after development- and air dried for complete solvent evaporation. Then, 0.2% (w/v) DPPH in MeOH was sprayed on to TLC plate after application or development of the samples.

Quantitative Assay: Stock solutions of each test sample was prepared to obtain a final concentration of 0.5 mg/ml in methanol, which were transferred in aliquots of 100 µl of each test solution to the first row of the 96 well plates. Ten fold serial dilutions in an equal amount of MeOH were prepared using a multichannel pipette and vortexed for 5 min. Then, 2 mg of DPPH was dissolved in 25 ml of methanol to obtain a stock reagent solution with a final concentration of 80 µg/ml. To initiate the reaction 100 µl of DPPH solution was added into each well and left for 30 min in dark. Vitamin C, BHT (butylated hydroxytoluene), and quercetin were used as positive controls at the same concentration, DPPH+MeOH served as negative control, and only MeOH as blank on the same test plate. The UV absorbance was read at 517 nm using a microplate spectrophotometer (BioTek, Powerwave) at room temperature. All experiments were performed in quadruplets in 2 different plates. Results were expressed as mean ± standard deviation (n = 8). For statistical evaluations and calculations Gen5 Data Analysis Software (BioTek) was used. Half maximal of the inhibitory concentration (IC_{50}) values were calculated using the formula $IC_{50} = [(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 the absorbance of the test sample.

Table 2. Antimicrobial activity results (MIC, mg/ml) of *Achillea* essential oils.

Microorganisms	Strain Numbers	AN	AT	Chloramphenicol	Ketoconazole
Escherichia coli	NRRL B-3008	>2	>2	0.0078	nt
Staphylococcus aureus	ATCC 6538	>2	>2	0.0019	nt
Pseudomonas aeruginosa	ATCC 27853	>2	>2	0.125	nt
Enterobacter aerogenes	NRRL 3567	>2	>2	0.0039	nt
Proteus vulgaris	NRRL B-123	0.5	0.5	0.0156	nt
Salmonella typhimurium	ATCC 13311	>2	1	0.0039	nt
Bacillus cereus	NRRL B-3711	>2	>2	0.031	nt
Candida tropicalis	NRRL Y-12968	0.5	>2	nt	0.25

nt: Not tested; AN: *A. nobilis* subsp. *neilreichii* essential oil; AT: *A. teretifolia* essential oil

Results and Discussion

Chemical Composition

The essential oils were obtained from the air dried aerial parts of *A. teretifolia* and *A. nobilis* subsp. *neilreichii* by hydrodistillation, which were immediately analyzed by both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) system. Overall, 100 essential oil components were identified. The main components of *A. teretifolia* essential oil were determined as 1,8-cineole (34%), camphor (11%), terpinen-4-ol (8%), and α-thujone (5%), which comprised an important fraction of oxygenated monoterpenes (>70%). Sixty nine components were identified representing 96% of the total *A. teretifolia* essential oil.

As a rare constituent, fragranyl acetate (32%) and its alcohol fragranol (24%), along with β-eudesmol (8%) were identified as the main components of *A. nobilis* subsp. *neilreichii* essential oil. In addition, the essential oil was also found to be rich in oxygenated monoterpenes (>60%), and 48 components were characterized with a gross sum of 85%. To the best of our knowledge, this is the first report on the essential oil composition of *A. nobilis* subsp. *neilreichii.* All identified components are listed in Table 1, and the chemical structures of the major components of both essential oils are given in the Figure.

Essential oil compositions from various *Achillea* species were previously reported by our research group (15,19,37-39). Mainly monoterpenes were identified as the major constituents. However, in a recent work on the essential oil composition of *A. schischkinii* Sosn. and *A. aleppica* DC. subsp. *aleppica*, 1,8-cineole was also reported as the main constituent (32.5% and 26.1%, respectively). The essential oil of *A. aleppica* subsp. *aleppica* was also found to be rich in bisabolol and its derivates, and showed significant anti-inflammatory and antinociceptive, and moderate antimicrobial activities (19).

According to a comprehensive work by Nemeth (24), a variation in the chemical composition of the essential oils within the genus *Achillea* in different plant parts seem to depend on the investigated species. The monoterpenes, such as 1,8-cineole, camphor, borneol, and α- and β-pinene were most abundant along with the sesquiterpenes, such as chamazulene, β-caryophyllene, and its oxide. Generally, domination of sesquiterpene derivatives compared to the monoterpenes was found in the vegetative parts of the plant.

In a previous study on the essential oil composition of the endemic plant *A. teretifolia*, 1,8-cineole (19.9%) was determined as the major component along with 42 compounds, representing 87% of the total essential oil as well as antimicrobial activity (16).

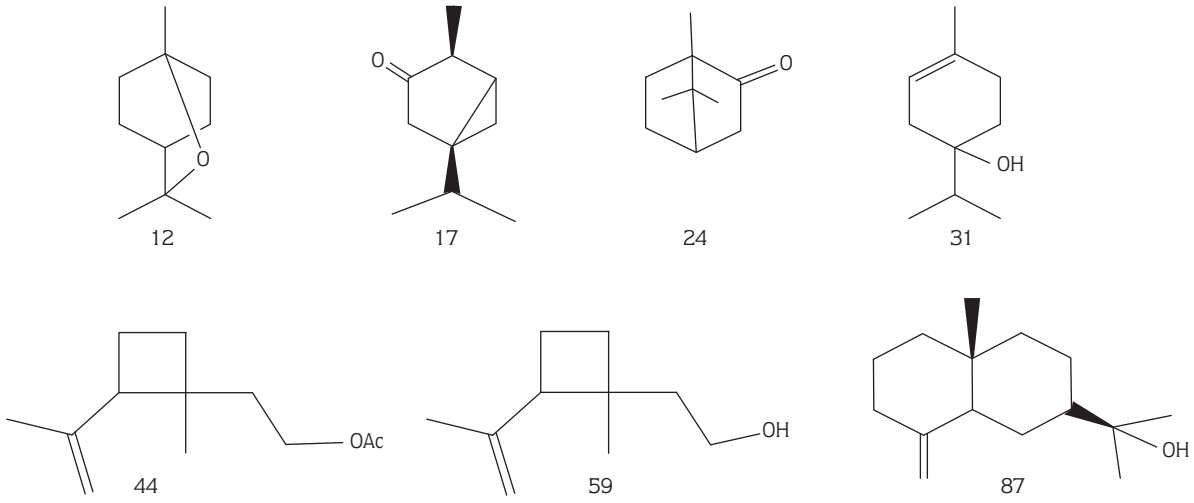

Figure. Major constituents of *Achillea* sp. (AT and AN) essential oils.

Antimicrobial and Free Radical Scavenging Activities

The essential oils were evaluated for their in vitro antimicrobial activities and their ability for scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals using microdilution techniques. Both essential oils were found fairly to be active against the tested human pathogenic microorganisms (MIC 0.5 to >2 mg/ml) and in the DPPH (IC_{50}> 0.5 mg/ml) assay when compared to references (Tables 2 and 3). However, it is interesting to note that the gram-negative pathogen *Proteus vulgaris* was more sensitive to both essential oils with a MIC of 0.5 mg/ml when compared to the other tested pathogens. *A. nobilis* subsp. *neilreichii* essential oil also inhibited the yeast-like pathogen *Candida tropicalis* -in the same range- and *Salmonella typhimurium*. Although oxygenated monoterpenes are the major components in both oils, other antagonistic compounds might possibly exist in both oils which lower both the antimicrobial and radical scavenging activity as observed.

It is well-known that the essential oils and their constituents possess good inhibitory effects against a wide spectrum of pathogens, as in the case of several *Achillea* essential oils (15,16-23,40). Depending on the chemical composition of the essential oil and the antimicrobial assays technique employed, the results obtained may display great variations from inactive to active. As in a previous work of Ünlü et al. (16), the antimicrobial activity of *A. teretifolia* essential oil was studied against 14 microorganisms and inhibitory effect was found against *Clostridium perfringens*, *Acinetobacter iwoffii* and *Candida albicans* showing MIC values of 0.28 to 2.25 mg/ml. On the other hand, it has been reported that gram-negative strains are generally less susceptible to essential oils due to the presence of an outer membrane surrounding the cell wall, which restricts diffusion of hydrophobic compounds, like essential oils, through the lipopolysaccharide covering among other possible mechanisms of action (41). This conclusion may be an explanation for the weak inhibitory effect determined in this study.

Previously, several *Achillea* essential oils were reported to possess good antioxidant activity in various test models (17,18,20,22). However, generally a higher antioxidant activity was reported for the aqueous and polar fractions of the essential oil bearing plants (13,18,20). Konyalıoğlu and Karamenderes reported that the infusions of *A. teretifolia* and *A. nobilis* subsp. *neilreichii* within 15 other *Achillea* species were found to be active on various antioxidant enzyme systems (13).

In a very recently published work, the antinociceptive and anti-inflammatory activities were reported for the ethanol extract of *A. nobilis* subsp. *neilreichii* (11).

In conclusion, the observed activity supports the ethno-medicinal uses of the title plants for the infection and other related complications. However, most of the activity studies on *Achillea* species lack the phytochemical study. The present work shows that essential oils from *A. teretifolia* and *A. nobilis* subsp. *neilreichii* are worthwhile for further bioassay guided investigations.

Table 3. The antioxidant activity results of *Achillea* essential oils.

Test substance	IC_{50} (mg/ml)
AN	> 0.500
AT	> 0.500
Vit C	0.008 ± 0.002
Vit E	0.022 ± 0.01
BHT	0.024 ± 0.002
Trolox	0.01 ± 0.01
Quercetin	0.005 ± 0.001

Mean ± Standard deviation (n = 8);
AN: *A. nobilis* subsp. *neilreichii* essential oil;
AT: *A. teretifolia* essential oil

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References

1. Duman H. *Achillea* L., In: Güner A, Özhatay N, Ekim T, Başer KHC. ed. Flora of Turkey and the East Aegean Islands. Vol. 11. Edinburgh University Press; 2000: pp. 158-159.
2. Huber-Morath A. *Achillea* L., In: Davis PH. ed. Flora of Turkey and the East Aegean Islands. Vol. 5. Edinburgh University Press; 1997: pp. 224-252.
3. Fujita T, Sezik E, Tabata M et al. Traditional medicine in Turkey, VII. Folk medicine in Middle and West Black Sea Regions. Economic Bot 49: 406-422, 1995.
4. Honda G, Yeşilada E, Tabata M et al. Traditional medicine in Turkey, VI. Folk medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydın provinces. J Ethnopharmacol 53: 75-87, 1996.
5. Sezik E, Yeşilada E, Honda G et al. Traditional medicine in Turkey, V. Folk medicine in Central Anatolia. J Ethnopharmacol 75: 95-115, 2001.
6. Sezik E, Yeşilada E, Tabata M et al. Traditional medicine in Turkey, VIII. Folk medicine in East Anotolia; Erzurum, Erzincan, Ağrı, Kars, Iğdır provinces. Economic Bot 51: 195-211, 1997.
7. Tabata M, Sezik E, Honda G et al. Traditional medicine in Turkey, III. Folk medicine in East Anatolia, Van and Bitlis Provinces, Int J Pharmacog 32: 3-12, 1994.
8. Yeşilada E, Honda G, Sezik E et al. Traditional medicine in Turkey, IV. Folk medicine in the Mediterranean Subdivision. J Ethnopharmacol 39: 31-38, 1993.
9. Baytop T. Therapy With Medicinal Plants in Turkey, Past and Present. Nobel Tıp Kitapevi. İstanbul; 1999.
10. Blumenthal M. The Complete German Commission E Monographs, American Botanical Council; Austin-Texas; 1999.
11. Karabay-Yavaşoğlu NU, Karamenderes C, Baykan S et al. Antinociceptive and anti-inflammatory activities and acute toxicity of *Achillea nobilis* subsp. *neilrechii* extract in mice and rats. Pharm. Biol 45: 162-168, 2007.
12. Küpeli E, Orhan İ, Küsmenoğlu Ş et al. Evaluation of anti-inflammatory and antinociceptive activity of five Anatolian *Achillea* species. Turkish J Pharm Sci 4: 89-99, 2007.
13. Konyalıoğlu S, Karamenderes C. The protective effects of *Achillea* L. species native in Turkey against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes J Ethnopharmacol 102: 221-227, 2005.
14. Karamenderes C, Apaydın S. Antispasmodic effect of *Achillea nobilis* L. subsp. *sipylea* (O. Schwarz) Bassler on the rat isolated duodenum. J Ethnopharmacol 84: 175-179, 2003.
15. Başer KHC, Demirci B, Demirci F et al. Composition and antimicrobial activity of the essential oil of *Achillea multifida*. Planta Med 68: 941-943, 2002.
16. Ünlü M, Daferera D, Dönmez D et al. Compositions and the *in vitro* antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea teretifolia* (Compositae). J Ethnopharmacol 83: 117-121, 2002.
17. Candan F, Ünlü M, Tepe B et al. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. (Asteraceae). J Ethnopharmacol 87: 215-220, 2003.
18. Sökmen A, Sökmen M, Daferera D et al. The *in vitro* antioxidant and antimicrobial activities of the essential oil and methanol extracts of *Achillea biebersteini* Afan. (Asteraceae) Phytother Res 18: 451-456, 2004.
19. İşcan G, Kırımer N, Kürkcüoğlu M et al. Biological activity and composition of the essential oils of *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. subsp. *aleppica*. J Agric Food Chem 54: 170-173, 2006.
20. Barış Ö, Güllüce M, Şahin F et al. Biological activities of the essential oil and methanol extract of *Achillea biebersteinii* Afan (Asteraceae). Turk J Biol 30: 65-73, 2006.
21. El-Shazly AM, Hafez SS, Wink M. Comparative study of the essential oils and extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. and *Achillea santolina* L. (Asteraceae) from Egypt. Pharmazie 59: 226-230, 2004.
22. Tuberoso CI, Kowalczyk A, Coroneo V *et al*. Chemical composition and antioxidant, antimicrobial, and antifungal activities of the essential oil of *Achillea ligustica* All. J Agric Food Chem 53: 10148-10153, 2005.
23. Filippi JJ, Lanfranchi DA, Prado S et al. Composition, enantiomeric distribution, and antibacterial activity of the essential oil of *Achillea ligustica* All. from Corsica. J Agric Food Chem 54: 6308-6313, 2006.
24. Nemeth E. Essential oil composition of species in the genus *Achillea*. J Essent Oil Res 17: 501-512, 2005.
25. Si XT, Zhang ML, Shi QW et al. Chemical constituents of the plants in the genus *Achillea*. Chem Biodiversity 3: 1163-1180, 2006.
26. Brendler T, Grünwald J, Janicke C. Herbal Remedies, Electronic Database. Version 5, Medpharm Scientific Publishers, Stuttgart, 2003.
27. Anon. Dictionary of Natural Products on CD-ROM, Ver.14.1 1982-2006, Chapman & Hall/CRC, 2006.
28. Koenig WA, Joulain D, Hochmuth DH. In: Hochmuth DH. ed. Convenient and Rapid Analysis of GC-MS, 2004.
29. Joulain D, König WA. The Atlas of Spectra Data of Sesquiterpene Hydrocarbons. E.B. -Verlag, Hamburg, 1998.
30. ESO 2000. The Complete Database of Essential Oils, Booleans Aroma Chemical Information Service. The Netherlands; 1999.
31. McLafferty FW, Stauffer DB. The Wiley/NBS Registry of Mass Spectral Data, J. Wiley and Sons: New York; 1989.
32. Jennings WG, Shibamoto T. Quantitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary GC. Academic Pres. New York; 1980.

33. İşcan G, Demirci F, Kırımer N et al. Antimicrobial screening: *Mentha piperita* essential oil. J Agric Food Chem 50: 3943-3946, 2002.

34. Koneman EW, Allen SD, Janda WM et al. Color Atlas and Textbook of Diagnostic Microbiology. Lippincott-Raven Publ. Philadelphia; 1997.

35. Kumarasamy Y, Fergusson ME, Nahar L et al. Bioactivity of moschamindole from *Centaurea moschata*. Pharmaceutical Biol 40: 307-310, 2002.

36. Sarker SD, Latif Z, Gray AI. Methods in Biotechnology. Vol. 20, Humana Press. Totowa; 2006.

37. Küsmenoğlu Ş, Başer KHC, Özek T et al. Constituents of the essential oil of *Achillea biebersteinii* Afan. J Essent Oil Res 7: 527-528, 1995.

38. Başer KHC, Demirci B, Kaiser R et al. Composition of the essential oil of *Achillea phrygia* Boiss.et Ball. J Essent Oil Res 12: 327-329, 2000.

39. Başer KHC, Demirci B, Duman D. Composition of the essential oils of two endemic species from Turkey: *Achillea lycaonica* and *A. ketenoglui*. Chem Nat Comp 37, 245, 2001.

40. Palic R, Stojanovic G, Naskovic T et al. Composition and antibacterial activity of *Achillea crithmifolia* and *Achillea nobilis* essential oils. J Essent Oil Res 15: 434-437, 2003.

41. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 10: 813-829, 2003.