RESEARCH ARTICLE

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Biosynthesis of selenium nanoparticles by Candida albicans and them antimicrobial effects

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ABSTRACT

This paper aims to testing the biological effectiveness of nanoparticles against types of pathogenic bacteria and fungi and opening promising horizons for the use of safe and effective alternatives with fewer side effects as alternatives to traditional antifungals or antibiotics to which pathogenic microorganisms have shown an ever-evolving resistance.

Keywords: Nanoparticles, Candida, Biosynthesis, Antimicrobial

INTRODUCTION

Cause Microorganisms in general and bacteria in particular cause various infections, some of which are infectious, which is one of the main reasons for the spread of these infections. (Ferreira et al., 2022).. cause Bacterial infection in death Millions of people all over the world(Hou et al., 2023)Bacteria are prokaryotic organisms, simple in structure, distributed, and highly resistant to extreme conditions, and these are the most important reasons for their wide spread. (Shu & Huang, 2022). After successful application of penicillin And The golden age of antibiotics has begun And Discovering a wide range of antimicrobial agents (Cunha et al., 2019). Many types of bacteria began to show a variety of resistance to these antibiotics(Hansson Brenthel, 2022) where This evolutionary and induced behavior provides bacteria with the ability to fight off drugs that are supposed to kill themAnd it resulted The so-called antimicrobial resistance crisis (AMR) (Puvaca & Frutos, 2021).

and hassave This resistance is the ability to develop various mechanisms that protect it from the effects of antibiotics(Pontes et al., 2022). And Although it is not a new problem but rather a feature of bacteria that has always been around acrisis amplified (AMR) (Giurazza et al., 2021). With the severe overuse and misuse of current antibiotics in healthcare, agriculture and livestock, this is one of the biggest challenges that must be addressed. (Schneider, 2021).

Review of references Nanotechnology

Humanity has gone through many scientific revolutions until it reached what we are experiencing today in terms of progress in various fields such asperhapsM corn, the development of space science, the development of the computer and its uses, and biotechnologies, as humanity witnessed in the last two decades the emergence of nanotechnology Nano technology (NT)(Boguszewicz et al., 2021).

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The word "nano" is a word of Greek origin and in the Greek language it is called "nanos". NanosIt means dwarf, The term Nano Refers to very subtle things a, And nanometres R or Nanometer (nm), that It is one billionth of a metre That is, one one billion nanometers(1 Nanometer = 10-9) (Al-Saadi, 2023). The philosophy of this technology Manufacture of materials whose dimensions are measured in nanometers, And This material has several unique properties due to a Her infinitesimals are small, she has capabilities apps Amazing (Kaddour & Othman, 2022), where Scientists believe Nanoparticles(NPs)The nanostructured materials originated during the Big Bang process of meteorites that led to the formation of the universe and the Earth(Barhoum et al., 2022), Substance use indicated then no first time by Richard P. Feynman1959 in his lecture entitled (Hussein & Aldujaili, 2022)There is plenty of room at the bottom And in 1974 the Japanese scientist formulated Norio Taniguchiterm Nanotechnology (NT) (Bisla et al., 2022). Nanotechnology has reached almost every sector and has amazed the world by offering many potential applications in this sectorT,Since nanostructured materials are known for their unique physical and chemical properties and and are improved performance preferred over their structured macro counterparts,(Chausali et al., 2022).

The use of chemicals and solvents used in chemical experiments are toxic to the biological system and the atmosphere, Advances in the molecular study of matter at the nanoscale have enabled the understanding, analysis, quantification and development of new materials, So he took refugea researchers to find asafer methods a For the production of nanomaterials, such as the use of fungi, bacteria or plants, and because chemical and physical methods are expensive and have many limitations. So damage Scientists have developed an approacha biologyacleanaeconomicallyaand frienda environment as alternative methods for the production of nanomaterials (Domb et al., 2021) Nanomaterials (NMs)

Nanoparticles NPs

Exist (NMs) mainly in two forms, either they are naturally, that is, they are of a nanoscale nature without any human intervention(Aljabali et al., 2022). For example, enzymes are substances ananoa naturally present in bodies a Living organisms (Dutta, 2012) as well as ribosomes and Golgi bodies are present nanomaterial sain a form naturally within bodies object salivea (Salhi, 2007). It also exists in form a factory aThere are several waysAhsa used to produce an ano materials there of the mechanical and the chemical fthe physical and vitality(Mushtaq et al., 2022).

I became NPs are important materials in many fields and industries thanks to their extremely size and shape-related properties. Currently, NPs and nanostructured materials are manufactured on a large scale and are indispensable to many industries. This fact enhances and supports research in biochemistry, biophysics, and biochemical engineering applications, more recently. Nanotechnology has been combined with other sciences manufacture new forms of nanomaterials that can be used(Barhoum et al., 2022).

Studies have shown that living organisms, especially microorganisms such as yeasts and a Afans and bacteria It has the ability and high efficiency to absorb and accumulate inorganic mineral ions in its surrounding environment(Rahman et al., 2019)., And the ability of these organisms to use their original biochemical processes to convert ions of inorganic materials into nanomaterials, and these complex processes lead us to a new field of research. (Vargas & Shon, 2019)

Classification of nanoparticles

The classification is appropriate NPs condition a essential a To highlight the properties of a class, no This is an order easy Where this classification depends on their morphology, size and characteristics (V.V. Singh, 2022) As there are multiple criteria for classification through which we can determine the appropriate classification and there is freedom in choosing the classification (Hossain et al., 2018)..

Firstly: Classification by origin According to origin

Based on the origin, nanoparticles can be classified into three categories (Khan & Hossain, 2022):-

natural nanoparticles Natura NPs -As the name suggests, these nanoparticles are mostly available in nature as ultrafine particles, without human intervention(Bals et al., 2022) Enzymes and some organelles are natural nanomaterials found inside living bodies (Al-Saadi, 2023).).

Accidental nanoparticles Accidental NPs - Occasional nanoparticles are emitted as byproducts ,like food processing and Power generation and engines And material welding And cigarette smoke In addition to Demolition of buildings, etc.(Courty et al., 2020)

manufactured nanoparticles Manufactured NPs - is now being manufactured NPsIn order to provide an improvement a To target properties that fit certain jobs.(Rayhan et al., 2022)

Secondly: Classification by dimensions

Basically all NPs It has at least one dimension jsqsB nanoscale (between 1 and 100 nanometers), It is classified into four types on the basis of dimensions which are described as follows (Dolez, 2015).

Zero dimensional nanoparticles Zero-dimensional NPs If all the outer dimensions of the nanoparticles are in the nanoscale, they Zero Dimensional Rating (He et al., 2019)(0 D) have applications several used in electronics due to its optical enhancement and transmission properties to The highest level of quantum confinement (Mishra et al., 2022).

One-dimensional nanoparticles One-dimensional NPs It consists of only one dimension within the measurement nanometers and other dimensions Outside nanoscale range(1 D)play These particles play a roleavitalain the construction of nanowires nanotubes, nanorods, nanobelts And etc., and serve as one of the main building blocks of the nano-hierarchy(Sahu et al., 2022)...

two-dimensional nanoparticles Two-dimensional NPs

They are particles that have two dimension swathing the measurement nanometer, These particles exhibit thin properties as in films, nanosheets or nanocoatings (Hossain et al., 2021) It has wide applications in nanostructures such as sensors, nanoreactors and carbon nanotubes(CNT)Common examples of such nanoparticles appear as a two-dimensional structure and consist of a two-dimensional layer of graphite rolled onto it forming a tubular structure. (Sun et al., 2022).

3D nanoparticles Three-dimensional NPs-

Be The three outer dimensions within The nanoscale meterj (Lee et al., 2022), Fibrous, multilayer and polycrystalline materials, as well as some powders, belong to this class, Structurally, they consist of low-dimensional nanoparticle elements as 3D nanoparticles that It is of paramount importance in Modern nanotechnology applications (Zhang et al., 2022)...

Third: classification NPs Based on the chemical composition

Classified NPs According to the chemical composition of the substance it is composed of,likeNPsMetallic, greasy, ceramic, carbon-based, etc(Lashari et al., 2022)..

In general, there are three basic methods for building nanoparticles:

Firstly: Chemical approach

Modalities include colloids sand slides BL films and liquid gel Sol-gel. This method is characterized by being safe and inexpensive, as it requires fewer devices compared to physical methods. It requires simple techniques that require low temperatures to be manufactured. Also, the quantities of nanomaterials manufactured in this way are large. Nanomaterials can be manufactured in different sizes and shapes.(Morán et al., 2023)...

Secondly :Physical approach

Include the use of physico-mechanical methods include Mechanical grinding methods where this

method involves the presence of a cylindrical container made of alloys Solid in which the granules to be crushed are placed By the presence of hard balls(Sharma et al., 2022). This is after emptying the container of air atmosphere and inject an inert gas instead to prevent oxidation of these granules, after which they are administered The ball mill has a high speed of 222 revolutions per minute in order to facilitate the grinding of large granules, soften them and reduce their dimensions to become smaller. from 100 nm, and there are physical methods for the production of nanomaterials such as the deposition method BaUse the sprayWaltersiB by thermal evaporation (Klein, 2020).

Third: Biological approach

Both chemical and physical methods It has a higher cost relatively a, preferred About an a method a chemical is done in Est Take aM Elements and compounds may be Same a Toxic effect and danger to researchers, as well may be exceed some This is amazing Risks to environment and the ashynesst TexistenceIn which(Saini & Ledwani, 2022),In addition to that some of the a Physical and chemical mechanisms lead to the production of nanoparticles no Tcon bThe desired shape, size and purity, as he doesSo it can be controlled It was a must Find a way Saferaf accuracy a And less expensivea(Husayn et al., 2023)..

That a biomethod Or what is known as the green method include obtain more homogeneous materials a with Fewer defects(Mech et al., 2022)., and in This is amazing method can be dones ynthesis nanoparticles by an object neighborhood may beMicroscopic like bacteria orthe mushroomYator the Tahacorevia plants orsunExtractIt came(Martins & Kaczerewska, 2021) This method relies on components from living organisms that are used as agents abbreviation a And Factors Envelope Hfor nanoparticlesAndaThey are these components aenzymes And the aacidosis LaMiniWallsugarstheMultiH So is the vitamins where excreteThese components kBiological factors and webquantitative bigIt has the ability to analyze minerals and thus has the ability and high efficiency in the reduction process.

That method that Complete with it production of nano particles she With in the natural functions of the organism District(Ali et al., 2020), and because micro organisms are A quick growth and development And the same Low cos tIt can also be easily controlled on its environment, so it is the most efficient and selective in the synthesis of NPs (Nasrollahzadeh et al., 2019). (Okeke et al., 2022)

The construction of nanomaterials can also be intracellular Or extracellular with the a Nazem from vinegaral Use cultures of easy-to-grow eukaryotes Simple biomass yeasts and LaAffan (Grasso et al., 2019).. that the Factors environmental Like hugging and the solution a The metallic ion affects me The size of the nanoparticles produced(Akl et al., 2020).

Safe sources for the production of nanoparticles NPs

When synthesizing nanomaterials using both chemical and physical methods, some pollutants are emitted. Therefore, scientists have resorted to producing nanomaterials in an environmentally friendly manner through biosynthesis by plants their extracts, or the use microorganisms.(Saravanan et al., 2022).where That plants, bacteria, yeasts or some organisms Tdecent minute may be by her nanoparticles(Jeevanandam et al., 2022)..

Firstly: Biosynthesis of NPs by plants

Nanoparticles are produced by biomass in plants extracted from the leaves and stems And flowers or from seeds(Chakraborty et al., 2022).,The mechanism that occurs to manufacture nanoparticles is in presence the metabolitesnanoparticles such as alkaloids, flavonoids Tanning substances and nourishing compounds Others act as reducing agents and stabilizing agents(El-Sherbiny & Sedki, 2019).

Secondly: Biosynthesis of NPs by microorganisms

by bacteria Bacteria are used in the production of nanomaterials for their ability to reduce metal ions mediated by such as a special reductase enzyme NADH Dependentor Reductase Nitro dependent reductase (Reddy et al., 2022).

by fungi Fungi bIts endurance and accumulation Vital to the elements as well as its high effectiveness In the secretion of enzymes outside the cell And capacity the asTsadIn addition to Ease of growing fungal biomass is another advantage no Fungi are used inSafe production of nanoparticles (Wu et al., 2022).

by yeasts Yeasts are an important model organism in molecular biology that are available as genetic tools for Talaheb Which makes it an exciting hostainterest Kimportant factorFor the manufacture of nanoparticles, yeasts work on types of Metallic nanoparticles, as these materials are manufactured outside the cell can accumulate inside cells Most yeast species quantitativeabigaof heavy metals, where The detoxification mechanism of yeast cells occurs via Glutathione And metallothione phytoclatinThis gives the yeastsThe ability to manufacture nanoparticles Methods for the synthesis of selenium nanoparticles are described (SeNPs) By fungi, including yeasts, and by biological methods, they are non-toxic and can be used successfully. They are simple, low-cost, energy-saving, and environmentally friendly, as they are non-polluting.(Fouda, Hassan, et al., 2022)..(Vijayakumar et al., 2022)Green nanoparticle biosynthesis is one of the most discussed topics in current nanotechnology (Thipe et al., 2022). All in all, many strains play a roleaWhateveraIn the food industry due to its ability to ferment sugar. A novel approach to using them could be the production of metallic nanoparticles and nanostructures via reductase enzymes inside or outside cells.

Due to the mass production of NPs As the ease of control of yeasts in laboratory conditions, the synthesis of many enzymes and the rapid growth using simple nutrients, the yeast strains possess more benefits.may excelon bacteria, Some studies have been performed to investigate the synthesis of nanoparticles using yeast To achieve this goal, by using eukaryotic systems, such as Candida glabrata And, one of the primary methods was achieved using biological materials.

Selenium

Selenium is one of the essential elements and is one of the micronutrients necessary for the proper biological processes of plants, animals and microbiologyH (Khanna et al., 2022)The word "selenium" is taken from the Greek word"selen" which refers to the moon goddess(Birmann et al., 2022) This element is essential for various metabolic processes, including protection against oxidative stress, and cardiovascular function. It has a role in maintaining physiological homeostasis in the body. Mhow mucha aN has a major role in the immune response and this in turn contributes to the particle's resistance to autoimmune diseases as well as the immune system's resistance to **HIVHIV** VerosCOVID-19 (Mal'tseva et al., 2022) (Chen et al., 2021)It is also necessaryaFor embryonic development in women and animalsT.(Mojadadi et al., 2021)

Biological applications of Se NPs Antimicrobial effect of NPs

Current treatments for bacterial infections consist mainly of combining traditional classes of antibiotics with newer drugs, the use of which is very limited amid concerns about the rise in antimicrobial resistance. (Ajose et 2022), Therefore the use of nanomaterials is presented as a suitable solution in particular selenium nanoparticles(See NPs) as one of the most promising nanoscale-based therapeutic agents for effective infection treatment.(Ferro et al., 2021)Green or biosynthesis represents a rapid, inexpensive, efficient and environmentally friendly approach with greater scalability and versatility. And abundance. (Rudramurthy et al., 2016)Ultimately clinical translation of SeNPs faces various obstacles including uncertain safety profiles in vivo, mechanisms of action and unclear regulatory frameworks. (Halwani, 2022). The use of nanoparticles in the treatment of infectionsand shorthandThe antimicrobial resistance crisis An efficient and environmentally friendly approach with more promise of adaptability and versatility. Ultimately, you face the clinical translation of SeNPs have different obstacles(Mubeen et al., 2021).

SeNPs acquire antifungal properties for various biological applications SeNPs can be used to treat fungal infections in immunocompromised patients(Ferro et al., 2021).It can also be used in the manufacture of anti-fungal clothing, in addition to the possibility of manufacturing antifungal wound dressings (Bisht & Phalswal, 2022) For example, different species of Candida yeast and Aspergillus filamentous fungi are found all over the world with a wide distribution. (Bafghi et al., 2021). Supposedly that Its diversity and growing growthconfercostsaexpensiveaon the health care system every yearAnd in all countries(León-Buitimea et al., 2021)., These fungi can act as opportunistic human pathogens in immunosuppressed or immunodeficient systems. (Gnat et al., 2021)., while these fungi have become somewhat resistant to antifungal drugs and are considered a therapeutic problem (Meade et al., 2021). The three main groups of widely used antifungal drugs include the azoles and polyene group and group Echinokanden(Matthews & Sandy, 2022) (Noël de Tilly & Tharmalingam, 2022)These drugs are used to treat fungal agents. Itraconazole is a widely used azole drug, but these microorganisms have become somewhat resistant to it.(Bafghi et al., 2021). Therefore, the researchers resorted to an alternative option, including manufacturingSeNPs were biosynthesized using standard strains such as C. albicans, A. flavus NPs, then these NPs were applied against different types of fungi and yeasts, on the other hand, the effect of biosynthetic **SeNPs** was compared antifungal drugs on the growth of different types of several fungal strains.(Bafghi et al., 2021a)., Given the antifungal effects of Biological NPs were found to have maximum activity even against some resistant strains(Hashem et al., 2021).

Preparation of nanocomposites Synthesis of Nanoparticles

has been manufacturedSeNpsBy sifting through the available isolates, eight isolates including different strains that were isolated from multiple sources were selected for the purpose of selecting a strain with the highest efficiency in producing the nanocomposite, where eight tubes containing a medium were inoculated.PDBwith yeastCandida albicans Each tube was inoculated with a different strain, then incubated for 48 hours. After incubation, a centrifugation process was performed for all eight tubes at a rate of 5000 cycles for 15 minutes. The supernatant contained organic proteins and reduced enzymes, while the sediment containing live yeast cells was neglected. Separation using filter paper(Hashemi et al., 2020)..

A solution was prepared by dissolvinggµ800 sodium seleniteNa2Seo4In 10 ml of distilled water, then 1 ml was taken from it and added to every 100 ml of the filtrate, then incubated for 24-48 hours at a temperature of 35-37 °C in a shaking incubator away from light.(Bafghi et al., 2021)...

After the incubation period, the color change was observed in four tubes from yellow to orange, unless there was any change in the color of the medium in the other four tubes, so the isolates in these tubes were neglected. extracellular nanoparticles(Srivastava & Mukhopadhyay, 2015)...

The same previous steps were repeated, but in larger quantities and for standard isolation only, where large quantities of the nanocomposite were produced, which underwent a centrifugation process, to get rid of the filtrate and dry the precipitate on drying paper and by means of the electric oven. After drying, the precipitate was ground to obtain a nanocomposite for which characterization and effectiveness tests were conducted vitality.

Characterization of nanoparticles Field emission scanning electron microscope (FESEM)

A field scanning electron microscope was used (FESEM) to characterize the morphological and size characteristics of nanoparticles in the electron microscopy unit of the College of Science/University of Tehran.

The sample was prepared by adding a small drop of the biosynthetic nanoparticle suspension onto silicon wafers.thenleft to dryuntilanalyzed by (SEM), wherethisThe microscope operates at accelerating voltages at 12.5-15 kV with various magnifications, in low vacuum mode, 5 mm spot

size and 5-10 mm working distances. (Zare et al., 2013).

Bioactivity of selenium nanoparticles SeNps antimicrobial activity Prepare the bacterial suspension

The bacterial suspension was prepared by culturing the bacteria on solid media and incubated at a temperature of 37 °C for 24 hours. The bacterial colony was transferred to a tube containing 5 ml of normal physiological saline solution using a sterile culture carrier, where the turbidity was measured by the Mc Farland device to obtain a standard vaccine up to to 0.5 to prevent accumulation of bacterial cells, then incubated for 10 minutes at a temperature of 37 °C.

Bacterial drug susceptibility test

The disc distribution method was used according to the guidelinesCLSITo calculate drug sensitivity on Mueller medium, Huntington agar and antigens were usedImipenem, Ticarcillin, Tobromycin, cefotaxim, Trimethoprim, Levofloxacin.

Mueller Hinton medium was inoculated with the bacterial suspension and the dishes were left to dry for 5 minutes, then the antibiotic tablets were placed on the media containing the bacterial suspension, with three replicates for each bacterial type, then incubated for 24 hours at a temperature of 37 °C, then the bacterial inhibition zone was calculated and taken as a measure of sensitivity.

At the same time, drilling was made on other dishes containing Mueller Henton's medium, which was inoculated with the bacterial suspension by means of a sterile cotton swab. Drilling was done with a sterile cork drill with a diameter of 9 mm for one hole, where 100 milliliters of the prepared concentrations were distributed.SeNpsdissolved in μL substanceDMSOThe volume of the solution was completed by adding 990 microliters of distillate, and the concentrations were as follows: 50, 100, 200, 300 microliters / ml, taking into account the addition of a hole forcontrolDistilled water is added to it. The mentioned experiment was

conducted with three replications for each bacterial type, then incubated for 24 hours at a temperature of 37 °C. The area of inhibition was measured to compare the effectiveness of the nanomaterials compared with the effectiveness of antibiotics.

SeNps antifungal activity Preparation of the fungal suspension

The fungal suspension was prepared according to the following method(Gelardi et al., 2014).By taking fungal colonies and cultivating them on mediumSDAAfter the activity of the young colonies, part of them were transferred by means of a culture carrier to a tube containing 5 ml of normal physiological salt solution, where the fungal cells are mixed well and with a rotating motion to get rid of all the lumps in the suspension, then the suspension is incubated for 10 minutes at a temperature of 37 °C, after that the suspension is spread on Container dishes on mediumBDAThen the antifungal antifungal tablets were placed directly on top of the fungal culture with three replications, and the were:clotrimazole, antifungals miconazole, ketoconazole and then incubated at 28 °C.

On the other hand, the center was drilled with a corkscrew to get five holes, one of which iscontrolAs for the rest of the pits, they included the concentrations prepared for the experiment, and the concentrations in the first two groups were 100, 200, 300, and 600.gu\MlRespectively, second group was 2, 3, 4 and 6mg\Ml(Magaldi et al., 2004)..Where the required quantity was added and then dissolved by 10lµ of substanceDMSOsolvent and then complete the volume by adding 990lµof distilled water, then incubated at a temperature of 28 °C for seven days, while observing the fungal growth, then the existing inhibition zones were measured.(Sami, 2019).

An additional experiment was conducted to calculate the synergistic activity of the antifungals after immersing them in one of the concentrations for three replicates, then the dishes were incubated at a temperature of 28 °C for a period of 7 days, and after the end of the period, the areas of inhibition were calculated.

Preparation of the nanocomposite SeNps by C.albicas

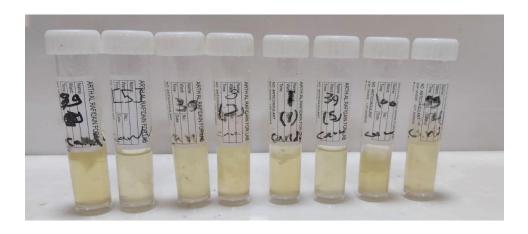
Some isolates available from C. albicans showed its ability to biosynthesize SeNps, where 8 isolates were selected to test the biosynthesis and extracellularity of SeNps, they were grown on sterile PDB medium and after the addition of sodium selenite (Na2Seo3) (0.004 g) per (5 mL) incubated under dark conditions at 28°C for 72 hours, in a rocking incubator at 160 rpm, with continuous visual observation of the change in color of the reaction, from pale yellow to yellowish orange changing to color Orange and dark red continuously during the incubation period, where four isolates showed a clear color change from yellow to orange-red. Mechanisms of biosynthesis of selenium in the form of nanoparticles occurs due to fungal reduction of selenite and selenates with the formation of Se NPs and proteins involved in these processes, and with increasing incubation period the color becomes darker and this indicates the formation of nanoparticles due to bioconsumption.

The color change that occurs when the

concentration of selenium ions drops toSeNPs allowing visual observation indicative of nanoparticle formation, which he observed(Torres et al., 2012). Who confirmed that changing the color of the medium to red is evidence of the extracellular production of

nanoparticles as a result of the consumption of selenium added to the medium as well as(Rasouli, 2019)Where he showed that the color change is the first sign of nanosynthesis outside the cells. He also confirmed that the color shift from pale yellow to red is the result of the consumption of selenium ions in the medium by yeast cells and(Allah et al., 2021)Who confirmed that changing the color of the culture to the red color resulted from the consumption of selenium by the cells, and this was proven by measuring the concentration of selenium ions in the culture, as he concluded that as the concentration of selenium ions decreased, the red color was dark in the middle and(Chitti Kondal Rao et al., 2022). Who watched the color change from pale yellow to red as an indication of the consumption of living cells of selenium ions and the production of nanoparticles and (Al-Shemmary et al., 2022)Who confirmed that the visual observation is one of the distinguishing characteristics of reducing mineral salts to nanoparticles to change the color of the solution, as viewing the color shift is an indication of the synthesisn.p.

And they proposed(Dhanjal & Cameotra, 2010). why mea The biosynthesis of nanoparticles involves a membrane-bound reductase enzyme, which is capable of producing Se shorthand through it Electron transfer enzymes.



Α



FIGURE (4-18) shows the color change caused by the extracellular synthesis of SeNps in the middle PDB planted in it C. albicans Aprior to biosynthesis SeNps B After the creation of SeNps

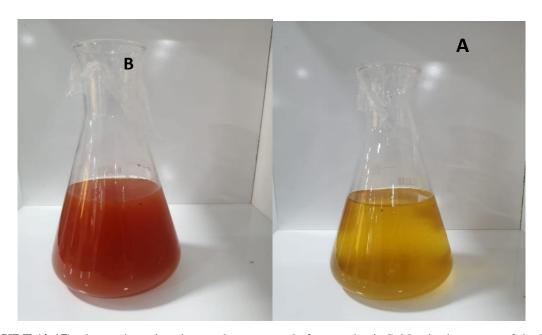


FIGURE (4-17): shows the color change that occurred after synthesis SeNps in the center of the PDB AbeforeBafter

Characterization of nanoparticles Field emission scanning electron microscope (FESEM)

A scanning electron microscope is one of the best microscopes To study the surface quality, where Cross section of the nanostructurejresult appeared the photomicrograph for FESEM on about facilitator, An image was usedSEM to determine the shape and size of the biosynthetic nanoparticles, where they were found in different sizes and using different magnification powers, and the results were examined, as the electronic

scanning of SeNPs showed that the basic shape of the particles is a rod and the particles were homogeneous in sizeand figTo some extent ,program was usedImage JTo measure the diameters of nanoparticles were the extentTheir diameters range between(46-69)nmAs in Figure (4-18). The results of these dimensions show convergence with what has been achieved (Chhabria & Desai, 2016). Where she wasSeNpsincluded in their studiesrodAnd the dimensions of its diameters are 59_+4.

В

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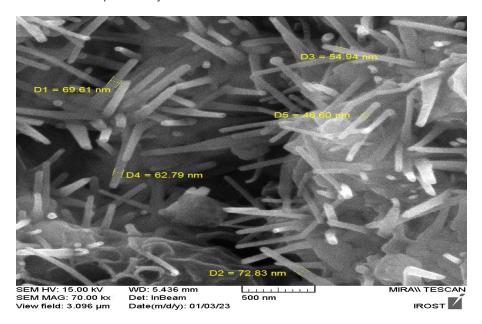
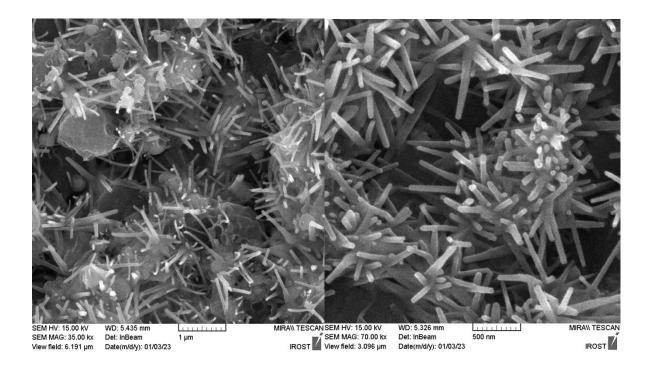


FIGURE (4-18): shows the shape and measurement of diametersSeNps



SeNps Biological Activity

Antimicrobial activity of SeNps

Selection of bacteria and fungi used in the study

A sensitivity test was conducted for two types of bacteria, one of which is Staphylococcus aureusaureus Staphylococcus It is Gram positive, while the other type is Gram positivePseudomonas aeruginosa Gram negative



FIGURE 4: shows the two types of bacteria used in the study



Minimal inhibitory concentration(MIC)

Serial concentrations of 10 nanocomposites were usedµg/mL (2, 4, 8, 16, 32, 64, 128, 256, 512, 1024) by the broth dilution method, where the MIC of the SeNps suspension against S. aureus was 64 µg/mL, although The effectiveness of SeNps against negative and positive bacteria, but this study proved more effective against positive, as this is due to the great difference in the nature of bacterial walls, including the presence of holes and peptidoglycan layers, these results showed a convergence to what was found(Huang et al., 2019). Whereas, the MIC of S.aureus bacteria was 66 µg/mL, and the results of the current study

were close to those of(Han et al., 2021).who mentioned it can be considered 40µg/mL is the MIC for S.aureus bacteria, but it may not be visible to the naked eye so they consider it to be 80 µg/mL as the MIC, either(Chitti Kondal Rao et al., 2022).They have come to thatThe MIC of S.aureus is 2.12 \pm 35.54 The difference in the shape and size of the nanoparticles is the main reason for the difference in the MIC of the same bacteria.

Calculation results are shown The MIC for P. aeruginosa is $128~\mu g/mL$. This result matched those of (Cremonini et al., 2016). And (Salem et

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al., 2022).which consolidated that mechanismsSeNPs lead to ROS production, cell barrier interaction (cell wall rupture permeability change), inhibition of protein and DNA synthesis, and metabolic gene expression as reported by(Eleraky et al., 2020). The antibacterial effect is often associated with the generation of reactive oxygen species (hydrogen peroxide, hydroxyl radicals, and superoxide ions) with metal-based nanoparticles. Several studies reported thatSe-NPs can produce ROS and this matches(Zhao et al., 2018).And(Nayak et al., 2021).. These forms of reactive oxygen species may impede DNA and amino acid replication, as well as degrade the cell membrane of bacteria, he said.(Hemeg, 2017).

Activity SeNps antibacterial

I showedBiosynthetic SeNps have antimicrobial properties. It was used to evaluate its ability to inhibit the growth of S.aureus bacteria, where the biological activity of SeNps was determined using the diffusion and etching method, using four concentrations of SeNps suspension and the concentrations were as follows: 50 µg/mL, 100 μ g/mL, 200 μ g/mL 300 μ g/mL, in addition to the presence of control of distilled water, showed the highest area of bacterial inhibition at the concentration of 300 µg/mL, where the inhibition rate for all replicates was 23.6667 mM, followed by the concentration of 200 µg/mL with an inhibition rate of 18.6667 mM, while the lowest concentration The most effective is the concentration of 100 µg/mL with an inhibition rate of 14.6667, while the concentration of 50 µg/mL did not show any effect against bacteria.

TABLE 1

concentration	С	50gμ\mL	100gµ∖mL	200gµmL	300gµ∖mL
Active mm	00_+00	00	14.6667	18.6667	23.6667*



FIGURE 5: shows the effect of SeNps against S.aureus bacteria

reach out(Vyas et al., 2018). to activity resultsSeNpsThe antimicrobialhighest percentageactivity is32 mMat a concentration of 100gµ\mLand least active at 25gµ\mLWhere

asuggestedwathat selenium may bind to the cell membrane surfacewhich changes whotransmittance finfluencebreathingavinegarAndj,It is also possible that selenium nanoparticles not only interact with a surfaceOnly it is possiblepenetrateaparticles within bacteria.

As for(Al-Shemmary et al., 2022)The results of their study showed that the highest inhibition zone forSeNpsanti-S. aureus It was at a concentration of 500gµ\mLwith an inhibitory activity of 22mM, followed by concentrations 300 and 400gµ\mLAnd actively 18mM, then a concentration of 200gµ\mLactively 17mMFinally, a concentration of 100 with an activity of 10mMWhere their study showed thatSeNpsownThe ability to pass through the cellAnd thereforepreventgrowthmostL positive bacteriagRam and negative thgArcher ,He attributed this anti-bacterial activity tosurface chargeFor the outer envelope of bacteria and its difference with chargeSeNPsWell, the perfect sizeforSeNpsWhich helps it penetrate the bacterial cell wall, and its effect on the internal processes of the cell. thev concludedconclusionImportant isthat sizeSeNPs has a significant effect on antibacterial activitythroughCrossingSeNps

membranecellularBacterial and cell wall easily, causing cell lysis, interfering with synthesisATP, affecting cell division, and all thatYaThis leads

to bacterial cell death.As they indicatedAmong the factors that contribute to pathogenesisYehbacteriaS. aureus It is an incremental development

inbiofilmsthroughPolymeric structures provide defenseTAntibiotics and host defense mechanisms, wheremaybefor **NPs** that destabilize biofilms by penetrating their own channelspassing by Almaand, One of the reasons behind selenium's ability to kill bacteria is its ability to stimulate intracellular oxidation, which leads to microbial death. This is in line with my opinion(Gunti et al., 2019)., where it was explainedactivityforSeNps Antibacterial restoretheactivitytheCatalysttol

glutathioneWallperoxidase in vivo to avoid Radical damage to cells and tissuesAnd this is what they proved(Ramos & Webster, 2012).

As for the activitySeNps against P. aeruginosa, the concentration of 300 gµ/mL showed activity with an inhibition rate of 18.3333 mM, followed by the concentration of 200 gµ/mL with an inhibition rate of 16.3333 mM, while the concentration of 100 gµ/mL showed the least activity, while the concentration of 50 gµ/mL did not show any activity. towards bacteria as shown in Table (4-).

TABLE 2

concentration	С	50gμ\mL	100gµ∖mL	200gµmL	300gµ∖mL
Active mM	00_+00	00	14,000	16.3333	18.3333

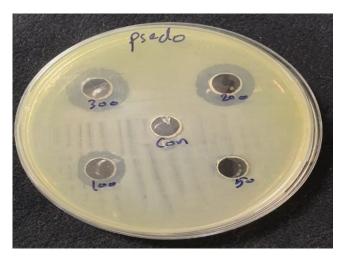


FIGURE 6: shows the effect SeNps against P. aeruginosa

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Where the study of (N. Singh et al., 2014). That the highest activity of SeNpsat a concentration of $100g\mu\mbox{mL}$ with an inhibition rate of 11mM, followed by concentrations 50 and $75g\mu\mbox{mL9}$ inhibition ratemM, and the lowest activity at concentration $25g\mu\mbox{mL7}$ inhibition ratemM.

As shown by a study(Fouda, Al-Otaibi, et al., 2022). For the highest activity of SeNpsat a concentration of 300gu\mLwith an inhibition rate of 19mMfollowed by a concentration of 200gµ\mLwith an inhibition rate 15mMFollowed by a concentration of 100gµ\mLwith an inhibition rate of 11mMThen 25gµ\mLwith an inhibition rate of 10mMThe lowest activity was a concentration of 12.5 with an inhibition rate of 9mMIn what concentration 6.5 did not appeargµ\mLany effectiveness.

The susceptibility test of antibiotics

The results of the susceptibility test showed that the bacteria had different sensitivity to the antibiotics used in The study, as shown in Table (4-) and Table (4-)

Table () shows resistanceS. Aurous While it was resistant to Cefotaxime, it was resistant to

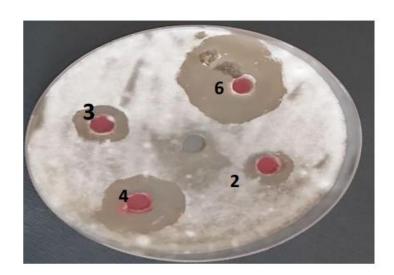
Ticarcillin, followed by resistance to Imipenem, then Levofloxacin, and to Trimethoprim, it was less resistant to it and less than to Tobromycin.

As shown P. aeruginosa Resistant to cefotaxime and trimethoprim and was sensitive to ticarcillin followed by sensitive to imipenem and least sensitive to levofloxacin and tobromycin.

Bacterial resistance to these antibiotics may be due to changesIt occurs in genes on the chromosome or as a result of a mutation that leads to the loss of proteins associated with penicillin or the loss of antibiotic activation,

Effectiveness SeNps towards fungi

Four concentrations were selected to measure the biological activity of SeNps fungusTricophyton spp.The concentrations were:ml/mg(2, 3, 4, 6) Where the focus score is 6ml/mgThe highest inhibition rate was 32.6667 mm, followed by concentration 4ml/mgWith an rate 31.0000 inhibition of mm, concentration 3ml/mgWith an inhibition rate of 19.6667 mm in what achieved concentration 2ml/mgThe lowest rate of inhibition is 10.6667 mm.



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