DF-776
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RESEARCH ARTICLE Agricultural Science Digest

Phytochemical Screening, Antimicrobial and Antioxidant Activity of Methanolic Extract of *Juniperus phoenicea* Leaves

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10.18805/ag.DF-776

ABSTRACT

Background: As part of the valorization of Algerian medicinal flora, *Juniperus phoenicea* has attracted attention due to its diverse pharmacological properties. The current investigation aims to evaluate the phytochemical profile, antimicrobial potential and antioxidant activity of the methanolic extract of *J. phoenicea* leaves.

Methods: *J. phoenicea*'s methanolic extract was subjected to various phytochemical tests. Antimicrobial activity was tested against six conventional pathogenic bacterial strains and one fungal strain using the disc-diffusion technique. The minimum inhibitory concentration was determined using the agar dilution method. Antioxidant activity was estimated by DPPH radical scavenging assay.

Result: The phytochemical screening of the methanolic extract indicated the presence of many secondary metabolite categories, including tannins, flavonoids, saponins and terpenoids. Antimicrobial activity was evaluated using the disc diffusion method and the bacterial strains *Staphylococcus aureus* and *Listeria monocytogenes* were shown to be the most sensitive. However, no antimicrobial activity was detected against *Pseudomonas aeruginosa, Escherichia coli* or the fungal strain *Candida albicans*. The minimal inhibitory concentration (MIC) was determined using the solid medium dilution technique and the results ranged from 1.56 to 12.5 mg/mL. The DPPH scavenging capability indicated that the methanolic extract has modest antioxidant activity, with an IC50 value of 0.82 mg/mL.

Key words: Antimicrobial activity, Antioxidant activity, *Juniperus phoenicea*, Methanolic extract, Phytochemical screening.

INTRODUCTION

For thousands of years, human have used various plant species found in its natural environment to treat and cure different diseases (Amrati *et al*., 2021; Mottaghipisheh *et al*., 2024). Plants contain a large variety of secondary metabolites, making them an immense source of chemically diverse substances with a wide range of biological activities (Erenler *et al*., 2019; Divekar *et al*., 2022; Gbenou *et al*., 2024). Among these bioactive constituents, alkaloids, terpenes and phenolic compounds are particularly noteworthy for their pharmacological importance and multiple health benefits (Kebede *et al*., 2021). Numerous epidemiological studies have highlighted that a diet rich in plant polyphenols may help reduce the risk of developing cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative disorders (Chang *et al*., 2022; Omidfar *et al*., 2023). Some of them are also used as ingredients in the food, pharmaceutical and cosmetic industries (Mayyas *et al*., 2021).

The alarming rise of antimicrobial resistance has intensified the global search for new, effective and safe antimicrobial agents from natural sources. Medicinal and aromatic plants have thus emerged as a promising alternative for developing novel bioactive molecules with fewer side effects (Khameneh *et al*., 2019; Aici and Benmehdi, 2021; Haile *et al*., 2022; Mishra *et al*., 2025).

The genus *Juniperus* (family Cupressaceae) is among the most taxonomically diverse conifer groups, comprising approximately 67 species and 28 varieties distributed mainly across subtropical and temperate regions of the Northern Hemisphere, with some extending into the Southern Hemisphere (Chelouati *et al*., 2023; Liu *et al*., 2024). *Juniperus phoenicea* is a resinous, aromatic shrub or small tree with a wide distribution across southern Europe, temperate and subtropical Asia, the Atlantic coast and North Africa (Ennajar *et al*., 2009; Mehira *et al*., 2021). This species is valued for its medicinal properties and traditional uses, leaves of *Juniperus phoenicea* are commonly prepared as a decoction to treat diabetes, diarrhea, rheumatism and digestive disorders. Furthermore, a combination of leaves and berries is traditionally employed as an oral hypoglycemic agent (Chelouati *et al*., 2023).

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How to cite this article: Derradjia, A., Deboucha, S. and Meskini, Z. (2025). Phytochemical Screening, Antimicrobial and Antioxidant Activity of Methanolic Extract of *Juniperus phoenicea* Leaves. *Agricultural Science Digest.* 1-6. doi: 10.18805/ag.DF-776.

Submitted: 17-07-2025 **Accepted:** 12-12-2025 **Online:** 05-01-2026

Algeria, with its remarkable diversity of climatic zones, harbors an exceptionally rich flora that includes numerous aromatic and medicinal species (Walas and Taib, 2022). This biodiversity offers a valuable reservoir of bioactive compounds with significant pharmacological potential.

In this context, the present study aims to evaluate the phytochemical composition, antimicrobial activity and antioxidant potential of the methanolic extract of *Juniperus phoenicea* leaves collected from northwestern Algeria. The results are expected to contribute to the valorization of this native species as a potential source of natural therapeutic agents.

MATERIALS AND METHODS

Plant material

Leaves of *J. phoenicea* were collected in March 2022 in the Sidi Khatab district of Relizane, Algeria. The plant was used after being identified and verified by a taxonomist from the Department of Natural and Life Sciences, University of Algies 1, Algeria. The leaves were air-dried at ambient temperature in a dark, well-ventilated environment, ground into a fine powder and stored in sterile amber glass bottles until further use.

Preparation of methanolic extract

The plant material was extracted using a soxhlet under continuous agitation for 24 hours. 30 mg of plant powder were dissolved in 100 mL of methanol. The obtained extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C until analysis (Zarei Yazdeli *et al.*, 2021). The extraction yield was calculated according to the formula:

$$Y\ (\%) = \frac{W_D}{P_P} \times 100$$

Where,

W_D= The weight of the dried extract.

P_P= The weight of the plant powder utilized.

Phytochemical screening

The methanolic extract was subjected to qualitative phytochemical analysis for the detection of major secondary metabolites including flavonoids, tannins, saponins, terpenoids and glycosides using standard procedures (Ingle *et al.*, 2017; Berreghioua and Ziane, 2025a). These methods are based on colorimetric or precipitation reactions characteristic of each compound class.

Test for flavonoids

An aqueous solution of the methanolic extract was treated with 10% ammonium hydroxide. Yellow fluorescence showed the presence of flavonoids.

Test for tannins

A 0.5 mg sample of the extract was boiled in 20 mL of distilled water then filtered. The addition of a few drops of 0.1% ferric chloride produced a brownish-green or blue-black colouration, confirming the presence of tannins.

Test for terpenoids

Five millilitres of extract were mixed with 2 mL of chloroform, followed by the careful addition of concentrated sulfuric acid. The formation of a brownish-red interface indicated the presence of terpenoids.

Test for saponins

Ten millilitres of the diluted extract were vigorously shaken in a graduated cylinder for 15 minutes. A stable 2 cm foam layer persisting for 15 minutes indicated the presence of saponins.

Test for glycosides

In total, 50 mg of extract was hydrolyzed with strong hydrochloric acid in a water bath for 2 hours before being filtered. The hydrolysate underwent the Borntrager test. To 2 mL of filtered hydrolysate was added 3 mL of chloroform. The chloroform layer was separated after shaking the mixture,then ammonia solution (10%) was added to it. Pink colouration suggests glycosides presence.

Antimicrobial activity

Preparation of standard strains

Six strains from the American type culture collection (ATCC) were kindly provided from the Laboratory of Valorization and Bioengineering of Natural Resources, University of Algiers 1, Algeria, to evaluate the antimicrobial properties of methanolic extract of *J. phoenicea*: *Staphylococcus aureus* 6538, *Bacillus subtilis* 6051, *Enterococcus faecalis* 29212, *Pseudomonas aeruginosa* 9027, *Escherichia coli* 8739, *Listeria monocytogenes* 19117 and *Candida albicans* 10231. Strains were revived in sterile Brain Heart Infusion broth (BHIB) and incubated at 37°C overnight.

Disc-diffusion method

Antimicrobial activity was evaluated using the disc diffusion method (Raoof *et al.*, 2019). Bacterial suspensions (10^8 CFU/mL) and yeast suspension (10^6 CFU/mL) were evenly spread on Mueller-Hinton agar and Sabouraud dextrose agar plates, respectively. Sterile Whatman paper discs (6 mm diameter) were impregnated with 10 µL of the methanolic extract at concentrations of 100, 50, 25 and 12.5 mg/mL (dissolved in DMSO) and placed on the inoculated agar surfaces. Plates were incubated at 37°C for 24 hours for bacterial strains and 48 hours for the fungal strain. Antimicrobial activity was expressed as the diameter (mm) of the inhibition zone, including the disc diameter. Discs containing only DMSO served as negative controls.

Agar dilution method

The minimum inhibitory concentration (MIC) of the methanolic extract was determined by the agar dilution method (Zarei Yazdeli *et al.*, 2021). Different concentrations of the extract (0.39 to 12.5 mg/mL) were incorporated into agar media. Standardized microbial inocula were applied to the agar surface and incubated under appropriate conditions. The MIC was defined as

the lowest extract concentration that completely inhibited visible microbial growth.

Antioxidant activity

The antioxidant activity was carried out using the DPPH radical scavenging assay (Bougandoura and Bendimerad, 2013). 0.5 µL of each methanolic extract solution at various concentrations (from 0.0125 to 5 mg/mL) were added to 1.95 mL of DPPH methanolic solution (0.025 g/L). After 30 minutes of incubation in the dark at room temperature, absorbance was measured at 515 nm against a corresponding blank. The positive control was a solution of ascorbic acid, a standard antioxidant, whose absorbance was measured under identical circumstances as the sample. The percentage of inhibition (I%) was calculated and the IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined by linear regression analysis.

Statistical analysis

The data obtained were analyzed using SPSS Statistical Software for Windows. All assays were performed in triplicate and quantitative variables were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical screening

The methanolic extract of *J. phoenicea* leaves was characterised by a brownish-green colour. The extraction yield is around 19.61%, which is considerably lower than the values reported by Medini *et al.* (2013) (28.77 and 39.23 %). However, Farahat (2020) reported that methanolic extract showed a yield of 9.8% for *J. phoenicea* leaves collected from Egypt. The yield of plant extracts can be affected by several parameters, it can vary depending on the chemical composition and physical characteristics of the plant material as well as the method and conditions under which the extraction was carried out (Dai and Mumper, 2010; Elsherif *et al.*, 2023; Berreghioua and Ziane, 2025b). Phytochemical analysis of *J. phoenicea* identified a range of bioactive compounds, including flavonoids, saponins, tannins and terpenoids. Likewise, the phytochemical screening of *J. phoenicea* from two different locations in Tunisia revealed the presence of tannins, flavonoids and saponins (Medini *et al.*, 2013). These phytochemicals have essential biological functions, saponins can lower cholesterol, modulate the immune system and inhibit tumor growth (Shraim *et al.*, 2021). However, a study by Makhloufi *et al.* (2014) did not detect tannins in *J. phoenicea* leaves, which contradicts the findings of the current study. Additionally, no glycosides were found in *J. phoenicea* leaves.

Antimicrobial activity

The antimicrobial screening of the methanolic extract of *Juniperus phoenicea* leaves against six bacterial strains and one *Candida albicans* strain revealed varying degrees of sensitivity. The inhibition zone diameters ranged from 9 mm to 19.5 mm (Table 1). *Listeria monocytogenes* and *Staphylococcus aureus* were the most sensitive strains, exhibiting the largest inhibition zones. In contrast, *Bacillus subtilis* and *Enterococcus faecalis* showed moderate inhibitory responses. However, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* were resistant to the extract's antimicrobial effect. These results indicate that Gram-positive bacteria are generally more sensitive than Gram-negative bacteria, as previously reported by Bouzouita *et al.* (2008) and Ait-Ouazzou *et al.* (2012). According to established criteria, an extract is considered active when it produces an inhibition zone ≥10 mm, whereas inhibition zones <7 mm are classified as inactive (Tekwu *et al.*, 2012; Draoui *et al.*, 2022). The higher sensitivity of Gram-positive bacteria can be explained by structural differences in the cell envelope. Gram-negative bacteria possess a complex, rigid outer membrane rich in lipopolysaccharides (LPS), which acts as an effective barrier limiting the diffusion of hydrophobic compounds. In contrast, Gram-positive bacteria lack this outer membrane and have a peptidoglycan layer whose relatively permeable structure does not provide sufficient protection against bioactive compounds (Burt, 2004).

Overall, our results are consistent with several previous studies. Similar to the findings of the present investigation, Draoui *et al.* (2022) reported that methanolic extract of *J. phoenicea* exhibited moderate to strong antibacterial

Table 1: Antimicrobial activity of methanolic extract of *J. phoenicea* leaves.

Strains	Diameter of the growth inhibition zone (mm)			
	Methanolic extract			
	100%	50%	25%	12,5%
S. aureus	19.5±00	18.5±0.70	13.5±0.70	12±1.41
E. coli	-	-	-	-
L. monocytogenes	19±0.71	17.5±0.70	16.5±0.71	15±00
P. aeruginosa	-	-	-	-
E. faecalis	10±1.41	10.4±0.71	09±00	10±1.41
B. subtilis	12±00	10.5±0.70	10.5±0.71	9±1.41
C. albicans	-	-	-	-

(-) Non-activity; 9 <IZ <14 mm: Moderate activity; 15 <IZ <19 mm: High activity; 19 mm <IZ: Strong activity.

activity against pathogenic microorganisms, with inhibition zone diameters ranging from 9 to 19 mm. Likewise, a study conducted in Libya in 2021 demonstrated that methanolic extract of *J. phoenicea* leaves showed a strong inhibitory effect against *S. aureus* (Alhadad *et al*., 2023). An additional study conducted in Libya reported a strong antibacterial activity of methanolic extract of *J. phoenicea* leaves against *S. aureus* and various wild strains of human pathogenic bacteria. In contrast to the findings of the present work, Elmhdwi *et al*. (2015) observed that the methanolic extract was also effective against *E. coli* and *P. aeruginosa*. On the other hand, *C. albicans* was found to be resistant, as previously reported by Zubi *et al*. (2025).

The minimum inhibitory concentration (MIC), defined as the lowest extract concentration that reduces microbial colony growth by 90%. The MIC was determined for strains that showed sensitivity using the disc diffusion assay. The results obtained show that *L. monocytogenes* and *S. aureus* had the lowest MIC values of 1.56 mg/mL and 3.12 mg/mL, respectively, while *E. faecalis* and *B. subtilis* exhibited higher MICs of 6.25 and 12.5 mg/mL, respectively. These results are consistent with previous findings reported in North Africa and the Mediterranean region. Draoui *et al*. (2022) reported MIC values between 0.78 and 6.25 mg/mL for methanolic extract of *J. phoenicea* against various Gram-positive and Gram-negative bacteria. Similarly, Zerrouki and Riazi (2021) observed a MIC of 10 mg/mL against *S. aureus* for hydromethanolic extract.

Antioxidant activity

The antioxidant capacity of the methanolic extract of *J. phoenicea* was determined using the DPPH technique. The results showed that the methanolic extract of *J. pheonicea* had a percentage of inhibition of around 90.59% (Fig 1). The synthetic molecule, ascorbic acid, showed a strong reduction at the first concentrations (Fig 2).

In a previous study, the percentage of inhibition for the methanolic extract of *J. pheonicea* leaves harvested in Tunisia was 95.89%, which is slightly higher than that obtained in our research (Medini *et al*., 2013). In another study, the maximum antiradical power of the methanolic extract of *J. pheonicea* was 62.41% (Al-Mustafa *et al*., 2021).

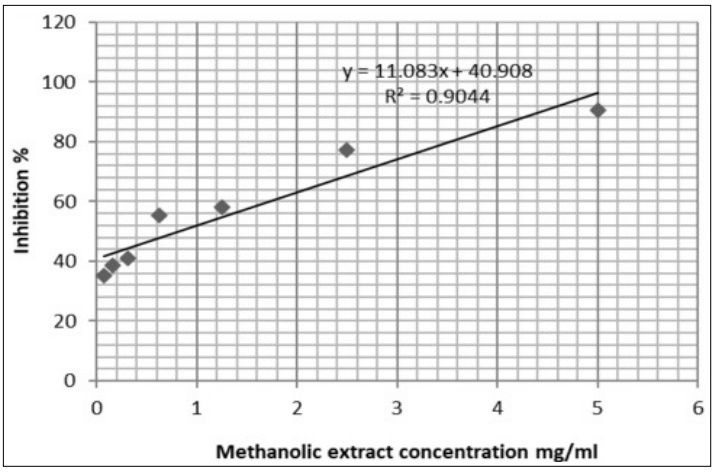

Fig 1: Percentage inhibition of DPPH by methanolic extract of *J. phoenicea*.

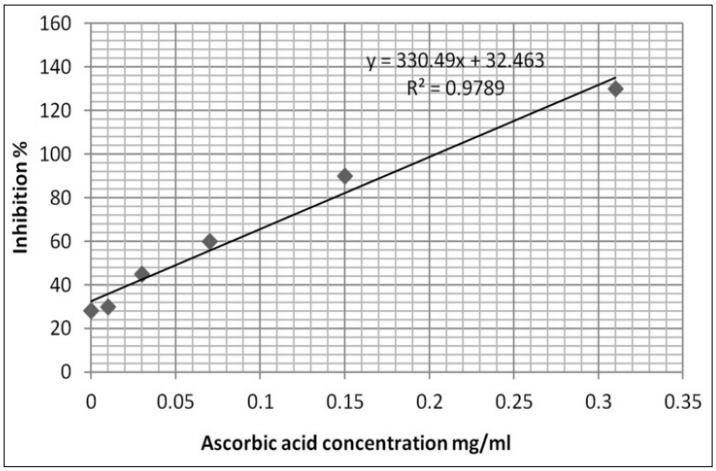

Fig 2: Percentage inhibition of DPPH by ascorbic acid.

This disparity might result from various extraction techniques, plant components, solvents or sample origins (Elsherif *et al.*, 2023).

The concentration required to block 50% of the DPPH radicals was also estimated. The IC_{50} value of the methanolic extract was determined to be 0.82 mg/mL. This indicates a moderate antioxidant activity, which remains lower than that of ascorbic acid (IC_{50}≈0.05 mg/mL). Antioxidant potency can vary depending on extraction methods and plant origin, as reported by Ennajar *et al*. (2009), *J. phoenicea* leaves showed a much lower IC_{50} (8.5±0.3 mg/L), highlighting these variations.

CONCLUSION

Medicinal plants continue to represent a reliable source of natural bioactive compounds with significant therapeutic potential. The present study demonstrated that the methanolic extract of *Juniperus phoenicea* leaves from northwestern Algeria exhibits notable antibacterial activity particularly against Gram-positive bacteria and moderate antioxidant capacity.

These findings support the traditional uses of *J. phoenicea* and highlight its potential as a natural source of antimicrobial and antioxidant agents. Future investigations should focus on quantitative phytochemical profiling, isolation of active compounds and elucidation of their mechanisms of action to better understand the pharmacological value of this species.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the content of this Manuscript. They report no financial, commercial, academic, or personal relationships that could be perceived as influencing the work presented. All authors have reviewed and approved this declaration.

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