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COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Achillea formosa* subsp. *amanica*

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The genus *Achillea* L. is a member of Asteraceae and includes approximately 110–140 species in the world with distribution especially in SE Europe and SW Asia and with extensions through Eurasia to North America [1]. It is represented by 48 species (54 taxa) (including *Otanthus* Hoffmanns. & Link and *Leucocyclus* Boiss.), which belong to five sections in Turkey [2–9]. The aerial parts of different species of the genus are widely used in folk medicine due to numerous pharmacological properties, such as anti-inflammatory, antioxidant, antispasmodic, antihemorrhoidal, stomactiche, antiseptic, and emmenagogue [10].

Achillea formosa (Boiss.) Sch. Bip. subsp. *amanica* (Rech. f.) Ehrend. & Y.P. Guo is one of the endemic taxa of the genus *Achillea.* To the best of our knowledge, no information is available concerning the essential oil of this taxon.

This study concerns the analysis of the essential oil of *A. formosa* subsp. *amanica* by gas chromatography/mass spectrometry (GC/MS) (see Table 1) and antimicrobial evaluation against Gram (+) and Gram (–) human pathogenic bacteria and the yeasts *Candida albicans* and *Candida tropicalis*. An agar dilution procedure [11] was used to determine the minimum inhibitory concentrations (MIC) of the essential oil against six different microorganisms (see Table 2).

Plant Material. The plant material for study was collected from Osmaniye province (Turkey), between Osmaniye and Yarpuz, from 1450 m altitude, during the flowering stage in 24.06.2004. Voucher specimens have been deposited in the Herbarium of Inonu University (INU) in Malatya, Turkey (Collector No. TA1769).

Isolation of the Essential Oil. Air-dried aerial parts of the plants were hydrodistilled for 3 h using a Clevenger-type apparatus. Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapor and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4°C until analysis by GC and GC/MS. The percentage yield (%) of the oil calculated on a moisture-free basis was 0.034% for the essential oil (v/w).

Chromatographic analysis and identification of compounds in the essential oil were carried out according to recently reported methods [12]. The individual compounds identified in the essential oil are given in Table 1.

Antimicrobial Assay. Antimicrobial activities of the essential oil were determined by using the agar dilution procedure outlined by the Clinical and Laboratory Standards Institute [11]. Minimal inhibitory concentrations for each compound were investigated against standard bacterial strains *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 and the yeasts *Candida albicans* and *Candida tropicalis* obtained from American Type Culture Collection (Rockville, MD) and the Department of Microbiology, Faculty of Medicine, Ege University (Turkey), respectively. Bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and yeasts strains were cultured on RPMI 1640 Broth (Sigma-Aldrich Chemie GmbH Taufkirchen, Germany). Their turbidities matched that of a McFarland No. 0.5 turbidity standard [13]. The stock solution of the essential oil was prepared in dimethylsulfoxide (DMSO), which had no effect on the microorganisms in the concentrations studied.

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TABLE 1. Composition of the Essential Oil TA 1769 (*Achillea formosa* subsp. *amanica*)

Compound	RRI	%	Compound	RRI	%
1,8-Cineole	1244	2.5	Caryophyllene oxide	1954	5.5
p-Cymene	1324	0.2	Methyl eugenol	1965	0.3
cis-Linalool oxide	1508	0.6	Salvial-4(14)-en-1-one	1971	0.9
Camphor	1584	4.7	*E*-Nerolidol	1979	0.7
Linalol	1605	0.9	Elemol	2011	5.1
Pinocarvone	1631	0.4	Hexahydrofarnesyl acetone	2036	0.8
6-Methyl-3,5-heptadien-2-one	1650	0.4	Spathulenol	2042	3.2
β-Caryophyllene	1655	0.6	τ-Cadinol	2071	3.2
Terpinen-4-ol	1656	3.8	Thymol	2074	2.9
α-Terpineol	1733	4.7	δ-Cadinol	2076	1.0
Borneol	1743	12.8	Carvacrol	2094	3.1
Piperitone	1763	0.3	α-Eudesmol	2104	2.1
Naphthalene	1776	1.0	β-Eudesmol	2110	5.0
γ-Cadinene	1786	0.5	Decanoic acid	2126	0.6
β-Damascanone	1832	0.4	*n*-Hexadecanoic acid	2763	11.6
p-Cymene-8-ol	1849	0.5	Total		81.4
E-Geranyl acetone	1853	1.1			

RRI: relative retention indices.

TABLE 2. Antimicrobial Activity of *Achillea formosa* subsp. *amanica* Essential Oil (MIC in μg/mL)

Microorganisms	Source	EO	ST
Enterococcus faecalis	ATCC 29212	50	0.78[a]
Staphylococcus aureus	ATCC 29213	25	0.39[a]
Escherichia coli	ATCC 25922	200	3.12[a]
Pseudomonas aeruginosa	ATCC 27853	200	> 75[a]
Candida albicans	Ege Univ. (TR)	12.5	> 12.5[b]
Candida tropicalis	Ege Univ. (TR)	12.5	> 12.5[b]

EO: *Achillea formosa* subsp. *amanica* Essential Oil; ST: standard agent; [a]ampicilin, [b]flucanazole.

All of the dilutions were done with distilled water. The concentrations of the tested compounds were 800, 400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL. Ampicilin and fluconazole from FAKO (Istanbul, Turkey) were used as a reference compound for the experiments. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of agar plates. All the inoculated plates were incubated at 35°C, and the results were evaluated after 16–20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the minimal inhibitory concentration (MIC).

Chemical Composition of the Essential Oils. Water-distillation of the dried aerial parts of *Achillea formosa* subsp. *amanica* gave a light yellowish oil of 0.034% (v/w). About 32 constituents (81.4% of the total oil) were identified by means of GC-MS analysis of the essential oils from *A. formosa* subsp. *amanica* (Table 1). The major components of *A. formosa* subsp. *amanica* were borneol (12.8%), *n*-hexadecanoic acid (11.6%), caryophyllene oxide (5.5%), and β-eudesmol (5.0%).

1,8-Cineole, camphor, borneol, and/or α-terpineol have been found as major compounds in many other *Achillea* species [14, 15]. Some differences in the quantity and quality of the oil composition among the *Achillea* species may be due to different subspecies, chemotype, and geographic and climatic factors.

Antimicrobial Activity. The *in vitro* antimicrobial tests of the essential oil from *Achillea formosa* subsp. *amanica* in question resulted in a range of growth inhibition patterns against pathogenic microorganisms (Table 2). The results showed that the essential oil was particularly effective against the yeasts *Candida albicans* and *Candida tropicalis* with the lowest MIC value (12.5 μg/mL), and moderately active against the Gram-positive bacteria *Staphylococcus aureus* and *Enterococcus faecalis*, and the Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* in the presence of the oil extracted from *Achillea formosa* subsp. *amanica*.

The *Achillea* genus is widespread all over the world, and many species of this genus have been used as traditional herbal medicines by local people. Phytochemical investigation of *Achillea* species has revealed that many components from this genus are highly bioactive. Therefore, phytochemical and biological studies of this genus should be intensified.

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