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RESEARCH ARTICLE

Bioactive Phenolic Compounds from Natural *Zygophyllum fabago* Plant: Isolation, Purification and Characterization

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Abstract

This study is to investigate the chemical constituents of the aerial parts of *Zygophyllum fabago* in Iraq. The compounds were isolated by silica gel and column chromatography from deferent extracts. Their structures were characterized by various spectroscopic data (HPLC, ^{1}H and ^{13}C-NMR) and compared with the literature. As a result, three phenolic compounds were isolated, and their structures were identified as Catechol, Coumarin and Resorcinol. Biological testing of the extracts against six different Gram-positive and Gram-negative bacteria showed significant results.

Keywords: *Zygophyllum fabago; Bioactive; Purification.*

Introduction

Natural products from medicinal plants have always been considered as a valuable source of new bioactive compounds in drug development studies. In traditional medicine, a wide range of substances can be extracted from plants and used for treatment of chronical and infectious diseases. Due to the high microbial resistance to the synthetic drugs as well as adverse effects, people turned to natural or herbal remedy.

The phytochemicals from plants regarded as safe and effective alternatives with less harmful effect. The World Health Organization (WHO) has estimated around 80% of the worlds populations depend on herbal medicine for their initial healthcare needs [1]. *Zygophyllum fabago* belongs to the Zygophyllaceae family. It is a small perennial herb with fleshy leaves and flowers; it is naturally available in the desert and steppe habitats of southwestern and central parts of Asia, South Africa, South of Europe and Australia [2].

In Iraq *Zygophyllum fabago* has been used in traditional medicine for treatment of various illnesses, such as fungal infections, cough, constipation, parasitic worms, inflammation and asthma [3].

Antimicrobial and antifungal activity has been reported for the same species [3-5]; the reason of such activities is due to their phytochemical constituents. Several classes of compounds including flavonoids, sterols, triterpenes, ketones, saponins, essential oils, esters and phenolic compounds have been isolated from diverse *Zygophyllum* species [6, 11]. Phenolic compounds have a large interest due to their wide range of biological properties. Chemically, phenolic compounds consist of aromatic ring connecting to one or more hydroxyl group. It is believed that such biological activity can be related to the chemical structure, especially the aromatic nucleus and the position of the hydroxyl groups [12, 14].

The present study aimed to isolation, purification and structure elucidation of some phytochemicals (phenolic compounds) that have been extracted from the aerial parts of the medicinal species of *Zygophyllum fabago*. Evaluation the biological activity of extracts against different Gram-positive and Gram-negative bacteria has been done.

Materials and Methods

Plant Collection

The aerial parts of *Zygophyllum fabago* plant were collected from Nineveh governate-Iraq in April 2017. The identification of the plant has been achieved by specialists in the college of agriculture and forestry, Mosul University, Mosul, Iraq.

Preparation of the Plant

Cleaning the plant from the soil and the suspended materials has been done under running distilled water three to four times. The aerial parts of the plant were dried in shade on a large filter paper at lab temperature and turned from time to time for 7 days to prevent the occurrence of rotting. The grinding process gave a fine powder which was kept in appropriate paper envelope [15].

Soxhlet Extraction

The ground sample (100 gm) is placed in an extraction thimble, soaked in a Soxhlet extractor using a sequential solvent system. Three solvents were used, starting from low to high polarity, namely hexane, ethyl acetate and ethanol (1000 mL of solvent / 100 g of sample). The extraction process continued for 72 hrs for each type of solvent, until the color of the solvent in the Soxhlet disappears. Thick dark crude extract was obtained after drying through rotary vacuum evaporator of each fraction. Each crude extract was kept in an amber vial and placed in the refrigerator [16].

Column Chromatography

Generally, the components of the crude extract from natural products are complex and vary. it is need additional separation and purification process to obtain pure and active fraction, mainly by using column chromatography. In this study, a vertical glass column (40 mm width - 60 mm length) was used for the separation. The packing material used was 200 g of silica gel (60–120 mesh size). Gradient elution method was followed to separate fractions from extract by using solvents from low polarity to high polarity in varying ratios. The flow rate was adjusted to 5 ml/min and 40 ml solvent was collected for each fraction [17].

Thin-Layer Chromatography (TLC)

Each fraction from above was collected separately and subjected to TLC plates (20 x 20 cm aluminum sheets coated with silica gel 60) to detect the existence of phytochemicals. Vanillin-conc. H_2SO_4 spray was used to develop the spots, dried in hot air by using a heat gun machine, the Rf value of each spot was calculated and compared with the standards. Fractions with the same Rf values were collected and concentrated using rotary evaporator. After drying, the weight was measured, and the condensed fractions were further analyzed by HPLC technique to detect the presence of phenolic compounds [17].

High Performance Liquid Chromatography (HPLC)

HPLC profiles of isolated fractions were determined by using acetonitril:water (80:20) mobile phase. All samples were analyzed through Shimadzu ODS-15M- C18 reverse-phase analytical column (250 - 4.6 mm) with binary gradient mode. U.V. Vis. Detector SPD-20A, injection volume 20 ml, total flow 1 ml/min, column oven temperature 60 °C and detection wavelength 280 nm. 40 mg of each extract were dissolved in 4 ml of methanol for the analysis and total run time was 10 min. Ascorbic acid, gallic acid, benzoic acid, coumarin, vanillin, catechol and resorcinol were used as standard solutions for the detection of phenolic compounds [18].

Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear magnetic resonance (NMR) spectroscopy has been used for the structure elucidation of isolated phenolic compounds. All samples were identified and characterized by using ^{1}H- and ^{13}C-NMR, via Bruker Avance 400 spectrometer in $CDCl_3$. ^{1}H-NMR spectra were normally run at 400 MHz, and ^{13}C-NMR spectra were run at 101 MHz. Chemical shifts are quoted in δ relative to the trace resonance of proton chloroform (δ_H 7.27 ppm, δ_C 77.0 ppm). The NMR spectra of the isolated phytochemicals were compared with the standards and showed a good agreement with those reported for the phenolic compounds (Catechol, Coumarin and Resorcinol).[19-21]

Obtained spectral data for the isolated phenolic compounds showed: **Catechol:** δ_H (400 MHz, $CDCl_3$) 6.66 (2H, br.dd, *J* 5.5, 3.4 Hz), 6.58 (2H, br.dd, *J* 5.6, 3.5 Hz), 2.88 (2H, s); δ_C (101 MHz, $CDCl_3$) 146.9, 120.9, 117.1; **Coumarin:** δ_H (400 MHz, $CDCl_3$) 7.73 (1H, d, *J* 10.8 Hz), 7.59 (1H, dd, *J* 7.8, 1.3 Hz), 7.44 (1H, td, *J* 7.4, 1.4 Hz), 7.22 – 7.17 (2H, m), 6.42 (1H, d, *J* 11.0 Hz); δ_C (101 MHz, $CDCl_3$) 161.8, 154.3, 143.1, 131.8, 128.4, 125.1, 119.6, 118.0, 114.0. and **Resorcinol:** δ_H (400 MHz, $CDCl_3$) 6.94 (1H, t, *J* 7.5 Hz), 6.32 (2H, dd, *J*

1.4, 7.5 Hz), 6.29 (1H, t, *J* 1.3 Hz), 3.71 (2H, s); δc (101 MHz, $CDCl_3$) 159.1, 131.6, 108.8, 102.7.

Biological Assay

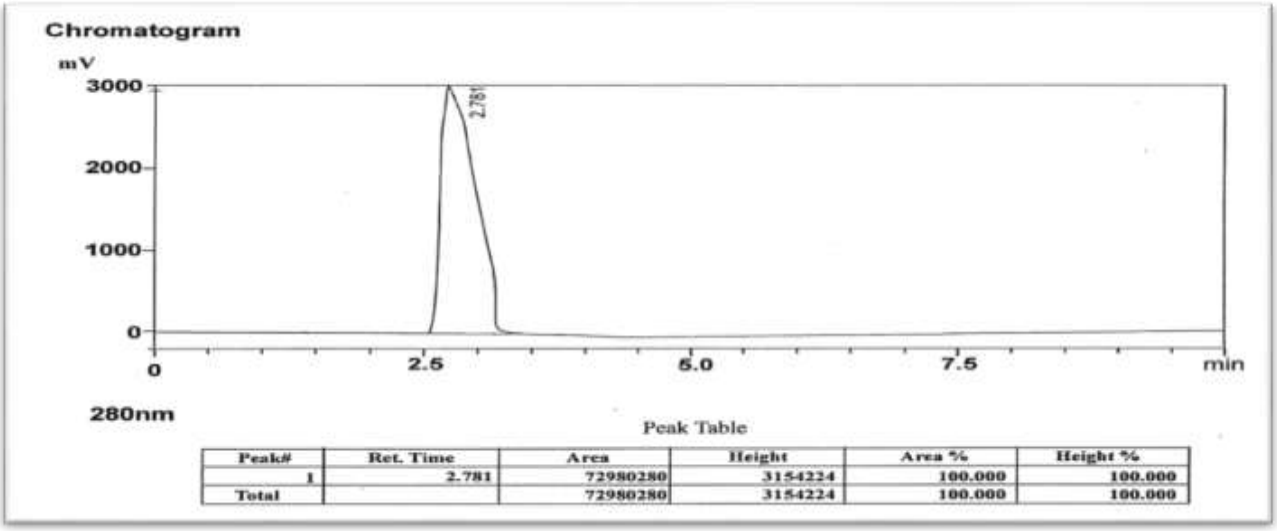
The isolated phenolic compounds have been subjected to biological evaluation. The antibacterial activity was examined against six Gram-positive and Gram-negative pathogenic bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella typhimurium*. The biological assay was carried out using the method described by Bauer *et al* [22].And compared to that of control samples.

Results and Discussion

The current study was aimed to the isolation of three phenolic compounds from the aerial parts of *Zygophyllum fabago* in Iraq. The chemical structure of the isolated compounds was elucidated clearly through HPLC chromatography and NMR spectroscopy. The spectroscopic data of the isolated compounds were showed a very good agreement with the standard respective data [14, 21].Antibacterial assay exhibited significant effects for the isolated compounds.

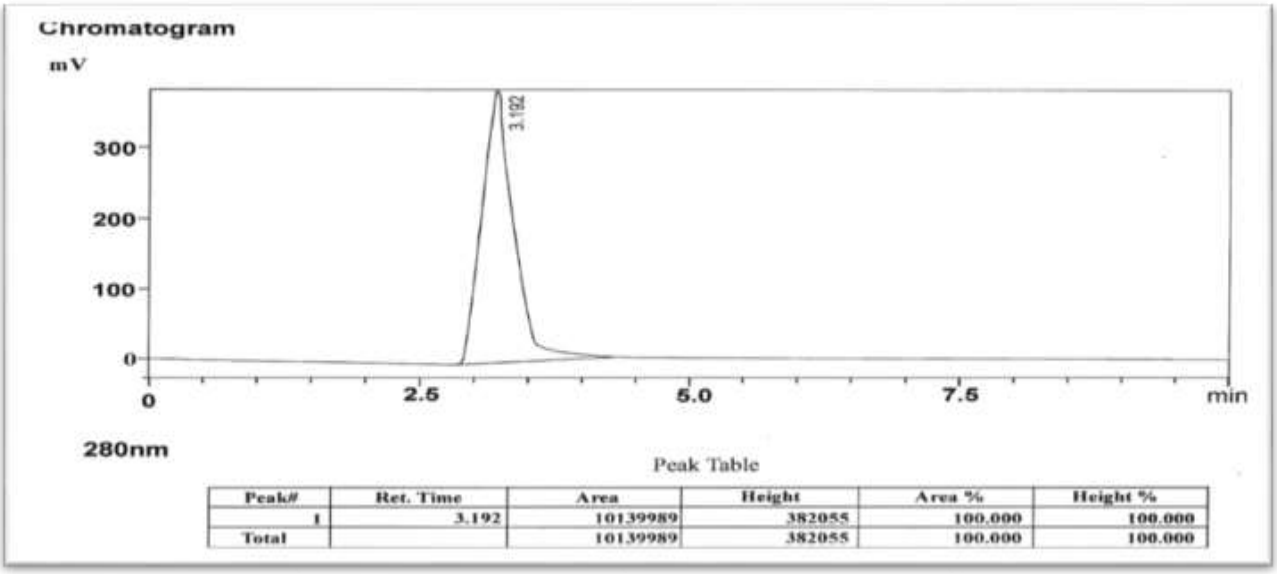
HPLC-analysis

The HPLC-DAD chromatogram of ethanolic extract of the aerial parts of *Zygophyllum fabago* in Iraq was compared with chromatogram of the phenolic standards. The comparison showed that ethanolic extract of the aerial parts of *Zygophyllum fabago* contain Catechol (2.78 min), Coumarin (3.24 min) and Resorcinol (2.72 min) at 280 nm. The highest quantity of phenolic compounds of this shrub was Catechol (27.71 mg/L), Coumarin (72.28 mg/L) and Resorcinol (100.00 mg/L) respectively. All data of HPLC-DAD chromatograms of the phenolic standards and ethanolic extracts showed in (Fig 1-5).



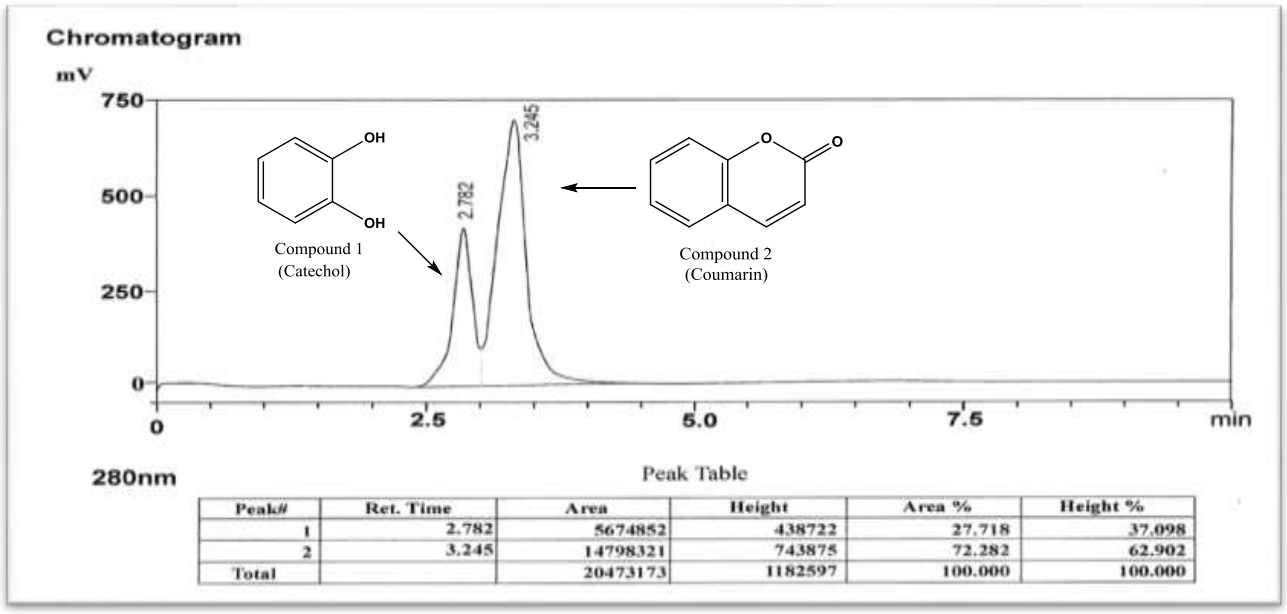
Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.781	72980280	3154224	100.000	100.000
Total		72980280	3154224	100.000	100.000

Figure 1: Standard HPLC-DAD chromatogram peak of Catechol at 280 nm



Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.192	10139989	382055	100.000	100.000
Total		10139989	382055	100.000	100.000

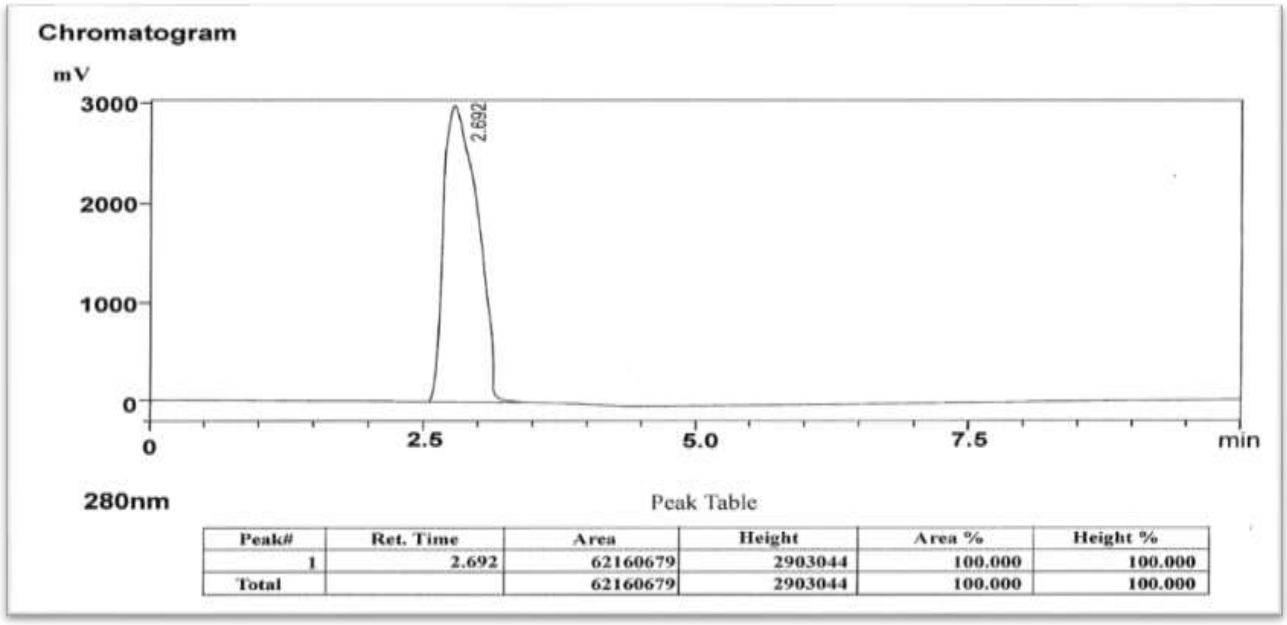
Figure 2: Standard HPLC-DAD chromatogram peak of Coumarin at 280 nm



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.782	5674852	438722	27.718	37.098
2	3.245	14798321	743875	72.282	62.902
Total		20473173	1182597	100.000	100.000

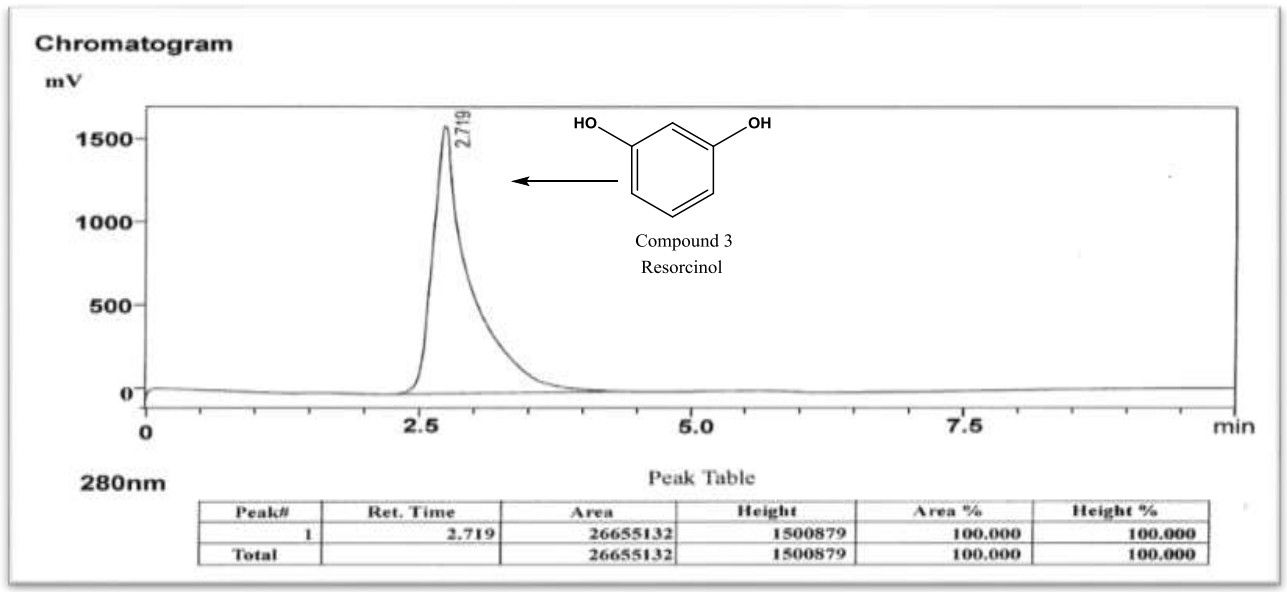
Figure 3: HPLC-DAD chromatogram peaks of the aerial part of *Zygophyllum fabago* in retention time for Catechol (2.78 min) & Coumarin (3.24 min) at 280 nm



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.692	62160679	2903044	100.000	100.000
Total		62160679	2903044	100.000	100.000

Figure 4: Standard HPLC-DAD chromatogram peak of resorcinol at 280 nm



Peak Table

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.719	26655132	1500879	100.000	100.000
Total		26655132	1500879	100.000	100.000

Figure 5: HPLC-DAD chromatogram peak of the aerial part of *Zygophyllum fabago* in retention time for Resorcinol (2.72 min) at 280 nm

NMR-analysis

The chemical structures of the isolated compounds were investigated by ^{1}H-NMR and ^{13}C-NMR spectroscopy. The structure of compound 1 (Catechol) was proven via ^{1}H-NMR, which clearly showed three signals.

Singlet at δ 2.88 ppm corresponding to the (OH-) groups, and two broad doublets of doublet related to the protons of (CH-) groups in the benzene ring, at δ 6.58 and 6.66 ppm. The ^{13}C-NMR exhibited three diverse peaks related to the aromatic ring appeared at δ 146.9, 120.9 and 117.1 ppm respectively.

Compound 2 (Coumarin) showed characteristic ^{1}H-NMR signals, which showed five signals in region δ 7.73-6.41 ppm belonging to coumarin core. The proton corresponding to the (CH-) group adjacent to the carbonyl group appeared as a doublet at δ 6.41 ppm. The next proton of the (CH-) group in the pyran ring came downfield around δ 7.73 ppm, while the protons of the (CH-) groups corresponding to the aromatic ring appeared as multiplet at δ 7.19, triple doublet at 7.44 and doublet of doublet at 7.59 ppm respectively.

The ^{13}C-NMR spectrum showed a downfield signal at δ 161.8 ppm corresponding to the carbonyl group. The signal of the carbon adjacent to the carbonyl group came around 114.0 ppm, while the remaining aromatic carbons ranged from δ 131.8 – 118.0 ppm. The signal of the carbon in the pyran ring appeared at δ 143.1 ppm. Once again, the ^{1}H-NMR spectrum of compound 3 (Resorcinol) revealed four signals. Singlet at δ 3.71 ppm corresponding to the hydroxyl groups, triplet at δ 6.29 ppm, doublet of doublet at δ 6.32 ppm and triplet at δ 6.94 ppm related to the aromatic protons.

The ^{13}C-NMR showed four different peaks corresponding to the aromatic ring appeared at δ 159.1, 131.6, 108.8 and 102.7. ppm respectively. All the data obtained were consistent with the literature and confirmed the structures of phenolic compounds catechol, coumarin and resorcinol [19, 21].

The Antibacterial Activity

In the present study, antibacterial activity has been done via disk diffusion method. Ethanolic extracts (F1&F2) of the aerial part of *Zygophyllum fabago* against (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella typhimurium* in 200, 100, 50, 25 and 12.5 mg/ml concentrations) were showed various inhabitation zone sizes. Different extracts were obtained from the aerial part of *Z.fabago* plant.

These extracts were fractionated by using silica gel column chromatography and solvents with variable polarity (hexane, ethyl acetate and ethanol). Two fractions (F1 and F2) were obtained. After completing the verification process via HPLC technique and NMR spectroscopy, the ethanolic extracts have found to possess three phenolic compounds (Catechol, Coumarin and Resorcinol). The inhibitory effect of these compounds against six types of Gram-positive and Gram-negative bacteria was determined by using antibiotics Amikacin (AMK) and Gentamicin (GEN) as control samples.

Table 1: The inhibition zone size in millimeter of *Zygophyllum fabago* of ethanolic extract (F1) against several Gram-positive and Gram-negative bacteria. Dish diameter is (6) millimeter

Concentration mg/mL	Bacteria					
	S. typhimurium	*P. vulgaris*	*E. coli*	*B. cereus*	*S. epidermidis*	*S. aureus*
200	18	21	26	20	26	24
100	15	17	24	17	21	20
50	13	15	21	14	19	17
25	10	12	18	12	13	15
12.5	8	9	15	8	10	8
Control						
Amikacin (amk)	23	25	24	24	22	25
Gentamicin (gen)	26	24	25	25	23	27

Table 2: The inhibition zone size in millimeter of *Zygophyllum fabago* of ethanolic extract (F2) against several Gram-positive and Gram-negative bacteria. Dish diameter is (6) millimeter

Concentration mg/mL	Bacteria					
	S. typhimurium	*P. vulgaris*	*E. coli*	*B. cereus*	*S. epidermidis*	*S. aureus*
200	27	20	25	27	23	28
100	25	18	22	22	20	25
50	20	13	20	21	18	24

25	19	10	17	18	13	20
12.5	17	8	12	13	9	16
Control						
Amikacin (amk)	23	25	24	24	22	25
Gentamicin (gem)	26	24	25	25	23	27

Fraction (F1-Catechol and Coumarin) showed the highest effect against E. coli and S.epidermidis (26 mm) which was higher than the used antibiotics, while the effect against S.aureus (24 mm) presented a similar effect to the same antibiotics.

Fraction (F2- Resorcinol) revealed the highest effect on S. aureus (28 mm) which was higher than the effect of the control antibiotics, also showed a high effect against S. typhimurium and B.cereus (27 mm) equally which was higher than the effect of the used antibiotics. All the results of antibacterial activity can be shown in (Table 1& 2). The reasonable explanation of such biological activity for such phenolic compounds is related to the presence of aromatic ring and (-OH) groups. Several mechanisms were suggested to explain the effect of these compounds on the bacterial growth inhibition, for instance, the weakening of the cytoplasmic membrane, the inhibition of extra cellular microbial enzymes, and the permeabilization of the cell membrane and/or direct actions on microbial metabolism [23].

Conclusions

Ethanolic extracts of the aerial part of Zygophyllu fabago in Iraq contains the following phenolic compounds: catechol, coumarin and resorcinol. The antibacterial activity of the extracts of this herbal revealed that this plant has a significant effect against several Gram-positive and Gram-negative pathogenic bacteria. In this study, the biological and physiological properties of the extracts of this shrub suggest that it is a proper candidate for more biological and pharmacological investigations.

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