

 Open Access

Article Information

Received: January 1, 2026

Accepted: January 14, 2026

Published: January 20, 2026

Authors' Contribution

AHAK & NAAA conceived and designed the study; AHAK & NAAA conducted experiments; HMI did statistical analysis; QYMA, AHAK and HMI wrote and revised the paper.



How to cite

Khalil, A.H.A., Al-ghori, N.A.A., Abdullah, Q.Y.M., Ibrahim, H.M., 2026. Phytochemical Profiling and Bioactivity of *Dodonaea viscosa* L. Leaf Extract. PSM Biol. Res., 11(1): 1-12.

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## Phytochemical Profiling and Bioactivity of *Dodonaea viscosa* L. Leaf Extract

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Abstract:

The increasing prevalence of drug-resistant pathogens necessitates the exploration of alternative therapeutic agents derived from natural sources. This study investigated the phytochemical composition, functional groups, antioxidant capacity, and antimicrobial potential of the ethanolic leaf extract of *Dodonaea viscosa* L. Qualitative phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, tannins, resins, and saponins in the extract. Fourier Transform Infrared (FT-IR) spectroscopy confirmed the presence of key functional groups, including phenolic hydroxyl (O-H), aliphatic C-H, aromatic C=C, and aliphatic C-O, which are characteristic of bioactive compounds. The extract demonstrated significant antioxidant activity in the DPPH free radical scavenging assay, achieving a maximum scavenging percentage of 93% at 20 µg/mL, which was comparable to that of the ascorbic acid control (95%). Antimicrobial assays showed notable dose-dependent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with Minimum Inhibitory Concentrations (MICs) of 6.25 mg/mL and 12.5 mg/mL, respectively. However, the extract was ineffective against *Pseudomonas aeruginosa* and *Candida albicans*. These findings suggest that the leaf extract of *D. viscosa* is a rich source of phenolic and flavonoid compounds with strong antioxidant and selective antibacterial properties, supporting its traditional medicinal use.

**Keywords:** *D. viscosa*, Leaves, Phytochemical screening, FT-IR spectrum analysis, Antioxidant activity, Antimicrobial.



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## INTRODUCTION

In recent years, plant scientists have become increasingly interested in phytochemical research due to the development of new methods and techniques. These advances have facilitated the production of more raw medicinal materials from plants, which are important in the pharmaceutical industry (Shahzad *et al.*, 2017; Iqbal and Ashraf, 2018). Medicinal plant materials are often called secondary products derived from certain plant parts, such as leaves, and their medical benefits result from a combination of these products (Krishnaraju *et al.*, 2005; Ali *et al.*, 2017; Ashraf and Iqbal, 2022; Farooq *et al.*, 2022; Morsi *et al.*, 2022). Pathogenic microorganisms are commonly treated with antibiotics derived from various sources. However, these antibiotics are often expensive and not readily available (Camporese *et al.*, 2003; Ashraf *et al.*, 2020). Moreover, options for pharmaceutical drugs to combat antibiotic-resistant bacteria are limited. To overcome this challenge, researchers have investigated the antimicrobial properties of various plants against antibiotic-resistant bacteria (Sukanya *et al.*, 2009; Iqbal and Ashraf, 2019). Yemen is a developing country (located in the southwestern part of the Arabian Peninsula) where traditional medicine plays a central role in Yemeni society, particularly the use of medicinal plants and herbs as primary healthcare resources for the treatment of inflammation, infections, and various other diseases (Fleurentin *et al.*, 1983; Schopen, 1983; Al-Dubaie and Al Khulaidi, 2005). This dependence on traditional remedies is largely attributed to the prevailing economic constraints faced by a substantial proportion of the population (Bazeeb, 1991).

Numerous studies have reported that various plant extracts, including *Dodonea viscosa* L., possess antimicrobial and antioxidant properties (Gulluce *et al.*, 2004; Mothana *et al.*, 2010; Salameh *et al.*, 2020; Aernan *et al.*, 2023; Olofu *et al.*, 2025). *D. viscosa* is a shrub that grows up to 2 meters tall and belongs to the Sapindaceae family. The genus *Dodonea* comprises approximately 68 species distributed worldwide (Chaudhary and al-Waṭānīyah, 2001).

Traditionally, *D. viscosa* has been used in folk medicine to treat fever and rheumatism (Getie *et al.*, 2003). Its leaves and roots are also used as natural pain relievers for toothaches and headaches (Getie *et al.*, 2003; Sandhya *et al.*, 2006). Additionally, previous research has highlighted that due to the presence of compounds such as flavonoids, tannins, saponins, essential oils, coumarins, gum, mucilage, and organic acids, *D. viscosa* exhibits antimicrobial, anti-inflammatory, antioxidant, and anti-ulcer effects (Getie *et al.*, 2003; Mothana *et al.*, 2010). In Yemen, *D. viscosa* is an important component of traditional medicine. The leaves are used to treat burns, wounds, rheumatism, and toothaches, whereas its bark is used to treat bleeding and digestive problems. In addition to its medicinal uses, local communities utilize *D. viscosa* to preserve food, especially milk, through a method known as Keba, which involves smoking milk with the *D. viscosa* stem (Al-Dubaie and Al Khulaidi, 2005). Although *D. viscosa* has a long history in Yemeni culture, there has been little scientific research on the local variety. Most previous studies have included *D. viscosa* in general screenings for antimicrobial and cytotoxic effects among Yemeni medicinal plants, mainly using methanol extracts (Mothana *et al.*, 2010; Al-Haj *et al.*, 2019). These studies reported that *D. viscosa* has antibacterial activity against *Staphylococcus aureus*; however, limited information is available on the efficacy of its ethanolic extracts. Since ethanol is a more compatible solvent and is often used in traditional preparations. Therefore, this study aimed to investigate the phytochemical content, antioxidant properties, and antimicrobial effects of the Yemeni variety of *D. viscosa* ethanolic leaf extract against selected human pathogens.

## MATERIALS AND METHODS

### Collection, classification, and preparation of plant material for extraction

Plant specimens and fully grown and mature leaf samples of *D. viscosa* (Figure 1) were collected from Qadas (latitude: 13°32'28.14"N, longitude: 44°01'20.51"E, altitude: 2483 m above sea level)

located in Taiz Governorate, Yemen. The plant specimens were identified and classified using available taxonomical references, specifically Wood (1997) and Al Khulaidi (2013). The plant specimen was assigned a herbarium voucher number (BHSS 710) and deposited in the Herbarium at the Faculty of Science, Sana'a University, for future reference. The collected leaves were thoroughly washed with distilled

water, cut into pieces, and air-dried in a shaded area for one week. The dried fruits were ground into a fine powder using a blender. The powdered leaves were stored in a refrigerator in airtight cellophane containers as stock samples until required for further analysis (Ibrahim *et al.*, 2023a; Alhadi *et al.*, 2025).



**Fig. 1.** *Dodonea viscosa* L.: leaves, Inflorescence and Fruits.

### Preparation of plant extracts

The plant extract was prepared using the method described by Nisar *et al.* (2015), with some minor changes. Approximately 50 g of *D. viscosa* leaf powder was soaked in 500 mL of 96% ethanol at a 1:10 (w/v) ratio. The mixture was left at room temperature for 24 h and then shaken continuously for 72 h using an electrical shaker to improve the extraction yield. The suspension was then centrifuged at 5000 rpm for 20 min (Eloff, 1999) to separate the supernatant. The filtrate was concentrated under reduced pressure using a rotary evaporator (Nisar *et al.*, 2015). The crude extract was dried in an oven at 44.5°C for 4 h to remove any remaining solvent (Abdullah *et al.*, 2012) and stored at 4°C until needed.

### Phytochemical Screening

Qualitative screening was performed to determine the phytochemical constituents of the

studied plant's crude ethanol extract, where the extraction was examined for the presence of alkaloids, resins, saponins, polyphenol compounds (flavonoids, tannins, and phenols), and essential oils.

### Detection of Alkaloids

To detect the presence of alkaloids, a portion of the powdered extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was then tested for alkaloids using Mayer's test. The formation of a yellow precipitate upon treatment with Mayer's reagent (Potassium Mercuric Iodide) indicates the presence of alkaloids (Tiwari *et al.*, 2011).

### Detection of Phenols

To examine the presence of phenols, a small amount of the powdered extract was dissolved in ethanol. The dissolved powdered extract was then treated with 3-4 drops of Ferric Chloride

solution. The appearance of a bluish-black color indicates the presence of phenols (Tiwari *et al.*, 2011).

#### Detection of Flavonoids

To detect the presence of flavonoids, a limited portion of the powdered extract was dissolved in ethanol, and 5 mL of dilute ammonia solution was added to the dissolved powdered extract, followed by the addition of drops of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). The appearance of a yellow coloration in the extract indicated the presence of flavonoids (Harborne, 1998).

#### Detection of Tannins

To examine the presence of tannins, a minimal amount of powdered extract was dissolved in ethanol, and a 1% gelatin solution containing 10% sodium chloride was added to the dissolved extract. The formation of a white precipitate indicates the presence of tannins (Makhawi and Hamadnalla, 2019).

#### Detection of Resins

To detect the presence of resin, 10 mL of 95% ethyl alcohol was added to 1 g of the powdered extract. The mixture was heated in a water bath until boiling for two minutes. Then, 10 ml of distilled water acidified with concentrated hydrochloric acid was added to the filtrate. The appearance of turbidity indicates the presence of resins (Al-Manhel and Niamah, 2015).

#### Detection of Saponins

To detect the presence of saponins, 0.2 g of the powdered extract was combined with 5 ml of distilled water in a test tube and heated until boiling. The appearance of a stable, creamy froth or a cluster of small bubbles signifies the presence of saponins in the extract (Alhadi *et al.*, 2025).

#### Detection of essential oils

To examine the presence of essential oils, a small amount of the powdered extract was dissolved in 2 mL of ethanol. Subsequently, 0.1 ml of diluted sodium hydroxide and a small amount of diluted hydrochloric acid were added

to the mixture. The formation of a white precipitate indicates the presence of essential oil (Ghalem and Ali, 2017).

#### Determination of Functional Groups by Fourier Transform Infrared (FT-IR) Spectroscopy

The functional groups present in the ethanolic leaf extract of *D. viscosa* were identified using IR spectroscopy. A portion of the powdered crude extract was mixed with Potassium Bromide (KBr) salt using a mortar and pestle. The mixture was then compressed into a thin pellet using a KBr press. The pellets were placed in the compartment of a Perkin-Elmer FT-IR spectrometer 410 and scanned across the IR range of 4000 to 400 cm<sup>-1</sup> (Ibrahim *et al.*, 2022).

#### Determination of Antioxidant Capacity Using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant activity of the *D. viscosa* leaf ethanolic extract was assessed using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay, as modified after Ibrahim *et al.* (2023b). In this assay, ascorbic acid served as the standard antioxidant, while a DPPH solution (50 mg DPPH/100 mL ethanol) served as the control. Approximately 0.5 ml of ethanolic DPPH solution was added to various concentrations (0.5, 1, 1.25, 1.5, 2, and 2.5 µg/ml) of ascorbic acid and different concentrations (5, 10, 15, and 20 µg/ml) of the *D. viscosa* leaf ethanolic extract. Both ascorbic acid and all extracts were dissolved in ethanol. The control and DPPH-treated samples (ascorbic acid and extract) were incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm using a Genova Life Science Analyser - Protein. The radical scavenging effect was calculated as a percentage using the formula:

$$\text{Radical scavenging effect (\%)} = (A_c - A_s) / A_c \times 100,$$

where  $A_c$  represents the absorbance of the control and  $A_s$  denotes the absorbance of the tested sample (ascorbic acid and all extracts) (Ibrahim *et al.*, 2023a).

## Determination of Antimicrobial Activities

### Tested Pathogens

The *D. viscosa* ethanolic leaf extract was tested for its antimicrobial properties against four significant human pathogens: a gram-positive bacterium (*Staphylococcus aureus*), two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and a fungal pathogen (*Candida albicans*). These microbial strains were obtained from the National Central Public Health Laboratory (NCPHL) in Sana'a, Yemen.

### Preparation of Standardized Inoculum

The microbial inocula were prepared by harvesting fresh cultures of the selected bacterial and fungal pathogens. For bacteria, 3–5 isolated colonies were suspended in sterile 0.85% saline solution. For fungi, the surface of a mature culture was gently probed with sterile saline to dislodge spores, which were then filtered through sterile gauze to remove hyphal fragments. The turbidity of these initial suspensions was adjusted to match the 0.5 McFarland standard, which corresponds to an approximate cell density of  $1 \times 10^8$  CFU/ml. This standardization was confirmed using a spectrophotometer by ensuring the optical density (OD) fell within the range of 0.08 to 0.1 at a wavelength of 600 nm for bacteria and 530 nm for fungi (Arya *et al.*, 2010).

### Antibacterial efficacy

The Agar well-diffusion method on Mueller-Hinton Agar (MHA) media was applied to determine the activity of *D. viscosa* ethanolic leaf extract against the selected bacterial pathogens (Iqbal *et al.*, 2015; Hussain *et al.*, 2016; Shahzad *et al.*, 2017). Four Petri dishes containing MHA media were prepared for each bacterial pathogen, with three plates assigned to the extract and one plate used as a positive control. Approximately  $1 \times 10^8$  CFU/ml of each tested bacterium was cultured in each petri dish (Arya *et al.*, 2010). Using a sterilized cork borer, 4 wells (5 mm in diameter) were created in the agar of ePetrietri dish. One well at the center was filled with 50  $\mu$ l of dimethyl sulfoxide

(DMSO) as a negative control. In contrast, the remaining wells (located at the corners) were filled with different concentrations the plant extracts (Ibrahim *et al.*, 2022). To assess the antibacterial activity of *D. viscosa* ethanolic leaf extract, a serial nine concentrations were prepared using DMSO to obtain concentrations of 400,300, 200,100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. Then, 50  $\mu$ l of each concentration (400,300, 200,100, 50, 25, 12.5, 6.25, and 3.125 mg/ml) of the extract was pipetted into the designated wells (modified after Ibrahim *et al.*, 2023a). For the positive control, a separate Petri dish was prepared containing the standard antibacterial agents Tetracycline (30 $\mu$ g), Ciprofloxacin (5  $\mu$ g), Erythromycin (15  $\mu$ g), and Gentamicin (10  $\mu$ g). The Petri dishes were incubated at 37°C for 24 h, and the diameters of the inhibition zones were measured in millimeters (modified after Alhadi *et al.*, 2025). This procedure was performed in triplicate for each bacterial species, and the results were expressed as the mean inhibition zone diameter (Ibrahim *et al.*, 2023b). Furthermore, the Minimum Inhibitory Concentration (MIC) of the plant extract was assessed against three selected human pathogenic bacteria: *S. aureus*, *E. coli*, and *P. aeruginosa*, following the methodologies outlined by Hiregoudar *et al.* (2011) and Ibrahim *et al.* (2022).

### Antifungal efficacy

The antifungal activity of *D. viscosa* ethanolic leaf extract against *C. albicans* was determined using the agar well-diffusion method on Sabouraud Dextrose Agar (SDA) as demonstrated by Ibrahim *et al.* (2023a). Four Petri dishes containing SDA were prepared (three testing plates for the *ethanolic* extract and one positive control plate). A sterilized swab was used to spread 100 microliters of *C. albicans* suspension ( $1 \times 10^8$  CFU/ml) over the SDA in each plate. Four wells (5 mm in diameter) were made in the agar media using a sterilized cork borer, one in the center, containing DMSO as a negative control. In contrast, the remaining wells (located at the corners) were filled with the different concentrations of the plant extracts (Ibrahim *et al.*, 2022). To determine the antifungal activity of *D. viscosa* ethanolic leaf

extract, a series of five concentrations was made using DMSO to obtain concentrations of 400,300, 200,100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. Then, 50  $\mu$ l of each concentration of the extract was pipetted into a well, while 50  $\mu$ l of DMSO served as a negative control (modified after Ibrahim *et al.*, 2023a). For the positive control, a separate Petri dish was prepared containing the standard antifungal agents: Miconazole (50  $\mu$ g), Nystatin (100  $\mu$ g), Voriconazole (1  $\mu$ g), and Itraconazole (8  $\mu$ g). Plates were incubated at 37°C for 48 hours before measuring the inhibitory zone for each concentration. This process was repeated three times, and the inhibition zone diameter (mm) results were obtained as a mean (Ibrahim *et al.* 2023a). Moreover, the Minimum Inhibitory Concentration (MIC) of the ethanolic leaf extract against *C. albicans* was assessed using the assay method outlined by Hiregoudar *et al.* (2011) and Ibrahim *et al.* (2022).

## RESULTS

### Phytochemical Screening

Table (1) presents the results of the phytochemical investigation, indicating that the ethanolic leaf extract of *D. viscosa* contains several active pharmacological components, such as alkaloids, phenols, flavonoids, tannins, resin, and saponin compounds. In contrast, essential oil is not present in the *D. viscosa* ethanolic leaf extract.

**Table 1.** Qualitative Phytochemical Constituents of *D. viscosa* Ethanolic Leaf Extract.

Sr. No.	Phytochemical constituents	<i>D. viscosa</i> Ethanolic Leaf Extract
1	Alkaloid	+ve
2	Phenols	+ve
3	Flavonoids	+ve

4	Tannin	+ve
5	Resin	+ve
6	Saponin	+ve
7	Essential oil	- ve

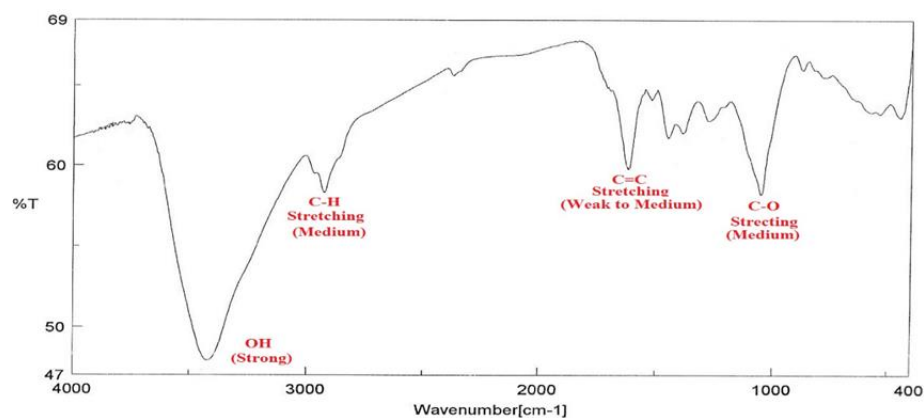
+ve: Present; -ve: Absent.

### Determination of Functional Groups by Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis identified four chemical and functional groups present in the ethanolic leaf extract of *D. viscosa*, as detailed in Table 2 and illustrated in Figure 2. The broad peak at 3848.26  $\text{cm}^{-1}$  (strong intensity) indicates the presence of the hydroxyl group stretching (O-H), suggesting the presence of pharmaceutical compounds, such as phenols and their derivatives, in the extract (Figure 2). In contrast, the peaks at 2925.48  $\text{cm}^{-1}$  (medium intensity) are attributed to the methylene (C-H, stretching) functional group, which is typically found in Aromatic and Alkenes compounds, indicating their presence in the ethanol leaf extract (Figure 2). Moreover, the peak observed at 1617.02  $\text{cm}^{-1}$  (weak to medium intensity) suggests the presence of Aromatic C=C (stretching) in the ethanol leaf extract (Figure 2). In addition, Figure 2 indicates that the peaks at 1050.1  $\text{cm}^{-1}$  (medium intensity) are due to the presence of Aliphatic-C-O (stretching).

**Table 2.** FT-IR Spectral Analysis of *D. viscosa* Ethanol Leaf Extract.

Sr. No.	Functional Group	Wavelength of Absorption $\text{cm}^{-1}$
1	Phenolic- O-H group (Stretching)	3848.26 $\text{cm}^{-1}$ (strong intensity)
2	Aliphatic-C-H (Stretching)	2925.48 $\text{cm}^{-1}$ (weak intensity)
3	Aromatic -C=C (Stretching)	1617.02 $\text{cm}^{-1}$ (weak intensity)
4	Aliphatic-C-O (Stretching)	1050.1 $\text{cm}^{-1}$ (medium intensity)

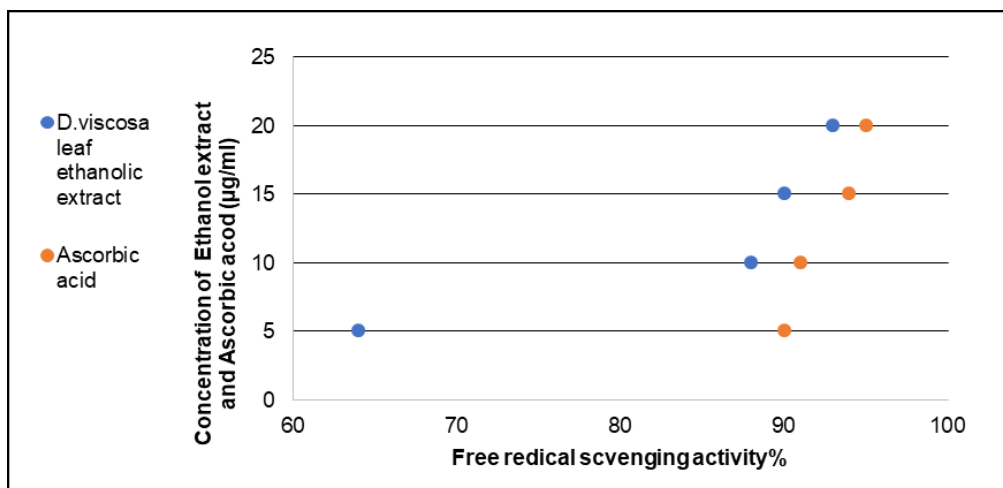


**Fig.2.** FT-IR spectrum analysis of *D. viscosa* Ethanol Leaf Extract.

### Determination of Antioxidant Capacity Using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Assay:

This method is based on the reduction of the stable free radical purple-colored DPPH in the presence of an antioxidant to the non-radical yellow-colored diphenyl picrylhydrazyl DPPH-H. Figure 3 illustrates the free radical scavenging

activity of the ethanolic leaf extract of *D. viscosa*, as measured using the DPPH assay. The extract demonstrated significantly higher antioxidant activity, with scavenging percentages of 93, 90, 88, and 64% at concentrations of 20, 15, 10, and 5  $\mu\text{g/ml}$ , respectively. In contrast, ascorbic acid showed 95, 94, 91, and 90 % radical scavenging at the same concentrations, respectively.



**Fig. 3.** Antioxidant Activity of *D. viscosa* Ethanol Leaf Extract and Ascorbic Acid.

### Antimicrobial activity

As shown in Table 3, the ethanol leaf extract of *D. viscosa* showed antibacterial activity against the selected gram-positive bacterium (*S. aureus*) and one of the two selected gram-negative

bacteria (*E. coli*). However, it did not exhibit any activity against the selected fungal pathogen (*C. albicans*). For *S. aureus*, the extract displayed mean inhibition zones of 7, 9, 11, 14, 17, 19, 20, and 22 mm at concentrations of 6.25, 12.5, 25, 50, 100, 200, 300, and 400 mg/ml, respectively

(Table 3). Against *E. coli*, the extract showed mean inhibition zones of 10, 12, 15, 19, 20, 22, and 24 mm at concentrations of 12.5, 25, 50, 100, 200, 300, and 400 mg/ml, respectively (Table 3). However, no antimicrobial activity was observed against *P. aeruginosa* (a gram-negative bacterium) and *C. albicans* by the extract (Table 3). In contrast, the antibacterial agents Tetracycline 30 µg (TE), Ciprofloxacin 5 µg (CIP), Erythromycin 15 µg (E), and Gentamicin 10 µg (GEN), which served as positive controls, illustrated antibacterial effects against the selected gram-positive and gram-negative bacteria (Table 3). Against *S. aureus*, the mean inhibition zones were 40, 16, 20, and 30 mm, respectively, whereas against *E. coli*,

the mean inhibition zones were 40, 50, 22, and 48 mm, respectively (Table 3). Against *P. aeruginosa*, the agents exhibited mean inhibition zones of 27, 55, 17, and 39 mm, respectively (Table 3). Additionally, the antifungal agents Miconazole 50 µg (MCZ), Nystatin 100 µg (NS), Voriconazole 1 µg (VRC), and Itraconazole 8 µg (ITZ) showed antifungal effects against *C. albicans*, with mean inhibition zones of 44, 28, 42, and 25 mm, respectively (Table 3). According to Table 3, the MIC of the ethanolic leaf extract was 6.25 mg/ml and 12.5 mg/ml against *S. aureus* and *E. coli*, with mean inhibition zones of 7 mm and 10 mm, respectively (Table 3).

**Table 3.** Inhibition zone diameters (mm) of *D. viscosa* Ethanol Leaf Extract against tested Pathogens.

Tested Bacterial Pathogens	Type	Extraction Concentration (mg/ml)									-ve Control (DMSO)	+ve Control (Antibiotics)				MIC
		400	300	200	100	50	25	12.5	6.25	3.125		TE	CIP	E	GEN	
		Inhibition Zone Diameter (mm)										Inhibition Zone Diameter (mm)				
<i>S. aureus</i>	G+ve	22	20	19	17	14	11	9	7	-	-	40	16	20	30	6.25
<i>E. coli</i>	G-ve	24	22	20	19	15	12	10	-	-	-	40	50	22	48	12.5
<i>P. aeruginosa</i>	G-ve	-	-	-	-	-	-	-	-	-	-	27	55	17	39	-
Tested Fungal Pathogen		Extraction Concentration (mg/ml)									-ve Control (DMSO)	+ve Control (Antibiotics)				MIC
		400	300	200	100	50	25	12.5	6.25	3.125		MCZ	NS	VRC	ITZ	
		Inhibition Zone Diameter (mm)										Inhibition Zone Diameter (mm)				
<i>C. albicans</i>		-	-	-	-	-	-	-	-	-	-	44	28	42	25	-

G +ve = Gram positive; G-ve = Gram negative; TE = Tetracycline (30 µg); CIP = Ciprofloxacin (5 µg); E = Erythromycin (15 µg); GEN= Gentamicin (10 µg); MCZ = Miconazole (50 µg); NS = Nystatin (100 µg); VRC= Voriconazole (1 µg) & ITZ = Itraconazole (8 µg)

## DISCUSSION

Table 1 shows that the ethanol leaf extract of *D. viscosa* contains several secondary metabolites with potential pharmacological uses. These include alkaloids, phenols, flavonoids, tannins, resins, and saponins. This finding aligns with earlier studies reporting flavonoids, saponins, alkaloids, and phenols (Lawal and Yunusa, 2013; Anode *et al.*, 2018), as well as tannins and resins (Orpin *et al.*, 2018) in *D. viscosa*. These results support the traditional medicinal use of *D. viscosa* leaves.

The FT-IR spectral analysis presented in Table (2) and Figure (2) indicates that the ethanol fruit extract of *D. viscosa* contains four functional groups: Phenolic O-H, Aliphatic C-H, Aromatic

C=C, and Aliphatic C-O. These groups are typical of various bioactive compounds that could contribute to the extract's pharmacological effects. These findings are consistent with those of AL-Azawi (2016), where he cited that Phenolic- O-H group, Aliphatic C-H, Aromatic - C=C, and Aliphatic-C-O were detected in methanolic *D. viscosa* leaf extract. He also reported the presence of an N-O symmetric group in the methanolic leaf extract of *D. viscosa*, which was not detected in the ethanolic leaf extract of *D. viscosa*, which may be due to variations in extraction methods or environmental conditions.

Previous research indicates that the high antioxidant activity observed in many plant extracts using the DPPH assay is closely linked

to the concentration of phenolic compounds, especially flavonoids. These compounds function as antioxidants by donating hydrogen atoms or electrons to the DPPH radical. This chemical reaction reduces the violet-colored DPPH radical to its stable yellow color form (DPPH-H), serving as a measurable indicator of radical-scavenging capacity (Huyut *et al.*, 2017). Consequently, the high free radical scavenging activities of the *D. viscosa* leaf extract are attributed to the presence of phenols and flavonoids in the ethanolic leaf extract and leave of *D. viscosa*. The antimicrobial assay results (Table 3) show that the ethanolic extract of *D. viscosa* leaves exhibited notable antibacterial activity, attributed to the presence of phenolic compounds and flavonoids (Al-Arnoot *et al.*, 2025), but its effects were selective. The extract produced a clear dose-dependent response against both *S. aureus* and *E. coli*. For *S. aureus*, the inhibition zones increased from 7 mm to 22 mm as the concentration increased from 6.25 to 400 mg/ml. *E. coli* was slightly more sensitive, with zones ranging from 10 mm to 24 mm as the concentration increased from 12.5 to 400 mg/ml. These results are consistent with earlier studies by Khurram *et al.* (2009) and Esmaeel and Kamil (2011), where they also found that the crude ethanolic extract of *D. viscosa* from the aerial parts (leaves and bark) had antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. However, *P. aeruginosa* was resistant, likely because its outer membrane is highly impermeable and its efflux pump systems are efficient, preventing many plant compounds from reaching effective intracellular concentrations (Lorusso *et al.*, 2022). The extract also showed no activity against *C. albicans*, which may be because fungi are generally more resistant to phenolic-rich plant extracts than bacteria (Oulahal and Degraeve, 2022).

## CONCLUSION

The study revealed that the ethanolic leaf extract of *Dodonaea viscosa* possesses significant medicinal properties, due to the presence of key compounds, including phenols and flavonoids.

These compounds were identified using chemical testing and Fourier-transform infrared (FT-IR) analysis. The extract exhibited strong antioxidant activity, effectively scavenging DPPH free radicals through hydrogen atom or electron donation. Additionally, it exhibited antibacterial activity against common bacteria, such as *S. aureus* and *E. coli*, with greater effectiveness at higher concentrations. However, it did not show any effect against *P. aeruginosa* or *C. albicans*. Overall, *D. viscosa* is a good candidate for natural antioxidant and antibacterial products.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the Biological Sciences Department Laboratories and the Central Laboratory at the Faculty of Science, Sana'a University, for their support. They also appreciate the invaluable contributions of the National Central Public Health Laboratory (NCPHL) in Sana'a.

## CONFLICT OF INTEREST

The authors hereby declare that they have no conflict of interest.

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