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Study of Silver, zinc oxide, and magnetite nanoparticles antimicrobial properties and its characterization techniques

UDAY PRATAP SINGH BHASKER
Research Scholar, Pharmacy School of Pharmaceutical Science, Shri Venkateshwara University, Gajraula District-Amroha, Uttar Pradesh
E-mail ID- udgs16@gmail.com

Dr. Umesh Kumar
Professor, Pharmacy School of Pharmaceutical Science, Shri Venkateshwara University, Gajraula District-Amroha, Uttar Pradesh
E-mail ID- uklatyan@yahoo.co.in

Abstract
In order to produce nanoparticles, green synthesis or bio-assisted technologies present an effective, low-toxic, economical, and ecologically acceptable method. The investigations on the green synthesis of Ag NPs, ZnO NPs, and Fe_3O_4 NPs, their antibacterial activity against various bacterial strains, and spectrum characterisation of these materials are summarised below. In the current work, biomolecules that can be employed as antimicrobial agents are produced using plant material.

Keywords: *Nano particles, Green synthesis, silver, zinc, Antimicrobial activity, Characterization*

Introduction
The unique physical, physiological, photonic, and biological properties of the nanoparticles can be adjusted to meet the requirements of numerous applications [1]. Additionally, due to the fact that natural processes occur at this scale and due to the ability of nanoparticles to modify the surface of living things, they are in line with their intended usage in the field of medicine [2]. The usefulness of nanostructured materials as antibiotic is now being intensively examined and investigated. It is well known that the surface energy of the nanostructures that come into touch with microbes influences their antimicrobial properties. The interface of the nanomaterials with the bacteria to take out a wide spectrum of antimicrobial actions is enhanced by their tiny size and high surface to volume ratio, or big surface area[3]. Because of their improved antibacterial activity, the composites made with metal oxide nanoparticles or monomers may have greater use. They also vary from the comparable larger particles in terms of increased chemical and physical qualities brought about by the symbiotic mixing of a number of constituents [4].

Problem Definition

The research is entitled as, "**Study of Silver, zinc oxide, and magnetite nanoparticles antimicrobial properties and its characterization techniques**".In the present study it is planned to exploit antibacterial and its spectral characterization techniques. Metal-based nanoparticles have been the subject of extensive research for a number of biomedical applications. Because they don't bind to a receptor molecule in the cell membrane, metal-based nanomaterials have semi-germ-toxic properties that make it harder for bacteria to develop resistance while broadening the range of their bactericidal effects. For this reason, the several iron nanoparticles study designs that have been implemented thus far in facultatively and facultatively germs have shown positive outcomes.

Objective

- To explore the significance of metal nanoparticles as an antimicrobial agent.
- To explore silver, zinc oxidespectral and characterization techniques.
- To explore the techniques of characterization of magnetite nanoparticle.

Scope of Study

A notable improvement in nanomedicine will be the straightforward green manufacturing of NPs with constrained dimensions and forms employing inter - and intra cloning, familial engineering techniques, and other biotechnological techniques. Experimental studies on these kind of living organisms, bio materials, or characteristics will be sped up, eliminating these perilous conservative forms. NPs with excellent "social responsibility, microelectronics, and electronic structure" generated from unlimited bio-resources and hypoallergenic operations all have much ramifications in science, health, engineering, and agricultural. While nanoscale has already been employed to cosmetics, green-produced NPs could pave the way for a new era in nano cosmetology. Aside from all of these options, competent technical direction or administration may be vital to the international economic growth post-COVID-19 sustainability.

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CHARACTERIZATIONTECHNIQUES:

Analytical techniques such as spectroscopic, microscopic, diffraction, and magnetic measurements were used to analyse green produced Ag NPs, ZnO NPs, and Fe_3O_4 NPs.

1. UV-Visible Spectroscopy

The UV-visible emission spectra of Gold Nanoparticles, Ag Nps, while Fe3O4 NPs were captured using a Quick Observer single beam LM-35 laser. UV-vis absorption spectra aid in the synthesis of Silver, Zinc, and Bifeo3. Different wavelengths with a range around 200 and 400 µm in length are used in Photothermal optical absorption. to an 800 nm using uv spectrophotometer Once sample compounds are illuminated with only an intensity that corresponds a plausible electric potential inside the molecules, a small amount of laser radiation is transferred when the proton is moved to a high-power shell. Using an infrared analyser, the bands where the absorbing takes place are documented. It is the recognisable liquid level of Ag Nanoparticles, Zno Nanoparticles, and Cofe2o4 NPs attributable to interface plasmons.

But since optic characteristics of particles are subject to height, shape, quantity, aggregate state, the diffusivity close to the surface of the particle, Spectrophotometry seems to be a helpful method for detecting, measuring, and analysing nanomaterials. Ag NPs, Zinc NPs, as Fe_3O_4 NPs, among others, have distinctive optical characteristics that engage strongly with certain photon wavelengths, setting the foundation for the field of plasmonics. Because various NPs contain distinctive colours, Spectrophotometry seems to be a potential method for characterising NPs. Employing Gold Nanoparticles as a catalysis to reduce phenol to contribute to health benzene in the context of NaBH4 and Uv detection to track that deterioration of Dye adsorption there in vicinity of ZnO catalyst at a later time, and the adsorption of MB dye on magnetite nanoparticles. The materials were dissolved in either suitable solvents or directly loaded in quartz cuvettes for recording the absorption spectra, with a blank cuvette containing only the solvent serving as a reference.

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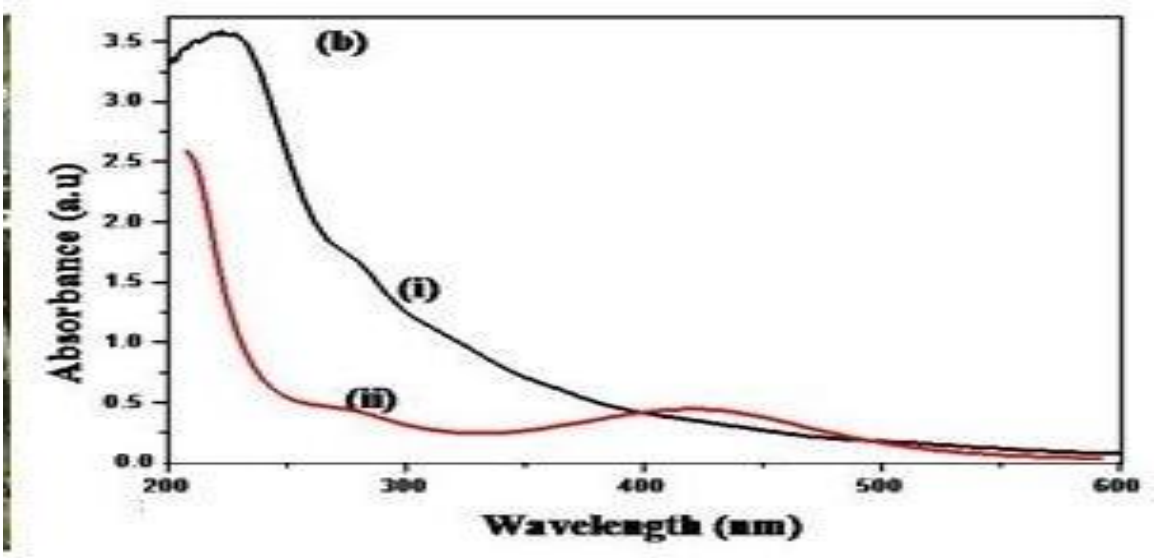

Figure 1: (b) UV-Vis spectra of (i) aqueous leaf extract of *FH LF* and (ii) synthesized Ag NPs.

2. Infrared Fourier Transform Spectroscopy (FTIR):

The functional groups contained in the plant extract that are responsible for the generation of Ag NPs, ZnO NPs, and Fe_3O_4 NPs by the usual KBr pellet method are identified using FTIR spectroscopy. FTIR spectroscopy is used to analyse the chemical structure of numerous inorganic chemicals and to perform qualitative analysis of phytochemical substances. FTIR spectra were collected over a wide range of temperatures.

A SHIMADZU-IR PRESTIGE-2 spectrometer was used to measure wavelengths between 400 and 4,000 cm-1. Infrared spectra are produced by a They are often seen as absorbed and emission spectroscopy there in ir region and represent a shift between the two wave numbers of a substance in its ground state. The phases responsible in light spectroscopy are connected to the translational changes in the protein. While various bridges and non - covalent interactions have diverse excitations, it is possible to recognise the presence of a certain bond or alkyl in a compound by identifying this specific wavelength as a fourier transform infrared line. A histogram of (percentage T) permeability vs frequency represents the emission spectra.

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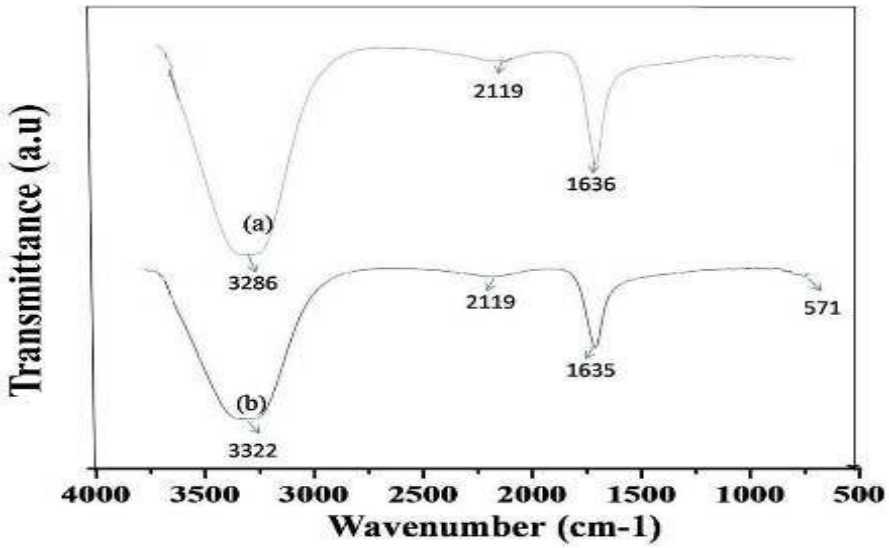

Fig 2. FTIR spectra of AG NPs

3. Transmission Electron Microscopy (TEM)

TEM (TEM model FEI TECNAI 20 U-Twin) was used to analyse the size and form of Ag NPs, ZnO NPs, and Fe_3O_4 NPs at accelerating voltages of 120 and 200 kV. The size distribution and nanostructures of Ag NPs, ZnO NPs, and Fe_3O_4 NPs may be determined using TEM, which is a helpful characterisation tool. The electron beam passes through the material during TEM investigation. The patterns on the sample are replicated by the transmitted beam. TEM samples were made by placing a drop of a very dilute solution of the nanoparticles in a suitable solvent and placing 2L on 300-mesh copper grids, followed by evaporation of the solvent. The grids were then vacuum-dried for an hour in preparation for TEM analysis. The crystalline phase of Ag NPs, ZnO NPs, and Fe_3O_4 NPs can be determined using selected area electron diffraction patterns (SAED). Image J was used to determine the average particle size of the produced Ag NPs, ZnO NPs, and Fe_3O_4 NPs (NIH, USA).

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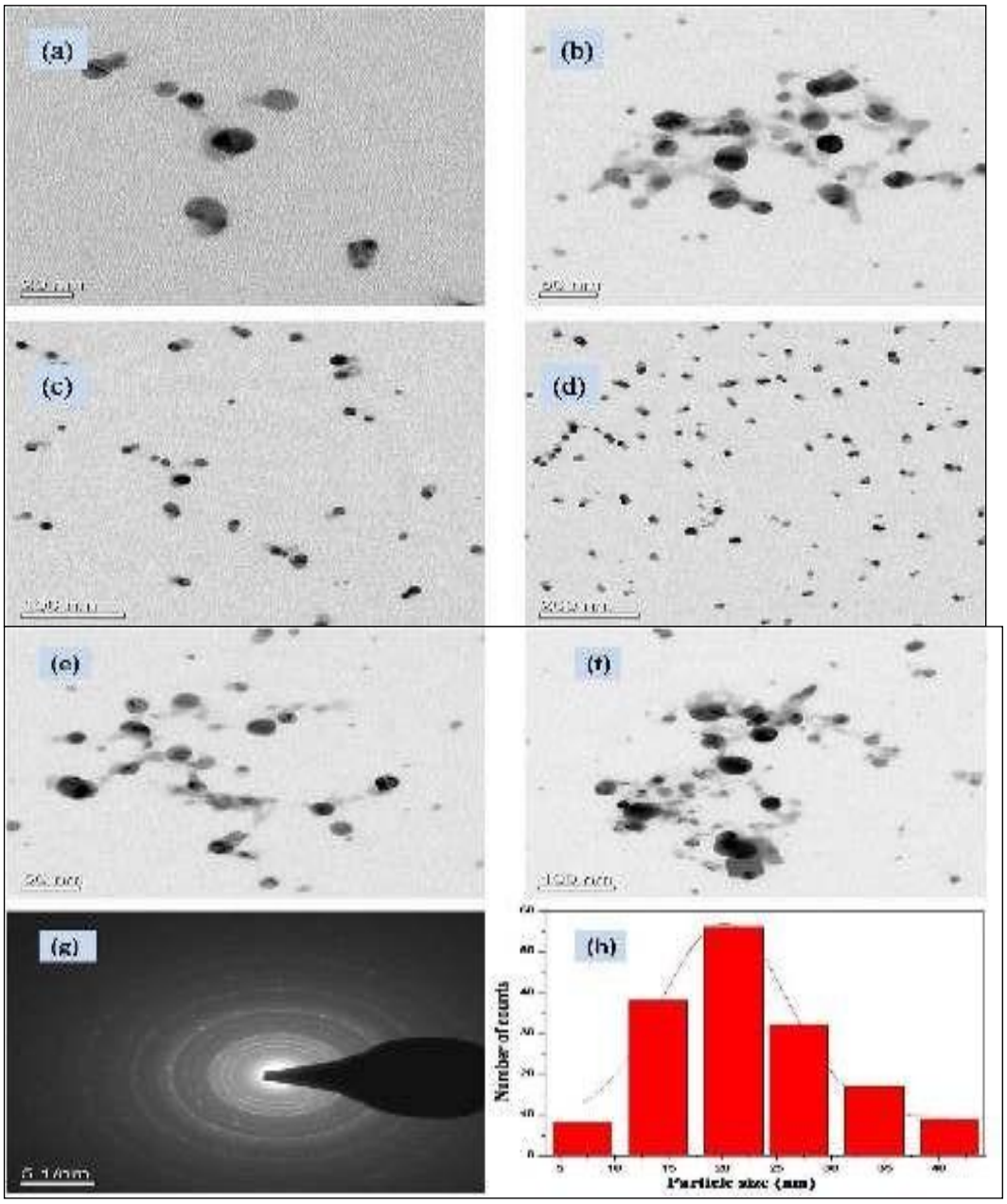

Figure 3: Shows TEM images of synthetic Ag NPs made from FH LF aqueous leaf extract (a-f), a selected area diffraction pattern, and a histogram of the particle size distribution.

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Antimicrobial research

Preparation of the media

Antibacterial investigations were conducted using the Mueller Hinton agar medium. It was created by dissolving manufactured Agar dust in deionized. His medium's pH was changed to 7 by adding 1N NaOH to it. Three test tubes (98 mm x internal diameter) and or the solution (95 mm x 30 cm) were pasteurised at 121 °C for 10 min using 15-pound pressure. Under sterile aerostatic arrangements, the properly sterilized mixture was chilled to 45 °C and then put onto Agar plate (100 µl).

Agar-well diffusion assay of produced Ag NPs using *ZA DC* aqueous bark extract

The agar-well diffusion method was used to study the antibacterial properties of Ag NPs. Using sterile cotton swabs, the standardised cultures of test microorganisms were equally dispersed across the surface of Mueller Hinton Agar plates. A sterile cork borer was used to make five wells (6 mm diameter) in each plate. In each plate, 50 mL of Ag NPs with varied concentrations (100 g / mL, 50 g / mL, and 25 g / mL) were applied to wells No.1, 2, and 3. 50 l of reference antibiotic solution (as a positive control) was added to well No.4. Reference antibiotics were streptomycin (25 g/mL). 50 l of plant extract containing 3% dimethyl sulpha oxide (DMSO) in a 1:1 ratio (as a negative control) was added to the middle well without Ag NPs. Compounds, antibiotics, and DMSO were let to permeate at room temperature for an hour. Each one of the plates were then sealed with lid and heated to 37°C for 24 hours. After treatment, platters were inspected to seek for a region where growth of bacteria was inhibited. The antimicrobial properties of the drugs were displayed as a proportion of both the diameter of approximately of minimum inhibitory concentration (mic, and the normal width of that interference vicinity was measured in millimeter. In two separate studies, the suppressive band widths from each ZNONps concentrations were evaluated in threefold, and the median values were calculated.

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Antimicrobialactivity

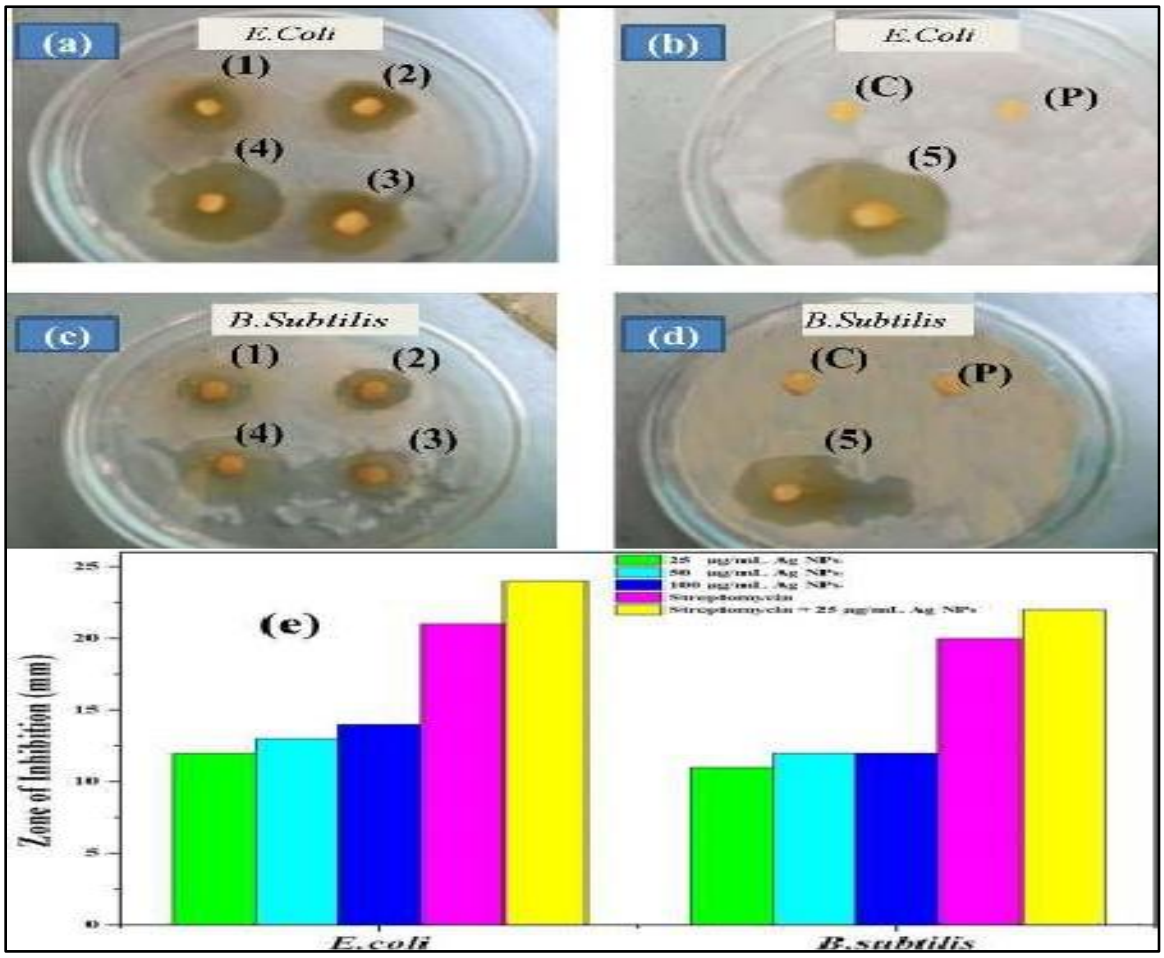

Figure 4: Shows the zone of activity of both the Ag^{+} Ions against with the *Escherichia coli* microorganisms *B.subtilis* and indeed the Month's supply viruses *E. coli* microorganisms. The antimicrobial activity of the synthetic Ag NPs was tested using a leaves extract of the FH LF Synthesized Ag NPs' antibacterial activity was examined using 3 percent DMSO as a control treatment. Above figure shows that neither the negative control nor the 5 percent leaf extract exhibited any antibacterial activity. Ag NPs' antimicrobial activity was discovered to vary on dosage. Ag NPs interact more frequently with bacterial proteins that contain sulphur, which causes cell death, thus increasing their antibacterial action as their concentration rises.

Table 1: FH LF aqueous leaf extract were used to make items Ag NPs, whom were examined in for their antiviral efficacy against the other Month's supply and Month's supply strains of bacteria.

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Bacteria	Inhibition Zone (mm)							
	DMSO Control	leaf extract	25 µg/mL	50 µg/mL	100 µg/mL	25 µg/mL (Streptomycin)	25 µg/mL Streptomycin + 25 µg/mL of Ag NPs	% Enhancement
E. Coli	0	0	12	13	14	21	24	1.03
B. subtilis	0	0	11	12	12	20	22	1.02

As a result, when comparing the Ag NP concentrations listed in Table 1, it was discovered that Ag NPs had antibacterial action against the *B. subtilis* (12 mm) and the Gram-negative bacteria *E. coli* (14 mm) at a concentration of 100 g/mL. Three main categories can be used to characterize how bacteria and Ag NPs interact. Electrostatic attraction between negatively charged Ag NPs and positive charge membrane proteins on the surface of bacteria may be the first strategy during their contacts (carboxylate stabilized). Another strategy would involve physicochemical modifications to the bacterial cell wall, which could result in the ejection of intracellular substances and cell death. Ag NPs are a third strategy that has the potential to cross bacterial membranes.

As a negative control, the antibacterial property of synthetic Ag NPs was examined using a 50 L mixture of 3 percent DMSO and 5% bark extract from ZA DC. The negative control lacked any evidence of antibacterial activity. Ag NPs' antibacterial activity was discovered to be dose-dependent. Ag NPs' antibacterial activity rises with Ag NP concentration because more Ag NPs bind with bacterial proteins that contain sulphur, which causes bacterial cell death [5-8]. Ag NPs were therefore shown to have the maximum bactericidal activities at a dose of 200 g/mL against the gram-positive bugs *B. subtilis* (cm diameter) and the gram-negative organisms *E. coli* (16 mm) (Table 1).

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Studies on antimicrobials

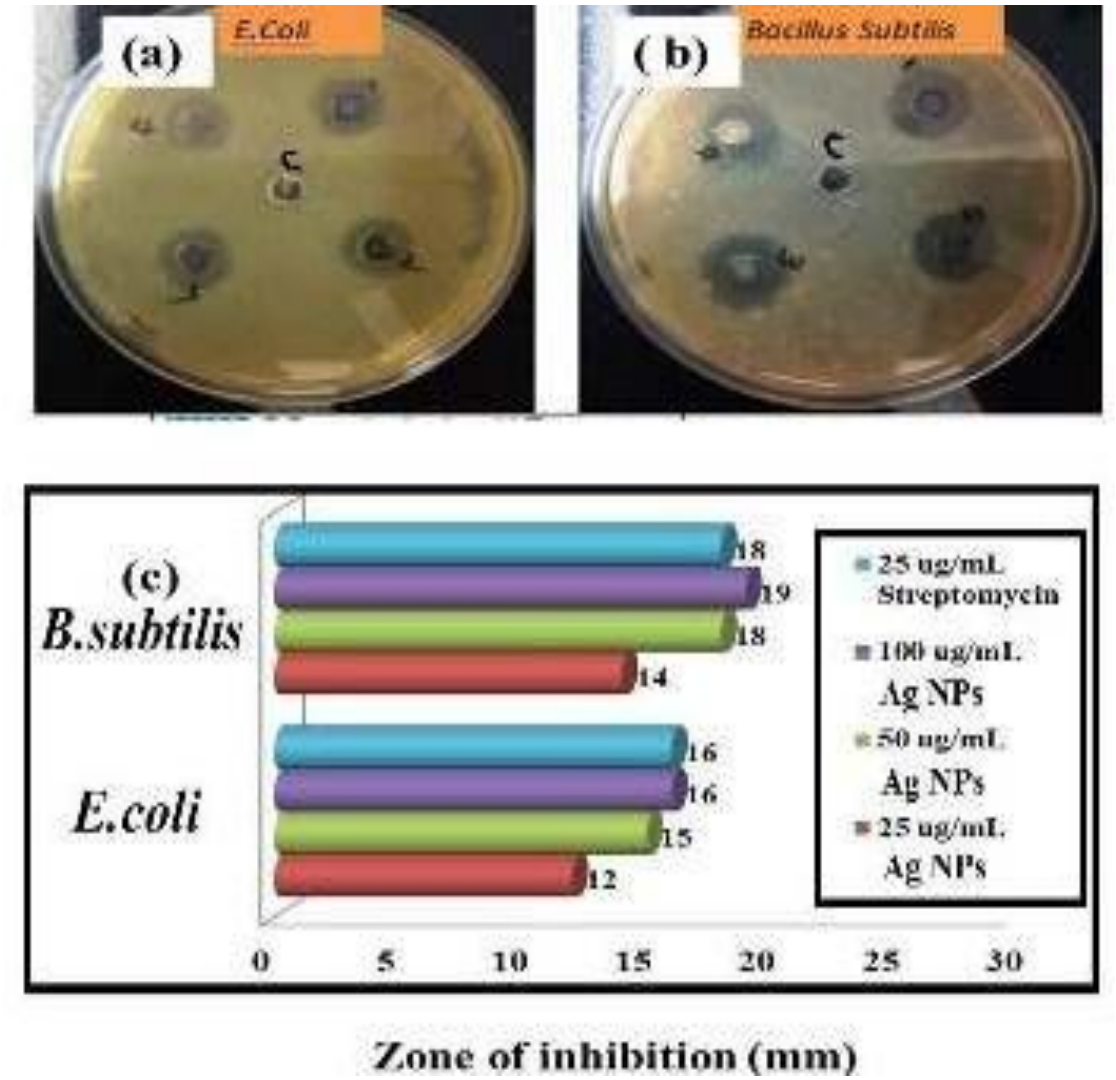

Figure 5: Synthesized Ag NPs have antibacterial action against the microorganisms Gram negative *E. coli*, Gram positive *B. subtilis*, and the effectiveness of disinfection (mm)

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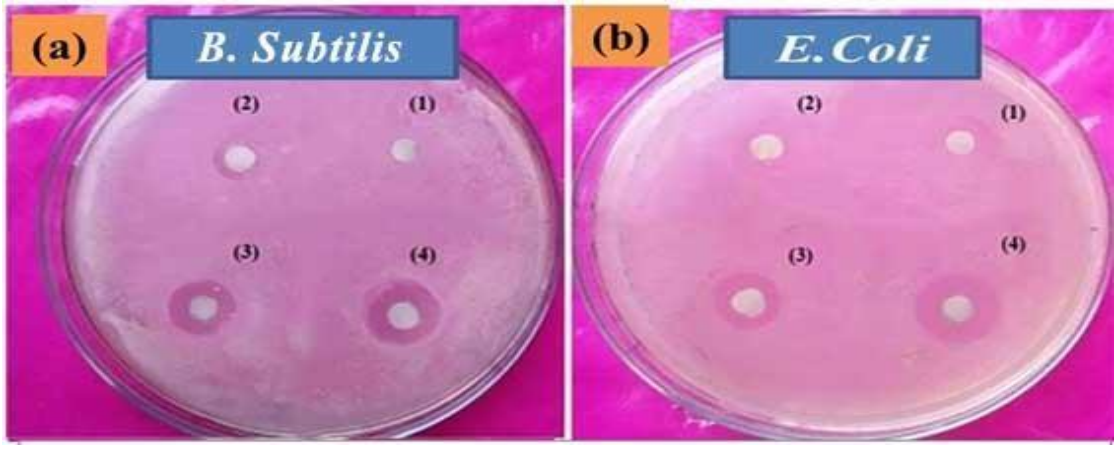
Table 2: Area of inhibition values based on produced Ag NPs' antibacterial activity against harmful microorganisms.

Bacteria	**Zone of inhibition (mm)**				
	25 (µg/mL)	**50 (µg/mL)**	**100 (µg/mL)**	**25 (µg/mL) reference drug (Streptomycin)**	**50µL of %Plant extract (3 % DMSO in 1:1 [Negative control])**
Bacillus subtilis	14	18	19	18	0
Escherichia coli	12	15	16	16	0

Using the agar well diffusion method, the antibacterial activity of ZnO NPs produced from the leaf extracts of ZA DC Linn.f. was examined against a variety of pathogenic organisms, including E. Coli MTCC 443 and B. subtillis MTCC 211. Figure 5 and Table 2 show the zone of inhibition was measured zones (mm) around each ZnO NP-filled well. The symbols 1, 2, 3 and 4 in Figure 5 (a) and (b) stand for DMSO, plant extract, 100 g/mL ZnO NPs, and 20 g/mL Streptomycin, respectively. The antibacterial activity was tested using DMSO, plant extract, 100 g/mL ZnO NPs, and 20 g/mL streptomycin (positive control).



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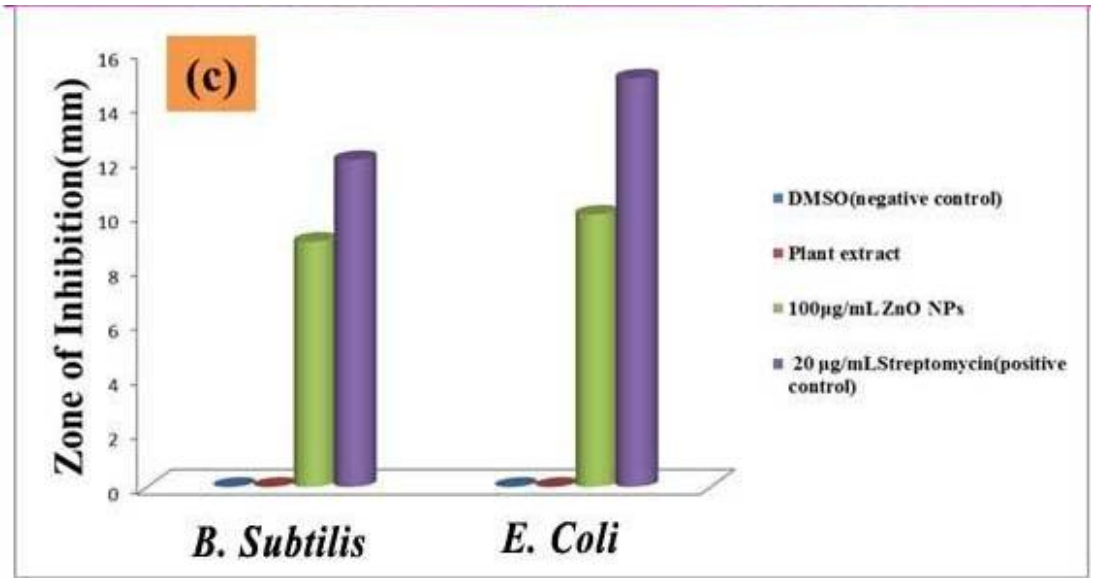

Figure 6: Antimicrobial property of produced ZnO NPs against Gram-positive bacteria B.subtilis, Gram-negative bacteria E. coli, and the effectiveness of ZnO NPs as disinfectants (mm).

Table 3: lists the area of inhibition values for Gram-negative and Gram-positive bacteria that were harmed by ZnO nanoparticles produced using a leaves extract of ZA DC.

Nameof the bacteria	**Zoneof inhibition(mm)**			
	DMSO (negativecontrol)	**Plantextract**	**100 (µg/mL)ofZnONps**	**20(µg/mL)Streptomycin (Positivecontrol)**
*Escherichiacoli(*Gram-negative*)*	0	0	10	15
Bacillussubtilis(Gram-positive)	0	0	9	12

ZnO nanoparticles have antibacterial effect against bacteria because they interact with bacterial proteins that contain sulphur, which interferes with cell respiration and causes ATP leaking, which causes cell death.As a result of (Table 3), ZnO NPs were discovered to have antimicrobial property against *B. subtilis* (9 mm) and Gram-negative bacteria *E. coli* (10 mm)

correspondingly at 100 g/mL concentration. This is caused by changes in the structure of bacterial cell walls, cell physiology, and metabolism.

Conclusion

Nanoparticle research is a focus of scientific study due to its vast range of probable applications in physiological, biochemical, and medical field and multidisciplinary fields. To help the long-term expansion of nanotechnology, potential threats will be assessed to fully grasp NP uses. The studies focus on the green synthesis of Ag NPs, ZnO NPs, and Fe3O4 NPs as well as their antibacterial activity against diverse bacterial strains and spectrum characterization.

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