



## Research Article

# Investigating the Bioactive Potential of *Ononis spinosa* L. Phytochemical Profiling and Antibacterial Effectiveness Evaluation

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**Abstract** | This study was conducted in order to investigate the antibacterial potency of the *Ononis spinosa* L. extracts. The phenolic compounds in the aerial parts of *Ononis spinosa* were examined using high performance liquid chromatography coupled with a diode array detector (HPLC-UV/DAD) technology, and were assessed due to their antimicrobial properties. The greatest total phenolic amount of the extracts, as measured by the Folin-Ciocalteu reagent procedure, was found to be  $43.03 \pm 1.02$  mg GAE/gDW in stem extract, while the utmost flavonoid content value was  $31.45 \pm 0.45$  mg QE/gDW in stem extract, as measured by aluminum chloride colorimetric method. Based on HPLC analysis, we find that epicatechin (5.1 mg/g) constituted the predominant compound in the aerial plant extract. The antibacterial capability of the extracts was determined against twelve strains of Gram-negative and Gram-positive bacteria. The strains evaluated were chosen for an antibacterial assay utilizing the *in vitro* disc diffusion and the minimum inhibitory concentration (MIC) assays. All examined extracts exhibited antibacterial potential, varying from moderate to strong, with the stem extract demonstrating the most pronounced effect, significantly inhibiting the development of *Staphylococcus aureus* recording diameter of inhibition zone, MIC, and MBC (minimal bactericidal concentration) of 30 mm, 4.68 mg/ml, and 9.36 mg/ml, respectively. The extracts from *Ononis spinosa* showed a high antibacterial efficacy, thus they may have potential applications in food and pharmaceutical products.

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## Introduction

A major future and present threat to global health, according to the World Health Organization

(WHO), is the rise of bacteria that are resistant to commonly used antibiotics (Farhadi *et al.*, 2018). Infectious illnesses produced by multidrug-resistant (MDR) bacteria have emerged as a significant

contemporary issue. The utilization of antibiotics has significantly diminished the prevalence of infectious diseases; yet, it has concurrently resulted in the emergence of drug-resistant bacteria (Varela *et al.*, 2021).

Many phenolic compounds, including flavonoids and phenolic acids, that plants produce in response to microbial infection, exhibit a wide range of antibacterial properties against various microorganisms, leading to the development of several effective pharmaceuticals (Othman *et al.*, 2019). Polyphenols demonstrate independent antibacterial properties and can also inhibit the antibiotic resistance of the pathogenic microorganisms or function synergistically with the traditional antimicrobial agents such as antibiotics (Nassarawa *et al.*, 2022). Furthermore, herbal formulations are relatively more economical, exhibit less adverse effects, and can complement other medicinal systems in addressing disorders induced by pathogenic microorganisms (Ignat *et al.*, 2013).

*Ononis spinosa* (Spiny Restharrow), a perennial herb of the *Fabaceae* family, is reported to thrive in both cultivated and wasteland areas in Algeria and many Mediterranean nations (Al-Eisawi, 1982). In traditional medicine, species of the *Ononis* genus display notable medicinal qualities for the treatment of various ailments. *Ononis spinosa* is utilized as a traditional therapy for urinary tract disorders and nephrolithiasis owing to its anti-inflammatory and diuretic properties, as well as for eczema (Öz *et al.*, 2017). Compounds extracted from the genus *Ononis* have demonstrated antibacterial (Orhan *et al.*, 2012) and antioxidant (Musa *et al.*, 2021) properties. The extract from *O. spinosa* demonstrated the ability to safeguard the stomach mucosa (Abbas *et al.*, 2021). Prior studies on *O. spinosa* led to the extraction of isoflavone glycosides (Benedec *et al.*, 2012), flavonoid-O-glycosides (Musa *et al.*, 2021), and triterpenes (Shaker *et al.*, 2004).

According to our knowledge, there are no publications about the characterization of the antibacterial activity related to phenolic and flavonoid contained in the extracts of aerial parts of *O. spinosa* from Algeria. The objectives of this study were to: (i) ascertain the complete chemical composition of methanolic extracts from stems and leaves utilizing HPLC-UV/DAD, and (ii) examine the efficacy “*in vitro*” antibacterial activity on the survival and growth of

selected bacteria through agar disc diffusion and broth microdilution techniques.

## Materials and Methods

### Plant materials

The whole plants of *O. spinosa* were collected in the spring of 2024 during flowering period from the Tessala mount region (northwest Algeria) (Latitude Nord: 35°16'886" N, Longitude Ouest: 00°46'392" W). Taxonomic identification was performed at the Laboratory of Plant Biodiversity: Conservation and Valorization of Djillali Liabes University, Sidi Bel Abbès, Algeria. After being air-dried at ambient temperature for 2 weeks, the plant materials were pulverized and preserved in a cold and dry environment, shielded from light, and maintained at 4 °C until required for future use.

### Sample preparation

A 10 g powder of the stem and leaf constituents of *O. spinosa* were subjected to extraction with 300 ml of methanol for 24 h. The resulting extract was sonicated for two intervals of 15 min and then filtered using Whatman no. 4 paper. Using a rotary evaporator, the methanol extract was patted dry to dryness at 40 °C. The dehydrated extracts were employed for all subsequent analyses (Stojković *et al.*, 2020).

### Total phenolic contents

The total phenolic content (TPC) was spectrophotometrically quantified using the Follin-Ciocalteu assay (Vermerius and Nicholson, 2006) with gallic acid as a standard. Diluted extracts (0–500 mg/ml) (1.6 ml) were combined with 0.2 ml of Follin–Ciocalteu reagent (diluted 5-fold with dis. water) and stirred vigorously for 3 min. 0.2 ml of sodium carbonate (10 % w/v) was included into the blend, which was then permitted to remain stationary for 30 min. at ambient temperature. The absorbance of the combination was quantified at 760 nm utilizing a UV-VIS spectrophotometer (T60U UV-Vis, PG Instruments Ltd, England) and represented in milligram gallic acid equivalents per gram of dry weight (mg GAE/gDW).

### Total flavonoids

The overall flavonoid contents (TFC) were determined using the complexation of Al<sup>3+</sup> with flavonoids (Topçu *et al.*, 2007). The collected extract underwent dilution in methanol to a concentration of 500 mg/ ml. A

calibration curve was established by diluting quercetin in methanol at concentrations that varied between 0 and 500 mg/ ml. The diluted quercetin extract (2.0 ml) was combined with 0.1 ml of a 10 % (w/v) aluminum chloride solution and 0.1 ml of a 0.1 mM potassium acetate solution. The amalgamation was maintained at ambient temperature around 30 min. The maximum absorbance of the combination was subsequently estimated at 415 nm utilizing a UV-VIS spectrophotometer (T60U UV-Vis, PG Instruments Ltd, England). Total flavonoid contents (TFC) were quantified in milligram quercetin equivalents per gram of dry weight (mg QE/g DW).

#### HPLC-UV/DAD analysis

Characterization of phenolic compounds from optimized aerial parts extracts of *O. spinosa* using HPLC-UV/DAD was carried out on an Agilent 1200 Infinity (Agilent Technologies, Santa Clara, CA, USA) series HPLC system connected to a diode array detector (DAD) (190–400 nm). The separation was executed utilizing a Zorbax C18 column (250 mm long×4.6 mm, *i.e.*, 5 µm). An automated system was used to inject the samples (5 µl). The column was regulated by a thermostat at 40 °C, and a 0.5 ml/ min flow rate was applied. As a whole, the mobile phase included of 0.1 % formic acid in water (solvent A) and acetonitrile (solvent B). The following multistep linear gradient was applied: 0.0 min/, 10 % A; 22.0 min., 50 % A; 32 min., 100% A; 40 min., 100 % A; 44.0 min., 10 % A; and 50.0 min., 10 % A. By analyzing the HPLC chromatograms of extracts obtained at various wavelengths between 210–430 nm and the associated UV absorption maxima for each reference chemical, wavelengths of 254, 280, and 330 nm were used for separation, identification, and quantitative analysis, respectively. Identification of polyphenols was conducted based on their chromatographic behavior and by comparison with the retention times (Rt) of standard compounds, when accessible, and information documented in the literatures, which may be used for tentative identification. The process of acquiring data was conducted using an Agilent ChemStation Software data system (Agilent Technologies, Santa Clara, CA, USA).

#### Antibacterial activity in vitro

**Bacterial strains:** Each extract was tested individually against a panel of bacterial strains, including *Proteus mirabilis*, *Acinetobacter baumannii*, *Streptococcus pneumoniae*, *Staphylococcus aureus* (ATCC25923),

*Pseudomonas aeruginosa* (ATCC25853), *Escherichia coli* (ATCC25922), *Bacillus cereus* (ATCC10876), and *Citrobacter freundii* (ATCC8090).

#### The disk diffusion assay

Dimethyl Sulfoxide (DMSO) was used to dissolve the extracts, resulting in a final concentration of 300 mg/ml. Pure cultures of each bacterial strain were submerged in sterile saline to achieve a turbidity equivalent to McFarland tube number 0.5 ( $1.5 \times 10^8$  cfu/ ml). A loopful from each adapted bacterium was inoculated up onto Muller Hinton (MH) agar using a cotton swab. Following drying, 100 µl of three distinct filter-sterilized (0.22 µm filter) extracts (100 % V/V) were injected into wells made using a sterile cork borer with a diameter of 6 mm in the MH agar. For 18 to 20 h, the plates were incubated at 37 °C. The positive antibacterial control was made using Gentamicin (Gen 10 %). The negative control consisted of pure DMSO. After incubation, the developing area of growth inhibition surrounding the wells was measured using a calibrated ruler to evaluate the antibacterial efficacy.

#### Minimum inhibitory concentration (MIC)

According to Boukada *et al.* (2023), the micro-well dilution method was used to determine the MIC values for each isolate. The first rows of microplates were filled with 50 µl of sample solutions (300 mg/ml) in DMSO. The mixtures were dispensed into their remaining wells contained in MH broth to form two-fold serial dilutions in the range of 300 to 4.68 mg/l. Afterward, 100 µl of the bacterial suspension was inoculated individually into each well. In order to verify the medium's sterility, the broth was injected into the first well, which served as a negative control. However, strain suspensions were employed to inoculate the final well, which served as a positive control. For duration of 24 h, the microplates were incubated at 37 °C. The bacterial growth was totally suppressed by the lowest flavonoid concentration, which was identified and reported as the MIC. Three replicates of each sample were used and the assay was conducted twice.

#### Minimum bactericidal concentration (MBC)

Approximately, 100 µl of mixture from each well that did not show any shift culture after 48 h were plated on nutrient agar (NA) to ascertain MBC. The plates were then incubated at 37 °C for 24 h. After incubation, there was no bacterial growth at the minimal amount when this subculture was classified as MBC (Boukada *et al.*, 2023).

### Statistical analysis

Triplicate runs of each test are conducted. The study's findings are presented as the mean with standard deviation ( $\pm$  SD). An examination of differences (ANOVA) was applied to the data from the different tests to assess their statistical significance.  $p$  values below 0.05 were considered significant.

## Results and Discussion

### Total phenolic and flavonoid contents of solvent extracts

The total phenol content of the extracts was quantified using the equation ( $y = 0.001x + 0.020$ ,  $R^2 = 0.996$ ), while the total flavonoid content was assessed using the equation ( $y = 2.619x + 0.022$ ,  $R^2 = 0.989$ ) derived from calibration curves. The quantities of total phenols and flavonoids present in *O. spinosa* extracts are presented in Table 1.

The total phenolics contents of the leaves and stems methanolic extracts of *O. spinosa* were 25.34 and 43.03 mg GAE/g DW, respectively. The results indicated that the stem extract possesses a greater total phenolic and flavonoid contents compared to the leaf extract. The aerial portion of *O. spinosa* has a significant amount of total phenolics, and while it is pertinent to compare our findings with other studies, to the best of our knowledge, no research has been conducted on this specific aerial section *O. spinosa*. For example, the total phenolic values were comparable to those reported in *O. spinosa* roots from Turkey that fell in the range  $3.09 \pm 0.01$  mg GAE/g (Orhan *et al.*, 2012). However, the values were much higher than that in *O. spinosa* roots from Bulgaria which fell in the range  $25.03 \pm 1.63$  mg GAE (Valyova *et al.*, 2008). Multiple investigations have been conducted on the phenolic content of various *Ononis* species. The total phenolic content of *O. natrix* in Tunisia was recorded as 51 mg GAE/g, whereas the flavonoid content was 14.76 QE/g.

### Chemical composition of Ononis spinosa extract

Typical UV-DAD chromatograms and the chemical composition of the methanolic extract of the *Ononis spinosa* aerial part are shown in Figure 1 and Table 2. Through comparing the retention durations of the extract components with their respective standards, 18 polyphenolic compounds were identified and quantified using HPLC-UV-DAD with standard curves in *O. spinosa*. The dominant compound was epicatechin (5.1 mg/g extract). In addition, seven

phenolic acids, mainly chlorogenic acid, o-coumaric acid, caffeic acid, protocatechuic acid, ferulic acid, vanillic acid, and p-coumaric acid), phenylethanoid (tyrosol), and nine flavonoids were distributed over the following subclasses: A flavanone (naringenin), two flavonols (rutin and quercetin), four flavones (luteolin, apigenin, luteolin-7-glucoside and apeginin-7-glucoside), and two flavan-3-ols (epicatechin and catechin), as well as other compounds (ascorbic acid).

**Table 1:** Total phenol and flavonoid contents of *Ononis spinosa* L. leaf and stem extracts.

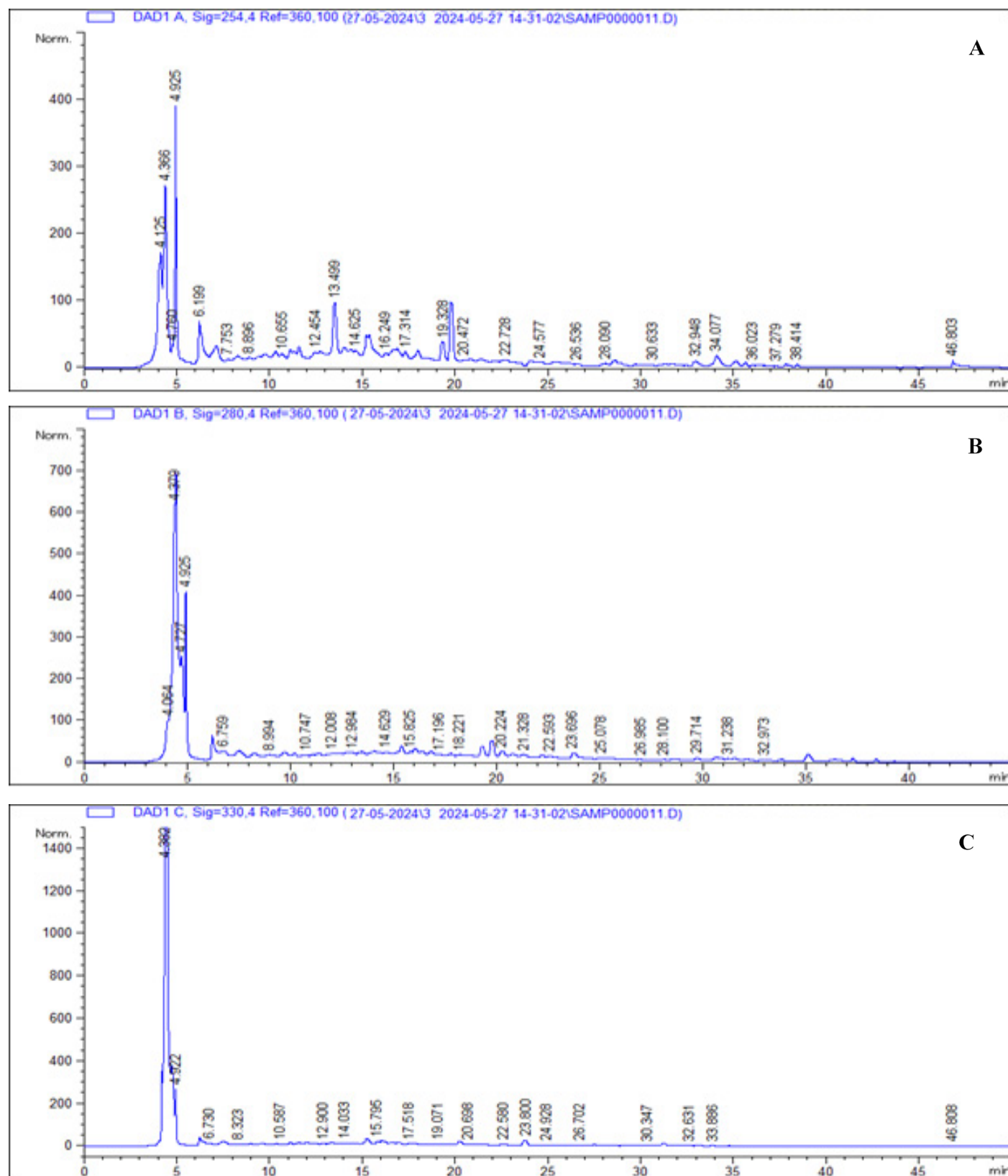
| Extracts | Total polyphenol content (mg GAE/g DW) | Total flavonoid content (mg QE/g DW) |
|----------|----------------------------------------|--------------------------------------|
| Leaf     | 25.34 $\pm$ 0.08                       | 12.06 $\pm$ 0.23                     |
| Stem     | 43.03 $\pm$ 1.02                       | 31.45 $\pm$ 0.45                     |

Values are expressed as means  $\pm$  Standard deviation (SD) of three parallel replicates.

**Table 2:** Phenolic profiles of the optimized extract of the *Ononis spinosa* L. stems and leaves using the HPLC-DAD analysis.

| N                        | Rt (min.) | Identified compound  | Molecular formula                               | Quantification (mg/g extract) | Wave-length (nm) |
|--------------------------|-----------|----------------------|-------------------------------------------------|-------------------------------|------------------|
| 1                        | 4.779     | Ascorbic acid        | C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>    | 0.070                         | 280              |
| 2                        | 10.313    | protocatechuic acid  | C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>    | 0.040                         | 280              |
| 3                        | 11.352    | Chlorogenic acid     | C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>  | 0.315                         | 280              |
| 4                        | 12.307    | Catechin             | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | 0.356                         | 254              |
| 5                        | 12.792    | Tyrosol              | C <sub>8</sub> H <sub>10</sub> O <sub>2</sub>   | 0.040                         | 280              |
| 6                        | 13.442    | Epicatechin          | C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>  | 5.857                         | 254              |
| 7                        | 13.575    | Caffeic acid         | C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>    | 0.056                         | 280              |
| 8                        | 14.362    | Vannilic acid        | C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>    | 0.084                         | 254              |
| 9                        | 15.352    | Rutine               | C <sub>27</sub> H <sub>30</sub> O <sub>16</sub> | 0.756                         | 254              |
| 10                       | 16.485    | Luteolin-7-glucoside | C <sub>21</sub> H <sub>20</sub> O <sub>11</sub> | 0.0625                        | 330              |
| 11                       | 16.881    | p-coumaric acid      | C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>    | 0.0421                        | 330              |
| 12                       | 18.208    | Apeginin-7-glucoside | C <sub>21</sub> H <sub>20</sub> O <sub>10</sub> | 0.0312                        | 330              |
| 13                       | 18.221    | Ferrulic acid        | C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>  | 0.0625                        | 280              |
| 14                       | 18.509    | o-coumaric acid      | C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>    | 0.0421                        | 280              |
| 15                       | 21.328    | Naringenin           | C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>  | 0.342                         | 280              |
| 16                       | 22.728    | Quercetin            | C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>  | 0.145                         | 254              |
| 17                       | 23.275    | Luteolin             | C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>  | 0.0375                        | 330              |
| 18                       | 25.290    | Apeginin             | C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>  | 0.0125                        | 280              |
| Total phenolic acids     |           |                      |                                                 | 0.64                          |                  |
| Total flavonoids         |           |                      |                                                 | 7.59                          |                  |
| Total phenolic compounds |           |                      |                                                 | 8.23                          |                  |
| Other constituents       |           |                      |                                                 | 0.11                          |                  |

Where; Rt: Retention time; nm: nanometer.



**Figure 1:** UV-DAD chromatograms of the aqueous-methanolic extract of *O. spinosa* aerial parts on a Zorbax C18 column. A: HPLC chromatograms measured at 254 nm, B: HPLC chromatograms recorded at 280 nm, C: HPLC chromatograms measured at 330 nm. The HPLC conditions are those optimized for qualitative analysis. Numbering of the retention times ( $R_t$ ) is the same as in Table 2.

Most of the identified phenolic compounds have previously been reported in *O. spinosa*, whereas new compounds, namely hydroxycinnamic acids

(chlorogenic acid and o-coumaric acid), flavonoids (catechins, epicatechin, rutin, naringin, and lutein-7-glucoside), and other constituents (ascorbic acid

and tyrosol), were reported in aerial part extraction for the first time. Nevertheless, some types of these compounds have been previously identified in other *Ononis* species, such as *O. arvensis* aerial parts (Denes *et al.*, 2015). However, the number of identified compounds is less reported by several other authors (Stojković *et al.*, 2020; Gampe *et al.*, 2021).

This variation may influence the phytochemical composition of the medicinal plants due to genetic and biological varieties, environmental conditions, and seasonal fluctuations (Biswas *et al.*, 2020; Ramasar *et al.*, 2022; Kayacetin, 2022). Pharmacological and nutritional studies have demonstrated that these discovered chemicals exhibit a range of advantageous activities, including antioxidant, bacteriostatic, anticancer, and anti-inflammatory effects, as documented in several previous studies reported by Al-Snafi, (2020) and Nardini (2022). Furthermore, the discovered chemicals, together with additional unidentified components, may contribute to the antibacterial activity observed in the *O. spinosa* extracts.

#### Antibacterial activity

The antibacterial activity of the methanolic extracts obtained from the examined plant parts was originally assessed using the disc diffusion assay against various bacterial strains, which comprise both Gram-positive and Gram-negative species commonly associated with infectious illnesses. The diameters of the inhibitory zones are presented in Table 3. All plant part extracts, shown different levels of antibacterial efficacy against all the tested bacterial strains.

**Table 3:** Diameter of inhibition zones of leaf and stem extracts from *Ononis spinosa* L.

| Bacterial strains       | Extracts   |           | Gentamicin |
|-------------------------|------------|-----------|------------|
|                         | Leaf       | Stem      |            |
| <i>A. bumannii</i>      | 09 ± 0.2   | 12 ± 0.4  | 18 ± 0.7   |
| <i>V. cholerae</i>      | 2.00 ± 0.5 | 10 ± 0.32 | 13 ± 0.4   |
| <i>P. mirabilis</i>     | 9.3 ± 0.5  | 10 ± 0.2  | 13 ± 1.1   |
| <i>K. pneumoniae</i>    | 14 ± 0.6   | 18 ± 0.4  | 0.00       |
| <i>S. aureus</i>        | 21 ± 0.33  | 30 ± 0.9  | 18 ± 0.9   |
| <i>P. aeruginosa</i>    | 8 ± 0.20   | 5 ± 0.3   | 0.00       |
| <i>E. coli</i>          | 16 ± 0.42  | 9 ± 1.00  | 14 ± 1.00  |
| <i>B. cereus</i>        | 6.8 ± 0.8  | 5 ± 0.5   | 6 ± 0.8    |
| <i>S. pneumoniae</i>    | 29 ± 0.4   | 29 ± 0.48 | 25 ± 1.2   |
| <i>S. typhimurium</i>   | 0.00       | 7 ± 1.6   | 10 ± 0.2   |
| <i>L. monocytogenes</i> | 0.00       | 13 ± 0.5  | 6 ± 0.6    |
| <i>C. freundii</i>      | 11 ± 0.4   | 17 ± 0.7  | 11 ± 0.5   |

Where; the inhibition zone diameters are expressed in mm ± SD.

The extracts of *Ononis spinosa* leaf and stem presented a strong activity against *Staphylococcus aureus* and *S. pneumoniae* with diameter of inhibition zone ranging between 21 and 29 mm, while the same plant parts extracts expressed a low activity against *P. aeruginosa* and *B. cereus*. In addition, our results showed that *V. cholerae*, *S. typhimurium* and *L. monocytogenes* were resistant to the leaf extract and presented moderate sensitivity to the stem extract. On the other hand, the antibacterial potential of the extracts was moderate (zone of inhibition ranging between 9 and 18 mm) against *K. pneumoniae*, *A. bumannii*, *P. mirabilis*, *E. coli*, and *C. freundii*. Stem extract was most active than leaf extract. Such disparities may be attributed to variations in the phenolic and flavonoid concentration, as indicated in Table 1.

The findings of this study indicate that there was no statistically significant difference observed in the susceptibility of Gram-positive and Gram-negative bacteria to the plant extracts. Previous literatures demonstrated that the Gram-positive bacteria demonstrated high sensitivity to the plant extracts compared to the Gram-negative bacteria, attributable to the hydrophobic lipopolysaccharide in the outer cell membrane that offers protection against many chemicals (Ghazghazi *et al.*, 2015; Karaman *et al.*, 2003). The previous studies validate the findings of the current study, indicating that the extracts exhibit greater efficacy against Gram-positive bacteria ( $p < 0.05$ ) such as *Staphylococcus aureus* and *S. pneumoniae* compared to Gram-negative bacteria such as *E. coli*.

In addition, the positive control the antibiotic gentamycin was more effective in controlling the bacterial strains than the *O. spinosa* extracts. Statistical analysis showed non-significant differences in efficacy between the extracts ( $p > 0.05$  for both plant parts). Statistical analysis also expressed highly significant differences among the different bacterial species ( $p < 0.05$ ), except for *S. typhimurium* and *L. monocytogenes*.

The antibacterial efficacy of the examined methanolic extracts was assessed through the assessment of the MIC values in relation to three Gram-positive and five Gram-negative bacteria. The obtained results demonstrated that the extracts exhibited differing levels of growth inhibition against the tested bacterial strains (Table 3). The greatest significant inhibitory effect was noted against *Staphylococcus aureus* and *S.*

*pneumoniae*, with a MIC and MBC of 4.68 and 9.36 mg/ ml, respectively. However, *V. cholerae*, *B. cereus*, and *P. aeruginosa* showed low activity with MIC values of 150 and 300 mg/ ml. Furthermore, *E. coli*, *P. mirabilis*, and *C. freundii* displayed MIC values ranging from 9.36 to 75 mg/ ml (Table 4).

Many *O. spinosa* extracts previously exhibited antibacterial activity, thus they demonstrated efficacy as powerful therapeutic agents. Results obtained by Ferrante *et al.* (2020) showed that the MIC of the *O. spinosa* extract on *B. cereus* and *Staphylococcus aureus* was 99.21 mg/ ml and 198.42 mg/ ml, respectively.

**Table 4:** Results of MIC and MBC of *Ononis spinosa* L. extracts (mg/ ml) against the tested bacterial strains.

| Bacterial strains       | MIC (mg/ml)  |              | MBC (mg/ml)  |              |
|-------------------------|--------------|--------------|--------------|--------------|
|                         | Leaf extract | Stem extract | Leaf extract | Stem extract |
| <i>A.umannii</i>        | 18.72        | 9.36         | 37.5         | 18.72        |
| <i>V. cholerae</i>      | 300          | 150          | >300         | > 300        |
| <i>P. mirabilis</i>     | 75           | 75           | 150          | 150          |
| <i>K. pneumoniae</i>    | 18.72        | 9.36         | 37.44        | 18.72        |
| <i>S. aureus</i>        | 18.72        | 4.68         | 18.72        | 9.36         |
| <i>P. aeruginosa</i>    | 150          | 300          | >300         | >300         |
| <i>E. coli</i>          | 9.36         | 37.44        | 18.72        | 150          |
| <i>B. cereus</i>        | 150          | 150          | >300         | >300         |
| <i>S. pneumoniae</i>    | 9.36         | 4.68         | 18.72        | 9.36         |
| <i>S. typhimurium</i>   | ND           | 75           | ND           | >300         |
| <i>L. monocytogenes</i> | ND           | 150          | ND           | >300         |
| <i>C. freundii</i>      | 75           | 75           | 150          | 300          |

Where; MIC: Minimum Inhibitory Concentration, MBC: Minimum bactericidal concentration.

Some authors have reported the antibacterial activity of alcoholic root extracts against uropathogenic *E. coli*, although the results showed no impact on proliferation of *E. coli* (125-1000 mg/ml), which contradict our minimum MIC and BMC values obtained against *E. coli* (9.36 mg/ml, 18.72 mg/ml, respectively) (Deipenbrock *et al.*, 2020).

Epicatechin was the main component existing in *O. spinosa* extract and previous studies have indicated its antibacterial action (Reygaert, 2014; Fathima and Rao, 2016). Furthermore, 3-O-octanoyl epicatechin, a derivative of epicatechin, exhibited antibacterial properties chiefly by disrupting the plasma membrane and have shown considerable antibacterial effectiveness against Gram-positive

bacteria, including both antibiotic-sensitive and resistant strains (Cushnie *et al.*, 2008).

Additional minor constituents in our extract, including chlorogenic acid, rutin, and quercetin, have been identified in previous investigations as possessing antibacterial properties (Rodríguez-Valdovinos *et al.*, 2021; Chen *et al.*, 2022; Veiko *et al.*, 2023). Nonetheless, the activity of a complex mixture cannot be readily ascribed to a single or a specialized component. Compounds, whether major or minor, present in the extract may elicit antibacterial action. Potential synergistic and antagonistic interactions among the biochemicals in the extract must be taken into account (Lopes-Lutz *et al.*, 2008).

## Conclusions and Recommendations

The obtained results indicated that the extracts from the aerial parts of *O. spinosa* displayed substantial antibacterial activity against several bacterial strains utilized in this investigation. Consequently, the findings of this study indicate that extracts from the aerial parts of *O. spinosa* may possess prospective applications as natural antimicrobials and antioxidants may therefore be suggested as novel sources of natural additives for the food and/or medicinal sectors. Chemical investigations revealed that the aerial portion of *O. spinosa* is a prospective source of bioactive substances, including epicatechin and phenolic compounds, whose qualities will be further investigated in future studies. We recommend the use of the *O. spinosa* methanolic extracts for future applications in the food and the pharmaceutical industries. However, cytotoxicity assays should be conducted prior to industrial application to ensure that these extracts are safe.

## Novelty Statement

This study provides a comprehensive investigation of the bioactive potential of *O. spinosa*, focusing on its phytochemical profile and assessing its antibacterial efficacy against a variety of pathogenic bacteria. Unlike the previous studies and to the best of our knowledge, this is the first report provides a detailed assessment of the plant's bioactive compounds and their specific antifibacterial potential, highlighting their therapeutic prospects, and paving the way for future applications in the development of natural product-based antibiotics.

## Author's Contribution

**Lakhdar Benalach:** Conceptualization, methodology, formal analysis, software, writing review and editing, supervision, writing original draft.

**Fadhela Boukada:** Conceptualization, methodology, formal analysis, software, writing – review and editing, validation.

**Kouider Cherifi:** Supervision, conceptualization, methodology

**Ali Latreche:** Resources, supervision, visualization.

**Ikram Messellem:** Methodology, formal analysis, software.

**Mohammed Bellatreche:** Formal analysis, Investigation, Software.

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The authors have declared no conflict of interest.

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