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*Original Research Article*

# Evaluation of the Antioxidant Activity and the Antibacterial Potential of *Dittrichia viscosa* (L.) against Nosocomial Pathogens

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ABSTRACT

*Dittrichia viscosa* (L.) Greuter, a Mediterranean medicinal plant widely used in traditional medicine, is renowned for its wealth of phytochemical and therapeutic potential. This study aimed to investigate the antioxidant activity and antibacterial potential of *Dittrichia viscosa* aerial parts and roots against nosocomial pathogens. The antibacterial activity was assessed against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains using the disc diffusion and microdilution assays. The antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), and total antioxidant capacity (TAC) assays. The extracts demonstrated strong antibacterial activity, particularly against *Staphylococcus aureus* and *Escherichia coli*, with minimum inhibitory concentration (MIC) ranging from 0.1562 to 5.0 µg/mL. Most of the extracts exhibited bactericidal effects, with minimum bactericidal concentration (MBC) equating the MIC for certain strains. Among the extracts, the decoction demonstrated the strongest antioxidant capacity, with $IC_{50}$ values of 15.68 ± 0.07 µg/mL, 36.32 ± 1.09 µg/mL, and 83.29 ± 13.89 µg/mL in the DPPH, ABTS, and FRAP assays, respectively, along with a TAC value of 12.40 ± 1.23 mg AAE/g extract. These results underline the ability of the plant to neutralize free radicals and reduce oxidative stress effectively.
The dual antibacterial and antioxidant activities of *D. viscosa* highlight its potential as a natural source of bioactive compounds for pharmaceutical applications. These findings position *D. viscosa* as a promising candidate for developing therapies targeting oxidative stress and multidrug-resistant infections, addressing the growing demand for alternative and sustainable treatment options.

***Keywords:*** *Dittrichia viscosa* (L.), Antioxidant, Antibacterial, Nosocomial pathogens.

## Introduction

Medicinal plants have long been the cornerstone of traditional medicine, providing a rich source of bioactive compounds with therapeutic potential. In recent years, the scientific community has increasingly focused on these natural resources to explore their pharmacological properties and identify novel compounds for applications in medicine, food preservation, and sustainable technologies.[1] Among these plants, *Dittrichia viscosa* (L.) Greuter (Figure 1) stands out as a particularly important species due to its diverse medicinal uses and widespread availability.[2, 3]

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*D. viscosa*, locally referred to in Morocco as "Tarehla" "Magramane" or "Safsag" is a perennial shrub native to the Mediterranean basin.[4] Thriving in varied habitats such as hillslopes, humid areas, and roadsides,[5] it is easily recognizable by its sticky leaves, distinctive aroma, and bright yellow flowers, which bloom from late summer to early autumn.[6, 7] Traditionally, this plant has been extensively used in folk medicine to treat various conditions, including microbial infections, inflammation, fever, and hypertension.[8-12] Its therapeutic effects are attributed to its rich phytochemical profile, including terpenoids, sesquiterpene lactones, flavonoids, tannins, and polyphenols, which exhibit antioxidant, antimicrobial, anti-inflammatory, and antiproliferative activities.[2]
Nosocomial infections, caused by antibiotic-resistant pathogens, represent a significant global health challenge, contributing to increased morbidity, mortality, and healthcare costs.[13] These infections are often linked to multidrug-resistant Gram-positive and Gram-negative bacteria, complicating treatment strategies and requiring the development of novel antimicrobial agents.[14] The emergence of these resistant strains underscores the urgent need for alternative therapeutic approaches, including plant-based bioactive compounds with potent antimicrobial properties.[15] Given its promising bioactive properties, *D. viscosa* has garnered scientific interest as a source of natural compounds for pharmaceutical and industrial applications.[8, 16] The aim of this study

was to evaluate the antioxidant and antimicrobial activities of *D. viscosa* aerial parts and roots. Antioxidant potential was assessed using four complementary assays: DPPH radical scavenging, ABTS radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and Total Antioxidant Capacity (TAC). On the other hand, the antimicrobial efficacy of *D. viscosa* extracts was tested against clinical isolates of some Gram-positive and Gram-negative bacterial strains provided by the Department of Food Microbiology and Hygiene at the National Institute of Hygiene (INH), Rabat, Morocco, and the Centers for Disease Control and Prevention (CDC).

This comprehensive investigation highlights the importance of *D. viscosa* as a medicinal plant with significant antioxidant and antimicrobial potential, contributing to its potential applications in healthcare and sustainable green technologies.


**Figure 1:** *Dittrichia viscosa* (L.) Plant in its natural habitat

## Materials and Methods

*Collection and identification of plant material*

The aerial parts and roots of *Dittrichia viscosa* (L) was collected in the region of Ouarzazate Drâa-Tafilalet, Morocco (Geographical coordinates: 30.924534, -7.001902) (Figure 2) in May 2023. The plant was identified and authenticated by a botanist from the Botany Department of the Scientific Institute of Rabat, Morocco. A voucher specimen was subsequently deposited in the herbarium with the voucher specimen number: RAB113637.

*Preparation of plant extract*

The aerial parts were dried in the laboratory while being protected from both light and moisture. The roots were also dried under similar conditions. The dried aerial parts (100 g) were extracted by successive maceration in 1 L each of cyclohexane, ethyl acetate, dichloromethane, acetonitrile, butanol, ethanol, and distilled water at room temperature for 24 h. The extracts were filtered, the filtrates were concentrated, and the extracts obtained were stored at 4°C until ready for use.

*In vitro evaluation of antimicrobial activity*

The evaluation of the antimicrobial activity of *D. viscosa* extracts was done using two methods: the disc diffusion assay and the microdilution assay.


**Figure 2:** Map of Morocco and Ouarzazate region

*Bacterial strains*

The bacterial strains used in this study included two Gram-positive and five Gram-negative bacteria (Table 1). These strains were selected for their clinical relevance, particularly for their roles in nosocomial and opportunistic infections. All strains were provided by the Department of Food Microbiology and Hygiene at the National Institute of Hygiene, Rabat, Morocco.

*Sample preparation for testing*

The extracts of *D. viscosa* were prepared as follows:

- Aqueous and decoction extracts were solubilized in sterile distilled water.
- Organic solvent extracts (cyclohexane, ethyl acetate, dichloromethane, acetonitrile, butanol, and ethanol extracts) were dissolved in **1**0% (v/v) dimethyl sulfoxide (DMSO).

The negative controls included sterile distilled water and 10% (v/v) DMSO to ensure that the solvents did not interfere with bacterial growth.

**Table 1:** Bacterial strains used in the study

| Gram Type | Bacterial Strain | ATCC Code |
|---|---|---|
| Gram-Positive | *Enterococcus faecalis* | ATCC® 19433 |
| | *Staphylococcus aureus* | ATCC® 35548 |
| Gram-Negative | *Escherichia coli* | ATCC® 11775 |
| | *Pseudomonas aeruginosa* | ATCC® 10145 |
| | *Legionella pneumophila* | ATCC® 33823 |
| | *ESBLs Escherichia coli* | Provided by CDC |
| | *ESBLs Klebsiella pneumoniae* | Provided by CDC |

*Disc diffusion assay*

The antimicrobial activity of the extracts was evaluated using the disc diffusion method on Mueller-Hinton agar (Oxoid CM0337), following CLSI guidelines (M07-A10).[17] Bacterial inocula were prepared by suspending fresh cultures in sterile 0.9% saline solution to a turbidity equivalent to 0.5 McFarland standard, corresponding to approximately $10^6$ CFU/mL.

The prepared bacterial suspensions were spread evenly over the agar plates using a sterile swab. Sterile paper discs (6 mm diameter) were

then impregnated with 50 µL of the extract solution (100 mg/mL) and placed onto the inoculated agar surface. Positive controls included Tetracycline (30 µg) and Ciprofloxacin (5 µg), while discs with sterile water and 10% (v/v) DMSO served as negative controls.
The plates were incubated at 37°C for 24 h, after which the diameters of the inhibition zones (including the disc diameter) were measured in millimeters using a digital caliper.[18]

*Microdilution assay for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*
The minimum inhibitory concentration (MIC) of the extracts was determined using the microdilution method in 96-well microplates according to the procedure described by Ellof (1998).[19] Serial twofold dilutions of the extracts were prepared in BHI (Oxoid CM1135) broth for all bacterial strains. Each well contained 100 µL of diluted extract solution and 10 µL of bacterial suspension, resulting in a final bacterial concentration of approximately $10^5$ CFU/mL.
The plates were incubated at 37°C for 24 h, and microbial growth was evaluated using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) as a viability indicator. After the incubation period, 10 µL of MTT solution (0.4 mg/mL) was added to each well, and the plates were incubated further for 10–30 minutes. The MIC was defined as the lowest concentration of the extract that completely inhibited visible microbial growth, indicated by the absence of a colour change in the wells.[19]
The minimum bactericidal concentration (MBC) was determined by transferring 50 µL from a microplate well showing no visible microbial growth onto Nutrient Agar (Oxoid CM0003). The plates were subsequently incubated at 37 °C for 24 h, and the lowest concentration at which no microbial growth was observed on the agar was recorded as the MBC.

*In vitro evaluation of antioxidant activity*
The antioxidant activity of *D. viscosa* extracts was assessed using four complementary assays, each based on different mechanisms of action. These included the DPPH radical scavenging assay, ABTS cationic radical ($ABTS^{\bullet+}$) scavenging assay, Ferric Reducing Antioxidant Power (FRAP) assay, and Total Antioxidant Capacity (TAC) assay. These assays measured the ability of the extracts to neutralize free radicals or reduce oxidative agents, providing a comprehensive evaluation of their antioxidant potential. All experiments were performed in triplicate.

*DPPH radical scavenging assay*
The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A methanol solution of DPPH (60 µM) was mixed with varying concentrations of the extracts (50 µL) of each extract at varying concentrations ranging from 0.19 to 6.09 mg/mL. The reaction mixture was incubated in the dark at room temperature for 20 min, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A, Peak Instruments Inc., USA). The percentage inhibition (I%) was calculated using the formula below (Eq. 1).[20, 21]

$$I\,\% = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \text{---------- Eq. 1}$$

The 50% inhibitory concentration ($IC_{50}$) was calculated from a regression analysis.

*ABTS radical scavenging assay*
ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals were generated by reacting 7 mM ABTS with 70 mM potassium persulfate and incubating the mixture in the dark for 16 h. The solution was diluted to an absorbance of 0.700 at 734 nm. To assess the radical scavenging activity, 100 µL of each extract at concentrations ranging from 0.19 to 6.09 mg/mL was added to 2 mL of ABTS solution, and the absorbance was measured at 734 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A, Peak Instruments Inc., USA). The $IC_{50}$ values were calculated to quantify the antioxidant potential.[20, 22]
*FRAP Assay*
The reducing power of the extracts was measured using the ferric reducing antioxidant power (FRAP) assay, which evaluates the reduction of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$). The reaction mixture consisted of 0.2 mL of each extract at concentrations ranging from 3.34 to 106.93 µg/mL, 2.5 mL of 0.2 M phosphate buffer (pH 6.6), and 2.5 mL of 1% (w/v) potassium ferricyanide, followed by the addition of ferric chloride. The absorbance of the blue-green complex formed was measured at 700 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A, Peak Instruments Inc., USA). The $IC_{50}$ values were determined.[20, 23]
*Total antioxidant capacity (TAC) assay*
The TAC assay was conducted using the phosphomolybdenum method. The extract was mixed with a reagent solution containing sulfuric acid, sodium phosphate, and ammonium molybdate. The mixture was heated at 95°C for 90 min, and absorbance was measured at 695 nm using a UV-Vis spectrophotometer (Peak Instruments® C-7200A, Peak Instruments Inc., USA). Extracts were tested at concentrations ranging from 3.34 to 106.93 µg/mL, and ascorbic acid (15 to 250 µg/mL). Results were expressed as mg ascorbic acid equivalent per gram of extract (mg AAE/g).[20, 24]

*Statistical analysis*
All experiments were conducted in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess differences among groups using GraphPad Prism 8.0 software.
Tukey's Honestly Significant Difference (HSD) post hoc test was used for multiple comparisons to determine which group means differed significantly from the reference standard. A p-value $< 0.05$ was considered statistically significant. Values marked with "s" indicate a statistically significant difference from the reference ($p < 0.05$), while values marked "ns" denote no significant difference.

## Results and Discussion

*Antimicrobial activity*
*Inhibition zone diameter*
The antibacterial activity of *D. viscosa* extracts against a variety of Gram-positive and Gram-negative bacterial strains was evaluated, and the results are summarized in Table 2. The data showed moderate to significant activity, particularly against Gram-positive bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus*. The inhibition zones for *Enterococcus faecalis* ranged from 14 mm to 20 mm. Tetracycline with inhibition zone diameter of 23 mm served as the positive control. Similarly, for *Staphylococcus aureus*, the extracts showed inhibition zones between 15 mm and 18 mm, slightly lower than the standard antibiotic tetracycline with inhibition zone diameter of 22 mm.
For Gram-negative bacteria, the activity was more variable. *Pseudomonas aeruginosa* exhibited inhibition zone diameter ranging from 10 mm to 16 mm, while *Escherichia coli* and *Klebsiella pneumoniae* showed inhibition zone diameter ranging from 12 mm to 20 mm. However, the extracts were generally less effective against Gram-negative strains compared to ciprofloxacin, which demonstrated inhibition zones up to 25 mm.
These findings are consistent with previously published studies. For instance, [25] reported a strong antibacterial activity of methanol extract of *D. viscosa* aerial parts, particularly against *Staphylococcus aureus* (25 ± 1 mm) and *Bacillus subtilis* (22 ± 1 mm).[25] Similarly, [26] found that the essential oil of *D. viscosa* exhibited significant activity against *Staphylococcus aureus* with mean inhibition zone diameter of 26.84 mm,[26] exceeding that obtained in the present study.
Regarding the Gram-negative bacteria, [8] noted limited efficacy of *D. viscosa* essential oils against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, but moderate activity against *Escherichia coli* with inhibition zone diameter of 9.5 ± 0.5 mm.[8] These results are comparable to those observed in in the present study, where *Escherichia coli* exhibited inhibition zone diameter of 12 mm to 13 mm.
Additionally, [27] demonstrated a potent antibacterial effects of polyphenolic extracts from *D. viscosa* against Gram-positive and Gram-negative bacteria, with inhibition zone

**Table 2:** Antibacterial activity of *D. viscosa* aerial parts and roots extracts: Zone of Inhibition (mm) against bacterial strains

| Bacterial strains | | Diameter of the zone of inhibition (mm) | | | | | | | | Standard Antibiotics disc (µg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | Ext. 8 | Tetracycline (30 µg) | Ciprofloxacin (5 µg) |
| Gram-positive | *Enterococcus faecalis* ATCC® 19433 | 20 | 14 | 15 | 15 | 17 | 14 | 15 | 16 | 23 | - |
| | *Staphylococcus aureus* ATCC® 35548 | 15 | 15 | 16 | 18 | 16 | 16 | 12 | 17 | 22 | - |
| Gram-negative | *Pseudomonas aeruginosa* ATCC® 10145 | 12 | 10 | 11 | 12 | 16 | 15 | 12 | 15 | 21 | 21 |
| | *Legionella pneumophila* ATCC® 33823 | 21 | 28 | 25 | 21 | 15 | 14 | 16 | 18 | - | 29 |
| | *Escherichia coli ATCC*® 11775 | 12 | 13 | 12 | 12 | 23 | 13 | 14 | 13 | - | 21 |
| | *ESBLs Escherichia coli* | 11 | 10 | 12 | 11 | 18 | 10 | 12 | 11 | - | 25 |
| | *ESBLs Klebsiella pneumoniae* | 18 | 16 | 13 | 10 | 20 | 9 | 10 | 10 | - | 25 |

***F:** Extract; **$F_1$:** Cyclohexane; **$F_2$:** Ethyl acetate ;**$F_3$:** Dichloromethane ; **$F_4$:** Acetonitrile ; **$F_5$:** Butanol ; **$F_6$:** Ethanol ; **$F_7$:** Aqueous ;**Ext.8:** Decoction*

diameter ranging from 10.8 mm to 21 mm.[27] These findings align with the results obtained in the present study, particularly for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, where moderate activity was observed.

Overall, the antibacterial activity of *D. viscosa* appeared to depend on the extraction method and the bacterial strain tested. Methanol extracts and essential oils exhibit the most significant antibacterial properties, with higher inhibition zone diameter and lower MIC values reported in the literature compared to aqueous or ethanol extracts. This highlights the importance of optimizing extraction methods to enhance the antibacterial potential of *D. viscosa*.

*Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of D. viscosa extracts*

The antibacterial activity of *D. viscosa* extracts as measured by the MIC and MBC are presented in Table 3 and Table 4, respectively. The results highlight notable efficacy against both Gram-positive and Gram-negative bacterial strains. The MIC values for *Enterococcus faecalis* ranged from 0.3125 to 5.0 µg/mL, while the MBC values ranged from 1.25 to 5.0 µg/mL. These findings are consistent with the results reported by [25], who observed MIC values for methanol extract of *D. viscosa* aerial parts between 0.25 and 2 µg/mL.[25] Additionally, the bactericidal activity of essential oils of *D. viscosa*, although moderate against this strain, has been documented in a previous study.[26]

For *Staphylococcus aureus*, the MIC values varied between 0.1562 and 2.5 µg/mL, and the corresponding MBC values ranged from 0.1562 to 5.0 µg/mL. These results align with the findings of [28], who demonstrated strong bactericidal activity of *D. viscosa* essential oils, with MIC values reported as low as 0.09 mg/mL.[28] Furthermore, the efficacy of methanol extracts against this strain has also been confirmed in the literature.[16]

Regarding Gram-negative bacteria, *Pseudomonas aeruginosa* exhibited MIC values between 0.3125 and 5.0 µg/mL, while the MBC values ranged from 0.3125 to 5.0 µg/mL. These results are comparable to those of [27], who reported MIC values of 0.39 mg/mL for polyphenolic extracts, highlighting their potential bactericidal activity.[27] Similarly, the results for *Escherichia coli* showed MIC values ranging from 0.3125 to 2.5 µg/mL and MBC values from 0.625 to 2.5 µg/mL. These findings are consistent with that of [16], who observed similar MIC values for methanol extract of *D. viscosa*.[16] However, the essential oils were less effective against *E. coli*, as reported by [8].

For *Klebsiella pneumoniae*, the MIC values varied from 0.3125 to 2.5 µg/mL, with MBC values ranging between 0.625 and 2.5 µg/mL. Comparable results were reported by [27], who noted significant bactericidal effects with MIC values ranging from 0.39 to 1.25 µg/mL.[27] The effectiveness of specific extracts, such as Acetonitrile and Butanol, against this resistant strain highlights their promising antibacterial potential.

Overall, the data presented in Tables 3 and 4 align closely with previously published findings, particularly for the methanol and polyphenolic extracts, which consistently demonstrated strong antibacterial activity. While Gram-positive strains, such as *Staphylococcus aureus* and *Enterococcus faecalis*, exhibited higher sensitivity, the results for Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, varied depending on the extract and strain-specific resistance mechanisms. These observations underscore the potential of *D. viscosa* as a source of bioactive compounds with broad-spectrum antibacterial properties.

*Antioxidant activity*

The antioxidant activity of *D. viscosa* extracts was evaluated using Total Antioxidant Capacity (TAC) and complementary assays (DPPH, ABTS, and FRAP). The results revealed distinct variations in antioxidant capacity based on the extraction methods and solvents used. Tables 5 and 6 below summarized the key results obtained for different extracts and methods.

**Table 3:** Minimum inhibitory concentration of *D. viscosa* aerial parts and roots extracts against bacterial strains

| Bacterial strain | | MIC (µg/mL) | | | | | | | | Standard Antibiotics (µg/mL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | Ext. 8 | Tetracycline | Ciprofloxacin |
| Gram-positive | *Enterococcus faecalis* ATCC® 19433 | 1.25 | 2.5 | 2.5 | 5.0 | 5.0 | 1.25 | 2.5 | 1.25 | 2.5 | - |
| | *Staphylococcus aureus* ATCC® 35548 | 2.5 | 0.3125 | 1.25 | 0.625 | 0.625 | 0.78 | 5.0 | 0.78 | 0.625 | - |
| Gram-negative | *Pseudomonas aeruginosa* ATCC® 10145 | 2.5 | 1.25 | 2.5 | 5.0 | 1.25 | 0.625 | 0.3125 | 1.25 | 2.5 | - |
| | *Legionella pneumophila* ATCC® 33823 | 0.625 | 1.25 | 2.5 | 2.5 | 1.25 | 2.5 | 2.5 | 0.625 | - | 0.625 |
| | *Escherichia coli ATCC*® 11775 | 1.25 | 0.625 | 2.5 | 0.3125 | 0.3125 | 2.5 | 0.625 | 1.25 | - | 1.25 |
| | *ESBLs Escherichia coli* | 1.25 | 5.0 | 1.25 | 2.5 | 0.625 | 2.5 | 5.0 | 5.0 | - | 5.0 |
| | *ESBLs Klebsiella pneumoniae* | 1.25 | 2.5 | 1.25 | 0.3125 | 0.625 | 0.625 | 1.25 | 2.5 | - | 5.0 |

***F:** Extract; **$F_1$:** Cyclohexane; **$F_2$:** Ethyl acetate ;**$F_3$:** Dichloromethane ; **$F_4$:** Acetonitrile ; **$F_5$:** Butanol ; **$F_6$:** Ethanol ; **$F_7$:** Aqueous ;**Ext.8:** Decoction*

**Table 4:** Minimum bactericidal concentration of *D. viscosa* aerial parts and roots extracts against bacterial strains

| Bacterial strain | | MBC (µg/mL) | | | | | | | | Standard Antibiotics (µg/mL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | Ext. 8 | Tetracycline | Ciprofloxacin |
| Gram-positive | *Enterococcus faecalis* ATCC® 19433 | 0.3125 | 5.0 | 2.5 | 2.5 | 5.0 | 2.5 | 5.0 | 5.0 | 2.5 | - |
| | *Staphylococcus aureus* ATCC® 35548 | 2.5 | 5.0 | 1.25 | 0.625 | 2.5 | 2.5 | 0.1562 | 0.1562 | 0.625 | - |
| Gram-negative | *Pseudomonas aeruginosa* ATCC® 10145 | 2.5 | 1.25 | 2.5 | 5.0 | 5.0 | 0.625 | 0.3125 | 2.5 | 2.5 | |
| | *Legionella pneumophila* ATCC® 33823 | 0.625 | 1.25 | 5.0 | 5.0 | 1.25 | 2.5 | 2.5 | 0.625 | - | 1.25 |
| | *Escherichia coli ATCC*® 11775 | 1.25 | 0.625 | 2.5 | 2.5 | 0.625 | 2.5 | 0.625 | 1.25 | - | 1.25 |
| | *ESBLs Escherichia coli* | 5.0 | 5.0 | 5.0 | 2.5 | 2.5 | 5.0 | 5.0 | 5.0 | - | 5.0 |
| | *ESBLs Klebsiella pneumoniae* | 2.5 | 5.0 | 2.5 | 0.3125 | 1.25 | 1.25 | 1.25 | 2.5 | - | 5.0 |

***F:** Extract; **$F_1$:** Cyclohexane; **$F_2$:** Ethyl acetate ;**$F_3$:** Dichloromethane ; **$F_4$:** Acetonitrile ; **$F_5$:** Butanol ; **$F_6$:** Ethanol ; **$F_7$:** Aqueous; **Ext.8:** Decoction*

In the DPPH radical scavenging assay, the decoction extracts of *D. viscosa* exhibited an $IC_{50}$ of 15.68 ± 0.07 µg/mL, highlighting their potent antioxidant activity. This is consistent with findings of [29], where ethyl acetate (EtOAc) extract of *D. viscosa* demonstrated the highest activity with an $IC_{50}$ of 0.6 µg/mL.[29] Similarly, [16] observed high activity using Soxhlet extraction for flower buds ($IC_{50}$ = 39.77 ± 0.23 µg/mL).[16] Comparatively, [30] reported weaker activity for the ethanol extracts ($IC_{50}$ = 768.06 ± 0.5 µg/mL),[30] while [31] found methanol extracts of *D. viscosa* with $IC_{50}$ = 23.33 µg/mL.[31] These findings underscore the superior antioxidant potential of aqueous and ethyl acetate extracts observed in the present analysis.

For the ABTS assay, aqueous decoction of *D. viscosa* aerial parts displayed an $IC_{50}$ of 33.78 ± 2.60 µg/mL, demonstrating a comparable performance to the methanol extract as reported by [31]. (2016) ($IC_{50}$ = 16.75 µg/mL).[31] [29] found that the ethyl acetate extract exhibited superior performance ($IC_{50}$ = 8.6 µg/mL),[29] while [30] reported lower activity for ethanol extract ($IC_{50}$ = 452.08 µg/mL).[30] The findings indicate that polar solvents, such as water and methanol, are particularly effective for extracting antioxidant compounds with high ABTS radical scavenging potential.

The results from the FRAP assay further confirmed the strong reducing power of the aqueous decoction, with a value of 83.29 ± 13.89 µg/mL. This aligns with the findings of [29], who reported high reducing power for ethyl acetate extract (634.8 ± 1.45 mg EAA/g DW),[29] and [32], who observed $IC_{50}$ values of 296.425 ± 3.3 mg TE/g for the ethanol leaf extract of *D. viscosa*.[32] These results emphasized the effectiveness of water-based extracts in releasing bioactive compounds with reducing properties.

**Table 5:** Antioxidant activity of *D. viscosa aerial parts and roots extracts*

| ***D. viscosa* extracts** | **DPPH $IC_{50}$ (µg/mL)** | **ABTS $IC_{50}$ (µg/mL)** | **FRAP $IC_{50}$ (µg/mL)** |
|---|---|---|---|
| $F_1$. Cyclohexane | 2330.14 ± 8.80$^{s}$ | 200.83 ± 61.91$^{s}$ | 253.13 ± 29.53$^{s}$ |
| $F_2$. Ethyl acetate | 137.90 ± 19.77$^{s}$ | 605.05 ± 2.34$^{s}$ | 431.98±19.82$^{s}$ |
| $F_3$. Dichloromethane | 219.16 ± 5.22$^{s}$ | 944.54 ± 134.36$^{s}$ | 580.55 ± 4684$^{s}$ |
| $F_4$. Acetonitrile | 40.17 ± 0.84$^{s}$ | 56.86 ± 10.08$^{ns}$ | 241.47 ± 7.59$^{s}$ |
| $F_5$. Butanol | 11.15 ± 0.17$^{ns}$ | 394.03 ± 93.03$^{s}$ | 221.09 ± 7.93$^{s}$ |
| $F_6$. Ethanol | 33.34 ± 0.26$^{s}$ | 158.29 ± 3.01$^{s}$ | 172.83 ± 6.32$^{s}$ |
| $F_7$. Aqueous | 20.15 ± 3.60$^{ns}$ | 33.78 ± 2.60$^{ns}$ | 130.25 ± 3.70$^{s}$ |
| Decoction | 15.68 ± 0.07$^{ns}$ | 36.32 ± 1.09$^{ns}$ | 83.29 ± 13.89$^{s}$ |
| Quercetin | 5.49 ± 0.01 | - | - |
| Ascorbic Acid | - | 10.07± 1.59 | - |
| $^{a}$BHT | - | - | 7.02 ± 0.01 |

***F:** Extract; **$^{a}$BHT:** Butylated hydroxytoluene; DPPH and ABTS assays used extract concentrations of 0.19–6.09 mg/mL; FRAP assay used extract concentrations of 3.34–106.93 µg/mL. Data represent the mean ± standard deviation of three independent experiments." Values marked with " **s:** Statistically significant compared to the reference standard for each test. ($p < 0.05$)" . **ns:** Not significantly different from the reference standard ($p < 0.05$).*

**Table 6:** Total antioxidant capacity (TAC) of *D. viscosa* aerial parts and roots extracts

| ***D. viscosa* extract** | **TAC value (mg AAE/g extract)** |
|---|---|
| $F_1$. Cyclohexane | 3.11 ± 0.01e,d |
| $F_2$. Ethyl acetate | 10.95 ± 1.30a,b,c |
| $F_3$. Dichloromethane | 12.79 ± 0.47b |
| $F_4$. Acetonitrile | 5.03 ± 0.49d |
| $F_5$. Butanol | 10.56 ± 0.19a,c |
| $F_6$. Ethanol | 9.06 ± 0.01c |
| $F_7$. Aqueous | 3.14 ± 0.06d |
| Decoction | 12.40 ± 1.23a,b |

***F:** Extracts were tested at concentrations ranging from 3.34 to 106.93 µg/mL. Values represent the mean ± standard deviation of three independent experiments. Values in the same column followed by different superscript letters (e.g., $^{a}$, $^{b}$, $^{c}$) indicate statistically significant differences ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test.*

With respect to the Total Antioxidant Capacity (TAC), the decoction extract showed a TAC value of 12.40 ± 1.23 mg AAE/g extract, which was comparable to dichloromethane extract (12.79 ± 0.47 mg AAE/g) and slightly lower than ethyl acetate extract (10.95 ± 1.30 mg AAE/g). [32] reported a TAC value of 133.02 ± 3.1 mg AAE/g for ethanol extract,[32] while [33] highlighted the superior TAC of ethyl acetate extracts from aerial parts (155.42 ± 3.54 mg BHT E/g dry extract).[33] These comparisons indicated that both the aqueous decoction and organic solvents such as ethyl acetate were effective in extracting compounds with high antioxidant capacity.

In conclusion, the comparative analysis demonstrated that the extraction method and solvent played a crucial role in determining the antioxidant activity of *D. viscosa*. Decoctions and aqueous extracts consistently exhibited strong performance across all assays, corroborating findings in the literature that highlighted the efficacy of polar solvents. The superior performance of ethyl acetate and methanol extract further underscores the versatility of *D. viscosa* as a source of natural antioxidants. These results provided a comprehensive understanding of its bioactive properties and potential applications.

## Conclusion

This study demonstrates the potent antibacterial and antioxidant activities of *D. viscosa* (L.) Greuter. The extracts showed significant efficacy against multidrug-resistant pathogens, with MIC and MBC

values confirming their bactericidal potential. Additionally, the antioxidant assays (DPPH, ABTS, FRAP, and TAC) highlighted the decoction extract as the most effective in neutralizing free radicals. The phytochemical richness of *D. viscosa*, particularly its phenolic and flavonoid contents, underpins its bioactivity. These findings position *D. viscosa* as a promising candidate for developing natural therapies targeting oxidative stress and nosocomial infections, encouraging further research into its pharmaceutical applications.

## Conflicts of Interest

The authors declare no conflicts 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.

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