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Comparison between AgNO_3 + PbNO_3 for medical application

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Abstract

There are several ways to prepare nanomaterials, including physical methods, biological methods, and chemical methods. In this research, a biological method was used to prepare silver nanoparticles using mint leaf extract, and nano-lead using cumin seed extract. Plant extracts are considered reducing agents, and the green synthesis method was used because this method is environmentally friendly, uncomplicated, and less expensive. Mint and cumin leaves were collected, dried, and then ground in a grinder. After that, the plant extract was prepared. The nanocomposites were prepared using the droplet method and were sent for examination to conduct the (XRD, FE-SEM, and UV) test on the samples. The results of the tests showed that we had obtained nanoparticles of silver and lead nanoparticles. The Uv test showed peaks indicating the presence of silver inside the sample and lead inside the other sample. Absorption, transmittance and energy gap testing were also performed.the results of the XRD test also showed that the grain size of the silver nanoparticles (19.6 nm) and that its crystal structure is of (cubic) type also showed that the grain size of the lead nanoparticles range between (16.6 nm_17.4nm) and that its crystal structure is of (cubic) type.The FE-Sem examination was conducted to determine the shape and morphological characteristics of the nanoparticles, and it was used to determine the particle size of the nanocomposites. The nanosize of the silver particles ranged from 46-82 nanometers, and the nanosize of the lead particles ranged from 99-138 nanometers. The resulting solutions were applied to the taken bacteria. From the eye (Pseudomonas lutea) and the extracts proved effective in killing bacteria. Cumin extract with lead nitrate was more effective than silver nitrate with mint extract.

Keywords: silver nitrate, lead nitrate

مقارنه بين نترات الفضة ونترات الرصاص للتطبيقات الطبية

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الملخص

هناك عدة طرق لتحضير المواد النانوية منها الطرق الفيزيائية والطرق البيولوجية والطرق الكيميائية، وفي هذا البحث تم استخدام الطريقة البيولوجية لتحضير جسيمات نانوية فضية باستخدام مستخلص أوراق النعناع، وجسيمات نانوية رصاص باستخدام مستخلص بذور الكمون، وتعتبر المستخلصات النباتية عوامل اختزال، وتم استخدام طريقة التخليق الأخضر لأن هذه الطريقة صديقة للبيئة وغير معقدة وأقل تكلفة، وتم جمع أوراق النعناع والكمون وتجفيفها ثم طحنها في مطحنة، وبعد ذلك تم تحضير المستخلص النباتي، وتم تحضير المركبات النانوية باستخدام طريقة القطرات وإرسالها للفحص لإجراء اختبار (XRD و FE-SEM و UV) على العينات، وأظهرت نتائج الاختبارات أننا حصلنا على جسيمات نانوية من الفضة والرصاص، وأظهر اختبار Uv قممًا تشير إلى وجود الفضة داخل العينة والرصاص داخل العينة الأخرى. كما تم إجراء اختبار الامتصاص والنفاذية وفجوة الطاقة، وأظهرت نتائج اختبار XRD أيضًا أن حجم حبيبات جسيمات النانو الفضية (19.6 نانومتر) وأن بنيتها البلورية من النوع (مكعب) وأظهرت أيضًا أن حجم حبيبات جسيمات النانو الرصاصية يتراوح بين (16.6 نانومتر_17.4 نانومتر) وأن بنيتها البلورية من النوع (مكعب). تم إجراء فحص FE-Sem لتحديد شكل وخصائص الجسيمات النانوية، وتم استخدامه لتحديد حجم جسيمات النانو المركبة. تراوح حجم جسيمات الفضة النانوية من 46-82 نانومتر، وتراوح حجم جسيمات الرصاص النانوية من 99-138 نانومتر. تم تطبيق المحاليل الناتجة على البكتيريا المأخوذة من العين (Pseudomonas lutea) وأثبتت المستخلصات فعاليتها في قتل البكتيريا. كان مستخلص الكمون مع نترات الرصاص أكثر فعالية من نترات الفضة مع مستخلص النعناع.

كلمات مفتاحية : نترات الفضة ، نترات الرصاص

Chapter one

1. Introduction:

The origin of the word "Nano" is derived from the Greek (Nanos), which means dwarf and means everything that is small[1].

Nanoscience is concerned with the study of the basic principles for the formation, synthesis and application of molecules and compounds whose size measurement does not exceed 100 nm. Since the nanometer is a SI unit of measurement and is equal to10^{-9} meters. The principle of this technique is based on capturing nanoparticles of the material, manipulating and moving them from their original positions to other positions and then combining them with atoms of other materials to form a crystal lattice to obtain materials with distinct nanoscale dimensions and high performance and desirable properties[2].

Nanotechnology has attracted great attention, as this technology is one of the new technologies that still needs a lot of research and studies that have become of great importance due to their unique properties and the breadth of their applications Nanotechnology, as stated in many nanotechnology research centers, is the technology of the next age, i.e. we can call our next age the "Nano age" This great field will be in many areas of industrial, agricultural and medical life, in the areas of transportation and aviation, in space and water technology, and in many important vital fields.at the end of the twentieth century, the world experienced a rapid development of technological science, after the invention of computers, which led to an electronic revolution, which made the competition between industrialized countries great[3].

The invention of the microscope contributed to the penetration into the small worlds of matter, which became clearer, and man was able to go deeper into those worlds from the living worlds, including living organisms and their components, and from the inanimate worlds, including the elements of matter and its components of atoms and molecules[4].

Nanotechnology is one of the most important technologies in multiple fields, as it is based on the synthesis of nanoparticles (NPs) ،Where these particles have different properties from the metals from which they were formed, based on the engineering of metal particles in various shapes and sizes, interest has increased in recent years in the production of metal materials Nanoparticles have applications in various fields such as biomedical, agricultural, environmental and industrial fields. Bio-preparation of nanoparticles (biosynthesis) is carried out using metabolites or plant extracts and one of the advantages of this method is that it is environmentally friendly, does not need energy, cheap and fast[5].

In our research, we will conduct the green synthesis process using two basic elements in nature, silver and lead, on *Pseudomonas*. Silver is an element symbolized by the letters "Ag", an abbreviation of the Latin word (Argentum) and attributed to the country of Argentina (the land of silver), where silver was found in large quantities. It is a white metal and can be polished and refined. The silver metal comes in transition group II of the periodic table, and its number is atomic weight is 47, its atomic weight is 107,868, and its specific gravity is 10.5. Silver melts at a temperature of 962 degrees Celsius, and reaches the boiling stage at a temperature of 2212 degrees Celsius[6].

Lead is a chemical element with symbol Pb and atomic number 82; It is located in the periodic table within the carbon group (the 2 fourteenth group; it is also the fourth group according to the numbering of the main groups). Lead is a heavy metal with a high density, and is normally found in a bluish-silver color, which quickly loses its luster to an opaque gray color when exposed to air.particular, lead oxide electronic properties, i.e., bandgap and hence color, depend largely on the lead to oxygen ratios and crystal structures of various polymorphs[7].

Pseudomonas luteola, characterized by its distinctive yellow pigment, is a gram-negative, motile, and strictly aerobic rod. Differentiating from other yellowpigmented non-fermenters due to its negative oxidase reaction, P. luteola is primarily found in environments with high moisture content [8].

1.1 Previous studies

- **In 2022, researcher Hadeel Adel [9].** exposed mint leaf extract (aqueous and methanol) to a solution of silver nitrate to produce nanoparticles as a reducing agent that caused the conversion of Ag+ ions into silver nanoparticles that produced a color shift. The peak that appeared between (400-550) indicated Nanometer transform infrared spectroscopy (FTIR) allows access to effective aggregates. The diameter of inhibition results for the silver particles of the methanolic extract are higher compared to the silver particles of the aqueous extract of this bacteria (for Acinetobacter baumannii).
- **In 2011, Shriram Joglekar along [10]**. in India, demonstrated a less expensive and environment-friendly method for rapid synthesis of lead nanoparticles using 0.5% aqueous extract of Jatropha curcas L. latex. The lead nanoparticles were characterized by spectroscopic analysis and showed a distinct peak at 218 nm. EDAX showed characteristic peaks of lead.

- **In 2023, Jassim Arif [11]**.used previously prepared lead nanoparticles (Pb-NPs). The results of XRD examinations showed small particles of lead nanoparticles in small proportions (8-37 nanometers) and its crystal structure (cubic). Bacteria were prepared using minimum inhibitory concentration methods. And inhibiting Staphylococc aerus bacteria. The MIC results showed that the nanomaterial was effective in killing the aforementioned bacteria.

- **In 2023, Arwa Emad [12]**.carried out the most common and least expensive method, which is extracting mint leaves and exposing them to silver nitrate at 60°, stirring continuously, placing the plant extract in a buret, and distilling it over the nitrate for two hours. The color changes gradually until the desired color is reached. Many tests were performed (FE-SEM, EDS, XRD, FTIR). The EDS examination showed the proportions of the substances present in the sample, where (Ag, O, Na, Mg, Cl, Ca) were obtained in different proportions, and that the most weight obtained was for the element silver. In addition, the resulting solution was applied to Bacteria (*Staphylococcus*) and has proven effective in killing them.

- **January 2023, Maryam Ahmed Ali [13]**. she discovered the bacteria *Pseudomonas luteola* in Iraq, the city of Babylon. A 51-year-old woman suffering from chronic urinary tract infections and kidney failure was examined. Samples were taken and examined. The results showed that the colonies were gray in color on blood agar and green on chromium agar. The gram-negative bacteria *Pseudomona luteola* are aerobic, non-spore-forming, motile, non-lactose-fermenting bacteria. They grow well on MacConkey agar, which is a non-lactose-fermenting agar, and does not form spores. Most clinical isolates are oxidase negative. On MacConkey agar, they produce yellow colonies. And distinguish it from other pseudomonads. In contrast to other fluorescent species, *P.luteola* neither reduces nitrate nor oxidizes xylose, making them easily distinguishable

- **In 2023 ,Researcher Enas Hammoud Abboud Diaa El-Din [14]**.conducted the current study with the aim of investigating the most important bacterial microorganisms causing eye infections in Karbala Governorate or determining their virulence. She collected several swabs from different areas of the eye infected with different types of bacterial infections, and the results showed the dominance of Staphylococcus aureus, then *Staphylococcus* epidermidis, then *Pseu. domonas* aeruginosa and many other species in varying proportions, an antibiotic susceptibility test was conducted for the most frequent bacterial isolates in these areas. Study, which is both *Staphylococcus aureus bacteria* and *Pseudomonas aeruginosa bacteria*. Most of the bacterial isolates were multi-resistant. The results of this study were that there was a significant correlation between the incidence of dry eye and systemic infections such as the respiratory system and diabetes, but no significant correlation was found between the age and sex of those infected the current study confirmed that Gram-positive bacterial species are more common than Gram-negative bacterial species in causing various eye infections.

1.2 Research Objective

- Synthesiz of silver (Ag) NPS and Studying the effect of silver nanopartical on (*Pseudomonas luteola*) bacteria
- Synthesiz of lead (Pb) NPS and Studying the effect of lead nanopartical on (*Pseudomonas luteola*) bacteria.
- Finding the lowest concentration of the nanomaterial that kills bacteria

Chapter two

2. Introduction

In this chapter, methods of manufacturing will also be discussed. Then we will talk about silver nitrate in brief and the mint leaf plant and its components and lead nitrate and Cuminum cyminum plant and its components. We will also talk about nano silver. In addition, we will write about devices and tests that can be used to identify nanomaterials.

2.1. Methods for preparing nanomaterials

There are many ways to manufacture nanomaterials, there are ways from top to bottom, and there are ways from bottom to top. The biological synthesis method was used to manufacture nanomaterials from mint leaf extract with silver nitrate to prepare silver nano particles. also the biological synthesis method was used manufacture nano materials from plant Cuminum cyminum extract with lead nitrate to prepare lead nanoparticles **[15]**.

2.1.1 Biological Methods Synthesis

Our key purpose is to highlight the biological synthesis of nanoparticles, because of the ease of its rapid synthesis and ecofriendly synthesis. Also, the toxicity and size characterization can be controlled. The variety of natural sources is there for nanoparticles synthesis, together with plants, fungi, yeast, bacteria, etc. Additionally, the unicellular and multicultural organisms are able to synthesize intracellular and extra cellular inorganic nanoparticles. The various sources of nanoparticles synthesis by biological methods in Nanoshel **[16]**.

1- Nanoparticles synthesis by plant extracts: Nanoshel uses plants in the synthesis of nanoparticles have drawn more interest of researchers because it provides single step biosynthesis process. Plants tender a superior option for synthesis of nanoparticles, as the protocols involving plant sources are free from toxicants; furthermore, natural capping agents are readily supplied by the plants. The production of gold and silver nanoparticles and many other nanoparticles, using Geranium extract, Aloe vera plant extracts, sundried Cinnamomum camphora and Azadiracta indica leaf extract is carried out in Nanoshel **[17].**

There are other ways to manufacture nanoparticles in the biological method.

We will only mention them.

2- Nanoparticles synthesis by bacteria

3- Nanoparticles synthesis by fungi

4- Nanoparticles synthesis by yeast

5- Nanoparticles synthesis by biological particles

2.2 Silver nanoparticles

Silver nanoparticles are nanoparticles of silver of between 1 nm and 100 nm in size. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Numerous shapes of nanoparticles can be constructed depending on the application at hand. Commonly used silver nanoparticles are spherical, but diamond, octagonal, and thin sheets are also common **[18]**.

Their extremely large surface area permits the coordination of a vast number of ligands. The properties of silver nanoparticles applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, biosafety, and biodistribution **[19]**.

2.3 Silver Nitrate

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Silver nitrate is a chemical compound with the formula $AgNo_3$. It consists of an ionic bond between the silver cation (Ag^+) and the nitrate anion (NO_3^-). Due to the ionic nature of this compound, it readily dissolves in water and dissociates into its constituent ions. Silver nitrate is a precursor to many compounds of silver, including the silver compounds used in photography. When compared to silver halides, which are used in photography due to their sensitivity to light, $AgNO_3$ is quite stable when exposed to light **[20]**.

2.3.1 Structure of $AgNO_3$

An illustration describing the structure of the silver nitrate molecule is provided below. It can be observed that silver has an oxidation number of +1 in this compound.

2.4 Mint plant

Mint is a perennial herb with very fragrant, toothed leaves and tiny purple, pink, or white flowers. There are many varieties of mint—all fragrant, whether shiny or fuzzy, smooth or crinkled, bright green or variegated. However, you can always tell a member of the mint family by its square stem. Rolling it between your fingers, you'll notice a pungent scent and think of candy, sweet teas, or maybe even mint juleps **[21]**.

Figure (1) Mint plant

As well as kitchen companions, mints are used as garden accents, ground covers, air fresheners, and herbal medicines. They're as beautiful as they are functional, and they're foolproof to grow, thriving in sun and shade all over North America. Since mint can be vigorous spreaders, you simply have to be careful where you plant it **[22]**.

2.4.1 Synthesis method using plant

The reduction of silver ions into silver nanoparticles has also been achieved using geranium leaves. It has been found that adding geranium leaf extract to

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silver nitrate solutions causes their silver ions to be quickly reduced and that the nanoparticles produced are particularly stable. The silver nanoparticles produced in solution had a size range between 16 *and* 40 *nm* [23].

In another study different plant leaf extracts were used to reduce silver ions.It was found that out of Camellia sinensis (green tea), pine,persimmon, ginko, magnolia, and platanus that the magnolia leaf extract was the best at creating silver nanoparticles. This method created particles with a disperse size range of 15 to 500 nm, but it was also found that the particle size could be controlled by varying the reaction temperature. The speed at which the ions were reduced by the magnolia leaf extract was comparable to those of using chemicals to reduce [24].

The use of plants, microbes, and fungi in the production of silver nanoparticles is leading the way to more environmentally sound production of silver nanoparticles [25].

2.5 lead nanoparticles

Lead is a chemical element with the symbol Pb (from the Latin plumbum) and atomic number 82. It is a heavy metal that is denser than most common substances. Lead is soft and malleable, and it also has a relatively low melting point. When freshly cut, the lead is silver with a touch of blue; It turns a dull gray color when exposed to air. Lead has the highest atomic number of any stable element [26].

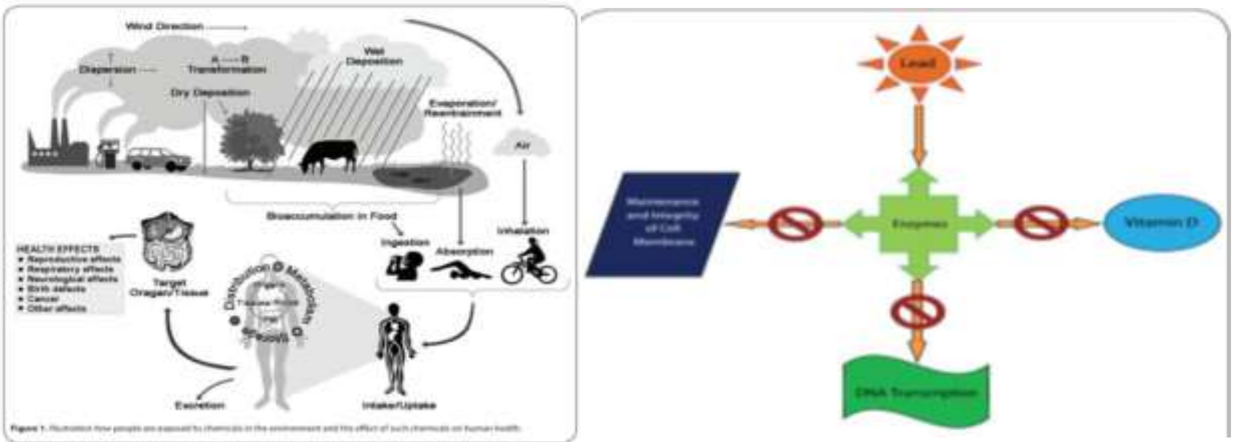

Figure (2): Illustration of the action of lead on enzymes, leading to the disruption of vitamin D synthesis, maintenance of cell mem- brane and DNA transcription

Lead nanoparticles are small particles of lead that are manufactured using various methods such as physical, chemical and green (biological) methods. These nanoparticles have unique properties and find applications in diverse fields. For example, lead oxide nanoparticles are used as X-ray contrast agents in in vitro imaging and in vivo. Lead nanoparticles (Pb-NPs) play a vital role in electronic devices especially sensors[27],[28].

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2.5.1 cumin plant

The cumin plant (Cumin) is one of these medicinal plants. Its scientific name is Cuminum L. cyminum. It is an annual herbaceous plant that belongs to the Apiaceae family (Divakara et al. (2013)). It has several therapeutic uses, as it works as a sterilizer, antiseptic, and carminative. From the intestines, it strengthens the stomach (Johri), (2011), it is used for diseases related to the stomach and intestines, bronchopulmonary disorders and respiratory disorders, a treatment for toothache, and a regulator of liver functions as well. Nasrallah (2012) and (Deepak, (2013), and cumin is considered one of the most important the plants antioxidant, regulates blood pressure, and helps absorb calcium **[29]**.

Figure (3) cumin plant

2.6 X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a versatile non-destructive analytical technique used to analyze physical properties such as phase composition, crystal structure and orientation of powder, solid and liquid samples **[30]**.

Many materials are made up of tiny crystallites. The chemical composition and structural type of these crystals is called their 'phase'. Materials can be single phase or multiphase mixtures and may contain crystalline and non- crystalline components. In an X-ray diffractometer, different crystalline phases give different diffraction patterns. Phase identification can be performed by comparing X-ray diffraction patterns obtained from unknown samples to patterns in reference databases **[31]**.

The most comprehensive compound database is maintained by ICDD (International Center of Diffraction Data). The relative strengths of the patterns from different phases in a multiphase mixture is used to determine the full composition of a sample **[32]**.

X-Ray Diffraction is the result of constructive interference between X-rays and a crystalline sample. The wavelength of the X-rays used is of the same order of magnitude of the distance between the atoms in a crystalline lattice. This gives rise to a diffraction pattern that can be analysed in a number of ways, the most popular being applying the famous Bragg's Law **[33]**.

$n\lambda = 2d \sin\theta$ (2.1)

which is used in the measurement of crystals and their phases.

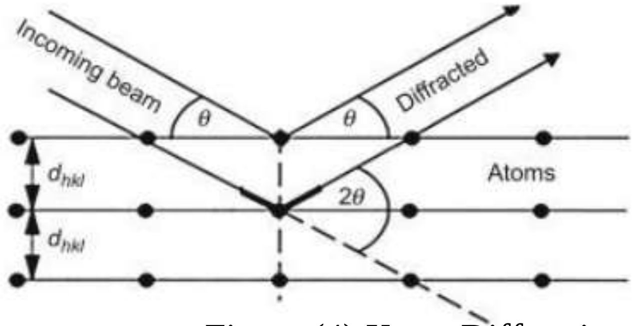

Figure (4) X-ray Diffraction (XRD)

The average grain size of lead nanoparticles can determine using the Debye-Scherrer equation:

$D_{KhI} = K\lambda / (B_{khI} \cos\theta)$ 2-2

D_{KhI} is the crystal size in the direction perpendicular to the lattice planes. hkl are Miller's indices for planes. K It is a numerical factor frequently referred to as the crystal form factor. λ is the wavelength of X-rays. B_{khI} is the width of the X-ray diffraction peak in radians (full width at half maximum). θ Bragg corner **[34]**.

2.7 Field emission scanning electron microscopy (FESEM)

Provides topographical and elemental information at magnifications of 10x to 300,000x, with virtually unlimited depth of field. Its advantages the ability to examine smaller-area contamination spots at electron accelerating voltages compatible with energy dispersive spectroscopy (EDS). Reduced penetration of low-kinetic-energy electrons probes closer to the immediate material surface. High-quality, low-voltage images with negligible electrical charging of samples (accelerating voltages ranging from 0.5 to 30 kilovolts). A field-emission cathode in the electron gun of a scanning electron microscope provides narrower probing beams at low as well as high electron energy, resulting in both improved spatial resolution and minimized sample charging and damage **[35]**.

2.8 UV spectroscopy

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs. AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light. In addition, UV-vis spectroscopy is fast, easy, simple, sensitive, selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is

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not required for particle characterization of colloidal suspensions. In AgNPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of silver nano particles in resonance with the light wave. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings. Observation of this peak—assigned to a surface plasmon—is well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm. The stability of AgNPs prepared from biological methods was observed for more than 12 months, and an SPR peak at the same wavelength using UV-vis spectroscopy was observed **[36]**.

Figure 5: Visible and ultraviolet absorption spectrum of silver nanoparticles

2.9 Pseudomonas luteola bacteria

Pseudomonas luteola is a Gram-negative, rod-shaped bacterium considered ecologically problematic and can cause pneumonia and urinary tract infections in humans.It is found in soil, water, and the respiratory tract of humans and animals. *P. luteola*, is a facultative anaerobe and can grow in the presence or absence of oxygen. This organism is typically found in the environment in low doses and does not cause illness. However, when *P. luteola* is found in high concentrations it can cause a variety of infections in both humans and other animals. The clinical forms of disease caused by this organism are pneumonia, urinary tract infections, skin infections, eye infections, and ear infections. In addition to these clinical forms, there have been several case reports describing cases of septicemia due to *P. luteola* in humans. This condition can cause sepsis, which is a serious condition in which there is, widespread inflammation throughout the body and can lead to death. It is estimated that there are between 10 million and 100 million cases of bacterial infection due to *P. luteola* each year around the world. *Pseudomonas* luteola is a Gram-negative, aerobic bacterium that is commonly found in water and soil. This bacterium is capable of causing diseases in both plants and animals. *P. luteola* can cause a number of diseases, including leaf spot, stem blight and downy mildew. There are also reports of this bacterium being responsible for food spoilage. In this case report, we isolated the of *P.luteola* form contact lens **[37]**.

2.10 Mechanism of effectiveness of nanosilver as an antibacterial

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After Ag-NPs bind to the cell surface, they accumulate in the cell wall pits, leading to disruption of the cell membrane [38]. Likewise, Ag-NPs also disrupt bacterial signal transduction by dephosphorylate tyrosine residues on peptide substrates, leading to termination of cell proliferation and apoptosis [39]. The antibacterial effect of Ag-NPs also depends on the dissolution state of Ag-NPs in the exposure medium. Synthetic and processing factors, such as the properties of the intrinsic Ag-NPs (shape, size, and capping factor) and the surrounding media (organic and inorganic components), directly affect the effectiveness of Ag-NPs. The thick cell wall of Gram-positive bacteria may reduce the penetration of Ag-NP into their cells; This is why Gram-negative bacteria are more susceptible to Ag-NPs. Biofilm formation in the oral environment protects bacteria from Ag and Ag-NPs by impeding their transport. The bioavailability and mobility of Ag-NPs in biofilm are determined by the diffusion coefficients of Ag-NPs. Ivan Sundi (2004) also studied the biocidal activities of Ag-NPs against E. coli and confirmed the "pit" formation in the cell wall of this model of Gram-negative bacteria. A significant increase in permeability occurred due to the accumulation of Ag-NPs in the bacterial membrane, which resulted in to cell death [40].

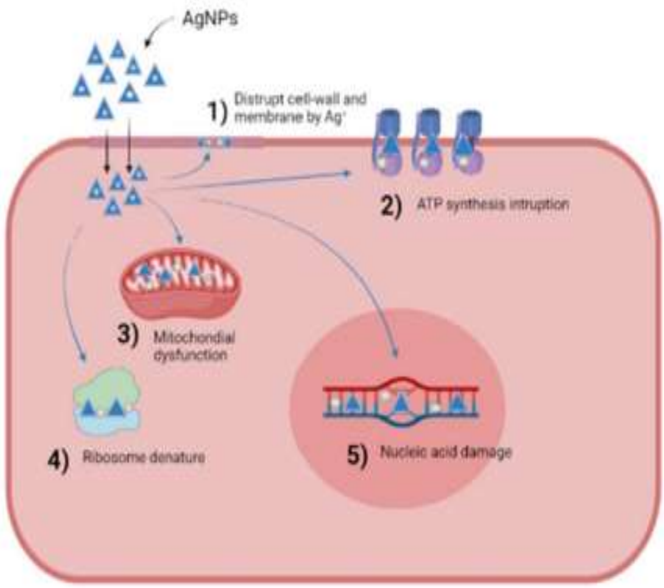

Figure (6): The antibacterial mechanism of action of (NPs-Ag): (1) disruption of the cell wall and membrane by silver ions released from NPs-Ag; (2) NPs-Ag inhibition of ATP synthesis; (3) dysfunction of Mitochondrial damage induced by Ag+ released from Ag NPs; (4) ribosome degradation by Ag+ NPs; (5) Ag+ NPs cause DNA damage through Ag+ incorporation [41].

Chapter Three

3. Materials and Methods

3.1 Materials

Laboratory Equipment and Apparatuses: The equipments and apparatus that were used throughout this study are listed in Table 3-1-1.

No.	Equipment & instrument	Company and Origin
1	Plastic Petri – dishes	Marienfeld (Germany)
2	Refrigerator	Esplf- Germany
3	Incubator	Gallenkamp (England)
4	Magnetic stirrer	IKA - Combimag RCT
5	Slides	Marienfeld (Germany)
6	Bacteriological Hood	(U.A.)
7	Autoclave	Littlesiter (U.R.)
8	Sensitive balance	Sortorius (Germany)
9	Loop	HIMEDIA (India)
10	Distillator Water	GFL (Germany)
11	1.5ml , 0.5ml and 0.2ml Tube	JET BIOFIL, Singapore
12	Centrifuge	Fisher Scientific, USA
13	Micropipette	Human, Germany
14	Vortex	Quality Lab System,England
15	Dentirak	
16	Bernard lamp	
17	Cotto swap	England
18	Conveying medium	England
19	White tube	England
20	Water bath	China
21	Silver (Ag)	
22	Lead (Pb)	
23	Ultrasound water bath	China

Table 1-3: Equipments and apparatus used in this study.

3.2 Culture Media used in the study:

The Culture Media used in the study are listed in Table 3-2-2

No.	Culture Media	Company and Origin
1	Mueller	Himedia (England)
2	Nutrient Agar	Himedia (England)
3	Pseudomonas luteola bacteria	

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Fig. (7) Equipments and apparatus used in this study

3.3 Method of work

3.3.1 Preparation of the plant extract(mint)

Preparing the plant extracts. Fresh leaves were collected from the leaves of the mint plant, Mentha spicata, on September 6, 2023, from the city of Khalidiya. After that, the leaves were washed several times with sterile distilled, deionized water, and then they were spread in a strainer away from sunlight and at room temperature. It was spread on dry paper and stirred several times to prevent it from rotting for a week, then it was crushed using a grinder to obtain a fine powder of mint leaves **[42]**.

Figure 8: A- Fresh mint, B- Ground mint

3.3.2 Preparation of the plant extract(cumin)

The seeds of Cuminum cyminum seeds were purchased from the local market (herbs and aromatic Abu zaydoun) in the latifiya area of Al-Salam neighborhood and washed several times by water to remove dust particles and then they were introduced to the incubator for 24 hours at a temperature of 37 ℃ and after taking them out of the incubator, spread them in a place to remove the remaining moisture from them and then grind them to form a powder [43].

Figure 9: A- Dried cumin after leaving the incubator,B- Ground cumin

3.4 Extraction of plant extract

3.4.1 Prepare mint extract method

The baker, thermometer, and gauze were used, and the baker were washed well with distilled water before starting the process of preparing the extract for the mint plants. Then 18 grams of mint and 300 ml of distilled water were used at a temperature of 42-50 ℃ for 35 minutes. The distilled water was mixed with the mint until it became liquid and then it was placed in the refrigerator for 24 hours.

After 24 hours had passed, we filtered the extract of mint several times through gauze to obtain the best quality of the extract. Then we used tubes to put the extract in it, so that each tube contained 10 ml of extract for each of mint, and then we performed the centrifugation process for the extract of mint. The mint extract was centrifuged for 20 minutes at a speed of 3500 rpm [44].

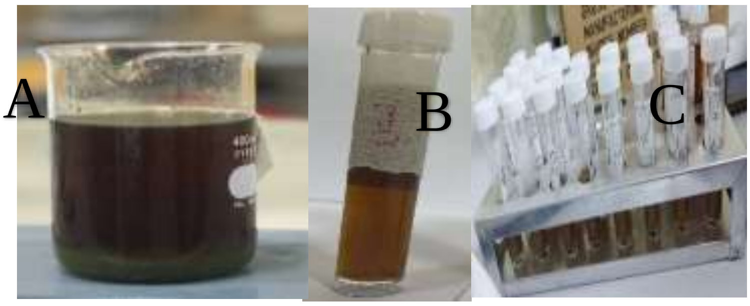

Figure 10: A- Prepare mint leaves before centrifugation.B-Mint leaf extract after centrifugation.C-place the plant extract inside the tubes

3.4.2 cumin extract method

The seed extract was prepared by mixing 64.32 grams of cumin and 300 ml of distilled water in a conical flask on a Stirling heating device for 35 minutes at a temperature of 35-40 ℃. To improve the mixing process, we close the mouth of the conical flask, then put the solution in the tripod for 24 hours, and after 24 hours have passed, we filtered the cumin extract several times through cheesecloth to obtain the best quality of the extract.

Then we used tubes to put the extract in, so that each tube contained 10 ml of cumin extract. Then we performed a centrifugation process for 20 minutes at a speed of 3500 rpm, then separated the supernatant, filtered it using a filter (filter paper), and took the solution **[45]**.

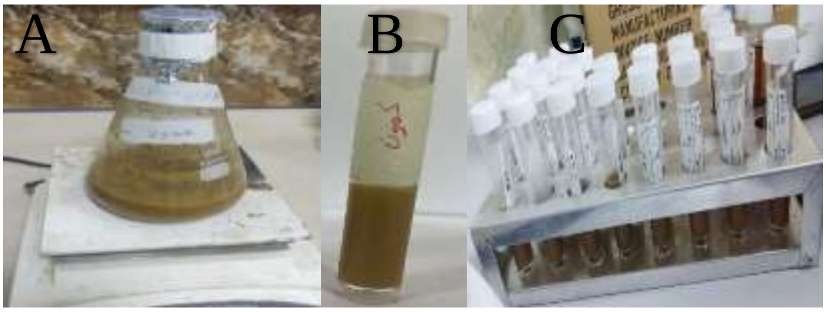

Figure 11: A- Prepare cumin.B- Prepare the cumin before centrifugation,C- Place the cumin extract into the tubes

3.5 methods prepare silver and lead nitrate

3.5.1 methods prepare silver nitrate

Silver nitrate should be prepared in a dark place away from light. Silver nitrate was prepared in light-insulating glass and covered with aluminum foil. Where (0.2 g) of silver nitrate was added to (100 ml) of deionized water **[46]**.

3.5.2 methods prepare lead nitrate

Lead nitrate should be prepared in a dark place away from light. Lead nitrate was prepared in light-insulating glass and covered with aluminum foil. Where (0.2 g) of lead nitrate was added to (100 ml) of deionized water **[47]**.

3.6 method load mint extract onto silver nitrate

We took 15 ml of mint leaf extract and took 10 ml of diluted silver nitrate. The temperature of the thermometer was measured using a pre-set between (60-70) ℃ as a specific range. After that, the silver nitrate was added in a dark container, the extract was added in a numbered buret, and the container containing the 10 ml of silver nitrate was placed on the thermometer. The burette containing the extract was placed over the nitrate container placed on the thermometer. The extract from the burette was added to the container by a drop method, and when the mixture turned to the desired color of bronth black, the process, which took 25 minutes was stopped **[48]**.

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Figure 12: Mint extract with silver nitrate during the calibration process

3.7 method load cumin extract on lead nitrate

20 ml of cumin extract was taken, 10 ml of diluted lead nitrate was taken, and the thermometer was set at 40 ℃, and using the dripping method with a burette for 25 minutes, and we got a color close to dark orange **[49]**.

Figure 13: cumin extract with lead nitrate during the calibration process

3.8 Prepare the sample to XRD and UV tests

A small amount of silver nitrate AgNO3 was placed with mint extract that had been previously prepared by distillation on a slide on the thermometer, and then the sample was waited until it dried. After that, the sample was removed from the thermometer and placed in a dish, and the address of each sample was then placed. The examination was repeated with lead nitrate and cumin extract, and it was prepared for the purpose of XRD examination. Also put the liquid Frome AgNO3+ plant extract 5ml in tube. PbNO3 with plant extract 5ml in tube to UV test.

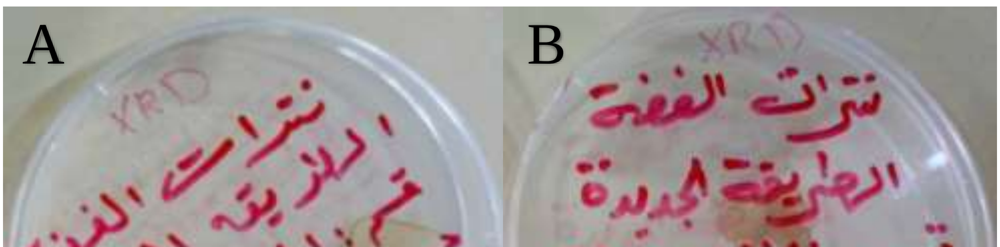

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Figure 14: A-Silver nitrate and mint extract slide the sample to XRD tests,B-lead nitrate and cumin extract slide the sample to XRD tests

3.9 method prepare the neutron agar medium

The culture media mentioned in Table (3-1-1) were prepared according to the manufacturer's instructions stated on the containers. They were then sterilized with an autoclave, then poured into sterile dishes and incubated at a temperature of 37°C for 24 hours to ensure that they were not contaminated. Then they were stored in the refrigerator at 4°C until use.

Agricultural Media are sterilized using an autoclave at a temperature of 121° and a pressure of 15 for 15 minutes.

Prepare this medium according to the company's instructions by dissolving 28 grams of the medium in a liter of distilled water, sterilize it with an autoclave, then leave it to cool to a temperature of (45-50) ℃. It is then poured into sterile dishes and tubes, and then used to grow and preserve bacterial isolates.

Figure 15: Neutron acar preparation process

3.10 Bacteria cultivation

Samples were collected from eye patients who use contact lenses. The sample was taken from a 25-year-old female who had symptoms of redness, burning, and inflammation of the cornea, taking the patient's information through the questionnaire form (age, gender), and cultivating them directly on the agricultural media that had been prepared in advance. Then it is transferred to the laboratory to be incubated for 24 hours at a temperature of 37°C.

Chapter foure

Results and discussion

4.1 UV Ultraviolet spectrum results (absorption, transmission and Energy gab)

4.1.1 UV Ultraviolet spectrum results(absorption)

UV-visble absorption spectrum of Ag and Pb nanoparticles within the wavelengths (200-1100) nm. The absorption of pb appears at a wavelength of 430nm is 0.32As for the second compound Ag the appears at a wavelength of 350nm is 0.16 and that mean the absorption of PbNO3 with cumin extract large thanAgNO3 with leaf mint extract.

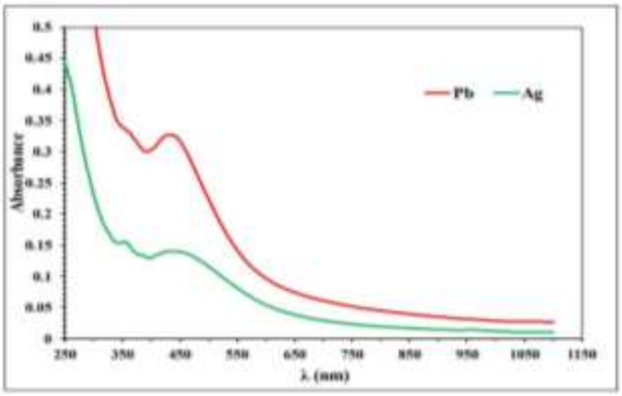

Figure (16): Absorption of nanomaterials for silver and lead

4.1.2 UV Ultraviolet spectrum results(transmission)

UV-visble transmittance spectrum of Ag and Pb nanoparticles within the wavelengths (200-1100) nm. The transmittance of Ag appears at a wavelength of 340nm at a rate of 95%. As for the second compound pb the transmittance appears clearly at a wavelength of 400nm at a rate of 80%. And that mean the transmission of pbNO3 with cumin extract less thanAgNO3 with leaf mint extract.

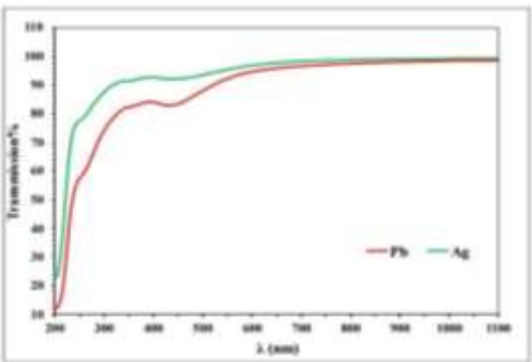

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Figure (17): transmittance of nanomaterials for silver and lead

4.1.3 UV Ultraviolet spectrum results (Energy gab)

The value of the optical energy gap was obtained by extending the straight line (tangent) segment to intersect the photon energy axis hν.it was found that the values of the energy gap for the compounds pb and Ag are close, ranging from (2.1-2.3) ev

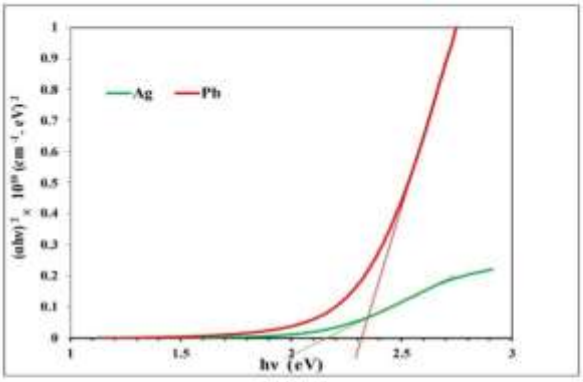

Figure (18): Energy gab of nanomaterials for silver and lead

4.2 X-ray Diffraction (XRD) of (lead and silver)

4.2.1X-ray Diffraction (XRD) of lead

The test results showed that the powder used is nano lead. The results of the XRD test also showed that the grain size of the lead nanoparticles ranges between (16.6-17.4) nm and that its crystal structure is of (Cubic) type as shown in the figure (19) and table (4.2). The particle size of lead was calculated using the equation (2-2).

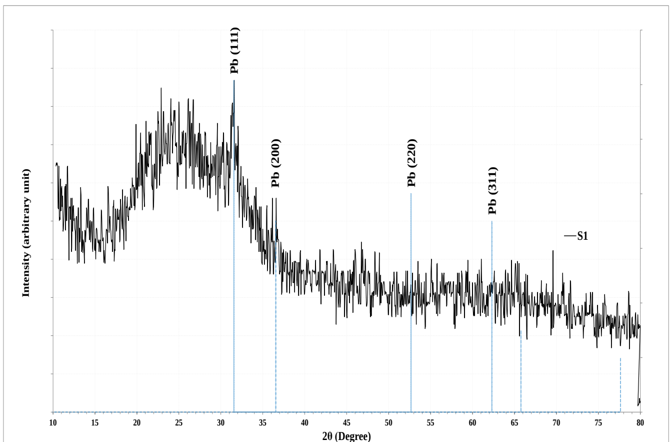

Figure (19): X-ray diffraction pattern of the prepared Pb nano particles

$$D_{KhI} = K\lambda / (B_{khI} \cos\theta) \qquad 2\text{-}2$$

Table 4.2: X-Ray parameters of the prepared Pb nano particles

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2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	C.S (nm)	hkl	Phase	card No.
31.5885	0.4732	2.8301	17.4	(111)	Pb	96-901-3419
36.5864	0.5027	2.4541	16.6	(200)	Pb	96-901-3419

4.2.2 X-ray Diffraction (XRD) of silver

The test results showed that the powder used is nano silver oxide. The results of the XRD test also showed that the grain size of the silver oxide nanoparticles (19.6 nm) and that its crystal structure is of (Cubic) type as shown in the figure (20) and table (4.2). The particle size of lead was calculated using the equation (2-2).

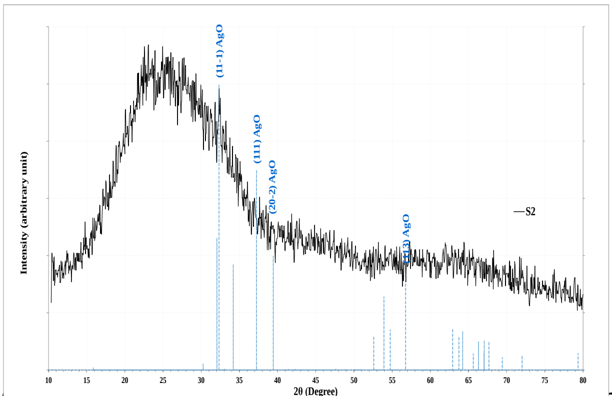

Figure (20): X-ray diffraction pattern of the prepared Ag nano particles

$$D_{KhI} = K\lambda / (B_{khI} \cos\theta) \qquad 2\text{-}2$$

Table 4.2: X-Ray parameters of the prepared Ag nano particles

4.3 Field emission scanning electron microscopy (FE-SEM)

2θ (Deg.)	FWHM (Deg.)	d_{hkl} Exp.(Å)	C.S (nm)	hkl	Phase	card No.
32.3446	0.4210	2.7656	19.6	(11-1)	AgO	96-900-8

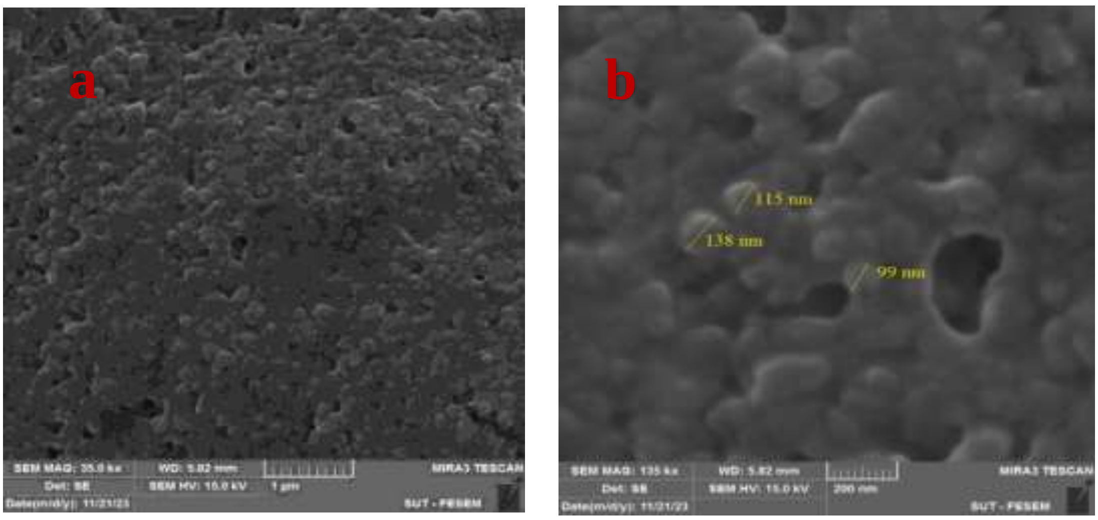

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Figure (21): FE-SEM images of Lead nanoparticles: a) magnifying image: b) We obtained lead nanoparticles and they were spherical in shape. There are also particles that are irregular in shape, but most of the particles obtained are spherical and the nanometer size ranges from (99-138) nm.

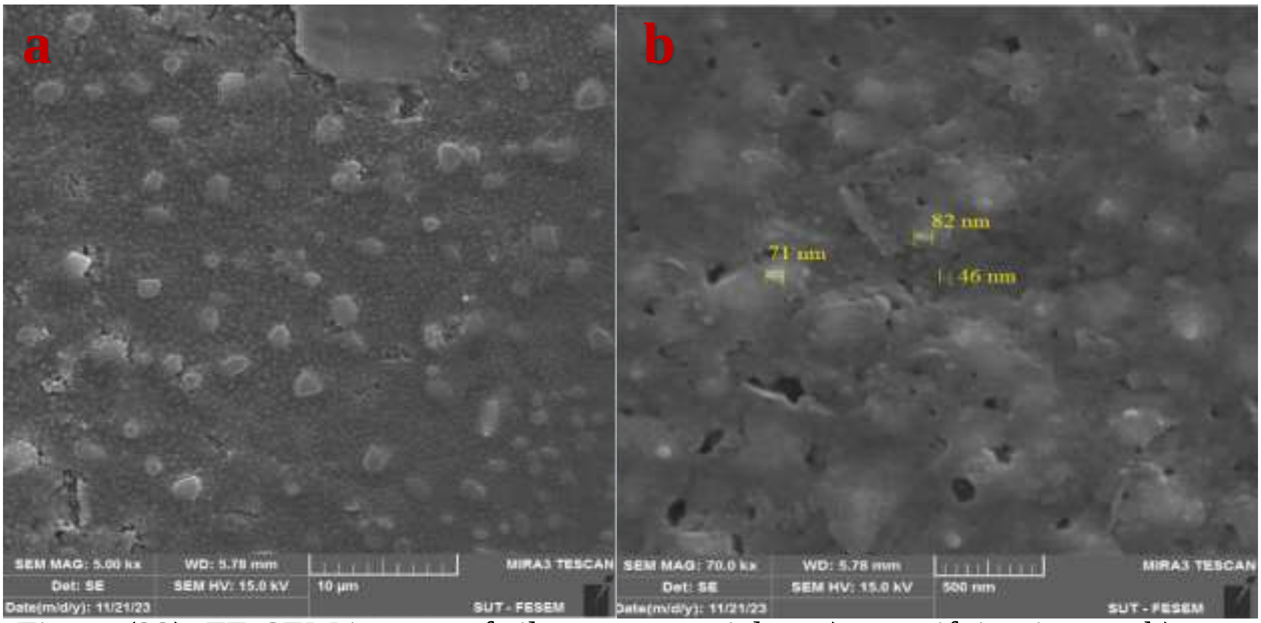

Figure (22): FE-SEM images of silver nanoparticles: a) magnifying image: b) We obtained silver nanoparticles and their shape was spherical and the size ranged from (46-82) nm.

4.4 Steps to culture bacteria and influence them using extracts:

We prepared two dishes: control 1 and control 2, It contains agar of agricultural medium, and nano-plant extracts have been spread on it (without bacteria) so that we can ensure that the extract is free of any bacteria or contamination. It was confirmed that the extracts were free of any external contamination or bacteria.

4.5 The effect of nanomaterial on bacteria

the method called well diffusion method. The plant extracts were placed in a water bath ultrasonic device for the purpose of homogenizing the material. After that, two dishes were prepared, each dish containing an agricultural medium called molar agar. A 3mm diameter perforator was taken. The dishes were perforated and a 100µm pipette was taken. As for the dish, silver nitrate was taken and placed in the first hole of the dish, and lead nitrate was taken and placed in the second hole of the second dish, after which they were placed in the incubator for 24 hours. Then, a zone measurement was taken for each area density stick [50].

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Solution	Inhibtion zone	average
Symbol	Zone diameter (mm)	
Control 1	The extract was grown on an agricultural medium and no bacterial growth was observed	
Control 2	The extract was grown on an agricultural medium and no bacterial growth was observed	
S_1----PbNO_3	23.48 mm	20.92 mm
S_2---- AgNO_3	18.37 mm	

Figure 23: Inhibtion of the lead nitrate +cumin extract+sliver nitrate+mint extract

4.6 Bacterial inhibition results

4.7 Results effect of nanoparticles on bacteria

By measuring the inhibition zones for both compounds, it was found that the measurement of the zone S1 (PbNO3) with cumin extract is 23.48 mm. With mint extract, S2 (AgNO3) is 18.37 mm. These results showed that PbNO3 with cumin extract has greater effectiveness than AgNO3 with mint extract in killing Bacteria. This is due to its nano-crystalline structure and its high ability to be absorbed into the target cell body.

3.8 Conclusions

- The results of the XRD test showed that the powder used is nano-silver oxide. The results of the XRD test also showed that the grain size of the silver oxide nanoparticles is (19.6 nm) and that its crystal structure is of the cubic type. It also showed that the grain size of the lead nanoparticles ranges between (16.6 nm_17.4 nm). Its crystal structure is cubic.
- A UV test was performed and peaks of absorbance, transmittance, and gap energy appeared. It was proven that the materials that were prepared are materials within the nanoscale range.

- Field emission scanning electron microscopy (FE-SEM) was used to determine the morphological characteristics of the nanoparticles. It was a form. The biosynthesized AgNps are spherical, with diameters ranging from (46 nm) to (82 nm) and falling within the nanoparticle boundaries. The shape of the biosynthesized PbNps was spherical and some particles were irregular in shape with diameters ranging from (99 nm) to (138 nm).In addition, a few aggregates were observed, and most of the nanoparticles appeared spherical in shape, and these results are consistent with what was obtained.
- The nano-lead loaded on cumin seeds and the nano-silver loaded on the mint plant showed effectiveness in killing the bacteria (Pseudomonas luteola) taken by swabbing it from the contact lenses of the eye of a person carrying this bacteria. There was effectiveness in killing that bacteria, as the measurement of the killed area was 23.48 mm with lead. Nanoparticles and 18.37mm silver nanoparticles.

According to the interpretation of the results and comparisons made, the effectiveness of lead was greater, making it a pesticide and antibacterial suitable for use in medical and commercial fields and in sterilization.

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