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ANTIMICROBIAL STUDY OF SILVER NANOPARTICLES AGAINST DIFFERENT MICROBIAL STRAINS

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Abstract: There is data that bacteria can be killed and suppressed by silver nanoparticles (Ag-NPs). A serious public health concern is the recent emergence of pathogenic bacterial resistance to antibiotics. On how Ag-NPs affect an antibiotic's ability to fight bacteria, there is no research. Here, we explain the process of reducing aqueous Ag+ ions using culture supernatants to produce metallic silver nanoparticles. The effectiveness of these nanoparticles in enhancing the antibacterial effects of several medications against Staphylococcus aureus and Escherichia coli is also evaluated in this study. In presence of Ag-NPs, the effect of antibacterial will be shown against test strains. . As a result, we created silver nanoparticles chemically and investigated how they affected microorganisms as antimicrobial agents in this work. Particle size has been recorded by PSA, and the produced nanoparticles have been analyzed using a variety of analytical techniques such UV-visible spectroscopy. Also identified was the zeta potential. X-Ray diffraction examination of the crystalline structure. All the characterization methods mentioned above have effectively demonstrated correct nanoparticle production. E. coli, Staphylococcus, and Pseudomonas were only a few of the bacteria against which the manufactured nanoparticles were tested.
Keywords: Antimicrobial, Silver Nanoparticles, Microbial Strains, Nanoscale Metal Particles, silver nanoparticles

I. INTRODUCTION

Microorganisms like bacteria, mould, yeast, and viruses that are present in theliving environment frequently infect people. There has been a lot of research done on antibacterial materials made of different organic and inorganic compounds.According to the unique physical and chemical characteristics of these materials, including their enzymatic activity, dielectric properties, electronic conductivity, antimicrobial activities, and electromagnetic qualities of nanoscale metal particles (Me-NPs).Among Me-NPs,(Ag-NPs) were discovered to have antibacterial and inhibitive actions. Comparing Ag-NPs to bulk silver metal having a large percentage of surface atoms and a high specific surface area are expected to be the results in higher antibacterial activity.(Shahverdi et al., 2007). Silver nanoparticles have remarkable antibacterial properties, making them a popular tool in biomedical sectors for preventing and treating infections. Due to their detrimental effects on biocompatibility, such as the thrombogeniceffect, silver nanoparticles as an antibacterial agent are increasingly acquiring more and more popularity for medical applications. Chemical reduction is the most widely used and most promising industrial preparation process for silver nanoparticlesowing to its flexibility of use and more yield.(Cao et al., 2010). Because of their effectiveness against a variety of bacteria, viruses, and fungi, silver nanoparticles (Ag NPs) have attracted a lot of attention. According to reports,(Ag NPs) having anti-bacterial activity against the Gm-positive (S. aureus, and E. faecalis) $Gm-negative (E. coli, Pseudomonas and pneumoniae) bacterias.(Calabrese et al., 2022).AgNPs are extensively employed in a variety of industries, including cotton textiles, food packaging, medical goods, and other industries. AgNPs can result in a number of issues for the environment and human health as a result of the increased use of AgNP products. Thus, we must know the toxicity of AgNPs and their mechanisms of toxicity better.(Nie et al., 2023). Thermal degradation of silver complexes, laser-mediated synthesized, microwave-assisted synthesizing, and biologically degradation techniquesare all used to create silver nanoparticles. The most often utilised salt for the production of AgNP is silver nitrate (AgNO3). Functional groups, like hydroxyl groups, have limiting roles.(Singla et al., 2022). They hypothesised that these nanoparticles would be safer than fungicides made of synthetic materials.(Dizaj et al., 2014). From at least 1000 BCE(before the common era), silver has been utilised as an antibacterial agent. Thus, the potential antibacterial activity of silver nanoparticles (AgNPs) was thoroughly researched. Ag NPs stick to cell membranes and create cavities, according to research into the antibacterial mechanism of these particles. They successfully limit protein activity inside the cell, changing the way that microbial cells function.(Burlibaşa et al., 2020). Due to their special properties, a unique substance called silver nanoparticles (AgNP) is antimicrobial, biosensing materials, super conductingand electrical in nature. These nanoparticles are said to be safe for usage on people and effective against to bacteria, viruses, and micro-organisms with no negative effects.(Azwatul et al., 2023).

II. MATERIAL AND METHODS USED

A. Synthesis of Ag nano-particles by chemical method

A very largest amount of NaBH4(sodium borohydrate) needed to stabilise the produced silver (Ag) nano-particles and reducesilver(Ag) ion. Drop by drop (about one drop per second) 0.001M silver-nitrate(AgNO3) was added to 30 mL(milliliter) of cool .002M NaBH4 (sodium borohydride).A magnetic-stirrer will be use to rapidly agitatereaction mixture. The solution turned pale yellow after 2 mL(milliliter) of silver-nitrate($AgNO_3$) will be add, orbrilliant yellow color with the added mixture of all ofsilver-nitrate($AgNO_3$).

$$AgNO_3 + NaBH_4\text{------ } Ag + 1/2\ H_2 + \text{‘}B_2 H_6 + NaNO_3$$

It takes roughly 2-3 minutes to complete the adding. After that, the stirring paused, and the stirrer immediately switched off. Reaction parameters like stirring time and relative reagent concentrations need to be skillfully managed to create consistent yellow crystalline silver. This yellow solution started to aggregate after adding all of the silver nitrate, the colour changed from brighter yellow to violet to grey.. Eventually, the colloid collapsed, releasing the nano-particles shown in figure 1 . After titrating along with silver-nitrate(AgNO3), the extract colour turned pale yellow. The created nanoparticles' light yellow colour indicated that almost all of the silver ions had undergone nanoparticle transformation. A light yellow color can be seen in aqueous solution of silver nanoparticles as a result of surface stimulation.(Padmavathi et al., 2022)

Fig. 1: Preparation of silver nano-particles

B. Characterization of silver nano-particles(AgNPs)

a) UV-Vis Spectrophotometry

Using a comparison of reaction mixtures, between 350-550 nm(nano-meter)utilising UV-visible spectrophotometry, the creation of AgNPs at various concentrations was first and foremost validated.

b) Particle Size Analyser PSA

PSA also known as DLS dynamic light scattering.Particle size analysis(PSA) and zeta potential measurements was done using the dynamic light scattering (DLS) technology. Using a scattering angle of 90°, nanoparticleswas diluted in deionized water at a 200 mg/mL(milligram per milliliter) concentration. Particles size measurements was then carried out at 0.1-10,000 nm range.Results of the analyses were presented asaverage zeta potential and average particle size , which were carried out in triplicate.

c) Zeta Potential

This potential is known as the ZP or electrokinetic potential.A colloid particle moving under an electric field has a potential at its sliding or shear plane. The electric potential of a surface is the work necessary to accelerate a unit positive charge from infinity to that surface. The ZP shows the possible discrepancy at sliding plane between the electrophoretically mobile particles' EDL (electric double layer) and the layer of dispersant around them.(Moradi et al., 2022)

d) Fourier Transform Infrared Spectrophotometry (FTIR)

The functional groups of phytochemicals found on the surface of AgNPs were examined using FTIR. In the transmittance mode, the existence of IR bands was detected when potassium bromide (KBr) crystals and resuspendedAgNPs powder were used as beam splitter in region of 40-400 cm^{-1} was examined.(Hanachi et al., 2022)

C. Disk Diffusion Method to evaluate antimicrobial activity

The different antibiotics were tested in test for antimicrobial action bacteria on agar culture plates using disc diffusion method. Traditional antibiotic discsto ascertain mixed effects, each and every conventional filter paper disc weresaturate with 10 micro L recently created silver NPs at the final concentration of 10ml Ag per disk.. Every test strain were cultivated as a single colony in liquidon a rotary shaker, medium at 358°C for an entire night.To make the inoculate, overnight cultures were diluted with 0.9% NaCl to a 0.5 standard, which was then placed in culture plates alongside , standard and created discs contains various concentrations of silver-

NPs. As test strains, we used pseudomonas,Staphylococcousaureus and Escherichiacoli (antimicrobial) isolates were collected. Similar tests performed using only Ag-NPs. The zones of inhibition were measured following an 18-hour incubation period at 35 degreeC. The assays were carried out .(Shahverdi et al., 2007).

III. RESULTS AND DISCUSSIONS

A. UV-Analysis

Fig.2.displays the silver nanoparticle UV-(vis)visible spectra graph. The significant peak will be at 390nm(nano-meter) was revealed in UV-vis spectrum. Agnano-particles responded to UV- rays better as result. The silver nanoparticles' conductivity was dramatically boosted, and this characteristic significantlyactivates their contact with bacteria**.**(Padmavathi et al., 2022)**.**

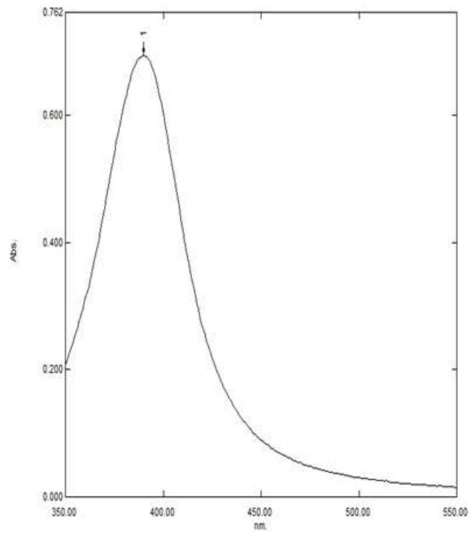

Fig. 2: UV Spectra of silver nanopartic

B. Analysis of Particle Size of Ag NPs

PSA (Particle Size Analyzer) examination confirmed the prepared sample's estimated size. The PSA micrograph that was taken; the peak will be in between 10 and 50 nm. It gives us the rough idea of the size of silver nanoparticles .(Yaseen et al., 2023)

C. Analysis of Zeta Potential

The stabalised NPs will be investigated using zeta-potential analysis. It confirmed the NPs' incredible stability. The high stabilization of NPs will demonstrate as- 32.1 mV zeta-potential around particle's surface. In the previous examination, we saw a negative charge on the Ag 2ONPs' surface, which represents the charge. The results show that long-term structural stability is possible for the synthesised Ag2 ONPs. The maintenance of the nanostructure that reflects the attributes of the nanoparticle based on its shape and size shall demonstrate nanoparticle stability. According to reports, NPs made from chemicals are more stable. The chemically produced silver NPs are discovered to be more stable and closely similar to prior results.(Sujatha et al., 2023)

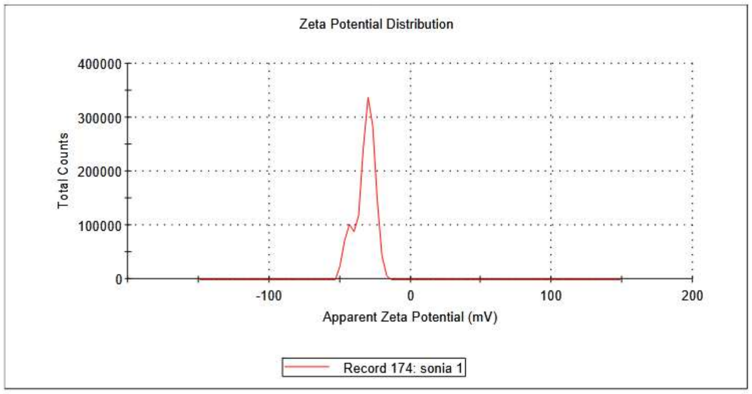

Fig. 3: Zeta Potential of silver nanoparticles

D. Analysis of FTIR (Fourier Transform Infra-red Spectroscopy)

The purpose of the FT-IR study for determine the AgNPs' surface chemistry and the involvement of active functional groups in AgNP production. (Yaseen et al., 2023). The absorption peak is at roughly 3326 cm^{-1}(centimetre) in the spectrum peaks of Ag(silver) NPs indicates –OH presence. Presence of C-H stretched vibrations is shown by peaks at about 2975 cm^{-1}. The 1654-cm^{-1} peak reveals C-C groups. The C-O groups are indicated by 1379 cm^{-1} and 1042 cm^{-1}. A 3252 cm^{-1}(centimeter)-wide absorption peak that indicates the existence of O-H groups. The existence of C-H groups is shown having peaks at 2930 cm^{-1} and 2860 cm^{-1}. Existence of (carbon-carbon) C-C functional groups is shown that peak will be at 1605 cm^{-1} , whereas 1017 cm^{-1} suggested FTIR study that the nitrile group is highly adsorbs on Ag NPs(nano-particles). In the FT-IR analysis, it is believed that there has been a significantinteraction of all functional groups, including alkyl group, -oh, and alcohol groups, is absorbed.(Liang et al., 2022)

E. Anti-microbial Activities of Silver NPs by particular strains

Gm-positive and gm-negative organisms helpful in investigate antibacterial activity of Ag NPs. Images of the Ag NPs' Zone of Inhibition Against Gram +ve and Gram -ve Organisms Fig4a,band c .The zone of inhibition diameter(ZOI) of Ag nano-particles against gm-positive and gm-negative organisms was shown in table1. Ag NPs were reported to exhibit superb antibacterial action against both bacteria. The size of the inhibition zone reveals how susceptible the bacteria are. In contrast to Gram ve bacteria, Gram +ve bacteria displayed a greater zone of inhibited growth, which suggests that Gm +ve bacteria was highly sensitive towards AgNPs.

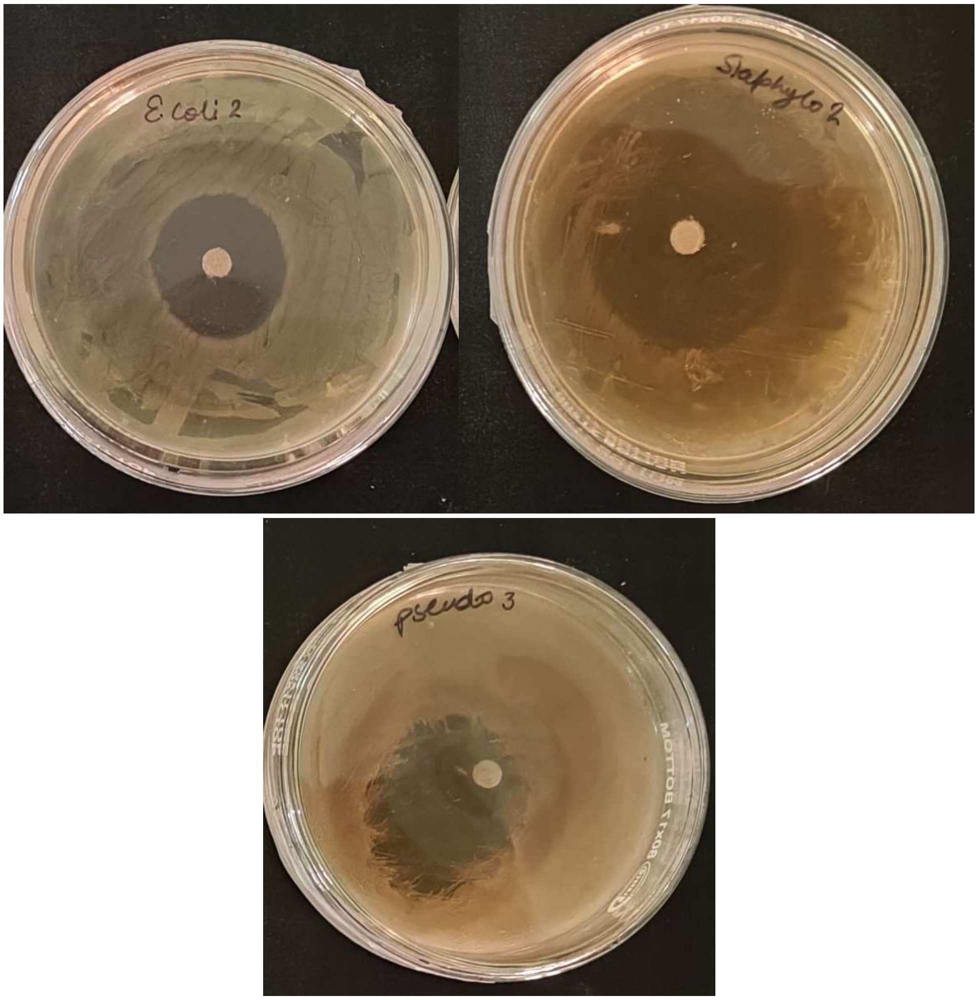

Fig. 4: antimicrobial activity ofAgNPs

- The Ag nano-particles have anti-bacterial properties that are helpful in treating a variety of topical disorders brought on by diverse pathogens. Green synthetic Ag NPs' antibacterial action is based on their ability to connect with released ions from nanoparticles.Small nanoparticles(NPs) typically having higher surface-to-volume and dispersion ratios, allowing for increased interaction with microbe surfaces. When Ag NPs are distinguished from other trace metals, hydroxyl radicals are produced. These radicals connect to DNA molecules, which damages vital proteins and leading disordering of the DNA structure. The results of the current investigation definitely show that Ag NPs demonstrated outstanding antibacterial action against the Gm +ve and Gm-ve pathogens. Due to small size, themore energetic active absorption, according to previously published studies, which kills organism activity. Ag NPs are demonstrated in the current research paper will more effective against Gm +ve and Gm-ve bacteria because of distinct outer cell wall shape. Highest zone of inhibition(ZOI) will be find in Ag NPs against pseudomonas,S.aeurus and E. coli.(Moradi et al., 2022).

Serial No.	Compound(AgNPs)	Gm(-ve) / Gm(+ve)Bacteria	Zone of inhibition (Diameter in mm)		
			150 µl	200µl	250µl

1.		E.coli (Gm –ve)	16	18	20
2.		Pseudomonas(Gm –ve)	17	19	21
3.		S.aureus (Gm+ve)	22	24	26

Table 1: By using Compound (AgNPs) zone of inhibition of bacterial strains at different concentrations

IV. CONCLUSIONS

Gm-positive and Gm-negative bacterias, along with mouldsand yeasts, impact humans. Silver nanoparticles (AgNPs), mostly chemical ones, offer powerful antibacterial characteristics in combating of microorganisms. More specifically, chemical biological AgNP production is safer and more effective than green AgNP production, primarily due to the higher concentration of AgNPs produced. The findings contribute to overcoming disease-related antibiotic resistance, boosting the economy, and ultimately enhancing human health.

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