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Antibacterial Activity of Silver Nanoparticles Synthesized from *Aloe Vera* Extract

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***Abstract*— *Material science has advanced significantly due to nanotechnology, which enables researchers to work with materials at the nanoscale to produce novel goods. It has been suggested that using plant extract nanoparticles biosynthesis is an economical, and environmentally benign process. Despite various chemical and physical approaches, green nanomaterial synthesis is the most contemporary. We used the Aloe vera plant to synthesize silver nanoparticles (AgNPs) and tested their antibacterial characteristics. UV-Vis spectroscopy, Scanning Electron Microscope (SEM), and X-ray diffraction were used to analyze the produced AgNPs. The XRD peaks, which were indexed, represented the face-centered cubic configuration of AgNp's. The exhibited peak indicates nanocrystalline-structured silver particles. The average particle size of bio-synthesized NPs which was between 30 and 35 nm is confirmed by SEM. The antimicrobial activity of our synthesized Nps was tested against bacteria (Escherichia. E. coli, Pseudomonas luteola, Bacillus Subtillis) as a function of nanoparticle concentration and the test was done by Disc diffusion method and antimicrobial activity was found to be quite robust. When the outcomes were compared to the effect of antibiotics like Ciprofloxacin 5, Vancomycin 30, and Ampicillin 10, these antibiotics were shown to be less effective than nanoparticles*

***Keywords*— Aloe Vera, Biosynthesis, Nanotechnology, Nanoparticles (NPs) Silver Nanoparticle (AgNPs).**

I. INTRODUCTION

The 'nano' is a Greek prefix that indicates 'dwarf' or minuscule and indicates 1 billionth of a meter (10^{-9} m) [1]. the study of structures and substances on a nanometer scale is known as Nanoscience. nanotechnology is a technology used in practical applications like electronic gadgets. Nanoscience dates back to the 5th century BC Greek and Democritus eras [2]. From the huge molecule to the small solid item to be a strong link between surface and volume, the nanoworld is the gateway between the atom and the solid. The nanoworld concept is based on the coming together of a diverse range of scientific and technological fields that were distinct earlier [3].

Nanotechnology is the study of the very small; it is the small-scale manipulation of material. Atoms and molecules have various functions at this scale and have various remarkable and intriguing uses. It provides opportunities for the development of materials, including medical applications [4].

In current material science, nanotechnology has been the most actively studied topic. Nanoparticles show entirely new and innovative properties based on certain qualities such as dimension, shape, and distribution [5, 6].

NPs differ in shape, size, and structure. It's between 1 and 100 nanometres and its shapes include round, hollow, spiral, flat, and uneven in shape with a tabular, conical hollow center. Surface variation might be uniform and uneven. Certain NPs are crystal solids that maybe loosely or densely packed and they are crystalline or amorphous. [7].

CLASSIFICATION OF NANOPARTICLES:

Carbon-based, inorganic, and organic NPs are the 3 subclasses of NPs.

ORGANIC NANOPARTICLES:

Organic NPs include dendrimers, micelles, liposomes, and ferritin, among others [8]. In addition to their typical traits including size, composition, surface morphology such as, and so on, their unique characteristics make them ideal organic nanoparticles, medication delivery systems may also employ organic NPs[9]. These NPs are environment-friendly. [10]. The medication carrying capacity, stability, and distribution mechanisms, such as liposomes, have a vacuous core and are delicate to temperature and targeted heat.

INORGANIC NANOPARTICLES:

Inorganic NPs didn't contain carbon. Inorganic NPs are not harmful. Biocompatible and hydrophilic inorganic nanoparticles, contrastingly with organic nanoparticles [11], inorganic NPs are extremely stable. Inorganic NPs are categorized into Metal and metal oxide NPs.

METAL-BASED NANOPARTICLES:

Metals are converted into nanometric-sized particles by constructive destructive methods [12]. Almost any metals can be converted into Nanoparticles [13]. Aluminum (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag), and zinc (Zn) theses metal are commonly used in the synthesis NPs [14]. Aside from their sizes, which range from 10-100nanometer. [7].

METAL OXIDE-BASED NANOPARTICLES:

The goal of metal oxide NPs is to correspond with relvant metals. [15]. Iron nanoparticles, for example, are oxidized to iron oxide nanoparticles. Iron oxide nanoparticles have a higher degree of reactivity than iron NPS. An improvement in efficiency and reactivity results in the generation of metal oxide NPs [16]. Zinc oxide, silicon dioxide, iron oxide, aluminum oxide, cerium oxide, titanium oxide, and magnetite are examples of metal oxide nanoparticles [7].

CARBON-BASED NANOPARTICLES:

Carbon-based nanomaterial is made entirely of carbon. Carbon-based NPs include fullerenes, graphene carbon nanotubes (CNT), carbon black, and carbon nanofibers.

METHODS FOR SYNTHESIS OF NANOPARTICLES:

Different techniques including chemical, physical, and biological techniques are used to create nanoparticles.

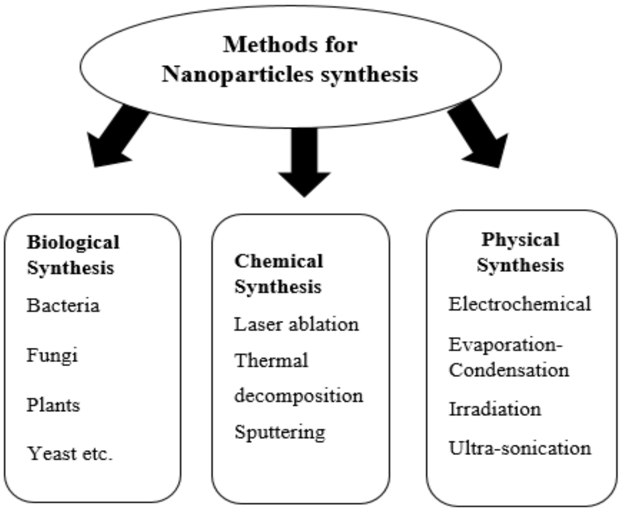

Fig.1. Methods for Synthesis of Nanoparticles

PHYSICAL METHOD:

physical methods used to make nanoparticles are UV irradiation, sonochemistry, laser ablation, and radiolysis. during the physical synthesis process, metal atoms evaporate. Immediately after this, condensation on various supports, where the metallic atoms rearrange as tiny collection of metals nanoparticles. using physical methods we may create NPs that are extremely pure and have a certain form [17]. Evaporation/condensation has previously been used to make nanoparticles of several materials such as Ag, Au, and fullerene using this physical technique [7]. Conversely, these techniques usually need highly advanced instruments, chemicals, and radiant heating, along with significant power consumption, leading to elevated operational expenses.[17].

CHEMICAL METHOD:

Mechanical milling, laser ablation, thermal decomposition, sputtering, etc. [17] are the chemical techniques for the synthesis of nanoparticles but demands a short period in order to produced enormous amount of NPs. The majority of chemicals employed in nanoparticle synthesis and stabilization are harmful and produce non environment-friendly wastes [5].

BIOLOGICAL METHOD:

There is a current demand to design environment-friendly synthesis techniques that do not have harmful compounds and have advantages over traditional processes that use harmful chemicals. To synthesize nanoparticles, biosynthesis employs bacteria, plant extracts, fungi, and other microbes, as well as precursors [18]. The need for ecofriendly NP synthetic procedures. Synthesis has led to an increasing interest in biological methods without using hazardous chemicals as byproducts and green nanotechnology has emerged. Because they don't contain hazardous compounds and offer natural capping agents, plants make a superior starting point for the creation of nanoparticles.[19].

Several plant extract has been used to accomplish the Green synthesis of NPs we use *Aloe vera* in this study.

ALOE VERA:

The succulent plant *Aloe vera* belongs to the Aloe genus [20]. There are roughly 500 species and many places in the world, and consider them invasive. The botanical names *aloe* (also from Greek) and *Vera* ("true") are both Latin. *Aloe* is commonly known by its common names which include *Chinese aloe*, *Cape aloe*, or *Barbados aloe.* Nicolass Laurens Burman described it in 1768 as *Aloe vera* in *Flora.* [21]. Aloe species are succulent perennial xerophytes with thick fleshy leaves that allow water to be stored in the well-known gel [22]. Aloe species thrive in semi-arid, warm conditions and best grow on dry, sandy, calcareous soil. Aloe, on the other hand, is not a cactus. Africa the Arab peninsula, Madagascar, and all the Indian Ocean islands are home to the Aloeis. Some of their species can be also found in the Asia California and in the United States [23].

Because of its plethora of biomolecules, *Aloe vera* is a fleshy plant that has therapeutic benefits [24][25]. The researchers were successful in synthesizing nanoparticles using microorganisms and extracts of plant-specific items such as *Aloe vera gel* to make silver and gold nanoparticles [26]. The nanoparticles were embedded into cotton fabric and screened for their antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus* [19]. Because of its antimicrobial, antioxidant, anti-inflammatory, and other functional qualities, *Aloe vera* is one of the oldest therapies for a variety of human maladies, as a result, it is found in favor in disciplines including, preserving food, sustainable packaging cosmetics, and pharmaceuticals [27].

SILVER NANOPARTICLES:

Several new products are using silver nanoparticles to generate antimicrobial action due to a large surface area [31]. Because of its dichroic property when integrated into glass, silver nanoparticles were of long-term interest for centuries. Because of their biocidal qualities, silver-based substances have been utilized as harmless, inorganic, and antimicrobial agents for ages in a variety of applications including wood preservatives and water purification [32]. AgNps are widely employed characteristic in catalysis, chemical sensing, biosensing, photonics, electronics, and pharmaceuticals due to their unique properties [33]. Antimicrobial activity is another application of AgNps. With a low toxicity profile, silver is a powerful antimicrobial. Silver and silver nanoparticles are most commonly used in scientific applications, such as topical ointments to prevent burns and open wound infections [34]. Owing to their intriguing physicochemical properties, biology, and medicine both greatly benefit from the use of AgNp [35]. Because of its remarkable aspects, researchers are now concentrating on metal nanoparticles, and nanostructure nanomaterial production [36]. The AgNps has piqued the curiosity of the scientific community due to their vast range of applications. Additionally, These AgNps are being used to effectively identify and treat cancer [37].

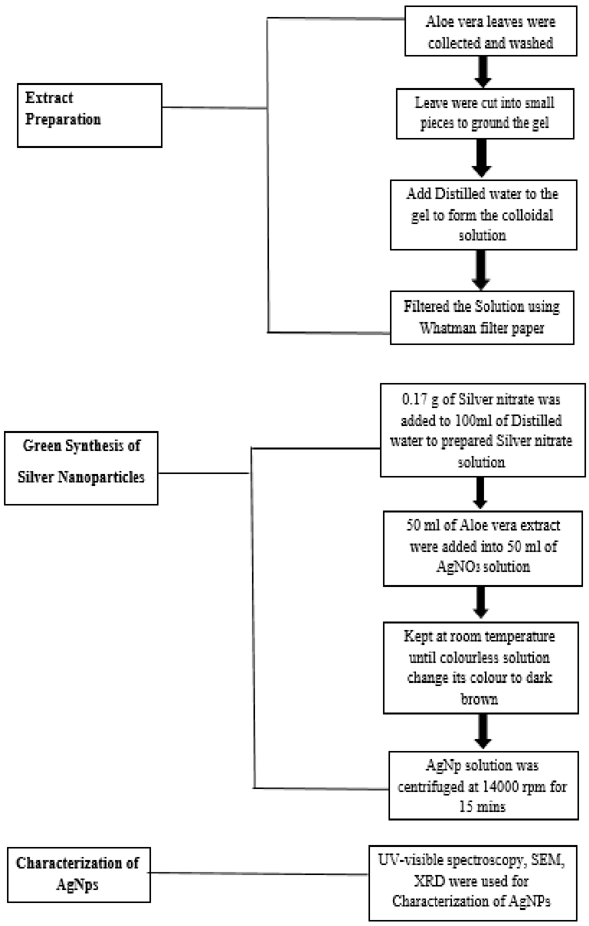

Fig 2. Schematic Diagram of Synthesis of Silver Nitrate

SAMPLE COLLECTION:

We harvested fresh *Aloe vera* leaves from the garden of Women University Mardan and *Escherichia coli, Pseudomonas luteola, and Bacillus Subtillis* were provided by the Central Lab of Women University Mardan.

SILVER NANOPARTICLES SYNTHESIS:

ALOE VERA EXTRACT PREPARATION:

Fresh *Aloe vera* leaves were collected and were washed two to three times with tap water to eliminate any dirt and dust, after surface sterilization the green peel was removed, and the leaves were chopped into minute pieces, a gel emerged. the gel was then blended to produce an aqueous solution which was sieved through Filter paper and the filtered leaf extract was then placed at room temperature.

PREPARATION OF SILVER NITRATE SOLUTION:

To make the solutin of 1mM Silver Nitrate, add 0.17 grams of silver nitrate in 100ml of distilled water. After stirring for a few minutes, the silver nitrate solution was prepared and covered with Aluminum foil.

SYNTHESIS OF SILVER NANOPARTICLES FROM *ALOE VERA* EXTRACT:

In a reaction process, both the AgNO3 solution and extract were taken in 1:1 concentration. To alter the pH of the medium 50ml of 1mM $AgNO_3$ solution was added into a flask having 50ml of plant extract and dissolved with 1% ammonia solution. The prepared solution was kept at room temperature the synthesis of spherical-shaped AgNP was indicated by the appearance of orange-yellow coloration. The solution was centrifuged and the Suprenant was eliminated from the prepared solution and the pellet was rinsed in ethanol and placed directly on a glass coverslip for air drying.

II. CHARACTERIZATION

UV-VISIBLE SPECTROSCOPY:

The AgNp's were studied using a UV-visible spectrophotometer (Uviline Connect Series 940) to compare the synthesis and kinetic properties of our designed nanoparticles. The spectrometer was equipped with "Spectra lab" software to collect and examine the results. The formation and stability of AgNp were seen in wavelengths between 300-550 nm. UV-Vis absorption spectra recorded the numerical data were plotted as a graph.

X-RAY DIFFRACTION METHOD (XRD):

The developed AgNp solution was centrifuged for 30 min at 14000 rpm. The Suprenant was removed with the help of a disposable syringe and the pellet was washed with ethanol and placed for air drying to get powder AgNps for XRD. X-ray diffraction (JDX 3532 Jeol Japan) Cu K radiation is used for the characterization of synthesized nanoparticles. The samples were imaged with the generator's current and voltage parameters set at 35 kilovolts and 25 milliamperes, and they scanned in the 15 to 700 C range. 0.04/sec was the scan rate.

SCANNING ELECTRON MICROSCOPE (SEM):

The JSM-5910 Jeol Japan SEM machine was utilized to characterize the mean particle size and shape of our synthesized nanoparticles. After sonicating the AgNP solution with distilled water, tiny droplets of these samples were applied on a slide and waited for it to dry. A tiny plated platinum layer was added to the samples to make them conductible. A vacuum of 10-5 torr was applied to the JSM-5910 Jeol Japan SEM machine. The microscope's accelerating voltage was maintained between 10 and 20 kV. The SEM micrograph gives the shape and size of our synthesized AgNPs.

ANTIBACTERIAL ACTIVITY:

Different bacterial strains including *Bacillus Subtillis, Escherichia coli, and Pseudomonas Luteola* were utilized antibacterial activity of synthestic ANPs was determined using Well Diffusion technique.

SUB-CULTURING:

To obtain the pure colonies, the bacterial strains were grown on nutrient agar and incubated at 37° Celsius for 24 hrs. Using the streaking technique, the bacteria were cultured on nutrient agar and incubated the cultures at 37° Celsius for 24 hrs. Inoculate the bacterial strain's single colony and keep it at 37° Celsius in the incubator for 24 hours.

WELL DIFFUSION METHOD:

MHA media was ready to use for the Well Diffusion technique to evaluated the produced silver nanoparticles' antimicrobial activity against bacterial species. The glass plates were filled with MHA medium and the inoculated bacterial strain using a disinfected cotton bud the holes were made with the assistance of micropipette blue tips and wells were filled with silver nanoparticle solution at different concentrations of 40, 60, and 80 µl/ml and incubated for 24hrs at 37°C. The antimicrobial action was determined by examining the region of embarrassment (mm) that was developed all around the discs.

III. RESULTS

The alteration in hue of silver nanoparticles is due to surface plasmon vibration. A visible color shift in the reaction medium was used to initially identify the silver nanoparticles. Over time, the intensity of the color grew to turn from orange-yellow to dark brown.

UV-VISIBLE SPECTRAL ANALYSIS:

The appearance of brown color is due to surface plasmon vibration. A surface plasmon absorption band with a maximal of 350 nano-meters was seen in brown. This indicates the AgNp absorbance at 350 nm-53 0nm as shown in Figure 5 and their structure was confirmed by SEM images.

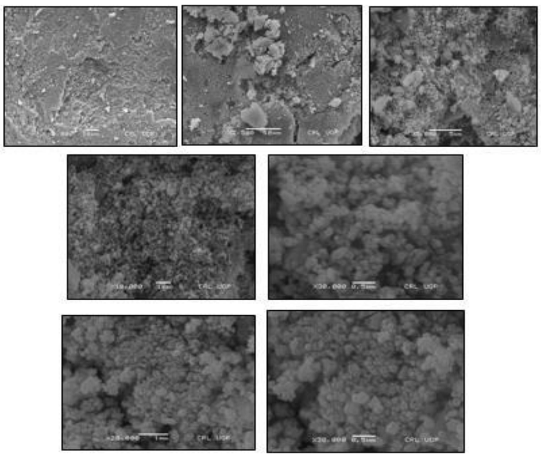

Fig 3. SEM images of nanoparticles at various magnifications

X-RAY DIFFRACTION ANALYSIS:

X-RD is utilized to examine the particle's overall oxidative condition throughout period, i.e., phase identification and depiction of the nanoparticles' crystal structure. XRD investigations were used to perform structural analysis. Figure 10 shows the XRD form of *Aloe vera* plant extract in the manifestation of AgNP. The face-centered cubic pattern of AgNP was represented by the XRD peaks, which were indexed. The nanocrystalline-shaped silver particles are indicated by the depicted peak.

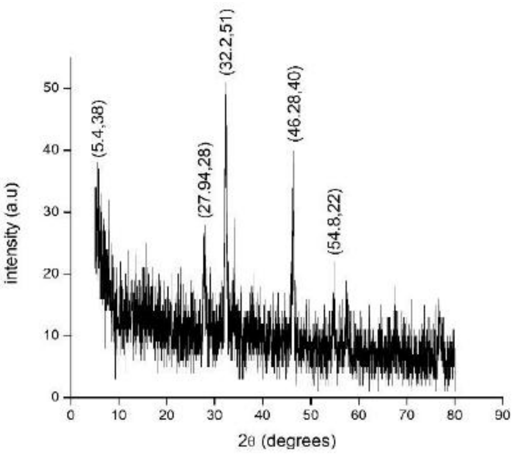

Fig.4. XRD Graph Of Silver Nanoparticles

4.4 ANTIBACTERIAL ACTIVITY OF THE AgNPs:

The antimicrobial characteristics of the AgNPs were concluded by monitoring the inhibition zones surrounding wells at various volumes of 40, 60, and 80 µl/ml, following 24 hrs of the incubation period at 37°C. The result revealed that sliver nanoparticles had an inhibition zone against *Escherichia. Coli, Pseudomonas luteola, Bacillus Subtilis*.The antibacterial activity enhanced as the concentration of AgNps was increased. Figure 11 shows Ag nanoparticles were used to create a clear zone of inhibition. *Bacillus Subtilis* relative to the most significant zone of inhibition as compared to other bacterial strains.

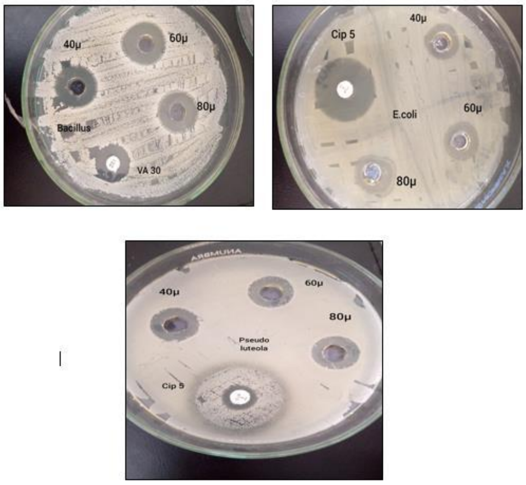

Fig 5. Antibacterial Activity of Nanoparticles with Different

Concentrations Ranging From 40 µL, 60 µL, and 80 µL

Table 1. The antimicrobial activity of silver nanoparticle synthesized using extract of Aloe vera

Bioactive agent		Inhibition Zone		
		Escherichia coli	Bacillus subtilis	Pseudomona luteola
Ag nanoparticles	40µl	13	18	12
	60µl	15	22	14
	80µl	16	21	16
Ciprofloxacin 5		22	nil	10
Vancomycin 30		Nil	17	Nil

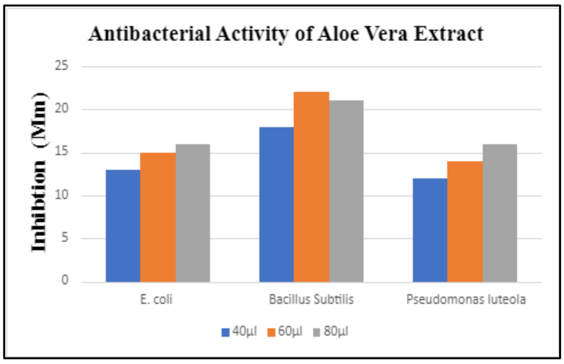

Fig 6. Antibacterial Activity of Nanoparticles with different

concentrations ranging from 40 µl, 60 µl, and 80 µl

IV. DISCUSSION

When AgNO3 solution was added to the *Aloe vera* extract the color was slightly changed into an orange-yellow color and over time, the intensity of the color grew to turn from yellowish to brown. Since within the increase in time of reaction from 10 to 15 mins from 10 hrs the colorless $AgNO_3$ solution turned pale yellow to deep red-brown during silver reduction (*Cotton Fabric*, n.d.). The study showed that further increases in reaction time such as 24 hrs and 48 hrs resulted in little change in color intensity, indicating that reaction time is complete. In this research study generated NPs were studied under UV-Vis spectrophotometer the peak absorbance was between 350 nm-530 nm and the maximum peak was observed at 350 mn. But in [38] study the Plasmon peak was observed at 450 nm and illustrates the making of AgNps. Another study showed that spectral peaks were between 430 nm to 530 nm [19].

According to SEM studies the size of synthesized AgNps was between 30 nm to 35 nm and is spherical and clumped together. According to [39] nanoparticles synthesized from The agglomerated nanoparticles were measured to be between 287.5 and 293.2 nm in size, although the usual particle dimension is predicted to be 70 nm. Moreover, Vélez et al., 2018 synthesized AgNps that were mostly spheroid within a usual size of 25 nm according to SEM studies.

The analysis of the nanoparticles we manufactured through Alovera extract was conducted through X-ray diffraction XRD. The XRD analysis shows the face-centered cubic crystalline structure. In another study the XRD examination of AgNPs produced Bragg reflection peaks at 111,200,200, and 311 were observed in the examination after biological reduction and stabilization with Alovera, these confirm that there is a cubic lattice structure centered on the nanoparticle face [19].

In this study nanoparticles synthesized from *Aloe vera* extract have strong antimicrobial properties towards *Escherichia. Coli,* (Gram-negative) *Pseudomonas luteola,* (Gram-negative) *Bacillus Subtilis* (Gram-positive), and the antibacterial activity was strong against *Bacillus Subtilis* as compared to the other two bacterial strains. From previous studies, it was observed that nanoparticles synthesized from Elettaria Cardamom the nanoparticles showed the inhibition zone but the maximum inhibition zone was shown against *Bacillus Subtilis* [40]

V. CONCLUSION

The following are the conclusions from the current research

- AgNps were effectively produced by the green synthesis method
- Organisms ranging from simple to very complex can be used to synthesize nanoparticles of the correct size and shape.
- UV-visible spectrophotometer, SEM, and XRD were used for detailed characterization of AgNps.
- We explored the antimicrobial efficacy of the AgNps we manufactured against bacteria (*Escherichia. E. coli*, *Pseudomonas luteola*, *Bacillus Subtillis*) at varying nanoparticle concentrations. The evaluation was conducted using Disc Diffusion method, revealing notably strong antimicrobial activity.
- When the outcomes were compared to the effect of antibiotics like Ciprofloxacin 5, Vancomycin 30, and Ampicillin 10, these antibiotics were shown to be less effective than nanoparticles.

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