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EXPLORING THE EFFICACY OF ACACIA CATECHU-MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES FOR INFECTION CONTROL IN BURN WOUNDS

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ABSTRACT

The present research explores the use of eco-friendly silver nanoparticles (AgNPs) created through a green method, employing *Acacia Catechu* extract, to combat infections in burn wounds. The successful synthesis of AgNPs was confirmed using UV-Visible spectroscopy, with the extract acting as a reducing and stabilizing agent. The AgNPs' size and shape were determined through Scanning Electron Microscopy and Zeta Sizer. These nanoparticles displayed potent antimicrobial properties against a range of bacteria commonly associated with burn wound infections, showing promising results in agar well diffusion assays and minimum inhibitory concentration (MIC) determination. The antimicrobial action involved disrupting microbial cell membranes and generating reactive oxygen species (ROS), leading to oxidative stress within the cells. The green synthesis approach presents a sustainable alternative, avoiding hazardous chemicals, and the natural plant extracts may contribute to wound healing and anti-inflammatory properties, further enhancing the therapeutic effectiveness of the AgNPs. This study underscores the potential of *Acacia Catechu*-mediated AgNPs as a valuable solution for preventing infections in burn wounds, prompting further investigation into their in vivo effectiveness, wound healing capabilities, and long-term stability. Ultimately, this research concludes that *Acacia Catechu*-mediated AgNPs hold promise for safer and more effective wound care

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INTRODUCTION

Silver nanoparticles (AgNPs) have drawn a lot of interest because of their distinctive characteristics and prospective uses in a variety of industries, including medicine [1]. However, limitations prevent the widespread application of conventional methods for producing silver nanoparticles. These techniques, including chemical reduction utilizing substances like citrate or sodium borohydride, can produce hazardous byproducts and are not eco-friendly. They can also be costly due to the requirement for specialized equipment and time-consuming due to the possibility that the nanoparticle size is not uniform. Traditional synthesis techniques are less suitable for specialized applications like the treatment of burn wounds due to these drawbacks [2].

Green technologies for the synthesis of silver nanoparticles have emerged as a viable approach for the creation of long-lasting burn wound dressings. Science has adopted environmentally friendly procedures as awareness of the negative effects of traditional synthesis techniques on the environment has grown. The use of natural extracts as reducing agents in a process called "green synthesis" presents a practical substitute for conventional chemical processes. These antibacterial silver nanoparticles from green synthesis are useful for avoiding infections and hastening the healing of burn wounds [3]. Additionally, their production adheres to the ideals of sustainable healthcare by reducing the use of hazardous chemicals and non-renewable resources [4]. This article examines green synthesis, its benefits for the manufacturing of silver nanoparticles, and its substantial contribution to the development of ecologically friendly and efficient burn products.

A medicinal plant called *Acacia Catechu*, also called *Catechu* or Black cutch, can be found throughout Asia. It is also scientifically refered as *Senegalia Catechu* belongs to the family *Fabaceae*. A wide variety of phytochemicals, including as flavonoids, tannins, and phenolic compounds, are present in the heartwood extract of *Acacia catechu*, which have considerable wound healing capabilities [5].

In the green manufacture of silver nanoparticles, the extract of *Acacia Catechu* heartwood has been used as a reducing and stabilizing agent. The bioactive substances in the extract would aid in the stabilization of the nanoparticles and the decrease of silver ions [6]. Additionally, the natural wound-healing abilities of *Acacia Catechu* heartwood extract can improve the therapeutic effects of silver nanoparticles [7]. According to reports, the extract possesses antioxidant, anti-inflammatory and pro-collagen synthesis properties, all of which are essential for accelerating the healing of wounds. These characteristics support the production of collagen, a crucial element of the extracellular matrix required for tissue regeneration and wound healing, as well as the reduction of oxidative stress, inflammation, and other negative health effects [8].

Burn wound care can be considerably aided by the synergistic interaction between the antibacterial capabilities of silver nanoparticles and the wound-healing properties of *Acacia Catechu* heartwood extract. While the extract's wound healing capabilities which are characterized by antioxidant and anti-inflammatory activities aid in a quicker and more effective healing process, the antibacterial activity of the silver nanoparticles helps prevent infection [9–11]. The development of enhanced burn wound dressings that not only fight infections but also encourage the best possible healing outcomes has a lot of potential because to this dual-action strategy.

The present study aims to employ an environmentally sustainable method for the production and analysis of silver nanoparticles which can be achieved by reduction of silver ions using the natural phytoconstituents of *Acacia Catechu* extract. Subsequently, our investigation will center on assessing the antimicrobial efficacy of these eco-friendly nanoparticles against bacterial strains responsible for infections in burn wound cases.

Through this study, we aim to shed light on the potential of these eco-friendly nanoparticles as an effective solution for combating such infections.

MATERIALS AND METHODS

Materials:

Chemicals: Black Catechu (Crude Extract of *Acacia Catechu* Heartwood), Pure Crystalline Silver Nitrate and Catechin Hydrate (Marker) were acquired from Research Lab Fine Chem Laboratories, Mumbai, India. AR grade Methanol was acquired from Loba Chemie Pvt.Ltd, Mumbai, India.

Methods:

Preparation of Different Extracts of *Acacia Catechu*:

Water and methanol were used as two separate solvents to create crude *Black Catechu* plant extracts. The solvents were chosen in accordance with the high extractive values discovered during preformulation investigations carried out in accordance withpharmacopoeial protocol [12]. The extracts were made using the Soxhlet extraction method. To summarise, Soxhlet extraction was performed using 5g of powdered crude drug and 100ml of the appropriate solvent. The resultant extracts were stored in the refrigerator to be used later.

Biosynthesis of Silver Nanoparticles:

Briefly, 0.0169 g of Silver Nitrate was dissolved in 100 ml of distilled water to create a 1Mm aqueous solution, which was then transferred to an amber-colored container. Then, a 1:9 ratio of each of the produced aqueous and methanolic extracts was added to the 1Mm Silver Nitrate extract. After that, the solutions were left to go through reduction for 48 hours. The darkening of solution colours served as an indicator of the reduction process and production of silver nanoparticles [7].

Evaluation of Silver Nanoparticle Synthesis Method:

Visual observations for stability and the assessment of the Surface Plasma Resonance (SPR) effect by UV- Visible Spectroscopy Studies (Jasco UV-VIS Spectrophotometer) were used to assess the biogenesis of silver nanoparticles [13,14]. The occurrence of an absorption peak in the 400–500 nm region provided more evidence of the SPR effect.

Characterization of Silver Nanoparticles:

The prepared silver nanoparticles were then centrifuged at 6000 rpm for 15 minutes after being filtered using Millipore 0.2 m cellulose acetate filters. The nanoparticulate residue was then cleaned, diluted with distilled water, and held for further evaluation after the supernatant was discarded.

Scanning Electron Microscopy Studies:

The FE-SEM (FEI Nova NanoSEM 450, Lincoln, USA) was used to examine the size and morphology of the AgNPs after they had been re-dissolved in molecular grade sterile water and drop cast onto silicon wafers for SEM examinations.

Zeta Potential Studies:

Estimating the surface charge of biosynthesized AgNPs, which represents their stability was done using the zeta potential measurement [15]. In order to dissolve aggregates of nanoparticles, the colloidal solution of AgNPs was 10-fold diluted with molecular grade sterile water and homogenised using a Probe Sonicator for 15 min at 20 Hz. After passing through a Millipore filter (0.22 m), the nanoparticle solution was examined using Zetasizer (Malvern Instruments Ltd., Malvern, United Kingdom).

Particle Size Distribution:

To ascertain the size distribution pattern of AgNPs produced during biosynthesis, the particle size distribution was examined. Zetasizer (Malvern Instruments Ltd., Malvern, United Kingdom) was used to analyse the colloidal suspension of AgNPs after it had been diluted 10 times with distilled water.

Fourier Transform Infrared Spectroscopy Studies:

The FTIR 4100 Jasco spectrometer was used to analyse the functional groups on the surface of AgNPs between the wavelengths of 4,000 and 400 cm-1. Dried nanoparticles were combined with potassium bromide (KBr) to provide a sample in powder form for FTIR measurement. The same analysis was performed on the dried plant extract, and the resulting spectrum was compared to that of biosynthesized nanoparticles.

Antimicrobial Activity

In Vitro Antimicrobial Activity through determination of zone of inhibition:

Using an agar-well diffusion experiment, the zone of inhibition (ZOI) brought on by antimicrobial AgNPs was identified. A little under 20 mL of solidified material were placed in presterilized glass petri plates together with a microbial culture. With the aid of a sterile corn borer with an 8 mm diameter, aseptic holes were made in the surface of the solidified media, and 100 l of each sample—AgNPs, 5% aqueous extract of *AcaciaCatechu*, distilled water, and negative control—was pipetted into each well. Both plates were then incubated at 37 °C for 24 hours before being checked for the presence of an inhibition zone. The specimens' level of antibacterial effectiveness was assessed using the diameter of the zone of inhibition in comparison to negative control [16,17].

Determination of Minimum Inhibitory Concentration (MIC):

By using a broth dilution experiment, the minimal inhibitory concentration was identified. As a solvent, DMSO was used to dissolve the test substance. For each chemical, a primary stock containing 10,000 g/ml was created. In sterile Muller Hinton Broth, a secondary stock weighing 1000 g/ml was made. Later, each compound's concentration was serially reduced from 250 g/ml to 0.9 g/ml. All of the diluted chemicals had the appropriate labels. Aseptic techniques were used to prepare the plates. The 96-well sterile plate was labelled in accordance with the planned experiment. The first well for each medication was loaded with 300ul of the medication (1000ug/ml in MHB). 100ul of sterile MHB were placed in each of the other wells. The first column was kept as the Growth Control (GC), containing media and organism only without drug, and the second column was kept as the Sterility Control (SC), containing media only without any drug or organism. 100ul of the drug from the first well was transferred aseptically to the second well and serially diluted until well number 10. 100ul of bacterial suspension with a 5 × 105 CFU/ml concentration was loaded from the second to the eleventh well after the medications had been serially diluted. The plates were sealed and incubated for 18 hours at 37 ° C [18]. After the incubation plates were examined for MIC using visual turbidity, a microplate reader (Read well Touch-2019) was used to analyse the results. The absorbance was taken at 600nm.

RESULTS AND DISCUSSION

Evaluation of Silver Nanoparticle Synthesis Method:

The visual examination of silver nanoparticles synthesis revealed that the biosynthesis of silver nanoparticles using methanol extract resulted in precipitation of the extract and therefore rendered the system unstable as shown in Figure 1.

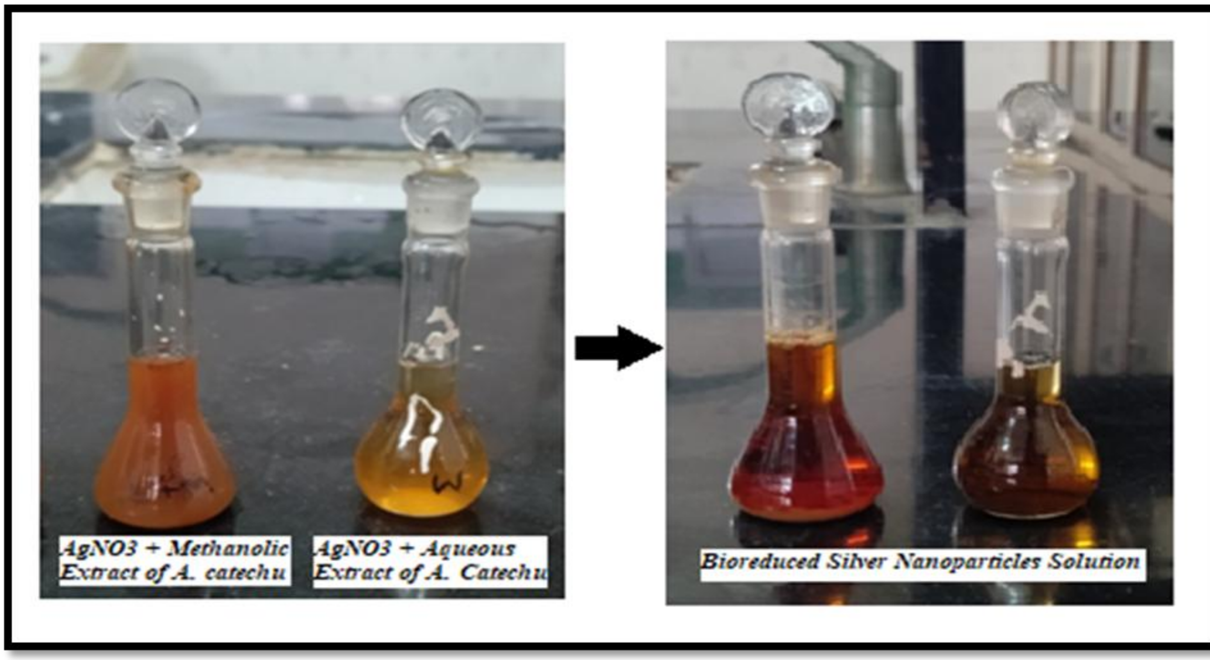

Figure 1- Bioreduction Of Silver Nitrate To Silver Nanoparticles using *A.Catechu* Extract in different solvents.

The bioreduced silver nanoparticles formed using aqueous extract of *Acacia Catechu* were found to be stable and were further evaluated for Surface Plasma Resonance effect using UV-Vis Spectroscopy. The SRP effect of Silver nanoparticles was confirmed by the presence of λ max at 452 nm as shown in the Figure 2.

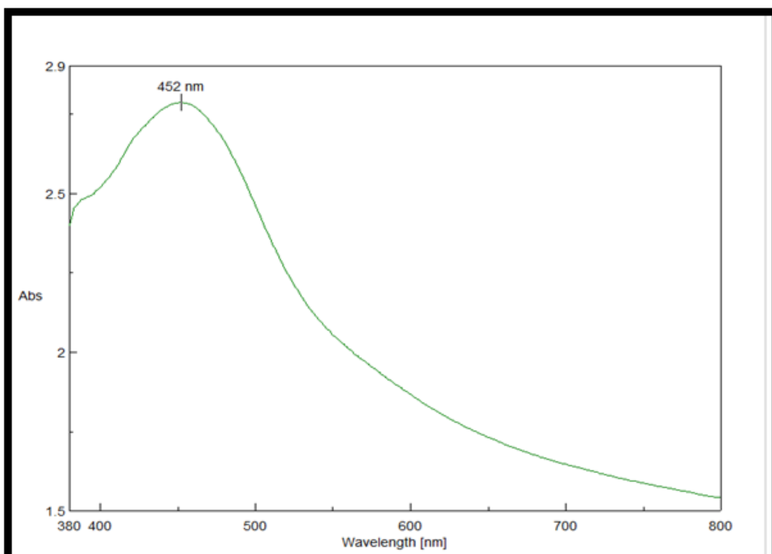

Figure 2- Maximum absorbance wavelength in UV Vis spectroscopy that confirms SRP effect.

Characterization of Silver Nanoparticles:

Scanning Electron Microscopy Studies:

The shape and size of nanoparticles was confirmed through FE SEM Studies. The Nanoparticles appeared to be spherical in shape when observed at 100000X magnification. The size of the nanoparticles were found to be in the range of 70 - 180nm as shown in Figure 3.

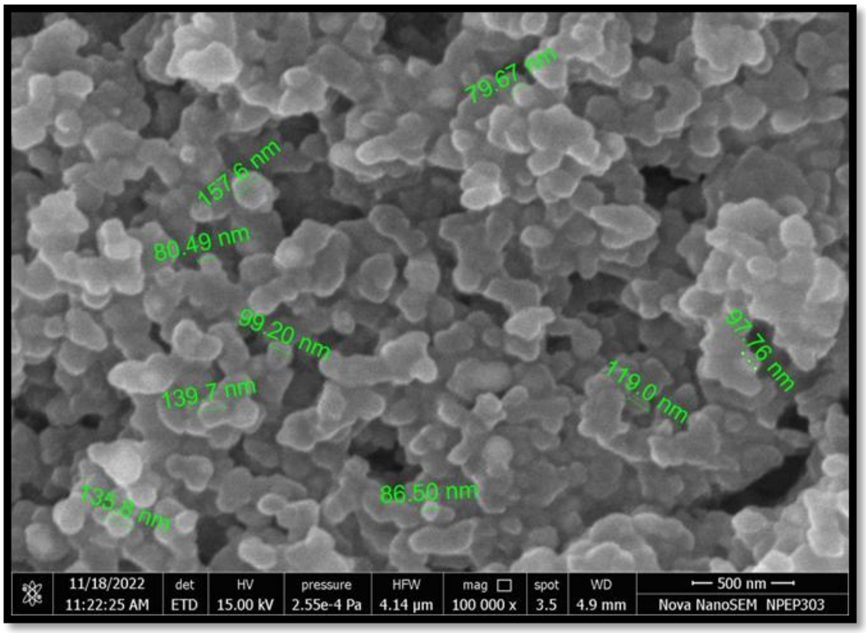

Figure 3- SEM image at 100000x magnification.

Zeta Potential Studies:

The Zeta Potential studies revealed that the biosynthesized Silver nanoparticles were negatively charged (−18.2 ±0.1024 mV) which was found to be in an acceptable range for stability. The zeta potential results are shown in Figure 4.

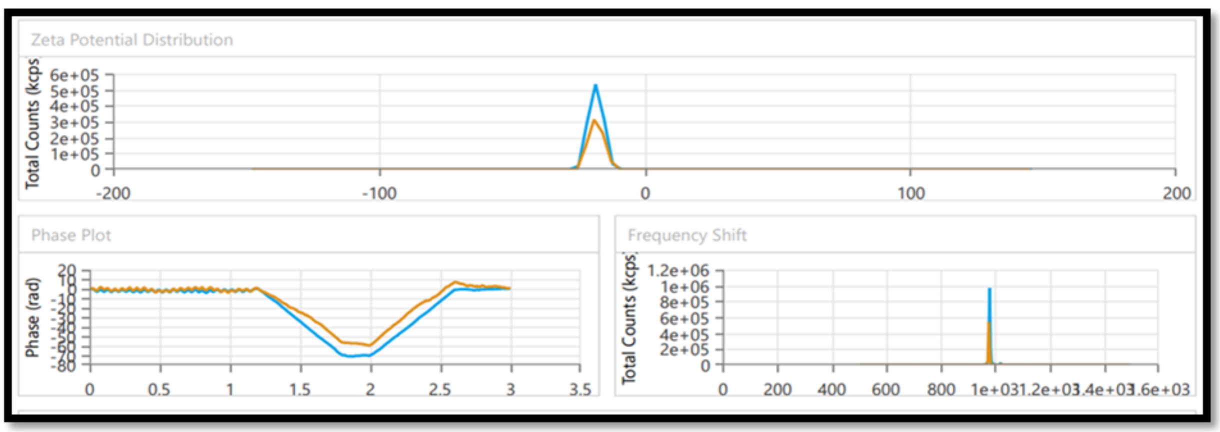

Figure 4- Results of zeta potential determination.

Particle Size Distribution (PSD):

The particle size distribution was analyzed using Malvern Zeta Sizer. The PSD was found to be around 112.5 nm in diameter. The results are shown in the Figure 5.

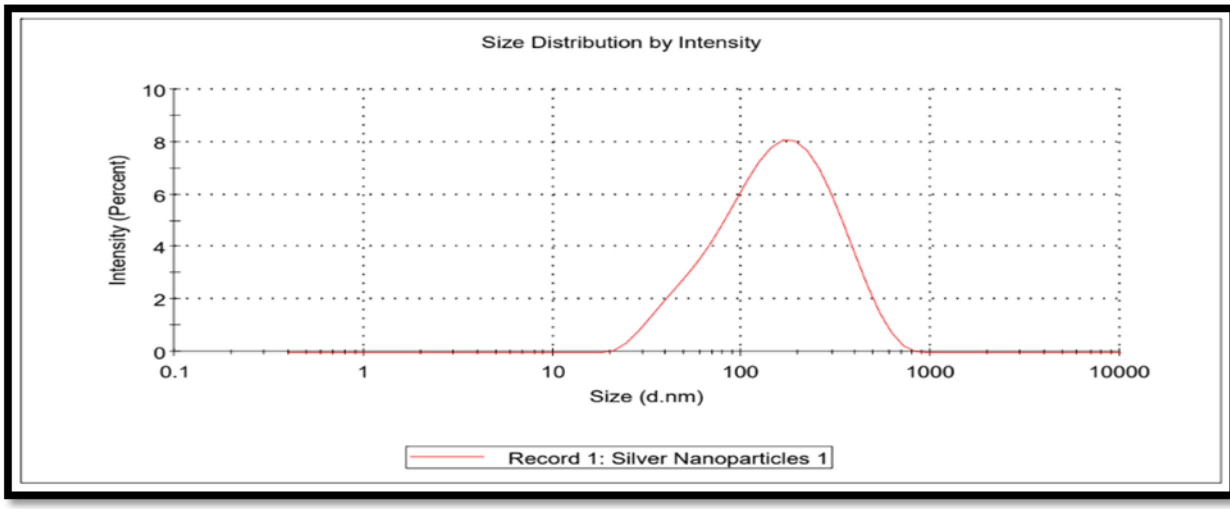

Figure 5- Results for particle size distribution of silver nanoparticles.

Fourier Transform Infrared Spectroscopy Study:

Major peaks in the silver nanoparticles' FT-IR spectrum were found at 3343 cm^{-1}, 2923 cm^{-1}, 2857 cm^{-1}, and 1608 cm^{-1}. The Ag NPs are stabilised in this spectrum by a prominent absorption signal peak at 3343 cm^{-1}, which is connected to the N-H bond of the amine groups of the greenly synthesised Ag NPs. Thus, the appearance of molecules peculiar to the N-H group confirm the conversion of AgNO3 to Ag. A sharp signal peak at 2923 cm^{-1} may be connected to the alkyne group in the biomolecules of the *A. Catechu* extract, while another peak at 1608 cm^{-1} is related to the stretching of C=O. The apparent peak at 2356 cm^{-1} in the herbal extract may correlate to tannin components' N-H and C-O stretching during the biosynthesis process [19]. The overlay of the FT-IR spectra of the biosynthesized AgNPs and the aqueous extract of *Acacia Catechu* are shown in the Figure 6.

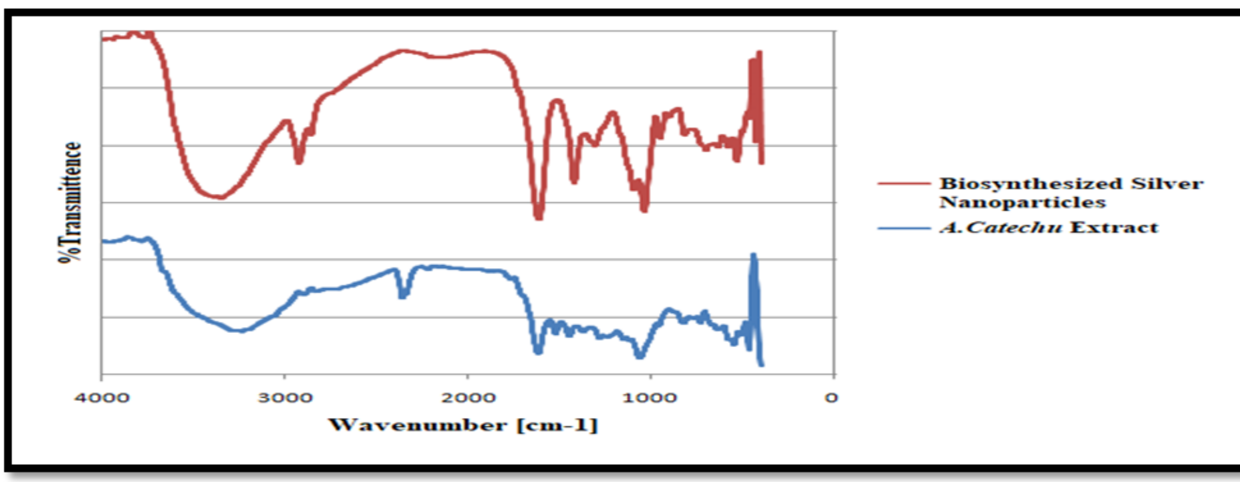

Figure 6- FT IR spectra of silver nanoparticles and *A.catechu* extract.

In Vitro Antimicrobial Activity:

The zone of inhibition of the biosynthesized AgNPs was found to be of 15 mm for *Staphylococcus aureus* and 14 mm for *Pseudomonas aeruginosa* as shown in the Figure 7. The comparable results are shown in Table 1.

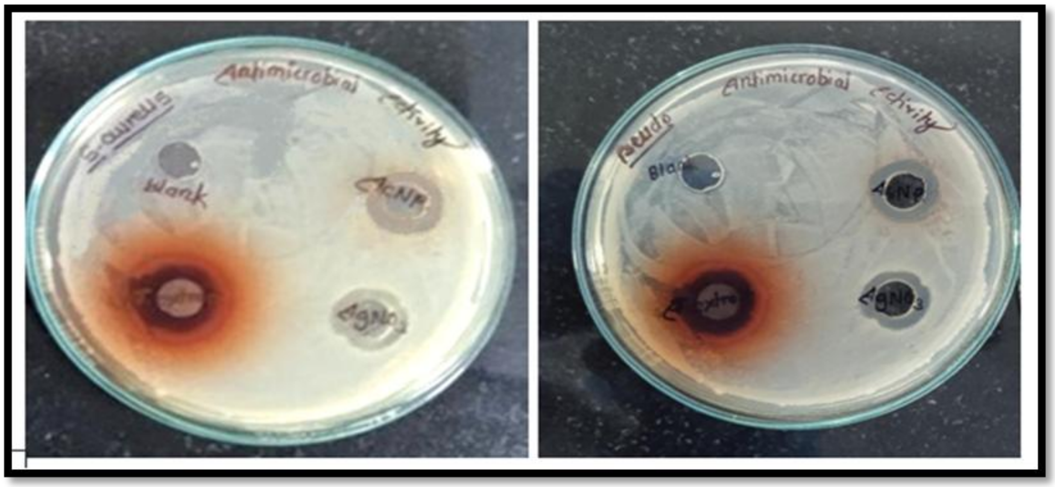

Figure 7- Zone of inhibition as observed for *Staphycoccus aureus* and *Pseudomonas aeruginosa*.

Table 1- Zone Of Inhibition of Samples.

Sample No.	Sample Name	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
1	Blank	No zone of Inhibition	No zone of Inhibition
2	*A.Catechu* extract	14mm	13mm
3	AgNO3	12mm	13mm
4	AC.NP(AgNP)	15mm	14mm

The Minimum Inhibitory Concentration of the AgNPs against *S. aureus* and *P. aeruginosa* was found to be **31.2** µg/ml and **62.5** µg/ml as shown in Table 2. These ranges were found to be comparable to that of topical Silver Sulfadiazine which has an MIC of 50 µg/ml for against both the strains [20].

Table 2- Minimum Inhibitory Concentration Values of Sample.

Compounds	MIC (ug/ml) for *S. aureus*	MIC (ug/ml) for *P. aeruginosa*
Acacia catechu extract	62.5	125
Silver nanoparticles (using above extract)	31.2	62.5
Combination of both	31.2	62.5

Burn wounds pose significant challenges due to their susceptibility to infection, which can severely impede the healing process and increase morbidity and mortality rates. Traditional approaches for infection control in burn wounds often rely on synthetic antimicrobial agents, which may have limitations such as toxicity, antibiotic resistance, and environmental concerns. Therefore, there is a growing need for the development of alternative, safe, and effective strategies. This study aimed to explore the potential of green-synthesized silver nanoparticles (AgNPs) using *Acacia Catechu* extract as a novel approach for infection prevention in burn wounds.

The results of our study demonstrated successful green synthesis of AgNPs using *Acacia Catechu* extract as a reducing and stabilizing agent. The formation of AgNPs was confirmed by UV-Vis spectroscopy, which showed a characteristic peak at 452nm. The average size and morphology of the nanoparticles were analyzed using Scanning Electron Microscopy and Particle Size Distribution, revealing spherical shape of the biosynthesized nanoparticles with an average particle diameter of ~112 nm. The stability of the synthesized AgNPs was assessed by determination of zeta potential which was found to be -18.2 mV.

The antimicrobial efficacy of the *Acacia Catechu*-mediated AgNPs was evaluated against pathogenic microorganisms commonly associated with burn wound infections. The AgNPs exhibited potent antimicrobial activity against both Gram-positive and Gram-negative bacteria. The zone of inhibition observed in the Agar well diffusion assay indicated the ability of AgNPs to hinder the growth of tested microorganisms. Furthermore, minimum inhibitory concentration (MIC) assays demonstrated concentration-dependent antimicrobial efficacy of the AgNPs, with lower MIC values indicating higher potency. In conclusion, The study demonstrates the successful green synthesis of AgNPs using *Acacia Catechu* extract and highlights their potential for infection prevention in burn wounds. The synthesized AgNPs exhibited potent antimicrobial activity against both, gram positive and gram negative pathogenic microorganisms. The use of *Acacia Catechu* extract as a reducing and stabilizing agent provides a sustainable and environmentally friendly approach for AgNP synthesis. Further studies are warranted to explore the in vivo efficacy, wound healing properties, and long-term stability of these green-synthesized AgNPs, with the ultimate goal of translating them into effective therapeutic strategies for burn wound management.

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COMPETING INTERESTS

The authors declare no conflict of interest.

ABBREVIATIONS

UV	Ultraviolet
IR	Infra Red
FT IR	Fourier Transform Infra red
AgNP	Silver Nanoparticles
AgNO3	Silver Nitrate
MIC	Minimum Inhibitory Concentration
DMSO	Dimethyl Sulfoxide
FE SEM	Field Emission Scanning Electron Microscopy
AC NP	Acacia Catechu mediated Silver Nanoparticles
mg	milligram
µg	microgram
ml	milli litre
µl	microlitre

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