

## Comparative Study of Three *Achillea* Essential Oils from Eastern Part of Turkey and their Biological Activities

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**Abstract:** Essential oils obtained by hydrodistillation were analyzed both by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The main constituents found in *Achillea* oil were as follows: *A. filipendulina* Lam.: 43.8% santolina alcohol, 14.5% 1,8-cineole and 12.5% *cis*-chrysanthenyl acetate; *A. magnifica* Hiemerl ex Hub.-Mor.: 27.5% linalool, 5.8% spathulenol, 5.5% terpinen-4-ol, 4.7%  $\alpha$ -terpineol and 4.7%  $\beta$ -eudesmol; *A. tenuifolia* Lam.: 12.4% artemisia ketone, 9.9% *p*-cymene, 7.1% camphor, 5.9% terpinen-4-ol, 4.7% caryophyllene oxide and 4.5%  $\alpha$ -pinene. Furthermore, the *Achillea* essential oils were evaluated for antimalarial and antimicrobial activities. *A. magnifica* and *A. filipendulina* oils showed strong antimalarial activity against both chloroquine sensitive D6 (IC<sub>50</sub>= 1.2 and 0.68  $\mu$ g/mL) and chloroquine resistant W2 (IC<sub>50</sub>= 1.1 and 0.9  $\mu$ g/mL) strains of *Plasmodium falciparum* without any cytotoxicity to mammalian cells up to IC<sub>50</sub>=47.6  $\mu$ g/mL against Vero cells, whereas *A. tenuifolia* oil showed no antimalarial activity up to a concentration of 20 mg/mL. All three *Achillea* oils showed no antibacterial activity against human pathogenic bacteria up to a concentration of 200  $\mu$ g/mL. *A. tenuifolia* and *A. magnifica* oils demonstrated mild antifungal activity against *Cryptococcus neoformans* (IC<sub>50</sub>= 45, 20 and 15  $\mu$ g/mL, respectively).

**Keywords:** Asteraceae; *Achillea filipendulina*; *A. magnifica*; *A. tenuifolia*; essential oil composition; antimalarial and antimicrobial. © 2017 ACG Publications. All rights reserved.

### 1. Plant Source

*Achillea* L. is a large genus belonging to the family Asteraceae. This genus is widely distributed in Anatolia and is represented by 59 species of which 31 are endemic for Turkey [1]. *Achillea* species comprise an important biological resource in folk medicine to treat various diseases and several of them are used for their pharmaceutical, cosmetic, and fragrance properties [3]. *Achillea* species are generally known as “Civanperçemi” and used for the treatment of gastrointestinal disorders in Anatolian folk medicine. In the present study, we investigated three *Achillea* species (*A. filipendulina* Lam., *A. tenuifolia* Lam., and *A.*

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*magnifica* Hiemerl ex Hub.-Mor. from eastern part of Turkey. Out of the three species, *A. magnifica*, an endemic species, is recognized by local names as “karcivani” and its dried flowering parts are mixed with honey and consumed for stomach disorders [4].

The plant material was identified by Prof. Dr. Z. Aytaç. Voucher specimens were deposited at the Herbarium of Gazi University, Faculty of Science and Letters. Detailed information on the plant materials used are given in Table 1.

## 2. Previous Studies

Essential oils of *Achillea* species have been the subject of several investigations. *A. millefolium* L. has been the most widely studied species because of its economic value and therapeutic properties. Previous phytochemical investigations of *Achillea* species have revealed the presence of sesquiterpene lactones, proazulenes, sesquiterpenoids, flavonoids, triterpenes, coumarins, peroxides, phenolic and polyacetylene compounds [5].

Although the essential oil composition of *A. filipendulina*, *A. tenuifolia* and *A. magnifica* have been recently reported in the literature and the antibacterial activity of *A. filipendulina* has been studied [5-10], the antimalarial activity of *A. tenuifolia* and antimicrobial activity of *A. tenuifolia* and *A. magnifica* were investigated for the first time.

## 3. Present Studies

*Isolation of the Essential Oil:* Essential oils were hydrodistilled from dried aerial parts for 3 h using a Clevenger apparatus. The yields were calculated on a dry weight basis as given in Table 1.

*GC-MS and GC-FID Analysis:* The GC-MS and GC-FID analysis were carried out with an Agilent 5975 GC-MSD and Agilent 6890N GC systems, respectively. Analysis conditions and identification of the oil components are similar to our earlier studies [1].

*Biological Activity:* The *in vitro* antimalarial [11] and antimicrobial [12] activity was performed as previously described. Antimalarial standards chloroquine (Aldrich-Sigma, ST, Louis, MO) and artemisinin (Aldrich-Sigma, ST, Louis, MO) and antimicrobial standards ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi were purchased from commercial sources.

**Table 1.** Information on the plant material and essential oils

<i>Achillea</i> sp.	Collection site	Altitude (m)	Collection period	Oil yield <sup>1</sup> (%)	ZA <sup>2</sup>
<i>A. filipendulina</i>	Şırnak-Şenova-Hakkari 79. km	1950	15.07.2001	0.80	ZA8195
<i>A. magnifica</i>	Malatya: Pütürge, 17.km	1100	12.07.2001	0.09	ZA8135
<i>A. tenuifolia</i>	Ağrı-Doğubeyazıt-Igdir, 13. km	1600	19.07.2001	0.20	ZA8272

<sup>1</sup> Essential oil yields are given on moisture-free basis.

<sup>2</sup> Voucher specimens were deposited at the Herbarium of GAZI (Gazi University, Faculty of Science)

Air-dried aerial parts of three *Achillea* species, *A. filipendulina* Lam., *A. magnifica* Hiemerl ex Hub.-Mor., and *A. tenuifolia* Lam., were analyzed by GC and GC-MS and the individual identified components with their relative percentages are given in Table 2.

In the oil of the *A. filipendulina*, 53 components were characterized representing 93% of the total oil. This oil was characterized by a relatively high content of santolina alcohol (43.8%). 1,8-Cineole (14.5%) and *cis*-chrysanthenyl acetate (12.5%) were found as other main constituents.

A total of 70 compounds were characterized in *A. magnifica* essential oil, representing 91.7% of the total oil with linalool (27.5%), spathulenol (5.8%), terpinen-4-ol (5.5%),  $\alpha$ -terpineol (4.7%) and  $\beta$ -eudesmol (4.7%) as main constituents.

**Table 2.** The composition of the essential oils of three *Achillea* species

RRI <sup>a</sup>	RRI <sup>b</sup>	Compound	Af (%) <sup>c</sup>	Am (%) <sup>c</sup>	At (%) <sup>c</sup>	IM <sup>d</sup>
1025 <sup>f</sup>	1032	$\alpha$ -Pinene	1.3	0.1	4.5	tR, MS
1036 <sup>g</sup>	1043	Santolinatriene	2.3	-	3.6	MS
1068 <sup>g</sup>	1076	Camphene	0.9	-	1.6	tR, MS
1082 <sup>g</sup>	1093	Hexanal	-	-	0.3	tR, MS
1117 <sup>f</sup>	1118	$\beta$ -Pinene	0.9	0.1	1.2	tR, MS
1122 <sup>f</sup>	1132	Sabinene	0.2	-	0.4	tR, MS
1122 <sup>g</sup>	1135	Thuja-2,4(10)-diene	-	-	0.1	MS
1160 <sup>f</sup>	1174	Myrcene	-	-	0.3	tR, MS
	1185	Isobutyl 2-methyl butyrate	-	-	0.1	MS
1192 <sup>g</sup>	1195	Dehydro-1,8-cineole	0.2	-	0.1	MS
1212 <sup>f</sup>	1203	Limonene	-	0.3	0.6	tR, MS
1211 <sup>g</sup>	1213	1,8-Cineole	14.5	1.9	2.3	tR, MS
1232 <sup>f</sup>	1244	2-Pentyl furan	-	-	0.1	MS
1245 <sup>f</sup>	1255	$\gamma$ -Terpinene	-	-	0.2	tR, MS
1281 <sup>f</sup>	1280	<i>p</i> -Cymene	1.5	0.4	9.9	tR, MS
	1285	Isoamyl isovalerate	0.1	-	-	MS
1282 <sup>f</sup>	1290	Terpinolene	-	-	0.1	tR, MS
	1296	Pentyl isovalerate	-	0.1	-	MS
	1355	1,2,3-Trimethyl benzene	0.1	-	-	MS
	1358	Artemisia ketone	-	-	12.4	MS
	1386	Octenyl acetate	-	-	0.2	MS
1395 <sup>g</sup>	1403	Yomogi alcohol	0.5	-	2.0	MS
	1405	Santolina alcohol	43.8	-	0.1	MS
	1429	Artemisyl acetate	0.2	-	0.2	MS
	1431	7 $\alpha$ -(H)-Silphiperfol-5-ene	-	-	0.3	MS
1446 <sup>g</sup>	1450	<i>trans</i> -Linalool oxide ( <i>Furanoid</i> )	-	1.0	-	MS
	1454	7 $\beta$ -(H)-silphiperfol-5-ene	-	-	0.1	MS
1548 <sup>g</sup>	1474	<i>trans</i> -Sabinene hydrate	0.2	2.3	1.8	MS
1454 <sup>g</sup>	1478	<i>cis</i> -Linalool oxide ( <i>Furanoid</i> )	-	0.8	-	MS
	1478	Linalool-7-oxide-3-one	-	0.2	-	MS
	1487	Isoneroloxide-I	-	-	0.3	MS
1458 <sup>f</sup>	1497	$\alpha$ -Copaene	-	t <sup>e</sup>	0.1	MS
	1499	$\alpha$ -Campholene aldehyde	0.1	t	0.1	MS
	1501	Silphiperfol-6-ene	-	-	tr	MS
1510 <sup>g</sup>	1510	Artemisia alcohol	0.3	-	3.6	MS
1515 <sup>h</sup>	1532	Camphor	0.6	1.3	7.1	tR, MS
1518 <sup>g</sup>	1541	Benzaldehyde	-	-	0.3	tR, MS
1543 <sup>h</sup>	1553	Linalool	0.1	27.5	0.1	tR, MS
	1556	<i>cis</i> -Sabinene hydrate	0.2	1.1	1.0	MS
	1562	Isopinocampone	0.1	-	-	MS
1584 <sup>g</sup>	1571	<i>trans-p</i> -Menth-2-en-1-ol	0.1	0.5	0.8	MS
1561 <sup>g</sup>	1582	<i>cis</i> -Chrysanthenyl acetate	12.5	-	-	MS
1575 <sup>h</sup>	1586	Pinocarvone	-	0.7	0.8	tR, MS
1579 <sup>h</sup>	1591	Bornyl acetate	1.4	-	0.1	tR, MS
1590 <sup>h</sup>	1600	$\beta$ -Elemene	-	-	0.5	MS
	1601	Nopinone	-	0.5	-	MS
1601 <sup>h</sup>	1611	Terpinen-4-ol	0.8	5.5	5.9	tR, MS
1608 <sup>h</sup>	1612	$\beta$ -Caryophyllene	-	-	0.2	tR, MS
1602 <sup>h</sup>	1616	Hotrienol	-	3.0	-	MS
1614 <sup>g</sup>	1638	<i>cis-p</i> -Menth-2-en-1-ol	0.1	0.4	0.6	MS
1631 <sup>h</sup>	1648	Myrtenal	0.1	1.4	-	MS
1651 <sup>h</sup>	1651	Sabinaketone	0.2	-	-	MS

	1655	Isobornyl propionate	-	-	0.1	MS
	1656	Chrysanthenyl isobutyrate	-	-	0.2	MS
1649 <sup>g</sup>	1661	Alloaromadendrene	-	0.1	-	MS
<i>Table 2 Continued</i>						
1659 <sup>g</sup>	1663	<i>cis</i> -Verbenol	0.1	-	0.3	tR, MS
	1665	<i>cis</i> -Sabinyl acetate	-	0.1	-	MS
1661 <sup>h</sup>	1670	<i>trans</i> -Pinocarveol	0.5	1.2	0.4	tR, MS
1679 <sup>g</sup>	1682	$\delta$ -Terpineol	0.1	0.3	-	MS
	1682	( <i>E</i> )-Ocimenol	-	-	0.1	MS
1680 <sup>g</sup>	1683	<i>trans</i> -Verbenol	0.3	-	1.6	MS
1666 <sup>g</sup>	1687	$\alpha$ -Humulene	-	0.1	-	tR, MS
1710 <sup>g</sup>	1689	<i>trans</i> -piperitol	0.1	0.2	-	MS
	1688	Selina-4,11-diene	-	0.3	-	MS
1694 <sup>g</sup>	1706	$\alpha$ -Terpineol	0.2	4.7	0.8	tR, MS
1699 <sup>g</sup>	1719	Borneol	2.9	0.2	0.5	tR, MS
1720 <sup>g</sup>	1725	Verbenone	t	-	0.1	tR, MS
1708 <sup>g</sup>	1726	Germacrene D	-	0.6	-	MS
1727 <sup>g</sup>	1741	$\beta$ -Bisabolene	-	0.2	-	MS
1725 <sup>g</sup>	1742	Geranial	-	0.4	-	tR, MS
1729 <sup>g</sup>	1748	Piperitone	-	-	1.4	MS
1733 <sup>g</sup>	1751	Carvone	-	-	t	tR, MS
	1758	<i>cis</i> -Piperitol	t	0.2	0.3	MS
	1760	Chrysanthenyl isovalerate II	-	-	0.1	MS
1762 <sup>g</sup>	1764	<i>cis</i> -Chrysanthenol	3.0	-	-	MS
1754 <sup>g</sup>	1766	Decanol	-	0.5	-	tR, MS
	1770	Isobornyl isovalerate	-	-	0.1	MS
1738 <sup>g</sup>	1770	<i>trans</i> -Linalool oxide ( <i>Pyranoid</i> )	-	0.2	-	MS
1773 <sup>g</sup>	1786	<i>ar</i> -Curcumene	-	-	0.2	MS
	1793	$\alpha$ -Campholene alcohol	-	-	0.2	MS
1784 <sup>g</sup>	1802	Cumin aldehyde	-	-	0.2	tR, MS
1790 <sup>h</sup>	1804	Myrtenol	0.3	3.0	0.1	MS
1803 <sup>g</sup>	1811	<i>trans-p</i> -Mentha-1(7),8-dien-2-ol	-	-	0.1	MS
1836 <sup>g</sup>	1845	<i>trans</i> -Carveol	0.2	-	0.2	tR, MS
1839 <sup>g</sup>	1857	Geraniol	-	0.9	-	tR, MS
1848 <sup>g</sup>	1864	<i>p</i> -Cymen-8-ol	0.1	0.2	0.4	tR, MS
	1889	Ascaridol	0.4	-	-	MS
	1902	Benzyl isovalerate	-	-	0.1	tR, MS
	1945	1,5-Epoxy-salvial(4)14-ene	-	0.6	-	MS
1955 <sup>g</sup>	1969	<i>cis</i> -Jasmone	-	0.4	-	MS
	2001	Isocaryophyllene oxide	-	-	0.5	MS
1962 <sup>h</sup>	2008	Caryophyllene oxide	0.1	0.9	4.7	tR, MS
2006 <sup>h</sup>	2029	Perilla alcohol	0.1	0.6	-	MS
2006 <sup>h</sup>	2030	Methyl eugenol	-	1.1	-	tR, MS
	2030	Presilphiperfolan-9 $\alpha$ -ol	-	-	0.1	MS
	2033	4 $\alpha$ -Hydroxy achipendol	0.2	-	-	MS
2016 <sup>h</sup>	2037	Salvial-4(14)-en-1-one	-	0.5	0.2	MS
2036 <sup>h</sup>	2050	( <i>E</i> )-Nerolidol	-	2.0	-	MS
	2056	13-Tetradecanolide	-	0.8	0.6	MS
	2061	$\beta$ - <i>trans</i> -Bejarol	-	1.0	-	MS
2047 <sup>h</sup>	2071	Humulene epoxide-II	-	-	0.4	MS
	2113	Cumin alcohol	0.2	-	0.1	tR, MS
	2118	$\alpha$ - <i>trans</i> -Bejarol	-	0.2	-	MS
	2122	<i>cis</i> -Bejarol	-	0.5	-	tR, MS
	2130	Salviadienol	-	0.3	-	MS
	2131	Silphiperfol-6-en-5-one	-	-	0.7	MS
2126 <sup>h</sup>	2144	Spathulenol	0.2	5.8	3.4	MS
	2174	Fokienol	-	0.4	-	MS

	2181	Isothymol	-	-	0.1	tR, MS
2176 <sup>g</sup>	2185	$\gamma$ -Eudesmol	-	0.9	-	MS
2165 <sup>g</sup>	2187	T-Cadinol	-	-	0.2	MS
<i>Table 2 Continued</i>						
2164 <sup>g</sup>	2198	Thymol	-	-	0.1	tR, MS
	2204	Eremoligenol	-	0.6	-	MS
	2210	Copaborneol	0.1	-	-	MS
	2214	$\alpha$ -Turmerol	-	-	0.3	MS
	2221	Isocarvacrol	-	-	0.3	tR, MS
	2232	$\alpha$ -Bisabolol	-	0.3	-	MS
2210 <sup>g</sup>	2239	Carvacrol	0.1	1.5	0.1	tR, MS
	2247	<i>trans</i> - $\alpha$ -Bergamotol	-	0.2	0.2	MS
2238 <sup>h</sup>	2257	$\beta$ -Eudesmol	0.1	4.7	-	MS
	2260	15-Hexadecanolide	-	1.8	0.6	MS
2278 <sup>h</sup>	2278	Torilenol	-	0.3	-	MS
	2298	Decanoic acid	-	1.0	0.3	tR, MS
	2300	Tricosane	0.2	-	-	tR, MS
	2369	Eudesma-4(15),7-dien-4 $\beta$ -ol	-	0.5	0.2	MS
	2392	Caryophylla-2(12),6-dien-5 $\beta$ -ol (= <i>Caryophyllenol II</i> )	-	-	0.6	MS
	2396	$\gamma$ -Dodecalactone	-	-	0.4	MS
	2500	Pentacosane	-	-	0.1	tR, MS
	2533	$\gamma$ -Costol	t	0.8	-	MS
	2541	Methyl nonadecanoate	-	0.5	-	MS
	2604	$\alpha$ -Costol	t	-	-	MS
	2606	$\beta$ -Costol	0.2	-	-	MS
	2607	Octadecanol	-	0.8	-	MS
	2622	Phytol	-	0.2	-	MS
	2700	Heptacosane	-	tr	0.2	tR, MS
<b>Total</b>			93.0	91.7	85.9	

<sup>a</sup>RRI indices from literature f [13], g [14], h [15], <sup>b</sup>RRI: Relative retention indices calculated against *n*-alkanes; <sup>c</sup>%: calculated from FID data; <sup>d</sup>IM: Identification Method; tR, identification based on the retention times (tR) of genuine standard compounds on the HP Innowax column; MS, tentatively identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data. <sup>e</sup>t: Trace (< 0.1 %); Af: *A. filipendulina*; Am: *A. magnifica*; At: *A. tenuifolia*.

The main components of *A. tenuifolia* oil were determined as artemisia ketone (12.4%), *p*-cymene (9.9%), camphor (7.1%), terpinen-4-ol (5.9%), caryophyllene oxide (4.7%) and  $\alpha$ -pinene (4.5%). 85 components were identified representing 85.9% of the total *A. tenuifolia* essential oil.

In the current study, these three *Achillea* essential oils were evaluated for their antimalarial and antimicrobial activities. *A. magnifica* and *A. filipendulina* oils showed strong antimalarial activity against both chloroquine-sensitive D6 (IC<sub>50</sub>= 1.2 and 0.68  $\mu$ g/mL) and chloroquine-resistant W2 (IC<sub>50</sub>= 1.1 and 0.9  $\mu$ g/mL) strains of *Plasmodium falciparum* when the compared to positive standards chloroquine (IC<sub>50</sub>= 0.018  $\mu$ g/mL for D6 and IC<sub>50</sub>= 0.16  $\mu$ g/mL for W2 clones) and artemisinin (IC<sub>50</sub>= 0.0037  $\mu$ g/mL for D6 and IC<sub>50</sub>= 0.0035  $\mu$ g/mL for W2 clones) without exhibiting any cytotoxicity at IC<sub>50</sub> values of 47.6, 15.867 and 5.288  $\mu$ g/mL against Vero cells. however, *A. tenuifolia* did not show antimalarial activity *Achillea* oils showed no antibacterial activity against human pathogenic bacteria up to a concentration of 200  $\mu$ g/mL. *A. tenuifolia* and *A. magnifica* oils demonstrated mild antifungal activity against *Cryptococcus neoformans* (IC<sub>50</sub>= 20 and 15  $\mu$ g/mL, respectively) and *A. tenuifolia* oil demonstrated weak antimycobacterial activity against *Mycobacterium intracellulare* with an IC<sub>50</sub> value of 200  $\mu$ g/mL. Bioassay-guided investigations are warranted out to identify active antimalarial compounds from *A. magnifica* and *A. filipendulina* essential oils.

To the best of our knowledge, this is the first report of chemical composition these three *Achillea* species from eastern (Malatya and Ağrı) and south-eastern (Şırnak) region of Turkey and their biological activities of *A. magnifica*, *A. tenuifolia* and *A. filipendulina* essential oils were evaluated.

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