

Research Article

Green synthesis and characterization of a nanocomposite *Petroselinum crispum* (PcNps) and evaluation of its effect on the inhibition of *Pseudomonas aeruginosa* before and after loading the antibiotic erythromycin

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Abstract

Pseudomonas aeruginosa is a multidrug-resistant bacteria. Therefore, it has become necessary to use new methods, including the process of delivering treatment using nanomaterials manufactured using green methods. The present study explores the antibacterial effects of a green-synthesized nanocomposite from *Petroselinum crispum* (parsley) against *Pseudomonas aeruginosa*. The nanocomposite was prepared using an environmentally friendly method and characterized using Atomic Force Microscopy (AFM) and Fourier Transform Infrared (FTIR) spectroscopy. AFM results revealed that the root mean square height (Sq) of the parsley extract was 138 nm, while the nanocomposite (PcNps) measured 111 nm, a difference of 27 nm. The texture direction (Std) of the extract was 933 nm, compared to 667 nm for the nanocomposite. The maximum peak height (Sp) for the nanocomposite was 279 nm, which increased to 293 nm after converting the parsley extract into the nanocomposite. The maximum pit height (Sv) was 654 nm for the extract and 374 nm for the nanocomposite. FTIR analysis confirmed that the antibiotic loading showed positive results, with significant shifts observed in the spectra before and after loading. The antibacterial activity of the nanocomposite was significantly enhanced when combined with the antibiotic. The inhibition zone reached 21.00 ± 1.41 mm ($p \leq 0.05$) with the antibiotic-nanocomposite combination. At a concentration of 5 μ g (C1 PcNps/ER T5), the inhibition zone was 17.75 ± 1.50 mm ($p \leq 0.005$), while at 10 μ g, the zone was 10.25 ± 0.25 mm. These results demonstrate the effectiveness of the green nanocomposite, especially in combination with antibiotics, for inhibiting bacterial growth and show its potential as an antimicrobial agent.

Keywords: AgNps nanocomposite, Erythromycin, Green synthesis, *Petroselinum crispum*, *Pseudomonas aeruginosa***INTRODUCTION**

The Pseudomonadaceae family of non-spore-forming, gram-negative, non-fermenting bacteria includes *Pseudomonas aeruginosa*. Most of the 0.5 to 1.0 μ m by 3 to 4 μ m *P. aeruginosa* cells have a single polar flagellum (Tural et al., 2019). *P. aeruginosa* is catalase- and oxidase-positive, and it has long been thought of as a strict aerobe since it cannot thrive in anaerobic environments because it needs oxygen as a terminal electron acceptor in metabolic pathways (Mohammad and Taher, 2019). With a wide temperature range for

growth, *P. aeruginosa* can grow at 37° C and room temperature just as well as clinically important *Pseudomonas* species can at 42° C (Linde et al., 2016). The elements of virulence Type IV pili and flagella of *P. aeruginosa*, as well as substrates that are known to encourage its growth, all play a significant role in the development of biofilms (Qin et al., 2022). Although it does not directly contribute to attachment or biofilm formation, alginate in biofilm formation serves as a barrier against macrophage phagocytosis (Muhsin et al., 2015). In addition to other cell-structure-related virulence markers, *P. aeruginosa* contains a single polar

flagellum that allows it to travel through liquid media in search of nutrients for survival and replication, moving in one direction over solid and somewhat solid surfaces is made possible by Type IV pili (Lefèvre *et al.*, 2015).

One of the biggest threats to human health is antibiotic-resistant microbes, which increase infection and mortality rates. Resistance genes play a significant role in making microorganisms more resistant to antibiotics; thus, alternative solutions, like nanocomposites, are the first line of defense against resistance development and antibiotic consumption (Huemer *et al.*, 2020). Nanotechnology is one of the most promising technologies scientists use to develop novel pharmaceuticals that transform medicine and treat various diseases. New and ongoing indications of nanomedicine's potential to improve human health and lengthen life expectancy have emerged (Kawasaki and Player, 2005). Depending on the production of micro-nano materials, which increase drug bioavailability, drug molecules are located in the intended location in the body where they can function most effectively and lower the rate of drug consumption (De Jong and Borm, 2008). Both chemical and physical processes could be utilized for preparing nanoparticles (NPs), but the chemicals employed in synthesizing nanomaterials are toxic and produce by-products that are not ecologically friendly (Li *et al.*, 2014). To synthesize NPs in an environmentally friendly way, biological entities like microorganisms or plant extracts need to be used instead of chemical synthesis.

The present study aimed to determine the antibacterial effects of green-synthesized nanocomposites derived from plant extracts against *Pseudomonas aeruginosa*, a bacterium known for its antibiotic resistance. The research focuses on using eco-friendly green synthesis methods as an alternative to conventional chemical processes involving toxic substances and harmful by-products. Additionally, the study explores the potential of nanotechnology in developing novel pharmaceuticals that enhance drug bioavailability, target specific areas of the body for maximum effectiveness, and reduce overall drug consumption. The goal was to assess the impact of these nanocomposites in combating antibiotic-resistant *Pseudomonas aeruginosa*, highlighting their role in overcoming the challenges posed by bacterial resistance to traditional antibiotics.

MATERIALS AND METHODS

Chemicals

Purchased and used without further purification were all chemicals. The antibiotic erythromycin and silver nitrate Ag(NO₃) 99.0%, USA, was from Pharco Corporation (Pharco Pharmaceutical Company (Egypt)), and distilled water was utilized to make aqueous extracts in all tests.

Synthesis of aqueous extracts of *Petroselinum crispum*

Fresh aerial portions of *P. crispum* (parsley) were obtained from Aldahan market in Karbala, (Iraq) and cleaned with tap water before being distilled to remove impurities. To get rid of any moisture, they have been allowed to dry for a few days at room temperature. After being sieved and dried, the plant components were ground into a fine powder using an electric blender. In order to deactivate the oxidase enzyme, 5 grams of parsley herb (CS) were added to 100 ml of distilled water and heated to 60 °C for 30 minutes. Whatman No. 1 filter paper was after that used to filter the Decoction. In the characterization and biological investigations, the filtrate (aqueous extract) served as a control for comparison (Helmy *et al.*, 2020).

Synthesis Ag NPs of *Petroselinum crispum*

Various aqueous extracts were added in a ratio of 2:10 to 1 mM (0.001 M) silver nitrate for synthesizing AgNPs. Two drops of 1 N NaOH were then added, and the mixture was maintained in a water bath at 60 °C for 10 minutes.

Microorganisms

This study's bacterial cultures came from the Microbial Type Culture Collection. Standard and diagnostic bacterial strains were identified as *P. aeruginosa*. These isolates were employed to assess the antimicrobial activity in vitro.. The Biology Department of Kerbala University's Education Faculty of Pure Sciences of Kerbala provided all the microorganisms used in the tests.

Experimental design

To measure the sensitivity of bacteria-prepared extracts and nanocomposite of culture, the groups were designed as follows for one treatment with two concentrations (10,20 µl), as the first treatment was considered a control group (T₁) treated with free extract Pc. In contrast, the second treatment (T₂) was treated with free nanocomposite PcNps. The third treatment (T₃) was treated with free antibiotics.

The fourth (T₄) was treated with the synergistic extract with the antibiotic *P.crispum*/ Erythromycin (Pc/ER), and the fifth (T₅) was treated with the nanocomposite loaded with the antibiotic *P.crispum*/ Nanoparticle Erythromycin (PcNps/ER). Disk Diffusion method was used to determine the sensitivity of microorganisms (Hussain *et al.*, 2020)

RESULTS AND DISCUSSION

The present study used infrared spectrophotometry to diagnose the extract of *P.crispum* (Pc), the prepared nanocomposite before and after loading the studied antibiotic Erythromycin. A peak between 2927.94-

3205.69 cm^{-1} regarding the stretching hydroxyl groups (O-H) as well as the amine group (N-H) found in compound (Pc) was displaced to the peaks between 2924.09-3182.55 cm^{-1} for the same groups. The extract's properties changed after the nanocomposite was prepared, as shown in Fig. (b/1). The results above were consistent with the findings of the study (Hussain and Khalaf, 2020), which indicates the change in the peaks of the hydroxyl (O-H) and amine (N-H) bonds after loading the antibiotic on the prepared nanocomposite. The preparation also succeeded because the triple superimposed peak of carbon 2322.29 cm^{-1} disappeared, and the peaks for the groups C = O and C = C were slightly altered. The peak at a frequency of 636.51 cm^{-1} was caused by the C-C group bond extending to a new location of 567.07 cm^{-1} in Fig. (1/b), indicating the presence of alkene bonds in a row. The existence of organic functional groups like alkanes, amine bonds, and aromatic compounds played a crucial role in the stability and production of AgNPs. Those bands, which relate to extended vibrational bands responsible for compounds like terpenoids and flavonoids, could thus be considered accountable for the efficient coverage and stabilization of the metallic bond of the silver element from the AgNO₃ compound (Hussain *et al.*, 2020). The loading of antibiotic (ER) on the extract of (PC) in the compound ER/ PC the latest stretched in the wave band between 2000-3600 cm^{-1} for the monomeric carboxyl and amine groups (OH, NH) respectively as well as the C-H group, as shown in Fig. 1c, stretching peak 3205.69, 2927.94 cm^{-1} in

Fig.1a) to the new site 3963.75, 2916.37 cm^{-1} overlapping peak 2322.29 cm^{-1} to site 2098.55 cm^{-1} , which indicates an interaction between the antibiotic and the extract and this indicates that the loading process was successful.

The results of the present study showed that the peaks of C, C=N, as well as C-C, C-O and C-N, respectively, in *P.crispum* (Fig. 1a, c), were in agreement with the findings of Mammate *et al.* (2023) whose results showed that the beams 1800-600 cm^{-1} achieved superposition and expansion. It also showed the results of the free study when loading the ER on the *P.crispum* nanocomposite (PCNp/ER); the results showed the presence of wave overlap as well as the development of new waves resulting from the stretching of the carboxyl and amine monomer groups and their displacement from their positions in the form (Fig. 1/d), as the beam stretched (3182.55, 2924.09) cm^{-1} . The shape of (Fig.1/b) to the new site (3441.01, 2873.94) cm^{-1} , respectively, and the emergence of two new bundles, namely (2962.66, 2927.94) cm^{-1} belong to the groups of the Erythromycin ER antibiotic groups, and this is an indication of the stability of the antibiotic loading with the nanocomposite. The C-O, C, and C-N groups which departed from their places before loading were among the changes in C = C, C = O, and C=N bundles, according to the data (Fig. 1/ b d). This is what was agreed upon by the study of Hussain and Khalaf (2020), which indicated that the success of the loading process would remove the arranged carbon double bonds after the nanocomposite is loaded with the

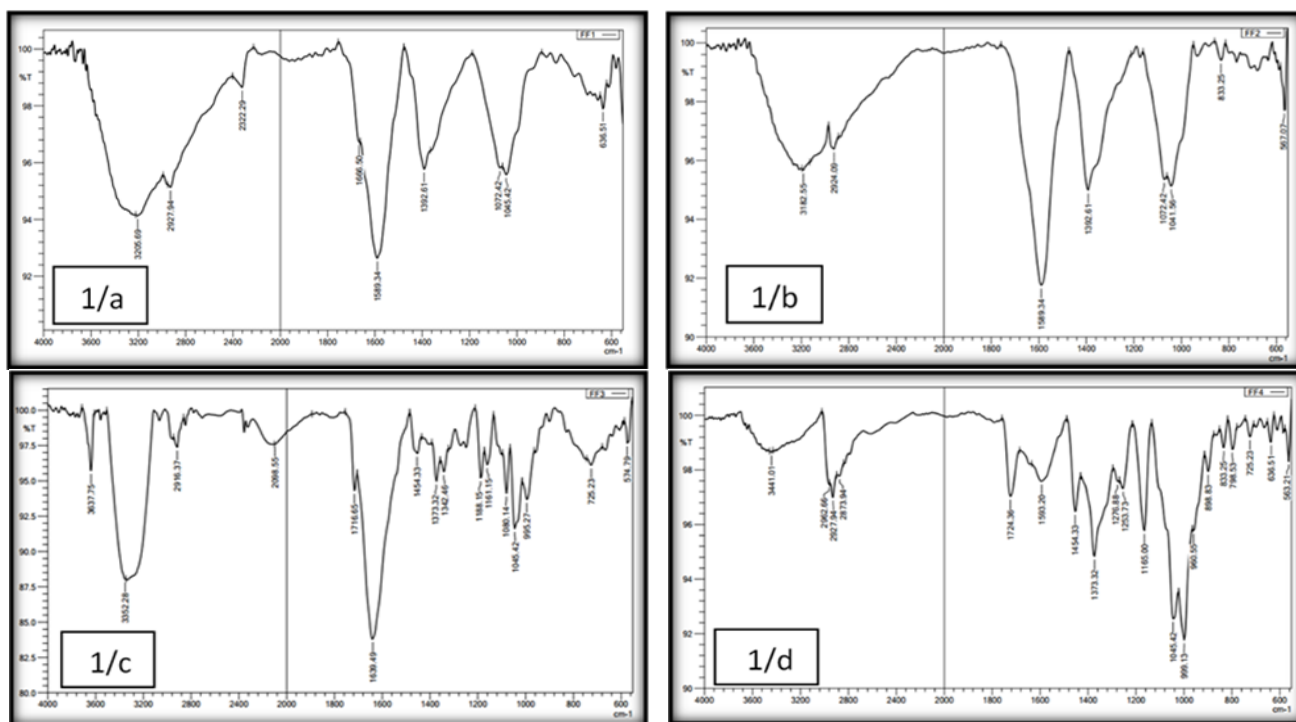


Fig. 1. FT-IR spectrum of *Petroselinum crispum* extract (1/a), free *Petroselinum crispum* nanocomposite (1/b), ER/PC (1/c), and PCNp/ER nanocomposite (1/d)

loaded compound, and here the compound was the antibiotic.

The roughness coefficient regarding the outer surface of *P. crispum* extract molecules is shown in Fig. (2/a) at 93.5 nm. After the extract was converted to a PcNps nanocomposite, Fig (2/b) shows that the compound's roughness coefficient increased to 98.4 nm, with a 4.9 nm difference between the two. This indicates that. The square root rate regarding the nanocomposite PcNps (111 nm) differs by 27 nm from the Root mean square height (Sq) of the Pc extract 138 nm. Following its transformation from the free extract, the nanocomposite exhibited crystal homogeneity. Following the transformation of the parsley extract into a nanocomposite, this extract achieved 293 nm in terms of the Texture Peak Direction Rate (Std) of the extract (Pc) and the PcNPs nanocomplex (Sp). The maximum pit height (Sv) in the parsley extract was 654 nm, but it was 374 nm in the nanocomposite.

The average volume ratio of the extract was recorded as the highest percentage (80%) for the volume 125 nm (Fig. (3/a)), while the volume ratio of the prepared nanocomposite (PcNPs) was about 80% in favour of the volume of 75 nm (Fig. 3/b), which is clear evidence of the formation regarding Nanomaterials from the extract of *P. crispum* A previous study also showed that the size of the nanoparticles is larger after loading the antibiotic than in the free form before loading, which is evidence of the success of the loading process (Hussain et al., 2020). Figure (2c,d) shows the outer surface of the particles of *P. etroselinum crispum* extract and PcNps nanoparticles before and after loading the Erythromycin on each of them. The roughness coefficient of ER was 114 nm, with a significant difference of 18.6 nm, which indicates an increase in the new crystal form resulting from the adherence of antibody molecules to the surface of the extract. The present study also agreed with the study that showed that physical

Table 1. Physical properties of nanocomposites and free compounds(μm) for *Petroselinum crispum* extract (Pc), free *Petroselinum crispum* nanocomposite (PcNps), ER/PC, and PCNp/ER nanocomposite

Physical properties of nanocomposites and free compounds	Pc (μm)	PcNps(μm)	Pc/ER(μm)	PcNps/ER(μm)
(Sa) Arithmetcal mean height	0.0984	0.0935	0.114	0.212
(Sq) Root mean square height	0.138	0.111	0.14	0.243
(Std) Texture direction	0.933	0.667	0.853	0.966
(Sp) Maximum peak height	0.279	0.293	0.352	0.398
(Sv) Maximum pit height	0.654	0.374	0.501	0.568

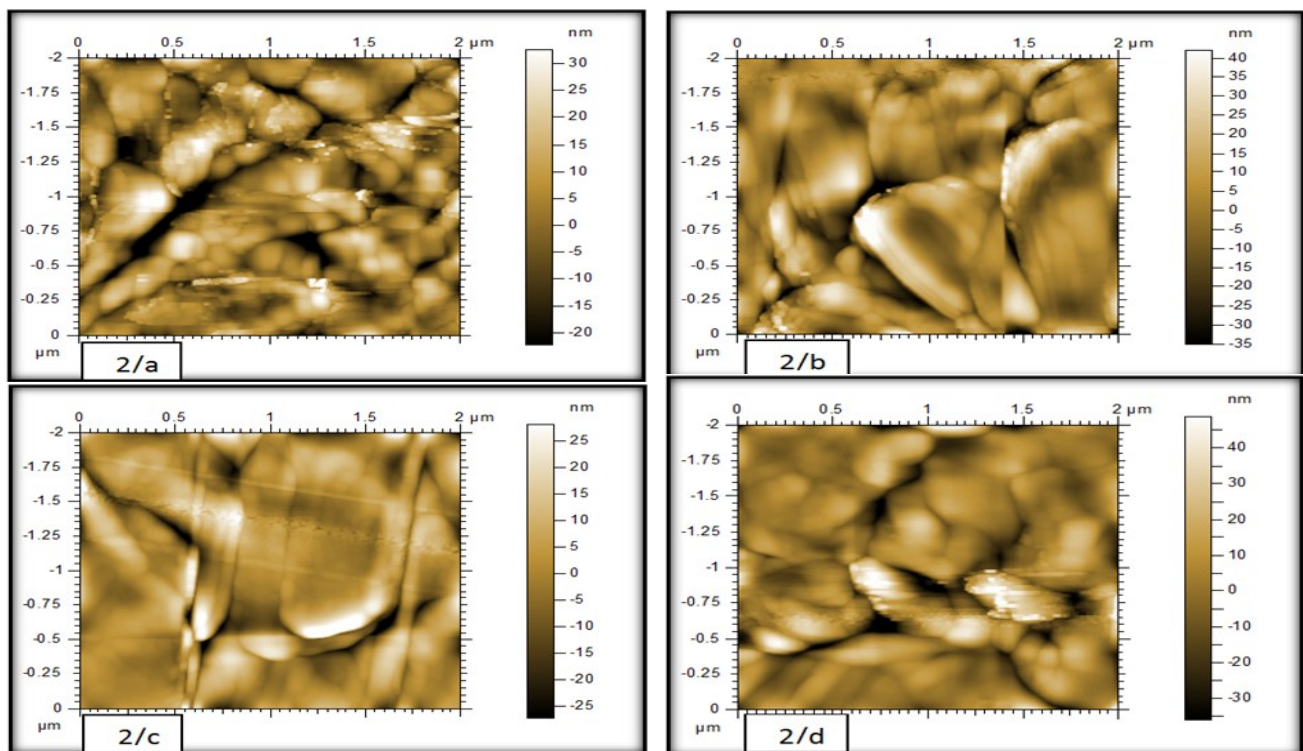


Fig. 2. Atomic force microscopy (AFM) of *Petroselinum crispum* extract (2/a), free *Petroselinum crispum* nanocomposite (2/b), ER/PC (2/c), and PCNp/ER nanocomposite (2/d)

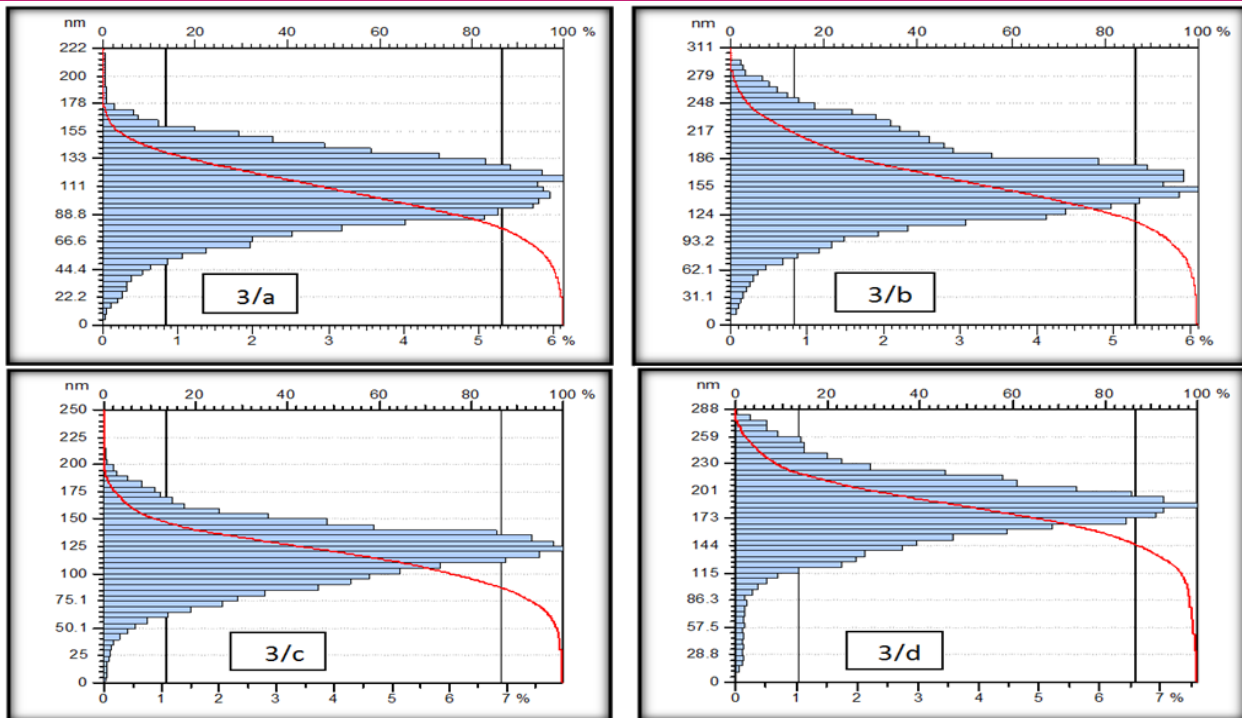


Fig. 3. Volume ratio of nanocomposites and free to *Petroselinum crispum* extract (3/a), free *Petroselinum crispum* nanocomposite (3/b), ER/PC (3/d), and PCNP/ER nanocomposite (3/d).

properties are important in the process of synthesizing the nanocomposite because they play a role in controlling the mechanism of controlling the release of loaded materials from the nano surfaces as well as changing the interaction kinetics between them because of its role in delivering the loaded materials on the nano surfaces with the least losses (Hamza *et al.*, 2022). While the roughness coefficient of the surface in the current study of the nanocomposite loaded with antibiotic ER/PCNPs was equal to 212 nm (Table 1), with a difference of 118.5 nm after it was 98.4 nm. The surface roughness, regular crystal structure, and surface homogeneity are all significantly influenced by the nano-surfaces (Youssef *et al.*, 2023). When loading the antagonist on the surface of the extract and the nanocomposite, the root means square height of ER/PCs and ER/PCNPs (243,140) nm extracts, respectively, and this increase is also a positive indicator of the homogeneity of the antagonist with the nanocomposite and the extract. The same applies to the rest of the criteria for the results of the physical properties of the prepared nanocomposite shown in Table 1, such as maximum peak height (Sp), texture direction (Std), root mean square height (Sq), maximum pit height (Sv) .

The size rate recorded a significant increase in the percentage of particles prepared from the extract and nanocomposite after loading the antibiotic on both the extract and the prepared nanocomposite. It reached approximately 165 nm by 65% for the extract Pc and 170 nm by a percentage that reached 70% (Fig. (3 c,d) which is important because it plays a role in the differ-

ence in the physical and chemical properties that compounds can provide within nanoscale sizes, unlike those provided by the same compounds in large sizes before and after downloading, as referred to by the study (Abdelfatah *et al.*, 2022).

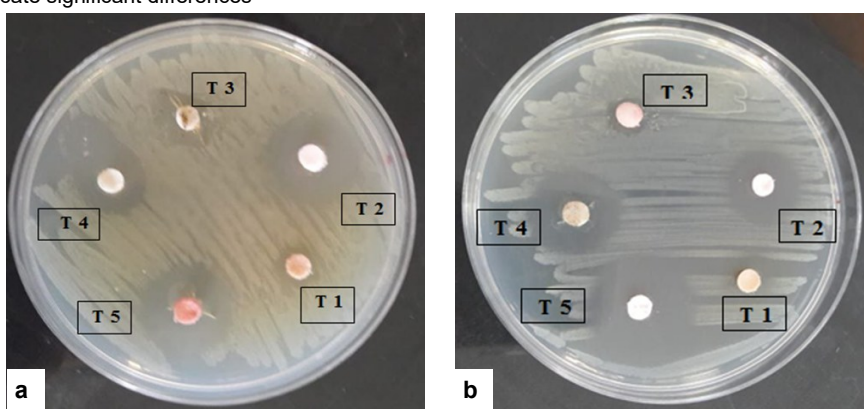
Table 2 shows that *P. aeruginosa* bacteria treated with *P. crispum* T1 plant extract at a concentration of C2 (g μ 10) 20 μ L with an area of inhibition of 1.25 ± 9.75 mm compared with the group treated with anti-ER (T₃) at a concentration of C2 /g μ 10 (20 μ L) which record 10.25 ± 0.25 mm, this indicates that many plant extracts were shown to possess antibacterial activity against microbial pathogens, (Fig.(4/a,b)), in both concentration 1,2 (C₁, C₂) respectively as studies have suggested including that of Linde *et al.* (2016). Studies have shown that these substances are lipophilic stimuli that can penetrate the phospholipid bilayer of the biofilm, changing the fluidity and permeability of the membrane that can eventually affect this type of bacteria (Vijaya-preetha *et al.*, 2021). The study showed that the preparation of nanocomposites in safe ways from organic compounds and in environmentally friendly synthesis methods gives results equal to synthesis by chemical methods, as indicated by the study of Hussain *et al.* (2020).

According to the results of a study which tested AgNPs' antibacterial activity against two types of *Staphylococcus aureus* and *Escherichia coli* bacteria with the use of the agar diffusion method, the inhibition rate reached 13.00 ± 0.81 mm when the case was treated with the nanocomposite. NPs synthesized by various plant ex-

Table 2. Measurement of the diameter of the inhibition zone mm upon free treatment and free Pc, PcNps nanocomposite before and after ER antibiotic loading in *Pseudomonas aeruginosa*

Treatment	concentration	Mean $\pm$ SE (mm) (C1= 10/ μ)	Mean $\pm$ SE(mm) (C2= 20/ μ)
Pc T ₁	C ₁ (g μ 5)	7.00 $\pm$ 0.80	9.75 $\pm$ 1.25
	C ₂ (10 g μ)	A	A
PcNps T ₂	C ₁ (g μ 3.5)	11.00 $\pm$ 0.81	13.00 $\pm$ 0.81
	C ₂ (7 g μ)	B	B
ER T ₃	C ₁ (7.5 g μ)	8.25 $\pm$ 0.95	10.25 $\pm$ 0.25
	C ₂ (15g μ)	A	A
Pc/ER T ₄	C ₁ (3.5 g μ)	13.00 $\pm$ 0.81	15.75 $\pm$ 0.95
	C ₂ (7 g μ)	B	C
PcNps/ER T ₅	C ₁ (3.5 g μ)	17.75 $\pm$ 1.50	21.00 $\pm$ 1.41
	C ₂ (7 g μ)	C	D
T ₅ /C1*T3/C ₂		10.25 $\pm$ 0.25	17.75 $\pm$ 1.50
T calculated = 8.42		df = 6	T table = 2.44 /1.94

* Different letters indicate significant differences

**Fig. 4.** a. Concentration (1), b. Concentration (2) of Inhibitory effect on the growth of *Pseudomonas aeruginosa* of (Pc T₁), free *Petroselinum crispum* nanocomposite (PcNps T₂), Erythromycin (ER T₃), *Petroselinum crispum* extract/ RE (ER/ Pc T₄), and *Petroselinum crispum* nanocomposite/ ER (PcNps/ER T₅).

tracts, such as *P. crispum* and *Beta vulgaris*, have shown activity against *S. aureus* (Al-Ebadi *et al.*, 2021). According to the study (Hussain and Khalaf, 2020), loading the antibiotic increases effectiveness. The present study observed the maximum inhibition rate when the antibiotic synergizes with the AgNps nanocomposite, where the inhibition rate was significant at $p \leq 0.05$ to 21.00 ± 1.41 mm. The results were consistent with the findings of Hussain and Khalaf (2020), which state that putting the anti-ER on the copper nanoparticle causes the rate of inhibition to nearly double. This aligns with the study of Snoussi *et al.* (2016). The present study also agrees with the results of a study (Almjalawi *et al.*, 2022) that concluded that Depending on the difference in charges, an ionic exchange occurs, which helps in the binding of nanoparticles to the surfaces of bacterial cell membranes and that the primary mechanisms by which organic compounds destroy bacterial cells because of the antimicrobial activities of NP metal are the production of ROS and free radicals. Additionally, their interactions with essential intracellular components (ribosomes, DNA, and RNA) alter and disrupt their biologically active processes, inhibiting bacterial growth (Almjalawi *et al.*, 2022). Numerous studies agree with

the present study's findings that have demonstrated AgNPs adherence and accumulation on the surface of bacteria, particularly Gram-negative bacteria, indicating that AgNPs have an inhibitory bioactivity for bacteria (Bruna *et al.*, 2021). The size created in the present study was within the limits that proved that there is an inhibitory effect for the bacteria under study, and this is what previous studies have concluded, concentration regarding NPs; the fact that AgNP activity is highly dependent on size, and the fact that AgNP accumulation on a cell membrane causes gaps in the bilayer, rendering it vulnerable to increased permeability and, ultimately, bacterial cell death. AgNPs smaller than thirty nanometers have been discovered to have maximum bactericidal activity against some types of bacteria. Smaller NPs can penetrate bacteria better (Vanitha *et al.*, 2017).

Conclusion

In the present study, nanotechnology was used by preparing a nanocomposite from the extract of *P. crispum* plant and diagnosing the nanocomposite using the technique of the FTIR and AFM before and after load-

ing the antibiotic Erythromycin and measuring the volumes in both cases. The preparation results showed that all sizes were within the ideal nanometer measurements, while the application results indicated the possibility of breaking the resistance of *P. aeruginosa* by obtaining areas of inhibition diameters on bacteria from the antibiotic in its free form and at concentrations relatively lower than usual with significant differences, which contributes to significantly increasing the breaking of the resistance of the studied bacteria. Despite the spread of bacterial antibiotic resistance, focus must be placed on discovering alternative methods to combat this growing phenomenon by inventing environmentally friendly materials safe for humans, controlling their mechanism of action, and targeting effective sites in microorganisms. This study may open the door to more applications of this technology by using other plants and different preparation methods that include different resistant bacterial species. It may also be helpful in developing the pharmaceutical properties of other antibiotics used to protect humans and their plants, increase animal productivity and preserve the environment.

Conflict of interest

The authors declare that they have no conflict of interest.

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