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Biological Activity Test of the Alcoholic Extract of the Leaves of *Catharanthus roseus* (L.) G.Don Treated with Sodium Chloride, Nitrogen, and Selenium in Inhibiting the Growth of Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

Aims This study aimed to determine the biological activity test of the alcoholic extract of the leaves of *Catharanthus roseus*.

Materials & Methods *Catharanthus roseus* was used in the implementation of the experiment and it included three factors: the first factor is watering the plants with three concentrations of sodium chloride salt (0, 50, and 100mmol) of sodium chloride, the second factor is three concentrations of nitrogen (urea) (0, 100 and 200mg.L^{-1}) and the third factor three concentrations of selenium (Na_2SeO_4; 0,25 and 50mg.L^{-1}), then tested the biological activity of plant leaf extracts from all treatments against Gram-positive Staphylococcus aureus and Gram-negative *Escherichia coli* bacteria through Measure the diameters of the areas of inhibition of bacterial growth.

Findings The results of the laboratory experiment showed that the extract of the leaves of triple combination plants (100mmol NaCl+200mg.L^{-1} N+50mg.L^{-1} Se) achieved the largest inhibition area for the growth of *E. coli* reaching 27.50mm compared to the control, which is 4.20mm, and plant leaf extract in the combination (100mmol NaCl+100mg.L^{-1} N+50mg.L^{-1} Se) recorded the largest inhibition area in *S. aureus* reached 50.27mm compared to the control which recorded 9.10mm.

Conclusion The plant leaf extract had an important role in inhibiting the growth of the bacteria studied, and the highest inhibition was observed in the interaction between the three factors salt, nitrogen, and selenium.

Keywords Alcoholic; Catharanthus; Sodium Chloride; Nitrogen; Selenium; Gram-Negative Bacteria

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Introduction

The plant *Catharanthus roseus* (L.) G.Don belongs to the oleander family Apocynaceae, which is also known as *Medagascar perwinikle*. It is a tropical and subtropical plant [1]. The Catharanthus genus includes eight species, seven of which are endemic to the island of Madagascar and one in India [2]. It is widely relied upon in the Indian Ayurvedic medicine system (a system that focuses on the medicinal potential of plants) for its important effects. The effectiveness of its leaf extract against bacterial pathogens is also due to the development of resistance of most bacterial pathogens to many available antibiotics [3]. In order to meet the increasing demand for medicinal plants and alternative medicine in the pharmaceutical industries, attention must be paid to the cultivation of these plants, but the prevailing soil salinity in many parts of the world poses a serious threat to plant production, so studying their ability to tolerate salinity is very important [4]. Soil salinity is one of the environmental factors that cause severe changes in plant growth, physiology, and metabolism, which lead to an increase in the accumulation of secondary metabolic products, including alkaloids. Therefore stresses are adopted as a strategy to increase the content of therapeutic secondary metabolites present in plants [5]. The known antimicrobial properties of both plant extracts and phytochemicals could be of great importance in developing new therapeutic approaches in combating emerging multidrug resistance among bacteria [6]. Parajakta *et al.* reported that the extract of Vinca leaves showed antibacterial properties and could be used as a prophylactic agent [3]. Moll *et al.* [7] selected seven medicinal plants that are frequently used in the traditional medicine system, including the *C. roseus*, *Andrographis paniculata*, *Adhatoda vasica*, *Vitex vegundo*, *Aloe vera*, *Flacortia ramontchi,* and *Nyctanthes arborristis* and tested against nine types of bacteria at concentrations of 300, 400 and 500µg/ml. The antibiotic kanamycin (30µg/ml) was selected for comparison. The minimum inhibitory concentration of the extracts was determined. Leaves of these plants, the results showed that all extracts have clear activities in inhibiting the growth of the bacteria studied. As Khalil [8] mentioned in his study measuring the inhibitory activity of the ethanolic leaf extract of plant *C. roseus* against two types of pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*). The plant has anti-bacterial properties, as the extracts showed high inhibitory activity for these species. The best inhibitory activity for the leaf extract with a concentration of 100 mg/ml *C. roseus* against *S. aureus* resulted in an inhibition area (15mm), followed by *E. coli* bacteria, where an inhibition area of 11mm was produced at 100 mg/ml of leaf extract compared to the control. It was concluded from his study that gram-positive bacteria are more sensitive than gram-negative bacteria. The medicinal efficacy of two types of plants, Mentha piperita and *C. roseus*, was studied against some pathogenic fungi and bacteria (*Pseudomonas aeruginosa*, *S. aureus* and *Ralstonia solanacearum*). The two plants gave positive results in inhibiting the growth of the bacterial species studied, and the most effect was against *S. aureus* at the concentration of the extract of 20%, and the extracts of Ain al-Bzon were more effective in inhibiting the growth of bacteria, as the area of growth inhibition increased significantly at this concentration of the aqueous extract and reached 36mm compared to the area of inhibition of the concentration 10%, which is the comparison concentration, which was 23% [5].

This study aimed to determine the biological activity test of the alcoholic extract of the leaves of *Catharanthus roseus*.

Materials and Methods

Preparation of the alcoholic extract

The dried leaves of the plant were ground using an electric grinder, then the crushed parts were sieved, the powder was collected, and left at a temperature of 45°C for 12 hours. A weight of 2.0g of dry powder was taken and added to 100ml of methanol and deionized water mixture at a ratio of 50:50 volume/volume and placed in a wave machine. Ultrasonic bath Shimadzu For 15 minutes to separate the active compounds from the plant powder, the extract was filtered to get rid of fibers and insoluble materials by (Sepack-18°C disposable filter). Methanol was withdrawn from the extract by a min rotary evaporator at 40°C. After the methanol was withdrawn, the aqueous extract containing the active substances was obtained for the alcohol-free bacterial study.

Test the bioactivity of the extract on bacteria

In the experiment, isolates of gram-positive *S. aureus* and gram-negative *E. coli*, which were diagnosed in the laboratories of the Ministry of Science and Technology in Baghdad using the Vitek 2 Compact System, were used to test the biological activity of the raw aqueous extract of the leaves of *C. roseus* by well drilling method in the medium of solid agar [3]. Bacteria were activated by transferring part of the growing colonies on a nutrient agar medium by Loop to test tubes containing 5ml of nutrient broth medium and incubated at 37°C for 24 hours. Then 1ml of this culture was transferred to 5ml of normal physiological solution. MacFarland (0.5) tubes to obtain a cell number equivalent to 1.8 x 108 /ml. Then 0.1ml of it was spread on Muller Hinton agar medium using a cotton swab and left to dry for 10 minutes at room temperature. Then 100µl of aqueous leaf extract was added and the dishes were incubated for 24 hours at 37°C. The diameter of the inhibition areas around the pits was measured in millimeters by a ruler, then the measurements were compared with the areas of inhibition around the

control pits, in which distilled water was used. 3 replicates were used for each treatment for each bacterial species, and the results were calculated by measuring the average for those replicates. Results and discussion Test the biological activity of the aqueous extract of the leaves of ain al-bazon plant treated with sodium chloride, nitrogen and selenium in inhibiting the growth of gram-positive and gram-negative bacteria (the diameter of the inhibition zone was based on mm).

The results were analyzed using the statistical program Genstat, the first version of 2010. All experimental data were subjected to statistical analysis (ANOVA) and the averages of the transactions were compared using Duncan's multiple range test at a probability level. 0.05

Findings

S. aureus

Table 1 shows the effect of the crude alcoholic extract of leaves of plants treated with sodium chloride on increasing the areas of inhibition of the growth of *S. aureus*. 17.38mm. The treatment of plants with nitrogen led to an increase in the area of inhibition of growth for this bacteria, and the highest area reached 32.17mm at a concentration of 100mg.L^{-1} in comparison with the inhibition area of the comparison, which amounted to 16.03mm. While the average diameter of the inhibition area decreased in the higher concentration of nitrogen. As for the addition of selenium, it also led to a significant increase in the diameter of the inhibition zone at a concentration of 50mg.L^{-1}, reaching 25.29mm compared to the control. The area of inhibition was 17.34mm. In the case of interaction between salt and nitrogen factors, the largest inhibition area reached 35.59mm and was recorded in the mixture (100mmol NaCl+100mg.L^{-1} N) compared to the lowest inhibition area reached 9.44mm in the mixture (0mmol NaCl+200mg.L^{-1} N).

When the salt and selenium factors were overlapping, the diameters of the inhibition zones increased and the largest inhibition zone reached 30.29mm in the combination (50mmol NaCl+50mg.L^{-1} Se) compared with the lowest inhibition in the combination (l0mmol NaCl+0mg.L^{-1} Se) which is 13.78mm. The interaction effect between the two factors nitrogen and selenium was positive in increasing the diameters of the inhibition zones, and the largest inhibition zone was observed in the combination (100mg.L^{-1} N+50mg.L^{-1} Se), which amounted to 44.21mm compared with the smallest inhibition area in the combination (0mg.L-1 N+0mg.L-^{1}Se) when it was 12.78mm. The results of the triple interaction indicate variations in the diameters of the inhibition zones, and the combination (l100mmol NaCl+100mg.L^{-1} N+50mg.L-^{1} Se) gave the largest inhibition area of 50.27mm compared to the inhibition area of the control extract, which was 9.10mm. (Figure 1)

Table 1) The effect of the alcoholic extract of the leaves of *C. roseus* plant treated with sodium chloride, nitrogen and selenium on measuring the diameter of the growth inhibition zone (mm) for *S. aureus*

NaCl X N	Con. Se			Con. N	Con. NaCl
	50	25	0		
13.17 de	15.27 l	15.13 l	9.10 b	0	0
29.54 b	40.37 c	26.13 f	22.13 i	100	
9.44 e	9.23 p	9.00 p	10.10 o	200	
13.79 de	14.17 m	14.10 m	13.10 n	0	50
31.38 ab	41.80 b	28.20 e	24.13 g	100	
15.16 de	16.13 k	15.13 l	14.20 m	200	
21.13 c	24.17 g	23.10 h	16.13 k	0	100
35.59 a	50.27 a	30.20 d	26.10 f	100	
17.53 cd	16.23 k	15.27 l	21.10 j	200	
Se X NaCl					
Average (NaCl)					Con.NaCl
17.38 c	21.62 ab	16.76 b	13.78 b		0
20.10 b	24.03 ab	19.14 b	17.14 b		50
24.75 a	30.29 a	22.86 ab	21.11 ab		100
Se X N					
Average (N)	50	25	0		Con.N
16.03 b	17.81 d	17.44 de	12.78 f		0
32.17 a	44.21 a	28.18 b	24.12 c		100
14.04 c	13.87 ef	13.13 f	15.13 def		200
	25.29 a	19.58 b	17.34 b		Average(Se)

Averages with similar letters that do not differ from each other within the main factors or their two- or three-way interactions according to Duncan's multiple test 0.05

E. coli

Table 2 shows the effect of irrigating plants with different concentrations of sodium chloride salt to significant effects on the average diameters of the inhibition zones for *E. coli* bacteria, as it increased in concentrations 100 and 50 mmol of sodium chloride to 20.82 and 19.01mm compared to the control, which recorded the lowest value. It is 13.14mm. The extract of plants sprayed with nitrogen had positive significant effects in increasing the average diameters of the inhibition zones, which rose to 21.95 and 17.62mm at concentrations 200 and 100mg.L^{-1}, respectively, compared with concentration 0mg.L^{-1}, which gave the lowest mean of inhibition zone diameter, which reached 13.45mm.

Similarly, the extract of plants sprayed with selenium had similar effects to nitrogen, so the average diameter of the inhibition zone increased to 19.95mm at a concentration of 50mg.L^{-1} compared to the concentration of 0mg.L^{-1}, which gave 15.16mm. The interaction of the two factors, sodium chloride, and nitrogen, caused significant effects in the increase of the inhibition zone average, which reached the highest value of 24.96mm in the combination (100mmol NaCl+200mg.L^{-1} N) and it did not differ significantly from the average in the combination (50mmol NaCl+200mg.L^{-1} N), in which the average inhibition area was 23.00mm, while the comparison rate was 8.81mm, which is the lowest rate recorded in this interference. The interaction of salt and selenium resulted in a significant increase in the rate of this trait, which reached the highest rate of 22.72mm in the combination (100mmol NaCl+50mg.L^{-1} Se) compared to the lowest value in the comparison, which amounted to 9.14mm.

As for the interaction of nitrogen and selenium factors, an improvement was achieved in the areas of inhibition, which increased the rate in the combination (200mg.L^{-1} N+50mg.L^{-1} Se) to 24.28mm, while it was in the extract of plants that were not treated with 10.83mm. The effect of the triple interaction was also positive, as the ratios of the diameters of the inhibition zones increased significantly, which increased the highest value to 27.50mm in the combination (100mmol NaCl+200mg.L^{-1} N+50mg.L^{-1} Se) compared to the lowest value was in the extract of the control plants, which was 4.20mm. (Figure 2).

Table 2) The effect of the alcoholic extract of the leaves of *C. roseus* plant treated with sodium chloride, nitrogen, and selenium on measuring the diameter of the growth inhibition zone (mm) of the *E. coli*

NaCl X N	Con.Se			Con.N	Con.NaCl
	50	25	0		
8.81 f	12.10 m	10.13 n	4.20 p	0	0
12.86 e	16.33 k	15.13 k	7.10 o	100	
17.78 cd	20.10 f	17.10 i	16.13 j	200	
14.53 e	15.23 k	15.27 k	13.10 l	0	50
19.52 bc	21.13 f	20.23 f	17.20 i	100	
23.00 a	24.23 b	22.63 c	22.13 d	200	
17.02 d	18.50 h	17.37 i	15.20 k	0	100
20.52 b	21.17 e	21.13 e	19.20 g	100	
24.96 a	27.50 a	24.17 b	22.20 d	200	
Se X NaCl					
Average (NaCl)					Con.NaCl
13.14 c	16.18 cd	14.12 d	9.14 e		0
19.01 b	20.20 abc	19.38 abc	17.48 bcd		50
20.82 a	22.72 a	20.89 ab	18.87 abc		100
Se X N					
Average (N)	50	25	0		Con.N
13.45 c	15.28 c	14.26 cd	10.83 d		0
17.62 b	19.54 b	18.83 b	14.50 cd		100
21.95 a	24.28 a	21.30 ab	20.16 b		200
	19.70 a	18.12 b	15.16 c		Average(Se)

Averages with similar letters that do not differ from each other within the main factors or their two- or three-way interactions according to Duncan's multiple tests 0.05

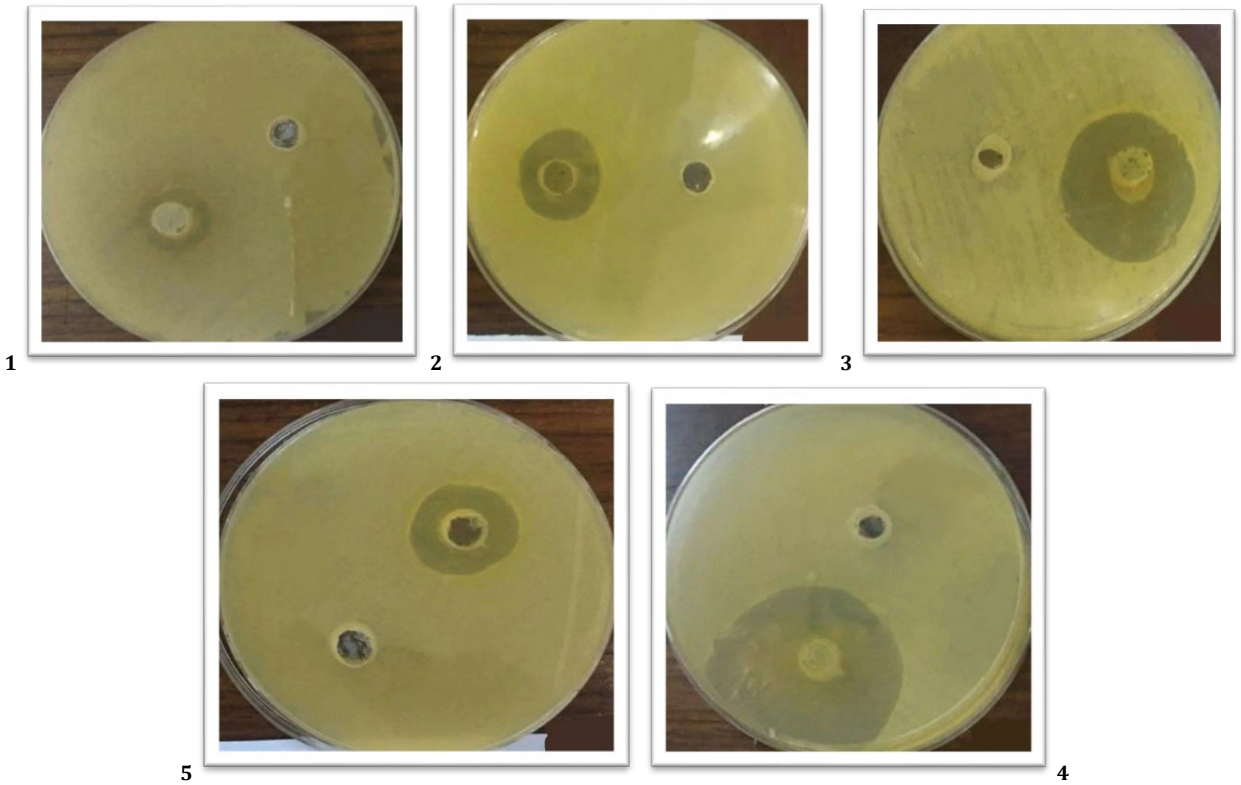

Figure 1) Effect of the alcoholic extract of the leaves of *C. roseus* plant treated with sodium chloride, nitrogen and selenium on the diameters of the areas of inhibition of growth of the gram-positive bacteria *S. aureus*; 1- Control treatment: (0mmol NaCl+0mg.L-1 N+0mg.L-1Se); 2- Treatment: (50mmol NaCl+100mg.L-1 N+50mg.L-1 Se); 3- Treatment:(50 mmol (NaCl+200mg.L-1N+50mg.L-1 Se); 4- Treatment: (100mmol NaCl+100mg.L-1 N+50mg.L-1Se); 5- Treatment: (100mmol NaCl+200mg.L-1 N+50mg.L-1 Se)

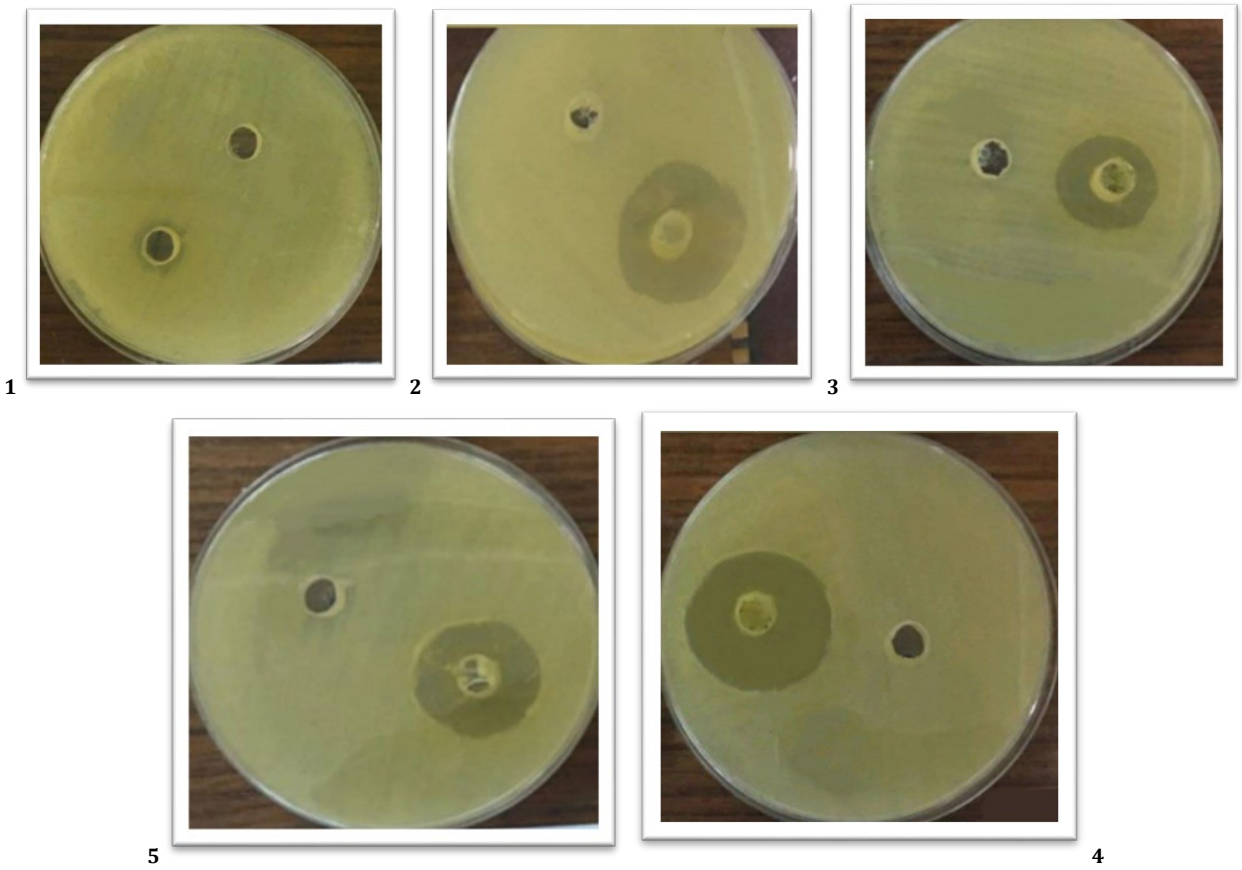

Figure 2) Effect of alcoholic extract of leaves of *C. roseus* plant treated with sodium chloride, nitrogen and selenium on the diameters of growth inhibition zones for Gram-negative bacteria *E. coli*; 1- Control treatment: (0mmol NaCl+0mg.L-1 N+0mg.L-1 Se); 2- Treatment: (50mmol NaCl+100mg.L-1 N+50mg.L-1 Se); 3- Treatment:(50mmol NaCl+200mg.L-1 N+50mg.L-1 Se); 4- Treatment: (100mmol NaCl+100mg.L-1 N+50mg.L-1 Se); 5- Treatment: (100mmol NaCl+200mg.L-1 N+50mg.L-1 Se)

Discussion

Recent studies on the anti-microorganism activity when studying the qualitative analysis of the chemical examination of the *C. roseus* plant revealed the presence of many chemicals in different parts individually and in different quantities in the plant and that the main components are the alkaloid vinblastine, vincristine, flavonoids and phenolic in addition to the presence of saponins, tannins and protein [9-11] that these compounds have conferred this plant a high potential against pathogenic microorganisms, The different treatments in our study achieved a clear inhibitory effect on the growth of the bacteria under study, and the inhibition areas in Gram-positive bacteria *S. aureus* were much larger than the inhibition areas of Gram-negative bacteria *E. coli*, and this result is similar to what Khalil [8] and Hussein & Amir [12] reported. This reason was attributed to the differences in the chemical composition of the plasma membrane and the structure of the cell wall for both types of bacteria. The diameters of the inhibition zones in Gram-negative *E. coli* bacteria were directly proportional to the accumulation of the active substance content in the leaf extracts that contained the highest concentration of alkaloids based on the treatments used in the experiment. While we notice in Gram-positive bacteria *S. aureus* that the diameters of the inhibition zones reached the maximum with the extracts with an average content of the active substance and not the highest. This may be due to the cell membrane and the nature and structure of the cell wall. The cell membrane is selectively permeable as it allows the passage of certain molecules and impedes the passage of other molecules. Depending on size, molecular weight, solubility in water or fat, and charge [13].

The inhibition of bacterial growth is due to the effect of alkaloids and other active compounds. Alkaloids have biological activity in preventing the growth of microorganisms due to their association with DNA, as well as their ability to stop the work of the enzyme RNA-polymerase and DNA-estrase, and also inhibit the process of cellular respiration [14] Alkaloids and tannins It was also known to include compounds that inhibit the QS process (Quorum-sensing) due to the role of this last process in the formation of the biofilm of the bacterial cell [15] (a sensing process through which several things are controlled, the most important of which is the transfer of genes responsible for resistance to life antigens and the virulence of bacteria and the

formation of Biofilm [16], as well as that plant extracts contribute to a decrease in the level of mRNA and gene expression of various pro-inflammatory mediators [17]. The results agreed with Bakht *et al.* [18] and Alhaithloul *et al.* [5] in the effect of *C. roseus* plant extract on different types of bacteria. As for other plant extracts, the results agreed with al-Mousawi [19]. In his study of the aqueous extract of Sidr leaves and alcohol of black grape seeds and lemon peel essential oil on Staphylococcus bacteria and al-Kinani [20] in Eucalyptus oil extract on the same bacteria. The latter two concluded that the ability of these extracts to inhibit the growth of bacteria is attributed to preventing the formation of the biofilm, which has an important role in pathogenesis, and therefore preventing the formation of this membrane leads to the inhibition of its growth.

Conclusion

The plant leaf extract had an important role in inhibiting the growth of the bacteria studied, and the highest inhibition was observed in the interaction between the three factors salt, nitrogen, and selenium.

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Conflicts of Interests: there is no conflict of interest.
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