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Green Synthesis of Silver Nanoparticles and Testing its Effectiveness in Inhibiting E. coli, Staphylococcus Aureus Isolated from Urinary Tract Infections of Pregnant Women

Fatima Mustafa Al-najar[1*], Arshad Mahdi Hamad[2], Suha M. Abed[3], Hayder Mudheher Abbas[4]

[1*,2,3,4]*Department of Biology, College of Science, Tikrit University, Tikrit, Iraq.*

Corresponding Email: [1]Fatima.m.mahdi@tu.edu.iq*

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Abstract:** The aim of the study was to identify the E. coli and S. aureus bacteria isolated from pregnant women with urinary tract infections that silver nanoparticles derived from green leaves inhibited. The silver nanoparticles showed inhibitory activity against these two types by Well diffusion method. towards bacteria. The result of the inhibition diameters by Well diffusion method were (35,33,30,25) for E.coli and (37,34,28,23) for S.aureus bacteria at concentrations (10,7.5,5,2.5)% respectively through the absence of colony growth on the surface of Muller Hunton agar plates at those concentrations. The use of nanoparticles as a result of their effective properties in inhibiting bacteria, provided that there are no side effects that affect its work. To make silver nanoparticles in an eco-friendly manner—that is, without heating the plant extract and solution or utilizing chemical catalysts during the reaction—one milliliter of strawberry leaf extract and fifty milliliters of silver nitrate solution—prepared at a concentration of one millimeter at room temperature for one to eight hours—were combined. Using UV spectrophotometers, scanning electron microscopy (SEM), XRD, and FTIR, it was determined that the preparation had begun when the mixture's color intensity increased after each of these eight hours had gone. The absorbance at 378 nm was visible in the UV-VIS spectrum. According to the results of scanning electron microscopy, the produced silver nanoparticles ranged in size from 37.35 nm. The biological method of producing silver nanoparticles in this low-cost and eco-friendly manner has shown promising results in preventing the growth of bacteria isolated from wound infections.

***Keywords:** Strawberries, Biosynthetic Agnps, Wound Healing, and Bioreducing Agents.*

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1. INTRODUCTION

The second most frequent bacterial illness in pregnant women after anemia is urinary tract infection(1), Urinary tract infection, especially in the case of non-symptomatic infection that is common in pregnant women, may develop into acute nephritis If not treated, which increases the risk of numerous complications for the mother and fetus. Urinary tract infection, especially in the case of non-symptomatic infection that is common in pregnant women, may develop into acute nephritis If not treated. (2). Although antibiotics have helped and are still helping to treat urinary tract infections, their efficacy has started to decline due to bacteria's growing capacity to resist them in a variety of ways, rendering the treatment ineffective. This resistance manifests itself dramatically when the use of antibiotics is increased arbitrarily. (3). Therefore, in order to treat UTI infections, new antimicrobial medications are required that are safe for healthy cells, efficient against bacteria resistant to antibiotics, inexpensive, and do not encourage bacterial resistance. The oxides of metal nanoparticles (MNPs) (ZnO, AgO, CuO, and CoO NPs) are the main components of inorganic nanoparticle technology, and they have garnered a lot of attention because of their potential for delivering specific drugs. [4], medical imaging [5], cancer therapy [6], limiting bacterial development [7].

Silver and its compounds have long been known to possess broad-spectrum antibacterial capabilities that are effective against bacteria, fungus, and viruses[8]. Compared to other minerals, it is less dangerous to mammals while being exceedingly deadly to microbes. [9]. and because using chemicals causes dangerous substances to build up on nanoparticle surfaces,[10]. A cheap, environmentally beneficial, and easily scaled-up synthesis strategy is using plant extracts [11]. a plant's extracts It has an advantage over other biological processes used for making nanoparticles in that it does not require the time-consuming step of maintaining cell cultures. [12].

2. RELATED WORKS

Numerous studies have employed plant extracts, particularly Ziziphora tenuior, to create silver nanoparticles. Olive (Olea europaea) tree [13], In accordance with a number of studies, silver ions interact with amino acids in the SH group and contribute to the inactivation of bacteria. [15] Disconnection of the respiratory chain's electron carrier, which prevents the activity of respiratory chain enzymes or affects the permeability of the protons and phosphates membrane. [16]. In this study, silver nanoparticles were produced, analyzed, and their potential for testing silver nanoparticles' antibacterial activities against those of strawberry (Fragaria ananassa) leaf extract were also tested.

3. METHODOLOGY

Collection Urine Sample

Samples of urine were gathered according to the (17) method, as 30 urine samples were collected from Salah El-Din General Hospital for the period from May to July 2022.

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Diagnostics of the Isolates under Study
Bacterial isolates were diagnosed by the following test methods

Microscopic Examination and Cultivation Characteristics
We investigated the phenotypic traits of the forming colonies, including color, size, shape, texture, edge shape, height, blood lysis, and whether or not they were fermented with lactose sugar. Following that, the isolates were stained with Gram stain and seen under a light microscope equipped with an oil lens to determine how the cells responded to the stain, as well as their form and arrangement and size; (18)

Biochemical Tests
Testing methods for identifying bacteria included urease production, mannitol fermentation, coagulase, novobiocin susceptibilityurease synthesis, indole, methyl red, voges-proskauer, citrate usage, Kligler iron agar, and culture on methylene and eosin blue medium (EMB) (19)

Vitek 2 Compact System
The ViteK device manufactured by the French company biomerieux was employed to validate the bacterial isolates' diagnosis that could not be diagnosed by traditional methods.

Green Synthesis of Agnps
Preparation of Strawberry (Fragaria Ananassa) Leaf Plant Extract
In order to make strawberry leaf plant extracts, 1 g of fresh strawberry leaves were mashed in 100 ml of distilled water. The liquid was then stirred for five minutes in a 500 ml beaker before being filtered using Whatman No. 1 filter paper. After that, the extracted product is kept in a refrigerator at 4 °C [20].

Making a Solution of 1 Milligram Silver Nitrate
To make silver nitrate at 1 mM concentrations, 0.0421 g of silver nitrate were dissolved in 100 ml of double-distilled water. To stop the silver from oxidizing naturally, the resulting solution is kept in opaque glass vials. [21].

Green Production of Silver Nanoparticles with Plant Extract from Strawberry (Fragaria Ananassa) Leaves
A milliliter of leaf extract from the strawberry (Fragaria ananassa) plant was mixed with fifty milliliters of a A 1 mM aqueous silver nitrate ($AgNO3$) solution was added, and the combination was let to remain at room temperature for eight hours in order to form silver nanoparticles [22]. After the nitrate from the silver $Ag+$ was reduced to the free reduced form, the solution changed color from yellow to black. Every hour, the solution's hue changed. The color intensity was measured as the reaction time increased following the reduction of $Ag+$ to silver nanoparticles by strawberry (Fragaria ananassa) plant leaf extracts.

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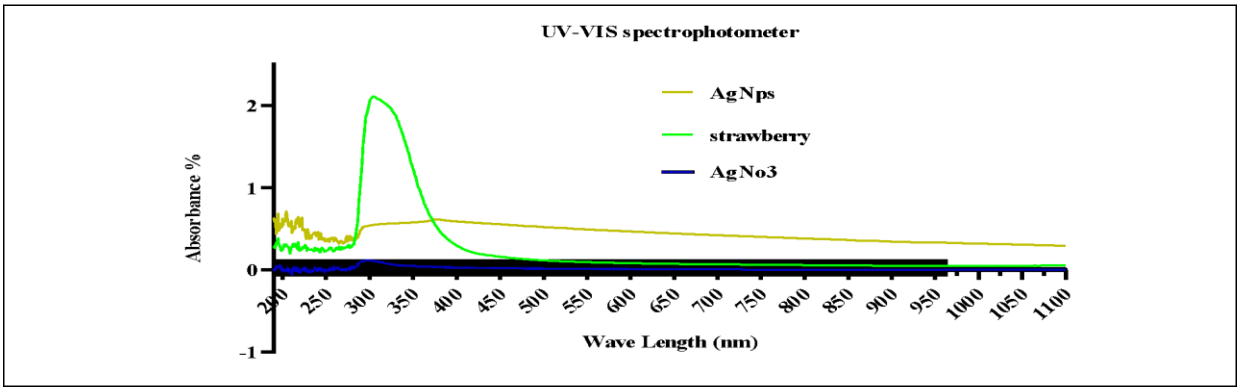
Silver Nanoparticles Produced Using Green Synthesis: Characterization Through Color Shift:
The color of the reaction mixture altered as observed with the naked eye. The production of silver nanoparticles was indicated by the color changing from yellow to dark brown [23].

UV-Visible (UV-Vis) Spectra
A Varian Cary-100 160 Konc spectrophotometer (Varian, Australia) was used to analyze the UV-visible spectra of the diluted samples at 230 V/50 Hz and between 200 and 800 nm [24].

Infrared Spectroscopy with Fourier Transform (FTIR)
FT-IR analysis was used to examine the functional groups present in the green-produced Ag-NPs (JASCO FT-IR 4100 spectrometer, Hachioji, Tokyo, Japan). High pressure was applied to a disk (KBr) that contained potassium bromide and 0.2 g of Ag-NPs powder. At a resolution of 4 cm–1, the 400–4000 cm–1 wavelength FT-IR spectra were examined [24].

Scanning Electron Microscope (SEM)
To image the optimized sample, a SEM (JSM 6390®, JEOL DATUM Ltd., Japan) was utilized. Every enhanced sample was lyophilized into a drop on tape that was then placed on an aluminum stub. A gold spit coater was then used to coat each drop with 400 A thick coatings of gold [25].

X-Ray Diffraction (XRD)
The characterization of the structure was studied to acquire information on particle size, crystal structure, and surface shape by means of a type (shimadzu 600) using a monochromatic copper beam with a wavelength (0.15406n) in operation (40 kV) and a current of 30 amperes and a scanning speed (0.02deg/s) and within an angular range of 20-(600) and with a ray entry hole of (0.3) mm diameter, The material under investigation was put in powder form on a glass slide with a silicon substrate on it [26].



4. RESULT AND DISCUSSION

Result
Bacterial Culture Results

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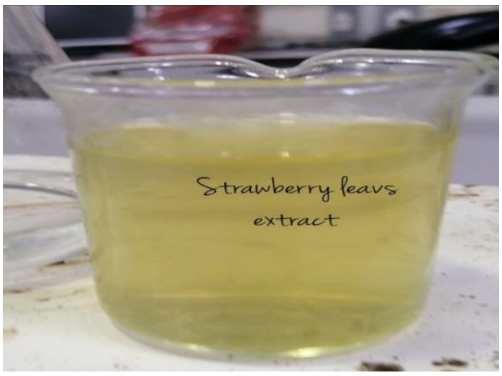
By examining the phenotypic traits of the bacterial colonies growing on the culture media, bacteria isolated from pregnant women's urine were identified and their microscopic and biochemical characteristics. Isolates of this type belong to the genus *Staphylococcus aureus*, while the number of isolates negative for gram-negative is 15 isolates by accepting the red safranin dye. It was found that this type of isolates belong to the genus *E.coli*, as they produce fermented pink colonies of lactose sugar on the MaCconkey agar medium, and they appeared in a shiny green color on the EMB medium.The results showed that the number of *E.coli* isolates reached 50%, and this percentage is close to studies conducted by researchers in Egypt and India (27) The percentage of isolation of these bacteria was 54.7% and 53.85%, respectively. The frequency of S. aureus bacterial isolation was also 50%, and this percentage is close to what was obtained by the researcher (28) as he isolated it by 55.9%.

By Color Change:
For eight hours at room temperature, color changes were seen in glass tubes containing a mixture of plant extract and silver nitrate at a concentration of 1 mM. There was a distinct change in color from bright yellow in the first hour to dark brown in the eighth. The initial indication of silver nanosynthesis is a color shift. [29]. as seen in Figure (1).

Figure 1: The color shift of silver nanoparticles made with the green synthesis method at room temperature

UV-Vis (Ultraviolet-Visible) Spectrum
used a UV-VIS spectrophotometer to evaluate the stability of silver nanoparticles based on colloidal liquid and produced from strawberry leaf extract. The UV-visible spectrograph of silver nanoparticles is shown in Figure (2).



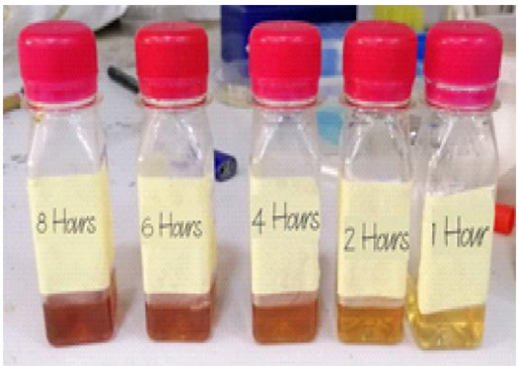


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Figure (2): Absorbance spectrum of AgNPs prepared by cold green synthesis ($AgNO_3$ and strawberry leaf extract)

The UV test was completed for the strawberry extract, in which the absorption value was at the highest peak at wavelength 304.50nm. While the absorption of ultraviolet rays for silver nitrate at the wavelength was 297.23 nm When mixing these two solutions according to the ratio mentioned in the preparation of silver nanoparticles, the wavelength of the resulting silver nanoparticles was 378 nm, which is an optimum value for nanosilver prepared by the green synthesis method [30].

Fourier Transform Infrared Spectroscopy (Ftir)

The green synthesis approach was employed in the production of silver nanoparticles. Three bands, one at 3429 cm-1, one at 2864 cm-1, one at 1741 cm-1, and one at 3429 cm-1, which belonged to the hydroxyl (OH) group, were seen in the infrared spectra of these particles. In addition to two bands that belong to the (NO2) group at frequencies (1516) and 1464) cm-1, a beam that belongs to the (CN) group at frequency 1383 cm-1, a beam that belongs to the (CN) group There is an appearance of the carboxylic acid carbonyl group (C=O) at frequency 1053 cm-1 and a beam associated with the (CN) group at frequency 515 cm-1 at frequencies between 1645 cm-1 and 1562 cm-1. For group (Ag-O), a beam from group (C-Ag) was detected at frequency 441 cm-1. The infrared (FT-IR) spectra of the silver nitrate salts revealed that the Ag-NO3 group had a band at frequency 443 cm-1 and the nitrate group NO3 had a band group at (1001, 1319, 1379) cm-1. is located in the hydroxyl group at (1753, 1770, 2883, 2989, 3030, 3294, 3392) cm-1. The hydroxyl (OH) group had a broad band at 3425 cm-1, the aliphatic (C-H) group had a band at 3380 cm-1, and the aromatic (AR) group had a band at 2350 cm-1, according to the infrared spectra of water-leaf strawberries isolated using the green synthesis method. For the carboxylic acid carbonyl group (C=O), a band from the group (C-O) occurs at frequency 1457 cm-1, a band from the group (C=C) appears at frequency 1627 cm-1, and a band from the amide carbonyl group (C=O) emerges at frequency 1719 cm-1. These frequencies originate from [31]. This is seen in Figure (3).

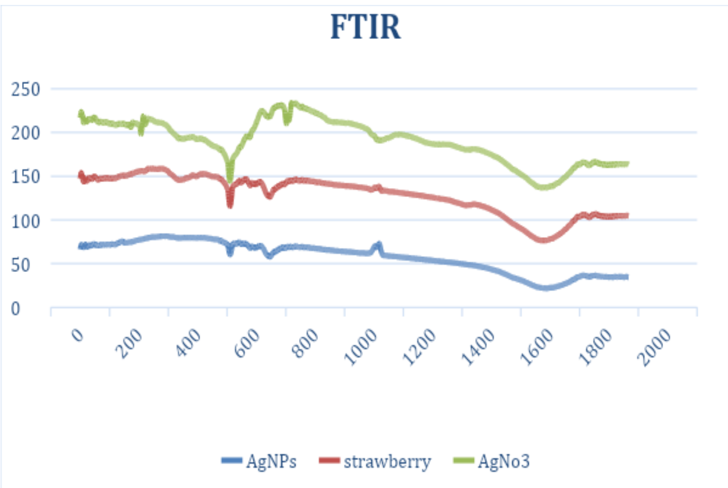

Figure (3): FTIR analysis of AgNPs made from AgNO3 and strawberry (Fragaria ananassa) leaves using a green synthesis (cold process) method

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EDX analysis and scanning electron microscopy (SEM) of FA-AgNPs

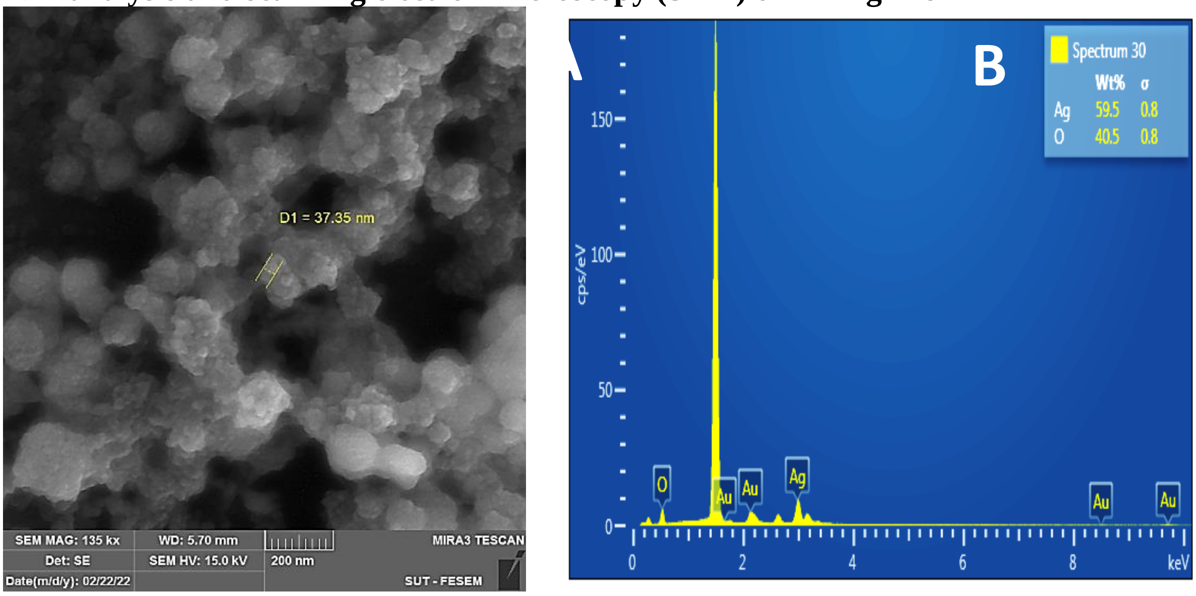

Figure 4 (A) demonstrates a SEM picture of the artificial FA-Ag NPs with a 1 µm scale bar, and Figure 4B displays the EDX spectrum for the FA-Ag NPs made from aqueous leaf extract. The energy is displayed in kilo electron volts on the horizontal axis, while the number of X-ray counts is displayed on the vertical axis.

SEM pictures revealed poly aggregation nanoparticles with a spherical and hexagonal form, with an average round size of 37.35 nm. (As seen in Figure 4A). Energy dispersive X-ray spectroscopy (EDX) investigation revealed the elemental mapping of the biogenic Ag NPs. An EDX analysis revealed the presence of oxygen (40.46%) and silver (59.54%) (Fig. 4 B). Two further peaks at 3.00 and 3.20 keV, respectively, were also associated with silver (Ag), while a prominent peak at 1.44 keV showed the presence of silver (Au). Our findings concurred with those of Okaiyeto et al., 2019 who discovered that silver and chloride were present in AgNPs prepared from an aqueous leaf extract of Oedera genistifolia [32]. As per an earlier study [33], a number of components found in the EDX analysis, including Si, Au, and Cl, act as capping agents for the biogenic AgNPs as a whole.

XRD, or X-Ray Diffraction

Figure 5 displays the XRD patterns of AgNPs made from AgNO3 and strawberry (Fragaria ananassa) leaves using the green synthesis (cold procedure). The conventional pattern and the tailored XRD pattern were contrasted [96-901-3054]; as we can see, the generated patterns feature peaks and intensities that correspond to AgNPs with a cubic crystal structure, while another peak refers to silver oxides NPs The COD card number for monoclinic Ag3O4 is [96-151-0026]. The angles and Miller's index are shown in this image, and apparent crystal systems. After being exposed to air for a while, the resultant solution developed oxidation, which led to the establishment of the intensities and preferred directions of the aforementioned peaks due to the nanosilver oxide. Numerous investigations have demonstrated that when Ag atoms disintegrate in liquid, they mix with oxygen atoms or

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radicals to generate Ag oxide nanoparticles (NPs). They went on to say that the temperature and partial pressure of oxygen determine the type of oxide that forms.

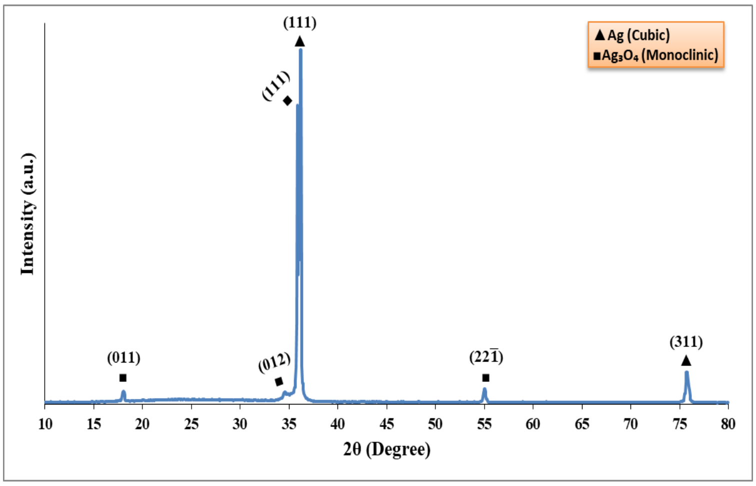

Figure (5): XRD pattern of AgNPs and Ag_3O_4 NPs prepared by using AgNO3 and strawberry (Fragaria ananassa) leaves for green synthesis (cold method)

Evaluation of the Silver Nanoparticles' Ability to Inhibit the Targeted Bacterial Species
Silver nanoparticles that were biosynthesized shown outstanding antibacterial efficacy against methicillin-resistant As shown in Figure 1, Staph. aureus had the following diameters: (37) mm for concentrations of (10) mM, (34) mm for concentrations of (7.5) mM, (28) mm for concentrations of (5) mM, and (23) mm for concentrations of (2.5) mM. While the results of the inhibition areas of Biosynthesized silver nanoparticles on *E.coli* bacteria were with a diameter of (35) mm for the concentration (10) mM, and with a diameter of (33) mm for the concentration (7.5) mM, and with a diameter of (30) mm for the concentration (5) mM, and a diameter of (25) mm for the as demonstrated in Figure (2) by the concentration (2.5) mM.

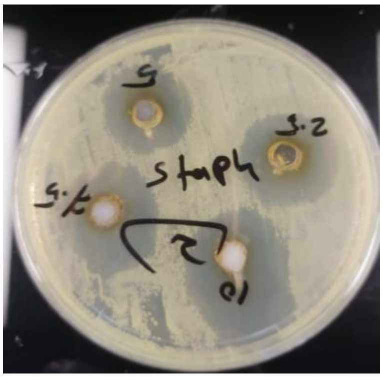	
A	B

Figure (6): (A) The Sensitivity of Methicillin-Resistant (B) The Sensitivity of E.Coli to Silver Nanoparticles_Staph.Aureus to Silver Nanoparticles

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Table (2): (Sensitivity of bacteria types to silver nanoparticles by Agar well diffusion method

Type of bacteria	Inhibition zone diameter (in mm) for silver nanoparticles (concentration in mM)			
	2.5	5	7.5	10
MRSA methicillin-resistant Staph.aureus	23	28	34	37
E.coli	25	30	33	35

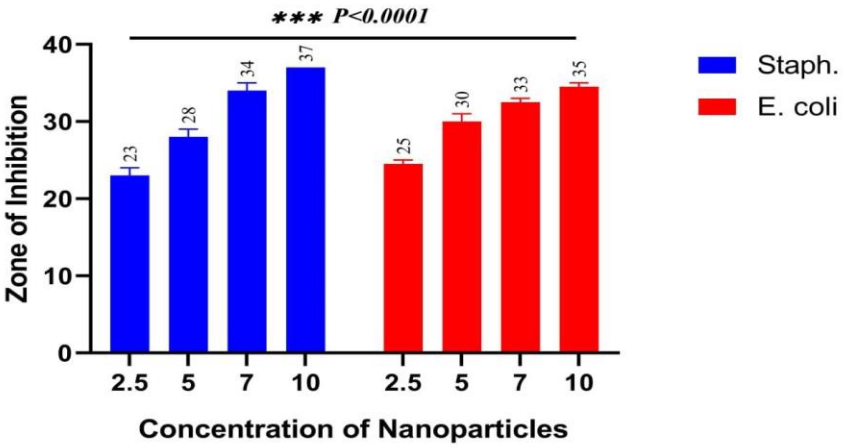

Figure (7): Column diagram of sensitivity of (Blue) Staphylococcus aureus and (Red) E.coli to different silver nanoparticle concentrations.

5. DISCUSSION

The green synthesis of nitrous silver from plant leaves produced results that demonstrated a high level of production efficiency. The results of the research showed that nano-silver manufactured from silver nitrate and strawberry leaf extract is highly efficient in manufacturing, a cheap, environmentally friendly and highly efficient process, and that this topic opens the horizons and gives hope for the production of nanoparticles. New types of antibiotics affecting pathogenic microorganisms. In our study, This result is consistent with what was stated by (34) where it was demonstrated that silver nanoparticles could be used to stop the growth of the bacteria S. aureus and E. coli by reacting with sulfur in the bacterial cell wall to alter the bacterial cell membranes. This finally caused the bacterial cell to die. The idea is the same even though there are numerous techniques to demonstrate bacterial growth in the presence of silver nanoparticles. Because these molecules have a positive charge and are coupled to the negative charge on the surface of the bacterial cell, there are theories explaining how nanoparticles can stop bacteria from growing. Additionally, the properties of the cell membrane are altered as molecules build up on its surface. The physical and chemical properties of the membrane can damage it and prevent it from carrying out specific functions, such as controlling respiration, electron transport, and osmosis (35)Also, Due to the buildup of silver nanoparticles in bacteria's plasma membrane and the generation of free radicals, the membrane function is disrupted, and the nanoparticles then penetrate the cell (36). Furthermore, a certain quantity of positively charged silver ions is released by the inside of the cell by the nanoparticles. These silver ions can attach to the bacterial ribosome and halt protein synthesis, or they can attach to the genetic material of the bacteria and destroy DNA that would otherwise replicate. It inhibits respiratory chain enzymes and cell proteins, causing the cell to lose its constituent parts, and attaches itself to the thiol group of proteins to produce free radicals that lead to cell death. (37).Both Gram-positive and Gram-negative microbes have been shown to be susceptible to membrane degradation and protein leakage when exposed to silver nanoparticles (AgO) [38]. Additionally, nanoparticles have the ability to reduce microbial biofilms, which lowers the likelihood of recurrent infections[39]. Glycolipids and glycopeptides are present in considerably higher concentrations in the cell walls of cancer cells than in normal cells because of their distinct receptor characteristics. Furthermore, by provoking programmed death in cancer cells, silver nanoparticles can damage their genetic makeup by upsetting genes involved in the cell cycle[40]. When the resulting silver ion binds to key macromolecules like DNA, RNA, and enzymes, it can cause damage to mitochondria and start the oxidation process [41]. Silver nanoparticles also affect the cancer cell's biofilm, preventing ATP generation and the respiratory chain [42].The selective nature of certain metabolic properties—such as the shape and metabolism of the cancer cells' surface receptors and their capacity to bind to different compounds—that distinguish cancer cells from normal cells also explains the effect on the cancerous line in comparison to the natural one, such as the MCF-7 cancer line [43]. Diffusion in the pits was used to quantitatively investigate antibacterial nanoparticles, and the results by[44] were consistent with the inhibitory zone being predominantly concentration-dependent. To reduce the prevalence and survival of viruses and other diseases, nanomaterials can be added to paints or coatings for walls, medical equipment, and other regularly touched surfaces like doorknobs, handrails, and so forth. Various nanocoatings and their possible application in public settings to lower infections are described in a recent review by [45].

6. CONCLUSION

The silver nanoparticles showed inhibitory activity against these two types by Well diffusion method. towards bacteria, The use of nanoparticles as a result of their effective properties in inhibiting bacteria, provided that there are no side effects that affect its work.

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