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Chemical Composition of the Essential Oils of *Juniperus* from Ripe and Unripe Berries and Leaves and Their Antimicrobial Activity

ALBERTO ANGIONI,*† ANDREA BARRA,‡† MARIA T. RUSSO,§ VALENTINA CORONEO,‡ SANDRO DESSÍ,‡ AND PAOLO CABRAS†

Dipartimento di Tossicologia, Università di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy, Dipartimento di Sanità Pubblica, Laboratorio di Igiene degli Alimenti, Università di Cagliari, Via Porcell 4, 09124 Cagliari, Italy, and Dipartimento di Scienze e Tecnologie Agro-Forestali e Ambientali, Piazza S. Francesco di Sales 7, 89061, Gallina (RC), Italy

The composition of the essential oil from ripe and unripe berries and leaves of *Juniperus oxycedrus L.* ssp. *oxycedrus*, *Juniperus phoenicea* ssp*. turbinata* and *Juniperus communis* ssp*. communis* was analyzed by GC-MS, and microbiological assays were carried out. Samples were collected in different localities (Sardinia, Italy) and hydro distilled. The yields ranged between 2.54% ± 0.21 (v\w dried weight) and 0.04% ± 0.00. A total of 36 components were identified. The major compounds in the essential oils were α-pinene, *β*-pinene, *δ*-3-carene, sabinene, myrcene, *β*-phellandrene, limonene, and D-germacrene. Both qualitative and quantitative differences between species and between different parts of the plant were observed. The essential oils and their major compounds were tested against *Candida albicans, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa,* and the minimum inhibitory concentration and minimum bactericidal concentration were determined. The results obtained led to a nonsignificant inhibitory effect, although all the essential oils from *Juniperus phoenicea* ssp*. turbinata* and the essential oil from leaves of *Juniperus oxycedrus* ssp*. oxycedrus* exhibited rather good or weak activity against *Candida albicans* and *Staphylococcus aureus.*

KEYWORDS: ***Juniperus* species; essential oil composition; *Candida albicans*; *Staphylococcus aureus***

INTRODUCTION

The genus *Juniperus*, which belongs to the Cucupressaceae family, includes many native plants of the Mediterranean regions. In Sardinia (Italy) the most widespread species are *J. oxycedrus L.* and J. *phoenicea* ssp. *turbinata*; only few specimens of *J. communis* are found on the mountains in the center of the island.

Only the berries from *Juniperus communis* are registered on the Italian Official Pharmacopoeia and used as a diuretic and balsamic (*1*). The essential oil, infusions, decoctions, and alcoholic extracts are used in different fields (pharmaceuticals, alcoholics, etc.) (*2*−*4*).

In the literature, many papers report on the composition of the essential oil from berries and leaves of the *Juniperus* species (*5*−*18*), but few studies have investigated their antimicrobial activity.

The essential oils from leaves and berries of *J. oxycedrus* ssp. *oxycedrus* from Greece exhibited activity against several microorganisms (*19*). Bonsignore et al. (*20*) reported that the essential oil from the heartwood of Sardinian *J. oxycedrus* showed activity against gram-positive bacteria and most of the screened blastomycetes. Digrak et al. (*21*) reported the activity of different solvent extracts of leaves bark and fruits of J. *oxycedrus*. No data were found on the biological properties and chemical composition of the essential oil of ripe berries of *J. phoenicea* ssp. *turbinata*.

The aim of this paper was: (a) to investigate the chemical composition of the essential oils from ripe and unripe berries and leaves of the *Juniperus* species from Sardinia and compare it with that of the essential oils from the *Juniperus* species from other Mediterranean countries; and (b) to assess the antimicrobial activity of the obtained essential oils and of their major compounds against *Candida albicans, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.*

EXPERIMENTAL PROCEDURES

Plant Material. Wild samples were collected in Sardinia between October and December in 2000 and 2001. *Juniperus phoenicea ssp. turbinata* and *Juniperus oxycedrus ssp. oxycedrus* were harvested in coastal areas in southeastern and southwestern Sardinia (Villasimius, Capoterra, Cala Mosca), and in the mountain areas of the Southwest (Monte Arcosu, Capoterra, S. Priamo), while *Juniperus communis* ssp. communis was harvested only in mountain areas (Laconi). The harvest

* To whom correspondence should be addressed. E-mail: aangioni@unica.it).
† Università di Cagliari.
‡ Laboratorio di Igiene degli Alimenti.
§ Piazza S. Francesco di Sales.

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Table 1. Humidity (% w/w) and Yields (% v/w) from Different Parts and Species of *Juniperus*

	J. *phoenicea ssp. turbinata*		J. *oxycedrus*		J. *communis*	
	yield[a]	% humidity	yield[a]	% humidity	yield[a]	% humidity
leaves	0.22 ± 0.08	42	0.04 ± 0.00	45	0.19 ± 0.04	47
ripe berries	2.54 ± 0.21	12	0.49 ± 0.13	21	0.32 ± 0.09	29
unripe berries	1.69 ± 0.44	25	1.07 ± 0.06	22	0.08 ± 0.01	49

[a] Expressed on 100 g dried weight.

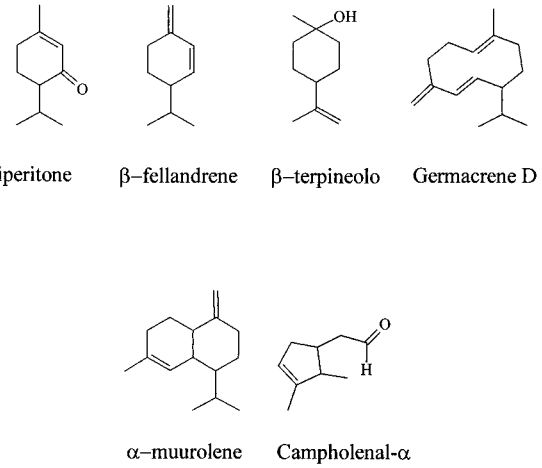

Figure 1. Chemical Structure of Compounds Not Available in the Market.

involved a random sampling from 10 trees; in each area, three different samples were collected for each species.

Prof. Mauro Ballero, of the Department of Botany, University of Cagliari, identified the species.

Essential Oil Distillation. Berries and leaves were selected and cleaned from impurity in the laboratory. The berries were then individually crushed. An aliquot of 100 g of samples (ripe berries, unripe berries, and leaves) were steam distilled in triplicate with a Clevenger-type apparatus according to the Italian Official Pharmacopoeia X (1). The essential oils were stored with anhydrous sodium sulfate in dark vials at 4 C°. The essential oils were dissolved (1%, v/v) in *n*-hexane before GC\MS analysis.

GC/MS Analysis. A Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a MS Detector HP 5971 A, an HP 7673 auto sampler, a split-splitless injector, and a MS ChemStation HP v. C.00.07, were used. The column was a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m × 0.25 mm; film thickness 0.25 μm; J&W Scientific Fisons, Folsom, CA). The injector and interface were operated at 200 and 280 °C, respectively. The oven temperature was programmed as follows: from 60 to 180 °C (3 °C/min), and isothermally held for 15 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 of 70 eV, scan rate 1.6 scan/sec, mass range 50−500, 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 the series of n-hydrocarbons, and computer matching against commercial (Adams, Nist 98)(*22*, *23*) and homemade library mass spectra made up of pure substances and components of known oils and MS literature data. The KI calculated were in agreement with that reported by Adams (*22*). A quantitative analysis of each oil component (expressed in percentages) was carried out by peak area normalization measurement. **Figure 1** reports the formula of some of the compound not available from the marketing.

Chemicals. α-Pinene, (−) camphene, α−copaene, *β*-pinene, myrcene, citronellol, myrtenyl-acetate, α-phellanderene, α-terpinene, α-terpinolene, *β*-thujone, trans-pinocarveol, terpinen-4-ol, α-terpineol, linalyl-acetate, α-cubebene, bornyl-acetate, *β*-caryophyllene, and caryophyllene-oxide were obtained from Aldrich, Acros, Fluka, (Milan, Italy); α-thujene, sabinene, *δ*-3-carene, limonene, linalool, α-terpinyl-acetate, and α-humulene were obtained from Extrasynthese (Genay, France); and camphor was from Carlo Erba, (Milan, Italy). All compounds were analytical standard grade. N-hexane was an analytical grade solvent, and Na_2SO_4 was analytical reagent grade Carlo Erba; (Milan, Italy).

Antibacterial Assays. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the nine oils and their five main components were determined using a broth microdilution method (*24*). All tests were performed in NB (neutral broth) for bacteria. All media were supplemented with DMSO (dimethyl sulfoxide) at a final concentration of 0.5%. Serial doubling dilutions of each oil or component were performed in a 96-well microtiter plate (Nunc, Copenhagen, Denmark) over the range of 3.5−900 μg/mL.

Overnight, broth cultures were prepared in NB and adjusted so that the final concentration in each well following inoculation was approximately 5.0×10^5 cfu (colony forming units)/mL. The concentration of each inoculum was confirmed using viable counts on TSA (tryptic soy agar) plates for bacteria. Positive and negative growth controls were included in every test.

The plates were incubated aerobically at 37 °C (except for *Candida albicans* 30 °C) for 24h and the MICs and MBCs determined. Bacterial growth was indicated by the presence of turbidity and a "pellet" on the well bottom. MICs were determined presumptively as the first well in ascending order that did not produce a pellet. To confirm MICs and establish MBCs, 25 μL of broth was removed from each well and inoculated on the TSA plates. After aerobic incubation at 37 °C overnight, the number of surviving organisms was determined. Each experiment was repeated at least three times for each oil or compound for each test concentration, and the modal MIC and MBC values were selected.

Staphylococcus aureus ATCC number 6538, *Escherichia coli* ATCC number 8739, *Pseudomonas aeruginosas* ATCC number 9027, and *Candida albicans* ATCC number 14053 were used for all experiments.

Antimicrobial Assay. *Paper Disk Assay.* The effectiveness of the essential oils of ripe berries of Juniperus turbinata and oxycedrus on eleven plant fungi, was evaluated. The fungi were *Botrytis cinerea, Cercospora beticola, Fusarium oxysporium lycopersici, Fusarium graminearum, Helminthosporium oryzae, Pythium ultimum, Pyricularia orizae, Rhizoctonia solani, Sclerotium rolfsii, Phytophthora capsici e Septoria tritici.*

Paper discs (12.7 mm diameter) were soaked in a solution of essential oil/acetone (1/1), and stored for several days at 24 °C in Petri dishes (30 mm diameter) containing potato dextrose agar, and fungal mycelia. All the experiments were performed in triplicate. Inhibition of fungal growth (dependent on the rate of fungi growth) for each test fungus was measured after treatment. Sensitivity of the fungal species to the oils was determined comparing the sizes of inhibitory zones (*24*). The mycelia growth was classified with (−) when mycelia exceed the paper border, (+-) when mycelia had a slow initial growth but later exceeded the paper border, and (+) when mycelia did not grow.

RESULTS AND DISCUSSION

Table 1 sum up the yields (v\w, dried weight) of essential oil and water content (% w\w) of the different samples of *Juniperus*. The yield obtained from the leaves of *J. phoenicea* ssp. *turbinata* was similar to *J. Communis* (≈0.2%), while *J. oxycedrus* exhibited a lower yield (0.04%).

Ripe berries of *J. phoenicea* ssp. *turbinata* showed a maximum yield of 2.54%, almost 5-fold higher than the other

two species. The lowest yield was obtained from the unripe berries of *J. communis* (0.08%).

Generally, the maximum yields were obtained by distilling ripe berries, except for *J. oxycedrus*, whose maximum yield was obtained by distilling unripe berries.

The water content (determined gravimetrically) of the leaves was similar in all analyzed *Juniperus* species (≈45%). Ripe berries of *J. phoenicea* ssp. *turbinata* showed water content of 12%, and unripe berries of *J. communis* of showed water content of 25%, half and 2-fold higher than that of the other species, respectively.

Essential Oil Composition from Different Parts of the Plants. Thirty-six components (91.39−98.57% of the total oil components) were identified.

***Juniperus phoenicea* ssp. *turbinata* (Table 2).** The monoterpenes content was higher in the ripe and unripe berries (95%, and 94.83%, respectively) than in the leaves (87.78%), while the content of alcohols and esters was higher in the leaves (1.20 and 3.22%, respectively) than in the ripe and unripe berries ≈ 0.5%.

The α-pinene content was higher in ripe and unripe berries (87.5 and 84.55%) than in leaves (48.90%). The δ-3-carene content was higher in the leaves (22.82%) than in the ripe or unripe berries (1.23 and 3.61%, respectively). β-Phellandrene showed the same behavior; γ-terpinene was not found in the leaves. The concentrations of sabinene, β-pinene, myrcene, α-phellandrene, and α-terpinene were similar in all samples.

Linalool and citronellol were only found in the leaves. Terpinen-4-ol was higher in the leaves than in the other parts; only traces of ketones were found.

Only the leaves possess moderate amounts of linalyl-acetate, while α-terpinyl-acetate was 4-fold higher in the leaves. The amounts of β-caryophyllene and α-humulene were almost equivalent in all parts of the plant. D-germacrene was 0.55% in the leaves versus 1.17 and 1.74% in ripe and unripe berries, α-muurolene was significantly higher in the leaves.

Data reported by R. P.Adams et al. (*8*) on the chemical composition of the essential oil of Spanish *Juniperus phoenicea* ssp. *turbinata* leaves showed that it was dominated by α-pinene (28.3%), β-phellandrene (25.3%), myrcene (7.2%), and α-phellandrene (4.1%); while limonene and δ-3-carene were absent. Rezzi S. et al. (9) reported the composition of the essential oil from leaves of *J. phoenicea* from Corsica. In *Juniperus* from Corsica, they found two different main compositions, identified as Cluster I and II, with the former rich in α-pinene (70%) and the latter rich in α-pinene (33.0%), β-phellandrene (21.1%), and α-terpinyl acetate (8.2%). The amount of δ-3-carene was low. In the essential oil from *Juniperus* leaves from Portugal, Cavaleiro (*10*) recognized three clusters based on the α-pinene/β-phellandrene ratio. Our results agreed with L. Falchi Delitala (*12*), who found that the essential oils from unripe berries of *Juniperus phoenicea* ssp. *mediterranea* (conspecific with *turbinata* as reported by Adams et al. (*8*)), harvested in north Sardinia, were dominated by α-pinene (93.8%), camphene (2.8%), β-pinene (2.07%), δ-3-carene (1.05%), limonene (0.03%), and γ-terpinene (0.21%).

When our data is compared with literature data, it emerges that the composition of the essential oil from leaves of Sardinian *Juniperus phoenicea* ssp. *turbinata* is different from all the other oils studied, i.e., Spanish, Corsican, and Portuguese oils. The composition of unripe berries is according to Delitala (*12*).

***Juniperus oxycedrus* ssp. *oxycedrus* (Table 3).** The content of monoterpenes was lower in the ripe and unripe berries (81.88 and 83.51%, respectively) than in the leaves (95.58%). The same behavior showed the esters being 0.06% in ripe and unripe berries and 0.14% in the leaves. No alcohols were found in the leaves. Sesquiterpenes were higher in ripe and unripe berries, (14.79 and 13.89%, respectively) than in the leaves 1.00%. In all samples, α-pinene was the main component (85.95% in leaves, 70.64% in ripe berries, and 62.26% in unripe berries). α-Thujene, δ-3-carene, α-phellandrene, and p-cymene were only found in the leaves. Sabinene, β-pinene, limonene, and (−) camphene were similar in all plant parts. γ-Terpinene was higher in the leaves than in the berries (1.4% versus 0.01%). The content of myrcene was higher in ripe and unripe berries than in the leaves (10.24 and 17.07% versus 1.20%). Only traces of alcohols, ketones, and esters were found in the berries.

The concentration of D-germacrene was higher in the ripe and unripe berries than in the leaves (12.86 and 13.71% versus

Table 2. Constituents (Area %) of the Oil of *Juniperus phoenicea* ssp. *turbinata*

compound	leaves (RA[a]%)	unripe berries (RA%)	ripe berries (RA%)
	monoterpenes		
	87.78	94.83	95.12
α-thujene[b]			
α-pinene[b]	48.90 ± 1.84	84.55 ± 0.14	87.54 ± 1.02
(–) camphene[b]	0.88 ± 0.13	0.39 ± 0.02	0.30 ± 0.02
sabinene[b]	0.24 ± 0.03	0.16 ± 0.01	0.21 ± 0.01
β-pinene[b]	0.67 ± 0.08	0.89 ± 0.04	0.78 ± 0.03
myrcene[b]	2.65 ± 0.44	2.10 ± 0.11	1.61 ± 0.59
α-phellandrene[b]	0.08 ± 0.08	0.02 ± 0.02	0.17 ± 0.14
δ–3-carene[b]	22.82 ± 5.83	3.61 ± 0.78	1.23 ± 0.48
α-terpinene[b]		0.03 ± 0.01	0.01 ± 0.01
p-cymene[b]	0.73 ± 0.19	0.04 ± 0.03	0.20 ± 0.02
limonene[b]			
β-phellandrene	10.01 ± 4.78	2.56 ± 0.28	2.81 ± 0.35
γ-terpinene[b]	0.18 ± 0.05	0.10 ± 0.02	0.04 ± 0.01
α-terpinolene[b]	0.62 ± 0.14	0.38 ± 0.07	0.22 ± 0.03
	alcohols		
	1.2	0.35	0.67
$\bar{\beta}$ -terpineol			
linalool[b]	0.33 ± 0.03		
trans-pinocarveol[b]	0.25 ± 0.09	0.02 ± 0.00	0.07 ± 0.07
terpinen-4-ol[b]	0.11 ± 0.02	0.05 ± 0.0.01	0.04 ± 0.02
α-terpineol[b]	0.33 ± 0.17	0.26 ± 0.07	0.51 ± 0.18
citronellool[b]	0.06 ± 0.11		
	terpene aldehydes		
	0.18	0.03	
campholenal-a	0.18 ± 0.05		0.03 ± 0.05
	ketones		
	0.34	0.16	0.18
camphor[b]	0.12 ± 0.05	0.12 ± 0.01	0.15 ± 0.02
piperiton	0.22 ± 0.08	0.04 ± 0.01	0.03 ± 0.03
β-thujone[b]			
	esters		
	3.22	0.63	0.51
linalyl acetato[b]	0.61 ± 0.18		
bornyl-acetate[b]			
myrtenyl-acetate[b]			
α-terpinyl acetate[b]	2.61 ± 0.31	0.63 ± 0.08	0.51 ± 0.09
Sesquiterpenes	1.69	2.57	2.07
α-cubebene[b]			
α-copaene[b]			
β-caryophyllene[b]	0.57 ± 0.07	0.60 ± 0.03	0.65 ± 0.04
α-humulene[b]	0.25 ± 0.14	0.23 ± 0.03	0.23 ± 0.01
D-germacrene	0.56 ± 0.11	1.74 ± 0.21	1.17 ± 0.12
α-muurolene	0.31 ± 0.32	0.02 ± 0.03	0.02 ± 0.01
	oxides		
caryophyllene oxide[b]			
	total identified		
	94.41	98.56	98.57

[a] Relative area. [b] Peaks identified by comparison with respective pure standards.

Table 3. (Area %) Constituents of the Oil of Juniperus *oxycedrus* ssp. *oxycedrus*

compound	leaves (RA[a]%)	unripe berries (RA%)	ripe berries (RA%)
monoterpenes			
	95.58	81.88	83.51
α-thujene[b]	0.11 ± 0.06		
α-pinene[b]	85.95 ± 0.28	62.26 ± 0.52	70.64 ± 1.13
(–)camphene[b]	0.26 ± 0.03	0.17 ± 0.02	0.18 ± 0.01
sabinene[b]	0.95 ± 0.07	0.16 ± 0.05	0.1 ± 0.00
β-pinene[b]	1.03 ± 0.23	1.06 ± 0.23	1.41 ± 0.03
myrcene[b]	1.20 ± 0.09	17.07 ± 1.7	10.24 ± 0.47
α-phellandrene[b]	0.22 ± 0.03		
δ–3-carene[b]	2.81 ± 1.06		
α-terpinene[b]			
p-cymene[b]	0.57 ± 0.21		
limonene[b]	0.83 ± 0.11	1.00 ± 0.04	0.83 ± 0.02
β-phellandrene			
γ-terpinene[b]	1.40 ± 0.11	0.01 ± 0.002	0.01 ± 0.001
α-terpinolene[b]	0.25 ± 0.1	0.15 ± 0.04	0.1 ± 0.00
alcohols			
		0.04	0.22
$\bar{\beta}$ -terpineolo			
linalool[b]		0.01 ± 0.02	0.02 ± 0.01
trans-pinocarveol[b]			0.12 ± 0.03
terpinen 4-ol[b]		0.02 ± 0.02	0.04 ± 0.01
α-terpineol[b]		0.01 ± 0.01	0.04 ± 0.01
citronellool[b]			
terpene aldehydes			
campholenal-a			
ketones			
		0.04	0.05
camphor[b]		0.04 ± 0.02	0.05 ± 0.01
β-thujone[b]			
piperiton			
esters			
	0.14	0.06	0.06
linalyl acetate[b]			
bornyl-acetate[b]	0.12 ± 0.07		
myrtenyl acetate[b]			
α-terpinyl acetate[b]		0.06 ± 0.09	0.06 ± 0.01
Sesquiterpenes	1.00	14.79	13.89
α-cubebene[b]			
α-copaene[b]			
β-caryophyllene[b]		0.44 ± 0.02	0.43 ± 0.05
α-humulene[b]		0.43 ± 0.03	0.43 ± 0.05
D-germacrene	0.77 ± 0.21	13.79 ± 1.82	12.86 ± 1.19
α-muurolene	0.23 ± 0.05	0.13 ± 0.05	0.17 ± 0.04
oxides			
caryophyllene oxide[b]			
total identified			
	96.68	96.80	97.73

[a] Relative area. [b] Peaks identified by comparison with respective pure standards.

Table 4. Constituents (Area %) of the Oil of Juniperus *communis* ssp. *communis*

compound	leaves (RA[a]%)	unripe berries (RA%)	ripe berries (RA%)
monoterpenes			
	83.21	84.2	81.14
α-thujene*	2.27 ± 0.13	0.56 ± 0.11	0.53 ± 0.06
α-pinene[b]	6.41 ± 0.41	52.91 ± 1.22	52.26 ± 0.94
(–) camphene[b]	0.04 ± 0.01	0.22 ± 0.02	0.22 ± 0.02
sabinene[b]	61.09 ± 5.06	13.73 ± 1.65	5.58 ± 0.82
β-pinene[b]	0.63 ± 0.08	2.98 ± 0.22	2.86 ± 0.03
myrcene[b]	2.57 ± 0.18	8.13 ± 0.50	15.32 ± 0.53
α-phellandrene[b]	0.1 ± 0.04		
δ–3-carene[b]			
α-terpinene[b]	1.79 ± 0.32	0.21 ± 0.02	
p-cymene[b]	1.15 ± 0.07		0.25 ± 0.04
limonene[b]	2.50 ± 0.27	3.81 ± 0.5	3.11 ± 0.25
β-phellandrene			
γ-terpinene[b]	3.32 ± 0.62	0.59 ± 0.13	0.52 ± 0.09
α-terpinolene[b]	1.34 ± 0.12	1.06 ± 0.22	0.49 ± 0.04
alcohols			
	12.33	1.13	1.51
$\bar{\beta}$ -terpineol	0.71 ± 0.05		
linalool[b]			
trans-pinocarveol[b]			
terpinen 4-ol[b]	10.67 ± 2.51	1.13 ± 0.51	1.51 ± 0.15
α-terpineol[b]	0.52 ± 0.06		
citronellool[b]			
terpene aldehydes			
campholenal-a			
ketones			
	0.29		
camphor[b]			
piperiton			
β-thujone[b]	0.29 ± 0.04		
esters			
	0.57	0.66	
linalyl acetate[b]			
bornyl acetate[b]	0.13 ± 0.04	0.66 ± 0.21	
myrtenyl acetate[b]	0.04 ± 0.01		
α-terpinyl acetate[b]	0.4 ± 0.26		
sesquiterpenes			
	1.27	10.02	8.75
α-cubebene[b]			1.25 ± 0.11
α-copaene[b]		0.65 ± 0.27	
β-caryophyllene[b]	0.12 ± 0.06	0.78 ± 0.15	
α-humulene[b]	0.11 ± 0.05	0.86 ± 0.18	0.81 ± 0.08
D-germacrene	0.75 ± 0.71	6.57 ± 1.94	6.69 ± 0.53
α-muurolene	0.14 ± 0.09	1.16 ± 0.33	
oxides			
	0.14		
caryophyllene oxide[b]	0.14 ± 0.06		
total identified			
	97.66	96.01	91.39

[a] Relative area. [b] Peaks identified by comparison with respective pure standards.

0.77%). Traces of β-caryophyllene and α-humulene were found in ripe and unripe berries.

Adams et al. (*7*) reported that the essential oil from leaves of Spanish J. *oxycedrus* ssp. *oxycedrus* was dominated by α-pinene (25−43%) and limonene (4,5−28%), with moderate amounts of β-pinene, myrcene, p-cymene, β-phellandrene, and malonyl-oxide. Guerra Hernandez et al. (5) reported that the essential oil from berries of *J. oxycedrus* from Spain was dominated by α-pinene (60.6%), myrcene (24.9%), γ-muurolene (5.19%), limonene (1.77%), β-pinene (1.08%), and other minor compounds, which are absent in the essential oil of the other species. Milos et al. (*6*) reported the composition of the essential oils of fresh leaves and unripe and ripe berries of *J. oxycedrus* from Croatia. The major compounds in the leaves were α−pinene (41.37%), malonyl oxide (12.29%), farnesol (8.60%), a mixture of dodecenyl acetate isomers (6.32%), sesquiterpenic hydrocarbon (4.40%), and dihydrofarnesal (3.35%). The major compounds in ripe and unripe berries were α−pinene (66.3−61.21%), unidentified sesquiterpenic hydrocarbon (9.87−5.22%), β−myrcene (4.90−5.73%), α−humulene (1.19−2.35%), bornyl acetate (1.88−1.40%), and δ−cadinene (1.32−1.22%).

The composition of the essential oil from the leaves of Sardinian *J. oxycedrus* is different from that of the essential oils of Spanish and Croatian *J. oxycedrus* found in the literature, especially in α−pinene and δ-3-carene content. On the contrary, our data on the essential oil from berries on the whole agree with Milos and Guerra Hernandez, except for the content

Table 5. MBC and MIC (μg/mL) of the Essential Oils of *Juniperus* and Major Components against *Candida albicans, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosas*

essential oils and major components tested	*Candida albicans* ATCC 14053		*Staphylococcus aureus* ATCC 6538		*Escherichia coli* ATCC 8739		*Pseudomonas aeruginosas* ATCC 9027	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
J. Phoenicea ssp. turbinata leaves	900	900	900		>900		>900	
J. Phoenicea ssp. turbinata unripe berries	900	900	900		>900		>900	
J. Phoenicea ssp. turbinata ripe berries	900	900	900		>900		>900	
J. Communis leaves	>900	>900	>900		>900		>900	
J. Communis unripe berries	>900	>900	>900		>900		>900	
J. Communis ripe berries	>900	>900	>900		>900		>900	
J. Oxycedrus leaves	900	900	225	450	>900		>900	
J. Oxycedrus unripe berries	>900	>900	>900		>900		>900	
J. Oxycedrus ripe berries	>900	>900	>900		>900		>900	
δ-3-carene	225	225	900		900		900	
sabinene	>900	>900	450		>900		>900	
terpinene-4-ol	>900	>900	>900		>900		>900	
β-myrcene	>900	>900	>900		>900		>900	
α- pinene	>900	>900	>900		>900		>900	

Table 6. Fungicidal Activity on Paper Disk Assay of the Essential Oils

	fungi										
essential oils	BOTCI	CERBE	FUSXL	FUSGR	HELOR	PYTUL	PYROR	SCLRO	RHISO	PHYCA	SEPTR
J. Phoenicea ssp. *turbinata*	±	–	–	–	–	–	–	–	–	–	–
J. Oxycedrus ssp. *oxycedrus*	–	–	±	–	–	–	–	–	–	±	–

of myrcene, which in our samples is at least double that found in the Croatian oil and half that in the Spanish oil.

***Juniperus communis* ssp. *communis* (Table 4).** The content of alcohols was lower in ripe and unripe berries (1.51 and 1.3%, respectively) than in the leaves 12.33%, while the content of sesquiterpenes was higher in ripe and unripe berries (8.75 and 10.02%, respectively) than in the leaves (1.27%). No esters were found in the ripe berries, while in leaves and unripe berries, the content was 0.57 and 0.66%, respectively.

The main component in the leaves was sabinene (61.09%), while in the unripe and ripe berries it was α-pinene (52.26 and 52.91%, respectively). Sabinene was higher in unripe than in ripe berries (13.73 versus 5.8%), while myrcene was higher in ripe berries (15.32 versus 8.13%).

The leaves were richer in α-thujene (2.17 versus 0.5 and 0.56%) and γ-terpinene (3.33 versus 0.52 and 0.59%). β-pinene was found higher in ripe and unripe berries than in leaves (2.86 and 2.98 versus 0.63%).

Terpinen-4-ol was 10-fold higher in the leaves than in the ripe or unripe berries (10.67 versus 1.5 and 1.13%). Only traces of ketones, esters, and oxides were found in the leaves.

The berries were richer in D-germacrene and α-humulene, which were 8-fold higher than in the leaves. Traces of other sesquiterpenes were found in all plant parts.

Catzopoulou (*14*−*16*) reported that the essential oil from the leaves of *J. communis* from northern Greece was dominated by α-pinene (41.3%) and sabinene (17.4%) and that the essential oil from the berries was dominated by α-pinene (27%), sabinene (13%), D-germacrene (10%), and myrcene (9%). Koukos (*25*) reported the composition of the essential oil from green and black berries from three different locations in northern Greece. He obtained yields 5- to 10-fold higher than that found in the literature, an extremely high variability in the quantitative composition, and any comparison can be done. From a qualitative point of view, the oil collected are comparable to the other data present in the literature. Caramiello et al. (*17*) list sabinene as the dominant component (41.4%) with 13.4% α-pinene and 8.7% terpinen-4-ol in the essential oil from the leaves of *J. communis* from the Northwestern Italian Alps. Kallio H. et al. (*13*) analyzed the composition of the essential oils of *J. communis* from ripe and unripe berries from Finland. They were dominated by α-pinene (18−58%), myrcene (7−23%), and γ-cadinene (5−13%). The composition of the essential oil from the leaves of Sardinian *J. communis* is different from that found in the Greek oils studied by Catzopoulou, especially in the content of α−pinene, sabinene, and terpinen-4-ol. The results obtained by Caramiello generally agreed with our finding, while the α−pinene and sabinene content was lower (9.2 and 44.7 versus 6.41 and 61.09%, respectively). The composition of the essential oil from Sardinian berries generally agrees with the data from Catzopoulou and Kallio, except in the γ-cadinene content, which was not found in our samples.

Bioassays. Table 5 reports the MIC and the MBC of the oils and components tested against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosas*. The oils extracted from the berries and the leaves of *J. communis* and from the berries of *J. oxycedrus* failed to inhibit the microorganisms at the highest concentration tested (MIC

and MBC >900 μg\mL), but the essential oils from the leaves of *J. phoenicea* ssp. *turbinata* and *J. oxycedrus* exhibited weak activity against *Candida albicans* (MIC and MBC 900 μg\mL) and weak and good activity against *Staphylococcus aureus* (MIC and MBC 900 μg\mL and MIC 225 and MBC 450 μg\mL, respectively). Among the components tested, only δ-3-carene was found to possess a moderate MIC and MBC against *Candida albicans* (225 μg\mL) and a weak activity against all the other tested microorganisms (MIC 900 μg\mL). Sabinene inhibited *Staphylococcus aureus* with an MIC of 450 μg\mL.

In conclusion, 90% of all the analyzed oils were found to be made up of monoterpenes. Only the essential oil from the leaves of *J. communis* had 10% alcohols, and the essential oils from ripe and unripe berries of J. *oxycedrus* and *J. communis* were found to have a relatively high percentage of sesquiterpenes. The chemical composition of the essential oils (**Tables 2**−**4**) was similar both in the different harvesting areas and in the different years, as confirmed by the low standard deviations obtained. Sardinian *Juniperus* oils are distinct from those from other countries. Major differences were found in the essential oils from leaves. This aspect could be due to local environmental conditions or to a different chemotaxonomy of the plants of *Juniperus* (*9*, *10*, *12*, *17*).

The antimicrobial activity of the tested oils was generally nonsignificant; only the essential oils from *Juniperus phoenicea* ssp. *turbinata* and from the leaves of *Juniperus oxycedrus* ssp. *oxycedrus* exhibited a relatively good or weak activity against *Candida albicans* and *Staphylococcus aureus*.

Microbiological Data. This preliminary test was carried out only on the essential oil of ripe berries of *Juniperus turbinata* and *oxycedrus*, the first containing δ-3-carene and the second not. **Table 6** reports the data from the paper test. Both types of *Juniperus* did not show inhibition against fungi growth, for this reason the experimentation was not carried over.

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