Chemical Composition and *in-vitro* Antimicrobial Activity of the Essential Oils of Three Greek *Achillea* Species

Prokopios Magiatis[a], Alexios-Leandros Skaltsounis[a], Ioanna Chinou[a] and Serkos A. Haroutounian[b,*]

[a] Laboratory of Pharmacognosy, Department of Pharmacy, University of Athens, Zografou 15771, Greece

[b] Chemistry Laboratory, Agricultural University of Athens, Iera odos 75, Athens 11855, Greece. Fax +30-1-5294265. E-mail: sehar@aua.gr

* Author for correspondence and reprint requests

Z. Naturforsch. **57c,** 287–290 (2002); received October 29/November 30, 2001

Achillea sp., Essential Oil, Antimicrobial Activity

The chemical composition of the essential oils of *Achillea holosericea*, *Achillea taygetea, Achillea fraasii* was determined by GC/MS analysis. Among the ninety-five assayed constituents, camphor, borneol and 1,8-cineol were found to be the major components. The *in-vitro* antimicrobial activity of these essential oils was evaluated against six bacteria indicating that the first is totally inactive, while the other two possess moderate to strong activities mainly against the Gram negative strains. The essential oil of *A. fraasii* was also active against the tested pathogenic fungi.

Introduction

Aromatic and medicinal plants produce a wide variety of volatile terpene hydrocarbons (aliphatic and cyclic) and their corresponding oxygenated isoprenoid derivatives and analogues. Mixtures of these substances, which are known as essential oils, can be isolated from diverse parts of plants by steam distillation. The antimicrobial properties of essential oils are well recognized for many years (Hammer *et al.*, 1999; Cosentino *et al.*, 1999; Daferera *et al.*, 2000), and their preparations are found applications as naturally occurring antimicrobial agents in pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology, food preservation etc. Thus, the discovery of essential oil preparations that possess antimicrobial activities has been the subject of many research investigations consequencing the screening of a wide variety of plant species. However, less attention was given to the activities of their main components in respect to their presence in the oils tested. The latter depends on a number of parameters such as environmental conditions, collection period, dehydration procedure, storage condition, isolation method.

Achillea is a genus of the well known medicinal plant family of Asteraceae and comprises by numerous species and wild-growing plants. Fifty of them have been identified as European species, mainly as typical plants of the Mediterranean area. Since several *Achillea* plants have been found to possess antiseptic and infection preventing properties (Mitich, 1990), many of them are being used as ingredients in medicinal and cosmetic preparations. Thus, there is a considerable research interest towards the assay of composition and/or biological properties of various *Achillea* essential oils.

Among the twenty-five *Achillea sp.* found in Greece (Tutin, 1968; Strid and Tan, 1991), *Achillea taygetea* Boiss & Heldr. is endemic at the mountains Taygetos and Parnon (south Pelopennese) (Strid and Tan, 1991), while *Achillea holosericea* Sibth. & Sm. and *Achillea fraasii* Schultz Bip. are distributed throughout the southern Balkan peninsula (Tutin, 1968). The essential oils of *A. holosericea* and *A. fraasii* have never been studied, while there is only one report on chemical analysis of the essential oil of *A. taygetea* which concern material obtained from plant which was cultivated in a botanical garden out of its natural habitat (Maffei *et al.*, 1994). Main objective of this study is to assay the main constituents of these three essential oils and carry out a comparative evaluation of their antimicrobial activities in respect to their chemical compositions.

0939–5075/2002/0300–0287 $ 06.00

Materials and Methods

Plant material

Achillea taygetea and *Achillea holosericea* were collected at Taygetos mountain (1700 m and 1600 m height, respectively) in July 2000. *Achillea fraasii* was collected at Tymfi mountain (2000 m height) in July 2000. A voucher specimen of each plant is deposited in the herbarium of the Division of Pharmacognosy, University of Athens (Tg001, Tg002, In007, respectively).

Preparation of essential oils

The fresh aerial parts (200 g of each of the above mentioned species) were submitted for 3 hours to steam distillation to afford 0.80, 0.22 and 0.24 ml of oil respectively (yields 0.40, 0.11, 0.12%). The three oils were dried over anhydrous sodium sulfate and stored at +4 °C in bottles covered with aluminum foil to prevent the negative effect of light.

GC/MS analysis

The GC/MS analysis of the essential oils was performed using a Hewlett-Packard 6890 series II gas chromatograph equipped with a HP-5 capillary column (30 m, 00.25 mm i.d., 0.25 μm film thickness) and a mass spectrometer 5973 of the same company which was operated in EI mode. Helium was the carrier gas at a flow rate of 1 ml/min. The injector was operated at 200 °C and the oven temperature was programmed as follows: 60 °C for 5 min, then gradually increased to 280 °C at 3 °C/min.

The identification of components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada, 1976) and those described by Adams (1995), as well as on comparison of their retention indices (Van den Dool and Kratz, 1963) with literature values (Adams, 1995).

Reference strains

The reference strains used in all antimicrobial assays were: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 227853) and the pathogens fungi *Candida albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801), *C. glabrata* (ATCC 28838)

Antimicrobial assay

The minimum inhibitory concentrations (MIC) of the oils and their main components were determined using the dilution technique (Janssen *et. al.*, 1987). Standard antibiotics (netilmicin, amo×icillin and clavulanic acid) were used in order to control the sensitivity of the tested bacteria, while intraconazole, 5-flucytocine and amphotericin were used in order to control the tested fungi. Technical data have been described previously (Aligiannis *et al.*, 2000).

Results and Discussion

Ninety-five phytochemicals have been identified by the GC/MS analysis as constituents of the essential oils. The main components and their retention indices are summarised in Table I, while the results of the antibacterial and antifungal activities of essential oils and their main components are presented in Table II.

Chemical analysis revealed that there are significant qualitative and quantitative differences among the oils tested. Camphor and borneol were the only major constituents present in all oil samples, with their highest percentage found in *A. taygetea* and *A. holosericea* (25.71% and 16.26% respectively). Furthermore, 1,8-cineole which is the major constituent of the essential oil of *A. taygetea* and is present in large amount in *A. fraasii* (11.94%) was detected only in trace amounts (0.69%) in the oil of *A. holosericea*. In regard to the previously reported content of the essential oil of *A. taygetea* (Maffei *et. al.*, 1994), it is interesting to point out that there are important quantitative differences indicating that environmental factors strongly influence its chemical composition. Thus, 1,8-cineole which was found to be the major constituent, previously was assayed in only 1.6% concentration. On the contrary, α-pinene, β-pinene and germacrene-D which were detected in highest percentage, in our samples were present at very low concentrations (<2%). Overall, chemical composition analyses indicate that *A. fraasii* and *A. holosericea* oils have a higher diversity of phytochemicals than *A. taygetea* oil.

Table I. Main constituents of the essential oils of the three *Achillea species*, as identified by GC/MS analysis and their retention indices.

Phytochemicals	*Achillea frasii*	*Achillea holosericea*	*Achillea taygetea*	*KI* [a]
α-Pinene	0.46	1.62	1.92	939
Camphene	0.31	1	3.85	953
β-Pinene	0.38	–	1.51	980
1,8-Cineole	11.94	0.69	26.63	1033
2,7-Dimethyl-4(E), 6-octadien-2-ol	8.29	–	–	1044
Benzenacetaldehyde	1.92	0.24	0.13	1043
Nonanal	–	4.91	–	1102
Chrysanthenone	1.22	–	–	1123
α-Campholene aldehyde	–	2.21	0.54	1125
Camphor	16.3	20.93	25.71	1143
Pinocarvone	–	3.63	0.27	1162
Borneol	8.62	16.26	6.72	1165
Terpin-4-ol	1.45	2.95	2.32	1177
α-Terpineol	2.33	0.4	3.5	1189
Germacrene-D	0.18	3.52	0.29	1480
Caryophyllene oxide	4.96	–	1.57	1581
Farnesyl acetate cis, trans	–	9.58	–	1818
6,10,14-Trimethyl-2-pentadecanone	0.27	1.68	–	1848

[a] *KI* = Kovats Index.

Table II. Antimicrobial activity (MIC mg/ml) of the essential oils of *Achillea species* and their main components.

Ess. Oil	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. Pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*C. glabrata*
Achillea holosericea	–	–	–	–	–	–	–	–	–
A. taygetea	4.86	4.25	3.67	2.90	3.23	2.87	1.67	1.23	1.17
A. frasii	6.35	6.87	3.21	3.87	4.23	4.96	–	–	–
Camphor	3.25	2.12	2.45	2.80	2.35	1.2	6.23	4.58	3.67
Caryophyllene oxide	0.073	0.90	0.87	1.23	2.43	5.20	–	–	–
1,8-Cineol	9.50	9.50	2.75	2.35	3.20	2.40	0.25	0.25	0.20
Intraconazole	–	–	–	–	–	–	1×10^{-3}	0.1×10^{-3}	1×10^{-3}
5-Flucytocine	–	–	–	–	–	–	0.1×10^{-3}	1×10^{-3}	10×10^{-3}
Amphotericin B	–	–	–	–	–	–	1×10^{-3}	0.5×10^{-3}	0.4×10^{-3}
Netilmicin	4×10^{-3}	4×10^{-3}	8.8×10^{-3}	8×10^{-3}	8×10^{-3}	10×10^{-3}	–	–	–
Amoxycillin	2×10^{-3}	2×10^{-3}	2.4×10^{-3}	2.8×10^{-3}	2.2×10^{-3}	2×10^{-3}	–	–	–
Clavulanic acid	0.5×10^{-3}	0.5×10^{-3}	1×10^{-3}	1.6×10^{-3}	1×10^{-3}	1.2×10^{-3}	–	–	–

The results of the antimicrobial activity assays indicated that the essential oil of *A. holosericea* was completely inactive. The essential oils of *A. taygetea* and *A. frasii* displayed moderate activities against all the tested bacterial strains, with the first being more active in all cases. Both oils exhibited higher activities against the Gram negative tested bacterial strains, while the Gram positive strains of *S. aureus* and *S. epidermidis* appeared as the most resistant ones. These results are in contrast with previous repoerts in the literature indicating that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria (Outtara *et al.*, 1997; Mangena and Muyima, 1999). In an attempt to rationalize these peculiar results, we have determined the antibacterial properties of their main constituents, and found that among the compounds tested, caryophyllene oxide is the most efficient, followed by camphor and 1,8-cineole. The antibacterial properties of caryophyllene oxide have been revealed previously (Ulubelen *et al.*, 1994), while 1,8-cineole and camphor are also known to possess some antimicrobial activities (Aligiannis *et al.*, 2000; Prudent *et al.*, 1993). Thus, the antibacterial properties of the essential oils *A. taygetea* and *A. frasii* are probably connected with their high content of 1,8-cineol and camphor. In the case of the essential oil of *A. frasii* however, the presence of caryophyllene oxide also contributes in a considerable degree to the observed activity. Finally, both oils of *A. holosericea* and *A. taygetea* were found inactive against the tested fungi, while the oil of *A. frasii* showed an interesting antifungal profile (MIC values 0.33-6.40 mg/ml).

The complete phytochemical profile of the essential oils of the three *Achillea species* as identified by GC/MS analysis and their retention indices are available to interested readers on request from the authors.

Acknowledgements

This work was financed by the European Union Project FAIR CT96–1781. Dr. T. Constantinidis is acknowledged for the identification of the plant material.

Adams R. (1995), Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing Co., Carol Stream, IL.

Aligiannis N., Kalpoutzakis E., Chinou I. B., Mitakou S., Gikas E. and Tsarbopoulos A. (2000), Composition and antimicrobial activity of the essential oils of five taxa of *Sideritis* from Greece. J. Agric. Food Chem. **49**, 811–815.

Cosentino S., Tuberoso C. I. G., Pisano B., Satta M., Mascia V., Arzedi E. and Palmas F. (1999), In-vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett. Appl. Microbiol. **29**, 130–135.

Daferera D. J., Ziogas B. N. and Polissiou M. G. (2000), GC-MS Analysis of essential oils of some Greek aromatic plants and their fungiotoxicity on *Penicillium digitatum.* J. Agric. Food Chem. **48**, 2576–2581.

Hammer K. A., Carson C. F. and Riley T. V. (1999), Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. **86**, 985–990.

Janssen A. M., Scheffer J. J. C. and Svendsen A. (1987), Antimicrobial activity of essential oils: a 1976–1986 literature review. Aspects of the test methods. Planta Med. **5**, 395–397.

Maffei M., Mucciarelli M. and Scannerini S. (1994), Essential oils from *Achillea* species of different geographic origin. Biochem. Syst. Ecol. **22**, 679–687.

Mangena T. and Muyima N. Y. O. (1999), Comparative evaluation of the antimicrobial activities of essential oils of *Artemisia afra, Pteronia incana* and *Rosmarinus officinalis* on selected bacteria and yeast strains. Lett. Appl. Microbiol. **28**, 291–296.

Massada Y. (1976), Analysis of Essential Oil by Gas Chromatography and Spectrometry. J. Wiley & Sons, New York.

Mitich L. W. (1990), Yarrow the herb of Achilles. Weed Technol. **4**, 451–453.

Outtara B., Simard R. E., Holley R. A., Piettte G. J. P. and Begin A. (1997), Antimicrobial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. **37**, 155–162.

Prudent D., Perineau F., Bessiere J. M., Michel G. and Bravo R. (1993), Chemical analysis, bacteriostatic, and fungistatic properties of the essential oil of the atoumau from Martinique (*Alpinia speciosa*). J. Ess. Oil. Res. **5**, 255–264.

Strid A. and Tan K. (1991), Mountain Flora of Greece, vol. **2**. Edinburgh University Press, Edinburgh, pp. 431–455.

Tutin T. G. (1968), Flora Europaea, vol **4** (Tutin T. G., Heywood V. H., Burgess N. A., Moore D. M., Valentine D. H., Walters S. M. and Webb D. A., eds). Cambridge University Press, Cambridge, pp. 159–165.

Van den Dool H. and Kratz P. D. (1963), A generalization of the retention index× system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. **11**, 463–471.

Ulubelen A., Topcu G., Eris C., Sőnmez U., Kartal M., Kurucu S. and Bozok-Johansson C. (1994), Terpenoids from *Salvia sclarea*. Phytochemistry **36**, 971–974.