Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2022, 12(5), 31-39, doi: 10.15421/2022_372

ORIGINAL ARTICLE

Antibacterial activity and identification by GC/MS of the chemical composition of essential oils of *Juniperus phoenecea* and *Juniperus oxycedrus* L. from Western Algeria: Tiaret province

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Received: *08 May, 2022; Manuscript No: UJE-22-63119;* ***Editor assigned:*** *11 May, 2022, PreQC No: P-63119;* ***Reviewed:*** *21 May, 2022, QC No: Q-63119;* ***Revised:*** *26 May, 2022, Manuscript No: R-63119;* ***Published:*** *31 May, 2022.*

Extraction by hydrodistillation and characterization by GC/MS of essential oils from the aerial part of the two species of the *Juniperus* genus: *Juniperus phoenecea* and *Juniperus oxycedrus*, harvested in the province of Tiaret; showed the presence of seventeen compounds in *J. oxycedrus* oil, the main constituents of which were: hydrocarbon monoterpenes (69.2%), α-pinene (29.1%), β-copaene (19.3%), β-pinene (17.6%), limonene (12.1%), α-fenchene (5.1%). The *J. phenecea* manifested thirteen components, the main constituents of which were: β-pinene (35.7%), α-pinene (21.3%), β-coepane (8.3%) and p-cymene (6, 6%) and segmaterpinene (3.7%). While the antimicrobial activity of the two essential oils carried out on bacteria referenced (Gram+) and (Gram-); showed good activity on all the bacteria tested, with the exception of the *Pseudomonas* strain which proved to be very resistant to *Juniperus phoeneceae* while the *Juniperus oxycedrus* oil is active on all the bacterial strains tested unlike the strain *staphylococcus*.

Keywords: *Juniperus phoenecea*, *Juniperus oxycedrus*, Essential oil, Chemical composition, GC/MS, Antimicrobial property.

Introduction

Junipers are important precursors in the dynamics of forest groupings in Mediterranean vegetation, but also develop under extreme ecological conditions (Quezel et Medail, 2003). The genus *Juniperus* is an integral part of arid and semi-arid ecosystems in the northern hemisphere (Farjon, 1992; Adams, 2008). In the Algerian flora, five species are present: *Juniperus oxycedrus* L., *Juniperus Sabina* L., *Juniperus thurifera* L., *Juniperus communis* L. and *Juniperus phoenecea* L. (Harhour, 2018; Quezel et Santa, 1963).

Juniperus phoenecea L., also called red cedar, is an evergreen coniferous shrub or small tree belonging to the *Cupressaceae* family. In Algeria, it covers an area of 227,000 ha (Louni, 1994). It is considered an important medicinal plant widely used in traditional medicine. Its leaves are used as a decoction to treat rheumatism and diabetes. In powder form it is used against bronchopulmonary diseases and as a diuretic (Bellakhder, 1997). The mixture with berries is used as an oral hypoglycaemic agent (Amer et al., 1994). Dried and powdered fruits can cure skin ulcers and abscesses (Le Floc'h, 1983).

Juniperus oxycedrus L. called Taga in Algeria (Quezel et Santa, 1962) is distributed in different parts of the world, including the Mediterranean regions. It can be found in the Tell, mainly associated with holm oaks (*Quercus ilex* L.), cork oaks (*Quercus suber* L.), and even Aleppo pines (*Pinus halepensis*), and on mountainous massifs. Not very demanding in terms of soil, it can be found mainly on limestone, at meso- and supra-Mediterranean levels, in a sub-humid bioclimate. It can appear very locally in semi-arid bioclimates (Boudy, 1950). This plant is used in traditional Algerian medicine as a diuretic, stomach tonic and disinfectant (Baba Aïssa, 1991).

Materials and Methods

Plant material

Specimens of *Juniperus phoenicea* were collected from Ain Bezzez in the Naddor massif (eastern of Tiaret province) and *Juniperus oxycedrus* was collected from the western forest of Tiaret. Fruits and leaves were collected during their flowering period in January 2022 (Fig. 1-3).

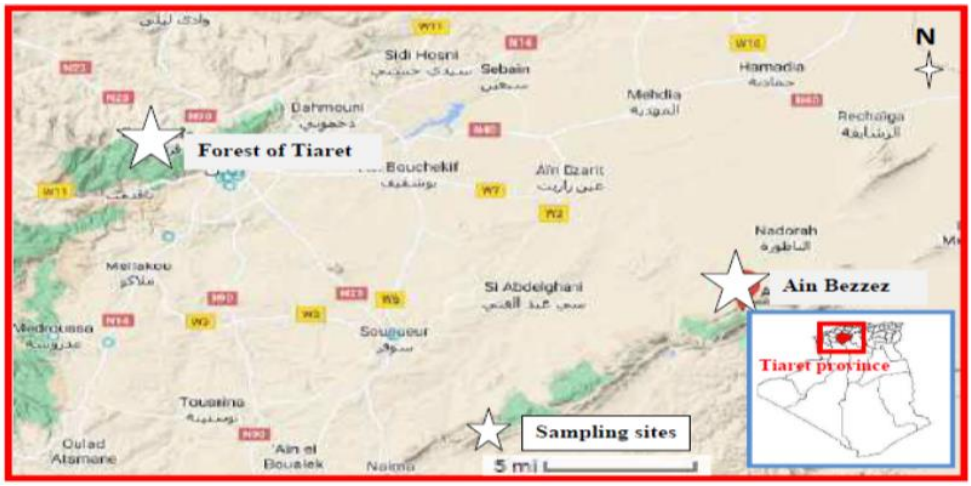

Fig. 1. Location of the sampling sites.

Fig. 2. *Juniperus oxycedrus*.

Fig. 3. *Juniperus phoenecea*.

Extraction of the essential oils

All samples were air-dried at room temperature in the dark and stored in paper bags in a cool, dry and dark place. Once the plants were dried, we hydro distilled them for 3 h using a Clevenger. The oil obtained was collected and dried by anhydrous sodium sulphate and collected in opaque glass bottles stored at 4-5°C. The yield was calculated on the basis of the dry weight of the samples in percent.

Analysis of essential oils

The essential oils of *Juniperus phoenecea* and *Juniperus Oxycedrus* were analysed by Heweltt Packard 6890 N gas chromatography, driven by a HP5MS capillary column of 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness. The analyses were carried out at isothermal temperatures, programmed at 60°C for 8 min, and then at a variable increase between 2°C and 250°C for 10 min. This operation was 113 minutes. The volume injected 0.2 µl at 250°C with one analysis mode. Scan Tic (35 to 600) and a solvent delay of 3.5 min at 280°C. The gas chromatograph is coupled to an Agilent 5973 mass spectrometer. The helium carrier gas was of N6.0 purity with a flow rate of 0.8 ml/min managed in split 1:100 mode. Ionisation was performed by electron impact at 70 ev, with an ion source temperature of 270°C. The results obtained were processed by *Workstation 8* software, supported by the software's internal database and by the NIST and AMDIS database. A series of alkanes was injected under the same operating conditions in order to calculate the retention index for each peak detected.

Antimicrobial activity

The sensitivity of microbial strains to essential oils was studied using the disc diffusion method against bacterial strains (Perez et al., 1990). The standards obtained were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11778, *Escherichia coli* ATCC 25922, *Pseudomenas aurus* ATCC 27853 and a yeast *Candidas albicans* ATCC 10231. Whatman sterile discs (6 mm diameter) were impregnated with 10 µL of essential oil and placed on the central surfaces of Müller Hinton agar inoculated with a bacterial or fungal suspension. After diffusion, the petri dishes were incubated at 37 C for 24 h for bacteria and at 25 C for 48 h for yeast, the diameters of the clear inhibition zones were measured with a caliper. All tests were performed in triplicate and the antibacterial sensitivity of the oils was expressed as the average of the inhibition diameters produced (in mm).

Determination of the minimum inhibitory concentration (MIC)

MIC is the lowest concentration of essential oil at which no bacterial entrainment is observed. The calculation of MIC was performed by the microdilution method (Andrews, 2001), using 96-well plates. A fixed volume of Mueller Hinton broth placed in each well followed by the presence of each strain tested and a dilution of the essential oil, with the exception of the positive control consisting of 100ul MH broth with 20 ul inoculum and the negative control consisting of MH broth alone. Plates were incubated for 24 hours, distinguishing susceptible from resistant strains for each concentration by the presence (+) or absence (-) of a deposit at the bottom of the well and the cloudiness.

Results and Discussion

Chemical composition of essential oils

GC-MS analysis (Table 1) (Fig. 4) of the essential oils obtained from *J. phenecea* with a yield of 0.8% showed the presence of seventeen identified compounds representing 90.3% of the total oil composition. The main compounds were β-pinene (35.7%), followed by α-pinene (21.3%) beta-copaene (8.3%), p-cymene (6.6%), γ-terpinene (3.7%), γ-muurlene (3.6%), (E)-β-caryophyllene (2.1%).

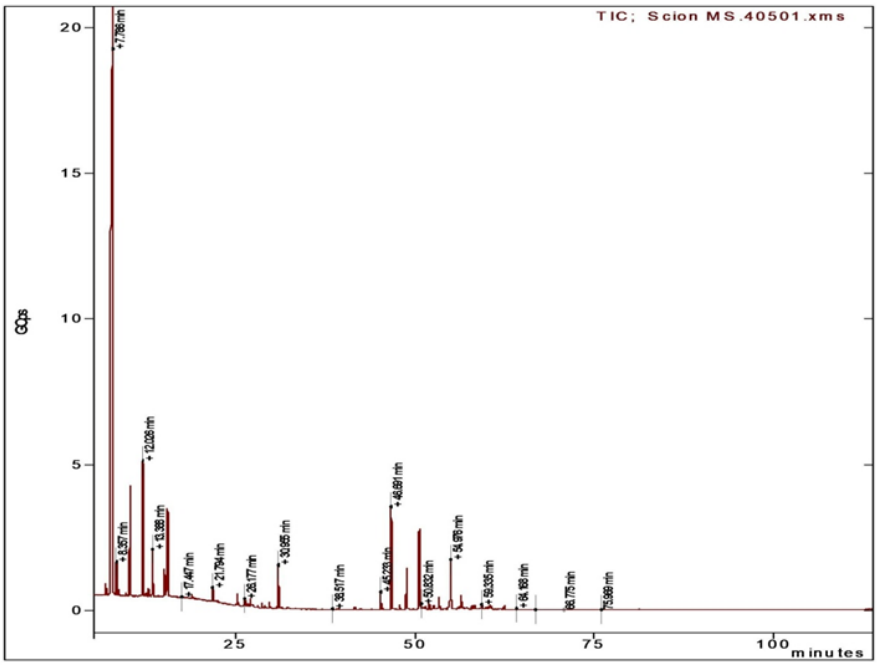

Fig. 4. GC-MS chromatogram of *J. Phoenicea* essential oil (Abundance versus retention time in min).

Many studies have been carried out on the chemical composition of *J. phenecea* oil in different regions. Derwich et al, 2001 in Tunisia, found that the yield of essential oil of *J. phoenecea* was 1.62% and the main compound in the aerial parts was α-pinene

(49.15%), followed by α-phyllandrene (7.39%), mycene (5.24%), β-pinene (3, 58%), linalool (2.54%), piperitone (1.56%), γ-terpinene (1.28%), trans-pinocarveole (1.23%) ρ-cymene (1.10%), α-terpineol (1.02%) and γ-cardinene (1.01%). By comparison of our results, we noted, the absence of α-pherlandrene and the presence of a β-pinene component (35.7%) as the first major component; however, β-myrcene and caryophyllene oxide and 4-Epi-Cubebol shows a lower yield in the order (1.3% and 0.1%, respectively). This variation could be due to geographical and bioclimatic factors of the provinces. On the other hand (Fouad et al., 2011) in Morocco revealed the presence of sixty-three volatile compounds that represent 52-92% of the total petroleum compositions. The main monoterpenes were α-pinene (26.7-78.7%) and δ-3carene (7.6-15.4%). According to (Ramdani et al., 2013) collected from Boutaleb (Setif), Boussâada (M'sila), Menâa and T'kout (Batna), and Elhadjab (Biskra) showed that the average yield of essential oil from the samples is 0.82%, The chemical composition of *J. Phoenecea* is dominated by the presence of a major product, α-pinene (36.3-55.9%). Three components are represented with high concentrations, terpinolene (0-13%), Δ3-carene (0-12.4%) and β-phellandrene (0-7.3%). Another work showed that the main components of *J. phenecea* leaf oil (Abdeli et al., 2018) were mainly composed of β-phellandrene (44.9%), α-pinene (20.3%), myrcene (8.2%), α-phellandrene (4.5%) p-cymene (3.0%) and limonene (2.5%). Harhour et al., 2018; found that the main constituents α-Pinene (67.7 4.3%) were the main compound oils, followed by δ-3-carene (26.8 2.3%) and α-cedrol (7 1.1%). From the comparison study between the cited works and the results found, we note the presence of several similar products at comparable concentrations, the only difference being the presence in the extracted oils of β-Pinene as the majority product, unlike the other studies where the majority product is α-Pinene.

Table 1. Chemical composition of essential oil of analysis *Juniperus Phoenecea*.

S. No	Compounds[a]	RI_a[b]	RI_a[c]	% [d]	Identification[e]
1.	α –pinene	939	942	21.3	RI,MS
2.	*α* –Fenchene	947	947	0.1	RI,MS
3.	Camphene	948	946	1.6	RI,MS
4.	β –Pinene	978	975	35.7	RI,MS
5.	α- Phelllandrene	1004	1001	1.3	RI,MS
6.	γ-Terpinene	1058	1055	3.7	RI,MS
7.	p-cymenene	1064	1067	6.6	RI,MS
8.	Terpinolene	1087	1089	0.9	RI,MS
9.	α – Campholenal	1125	1122	0.7	RI,MS
10.	Camphor	1138	1142	1.3	RI,MS
11.	α- Terpineol	1189	1193	1.6	RI,MS
12.	(E)-β- Caryophellene	1421	1419	2.1	RI,MS
13.	β- Copaene	1435	1431	8.3	RI,MS
14.	γ-Muurolene	1473	1474	3.6	RI,MS
15.	4-Epi-Cubebol	1493	1490	0.1	RI,MS
16.	γ-Cadinene	1517	1514	0.1	RI,MS
17	Oxyde de Caryophellenes	1582	1583	1.3	RI,MS
	Total identification %				90.3
	Monoterpene hydrocarbons				71.2
	Oxygenated monoterpenes				3.6
	Sesquiterpene hydrocarbons				14.1
	Oxygenated sesquiterpenes				1.4

Legend: [a]Order of elution are given on the apolar (DB5) column, [b]Retention indices of literature on the apolarcolumn (RIa) reported from the literature, [c]Retention indices on the apolar (DB5) column (RIa), [d]percentage, [e]RI: Retention Indices; Mass Spectrometry in electronic impact mode.

GC-MS analysis (Table 2) (Fig. 5) of *J. Oxycedrus* oil with a yield of 1.8% showed the presence of thirteen identified compounds representing 90.3% of the total oil composition. The main compounds were α-pinene (29.1%), β-copaene (19.3%), β-pinene (17.6%), limonene (12.1%), α-fenchene (5.1%), (E)-β-caryophyllene (3.7%) and β-ylangene (2.5%). Many studies have identified the chemical composition of J. oxycedrus oil in different regions. *J. oxycedrus* from Djelfa (Algeria) (Dob et al., 2006) showed that the main components were trans-pinocarveol (7.0%), cis-verbenol (6.3%) and manoyl oxide (6.0%), respectively. The main constituents identified by Fadel et al. in 2019 were manoyl oxide (23.5%), pentadecane-2-enene 6Z (12.6%), abietatriene (8.0%), abieta-8,11,13-triene-7-one (6.5%), cubebol (4.6%), epi-torilenol (3.8%) and a-cadinol (2.6%). Regarding the work of (Boudiba et al., 2021), the main constituents found in the fruit oil were α-pinene (57.5%), α-amorphene (9.0%), β-myrcene (8.0%), δ-cadinene (1.7%), manoyloxide (1.5%) and zonarene (1.4%). Several studies on the same plant from different parts of the world collected in

El pennon (Spain) (Adams, 1998) revealed that the main compounds were a-Pinene (41.3%) (a-Phellandrene (8.2%) p-Cymene (6.2%). From Crysortis (Greece) (Adams et al., 1999) found that the main components a-Pinene (42.7%), Limonene (17.1%), d-3-Carene (13.7%). Angioni et *al.,* 2003; from Sardinia (Italy) the main constituents were a-Pinene (85.6%) d-3-Carene (2.8%) c-Terpinene (1.4%). According to (Loizzo et al., 2007); *J. oxycedrusssp. oxycedrus* berry oil was characterised by high contents of α-pinene (27.4%) and β-myrcene (18.9%). Other important compounds were α-phellandrene (7.1%), limonene (6.7%) and δ-cadinene (2.2%). For Alan et al., 2016; the main compounds in the oil of *J. oxycedrus* from Turkey were manoyl oxide (32.8%) and caryophyll oxide (11.9%) in the leaf oil, myrcene (44.6%), a-pinene (19.9%) and D-germ (15.5%) in the berry oil, and manoyl oxide (35.4%) and caryophyll oxide (16.8%) in the twigs.

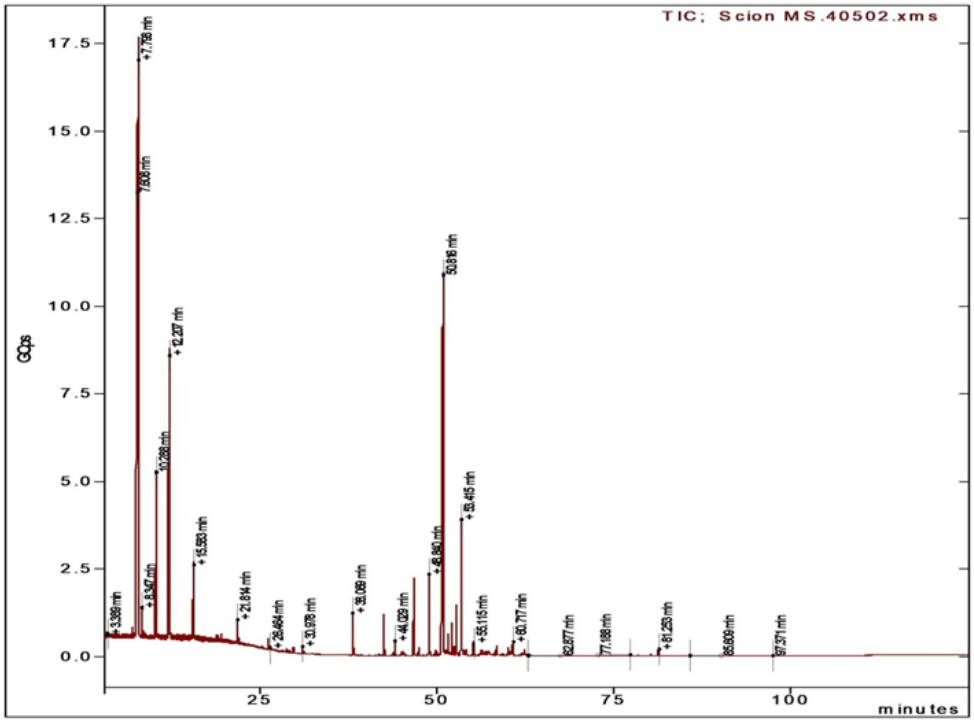

Fig. 5. GC-MS chromatogram of *J. Oxycedrus* essential oil (Abundance versus retention time in min).

Table 2. Chemical composition of essential oil of analysi*s Juniperus Oxycedrus*.

S.No	Compounds[a]	RI_a[b]	RI_a[c]	%[d]	Identification[e]
1.	α-Thujene	932	929	2,3	RI,MS
2.	*α*–pinene	939	937	29,1	RI,MS
3.	α-Fenchene	947	945	5,1	RI,MS
4.	Camphene	948	950	0,9	RI,MS
5.	Sabinene	971	970	2,1	RI,MS
6.	β-pinene	978	976	17,6	RI,MS
7.	Limonene	1021	1020	12,1	RI,MS
8.	β- Ylangene	1375	1373	2,5	RI,MS
9.	(E)- β- Caryophellene	1421	1419	3,7	RI,MS
10.	β- Copaene	1435	1137	19,3	RI,MS
11.	γ-Muurolene	1473	1471	1,6	RI,MS
12.	4-Epi-Cubebol	1493	1492	0,3	RI,MS
13.	γ-Cadinene	1517	1515	0,1	RI,MS
	Total identification %				96.7
	Monoterpenehydrocarbons				69.2
	Sesquiterpenehydrocarbons				27.2
	Oxygenatedsesquiterpenes				0.3

Legend: [a]Order of elution are given on the apolar (DB5) column, [b]Retention indices of literature on the apolarcolumn (RIa) reported from the literature, [c]Retention indices on the apolar (DB5) column (RIa). [d]Percentage, [e]RI: Retention Indices; Mass Spectrometry in electronic impact mode.

The antimicrobial activity

The results of the aromatogram of *J. phoenecea* and *J. oxycedrus* essential oils against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus* and *Candida Albicans*: ATCC10231 are shown in Table 3.

Table 3. Antibacterial activity by zone of inhibition of *J. phoenecea* and *J. oxycedrus* oils.

Microorganisms	**EO *J. Phenecea***	**EO *J. Oxycedrus***
Strains	D (mm)	D (mm)
Escherichia coli	20 mm	30 mm
Staphylococcus aureus	47 mm	20 mm
Bacillus cereus	20 mm	25 mm
Pseudomonas aeruginosa	28 mm	21 mm
Candidas abicans	28 mm	30 mm

J. phoenecea oil exerts strong antibacterial activity against the referenced strains tested. It was found that *Staphylococcus aureus* ATCC11778 seems to be extremely most sensitive to *J. phoenecea* oil tested with a diameter of 47 mm and moderately sensitive with our oil against strains *(Pseudomonas aeruginosa:* ATCC10231 and *Candida albicans* yeast: ATCC10231) with a diameter of 28mm and weakly sensitive with *Escherichia coli*: ATCC25922 and *Bacillus cereus*: ATCC25923 with a diameter of 20 mm.
The results are read as follows (Djabou et al., 2013).

- Resistant (-): diameter ≤ 8 mm
- Moderately sensitive (+): diameter between 8 and 14 mm.
- Sensitive (++): diameter between 14 and 20 mm.
- Extremely sensitive (+++): diameter >20 mm.

Actually, the essential oil of *J. oxycedrus* berries showed different inhibitory activities on the strains. Especially against *Staphylococcus aureus* ATCC11778, exerted medium activity with a diameter of 20 mm, on the other hand phoenecea oil significantly inhibited the growth of *Staphylococcus aureus* ATCC11778. While a significant and higher activity than phoenecea oil was found against *Escherichia coli*: ATCC25922 and Candidas albicans: ATCC10231 with a diameter of 30 mm and against *Bacillus cereus*: ATCC25923 with a diameter of 25 mm. *Pseudomonas aeruginosa*: ATCC10231 which is also a gram negative bacterium showed the smallest diameter of 21 mm against the oxycedar oil. The inhibitory power of the essential oils was confirmed by MIC tests. The minimum inhibitory concentrations (MIC) in µg/ml of the essential oils of *J. phoenicea* and *J. oxycedrus* obtained by the direct contact method are shown in Table 4 and 5, where they were strongly correlated with the diameters of the inhibition zones. According to the sensitivity, the lowest MIC (3.12 µg/mL) was obtained for the essential oil of *J. phoenecea* on the strains of *Pseudomonas aeruginosa*: ATCC10231 and *Bacillus cereus*: ATCC25923. In the case of *J. oxycedrus* essential oil, the MIC value was not determined against the *Staphylococcus aureus* strain ATCC11778, which proved to be extremely resistant.

Table 4. MIC of *J. phoenecea* oil.

Strain	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.56**	**0.78**	**0.3**	**0.1**	**0**	**T+**	**T-**
E. coli	-	MIC	+	+	+	+	+	+	+	+	+	-
Pseudomenas	-	-	-	-	MIC	+	+	+	+	+	+	-
Staph	-	-	MIC	+	+	+	+	+	+	+	+	-
Bacillus	-	-	-	-	MIC	+	+	+	+	+	+	-
Candida	-	-	MIC	+	+	+	+	+	+	+	+	-

Table 5. MIC of *J.* Oxycedrus oil.

Strain	**50**	**25**	**12.5**	**6.25**	**3.12**	**1.56**	**0.78**	**0.3**	**0.1**	**0**	**T+**	**T-**
E. coli	MIC	+	+	+	+	+	+	+	+	+	+	-
Pseudomenas	-	MIC	+	+	+	+	+	+	+	+	+	-
Staph	+	+	+	+	+	+	+	+	+	+	+	-
Bacillus	MIC	+	+	+	+	+	+	+	+	+	+	-
Candida	-	MIC	+	+	+	+	+	+	+	+	+	-

Our results reveal that our plant oil collected from the Tiaret region showed a good inhibitory activity on Gram-negative bacteria such as *Pseudomonas aeruginosa*: ATCC10231 and Gram-positive bacteria *Staphylococcus aureus*: ATCC11778; on the other hand, it is weakly active on the *Escherichia coli* strain: ATCC25922. The bacteria most sensitive to the action of the essential oils studied in our study are the Gram-positive bacteria. According to the zones of inhibition and MIC values generated by the essential oils studied, the essential oil of *J. phoenecea* presents the best activity on all the strains tested. Of the two oils tested, the antimicrobial

activities were recorded on all the strains are almost in agreement with those reported by (Rahhal et al., 2019) in which it was shown against *Staphylococcus aureus* by *J. oxycedrus* oil. On the other hand, according to the study of (Sela et al., 2013) revealed lower activities when compared to the activity found in our study which enhances the antibacterial effect of J. *J. oxycedrus* oil (<20 mm) towards *Escherichia coli*, *Pseudomonas aeruginosa* and *Candidas albicans* strains. The inhibitory effect of *J.phenecea* oil against the strains shows significant results compared to the studies of (Rahhal et al., 2019, Mazari et al., 2010; Ennajar et al., 2009) shows no antibacterial activity against *Escherichia coli*. Other works such as (Abdeli, 2018) show significant results against *Bacillus* and *Candidas albicans* by a diameter of 34.67 mm and against *Escherichia coli*, with a similar effect to our results on *Candidas albicans* (Bouyahyaoui et al., 2016). These results are difficult to compare as the methods used are different. The choice of the extraction protocol, other factors such as climate, region and harvesting period of the species studied..., etc., are all factors that may influence the results. Therefore, the structural of the cell wall of (Gram+) bacteria is less complex than that of (Gram-) bacteria. This structural difference makes it less sensitive to the effects of essential oils and plant extracts (Kalemba and Kunicka, 2003). According to (Nikaido, 2003); Gram- negative bacteria have the outer membrane as an effective permeability barrier; the lipopolysaccharide, thanks to its negative surface charges prevents the diffusion of hydrophobic molecules, while Gram+ positive bacteria are less protected because the wall is formed by a peptidoglycan layer only which hinders the diffusion of molecular weights at 50 KD. The chemical composition of the essential oils studied for their antibacterial activity is dominated by the presence of hydrocarbon molecules. Many works have also confirmed that the activity of essential oils is related to most of the compounds and to a possible synergy between the components (Oussou et al., 2008; Oussou et al., 2010; Saint, 2003, Kalemba and Kunicka, 2003).

Conclusion

The essential oil of the aerial part of *J. oxycedrus* is mainly dominated by the hydrocarbon monoterpene class (69.2%). It should be noted that this family is characterised by the abundance of α-pinene (29.1%) and β-pinene (17.6%). The hydrocarbon sesquiterpenes (27.2%) are clearly dominated by β-copaene (19.3%). While, the *J. phoenecea* essential oil profile is also characterised by its richness in hydrocarbon monoterpenes (71.2%) with β-pinene (35.7%) as the majority compound. The results of this work show that the variation in the chemical composition of *J. phoenecea* and *J. oxycedrus* essential oils in the Tiaret region may be due to various factors such as: collection region, harvesting season, species and subspecies, plant parts and fruit maturity, as well as to the extraction method used, genetic background of the species or environmental factors such as altitude, climate, soils and precipitations. The microbiological study revealed that our oils possess antimicrobial properties, which can be used as natural antimicrobial agents for human and especially infectious pathologies and also as food or in medicinal formulations. In addition, the presence of major compounds such as terpenes (α-pinene, β-pinene, limonene, sabinene) in these oils, they promote it and give it this faculty to have this strong antimicrobial and antifungal activity very interesting.

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Citation:

Boukhaloua, A.H.A., Berrayah, M., Bennabi, F., Ayache, A., Abdeldjebar, F. (2022). Antibacterial activity and identification by GC/MS of the chemical composition of essential oils of *Juniperus phoenecea* and *Juniperus oxycedrus* L. from Western Algeria: Tiaret province. *Ukrainian Journal of Ecology.* 12:31-39.