Chemical Composition and Antimicrobial Activity of Essential oil from Scales of Moroccan *Juniperus phoenicea*

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Doi-10.51129/ujpah-june2021-30-1(1)

Abstract-The essential oil of *Juniperus phoenicea* was obtained by hydrodistillation method using a Clevenger-type apparatus with a yield of 1.9 % and was analyzed by gas chromatography coupled to a mass spectrometer (GC-MS). Twelve volatile compounds were identified representing 99,85% of the total oil composition, while the α-pinene (78,31%), β-Myrcene (11,92%) and limonene (3,96%) were the major compounds. This essential oil was evaluated as an antibacterial and antifungal agent. The result showed that the oil presents a high biological activity as an antibacterial agent against the three tested strains Escherichia coli, Staphylococcus aureus and Pseudomonas aeroginos. It's also active as an antifungal agent against the Candida albicans with a zone inhibition of 28 mm.

Keywords: Medicinal plant, *Juniperus, phoenicea,* Essential oil, Chemical composition, Antimicrobial activity.

Introduction

The history of aromatic and medicinal plants is associated with the evolution of civilizations[1]. Since ancient times and in all parts of the world, these plants have been always occupied an important source of medicine[2].In addition, it was found that their applications are in different areas like cosmetics, perfumes and food[3].The evaluation of phytotherapeutic properties such as an antimicrobial, remains a very interesting task and useful, especially for plants of rare use or less frequent, if not unknown, in medicine and folk medicinal traditions. These plants represent a new source of active compounds[4].

The genus *Juniperus*(Cupressaceae)is an aromatic plant. It has a large number of specie sat about 60[5], increasing rapidly in abundance in arid and semiarid regions, trees hrub ecosystems throughout the Northern Hemisphere[6,7]. They occupy an important place native of the Mediterranean basin[8].This tree reaches commonly 4-8 m height, presents in the form of several species. In Moroccan flora, four species are present as J*uniperus oxycedrus, Juniperus communis, Juniperus phoenicea* and *Juniperus thurifera*[9].The genus *Juniperus* has been widely used in Morocco folk medicine for stomach tonic, diarrhea, rheumatism, and as under protection[10]. The *Juniperus phoenicea* popularly known as "Arâar" is also used as a decoction against rheumatism and diabetes, while dried and powdered fruit can cure skin ulcerations[10]. Its essential oil contains diversity of substances giving them important biological activities such as an antibacterial[11,12], antifungal[11,13], antioxidant[11,14] and cytotoxic against cell lines[12,13].

In continuation of our work on valorization of natural products[15,17], the aim of this study is to extract the essential oil (E.O) from scales of *J. Phoenicea* colletted from the region of Ifran in Morocco using hydro distillation method, and to determine its chemical composition by GCMS and evaluate its antibacterial and antifungal activities.

Material and Methods

Plant material

The cones used in this study were collected from the region of Ifran in the Middle Atlas Mountain during the month of January. The botanical identification was achieved by the

National Scientific Institute (Rabat) where voucher specimen (RAB-104243) was deposited in the herbarium. In the region studied, we have chosen 10 trees. From each tree we took about 2 kg of cones and prepared 10 individual samples. After we made a heterogeneous sample by mixing 500g of each tree cones (about 5 Kg), it was submitted to the air-drying in the shade for two weeks at room temperature and powdered. The moisture level was 12%.

Analysis by gas chromatography-mass spectrometry (GC/MS)

The essential oil of *Juniperus phoenicea* was analyzed by gas chromatography coupled with GC/MS mass spectrometer. This technique is widely used in the qualitative and quantitative analysis of essential oils. The apparatus used is Shimadzu GC-2010 equipped with a BP-5 capillary column (30m x 0.25mm I.D; 0.25mm film thickness); coupled with a Shimadzu QP-2010 Plus mass spectrometer. The carrier gas is helium set at a flow rate of 1ml/min and adjusted at a linear velocity of30 cm/s. The oven was programmed as follows: from 60 to 200°C at 3°C/min, then at 15°C/min to 280°C, then maintained for 10min at this temperature. The injected volume was about 0.1 µl.

Microorganism and culture material

The microbial strains used for the antimicrobial activity test are: Escherichia coli ATCC 25922,Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853 and a yeast Candida albicans ATCC 10231.The bacterial strains provided are vivified by a culture of one night, the bacteria are subcultured in a sterile biokar nutrient medium and the albican yeast is subcultured in a YPG medium (yeastextract, peptone, glucose). Biokar steril then is incubated for 18 to 24 hours in an oven under a temperature of 37 °C. The bacterial load is suitable for analysis by the following method: a colony of each bacterial strain is imposed using a sterile loop and incorporated into a sterile tube of physiological saline (NaCl at 0.99%). The density of this solution was compared to the turbidity of the solution to 0.5 McFarland [18],The absorbance of this bacterial suspension at 625 nm is adjusted to have an optical density of 0.08 to 0.12 which corresponds to a concentration bacterial 10^8 CFU / ml, the suspension is diluted by a factor of 2 to have a bacterial concentration of 10^6 CFU / ml. By cons for the yeast strain, agar surface was scraped and introduced into 9 ml of physiological saline, three decimal dilutions are prepared and the latter is used to test the antibacterial activity.

Micro-dilution : Calculation of the minimum inhibitory concentration, to determine the minimum inhibitory concentration of J. phoenicea's E.O, the sterile Elsa microtiter plates are prepared as follows: the first 11 columns are filled with 100 µl of MULER HILTON medium and 50 µl diluted with a factor of (2) of the following emulsion: (since our organic compound is insoluble in water, we solubilized 20 mg of the tested compounds in a volume of 1 ml composed of 0.5 ml of the sterile medium and 0.5 ml of hexane), the microplate wells are then inoculated for each line, a bacterial strain. The test was replicated three times. The plates were incubated for 18 to 24 hours. The reading of the results was defined by the Resazurin staining test, it responded by giving a red color in the presence of bacterial growth.

Results and Discussion

Chemical composition of the essential oil (E.O) of *Juniperus phoenicea*

The essential oil from scales of *Juniperus phoenicea* was obtained by hydrodistillation with a good yield 1.9% compared to previous work[19]. According to the chemical analyses, twelve compounds were identified in the essential oil using a gas chromatography mass GC-MS, representing 99.85% of the total oil (Table-1). Monoterpenes hydrocarbons were the predominant chemical class with the α-Pinene (78.31%) as the major constituent, followed by β-myrcene (11.92%) and limonene (3.96%).

Table-1 Chemical composition of *Juniperus phoenicea* populations.

N°	Identified Compounds	RT	Yield %
1	α-phellandrene	5.688	0.07
2	α-pinene	5.898	**78.31**
3	thujene	6.323	0.14
4	β-phellandrene	7.027	0.22
5	β -pinene	7.152	2.43
6	β -myrcene	7.526	**11.92**
7	3-carene	8.226	1.67
8	limonene	8.871	**3.96**
9	ocimene	9.552	0.29
10	4-carene	11.159	0.56
11	2-methyl - 6-methylene - 7-Octen - 4-ol	15.560	0.16
12	thymol methyl ether	17.881	0.12
Total monoterpenes hydrocarbons			99.85%

Antibacterial activity of Essential oil (E.O)

The in vitro antibacterial activity of this essential oil against three pathogenic strains Gram-positive(Staphylococcus aureus) and Gram-negative (Escherichia coli and Pseudomonas aeroginosa) was assayed using the disc diffusion method by measuring inhibition zone diameter (Figure-1).

The essential oil of *Juniperus phoenicea* tested showed significant antibacterial activity which was extremely active on all tested bacteria compared to those of the antibiotics (Penicilline and Novobiocine), with inhibition zones ranging from 22 to 28 mm (Table- 2).The high antibacterial potential may due to the presence of α-pinene (78.31%)[20]. In essential oil of *Juniperus phoenicea,*

preliminary screening revealed that the EO were effective against all tested bacteria. To accomplish this, additional minimum inhibitory concentration (MIC) assays were performed using the liquid serial dilution method of Mueller Hinton (MH) . MICs varied from 0.937 mg/ml to 0.117 mg/ml. (Table-3).The results proved that E.O of *Juniperus phoenicea* has a good antimicrobial activity for all the three strains tested. Our observations are in consistent with the results obtained by Cosentino et al.[21]. They showed that the minimum inhibitory concentration of the E.O of *Juniperus phoenicea* was 0.9 mg/ml towards the different bacterial strains.

Table -2 Results of antibacterial activities of *J. Phoenicea* Essential Oil (E.O)

	J. phoenicea	**Positif control**		**Negatif control**
	E.O	Penicilline	Novobiocine	Ethanol
Escherichia coli ATCC 25922	22	9	13	-
Staphylococcus aureus. ATCC 25923	28	34	12	-
Pseudomonas aeroginosa. ATCC 27853	24	9	15	-

- :No activity

Table -3 Minimum Inhibitory Concentration of *J. Phoenicea* E.O

	E. Coli	***S. Aureus***	***P. Aeroginosa***
MIC (mg/ml)	0.234	0.937	0.117

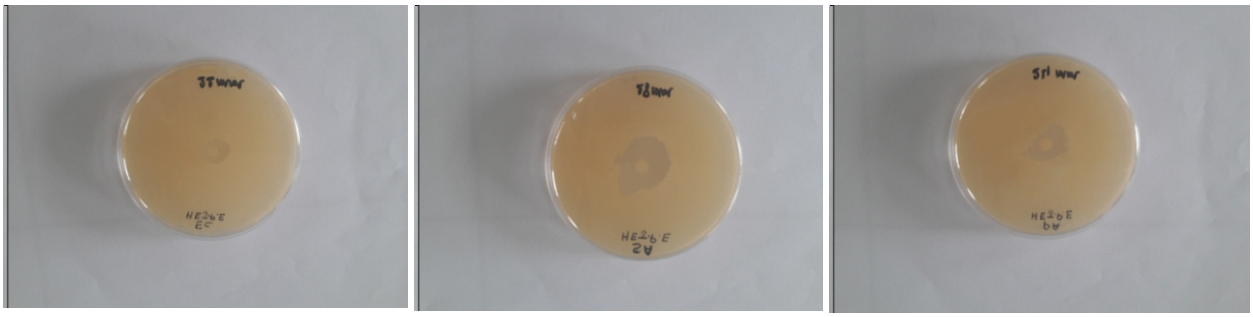

Figure-1 The inhibition zone of the E.O of *J. phoenicea* against the three strains *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*

Antifungal activity

The E.O isolated from the scales of *J. phoenicia* were tested for antifungal activity against *C.albicans,* and their fungistatic effects tested with an inhibition diameter of 28 mm compared to the commercial antifungal Metrazol which has more significant action on *C. albicans*(Table-4).The evaluation of the inhibitory effects of E.O allowed us to deduce that they are very active against the tested *C.albicans* yeast. This activity is probably due to the chemical composition of the oil rich in α-pinene, which is also in accordance with the work of Yarelis et al. This indicated that E.O moderately reduce the development of *C. Albicans*[22].

Table-4 Results of antifungal activity of *J. phoenicea* essential oil (E.O)

	J. phoenicea	Control positif	Control negatif
	E.O	Metr azol	Methanol
Candida albicans	28 mm	29 mm	-

- :No activity

Conclusion

The present study provides the chemical composition and antimicrobial activity of the essential oil *J. phoenicea,* the aromatic plant growing in Morocco. The chemical composition of the essential oil extracted from scales of *J. phoenicea* using hydrodistillation method indicate the predominance of α-pinene(78.31%), β-myrcene (11.92%) and limonene (3.96%). The extracted oil on the monoterpenoids is 99.85%. The chemical composition of the essential oil is in good agreement with literature[23].The oil demonstrates a better inhibitory effect against the bacterial and fungal strains study which was deduced from the size variation of the inhibiting zone. This may due to the significant presence of α-pinene in the scale.

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