Trop J Nat Prod Res, December 2023; 7(12):5633-5638

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Composition, Antibacterial and Antifungal Activities of Essential Oil of *Juniperus phoenicea* from Fez-Meknes Region, North Central Morocco

Samara Yacine Khalil[1]*, Beniaich Ghada[2], El Abdali Youness[3], Kaouia Samiha[1], Flouchi Rachid[4,5], Moubchir Tarik[6], Allali Aimad[5,7], Khadmaoui Abderrazzak[1]

[1]*Biology and Health Laboratory, Department of Biology, Faculty of Sciences, Ibn Tofail University, Kenitra, Morocco.*
[2]*Department of Biology, Laboratory of Engineering, Electrochemistry, Modeling and Environment (LIEME), Faculty of Sciences, Dhar EL Mahraz, Sidi Mohamed Ben Abdellah University, Fez, Morocco.*
[3]*Laboratory of Biotechnology, Environment, Agrifood and Health, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez 30050, Morocco.*
[4]*Microbial Biotechnology and Bioactive Molecules Laboratory, Sciences and Technologies Faculty, Sidi Mohamed Ben Abdellah University, Fez, Morocco.*
[5]*High Institute of Nursing Professions and Health Techniques annex Taza, Fez, Morocco.*
[6]*Polyvalent Team in Research and Development, Department of Biology, Sultan Moulay Slimane University, Polydisciplinary Faculty Beni Mellal, BP: 592, 23030, Béni Mellal, Morocco.*
[7]*Laboratory of Plant, Animal, and Agro-industry Productions, Faculty of Sciences, University of Ibn Tofail, Kenitra, Morocco.*

ARTICLE INFO

Article history:
Received 21 June 2023
Revised 04 November 2023
Accepted 07 December 2023
Published online 01 January 2024

ABSTRACT

Morocco's climate, characterized by a diverse range of ecosystems, including coastal areas, mountains, and deserts, provides favorable conditions for the growth of a wide variety of plants. Many of these plants contain active chemicals with various biological activities, including antibacterial and antifungal activities. For this reason, the study aim to investigate the antimicrobial activity of the essential oil of *Juniperus phoenicea* leaves and twigs collected from the central region of Morocco, specifically Fès-Meknès. The essential oil (EO) was obtained by hydrodistillation using the Clavenger-type apparatus. The EO was subsequently subjected to gas chromatographic-mass spectrometric (GC-MS) analysis. The antimicrobial activity of the essential oil was evaluated using the agar disk diffusion method and determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations. GC-MS analysis revealed that the major compounds of *Juniperus phoenicea* EO are *α*-pinene (43.61%) and manoyl oxide (11.50%). The results of the antimicrobial activity evaluation showed that *Juniperus phoenicea* EO exhibited strong antibacterial activity against both Gram-negative and Gram-positive bacteria, notably *E. coli* (ATB: 97) and *E. coli* (ATB: 57), with inhibition zone diameters of 23.67 ± 1.15 mm and 23.33 ± 2.89 mm, respectively. The lowest MIC value was 0.1 µL/mL. In addition, *Juniperus phoenicea* EO displayed strong antifungal activity, particularly against *F. oxysporum*, *A. alternate*, and *C. albicans* with inhibition zone diameters of 40.61 ± 1.53 mm, 17.33 ± 1.52 mm, and 10.5 ± 1.80 mm, respectively. The antibacterial and antifungal properties of *Juniperus phoenicea* essential oil could be attributed to the distinctive chemical composition.

Keywords: *Juniperus phoenicea*, Essential oil, Antibacterial, Antifungal, Phytochemical.

Introduction

Nature is essentially made up of plants, which served as food for man and animals. In addition to this nutritive function, man discovered many other functions that plants have, particularly, the power of healing, which had long been known to our ancestors since antiquity. This later became embedded in traditional medicine, with all its recent advances.[1,2] The study of medicinal plants and their traditional usage in many parts of the globe has seen an increase in recent decades. In fact, according to the World Health Organization (WHO), about 80% of the world's population living in rural communities still use traditional medicine for their primary health care need.[3]

*Corresponding author. E mail: yacinesamarakhalil@gmail.com
Tel: +212 678134873

Citation: Samara YK, Beniaich G, El abdali Y, Kaouia S, Flouchi R, Moubchir T, Allali A, Khadmaoui A. Phytochemical Composition, Antibacterial and Antifungal Activities of Essential Oil of *Juniperus phoenicea* from Fez-Meknes Region, North Central Morocco. Trop J Nat Prod Res. 2023; 7(12):5633-5639. http://www.doi.org/10.26538/tjnpr/v7i12.39

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Traditional medicine and the use of medicinal plants for the treatment of different ailments have been shown to offer significant economic advantages.[4]

Morocco's particular geographical location, in addition to its physiographic and bioclimatic diversity, are variables that explain the rich and diverse Moroccan flora, particularly in terms of medicinal and aromatic plants.[5] Of these medicinal and aromatic plants, more than 4200 species and subspecies have been listed, with around a hundred as endemic.[6] These plants are a huge source of bioactive compounds that are used in the perfume, culinary, cosmetics, and pharmaceutical industries.[7]

As secondary metabolites, essential oils are present in almost all plants.[8] They are complex mixture made up of tens or hundreds of chemicals with antibacterial, antifungal, antiviral, antiparasitic, insecticidal, antioxidant, and anticancer properties. There are presently roughly 3000 essential oils, of which about 300 are commercially available, mostly for the pharmaceutical, agronomic, food, and cosmetic industries.[9]

In recent time, antibiotic resistance has emerged as a profound challenge to public health. It results in significant crises in many hospitals globally and contributes to the prevalence of hospital-acquired infections. As a result, the pursuit of anti-infective agents has become an imperative requirement.[10] This underscores the need to explore the use of naturally occurring antimicrobial agents.[11]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The Fez-Meknes region of Morocco presents a distinctive floristic and faunistic diversity. Despite these potentials, the ecosystems of this region are particularly threatened by several anthropic and climatic factors, and may no longer provide the services essential to the socio-economic development of this region, especially for the population dependent on its natural resources.[12] Consequently, it is essential to preserve the worth of specific medicinal plants native to this region, while also providing scientific validation for their traditional usage.

Although, a prevalent plant in the Fès-Meknès region, the red juniper (*Juniperus phoenicea* L., family; Cupressaceae), a compact Mediterranean tree known as "Arar," exhibits a broad geographical distribution from Portugal to Saudi Arabia and numerous other regions. Its origins can be traced to Algeria, Morocco, Tunisia, the Canary Islands, and regions within North Africa.[13] Unfortunately, due to its utilization for fuel and in the production of commercial charcoal, the red juniper is progressively diminishing in availability.

The *Juniperus* genus is widely used in traditional medicine due to the presence of diverse chemical compounds.[14] They are used in the treatment of a variety of ailments such as diabetes, diarrhea, common cold, rheumatism and bronchopulmonary problems. They also have hepatoprotective and anti-obesity effects. An oral hypoglycemic agent have been produced by combining the leaves and berries of this plant, while the powdered dried fruits are used to treat skin lesions.[15] Parts such as cones and branches, especially infused young shoots have diuretic, digestive and soothing properties.[15] They are beneficial against urinary tract infections and dermatological disorders, and have been used to combat toxic effects on the liver and kidneys.[16] Juniper is also renowned for its antiseptic properties. Plants of the *Juniperus* genus are also used as spices in various cuisines, to flavor alcoholic beverages and in cosmetics.[17] Red juniper or Phoenician juniper twigs, leaves, and fruits are also used in medicinal preparations for their antibacterial properties, which are attributed to their essential oil contents.[18]

Despite the extensive investigations conducted so far, information on the antimicrobial properties of the essential oil derived from *Juniperus phoenicea* in the Fez-Meknes region is limited. Therefore, the aim of the present study is to investigate the phytochemical composition of *Juniperus phoenicea* essential oil and to evaluate its antimicrobial activity against various bacterial and fungal strains.

Material and Methods

Collection and identification of plant material

The aerial part of *Juniperus phoenicea* was collected in May, 2022 from de Boulemane (33°29'47N; 3°48'36 "W) in Fez-Meknes region in North-Central Morocco. The taxonomic identification of the plant material was done in the laboratory of the National Agency for Aromatic and Medicinal Plants (ANPAM) of Taounate. Herbarium specimen with voucher number BD01/11281 was deposited in the agency's herbarium.

Extraction of essential oil

The aerial part (leaves and twigs) of *Juniperus phoenicea* was air-dried in the shade at ambient temperature. The dried plant material (100 g) was subjected to hydrodistillation for 3 h at 120°C in a Clevenger-type apparatus to extract the essential oil (EO). The essential oil was subsequently dried with anhydrous sodium sulfate and then preserved in a refrigerator at 4°C until ready for use.[19]

GC-MS Analysis of the essential oil

The analysis of the EO was carried out by gas chromatography coupled to mass spectrometry [GC-MS-TQ8040 NX (Shimadzu brand)] with TRIPLE quadrupole as detector, and a column (Apolar capillary RTxi- 5 Sil MS - 30m x 0.25mm ID x 0.25 µm). The carrier gas used was Helium, the injection volume was 1 µL, the source ion temperature was 200°C, and the Interface temperature was 280°C. The analysis was performed in splitless injection mode with a split opening at 2.5 min, using an injection temperature of 250°C and a pressure of 37.1 kPa. The analysis was performed for 50 minutes with an initial temperature of 50°C for 2 min. The Ramp 1 carried out at 5°C/min up to 160°C for 2 min, and the Ramp 2 at 5°C/min up to 280°C for 2 min. Methanol was used as the dilution solvent.

Antimicrobial activity screening

Bacterial strains and growth media

Five bacterial strains namely; *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* (ATB: 57) B6N, *Escherichia coli* (ATB: 97) BGM and *Pseudomonas aeruginosa* were used in this study. Muller-Hinton Broth (MHB) and Miller-Hinton Agar (MHA) provided by VWR Chemicals were used as growth media for the bacteria. For the microbial suspension, a fresh 18–24 h culture of bacteria was placed in a 0.9% NaCl solution, and the optical density was measured at 625 nm using a UV-Visible spectrophotometer, the absorbance was adjusted to between 0.08 and 0.1, which corresponds to suspensions containing 10^7-10^8 CFU/mL.

Determination of the inhibition zone

The antibacterial properties of *Juniperus phoenicea* EO was evaluated by the disk diffusion method. Petri dishes containing Muller-Hinton agar (MHA) were inoculated with the test bacterial strains. Then, Whatman paper discs (6 mm diameter) impregnated with 20 µL of *Juniperus phoenicea* EO were placed on the inoculated agar. The Petri dishes were incubated at 37°C for 24 h. Negative and positive controls containing 0.2% agar, and streptomycin (0.02 mg/disc), respectively were used under the same conditions. After incubation, the inhibition zone diameter and percentage inhibition were evaluated.[20]

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC were evaluated according to the method previously described,[20,21] with slight modifications. Each well of a 96-well microplate was filled with 100 µL of Mueller-Hinton growth medium (MHB), then 100 µL of EO diluted in DMSO (40% v/v) was added to the first well, and a microdilution was done by transferring 100 µL from the first well to the next well, and so on, to obtain concentrations ranging from 80 to 0.67 mg/mL. In addition, an essential oil-free control was made using MHB with DMSO (40% v/v). Then, aliquots of 100 µL of the bacterial suspension of each previously prepared strain (10^7 CFU/mL) were added to all the wells except the last one (negative control). The plate was incubated at 37°C for 24 h. The MIC which is the lowest concentration of EO that inhibited clearly apparent bacterial growth was obtained after 24 h incubation. Aliquots of each tube showing no growth were spread on MHA plates to estimate the minimum bactericidal concentration (MBC). Three tests were performed to determine MIC and MBC, both measured in microlitre per milliliter (µL/mL). From the MBC/MIC ratio, antimicrobials can be classified as bactericidal (MBC/MIC < 4) or bacteriostatic (MBC/MIC > 4).[22]

Antifungal activity screening

Fungal strains and growth medium

The antifungal activity was tested against five fungal strains including; *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Fusarium oxysporum* and *Alternaria alternata*. All the fungal strains were pathogenic and drug resistant. The fungal strains were cultured on sabouraud dextrose agar at 35°C for 7 days. The optical density of the fungal cell suspension in sterile physiological solution (NaCl 0.9%) was measured using a spectrophotometer at 530 nm. The absorbance of the cell suspension was adjusted to 0.8-0.12 corresponding to a cell density of 0.4-5 x 10^4 CFU/mL.

Disc diffusion assay

The Sabouraud dextrose agar plate was covered with 1 mL of inoculum. Filter discs of 6 mm diameter (Whatman No. 3 paper) impregnated with 15 µL of EO were then placed on the inoculated Sabouraud dextrose agar. The plates were incubated at 35°C for 48 h, after which antifungal activity was assessed by examining the inhibition zone diameter (IZD). All examinations were performed in triplicate. Results were classified as resistant (IZD < 6 mm), intermediate (IZD between 6 and 13 mm), and susceptible (IZD > 13 mm). Inhibition zone diameters were given as mean ± standard deviation (NCCLS M38-A2, 2008).[21]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Statistical analysis

Mean values and standard deviations were computed using GraphPad Prism 8 software (California, USA). Statistical analysis for all the tests were performed using one-way ANOVA followed by Tukey test. A probability value of $p < 0.05$ was applied to determine significant difference.

Results and Discussion

Extraction yield of Juniperus phoenicea essential oil

The hydrodistillation of *Juniperus phoenicea* aerial part yielded an essential oil with a characteristic aroma, amounting to 1.10 ± 0.03 mL/100 g of dry matter. This yield is comparatively lower than the 2% reported by Ennajar *et al.* (2009)[23] in Southern Tunisia but falls within the same range as the EO obtained from the same plant collected in Central-Western Tunisia, which gave a yield of 1%. However, this yield exceeds that obtained from red juniper. Several factors, such as environmental conditions (moisture and soil type), plant age, the plant part used, the stage of the vegetative cycle, or the timing of harvest, can significantly influence the yield of essential oil of several species.[24,25]

Chemical composition of Juniperus phoenicea essential oil

The study also involved the GC-MS analysis of EO extracted from *Juniperus phoenicea* leaves and twigs collected in the Fez-Meknes region. The results as shown in Tables 1 and 2, revealed the presence of 30 different constituents in the EO. These constituents exhibited varying concentrations, ranging from 0.61% to 43.61%. The most abundant compounds were α-pinene (43.61%), manoyl oxide (11.50%), hexanedioic acid (4.41%), caryophyllene oxide (4.34%), and trans-verbenol (3.16%). The other compounds were detected in lower concentrations. Generally, the main class of compounds identified in *Juniperus phoenicea* EO were monoterpenoids, hydrocarbons, oxygenated monoterpenes, and sesquiterpenes.

One of the most bioactive components of red juniper is α-pinene which has also been found in different proportions in the EO of *Juniperus phoenicea* in the Mediterranean countries. According to the study of Angioni *et al.* (2003),[26] α-pinene (49.2%) was the most prevalent compound of *Juniperus phoenicea* EO, followed by α-phellandrene (7.4%), myrcene (5.2%), *β*-pinene (3.6%) and linalool (2.5%). Monoterpenes were found to be the most prevalent class of compounds in *Juniperus phoenicea* EO according to the study of Barrero *et al*. (2006).[27] The major constituents within this group were α-pinene, making up 35.66% of the composition, and δ-3-carène, accounting for 35.46%. Alpha pinene was also found to be the dominant component (74.03%) of the same EO according to the findings of Mansouri *et al.* (2010).[28] In addition to these compounds, a number of other compounds were found in low concentrations in *Juniperus phoenicea* EO, these include germacrene B, E-*β*-caryophyllene, myrcene, *β*-phellandrene, linalool, α-terpineol and citronellol.

In the current study, the oxygenated monoterpenes, which are the primary chemical class of compounds in *Juniperus phoenicea* EO, were discovered to be the most prevalent of the many components. Most essential oils are composed of monoterpenes (C10), a class of chemically unstable compounds that prevent the development of germs, protect plants from parasites, and attract animal pollinators.[29] In general, the composition of essential oils changes depending on numerous factors. The existing intra-specific variability within the species *Juniperus phoenicea* could be of geographical, genetic,[30] seasonal,[25] or even ecological (soil, moisture) origin.[31]

Alpha pinene is highly sought after in the international market due to its antibacterial, anti-inflammatory, antiviral, expectorant, sedative, herbicidal, insect repellent, and flavor-enhancing properties.[32] Consequently, *Juniperus phoenicea* has the potential to serve as a significant source of this valuable active ingredient. This opens up promising opportunities for its utilization in various industries, including food, cosmetics, pharmaceuticals, and wood preservation industries.[28] In fact, this component is used in the manufacture of many products (such as perfumes and vitamin E) and is of great interest to the chemical and cosmetic industries.[24]

Antibacterial activity of Juniperus phoenicea essential oil

In this study, the antibacterial activity of *Juniperus phoenicea* EO was assessed *in vitro*, against five bacterial strains qualitatively using the disc diffusion method and quantitatively by the microdilution method to measure the MIC and MBC. The test strains are pathogenic and antibiotic resistant. They are prevalent cause of many diseases in Morocco, and treatment of infections due to these organisms can pose significant challenges.[33]

Table 1: Constituents of essential oil of *Juniperus phoenicea*

Peak	RT (min)	Compound	Percentage composition
1	7.989	*α*-Pinene	43.61%
2	12.593	*β*-Linalool	0.83%
3	12.904	Linalool	2.88%
4	13.464	Thujone	0.82%
5	13.961	Cis-Verbenol	0.68%
6	14,177	Trans-*α*-Pinen-4-ol	1.04%
7	14.312	Trans-Verbenol	3.16%
8	15.750	Myrtenal	1.37%
9	16.093	Verbenone	1.52%
10	17.441	Cis-4-Decenol	0.68%
11	20,833	Sobrerol 8-acetate	1.22%
12	21.128	*β*-Elemene	1.18%
13	21.955	Caryophyllene	2.01%
14	22.152	Germacrene B	1.18%
15	24.183	Shyobunol	0.82%
16	24.866	Geranyl-α-terpinene	1.55%
17	25.022	Spathulanol	1.08%
18	25.171	Elemol	2.31%
19	26.244	Caryophyllene oxide	4.34%
20	27.526	Isospathulenol	2.04%
21	28.297	Ledol	2.55%
22	29.816	(-)-Spathulenol	0.83%
23	31.253	Alloaromadendrene oxide-(2)	1.83%
24	32.286	Caryophyllene epoxide	1.41%
25	32.410	Rosifoliol	0.61%
26	32.605	Eudesma-4,11-dien-2-ol	0.63%
27	32.685	*β*-Oplopenone	0.96%
28	36.392	Manoyl oxide	11,50%
29	40.151	Néo-Abienol	0.94%
30	43.429	Hexanedioic acid, bis(2-ethylhexyl) ester	4.41%
Total identified			100%

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

As presented in Table 3, *Juniperus phoenicea* EO exhibited antibacterial activity against both Gram-negative and Gram-positive bacteria. It had a significant inhibitory effect on susceptible bacteria, as evidenced by inhibition zone diameters ranging from 12.33 to 23.67 mm.

Juniperus phoenicea EO exhibited the highest activity against *E. coli* (ATB:97) BGM strain, with an inhibition zone diameter of 23.67 ± 1.15 mm. It also demonstrated effective antibacterial activity against *E. coli* (ATB:57) B6N (23.33 ± 2.89), *B. subtilis* (22.66 ± 2.51 mm), *S. aureus* (14.00 ± 1.73 mm) and *P. aeruginosa* (12.33 ± 1.52 mm).

The results of the MIC and MBC evaluation which is a measure of the bacteriostatic and bactericidal effect of *Juniperus phoenicea* EO are presented in Table 3. From the results, the MIC values ranged from 0.1 to 0.39 µL/mL, and the MBC ranged from 0.19 to 1.3 µL/mL for the bacterial strains tested.

Derwich *et al.* (2010)[34] observed higher MIC and MBC compared to the present study. Consequently, it can be inferred that the studied *Juniperus phoenicea* EO effectively inhibited the growth of all the tested bacterial strains at low concentrations. Additionally, *Juniperus phoenicea* EO exhibited significant bactericidal activity against all the test bacterial strains which compares with the reference values as indicated by MBC/MIC ratio of less than 4.[35] Other studies on Juniper have yielded similar results, indicating a strong bacterial growth inhibitory effect of *J. phoenicea* EO.[34,23]

As reported by Derwich *et al.* (2010),[34] *E. coli* exhibited the most substantial inhibition zone diameter (34 mm) when exposed to *J. phoenicea* EO, while *S. aureus* displayed inhibition zone diameter of 24 mm. These findings were corroborated by the MIC values. Contrary to previous assumptions, it is noteworthy that Gram-positive bacteria do not demonstrate greater sensitivity to plant essential oil compared to Gram-negative bacteria.[36]

Previous research has indicated that *E. coli* exhibits greater sensitivity to various chemicals when compared to other Gram-positive bacteria. This heightened sensitivity of *E. coli* in contrast to the other microorganisms under investigation may be attributed to the presence of linalool (3.8%) in the analyzed EO.[34] Although, α-pinene is the predominant compound in *J. phoenicea* EOs worldwide, including Morocco,[35] its concentration in the studied EO varied. The antibacterial efficacy of this EO has been linked to the elevated levels of α-pinene, alongside the overall chemical composition of the EO, as previously reported.[34] It is worthy to note that the antimicrobial properties of α-pinene, have been elucidated.[36]

This study underscores the potential utility of *J. phoenicea* EO in enhancing food safety, primarily owing to its robust antibacterial activity particularly against pathogenic bacteria. The utilization of *J. phoenicea* EO as a potential weapon against antibiotic-resistant bacteria has gained substantial attention in recent decades,[34] and our investigation has revealed distinct responses to microorganisms that had been previously examined in other studies. To counteract the effects of *J. phoenicea* EO, it is plausible that the metabolic activities of specific bacteria may interact synergistically with the diverse components in the oil. In particular, α-pinene, a terpene hydrocarbon, has been consistently associated with the antibacterial activity of essential oil in numerous studies.[37] The efficacy of essential oils fluctuates based on their concentration and the type of bacteria. The variations in the sensitivity of bacterial to the essential oil may be linked to alterations in the concentration of the essential oil components by the specific cell wall and cell membrane characteristics of the bacteria.

Table 2: Chemical structures of the major compounds of *Juniperus phoenicea* essential oil

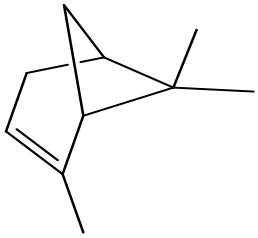
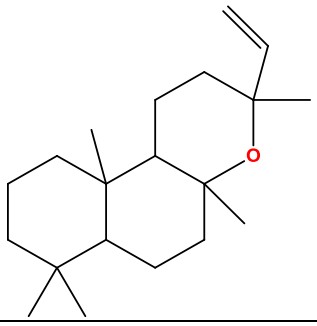
Major compounds	Relative percentage	Chemical formula	Chemical structure
Pinene	43.61%	$C_{10}H_{16}$	
Manoyl oxide	11.50%	$C_{20}H_{34}O$	

Table 3: Antibacterial activity of *Juniperus phoenicea* essential oil

Bacterial strains	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)	Inhibition zone diameter (IZD)
E. coli (ATB:57) B6N	0.39 µL/mL	0.39 µL/mL	23.33 ± 2.89 mm
E. coli (ATB:97) BGM	0.1 µL/mL	0.19 µL/mL	23.67 ± 1.15 mm
S. aureus	0.195 µL/mL	0.78 µL/mL	14.00 ± 1.73 mm
B. Subtilis	0.195 µL/mL	0.78 µL/mL	21.33 ± 2.08 mm
P. aeruginosa	0.33 µL/mL	1.3 µL/mL	12.33 ± 1.52 mm

Table 4: Antifungal activity of *Juniperus phoenicea* essential oil

Fungal strains	Inhibition zone Diameter (IZD)
A. fumigatus	NI
C. albicans	10.5 ± 1.80 mm
A. niger	NI
F. oxysporum	40.61 ± 1.53 mm
A. alternata	17.33 ± 1.52 mm

Inhibition zone includes diameter of disk (6 mm). NI: No inhibition of growth.

The ability of the essential oil to disrupt the permeability barrier of cell membrane structures and the resultant loss of chemiosmotic control are the most probable explanations for its lethal activity.[23]
The above observations substantiate the extensive historical use of *Juniperus phoenicea* in traditional medicine as antibacterial agent. Consequently, this plant may be viewed as a readily accessible alternative to conventional antibacterial medications.

Antifungal activity of Juniperus phoenicea essential oil
The antifungal properties of essential oil of *Juniperus phoenicea* from Fez-Meknes Region were evaluated against four pathogenic fungi using the disk diffusion method, and the results obtained are presented in Table 4. Analysis of the antifungal activity of *Juniperus phoenicea* EO reveals variable results depending on the fungal strains tested. Firstly, no significant inhibition was observed against *A. fumigatus* and *A. niger*, indicating a lack of antifungal effect of the essential oil on these fungal strains. In contrast, the essential oil showed strong antifungal activity against *F. oxysporum*, with inhibition zone diameter of 40.61 ± 1.53 mm. Moderate antifungal activity was observed against *C. albicans* (10.5 ± 1.80 mm) and *A. alternata* (17.33 ± 1.52 mm).
These results suggest that the efficacy of *Juniperus phoenicea* EO varies according to the strain of fungus, which could have important implications for its potential use as an antifungal agent. The antifungal activity observed for *Juniperus phoenicea* essential oil agrees with the findings from other studies, wherein essential oils extracted from the aerial parts of *Juniperus phoenicea* exhibited inhibitory effects against all tested fungi at low concentrations.[28,38]

Conclusion

Essential oil extracted from *Juniperus phoenicea* in the Fès-Meknès region was found to be rich in compounds such as α-pinene and manoyl oxide. This essential oil has demonstrated antibacterial properties against various strains, notably *E. coli*, and antifungal activity against *F. oxysporum, A. alternata* and *C. albicans*. The unique chemical composition of this essential oil largely explains its effectiveness. As a result, its potential application in the fields of bioconservation, cosmetics, and pharmaceuticals holds promise.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Mikou K, Rachiq S, Oulidi AJ. Ethnobotanical survey of medicinal and aromatic plants used by the people of Fez in Morocco. Phytothér. 2016; 14(1):35-43.
2. Allali A, Bourhia M, Hadin H, Rezouki S, Salamatullah AM, Soufan W, Hail ZR, Ouahmane L, El abdali Y, Eloutassi N, Fadli M. Essential oils from *Artemisia herba alba* Asso., *Maticaria recutita* L., and *Dittrichia viscosa* L. (asteraceae): A promising source of eco-friendly agents to control *Callosobruchus maculatus* fab. Warehouse pest. J Chem. 2022; 2022:1-14.
3. Zeggwagh AA, Lahlou Y, Bousliman Y. Survey of toxicological aspects of herbal medicine used by a herbalist in Fes, Morocco. The Pan Afr Med J. 2013; 14:125-125.
4. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J Ethnobiol Ethnomed. 2006; 2 (1):1-10.
5. El Abdali Y, Allali A, Agour A, Mikou K, Lahkimi A, Eloutassi N, Bouia A. Phytochemical screening, and in vitro antiradical and immunostimulant potential of *Linum usitatissimum* L. Pharmacologyonline. 2021; 3: 602-614.
6. Hseini S and Kahouadji A. Ethnobotanical study of medicinal flora in the Rabat region (western Morocco). Lazaroa. 2007; 28:79–93.
7. Beniaich G, Salim R, Ech-chihbi E, El-Hajjaji F, Rais Z, Abdellaoui A, Taleb M. Ethnobotanical survey about medicinal plants used in the traditional treatment of insomnia, asthenia, and oral and gum infections in the region of Fez-Meknes, Morocco. Environ Sci Pollut Res. 2021; 29(1):133-145.
8. Chen F, Liu S, Zhao Z, Gao W, Ma Y, Wang X, Yan S, Luo D. Ultrasound pre-treatment combined with microwave-assisted hydrodistillation of essential oils from *Perilla frutescens* (L) Britt. Leaves and its chemical composition and biological activity. Ind Crops Prod. 2020; 143:1-8.
9. Lee J, Chae K, Ha J. Regulation of obesity and lipid disorders by herbal extracts from *Morus alba, Melissa officinalis*, and *Artemisia capillaris* in high-fat diet-induced obese mice. J Ethnopharmacol. 2008; 115(2):263-270.
10. Allali A, El abdali Y, Rezouki S, El moussaoui A, Bourhia M, Salamatullah AM, Alzahrani A, Alyahya HK, Albadr NA, Nafidi HA, Ouahmane L, Fadli M. Chemical composition and antifungal, insecticidal and repellent activity of essential oils from *Origanum compactum* Benth. Used in the Mediterranean diet. Front Plant Sci. 2022; 13: 798259.
11. Allali A, Rezouki S, Fadli A, El Moussaoui A, Mohammed B, Salamatullah A, Alzahrani A, Alyahya H, Albadr N, Agour A, El Barnossi A, and Eloutassi N. Chemical Characterization and Antioxidant, Antimicrobial, and Insecticidal Properties of Essential Oil from *Mentha pulegium* L. Evid Based Complement Altern Med. 2021; 2021:1-12.
12. Amaghnouje A, Slighoua M, Es-safi I, ElJaoudi R, Elyoubi A, Bousta D, Grafov A. Ethnobotanical Survey of Medicinal Plants Used in the Traditional Treatment of Depression and Anxiety in Fez-Meknes Region. Phytothér. 2020; 18(3-4):220-229.
13. Nedjimi B, Beladel B, Guit B. Multi-element determination in medicinal juniper tree (*Junipurus phonicea*) by instrumental neutron activation analysis. J Radiat Res Appl Sci. 2015; 8:243-246.
14. Tahar D and Dahmane D. Chemical composition of the essential oil of *Juniperus phoenicea* L. from Algeria. J Essent Oil Res. 2008; 20(1):15-20.
15. Qnais EY, Abdulla YY, Abu Ghlyun P. Antidiarrheal effect of *Juniperus phoenicea* L. leaves extract in rats. J Biol Sci. 2005; 8(6):867-871.
16. Ghouti D, Rached W, Abdallah M, Pires T, Calhelha R, Alves MJ, Lazzouni HA, Barros L, Isabel M, Ferreira I.

Phenolic profile and *in vitro* bioactive potential of Saharan *Juniperus phoenicea* L. and *Cotula cinerea* (Del) growing in Algeria, Food Funct. 2018; 9(9):4664-4672.
17. Medini H, Elaissi A, Khouja ML, Piras A, Porcedda S, Falconieri D, Marongiu B, Chemli R. Chemical composition and antioxidant activity of the essential oil of *Juniperus phoenicea* L berries. Nat Prod Res. 2011; 25:1695–1706.
18. Bouil L. Notes on the traditional plant tar extraction technique. Mashrq and Maghreb III Algeria project. 2007; 2:4-6.
19. El abdali Y, Beniaich G, Mahraz AM, El Moussaoui A, Bin Jardan YA, Akhazzane M, Chebaibi M, Nafidi HA, Eloutassi N, Bourhia M, Bouia A. Antibacterial, Antioxidant, and in silico NADPH Oxidase Inhibition Studies of Essential Oils of *Lavandula dentata* against Foodborne Pathogens. Evid-Based Complement Altern Med. 2023; 2023:1-12.
20. El abdali Y, Mahraz AM, Beniaich G, Mssillou I, Chebaibi M, Bin Jardan YA, Lahkimi A, Nafidi HA, Aboul-Soud MAM, Bourhia M, Bouia, A. Essential oils of *Origanum compactum* Benth: Chemical characterization, in vitro, in silico, antioxidant, and antibacterial activities. Open Chem. 2023; 21:20220282.
21. El Moussaoui A, Bourhia M, Jawhari FZ, Salamatullah AM, Ullah R, Bari A, Majid Mahmood H, Sohaib M, Serhii B, Rozhenko A, Aboul-Soud MA. Chemical profiling, antioxidant, and antimicrobial activity against drug-resistant microbes of essential oil from *withania frutescens* l. Appl Sci. 2021; 11(11):51-68.
22. Bertella A, Benlahcen K, Abouamama S, Pinto DC, Maamar K, Kihal M, Silva AM. *Artemisia herba-alba* Asso. essential oil antibacterial activity and acute toxicity. Industr Crop Prod. 2018; 116:137-143.
23. Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Abderraba M, Raies A, Romdhane M. Chemical composition and antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L.(Cupressacees). J Food Sci. 2009; 74(7):364-371.
24. Ghanmi M, Satrani B, Chaouch A, Aafi A, Abid AE, Ismaili MR, Farah A. Chemical composition and antimicrobial activity of turpentine from maritime pine (*Pinus pinaster*) and Aleppo pine (*Pinus halepensis*) from Morocco. Acta Botanica Gallica. 2007;154(2):293-300.
25. Rezouki S, Allali A, Eloutassi N, Fadli M. Biotechnological valorization of aromatic plants in Morocco: impact of extraction methods on the yield and chemical composition of *Origanum compactum* Benthof Taounate (Northern Morocco). Plant Cell Biotechnol Mol Biol. 2020; 21:61-62.
26. Angioni A, Barra A, Maria T, Russo MT, Coroneo V, Sandro Dessia S, Cabras P. Chemical composition of the essential oils of Juniperus from ripe and unripe berries and leaves and their antimicrobial activity. J Agric Food Chem. 2003; 51:3073–3078.
27. Barrero AF, Herrador MM, Arteaga P, Quilez del Moral JF, Sanchez-Fernandez E, Akssira M, Aitigri M, Mellouki F, Akkad S. Chemical composition of the essential oil from the leaves of *Juniperus phoenicea* L. North Afr J Essent Oil Res. 2006; 18:168–169.
28. Mansouri N, Satrani B, Ghanmi M, El Ghadraoui L, Aafi A. Chemical and biological study of *Juniperus phoenicea* ssp. lycia and *Juniperus phoenicea* ssp. Turbinata essential oils from Morocco. Biotechnol Agron Soc Environ. 2010; 15(3):415-424.
29. Lüttge U, Kluge M, Bauer G. Botany: basic treatise. Ed. Tec. & doc. Lavoisier, Paris. 1992. 205-218 p.
30. El Ajjouri M, Satrani B, Ghanmi M, Aafi A, Farah A, Rahouti M, Amarti F, Aberchane M. Antifungal activity of essential oils of *Thymus bleicherianus* Pomel and *Thymus capitatus* (L.) Hoffm. & Link against wood-rotting fungi. Biotechnol Agron Soc Environ. 2008; 12(4):345-351.
31. Aljaiyash A, Kasrati A, Alaoui Jamali C, Chaouch A. Effect of cultivation on chemical composition and bioactivities of essential oils from *Artemisia herba-alba* Asso grown in Morocco. Biochem Syst Ecol. 2018; 81:74–79.
32. Duke JA. Phytochemical Database. USDA – ARS – NGRL, (ed), Beltville Agricultural Research Center, Belstville, Maryland. Edward P.Claus., Varro E.T., Lynn R. B. Pharmacognosy, sixth edition, LEA et Febiger, 1998. 184-187 p.
33. Bencheqroun HK, Ghanmi M, Satrani B, Aafi A, Chaouch A. Antimicrobial activity of essential oils from Artemisia mesatlantica, an endemic plant of Morocco. Bulletin de la Société Royale des Sciences de Liège. 2012; 81:4-21.
34. Derwich E, Benziane Z, Boukir A. Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. Int J Agric Biol. 2010a; 12:199–204.
35. Derwich E, Benziane Z, Boukir A. GC/MS analysis and antibacterial activity of the essential oil of *Mentha pulegium* grown in Morocco. Res J Agric Biol Sci. 2010b; 6(3):191–198.
36. Akermi M, Moussaoui A, Makhloufi A, Dalil H. Phytochemistry, antimicrobial activities of the essential oils of the branches of *Juniperus phoeniceain* Bechar (Algeria). Appl Biol Saharan Areas. 2017; 1(2):1-7.
37. Alessandra LO, Roberta BL, Fernando AC, Marcos NE. Volatile compounds from pitanga fruit (*Eugenia uniflora* L.). Food Chem. 2005; 99:1–5.
38. Ennajar M, Bouajila J, Lebrihi A, Mathieu F, Savagnac A, Abderraba M, Raies A, Romdhane M. The influence of organ, season and drying method on chemical composition and antioxidant and antimicrobial activities of *Juniperus phoenicea* L. essential oils. J Sci Food Agric. 2010; 90:462–470.