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Eco-Friendly Synthesis Of Selenium Nanoparticles With Detect Antibacterial Activity On Bacterial Isolates From Diabetic Foot Ulcer

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Abstract

Background: *Due to the abuse of antibiotics, numerous bacterial species exhibited a high resistance to the routinely used antibiotics, particularly in prevalent bacterial species such as E. coli, S. aureus, S. pyogenes, E. faecalis, K. pneumoniae, A. baumannii, P. mirabilis, and P. aeruginosa. Which result in a challenges in controlling and treatment of Diabetic Foot Ulcers (DFUs) caused by certain bacterial species.Therefore today, the scientific media and scientist attempt to find an alternative antibacterial agent with fewer adverse effects and lower bacterial resistance, which is during the recent years provided by nanotechnology by eco-friendly synthesis of nanoparticles which showed a significant result on pathogenic bacteria in many studies including the current study.*

Objective: *this study aims to synthesis of selenium nanoparticles eco-friendly and indicate their antibacterial activity against antimicrobial resistant bacteria in DFU patients.*

Materials and methods: *150 specimens of different age groups for both genders from clinical source (diabetic foot ulcer) were collected, between the beginning of November 2024 and end of February 2025 patients of Baqubah Teaching Hospital in Diyala, after cultured in the cultures media, the total clinical isolates were 130 isolates of a different genus of bacteria.*

Results: *The results showed that among gram negative bacteria five species have been isolated from DFU are K. pneumoniae 30 (23%), E. coli 19 (14.6%), P. aeruginosa 20(15.4%), A. baumannii 13 (10%), and P. mirabilis10 (7.7%). while gram positive bacteria were S. aureus25 (19.2%), S. pyogenes 7 (5.4%) and E. faecalis 6 (4.6%). Atomic Force Microscopy (AFM) was used to determine the average size and shape of the nanoparticles, which came out to be (20.40 –56.8) nm. The Se NPs have a smooth surface texture and a spherical shape, according to scanning electron microscopy (SEM). The wavelength range was evaluated using UV–visible spectroscopy (UV-Vis), which investigates a noticeable peak at 420 nm. X-ray diffraction device was used to study the bio-synthesized Se nanoparticles in order to determine their average particle size and crystalline nature which was in average of 20.092nm. Fourier Transform Infrared Spectroscopy (FTIR) shows that the reduction and capping processes are made possible by several functional groups found in biomolecules. Five concentrations of Se NPs (10, 20, 40, 80 and 160) mg/ml were evaluated against isolates that were multiple drug-resistant (MDR). The results demonstrated that at the highest concentration 160 mg/ml Se NPs showed the highest efficiency against K. pneumoniae, S. aureus, S. pyogenes and E. faecalis with 26 mm, followed by E. coli, P. aeruginosa, P. mirabilis and A. baumannii, which were (25, 24, 24 and 24) mm respectively. On the other hand, the same isolates showed the smallest zone inhibition at 10 mg/ml to be (18, 16, 16, 18, 15, 15 18 and 13) mm respectively.*

Conclusion: *The study shown that The Zingiber officinale aqueous extract displays a significant action against Gram-negative bacteria, however the antibacterial efficacy of selenium nanoparticles (Se NPs) surpassed that of Zingiber officinale extracts.*

Key words: *DFU, MDR, nanotechnology, Nanoparticles, Se NPs, Ecofriendly synthesis.*

INTRODUCTION

Elevated blood sugar levels and diabetes that is untreated increase the possibility of a diabetic foot an infection, which can lead to numbness and a lack of feeling in the feet due to nerve damage and inadequate blood flow. If infections, ulcers, and other severe effects aren't treated in a timely manner, the feet of diabetics may have to be cut off [1].

Due to ancient times, plants that are medicinal have been found and utilized in traditional medical practices, as well as in pharmaceuticals for the treatment of a variety of illnesses. Plants synthesize hundreds of chemical substances that serve a variety of purposes, such as protecting against insects, fungi, diseases, and mammals that eat plants [2]. Because of its anti-inflammatory properties and antioxidant qualities, ginger—scientifically known as Zingiber officinale has demonstrated encouraging potential in accelerating wound healing. According to studies, ginger extract may improve the structure and function of the skin, which could lessen the number of ulcers in regions at risk that do not heal. Furthermore, a crucial component of ginger, gingerol, may promote the development of new blood vessels in injured skin, improving healing of wounds [3].

A tiny particle, also known as an ultrafine particle, is a particle of matter that has an outside diameter of between one and one hundred nanometers (nm). Due primarily to their minuscule size and enormous surface area, nanoparticles often display unique size-dependent characteristics. When a particle's size reaches the nanoscale, with a typical length scale near or less than the wavelength of de Broglie or a wavelength of light, the normal boundary features of the crystal particle are abolished. According to earlier research, there is little proof that supports the use of ginger in general health and dermatology. Ginger is utilized because of its anti-inflammatory in nature anti-oxidant, anti-cancer, and wound-healing properties. The strength of the data should guide the use of ginger in many medical and dermatological contexts as well as in the pharmaceutical sector. There is now significant suggestion that ginger reduces inflammation, aging, cancer, and wound recovery [4].

With its potential antibacterial properties, selenium nanoparticles (Se NPs) may prove to be a useful treatment for ulcers in diabetic feet. They have potent antibacterial qualities that lower the bacterial burden and hasten the healing of diabetic lesions. Additionally, Se NPs can speed up wound healing by working in concert with other therapies like platelet-rich plasma (PRP) [5].

MATERIALS AND METHODS

Collection of specimens

150 specimens of different age groups for both genders of patients with diabetic foot ulcer were collected between the beginning of November 2024 and end of February 2025 at Baqubah Teaching Hospital in Diyala.

Culturing of specimens

All samples were cultured on MacConkey agar for gram negative bacteria and blood agar for gram positive bacteria that supplies the nutrients needed for bacterial growth. After covering, all agar plates were incubated for 24 hours at 37°C and then examined for growth in bacteria.

Isolation and identification of bacteria

Bacteria were isolated and identified using morphological characteristics of the colonies, microscopic examination of bacterial cells, and biochemical tests including IMViC test , catalase test, oxidase test, Urease utilization test, coagulase test, hemolysin test and motility test. The identification then verified by using the Vitek-2 method.

Collection of plant

Zingiber officinale roots were collected from local market and have been diagnosed by Prof. Dr. Khazal. D. Wadi from the College of Sciences\ University of Diyala \ specific specialization in plant taxonomy.

Preparation of Zingiber officinale roots

The Zingiber officinale roots was taken to the laboratory, purified with distilled water, and dried at oven at 30 °C for four days. They were then crushed with an electric grinder to produce a fine powder and stored in a plastic bag until required [6].

Preparation of aqueous extract of Zingiber officinale roots

The aqueous extract was prepared by dissolving 50g of the seed powder in 100 ml of hot water, Whatman No. 1.5 filter sheets were used to separate the necessary filtrates from the solid residues after 24 h. at room temperature and 150 rpm of agitation. To obtain the crude extracts, all

filtrates were pre-concentrated using a rotary evaporator set to lower pressure and a temperature of (40–60) °C. The extract was stored at 4°C until used [7].

Determination of the antimicrobial activity of Zingiber officinale aqueous extract

The determination method was performed according to [8].

1- A number of bacteria colonies were transported by loop to prepare the suspended bacteria and put it in tubes contain brain heart infusion broth to activate the bacteria. The tubes were incubated for (18 - 24) h. at 37 °C. The suspended bacteria were compared to the standard MacFarland solution (1.5 x 10^8) CFU/ml. After that the bacteria suspended was spread by sterile swab, it was spread on the plates containing Muller Hinton agar and then left the plate for a while to dry.

2- A wells were made in the culture media by using sterilized a cork borer

3- 100 µl of the aqueous extract of Zingiber officinale roots were added to each well individually by micropipette.

4- The effectiveness of each concentration (160, 80, 40, 20, 10 mg/ml) was determined by measuring the diameter of the inhibition zone around each well by using a scale ruler.

Biosynthesis of Se NPs

To prepare Selenium nanoparticles (Se NPs), 2.5 g of Se nitrate ($SeNO_3$) was dissolved in 50 ml of deionized water using a magnetic stirrer (800 rpm) at room temperature. Then, 100 ml of Zingiber officinale plant extract was added to the precursor solution. After 72 h., 0.5 M sodium hydroxide (NaOH) was added to the above solution, and the color of the solution turned brown. The precipitate is separated by using centrifuge and washed with water and ethanol 5 times. The drying process was carried out using an oven at 40°C [9].

Characterization of Selenium nanoparticles (Se NPs)

The characterization and the identification of specific functional groups inside the Ag NPs were performed using Fourier Transform Infrared Spectroscopy (FTIR) from Shimadzu (Germany)[10]. Scanning electron microscopy (SEM) was utilized to ascertain the morphology, dimensions, and size distribution of [11]. The UV-Vis spectrophotometer is an efficient, direct, and sensitive method for analyzing silver nanoparticles, and the reduction of pure Ag+ ions was assessed by measuring the UV-Visible spectrum of the reaction medium [12]. The size, surface texture, and granular volume of the Ag nanoparticles were assessed using Atomic Force Microscopy (AFM) [13].

Transmission electron microscopy (TEM), provides the most precise and high-resolution imaging data about the size, shape, morphology, state of aggregation, and distribution of nanoparticles at nanometer resolution [14].

Antibacterial activity of Se NPs

According to [15], research was done on the effectiveness of selenium nanoparticles against both gram positive and gram negative bacteria, first 300 mg of NPs powder were dissolved in 100 ml of distilled deionized water to create Se NPs stock, which was then concentrated to 200 mg/ml. To ensure complete dissolution of the powder, the solution was heated to 45°C in a water bath and a vortex was used during the dissolution process. From stock, five distinct concentrations (160, 80, 40, 20 and 10) mg/ml were prepared, following comparison using the streak method on a McFarland tube 1.5 x 10^8 CFU / ml, the bacteria were cultured on Muller-Hinton agar, and six wells (5 mm) were created in the plate using a sterile cork drill. Five distinct concentrations of Se NPs containing 100 µl were added into the wells, while the sixth well was remained under control by adding 100µl ddH_2O, the plates then Incubated at 37°C for a 24h.

RESULTS AND DISCUSSION

Distribution of specimens according to gender

Out of a total of 150 specimens collected, 58 (38.7%) were from females patients, and 92 (61.3%) were from males patients. Among the female's specimens, bacterial growth was observed in 47 cases, representing 31.3% of the total specimens. In contrast, 11 female specimens showed no bacterial growth, accounting for 7.3% of the overall total. For the males group, 68 specimens

showed bacterial growth, which represents 45.3% of the total, while 24 specimens had no growth, accounting for 16% of the 92 (61.3%).

Table (1): Distribution of bacterial growth based on gender

Sex	Growth (%)	No growth (%)	Total specimens (%)	P value
Female	47 (31.3%)	11 (7.3%)	58 (38.7%)	< 0.01* χ^2 (7.7)
Male	68 (45.3%)	24 (16%)	92 (61.3%)	
Total	115 (76.7%)	35 (23.3%)	150 (100%)	

The results of the current study were consistent with [16] which they reached through their meta-analysis study that male's patients with Diabetic Foot Ulcer (DFU) had a significantly higher incidence than females, [17] who reported that the majority of patients with DFU were males (72.7%) while the females were 27.3%. Local study by [18] demonstrates that males with DFU were 67% and females were 33%.

Distribution of specimens according to the bacterial species

Following the bacteriological examination, biochemical tests and VITEC compact 2 systems was done for confirmation, the result showed that K. pneumoniae 30 (23%), E. coli 19 (14.6%), P. aeruginosa 20(15.4%), A. baumannii 13 (10%), and P. mirabilis10 (7.7%). while gram positive bacteria were S. aureus 25 (19.2%), S. pyogenes 7 (5.4%) and E. faecalis 6 (4.6%).

Table (2): Distribution of Bacteria according to gram type and species

Isolated bacteria	No (%)	P value	Species of bacteria	No (%)	P-value
Gram negative	92 (70.8%)	χ^2 (22.4) < 0.001**	K. pneumoniae	30 (23%)	χ^2 (32.4) < 0.001**
			E. coli	19 (14.6%)	
			P. aeruginosa	20 (15.4%)	
			A. baumannii	13 (10%)	
			P. mirabilis	10 (7.7%)	
Gram positive	38 (29.2%)		S. aureus	25 (19.2%)	
			S. pyogenes	7 (5.4%)	
			E. faecalis	6 (4.6%)	
Total	130 (100%)			130 (100%)	

The findings of the present study demonstrate a clear concordance with those reported by [19] in which gram-negative bacteria constituted the predominant group of isolates, accounting for 78.6% of all identified strains, while Gram-positive bacteria represented only 21.4%. This consistency between the two studies supports the growing consensus in the literature that Gram-negative bacteria are increasingly dominant in chronic wound infections, particularly in diabetic foot ulcers.

Antibacterial Susceptibility test

An antibiotic susceptibility test was performed on eight species of pathogenic bacteria, assessing sensitivity against different families of antibiotics for each bacterium, as published by CLSI [20].To assess the sensitivity of isolates or their resistance to antibiotics prevalent in healthcare settings, these drugs were selected due to their common application in treating bacterial illnesses.

Antibacterial Susceptibility test of P. aeruginosa

The results of the current study shown in the figure (1) indicated that from the total 20 isolates of P. aeruginosa showed resistance to Piperacillin 14(70), Ceftazidim 13(65%), Azetronam 12(60), Imipenem 12(60), Piperacillin- tazobactam 10(50%), Ciprofloxacin 8(40%), Ofloxacin 8(40%), Amikacin 6(30%), Meropenem 6(30%), Norfloxacin 5(25%).

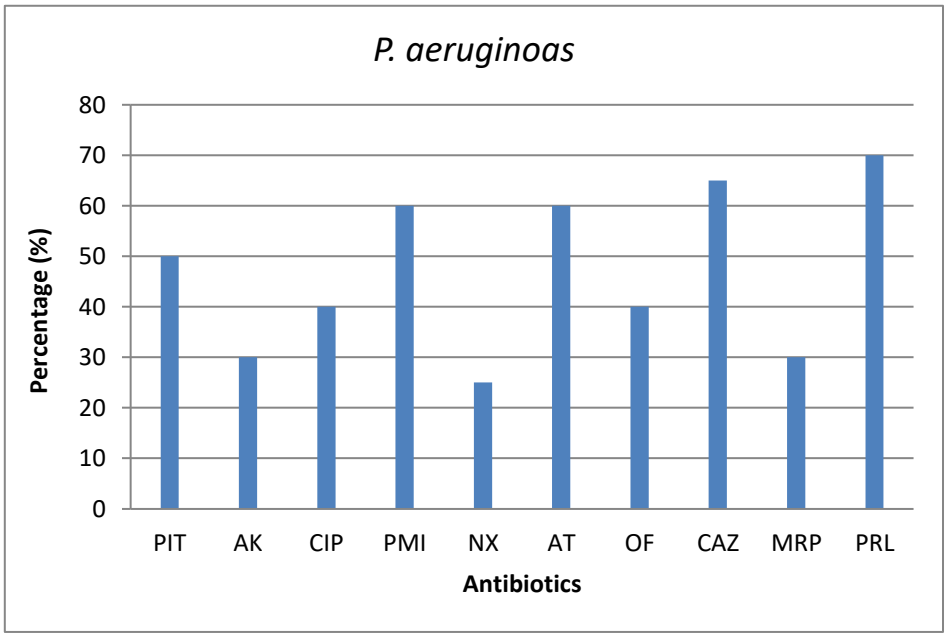


Figure (1): the percentages of antibiotic resistance of P.aeruginosa
Piperacillin-tazobactam (PIT), Amikacin (AK), Ciprofloxacin (CIP), Imipenem (IMI), Norfloxacin (NX), Azetronam (AT), Ofloxacin (OF), Ceftazidime (CAZ), Meropenem (MRP), Piperacillin (PRL)

The present study results were in good agreement with a study conducted by [21] found that piperacillin was resisted in only 23 of P. aeruginosa isolates. A higher percentage of ceftazidime resistance was found by [22] where it was 87.5% and very close ratio of resistance to aztreonam where it was 54.2%. [23] found a lower percentage of Imepinem and Meropenem resistance where it was only 30 % and 34%.

Antibacterial Susceptibility test of K.pneumoniae

The results of the current study shown in the figure (2) indicated that from the total 30 isolates of K.pneumoniae showed resistance to Piperacillin 29 (96%), Cefoxitin 27(90%), Cefotaxime 22(73%), Cefepem 19(63%), Ciprofloxacin 18(60%) Co-trimoxazole 17(56%), Imipenem 15(50%), Amikacin 10(33%), tetracyclin 10(33%), Piperacillin-tazobactam 9(30%), Amoxillin-clavulanate 8(26%).

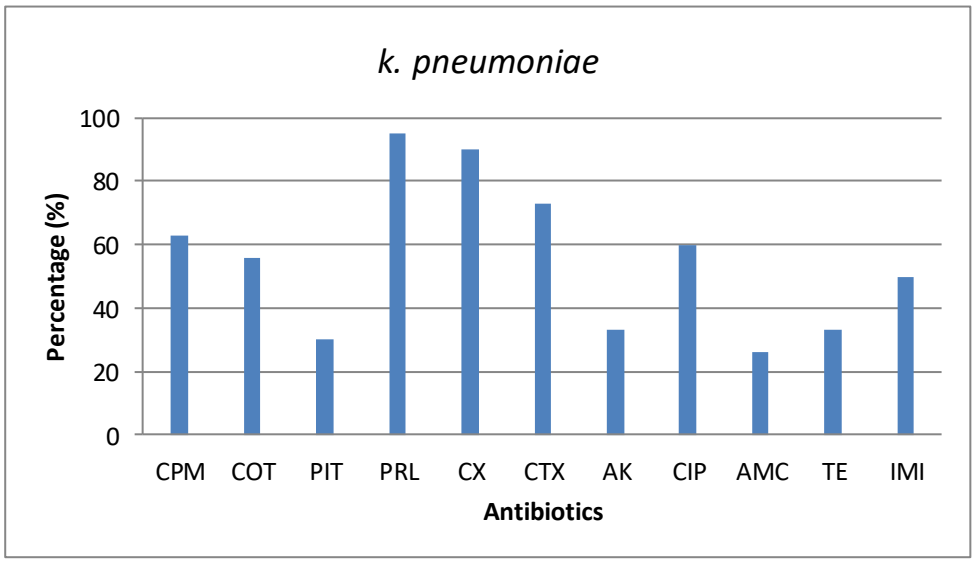


Figure (2): the percentages of antibiotic resistance of K.pneumoniae

Piperacillin (PIP), Cefoxitin (CX), Cefotaxime (CTX), Cefepem (CPM), Ciprofloxacin (CIP), Co-trimoxazole (COT), Imipenem (IPM), Amikacin (AK),Tetracyclin (TE), Piperacillin-tazobactam (PIT), Amoxillin-clavulanate (AMC)

carbapenems are often reserved for MDR infections, their declining efficacy in this context is concerning. These findings were consistent with those reported by [18] and [24], who also documented a resistance rate of K. pneumoniae to cefepime which was 55.6% while he found that the resistance to cefotaxime and cefoxitin were 94.4% and 55.5% respectively. And also found a close resistance ratio of imipenem which were 22.2% and 36.5% respectively, and low resistance of amikacin by 16.7% and 21.9% respectively.

Antibacterial Susceptibility test of E.coli

The antimicrobial susceptibility profile of E. coli isolates obtained from DFU specimens exhibited a heterogeneous resistance landscape across a wide range of antibiotics as Cefoxitin 17(89%), Cefotaxime 14(73%), Cephepem 14 (73%), Piperacillin 13(68%), Ciprofloxacin 12 (63%), Tetracyclin 10(52%), Piperacillin-tazobactam 9(47%), Co-trimoxazole 9(47%), Amoxillin-clavulanate 8(42%), Imipenem 9(42%), Amikacin 3(15%).

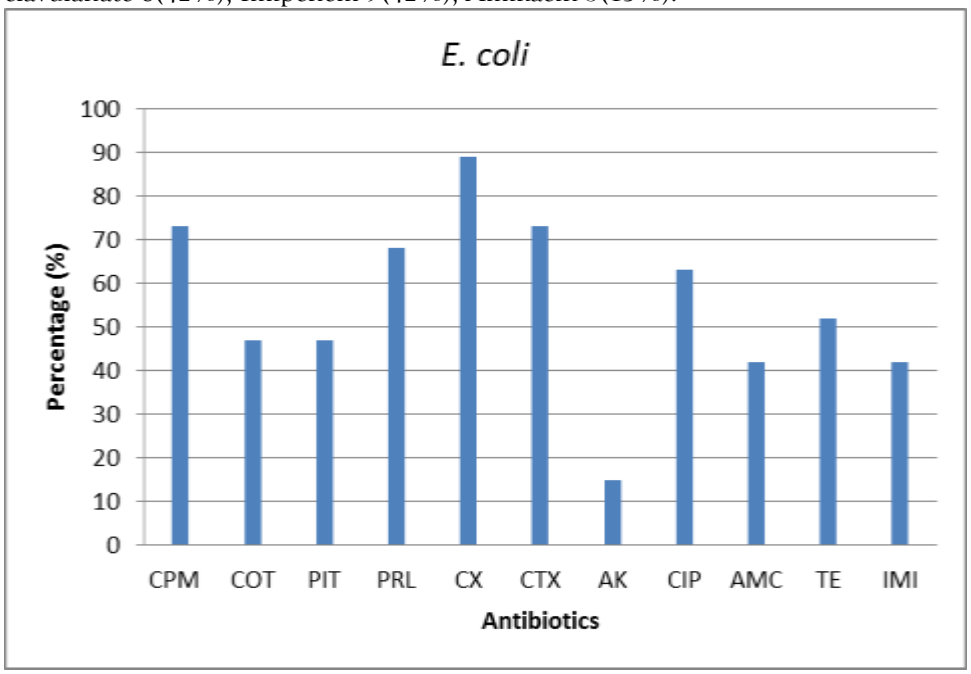


Figure (3): the percentages of antibiotic resistance of E. coli

Piperacillin-tazobactam (PIT), Amikacin (AK), Ciprofloxacin (CIP), Imipenem (IMI), Norfloxacin (NX), Azetronam (AT), Ofloxacin (OF), Ceftazidime (CAZ), Meropenem (MRP), Piperacillin (PRL)

The resistance to third-generation cephalosporins, such as cefotaxime and fourth-generation cefepime, was notably high, at 73.7% and 63.2% respectively. This strongly suggests the probable presence of extended-spectrum β-lactamase (ESBL) producing strains, a well-recognized phenomenon in E. coli, particularly among chronic wound isolates where [22] reached results that were similar to most of resistance percentage of E. coli, he found that cefotaxime, cefepime, piperacillin-tazobactam, imipenem, amikacin, ciprofloxacin, and cotrimoxazole were resisted in 74%, 100%, 33%, 23.8%, 28.8%, 38%, and 90% of E. coli isolates respectively. recurrent antibiotic exposure is common [25].

Antibacterial Susceptibility test of A. baumannii

The results of the current study shown in the figure (4) indicated that from the total 13 isolates of A. baumannii showed resistance to Piperacillin 13(100%), piperacillin-tazobactam 13 (100%), Cefotaxim13 (100%), Ciprofloxacin 13(100%), Imipenem 12 (92), Meropenem 11(84%), Tetracyclin 8(61%), Gentamicin 8(61%), Amikacin 5(38%), Co-trimoxazole 4(30%).

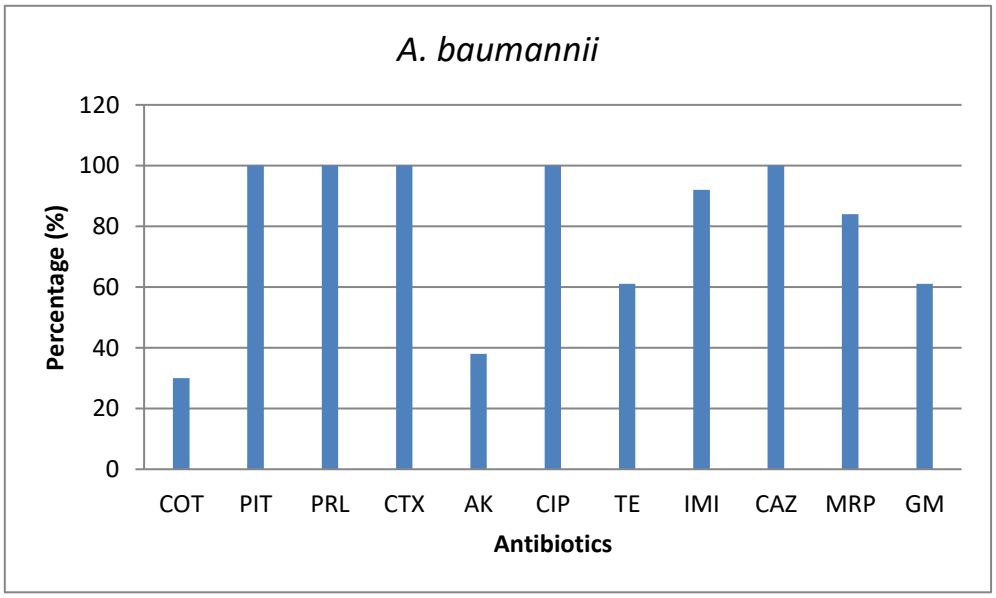


Figure (4): the percentages of antibiotic resistance of A. baumannii
Co-trimoxazole (COT), Piperacillin-tazobactam (PIT), Piperacillin (PRL), Cefotaxime (CTX), Amikacin (AK), Ciprofloxacin (CIP), Tetracycline (TE), Imipenem (IMI), Ceftazidim (CAZ), Meropenem (MRP), Gentamicin (GM).

The data show that the pathogen exhibits extremely high resistance rates across nearly all tested antibiotics, with total resistance (100%) observed against piperacillin, piperacillin-tazobactam, cefotaxime, ceftazidime, and ciprofloxacin. These antibiotics are commonly used for empirical treatment of Gram-negative infections, particularly in hospital settings, and their complete failure here highlights the severity of resistance in A. baumannii strains associated with chronic diabetic wounds [26]. High resistance rate of antibiotics was also found by [19] in which imipenem, meropenem, amikacin, cotrimoxazole, cefotaxime, ceftazidime, piperacillin, and ciprofloxacin were resisted by 92.3%, 88.6%, 97%, 94.3%, 97%, 100%, 98.6%, and 94.3% respectively.

Antibacterial Susceptibility test of P.mirabilis

The results of the current study shown in the figure (5) indicated that from the total 10 isolates of P.mirabilis showed resistance to Cefotaxim 8(80%), Cefoxitin 8(80%), Tetracyclin 8(80%), Imipenem 7(70%), Ciprofloxacin 6(60%), Co-trimoxazole 6(60%), Cefepem 5(50%), Piperacillin 4(40%), Amoxillin-culvanate 4(40%), Piperacillin-tazobactam 3(30%), Amikacin 1(1%).

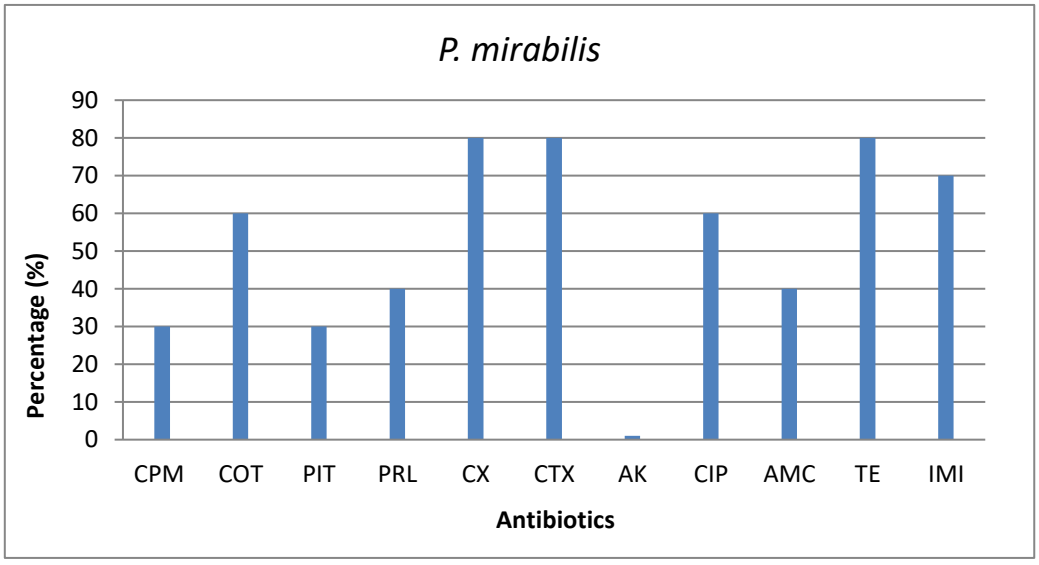


Figure (5): the percentages of antibiotic resistance of P. mirabilis

Co-trimoxazole (COT), Piperacillin-tazobactam (PIT), Piperacillin (PRL), Cefotaxime (CTX), Amikacin (AK), Ciprofloxacin (CIP), Tetracycline (TE), Imipenem (IMI) Cefotaxin (CX), Cefepem (CPM), Amoxillin-culvanate (AMC)

Among the β-lactam antibiotics, cefotaxime and cefoxitin showed the highest resistance rates, at 80%, with only 20% sensitivity for both. This substantial resistance likely reflects the production of extended-spectrum β-lactamases (ESBLs), rendering many cephalosporins ineffective against P. mirabilis in DFU settings [27].

75% of P. mirabilis isolates were resist to cefotaxime was reported by the previous study. While cefepime showed 50% resistance in which it consistent with the findings of [24] who reported that 36.6% resistance of P. mirabilis to cefepime. Piperacillin performed better, with 60% sensitivity and 40% resistance, although its use should still be based on susceptibility testing.

Antibacterial Susceptibility test of S. aureus

The results of the current study shown in the figure (6) indicated that from the total 25 isolates of S. aureus showed resistance to Clarithomycin 16(64%), Tetracyclin 12(45%), Norfloxacin 11(44%), Nitrofuranation 11(44%), Levofloxacin 10(40%), Ofloxacin 10 (40%), Rifampicin 9(36%), Co- trimoxazole 9(36%), Gentamicin 8(32%), Clindamycin 8 (32%), Cloramphenicole 0(0%).

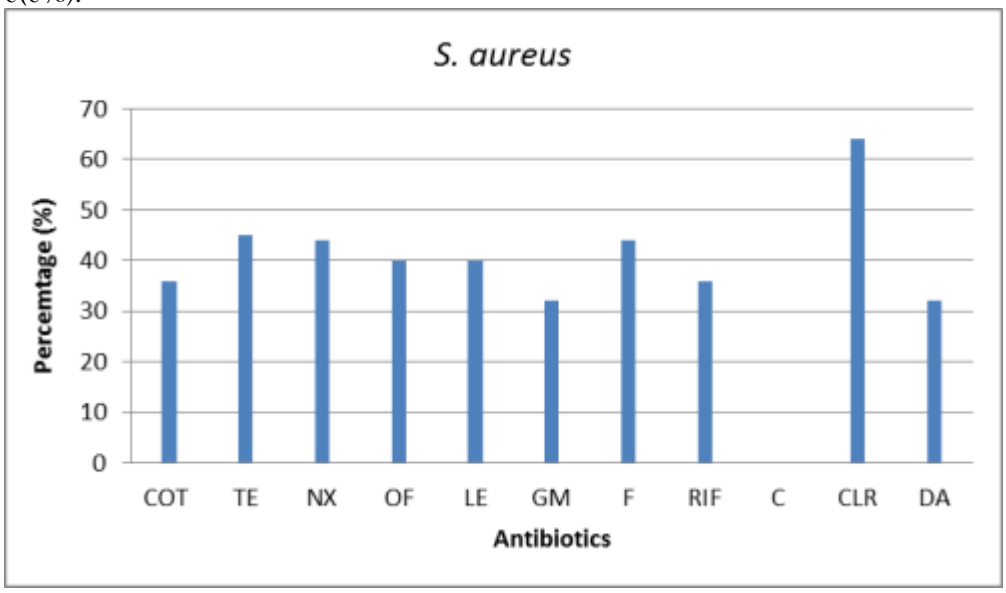


Figure (6): the percentages of antibiotic resistance of S.aureus
Gentamicin (GM), Tetracyclin (TE), Norfloxacin (NX), Ofloxacin (OF), Levofloxacin (LE), Co-trimoxazole (COT), Nitrifuranation (F), Rifampicin (RIF), Colramphenicole (C), Clarithomycin (CLR), Clindamycin (DA)

Among the tested antibiotics, chloramphenicol demonstrated the highest efficacy with 100% sensitivity and 0% resistance or intermediate response, reaffirming its bacteriostatic activity against S. aureus, this finding consistent with [22] who also found 0% of resistance, and 78%, 87.5%, 38%, and 66% resistance to gentamicin, cotrimoxazole, clindamycin, and norfloxacin respectively.While not commonly used due to safety concerns (notably bone marrow suppression) [28].

While [29] found consistent ratio of tetracycline resistance by 40.4%, on the other hand, he reported low resistance to rifampin by S. aureus isolates which was only 5.6%.

Antibacterial Susceptibility test S. pyogens

The results of the current study shown in the figure (7) indicated that from the total 25 isolates of S. pyogens showed resistance to Tetracyclin 4(57%), Gentamicin 4(57%), Co-trimoxazole 4(57%), Amikacin 3(42%), Norfloxacin 3(42%), Nitrofuranation 3(42%), Rifampicin 3(42%), Doxycyclin 2(28%), Ofloxacin 1(14%), Levofloxacin 1(14%), Cloramphenicole 1 (14%).

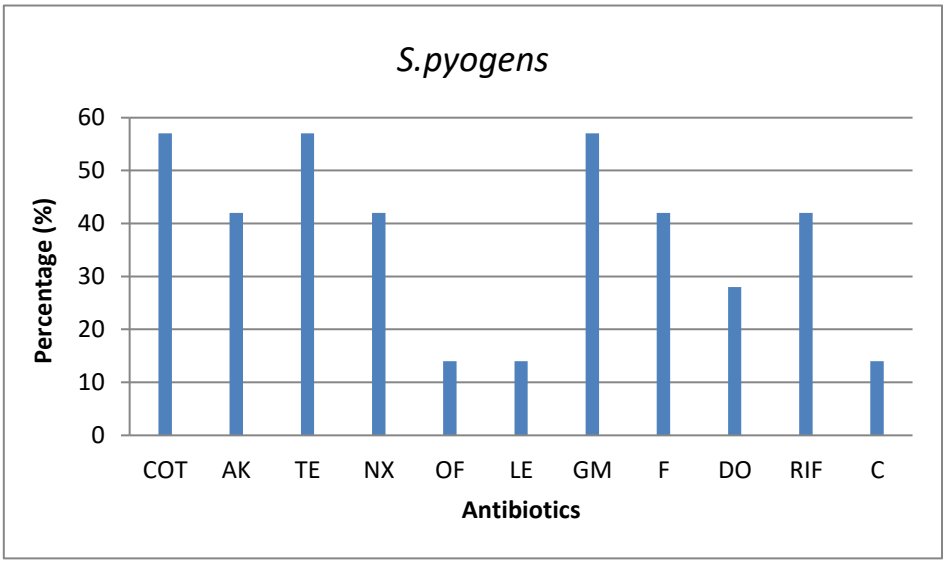


Figure (7): the percentages of antibiotic resistance of S. pyogens
Gentamicin (GM), Tetracyclin (TE), Norfloxacin (NX), Ofloxacin (OF), Levofloxacin (LE), Co-trimoxazole (COT), Nitrifuranation (F), Rifampicin (RIF), Colramphenicole (C), Doxycyclin (DO), Clindamycin (DA)

Rifampin presented a balanced profile with 42.9% sensitivity, 42.9% resistance, and 14.3% intermediate, raising caution against monotherapy due to resistance emergence. Doxycycline was among the better-performing oral options with 71.4% sensitivity, though not without resistance (28.6%).

The most promising result came from chloramphenicol, with 85.7% sensitivity and only 14.3% resistance. Despite its limited use in clinical practice due to safety concerns [28].

Interestingly, [22] found that all isolates of S. pyogenes were 100% resisted to all antibiotics, this is because of limited number of S. pyogenes isolated from DFU cases which may give unrealistic results.

Antibacterial Susceptibility test E. faecalis

The results of the current study shown in the figure (8) indicated that from the total 6 isolates of E. faecalis showed resistance to Rifampicin 5(83%), Tetracyclin 3(50%), Levofloxacin 3(50%), Gentamicin 3(50%), Co-trimoxazole 2(33%), Amikacin 2(33%), Ofloxacin 2(33%), Norfloxacin 1(16%), Nitrofuranation 1(16%), Doxycyclin 0(0%), Cloramphenicole 0(0%).

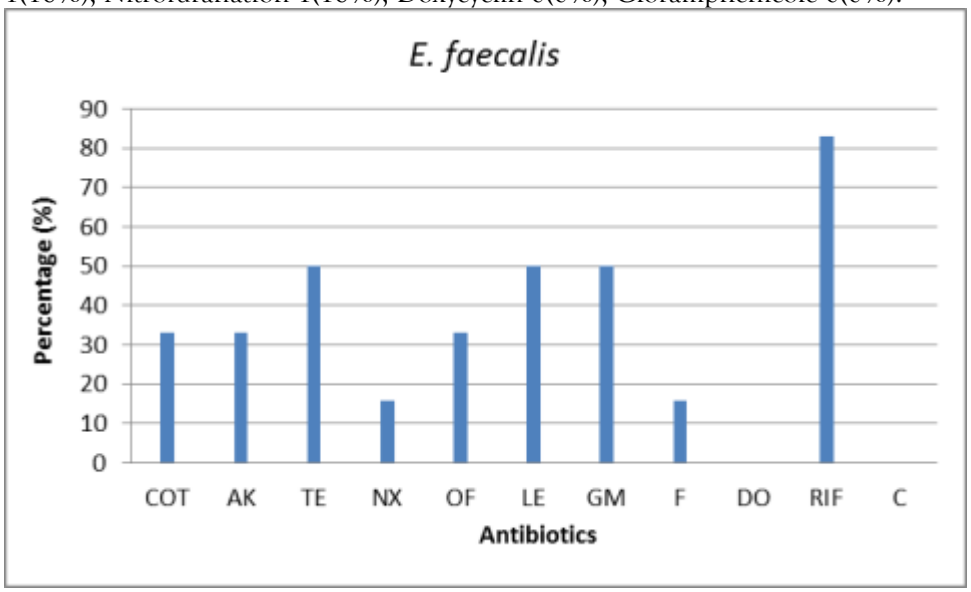


Figure (8): the percentages of antibiotic resistance of E. faecalis

Gentamicin (GM), Tetracyclin (TE), Norfloxacin (NX), Ofloxacin (OF), Levofloxacin (LE), Co-trimoxazole (COT), Nitrifuranation (F), Rifampicin (RIF), Colramphenicole (C), Doxycyclin (DO), Clindamycin (DA)

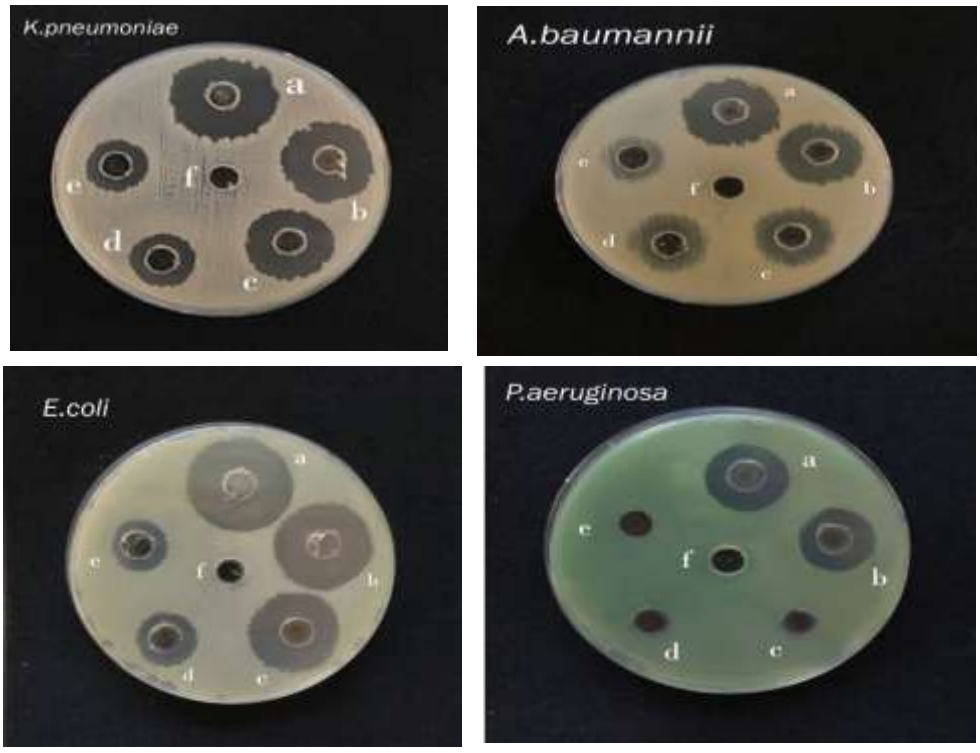
Among the most effective antibiotics were doxycycline and chloramphenicol, both exhibiting 100% sensitivity and 0% resistance. These findings are of significant clinical interest, especially considering the oral availability and affordability of doxycycline, these results were consistent with the finding of [22] who found 100% of S. pyogenes were sensitive to chloramphenicol but 100% resistance to doxycycline. Chloramphenicol, though highly effective, is less favored due to its hematologic toxicity profile but may be reconsidered in multidrug-resistant cases under close monitoring [28].

Antibacterial activity of aqueous extract of Zingiber officinale against pathogenic bacteria

Table (3), figure (9) summarizes the inhibition zone diameters observed for various bacterial isolates at five serial concentrations (160, 80, 40, 20 and 10) mg/ml of the tested compound using the agar well diffusion method. The results demonstrated a concentration-dependent antibacterial effect across all tested organisms, with inhibition zones decreasing progressively with lower concentrations.

Table (3): Effect of aqueous extract of Zingiber officinale on bacterial growth.

Bacterial isolates	Inhibition zone (mm)				
	160 mg/ml	80 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml
K. pneumoniae	22 mm	21 mm	18 mm	13 mm	12 mm
E. coli	21 mm	20 mm	18 mm	12 mm	11 mm
P. aeruginosa	16 mm	15 mm	0 mm	0 mm	0 mm
A. baumannii	19 mm	17 mm	14 mm	14 mm	12 mm
P. mirabilis	20 mm	18 mm	14 mm	13 mm	12 mm
S. aureus	22 mm	21 mm	18 mm	15 mm	14 mm
S. pyogenes	22 mm	21 mm	19 mm	17 mm	14 mm
E. faecalis	24 mm	22 mm	20 mm	16 mm	12 mm



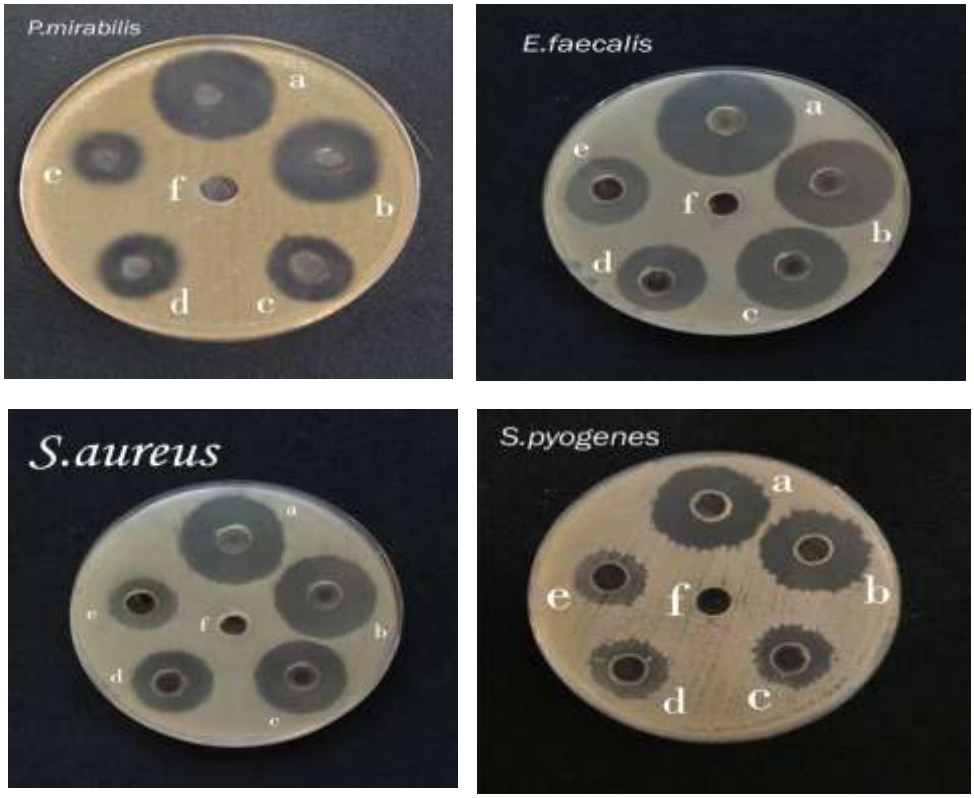


Figure (9): The inhibition zone aqueous extract of Zingiber officinale on Bacterial isolates (a=160, b=80, c=40, d=20, e=10, f= Control)

In general, Gram-positive isolates (S. aureus, S. pyogenes, and E. faecalis) tended to exhibit stronger inhibition compared to most Gram-negative bacteria, with the exception of K. pneumoniae. These findings suggest that the tested agent may have higher efficacy against Gram-positive organisms, and that its antibacterial potential is significantly influenced by concentration.

Many bioactive compounds in ginger have been identified, such as phenolic and terpene compounds. The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the various bioactivities of ginger [30].

In Zingiber officinale Gingerols have been investigated for analgesic, sedative, antipyretic, antibacterial, and gastrointestinal tract motility effects, They have been found to inhibit Gram-positive and Gram-negative bacteria.

Ginger possessed potent antibacterial as well as to a lesser degree, antifungal qualities. According to in vitro research, ginger's active ingredients prevented colon bacteria from growing. Escherichia coli, Salmonella, Proteus species, Staphylococci, and Streptococci were all inhibited in their growth by ginger. Ginger should therefore affect the growth of Bacillus cereus, which primarily produced nausea and diarrhea [31].

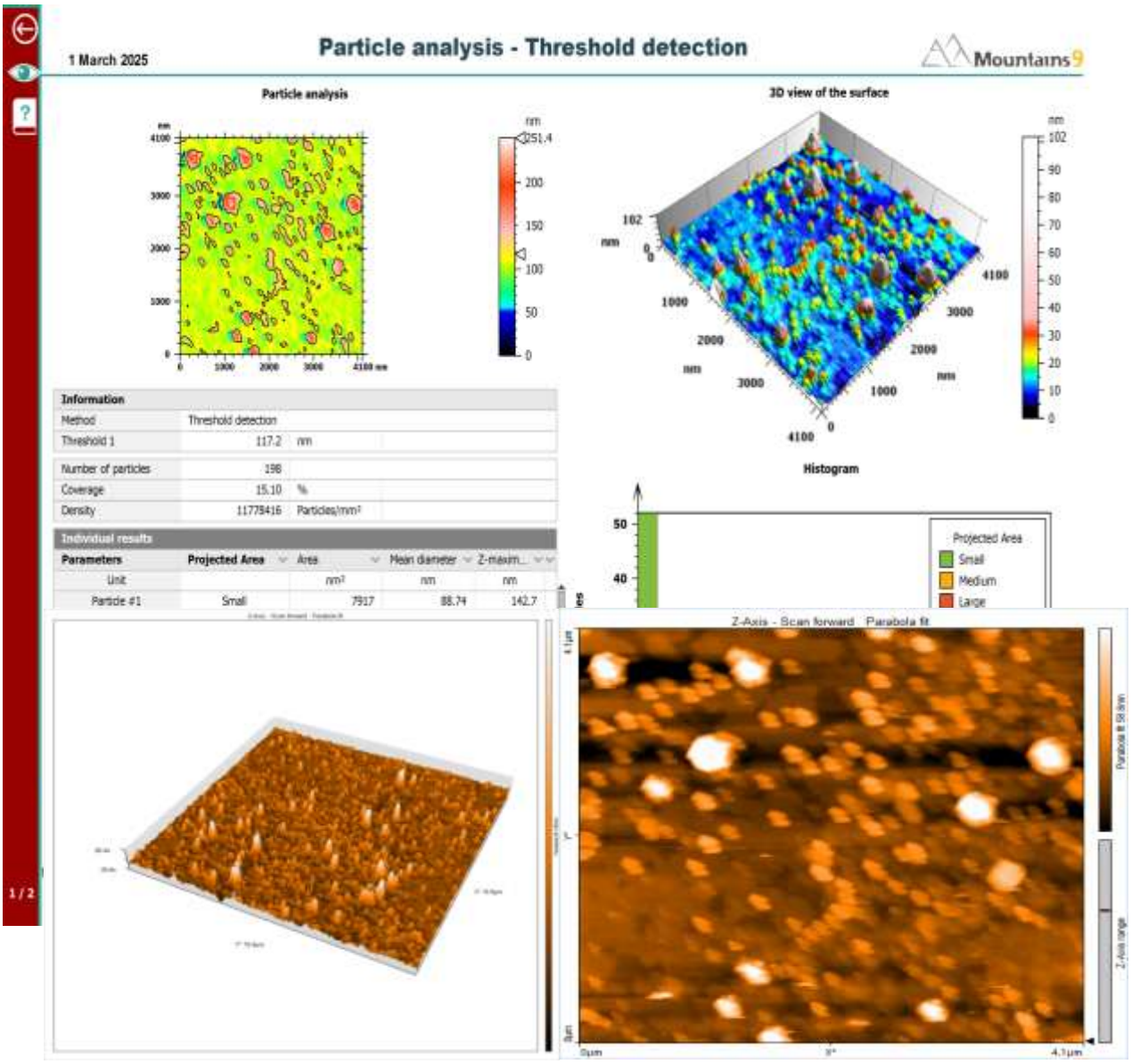
A study done by [32] demonstrated that ginger's aqueous extract had the strongest antibacterial activity against Proteus vulgaris, Streptococcus pyogenes, Klebsiella pneumonia, and Staphylococcus aureus, which in good agreement with the present study results .

Characterization of selenium nanoparticles

Atomic Force Microscopy (AFM)

The AFM technique was used to investigate the morphology of Se NPs. The Se NPs histogram and 3D AFM pictures are shown in Figure (10) indicates that the highest height of Se NPs is 125.7 nm, its root mean square (RMS) is 8.81 nm, and its peak density is 3.77x10-6, all of which point to the construction of a nanostructure. The average diameters of Se NPs biosynthesized by Zingiber officinale was (18.15) nm and the calculated size of nanoparticle was ranged between

(20.40 –56.8) nm. These findings revealed that Zingiber officinale extract was effective in creating smaller NPs by showing that the produced particles were ultrafine particles with a diameter of less than 100 nm.



Scanning Electron Microscopy (SEM) analysis

A type of electron microscope that creates an image of the sample by scanning with a high-energy electron beam in a raster scan pattern, was used to measure the size and form of the silver and Se NPs. The images illustrate that some of the nanoparticles were well separated from each other, while most of them were in a lumpy form. This agglomeration is due to the electrostatic effects, this behavior is consistent with a similar behavior to nanoparticle agglomeration of another studies. The current results table (4), figure (11) revealed that Se NPs were spherical shaped and the average of the particles size ranged from 33.20 to 53.70 nm, these results are in good agreement with [33].

Table (4): particle size (nm) of Se NPs biosynthesized by Zingiber officinale

Particles Size (nm)	Particles Size (nm)	Particles Size (nm)
33.20	40.42	47.65
34.22	41.20	48.98
34.15	42.36	49.50
35.5	43.33	50.26
36.8	44.73	51.70
38.40	45.22	53.70
40.30	46.72	

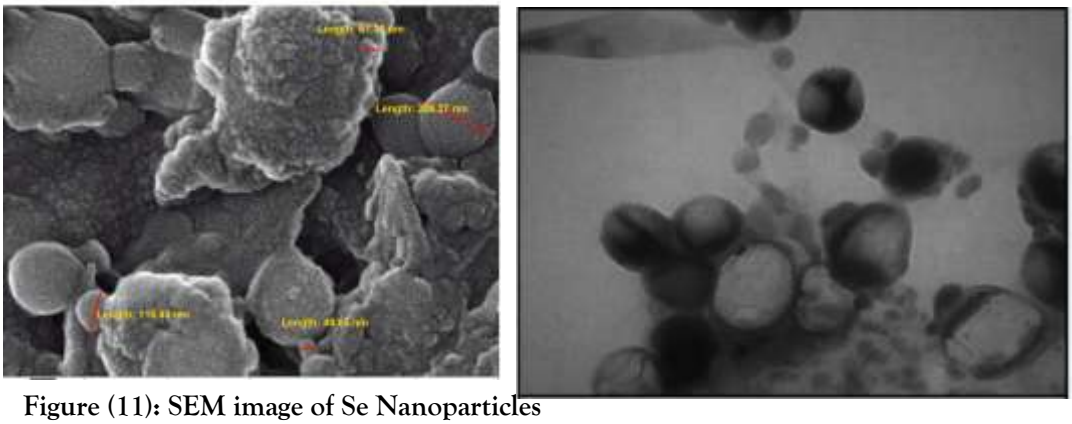


Figure (11): SEM image of Se Nanoparticles

UV–Visible spectroscopy

The most crucial method for identifying and characterizing nanoparticles is the UV-vis spectrum. The wavelength of biosynthesized Se NPs was measured between 200 and 800 nm, and the greatest absorption peak was 263 nm, figure (12). The current result agreed with [34] who showed that a strong absorption peak was observed between 250-280 nm with maxima at 265 nm, confirming the presence of selenium in the samples.

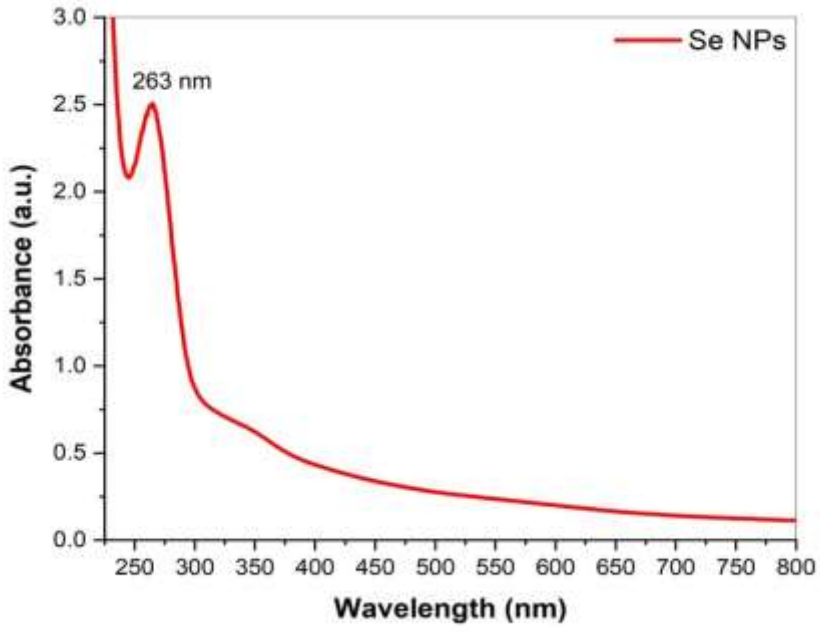


Figure (12): UV-Vis spectrum of synthesized Se NPs

X-ray Ddiffraction (XRD)

X-ray diffraction device was used to study the bio-synthesized Se nanoparticles in order to determine their average particle size and crystalline nature. Captured using an X-ray diffraction device called Panalitycal X'pert Pro MRD. Figure (13) showed the prominent peaks combining the diffraction values obtained at the 2θ levels 23.4° (100), 29.6° (101), 43.6° (012), and 55.5° (003), which were related to the peaks of Jhoint Committee on Powder Diffraction Station (JCPDS) card No.(01-086-2246) standard.

The average size (D) of the nanoparticles table (5) was determined using Scherrer's equation $D=k\lambda/\beta\cos\theta$

Where θ is Bragg's diffraction angle, λ is the X-ray wavelength, β is the width in radians of the peak caused by the size effect, and θ is a constant with a value of approximately 0.9. In particular, the line width of the (101) XRD peak was used to assess the sample's particle size [35].

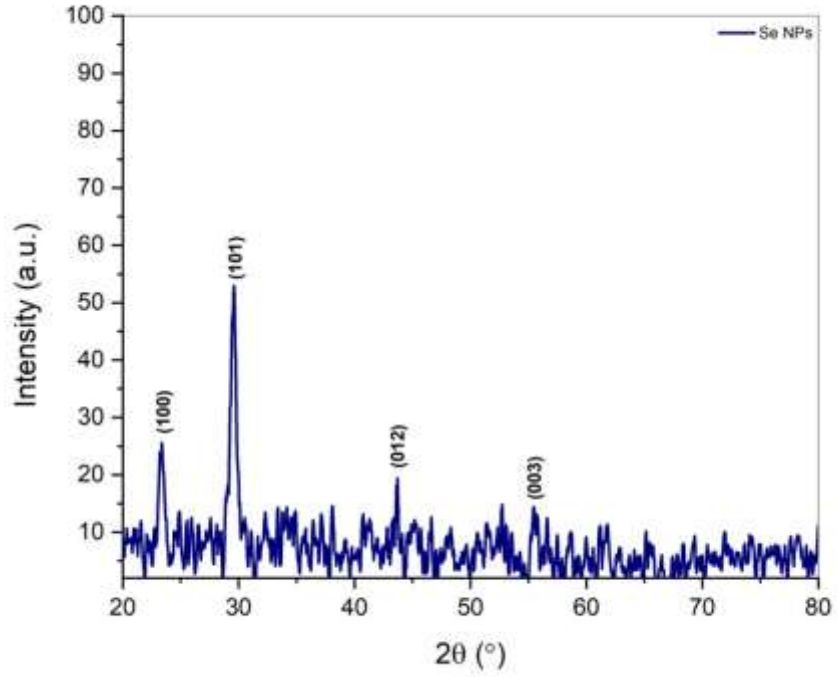


Figure (13): XRD spectra of Se NPs.

Table (5): The crystallite size of Se NPS

2 θ	hkl	FWHML	2 theta (Rad.)	FWHM (Rad)	Crystallite Size AgNPs (nm)	Average crystallite size (nm)
23.31855	100	0.47232	0.203493	0.008	17.167	20.092nm
29.61158	101	0.31488	0.25841	0.005	26.086	
43.73603	012	0.47232	0.381669	0.008	18.117	
55.49707	003	0.47232	0.484303	0.008	18.998	

Transmission electron microscope (TEM)

Selenium NPs aggregation confirms that the generated particles are capped, as was previously stated in the section on chemical interactions in FTIR. The TEM analysis of the size and form of the biosynthesized Se NPs revealed that they were roughly sphere-shaped with minimal agglomerations. Se NPs ranged in size from 5 to 60 nm figure (4-14).

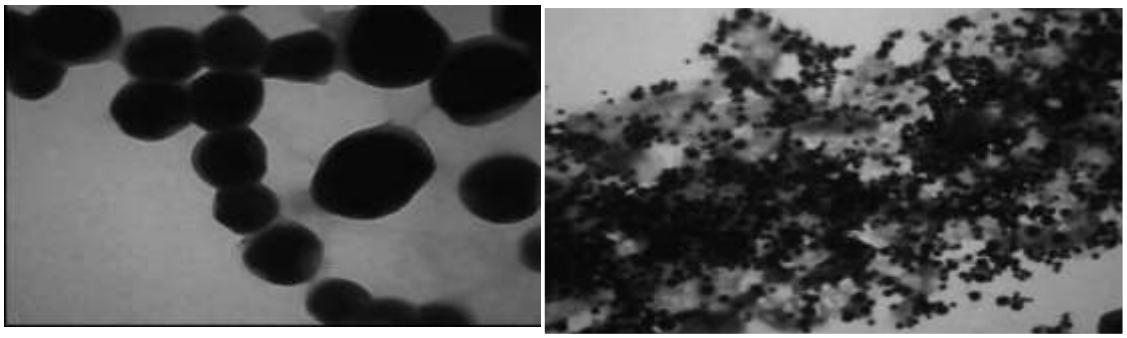


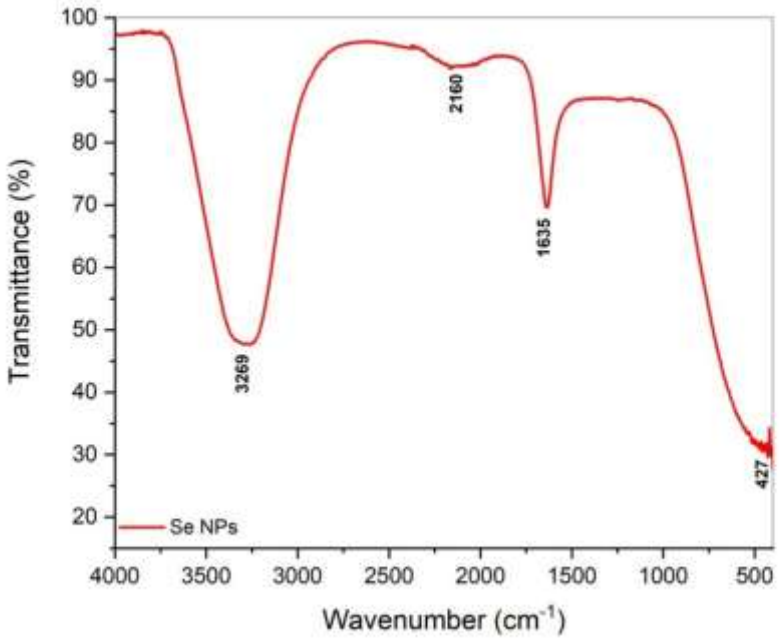
Figure (14): (A) Selenium nanoparticles (B) TEM image of Zingiber officinale with Se nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis of Zingiber officinale extract was done as shown in Figure (15), a wide peak centered at 3269 cm^{-1} could be assigned to the presence of probable the N-H stretching amine vibration and hydroxyl O-H stretching vibrations associated with amines, phenolic chemicals and carbohydrates, while the peak observed at 2160 cm^{-1} may be evidence to the presence of C ≡C and C≡N stretching vibrations associated with alkynes and nitrile.

The peak observed at 1635 cm^{-1} could be related to C=C stretching vibrations associated with α, β-unsaturated ketones, or alkenes (cyclic alkenes or conjugated alkene) these results are in good agreement with [36].

Based on these results, gelatin and glucose Species were able to adhere to the surface of the produced nanoparticles. Accordingly, the extract's bioactive phytochemicals—such as polyphenols, flavonoids, tannins, amino acids, and sugars—may contribute to the bioreduction of Se NPs into corona, as demonstrated by FTIR analysis. These phytochemicals also serve as a surface stabilizing agent for Se NPs and enhance their biocompatibility. Because the capping prevents particle aggregation, the particles are stabilized in the medium.



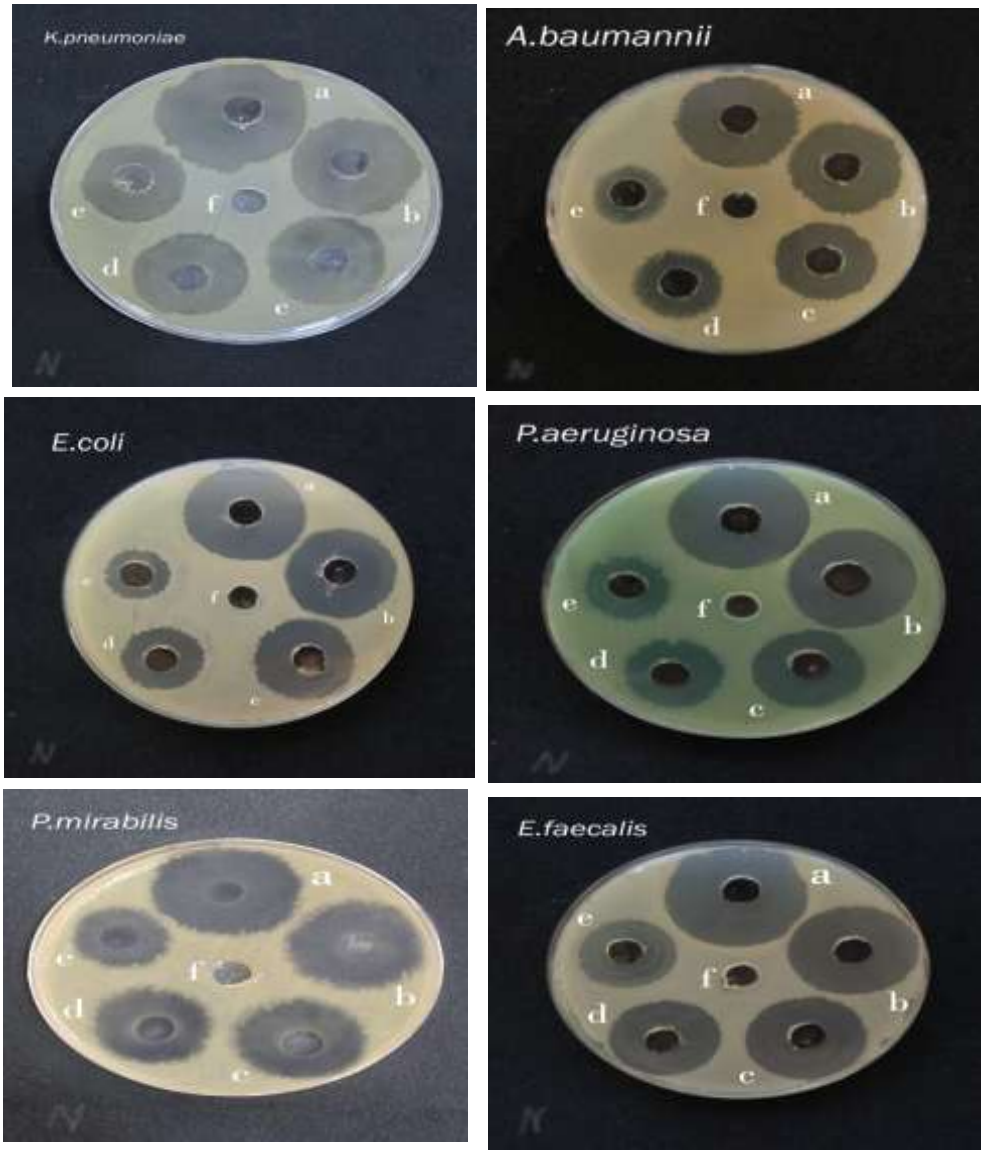
(15): FTIR spectra of functional groups of the Se NPs

The antibacterial activity of Se NPs from the aqueous extract of Zingiber officinale

Table (6), figure (16) presents the results of the antibacterial activity against eight bacterial isolates commonly associated with diabetic foot ulcers, assessed using the agar well diffusion method across a range of concentrations (10–160) mg/ml. The data reveal a clear concentration dependent inhibitory effect, where the diameter of the inhibition zones decreased progressively as the concentration of the compound was reduced.

Table (6): Effect of Selenium nanoparticles on bacterial growth.

Bacteria	Inhibition zone (mm)				
	160 mg/ml	80 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml
K. pneumoniae	26 mm	24 mm	21 mm	20 mm	18 mm
E. coli	25 mm	22 mm	20 mm	17 mm	15 mm
P. aeruginosa	24 mm	22 mm	19 mm	17 mm	15 mm
A. baumannii	24 mm	20 mm	18 mm	16 mm	13 mm
P. mirabilis	24 mm	22 mm	20 mm	19 mm	18 mm
S. aureus	26 mm	23 mm	21 mm	19 mm	16 mm
S. pyogenes	26 mm	23 mm	21 mm	19 mm	16 mm
E. faecalis	26 mm	24 mm	22 mm	20 mm	18 mm



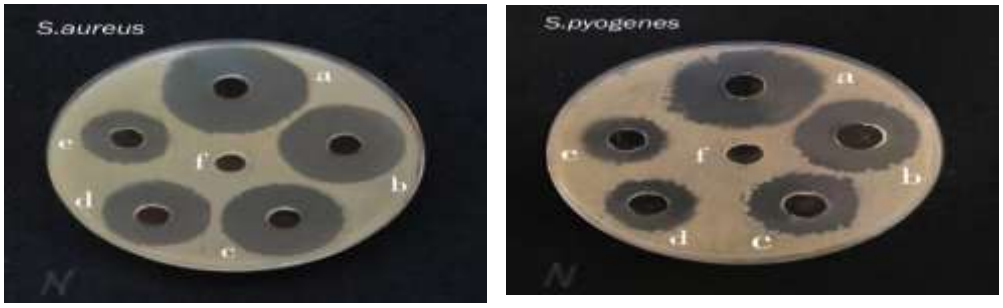


Figure (16): The inhibition zone of Se NPs on Bacterial isolates (a=160, b=80, c=40, d=20, e=10, f= Control)

Among Gram-positive isolates, S. aureus, S. pyogenes, and E. faecalis responded favorably to the compound, with inhibition zones ranging from (16–26) mm for S. aureus and S. pyogenes, and from (18–25) mm for E. faecalis. These values reflect a generally good level of sensitivity, especially at higher concentrations.

Overall, the results suggest that the tested agent possesses broad-spectrum antibacterial activity, with particularly high efficacy against E. coli, k.pneumoniae and P. aeruginosa, and sustained inhibition of both Gram-positive and Gram-negative pathogens across all concentrations, results of the current study are in agreement with a study conducted by [37], showed that Se NPs had a noticeable antibacterial effect on k.pneumoniae, E. coli, S.auerus, P. aeruginosa.

A two-way ANOVA test was conducted to evaluate the effect of both the treatment type (plant extract vs. nanoparticles) and concentration on the antimicrobial activity, as measured by the inhibition zone diameters against bacterial isolates. The results revealed a statistically significant effect for the treatment type ($F = 32.84$, $p < 0.001$), indicating that the overall antimicrobial performance of the nanoparticles was significantly superior to that of the crude extract across all tested concentrations, table (7).

Table (7): Comparison study between plant extract and nanoparticle activity

Test	Statistic	P-value
Two-way ANOVA (Effect of Type)	32.84	< 0.001**
One-way ANOVA (Effect of Extract Concentration)	7.09	< 0.001**
One-way ANOVA (Effect of NPs Concentration)	30.48	< 0.001**

Furthermore, one-way ANOVA tests were performed separately to assess the influence of different concentrations on the antimicrobial efficacy of each treatment. In the case of the plant extract, concentration exhibited a significant impact ($F = 7.09$, $p < 0.001$), suggesting a dose-dependent increase in inhibition zone diameters, wherein higher concentrations were generally associated with enhanced antimicrobial activity.

Similarly, for the nanoparticles, the one-way ANOVA also demonstrated a highly significant effect of concentration ($F = 30.48$, $p < 0.001$), indicating a strong concentration-dependent response. This finding affirms that the nanoscale formulation maintained high antimicrobial efficacy even at lower doses, but still exhibited a statistically significant increase with rising concentrations.

These results collectively highlight the enhanced potency of nanoparticle formulations and underscore the critical role of concentration in modulating antimicrobial responses. The statistical significance across both treatment types supports the reliability of the findings and their potential relevance in the development of novel antibacterial therapies for managing diabetic foot infections.

Determination of Minimum Inhibition Concentration (MIC) of aqueous extract of Zingiber officinale and Se NPs

The MIC values presented in Table (8) reflect the minimum inhibitory concentrations of both the aqueous extract and selenium nanoparticles (Se NPs) required inhibiting the visible growth of various bacterial isolates obtained from diabetic foot ulcer. The findings demonstrate

variability in antimicrobial susceptibility among different bacterial species, reflecting potential differences in structural, metabolic, and resistance profiles.

Table (8): Minimum Inhibitory Concentration of Zingiber officinale extract and nanoparticles

Bacterial isolates	MIC of Zingiber officinale aqueous extract	MIC of Se NPs
K. pneumonia	12.5 µg /ml	6.12 µg /ml
E. coli	12.5 µg /ml	3.12 µg /ml
P. aeruginosa	25 µg /ml	6.12 µg /ml
A. baumannii	50 µg /ml	6.12 µg /ml
P. mirabilis	50 µg /ml	6.12 µg /ml
S. aureus	25 µg /ml	1.5 µg /ml
S. pyogenes	25 µg /ml	1.5 µg /ml
E. faecalis	12.5 µg /ml	3.12 µg /ml

The MIC of Zingiber officinale ranged from (12.5 to 50) µg /ml, while the MIC of the Se NPs ranged from (1.5 to 6.12) µg /ml, in Zingiber officinale E. coli, K. pneumonia and E. faecalis showed the showed the highest sensitivity at the concentration 12.5 µg /ml, followed by P. aeruginosa and S. aureus at 25µg/ml followed by P. mirabilis and A. baumannii which showed less sensitivity at the concentration 50 µg/ml respectively. The MIC of Se NPs against S.aureus and S. pyogenes showed the highest sensitivity at concentration 1.5 µg /ml, followed by E. coli and E. faecalis at 3.12 µg /ml concentration. While K. pneumonia, P. aeruginosa, A. baumannii and P. mirabilis showed the lowest sensitivity at concentration 6.12 µg /ml.

Overall, while Se NPs did not consistently outperform the aqueous extract across all species, the data support their role as a potential antimicrobial alternative, particularly for bacteria such as K. pneumoniae and E. coli. However, the elevated MIC observed in P. aeruginosa against Se NPs warrants further investigation into possible resistance mechanisms and optimization of nanoparticle formulation.

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