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Research Article

Comparison of Antibacterial and Antioxidant Activities of Ethanolic Extracts of Four Plant Species Selected from South of Saudi Arabia

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ABSTRACT

One of the most ancient human medical techniques is the use of plants to treat, prevent, and cure diseases. These plants can manufacture a wide variety of natural chemicals. The present study aimed to evaluate the antibacterial activity and antioxidant capacity of ethanolic extract of four plant species (*Zizyphus lotus, Lavandula dentata, Ruta graveolens,* and *Dodonaea viscosa*). Using disc diffusion and serial dilution procedures, the antibacterial abilities of these EtPEs were evaluated. The antioxidant properties were evaluated by the FRAP method and the Folin-Ciocalteu technique was used to measure the total phenolic content. Different plant extracts showed different inhibitory effects on the tested bacteria in a dose-dependent manner. Among the tested plant extracts, *D. viscose* exhibited the highest antibacterial activity against *P. vulgaris* and *S. aureus*, with a minimum inhibitory concentration (MIC) value of 0.5 mg/ml. On the other hand, *R. graveolens* displayed the highest quantity of phenolic compounds and demonstrated the highest antioxidant activity. Notably, there was a positive correlation observed between the antioxidant activity of the plant extracts and their total phenolic content. In conclusion, the findings of this study suggest that the tested plant extracts hold potential as promising sources of natural antibacterial and antioxidant agents.

Key words: Antibacterial, Antioxidants, Extraction, Total phenols, Medicinal plants.

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INTRODUCTION

The search for new antiradical and antibacterial compounds remains a problem for modern science due to the high toxicity of synthetic compounds. Since antiquity, plants containing active substances have been used worldwide in traditional medicine and considered a source of potential and powerful drugs.[1-4] Naturally, Plant tissues are rich in therapeutically active components of plant secondary metabolites. The chemical analysis and purification of plant extracts with purported therapeutic characteristics have produced a huge number of refined substances that have shown to be indispensable in the usage of modern medicine.[5-7] Only a small portion of the estimated 250,000–500,000 plant species have been subjected to biological or pharmacological screening, and the potential of higher plants as a source for novel medications is still unexplored.[8] Utilizing antimicrobials produced from plants may help reduce the need for antibiotics and minimize the chances of antibiotic resistance in foodborne pathogenic microorganisms.[6,7]

Plants create a vast variety of secondary metabolites that act as substances that protect them from herbivores, microorganisms, and other plants. Secondary metabolites typically display a wide range of biological and pharmacological characteristics.[5,8-10] As a result, certain plants or products derived from them have been utilized to treat illnesses, infections, and other health disorders. The treatment of infections and health disorders with herbal medicines usually involves active natural products mostly of low molecular weight of great structural diversity.[11,12] plants are frequently employed as dietary supplements. Their significance as a source of natural antioxidants is particularly intriguing. Exploiting less expensive and safer sources of antioxidants from natural sources, particularly from plants, is of interest nowadays due to rising safety concerns associated with the consumption of synthetic antioxidants.[11,13,14]

Four different plant species were used in this study, the first species is *Zizyphus lotus*, and this genus belongs to the buckthorn family (Rhamnaceae). It is a genus of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world.[15] *Z. lotus* has been widely used as a homemade ingredient to cure many diseases in folk medicine. The antimicrobial activity of *Z. lotus* has been shown to have activity against bacteria and fungi and other pathogens that are normally quite resistant.[16-18]

The genus *Lavandula* contains at least 28 different species. Among the more common species believed to have medicinal value is *Lavandula dentata*. Different lavender species have variable antibacterial effects, depending on the concentration of specific chemical constituents.[19,20] *Ruta graveolens* has been extensively studied as a medicinal plant due to its rich phytochemical content, such as furanocoumarins and flavonoids. *Ruta graveolens* is an aromatic shrub belonging to the family *Rutaceae* and is commonly known as rue. The plant extract is used

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to treat inflammation and ulcers. This plant extract exerts cytotoxic, antibacterial, and antihelminthic. Plant extracts are also used in the treatment of reproductive disorders.[21,22] *Dodonaea viscosa* is a medicinal plant used in folk medicine to treat various ailments; the leaves have traditionally been administered to treat wounds, fever, malaria, cold, and arthritis.[23-25]

This study aimed to examine the antibacterial and antioxidant activities of ethanolic extracts derived from representative plant species belonging to the Rutaceae, Rhamnaceae, Sapindaceae, and Lamiaceae families, which include the predominant aromatic plants found in the southern region of Saudi Arabia. Specifically, the leaves of four distinct species (*Z. lotus*, *L. dentata*, *R. graveolens*, and *D. viscosa*) were investigated for their respective properties.

MATERIAL AND METHODS

Plant material

Four plant species indigenous to the flora of the South of Saudi Arabia, and commonly used as medicinal herbs in traditional local medicine. The fresh samples of four medicinal plants were collected from their natural environment (*Zizyphus lotus, Lavandula dentata, Ruta graveolens,* and *Dodonaea viscosa*). The plants were identified by Prof. Saleh Al-Quran who is a plant taxonomist in the Department of Biology, Faculty of Sciences, Mu'tah University, Jordan. The botanical data of the selected plants are shown in Table 1.

Preparation of plants and extraction method

Before drying, the fresh samples were cleaned of any dirt using tap water. The samples were blended into a fine powder after drying at room temperature in the shade for 10 days. Twenty-five grams of the plant powder was mixed with 250 mL of 80% ethanol to begin the extraction procedure. On a rotary shaker, the mixture was left at room temperature for 36 hours. The extracts were then dried using a rotary evaporator under reduced pressure after being filtered using 0.45 mm filter paper and centrifuged at 5000 rpm for 15 min. Each extract was dissolved in 200 mg/mL of 10% dimethylsulphoxide (DMSO) in ethanol (v/v) and kept at 4°C.

Test microorganisms

Four strains of bacteria were used in this study for the evaluation of antibacterial activities. The strains included *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus vulgaris* ATCC 25924 and *Staphylococcus aureus* ATCC 25923. These bacterial species were sourced from the Microbiology Research Laboratory at Mutah University in Alkarak, Jordan.

Table 1: The botanical information about the four-plant species selected in this study.

Scientific name	Family	Common name	Parts used
Ziziphus lotus	Rhamnaceae	Lotus	leaves
Lavandula dentata	Lamiaceae	lavender	leaves
Ruta graveolens	Rutaceae	Ruta	leaves
Dodonaea viscose	Sapindaceae	Hopseed	leaves

Table 2: Dry weight and (%) yield of ethanol plant extracts.

Name of plant	Dry weight (g)	Yield Percentage of EtPEs (%) *
Dodonaea viscose	0.04	3.36 %
Ziziphus lotus	0.06	4.54 %
Lavandula dentata	0.06	5.12%
Ruta graveolens	0.04	3.7%

* Yield Percentage of plant extract (%) = (dry weight of crude extract / initial weight of plant sample) x 100%.

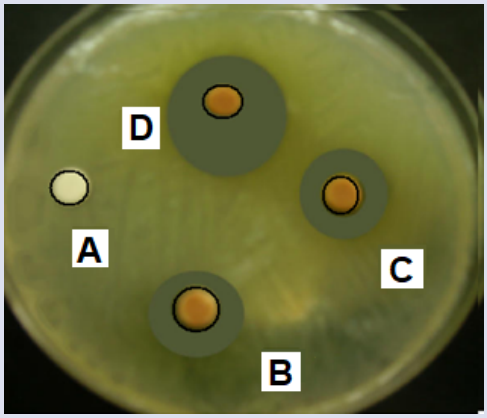

Figure 1: Antibacterial activity of *R. graveolens* extract: (A) Negative control disc with 10µl of the solvent (10 %DMSO); (B)100 µg of crude extract; (C) 200 µg of crude extract; and (D) 400 µg of crude extract.

Antibacterial properties of ethanolic plant extracts (EtPEs)

The antibacterial properties of the four extracts were investigated *in vitro* against the four bacterial species using the disc diffusion assay.[26,27] Mueller Hinton agar (Hi-Media, India) was used as the growth medium. The antibacterial action was examined at three different concentrations (100µg, 400µg, and 400µg). The sterilized media was poured up to 3/4th of the petri dish and bacterial cultures were spread uniformly. Sterile antimicrobial susceptibility discs (6 mm diameter) were loaded with 10 µL of each EtPEs and placed on the inoculated plates. For each bacterial strain, a negative control disc loaded with 10 µL of 10% DMSO in methanol was included. Streptomycin and tetracycline discs were used as positive controls. The cultures were incubated at 37°C for 24h. The antibacterial activity of each EtPEs was determined according to the size of the inhibition zone around each disc and the inhibition zones were measured in millimeters.

The Minimum Inhibitory Concentration (MIC)

According to the guidelines set by the National Committee for Clinical Laboratory Standardization, the MIC values were calculated using the broth dilution method. A standard initial inoculum (5×10^5 cells/mL) was used for all trials. The cell suspension was supplemented with EtPEs to achieve a concentration range of 2 to 0.06 mg/ml. The tubes were cultured in an orbital shaker incubator at 37 °C and 150 rpm. Using a spectrophotometer, the OD_{600} was measured after 24 hours of incubation. The lowest EtPEs concentration that prevents at least 90% of growth was identified as the MIC.[28,29]

Determination of total phenolic content (TPC)

The TPC of all four plant extracts was determined by using Folin-Ciocalteu method. A standard gallic acid curve was constructed by preparing different concentrations of Gallic acid and used to estimate the TPC in each extract as Gallic acid equivalents (GAE). In a test tube, 5 mL of Folin-Ciocalteu's reagent was mixed with 1 ml of the EtPEs. Afterward, 4 mL of sodium carbonate (7.5%) was added. The tube's contents were well mixed before being incubated at room temperature for 30 minutes. The absorbance of each mixture was then measured at 760 nm.[30]

Determination of antioxidant activity

The total antioxidant activity of four plants in southern Saudi Arabia is measured by the Ferric Reducing Antioxidant Power (FRAP) assay (Benzie & Strain, 1999). FRAP assay used antioxidants as reductants

in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. Antioxidants are evaluated as reductants of Fe^{3+} to Fe^{2+} which is chelated by TPTZ (2,4,6-tripyridly-s-triazine) to form a Fe^{2+}–TPTZ complex absorbing at 593 nm.

Fe (III)–TPTZ + antioxidant Fe (II)–TPTZ + oxidized antioxidant

According to (Benzie and Strain 1999)[31] the working FRAP reagent was prepared: acetate buffer 300mM pH 3.6 (weight 3.1g sodium acetate trihydrate and added 16 ml acetic acid and complete the volume to 1L with distilled water), 20 mM $FeCL_3$, 10 mM TPTZ in 40 mM HCL. Mixing acetate buffer, $FeCL_3$, and TPTZ in the ratio 10:1:1 at the time of use. Mix 10 µl of plant extract to 3ml of working FRAP reagent and absorbance at 593 nm after placing the sample in a water bath for 4 minutes. A standard curve was prepared using different concentrations (100 µM- 1000 µM) of Ascorbic Acid.

RESULTS

This article describes the antibacterial and antioxidant activities of plants used previously in traditional medicine in South Saudi Arabia. A total of 4 extracts from 4 different plant species were tested. The results of dry weight and the percentage of yield of Ethanol crude plant extracts were shown in Table 2.

Determination of antibacterial activity of ethanolic plant extracts

Table 3 presents the antibacterial activity of the plant species investigated in this study. A control disc containing the solvent was included on the same plate, which demonstrated no inhibition of bacterial growth, as illustrated in Figure 1. Additionally, the antibacterial activity was evaluated through the broth dilution method to determine the minimum inhibitory concentration (MIC) values of the EtPEs against the tested bacterial species. The assessed EtPEs exhibited varying levels of antibacterial activity.

Effect of different concentrations of plant extracts on bacterial growth

The growth of bacterial strains in nutrient broth was assessed using various concentrations of the studied plant extracts. The results, depicted in Figure 2, demonstrated a dose-dependent inhibitory effect of all plant extracts on the tested bacteria.

MIC values of EtPEs against bacterial growth

The MIC values were between 0.5-3.75 mg/ml for *Z. lotus*, *L. dentata*, *R. graveolens,* and *D. viscosa*. However, the MIC values of *L. dentata* extract against all the tested bacterial species were the highest. While the ethanolic extracts of *D. viscosa* showed that lower MIC than other plant extracts as shown in Table 4. *Z. lotus* and *D. viscosa* have the lowest MIC values compared to *L. dentata* and *R. graveolens*.

Total phenolic content (TPC)

Polyphenolic compounds are recognized for their antioxidant activity. Figure 3 displays the total polyphenolic content (TPC) of the EtPEs. Among the studied species, *R. graveolens* extract exhibited the highest level of polyphenols, with a TPC of 750 mg GA/g dry extract. Following closely were *Z. lotus* and *L. dentata* with TPC values of 730 mg GA/g dry extract and 725 mg GA/g dry extract, respectively. On the other hand, *D. viscosa* displayed the lowest TPC, measuring 650 mg GA/g dry extract.

Determination of the antioxidant activity of crude plant extracts

Ascorbic acid was chosen as a standard for this experiment due to its well-known antioxidant properties. Table 5 presents the Ascorbic Acid Equivalents Antioxidant Capacity (AEAC) of the ethanolic extracts of the plants, determined using the FRAP assay. The results revealed that the ethanolic extracts of *R. graveolens* exhibited the highest activity, with a value of 3.37 ± 0.113 M/g of dry weight. Conversely, the ethanol extracts of *D. viscosa* displayed lower antioxidant activity compared to the other plant extracts.

DISCUSSION

The emergence of antibiotic resistance in pathogenic microorganisms has been attributed to the improper use of commercial antimicrobial drugs, which are commonly prescribed to treat a variety of diseases. This has increased the demand for substitute antimicrobial compounds. As a consequence, scientists have been motivated to investigate various plant sources for new antimicrobial compounds. Several factors, including the need for effective treatments against drug-resistant microbes and the possibility of discovering novel antimicrobial compounds, have contributed to this endeavor.[32,33]

In the present study, the antibacterial assays consistently demonstrated that all the tested EtPEs exhibited activity against the tested microorganisms. The gram-negative bacteria, including *E. coli*, *P. vulgaris*, and *K. pneumoniae*, as well as the gram-positive bacterium *S. aureus*, were exposed to the ethanolic extracts of *D. viscosa*, *Z. lotus*, *L. dentata*, and *R. graveolens*. Notably, the antibacterial activity displayed a positive correlation with the concentration of the plant extracts, indicating that higher concentrations led to increased inhibitory effects against the microorganisms. As determined by both the broth dilution and disc diffusion assay methodologies, the ethanolic extracts of *D. viscosa* exhibited the highest antibacterial activity of all the tested plant extracts. It has been previously reported that the aqueous extracts of *D. viscosa* displayed exceptional antimicrobial activity against many

Table 3: Antibacterial activity of three different concentrations of EtPEs of *D. viscosa, Z. lotus, L. dentata*, and *R. graveolens* against the examined bacterial species. Data are shown as Mean± standard deviation, n=3.

Bacteria	Zone of inhibition (mm)*											
	D. viscosa			Z. lotus			L. dentata			R. graveolens		
	100 µg /disc	200 µg /disc	400 µg /disc	100 µg /disc	200 µg /disc	400 µg /disc	100 µg /disc	200 µg /disc	400 µg /disc	100 µg /disc	200 µg /disc	400 µg /disc
***E. coli* ATCC 25922**	11.3±0.57	19.6±0.75	24±1	12±1.52	19.6±1.52	23.6±1.15	7.6±1.15	10.6±1.15	18.3±1.52	7.67±1.15	9±1	16.3±1.52
***K. pneumonia* ATCC 13883**	10.3±1.15	19.6 ±1.15	24±1	8.3±1.15	16.6±1.52	21.6±0.57	7.8±0.57	9.3±1.52	17±2	7.67± 1.12	9.3±1.52	15.6±1.15
***P. vulgaris* ATCC 25924**	15.3±0.57	19.6±0.75	25.3±0.57	8±1	21.6±1.52	24.3±1.52	8±1	9.6±1.52	20±2	8.3±1	11.6±0.57	18.3±1.15
***S. aureus* ATCC 25923**	17.6±1	21±1	26±0.57	15.3±1.52	21±1	25.6±1.52	9±1.73	19±2	24.3±1.52	8.6±1.52	12.3±1.15	21.6±2.08

* Inhibition zone (mm) is the mean value of three independent experiments; Diameter of the inhibition zone (mm) includes the diameter of the disc (6 mm).

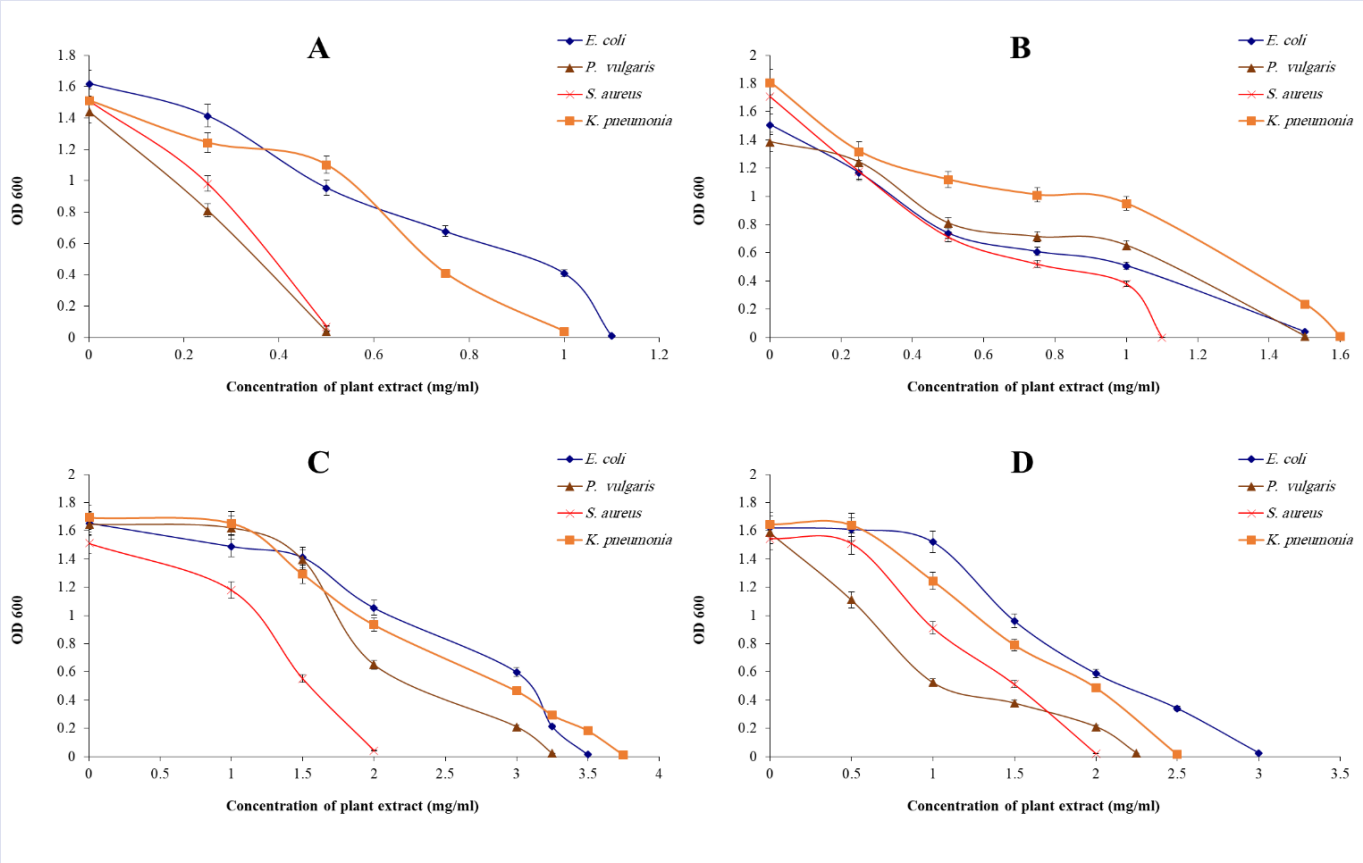

Figure 2: Effect of different concentrations of EtPEs on growth of different bacterial species: (A) *D. viscosa*; (B) *Z. lotus*; (C) *L. dentata*; and (D) *R. graveolens*. Data are shown as Mean± standard deviation, n=3.

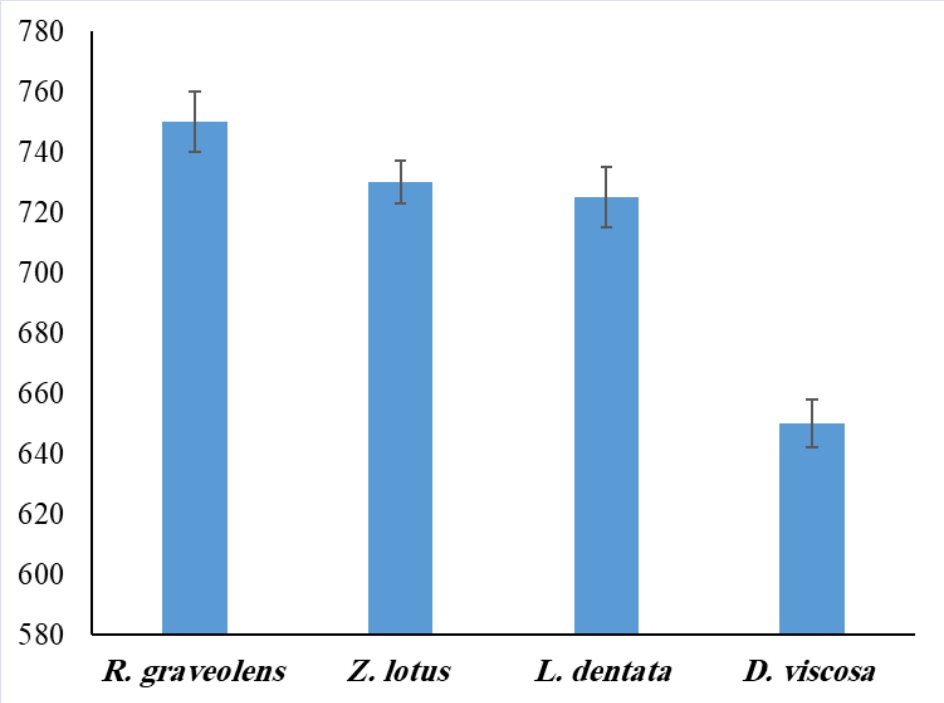

Figure 3: Total phenolic contents of EtPEs expressed as GAE. Data are shown as Mean± standard deviation, n=3.

Table 4: MIC values of EtPEs against bacterial growth.

	Minimal inhibition concentration (MIC) (mg/ml)			
	Z. lotus	L. dentata	R. graveolens	D. viscosa
E. coli	1.5	3.5	3	1.1
K. pneumonia	1.6	3.75	2.5	1
P. vulgaris	1.5	3.25	2.25	0.5
S. aureus	1.1	2	2	0.5

Table 5: Ferric reducing antioxidant power of EtPEs expressed as ascorbic acid equivalents antioxidant capacity (AEAC). Data are shown as Mean± standard deviation, n=3.

Plant	FRAP-AEAC (M/g of dry weight)
D. viscose	2.09 ± 0.126
Z. lotus	2.65 ± 0.238
L. dentata	3.25 ± 0.121
R. graveolens	3.37 ± 0.113

pathogenic microorganisms including bacteria and fungi, as well as a broad spectrum of antibiofilm activity.[34] Additionally, in this study, the ethanolic extract of *Z. lotus* demonstrated significant antibacterial activity, particularly against *S. aureus* and *P. vulgaris*. These findings emphasize the potential of both *D. viscosa* and *Z. lotus* as sources of antimicrobial compounds, specifically targeting these bacterial strains.

The findings of the current investigation showed that Gram-positive bacterium, *S. aureus*, was more susceptible to the tested EtPEs than Gram-negative bacteria.[35] This is consistent with earlier research that found Gram-negative bacteria are typically more resistant to conventional antibiotics than Gram-positive bacteria.[36] The lipopolysaccharide layer in the outer membrane of Gram-negative bacteria is known to serve as a strong barrier that reduces the permeability to many substances, therefore these differences in susceptibility are primarily due to the cell wall structure.[37,38]

Furthermore, the current study demonstrated that the tested plant extracts exhibited significant antioxidant activities. This observation aligns with existing literature that highlights the presence of diverse antioxidant compounds in plants. Phenolic compounds, nitrogen compounds, vitamins, terpenoids, and other endogenous metabolites are well-known constituents of plants that possess rich antioxidant activity.[14,22] The findings of this study further contribute to the understanding of the antioxidant potential inherent in these plant extracts. Several assays have been developed to measure the antioxidant capacity *in vitro*. In this study the antioxidant activity of the EtPEs was evaluated by the ferric reducing antioxidant power (FRAP). The FRAP assay is a simple procedure that measures the total antioxidant levels in a sample. It utilizes the reducing potential of the antioxidants to react with a ferric tripyridyltriazine (Fe^{+3}-TPTZ) complex and produce a colored ferrous tripyridyltriazine (Fe^{+2}-TPTZ) form (Benzie and Strain, 1999).[31] The total antioxidant activity of extracts using the FRAP assay indicated that the ethanolic extract of *R. graveolens* showed the highest activity. However, *D. viscosa* ethanolic extract showed lower activity. This difference in the antioxidant capacity might be attributed to the differences in the total polyphenolic content of the plant extracts. It has been reported previously that there is a strong correlation between the antioxidant capacity and the Total Polyphenolic Content of the plant extracts.[36,39,40] The collective evidence suggests that phenolic compounds are major contributors to the overall antioxidant potential of the tested plant extracts.

CONCLUSION

New antibacterial and antioxidant agents are required because bacteria are becoming more resistant to treatment as well as the tendency to reduce the use of chemical additives. Without a doubt, the alternative is the world of plants and the extracts made from them, which are becoming more and more popular among consumers because of their natural origin. In this study, the extract of *R. graveolens* is considered an interesting source of natural antioxidant compounds. In addition, all the studied plant species showed antibacterial activity, and these results suggest that promising therapeutic compounds could be isolated and purified from these plant extracts.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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