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# Essential Oil Composition and Antimicrobial Activities of *Achillea biserrata* M. Bieb. and *Achillea salicifolia* Besser subsp. *salicifolia* Collected in Turkey

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Aerial parts of *Achillea biserrata* (collected from Gumushane between Trabzon) and *A. salicifolia* subsp. *salicifolia* (collected from Ardahan between Gole) were subjected to hydrodistillation to yield their essential oils and were analyzed by GC and GC-MS. The main components of tested essential oils were camphor (36.80-55.26 %), 1,8-cineol (19.35-22.75 %), camphene (16.41-3.23 %) and artemisia alcohol (14.28-3.17 %), respectively. The antibacterial and antifungal activities of tested essential oils were evaluated against *Escherichia coli* ATCC 25292, *Staphylococcus aureus* ATCC 6538, *Proteus vulgaris* NRRL 123, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* NRRL 3567, *Listeria monocystogenes* ATCC 7644, *Serratia marcescens*, *Candida albicans, Penicillium expansum, Aspergillus flavus, A. niger* and *Alternaria brassicola*. All tested bacteria and *Candida albicans* were inhibited by the essential oils. The essential oils showed weak antifungal activity against all microfungi tested. Nevertheless, it is found that *P. expansum* was not inhibited by any essential oils used in this study. *Aspergillus niger* was also not inhibited by *A. biserrata. Alternaria brassicola* was the most sensitive microfungi against all the tested essential oils.

**Key Words: *Achillea biserrata, A. salicifolia* subsp. *salicifolia*, Essential oil, Chemical composition, Antibacterial and Antifungal activity.**

## INTRODUCTION

The genus *Achillea* L. (family Asteraceae) comprises about 137 species throughout the world, represented by 43 species (49 taxa) of which 22 are endemic to Turkey[1-3]. The aerial parts of different species of the genus are widely used in folk medicine due to numerous medicinal properties, such as antiinflammatory, antispasmodic, antihemorrhoidal, stomachic, antiseptic and emmenagogue[4,5]. Several *Achillea* spp. have been studied for their essential oils by researchers[4,6-8].

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Studies on the chemical composition of *Achillea* spp. essential oil report a variegated pattern of composition. Most of the *Achillea* essential oils contain large amount of 1,8-cineole, camphor and borneol[9-11] while some oils show as their major components camphene, piperitone, α-thujone[12,13]. A survey of the literature shows that extracts of *Achillea* spp. exhibit antimicrobial properties against human and plant pathogenic bacteria, fungi and yeast and some studies have also shown that essential oils from *Achillea* spp. may be used as antimicrobial agents for various uses with several medicinal applications[14,15].

The aim of this paper is to present the chemical composition and antimicrobial activity of the *Achillea* essential oils, which may lead to its use in medicine.

## EXPERIMENTAL

**Plant Material and isolation of the oils:** Aerial parts of *Achillea biserrata* M. Bieb. and *Achillea salicifolia* Besser subsp. *salicifolia* collected in the blooming stage from Gumushane between Trabzon and Ardahan between Gole in Turkey. Air dried aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus. A voucher specimen of each plant is deposited in the herbarium of the Department of Biology, Inonu University, Malatya, Turkey.

**Gas chromatography:** The gas chromatography analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m × 0.25 mm i.d. 0.25 μm film thickness) was used. Helium was used as a carrier gas (1.4 mL/min). The column was temperature programmed as follows: 5 min at 45 °C; then at 3 °C/min to 220 °C and held for 10 min. The injector and detector temperatures were to 220 and 250 °C, respectively. Injection was carried out automatic mode. Samples [0.5 μL of the oil solution in hexane (1:100)] were injected by the splitless technique into helium carrier gas. Peak areas and retention times were measured by Electronic Integration.

**Gas chromatography mass spectrometry (GC/MS):** GC/MS analyses of the essential oils were carried out on Hewlett Packard 5970A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature programme and injection were performed as described above. Injection was carried out automatic mode. Library search was carried out using Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR. EI mass spectra were measured at 70 eV ionization voltage over the mass range 10-400 μ.

The constituents of the oils were identified by matching their mass spectra and relative retention indices (RRI) and the components identified in the oils are listed in Table-1.

**Antimicrobial screening:** Growth inhibitory activities of the essential oils were tested against 7 bacteria, 1 yeast and 4 filamentous fungi by disc diffusion method[16]. Suspension of the tested microorganisms ($10^8$ CFU/mL) was spread on the solid media plates. Each test solutions are prepared in DMSO. Then filter paper discs (6 mm in diameter) were soaked with 20 μL of the stock solutions and placed on the

TABLE-1
INFORMATION ON *Achillea* sp. AND ESSENTIAL OILS COMPOSITION

| | | *Achillea salicifolia* subsp. *salicifolia* | *Achillea biserrata* |
|---|---|---|---|
| Collection site and date | | Ardahan between Gole, 1900 m, 12-08-2004 | Gumushane between Trabzon, 1600 m, 31-08- 2004 |
| Collector Number | | B.Y. 15884 | T.A. 1605 |
| RRI | Components | Yield (%) | Yield (%) |
| 1036 | α-Pinene | 0.46 | 2.8 |
| 1053 | Camphene | 3.23 | 16.41 |
| 1089 | Sabinene | 0.83 | – |
| 1150 | 2,5,5-Trimethyl-3,6-heptadien-2-ol | 4.38 | – |
| 1216 | 1,8-Cineol | 22.75 | 19.35 |
| 1224 | γ-Terpinene | 0.25 | – |
| 1362 | Artemisia alcohol | 3.17 | 14.28 |
| 1408 | Camphor | 55.26 | 36.80 |
| 1445 | *trans*-2-Caren-4-ol | 0.29 | – |
| 1472 | Borneol | – | 2.46 |
| 1485 | Terpinene-4-ol | 2.98 | 2.18 |
| 1538 | α-Terpineol | 2.47 | – |
| 1746 | Bornyl acetate | 1.99 | – |
| 2132 | Spathulenol | – | 3.49 |
| 2138 | α-Humulene | 0.16 | – |
| 2142 | Geranyl isovalerate | 0.19 | – |
| 2238 | Caryophyllene oxide | 0.94 | – |
| 2257 | β-Selinene | 0.65 | – |
| 2495 | Δ-Cadinene | – | 0.55 |
| 2609 | β-Eudesmol | – | 1.68 |

inoculated plates. After keeping at 2 ºC for 2 h, they were incubated at 37 ºC for 24 h for bacteria and *Candida albicans*. The diameter of the inhibition zones were measured in millimetres (Table-2).

TABLE-2
ANTIBACTERIAL SCREENING ACCORDING TO THE AGAR DISC DIFFUSION METHOD (mm)

| Microorganisms | Source | *A. biserrata* | *A. salicifolia* subsp. *salicifolia* | Standard |
|---|---|---|---|---|
| *Enterobacter aerogenes* | NRRL 3567 | 9 | 8 | 22[a] |
| *Escherichia coli* | ATCC 25292 | 9 | 7 | 22[a] |
| *Pseudomonas aeruginosa* | ATCC 27853 | 9 | 8 | 23[a] |
| *Proteus vulgaris* | NRRL 123 | 8 | 7 | 24[a] |
| *Staphylococcus aureus* | ATCC 6538 | 10 | 10 | 22[a] |
| *Listeria monocystogenes* | ATCC 7644 | 7 | 8 | 24[a] |
| *Serratia marcescens* | Clinic isolate | 9 | 6.5 | 24[a] |
| *Candida albicans* | Clinic isolate | 9 | 8 | 34[b] |

[a]Chloramphenicol; [b]Ketoconazol.

**Determination of minimum inhibitory concentration (MIC):** The antimicrobial action of the oils was investigated by microdilution broth susceptibility assay[16] with the following microorganisms *i.e.*, *Escherichia coli* ATCC 25292, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter aerogenes* NRRL 3567, *Proteus vulgaris* NRLL 123, *Listeria monocystogenes* ATCC 7644, *Serratia marcescens* (Clinic isolate) and *Candida albicans* (Clinic isolate). Stock solutions of essential oils were prepared in dimethyl sulfoxide. Serial dilutions of essential oils were prepared using sterile distilled water placed in 96-well microtiter plates. Freshly grown bacterial suspensions in double strength Mueller Hinton Broth (Merck) and yeast suspension of *Candida albicans* in yeast medium were standardized $10^8$ CFU/mL (McFarland No: 0.5 ). 100 µL of each microbial suspension was then added to each well. The wells including sterile distilled water only served as growth control. The last row containing only the serial dilutions of antibacterial agent without microorganism was used as negative control. After incubation at 37 ºC for 24 h, the first well without turbidity was determined as the minimal inhibitory concentration (MIC) (Table-3).

TABLE-3
ANTIBACTERIAL AND ANTICANDIDAL ACTIVITIES OF *A. biserrata* AND *A. salicifolia* subsp. *salicifolia* ESSENTIAL OILS (MIC) (µg/mL)

| Microorganisms | Source | *A. biserrata* | *A. salicifolia* subsp. *salicifolia* | Standard | DMSO |
|---|---|---|---|---|---|
| *Enterobacter aerogenes* | NRRL 3567 | 250 | 500 | -[a] | + |
| *Escherichia coli* | ATCC 25292 | 250 | 500 | -[a] | + |
| *Pseudomonas aeruginosa* | ATCC 27853 | 500 | 500 | -[a] | + |
| *Proteus vulgaris* | NRRL 123 | 500 | 500 | -[a] | + |
| *Staphylococcus aureus* | ATCC 6538 | 250 | 500 | -[a] | + |
| *Listeria monocystogenes* | ATCC 7644 | 1000 | 500 | -[a] | + |
| *Serratia marcescens* | Clinic isolate | 500 | 1000 | -[a] | + |
| *Candida albicans* | Clinic isolate | 500 | 500 | -[b] | + |

[a]Chloramphenicol; [b] Ketoconazol.

**Antifungal studies:** In order to obtain conidia, the fungi were cultured on Czapex Dox Agar and Malt Extract Agar medium in 9 cm Petri dishes at 25 ºC, for 10 d. Harvesting was carried out by suspending the conidia in a 1 % (w/v) sodium chloride solution contain 5 % (w/v) DMSO. The spore suspension was then filtered and transferred into tubes and stored at -20 ºC, according to Hadecek and Greger[17].

Screening for antifungal activities of the stock solution of the essential oils were performed qualitatively using the disc diffusion method (Table-4) against saprophytic fungi namely *Aspergillus flavus* Link ex Gray, *A. niger* Tiegh. *Penicillium expansum* Link ex Gray and *Alternaria brassicola* (Berk.) Sacc. cultured on Malt Extract and Czapex Dox Agar medium. For this reason one loop drop the spore suspension was applied onto the centre of the Petri dishes.

TABLE-4
ANTIFUNGAL ACTIVITIES OF *A. biserrata* AND *A. salicifolia* subsp. *salicifolia* ESSENTIAL OILS (INHIBITION %)

| Microfungi | *A. biserrata* | | | *A. salicifolia* subsp. *salicifolia* | | | Ketoconazol | | |
|---|---|---|---|---|---|---|---|---|---|
| | C | T | Inh. (%) | C | T | Inh. (%) | C | T | Inh. (%) |
| *Alternaria brassicola* | 45 | 10 | 77.77 | 45 | 10 | 77.77 | 45 | 22 | 51.11 |
| *Aspergillus flavus* | 55 | 8 | 85.45 | 55 | 35 | 36.36 | 55 | 9 | 83.63 |
| *Aspergillus niger* | 20 | - | 100 | 20 | 15 | 25 | 20 | 18 | 10 |
| *Penicillum expansum* | 50 | - | 100 | 50 | - | 100 | 50 | 8 | 84.00 |

C = Diameter of fungal growth on the control; T = Diameter of fungal growth on the test plate; Inh. = Inhibition.

20 µL stock solution of the essential oil was applied onto sterile paper discs (6 mm in diameter) and placed in the Petri dishes and incubated at 25 ºC for 3 d. The test solution is prepared in DMSO. The inhibition of fungal growths expressed in percentage terms was determined on the growth in test plates compared to the respective control plates as given % inhibition[18].

$$\text{Inhibition (\%)} = 100 (C-T) / C$$

where C: Diameter of fungal growth on the control, T: Diameter of fungal growth on the test plate).

The activities of the essential oils have been compared with the activity of standard fungicide ketoconazole.

## RESULTS AND DISCUSSION

Dried aerial parts of *Achillea biserrata* and *Achillea salicifolia* subsp. *salicifolia* yielded 0.07 and 0.08 % oil with camphor (36.80-55.26 %), 1,8-cineol (19.35-22.75 %), camphene (16.41-3.23 %) and artemisia alcohol (14.28-3.17 %) as main constituents, as shown in Table-1. Compared with other *Achillea* species[9,19,20] the species *Achillea biserrata* and *Achillea salicifolia* subsp. *salicifolia* have a low oil yield. GC and GC-MS analysis enabled the identification of a total of 10 constituents in *Achillea biserrata* and 16 constituents in *Achillea salicifolia* subsp. *salicifolia* oil, amounting to100 % of the total oils.

In the oils of other members of Ptarmica group the most abundant components were: borneol (20.3 %, 29.6 %) in *A. lingulata*[12,19], camphor (33.9 %) and sabinyl acetate (39.9 %) in *A. serbica*[20] and were camphor (18.3 %) and 1,8-cineole (11.9 %) in *A. frasii*[21]. Tables 2 and 3 show the results of antimicrobial assay. This result was expected because the components of the oil have known antimicrobial activity (camphor, 1,8-cineole). Both the Gram-positive and Gram-negative strains of bacteria had approximately equal non-resistance to the oils. However the *Achillea biserrata* essential oil showed high activity against the *Aspergillus flavus* and *Aspergillus niger* than the *A. salicifolia* subsp. *salicifolia* and fungicide ketoconazole which was used as the standard for comparison.

The tested plant essential oils appear to be effective against a wide spectrum of microorganisms, both pathogenic and saprophytic. These compounds may be able to control a wide range of microbes but there is also the possibility that they cause an imbalance in the gut microflora. Essential oils, which often contain the principal aromatic and flavouring components of herbs and spices, if added to foodstuffs, would cause no loss of organoleptic properties, would retard microbial contamination and therefore reduce the onset of spoilage[23].

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