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COMPARISON OF THE ANTIMICROBIAL ACTIVITY AND THE ESSENTIAL OIL COMPOSITION OF *Juniperus oxycedrus* SUBSP. *macrocarpa* AND *J. oxycedrus* SUBSP. *rufescens* OBTAINED BY HYDRODISTILLATION AND SUPERCRITICAL CARBON DIOXIDE EXTRACTION METHODS

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Different *Juniperus* species have been used in traditional medicine for centuries as incense, diuretic, remedy for indigestion, and as a tar resource. *Juniperus oxycedrus* L. belongs to the genus *Juniperus*. The main use of *J. oxycedrus* is to prepare the so-called cade oil (also known in pharmacy as juniper tar) by destructive distillation of the branches and wood of the plant. This empyreumatic oil has been widely employed in human and veterinary dermatology to treat chronic eczema and other skin diseases [1], and rectified cade oil is used as a fragrance component in soaps, detergents, creams, lotions, and perfumes [2].

In Tunisia, *Juniperus oxycedrus* L. comprise two subspecies: *Juniperus oxycedrus* L. subsp. *rufescens* (L.K) Deb. which is characterized by a subglobular reddish cone (diameter 3–10 mm) having in their vertex a deep triangular depression. It is widespread in the scrubs and hilly forests especially in the continental localities.

J. oxycedrus L. subsp. *macrocarpa* (S. & SM.) Ball. has voluminous berries (diameter 2.0–2.5 cm) with a reddish brown colour and a slight depression in their summit. It occurs the maritime sand hill and hilly forests [3].

The essential oil of *J. oxycedrus* is obtained by hydrodistillation of leaves, berries, or wood. Although these oils are usually characterized by a high content of α-pinene, whatever the subspecies, the origin and the extractions process are responsible for the variation in the chemical composition of the essential oils [4].

Previous studies on the chemical composition of the hydrodistilled or supercritical extracted oil leaves (subspecies not specified, ssp. *oxycedrus*, ssp. *badia*, and ssp. *macrocarpa*) have been reported by others [5–9] but up to now no study has been reported on the chemical composition of *Juniperus oxycedrus* ssp. *rufescens*.

The main methods to obtain essential oils from the plant materials are hydrodistillation (HD), steam distillation (SD), and solvent extraction (SE). Among these methods, HD has been the most common approach to extract the essential oils from the medicinal herbs/plants. However, in order to reduce the extraction time and possibly improve the extraction yield, to enhance the quality of the extraction, new approaches such as microwave-assisted extraction (MAE), pressurized solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction have also been sought [10, 11].

Supercritical fluid extraction (SFE) has gained increasing attention over the traditional techniques in the recovery of edible and essential oils, as the use of a nontoxic and volatile fluids in SFE such as CO_2 protects the extracts from thermal degradation and solvent contamination [12].

In view of the increasing environmental and health concern over the use of organic solvents in the extraction of natural products, there has been growing interest in using supercritical fluids.

SFE has been applied to a wide range of nonpolar biologically active constituents from natural products, including essential oils, other flavor and fragrance compounds, medicinal compounds, carotenes, and alkaloids [13, 14].

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TABLE 1. Kovats Indices and Chromatographic Area Percentages of Compounds Identified in the SFE Extracts, Obtained at 90 bar and 50°C, and in the HD Oils of Leaves of *J. oxycedrus* ssp. *macrocarpa*

Compound	I_K	HD	SFE	Compound	I_K	HD	SFE
α-Pinene*	962	23.8	2.4	Caryophyllene oxide*	1570	1.6	1.6
Myrcene*	1007	1.1	–	α-Cadinal	1636	2.6	3.3
Δ^3-Carene*	1025	1.6	–	Acorenone	1670	1.6	2.5
p-Cymene*	1038	2.5	0.7	(*Z,E*)-Farnesol	1707	1.9	2.9
β-Phellandrene*	1043	6.1	1.7	Sandaracopimara-8(14)-15-diene	1955	17.6	31.3
meta-α-Terpineol	1194	0.7	1.3	Bifloratriene	1974	0.7	1.2
α-Terpinyl acetate*	1359	1.9	0.5	Kaurene	2012	4.1	5.6
(*E*)-Caryophyllene*	1422	1.2	1.1	Abietadiene	2036	2.7	4.0
cis-Muurola-3,5-diene	1452	1.0	0.9	Octadecanol	2069	–	3.8
Germacrene D isomer	1473	2.1	2.0	Sclareol	2197	–	2.5
γ-Muurolene	1478	5.2	5.7	*cis*-Totarol	2227	–	1.8
trans-Cadinene	1510	1.2	1.3	*trans*-Totarol	2240	1.0	4.5
cis-Calamenene	1520	3.6	1.7	Larixol	2262	–	1.0
Occidentalol	1546	1.7	1.6	3-α-Hydroxymanool	2277	–	1.5

*Identification by comparing mass spectra (MS), retention Indices (I_R), and injection of authentic compound (Inj).

TABLE 2. Kovats Indices and Chromatographic Area Percentages of Compounds Identified in the SFE Extracts, Obtained at 90 Bar and 50°C, and in the HD Oils of Leaves of *J. oxycedrus* ssp. *rufescens*

Compound	I_K	HD	SFE	Compound	I_K	HD	SFE
α-Pinene*	962	12.2	1.2	5-*neo*-Cedranol	1673	1.9	0.7
Δ^3-Carene*	1025	1.2	–	(*Z,E*)-Farnesol	1707	1.9	0.9
β-Phellandrene*	1043	1.9	0.7	Cembrene	1900	2.0	–
(*E*)-Caryophyllene*	1422	4.7	1.8	Sandaracopimara-8(14)-15-diene	1955	4.6	4.0
α-Humulene*	1455	2.0	0.6	Bifloratriene	1974	1.2	0.9
γ-Muurolene	1478	1.3	–	Phyllocladene	2009	3.6	19.5
Germacrene D	1482	11.7	4.0	Kaurene	2012	4.4	4.4
trans-Cadinene	1510	1.7	0.7	Abietadiene	2036	9.8	8.6
cis-Calamenene	1520	3.4	0.8	Octadecanol	2069	0.9	4.0
Occidentalol	1546	1.0	0.4	Abieta-8(14),13(15)-diene	2095	4.5	15.9
Caryophyllene oxide	1570	1.5	0.7	Sclareol	2197	0.2	2.9
1,10-di-epi-Cubenol	1610	2.2	–	*cis*-Totarol	2227	0.5	4.7
α-Cadinal	1636	1.8	1.3	*trans*-Totarol	2277	0.2	2.8
14-Hydroxy-9-epi-(*E*)-caryophyllene	1655	5.2	0.9	Larixol	2240	–	1.5
Acorenone	1670	1.2	1.3	3-α-Hydroxymanool	2262	0.2	2.9

*Identification by comparing mass spectra (MS), retention Indices (I_R), and injection of authentic compound (Inj).

TABLE 3. Biological Activity of *Juniperus oxycedrus* ssp. *macrocarpa* and *J. oxycedrus* ssp. *rufescens* Essential Oil Obtained by Supercritical Extraction (SFE) and Hydrodistillation (HD)

	J. macrocarpa HD	*J. macrocarpa* SFE	*J. rufescens* HD	*J. rufescens* SFE
*Staphylococcus aure*us ATCC 2913	2.5	0.6	2.5	0.6
Enterococcus faecali ATCC 24912	>10	>10	>10	>10
Escherichia coli ATCC 25922	>10	>10	>10	>10
Pseudomonas aeruginosa ATCC 27853	>10	>10	>10	>10
Candida albicans ATCC 2091	0.0125	0.0125	0.0125	0.0125

Antibiotic activity is given as MIC, the minimal inhibitory concentration (% v/v).

In the present work the essential oils obtained by hydrodistillation of *J. oxycedrus* ssp. *macrocarpa* and *J. oxycedrus* ssp. *rufescens* leaves were used for comparison of their chemical composition, and we continue with a study on the extraction and characterization of the SFE extracts derived from species belonging to the genus *Juniperus*, widespread in Sardinia and Tunisia: *Juniperus oxycedrus* ssp. *macrocarpa* (Table 1) and *Juniperus oxycedrus* ssp. *rufescens* (Table 2). An attempt at testing the antimicrobial activity of the essential oils extracted was made (Table 3).

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