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Article

Phytochemical Analysis and Evaluation of Antifungal and Antioxidant Activities of Essential Oil of Fruits from *Juniperus oxycedrus* L. Obtained from Morocco

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The present study was aimed at conducting phytochemical analysis and evaluating the *in vitro* antifungal and antioxidant activities of the essential oil obtained from the fruits of *J. oxycedrus* L. Hydro-distillation was used to extract the essential oil from the fruits of *Juniper oxycedrus.* The essential oil was analyzed using gas chromatography with a flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). The antioxidant activity of the essential oil against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was determined *in vitro* using varying concentrations of the essential oil and vitamin C as a standard antioxidant compound. A disc diffusion test was employed to evaluate the antifungal activity of the essential oil against two test fungal strains, *Penicillium citrinum*, and *Aspergillus niger.* The results revealed that 49 constituents were identified in fruit oil, representing 91.56% of the total oil and the yield was 1.58%. Juniper fruit oil was characterized by having high contents of β-pinene (42.04%), followed by limonene (15.45%), sabinene (9.52%), α–pinene (5.21%), (E)-caryophyllene (3.77%), ρ-cymene (1.56%), caryophyllene oxide (2.02%), and myrcene (1.02%). The radical scavenging activity (% inhibition) of the essential oil was highest (81.87± 2.83%) at a concentration of 200 µg/mL. The essential oil of *J. oxycedrus* exhibited antifungal activity against *A. niger* and *P. citrinum* with minimum inhibitory concentration values (MIC) ranging from 2.89 to 85.01 µl/mL. The findings of the study reveal that the antioxidant and antifungal properties of *J. oxycedrus* essential oil and their chemical composition are significantly correlated.

Keywords: *Juniperus oxycedrus* L.. Fruits. GC/MS. Antifungal. Antioxidant activities.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because of their biochemical constituents, which have therapeutic significance (Alotaibi *et al.*, 2021). Essential oils are valuable natural products used as raw materials in a variety of industries, including perfumes, cosmetics, aromatherapy, phototherapy, spices, and nutrition (Nazzaro *et al.*, 2020). *Juniper oxycedrus L.* (Cupressaceae) is a plant found in Morocco, which is widely utilized in traditional medicine for the treatment of different infectious diseases. It is a small tree endemic to Portugal's northern areas near the Mediterranean Sea. The plant is also native to North Africa, specifically Algeria and Morocco, as well as the Canary Islands (Gaussen, 1968). It also includes Turkey (Coode, Cullen, 1965; Sezik *et al.*, 2005), Colombia (Fretz, Sydnor, Cobbs, 1976), Spain (Adams *et al.*, 1987) and Greece (Stassi *et al.*, 1996;

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Koukos *et al*., 2002). The essential oil from the leaves of *J. oxycedrus* has been reported in various regions from Lebanon (Loizzo *et al*., 2007), Corsica (Boti *et al*., 2006) and Croatia (Milos, Radonic, 2000).

According to World Health Organization (WHO) reports, approximately 80% of people in developing countries rely on medicinal plants for health care (Picking, 2017). Essential oils and their constituents are widely utilized as constituents of many medical goods, as flavouring additives in the food industry, in cosmetics as scents, and in the pharmaceutical sector (El-Shemy, 2018). The oil extracted from *J. oxycedrus* was used in dermatology to treat chronic eczema and other skin diseases, while the rectified oil was used as a fragrance component in detergents, soaps, creams, and lotions (Leung, Foster, 1996). For many centuries, essential oils from different parts of junipers have been used for fragrance, flavouring, medicinal, antimicrobial, insecticidal, and cosmetic purposes (Orhan, 2019). *Juniperus oxycedrus* fruit has been widely used to treat gastrointestinal disorders and common colds, as an expectorant in cough to treat joint calcinosis, and as a diuretic to pass kidney stones against urinary inflammations and hemorrhoids. It has also been utilized as a hypoglycemic agent and is commonly used as flavoring spice, particularly for various types of meat dishes (Orhan *et al*., 2011).

Several studies on the chemical composition of essential oils of *J. oxycedrus* from various parts of the world have been reported (El-Abid *et al*., 2019; Llorens-Molina, Ygueravide, Vacas, 2019; Semerdjieva *et al*., 2019). Antioxidants are very important for the defense of a living system against oxidative stress. The addition of antioxidants to food products is gaining popularity as a strong means for extending the shelf-life of products and for decreasing nutritional losses by preventing or slowing the oxidation process (Tsuda *et al*., 1994). The most commonly applied antioxidants in the food industry are synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA).

The aims of this study were to conduct a phytochemical analysis and evaluate the antifungal and antioxidant properties of essential oil extracted from the fruits of *J. oxycedrus* obtained from a Morocco.

MATERIAL AND METHODS

Chemicals

The chemicals and standards used in this study were of analytical grade unless otherwise specified. Hexane diluted with distilled water at 10% (prepared for the dilution of essential oil), methanol, sulfuric acid, anhydrous sodium sulfate, series of alkanes (C_4–C_{28}) standards, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were purchased from Sigma-Aldrich (St. Louis, MI, USA). The fungal strains were obtained from the Microbiology Laboratory, Faculty of Medicine and Pharmacy, Fez, Morocco.

Plant materials

The fruits of juniper were collected during March 2020 in the Atlas median region (Taferdoust), 15 kilometers south-east of Boulmane (latitude: 25° 31'11" longitude: 5° 22' 21" elevation: 2100 m) in Morocco. With an annual average temperature of 20°C, the climate was semi-desertic with a strong continental influence. The specimens were then air-dried for 16 days. The plant was identified by Dr. Elhoussine Derwich, and subsequently separated from the other specimen and deposited under the voucher number JOF202003 in the Faculty of Medicine and Pharmacy, University Sidi Mohamed Ben Abdellah, Morocco.

Extraction of essential oil from the fruits of *Juniper oxycedrus*

The essential oil was extracted from the fruits of *Juniper oxycedrus* using a Clevenger-type apparatus (Clevenger, 1928) at the Faculty of Sciences in Fez, Morocco. It took 2.5 hours to extract 200 g of fruits in 1400 mL of distilled water. The yellowish oil (0.5 mL) for fruits was dissolved in hexane and dried over anhydrous sodium sulfate. Following yield determination and filtration, the solvent was removed by pressure distillation in a rotary evaporator at 35°C, and pure oil was stored at 4°C in darkness until the start of the analysis.

The amount of oil obtained from each plant material was calculated with the equation:

Oil (% v/w) = observed volume of oil (mL)/ weight of the sample (g) x 100.

Phytochemical screening of the essential oil from the fruits of *Juniper oxycedrus*

The analysis of the essential oil obtained from the fruits of *Juniper oxycedrus* was performed by gas chromatography with a flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS) at the City of Innovation, Sidi Mohamed Ben Adellah University, Fez, Morocco.

Gas chromatography analysis of the essential oil from the fruits of *Juniper oxycedrus*

The gas chromatography (Trace GC-ULTRA, S/N 20062969, Thermo Fischer, France) used in this study was equipped with a flame ionization detector (GC-FID), as well as a Varian capillary column test report CP 7770 (CP-SIL- 5 CB; 50 m length, 0.32 mm inside diameter, 0.45 mm outside diameter, and film thickness 1.20 m). The column temperature was initially kept at 40°C for 2 minutes before progressively increasing it to 260°C at a rate of 5 °C/min for 10 minutes. The injector temperature was set at 250°C, and one of the detectors (FID) at 270°C. The gas vector (nitrogen) debit was set to mL/min. The volume of the injected specimen was 0.5 µL of diluted oil in 10% hexane solution. The percentage of each constituent in the oil was determined by the area of the peaks. Gas phase chromatography (Trace GC-ULTRA, S/N 20062969, Thermo-Fischer-France) was used in conjunction with mass spectrometry (PolarisQ, S/N 210729, Thermo Fischer-France) to identify various chemical components. Varian capillary column test report CP 7770 (CP-SIL-5 CB; 50 m length, 0.32 mm inside diameter, 0.45 mm outside diameter, and film thickness 1.20 m) was used. The column temperature was set to rise at a rate of 5°C/min from 40 to 260°C. The injector temperature was set at 250°C, and one of the detectors (PolarisQ) was at 200°C. Ionization of the sample components was performed in electron ionization mode (EI, 70 eV). The debit of the gas vector (Helium) was set at 1 mL/min. The temperature of the transfer line was 300°C. At a rate of 2.9 scans/s, the mass range of 40 to 650 amu was scanned. The injected specimen included 1 µL of diluted oil in 10% hexane solution. The constituents of essential oil were identified by comparing their retention indices, calculated in relation to the retention time of a series of linear alkanes (C_4-C_{28}), with those of reference products and by comparing their retention indices with those of the chemical components reported by Adams (2001), in comparison with their spectra with those presented in a library (NIST-MS Search Version 2.0) and by comparing literature data in the same conditions (**IR).

DPPH free radical scavenging assay of the essential oil from *Juniper oxycedrus*

The DPPH (2,2-diphenyl1-1-picryl-hydroxyl radical) scavenging activity of the extracts from *J. oxycedrus* was determined according to the procedure described by Ramy *et al.* (2010) with some modifications. The spectrophotometric activity of plant essential oil against the stable DPPH radical was determined (Brand-Williams, Cuvelier, Berset, 1995). A UV/visible light spectrophotometer was used to quantify the colorimetric changes (from deep violet to light yellow) when DPPH was reduced. Using the stable radical DPPH, the antioxidant properties of essential oil were evaluated in terms of hydrogen donating or radical scavenging ability. Forty microliters of various concentrations (25, 50, 75, 100, 150, and 200 µg/mL) of the essential oil in dimethyl sulphoxide (DMSO), as well as vitamin C (as a standard antioxidant compound), were placed in appropriate tubes. Then, 4 ml of 0.004% methanolic solution of DPPH was added to each tube to give final concentrations (25, 50, 75, 100, 150, and 200 µg/mL). The tests were performed in triplicate. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 1 h for all samples. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH radical without antioxidants (the control) was measured. Special attention was given to minimize the loss of free radical activity of the DPPH radical stock solution. Radical scavenging activity was expressed as a percentage inhibition of the DPPH radical and was calculated according to the equation (Albayrak *et al.*, 2010):

% inhibition = (Control absorbance - Sample absorbance/ Control absorbance) x 100.

The extract concentration that provides 50% inhibition (IC_{50}) was calculated from the plotted graph of inhibition percentage against extract concentration.

Evaluation of the antifungal activity of the essential oil from *Juniper oxycedrus*

The antifungal activity of the essential oil extracted from *J. oxycedrus* fruits against two fungal strains (*Aspergillus niger* and *Penicillium citrinum*, from the Microbiology Laboratory, Faculty of Medicine and Pharmacy, Fez, Morocco) was determined by measuring the zone of inhibition and the minimum inhibitory concentration (MIC). The values were determined according to published procedures (Adiguzel *et al*., 2002; Gul, Ojanen, Hanninen, 2002) with minor modifications. Antifungal test was then carried out by the disc diffusion method (Murray *et al*., 1995), using 50 mL of suspension containing 52 spores/mL of fungi spread on potato dextrose agar (PDA). The discs (6 mm in diameter) were impregnated with 10 µL of essential oil and placed on the inoculated agar. Negative controls were prepared using the same solvents used to dissolve the plant extract. Ofloxacin (20 µg per disc) and sulbactam (30 µg) + cefoperazone (70 µg) (100 µg/disc) were used as positive reference standards to determine the sensitivity of one isolate in each test microbial species. The cultures were incubated at 27°C for 72 h. The test fungal strains were *Aspergillus niger* and *Penicillium citrinum*, and the assays were performed in duplicate.

RESULTS AND DISCUSSION

Identification of essential oil constituents

The retention time and chemical composition of the essential oils extracted from *J. oxycedrus* are presented in Figure 1 and Table I. The constituents of *J. oxycedrus* from Morocco are presented in the order in which they were eluted on the CP-SIL- 5 CB column (Figure 1). A total of forty-nine volatile compounds, representing 84.05 % of the total composition, were identified in the leaf oil, as shown in Table I. The most abundant components found in the leaf oil were β-pinene (42.04%), followed by limonene (15.45%), sabinene (9.52%), α–pinene (5.21%), (E)-caryophyllene (3.77%), ρ-cymene (1.56%), caryophyllene oxide (2.02%), myrcene (1.02%), and β phellandrene (1.01%). The essential oil yield of fruits from *J. oxycedrus* collected in Morocco's Atlas median region (Taferdoust) was 1.58% in this study. It is relatively higher than those of other plants used industrially as a source of essential oils, which include *Tetraclinis articulata* (0.22%) (Bourkhiss *et al*., 2000), *Juniperus thurifera* (0.8%) (Achak *et al*., 2009), *Juniperus oxycedrus* (1.14%) (Salido *et al*., 2002), *Artemisia herba-alba* (0.59%), *Artemisia absinthium* (0.57%) and *Artemisia pontica* (0.31%) (Derwich, Benziane, Boukir, 2009). Also, essential oil from *J. oxycedrus* was higher than that of lavender (0.8-2.8%), menthe (0.5-1%), néroli (0.5-1%), laurel (0.1-0.35%) (Edward *et al*., 1987), and Artemisia (0.65%) (Akrout *et al*., 2001). In contrast, the yield of essential oil from *J. oxycedrus* was lower than that extracted from *J. occidentalis* in the study conducted by Adams (1987), which was 2.3% and that of *J. oxycedrus* in Pindos from Greece, with a yield of 2.21% (Milos, Radonic, 2000).

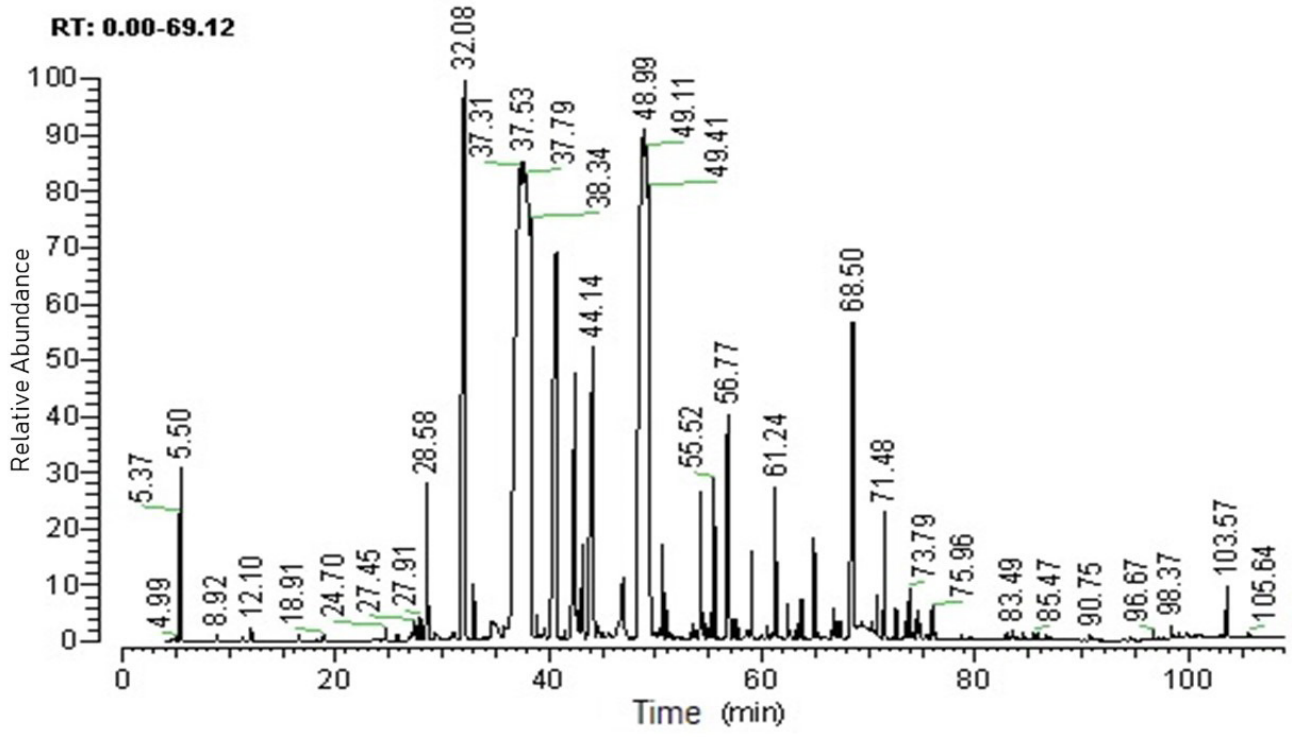

FIGURE I - Chromatogram of fruit essential oil from *Juniperus oxycedrus L.*

TABLE I - Chemical composition of fruit essential oil from *Juniperus oxycedrus L.*

Peak	Compounds	*RI	**RI	Area (%)	**Mass range (m/z)
1	α -pinene	924	924	5.21	*(136),93,91,136,121,77,92,79,43,41,105*
2	camphene	933	932	0.60	*(136),93,79,91,77,41,121,80,94,107,39*
3	β -pinene	938	938	42.04	*(136),93,91,136,121,77,92,79,43,41,105*
4	myrcene	948	947	1.02	*(136),41,93,69,39,27,53,79,77,67,91*
5	α -phellandrene	954	954	0.88	*(136),93,77,91,136,79,94,41,80,92,39*
6	σ-ocimene	958	959	01.08	*(136),93,41,27,39,79,80,77,43,29,91*
7	β phellandrene	964	964	1.01	*(136),93,77,91,136,79,94,41,80,92,39*
8	β -thujene	973	973	0.06	*(136),93,41,91,77,79,39,27,69,94,43*
9	sabinene	983	984	9.52	*(136),93,41,91,77,79,39,27,69,94,43*
10	γ-terpinene	988	988	0.17	*(136),93,91,121,77,92,79,43,41,105*
11	sabina ketone	1001	1001	0.05	*(138),81,96,95,55,41,67,43,39,68,82*
12	3-carene	1004	1003	0.61	*(136),93,91,79,77,92,121,80,136,94,105*
13	limonene	1018	1019	15.45	*(136),68,93,39,67,41,27,53,79,94,92*
14	ρ-cymene	1032	1032	1.56	*(134),119,134,91,120,117,41,77,39,65,115*

TABLE I - Chemical composition of fruit essential oil from *Juniperus oxycedrus L.*

Peak	Compounds	*RI	**RI	Area (%)	**Mass range (m/z)
15	caryophyllene oxide	1506	1508	2.02	*(220),43,41,79,93,91,95,69,55,67,81*
16	terpinolene	1042	1042	0.08	*(136),93,121,91,136,79,77,105,39,41,107*
17	1,8-Cineole	1059	1058	0.20	*(154),43,93,81,71,69,84,68,108,41,55*
18	β-thujone	1062	1063	0.05	*(152),110,81,95,67,68,41,69,109,55,70*
19	carvone	1190	1192	0.02	*(150),82,54,39,93,108,53,107,41,79,91*
20	verbenone	1119	1119	0.02	*(150),107,91,39,135,41,80,150,27,79,55*
21	α- terpineol	1133	1132	0.18	*(154),59,93,121,136,81,43,68,95,67,41*
22	terpinen-4-ol	1137	1138	0.03	*(154),71,111,93,43,86,41,69,55,68,154*
24	myrtenal	1136	1136	0.05	*(150),79,107,108,106,77,91,41,105,39,27*
25	myrtenol	1191	1191	0.08	*(152),79,91,108,41,93,43,119,77,39,67*
26	verbenol	1126	1125	0.08	*(125),109,41,94,81,39,69,55,91,43,57*
27	pinocarvone	1114	1114	0.06	*(150),81,53,108,41,69,107,79,39,27,150*
28	borneol	1128	1128	0.84	*(154),95,41,110,93,55,67,139,121,96,69*
29	carveol	1206	1208	0.95	*(152),91,119,77,134,117,92,39,109,65,93*
30	β –copaene	1221	1221	0.43	*(204),161,119,105,93,41,91,92,81,120,204*
31	sabinenyl acetate	1224	1223	0.57	*(194), 92,91,81,41,134,55,109,79,43,53*
32	bornyl acetate	1267	1267	0.43	*(196), 95,43,93,436,121,41,80,55,108,69*
33	α -terpinyl acetate	1333	1332	0.45	*(196),43,121,93,136,68,41,59,67,81,79*
34	cadinene-3,9-diene	1440	1441	0.41	*(204),161,189,204,105,91,133,119,95,41,81*
35	geraniol	1228	1228	0.33	*(154),69,41,68,29,93,123,67,70,84,55*
36	trans-pinocarveol	1321	1321	0.11	*(152),92,91,70,55,41,83,79,134,69,119*
37	γ-cadinene	1430	1429	0.14	*(204),161,189,204,105,91,119,133,27,55*
38	β-muurolane	1419	1419	0.01	*(208)109,95,41,55,81,165,83,69,67,164*
39	selinane	1432	1433	0.08	*(208),109,95,81,55,96,69,83,67,165,97*
40	α-cedrol	1543	1542	0.20	*(222),95,150,151,43,41,81,69,55,107,93*
41	β –humulene	1578	1578	0.41	*(204),93,80,41,121,92,43,55,67,91,147*
42	humulene	1579	1580	0.08	*(204),93,80,41,121,92,43,55,67,91,147*
43	germacrene D	1505	1507	1.50	*(204),161,105,91,41,119,79,81;93,77,27*
44	β -cubenol	1645	1645	0.49	*(222),161,105,119,41,81,93,79,93,55,59*
45	farnesol	1710	1711	0.25	*(222),69,81,41,93,95,68,109,67,55,107*

TABLE I - Chemical composition of fruit essential oil from *Juniperus oxycedrus L.*

Peak	Compounds	*RI	**RI	Area (%)	**Mass range (m/z)
46	manoyl oxide	1978	1978	0.07	*(290),275,257,81,192,55,137,177,95,67,43*
47	E-caryophyllene	1984	1985	3.77	*(204),93,133,91,41;79,69,105,107,120,77*
48	ethyl linoleate	2193	2192	0.08	*(308),67,81,41,55,95,54,45,68,82,69*
49	cadalene	1706	1705	0.06	*(198),183,198,168,184,153,165,152,167,169,141*
Total Identified Compounds (%)				91.56	
Yields (%v/w)				1.58	

* RI: Retention indices were determined by GC-FID on a CP-SIL- 5 CB column

**RI: Literature retention index

*** Mass range (m/z) was determined by mass spectrometry (PlarisQ).

The chemical composition revealed was similar to those of other *J. oxycedrus* essential oils analyzed in Lebanon by Loizzo *et al.* (2007), Espagne (Velasco-Negueruela *et al.*, 2003), Egypt (El-Ghorab *et al.*, 2008), Tunisia (Ennajar *et al.*, 2009), and in Europe by Milos and Radonic (2000), with α-pinene as the major component. Farjon (2005) investigated the cryptic speciation of *J. deltoids* and *J. oxycedrus* in the Mediterranean, collecting samples from Morocco, Portugal, Spain, France, Italy, Southern Greece, Northern Greece, and Turkey. They reported that α-pinene was the most abundant compound (45.3%, 47.3%, 40.9%, 53.2%, 19.3%, 19.7%, 27.4%, and 32.7%, respectively). The compound δ-cadinene was found to be the most abundant component in the essential oil of *J. oxycedrus* wood studied in Spain, France, and Italy (Barrero *et al.*, 1993). The berry oil of *J. oxycedrus* was studied in Greece from two different locations: Holomontas and Pindos, with the major components identified as α-myrcene (23.4%) and citronellol (26.8%) (Koukos *et al.*, 2002), and of *Juniperus occidentalis*, with the major commercially important compounds identified as α-cedrene (8.8 %), β-cedrene (2.6 %), thujospene (18.9 %), cuparene (1.5 %), cedrol (38.9 %), and widdrol (1.6%) (Adams, 1987).

Marongiu *et al.* (2003) examined samples collected in Sardinia and discovered the presence of δ-cadinene, 1-epi-cubenol (12.5%), cubenol (10.5%), α-muurolol (4.8%), α-cadinol (3.7%), and α-humulene (3.2%). This species has been the subject of extensive investigations (Magorzata *et al.*, 2007; Massei, Watkins, Hartley, 2006; Consentino *et al.*, 2003). In this study, the yield and total oil content of the essential oil of *J. oxycedrus* obtained in Morocco's Atlas median region was 1.58% and 91.56%, respectively. The essential oil yield of *J. oxycedrus* fruits is relatively higher than that of other plants studied in Sardinia, Italy (0.04-2.54%) (Angioni *et al.*, 2003) and Holomontas, Greece (0.97%) (Koukos *et al.*, 2002). Other studies on the essential oil of *J. oxycedrus* extracted from branches in Serbia and Bulgaria found yields ranging from 0.06 to 0.24% (Semerdjieva *et al.*, 2019), which was lower than the results of this study. In the same context, the results of a study on the essential oil of this species conducted by Fadel *et al.* (2019), revealed a yield of 0.02%. A study on the essential oil composition of *J. oxycedrus* ssp. *oxycedrus* berries according to their ripening stage, on the other hand, revealed a variance in yield ranging between 0.8 and 2.6% (Llorens-Molina, Ygueravide, Vacas, 2019). The essential oil content showed variations in plants of different geographical origins and also in different parts of the tree. Milos and Radonic (2000) studied the essential oil composition in fresh needles, and green and mature berries of *J.*

oxycedrus collected in Croatia. They reported that the number of compounds was 36, 15, and 22, and the total oil obtained was 94.90, 94.33, and 90.94%, respectively.

In Portugal, Cavaleiro *et al.* (2000) studied the composition and variability of the essential oils of *J. navicularis* leaves and berries. The results revealed that the composition was characterized by α-Pinene (6.3-38.0%), limonene (7.0-34.6%), α-phellandrene (2.2–13.1%), and *p*-cymene (4.8–10.3%) as the major constituents of the oils from leaves. Also, β-myrcene (25.8%) and α-pinene (24.4%) were the major constituents of the oil from berries. Other research on the chemistry of *J. oxycedrus* from Lebanon (Loizzo *et al*., 2007) found significant differences in the essential oil content of berries and wood, which include α-pinene (27.4%) and δ-cadinene (14.5%), respectively. The essential oils obtained from *J. excelsa* berries and leaves in Turkey were 56.1% of the oil and the major compounds identified were α-pinene (34.0%), cedrol (12.3%), L-verbenol (5.4%), and D-verbenol (4.4%) from berries, Meanwhile, there was 63.2% of the oil from the leaves, and the major constituents were α-pinene (29.7%), cedrol (25.3%), α-muurolene (4.4%), and 3-carene (3.8%) (Topçu *et al*., 2005). In-depth research reveals that earlier studies have focused on the variance in the quantitative and qualitative content of the leaves, particularly the berry oil (Papadoupoulou, Kokous, 1995).

Antioxidant activity of the essential oil from the fruits of *Juniper oxycedrus*

The free radical scavenging activity of *J. oxycedrus* essential oil as determined by the DPPH method is presented in Table II. The concept of scavenging stable DPPH free radicals can be utilized to quickly assess anti-oxidative capabilities. As the radical is scavenged by antioxidants via hydrogen donation to create the stable DPPH-H molecule, the absorbance falls as the colour changes from purple to yellow (Mclafferty, Stauffer, 1989). The ability of antioxidants to donate hydrogen was assumed to be responsible for their influence on DPPH radical scavenging activity. The active chemicals found in essential oils from aromatic plants are primarily responsible for their antioxidant properties. This could be due to the high percentage of key elements, but it could also be due to the existence of other constituents in minor amounts or to the synergy between them. The antioxidant properties of essential oils of *J. oxycedrus* were investigated in this study using the DPPH radical scavenging assay in comparison to vitamin C as a reference antioxidant component (Table II). All experiments were conducted in duplicate. The data were presented as means ± SD. When compared to vitamin C (standard antioxidant compound), the essential oil of *J. oxycedrus* was found to have good antioxidant capabilities.

TABLE II - Scavenging activity (%) of *Juniperus oxycedrus* essential oils and vitamin C at different concentrations by the DPPH method

	Concentration (µg/mL)	% Inhibition of DPPH	IC50 (µg/mL)
Juniperus oxycedrus L.	25	10.45± 0.09	48.08
	50	18.58 ± 1.15	
	75	40.05 ± 1.02	
	100	48.10± 1.10	
	150	55.15± 0.57	
	200	81.87± 2.83	

TABLE II - Scavenging activity (%) of *Juniperus oxycedrus* essential oils and vitamin C at different concentrations by the DPPH method

	Concentration (µg/mL)	% Inhibition of DPPH	IC50 (µg/mL)
Vitamin C	25	39.81 ± 0.56	36.18
	50	59.11 ± 1.78	
	75	72.55 ± 1.89	
	100	78.10 ± 1.58	
	150	82.05 ± 0.55	
	200	86.11± 1.11	

DPPH: 1,1-diphenyl-2-picrylhydrazyl

The results from Table II indicate that the radical scavenging activity (% inhibition) of the essential oil from *J. oxycedrus* was the highest (81.87± 2.83%) at a concentration of 200 µg/mL. The scavenging activity of essential oil was observed to increase with increasing essential oil concentrations. When compared to the standard, all of the tested compounds had lower DPPH radical scavenging activity. The results show that a concentration of 200 ppm of *J. oxycedrus* essential oil inhibited DPPH (81.87 2.83%) nearly as well as the same concentration of vitamin C (86.11 1.11%). The highest IC_{50} value was observed in vitamin C (36.18µg/mL). In this study, the antioxidant activity of essential oil of *J. oxycedrus* collected from the Taferdoust region of Morocco was characterized by IC_{50} (48.08µg/mL). This was relatively higher than the IC_{50} of other plants such as *Mentha piperita* studied in Meknes, Morocco, with an IC_{50} value of 53.67µg/mL (Derwich, Benziane, Boukir, 2009). The essential oil of *J. oxycedrus* were able to reduce the stable, purple-coloured radical DPPH into yellow-coloured DPPH, achieving a 50% reduction with IC_{50} values. This could be due to the chemical composition of the essential oil since it contained primarily monoterpene hydrocarbons such as α-pinene, limonene (5.02%), and β-pinene. These chemicals have been shown to have weak antioxidant activity (Deba *et al.*, 2008; Tepe *et al.*, 2005). The main component of this essential oil (β-pinene) was tested for antioxidant activity using the DPPH method in the same context. The results showed low antioxidant activity (IC_{50}=12.46 ± 0.17 mg/ml) when compared to the essential oil in the present study (Rodrigues, Gonçalves, Vieira, 2021). In the meantime, Wang *et al.* (2008) discovered that β-pinene inhibited the DPPH test by 46.21+2.24%, and the β-carotene bleaching test by 94.49+0.61%.

The variation in DPPH radical scavenging activity between *J. oxycedrus* essential oil and other plant essential oils is due to the chemical composition of each essential oil. Furthermore, the oil used in this study contained chemical components such as β-pinene (42.04%), followed by limonene (15.45%), sabinene (9.52%), α–pinene (5.21%), (E)-caryophyllene (3.77%), ρ-cymene (1.56%), caryophyllene oxide (2.02%), myrcene (1.02%) and β phellandrene (1.01%). The essential oil of *J. oxycedrus* contained monoterpenes and oxygenated terpenes such as α-pinene, sabinene, limonene, β–pinene, caryophyllene oxide, and myrcene. Furthermore, when attempting to correlate the observed activity with the chemical composition of the oils, it is worth noting the work of Ruberto and Baratta (2002), who investigated the antioxidant activity of the chemical compositions of 98 pure essential oils. They discovered that monoterpene hydrocarbons had a significant protective effect, with several variants due to the different functional groups. Furthermore, El-Massry *et al.* (2002) demonstrated that essential oils high in non-phenolic molecules have antioxidant properties.

Antifungal activity of the essential oil from the fruits of *Juniper oxycedrus*

Table III depicts the results of the antifungal studies of the essential oil from the fruits of *J. oxycedrus*. The oil extract showed antifungal activity against fungal strains. In the agar disc diffusion experiment, the oil was found to be active against *A. niger* at a minimum inhibitory concentration (MIC) of 85. 01 µl/mL. According to the results, A. *niger* was the most sensitive test strain to the oil of *J. oxycedrus*, having the highest inhibitory zone (14.89 mm). With a MIC of 2.89 µl/mL, only minor activity was observed. These antifungal metabolites were also found in essential oil extracted from the fruits of *J. oxycedrus*. Furthermore, the results showed that *J. oxycedrus* was particularly active against *P. citrinum*, with a maximum zone of inhibition (12.98 mm). With a MIC of 2.89 µl/mL, only minor activity was recorded.

TABLE III - Antifungal activity of fruit essential oil from *Juniperus oxycedrus*

Micro-organism	*MIC (µl /mL)	**Disc diffusion assay (inhibition zone mm)
A. niger	2.89	3.25
	12.25	4.56
	20.89	5.89
	48.05	8.32
	52.89	9.19
	68.32	11.23
	81.79	11.65
	85.01	14.89
P. citrinum	2.89	2.02
	12.25	3.12
	20.89	3.20
	48.05	4.08
	52.89	6.56
	68.32	6.70
	81.79	10.25
	85.01	12.98

*MIC: Minimal inhibitory concentration, concentration range: 2.89 to 85.01 µl/mL;

**Disc diameter 6 mm average of two consecutive trials

A wide range of secondary metabolites have been shown to have antibacterial properties, particularly monoterpene hydrocarbons (α-pinene and β-pinene) (Dorman, Deans, 2000). Antimicrobial activities of essential oils have been primarily explained by terpenes with aromatic rings and phenolic hydroxyl groups capable of forming hydrogen bonds with the active sites of target enzymes. Although, other active terpenes, as well as alcohols, aldehydes, and esters, can contribute to the overall antimicrobial effect (Belletti *et al.*, 2004). The difference in antifungal activity is due to either higher concentration of the same chemical or different chemical compositions between plants. Several studies have been undertaken to better understand the mechanism of action of plant extracts and essential oils, however the mechanism is still unclear. Omidbeygi *et al.* (2007) proposed that essential oil and extract components that pass the cell membrane interact with the membrane's enzymes and proteins, causing a flux of protons towards the cell exterior, causing alterations in the cells and eventually death. According to Cristani *et al.* (2007), antimicrobial activity is connected to terpenes' ability to impact not just permeability but also other functions of cell membranes. These chemicals may cross cell membranes, penetrating the cell's core and interacting with important intracellular locations. Furthermore, β-pinene has been studied for its antifungal and antimicrobial activities. It has shown good potential in antifungal activities, such as in the studies conducted by Silva *et al.* (2012) and Salehi *et al.* (2019). They conducted numerous tests on the biological activities of this compound and its enantiomer, including β-pinene's antifungal activity, which appeared to be very important in terms of fungal strain inhibition.

CONCLUSION

The chemical analysis in this study allowed the identification of about 91.56% of the total volatile products for *J. oxycedrus*, as well as 48 volatile compounds. The most abundant constituent in fruits was α-pinene (42.04%), with an essential oil yield of 1.58%. The findings of the present study reveal that the essential oil of *J. oxycedrus* tested positive for antioxidant capacity when compared to a vitamin C standard. Essential oil from the fruits of *J. oxycedrus* was also found to have antifungal activity against fungal strains. There is a significant

relationship between the antioxidant capabilities and antifungal activity of *J. oxycedrus* essential oil and their chemical composition.

AUTHOR'S CONTRIBUTIONS

The physicochemical characterization of essential oil of *J. oxycedrus* by gas chromatography/ mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID) was performed by Elhoussine Derwich and Badiaa Lyoussi. The evaluation of the antioxidant activity was performed by Ibrahim Mssillou and Ghizlane Nouioura. Ahmed Elfallaki Elidrissi, Abdelkrim Agour, and Meryem Tourabi conducted the experiment involving the antifungal effects of essential oil against the *A. niger* and *P. citrinum* test fungal strains.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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