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-chrysanthényl acetate; A. magnifica Hiemerl ex Hub.-Mor.: 27.5% linalool, 5.8% spathulenol, 5.5% terpinen-4-ol, 4.7% α-terpineol and 4.7% β-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% α-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 (IC50 = 1.2 and 0.68 μg/mL) and chloroquine resistant W2 (IC50 = 1.1 and 0.9 μg/mL) strains of Plasmodium falciparum without any cytotoxicity to mammalian cells up to IC50=47.6 μg/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 μg/mL. A. tenuifolia and A. magnifica oils demonstrated mild antifungal activity against Cryptococcus neoformans (IC50= 45, 20 and 15 μg/mL, respectively).

Keywords: Asteraceae; Achillea filipendulina; A. magnifica; A. tenuifolia; essential oil composition; antimalarial and antimicrobial. © 2018 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 “karcivanı” and its dried flowering parts are mixed with honey and consumed for stomach disorders [4].

The plant material was identified by Prof. Dr. Z. Aytąc. 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

| Achillea sp.    | Collection site                      | Altitude (m) | Collection period | Oil yield (%) | ZA²  |
|-----------------|--------------------------------------|--------------|-------------------|--------------|------|
| *A. filipendulina* | Şırnak-Şenova-Hakkari 79. km          | 1950         | 15.07.2001        | 0.80         | ZA8195 |
| *A. magnifica*   | Malatya: Pütürge, 17.km              | 1100         | 12.07.2001        | 0.09         | ZA8135 |
| *A. tenuifolia*  | Ağrı-Doğubeyazıt-Igdır, 13. km       | 1600         | 19.07.2001        | 0.20         | ZA8272 |

¹ Essential oil yields are given on moisture-free basis.
² 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%), α-terpineol (4.7%) and β-eudesmol (4.7%) as main constituents.

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

| RRIa | RRIb | Compound                        | Af (%)c | Am (%)c | At (%)c | IMd |
|------|-------|---------------------------------|---------|---------|---------|-----|
| 1025f | 1032  | α-Pinene                        | 1.3     | 0.1     | 4.5     | tR, MS |
| 1036g | 1043  | Santolinatriene                 | 2.3     |         | 3.6     | MS   |
| 1068g | 1076  | Camphene                        | 0.9     |         | 1.6     | tR, MS |
| 1082g | 1093  | Hexanal                         |         |         | 0.3     | tR, MS |
| 1117f | 1118  | β-Pinene                        | 0.9     | 0.1     | 1.2     | tR, MS |
| 1122f | 1132  | Sabinene                        | 0.2     |         | 0.4     | tR, MS |
| 1122g | 1135  | Thuj-2,4(10)-diene              | -       |         | 0.1     | MS   |
| 1160f | 1174  | Myrcene                         | -       |         | 0.3     | tR, MS |
|       | 1185  | Isobutyl 2-methyl butyrate      | -       |         | 0.1     | MS   |
| 1192g | 1195  | Dehydro-1,8-cineole             | 0.2     |         | 0.1     | MS   |
| 1212f | 1203  | Limonene                        | -       | 0.3     | 0.6     | tR, MS |
| 1211g | 1213  | 1,8-Cineole                     | 14.5    | 1.9     | 2.3     | tR, MS |
| 1232f | 1244  | 2-Pentyl furan                  | -       |         | 0.1     | MS   |
| 1245f | 1255  | γ-Terpine                       | -       |         | 0.2     | tR, MS |
| 1281f | 1280  | p-Cymene                        | 1.5     | 0.4     | 9.9     | tR, MS |
| 1285  |       | Isoamyl isovalerate             | 0.1     |         |         | MS   |
| 1282f | 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   |
| 1395g | 1403  | Yomogi alcohol                  | 0.5     |         | 2.0     | MS   |
| 1405  |       | Santolina alcohol               | 43.8    |         | 0.1     | MS   |
| 1429  |       | Artemisylacetate                | 0.2     |         | 0.2     | MS   |
| 1431  |       | 7α-(H)-Silphiperfol-5-ene       | -       |         | 0.3     | MS   |
| 1446f | 1450  | trans-Linalool oxide (Furanoid)  | -       | 1.0     | -       | MS   |
| 1454  |       | 7β-(H)-silphiperfol-5-ene       | -       |         | 0.1     | MS   |
| 1548g | 1474  | trans-Sabinene hydrate          | 0.2     | 2.3     | 1.8     | MS   |
| 1478  |       | Linalool-7-oxide-3-one          | -       | 0.2     | -       | MS   |
| 1487  |       | Isomeroloxide-I                 | -       |         | 0.3     | MS   |
| 1458f | 1497  | α-Copaene                       | -       | t6      | 0.1     | MS   |
| 1499  |       | α-Campholene aldehyde           | 0.1     | t       | 0.1     | MS   |
| 1501  |       | Silphiperfol-6-ene              | -       |         | tr      | MS   |
| 1510g | 1510  | Artemisia alcohol               | 0.3     |         | 3.6     | MS   |
| 1515h | 1532  | Camphor                         | 0.6     | 1.3     | 7.1     | tR, MS |
| 1518g | 1541  | Benzaldehyde                    | -       |         | 0.3     | tR, MS |
| 1543h | 1553  | Linalool                        | 0.1     | 27.5    | 0.1     | tR, MS |
| 1556  |       | cis-Sabinene hydrate            | 0.2     | 1.1     | 1.0     | MS   |
| 1562  |       | Isopinocamphene                 | 0.1     |         | -       | MS   |
| 1584g | 1571  | trans-p-Menth-2-en-1-ol          | 0.1     | 0.5     | 0.8     | MS   |
| 1561g | 1582  | cis-Chrysanthenyl acetate       | 12.5    |         | -       | MS   |
| 1575h | 1586  | Pinocarvone                     | -       | 0.7     | 0.8     | tR, MS |
| 1579h | 1591  | Bornyl acetate                  | 1.4     |         | 0.1     | tR, MS |
| 1590h | 1600  | β-Elemene                       | -       |         | 0.5     | MS   |
| 1601h | 1611  | Norpinone                       | -       | 0.5     | -       | MS   |
| 1601h | 1611  | Terpinen-4-ol                   | 0.8     | 5.5     | 5.9     | tR, MS |
| 1608h | 1612  | β-Caryophyllene                 | -       |         | 0.2     | tR, MS |
| 1602h | 1616  | Hotrienol                       | -       | 3.0     | -       | MS   |
| 1614g | 1638  | cis-p-Menth-2-en-1-ol           | 0.1     | 0.4     | 0.6     | MS   |
| 1631h | 1648  | Myrtenal                        | 0.1     | 1.4     | -       | MS   |
| 1651h | 1651  | Sabinaketone                    | 0.2     |         | -       | MS   |
|   |   |   |   |   |
|---|---|---|---|---|
| 1655 | Isobornyl propionate | - | - | 0.1 | MS |
| 1656 | Chrysanthenyl isobutyrate | - | - | 0.2 | MS |
| 1649s | Alloaromadendrene | - | 0.1 | - | MS |
| 1659s | cis-Verbenol | 0.1 | - | 0.3 | tR, MS |
| 1665 | cis-Sabinyl acetate | - | 0.1 | - | MS |
| 1661h | trans-Pinocarveol | 0.5 | 1.2 | 0.4 | tR, MS |
| 1679s | δ-Terpineol | 0.1 | 0.3 | - | MS |
| 1682 (E)-Ocimeno | - | - | 0.1 | MS |
| 1680s | trans-Verbenol | 0.3 | - | 1.6 | MS |
| 1666s | α-Humulene | - | 0.1 | - | tR, MS |
| 1710s | trans-piperitol | 0.1 | 0.2 | - | MS |
| 1688 | Selina-4,11-diene | - | 0.3 | - | MS |
| 1694s | α-Terpineol | 0.2 | 4.7 | 0.8 | tR, MS |
| 1719 | Borneol | 2.9 | 0.2 | 0.5 | tR, MS |
| 1720s | Verbenone | t | - | 0.1 | tR, MS |
| 1708s | Germacrene D | - | 0.6 | - | MS |
| 1727s | β-Bisabolene | - | 0.2 | - | MS |
| 1725s | Geranial | - | 0.4 | - | tR, MS |
| 1729s | Piperitone | - | - | 1.4 | MS |
| 1733s | Carvone | - | - | t | tR, MS |
| 1758 | cis-Piperitol | t | 0.2 | 0.3 | MS |
| 1760 | Chrysanthenyl isovalerale II | - | - | 0.1 | MS |
| 1762s | cis-Chrysanthene | 3.0 | - | - | MS |
| 1754s | Decanol | - | 0.5 | - | tR, MS |
| 1770 | Isobornyl isovalerale | - | - | 0.1 | MS |
| 1738s | trans-Linalool oxide (Pyranoid) | - | 0.2 | - | MS |
| 1773s | ar-Curcumene | - | - | 0.2 | MS |
| 1793 | α-Camphene alcohol | - | - | 0.2 | MS |
| 1784s | Cumin aldehyde | - | - | 0.2 | tR, MS |
| 1790h | Myrtenol | 0.3 | 3.0 | 0.1 | MS |
| 1803s | trans-p-Mentha-1(7),8-dien-2-ol | - | - | 0.1 | MS |
| 1836s | trans-Cardolve | 0.2 | - | 0.2 | tR, MS |
| 1848s | p-Cymen-8-ol | 0.1 | 0.2 | 0.4 | tR, MS |
| 1889 | Ascaridol | 0.4 | - | - | MS |
| 1902 | Benzyl isovalerale | - | - | 0.1 | tR, MS |
| 1945 | 1,5-Epoxy-salvial(4)14-ene | - | 0.6 | - | MS |
| 1955s | cis-Jasmon | - | 0.4 | - | MS |
| 2001 | Isoarylophene oxide | - | - | 0.5 | MS |
| 1962h | Caryophyllene oxide | 0.1 | 0.9 | 4.7 | tR, MS |
| 2006h | Perilla alcohol | 0.1 | 0.6 | - | MS |
| 2006h | Myrtenol | 0.3 | 3.0 | 0.1 | MS |
| 2001 | Isoarylophene oxide | - | - | 0.5 | MS |
| 1962h | Caryophyllene oxide | 0.1 | 0.9 | 4.7 | tR, MS |
| 2006h | Perilla alcohol | 0.1 | 0.6 | - | MS |
| 2006h | Methyl eugenol | - | 1.1 | - | tR, MS |
| 2018 | 4α-Hydroxy chiphendol | 0.2 | - | - | MS |
| 2016h | Salvial-4(14)-en-1-one | - | 0.5 | 0.2 | MS |
| 2036h | (E)-Nerolidol | - | 2.0 | - | MS |
| 2056 | 13-Tetradecanolide | - | 0.8 | 0.6 | MS |
| 2061 | trans-Bejarol | - | 1.0 | - | MS |
| 2047h | Humulene epoxide-II | - | - | 0.4 | MS |
| 2113 | Cumin alcohol | 0.2 | - | 0.1 | tR, MS |
| 2118 | α-trans-Bejarol | - | 0.2 | - | MS |
| 2122 | cis-Bejarol | - | 0.5 | - | tR, MS |
| 2130 | Salvadienol | - | 0.3 | - | MS |
| 2131 | Silphiperfol-6-en-5-one | - | - | 0.7 | MS |
| 2126h | Spathulenol | 0.2 | 5.8 | 3.4 | MS |
| 2174 | Fokienol | - | 0.4 | - | MS |
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 *α*-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$_{50}$= 1.2 and 0.68 µg/mL) and chloroquine-resistant W2 (IC$_{50}$= 1.1 and 0.9 µg/mL) strains of *Plasmodium falciparum* when the comported to positive standards chloroquine (IC$_{50}$= 0.018 µg/mL for D6 and IC$_{50}$= 0.16 µg/mL for W2 clones) and artemisinin (IC$_{50}$= 0.0037 µg/mL for D6 and IC$_{50}$= 0.0035 µg/mL for W2 clones) without exhibiting any cytotoxicity at IC$_{50}$ values of 47.6, 15.867 and 5.288 µg/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 µg/mL. *A. tenuifolia* and *A. magnifica* oils demonstrated mild antifungal activity against *Cryptococcus neoformans* (IC$_{50}$= 20 and 15 µg/mL, respectively) and *A. tenuifolia* oil demonstrated weak antimycobacterial activity against *Mycobacterium intracellulare* with an IC$_{50}$ value of 200 µg/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.
Essential oil composition of Achillea species and their biological activities

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