Regular Article

Antimicrobial activity of essential oils of *Juniperus phoenicea* from North Western Algeria

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Abstract

Juniperus phoenicea (Family: Cupressaceae) is an evergreen tree widely distributed in North Africa including Algeria. The aim of this investigation was to analyse the antimicrobial potential of essential oils from *J. phoenicea* on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Streptococcus* sp, *Bacillus* sp and *Candida albicans* using wells and discs diffusion methods. Broth dilution method was utilized to study the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The results showed a variable degree of antimicrobial activity. The diameters of inhibition zones for all test organisms were in the ranges of 7–21 mm, while MIC was from 62.5 to >500 µl/ml and MBC from 250 to >500 µl/ml. The highest antimicrobial activities were observed against Gram positive bacteria followed by Gram negative ones then *Candida albicans*. The findings provide the evidence that *J. phoenicea* as a good medicinal plant for further investigations.

Key words:

Juniperus phoenicea, essential oils, microorganisms, antimicrobial activity

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Introduction

Antibiotic resistance by microorganisms is turning into an expanding health concern and the need to find new antimicrobial agents is highly demanding. One approach includes the scan for new remedial operators with novel methods of activity from natural resources like plants and plant based products like secondary metabolites (Brantner and Grein, 1996). Medicinal and aromatic plants are generally utilized as a part of traditional antimicrobial agents and their essential oils, blends volatile compounds have been known to have antibacterial and antifungal properties. Past works have proposed that few fundamental oils indicated imperative antimicrobial actions against numerous pathogens (Pinto et al., 2003; Salgueiro et al., 2003; Pina-Vaz et al., 2004).

In Mediterranean regions, there are many native plants which belongs to the genus *Juniperus*, of Cupressaceae family. The flora of Algeria lists two sections and five *Juniperus* species; Sect. *Oxycedrus* (*J. communis*, *J. oxycedru*), and *Sabina* (*J. thurifera*, *J. phoenicea*, *J. sabina*) (Quezel and Santa, 1963; Maire, 1967; Adams et al., 2003). *J. phoenicea* is a shrub or a small tree which is believed to be originated in northern lands bordering the Mediterranean Sea from Portugal to Palestine and also considered as native to North Africa and mainly found in Libya, Algeria, Morocco and Canary Islands (Alfitori et al., 2014).

In traditional medicine, this plant is considered as highly valuable medicinal plant with properties like treatment in diarrhea,

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rheumatism and diabetes (Bellakhder, 1997; Allali et al., 2008).

This plant has been used as steam inhalant for bronchitis and to control arthritis, berries of this plant is used as an oral hypoglycaemic agent (Amer et al., 1994), whereas the leaves are used against bronco-pulmonary disease and as a diuretic (Bellakhder, 1997).

The chemical variability of the essential oil of *J. phoenicea* var. *turbinata* is already reported from Algeria (Bekhechi et al., 2012). The aim of this paper was to assess the antimicrobial activity of the obtained essential oils against *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus sp*, *Bacillus sp* and *Candida albicans*.

Materials and methods

Plant Material

J. phoenicea leaves were collected from the north-western part of Algeria, in the Mद्रousa region, province of Tiaret. They were identified by Dr. Belgherbi Benamar, Mascara University (Algeria).

Extraction of essential oils

100 g of the dry leaves of *J. phoenicea* were subjected to hydro distillation for 3 h with 500 ml distilled water using a Clevenger-type apparatus. The prepared volatile oils were dehydrated over anhydrous sodium sulphate and stored in dark vials in refrigerator at 4°C until analyzed. Essential oil yield was 0.048% (w/w).

Microbial strains

The antimicrobial activity was individually tested against a panel of microorganisms, including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 700603), obtained from Microbiology laboratory, Tlemcen university (Algeria), *Streptococcus sp* and *Bacillus sp* (from clinical sample) supplied by Medicinal analysis laboratory, hospital of Mohammadia (province of Mascara, Algeria), and *Candida albicans* (ATCC 10231) provided from Microbiology laboratory, Oran university (Algeria).

Determination of antimicrobial activity

Agar diffusion method

This method was carried out according to Mazari et al. (2010) with modification. Mueller-

Hinton agar was used for cultivation of bacteria, and Sabouraud for cultivation of yeast. In this method, pre-sterilized paper discs (6 mm in diameter) were impregnated with 10 µl of each oil dissolved in DMSO and applied on the surface of agar plates freshly seeded with standard inoculums of young cultures, 18-24 h old bacteria and yeast. The DMSO solvent was used as the negative control. Standard antibiotics Gentamycin (10 µg/disk) was used as positive controls for bacteria and Amphotericin B (20µg/disk) for *C. albicans*. The plates of test organisms were then incubated at 37°C for 24 h for bacteria and 30°C for 24 h for yeast. At the end of the incubation period, the diameters of inhibition zones were measured in millimeters.

Agar-well diffusion method

The agar well-diffusion method was followed to determine the antimicrobial activity as reported by Reddy et al. (2013) with modification. Mueller Hinton and Sabouraud plates were swabbed (sterile cotton swabs) with microorganism cultures. Wells were made on the agar surface with 6mm cork borer. Different dilutions of the essential oils (Pure, 1/2, 1/4, 1/8, 1/16, and 1/32) were added with a sterile syringe into the wells. The plates were incubated at 37°C for the bacteria and 30°C for the yeast, for 24 h. Negative and positive controls were used. The inhibition zones formed around the wells were measured in millimeters.

Determination of minimum inhibitory concentration (MIC) and minimum microbiocidal concentration (MBC) of essential oil

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each tested essential oil were determined using a broth dilution method (Almeida et al., 2013; Riahi et al., 2013) and as explained by Bachir et al. (2017).

Results and discussions

The crude essential oil of *J. phoenicea* showed antimicrobial activities against all the tested bacteria and *C. albicans*. It is shown that the susceptibility of the bacteria and *C. albicans* to the essential oil on the basis of inhibition zone diameters were differed based on the method used and microorganism (Figure 1 and Table 1).

The diameters of inhibition zone values (DIZ) of essential oil of *J. phoenicea* showed significant variation. There was moderate antimicrobial effect in essential oil against the microorganisms. The highest activity was observed on bacteria. The DIZ for bacterial strains and *C. albicans* were in the ranges of 7–21 mm and 8–19 mm respectively (Figure 1 and Table 1). This activity was highest at pure oil and was observed to decrease with concentration. This result was in accordance with previous studies which reported that bacterial strains were generally more sensitive to the essential oils than yeasts (Erkmen and Özcan, 2004; Helal et al., 2006; Obame et al., 2008).

In this study, we noted that Gram-positive bacteria (*S. aureus*, *Streptococcus. sp* and *Bacillus. sp*) were more susceptible to *J. phoenicea* essential oil than Gram-negative bacteria (*K. pneumoniae*, *E. coli* and *P. aeruginosa*). As shown in Figure 1 and Table 1, *S. aureus* was the most sensitive of the microorganisms to the *J. phoenicea* essential oil (inhibition zone = 21 mm), while, *P. aeruginosa* was the most resistant strain tested against this essential oil.

Our results are in good agreement with the findings of Cantore et al. (2004) and Zarai et al. (2012). The low susceptibility of Gram-negative

bacteria could be attributed to the presence of hydrophobic lipopolysaccharide in their outer membrane which provides protection against different agents (Kordali et al., 2005; Sepahvand et al., 2014; Shahi et al., 2002; Alves-Silva et al., 2013; Shahbazi, 2015).

Table 2 reports the MIC and the MBC of *J. phoenicea* oils against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus sp*, *Bacillus sp*, and *Candida albicans*. The oils extracted from the leaves of *J. phoenicea* exhibited weak activity against *Escherichia coli* (MIC >500µl/ml), moderate activity against *Bacillus sp* (MIC 125µl/ml and MBC >500µl/ml), and *Candida albicans* (MIC 125µl/ml and MBC >500µl/ml) high activity against *Streptococcus sp* (MIC 62.5µl/ml and MBC 500µl/ml), and the strongest was shown on *Staphylococcus aureus* (MIC 62.5µl/ml and MBC >250µl/ml).

Antimicrobial activity of the essential oils of *J. phoenicea* against many pathogens have been reported by many authors from many countries in the world, and really diverse results have been reported.

There is report by Angioni et al. (2003) which shows that the essential oils from the leaves of *J. phoenicea* ssp. *turbinata* exhibited weak activity against *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Table 1. Diameters inhibition zones (mm) of essential oil of *J. phoenicea* against test organisms with disc and wells diffusion methods.

Strains	Method	Gent	DMSO	Growth inhibition diameter (mm)					
				pure	1/2	1/4	1/8	1/16	1/32
<i>K. pneumoniae</i>	Disc	30	NI	12	7	NI	NI	NI	NI
	Wells	33	NI	14	10	NI	NI	NI	NI
<i>E. coli</i>	Disc	20	NI	16	7	7	NI	NI	NI
	Wells	24	NI	18	10	7	NI	NI	NI
<i>P. aeruginosa</i>	Disc	17	NI	8	7	NI	NI	NI	NI
	Wells	26	NI	10	7	NI	NI	NI	NI
<i>S. aureus</i>	Disc	27	NI	19	12	10	8	7	NI
	Wells	25	NI	21	19	11	11	NI	NI
<i>Streptococcus sp</i>	Disc	23	NI	17	13	11	9	7	NI
	Wells	23	NI	20	15	14	13	11	NI
<i>Bacillus sp</i>	Disc	28	NI	17	12	9	7	7	NI
	Wells	25	NI	16	14	10	7	7	NI
<i>C. albicans</i>	Disc	25	NI	17	NI	NI	NI	NI	NI
	Wells	NI	NI	19	10	8	NI	NI	NI

NI: no inhibition; Gent: Gentamycin

Fig. 1. Effect of essential oil of *J. phoenicea* on some microorganisms using wells and discs diffusion techniques.

Table 2. MBC and MIC ($\mu\text{L}/\text{mL}$) of the essential oils of *J. phoenicea* against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp, *Bacillus* sp and *Candida albicans*.

Strains	MIC	MBC
<i>E. coli</i> ATCC25922	>500 $\mu\text{L}/\text{mL}$	-
<i>S. aureus</i> ATCC25923	62.5 $\mu\text{L}/\text{mL}$	250 $\mu\text{L}/\text{mL}$
<i>Streptococcus</i> sp	62.5 $\mu\text{L}/\text{mL}$	500 $\mu\text{L}/\text{mL}$
<i>Bacillus</i> sp	125 $\mu\text{L}/\text{mL}$	500 $\mu\text{L}/\text{mL}$
<i>C. albicans</i> ATCC 10231	125 $\mu\text{L}/\text{mL}$	>500 $\mu\text{L}/\text{mL}$

Bouzouita et al. (2008) found that *J. phoenicea* oil shows inhibition effect against *Klebsiella oxytoca*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae* and *Geotrichum candidum*. Derwich et al. (2010) used essential oils of *J. phoenicea* to evaluate their activity on *Escherichia coli*, *Staphylococcus aureus*, *Staph. intermedius*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus mutans*. *E. coli* was the most sensitive strain with the strongest inhibition zone (34 mm), followed by *S. aureus*, *S. intermedius* and *K. pneumoniae*, 24, 18 and 14 mm, respectively. Modest activities were observed against *P. aeruginosa*, *B. subtilis* and *S. mutans* with inhibition zones of 10, 10 and 8 mm. The minimum inhibitory concentrations (MIC) ranging from 0.02 to 0.40 mg per mL. Mazari et al. (2010) examined the antimicrobial effect of *J. phoenicea* essential oil by MIC and disk diffusion methods on five bacteria (3 Gram-positive and 2 Gram-negative), and 3 fungi and reported that the essential oil had high antibacterial activity against *Enterococcus faecalis* with inhibition zone of 15.6 mm and MIC value of 7 $\mu\text{L}/\text{mL}$, and moderately

reduced the growth of *Aspergillus flavus* and *Fusarium oxysporum*.

Ait-Ouazzou et al. (2012) tested the essential oils of *J. phoenicea* on four Gram-positive, namely *Staphylococcus aureus*, *Enterococcus faecium*, *Listeria monocytogenes* 4b and *L. monocytogenes* EGD-e, and three Gram negative bacteria: *Salmonella Enteritidis*, *Escherichia coli* O157:H7, and *Pseudomonas aeruginosa*. The essential oil of *J. phoenicea* showed both, bacteriostatic and bactericidal activity only against the four Gram-positive strains, with diameter zone inhibition ranging from 13.1 mm (*E. faecium*) to 15.8 mm (*S. aureus*), and MIC values ranging from 0.5 $\mu\text{L}/\text{mL}$ (*S. aureus*) to 15 $\mu\text{L}/\text{mL}$ (*L. monocytogenes* EGD-e) and MBC values of 10 $\mu\text{L}/\text{mL}$ (*S. aureus* and *L. monocytogenes* 4b), 15 $\mu\text{L}/\text{mL}$ (*L. monocytogenes* EGD-e), and 30 $\mu\text{L}/\text{mL}$ (*E. faecium*). In other study, Ramdani et al. (2013) tested essential oils of *J. phoenicea* of five localities from eastern Algeria against both Gram positive (*Enterobacter cloacae* ATCC 13047, MRSA (Methicillin-resistant *Staphylococcus aureus*), *Staphylococcus aureus* ATCC 25923) and six Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas syringae*, *Salmonella* sp, *Serratia liquefaciens*

ATCC 27592, *Serratia marcescens* ATCC 14756, *Shigella* sp), and found that oil of the population of Elhadjaz shows a significant activity on all the bacteria tested. Elsewhere, Elmhdwi et al. (2015) reported that *J. phoenicea* extracts (70% methanol, 70% ethanol and 70% acetone) at concentration of 20%, 30% and 40% were effective against both Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Acinetobacter* sp.).

The antimicrobial activity of essential oils depends on their chemical composition (Teixeira et al., 2012). Their biological activities are often attributed to their major components (Ait-Ouazzou et al., 2012). In previous reports, the antimicrobial activity of essential oils of *Juniperus phoenicea* can be attributed to the presence of high concentration of α -pinene (Mazari et al., 2010; Ramdani et al., 2013; Elmhdwi et al., 2012) on the chemical composition of *J. phoenicea* oil from Algeria. α -pinene has been found to have relatively strong antimicrobial properties against many important pathogens and spoilage organisms (Bourkhiss et al., 2007; Safaei-Ghomi and Ahd, 2010).

It crushes the cellular integrity of Gram positive microbes and inhibit respiratory action in yeast mitochondria and had some antifungal movement however Gram negative bacteria were more resistant to it (Andrews et al., 1980). In addition, other compounds such as β -Phellandrene, D-germacrene and α -terpinyl acetate which have antimicrobial properties may be responsible for this activity (Hernández et al., 2008; Ngassapa et al., 2003; Simic et al., 2002). However, the parts with lower concentrations, may likewise be adding to the antimicrobial activity of the oil. In this manner, the synergistic impacts of the different major and minor parts of the basic oils ought to be contemplated to represent the oil organic action (Burt, 2004).

Conclusion

The essential oils of *J. phoenicea* leaves hindered the development of both bacteria and *C. albicans* and shows both a bacteriostatic and bactericidal impacts on tried microorganisms. Our outcomes legitimize the utilization of this plant by conventional healers in the treatment of specific sicknesses and recommend that *J. phoenicea* oils could be filled in as a vital

characteristic option for antimicrobial drug development research.

Author contributions

All authors contributed equally in the study and preparation of article. All authors approved the final version of the manuscript for publication.

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