JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Composition and Antioxidant, Antimicrobial, and Antifungal Activities of the Essential Oil of *Achillea ligustica* All.

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The chemical composition of the essential oil from flowering tops of *Achillea ligustica* All. was studied. Samples were collected in different localities of Sardinia (Italy) and hydrodistilled both with Clevenger-type and with simultaneous distillation−extraction apparatus. The yields ranged between 0.88 ± 0.06 and 0.43 ± 0.02% (vol/dry wt). The essential oils were analyzed by GC-MS, and a total of 96 components were detected. From a qualitative point of view, irrelevant differences between samples were observed. Strong chemical variability depending on the origin of the samples was observed. The major compounds found were santolina alcohol (6.7−21.8%, for the first time detected in *A. ligustica*), borneol (3.4−20.8%), sabinol (2.1−15.5%), *trans*-sabinyl acetate (0.9−17.6%), α-thujone (0.4−25.8%), and, among sesquiterpenes, viridiflorol (0.7−3.6%). No significant differences were detected between essential oils extracted by hydrodistillation and simultaneous distillation−extraction with CH_2Cl_2 and *n*-hexane. Antioxidant activity as DPPH radical scavenging activity was expressed in TEAC and ranged between 0.40 and 0.88 mmol/L. The antimicrobial and antifungal activities were investigated on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Penicillium commune*, *Fusarium oxysporum*, *Rizoctonia solani*, and *Aspergillus flavus*, showing low activity.

KEYWORDS: ***Achillea ligustica* All.; essential oil; GC-MS analysis; DPPH radical scavenging activity; antimicrobial and antifungal activities**

INTRODUCTION

Ligurian yarrow (*Achillea ligustica* All.) is a perennial, pubescent herbaceous plant belonging to the family of Asteraceae. It grows spontaneously throughout the western Mediterranean region, and in Italy grows especially in the Tyrrhenian area from Liguria to Sicily. The plant is not higher than a meter, with pinnati-partite leaves, a slightly aromatic scent, and a typically bitter taste. Flowers are arranged in flat-topped clusters, and both disk and ray flowers are small and white, like those of the common yarrow (*Achillea millefolium* L.) (*1*−*3*).

A. ligustica grows in different areas of Sardinia (Italy) usually from sea level to 1000 m, preferring sunny or partly shaded grassy fields or roadsides and other edges. It blooms at the beginning of summer. It has been used in traditional Sardinian medicine since ancient times, mainly as an anthelmintic, against gastric pains and neuralgias, and as an anti-inflammatory on skin diseases. Moreover, "magical uses", especially against bad luck, are reported (*1*, *4*).

Essential oils from different species of yarrow have been widely investigated, and herbal, food, and cosmetic uses are reported in many scientific papers. The chemical composition of the volatile fraction of *A. ligustica* in the scientific literature was found in only three papers (*5*−*7*). Tzakou et al. (*5*) reported data about one sample collected in Greece, and they obtained essential oils from leaves and flowers. By GC-MS analysis the main compound of both oils was linalool (28.2 and 70.8%, respectively). Also, Maffei et al. (*6*) and Bader et al. (*7*) analyzed one sample, the first from northern Italy and the second from Sicily. Maffei et al. found as the main compound artemisia ketone in full blooming plant (43.9%), whereas Bader et al. investigated the leaves and the main compound was terpinen-4-ol (19.3%). None of the three papers specifies which compound was identified by comparison with pure standard or library spectra and literature data.

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10.1021/jf0518913 CCC: $30.25
Published on Web 11/24/2005

Table 1. Information about the *A. ligustica* Samples

sample	collecting area	date	yield[a] (% ± SD) (vol/dry wt), Clevenger
1	Iglesias (lago Corsi)	June 11, 2004	0.73 ± 0.02
2	Fluminimaggiore (passo Bidderdi)	June 11, 2004	0.64 ± 0.04
3	Dolianova	June 13, 2004	0.43 ± 0.02
4	Serpeddì (Genna Manunga)	June 16, 2004	0.53 ± 0.02
5	Burcei (Monte Forrà)	June 16, 2004	0.64 ± 0.02
6	San Nicolò Gerrei	June 23, 2004	0.56 ± 0.02
7	Aritzo (monte Texile)	July 2, 2004	0.51 ± 0.03
8	Fonni-Desulo (Tascusì)	July 2, 2004	0.88 ± 0.06

[a] Mean value of triplicate data.

The aims of the present paper were (a) to characterize the chemical composition of essential oils of wild Ligurian yarrow from Sardinia, (b) to evaluate their antioxidant activity, and (c) to investigate the antifungal and antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Penicillium commune*, *Fusarium oxysporum*, *Rizoctonia solani*, and *Aspergillus flavus*.

MATERIALS AND METHODS

Plant Material. Wild samples were collected in blooming state (in the same flowering conditions) over June and July 2004, in eight different localities of Sardinia, from 300 to 1000 m above sea level. The harvest involved a random-block design sampling, and 2.0 kg amounts of aerial parts were collected in each area. After harvest, samples were carried in jute bags at 25 °C to the laboratory, cleaned from impurity, and hydrodistilled immediately. A 5 g amount was dried at 105 ± 1 °C for 90 min to verify the water content. **Table 1** shows sample origin and the essential oil yields (volume/dry weight, v/w). The specimens were identified and deposited in the Herbarium of University Botanical Garden of Cagliari (Italy).

Reagents and Standards. α-Pinene, camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, 1,8-cineole, γ-terpinene, sabinene hydrate, α-terpinolene, α- + β-thujone, chrysanthenone, camphor, borneol, terpinen-4-ol, myrtenal, α-terpineol, bornyl acetate, thymol, eugenol, β-caryophyllene, *allo*-aromadendrene, *trans*-nerolidol, caryophyllene oxide, α-bisabolol, *n*-alkanes (from C_8 to C_{20}), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylcromano-2-carboxylic acid (Trolox), and Na_2SO_4 were from Fluka and Sigma-Aldrich (Milan, Italy); α-thujene, sabinene, santolina alcohol, limonene, linalool, artemisia ketone, chamazulene, and *cis*-jasmone were obtained from Extrasynthése (Genay, France). All compounds were analytical grade standards. Dichloromethane, *n*-hexane, and ethyl acetate were analytical grade solvents (Merck, Milan, Italy).

Essential Oil Distillation. An aliquot of 250 g of samples (flowering tops with 10−15 cm of stalk and a few small leaves) was hydrodistilled in triplicate with a Clevenger-type apparatus according to the Italian Official Pharmacopoeia (*8*) for 2 h. On four samples of *A. ligustica* also simultaneous micro steam distillation−extraction with solvents lighter (*n*-hexane, L-SDE) and heavier (dichloromethane, H-SDE) than water have been performed with specific apparatus (Chrompack-Varian, Milan, Italy, cod. 16050 and 16051, respectively) for 2 h. The essential oils were dehydrated with anhydrous sodium sulfate and stored in dark vials at 4 C°.

Gas Chromatography−Mass Spectrometry. A Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a MS detector HP 5971 A, an HP 7673 autosampler, a split−splitless injector, and a MS ChemStation HP v. A.03.00 was used. The column was a fused silica capillary DB-5MS (5% phenylmethylpolysiloxane, 30 m × 0.25 mm; film thickness = 0.25 μm; J&W Scientific-Agilent, Folsom, CA). The injector and interface were operated at 100 and 280 °C, respectively. The oven temperature was programmed as follows: 60 °C for 5 min, raised to 140 °C (2 °C/min), raised to 250 °C (5 °C/min), and isothermally held for 5 min for a total run of 62 min. Helium was the carrier gas at 0.9 mL/min; the sample (1 μL) was injected in the split mode (1:20). MS conditions were as follows: ionization voltage, 70 eV; scan rate, 1.6 scan/s; mass range, 50−550; ion source temperature, 180 °C.

The oil components were identified by comparison of their relative retention times with those of authentic samples or by comparison of their retention index (RI) relative to a series of *n*-hydrocarbons. Computer matching against commercial (Adam, Nist 98, MassFinder 2.1) (*9*−*11*) and homemade library mass spectra made up of pure substances and components of known oils, as well as MS literature data, was also used for the identification. The KI values calculated were in agreement with those reported by Adams (*9*).

Quantitative Analysis. The essential oils obtained by hydrodistillation with a Clevenger-type apparatus were diluted (1%, v/v) in *n*-hexane before GC-MS analysis, whereas those obtained by L-SDE and H-SDE were injected without any dilution.

Quantitative analysis of each essential oil component (expressed in percentages) was carried out by peak area normalization measurement.

Antioxidant Activity. A spectrophotometric analysis that used DPPH and comparison with the Trolox calibration curve have been performed. Ten microliters of essential oil was dissolved in 3 mL of ethyl acetate. A calibration curve in the range 0.2/0.4/1.0/2.0/4.0 mmol/L has been prepared for the Trolox, and data have been expressed in Trolox equivalent antioxidant capacity (TEAC, mmol/L). The spectrophotometric readings have been carried out with a Cary 50 spectrophotometer (Varian, Milan, Italy) at 517 nm using a 10 mm quartz cuvette.

Antimicrobial and Antifungal Activities. The antimicrobial activity of *A. ligustica* essential oil was tested against a panel of microorganisms including four ATCC strains: *S. aureus* (6538), *E. coli* (8739), *P. aeruginosa* (9027), and *C. albicans* (14053). The microbiological assays were carried out using the agar disk diffusion method. The paper disks (6 mm in diameter) were impregnated with 20 μL of the essential oil and placed on the inoculated plates with a suspension of the tested microorganism [0.1 mL of 10^8 colony-forming units (cfu)/mL]. The plates were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeast. The diameters of the inhibition zones were measured in millimeters (*12*). All of the assays were performed in duplicate. For each of the above-mentioned strains, the minimum inhibitory concentration (MIC) was determined using a broth microdilution method. Stock standard solutions at 1% in dimethyl sulfoxide (DMSO) were prepared for each oil. Geometric solutions of the oil, ranging from 3.5 to 900 μL/mL, were prepared by dilution in a 96-well microtiter plate. The bacterial suspensions in Mueller Hinton broth with the exception of the yeast (Sabouraud dextrose broth) were added in the microwells at the concentration of 5.0×10^5 cfu/mL. The concentration for each inoculum was verified in plates containing agar plate count agar (PCA). The plates were incubated aerobically at 37 °C for 24 h (except for the yeast, *C. albicans*, which was incubated at 30 °C for 48 h). The antifungal activity was investigated against *F. oxysporum*, *R. solani*, *P. commune*, and *A. flavus*, isolated and identified from feeds in the Laboratory of Hygiene of Cagliari University. The tests were carried out by insemination, with mycelia fragments of 6 mm in diameter (10 day hold), in Petri dishes containing potato dextrose agar (PDA). After the addition of the essential oil (900 μL/mL), the plates were sealed with Parafilm M to avoid the dispersion of the oil and incubated in the dark at 22 °C. Control samples with the mycelia in PDA and distilled water were subjected to the same treatments. The effectiveness of the treatments was evaluated by measuring the average diametric growth of the colonies at 4, 8, and 12 days after the inoculation. The percentage of inhibition was calculated according to the equation of Zygadlo and Guzman (*13*)

$$I = 100(C - T)C^{-1}$$

where I = inhibition, C = average diameter of fungi grown in PDA + water, and T= average diameter of fungi cultivated in PDA + essential oil. All of the test were performed three times.

Table 2. Percentage Composition (Relative Area) of the Essential Oils of *A. ligustica*

			sample											
no.	KI[§]	compound	1	2	3	4	5	6	7	8	mean	±SD	min	max
1	781	2-methylbutan-1-ol[b]	0.1	0.1	t	0.1	0.1	0.1	0.1	0.1	0.1	0.0	t	0.1
2	796	1-octene[b]	0.1	0.1	0.1	0.1	t	0.1	0.1	0.1	0.1	0.0	t	0.1
3	855	(*E*)-3-hexenol[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
4	894	1-nonene[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1
5	905	santolina triene[b]	2.0	0.9	1.1	0.8	0.8	1.7	1.1	1.7	1.3	0.5	0.8	2.0
6	927	tricyclene[b]	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2
7	929	α-thujene	0.1	0.1	t	0.1	0.1	0.1	0.1	0.1	0.1	0.0	t	0.1
8	937	α-pinene	1.4	1.2	1.7	0.8	1.1	0.6	1.0	0.9	1.1	0.3	0.6	1.7
9	953	camphene	3.1	1.5	3.6	1.4	1.6	0.7	0.9	0.8	1.7	1.1	0.7	3.6
10	964	benzaldehyde[b]	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.2
11	975	sabinene	2.4	5.2	1.0	2.3	2.8	2.2	2.3	3.2	2.7	1.2	1.0	5.2
12	979	β-pinene	0.9	3.0	3.4	0.9	0.7	0.3	3.4	0.8	1.7	1.3	0.3	3.4
13	990	myrcene	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2
14	996	yomogi alcohol[b]	0.2	0.4	0.2	0.3	0.2	0.5	0.2	0.4	0.3	0.1	0.2	0.5
15	1004	α-phellandrene	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.2
16	1017	α-terpinene	0.3	1.1	0.3	0.6	0.6	0.3	0.5	0.5	0.5	0.3	0.3	1.1
17	1026	*p*-cymene	1.0	0.9	1.0	0.7	0.8	0.7	1.1	0.7	0.9	0.2	0.7	1.1
18	1029	limonene	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.3
19	1033	1,8-cineole	2.9	1.0	2.1	1.7	1.7	5.4	4.6	3.4	2.9	1.5	1.0	5.4
20	1034	santolina alcohol	21.1	10.2	11.7	6.7	9.7	21.8	14.4	13.7	13.7	5.4	6.7	21.8
21	1058	γ-terpinene	0.7	2.1	0.6	0.6	0.7	0.9	1.1	1.0	1.0	0.5	0.6	2.1
22	1059	artemisia ketone	2.0	7.6	2.2	5.3	1.6	6.8	0.3	2.7	3.6	2.7	0.3	7.6
23	1068	3-hexenyl butanoate[b]	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.2
24	1070	*cis*-sabinene hydrate	0.3	0.6	0.2	0.4	0.2	0.2	0.3	0.5	0.3	0.2	0.2	0.6
25	1079	artemisia alcohol[b]	0.5	0.6	0.3	0.3	0.2	0.8	0.2	0.5	0.4	0.2	0.2	0.8
26	1084	terpinolene	0.1	0.4	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.4
27	1090	isobutyl tiglate[b]	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.1	0.2
28	1097	2-methylbicyclo[2.2.1]hept-2-ene[b]	0.9	1.1	0.6	0.9	1.5	0.2	0.3	0.2	0.7	0.5	0.2	1.5
29	1102	linalool	3.8	10.4	2.9	3.0	3.0	2.1	5.2	4.7	4.4	2.6	2.1	10.4
30	1106	α-thujone	1.6	1.1	6.3	11.3	25.8	0.4	0.8	0.5	6.0	8.9	0.4	25.8
31	1107	pentyl isovalerate[b]	0.3	0.2	0.3	0.2	0.1	0.3	0.3	0.2	0.2	0.1	0.1	0.3
32	1113	unknown	0.3	0.2	0.3	0.3	0.6	0.4	0.1	0.2	0.3	0.2	0.1	0.6
33	1117	β-thujone	0.4	0.5	1.2	2.5	4.3	2.1	1.2	1.4	1.7	1.3	0.4	4.3
34	1121	chrysanthenone	5.8	3.9	4.7	6.5	12.8	5.3	1.5	2.5	5.4	3.4	1.5	12.8
35	1122	*cis*-*p*-menth-2-en-1-ol[b]	0.1	0.3	t	0.6	t	0.1	1.0	0.7	0.5	0.4	t	1.0
36	1139	sabinol[b]	3.8	5.9	4.2	11.6	2.1	9.7	11.3	15.5	8.0	4.7	2.1	15.5
37	1142	*trans*-*p*-menth-2-en-1-ol[b]	0.1	0.2	t	0.5			0.6	0.4	0.4	0.2		0.6
38	1144	camphor	5.4	3.4	5.8	3.2	2.7	0.7	1.6	2.6	3.2	1.7	0.7	5.8
39	1155	sabina ketone[b]	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.0	0.1	0.2
40	1162	*trans*-chrysanthenol[b]	t	0.9	t	1.1	0.1	-	0.6	0.5	0.5	0.4	-	1.1
41	1163	pinocarvone[b]	0.1	0.2	0.2	t	0.1	0.6	0.1	0.1	0.2	0.2	t	0.6
42	1164	*cis*-chrysanthenol[b]	0.1	0.2	1.1	0.2	2.6	0.3	0.3	0.1	0.6	0.9	0.1	2.6
43	1168	borneol	15.0	6.5	20.8	6.2	8.5	3.4	4.4	3.9	8.6	6.2	3.4	20.8
44	1177	terpinen-4-ol	2.1	6.1	1.8	3.3	1.9	1.8	3.0	2.4	2.8	1.4	1.8	6.1
45	1184	unknown	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.2
46	1187	myrtenol[b]	0.1	0.1	t	0.1	0.1	0.1	0.1	0.1	0.1	0.0	t	0.1
47+48	1190	myrtenal + α-terpineol	0.9	1.4	1.1	1.2	0.9	1.4	2.1	1.0	1.3	0.4	0.9	2.1
49	1203	*trans*-piperitol[b]	0.2	0.2	0.1	0.3	0.1	0.1	0.5	0.3	0.2	0.1	0.1	0.5
50	1207	*trans*-carveol[b]	0.1	0.1	0.2	0.1	0.5	0.1	0.1	0.1	0.2	0.1	0.1	0.5
51	1228	*trans*-chrysanthenyl acetate[b]	0.3	0.2	0.3	0.4	0.3	0.1	0.3	0.4	0.3	0.1	0.1	0.4
52	1231	unknown	t	0.2	0.1	0.1	0.1	0.1	t	t	0.1	0.0	t	0.2
53	1250	piperitone[b]	0.1	0.1	0.3	1.4	0.2	0.1	1.5	1.1	0.6	0.6	0.1	1.5
54	1253	*cis*-chrysanthenyl acetate[b]	0.5	0.1	0.7	0.8	0.2	3.0	0.1	0.5	0.7	1.0	0.1	3.0
55	1279	bornyl acetate	5.2	2.4	2.1	2.9	0.3	2.0	1.5	1.2	2.2	1.4	0.3	5.2
56	1282	lavandulyl acetate[b]						0.2			0.2			0.2
57	1283	*trans*-sabinyl acetate[b]	2.7	5.4	2.6	6.8	0.9	8.8	14.8	17.6	7.5	6.0	0.9	17.6
58	1284	thymol	1.9	2.3	1.7	4.5	0.6	3.8	3.9	4.4	2.9	1.4	0.6	4.5
59	1298	unknown	0.1	0.1	0.2	t	0.1	0.3	0.1	t	0.2	0.1	t	0.3
60	1348	eugenol	0.3	0.2	0.3	0.2	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.3
61	1391	*cis*-jasmone	0.2	0.1	0.1	0.1	0.4	0.1	0.1	0.1	0.2	0.1	0.1	0.4
62	1417	β-caryophyllene	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.2
63	1453	*allo*-aromandendrene	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2
64	1462	unknown	0.2	0.1	0.3	0.2	t	0.2	0.1	0.1	0.2	0.1	t	0.3
65	1468	β-chamigrene[b]	0.1	t	t	t	0.1	t	0.1	t	0.1	0.0	t	0.1
66	1473	unknown	t	t	0.2		t	t	0.1	0.1	0.1	0.1		0.2
67	1474	γ-muurolene[b]	0.7	1.2	0.6	0.2	0.7	0.4	0.9	0.6	0.7	0.3	0.2	1.2
68	1475	*ar*-curcumene[b]	0.1	0.1	0.1	t	0.1	t	0.1	0.1	0.1	0.0	t	0.1
69	1477	germacrene D[b]	0.1	t	0.1	t	0.1	t	0.1	t	0.1	0.0	t	0.1
70	1488	bicyclogermacrene[b]	0.1	0.1	t	t	t	0.1	0.1	t	0.1	0.0	t	0.1
71	1510	δ-amorphene[b]	0.1	0.3	0.1	t	0.2	0.1	0.2	0.2	0.2	0.1	t	0.3
72+73	1542	α-calacorene[b] + *cis*-dracunculifolol[b]	0.1	0.1	0.2		0.1	0.1	0.1		0.1	0.0		0.2
74	1552	α-agarofuran[b]	t	0.1	0.1			t	0.1	0.1	0.1	0.0		0.1
75	1573	*trans*-nerolidol	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.2
76	1580	ledol[b]	0.1	t	0.1	0.1	t	0.1	0.1	t	0.1	0.0	t	0.1

Table 2. (Continued)

			sample											
no.	KI[§]	compound	1	2	3	4	5	6	7	8	mean	±SD	min	max
77+**78**	1594	*ar*-tumerol[b] + caryophyllene oxide	0.2	0.1	0.2	0.1	t	0.2	0.4	0.1	0.2	0.1	t	0.4
79	1603	unknown	0.2	0.2	0.4		0.1		0.1	0.1	0.2		0.1	0.4
80	1612	viridiflorol[b]	2.1	1.3	2.5	2.6	1.1	3.6	2.7	0.7	2.1	1.0	0.7	3.6
81	1614	guaiol[b]	0.2	0.2	0.4	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.4
82	1621	unknown	0.3	0.2	0.5	0.3	0.1	0.4	0.4	0.1	0.3	0.1	0.1	0.5
83	1631	unknown	0.1	t	0.3	0.2	0.1	0.2		0.1	0.2	0.1		0.3
84	1635	zingiberenol[b]	0.2	0.3	0.3	0.1	t	0.1	0.3	0.1	0.2	0.1	t	0.3
85	1647	1-epicubenol[b]	0.1	0.7	t	0.1	0.7	0.4	0.9	0.4	0.5	0.3	t	0.9
86	1652	γ-eudesmol	0.4	0.3	t	0.1	0.2	t	0.3	0.4	0.3	0.1	t	0.4
87	1656	caryophilla-4(14),8(15)-dien-5-β-ol[b]	0.2	0.1	0.2	0.2	t	0.1	0.2		0.2	0.1		0.2
88	1663	7-epi-α-eudesmol[b]	0.1		0.1	t	0.1		0.2	0.1	0.1	0.0		0.2
89	1674	β-eudesmol[b]	0.1	1.3	0.7	0.1	t	0.1	0.1	0.1	0.4	0.5	t	1.3
90	1675	himachalol[b]	0.1	t		t			0.1	0.2	0.1	0.1		0.2
91	1684	unknown		0.1	t	t	0.3	0.1			0.2	0.1		0.3
92	1691	unknown	0.1	0.1	0.2			t		0.1	0.1	0.1		0.2
93	1704	α-bisabolol	0.5	0.6	0.7	0.2	0.1	0.4	0.6	0.3	0.4	0.2	0.1	0.7
94	1741	chamazulene	0.1	t	0.1	t	0.1	0.1	t	t	0.1	0.0	t	0.1
95	1750	mint sulfide[b]				0.2	0.1	t	0.1	t	0.1	0.1		0.2
96	1755	6*S*,7*R*-bisabolone[b]	0.1	t	t	t	t	t	t	0.1	0.1	0.0	t	0.1
total			100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0				

[a] Relative to C_8–C_{20} *n*-alkanes on the DB5-MS column. [b] Identification by mass spectrum + retention index (KI). [t] Trace (<0.05%).

Statistical Analysis. Analysis of variance (ANOVA) was carried out, and the average values have been compared with the Duncan test at $P \leq 0.05$ using GenStat v 7.1 software (VSN International Ltd., Herts, U.K.).

RESULTS AND DISCUSSION

Essential Oil Composition. Table 2 shows the percentage composition of the eight samples obtained by hydrodistillation with a Clevenger-type apparatus. Ninety-six components were detected, and 36 of them were identified by comparison with pure standards (KI and mass spectra matching); 49 compounds were identified by comparing both their KI and mass spectra with literature data and commercial mass spectra library. For the other 11 compounds (0.5−3.8% of the total oil components) not enough information was found, and no possible attribution was possible (unknown compounds).

The eight essential oil samples of *A. ligustica* have shown a homogeneous qualitative composition, whereas strong percentage variability depending on the origin of the samples was observed.

Among the monoterpenes, the most representative compound is the santolina alcohol with percentages ranging from 6.7 to 21.8%. This compound is an irregular terpene, a category of typical structures of the Asteraceae that include also the santolina triene (0.8−2.0%) and the artemisia ketone (0.3−7.6%). This is the first time that santolina alcohol has been found in *A. ligustica* essential oils. Borneol, another monoterpenic alcohol, is present in high amount ranging from 3.4 to 20.8%. A group of correlated structure compounds is sabinol (2.1−15.5%) and its acetic ester (sabinyl acetate, 0.9−17.6%), sabinene (1.0−5.2%), sabinene hydrate (0.2−0.6%), and sabina ketone (maximum 0.2%). Among the ketones the high amount of thujone should be noted, with the prevalence of α-isomer (0.4−25.8%) followed by the β-isomer (0.4−4.3%). Moreover, chrysanthenone (1.5−12.8%), camphor (0.7−5.8%), and piperitone (0.1−1.5%) were found. A ketone of particular nature is *cis*-jasmone ($C_{11}H_{16}O$), present at up to 0.4%. It is important to note that the epoxide 1,8-cineole can be detected up to 5.4%, but with an average value of 2.9 ± 1.5%. Also, aldehydes and esters are present. The hydrocarbons are present in rather low amounts: camphene (0.7−3.6%), β-pinene (0.3−3.4%), γ-terpinene (0.6−2.1%), α-pinene (0.6−1.7%), α-terpinene (maximum 1.1%), and all the others below 0.3%. The only aromatic hydrocarbon is the *p*-cymene (0.7−1.1%).

Among the sesquiterpenes, the most represented are the alcohols such as viridiflorol (0.7−3.6%) followed by β-eudesmol (0.1−1.3%), α-bisabolol (maximum 0.7%), guaiacol (maximum 0.4%), and *trans*-nerolidol and ledol (maximum 0.2 and 0.1%, respectively). Between oxides is the caryophyllene oxide (maximum 0.4%), although it coeluted with turmerol (*ar*) . The monocyclic hydrocarbons are represented by *ar*-curcumene (maximum 0.1%), the bicyclic by γ-muurolene (maximum 1.2%), δ-amorphene (maximum 0.3%), and β-caryophyllene (maximum 0.2%), and the tricyclic by *allo*-aromadendrene (maximum 0.2%). The macrocyclic hydrocarbons are represented by germacrene D (maximum 0.1%) and bicyclogermacrene (maximum 0.1%). Chamazulene has been found in all samples with a maximum value of 0.1%, but all of the obtained oils showed a blue coloration, due therefore to other compounds of azulenic nature.

Comparison with literature data on Ligurian yarrow is not easy: a Greek oil from flowers contained 70.8% of linalool, whereas that from leaves contained 28.2% (*5*); an oil from a northern Italy blooming plant was characterized by artemisia ketone 43.9% (*6*), whereas the main compounds of a Sicilian oil obtained from leaves (*7*) were terpinen-4-ol (19.3%), carvone (8.9%), and γ-terpinene (7.2%). Strong variability from qualitative and quantitative points of view is present, and this can be due to the different distilled parts of the plant or the geographical origin of the samples.

Table 3 shows the comparison between the essential oils of four samples of Ligurian yarrow obtained by hydrodistillation (HD) with the Clevenger-type apparatus and by simultaneous distillation−extraction (SDE) in the presence of organic solvents. This experimentation has been carried out to verify if SDE can have some practical advantage with respect to hydrodistillation. The SDE technique, in fact, according to the essential oil amount in the plant, needs only a few grams of plant for the extraction. On the other hand, the presence of organic solvents can extract preferentially some classes of compounds. The detected com-

Table 3. Comparison between Essential Oils (Area Percent[a]) Obtained by HD and SDE

		sample											
compound	class	3	3 H-SDE	3 L-SDE	5	5 H-SDE	5 L-SDE	7	7 H-SDE	7 L-SDE	8	8 H-SDE	8 L-SDE
monoterpenes	hydrocarbons	14.1a	6.8b	7.5b	10.2c	7.7b	8.1b	12.8a	8.9cb	6.2b	10.9c	8.5b	5.6b
	alcohols	45.3a	46.4a	45.8a	38.9b	33.5c	34.7cb	47.2a	36.6bc	42.0b	48.3a	45.3a	42.5ab
	aldehydes	t	t	t	t	t	t	t	t	t	t	t	t
	ketones	20.8a	14.3b	13.7b	30.3c	36.1d	35.8d	7.1e	7.3e	6.8e	11.0f	17.9g	16.4gb
	esters	5.7a	15.4b	15.0b	10.9c	6.9d	6.3ad	16.7eb	17.2eb	20.4e	19.7e	19.9e	18.7e
	oxides	2.1a	2.4a	3.7b	1.7c	1.7c	1.9ac	4.6d	1.3e	1.2e	3.4a	3.1b	1.7c
	total	88.0a	85.2a	85.7a	92.0ac	85.9a	86.8a	88.4a	71.3b	76.6b	93.3ca.	94.7c	84.9a
sesquiterpenes	hydrocarbons	1.4a	0.3b	0.3b	0.4b	1.2a	1.1ae	2.0c	0.7d	1.0e	1.1a	1.1ae	3.4f
	alcohols	5.2a	12.0b	12.3b	3.8c	7.9d	7.4d	5.9a	21.9e	19.9e	2.5f	3.3c	9.7b
	ketones	t	t	t	t	t	t	t	3.0a	0.2b	0.1b	t	t
	oxides	0.3a	0.3a	0.3a	0.1b	0.3a	0.3a	0.5c	1.3d	0.6c	0.2ab	t	0,2ab
	azulen	0.1a	0.1a	0.1a	t	0.2b	0.1a	t	t	t	t	t	t
	total	7.0a	12.7b	13.0b	4.5c	9.5d	8.9d	8.5de	26.9e	21.8f	3.9c	4.4c	13,3b
others	hydrocarbons	0.4a	0.1b	t	0.3a	t	t	0.3a	t	t	0.4a	t	t
	aldehydes	0.1a	t	t	0.1a	0.1a	0.1a	0.1a	t	t	0.1a	t	t
	alcohols	0.1a	0.2b	0.2b	0.2b	0.1a	0.1a	0.2b	0.1a	0.1a	0.2b	0.1	t
	esters	0.6a	0.4b	0.4b	0.4b	0.4b	0.4b	0.5ab	0.3b	0.2c	0.4b	0.2c	0.2c
	total	1.2a	0.7b	0.6b	0.9c	0.6b	0.6b	1.1a	0.4d	0.4d	1.1a	0.4d	0.3d
unknowns	total	3.8a	1.4b	0.7c	2.6d	3.9a	3.7a	2.0e	1.4b	1.3b	1.7be	0.5a	1.5b
total		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a] Values within a line for each compound having different letters are significantly different from each other at $p < 0.05$. t = trace (<0.05%).

pounds are the same 96 listed in **Table 2**, but it has been preferred to group them in homogeneous classes to make easier the comparison. It has been observed that the nonterpenic compounds, especially hydrocarbons, strongly diminish. With regard to terpenic compounds, no statistical differences can be noted except for the hydrocarbons, where the reduction can be >50%. Sesquiterpenes show a general increase, but the different classes have not the same behavior. For instance, the total amount of hydrocarbons sometimes increases and sometimes decreases, but δ-amorphene always increases, sometimes even by >4 times. Alcohols show a clear increasing trend, and among the components *trans*-nerolidol and viridiflorol increase in some cases by a factor of 10.

No significant difference between the microdistillation with heavier solvents and the one with lighter solvents has been observed.

DPPH Radical Scavenging Activity of Essential Oils. As far as the antioxidant activity is concerned, the various essential oil samples of Ligurian yarrow have shown values of TEAC ranging between 0.40 and 0.88 mmol/L, with samples 1 and 2 having the higher values (0.79 and 0.88 mmol/L, respectively). **Table 4** shows some TEAC values of essential oil obtained from other typical species of the Sardinian flora. Such samples were collected in the period 2004−2005 and were freshly hydrodistilled soon after harvesting. It can be noted that essential oils from *A. ligustica* show average values that give an idea of the interesting antioxidant activity of such oils and that can be taken advantage of to realize nutraceutical products or additives for the food industry (*14*).

Antimicrobial and Antifungal Activities of Essential Oils. Results obtained from the disk diffusion method indicate that *C. albicans* is the most sensitive microorganism tested in the presence of the oil extracted from *A. ligustica*, although the results of the antimicrobial activity assays indicated that the essential oil of *A. ligustica* showed low inhibitory activities against *S. aureus* (6538), *E. coli* (8739), *P. aeruginosa* (9027), and *C. albicans* with MIC values always >900 μg/L (**Table 5**). The results of antifungal activity assay showed that the oil of *A. ligustica* was inactive against the tested agricultural pathogenic fungi (*F. oxysporum*, *R. solani*, *P. commune*, and *A. flavus*).

Table 4. Antioxidant Activity (TEAC)

		TEAC[a] (mmol/L)			
essential oil	no. of samples	mean	±SD	min	max
Rosmarinus officinalis L.	12	0.17	0.03	0.13	0.24
Eucaliptus globulus Labill. and *E. camaldulensis* Dehn.	10	0.18	0.02	0.16	0.20
Achillea ligustica All.	8	0.69	0.13	0.40	0.88
Lavandula stoechas L.	8	1.15	0.06	1.06	1.24
Myrtus communis L.	14	1.62	0.25	1.22	1.92
Thymus capitatus (L.) Hoff. & Link.	8	11.38	0.47	10.71	12.03

[a] TEAC is the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to that of the dilution of the essential oils.

Table 5. Antimicrobial Activity of the Essential Oil of *A. ligustica* Using Agar Disk Diffusion Method

microorganism	DD[a] (mm)	MIC[b] (μg/mL)
Staphylococcus aureus	10	>900
Escherichia coli	12	>900
Pseudomonas aeruginosa	0	>900
Candida albicans	20	>900

[a] Diameter of zone of inhibition. [b] Minimal inhibitory concentration by broth microdilution method.

In conclusion, the low tenor of monoterpenic hydrocarbons in *A. ligustica* is an interesting aspect, taking into account the generally insufficient antibacterial and antioxidant activities of such compounds. The most representative compound of *A. ligustica*, the santolina alcohol, has never been detected in *A. millefolium* L. It is important to observe from the toxicological

point of view that samples 3−5 show high tenors of thujone, in particular the α-isomer. This compound can cause epileptic convulsions with generalized vasodilatation, hypotension, and bradycardia (*15*−*17*). In the food industry, as an example, thujone amount in foodstuffs and beverages has been fixed to a maximum of 0.5 mg/kg, with some exceptions for alcoholic drinks (Council Directive 88/388/EEC, 15/7/88). Therefore, in the evaluation of these problems, it would be opportune to exclude from the alimentary and therapeutic use those samples with elevated tenors of α- +*β*-thujone. Comparison with the literature data on *A. millefolium* L. (*18*−*23*) has proved that the greatest part of the compounds are the same; moreover, the ligustica yarrow being a proazulenic species, it is expected to possess a similar therapeutic activity.

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Received for review August 3, 2005. Revised manuscript received October 25, 2005. Accepted October 30, 2005.

JF0518913