# Phytochemical, antioxidant and antibacterial study of essential oils of the leaves and fruits of *Juniperus Phoenicea*

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**Abstract:** Extracts from aromatic and medicinal plants contain a variety of phenolic compounds as well as essential oils (EOs) which are believed to have antioxidant and microorganism inhibiting activities.

Essential oils of leaves and fruits of *Juniperus Phoenicea* collected in the region of Midelt (Morocco) were obtained by the technique of hydrodistillation and analyzed by Gas Chromatography coupled to Mass Spectrometer (GC-MS).

The yield of essential oil is variable depending on the part of the plant studied; it is 0.5% for leaves and 0.14% for fruits. The main compounds of the essential oil of the leaves are α- pinene, Caryophyllene and β- phellandrene while the EO of the fruits is largely dominated by α- pinene.

These EOs have a marked antioxidant activity but still remains lower than that of the decocted leaves and fruits of the same plant studied, this activity measured by the DPPH method.

An antibacterial activity of these EOs was also demonstrated by the aromatogram method, with a strong inhibitory activity for the fruit EO compared to that of the leaves.

**Keywords:** Antibacterial activity, Antioxidant activity, GC-MS, *Juniperus Phoenicea*

## Introduction:

Oxidative stress and microbial antibiotic resistance are two major global problems.

Oxidative stress is a profound imbalance between pro-oxydants and antioxidants, caused by an endogenous or exogenous production of oxygenated free radicals, which leads to irreversible cellular damage. (Sies 1997)

The excess of free radicals not neutralized by the means of defense is very harmful for the essential macro-biomolecules (leading to abnormalities in gene expression, cell proliferation

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or death, immune disorders...) (Favier 2003), by causing the appearance of various diseases such as neurodegenerative diseases (Alzheimer...), cardiovascular diseases, cancers, aging of the skin... (Favier 2006); this sparks the search for new antioxidant remedies.

Indeed, natural antioxidants are the subject of much research and a new breath towards the exploitation of secondary metabolites generally and polyphenols particularly, to fight malignant diseases such as cancer, noting that the powerful effectiveness of these substances to neutralizing free radicals is mainly due to their phenolic structures. (Belyagoubi 2011).

The misuse of antibiotics and their increased use have resulted in the appearance of certain forms of resistance of microbial strains to antibiotics (Goossens and al. 2005), Indeed, certain bacterial strains manage to re-establish multi-resistance against several antibiotics at the same time giving rise to what is called multi-antibiotic resistant bacteria. (Chaudhary 2016).

Faced with this problem, many studies have been realized to develop alternative molecules effective against these microbial strains. Aromatic and medicinal plants (AMP) constitute an important source of bioactive molecules that could be exploited in the therapy of infectious diseases (Talbaoui and al. 2012; Griffin and al. 1999).

AMPs are a great source of natural antioxidants and antimicrobials. Indeed, different aromatic plants are characterized by the biosynthesis of odorous molecules (essential oils) known for a long time for their antiseptic and therapeutic activities in traditional medicine (El Kalamo Uni 2010).

Thanks to Moroccan Mediterranean climate and its geomorphological characteristics, it leads to suitable conditions for the development of rich and varied vegetation that includes an important potential in aromatic and medicinal plants often endemic that are reflected in its very diverse vegetation. (Morocco-AMP 2006).

Junipers have a significant place in the Mediterranean flora, in Morocco there are four species, which are *Juniperus Oxycedrus*, *Juniperus Communis*, *Juniperus Phoenicea*, *and Juniperus Thurifera*. (Benabid 2000; Quezel and Medial 2003; Mansouri and al. 2011a).

*Juniperus Phoenicea (J. Phoenicea*) of the Cupressaceae family is a species widely used in traditional medicine: the leaves are used in the form of decoction to treat diabetes, diarrhea and rheumatism, they are used against bronco-pulmonary diseases and as diuretic (Bellakhder 1997), while the dried and powdered fruits can heal skin ulcers and abscesses. (Uphof 1968; Le Floch 1983; Qnais and al. 2005; Aouadhi 2010). The mixture of leaves and fruits of *J. Phoenicea* are used as an oral hypoglycemic. (Bellakhder 1997; Alejandro and al. 2004).

*J. phoenicea* consists mainly of an essential oil rich in terpene hydrocarbons, among which we find mainly pinene and terpineol, as well as a bitter principle, juniperin. (Aouadhi 2010).

The yield of EOs from *J. phoenicea* is variable depending on the subspecies and the part used of this plant, it is acceptable and can be profitable on an industrial scale. Moreover, the majority compounds of these oils present several interesting biological activities. (Adams 1996; Stassi and al. 1996; Akrout 2001; Glulluce and al. 2007). EOs from leaves and cones that have been shown to possess primarily antimicrobial activity. (Ait-Ouazzou and al. 2012).

In the region of Midelt in Morocco, the *j. phoenicea* is more responded; this plant and mainly its leaves are frequently used in traditional medicine for the treatment of several diseases such as diseases of the digestive tract.

**Objective**

The objective of this work is to extract the essential oils from the leaves as well as from the fruits of *Juniperus Phoenicea* harvested in the region of Midelt - Morocco, and to determine their chemical composition, in order to evaluate the antioxidant activity of the extracts and essential oils of Juniperus Phoenicea and the antibacterial activity of the HEs of the same plant.

**Materials and methods:**

1. **Plant material**

The plant of *J. Phoenicea ssp* (Turbinata) was harvested during the period of March 2014 in the region of Midelt Morocco, it was placed in a paper and put in a well-ventilated place and exposed to the light of day and to the protected from the sun to preserve its aromatic compounds. After the plant has dried, the leaves are isolated from the fruits to work with each part of the plant separately.

2. **Extraction process of essential oils**

The essential oils of leaves and fruits of *J. Phoenicea* have been obtained by the hydrodistillation technique (Lucchesi 2005).

For the realization of our hydrodistillation, we have first prepared 500g of the dry leaves and 700g of the equal dry fruits of *J. Phoenicea*, then these quantities have been introduced in the flask of 2000ml for each of the two experiments, we added 800ml of distilled water for the leaves and about 700 ml for the fruits. The essential oils obtained stored in opaque and tightly closed tubes and stored at a temperature of 4° to 5°C until their use.

The second product is nothing other than the solution collected in the flask where the part of the plant studied was soaked, this product called the decoctate.

### 3. Determination of the yield of essential oils

The yield of EO for each sample corresponds to the ratio between the quantity of EO extracted and the mass of dry plant material to be treated. The yield is expressed as a percentage (%) and calculated by the following formula:

$$R\% = (Me/Mp) * 100$$

R%: yield of essential oil in %, Me: the mass of EO in grams (g), Mp: the mass of the plant material in grams (g).

### 4. Analysis of the chemical composition of essential oils (EOs) by GC/MS

The phytochemical study of the HEs was carried out at the Regional University Interface Centre (RUIC). The separation and identification of the different chemical compounds of the essential oils extracted from the leaves and fruits of J. Phoenicea were carried out by phase chromatography gaz (Ultra GC Trace), coupled to a mass spectrometer of the type (PolarisQ), Ion trap in electronic impact mode (EI) with an ionization energy of 70 eV. The column used is an apolar capillary column in silica type (Wcot Fused Silica), stationary phase (CP-SIL5CB), 50 m length with a diameter of 0.32 mm and 1.25 µm thicknesses. The temperature of the column is programmed from 40 to 280 ºC at a rate of 3 ºC/min. The temperature of the injector is fixed at 240 ºC and that of the detector (ionization source) is 200 ºC. The flow rate of the carrier gas (Helium) is fixed at 1 ml/min. The volume of the sample injected is 1µl of the oil diluted in Hexane. The constituents of the essential oil were identified by comparing their mass spectra with those listed in a library of type (NIST-MS). (Adams 2007).

### 5. The antioxidant power of *Juniperus Phoenicea* extracts

#### 5.1. Evaluation of the antioxidant activity by the DPPH method

The antioxidant activity in vitro was evaluated by measuring the scavenging power of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant power of extracts from leaves and fruit (essential oils and decocts) were estimated by comparison with butylated hydroxytoluene (BHT).

In our study, The DPPH test is carried out at room temperature, this making it possible to eliminate any risk of thermal degradation of thermolabile molecules, it is carried out by following the method described by Burits and Bucar (2000).Where 250µl of each of the ethanolic solutions of the EOs tested at different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml, 125 mg/ml, and 250mg/ml for the leaves of *J. Phoenicea*; and 500mg/ml, 1000mg/ml, 1500mg/ml, and 2000 mg/ml for the fruits of J. Phoenicea), likewise 250 µl of the decoctate tested at different concentrations (156 µg/ml, 312 µg/ml, 625 µg/ml, 1250

µg/ml, 2500 µg/ml, and 5000 µg/ml for the leaves of J. Phoenicea; and 2760 µg/ml, 5520 µg/ml, 11042 µg/ml, 22083 µg/ml, 44167 µg/ml, and 88330 µg/ml for the fruits of *J. Phoenicea*) are mixed with 1.5 ml of an ethanolic solution of DPPH and 125µl of 96 % ethanol. After an incubation period of 30 minutes at laboratory temperature, the absorbance is read at 515nm.The inhibition of the free radical DPPH by BHT (a phenol which is in the form of yellowish white crystals, it is used for its antioxidant properties in cosmetics; it is a strong antioxidant) was also analyzed for comparison. The Parameters for calculating the antioxidant activity are determined for BHT, essential oils and decocts.

**5.2. Parameters for calculating antioxidant activity**

- **Percent inhibition:** the percent inhibition (I%) of DPPH is calculated as follows:

$$I\% = {}^{(Ab - As)}/_{Ab} * 100$$

With: I%: Percent inhibition, Ab: Absorbance of blank (DPPH in Ethanol), As: Absorbance of the test sample.

- **$IC_{50}$ :** This is the concentration of antioxidants necessary to reduce the initial concentration of 50% of free radicals DPPH; it is inversely related to the antioxidant capacity.
- Anti-radical power ARP: it is calculated by the following formula:

$$ARP = {}^{1}/_{IC_{50}}$$

**6. The antibacterial power of essential oils**

**6.1. The microorganisms used**

The choice of bacteria was based on five strains common in human pathology: *Klebsiella Pneumoniae, Proteus, E.coli* and *Pseudomonas* which are gram negative bacteria, and *Staphylococcus aureus* which is a gram positive bacterium.

These strains are placed in sterile tubes containing liquid culture medium (nutrient agar) and incubated at 37 ºC.

**6.2. The culture medium used**

The culture medium used for antibacterial activity is LB nutrient agar (lysogenic broth). We distinguish: the liquid medium which was used for the preparation of the inoculum of the strains, and the solid medium which was used for the culture of the bacteria and allows to work easily with the method employed.

### 6.3. Antibiotics used

We used five control antibiotics to test the sensitivity of the bacterial strains including: Nitrofurantoin (300µg): F-300, Pristinamycin (15µg): PT-15, Cefotaxine (30µg): CTX-30, Vancomycin (30µg): VA-30, Levofloxacin (5µg): LVX-5.

### 6.4. The aromatogram method

In our study, we used the solid medium aromatogram method (Gurini and Carrei 1999; Benzaggouta 2004) for the evaluation of the antibacterial activity of essential oils; 250µl of inoculum is placed on each dish and then spread this suspension on the agar surface. After sterile filter paper discs 6 mm in diameter (Wattman paper) soaked with 20 µl of essential oil and antibiotic discs were placed on the surface of nutrient agar inoculated with the bacterial suspension. After incubation at 37 ºC for 18 to 24 hours, sensitivity was assessed by measuring the inhibition diameter.

The results obtained are compared with those of the antibiotics (already mentioned) tested on the same bacterial strains and by the same method.

**Results and discussion:**

**I. Essential oil yield and chemical composition**

The yields of EOs extracted from leaves and fruits are 0.5 % and 0.14 % respectively. For leaves the yield is almost similar to that obtained for the same species in Tunisia (0.5 to 0.9%) (Vitti and al. 2005; Wallace 2004; Williams and al. 2009). It remains higher than that of red juniper from Greece (0.21%) (Adams and al. 1996), of the subspecies Turbinata from Spain (0.3%) (Adams and al. 1996), from Egypt (0.36 %) (El-Sawi and al. 2007). For fruits, the yield is lower than that from the fruits with 0.96 % for Egypt (El-Sawi and al. 2007).

This variation in yield can be explained by different factors such as the age of the plant, the harvest period, and the specific geographical location of the species...

**I.2. the chemical composition of essential oils**

**I.2.1. the leaves of *Juniperus Phoenicea***

Figure 1 shows the rate of the different compounds of the essential oils of the leaves of *J. phoenicea* and Table 1 shows the chemical composition of these essential oils as well as their major compound, their chemical formulas but also the total number of peaks, their retention time (RT) and the area of each peak.

Indeed, the sample of the essential oil from the leaves of *J. Phoenicea* analyzed contains 12 components with a yield of 0.5 %. It is wealth of terpene components ($C_{10}H_{16}$) and

sesquiterpene components ($C_{15}H_{24}$) and many others. But still, it informs us about the majority compounds. These include α-pinene (60.21 %), Caryophyllene (11.63%), β-phellandrene (4.48%), 3- carene (3.51 %), Limonene (3.11 %), Myrcene (2.47 %), β-pinene (2.15 %), and others.

**Figure 1:** Chromatographic profile of the EO of the leaves of J. Phoenicea

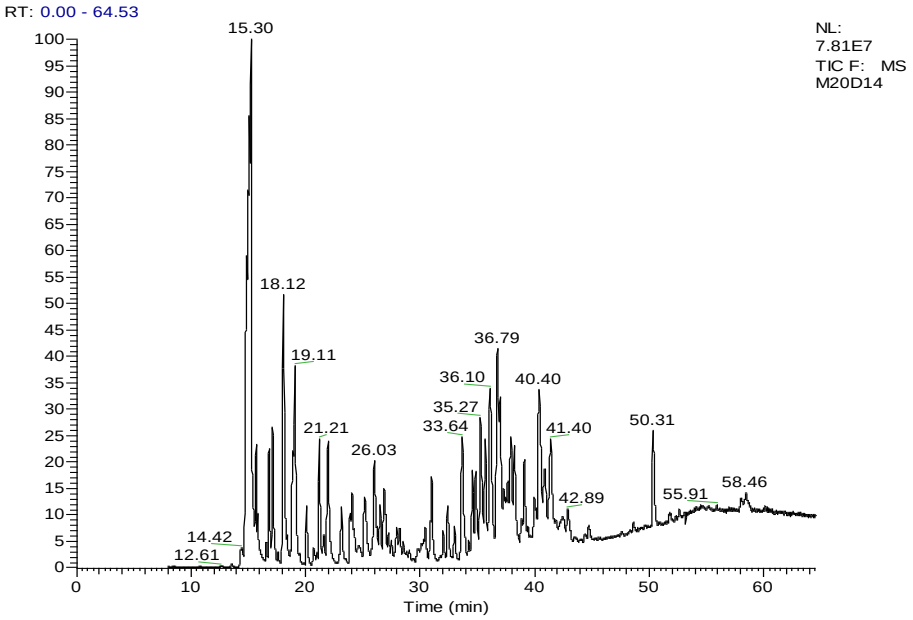



**Table 1**: Chemical composition of the EOs of the leaves of J. phoenicea; RT: retention time (in min); Air: chromatographic peak areas

| Peaks | RT (min) | Chemical names of compounds | Chemical formulas | Area (%) |
|---|---|---|---|---|
| 1 | 15.3 | α-pinene | C10H16 | 60.21 |
| 2 | 21.21 | β –pinene | C10H16 | 2.15 |
| 3 | 24.08 | Myrcene | C10H16 | 2.47 |
| 4 | 25.15 | α-phellandrene | C10H16 | 1.66 |
| 5 | 26.03 | 3- carene | C10H16 | 3.51 |
| 6 | 31 | p- cymene | C10H14 | 2.12 |
| 7 | 33.64 | Limonene | C10H16 | 3.11 |
| 8 | 35.27 | β- phellandrene | C10H16 | 4.48 |
| 9 | 36.1 | Terpenoline | C10H16O | 3.49 |
| 10 | 37.94 | α-terpinyl acetate | C12H20O2 | 2.83 |
| 11 | 40.4 | Caryophyllene | C15H24 | 11.63 |
| 12 | 50.31 | Germacrene- D | C15H24 | 2.36 |

The chemical composition of essential oil of the leaves is almost identical to that obtained by Ait Ouazou and al. (2012), who examined the chemical composition of the EO of J. Phoenicea leaves from Morocco, but with different percentages. They found that the predominant constituents are: α- pinene, (24.9 %), β- phellandrene (24.4 %), α- terpinyl acetate (12.9 %), Myrcein (4 %), α- phellandrene (3.7 %), germacrene D (2.4 %), terpenolene (1.9 %), and β-Caryophyllene (1.2 %).

These results are different from those obtained by Rezzi and al. (2001), who examined the chemical composition of EO from the leaves of J. Phoenicea of Corsica. They found 30 components with a predominance of α- pinene, β- phellandrene, and terpinyl acetate.

Mansouri and al. (2011b) were obtained 28 constituents for the EO of J. Phoenicea from Morocco.

This difference in composition is probably due to various conditions including environment, genotype, geographic origin, harvest time, location and duration and temperature of drying, and method of extraction. In addition, in our study, only the leaves of the plant of *J. Phoenicea* were used, while in the work of Mansouri and al. (2011b), they used a mixture of twigs and leaves of this plant.

Nevertheless, a comparison on the majority compounds and on the yields of essential oil of the leaves of *J. Phoenicea* in the main producing countries of the Mediterranean basin in general shows particular differences (Table 3).

**Table 3**: Comparative table of the major constituents (data in %) and the EO yields of he leaves of *J. Phoenicea*.

| Country | Yield | Components | Authors |
|---|---|---|---|
| Portugal | 0,41% | α-pinene (34.1%), β-phelandren(19.2%), β-Caryophyllene (0.22%). | Robert and al (1996) |
| Spain | 0,66% | α-pinene 53.5% β-phelandren 5.9% β-Caryophyllene 1.0% | |
| Greece | 0,58% | α-pinene 41.8%), β-phelandren 0.5%, β-Caryophyllene 3.5%. | |
| Egypt | 0,36 % | α-pinene 39.30%, α-Cedrol 31.23%, Sabinene 24.29%. | El-Sawi and al (2007) |
| Tunisia | 0,5 % | α -Pinene 59,1%, Myrcene 1,14 %, Linalool 3,34 % | Bouzouita and al (2008) |
| Algeria | 0,8 % | α-pinene 40.2 %, β-phellandren 14,1 %, β-pinene 2,0% | Dob |

| | | | |
|---|---|---|---|
| (Djelfa) | | | and al (2008) |

### I.2.2. the fruits of *Juniperus Phoenicea*

Also for the essential oil of the fruits of *J. Phoenicea* the first peaks appeared after 8 minutes. But the only major compound with a yield of 0.14 % appeared at 15.59 min with a very high abundance as shown in Figure 2 and Table 2, it is the terpene compound; α-pinene ($C_{10}H_{16}$), with an area of 100 %.

These essential oils were also dominated by mono-terpene compounds rather than sesqui-terpene compounds.

**Figure 2:** Chromatographic profile of the EO of the fruits of *J. Phoenicea*

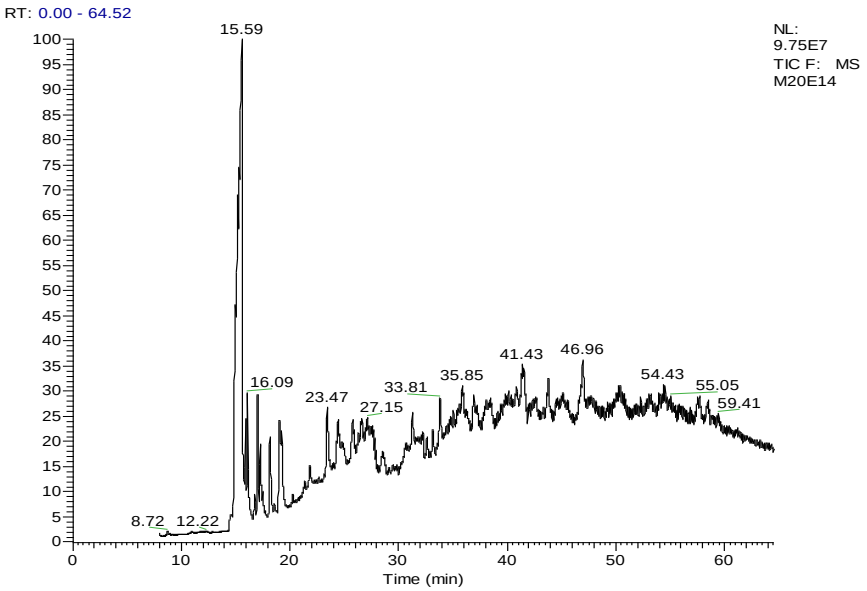



Table 2: Chemical composition of the EOs of the fruits of *J. phoenicea*; RT: retention time (in min); Air: chromatographic peak areas

| Peaks | RT (min) | Chemical names of compounds | Chemical formulas | Area (%) |
|---|---|---|---|---|
| 1 | 15.59 | α-pinene | C10H16 | 100 |

The EO of the fruits of *J. Phoenicea* are largely dominated by α- pinene, Mansouri and al. (2011b), were also found a dominance of α- pinene for the EO of the fruits of J. Phoenicea subspecies Turbinata from Morocco, and a low percentage for β- pinene.

## II. Antioxidant activity

### II.1. Determination of the percentage inhibition (I %)

The results obtained during the test for measuring the percentage of inhibition of the free radical DPPH by extracts from the studied plant and by the BHT, are represented in figures 3, 4, and 5.

**Figure 3:** The percentage inhibition (I %) of DPPH by the decoctate (left) and by EO of the leaves of *J. Phoenicea* (right)

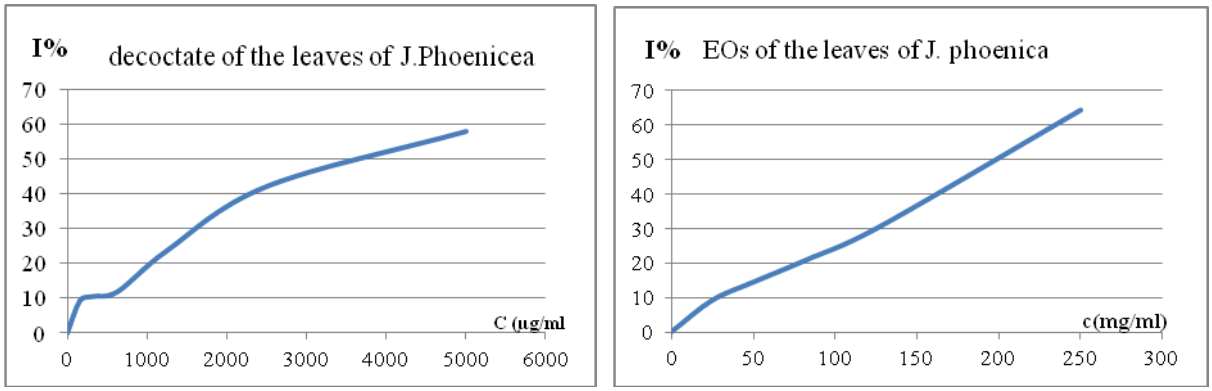



**Figure 4:** The percentage inhibition (I %) of DPPH by the decoctate (left) and by EO of the fruits of *J. Phoenicea* (right)

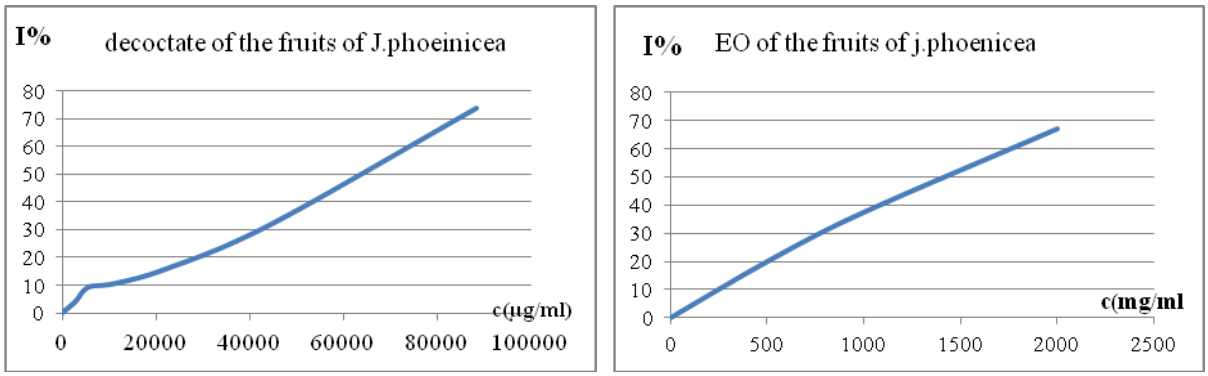
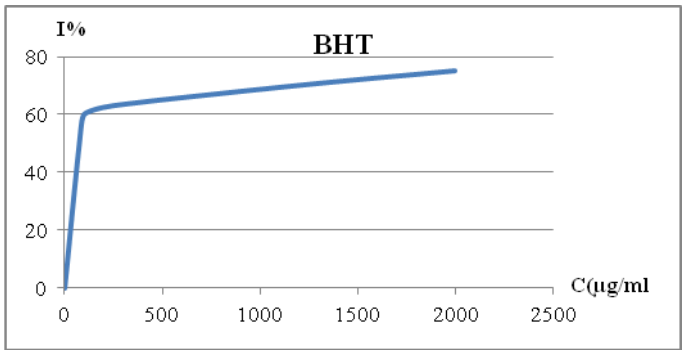



Figure 5: The inhibition percentage of DPPH by BHT




The percentage inhibition of the free radical DPPH increases with increasing concentration of EO and Decoctate from leaves and fruits of *J. Phoenicea* and also of BHT.

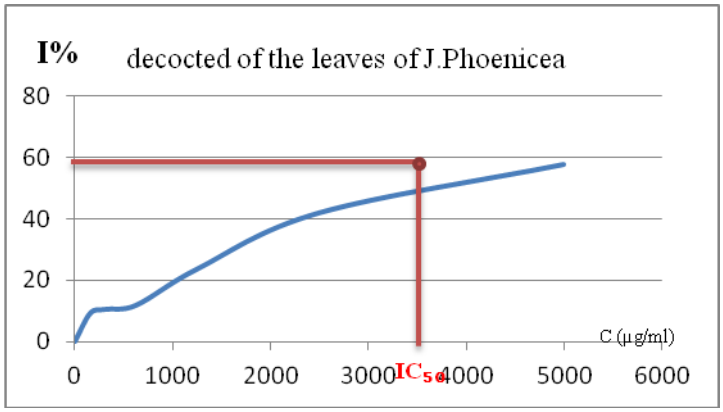
It is observed that the percentage inhibition of DPPH by EO and by decocts remains lower than that of BHT for all the concentrations used.

We note for the extracts of leaves and fruits of *J. Phoenicea* that the inhibition percentage of DPPH by the Decoctate is more important than that of the essential oils.

### II.2 Determination of the IC50

IC50 is inversely related to the antioxidant capacity of a compound, as it expresses the quantity of antioxidant required to decrease the concentration of the free radical by 50%. The lower the IC50 value, the greater the antioxidant activity of a compound. (Hebi and Eddouks 2016). Figure 6 shows an example of how to calculate IC50.

**Figure 6:** determination of the IC50 for decocted leaves of *J. phoenicea*




The $IC_{50}$ values for the essential oil and for the decoction of the leaves and fruits of J. Phoenicea are shown in Table 4.

**Table 4:** IC50 values of extracts from leaves and fruits of J. Phoenicea and that of BHT

| | **Sample** | **IC50** |
|---|---|---|
| Leaves | Decoctate | 3.6 mg/ml |
| | EO | 210mg/ml |
| Fruits | Decoctate | 63 mg/ml |
| | EO | 1400 mg/ml |
| Control | BHT | 0.075mg/ml |

From these results we notice that BHT is the most effective antioxidant with an IC50 of 0.075 mg/ml, while the antioxidant activity of decoctate is higher than that of EO for leaves and fruits, and that the antioxidant activity of leaves is higher than that of fruits of *J. Phoenicea*.

### II.3. Anti-radical power ARP

The higher the ARP values, the greater the antioxidant power. Tables 5 and 6 summarize the ARP values obtained.

**Table 5:** ARP and $IC_{50}$ values of extracts from leaves of *J. Phoenicea* and BHT

| | | **IC50** | **ARP= 1/ IC50** |
|---|---|---|---|
| **Leaves of *J. Phoenicea*** | Decoctate | 3.6mg/ml | 0.28 |
| | EO | 210mg/ml | 0.0048 |
| **BHT** | | 0.075 | 13.33 |

**Table 6:** ARP and **IC50**values of extracts from fruits of *J. Phoenicea* and BHT

| | | **$IC_{50}$** | **ARP= 1/ $IC_{50}$** |
|---|---|---|---|
| **Fruits of J.Phoencea** | Decocted | 63mg/ml | 0.016 |
| | EO | 1400mg/ml | 0.00071 |
| **BHT** | | 0.075 | 13.33 |

For extracts from the leaves of J. Phoenicea, it is observed that the decoctate has a higher anti-radical power when added to the essential oil.

Also for the extracts of the fruits of *J. Phoenicea*, we notice that the anti-radical power of decoctate is more important when added to the essential oil.

From the above results, it is noticed that the essential oils and decocts of the leaves of *J. Phoenicea* have a higher antioxidant activity than the fruits of the same species

So we can say that our extracts have antioxidant activity and that the possibility of trapping the free radical DPPH by BHT is very strong.

The high antioxidant activity of BHT can be explained by the nature of this product since it is an excellent synthetic antioxidant can easily form a very stable radical. (Marilou 2016).

The antioxidant power of the EO from leaves of J. Phoenicea can be explained by their high content of mono-terpene compounds like α-pinene and β-phelandren and sesqui-terpene compounds like Caryophyllene. While for the EO of the fruits of J. Phoenicea, we can say that α-pinene which is responsible for this antioxidant activity.

Also for the study carried out by Bouzouita and al. (2008) found that the essential oil of *J. phoenicea* revealed an antioxidant property comparable to that of δ- tocopherol.

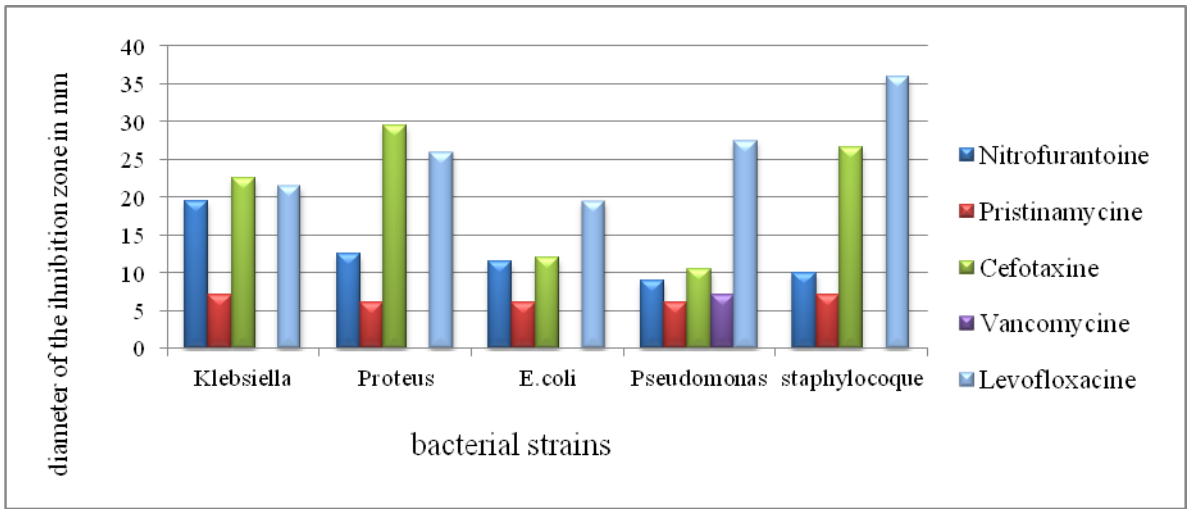
For the study carried out by Soltani and al. (2017). Also found that the antioxidant capacity of extracts of *J. phoenicea* measured by the FRAP method (Benzie and Strain 1996; Ou and al. 2002) is important for twig extracts, followed by leaves extracts, while berry extracts show relatively moderate activity.

## III. Antibacterial power

### III.1 The antibacterial power of antibiotics

The results of the sensitivity of the bacteria studied to one or more antibiotics are presented in figure 7.

**Figure 7:** Comparison of the results of antibiograms with control antibiotics




- *Klebsiella pneumoniae*: presented high sensitivity to Nitrofurantoin with a diameter of the inhibition zone of ID =19.5 mm, to Cefotaxin with ID = 22.5 mm, and to levofloxacin with ID =21.5 mm, and resistant to Pristinamycin with ID = 7 mm.
- Proteus: showed moderate sensitivity to Nitrofurantoine (ID = 12.5 mm), and important to Cefotaxine (ID = 29.5 mm) and levofloxacin (ID = 26 mm), while it is resistant to Pristinamycin (ID = 6 mm).
- *E. coli*: the inhibitory action of Nitrofurantoine and Cefotaxine is intermediate (ID =11.5 mm for the first antibiotic and DI= 12 mm for the second), while this action is weak for Pristinamycin (ID = 6 mm), while this action is important for Levofloxacin (ID = 19.5 mm).
- *Pseudomonas*: presented a high sensitivity to Levofloxacin (ID = 27.5mm), while it presented a low sensitivity to Nitrofurantoine (ID = 9mm) and to Cefotaxine (IDI = 10.5mm), but it is resistant to Pristinamycin (ID = 6mm) and to Vancomycin (ID = 7mm).
- Staphylococcus: presented a very high sensitivity to levofloxacin (ID = 36 mm), and an equally high sensitivity to Cefotaxin (ID = 26.5 mm), and low to Nitrofurantoin (ID = 10 mm), but it is resistant to Pristinamycin (ID = 7 mm).

### III.2 The antibacterial power of essential oils

The observations made on the effect of EOs from the leaves and fruits of *J. Phoenicea* on the growth of the bacterial strains tested are represented in Table 7 and Figure 8.

**Figure 8:** The comparison of the antibacterial activity of essential oils from the leaves and fruits of *Juniperus Phoenicea*.

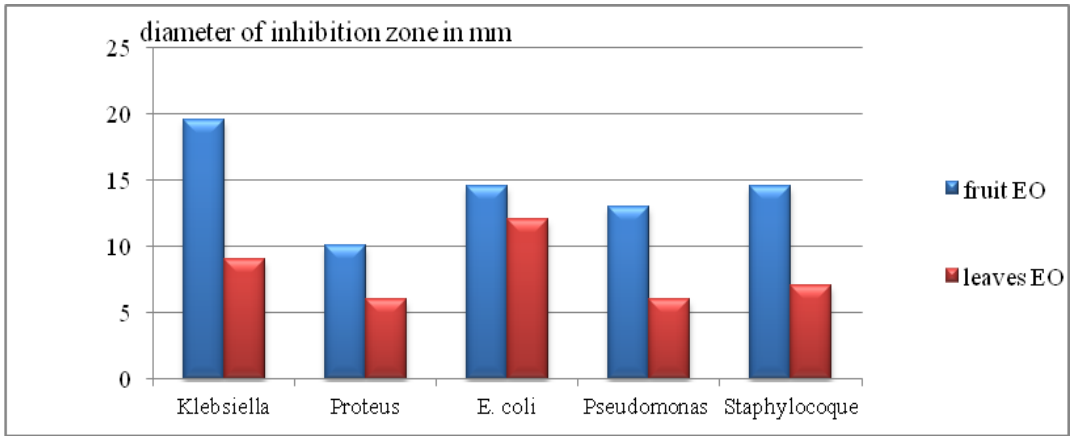



**Table 7:** Inhibition diameters (ID) for essential oils the from leaves and fruits of *J. Phoenicea*

| | Klebsiella | Proteus | E. coli | Pseudomonas | Staphylocoque |
|---|---|---|---|---|---|
| ID for Fruit EO | 19,5 mm | 10 mm | 14,5 mm | 13 mm | 14,5 mm |
| ID for leaves EO | 9 mm | 6 mm | 12 mm | 6 mm | 7 mm |

From the results obtained it is noted that:

- The EO of the fruits of *J. Phoenicea* presented an important inhibitory effect by supplying the essential oil of the leaves of *J. Phoenicea* against all the strains tested.
- The EO of the fruit of *J. Phoenicea* has a strong inhibitory activity for Klebsiella (ID = 19.5mm) and is equal to that of the control antibiotic (Nitrofurantoin); it also has a strong inhibitory activity for E.coli and staphylococcus, while it has a moderate inhibitory activity for the other strains (ID of 10 to 13 mm).
- The EO of leaves of *J. Phoenicea* has a moderate inhibitory activity for E.coli (ID = 12 mm) and for Klebsiella (ID = 9mm).

Likewise, Derwich and al. (2010), found that *E.coli* was the most sensitive strain tested to EO of the leaves of *J. Phoenicea* but with an inhibition zone of 34mm.

On the other hand, Angioni and al. (2003), also reported that EO of the leaves of *J. Phoenicea* leaves presented a low activity against *S. aureus* but no activity against *E.coli* or *Pseudomonas*.

In general, it is found that the EO of the fruits of J. Phoenicea has a major inhibitory activity against the strains tested, so we can say that it is the majority compound of this EO; α- pinene; which is the responsible for this antibacterial activity since it is the only majority compound of EOs in fruit with a yield of 0.14.

Derwich and al. (2010), supposed that the antibacterial activity shown by the EO of *J. Phoenicea* was due to the abundance of α- pinene. Cha and al. (2007); Hajlaoui and al. (2009) have been shown that the chemical constituent α -pinene is an effective compound against some food-borne pathogens.

**Conclusion**

Analysis by GC/MS of the EOs of the two parts (leaves and fruits) of *Juniperus phoenicea* collected in the region of Midelt-Morocco demonstrated a variation in the yield and chemical composition of the EOs of the two parts of the plant studied and which is rich in terpene compounds especially α-pinene.

The results obtained during this study show the existence of an antioxidant activity for the decocts and EOs of the leaves and fruits of *J. phoenicea*, noting that the antioxidant activity of the extracts of the leaves is greater than that of the fruits. In addition to the presence of antibacterial properties for the Eos of the leaves and fruit of the same plant; the EO of the fruit of *J. phoenicea* presented a significant inhibitory activity against the five bacterial strains, while the EO of the leaves of *J. phoenicea* presented a significant inhibitory effect against the bacterial strain E. coli and a remarkable effect against the Klebsiella strain.

Finally, the objective of this study was achieved since all the results obtained clearly demonstrate the antioxidant power and the antibacterial activity of the Eos obtained from the leaves and fruits of *J. phoenicea*.

So we can conclude that the EOs extracted from *J. Phoenicea* could be considered as possible alternatives for synthetic antibiotics and as natural antioxidants for use in the pharmaceutical industry as well as in the food industry.

**Acknowledgements:**

The authors would like to thank the Regional University Interface Center, Sidi Mohamed Ben Abdellah University, Morocco, for providing GC-MS setups.

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