

Preparation of Nanoparticles of Selenium / Zinc Oxide Reductase in an Environmentally Friendly Green Synthesis Method and Studying Their Effect on *Pseudomonas Aeruginosa*

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Abstract :

The current study included testing the two nanocomposites (curcumin / zinc oxide Nps) (curcumin / selenium Nps) on *Pseudomonas aeruginosa* which isolated from burn injuries and surgical wounds, after it was diagnosed by manual and biochemical methods. Two nanocomposites, namely (curcumin / zinc oxide Nps) and (curcumin / selenium Nps) were prepared and charecterised by: UV-Visible spectroscopy and Fourier-transform infrared spectroscopy, infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscope (SEM), and evaluation of the inhibitory efficacy of nanocomposites and free curcumin in addition to several types of antibiotics (gentamycin ,imipenem). It was highly effective against the studied bacteria. The MIC test was performed by broth dilution method, and it was found that the minimum inhibitory concentration of curcumin was 500 µg / ml. And the MIC for cur / zno NPs was 2000 µg / ml. Whereas, the MIC for cur / se NPs was 250. The results also indicated an increase in the effect of nanocomposites on the bacteria compared to the free curcumin, and this increase resulted from the synergistic action between the two components of the nanocomposite. The cur / zno Nps results were close to those of gentamycin at 8 mg / ml, and the cur / se Nps results were close to those of the imipenem antibiotic for *Pseudomonas aeruginosa*.

Introduction

Infectious diseases resulting from infection with microorganisms in general and bacteria in particular are the most common diseases around the world and it causes millions of deaths every year (Singh, Krishnamurthy, & Mathew, 2014). There are many bacterial species that are capable of causing diseases to humans and various living organisms as a result of their possession of a number of structural, chemical or genetic characteristics that can constitute a pathogen for humans and other organisms and these factors can be called together as virulence factors that help The bacterial cell causes damage to the host's body, such as producing toxins and a number of other enzymes (Murray *et al.*,2020).

The genus *Pseudomonas aeruginosa* is characterized as one of the bacterial genera widespread in nature and in different environmental conditions as it can be isolated from the bodies of different organisms such as humans, various animals and plants, as it is characterized by the ability to withstand the lack of food in the environment in which it is located as well as its resistance to different environmental conditions and As it can be found in the natural environment and sterile hospital environment, according to CDC and data collected between 1968 - 1998, *pseudomonas* is the fifth most inflammatory contaminant in US hospitals(Lister *et al.*, 2009). *P. aeruginosa* is an opportunistic pathogen that affects immunocompromised patients who are placed in intensive care units after surgery, and those with burns and lung diseases, particularly cystic fibrosis(Awan *et al.*, 2019). The excessive and frequent use of antibiotics has made the emergence of resistant generations and strains of bacteria a global problem (Kariminik & Nazoori, 2018).

From this context, it can be said that there are two main challenges that health organizations and treatment centers faces in the world today: the spread of infection in hospitals and health centers around the world, its rapid transmission from one country to another, and the emergence of new strains and genus of resistant pathogenic bacteria to existing antibiotics. antibiotic resistance bacteria (Niakan *et al.*, 2019). Therefore, studies are heading to solve this problem by searching for alternatives such as nanomaterials for some metals and their oxides, which have appeared as a promising alternative to antibiotics in the last few years (Kariminik & Nazoori, 2018). Many of the elements and metals that were classified as safe for micro-organisms in the natural state showed high toxicity at the nanoscale level, due to the increase in the specific surface area and the increase in the high reactivity of these

particles (Emami-Karvani & Chehrizi, 2011). Also, parts of some plants and their alcoholic and aqueous extracts were used as antidotes and treatments for cases of bacterial infections in general, as a result of the effective compounds they contain with therapeutic properties (Lawhavinit *et al.*, 2010). Turmeric is one of the spices obtained from the *Curcuma longa* L. plant and one of the plants widely used in the medical field because it contains phenolic compounds and antioxidants, which can make its use available to treat fungal bacterial infections, fight cancer and analgesic pain. Ulcerative treatment (Altunatmaz *et al.*, 2016).

Turmeric plant extract contains sesquiterpene compounds resulting from the breakdown of the terpene molecule and polyphenols called curcuminoids, as well as ferulic acid derivatives responsible for most of the medicinal efficacy of the turmeric plant, and antioxidants are likely to contribute to the inhibition of The growth and development of tumors, as well as its antisclerotic action, as many studies have indicated its anti-microbiological effectiveness, especially with oral bacteria (Czernicka *et al.*, 2019). the statistics indicate that it is safe when consuming 8 grams of it per day for a period of 3 consecutive months for healthy people, and it was safe and showed therapeutic ability when used at a dose of 8 grams per day with the compound gemcitabine used to treat pancreatic cancer (Teow *et al.*, 2016). The effectiveness of curcumin against microorganisms appears on two different levels, as it can be used to prevent and treat wounds from pathogenic bacteria contaminating them, and it can also be used as a protective material for preserved food against different pathogens, and it has been evaluated by the FDA's expert committee and World Health Organization (WHO) as a natural food additive extracted from plants and it can be manufactured chemically, but when chemically manufactured it cannot be added to food (Altunatmaz *et al.*, 2016). Nanoparticles are defined as tiny particles of at least one dimension with a size ranging from 1- 100 nm (Christian *et al.*, 2008). Nanoparticles have unique properties in electronic, magnetic and chemical stimulation, they are characterized by high stability and little reactivity, and they have biocompatibility, they also lack toxicity and come in many forms, and this is what makes them widely applied in various fields of biomedicine such as diagnosis, treatment of cancers and Other diseases, drug manufacturing, and gene delivery, and some metal nanoparticles have anti-viral, bacterial, fungi, and some tumor properties (Vetchinkina *et al.*, 2018).

Selenium nanoparticles are more attractive to researchers compared to other minerals, due to its low toxicity compared to organic and inorganic nanoparticles, as inorganic nanoparticles are more toxic than organic, and selenium nanoparticles have important biological properties as it is characterized by its small size, which facilitates its absorption by cells. It can also be used in the nonoxidation state of Se0, which is less toxic compared to other oxidation states (Se + IV, Se + VI), and although it is unstable and may become ineffective quickly, but addressing this problem by encapsulating it with a substance that expresses From nano-vehicles such as chitosan (CS) (Hosnedlova *et al.*, 2018), nanoparticle selenium is a good choice for use as an anti-microbial growth due to its unique phenotypic and chemical properties, It was used as a preservative by coating the metal cans used to preserve food in it (Rajan *et al.*, 2019). It has been shown that it binds to the cell envelope by means of the chemisorption mechanism to penetrate the cells via lipopolysaccharides and through the change in the composition of the three enzymes: prelipoprotein diacylglycerol transferase, lipoprotein signal, apolipoprotein N-acyltransferase and peptidase which play a pivotal role in maintaining cell life (Guisbiers *et al.*, 2016).

ZnO-NPs is an antimicrobial agent for many pathogenic and food-spoiling microorganisms, and ZnONPs is characterized by its wide applications as an anti-microbial agent compared to nanoparticles of other metals. Of reactive oxygenation and disruption of cell membranes as a result of accumulation of ZnO-NPs (Souza *et al.*, 2019). And zinc oxide nanoparticles have the ability to inhibit the growth of Gram negative and Gram positive bacteria (Ugaili *et al.*, 2014). To test the effectiveness of ZnO-NPs in controlling pathogenic bacteria, Lee *et al.* (2014) studied its effect on the growth of *Pseudomonas aeruginosa* and its virulence factors and colony formation, and the results showed the ability of nanoxide to inhibit pyocyanin production. Before the bacteria, the production of the hemolysin enzyme that degraded red blood cells decreased, in addition to the fact that the product of it decreased its effectiveness in breaking down blood cells. The results also showed a decrease in colony building by 95% due to the presence of the nanocomposite.

Materials and Methods

preparation of Cur/ZnO nanoparticle was done by following the method described by (Bashi *et al.* 2013) with some modification in preparing ,1 gram of curcumin was added to a solution of zinc

oxide (resulting from dissolving 1 g of zinc oxide in 50 ml of distilled water). The mixture was stirred by a magnetic stirrer at room temperature for 24 hours and then the mixture was placed in the vibrating incubator at a temperature of 40 ° C for 18 hours. The color of the solution changed from pale yellow to reddish yellow. The precipitate was separated by a centrifuge and then washed with distilled water to remove the ions several times, and then the residue was dried in an electric oven at a temperature of 50 ° C, and then grinded with good ceramic mortar to obtain a fine powder and it was stored in the refrigerator until it was used in the study. and to prepare Cur/Se nanoparticle 1 gram of curcumin was added to 2 ml of selenium solution (prepared by dissolving 0.263 grams (0.01 mol) in 100 ml of distilled water) and then placed on a magnetic stirrer at a temperature of 40 ° C. The color of the solution changes from pale yellow to reddish yellow, then the permeability of the solution is measured in the Spectrophotometer, after that the solution is placed in the shaker incubator for a period of 24 hours, and the residue is separated by a centrifuge and finally washed with distilled water Several times after that it was dried at a temperature of 50 ° C, then grinded well to obtain a fine powder and stored in the refrigerator until it was used in the study.

To characterize the Cur/ZnO, Cur/Se nanoparticle four exams was used. **first**, UV-Visible Spectroscopy, were Curcumin / selenium nanoparticles characterized by dissolving 500 mg of the prepared substance in 4 ml of distilled water, then subjected to ultrasound for 15 minutes, and then measured in an ultraviolet spectrometer, as both visual examination and absorbance measurements were detected. The apparatus was cleared by means of Distilled water was then screened with a wavelength range of 200-800nm. This test is used to confirm the formation of curcumin / selenium nanoparticles. **second**, X-ray Diffraction Analysis, this exam was used to explains the difference in layer thickness before before and after the intercalation process by using Brack's law $n\lambda = 2d\sin\theta$ to get the value of the layer thickness

Where:

n: the order of the crystal plane from which the X-ray diffraction is. λ :

The wavelength of the X-ray used (copper tube).

θ : the diffraction angle of the X-ray.

d: the vertical crystal distance between two parallel planes.

Third, the scanning electron microscope (SEM) was used to determine The surface morphology of the two nanocomposites, which done by dispersed and dried NPs were placed over an aluminum heel containing a double-stick conductive carbon tape and coated with gold under an atmosphere of argon using a spray coating. **Fourth**, the Fourier-transform infrared spectroscopy (FTIR) which is a unique technology that allows to perform biochemical analysis of biological samples, functional properties, and structural information of a compound. The plant extract has distinct peaks in the FTIR spectrum, indicating the presence or absence of functional groups.

The infrared spectrum of each compound of the free nanocomposites under study as well as the free form therapeutic materials as well as the nanocomposites were studied after loading the treatment on them, as a tablet of each of these compounds was made with potassium bromide (KBr) after being well ground and measured. The infrared spectrum in the range of wave numbers (400-4000) cm^{-1} . All visible beams were fixed as most of the main beams were diagnosed.

After the nanocomposites was examined, the Minimum Inhibitory Concentration (M.I.C.) was determined by broth dilution method as following:

1. same quantities of bacteria were grown in a liquid medium, which is Muller Hinton Broth, in sterile test tubes with the number of concentrations required to be prepared from the antagonist (the number of bacteria is 10^5 - cells / ml).

2. Adding increased concentrations, as the first tube (No. 1) contains the concentration (zero) of the nanomaterial, then it is followed by the second tube (No. 2) that contains the lowest concentrations of the nanomaterial, followed by the third tube (No. 3) that contains the concentration of twice as much It is present in tube No. 2 and so on for the rest of the tubes. Concentrations of 62.5-2 mg were used.

3. All tubes are incubated at a temperature suitable for the studied bacteria. The tubes are examined after incubation to identify any of them that contain growth as an indication of turbidity. The clear tubes indicate no growth due to the effectiveness of the antagonist.

4. The first clear tube that comes after a series of cloudy tubes is tested so that the concentration of the nanoparticle in it is the MIC.

The last step in this work is determination the Antibacterial Activity of Cur/ZnO, Cur/se NPs by Disk Diffusion Assay as Morello et al., (2006) mentioned and according to the following steps:

1. The bacterial isolates were activated on a nutrient agar and were incubated in the incubator at 37 ° C for 24 hours.

2. A small amount of the growing bacteria colonies were transferred by the Loop to tubes containing the physiological saline solution and mixed well. The turbidity of the growth was compared with the turbidity of the standard McFarland standard 0.5: turbidity solution.

3. 20 µl of the bacterial suspension was placed by a micropipette, then spread by a glass diffuser (Lshape) on the surface of the Muller-Hinton agar homogeneously, after which the dishes were left to dry at room temperature for a period of (10-15) minutes. .

4. Sterile tablets were prepared and saturated with serial concentrations of nanocomposites (8,6,4,2 mg / ml) by means of a micropipette, then these tablets were transferred with sterile forceps to the dishes, then the dishes were incubated at a temperature of 37 ° C for 24 hours. As for the antibiotic tablets, they were transferred directly to the plates and incubated in the same way.

5. The results were read by measuring the diameters of the damping zones in mm around each disc.

results

UV-Visible Spectroscopy for cur/se Nps show that The change in the color of the prepared solution from yellow to reddish orange is due to the plasmon resonance peak, which lies between the 300 nm-450 nm (Snigdha, Sugathan, & Mathew, 2017). The examination is used to show the type of plasmon on the surface of the nanomaterial, When the wavelength nm is plotted on the x-axis versus the absorption on the y-axis, we see the height of the plasmon as in Figure (1). The emergence of the high peak is the spectral pattern that occurs due to the excitation of the local surface plasmons that cause strong scattering of light by an electric field of a specific wavelength where the resonance phenomenon occurs (Deepa & Ganesan, 2013). The rise starts from (300 nm) and is in the form of a parabolic oval from the top and has a peak representing the highest absorption located at (450 nm) and then falls after. The oval shape with its head up and its base down is evidence of a nanomaterial that begins to be affected when exposed to visible ultraviolet rays. The apparent beams that were observed in the nanocomposites of the main compounds (curcumin + selenium) clearly indicate that the interaction between the two components occurred and the formation of a stable nanocomposite.

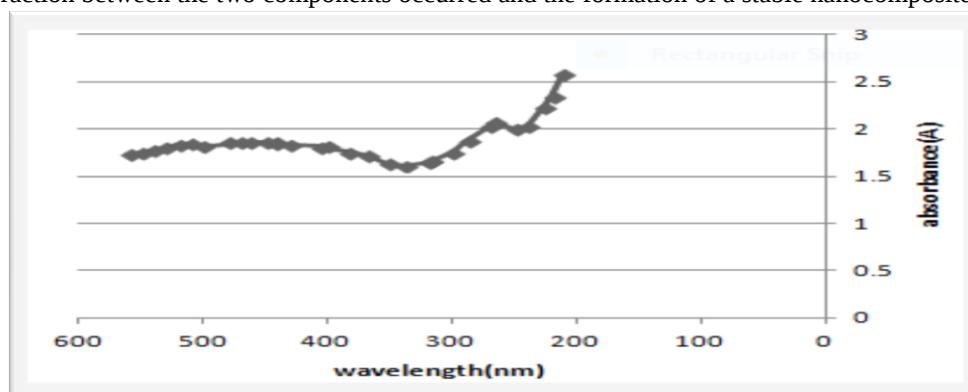


Figure 1: UV-vis spectroscopy of the cur-se Nps nanocomposite

Based on the X-ray Diffraction Analysis for cur/se nps The spectrum in Figure (2) shows that the angles before 30 ° are indicative of selenium, which means that curcumin has reduced selenium to a

nanocomposite and its beams appeared in a clear and sharp form, which are 19.72° , 25.13° , 36.33° , 44.44° and with a crystalline distance of 674.80, 1000.00, 53.91 and 55.07 nm respectively.

And from the X-ray Diffraction Analysis for cur/zno Nps We note that the spectrum in Figure (3) is zinc oxide, and curcumin was not clear between the layers and did not give crystals, perhaps because it is squeezed horizontally, so it does not give height to the crystals, but it is very clear with respect to zinc oxide, which is at the angles 34.73° , 47.81° , 52.33° , 56.71° , 62.33° , 67.36° . With crystal distances of 1000.00, 221.75, 16.52, 213.27, 536.37, and 375.84 nm respectively, there is one peak that indicates curcumin at 26.23° and a crystalline distance of 38.14 nm.

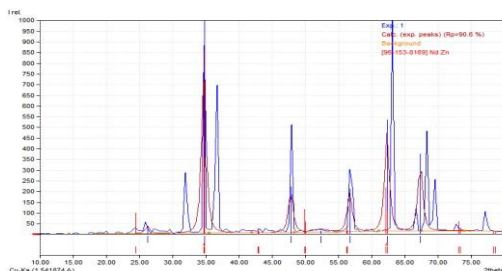


Figure (3) X-ray diffraction (XRD) spectrum for zinc oxide (ZnO) formed by curcumin. (seNPs) formed by curcumin.

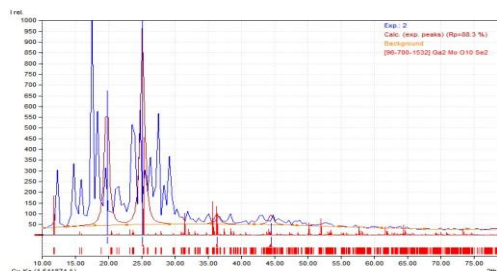


Figure (2) X-ray diffraction (XRD) spectrum for selenium nanoparticles

the second exam was Scanning Election Microscope for cur/zno Nps and it was clearly shown In figure (4), the appearance of ZnO as a hexagonal crystal in Image No. (1) was enlarged in Image No. (2) and appeared in the form of layers of zinc oxide, perhaps jammed between curcumin, but not completely clear. It was also enlarged in Image No. (3) and granules appeared to us. Zinc oxide is clear on the surface, and in picture No. (4) one molecule was taken and confined between two lines and its diameter was calculated, which is at d1 it is 61.32 nm and at d2 it is 26.49 nm and at d3 it is 17.37 nm is an indication of the occurrence of a reduction due to the presence of zinc oxide particles of different sizes, which It means that curcumin has reduced zinc oxide (ZnO) to molecules of different sizes.

Fig. No. (5) shows the Scanning Election Microscope for cur/se nps , Image No. (1) shows the layers of precipitated selenium reduced in the form of clear crystals. In pictures No. (2) the layers were enlarged and above the layers granules or balls of nanoparticles appeared. In Image No. (3) they have clusters. The balls are in the shape of a cauliflower, in picture No. (4) certain balls were taken and limited between two lines and the measurements in d1 were 45.59 nm and d2 equal to 24.60 nm and at d3 equal to 31.23 nm indication p is that the reduction is not homogeneous and it shows molecular sizes between (24-45 nm) This indicates that the reduction is either in the form of selenium metal or selenium oxide (selenium dioxide)

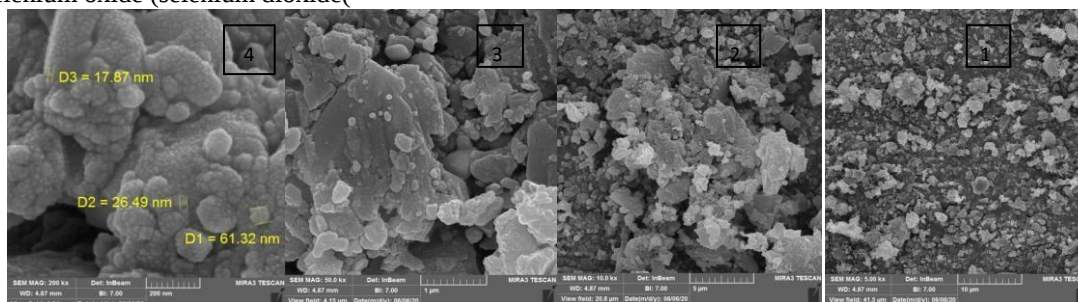


Figure (4) the cur-zno Nps nanocomposite under a scanning electron microscope (SEM)

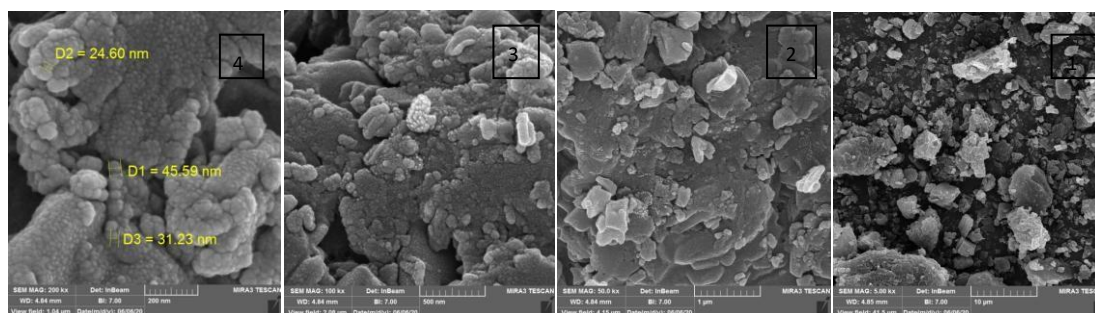


Figure (5): the cur-se Nps nanocomposite under a scanning electron microscope (SEM).

The next examination that the samples underwent was The ftir technique which is one of the important techniques for viewing the effective groups on the surface, and from these groups we infer the process of manufacturing nanocomposites, in the selenium spectrum as shown in Figure (6) We see the existing beams which are 3439 cm⁻¹ low intensity beam (2922, 2854, 2360,2342) cm⁻¹, and these beams are due to the synthesis of nanocomposites from selenium nanoparticles that have been reduced with curcumin juice; Where a process of stabilizing and reduction occurred by the action of the compounds present in the curcumin juice, as the compounds that act on the reduction act first and then those that fix the nanoparticles..

The beams (1457, 1636, 1734) cm⁻¹ are attributed to the vibration of the O-H bond resulting either from water or from alcohol. As for the amino NH groups, the bond elongation is 3439 cm⁻¹. Elsewhere, the beam (1734) cm⁻¹ means the presence of O-H and. N-H beams at (2800-2900) cm⁻¹ are attributed to the compounds containing the C-H bond. The beam at (2360) cm⁻¹ is attributed to the carbonyl COO bond, the beam at (2842) cm⁻¹ is specific to the amine group of NH. The beams at (1457,1057) cm⁻¹ are due to the absorption of the C-H bond. The last beam, between (600-400) cm⁻¹, is attributed to the formation of the nanocomposite of selenium, which is either selenium oxide or selenium dioxide.

The peaks corresponding to the expansion and vibration of the bonds in NH₂, COOH, CH₂, O-H indicate the presence of reducing groups that have reduced and fixed selenium molecules in the form of a nanocomposite, as three-dimensional particles were formed (Sampath et al., 2013).

Secondary metabolites are the main agents of nanoparticle biosynthesis, and plant extracts contain phenol, alcohol, amine, carboxylic acid, alkaloids and terpenoids responsible for reducing and stabilizing nanoparticles (Jha et al.). al., 2009). in cur/zno spectrum in Figure (7) many packages, 3400,2800,2900, 1600, 1450, 1200 cm⁻¹ that are attributed to the hydroxyl (OH), amine (NH₂) and carboxylate (COOH) groups, meaning we have a mixture of hydrocarbon compounds, amino acids or acids. Carboxylic works on the process of reduction and fixation, the bundles returning to the curcumin juice are visible between the layers of zinc, the bundle between 400-600 cm⁻¹ is due to zinc oxide, an indication of the presence of these compounds between the layers of zinc oxide.

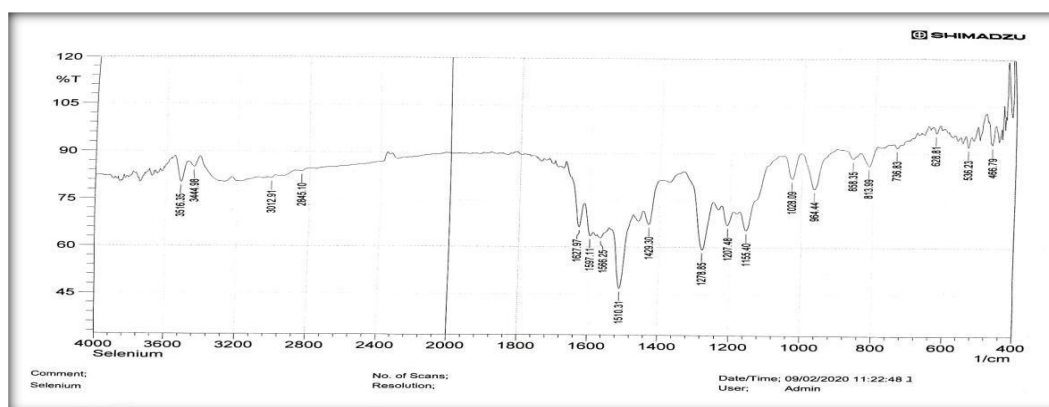


Figure (6): FT-IR spectrum of nano-selenium formed by curcumin.

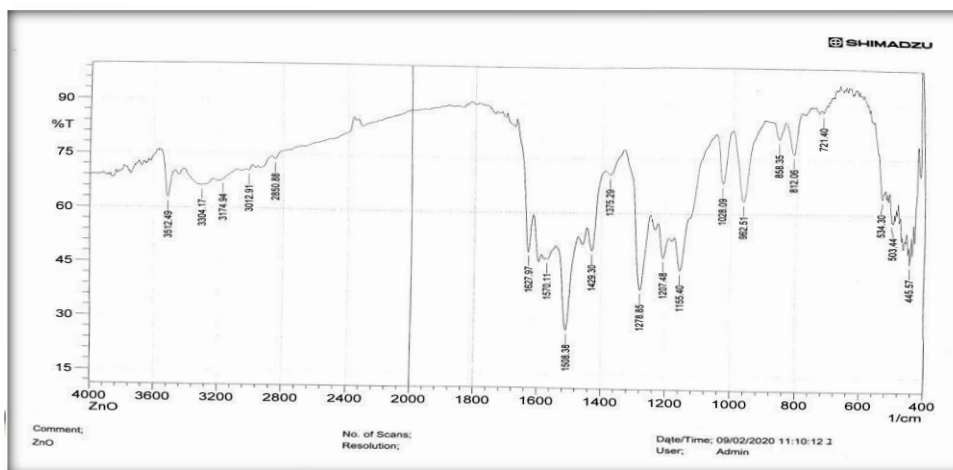


Figure (7): FT-IR spectrum of nanostructured zinc oxide formed by curcumin.

50 bacterial isolates were collected from *P.aeruginosa*. These isolates included swabs from burn injuries and surgical wounds, and they were collected from the Turkish Hospital in Babylon and Musayyib General Hospital. The agricultural and biochemical characteristics were studied in addition to the API20e diagnostic kit, where the germ colonies appeared large and clear and had a smell similar to the grapes smell, most of which produced the dye. Isolates were grown on MacConkey agar, as the colonies appeared pale, indicating their inability to ferment the sugar lactose.(Baron et al., 2007), on the solid cetrimide agar medium containing Cetrimide at a rate of (0.03), the isolates grew as this percentage does not affect the *Pseudomonas aeruginosa* but inhibits the growth of the rest of the germs ,The colonies appeared in the greenish yellow that is due to the production of the pigment pyoverdine,Or the greenish blue that returns to the production of the pigment pyocyanin, which fluoresces when exposed to UV-light (Sudhakar et al., 2015). When grown on solid blood agar only, Blactamase was completely analyzed (Procop et al., 2017). As for the results of the microscopic examination after staining with Gram stain, the cells of this germ appeared in the form of Gramnegative bacillus cells, and this is consistent with what was mentioned byCappuccino et al., (2018). The shape of the bacteria can be observed under the microscope after staining in Figure (8).

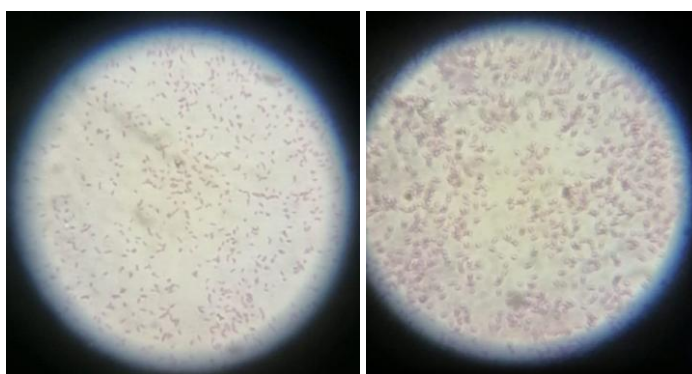


Figure (8) stained *Pseudomonas aeruginos* Under the microscope.

The API 20e diagnostic kit was used to give an accurate diagnosis. It is a plastic strip containing microtubules containing dried materials and is an effective system for diagnosing *P. aeruginosa* (Shayegani et al., 1978). It was found through the results that all the isolates obtained in this study are *P.aeruginosa*, after analyzing the results and comparing them with the index for the test.

After characterization of the bacteria the Minimum Inhibitory Concentration (M.I.C.) was done by using 6 serial concentrations of nanocomposites $\mu\text{g} / \text{ml}$ (2,1,500,250,125,62.5) by the broth dilution method, the MIC of free curcumin was 500 $\mu\text{g} / \text{ml}$ for *Pseudomonas aeruginosa*, and its mic was 256 $\mu\text{g} / \text{ml}$ according to Asadian *et al.* (2019). and (125 $\mu\text{g} / \text{ml}$) according to Gholami *et*

al.(2020). The MIC for cur / zno NPs was 2000 µg / ml. While the MIC for cur / se NPs was 250 µg / ml, Boroumand *et al.* (2019) indicated that the MIC was 250 when using Ascorbic acid as a reducing agent for selenium instead of curcumin.

Table (1): LSD inhibition diameters for curcumin, cur / zno Nps, cur / se Nps, IMP.10 and CN.30 in *pseudomonas aeruginosa*.

pseudomonas aeruginosa					
	lsd	Mean &Std. Deviation	concentration	materials	Bacterial name
lsd	0.58	12.22±1.44	2 mg/ml	curcumin	pseudomonas aeruginosa
		12.22±1.44	Total		
0.26		7.57±1.03	2 mg/ml	curcumin/zno NPs	
		9.37±1.11	4 mg/ml		
		11.13±1.19	6 mg/ml		
		12.93±1.22	8 mg/ml		
		10.24±2.31	Total		
0.35		10.13±1.47	2 mg/ml	curcumin/selenium NPs	
		13.13±1.51	4 mg/ml		
		15.97±1.79	6 mg/ml		
		18.96±1.36	8 mg/ml		
		14.55±3.62	Total		
		24.56±4.37	2 mg/ml	IMP.10	
		24.56±4.37	Total		
		16.88±4.44	2 mg/ml	CN.30	
		16.88±4.44	Total		
	0.62	14.27±6.68	2 mg/ml	Total	
		11.25±2.30	4 mg/ml		
		13.55±2.86	6 mg/ml		

		15.95±3.28	8 mg/ml
		12.25±2.45	4 mg/ml
		14.71±2.87	6 mg/ml
		17.20±3.59	8 mg/ml

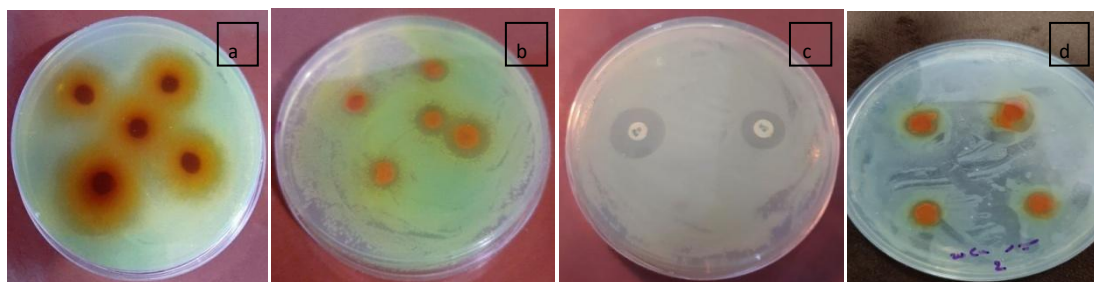


Fig. (9) Inhibitory diameters of all compounds used in the research on Mueller Hinton agar. (a) cur / se Nps (b) cur / zno Nps. (c) CN + IMP. Antibiotic. (d) cur on *p.aeruginosa*.

After comparing the values of the inhibition diameters of the antibiotic Gentamycin 30 imipenem10, it was found that the cur / zno Nps, cur / se Nps nanocomposites are good inhibitors of bacterial growth and show anti-bacterial activity similar to the activity of the studied antibiotics at certain concentrations, where the results of cur / zno Nps are close. From the results of gentamycin antibiotic at a concentration of 8 mg / ml and the cur / se Nps results are close to the results of the antibiotic imipenem, and the percentage of resistant and sensitive isolates can be seen in Table (2) and Fig. (10).

Table No. (2) represents the rates of sensitivity and resistance of bacteria to antibiotics

		Observed N	%	Q2	p.value
IMP.10	sensitive	124	0.83	64.03	0.00
	resist	26	0.17		
CN.30	sensitive	90	0.60	6.00	0.01
	resist	60	0.40		

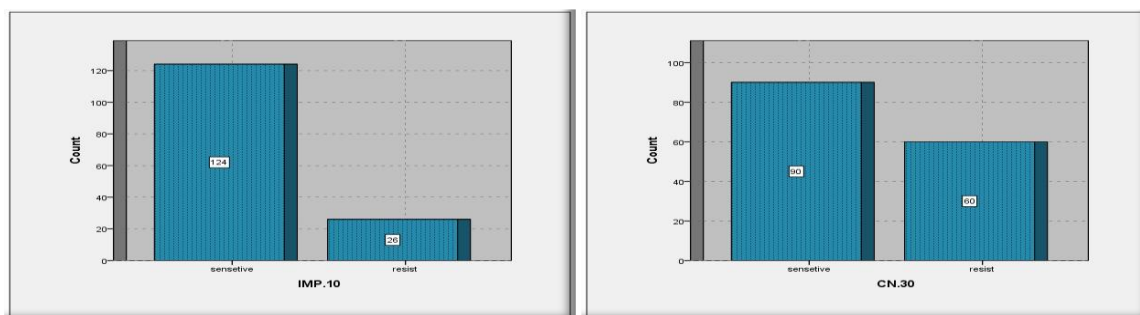


Figure (10) Sensitivity and resistance of bacteria to each antibiotic studied

Discussion

It has been shown that curcumin has an anti-bacterial effect, as it has shown its ability to weaken the bacterial membrane, Regardless of its response to the Gram stain, leading to its degradation (Tyagi et al., 2015). We find that the inhibition ratio of cur / zno is higher than curcumin alone . It is worth noting that the preparation of the nanomaterial was using 1 g of curcumin to 1 g of zinc oxide. That is, the free curcumin concentration was double the concentration of the cur / zno nanocomposite, this means that there is an increase in the effect of the nanocomposite on bacteria compared with curcumin alone. This increase is the result of the synergistic action between curcumin and zinc oxide. The study of Perera *et al.* (2020) mentioned that the inhibition ratio of cur / zno is higher than curcumin alone, indicates that the degree of efficacy of nanomaterials depends on the size. Therefore, the nanomaterial gives better results than the free material, as it gave high cur / zno Nps inhibition results, indicating that there is an additive effect between zinc oxide and curcumin, so this mixture can be used. As an antibacterial and medicinal drug. Curcumin is hydrophobic and therefore strongly binds to the bacterial membrane and provides a platform to increase the activity and effectiveness of nanocomposites (Snigdha et al., 2017). The main proposed mechanisms for cur / zno Nps include the destruction of bacterial cells through the generation of reactive oxygen species and the release or release of ions (Zn²⁺). (Sirelkhatim et al. 2015; Augustine et al. 2014).

It is possible that zinc oxide nanocomposites have toxic effects at high concentrations, as well as curcumin on the other hand, despite its therapeutic properties, has weak bioavailability (Rasmussen et al. 2010). Thus, the current study benefited from the best of both substances from Therapeutic effect even at low concentrations, knowing that the use of lower concentrations of these substances reduces or eliminates the toxicity dependent on these concentrations. It can also be observed in Table No. (1) that there is a direct relationship, i.e. the higher the concentration, the greater the inhibition rate. This was also indicated by Rajeshkumar *et al.*(2018). Nanoparticle selenium prepared by green synthesis and reduced with Ascorbic acid gave inhibitory droplets estimated at 9.25 for *Pseudomonas aeruginosa* according to Boroumand and his cohorts (2019).

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