Pharmaceutical Biology, 2009; 47(1): 86–91

informa
healthcare

ORIGINAL ARTICLE

Evaluation of antimicrobial properties of *Achillea L.* flower head extracts

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Abstract

Thirty-nine extracts obtained from flower heads of 13 *Achillea* species [*A. multifida* (DC.) Boiss., *A teretifolia* Waldst.&Kitt., *A. schischkinii* Sosn., *A. setacea* Waldst.&Kitt., *A. crithmifolia* Waldst.&Kitt., *A. falcata* L., *A. biebersteinii* Afan, *A. coarctata* Poir., *A. millefolium* L. subsp. *pannonica* (Scheele) Hayek., *A. clypeolata* Sm., *A. kotschyi* Boiss. subsp. *kotschyi*, *A. phyrigia* Boiss.&Bal,. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formánek] were evaluated for antimicrobial activity against *Escherichia coli* ATCC 29908, hemorrhagic *E. coli* (O157:H7) RSSK 232, *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 43300 (methicillin/oxacillin-resistant), *S. aureus* ATCC 6538/P, *Streptococcus epidermidis* ATCC 12228, *Salmonella typhimurium* CCM 5445, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeroginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 90028. The minimum inhibitory concentrations (MICs) were determined for all extracts against the tested organisms. Hexane extracts of *A. coarctata* and *A. setacea* showed antibacterial activity against *E. faecalis* (MIC=31.25 and 62.5 µg/mL, respectively). Chloroform extracts of a number of *Achillea* species showed selective activity against the tested bacteria isolates; MICs for the most active species (*A. teretifolia*, *A. multifida*) were found to range from 50 to 75 µg/mL against *S. aureus*, *S. epidermidis*, and *S. typhymurium*. All of the extracts were inactive against *C. albicans* at the tested concentrations. The study has shown that several *Achillea* species possess antibacterial activity, which may yield novel antibacterial compounds with potential use as phytotherapeutics.

Keywords: *Achillea; antibacterial; antifungal; Asteraceae*

Introduction

Researchers have for many decades been trying to develop new broad-spectrum antibiotics against the infectious diseases caused by bacteria, fungi, viruses, and parasites. Prolonged usage of these broad-spectrum antibiotics has led to the emergence of drug resistance. There is a tremendous need for novel antimicrobial agents from different sources. To treat infectious diseases, man has for centuries used plants, many of which are still used today as traditional medicines. Screening of plant extracts with validated methods is a primary source of discovery, in terms of identifying potentially useful molecules against infectious diseases.

The genus *Achillea* L. (Asteraceae) is represented by about 85 species found in the Northern hemisphere, mostly in Europe and Asia (Könemann, 1999). The name of the genus originates from the ancient use as a wound-healing remedy by the Trojan hero Achilles (Benedek & Kopp, 2007). The aerial parts of *A. millefolium* L., a well-known species among the members of *Achillea*, are commonly used in European traditional medicine for the treatment of gastrointestinal disorders and hepatobiliary complaints, as well as for wound healing and skin inflammations (Benedek & Kopp, 2007). *Achillea* is represented in Turkey with 46 taxa, 25 of which are endemic (Wagenitz, 1975; Güner et al., 2000). Various species of the genus are traditionally used in Turkey for wound healing, against diarrhea and flatulence, as a diuretic, as emmenagog agents, and for abdominal pain (Baytop, 1999; Sezik & Yesilada, 1999; Sezik et al., 2001).

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(Received 7 May 2008; revised 10 July 2008; accepted 19 July 2008)

ISSN 1388-0209 print/ISSN 1744-5116 online © 2009 Informa UK Ltd
DOI: 10.1080/13880200802448682

Beside essential oils, the constituents of *Achillea* are mainly sesquiterpene lactones, flavonoids, and phenolic acids. Reports on the phytochemistry of the *Achillea* species chosen for the present study are summarized in Table 1. Previously, it was shown that infusions prepared from *Achillea* species growing in Turkey have an antioxidant capacity and protective effects against H_2O_2-induced oxidative damage in human erythrocytes and leukocytes, which is consistent with their flavonoid and total phenol contents (Konyalıoglu & Karamenderes, 2004, 2005). The antibacterial and antifungal activities of the essential oil of *A. nobilis* subsp. *neilreichii* (Karamenderes et al., 2007) have been reported. *In vivo* antinociceptive and anti-inflammatory activities and the acute toxicity of the ethanol extract prepared from these species have also been studied (Karabay-Yavasoglu et al., 2007). However, the biological properties of these species have not been completely elucidated.

Table 1. Phytochemical studies on selected *Achillea* species.

Plant species	Contents	References
A. multifida	essential oil	Baser et al. (2002)
A. teretifolia	essential oil	Unlu et al. (2002)
A. schischkinii	sesquiterpenes, flavonoids, and cumarins	Ulubelen et al. (1987, 1988)
	essential oil	Donmez et al. (2005)
A. setacea	sesquiterpenes, a monoterpene triol, and flavone glycosides	Kubelka et al. (1999); Todorova et al. (2000); Marchart & Kopp (2003)
	essential oil	Unlu et al. (2002)
A. crithmifolia	sesquiterpene lactones	Milosavljevic et al. (1991, 1994); Todorova et al. (1998, 2000)
	essential oil	Maffei et al. (1994); Karamenderes et al. (2002); Kowalczyk et al. (2003); Palic et al. (2003)
A. falcata	amides	Hofer et al. (1986)
	essential oil	Kurkcuoglu et al. (2003); Senatore et al. (2005)
A. biebersteinii	ionone glucoside and terpenoids	Mahmoud & Al-Shihry (2006)
	flavonoids	Valant-Vetschera & Wollenweber (1996); Marchart & Kopp (2003)
	essential oil	Jaimand & Rezaee (2001); Bader et al. (2003); Sokmen et al. (2004); Esmaeili et al. (2006)
A. coarctata	sesquiterpene lactones	Todorova & Tsankova (2001)
	essential oil	Toker et al. (2003)
A. millefolium subsp. *pannonica*	essential oil	Karamenderes et al. (2002)
A. clypeolata	sesquiterpenes, diterpenes, flavonoids, and cumarins	Aljancic et al. (1996); Todorova et al. (1998); Todorova & Tsankova (1999); Werner et al. (2007)
	essential oil	Simic et al. (2005)
A. kotschyi subsp. *kotschyi*	essential oil	Karamenderes et al. (2002)
A. phyrigia	essential oil	Baser et al. (2000)
A. nobilis subsp. *neilreichii*	essential oil	Karamenderes et al. (2007)

In the present study, we investigated the antifungal and antibacterial activities of flower head extracts prepared from 13 Turkish *Achillea* species [*A. multifida* (DC.) Boiss., *A. teretifolia* Waldst. & Kitt., *A. schischkinii* Sosn., *A. setacea* Waldst. & Kitt., *A. crithmifolia* Waldst. & Kitt., *A. falcata* L., *A. biebersteinii* Afan, *A. coarctata* Poir., *A. millefolium* subsp. *pannonica* (Scheele) Hayek., *A. clypeolata* Sm., *A. kotschyi* Boiss. subsp. *kotschyi*, *A. phyrigia* Boiss. & Bal. and *A. nobilis* L. subsp. *neilreichii* (Kerner) Formánek] to explore the beneficial effects of these species. As far we know, the antimicrobial activity of the *n*-hexane, chloroform, and methanol extracts of these 13 taxa have not previously been investigated, except for the methanol extract of *A. biebersteinii*.

Materials and methods

Plant materials

Plants were collected during the flowering period in 2000 and 2001 from various locations within Turkey and identified by Professor Dr. Ozcan Secmen from Ege University, Faculty of Science, Department of Biology, Section of Botany, Bornova, Turkey. Voucher specimens are kept in the IZEF Herbarium of Ege University, Faculty of Pharmacy, Department of Pharmaceutical Botany (Table 2).

Preparation of the extracts

Dried flower heads of plants (about 50 g) were shaken sequentially in *n*-hexane, chloroform, and methanol for 16 h (10 mL/g, for each) at room temperature. Samples were sonicated separately for 15 min twice and extracts then filtered. The combined extracts were dried under reduced pressure at 40°C. The extracts were weighed and stored at +4°C for further experiments.

Antimicrobial activity test

In vitro antimicrobial studies were carried out against nine bacteria strains (*Escherichia coli* ATCC 29908, *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538/P, *Streptococcus epidermidis* ATCC 12228, *Salmonella typhimurium* CCM 5445, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212), two specific pathogenic strains [methicillin/oxacillin-resistant

Table 2. The collection sites, voucher numbers, and yields of various extracts of *Achillea* species in Turkey.

Plant material	Voucher no.	Collection site	Solvent	Yield of extract (% of dry weight)
A. multifida (DC.) Boiss. [E]	IZEF5598	Bursa, Uludag	*n*-hexane	1.37
			chloroform	4.86
			methanol	4.81
A. teretifolia Waldst.&Kitt.	IZEF5497	Nigde, Altunhisar	*n*-hexane	1.08
			chloroform	4.50
			methanol	5.22
A. schischkinii Sosn. [E]	IZEF5503	Sivas, Karacaören	*n*-hexane	1.37
			chloroform	2.46
			methanol	3.08
A. setacea Waldst.&Kitt.	IZEF5476	Kırklareli, Saray	*n*-hexane	1.15
			chloroform	2.94
			methanol	3.82
A. crithmifolia Waldst.&Kitt.	IZEF5477	Kırklareli, Kıyıköy	*n*-hexane	2.09
			chloroform	2.61
			methanol	4.75
A. falcata L.	IZEF5509	Burdur, Elmalıyurt	*n*-hexane	0.59
			chloroform	1.60
			methanol	2.73
A. biebersteinii Afan	IZEF5501	Konya, Aksaray	*n*-hexane	0.93
			chloroform	1.44
			methanol	5.66
A. coarctata Poir.	IZEF5473	Tekirdag, Ganos Mountain	*n*-hexane	1.37
			chloroform	2.08
			methanol	5.16
A. millefolium subsp. *pannonica* (Scheele) Hayek.	IZEF5481	Kırklareli, İgneada	*n*-hexane	1.78
			chloroform	2.62
			methanol	3.39
A. clypeolata Sm.	IZEF5479	Kırklareli, Vize	*n*-hexane	0.79
			chloroform	1.69
			methanol	2.88
A. kotschyi Boiss. subsp. *kotschyi*	IZEF5505	Erzurum, Oltu	*n*-hexane	1.36
			chloroform	1.80
			methanol	1.97
A. phyrigia Boiss.&Bal.[E]	IZEF5498	Kırsehir, Mucur	*n*-hexane	0.84
			chloroform	2.55
			methanol	3.48
A. nobilis L.subsp. *neilreichii* (Kerner) Formánek	IZEF5510	Burdur, Elmalıyurt	*n*-hexane	1.19
			chloroform	4.16
			methanol	4.98

[E] = endemic species.

S. aureus ATCC 43300 (MORSA) and hemorrhagic *E. coli* (O157:H7) RSSK 232] and a yeast (*Candida albicans* ATCC 90028), which were obtained from the Microbiology and Pharmaceutical Microbiology Departments, Ege University.

Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by NCCLS (2003) with some modifications. Dilution series of the extracts were prepared from 2.5 to 0.5 mg/mL in test tubes and then transferred to the broth in 96-well microtiter plates. Final concentrations in the medium were 250 to 25 μg/mL. Before inoculation of the test organisms, the bacteria strains and yeast strain were adjusted to 0.5 McFarland standards and diluted 1:100 (v/v) in Mueller–Hinton broth and Saboraud dextrose. Plates were incubated at 35°C for 18–24 h and at 30°C for 48 h for the yeast. All the tests were performed in broth and repeated twice. The MIC was defined as the lowest concentration that showed clear against a black background (no visible growth). Samples from clear wells were subcultured by plotting on to Mueller–Hinton agar. Ampicillin, streptomycin, and linesolide were used as standard antibacterial agents, whereas nystatin was used as a standard antifungal agent. All antibiotics

were purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA), and dilutions were prepared at concentrations ranging from 128 to 0.25 μg/mL in microtiter plates.

Results and discussion

The three extraction solvents were used in a sequential manner in order to extract nonpolar (n-hexane), intermediate (chloroform), and polar (methanol) compounds from the plants of interest (Table 2). These 39 extracts obtained from 13 *Achillea* species were evaluated for their *in vitro* antimicrobial activities against nine bacteria strains, two specific pathogenic bacteria strains, and a fungus. The results are displayed in Table 3.

Five plants showed antibacterial activity against *E. faecalis* at tested concentrations. Results indicated that the hexane extracts of *A. coarctata*, *A. setacea*, *A. biebersteinii*, *A. phyrigia*, and *A. falcata* showed mild to low antibacterial activity against *E. faecalis* (MIC=31.25, 62.50, 125, 125, and 250 μg/mL, respectively). Additionally, the chloroform extract of *A. multifida* showed moderate activity against *S. aureus* (ATCC 6538/P) and *S. epidermidis* (MIC=50 μg/mL for each). The chloroform extract of *A. teretifolia* exhibited antibacterial activity against *S. aureus* (MORSA), *S. epidermidis*, and *S. typhimurium* at the same concentration (MIC=50 μg/mL). *A. setacea* inhibited MORSA with MIC value of 50 μg/mL. Only the chloroform extracts of *A. multifida* and *A. teretifolia* showed antibacterial activity against hemorrhagic *E. coli* (MIC=100 and 250 μg/mL, respectively). All of the extracts were inactive against *C. albicans*. We observed no antimicrobial activity against tested organisms for any extract of *A. millefolium* subsp. *pannonica*.

Although some reports have focused on determining the antimicrobial activity of essential oils (Baser et al., 2002; Unlu et al., 2002; Candan et al., 2003; Sokmen et al., 2004; Senatore et al., 2005; Simic et al., 2005; Iscan et al., 2006; Karamenderes et al., 2007), information on the antimicrobial activity of *Achillea* extracts is limited. *A. millefolium* represents two subspecies of flora in Turkey: subsp. *millefolium* and subsp. *pannonica* (Wagenitz, 1975). Because of nonspecified subspecies names, exact plant materials are not known in some studies. Only the antimicrobial activity of the essential oil and methanol extracts of *A. millefolium* subsp *millefolium* from Turkey have been previously reported (Candan et al., 2003). Water-insoluble fractions of the methanol extract of this plant showed moderate activity against *Clostridium perfringens* and *C. albicans* by the disk diffusion method; the hexane–ether–methanol (1:1:1, v/v) extract of

Table 3. Antimicrobial activity of *Achillea* extracts.

Achillea species	Extract	EC	SA1	SA2	SE	ST	BC	PA	EF
A. multifida	chloroform	100	50	75	50	75	250	250	–
	methanol	–	200	–	–	–	187.5	–	–
A. teretifolia	chloroform	250	50	50	50	50	250	250	–
	methanol	–	–	125	–	–	187.5	–	–
A. schischkinii	methanol	–	–	162.5	–	–	200	–	–
A. setacea	*n*-hexane	–	–	–	–	–	–	–	62.5
	chloroform	–	100	50	87.5	250	–	–	–
	methanol	–	250	75	–	–	225	–	–
A. crithmifolia	chloroform	–	250	250	100	250	–	–	–
	methanol	–	100	100	–	–	200	–	–
A. falcata	*n*-hexane	–	–	–	–	–	–	–	250
	methanol	–	225	150	–	–	200	–	–
A. biebersteinii	*n*-hexane	–	–	–	–	–	–	–	125
	methanol	–	225	162.5	–	–	–	–	–
A. coarctata	*n*-hexane	–	–	–	–	–	–	–	31.25
A. clypeolata	methanol	–	100	200	–	–	100	–	–
A. kotschyi subsp. *kotschyi*	methanol	–	187.5	150	200	–	100	–	–
A. phyrigia	*n*-hexane	–	–	–	–	–	–	–	125
	methanol	–	250	100	–	–	200	–	–
A. nobilis subsp. *neilreichii*	chloroform	–	–	–	–	–	–	–	250
	methanol	–	250	125	200	–	200	–	–
Streptomycin		4.0	4.0	4.0	4.0	16	4.0	1.0	NT
Ampicillin		4.0	0.25	32	2.0	1.0	8.0	16	NT
Linesolide		NT	NT	NT	NT	NT	NT	NT	1.95

Values given are the minimum inhibitory concentrations in μg/mL. Results are shown only for the active plant extracts (– indicates not active at the test concentration of 250 μg/mL; NT means not tested). EC = *Escherichia coli* RSSK 232 (hemorrhagic, O157:H7); SA1 = *Staphylococcus aureus* ATCC 6538/P; SA2 = *S. aureus* ATCC 43300 (methicillin/oxacillin-resistant); SE = *Streptococcus epidermidis* ATCC 12228; ST = *Salmonella typhimurium* CCM 5445; BC = *Bacillus cereus* ATCC 7064; PA = *Pseudomonas aeroginosa* ATCC 27853; EF = *Enterococcus faecalis* ATCC 29212.

A. millefolium was found to be mildly active against *E. coli*, *P. aeruginosa*, *S. aureus*, *Salmonella enteridis*, *Aspergillus niger*, and *C. albicans* (Stojanovic et al., 2005). In another study, the methanol extract and its water-insoluble part of *A. biebersteinii* were evaluated for their antimicrobial activities *in vitro* (Sokmen at al., 2004) and found to be active on *B. cereus*, *C. perfringens*, and *C. albicans*. We did not observe any activity of *A. millefolium* subsp. *pannonica* and *A. biebersteinii* extracts at tested concentrations against *E. coli*, *S. aureus*, and *C. albicans*; this discrepancy most likely reflects the differences in plant subspecies, antimicrobial assay, extraction methods, and also in microbial strains.

The genus *Achillea* has been extensively studied in regard to its flavonoids (Valant-Vetschera & Wollenweber, 1996; Marchart & Kopp, 2003; Benedek et al., 2008) and sesquiterpene lactones (Todorova & Tsankova, 2001; Werner et al., 2007; Benedek et al., 2008). Flavonoids were demonstrated to possess antimicrobial properties and several investigations have examined the relationship between flavonoid structure and antibacterial activity (Aljancić et al., 1999; Cushnie & Lamb, 2006). Promising evidence has clearly shown that sesquiterpene lactones derived from several different plant species have significant antimicrobial activity *in vitro* (Neerman, 2003). The observed activity of the plants studied herein might be due to the presence of sesquiterpene lactones and flavonoids, and also possibly due to synergistic interactions between the components of these extracts.

In conclusion, to our knowledge, this is the first report of the antimicrobial activity by MIC of different extracts of several *Achillea* species. Active species such as *A. coarctata*, *A. setacea*, *A. multifida*, and *A. teretifolia* are good candidates for bioactivity-guided isolation studies, in order to obtain pure metabolites which might be used in the treatment of infectious diseases caused by pathogenic bacteria.

Acknowledgements

The authors thank Professor Dr U. Zeybek, Dr B. Ozturk, and Dr A. Aslan for providing some of plant materials; Professor Dr O. Secmen for the identification of the plants; and Dr P. Ballar for her critical review of the manuscript.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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